Cancer Treatment and Research
Series Editor: Steven T. Rosen

Andrew M. Evens
Kristie A. Blum *Editors*

Non-Hodgkin Lymphoma

Pathology, Imaging, and Current Therapy

Indexed in PubMed/Medline

Springer

Cancer Treatment and Research

Volume 165

Series editor
Steven T. Rosen, Duarte, CA, USA

More information about this series at http://www.springer.com/series/5808

Andrew M. Evens · Kristie A. Blum
Editors

Non-Hodgkin Lymphoma

Pathology, Imaging, and Current Therapy

Springer

Editors
Andrew M. Evens
Division of Hematology/Oncology
Tufts Medical Center
Boston, MA
USA

Kristie A. Blum
Division of Hematology
Ohio State University Comprehensive
Cancer Center
Columbus, OH
USA

ISSN 0927-3042
Cancer Treatment and Research
ISBN 978-3-319-13149-8 ISBN 978-3-319-13150-4 (eBook)
DOI 10.1007/978-3-319-13150-4

Library of Congress Control Number: 2014956714

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Contents

Epidemiology and Etiology of Non-Hodgkin Lymphoma

Brian C.-H. Chiu and Ningqi Hou

Abstract

Non-Hodgkin lymphoma (NHL) consists of many histologically and biologically distinct lymphoid malignancies with poorly understood, but possibly distinct, etiologies. The patterns of incidence and time trend vary not only by age, sex, and race/ethnicity in the USA, but also show significant geographic differences, suggesting the potential role of infectious agents, environmental factors, and lifestyle factors in addition to host genetic status in the development of NHL. Important pathogenetic mechanisms include immune modulation and chronic antigen stimulation. Epidemiologic studies in the past two decades have provided intriguing new insights on the possible causes of lymphoma and support the idea that there is some mechanistic commonality of lymphomagenesis, but significant etiologic heterogeneity clearly exists. This review presents a summary of the current understanding of the descriptive epidemiology and etiology of NHL and suggests areas of focus for future epidemiologic research.

Keywords

Epidemiology · Lymphoma · Immunomodulation · Infections · Diet · Alcohol · Tobacco · Obesity · Reproductive factors · Occupation · Chemical exposures · Blood transfusion · Autoimmune disease · Allergy · Medications · Radiation · Hair dyes · Genetics

B.C.-H. Chiu (✉) · N. Hou
Department of Public Health Sciences, University of Chicago, 5841 South Maryland Avenue, MC 2000, Chicago, IL 60637, USA
e-mail: bchiu@uchicago.edu

B.C.-H. Chiu
University of Chicago Comprehensive Cancer Center, Chicago, IL, USA

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_1

Contents

1 Introduction

Non-Hodgkin lymphomas (NHL) account for about 4.2 % of new cancer diagnoses in the USA [1]. It is the seventh most commonly diagnosed cancer in both men and women in the USA [2], with approximately 70,800 new cases (38,270 men and 32,530 women) expected in 2014 [1]. NHL is a heterogeneous group of malignancies that arises from two distinct lymphocyte types, B or T lymphocytes, at various stages of differentiation [3]. While 60–75 % of NHL develops or presents in the lymphoid tissues, such as lymph nodes, spleen, and bone marrow, it can occur in almost any tissue and ranges from the more indolent follicular lymphoma to the more aggressive diffuse large B-cell and Burkitt's lymphomas [4]. Incidence rates of NHL almost doubled between 1970 and 1990, but have stabilized since the late 1990s among general populations [2, 5]. The increases have been more pronounced in whites, males, the elderly, and those with NHL diagnosed at extranodal sites. Patterns of occurrence and intensive research efforts in the past two decades strongly suggest the role of environmental effects and considerable etiologic variation among NHL subtypes. This review presents the descriptive epidemiology of NHL and summarizes current knowledge about the possible etiology of NHL, with a focus on NHL subtypes for which data are available.

2 Descriptive Epidemiology

2.1 Histologic Classification and Disease Sites

NHL is presently classified according to the fourth edition of the World Health Organization (WHO) classification of tumors of hemopoietic and lymphoid tissues

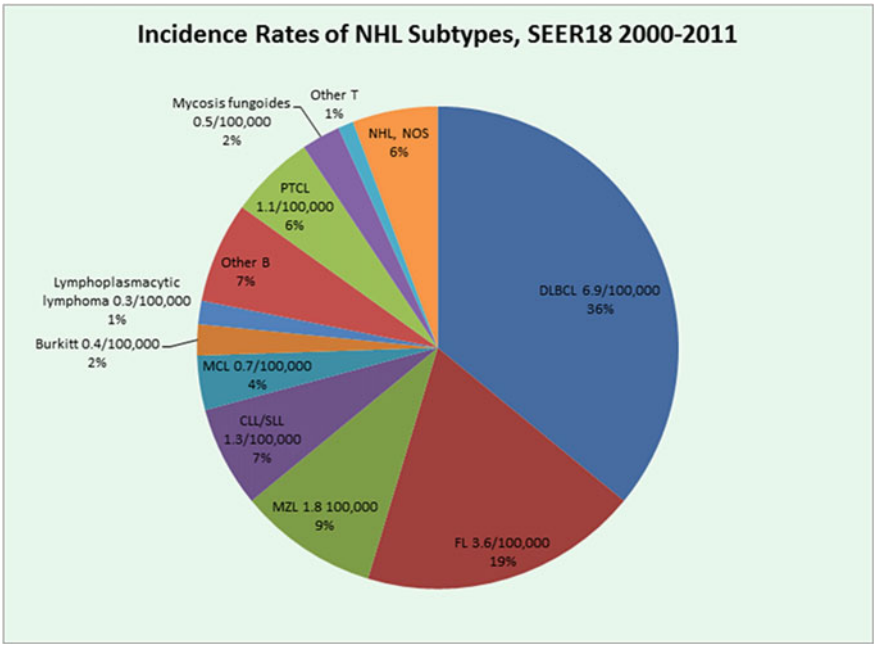


Fig. 1 Incidence Rates of NHL subtypes in the USA, 2000–2011, Surveillance, Epidemiology, and End-Results Program (Surveillance Research Program, National Cancer Institute SEER*Stat software (www.seer.cancer.gov/seerstat), Version 8.1.5. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) Database: Incidence-SEER 18 Registries Research Data, Nov 2013 Submission (2000–2011)

that distinguishes between precursor and mature neoplasms corresponding to stages of differentiation [3]. Approximately 85–90 % of all lymphomas arise from B lymphocytes and the remainder derives from T lymphocytes or NK lymphocytes [3]. The two most common types of NHL are diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma, accounting for approximately 35 and 20 % of all lymphomas, respectively (Fig. 1) [2, 3, 6]. Nodal disease accounted for approximately 65–70 % of all lymphomas in the USA [2]. The incidence of extranodal disease has increased rapidly during the 1980s and early 1990s and is now accounts for 20–30 % of all cases, with the most common sites of origin the skin, the gastrointestinal tract, and the central nervous system [2, 6–8].

2.2 Incidence

The annual incidence rate of NHL from 2007 to 2011, estimated from the Surveillance, Epidemiology, and End-Results (SEER) Program of the National Cancer Institute, was 19.7 cases per 100,000 persons, and it increased exponentially with age (9.3 per 100,000 persons under 65 years and 91.5 per 100,000 persons age

65 years and older) [2]. The overall incidence of NHL is about 50 % higher in men (23.9 per 100,000) than in women (16.4 per 100,000) in the USA [2], and this increased risk for men is seen in other countries as well [2, 6]. Male predominance in incidence was seen in most histologic subtypes with Burkitt lymphoma and mantle cell lymphoma exhibited the most marked excess among men (men vs. women rate ratios of 4 and 3, respectively) [6, 9]. The overall NHL incidence rates remained largely unchanged during 2001–2010 among women, but increased at the rate of 0.5 % per year among men.

In the USA, the incidence of NHL varies by race/ethnicity, with non-Hispanic whites (21 per 100,000 persons) at higher risk than blacks (14.3 per 100,000), Asian/Pacific Islanders (13.1 per 100,000) and Hispanics (17.8 per 100,000) during 2007–2011 [2]. Most histologies, particularly low-grade lymphoma and follicular lymphoma, are more common in whites than in blacks [9]. Only peripheral T-cell lymphoma (PTCL), mycosis fungoides, and Sezary syndrome are more common in blacks than in whites. There is also substantial variation in both incidence and histologic subtypes around the world. NHL is most common in developed countries, with the USA and Australia having one of the highest rates worldwide, followed by Europe (Fig. 2) [10]. In contrast, incidence rates are generally lowest in eastern and southern Asia (2–3 per 100,000). There are also marked differences in the distribution of lymphoma subtypes across geographic regions. Compared with North America and Western European countries, Asian countries tend to have higher incidences of mature T-/natural killer (NK)-cell lymphomas and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type (MALT lymphoma) and lower rates of follicular lymphoma, CLL/SLL, and Hodgkin lymphoma [11–15]. This geographic and racial/ethnic heterogeneity suggest that infectious, environmental, and lifestyle factors are important in addition to host factors in the etiology of certain subtypes of NHL [8, 16, 17].

2.3 Time Trends

The incidence of NHL has changed substantially in the past four decades in both the USA (Fig. 3) and in other countries [2, 6, 8, 18]. In the USA, the incidence almost doubled between 1970 (10.2 per 100,000) and 1990 (18.5 in 1990), and the increase has been more pronounced in whites, males, the elderly, and those with NHL diagnosed at extranodal sites [5, 7, 8]. Some of this increase may be due to improved diagnostic techniques, effect of the human immunodeficiency virus (HIV) epidemic, and immunosuppressive therapies. While the overall incidence rates stabilized between 1995 and 2010 (about 19 per 100,000), NHL rates among HIV-unaffected individuals increased 1.4 % per year during 1992 and 2003, before stabilizing in mid-2000s [19]. This slow increase of NHL incidence in HIV-unaffected individuals is largely unexplained.

Studies have reported diverse trends by NHL subtypes (Fig. 3). From 1992 to 2001, DLBCL and follicular lymphoma increased 1.4 and 1.8 % per year, respectively, whereas rates of CLL/SLL declined 2.1 % per year. The rates for

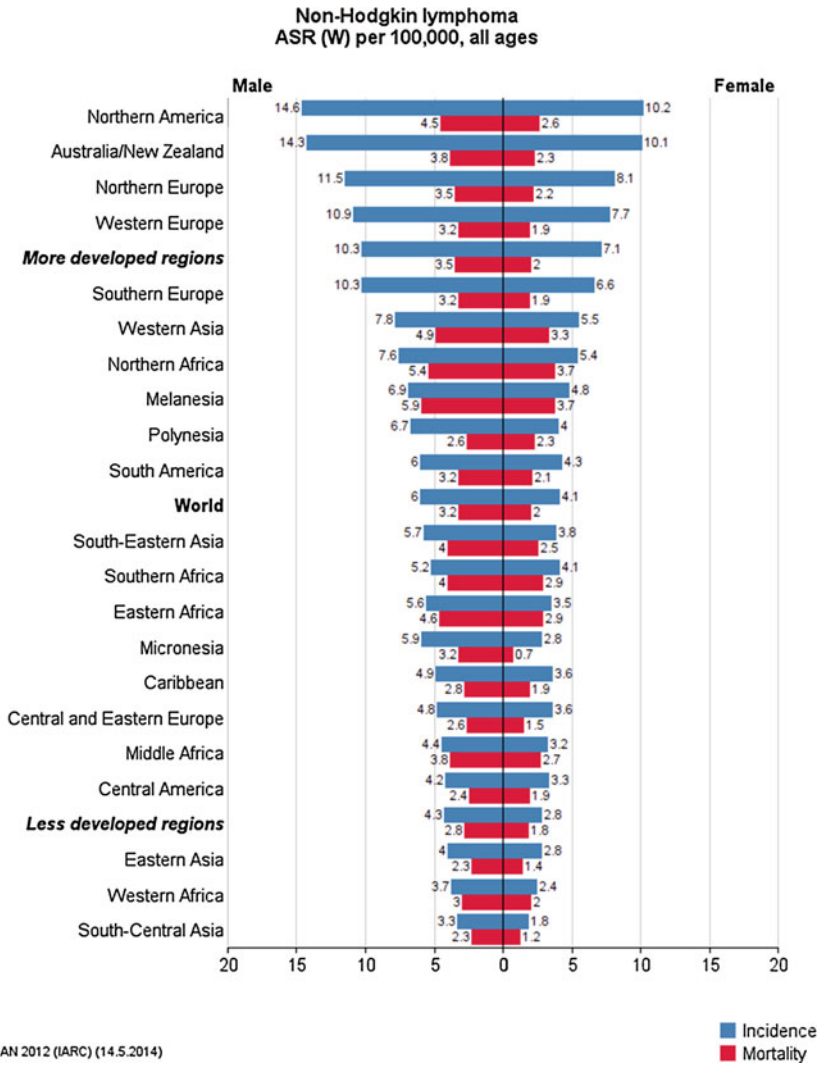


Fig. 2 Incidence and mortality of NHL in different parts of the world (Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed on day/month/year)

DLBCL and follicular lymphoma in the general population appear to have stabilized since mid-2000s, independent of HIV [19]. During 2002–2011, incidence rates increased significantly for marginal zone lymphoma (1.7 % per year) and mantle cell lymphoma (1.7 % per year), with white elderly men seeing the most

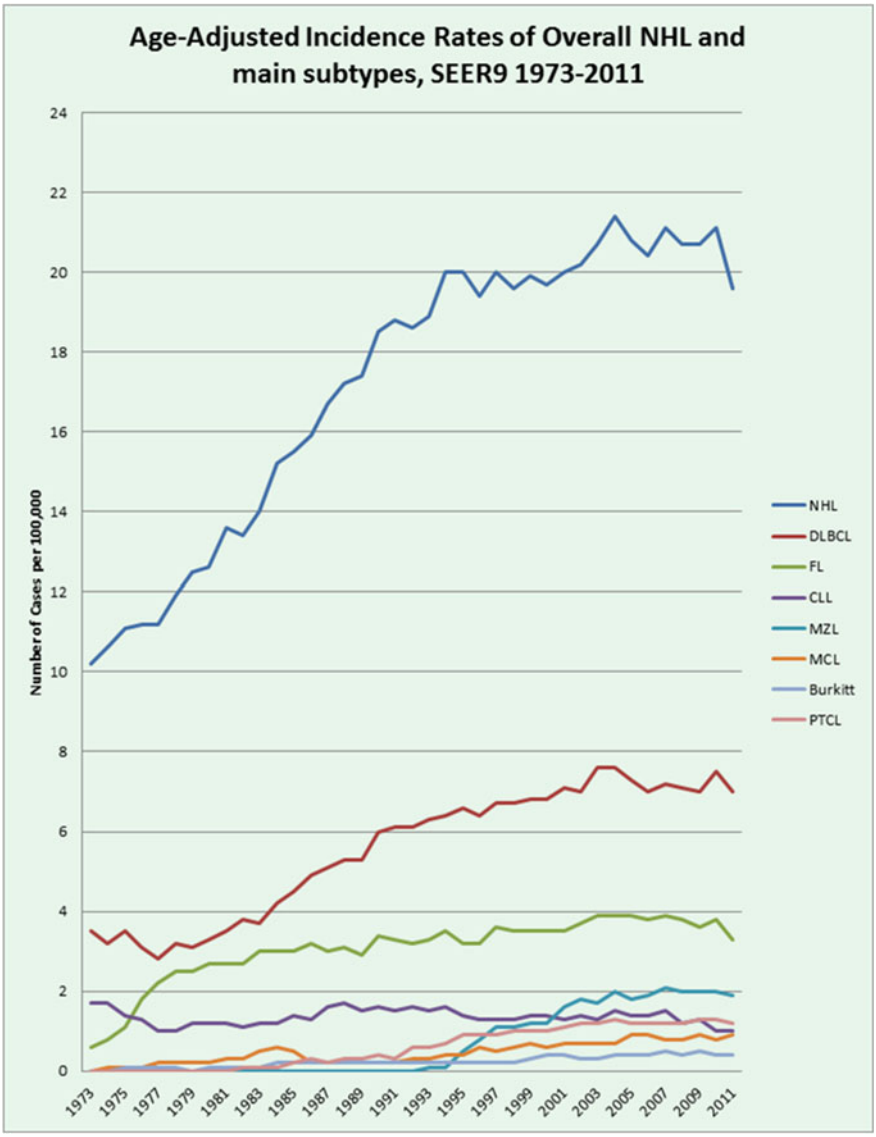


Fig. 3 Incidence rates of overall NHL and main histologic subtypes in the USA, 1973-2011, Surveillance, Epidemiology and End-Results Program (Surveillance Research Program, National Cancer Institute SEER*Stat software (www.seer.cancer.gov/seerstat), Version 8.1.5. Surveillance, Epidemiology, and End-Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence-SEER 9 Registries Limited-Use Data, Nov 2013 Submission (1973–2011)

striking increase [2, 20–22]. These time trends are difficult to assess due to the recent recognition of these two entities as distinct subtypes.

Primary extranodal disease has increased more rapidly than nodal disease since the 1970s [7, 23]. Incidence rates increased 3.0–6.9 % per year for extranodal cases compared to only 1.7–2.5 % per year for nodal cases, with the largest increase occurring in the brain and other areas of the central nervous system (224 %). The increase in extranodal lymphomas is, in part, a consequence of improved diagnostic tools and the application of modern immunophenotypic and molecular methods [24]. Although primary central nervous system lymphomas are rare, there has been a threefold increase in incidence. The dramatic increase in NHL of the central nervous system warrants investigation, although the rates have begun to decrease since the mid-1990s [25], most likely due to the decline in the incidence of acquired immunodeficiency syndrome (AIDS) [26].

Intensive research efforts have been made in the past two decades to understand factors that might account for the incidence patterns and trends. This effort is strengthened by the initiation of several consortia, such as a large International Lymphoma Epidemiology Consortium (InterLymph) and the EPILYMPH study in six European countries that have allowed a detailed examination of NHL subtype-specific association and the potential for etiologic heterogeneity as well as the assessment of less prevalent exposures [27–30]. The following section will review some of the established and postulated risk factors for the development of NHL with an emphasis on epidemiologic findings reported in the past two decades.

3 Etiology

3.1 Immune Modulation

Congenital and acquired states of immunosuppression are the strongest factor known to increase NHL risk [31]. These conditions include ataxia-telangiectasia, Wiskott-Aldrich syndrome, common variable hypogammaglobulinemia, X-linked lymphoproliferative syndrome, and severe combined immunodeficiency [32]. Epstein-Barr virus (EBV) appears to be an important cofactor, and host defects in immune regulation resulting in uncontrolled infection and proliferation of B-lymphocytes likely contribute to the development of NHL.

Acquired immunodeficiency states such as HIV infection are associated with 75- to 100-fold increased risk of NHL compared with the general population [19, 33], although recent data in the post-HARRT (highly active antiretroviral therapy) era suggest it has decreased [34, 35]. These NHLs are usually high-grade and often present with extranodal disease. Increased risk varied by NHL subtypes, ranging from 30-fold, 50-fold, and 1020-fold for DLBCL, Burkitt lymphoma, and central nervous system lymphoma, respectively [34]. The occurrence of NHL in HIV-infected persons has been attributed to deficient immune surveillance of oncogenic herpesviruses, such as EBV and human herpesvirus 8, as well as defective immune regulation and chronic antigenic stimulation due to other infections [36].

Patients who are treated with immunosuppressive drugs following solid organ transplant or hematopoietic stem cell transplant are at substantially increased risk (30–50 times) for NHL [37–39], particularly during the first year after transplant [40, 41]. The risk varied widely across subtypes and appeared markedly elevated for DLBCL, marginal zone lymphoma, lymphoplasmacytic lymphoma, and NK/T-cell lymphoma [37–39]. Chronic antigenic stimulation induced by the graft and significant immunosuppression associated with EBV infection are the probable mechanisms. Polyclonal or monoclonal B-cell proliferations are seen in transplant patients, but these often regress when immunosuppressive therapy is stopped. However, the proliferation may persist and evolve into an aggressive NHL. Loss of control of persistent EBV infection caused by the immunosuppressive therapy appears to be important to this process.

Patients who receive chemotherapy and/or radiation are also at increased risk for developing subsequent secondary NHL [42, 43]. In the SEER database, NHL risk was increased after initial radiotherapy for all solid cancers combined, non-small cell lung cancer, and prostate cancer [42]. Risk increased with longer latency after radiotherapy, but there was no clear pattern by NHL subtype or age.

Epidemiologic studies concerning a history of blood transfusion and the subsequent development of NHL have produced contradictory findings. A meta-analysis including 14 studies showed that blood transfusion was associated with a 20 % increase in the risk of NHL overall that was limited to cohort studies [44]. The association was similar for men and women as well as for transfusions given before or after 1992. In contrast, case–control studies have demonstrated no association of NHL with transfusion [45, 46]. A recent large pooled analysis from InterLymph found an inverse association between transfusion history and risk of DLBCL [47], follicular lymphoma [48], and CLL/SLL [49]. Bias cannot be ruled out because these results are inconsistent with the hypothesis that the immunosuppressive effects of allogeneic blood transfusion and infections caused by blood-borne organisms would likely increase the risk of NHL [50].

An increased incidence of gastrointestinal lymphomas is seen in patients with celiac (nontropical) sprue and inflammatory bowel disease, particularly Crohn's disease [5]. Sjogren's syndrome has been associated with NHL overall, particularly follicular lymphoma [48], DLBCL [51], marginal zone lymphoma [52, 53], and lymphoplasmacytic lymphoma/Waldenstrom's macroglobulinemia (LPL/WM) [54]. Systemic lupus erythematosus and rheumatoid arthritis have also been associated with B-cell lymphoma [53]. It remains unclear whether the excess risk is due to immunosuppressive drugs to treat these autoimmune conditions or the condition itself.

3.2 Viruses

Several viruses have been implicated in the pathogenesis of NHL, including EBV, human T-cell lymphotrophic virus (HTLV-1), Kaposi sarcoma-associated herpesvirus (KSHV, also known as human herpesvirus 8), and hepatitis C virus (HCV).

3.2.1 Epstein-Barr Virus (EBV)

Infection with EBV is highly prevalent in the adult population, with approximately 90 % of individuals in developed countries having evidence of previous infection by age 40 years [31]. In healthy individuals, equilibrium exists between latent EBV infection and the host's immune system. In immunocompromised patients (i.e., organ transplant and HIV infection), however, control mechanisms are impaired which could lead to EBV-driven B-cell proliferation and ultimately the development of B-cell lymphoma [18, 55]. EBV has been associated with Burkitt lymphoma (particularly in areas of Africa where the virus is endemic), Hodgkin lymphoma, lymphoma in immunocompromised patients, sinonasal lymphoma in Asia and South America, and sporadically in other NK/T-cell lymphomas (predominantly occurring in Asia) [31].

3.2.2 Human T-Cell Lymphotrophic Virus (HTLV-1)

HTLV-1 is a human retrovirus that establishes a latent infection via reverse transcription in activated T-helper cells. Infection with HTLV-1, especially in early childhood, is strongly related to adult T-cell leukemia/lymphoma in the Caribbean and Japan, where infection is endemic [56]. The cumulative lifetime risk in infected individuals is estimated to be approximately 5 % [57], suggesting a multistage process of T-cell transformation and involvement of additional pathogenetic factors [58]. An HTLV-1-like provirus has been detected in some patients with mycosis fungoides, although findings have been inconsistent.

3.2.3 Kaposi Sarcoma-associated Herpesvirus (KSHV)

KSHV-like DNA sequences are frequently detected in primary effusion lymphomas, in patients with Kaposi sarcoma, and in those with multicentric Castleman disease-plasmablastic lymphoma [6] . These herpesvirus 8 (HHV-8)-related NHL subtypes are associated almost exclusively with HIV infection in settings of profound immunosuppression, particularly primary effusion lymphomas [59]. It has also been seen in the absence of immunodeficiency in areas of high HHV-8 seroprevalence, such as the Mediterranean [18].

3.2.4 Hepatitis C Virus (HCV)

Several studies have linked HCV to NHL, but results are not entirely consistent. A positive association between HCV and B-cell NHL was found in some studies [60, 61], but not others [62, 63]. A study in Southern Italy showed a higher incidence of HCV infection in high-grade NHL than in low-grade NHL [64], whereas other studies report a higher incidence in low-grade NHL [61]. A meta-analysis of 18 studies found positive associations for both B- and T-cell NHL [65], while pooled analyses of individual-level data in 20 case–control studies from InterLymph reported excess risk for DLBCL [47], marginal zone lymphoma [52], CLL/SLL [49], and LPL/WM [54], but not follicular lymphoma [48, 66]. EPILYMPH also reported a positive association with DLBCL [67]. Geographic variability in HCV seroprevalence may account for some of the inconsistency in that positive

associations tend to be reported from geographical areas with high HCV seroprevalence such as Italy and Japan, whereas no associations were noted mostly in studies from northern Europe, northern USA, or Canada where HCV seroprevalence is low [18].

3.3 Bacterial Infections

Chronic gastric infection with *H. pylori* has been linked to the development of low-grade, gastric mucosa-associated lymphoid tissue (MALT) lymphoma [68, 69]. A pooled analysis of 1,052 marginal zone lymphoma cases and 13,766 controls in 12 case–control studies from the InterLymph found a positive association between self-reported peptic ulcers and risk of extranodal marginal zone lymphoma, but not nodal or splenic marginal zone lymphoma [52]. Eradication of *Helicobacter pylori* has been shown to result in the regression of MALT lymphoma [70]. Infection with *B. burgdorferi,* the causative agent in Lyme disease, has been detected in about 35 % of patients with primary cutaneous B-cell lymphoma [71]. A near-complete remission of a primary marginal zone B-cell lymphoma was observed after eradication of *Borrelia burgdorferi* with antibiotic treatment [72]. Studies have also linked *C. psittaci* to ocular adnexal marginal zone lymphoma [73]. This infection, however, is highly specific and does not reflect a subclinical infection widespread among the general population [5]. Herpes zoster has also been associated with Hodgkin lymphoma and NHL [74–76]. Findings on infectious agents are consistent with the idea of chronic antigenic stimulation or inflammation in the pathogenesis of NHL.

3.4 Lifestyle Factors

3.4.1 Tobacco

Cigarette smoking appears to have no association [77–79] or only a weak association [80–82] with NHL. A meta-analysis of 50 studies reported that ever smoking was associated with a higher risk of NHL, mainly because of the association with T-cell NHL [83]. Some studies, however, have linked smoking with a higher risk of follicular lymphoma [82, 84–86] and high-grade NHL including diffuse large B-cell NHL [80, 82]. Pooled analyses of 20 case–control studies from the InterLymph found that cigarette smoking was positively associated with risk of central nervous system, testicular and cutaneous DLBCL [47], follicular lymphoma [48], LPL/WM [54], and mycosis fungoides and Sezary syndromes (MF/SS) [87], but inversely associated with risk of CLL/SLL [49] and hairy cell leukemia [88]. Because greater smoking exposure was found to be associated with a higher frequency of t(14;18), a translocation that occurs commonly in follicular lymphoma, in healthy individuals [89], two studies specifically evaluated cigarette smoking and risk of t(14;18)-positive NHL but found no clear association [90, 91]. A recent study suggested that

while exposure to environmental tobacco smoke is not associated with NHL overall, it was associated with a higher risk of follicular lymphoma for both children and adults, and a lowered risk of DLBCL in adults [92].

3.4.2 Alcohol Use

Several epidemiologic studies have evaluated alcohol use and risk of NHL, but the findings are not entirely consistent. Alcohol use has been linked to an increased risk [93], a lower risk [94–98], or no effect on the risk of NHL [99]. Studies that assessed the association by type of alcoholic beverages or by subtype of NHL have also reported conflicting results. A large InterLymph NHL subtypes project [30] found an inverse association between ever drinkers and risk of many subtypes of NHL, including DLBCL [47], follicular lymphoma [48], marginal zone lymphoma [52], peripheral T-cell lymphomas [100], and sporadic Burkitt lymphoma [101], but most findings lack clear dose-response. The EPILYMPH study did not observe an association for NHL overall or across histologic subtypes, but found an inverse association in men [79].

3.4.3 Diet

The role of dietary intake and NHL risk has been reviewed elsewhere [102, 103]. Some studies found positive associations with intake of meat [104, 105], particularly red meat [104, 106, 107], whereas no association was reported by others [108–110]. Fish consumption has been associated with a lower risk of NHL [111, 112], although null results were also reported [105, 106, 108]. There are multiple pathways through which meat intake might impact NHL risk, including modulating the immune response through meat and its constituents (e.g., fat and protein), carcinogens, and mutagens [104, 113]. An excess risk of NHL has been associated with a higher intake of dietary fat, including total fat, animal fat, saturated fat, and trans fatty acids [104–107, 111, 112]. Evidence for total and animal protein intakes was less consistent. Findings on meat mutagens and NHL risk are also not entirely consistent [107, 109]. One recent study reported that phytanic acid, a saturated fatty acid obtained primarily through the consumption of ruminant meat and dairy products, is positively associated with risk of NHL, especially follicular lymphoma and CLL/SLL [114].

Dietary intake of fruit and vegetables has received great attention in the prevention of NHL because antioxidants and other constituents in these foods are thought to influence immune function and to inhibit oxidative processes involved in carcinogenesis and cell proliferation [103, 115]. Epidemiologic studies have reported inverse associations between the risk of NHL and a higher intake of all vegetables combined [116, 117], green leafy vegetables [108, 116], or cruciferous vegetables [116], but others have found no associations [104, 105, 118, 119] or even a suggestive positive association with green leafy vegetables [118]. A recent study found that higher circulating carotenoids prior to diagnosis are associated with reduced risk of NHL [120].

Dietary patterns and NHL risk were evaluated in one cohort [121] and one case–control study [122]. The Multiethnic cohort reported that the vegetable pattern was inversely related to risk in Caucasian women, whereas the fat and meat pattern was associated with a fivefold higher risk of follicular lymphoma in men [121]. A population-based case–control study found that a dietary pattern high in meats, fats, and sweets is associated with an increased risk of overall NHL, follicular lymphoma, DLBCL, and marginal zone lymphoma [122].

3.4.4 Anthropometric Measures

Obesity is associated with chronic, low-grade inflammation, and specific immune modulations including changes in cytokine profiles that may predispose to NHL [123]. Several studies have found a significant positive association between obesity and NHL risk [96, 124, 125], whereas others reported null associations with body mass index [126–128] or central obesity [129–131]. Excess risk of DLBCL was linked to obesity [126, 132, 133] and severe obesity [131]. The InterLymph NHL Subtypes Project reported a positive association between higher young adult body mass index and risk of diffuse large lymphoma [47] and follicular lymphoma [48]. Usual adult height was linked to risk of CLL/SLL [49] and sporadic Burkitt lymphoma [101].

3.4.5 Hair Dyes

Hair coloring products contain compounds that are mutagenic and carcinogenic in animals [134]. Several studies reported excess NHL risk associated with the use of hair dyes, particularly long-term use of dark permanent dyes [135–137]. A pooled analysis from InterLymph found excess risk of follicular lymphoma and CLL/SLL, but not other subtypes, in women who started use before 1980 [137]. These findings were supported by recent reports from the InterLymph with more than four times as many cases and controls [48, 49].

3.4.6 Ultraviolet (UV) Radiation

Exposure to sunlight and other sources of UV radiation, with possible immunosuppressive effects, has been suggested as a risk factor for NHL [138]. Recent studies estimated personal sun exposure with questionnaires instead of using latitude as a proxy reported an inverse association in general [139–142], particularly with regard to recreational sun exposure [139]. Pooled analyses from InterLymph found that the inverse association with recreational sun exposure was more evident for follicular lymphoma [48, 143] and DLBCL [47, 143]. This inverse association has been suggested to be due partly to effects on the immune function from sun exposure [144] or vitamin D production [145]. While low serum concentration of the vitamin D metabolite, 25-hydroxyvitamin D, has been found as an independent poor prognostic factor in patients with NHL [146], particularly CLL [147], DLBCL, and T-cell lymphoma [148], circulating 25-hydroxyvitamin D was not associated with risk of NHL in the European Prospective Investigation into Cancer and Nutrition (EPIC) study [149] and a pooled analysis of 10 cohorts in the cohort consortium [150].

3.5 Occupational Exposures

A number of occupations have been associated with increased risk for the development of NHL, including farmers, pesticide applicators, benzene workers, rubber workers, petroleum refinery workers, dry cleaners, firefighters, and chemists [6, 18, 151–153]. The InterLymph Subtypes Project reported excess risk of DLBCL in persons who worked as field crop/vegetables farmer, seamstress/embroiderer, and driver/material handling equipment operator [47], follicular lymphoma and employment as a spray painter [48], marginal zone lymphoma and metalworker occupation [52], CLL/SLL in persons worked as hairdresser [49], LPL/WM and occupation as medical doctor [54], sporadic Burkitt lymphoma and employment as a cleaner [101], adult acute lymphocytic leukemia in leather and sewing/embroidery workers [154], mycosis fungoides and Sezary syndrome in crop/vegetables farmers, painters, wood workers, and general carpenters [87], and PTCL in persons worked as textile worker and electrical fitter [100]. Common exposures in these occupations include benzene, pesticides, herbicides, and other organic solvents [6, 18, 155]. However, mechanisms linking these exposures to specific NHL subtypes remain to be determined.

Epidemiologic studies suggest that an excess risk of NHL among farmers is related to the use of phenoxyacetic acid herbicides, organophosphate insecticides, and fertilizers [151]. Pesticides have also been associated specifically with follicular NHL and small lymphocytic NHL [156–158]. Two studies evaluating the pesticide-NHL association according to the t(14;18) status of the NHL found that t(14;18)-positive NHL was associated with farming and pesticides, whereas there were no such associations with t(14;18)-negative NHL [159, 160]. Solvents have been associated with an increased risk of NHL, especially in occupational studies of rubber workers, aircraft maintenance workers, and dry cleaners [161]. A large EPILYMPH study found excess risk for follicular lymphoma and CLL [162]. A recent case–control study in Connecticut reported that polymorphism in IL10 (rs1800890) modified the association between occupational exposure to organic solvents and the risk of DLBCL [163].

3.6 Host Factors

3.6.1 Familial Aggregation

A history of NHL or other hematolymphoid cancer in close relatives has repeatedly been shown to increase the risk of NHL by 2- to 3-fold [164–166], a stronger association than is estimated for most of the other suspected risk factors. A pooled analysis from InterLymph reported that NHL risk was elevated for individuals who reposted a first-degree relative with NHL, especially among those who reported a brother with NHL [167]. A recent InterLymph pooled analysis with an additional cases and controls confirmed these earlier findings and further linked family history of NHL to risk of DLBCL [47], follicular lymphoma [48], and marginal zone

lymphoma [52]. Familial aggregation has been associated with an inherited defect of immune function in some instances, but no such abnormality can be discerned in most families [31]. Lymphomas may also cluster within families, not because of an inherited susceptibility, but because of shared environmental determinants [95, 164, 168].

3.6.2 Genetics

Numerous studies implicate the role of genetic variants that promote B-cell survival and growth with increased risk of NHL [169]. For example, NHL risk has been linked to genetic variation in various pathways, including one-carbon metabolism, cytokine, innate immunity, oxidative stress, and apoptotic and DNA repair pathways as well as in the HLA region [169, 170]. Two recent genome-wide association studies (GWAS) identified associations between FL risk and three variants within the HLA region, one at 6p21.33 (rs6457327) [171] and three at 6p21.32 (rs10484561, rs7755224, and rs2647012) [172]. Another study found that rs10484561 was also associated with risk of DLBCL [172], suggesting some shared biological mechanisms of susceptibility between these two common NHL subtypes. Another study reported that the TAP2 coding SNP rs2411447 at 6p21.4 was strongly associated with follicular lymphoma and, to a lesser extent, DLBCL [173]. Findings from the Population Architecture using Genomics and Epidemiology (PAGE) consortium further support a shared genetic susceptibility between follicular lymphoma and DLBCL, particularly involving variants in the major histocompatibility complex region [174]. The 6p21.3 also emerged as a potential susceptibility locus associated with familial CLL [175]. A GWAS of CLL further identified 4 highly correlated intronic variants within the IRF8 gene that were associated with CLL [176]. This association is specific to CLL, with little evidence for association across the other common NHL subtypes.

A large pooled InterLymph study reported that common polymorphisms of TNF and IL10, which are both key cytokines for inflammatory response and T-helper balance, were associated with risk of NHL, particularly for diffuse large B-cell lymphoma, but not for follicular lymphoma [177]. Studies evaluating the associations of SNPs in folate-metabolizing genes with NHL risk have reported inconsistent findings [178–180].

4 Conclusions

After two decades of steep increase in the incidence of NHL in the USA, the overall NHL incidence rates appear to have stabilized in the early 1990s due primarily to a decline in the AIDS incidence. NHL rates among HIV-unaffected individuals, however, continued to increase throughout the 1990–2009. The temporal trends varied by histologic subtypes. The incidence of NHL and the distribution of histologic subtypes not only show significant geographic differences and distinct time trends but also vary by age, sex, and race/ethnicity. These incidence patterns and

time trend, although poorly understood, strongly suggest that infectious, environmental, and lifestyle factors in addition to host factors are important in the etiology of NHL as well as certain NHL subtypes.

Intensive research efforts, including international consortia, in the past two decades, have led to a better understanding of the causes of NHL in that there is some mechanistic commonality of lymphomagenesis, but it has also become apparent that significant etiologic heterogeneity exists [8, 29, 181]. It is more likely that a compilation of immune function, genetic host susceptibility, and environmental and lifestyle factors will identify the most robust profiles for lymphoma risk [182]. Continued epidemiologic research to rigorously evaluate the interplay between these factors in NHL etiology is warranted and critically needed.

Cytogenetics, fluorescence in situ hybridization (FISH), immunophenotyping, and gene-rearrangement studies are increasingly being used to diagnose and characterize NHL [183]. New techniques, such as FISH and gene expression profiles, have made the identification of genetic abnormalities possible in routine paraffin-embedded tissue [184]. Because many WHO-defined NHL subtypes remain heterogeneous at the molecular level, future epidemiologic studies should collect not only peripheral blood but also tissues and incorporate these new technologies to investigate specific etiologic factors that are associated with well-defined homogeneous molecular subtypes of NHL.

References

1. American Cancer Society (2014) Cancer facts and figures 2014. Atlanta, GA
2. Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF et al (2014). SEER cancer statistics review, 1975–2011. National Cancer Institute. Bethesda, MD. http://seer.cancer.gov/csr/1975_2011/, based on November 2013 SEER data submission, posted to the SEER web site, April 2014
3. Swerdlow SH, Campo E (2008) WHO classification: pathology and genetics of tumors of haematopoietic and lymphoid tissues. IARC Press, Lyon
4. Shankland KR, Armitage JO, Hancock BW (2012) Non-Hodgkin lymphoma. Lancet 380 (9844):848–857
5. Evens AM, Winter JN, Gordon LI, Chiu BC-H, Tsang R, Rosen ST (2013). Non-Hodgkin lymphoma. In: Pazdur R, Wagman LD, Camphausen KA, Hoskins WJ (eds) Cancer management: a multidisciplinary approach. UBM Medica, Norwalk, CT
6. Hartge P, Wang SS, Bracci PM, Devesa SS, Holly EA (2006) Non-Hodgkin lymphoma. In: Schottenfeld D, Fraumeni JFJ (eds) Cancer epidemiology and prevention, 3rd edn. Oxford University Press, New York, pp 898–918
7. Groves FD, Linet MS, Travis LB, Devesa SS (2000) Cancer surveillance series: non-Hodgkin's lymphoma incidence by histologic subtype in the United States from 1978 through 1995. J Natl Cancer Inst 92(15):1240–1251
8. Chiu BC, Weisenburger DD (2003) An update of the epidemiology of non-Hodgkin's lymphoma. Clin Lymphoma 4(3):161–168
9. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS (2006) Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. Blood 107 (1):265–276

10. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C et al GLOBOCAN 2010 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No.10. 2012. Available from http://globocan.iarc.fr
11. Biagi JJ, Seymour JF (2002) Insights into the molecular pathogenesis of follicular lymphoma arising from analysis of geographic variation. Blood 99(12):4265–4275
12. Lee MY, Tan TD, Feng AC, Liu MC (2006) Clinicopathological analysis of 598 malignant lymphomas in Taiwan: seven-year experience in a single institution. Am J Hematol 81 (8):568–575
13. Yang QP, Zhang WY, Yu JB, Zhao S, Xu H, Wang WY et al (2011) Subtype distribution of lymphomas in Southwest China: analysis of 6,382 cases using WHO classification in a single institution. Diagn Pathol 6:77
14. Yoon SO, Suh C, Lee DH, Chi HS, Park CJ, Jang SS et al (2010) Distribution of lymphoid neoplasms in the Republic of Korea: analysis of 5,318 cases according to the World Health Organization classification. Am J Hematol 85(10):760–764
15. Laurini JA, Perry AM, Boilesen E, Diebold J, Maclennan KA, Muller-Hermelink HK et al (2012) Classification of non-Hodgkin lymphoma in Central and South America: a review of 1,028 cases. Blood 120(24):4795–4801
16. Clarke CA, Glaser SL, Gomez SL, Wang SS, Keegan TH, Yang J et al (2011) Lymphoid malignancies in U.S. Asians: incidence rate differences by birthplace and acculturation. Cancer Epidemiol Biomark Prev 20(6):1064–1077
17. Herrinton LJ, Goldoft M, Schwartz SM, Weiss NS (1996) The incidence of non-Hodgkin's lymphoma and its histologic subtypes in Asian migrants to the United States and their descendants. Cancer Causes Control 7(2):224–230
18. Ekstrom-Smedby K (2006) Epidemiology and etiology of non-Hodgkin lymphoma–a review. Acta Oncol 45(3):258–271
19. Shiels MS, Engels EA, Linet MS, Clarke CA, Li J, Hall HI et al (2013) The epidemic of non-Hodgkin lymphoma in the United States: disentangling the effect of HIV, 1992–2009. Cancer Epidemiol Biomark Prev 22(6):1069–1078
20. Aschebrook-Kilfoy B, Caces DB, Ollberding NJ, Smith SM, Chiu BC (2013) An upward trend in the age-specific incidence patterns for mantle cell lymphoma in the USA. Leuk Lymphoma 54(8):1677–1683
21. Liu L, Wang H, Chen Y, Rustveld L, Liu G, Du XL (2013) Splenic marginal zone lymphoma: a population-based study on the 2001–2008 incidence and survival in the United States. Leuk lymphoma 54(7):1380–1386
22. Zhou Y, Wang H, Fang W, Romaguer JE, Zhang Y, Delasalle KB et al (2008) Incidence trends of mantle cell lymphoma in the United States between 1992 and 2004. Cancer 113 (4):791–798
23. Devesa SS, Fears T (1992) Non-Hodgkin's lymphoma time trends: United States and international data. Cancer Res 52(19 Supplement):5432s–5440s
24. Olson JE, Janney CA, Rao RD, Cerhan JR, Kurtin PJ, Schiff D et al (2002) The continuing increase in the incidence of primary central nervous system non-Hodgkin lymphoma: a surveillance, epidemiology, and end results analysis. Cancer 95(7):1504–1510
25. Kadan-Lottick NS, Skluzacek MC, Gurney JG (2002) Decreasing incidence rates of primary central nervous system lymphoma. Cancer 95(1):193–202
26. O''eill BP, Decker PA, Tieu C, Cerhan JR (2013) The changing incidence of primary central nervous system lymphoma is driven primarily by the changing incidence in young and middle-aged men and differs from time trends in systemic diffuse large B-cell non-Hodgkin's lymphoma. Am J Hematol 88(12):997–1000
27. Bassig BA, Lan Q, Rothman N, Zhang Y, Zheng T (2012) Current understanding of lifestyle and environmental factors and risk of non-hodgkin lymphoma: an epidemiological update. J Cancer Epidemiol 2012:978930
28. Boffetta P, Armstrong B, Linet M, Kasten C, Cozen W, Hartge P (2007) Consortia in cancer epidemiology: lessons from InterLymph. Cancer Epidemiol Biomark Prev 16(2):197–199

29. Morton LM, Wang SS, Cozen W, Linet MS, Chatterjee N, Davis S et al (2008) Etiologic heterogeneity among non-Hodgkin lymphoma subtypes. Blood 112(13):5150–5160
30. Morton LM, Sampson JN, Cerhan JR, Turner JJ, Vajdic CM, Wang SS et al (2014) Rationale and design of the International Lymphoma Epidemiology Consortium (InterLymph) non-Hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014(48):1–14
31. Rabkin CS, Ward MH, Manns A, Blattner WA (1997) Epidemiology of non-Hodgkin's lymphomas. In: Magrath IT (ed) The non-Hodgkin's lymphomas, 2nd edn. Oxford University Press, New York, pp 171–186
32. Filipovich AH, Mathur A, Kamat D, Shapiro RS (1992) Primary immunodeficiencies: genetic risk factors for lymphoma. Cancer Res 52(19S):5465–5467
33. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM (2007) Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet 370(9581):59–67
34. Engels EA, Pfeiffer RM, Goedert JJ, Virgo P, McNeel, Timothy S, Scoppa SM et al (2006) Trends in cancer risk among people with AIDS in the United States 1980–2002. AIDS (London, England) 20(12):1645–1654
35. Shiels MS, Cole SR, Wegner S, Armenian H, Chmiel JS, Ganesan A et al (2008) Effect of HAART on incident cancer and noncancer AIDS events among male HIV seroconverters. J Acquir Immune Defic Syndr 48(4):485–490
36. Palackdharry CS (1994) The epidemiology of non-Hodgkin's lymphoma: why the increased incidence? Oncology 8(8):67–78
37. Clarke CA, Morton LM, Lynch C, Pfeiffer RM, Hall EC, Gibson TM et al (2013) Risk of lymphoma subtypes after solid organ transplantation in the United States. Br J Cancer 109 (1):280–288
38. Gibson TM, Engels EA, Clarke CA, Lynch CF, Weisenburger DD, Morton LM (2014) Risk of diffuse large B-cell lymphoma after solid organ transplantation in the United States. Am J Hematol 89(7):714–720
39. Quinlan SC, Morton LM, Pfeiffer RM, Anderson LA, Landgren O, Warren JL et al (2010) Increased risk for lymphoid and myeloid neoplasms in elderly solid-organ transplant recipients. Cancer Epidemiol Biomark Prev 19(5):1229–1237
40. Jiang Y, Villeneuve PJ, Fenton SS, Schaubel DE, Lilly L, Mao Y (2008) Liver transplantation and subsequent risk of cancer: findings from a Canadian cohort study. Liver Transpl 14(11):1588–1597
41. Jiang Y, Villeneuve PJ, Wielgosz A, Schaubel DE, Fenton SS, Mao Y (2010) The incidence of cancer in a population-based cohort of Canadian heart transplant recipients. Am J Transplant 10(3):637–645
42. Kim CJ, Freedman DM, Curtis RE, Berrington de Gonzalez A, Morton LM (2013) Risk of non-Hodgkin lymphoma after radiotherapy for solid cancers. Leuk Lymphoma 54(8): 1691–1697
43. Krishnan B, Morgan GJ (2007) Non-Hodgkin lymphoma secondary to cancer chemotherapy. Cancer Epidemiol Biomark Prev 16(3):377–380
44. Castillo JJ, Dalia S, Pascual SK (2010) Association between red blood cell transfusions and development of non-Hodgkin lymphoma: a meta-analysis of observational studies. Blood 116(16):2897–2907
45. Chow EJ, Holly EA (2002) Blood transfusions as a risk factor for non-Hodgkin's lymphoma in the San Francisco Bay Area: a population-based study. Am J Epidemiol 155(8):725–731
46. Maguire-Boston EK, Suman V, Jacobsen SJ, Moore SB, Habermann TM, Cerhan JR et al (1999) Blood transfusion and risk of non-Hodgkin's lymphoma. Am J Epidemiol 149 (12):1113–1118
47. Cerhan JR, Kricker A, Paltiel O, Flowers CR, Wang SS, Monnereau A et al (2014) Medical history, lifestyle, family history, and occupational risk factors for diffuse large B-cell lymphoma: the InterLymph non-hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014(48):15–25

48. Linet MS, Vajdic CM, Morton LM, de Roos AJ, Skibola CF, Boffetta P et al (2014) Medical history, lifestyle, family history, and occupational risk factors for follicular lymphoma: the InterLymph non-hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014 (48):26–40
49. Slager SL, Benavente Y, Blair A, Vermeulen R, Cerhan JR, Costantini AS et al (2014) medical history, lifestyle, family history, and occupational risk factors for chronic lymphocytic leukemia/small lymphocytic lymphoma: the InterLymph non-hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014(48):41–51
50. Cerhan JR, Wallace RB, Folsom AR, Potter JD, Munger RG, Prineas RJ (1993) Transfusion history and cancer risk in older women. Ann Intern Med 119:8–15
51. Smedby KE, Askling J, Mariette X, Baecklund E (2008) Autoimmune and inflammatory disorders and risk of malignant lymphomas–an update. J Intern Med 264(6):514–527
52. Bracci PM, Benavente Y, Turner JJ, Paltiel O, Slager SL, Vajdic CM et al (2014) Medical history, lifestyle, family history, and occupational risk factors for marginal zone lymphoma: the InterLymph non-hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014 (48):52–65
53. Ekstrom Smedby K, Vajdic CM, Falster M, Engels EA, Martinez-Maza O, Turner J et al (2008) Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph consortium. Blood. 111(8):4029–3408
54. Vajdic CM, Landgren O, McMaster ML, Slager SL, Brooks-Wilson A, Smith A et al (2014) Medical history, lifestyle, family history, and occupational risk factors for lymphoplasmacytic lymphoma/waldenstrom's macroglobulinemia: the InterLymph non-hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014(48):87–97
55. Oertel SH, Riess H (2002) Immunosurveillance, immunodeficiency and lymphoproliferations. Recent Results Cancer Res 159:1–8
56. Cleghorn FR, Manns A, Falk R, Hartge P, Hanchard B, Jack N et al (1995) Effect of human T-lymphotropic virus type I infection on non-Hodgkin's lymphoma incidence. J Natl Cancer Inst 87(13):1009–1014
57. Kondo T, Kono H, Miyamoto N, Yoshida R, Toki H, Matsumoto I et al (1989) Age- and sex-specific cumulative rate and risk of ATLL for HTLV-I carriers. Int J Cancer 43 (6):1061–1064
58. Giam CZ, Jeang KT (2007) HTLV-1 tax and adult T-cell leukemia. Front Biosci 12:1496–1507
59. Knowles DM, Cesarman E (1997) The Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) in Kaposi's sarcoma, malignant lymphoma, and other diseases. Ann Oncol 8 (Suppl 2):123–129
60. Engels EA, Chatterjee N, Cerhan JR, Davis S, Cozen W, Severson RK et al (2004) Hepatitis C virus infection and non-hodgkin lymphoma: results of the NCI-seer multi-center case-control study. Int J Cancer 111(1):76–80
61. Hausfater P, Rosenthal E, Cacoub P (2000) Lymphoproliferative diseases and hepatitis C virus infection. Ann Med Interne 151(1):53–57
62. Ohsawa M, Shingu N, Miwa H, Yoshihara H, Kubo M, Tsukuma H et al (1999) Risk of non-Hodgkin's lymphoma in patients with hepatitis C virus infection. Int J Cancer 80(2):237–239
63. Pioltelli P, Gargantini L, Cassi E, Santoleri L, Bellati G, Magliano EM et al (2000) Hepatitis C virus in non-Hodgkin's lymphoma. A reappraisal after a prospective case-control study of 300 patients. Lombart study group of HCV-lymphoma. Am J Hematol 64(2):95–100
64. Montella M, Crispo A, de Bellis G, Izzo F, Frigeri F, Ronga D et al (2001) HCV and cancer: a case-control study in a high-endemic area. Liver 21(5):335–341
65. Dal Maso L, Franceschi S (2006) Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. Cancer Epidemiol Biomark Prev 15 (11):2078–2085
66. de Sanjose S, Benavente Y, Vajdic CM, Engels EA, Morton LM, Bracci PM et al (2008) Hepatitis C and non-Hodgkin lymphoma among 4,784 cases and 6,269 controls from the

International Lymphoma Epidemiology Consortium. Clin Gastroenterol Hepatol 6(4): 451–458
67. Nieters A, Kallinowski B, Brennan P, Ott M, Maynadie M, Benavente Y et al (2006) Hepatitis C and risk of lymphoma: results of the European multicenter case-control study EPILYMPH. Gastroenterology 131(6):1879–1886
68. Wotherspoon AC (1998) Gastric lymphoma of mucosa-associated lymphoid tissue and *Helicobacter pylori*. Annu Rev Med 49:289–299
69. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E et al (1994) *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 330(18):1267–1271
70. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M et al (1993) Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet 342(8871):575–577
71. Goodlad JR, Davidson MM, Hollowood K, Ling C, MacKenzie C, Christie I et al (2000) Primary cutaneous B-cell lymphoma and *Borrelia burgdorferi* infection in patients from the Highlands of Scotland. Am J Surg Pathol 24(9):1279–1285
72. Roggero E, Zucca E, Mainetti C, Bertoni F, Valsangiacomo C, Pedrinis E et al (2000) Eradication of *Borrelia burgdorferi* infection in primary marginal zone B-cell lymphoma of the skin. Hum Pathol 31(2):263–268
73. Guidoboni M, Ferreri AJ, Ponzoni M, Doglioni C, Dolcetti R (2006) Infectious agents in mucosa-associated lymphoid tissue-type lymphomas: pathogenic role and therapeutic perspectives. Clin Lymphoma Myeloma 6(4):289–300
74. Anderson LA, Atman AA, McShane CM, Titmarsh GJ, Engels EA, Koshiol J (2014) Common infection-related conditions and risk of lymphoid malignancies in older individuals. Br J Cancer 110(11):2796–2803
75. Tavani A, La Vecchia C, Franceschi S, Serraino D, Carbone A (2000) Medical history and risk of Hodgkin's and non-Hodgkin's lymphomas. Eur J Cancer Prev 9(1):59–64
76. Liu YC, Yang YH, Hsiao HH, Yang WC, Liu TC, Chang CS et al (2012) Herpes zoster is associated with an increased risk of subsequent lymphoid malignancies—a nationwide population-based matched-control study in Taiwan. BMC Cancer 12:503
77. Adami J, Nyren O, Bergstrom R, Ekbom A, Engholm G, Englund A et al (1998) Smoking and the risk of leukemia, lymphoma, and multiple myeloma (Sweden). Cancer Causes Control 9(1):49–56
78. Willett EV, Smith AG, Dovey GJ, Morgan GJ, Parker J, Roman E (2004) Tobacco and alcohol consumption and the risk of non-Hodgkin lymphoma. Cancer Causes Control 15 (8):771–780
79. Besson H, Brennan P, Becker N, Nieters A, De Sanjose S, Font R et al (2006) Tobacco smoking, alcohol drinking and non-Hodgkin's lymphoma: a European multicenter case-control study (Epilymph). Int J Cancer 119(4):901–908
80. Herrinton LJ, Friedman GD (1998) Cigarette smoking and risk of non-Hodgkin's lymphoma subtypes. Cancer Epidemiol Biomark Prev 7(1):25–28
81. Talamini R, Polesel J, Montella M, Maso LD, Crispo A, Spina M et al (2005) Smoking and non-Hodgkin lymphoma: case-control study in Italy. Int J Cancer 115(4):606–610
82. Stagnaro E, Tumino R, Parodi S, Crosignani P, Fontana A, Masala G et al (2004) Non-Hodgkin's lymphoma and type of tobacco smoke. Cancer Epidemiol Biomark Prev 13 (3):431–437
83. Sergentanis TN, Kanavidis P, Michelakos T, Petridou ET (2013) Cigarette smoking and risk of lymphoma in adults: a comprehensive meta-analysis on Hodgkin and non-Hodgkin disease. Eur J Cancer Prev 22(2):131–150
84. Besson H, Renaudier P, Merrill RM, Coiffier B, Sebban C, Fabry J et al (2003) Smoking and non-Hodgkin's lymphoma: a case-control study in the Rhone-Alpes region of France. Cancer Causes Control 14(4):381–389
85. Morton LM, Hartge P, Holford TR, Holly EA, Chiu BC, Vineis P et al (2005) Cigarette smoking and risk of non-Hodgkin lymphoma: a pooled analysis from the International

Lymphoma Epidemiology Consortium (InterLymph). Cancer Epidemiol Biomark Prev 14 (4):925–933
86. Schollkopf C, Smedby KE, Hjalgrim H, Rostgaard K, Gadeberg O, Roos G et al (2005) Cigarette smoking and risk of non-Hodgkin's lymphoma–a population-based case-control study. Cancer Epidemiol Biomark Prev 14(7):1791–1796
87. Aschebrook-Kilfoy B, Cocco P, La Vecchia C, Chang ET, Vajdic CM, Kadin ME et al (2014) Medical history, lifestyle, family history, and occupational risk factors for mycosis fungoides and sezary syndrome: the InterLymph non-hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014(48):98–105
88. Monnereau A, Slager SL, Hughes AM, Smith A, Glimelius B, Habermann TM et al (2014) Medical history, lifestyle, and occupational risk factors for hairy cell leukemia: the InterLymph non-hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014 (48):115–124
89. Bell DA, Liu Y, Cortopassi GA (1995) Occurrence of bcl-2 oncogene translocation with increased frequency in the peripheral blood of heavy smokers. J Natl Cancer Inst 87 (3):223–224
90. Chiu BC, Dave BJ, Blair A, Gapstur SM, Chmiel JS, Fought AJ et al (2007) Cigarette smoking, familial hematopoietic cancer, hair dye use, and risk of t(14;18)-defined subtypes of non-Hodgkin's lymphoma. Am J Epidemiol 165(6):652–659
91. Schroeder JC, Olshan AF, Dent RB, Weinberg CR, Yount B, Cerhan JR et al (2002) A case-control study of tobacco use and other non-occupational risk factors for t(14;18) subtypes of non-Hodgkin's lymphoma (United States). Cancer Causes Control 13(2):159–168
92. Diver WR, Teras LR, Gaudet MM, Gapstur SM (2014) Exposure to environmental tobacco smoke and risk of non-Hodgkin lymphoma in nonsmoking men and women. Am J Epidemiol 179(8):987–995
93. Chang ET, Clarke CA, Canchola AJ, Lu Y, Wang SS, Ursin G et al (2010) Alcohol consumption over time and risk of lymphoid malignancies in the California teachers study cohort. Am J Epidemiol 172(12):1373–1383
94. Chiu BC, Cerhan JR, Gapstur SM, Sellers TA, Zheng W, Lutz CT et al (1999) Alcohol consumption and non-Hodgkin lymphoma in a cohort of older women. Br J Cancer 80 (9):1476–1482
95. Chiu BC, Weisenburger DD, Cantor KP, Zahm SH, Holmes F, Burmeister LF et al (2002) Alcohol consumption, family history of hematolymphoproliferative cancer, and the risk of non-Hodgkin's lymphoma in men. Ann Epidemiol 12(5):309–315
96. Holly EA, Lele C, Bracci PM, McGrath MS (1999) Case-control study of non-Hodgkin's lymphoma among women and heterosexual men in the San Francisco Bay Area, California. Am J Epidemiol 150(4):375–389
97. Morton LM, Holford TR, Leaderer B, Zhang Y, Zahm SH, Boyle P et al (2003) Alcohol use and risk of non-Hodgkin's lymphoma among Connecticut women (United States). Cancer Causes Control 14(7):687–694
98. Lim U, Morton LM, Subar AF, Baris D, Stolzenberg-Solomon R, Leitzmann M et al (2007) Alcohol, smoking, and body size in relation to incident Hodgkin'' and non-Hodgkin's lymphoma risk. Am J Epidemiol 166(6):697–708
99. De Stefani E, Fierro L, Barrios E, Ronco A (1998) Tobacco, alcohol, diet and risk of non-Hodgkin's lymphoma: a case-control study in Uruguay. Leuk Res 22(5):445–452
100. Wang SS, Flowers CR, Kadin ME, Chang ET, Hughes AM, Ansell SM et al (2014) Medical history, lifestyle, family history, and occupational risk factors for peripheral T-cell lymphomas: the InterLymph non-hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014(48):66–75
101. Mbulaiteye SM, Morton LM, Sampson JN, Chang ET, Costas L, de Sanjose S et al (2014) Medical history, lifestyle, family history, and occupational risk factors for sporadic burkitt lymphoma/leukemia: the InterLymph non-hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr. 2014(48):106–114

102. Cross AJ, Lim U (2006) The role of dietary factors in the epidemiology of non-Hodgkin's lymphoma. Leuk Lymphoma 47(12):2477–2487
103. Skibola CF (2007) Obesity, diet and risk of non-Hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev 16(3):392–395
104. Chiu BC, Cerhan JR, Folsom AR, Sellers TA, Kushi LH, Wallace RB et al (1996) Diet and risk of non-Hodgkin lymphoma in older women. JAMA 275(17):1315–1321
105. Purdue MP, Bassani DG, Klar NS, Sloan M, Kreiger N (2004) Dietary factors and risk of non-Hodgkin lymphoma by histologic subtype: a case-control analysis. Cancer Epidemiol Biomarkers Prev 13(10):1665–1676
106. Zhang S, Hunter DJ, Rosner BA, Colditz GA, Fuchs CS, Speizer FE et al (1999) Dietary fat and protein in relation to risk of non-Hodgkin's lymphoma among women. J Natl Cancer Inst 91(20):1751–1758
107. Aschebrook-Kilfoy B, Ollberding NJ, Kolar C, Lawson TA, Smith SM, Weisenburger DD et al (2012) Meat intake and risk of non-Hodgkin lymphoma. Cancer Causes Control 23 (10):1681–1692
108. Chang ET, Smedby KE, Zhang SM, Hjalgrim H, Melbye M, Ost A et al (2005) Dietary factors and risk of non-hodgkin lymphoma in men and women. Cancer Epidemiol Biomarkers Prev. 14(2):512–520
109. Cross AJ, Ward MH, Schenk M, Kulldorff M, Cozen W, Davis S et al (2006) Meat and meat-mutagen intake and risk of non-Hodgkin lymphoma: results from a NCI-SEER case-control study. Carcinogenesis 27(2):293–297
110. Daniel CR, Sinha R, Park Y, Graubard BI, Hollenbeck AR, Morton LM et al (2012) Meat intake is not associated with risk of non-Hodgkin lymphoma in a large prospective cohort of U.S. men and women. J Nutr 142(6):1074–1080
111. Charbonneau B, O''onnor HM, Wang AH, Liebow M, Thompson CA, Fredericksen ZS et al (2013) Trans fatty acid intake is associated with increased risk and n3 fatty acid intake with reduced risk of non-hodgkin lymphoma. J Nutr 143(5):672–681
112. Zheng T, Holford TR, Leaderer B, Zhang Y, Zahm SH, Flynn S et al (2004) Diet and Nutrient Intakes and Risk of Non-Hodgkin's Lymphoma in Connecticut Women. Am J Epidemiol 159(5):454–466
113. Davis S (1992) Nutritional factors and the development of non-Hodgkin's lymphoma: a review of the evidence. Cancer Res 52(19 Suppl):5492s–5495s
114. Ollberding NJ, Aschebrook-Kilfoy B, Caces DB, Wright ME, Weisenburger DD, Smith SM et al (2013) Phytanic acid and the risk of non-Hodgkin lymphoma. Carcinogenesis 34 (1):170–175
115. Calder PC, Kew S (2002) The immune system: a target for functional foods? Br J Nutr 88: S165–S177
116. Kelemen LE, Cerhan JR, Lim U, Davis S, Cozen W, Schenk M et al (2006) Vegetables, fruit, and antioxidant-related nutrients and risk of non-Hodgkin lymphoma: a National Cancer Institute-Surveillance, epidemiology, and end results population-based case-control study. Am J Clin Nutr 83(6):1401–1410
117. Talamini R, Polesel J, Montella M, Dal Maso L, Crovatto M, Crispo A et al. (2006) Food groups and risk of non-Hodgkin lymphoma: a multicenter, case-control study in Italy. Int J Cancer 118(11): 2871–2876
118. Rohrmann S, Becker N, Linseisen J, Nieters A, Rudiger T, Raaschou-Nielsen O et al (2007) Fruit and vegetable consumption and lymphoma risk in the European prospective investigation into cancer and nutrition (EPIC). Cancer Causes Control 18(5):537–549
119. Thompson CA, Habermann TM, Wang AH, Vierkant RA, Folsom AR, Ross JA et al (2010) Antioxidant intake from fruits, vegetables and other sources and risk of non-Hodgkin's lymphoma: the Iowa Women's health study. Int J Cancer 126(4):992–1003
120. Ollberding NJ, Maskarinec G, Conroy SM, Morimoto Y, Franke AA, Cooney RV et al (2012) Prediagnostic circulating carotenoid levels and the risk of non-Hodgkin lymphoma: the Multiethnic Cohort. Blood 119(24):5817–5823

121. Erber E, Maskarinec G, Gill JK, Park SY, Kolonel LN (2009) Dietary patterns and the risk of non-Hodgkin lymphoma: the multiethnic cohort. Leuk Lymphoma 50(8):1269–1275
122. Ollberding NJ, Aschebrook-Kilfoy B, Caces DB, Smith SM, Weisenburger DD, Chiu BC (2013) Dietary patterns and the risk of non-hodgkin lymphoma. Public Health Nutr 1–7
123. Marti A, Marcos A, Martinez JA (2001) Obesity and immune function relationships. Obes Rev 2(2):131–140
124. Cerhan JR, Bernstein L, Severson RK, Davis S, Colt JS, Blair A et al (2005) Anthropometrics, physical activity, related medical conditions, and the risk of non-hodgkin lymphoma. Cancer Causes Control 16(10):1203–1214
125. Skibola CF, Holly EA, Forrest MS, Hubbard A, Bracci PM, Skibola DR et al (2004) Body mass index, leptin and leptin receptor polymorphisms, and non-hodgkin lymphoma. Cancer Epidemiol Biomark Prev 13(5):779–786
126. Chang ET, Hjalgrim H, Smedby KE, Akerman M, Tani E, Johnsen HE et al (2005) Body mass index and risk of malignant lymphoma in Scandinavian men and women. J Natl Cancer Inst 97(3):210–218
127. Fernberg P, Odenbro A, Bellocco R, Boffetta P, Pawitan Y, Adami J (2006) Tobacco use, body mass index and the risk of malignant lymphomas–a nationwide cohort study in Sweden. Int J Cancer 118(9):2298–2302
128. Tulinius H, Sigfusson N, Sigvaldason H, Bjarnadottir K, Tryggvadottir L (1997) Risk factors for malignant diseases: a cohort study on a population of 22,946 Icelanders. Cancer Epidemiol Biomark Prev 6(11):863–873
129. Cerhan JR, Janney CA, Vachon CM, Habermann TM, Kay NE, Potter JD et al (2002) Anthropometric characteristics, physical activity, and risk of non-Hodgkin's lymphoma subtypes and B-cell chronic lymphocytic leukemia: a prospective study. Am J Epidemiol 156 (6):527–535
130. MacInnis RJ, English DR, Hopper JL, Giles GG (2005) Body size and composition and the risk of lymphohematopoietic malignancies. J Natl Cancer Inst 97(15):1154–1157
131. Willett EV, Morton LM, Hartge P, Becker N, Bernstein L, Boffetta P et al (2008) Non-Hodgkin lymphoma and obesity: a pooled analysis from the InterLymph Consortium. Int J Cancer 122(9):2062–2070
132. Larsson SC, Wolk A (2007) Obesity and risk of non-Hodgkin's lymphoma: a meta-analysis. Int J Cancer 121(7):1564–1570
133. Larsson SC, Wolk A (2011) Body mass index and risk of non-Hodgkin's and Hodgkin" lymphoma: a meta-analysis of prospective studies. Eur J Cancer 47(16):2422–2430
134. Ames BN, Kammen HO, Yamasaki E (1975) Hair dyes are mutagenic: identification of a variety of mutagenic ingredients. Proc Natl Acad Sci USA 72(6):2423–2427
135. de Sanjose S, Benavente Y, Nieters A, Foretova L, Maynadie M, Cocco PL et al (2006) Association between personal use of hair dyes and lymphoid neoplasms in Europe. Am J Epidemiol 164(1):47–55
136. Zhang Y, Holford TR, Leaderer B, Boyle P, Zahm SH, Flynn S et al (2004) Hair-coloring product use and risk of non-Hodgkin's lymphoma: a population-based case-control study in Connecticut. Am J Epidemiol 159(2):148–154
137. Zhang Y, Sanjose SD, Bracci PM, Morton LM, Wang R, Brennan P et al (2008) Personal use of hair dye and the risk of certain subtypes of non-Hodgkin lymphoma. Am J Epidemiol 167 (11):1321–1331
138. Melbye M, Adami HO, Hjalgrim H, Glimelius B (1996) Ultraviolet light and non-Hodgkin's lymphoma. Acta Oncol 35(6):655–657
139. Hartge P, Lim U, Freedman DM, Colt JS, Cerhan JR, Cozen W et al (2006) Ultraviolet radiation, dietary vitamin D, and risk of non-Hodgkin lymphoma (United States). Cancer Causes Control 17(8):1045–1052
140. Hughes AM, Armstrong BK, Vajdic CM, Turner J, Grulich AE, Fritschi L et al (2004) Sun exposure may protect against non-Hodgkin lymphoma: a case-control study. Int J Cancer 112 (5):865–871

141. Smedby KE, Hjalgrim H, Melbye M, Torrang A, Rostgaard K, Munksgaard L et al (2005) Ultraviolet radiation exposure and risk of malignant lymphomas. J Natl Cancer Inst 97 (3):199–209
142. Weihkopf T, Becker N, Nieters A, Mester B, Deeg E, Elsner G et al (2007) Sun exposure and malignant lymphoma: a population-based case-control study in Germany. Int J Cancer 120 (11):2445–2451
143. Kricker A, Armstrong BK, Hughes AM, Goumas C, Smedby KE, Zheng T et al (2008) Personal sun exposure and risk of non Hodgkin lymphoma: a pooled analysis from the InterLymph Consortium. Int J Cancer 122(1):144–154
144. Ullrich SE (1996) Does exposure to UV radiation induce a shift to a Th-2-like immune reaction? Photochem Photobiol 64(2):254–258
145. Reichrath J, Rappl G (2003) Ultraviolet light (UV)-induced immunosuppression: is vitamin D the missing link? J Cell Biochem 89(1):6–8
146. Aref S, Ibrahim L, Azmy E (2013) Prognostic impact of serum 25-hydroxivitamin D [25 (OH)D] concentrations in patients with lymphoid malignancies. Hematology 18(1):20–25
147. Shanafelt TD, Drake MT, Maurer MJ, Allmer C, Rabe KG, Slager SL et al (2011) Vitamin D insufficiency and prognosis in chronic lymphocytic leukemia. Blood 117(5):1492–1498
148. Drake MT, Maurer MJ, Link BK, Habermann TM, Ansell SM, Micallef IN et al (2010) Vitamin D insufficiency and prognosis in non-Hodgkin's lymphoma. J Clin Oncol 28 (27):4191–4198
149. Luczynska A, Kaaks R, Rohrmann S, Becker S, Linseisen J, Buijsse B et al (2013) Plasma 25-hydroxyvitamin D concentration and lymphoma risk: results of the European prospective investigation into cancer and nutrition. Am J clin Nutr 98(3):827–838
150. Purdue MP, Freedman DM, Gapstur SM, Helzlsouer KJ, Laden F, Lim U et al (2010) Circulating 25-hydroxyvitamin D and risk of non-hodgkin lymphoma: Cohort Consortium Vitamin D pooling project of rarer cancers. Am J Epidemiol 172(1):58–69
151. Boffetta P, de Vocht F (2007) Occupation and the risk of non-Hodgkin lymphoma. Cancer Epidemiol Biomark Prev 16(3):369–372
152. Cocco P, Satta G, D''ndrea I, Nonne T, Udas G, Zucca M et al (2013) Lymphoma risk in livestock farmers: results of the Epilymph study. Int J Cancer 132(11):2613–2618
153. Espinosa A, Zock JP, Benavente Y, Boffetta P, Becker N, Brennan P et al (2013) Occupational exposure to immunologically active agents and risk for lymphoma: the European Epilymph case-control study. Cancer Epidemiol 37(4):378–384
154. Skibola CF, Slager SL, Berndt SI, Lightfoot T, Sampson JN, Morton LM et al (2014) Medical history, lifestyle, family history, and occupational risk factors for adult acute lymphocytic leukemia: the InterLymph non-Hodgkin lymphoma subtypes Project. J Natl Cancer Inst Monogr 2014(48):125–129
155. Cocco P, Satta G, Dubois S, Pili C, Pilleri M, Zucca M et al (2013) Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study. Occup Environ Med 70 (2):91–98
156. Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM et al (1992) Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res 52(9):2447–2455
157. Zahm SH, Blair A. Pesticides and non-Hodgkin's lymphoma. Cancer Res. 52(19 Supplement):5485s–5488s
158. Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP et al (1990) A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1(5): 49–356
159. Chiu BC, Dave BJ, Blair A, Gapstur SM, Zahm SH, Weisenburger DD (2006) Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-Hodgkin lymphoma. Blood 108 (4):1363–1369

160. Schroeder JC, Olshan AF, Baric R, Dent GA, Weinberg CR, Yount B, et al. (2001) Agricultural risk factors for t(14;18) subtypes of non-Hodgkin's lymphoma. Epidemiology 12(6):701-709
161. Pimental D, McLaughlin L, Zepp A (1993) Environmental and economic impacts of reducing US agricultural pesticide use. In: Pimental D (ed) Handbook of pest management in agriculture, 1st edn. CRC Press, Boca Raton, pp 679–680
162. Cocco P, t''annetje A, Fadda D, Melis M, Becker N, de Sanjose S et al (2010) Occupational exposure to solvents and risk of lymphoma subtypes: results from the Epilymph case-control study. Occup Env Med 67(5):341–347
163. Deng Q, Zheng T, Lan Q, Lan Y, Holford T, Chen Y et al (2013) Occupational solvent exposure, genetic variation in immune genes, and the risk for non-Hodgkin lymphoma. Eur J Cancer Prev 22(1):77–82
164. Linet MS, Pottern LM (1992) Familial aggregation of hematopoietic malignancies and risk of non-Hodgkin's lymphoma. Cancer Res 52S:5468–5473
165. Paltiel O, Schmit T, Adler B, Rachmilevitz EA, Polliack A, Cohen A et al (2000) The incidence of lymphoma in first-degree relatives of patients with hodgkin disease and non-Hodgkin lymphoma: results and limitations of a registry-linked study. Cancer 88 (10):2357–2366
166. Casey R, Brennan P, Becker N, Boffetta P, Cocco P, Domingo-Domenech E et al (2006) Influence of familial cancer history on lymphoid neoplasms risk validated in the large European case-control study epilymph. Eur J Cancer 42(15):2570–2576
167. Wang SS, Slager SL, Brennan P, Holly EA, De Sanjose S, Bernstein L et al (2007) Family history of hematopoietic malignancies and risk of non-Hodgkin lymphoma (NHL): a pooled analysis of 10,211 cases and 11,905 controls from the International Lymphoma Epidemiology Consortium (InterLymph). Blood 109(8):3479–3488
168. Zhu K, Levine RS, Brann EA, Gu Y, Caplan LS, Hall I et al (2001) Risk factors for non-Hodgkin's lymphoma according to family history of haematolymphoproliferative malignancies. Int J Epidemiol 30:818–824
169. Skibola CF, Curry JD, Nieters A (2007) Genetic susceptibility to lymphoma. Haematologica 92(7):960–969
170. Bateman AC, Howell WM (1999) Human leukocyte antigens and cancer: is it in our genes? J Pathol 188(3):231–236
171. Skibola CF, Bracci PM, Halperin E, Conde L, Craig DW, Agana L et al (2009) Genetic variants at 6p21.33 are associated with susceptibility to follicular lymphoma. Nat Genet 41 (8):873–875
172. Smedby KE, Foo JN, Skibola CF, Darabi H, Conde L, Hjalgrim H et al (2011) GWAS of follicular lymphoma reveals allelic heterogeneity at 6p21.32 and suggests shared genetic susceptibility with diffuse large B-cell lymphoma. PLoS Genet 7(4):e1001378
173. Cerhan JR, Fredericksen ZS, Novak AJ, Ansell SM, Kay NE, Liebow M et al (2012) A two-stage evaluation of genetic variation in immune and inflammation genes with risk of non-Hodgkin lymphoma identifies new susceptibility locus in 6p21.3 region. Cancer Epidemiol Biomark Prev 21(10):1799–1806
174. Lim U, Kocarnik JM, Bush WS, Matise TC, Caberto C, Park SL et al (2014) Pleiotropy of cancer susceptibility variants on the risk of non-Hodgkin lymphoma: the PAGE consortium. PLoS one 9(3):e89791
175. Slager SL, Rabe KG, Achenbach SJ, Vachon CM, Goldin LR, Strom SS et al (2011) Genome-wide association study identifies a novel susceptibility locus at 6p21.3 among familial CLL. Blood 117(6):1911–1916
176. Slager SL, Achenbach SJ, Asmann YW, Camp NJ, Rabe KG, Goldin LR et al (2013) Mapping of the IRF8 gene identifies a 3'TR variant associated with risk of chronic lymphocytic leukemia but not other common non-Hodgkin lymphoma subtypes. Cancer Epidemiol Biomark Prev 22(3):461–466

177. Rothman N, Skibola CF, Wang SS, Morgan G, Lan Q, Smith MT et al (2006) Genetic variation in TNF and IL10 and risk of non-Hodgkin lymphoma: a report from the InterLymph Consortium. Lancet Oncol 7(1):27–38
178. Niclot S, Pruvot Q, Besson C, Savoy D, Macintyre E, Salles G et al (2006) Implication of the folate-methionine metabolism pathways in susceptibility to follicular lymphomas. Blood 108 (1):278–285
179. Rudd MF, Sellick GS, Allinson R, Matutes E, Catovsky D, Houlston RS (2004) MTHFR polymorphisms and risk of chronic lymphocytic leukemia. Cancer Epidemiol Biomark Prev 13(12):2268–2270
180. Skibola CF, Forrest MS, Coppede F, Agana L, Hubbard A, Smith MT et al (2004) Polymorphisms and haplotypes in folate-metabolizing genes and risk of non-Hodgkin lymphoma. Blood 104(7):2155–2162
181. Morton LM, Slager SL, Cerhan JR, Wang SS, Vajdic CM, Skibola CF et al (2014) Etiologic heterogeneity among non-hodgkin lymphoma subtypes: the InterLymph non-Hodgkin lymphoma subtypes project. J Natl Cancer Inst Monogr 2014(48):130–144
182. Evens AM, Chiu BC (2008) The challenges of epidemiologic research in non-Hodgkin lymphoma. JAMA 300(17):2059–2061
183. Turner JJ, Morton LM, Linet MS, Clarke CA, Kadin ME, Vajdic CM et al (2010) InterLymph hierarchical classification of lymphoid neoplasms for epidemiologic research based on the WHO classification (2008): update and future directions. Blood 116(20): e90–e98
184. Rimsza LM, Leblanc ML, Unger JM, Miller TP, Grogan TM, Persky DO et al (2008) Gene expression predicts overall survival in paraffin-embedded tissues of diffuse large B-cell lymphoma treated with R-CHOP. Blood 112(8):3425–3433

Pathology of B-Cell Lymphomas: Diagnosis and Biomarker Discovery

Sarah L. Ondrejka and Eric D. Hsi

Abstract

The diagnosis of B-cell non-Hodgkin lymphomas has changed significantly over the past few decades as new immunophenotypic markers, molecular subtype classification schemes, and novel biomarkers have emerged. Meanwhile, there has been an increasing emphasis on individualizing treatment approaches in accordance with a biologic heterogeneity that has been uncovered within many of the individual B-cell lymphoma entities. The application of high-throughput genomic sequencing to B-cell lymphomas has yielded large amounts of valuable information. The data encompass discoveries essential to an understanding of pathogenesis, clonal or tumoral evolution, and identification of biomarkers that may be useful for prognostic or therapeutic considerations. The following review discusses several of the more common, primarily tissuebased B-cell lymphomas, with a focus on pathologic classification and certain phenotypic characteristics or genetic lesions that apply to refinement of diagnosis and therapy.

Keywords

B-cell lymphoma · Biomarker

S.L. Ondrejka · E.D. Hsi (✉)
Cleveland Clinic—Robert J. Tomsich Pathology and Laboratory Medicine Institute,
9500 Euclid Ave, L3, Cleveland, OH 44195, USA
e-mail: hsie@ccf.org

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_2

Contents

1 Introduction

B-cell lymphoma diagnosis has conventionally incorporated morphologic, immunophenotypic, and genetic findings for classification into specific disease categories. Despite this, the clinical behavior within each B-cell lymphoma subtype is variable and reflects a biologic heterogeneity that is increasingly recognized with the application of unbiased molecular characterization. Recent discovery of new biomarkers has led to improvements in diagnosis and prognosis in B-cell lymphomas. Massively parallel sequencing technologies have identified recurrent mutations that target important cellular pathways, and this has deepened our understanding of the oncogenic mechanisms of many of these neoplasms. Aside from providing insights into pathogenesis, identification of biomarkers that might provide clues to new therapeutic options or targeted therapies with lower toxicity would be of great benefit to patients with B-cell lymphomas. An additional benefit of useful biomarkers is that they might serve to predict disease course, in order to inform individualized treatment decisions. This review will cover some of the more common, primarily tissue-based B-cell lymphomas, progressing from small B-cell entities to the diffuse aggressive B-cell lymphomas. Pertinent pathologic features and current classification issues will be addressed along the way, including phenotypic (Table 1) and genetic considerations for diagnosis and disease management. Potential biomarkers that have surfaced over the last few years will be considered along with each entity, in light of the feasibility of application in routine clinical settings (Table 2). Finally, a discussion of perhaps the most promising discoveries out of next-generation sequencing studies will provide a framework for ongoing literature review as the field advances.

Table 1 Expected immunophenotype of selected B cell non-Hodgkin lymphomas

	Expected immunophenotype
Follicular lymphoma	CD19+, CD20+, IgM ± IgD, monotypic sIg+, CD10+, bcl-6+, bcl-2 ±, HGAL, LMO2; associated with CD21+/CD23+ follicular dendritic cell meshworks
Lymphoplasmacytic lymphoma	CD19+, CD20+, monotypic sIg+, IgM, CD22+, CD79a+, CD5 ∓; plasma cells have monotypic cytoplasmic Ig
Mantle cell lymphoma	CD19+, CD20+, monotypic sIg+, IgM ± IgD, CD5+, CD23− or weakly+, cyclinD1+, SOX11± , LEF1 −
Diffuse large B cell lymphoma, nos	CD19+, CD20+, monotypic sIg+ or absent sIg, BCL2± . MYC+ in some cases of typical DLBCL, nos, and in BCL-U
• Germinal center B like	Hans: (CD10+) or (CD10−/bcl-6+/MUM1−) Choi: (GCET+/MUM1−) or (GCET−/CD10+) or (GCET−/CD10−/BCL6+/FOXP1−)
	Tally: Scoring system favors CD10, GCET, and LMO2 >30 %
• Non-germinal center B like	Hans: (CD10−/BCL6−) or (CD10−/BCL6+/MUM1+) Choi: (GCET+/MUM1+) or (GCET−/CD10−/BCL6+/FOXP1+) or (GCET−/CD10−/BCL6−)
	Tally: Scoring system favors MUM1, FOXP1, and LMO2 <30 %
Primary mediastinal (thymic) large B-cell lymphoma	CD19+, CD20+, CD22+, CD79a+, MAL+, cREL+, CD200, MUM1, CD23, BCL2±, BCL6±, CD30± (weak, heterogeneous), surface Ig weak to absent
Burkitt lymphoma	CD19+, CD20+, CD10+, BCL6+, BCL2-, monotypic sIg +, MUM1−, MYC+, Ki67 > 95 %. Cases associated with EBV: EBV-encoded RNA+/EBNA-1+ and LMP1−/EBNA-2−

2 Practical Points

Diagnosis of B-cell lymphoma is best achieved with excisional or incisional lymph node or extranodal tissue biopsy. Examination of the fresh biopsy tissue (by touch imprint or frozen section) clinically suspected of lymphomatous involvement at the time of the procedure allows proper allocation of fresh tissue for flow cytometry and cytogenetic studies. While fine-needle aspiration can result in procurement of fresh cells for ancillary flow cytometry testing, it is not ideal for definitive lymphoma classification since this requires assessment of spatial and architectural pattern recognition that is lost in FNA samples. Similarly, needle core biopsies may not show important architectural features and may result in a non-definitive diagnosis, misclassification, or even misdiagnosis. Formalin fixation is the standard, and through technical advances over the past 20 years, formalin-fixed paraffin-embedded tissues can now be used for detailed immunophenotyping by immunohistochemistry (essential in modern practice), fluorescence in situ hybridization for

Table 2 Recently investigated molecular biomarkers in selected B-cell non-Hodgkin lymphomas

	Molecular biomarker	Useful method for detection	Significance
Lymphoplasmacytic lymphoma	*MYD88* L265P mutation	Allele-specific PCR	Fairly sensitive and specific for LPL/WM and IgM MGUS diagnosis. May be predictive of disease burden and indicate risk of progression in IgM MGUS
Mantle cell lymphoma	SOX11 transcription factor overexpression	Immunohistochemistry (especially monoclonal anti-SOX11^{MRQ-58}, Cell Marque, Rocklin, CA)	Good biomarker for MCL diagnosis, including cyclin D1-negative variant. May be a useful prognostic parameter, but studies are conflicting. May be a promising prospect for MRD analysis. Suppresses terminal B-cell differentiation and drives angiogenesis in MCL [97].
Diffuse aggressive B-cell lymphoma	"double-hit" genotype (dual *BCL2* and *MYC* translocations are most common)	Fluorescence in situ hybridization (FISH) detects genetic translocation	Prognostic biomarker (worse progression-free and overall survival). Double-protein-positive (MYC and BCL2 protein by immunohistochemistry) independently predicts a worse survival in rituximab–CHOP-treated patients with DLBCL
Diffuse large B-cell lymphoma	Germinal center B cell-like (GCB) versus activated B cell-like (ABC) gene expression profile	Several immunohistochemistry—based algorithms, or new multiplex gene expression assay (ICEPlex® system, PrimeraDx, Mansfield, MA)	Prognostic and biologically significant. The ABC subtype has a worse outcome and indicates activation of the B-cell receptor and NF-κB pathways. This may also predict response to targeted therapies and support clinical trials

specific chromosomal rearrangements or numerical abnormalities, and DNA- or RNA-based testing (such as gene rearrangement, other amplification-based, expression, or sequencing studies). To summarize, excisional lymph node biopsy with a small portion sent for flow cytometric analysis provides the best guarantee of arriving at a specific diagnosis, particularly if an adequate lymphoid sample can be confirmed at the time of surgery.

3 Follicular Lymphoma

Follicular lymphoma (FL) is a common B-cell lymphoma that accounts for 20 % of non-Hodgkin lymphomas in the USA and Western Europe. It has a median age of 60 years and female predominance. Patients usually present with painless, slowly progressive lymphadenopathy and higher stage (Ann Arbor III–IV) with few exhibiting B symptoms. Despite its prototypic clinical course, it has been known for some time that the disease is variable with some patients experiencing waxing and waning symptoms, while others might succumb following early transformation to a high-grade lymphoma [47].

Histopathologically, this lymphoma subtype effaces the nodal architecture with at least a partially follicular pattern. The cytologic composition is a mixture of centrocytes and centroblasts in varying proportions, and grading is based on the number of centroblasts per 400× microscopic field (grades 1—3B). The phenotype is that of a germinal center B cell (GCB) with expression of BCL6, CD10, LMO2, and HGAL in most cases [40, 118]. The genetic features include clonally rearranged immunoglobulin genes and t(14;18)(q32;q21) that leads to aberrant *BCL2* expression. Alternatively, *BCL2* can be duplicated/amplified or translocated to one of the immunoglobulin light-chain genes, but the proportion of cases that have *BCL2* rearrangements decreases with increasing cytologic grade. The largest proportion of FL in North America and Western Europe has low-grade cytology, t(14;18)(q32;q21), and CD10 and BCL2 protein coexpression [37]. FISH seems to be the most sensitive method for detection of *BCL2* translocation [107].

FL is incurable but relatively indolent. The outcome can be predicted by the FL-specific International Prognostic Index (i.e., FLIPI), which was developed for all FL histologies, and includes a point system based on 5 factors (age > 60, stage III/IV, LDH > upper limit of normal, nodal groups > 4, and Hgb < 12 [96]. This was revised to the FLIPI-2 which was designed in the rituximab era and took into account the mass diameter size, as well as using progression-free survival as an endpoint instead of overall survival [25].

The clinical heterogeneity in FL was investigated by gene expression profiling. This led to the discovery of the importance of the microenvironment in predicting survival. Within a large cohort of FL, a survival predictor was generated based on genes that were found to reflect the nonmalignant immune cells (such as T cells, macrophages, and dendritic cells) that were flow sorted from the tumor [16]. A separate gene expression study using different selection criteria and endpoints found

differences between transformed and non-transformed FL, notably possession of an activated follicular hyperplasia versus a downregulated immune response signature, respectively. They also found that characterization of the immune state by immunohistochemistry was a sensitive way to assess the microenvironmental features [31]. However, there was little overlap in terms of a clinically useful gene set that could be exploited. Additional immunohistochemistry-based studies as a surrogate for GEP, in an attempt to characterize the immune cell composition (lymphoma-associated macrophages, "polarized macrophages," and T-cell helper or regulatory subsets) for prognostic benefit, yielded conflicting results, especially when this was extended to prospective studies of patients on monoclonal antibody therapy. Some were able to stratify groups based on outcome [102, 111], while others showed no association with immune makeup and overall survival [99]. Differences in results may be explained by differences in patient selection, treatment regimens, or technical issues. Regardless, there is evidence that the microenvironmental composition in FL shifts during disease progression and is likely important for tumor survival [50].

FL, along with other B-cell malignancies, possesses many genetic alterations that are being defined and characterized as to biologic and clinical significance. Recently, mutations in genes associated with chromatin modification (*EZH2*, 20 % of FL) and histone methytransferases (*MLL2*, 89 % of FL; *CREBBP*, 32 % of FL) were discovered using high-throughput sequencing technologies [61, 85]. *MLL2* has a high mutational frequency and was thus hypothesized to cooperate with other mutations to increase genetic instability. *CREBBP* mutation attenuates acetylation of *BCL6*, resulting in an increase in activity of that oncogene and altered expression of *BCL6* target genes [74]. Importantly though, to understand the significance of each mutation in lymphomagenesis, a pursuit of the clonal/subclonal architecture of FL demonstrated that *IGH@/BCL2* frequently underlies the disease (assumed to be the primary event), while *CREBBP* is a candidate driver mutation and an important secondary event. *MLL2* and *TNFRSF14* were considered tertiary accelerator mutations [35].

To summarize, the FLIPI remains the most widely used prognosticator for FL at diagnosis. Cytologic grade is not prognostic in many studies, but FL grade 3B (comprised almost entirely of large centroblasts) shares biologic features more akin to diffuse large B-cell lymphoma [41], and a recent large retrospective review found that FL grade 3B has a higher mortality and a distinctly different clinical course (more aggressive, but curable) in comparison with grades 1–2 and 3a [112]. Studies using array comparative genomic hybridization to detect DNA copy number alterations or using gene expression profiling revealing lesions of *TP53* or *CKND2A*, or increased *MYC* expression levels, have approximated risk of transformation. However, this is not routinely done, and risk is not consistently predictable for all samples, precluding its clinical utility [18, 27]. At present, there is no prospectively validated clinical tool with which to use gene expression profiling in FL to evaluate the microenvironment for prediction of prognosis. High-throughput sequencing is an avenue that will likely be utilized as a part of routine diagnostics in the near future, now that it is feasible on formalin-fixed paraffin-embedded tissues. Uncovering the clonal architecture of neoplasms can help to

identify therapeutic targets by helping to focus on what mutations might be subclonal (and less effective for a targeted therapy) as opposed to a founder, driver, or accelerator mutation [35]. These shed light on new targets for therapies such as demethylating agents, histone- or chromatin-modifying therapies, and small-molecule inhibitors but also might be powerful to predict whether a patient's individual tumor might be responsive to these therapies based on its stage of clonal evolution.

4 Lymphoplasmacytic Lymphoma

Lymphoplasmacytic lymphoma (LPL) is a small B-cell neoplasm of older adults that usually affects the bone marrow, and less often involves spleen or lymph nodes [101]. It is characteristically accompanied by a serum IgM monoclonal paraprotein. Clinically, it is associated with hyperviscosity and Waldenström macroglobulinemia (WM), defined as bone marrow involvement with monoclonal B cells of LPL type and serum IgM paraproteinemia at any level [73]. While the pathologic features can be distinctive, it shares features with other types of small B-cell lymphomas, and genetic abnormalities such as deletion of 6q occurring most commonly in LPL are neither sensitive nor specific in assisting the diagnosis [9].

Recently, whole-genome sequencing identified a recurrent somatic mutation in the myeloid differentiation response gene 88 (*MYD88* L265P) present in approximately 91 % of patients with WM, which is absent in multiple myeloma and rarely present in splenic marginal zone lymphoma (approximately 6 %) [29, 105]. The mutation itself was initially discovered in DLBCL (29 % of activated B-cell-like DLBCL vs. 1.4 % germinal center-like, and 9 % of gastric MALT lymphomas), where it was shown to promote cell survival via an IRAK kinase–protein complex leading to JAK-STAT3 activation, NF-KB signaling, and interleukin/interferon secretion [64]. It is prevalent among other types of aggressive B-cell lymphomas with an activated B-cell-like immunophenotype, such as primary central nervous system lymphoma [60] and primary cutaneous DLBCL, leg type [76].

The *MYD88* L265P is not entirely specific for LPL in small B-cell lymphomas. It can be identified using high-sensitivity allele-specific PCR in a small percentage of splenic marginal zone lymphoma and a very small minority of chronic lymphocytic leukemia, as well as one reported case of hairy cell leukemia. [22, 46, 69]. It is also present in 50–87 % of IgM MGUS [46, 54]. Aside from its use in diagnosis of LPL/WM, recurrent somatic mutations in WM such as *MYD88* and, to a lesser degree, in the more recently discovered chemokine receptor *CXCR4* (27 %) may determine clinical presentation, progression, and/or overall survival. For patients with IgM MGUS, presence of *MYD88* L265P was associated with greater disease burden and an increased risk of disease progression, such that it might prove to be a useful biomarker in evaluation of this disease [109]. The Bruton tyrosine kinase (BTK) inhibitor ibrutinib was shown to inhibit MYD88-BTK complexes and thus support lymphoplasmacytic cell survival in patients with WM [117]. Presence of the *CXCR4* mutation was shown to be associated with resistance to ibrutinib by

mediating signaling pathways resistant to growth suppression in the presence of CXCR4 ligand [11]. Nonetheless, in a phase I study, ibrutinib therapy showed promising results in patients with WM [2]. Detection of *MYD88* L265P in the peripheral blood of patients with WM and IgM MGUS by quantitative allele-specific PCR is an area of recent interest, as the delta Ct might predict disease burden in the bone marrow and might obviate the need for bone marrow aspiration/biopsy-based monitoring of patients with WM/IgM MGUS [116]. It may also have utility in minimal residual disease (MRD) assessment. Thus, *MYD88* L265P has proven its utility not only as a diagnostic marker, but as a biomarker for disease burden and/or progression, and it is a fairly simple assay to implement in a clinical laboratory setting.

5 Mantle Cell Lymphoma

Mantle cell lymphoma represents 2–8 % of non-Hodgkin lymphomas in the United States. It presents in the seventh decade (median age 63 years) and is characterized by a widespread nodal presentation (Ann Arbor stage III/IV) accompanied by B symptoms, with a high frequency of Waldeyer's tonsillar ring involvement, and often with splenic involvement. Some patients have gastrointestinal manifestations in the form of multiple intestinal lymphomatous polyposis. Staging bone marrow examinations are frequently positive, and occasionally, circulating lymphoma cells are identifiable by morphology and flow cytometric analysis [100]. The median survival has increased over the past 30 years and is now estimated to be 4–5 years. MCL is typified by a high response to frontline therapies with development of chemoresistance at the time of relapse [33].

Various histopathologic growth patterns of lymph node involvement include mantle zone, nodular, and diffuse, in addition to a relatively recently described, extremely rare "in situ" pattern of localization [1, 12]; Weisenburger et al. [113]. The neoplastic cells are monomorphous small lymphocytes with nuclear irregularities and scant cytoplasm, with a distinctive B-cell immunophenotype (CD19+, CD20+, sIg+, CD5+, CD10−, CD23− or subset+, IgM ±IgD, cyclinD1+). Less commonly, blastoid or pleomorphic variants with lymphoblast-like or centroblast-like cytologic features and numerous mitoses can occur, either de novo or as histologic progression of usual mantle cell lymphoma [72]. MCLs with a higher proliferative index as estimated by Ki-67 immunohistochemistry or by mitotic index have a worse overall survival, regardless of the cytologic variant [103].

The genetic hallmark of mantle cell lymphoma is t(11;14)(q13;q32). As a result of this translocation, the cyclin D1 gene (*CCND1*) on chromosomal region 11q13 is translocated into the *IGH@* locus, where it comes under regulatory control of the *IGH@* enhancer sequences, resulting in overexpression of cyclin D1 protein [93]. This leads to constitutive cell cycle dysregulation, which together with secondary alterations in DNA damage response and with activation of cell survival signals underlies the pathogenesis of MCL. Less often, other partner loci such as either of

the immunoglobulin light-chain genes (κ, 2p11; λ, 22q11) are joined to *CCND1* [51]. While an in-depth discussion of cyclin D1-negative mantle cell lymphoma is beyond the scope of this chapter, it is an increasingly studied variant that can be recognized by typical morphologic and phenotypic features of MCL and a shared global genomic profile, with the exception of cyclin D1 [28]. A recent study found that in over half of the cyclin D1-negative MCL variants, a *CCND2* rearrangement was detected predominantly involving one of the Ig light-chain genes. Still, the molecular mechanism for cyclin D1-negative mantle cell lymphomas is only partially revealed. The clinical and biologic behavior of the cyclin D1-negative mantle cell lymphomas was similar to that of conventional MCL, indicating that CCND2 rearrangement can be a biomarker that indicates a need for intensive chemotherapy [88]. In addition to cyclin D1 translocation, there are numerous secondary genetic alterations in mantle cell lymphoma that contribute to its pathogenesis including deletion of the 9p21 locus (thereby *CKDN2A* and affecting cell cycle control via INK4a/CDK4/RB1), point mutation or deletion of *RB1* or *TP53*, other gene amplification, or *ATM* deletion contributing to genomic instability [45]. Finally, somatic mutational data generated by whole-genome or exome sequencing technologies have identified significantly mutated genes in MCL including known drivers of pathogenesis and others that play a role in anti-apoptosis or chromatin modification. *NOTCH2* mutations were discovered and correlated with a poor prognosis. Additional studies are needed to further understand mutational profiles in the setting of disease progression and significance for biologic therapies [6].

The Mantle Cell Lymphoma International Prognostic Index (MIPI) can accurately stratify patients into low-, intermediate-, and high-risk groups. Assessment of the tumor cell proliferative index by Ki67 yielded valuable prognostic information independent of the MIPI, but combined with the prognostic index, a "biologic MIPI" can serve as a prognostic guide for risk-adapted therapy [20, 42]. So far, TP53 appears to be the only significant independent molecular marker to add additional prognostic value to the MIPI in multivariate analysis [66].

While mantle cell lymphoma is typified by overall short survival and relatively aggressive disease course, it is now recognized that an indolent form of mantle cell lymphoma exists. It can be identified clinically as a subgroup of patients with non-nodal disease who may present with splenomegaly and leukemic involvement. This has been associated with mutated IGVH genes and lack of CD38 expression [70]. These patients have been shown to have a favorable prognosis with a median survival of 79 months compared to a group of nodal-based lymphoma with median OS of 30 months [70]. Indeed, patients with what could be considered a monoclonal B-cell lymphocytosis with t(11;14) have been identified [68]. Furthermore, we now realize that patients do not need to be treated at diagnosis and may do well with a "wait and watch" strategy [58]. Several independent studies have identified recurrent biologic and clinical features that may identify this indolent subtype, including non-nodal leukemic presentation, hypermutated immunoglobulin heavy-chain variable genes (IGVH, indicating derivation from post-GCBs), simple karyotype, and stable disease with longer survival [70, 84]. These features were reinforced by gene expression profiling in a study that identified a molecular

signature of 13 genes that was highly expressed in conventional mantle cell lymphoma as compared to indolent MCL. Within the gene set, the transcription factor SOX11 was particularly overexpressed in conventional mantle cell lymphoma and confirmed by protein expression [26]. A separate outcomes-based study divided patients into groups based on degree of IGHV mutations from germline. There was a significant difference in overall survival between those with high and low mutational load. The highly mutated cases had a better survival, were preferentially SOX11 negative, had lower genomic complexity, and were more often non-nodal in disease distribution [63]. Furthermore, studies of patients with a monoclonal B-lymphocytosis-like presentation of cyclin D1-positive MCL were more often SOX11 negative as compared with symptomatic, nodal cases [24, 68].

While most cohorts of indolent patients showed a predilection for SOX11 negativity, a population-based cohort study published conflicting results with a shorter overall survival in SOX11-cases, but the significance was lost on multivariate analysis [67]. Another study among patients treated with dose-intensive (i.e., hyper-CVAD based) regimens found that high SOX11 expression was associated with improved survival [52]. Lack of uniformity among studies and difference in selection criteria of indolent populations add to the difficulty in drawing an overall conclusion from these data.

SOX11 has been recognized in the last several years as a reliable biomarker in identification of mantle cell lymphoma as well as the cyclin D1-negative conventional or blastoid variants [21, 62, 120]. Several new monoclonal antibodies are commercially available to incorporate into routine diagnostic practice [97]. It has been suggested that SOX11 by itself should not be considered to be a prognostic parameter but rather used as a biomarker that may help to recognize either cyclin D1-negative MCL or a subtype of MCL with different biologic and clinical features. Nonetheless, study of SOX11 target genes is an active area of interest and it remains to be determined whether SOX11 could be a useful prognostic marker.

With a broad range of treatment options for patients with small B-cell lymphomas, some patients achieve a long-lasting remission. However, the majority suffer a relapse that might be heralded by very low levels of residual lymphoma cells. Detection of MRD has been expanded from acute lymphoblastic leukemia to B-cell lymphomas, including MCL, in an increasingly important tool for risk prediction [77]. This can be achieved via various modalities including flow cytometric analysis, reporting sensitivities of approximately 10^{-3} [8]. PCR-based methods have made great strides in achieving optimal sensitivity. Real-time quantitative PCR using allele-specific primer design has standardization guidelines [108] and a high sensitivity (10^{-5}), superior to the sensitivity of immunoglobulin heavy-chain PCR analysis using consensus primers and overcoming inherent limitations such as ongoing somatic mutation [10]. An ideal, MCL-specific target is the t(11;14) translocation, which can be highly sensitive (10^{-5}) by nested PCR, but the translocation is PCR detectable in only 25–40 % of cases [4, 36]. A promising prospect, SOX11, as a MRD marker for MCL was studied using mRNA-specific quantitative PCR (qPCR) technology in longitudinal peripheral blood samples of MCL patients. The researchers were able to correlate quantifiable level of SOX11

expression with clinical status and with level of t(11;14) by qPCR. Still, patient selection and validation over larger cohorts of patient groups will be required before this could be considered for clinical use [95].

In summary, cyclin D1 and SOX11 remain good biomarkers for the diagnosis of MCL, including cyclin D1-negative MCL. The MIPI is a good clinical risk stratification tool, and assessment of proliferation and TP53 provide added prognostic information. Further work on defining additional useful prognostic biomarkers will require validation in uniformly treated patient populations.

6 Diffuse Large B-Cell Lymphoma, Not Otherwise Specified

This section covers diffuse large B-cell lymphoma (DLBCL), which represents the most common type of non-Hodgkin lymphoma (30–40 % of adult non-Hodgkin lymphomas) and can arise de novo or from transformation of a preexisting low-grade lymphoma. Patients usually present with a rapidly enlarging mass that is FDG avid on positron emission tomography imaging. Occurrence at lymph node sites is most common, but it can arise in virtually any location in the body and is common at extranodal sites such as the gastrointestinal tract or skin. Patients are most often treated with anthracyclin-containing multiple-agent chemotherapy regimens that include rituximab, and there is an approximately 55 % 5-year survival [65], but there is much variability in that percentage depending on other prognostic factors. The International Prognostic Index (IPI) is a valuable tool that can separate patients in distinct prognostic risk groups [94], and it has been revised and validated in the rituximab era [92].

From a morphologic standpoint, the recognized variants of DLBCL, not otherwise specified (NOS), include centroblastic, immunoblastic, and anaplastic. Rarer variants also exist and are important for recognition by the pathologist. The immunoblastic variant (being composed of at least 90 % immunoblasts) has been associated with poorer event-free and overall survival [7]. Other special categories of large B-cell lymphoma (without plasmablastic features) that are beyond the scope of this discussion include T cell/histiocyte-rich large B-cell lymphoma, primary DLBCL of the central nervous system, primary cutaneous DLBCL, leg type, EBV-positive DLBCL of the elderly, DLBCL associated with chronic inflammation, lymphomatoid granulomatosis, and intravascular large B-cell lymphoma, along with other categories of large B-cell lymphoma in association with immunodeficiency.

To better understand the heterogeneity of this lymphoma, gene expression profiling has identified “cell-of-origin” molecular subgroups of DLBCL that are similar to the profiles of different normal B-cell counterparts. One subgroup retained the gene expression program of the GCB and was thus termed “GCB like,” while the other group expressed genes that were induced during activation of peripheral blood B cells (“activated B cell like,” ABC) [3]. Aside from providing

insight into additional molecular alterations and activated pathways within the subgroups, distinct differences in overall survival between patients with GCB versus ABC subgroups were observed. The ABC type was shown to have an inferior outcome compared with the GCB type, even in the rituximab era. The subgroup occurs in an older proportion of patients (>70 years) and has biologic significance for sustained activation of the NF-κB pathway [55]. Since then, subsequent studies have validated this classification approach with large numbers of patients and have selected smaller gene sets to identify groups [14, 82, 91, 115]. To more easily identify a molecular subtype, immunohistochemical staining algorithms were developed based on the results of gene expression profiling [13, 38, 59]. When performed in qualified laboratories, these algorithms can show very good concordance with the GEP classifier [81]. The COO concept as a prognostic biomarker is relevant with modern immunochemotherapy [82, 110]. However, in addition, it may gain added relevance as a predictive marker. Given that the ABC-like DLBCLs demonstrate mutations that activate the B-cell receptor signaling pathway and ultimately NFkB, development of targeted therapies with preferential activity in the ABC subtype may call for knowledge of COO subtype in order to assist in therapy selection. Preliminary data support this approach, and trials are underway and in the planning phases that require COO determination as part of eligibility requirements [114].

Recent attention has been drawn to the importance of translocations involving *MYC*, *BCL2*, and *BCL6*. Apart from identifying some cases of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma (BL, see below), these translocations may have prognostic significance in DLBCL, NOS. The most frequent genetic finding in DLBCL, NOS, is a rearrangement of *BCL6* (3q27, 30–40 %), which occurs more often in the ABC subgroup, whereas *BCL2* and *MYC* translocations are usually associated with GCB DLBCL. In particular, *MYC* translocations occur in approximately 10 % of DLBCL and portend a dismal prognosis, either as a single genetic insult or as additional hits involving *BCL2*/*MYC*, *BCL6*/*MYC* ("double-hit lymphomas"), or the rare *BCL2*/*BCL6*/*MYC*-rearranged "triple-hit" lymphomas [5]. This includes cases with *IGH@BCL2*/t(14;18)(q32;q21) plus extra *MYC* signals or amplification, as well as cases with *MYC* rearrangements with extra *BCL2* copies [57]. However, there is still controversy as to whether *MYC* rearrangements in DLBCL are truly an independent predictor of prognosis. Studies of risk stratification in patients treated with rituximab–CHOP found confirmed *MYC* translocation as predictive of progression-free survival and overall survival on multivariate analysis [89] and also showed a significant association with treatment resistance [106]. Similarly, a Southwest Oncology Group Study of high-grade morphologic features and MYC protein expression by immunohistochemistry [15] found MYC to be a poor prognostic factor on multivariate analysis and independent of morphology, highlighting the importance of staining for this protein in routine clinical practice. However, other studies have questioned the prognostic power of MYC as a single abnormality. In one study examining BCL2 and MYC protein expression, the prognostic impact of *MYC* was due to the confounding effect of BCL2/MYC double-protein-positive

cases, and *MYC* positivity alone did not impact overall survival [43]. Another study showed that isolated *MYC* rearrangements have weaker prognostic relevance than isolated *BCL2* translocations [110]. In an attempt to clarify this issue, a recent systematic review and meta-analysis that included 24 enrolling clinical trials confirmed the prognostic relevance of isolated *MYC* aberrations (including amplifications) in terms of both protein and mRNA expression; however, interpretation of the data is challenged by the heterogeneity of the included studies [121].

More recently, it was found that MYC and BCL2 protein coexpression predicts survival in patients with DLBCL that are treated with rituximab (R)–CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone). While high MYC protein expression by IHC is often (but not invariably) associated with a *MYC* translocation (and thus an adverse risk), the negative impact of MYC protein expression on progression-free and overall survival was shown only when BCL2 protein was coexpressed. This was significant even after adjusting for high-risk features in the IPI score [48]. The practical use of an immunohistochemical "double-hit score" was demonstrated in a study that validated shorter overall and progression-free survival in patients with high BCL2/MYC protein coexpression independent of other variables and was able to identify patients with high-risk "double-hit biology" from within an independent cohort of DLBCL [34]. When examining these biomarkers in DLBCL patients according to the cell of origin, the level of positive protein expression was a strong independent predictor of overall survival in patients with GCB, but not the non-GCB subtype DLBCL, with high BCL2 (>30 %) and MYC (>50 %) coexpression correlating with the worst outcome [75]. Because of its wide availability, assessment of BCL2 and MYC expression by immunohistochemistry is becoming a routine part of the pathology report to provide additional prognostic information to clinicians.

7 Primary Mediastinal Large B-Cell Lymphoma

Primary mediastinal large B-cell lymphoma (PMBL) arises within the mediastinum from B cells of probable thymic origin. Patients are young adults with a median age of 35 and a female predominance. They may present with a bulky anterosuperior mediastinal mass that can secondarily invade nearby organs such as lungs or directly extend to local lymph nodes, but it rarely disseminates. It is important to exclude primary lymph node involvement at other sites, as well as bone marrow involvement, to avoid misdiagnosis of a systemic DLBCL that might secondarily involve the mediastinum [30].

The microscopic features of this lymphoma type vary from case but can take the form of a diffuse proliferation of intermediate to large cells with a moderate amount of clear cytoplasm and either ovoid or sometimes slightly more pleomorphic nuclei. Compartmentalizing alveolar sclerosis is a prominent feature in many cases. The phenotype includes most B-cell antigens including IRF4/MUM1, positive for CD23, with low to absent immunoglobulin and CD10 expression, sometimes

heterogeneous/weak CD30 expression, and MAL antigen. Frequent karyotypic abnormalities include chromosomal aberrations for 2p16.1, 9p24.1, and 8q24 [71]. A large study of B-cell lymphomas demonstrated rearrangement of the MHC class II transactivator *CIITA* (16p13.13) in PMBL (29/77, 38 %) [98]. The implication of such an aberration might be that the tumor can then escape from immune surveillance by downregulation of HLA class II associated proteins, among other mechanisms. An association was found between *CIITA* gene fusions, and reduced HLA-DR protein expression was demonstrated [23]. Additional research is needed to identify biomarkers for prognosis in PMBL, though one recent study found MUM1 protein expression to correlate independently with a decreased overall survival [19].

It recognized that some lymphomas have combined features of both classical Hodgkin lymphoma and DLBCL, particularly nodular sclerosis classical Hodgkin lymphoma and PMBL [104]. Furthermore, some patients were reported to develop large B-cell lymphoma after treatment for Hodgkin lymphoma, while others reported composite Hodgkin/large B-cell lymphoma, and thus the designation "mediastinal gray-zone lymphoma" [32, 119]. While PMBL lacks truly distinctive morphologic features that might otherwise distinguish it from conventional DLBCL, identification of gene expression signature unique to this entity was pivotal in the molecular classification of large B-cell lymphoma. In fact, when gene expression profiling was used to establish a more precise molecular diagnosis of PMBL, it emerged as an entity that primarily affected younger patients and identified a subgroup with a better prognosis compared to other DLBCLs. Gene profiling supported the suspected relationship between PMBL and Hodgkin lymphoma in that over one-third of the genes that were part of the PMBL signature were characteristic of Hodgkin lymphoma cells [83]. Furthermore, the epigenetic profile of PMBL was found to be distinct and separate from DLBCL, and analysis of methylation patterns supported an intermediate position between DLBCL and classical Hodgkin lymphoma [23].

8 Burkitt Lymphoma

BL is a highly aggressive mature B-cell neoplasm that makes up 40 % of non-Hodgkin lymphomas in youth under age 20. It presents as a rapidly enlarging mass, commonly involving extranodal sites, and exists in three different clinical variants. The endemic form predominantly affects young children in equatorial Africa and is usually associated with Epstein-Barr viral infection, and the tumors are often localized to the jaw or facial bones, kidneys, or abdominal region. The sporadic type occurs in a broad geographic distribution that includes North America and European countries, and often affects the abdomen or terminal ileum of immunocompetent children. The third type is immunodeficiency related, and it is known for its association with HIV. Rarely, a leukemic phase exists, either in patients with

bulky disease or very uncommonly as a de novo peripheral blood/bone marrow leukemia phenomenon [56].

The neoplastic infiltrate is composed of intermediately sized, monotonous B cells with numerous mitoses and tingible body macrophages imparting a "starry sky" appearance. BL cells resemble germinal center centroblasts by cytomorphologic grounds and express pan B-cell antigens and surface immunoglobulin, plus CD10 and BCL6. BCL2 is characteristically negative or only weakly positive [86].

The vast majority of cases have a detectable *MYC* translocation (8q24) to the immunoglobulin heavy-chain region (14q32), or to the kappa light-chain gene (2p11) or lambda-chain gene (22q11). In some cases, a *MYC* cannot be demonstrated by FISH, but in these cases, differences in the *MYC* breakpoint or subdetectable insertions/deletions in the *MYC* gene may elude detection. Thus, a negative test result does not exclude the diagnosis. Similarly, it is important to remember that other types of lymphomas can harbor a *MYC* rearrangement, such as DLBCL discussed previously, as well as mantle cell lymphoma and FL can acquire a *MYC* translocation as it transforms to a more aggressive tumor [18, 39].

Gene expression profiling has revealed a distinct molecular phenotype for BL [17] and that BL can be identified with certainty on the basis of a number of MYC target genes, involved in germinal center differentiation, NF-KB activation, and MHC class I molecules [44]. The genetic makeup of BL was further elucidated by using deep sequencing technologies which identified *ID3* as an important tumor suppressor gene in BL that appears to serve as a negative regulator of *MYC* [80, 90].

9 B-Cell Lymphoma, Unclassifiable, with Features Intermediate Between Diffuse Large B-Cell Lymphoma and Burkitt Lymphoma

The WHO 2008 category of B-cell lymphoma, unclassifiable, incorporates cases with morphologic, immunophenotypic and genetic features that are intermediate between DLBCL and BL (WHO 2008 classification). The existence of this category is supported by gene expression and karyotypic/genomic profiling, which can identify a "molecular Burkitt" signature [17, 44, 87]. Classification of these cases at the practice level is problematic, since the tools used to define them are not readily available in clinical laboratories. These types of case were likely included in former designations such as "small non-cleaved, non-Burkitt" (Working Formulation), "atypical Burkitt lymphoma," and "high-grade B-cell lymphoma, Burkitt-like" (Revised European and American Lymphoma Classification) or were loosely referred to as "DLBCL with high-grade features," "gray-zone lymphoma," or "diffuse aggressive B-cell lymphoma." Genetically defined "double-hit" lymphomas are included in this category of B-cell lymphoma, unclassifiable (BCL-U) [49]. These can be recognized with currently available clinical laboratory tests such as FISH testing. Thus, assessment of *MYC, BCL2*, and *MYC* translocation should be performed in all diffuse aggressive B-cell lymphomas suspected of being a double-

hit lymphoma. One practical way to screen is to perform MYC IHC given its ability to predict presence of *MYC* translocation and to perform FISH confirmation (for all three targets) in cases with high nuclear MYC (>40–50 %) expression.

This still leaves a difficult group of cases that belong to this category for which we cannot yet definitively recognize by current laboratory tests. The category should then be used only for cases in which the clinical, morphologic, phenotypic, and/or genetic evidence cause a truly inscrutable diagnostic conundrum between DLBCL and BL [79]. An example may be cases with morphologic features suggestive but insufficient for BL in which a BL-like (CD10+/BCL2 weak-negative) phenotype is seen, in the setting of a demonstrated immunoglobulin gene/*MYC* translocation with complex karyotype.

10 Next-Generation Sequencing for Minimal Residual Disease Monitoring and Mutation Detection

Many advances have been made in the treatment of B-cell lymphoma, and a need exists for post-treatment assessment and monitoring of treated patients as a means to identify an early relapse or predict the potential for relapse. MRD can be detected by cytogenetics, flow cytometry, or PCR-based methods. PCR-based MRD detection by detection of immunoglobulin heavy-chain and/or T-cell receptor gene rearrangements using tumor-specific primers has potential to be a sensitive means of assessment [78]. Next-generation sequencing was shown to be a feasible tool in MRD detection in MCL, with comparable results to real-time quantitative PCR [53]. Identification of an ideal MRD marker/target is of utmost importance in establishing a useful assay, and difficulties might arise due to ongoing somatic hypermutation or clonal evolution. Certainly, next-generation sequencing on routine patient samples will be feasible in the next several years, and it may provide valuable diagnostic information and prognostic information and help guide individualized therapies by identifying biologic pathways that can be targeted with new therapies. The challenge will be to refine them for use in routinely processed tissues and apply these techniques to highly annotated clinical data sets in order to begin to rationally use this complex data for prognosis and prediction.

References

1. Adam P, Schiefer AI, Prill S, Henopp T, Quintanilla-Martinez L, Bosmuller HC, Chott A, Fend F (2012) Incidence of preclinical manifestations of mantle cell lymphoma and mantle cell lymphoma in situ in reactive lymphoid tissues. Mod Pathol 25(12):1629–1636
2. Advani RH, Buggy JJ, Sharman JP, Smith SM, Boyd TE, Grant B, Kolibaba KS, Furman RR, Rodriguez S, Chang BY et al (2013) Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. J Clin Oncol 31(1):88–94

3. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X et al (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403(6769):503–511
4. Andersen NS, Donovan JW, Borus JS, Poor CM, Neuberg D, Aster JC, Nadler LM, Freedman AS, Gribben JG (1997) Failure of immunologic purging in mantle cell lymphoma assessed by polymerase chain reaction detection of minimal residual disease. Blood 90 (10):4212–4221
5. Barrans S, Crouch S, Smith A, Turner K, Owen R, Patmore R, Roman E, Jack A (2010) Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. J Clin Oncol 28(20):3360–3365
6. Bea S, Valdes-Mas R, Navarro A, Salaverria I, Martin-Garcia D, Jares P, Gine E, Pinyol M, Royo C, Nadeu F et al (2013) Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. Proc Natl Acad Sci USA 110(45):18250–18255
7. Bernd HW, Ziepert M, Thorns C, Klapper W, Wacker HH, Hummel M, Stein H, Hansmann ML, Ott G, Rosenwald A et al (2009) Loss of HLA-DR expression and immunoblastic morphology predict adverse outcome in diffuse large B-cell lymphoma—analyses of cases from two prospective randomized clinical trials. Haematologica 94(11):1569–1580
8. Bottcher S, Ritgen M, Buske S, Gesk S, Klapper W, Hoster E, Hiddemann W, Unterhalt M, Dreyling M, Siebert R et al (2008) Minimal residual disease detection in mantle cell lymphoma: Methods and significance of four-color flow cytometry compared to consensus IGH-polymerase chain reaction at initial staging and for follow-up examinations. Haematologica 93(4):551–559
9. Braggio E, Dogan A, Keats JJ, Chng WJ, Huang G, Matthews JM, Maurer MJ, Law ME, Bosler DS, Barrett M et al (2012) Genomic analysis of marginal zone and lymphoplasmacytic lymphomas identified common and disease-specific abnormalities. Mod Pathol 25(5):651–660
10. Bruggemann M, Droese J, Bolz I, Luth P, Pott C, von Neuhoff N, Scheuering U, Kneba M (2000) Improved assessment of minimal residual disease in B cell malignancies using fluorogenic consensus probes for real-time quantitative PCR. Leukemia 14(8):1419–1425
11. Cao Y, Hunter ZR, Liu X, Xu L, Yang G, Chen J, Patterson CJ, Tsakmaklis N, Kanan S, Rodig S, et al (2014) The WHIM-like CXCR4 somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of waldenstrom's macroglobulinemia. Leukemia Jun 10. [Epub ahead of print]
12. Carvajal-Cuenca A, Sua LF, Silva NM, Pittaluga S, Royo C, Song JY, Sargent RL, Espinet B, Climent F, Jacobs SA et al (2012) In situ mantle cell lymphoma: Clinical implications of an incidental finding with indolent clinical behavior. Haematologica 97(2):270–278
13. Choi WW, Weisenburger DD, Greiner TC, Piris MA, Banham AH, Delabie J, Braziel RM, Geng H, Iqbal J, Lenz G et al (2009) A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res 15 (17):5494–5502
14. Collie AM, Nolling J, Divakar KM, Lin JJ, Carver P, Durkin LM, Hill BT, Smith MR, Radivoyevitch T, Kong LI et al (2014) Molecular subtype classification of formalin-fixed, paraffin-embedded diffuse large B-cell lymphoma samples on the ICEPlex system. Br J Haematol 167(2):281–285
15. Cook JR, Goldman B, Tubbs RR, Rimsza L, Leblanc M, Stiff P, Fisher R (2014) Clinical significance of MYC expression and/or "high-grade" morphology in non-Burkitt, diffuse aggressive B-cell lymphomas: A SWOG S9704 correlative study. Am J Surg Pathol 38 (4):494–501
16. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, Fisher RI, Braziel RM, Rimsza LM, Grogan TM et al (2004) Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med 351(21):2159–2169

17. Dave SS, Fu K, Wright GW, Lam LT, Kluin P, Boerma EJ, Greiner TC, Weisenburger DD, Rosenwald A, Ott G et al (2006) Molecular diagnosis of Burkitt's lymphoma. N Engl J Med 354(23):2431–2442
18. Davies AJ, Rosenwald A, Wright G, Lee A, Last KW, Weisenburger DD, Chan WC, Delabie J, Braziel RM, Campo E et al (2007) Transformation of follicular lymphoma to diffuse large B-cell lymphoma proceeds by distinct oncogenic mechanisms. Br J Haematol 136 (2):286–293
19. De Mello CA, De Andrade VP, De Lima VC, Carvalho AL, Soares FA (2011) Prognostic impact of MUM1 expression by immunohistochemistry on primary mediastinal large B-cell lymphoma. Leuk Lymphoma 52(8):1495–1503
20. Determann O, Hoster E, Ott G, Wolfram Bernd H, Loddenkemper C, Leo Hansmann M, Barth TE, Unterhalt M, Hiddemann W, Dreyling M et al (2008) Ki-67 predicts outcome in advanced-stage mantle cell lymphoma patients treated with anti-CD20 immunochemotherapy: results from randomized trials of the European MCL network and the German low grade lymphoma study group. Blood 111(4):2385–2387
21. Dictor M, Ek S, Sundberg M, Warenholt J, Gyorgy C, Sernbo S, Gustavsson E, Abu-Alsoud W, Wadstrom T, Borrebaeck C (2009) Strong lymphoid nuclear expression of SOX11 transcription factor defines lymphoblastic neoplasms, mantle cell lymphoma and Burkitt's lymphoma. Haematologica 94(11):1563–1568
22. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P (2000) Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 343(26):1910–1916
23. Eberle FC, Rodriguez-Canales J, Wei L, Hanson JC, Killian JK, Sun HW, Adams LG, Hewitt SM, Wilson WH, Pittaluga S et al (2011) Methylation profiling of mediastinal gray zone lymphoma reveals a distinctive signature with elements shared by classical Hodgkin's lymphoma and primary mediastinal large B-cell lymphoma. Haematologica 96(4):558–566
24. Espinet B, Ferrer A, Bellosillo B, Nonell L, Salar A, Fernandez-Rodriguez C, Puigdecanet E, Gimeno J, Garcia-Garcia M, Vela MC et al (2014) Distinction between asymptomatic monoclonal B-cell lymphocytosis with cyclin D1 overexpression and mantle cell lymphoma: From molecular profiling to flow cytometry. Clin Cancer Res 20(4):1007–1019
25. Federico M, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, Vitolo U, Pro B, Pileri S, Pulsoni A, Soubeyran P et al (2009) Follicular lymphoma international prognostic index 2: A new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol 27(27):4555–4562
26. Fernandez V, Salamero O, Espinet B, Sole F, Royo C, Navarro A, Camacho F, Bea S, Hartmann E, Amador V et al (2010) Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res 70(4):1408–1418
27. Fitzgibbon J, Iqbal S, Davies A, O'shea D, Carlotti E, Chaplin T, Matthews J, Raghavan M, Norton A, Lister TA et al (2007) Genome-wide detection of recurring sites of uniparental disomy in follicular and transformed follicular lymphoma. Leukemia 21(7):1514–1520
28. Fu K, Weisenburger DD, Greiner TC, Dave S, Wright G, Rosenwald A, Chiorazzi M, Iqbal J, Gesk S, Siebert R et al (2005) Cyclin D1-negative mantle cell lymphoma: A clinicopathologic study based on gene expression profiling. Blood 106(13):4315–4321
29. Gachard N, Parrens M, Soubeyran I, Petit B, Marfak A, Rizzo D, Devesa M, Delage-Corre M, Coste V, Laforet MP et al (2013) IGHV gene features and MYD88 L265P mutation separate the three marginal zone lymphoma entities and waldenstrom macroglobulinemia/ lymphoplasmacytic lymphomas. Leukemia 27(1):183–189
30. Gaulard P, Harris NL, Pileri SA, Kutok J, Stein H, Kovrigina AM, Jaffe ES, Moller P (2008) Primary mediastinal (thymic) large B cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al (eds) WHO classification of tumours of the haematopoietic and lymphoid tissues, 4th edn. IARC, Lyon, 250 p
31. Glas AM, Knoops L, Delahaye L, Kersten MJ, Kibbelaar RE, Wessels LA, van Laar R, van Krieken JH, Baars JW, Raemaekers J et al (2007) Gene-expression and immunohistochemical

study of specific T-cell subsets and accessory cell types in the transformation and prognosis of follicular lymphoma. J Clin Oncol 25(4):390–398
32. Gonzalez CL, Medeiros LJ, Jaffe ES (1991) Composite lymphoma. A clinicopathologic analysis of nine patients with Hodgkin's disease and B-cell non-Hodgkin's lymphoma. Am J Clin Pathol 96(1):81–89
33. Goy A, Kahl B (2011) Mantle cell lymphoma: The promise of new treatment options. Crit Rev Oncol Hematol 80(1):69–86
34. Green TM, Young KH, Visco C, Xu-Monette ZY, Orazi A, Go RS, Nielsen O, Gadeberg OV, Mourits-Andersen T, Frederiksen M et al (2012) Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 30 (28):3460–3467
35. Green MR, Gentles AJ, Nair RV, Irish JM, Kihira S, Liu CL, Kela I, Hopmans ES, Myklebust JH, Ji H et al (2013) Hierarchy in somatic mutations arising during genomic evolution and progression of follicular lymphoma. Blood 121(9):1604–1611
36. Gribben JG, Neuberg D, Freedman AS, Gimmi CD, Pesek KW, Barber M, Saporito L, Woo SD, Coral F, Spector N (1993) Detection by polymerase chain reaction of residual cells with the bcl-2 translocation is associated with increased risk of relapse after autologous bone marrow transplantation for B-cell lymphoma. Blood 81(12):3449–3457
37. Guo Y, Karube K, Kawano R, Yamaguchi T, Suzumiya J, Huang GS, Ohshima K (2005) Low-grade follicular lymphoma with t(14;18) presents a homogeneous disease entity otherwise the rest comprises minor groups of heterogeneous disease entities with Bcl2 amplification, Bcl6 translocation or other gene aberrances. Leukemia 19(6):1058–1063
38. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Muller-Hermelink HK, Campo E, Braziel RM, Jaffe ES et al (2004) Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103(1):275–282
39. Haralambieva E, Boerma EJ, van Imhoff GW, Rosati S, Schuuring E, Muller-Hermelink HK, Kluin PM, Ott G (2005) Clinical, immunophenotypic, and genetic analysis of adult lymphomas with morphologic features of Burkitt lymphoma. Am J Surg Pathol 29 (8):1086–1094
40. Harris N, Swerdlow S, Jaffe E, Ott G (2008) Follicular lymphoma. In: Swerdlow S, Campo E, Harris N, et al (eds) WHO classification of tumours of the haematopoietic and lymphoid tissues, 4th edn. IARC, Lyon, 220 p
41. Horn H, Schmelter C, Leich E, Salaverria I, Katzenberger T, Ott MM, Kalla J, Romero M, Siebert R, Rosenwald A et al (2011) Follicular lymphoma grade 3B is a distinct neoplasm according to cytogenetic and immunohistochemical profiles. Haematologica 96 (9):1327–1334
42. Hoster E, Dreyling M, Klapper W, Gisselbrecht C, van Hoof A, Kluin-Nelemans HC, Pfreundschuh M, Reiser M, Metzner B, Einsele H et al (2008) A new prognostic index (MIPI) for patients with advanced-stage mantle cell lymphoma. Blood 111(2):558–565
43. Hu S, Xu-Monette ZY, Tzankov A, Green T, Wu L, Balasubramanyam A, Liu WM, Visco C, Li Y, Miranda RN, et al (2013) MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from the international DLBCL rituximab-CHOP consortium program. Blood 121(20):4021–4031; quiz 4250
44. Hummel M, Bentink S, Berger H, Klapper W, Wessendorf S, Barth TF, Bernd HW, Cogliatti SB, Dierlamm J, Feller AC et al (2006) A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med 354(23):2419–2430
45. Jares P, Colomer D, Campo E (2012) Molecular pathogenesis of mantle cell lymphoma. J Clin Invest 122(10):3416–3423
46. Jimenez C, Sebastian E, Chillon MC, Giraldo P, Mariano Hernandez J, Escalante F, Gonzalez-Lopez TJ, Aguilera C, de Coca AG, Murillo I et al (2013) MYD88 L265P is a

marker highly characteristic of, but not restricted to, waldenstrom's macroglobulinemia. Leukemia 27(8):1722–1728
47. Johnson PW, Rohatiner AZ, Whelan JS, Price CG, Love S, Lim J, Matthews J, Norton AJ, Amess JA, Lister TA (1995) Patterns of survival in patients with recurrent follicular lymphoma: A 20-year study from a single center. J Clin Oncol 13(1):140–147
48. Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, Rogic S, Scott DW, Tan KL, Steidl C, Sehn LH et al (2012) Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 30(28):3452–3459
49. Kluin P, Harris N, Stein H, Leoncini L, Raphael M, Campo E, Jaffe E (2008) B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and burkitt lymphoma. In: Swerdlow S, Campo E, Harris N, et al (eds) WHO classification of tumours of the haematopoietic and lymphoid tissues, 4th edn. IARC, Lyon, 265 p
50. Koch K, Hoster E, Unterhalt M, Ott G, Rosenwald A, Hansmann ML, Engelhard M, Hiddemann W, Klapper W (2012) The composition of the microenvironment in follicular lymphoma is associated with the stage of the disease. Hum Pathol 43(12):2274–2281
51. Komatsu H, Iida S, Yamamoto K, Mikuni C, Nitta M, Takahashi T, Ueda R, Seto M (1994) A variant chromosome translocation at 11q13 identifying PRAD1/cyclin D1 as the BCL-1 gene. Blood 84(4):1226–1231
52. Kuo PY, Leshchenko VV, Fazzari MJ, Perumal D, Gellen T, He T, Iqbal J, Baumgartner-Wennerholm S, Nygren L, Zhang F et al (2014) High-resolution chromatin immunoprecipitation (ChIP) sequencing reveals novel binding targets and prognostic role for SOX11 in mantle cell lymphoma. Oncogene. doi:10.1038/onc.2014.44
53. Ladetto M, Bruggemann M, Monitillo L, Ferrero S, Pepin F, Drandi D, Barbero D, Palumbo A, Passera R, Boccadoro M et al (2014) Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. Leukemia 28 (6):1299–1307
54. Landgren O, Staudt L (2012) MYD88 L265P somatic mutation in IgM MGUS. N Engl J Med 367(23):2255–2256; author reply 2256–7
55. Lenz G, Staudt LM (2010) Aggressive lymphomas. N Engl J Med 362(15):1417–1429
56. Leoncini L, Raphael M, Stein H, Harris N, Jaffe E, Kluin P (2008) Burkitt lymphoma. In: Swerdlow S, Campo E, Harris N, et al (eds) WHO classification of tumors of the hematopoietic and lymphoid tissues, 4th edn. IARC, Lyon, 262 p
57. Li S, Lin P, Fayad LE, Lennon P, Miranda RN, Yin CC, Lin E, Medeiros LJ (2012) B-cell lymphomas with MYC/8q24 rearrangements and IGH@BCL2/t(14;18)(q32;q21): an aggressive disease with heterogeneous histology, germinal center B-cell immunophenotype and poor outcome. Mod Pathol 25(1):145–156
58. Martin P, Chadburn A, Christos P, Weil K, Furman RR, Ruan J, Elstrom R, Niesvizky R, Ely S, Diliberto M et al (2009) Outcome of deferred initial therapy in mantle-cell lymphoma. J Clin Oncol 27(8):1209–1213
59. Meyer PN, Fu K, Greiner TC, Smith LM, Delabie J, Gascoyne RD, Ott G, Rosenwald A, Braziel RM, Campo E et al (2011) Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. J Clin Oncol 29(2):200–207
60. Montesinos-Rongen M, Godlewska E, Brunn A, Wiestler OD, Siebert R, Deckert M (2011) Activating L265P mutations of the MYD88 gene are common in primary central nervous system lymphoma. Acta Neuropathol 122(6):791–792
61. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, Johnson NA, Severson TM, Chiu R, Field M et al (2011) Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature 476(7360):298–303
62. Mozos A, Royo C, Hartmann E, De Jong D, Baro C, Valera A, Fu K, Weisenburger DD, Delabie J, Chuang SS et al (2009) SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. Haematologica 94(11):1555–1562

63. Navarro A, Clot G, Royo C, Jares P, Hadzidimitriou A, Agathangelidis A, Bikos V, Darzentas N, Papadaki T, Salaverria I et al (2012) Molecular subsets of mantle cell lymphoma defined by the IGHV mutational status and SOX11 expression have distinct biologic and clinical features. Cancer Res 72(20):5307–5316
64. Ngo VN, Young RM, Schmitz R, Jhavar S, Xiao W, Lim KH, Kohlhammer H, Xu W, Yang Y, Zhao H et al (2011) Oncogenically active MYD88 mutations in human lymphoma. Nature 470(7332):115–119
65. Niitsu N (2010) Current treatment strategy of diffuse large B-cell lymphomas. Int J Hematol 92(2):231–237
66. Nordstrom L, Sernbo S, Eden P, Gronbaek K, Kolstad A, Raty R, Karjalainen ML, Geisler C, Ralfkiaer E, Sundstrom C et al (2014) SOX11 and TP53 add prognostic information to MIPI in a homogenously treated cohort of mantle cell lymphoma—a nordic lymphoma group study. Br J Haematol 166(1):98–108
67. Nygren L, Baumgartner Wennerholm S, Klimkowska M, Christensson B, Kimby E, Sander B (2012) Prognostic role of SOX11 in a population-based cohort of mantle cell lymphoma. Blood 119(18):4215–4223
68. Ondrejka SL, Lai R, Smith SD, Hsi ED (2011) Indolent mantle cell leukemia: A clinicopathological variant characterized by isolated lymphocytosis, interstitial bone marrow involvement, kappa light chain restriction, and good prognosis. Haematologica 96 (8):1121–1127
69. Ondrejka SL, Lin JJ, Warden DW, Durkin L, Cook JR, Hsi ED (2013) MYD88 L265P somatic mutation: Its usefulness in the differential diagnosis of bone marrow involvement by B-cell lymphoproliferative disorders. Am J Clin Pathol 140(3):387–394
70. Orchard J, Garand R, Davis Z, Babbage G, Sahota S, Matutes E, Catovsky D, Thomas PW, Avet-Loiseau H, Oscier D (2003) A subset of t(11;14) lymphoma with mantle cell features displays mutated IgVH genes and includes patients with good prognosis, nonnodal disease. Blood 101(12):4975–4981
71. Oschlies I, Burkhardt B, Salaverria I, Rosenwald A, d'Amore ES, Szczepanowski M, Koch K, Hansmann ML, Stein H, Moller P et al (2011) Clinical, pathological and genetic features of primary mediastinal large B-cell lymphomas and mediastinal gray zone lymphomas in children. Haematologica 96(2):262–268
72. Ott G, Kalla J, Ott MM, Schryen B, Katzenberger T, Muller JG, Muller-Hermelink HK (1997) Blastoid variants of mantle cell lymphoma: Frequent bcl-1 rearrangements at the major translocation cluster region and tetraploid chromosome clones. Blood 89 (4):1421–1429
73. Owen RG, Treon SP, Al-Katib A, Fonseca R, Greipp PR, McMaster ML, Morra E, Pangalis GA, San Miguel JF, Branagan AR et al (2003) Clinicopathological definition of waldenstrom's macroglobulinemia: Consensus panel recommendations from the second international workshop on waldenstrom's macroglobulinemia. Semin Oncol 30(2):110–115
74. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, Kasper LH, Lerach S, Tang H, Ma J et al (2011) Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature 471(7337):189–195
75. Perry AM, Alvarado-Bernal Y, Laurini JA, Smith LM, Slack GW, Tan KL, Sehn LH, Fu K, Aoun P, Greiner TC et al (2014) MYC and BCL2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with rituximab. Br J Haematol 165 (3):382–391
76. Pham-Ledard A, Cappellen D, Martinez F, Vergier B, Beylot-Barry M, Merlio JP (2012) MYD88 somatic mutation is a genetic feature of primary cutaneous diffuse large B-cell lymphoma, leg type. J Invest Dermatol 132(8):2118–2120
77. Pott C (2011) Minimal residual disease detection in mantle cell lymphoma: Technical aspects and clinical relevance. Semin Hematol 48(3):172–184
78. Pott C, Bruggemann M, Ritgen M, van der Velden VH, van Dongen JJ, Kneba M (2013) MRD detection in B-cell non-Hodgkin lymphomas using Ig gene rearrangements and

chromosomal translocations as targets for real-time quantitative PCR. Methods Mol Biol 971:175–200
79. Quintanilla-Martinez L, de Jong D, de Mascarel A, Hsi ED, Kluin P, Natkunam Y, Parrens M, Pileri S, Ott G (2009) Gray zones around diffuse large B cell lymphoma. Conclusions based on the workshop of the XIV meeting of the European association for hematopathology and the society of hematopathology in Bordeaux, France. J Hematop 2(4):211–236
80. Richter J, Schlesner M, Hoffmann S, Kreuz M, Leich E, Burkhardt B, Rosolowski M, Ammerpohl O, Wagener R, Bernhart SH et al (2012) Recurrent mutation of the ID3 gene in Burkitt lymphoma identified by integrated genome, exome and transcriptome sequencing. Nat Genet 44(12):1316–1320
81. Rimsza LM, Wright G, Schwartz M, Chan WC, Jaffe ES, Gascoyne RD, Campo E, Rosenwald A, Ott G, Cook JR et al (2011) Accurate classification of diffuse large B-cell lymphoma into germinal center and activated B-cell subtypes using a nuclease protection assay on formalin-fixed, paraffin-embedded tissues. Clin Cancer Res 17(11):3727–3732
82. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, Smeland EB, Giltnane JM et al (2002) The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 346 (25):1937–1947
83. Rosenwald A, Wright G, Leroy K, Yu X, Gaulard P, Gascoyne RD, Chan WC, Zhao T, Haioun C, Greiner TC et al (2003) Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. J Exp Med 198(6):851–862
84. Royo C, Navarro A, Clot G, Salaverria I, Gine E, Jares P, Colomer D, Wiestner A, Wilson WH, Vegliante MC et al (2012) Non-nodal type of mantle cell lymphoma is a specific biological and clinical subgroup of the disease. Leukemia 26(8):1895–1898
85. Ryan RJ, Nitta M, Borger D, Zukerberg LR, Ferry JA, Harris NL, Iafrate AJ, Bernstein BE, Sohani AR, Le LP (2011) EZH2 codon 641 mutations are common in BCL2-rearranged germinal center B cell lymphomas. PLoS ONE 6(12):e28585
86. Said JW (2013) Aggressive B-cell lymphomas: How many categories do we need? Mod Pathol 26(1):S42–S56
87. Salaverria I, Zettl A, Bea S, Hartmann EM, Dave SS, Wright GW, Boerma EJ, Kluin PM, Ott G, Chan WC et al (2008) Chromosomal alterations detected by comparative genomic hybridization in subgroups of gene expression-defined Burkitt's lymphoma. Haematologica 93(9):1327–1334
88. Salaverria I, Royo C, Carvajal-Cuenca A, Clot G, Navarro A, Valera A, Song JY, Woroniecka R, Rymkiewicz G, Klapper W et al (2013) CCND2 rearrangements are the most frequent genetic events in cyclin D1(−) mantle cell lymphoma. Blood 121(8):1394–1402
89. Savage KJ, Johnson NA, Ben-Neriah S, Connors JM, Sehn LH, Farinha P, Horsman DE, Gascoyne RD (2009) MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. Blood 114 (17):3533–3537
90. Schmitz R, Young RM, Ceribelli M, Jhavar S, Xiao W, Zhang M, Wright G, Shaffer AL, Hodson DJ, Buras E et al (2012) Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. Nature 490(7418):116–120
91. Scott DW, Wright GW, Williams PM, Lih CJ, Walsh W, Jaffe ES, Rosenwald A, Campo E, Chan WC, Connors JM et al (2014) Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue. Blood 123 (8):1214–1217
92. Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P, Klasa R, Savage KJ, Shenkier T, Sutherland J et al (2007) The revised international prognostic index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood 109(5):1857–1861

93. Seto M, Yamamoto K, Iida S, Akao Y, Utsumi KR, Kubonishi I, Miyoshi I, Ohtsuki T, Yawata Y, Namba M (1992) Gene rearrangement and overexpression of PRAD1 in lymphoid malignancy with t(11;14)(q13;q32) translocation. Oncogene 7(7):1401–1406
94. Shipp MA (1993) A predictive model for aggressive non-Hodgkin's lymphoma. The international non-Hodgkin's lymphoma prognostic factors project. N Engl J Med 329 (14):987–994
95. Simonsen AT, Sorensen CD, Ebbesen LH, Bodker JS, Bentzen HH, Nyvold CG (2014) SOX11 as a minimal residual disease marker for mantle cell lymphoma. Leuk Res 38 (8):918–924
96. Solal-Celigny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, Au WY, Bellei M, Brice P, Caballero D et al (2004) Follicular lymphoma international prognostic index. Blood 104(5):1258–1265
97. Soldini D, Valera A, Sole C, Palomero J, Amador V, Martin-Subero JI, Ribera-Cortada I, Royo C, Salaverria I, Bea S et al (2014) Assessment of SOX11 expression in routine lymphoma tissue sections: Characterization of new monoclonal antibodies for diagnosis of mantle cell lymphoma. Am J Surg Pathol 38(1):86–93
98. Steidl C, Shah SP, Woolcock BW, Rui L, Kawahara M, Farinha P, Johnson NA, Zhao Y, Telenius A, Neriah SB et al (2011) MHC class II transactivator CIITA is a recurrent gene fusion partner in lymphoid cancers. Nature 471(7338):377–381
99. Sweetenham JW, Goldman B, LeBlanc ML, Cook JR, Tubbs RR, Press OW, Maloney DG, Fisher RI, Rimsza LM, Braziel RM et al (2010) Prognostic value of regulatory T cells, lymphoma-associated macrophages, and MUM-1 expression in follicular lymphoma treated before and after the introduction of monoclonal antibody therapy: A southwest oncology group study. Ann Oncol 21(6):1196–1202
100. Swerdlow S, Campo E, Seto M, Muller-Hermelink HK (2008a) Mantle cell lymphoma. In: Swerdlow S, Campo E, Harris N, et al (eds) WHO classification of tumours of the hematopoietic and lymphoid tissues, 4th edn. IARC, Lyon, 229 p
101. Swerdlow S, Berger F, Pileri S, Harris N, Jaffe E, Stein H (2008b) Lymphoplasmacytic lymphoma. In: Swerdlow S, Campo E, Harris N, et al (eds) Of tumours of haematopoietic and lymphoid tissues, fourth edition, 4th edn. 194 p
102. Taskinen M, Valo E, Karjalainen-Lindsberg ML, Hautaniemi S, Meri S, Leppa S (2010) Signal transducers and activators of transcription 5a-dependent cross-talk between follicular lymphoma cells and tumor microenvironment characterizes a group of patients with improved outcome after R-CHOP. Clin Cancer Res 16(9):2615–2623
103. Tiemann M, Schrader C, Klapper W, Dreyling MH, Campo E, Norton A, Berger F, Kluin P, Ott G, Pileri S et al (2005) Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): a clinicopathological study from the European MCL network. Br J Haematol 131(1):29–38
104. Traverse-Glehen A, Pittaluga S, Gaulard P, Sorbara L, Alonso MA, Raffeld M, Jaffe ES (2005) Mediastinal gray zone lymphoma: The missing link between classic Hodgkin's lymphoma and mediastinal large B-cell lymphoma. Am J Surg Pathol 29(11):1411–1421
105. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, Sheehy P, Manning RJ, Patterson CJ, Tripsas C et al (2012) MYD88 L265P somatic mutation in waldenstrom's macroglobulinemia. N Engl J Med 367(9):826–833
106. Tzankov A, Xu-Monette ZY, Gerhard M, Visco C, Dirnhofer S, Gisin N, Dybkaer K, Orazi A, Bhagat G, Richards KL et al (2013) Rearrangements of MYC gene facilitate risk stratification in diffuse large B-cell lymphoma patients treated with rituximab-CHOP. Mod Pathol 27(7):958–971
107. Vaandrager JW, Schuuring E, Raap T, Philippo K, Kleiverda K, Kluin P (2000) Interphase FISH detection of BCL2 rearrangement in follicular lymphoma using breakpoint-flanking probes. Genes Chromosomes Cancer 27(1):85–94
108. van der Velden VH, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grumayer ER, Flohr T, Sutton R, Cave H, Madsen HO et al (2007) Analysis of minimal residual disease by

Ig/TCR gene rearrangements: Guidelines for interpretation of real-time quantitative PCR data. Leukemia 21(4):604–611
109. Varettoni M, Zibellini S, Arcaini L, Boveri E, Rattotti S, Pascutto C, Mangiacavalli S, Gotti M, Pochintesta L, Paulli M et al (2013) MYD88 (L265P) mutation is an independent risk factor for progression in patients with IgM monoclonal gammopathy of undetermined significance. Blood 122(13):2284–2285
110. Visco C, Tzankov A, Xu-Monette ZY, Miranda RN, Tai YC, Li Y, Liu WM, d'Amore ES, Li Y, Montes-Moreno S et al (2013) Patients with diffuse large B-cell lymphoma of germinal center origin with BCL2 translocations have poor outcome, irrespective of MYC status: A report from an international DLBCL rituximab-CHOP consortium program study. Haematologica 98(2):255–263
111. Wahlin BE, Aggarwal M, Montes-Moreno S, Gonzalez LF, Roncador G, Sanchez-Verde L, Christensson B, Sander B, Kimby E (2010) A unifying microenvironment model in follicular lymphoma: Outcome is predicted by programmed death-1–positive, regulatory, cytotoxic, and helper T cells and macrophages. Clin Cancer Res 16(2):637–650
112. Wahlin BE, Yri OE, Kimby E, Holte H, Delabie J, Smeland EB, Sundstrom C, Christensson B, Sander B (2012) Clinical significance of the WHO grades of follicular lymphoma in a population-based cohort of 505 patients with long follow-up times. Br J Haematol 156 (2):225–233
113. Weisenburger DD, Kim H, Rappaport H (1982) Mantle-zone lymphoma: A follicular variant of intermediate lymphocytic lymphoma. Cancer 49(7):1429–1438
114. Wilson W, et al (2012) The bruton's tyrosine kinase (BTK) inhibitor, ibrutinib (PCI-32765), has preferential activity in the ABC subtype of relapsed/refractory de novo diffuse large B-cell lymphoma: Interim results of a multicenter, open-label, phase 2 study. Proc Ash (Abstract 686)
115. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM (2003) A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci USA 100(17):9991–9996
116. Xu L, Hunter ZR, Yang G, Cao Y, Liu X, Manning R, Tripsas C, Chen J, Patterson CJ, Kluk M, et al (2014) Detection of MYD88 L265P in peripheral blood of patients with waldenstrom's macroglobulinemia and IgM monoclonal gammopathy of undetermined significance. Leukemia 28(8):1698–704.
117. Yang G, Zhou Y, Liu X, Xu L, Cao Y, Manning RJ, Patterson CJ, Buhrlage SJ, Gray N, Tai YT et al (2013) A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of bruton tyrosine kinase in waldenstrom macroglobulinemia. Blood 122 (7):1222–1232
118. Younes SF, Beck AH, Lossos IS, Levy R, Warnke RA, Natkunam Y (2010) Immunoarchitectural patterns in follicular lymphoma: Efficacy of HGAL and LMO2 in the detection of the interfollicular and diffuse components. Am J Surg Pathol 34(9):1266–1276
119. Zarate-Osorno A, Medeiros LJ, Longo DL, Jaffe ES (1992) Non-Hodgkin's lymphomas arising in patients successfully treated for Hodgkin's disease. A clinical, histologic, and immunophenotypic study of 14 cases. Am J Surg Pathol 16(9):885–895
120. Zeng W, Fu K, Quintanilla-Fend L, Lim M, Ondrejka S, Hsi ED (2012) Cyclin D1-negative blastoid mantle cell lymphoma identified by SOX11 expression. Am J Surg Pathol 36 (2):214–219
121. Zhou K, Xu D, Cao Y, Wang J, Yang Y, Huang M (2014) C-MYC aberrations as prognostic factors in diffuse large B-cell lymphoma: A meta-analysis of epidemiological studies. PLoS ONE 9(4):e95020

Pathology of T-Cell Lymphomas: Diagnosis and Biomarker Discovery

Alejandro Ariel Gru

Abstract

T-cell lymphomas are a group of predominantly rare hematologic malignancies that tend to recapitulate different stages of T-cell development, in a similar way that B-cell lymphomas do. As opposed to B-cell lymphomas, the understanding of the biology and the classification of T-cell lymphomas are somewhat rudimentary, and numerous entities are still included as 'provisional categories' in the World Health Classification of hematolopoietic malignancies. A relevant and useful classification of these disorders have been difficult to accomplish because of the rarity nature of them, the relative lack of understanding of the molecular pathogenesis, and their morphological and immunophenotypical complexity. Overall, T-cell lymphomas represent only 15 % of all non-Hodgkin lymphomas. This review is focused on addressing the current status of the categories of mature T-cell leukemias and lymphomas (nodal and extranodal) using an approach that incorporates histopathology, immunophenotype, and molecular understanding of the nature of these disorders, using the same philosophy of the most recent revised WHO classification of hematopoietic malignancies.

Keywords

T-cell lymphoma · Non-Hodgkin lymphoma · WHO classification

A.A. Gru (✉)
Department of Pathology and Dermatology,
Divisions of Hematopathology and Dermatopathology, Cutaneous Lymphoma Program,
The Ohio State University Wexner Medical Center,
Richard Solove 'The James' Comprehensive Cancer Center,
333 W 10th Ave, Columbus, OH 43210, USA
e-mail: Alejandro.gru@osumc.edu

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_3

Contents

1 Introduction

T-cell lymphomas are a group of predominantly rare hematologic malignancies that tend to recapitulate different stages of T-cell development, in a similar way that B-cell lymphomas do. As opposed to B-cell lymphomas, the understanding of the biology and the classification of T-cell lymphomas is somewhat rudimentary, and numerous entities are still included as 'provisional categories' in the World Health Classification of hematopoietic malignancies [1]. A relevant and useful classification of these disorders has been difficult to accomplish because of their rarity, the relative lack of understanding of the molecular pathogenesis, and their morphological and immunophenotypical complexity [2]. Overall, T-cell lymphomas represent only 15 % of all non-Hodgkin lymphomas [2, 3].

In the recent years, many developments in immunology and molecular biology have provided tools to subclassify these disorders, using an approach that will benefit targeted therapy. In this sense, peripheral T-cell lymphomas (PTCL) not otherwise specified, an old waste basket category with an overall poor prognosis, have emerged with subsets of follicular T-helper (FTH) differentiation [2, 4] with an overall better prognosis. In addition, studies have determined an important prognostic value in distinguishing cases of ALK− anaplastic large cell lymphoma (ALCL) from PTCL as the former appear to have better prognosis and could benefit from certain forms of therapy [3–8]. Certain site-specific forms of ALCL (not yet incorporated into the WHO classification), such as ALCL associated with breast implants, can be completely indolent and not require additional forms of therapy other than removing the implants [9, 10]. The classification schemes that, are already difficult and of limited value for systemic T-cell lymphomas, are even more problematic for the cutaneous T-cell lymphomas (CTCL), where other than mycosis fungoides (MF), Sézary syndrome (SS), cutaneous ALCL, and subcutaneous panniculitis-like T-cell lymphoma (SPTCL), the remaining subtypes are currently still at the 'provisional' stage.

This review is focused on addressing the current status of the categories of mature T-cell leukemias and lymphomas (nodal and extranodal) using an approach that incorporates histopathology, immunophenotype, and molecular understanding of the nature of these disorders, using the same philosophy of the most recent

Table 1 WHO classification of mature T/NK-cell leukemias and lymphomas

Mature leukemias
• T-cell prolymphocytic leukemia
• T-cell large granular lymphocytic leukemia
• Chronic lymphoproliferative disorders of NK-cells
• Aggressive NK-cell leukemia
• Adult T-cell leukemia/lymphoma
• Sezary syndrome
Mature Lymphomas
• Extranodal NK/T-cell lymphoma, nasal type
• Enteropathy-associated T-cell lymphoma
• Hepatosplenic T-cell lymphoma
• Peripheral T-cell lymphoma, not otherwise specified
• Angioimmunoblastic T-cell lymphoma
• Anaplastic large cell lymphoma (ALCL), ALK positive
• Subcutaneous panniculitis-like T-cell lymphoma
• Mycosis fungoides
• Primary cutaneous anaplastic large cell lymphoma (C-ALCL)
• Lymphomatoid papulosis
Provisional categories
• Primary cutaneous gamma–delta T-cell lymphoma
• Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma
• Primary cutaneous CD4−positive small/medium T-cell lymphoma
• Anaplastic large cell lymphoma, ALK negative

revised WHO classification of hematopoietic malignancies. The current accepted and provisional categories of T-cell and NK-cell lymphomas are detailed in the Table 1 [1, 11].

1.1 Mature T-Cell Leukemias

1.1.1 T-Cell Prolymphocytic Leukemia (T-PLL)

Clinical and Epidemiologic Features

T-cell prolymphocytic leukemia is a rare, predominantly aggressive disorder, accounting for 2 % of the mature lymphoid leukemias. The median age at presentation is 65 years, and clinically, most patients have generalized disease with hepatosplenomegaly, generalized adenopathy, and a striking lymphocytosis (>100 × 10^9/l) [12–16]. Cutaneous dissemination is very common, and particularly in the form of a facial rash, which can be present in up to 20 % of patients. Sometimes, the rash is the first clue to the diagnosis [13, 17, 18]. Serologies for

HTLV are negative, but rare cases have been reported as positive [19]. The prognosis has been a matter of debate: Most cases have an aggressive course, with a survival of less than 1 year, but others have shown a more indolent clinical course [13–15, 20].

Histopathology, Immunophenotypic, and Molecular Features

The morphologic features (Fig. 1) of T-PLL are vital to make a diagnosis. It is recognized that this type of leukemia can have a broad morphologic spectrum of findings: In half of the cases, the cells have a round-to-oval nucleus, and the remainder can be irregular and somewhat cerebriform [14, 21]. Most cases show a predominance of prolymphocytic cells with condensed chromatin and prominent nucleoli. The cytoplasm is basophilic and agranular and can show 'blebs.' In 20 % of cases, the cells are much smaller and with more inconspicuous nucleoli. This is referred to as 'small cell variant of T-PLL.' A cerebriform variant, morphologically similar to the cells of SS can be seen in 5 % of cases [12–14, 16, 22]. Tissue diagnosis is not essential for its diagnosis: In the bone marrow, there are diffuse and interstitial infiltrates accompanied almost invariably by reticulin fibrosis. In most lymph node biopsies, there is an abnormal paracortical expansion of neoplastic T cells. Skin should be evaluated carefully: The infiltrates in T-PLL tend to be superficial and deep with a perivascular and interstitial distribution and spare the epidermis, as opposed to most primary CTCL [17, 18, 23].

Immunophenotypically, T-PLL is a mature T-cell leukemia (TdT and CD1a negative), while CD2, CD3, and CD7 are positive. Nearly 80 % of cases express CD4 and, of those, 65 % do not have coexpression of CD8 (CD4+/CD8−), 21 % have coexpression of both CD4+/CD8+, approximately 17 % were CD8+/CD4− [12], and 3.4 % are CD4−/CD8−. Rare cases can show a switch from CD4 to CD8 [22, 24]. Most cases have high levels of CD52 expression, providing a rational for treatment with alemtuzumab [15, 25–28]. CD25, CD38, and HLA-DR are variably expressed. The distinctive coexpression of CD4/CD8, weak membrane CD3, and strong CD7 suggests that T-PLL represents an intermediate stage of differentiation between a cortical thymocyte and a circulating mature T cell. Nearly all cases will have rearrangements of the TCR-β- or γ-genes.

T-PLL shows complex cytogenetic aberrations: Inversion (14)(q11;q32) is characteristic of the disease and present in more than 2/3 of cases [14, 15]. Tandem translocations between the 2 chromosomes 14, t(14;14) can also occur. The rearrangements involve the *TCR-α* and protooncogene *TCL-1*. 20 % of patients have a t (X;14) translocation. Abnormalities involving both arms of chromosome 8 are frequent, and overexpression of the c-myc protein is found in cases with iso8q. While the 14q abnormality and trisomy 8q are common in Western countries, they are rarely seen in Japan [20]. Although 11q23 abnormalities are seldom detected on cytogenetics, molecular analysis frequently detects mutations of the *ATM* gene [29]. Since these cytogenetic aberrations are believed to be incapable of leukemogenesis, more recently mutations in *JAK1* and *JAK3* have been found in up to 34 % of cases of T-PLL [30, 31]. JAK3 is directly linked to T-cell maturation. Abnormalities of

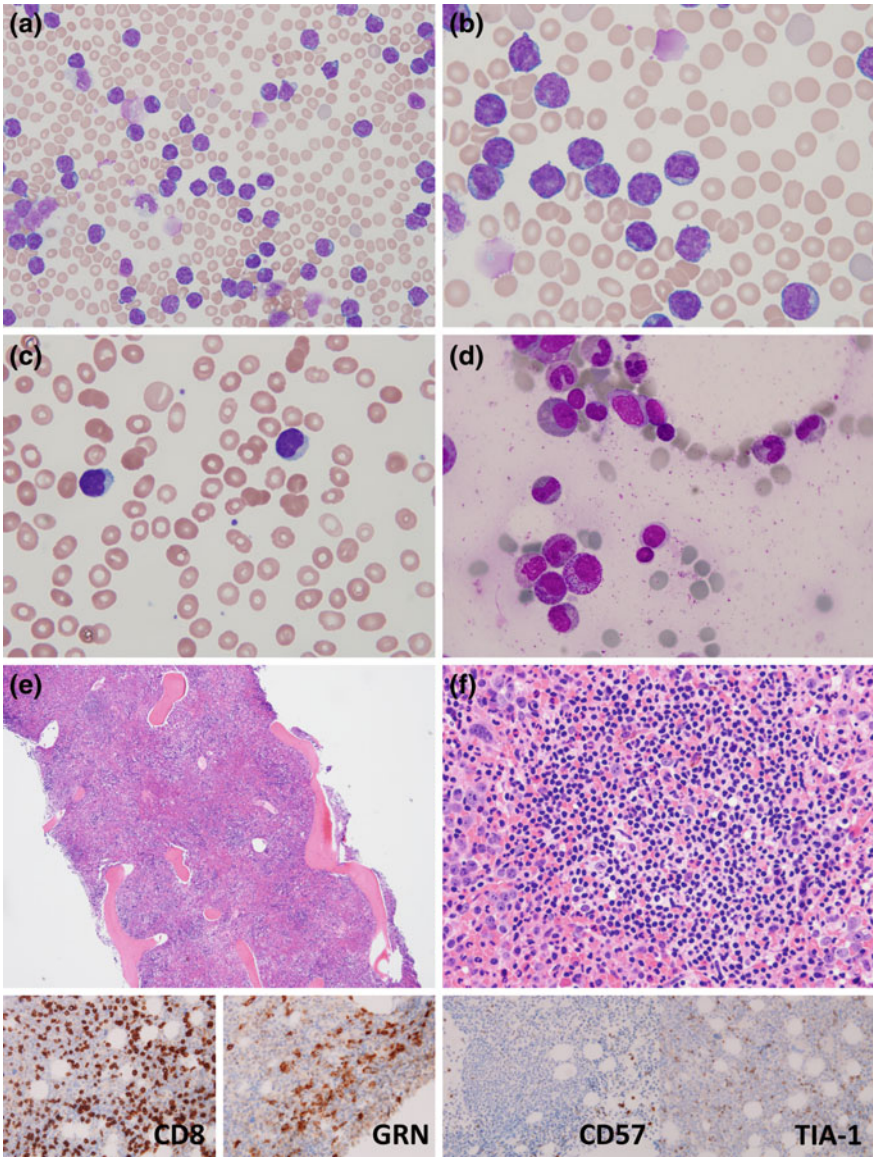


Fig. 1 **a**–**b**, T-cell prolymphocytic leukemia. The small-to-medium-sized cells show nuclear contour irregularities and 'blebbing,' with distinctive prominent nucleoli. **c** (PBL) and **d** (aspirate) T-LGL. The cells are small to medium, with irregular borders, with abundant cytoplasm with granules. **e** and **f** T-LGL involving the bone marrow. Interstitial and nodular aggregates of lymphoid cells. The atypical cells are positive for CD8, granzyme B (GRN), CD57 (weak), and TIA-1. The pattern of involvement in sinusoidal

chromosomes 22q11, 13q, 6q, 9p, 12p, 11p11-p14, and 17p have also been shown by chromosome analysis and aCGH [14, 32].

1.1.2 T-Cell Large Granular Lymphocytic Leukemia (T-LGL)

Clinical and Epidemiologic Features

T-LGL accounts for less than 5 % of all mature leukemic lymphoproliferative disorders [2, 33–35] with a mean age of 50–60. There is a slight higher prevalence of the disease in Asians, where appears to be more commonly associated with anemia [36, 37]. T-LGL is commonly associated with rheumatoid arthritis (20 % of cases) and some lymphoproliferative disorders such as CLL/SLL and monoclonal B-cell lymphocytosis (10–20 %). Other rheumatologic processes can be seen in association, and nearly half of patients with T-LGL have a positive rheumatoid factor or polyclonal hypergammaglobulinemia [38–46]. Rare cases present after transplantation [47, 48].

Originally, a large granular lymphocytosis of $>2 \times 10^9$/L was proposed as a diagnostic criterion but, since a third of cases show $<1 \times 10^9$/L, this is no longer needed for a diagnosis [49, 50]. Bone marrow involvement is seen in 75 % of cases and mild splenomegaly in 15–50 %. Rarely, there might be pulmonary hypertension, which appears to be linked to damage to the vascular endothelium [51]. Neutropenia is a very common feature and present in 60–70 % of cases, while thrombocytopenia is very rare. Anemia can be as frequent as neutropenia, and some cases can present with red cell aplasia [52]. T-LGL does not involve the lymph nodes, and if present, the diagnosis should be reconsidered [53].

Histopathology, Immunophenotypic, and Molecular Features

The classic morphologic features of T-LGL (Fig. 1) account the presence of lymphoid cells with abundant cytoplasm (which represent the 'large' character of the cells), minimal nuclear enlargement, nuclear contour irregularities, and the presence of cytoplasmic granules [21]. However, in some cases, the cytoplasm might be sparse and the granules not as prominent. The pattern of bone marrow involvement is sinusoidal (80 % of cases), which is best demonstrated with immunostains. However, interstitial aggregates of reactive T cells can be seen in up to 15–50 % of cases [54, 55]. In the spleen, there are both red pulp and perivascular white pulp involvements [54, 55]. Likewise, in the liver, there is usually a sinusoidal infiltrate of abnormal T cells. It is widely accepted that large cell transformation is not a feature of T-LGL.

Immunophenotypically, T-LGL is a disease of CD8+ cytotoxic T cells with TCRαβ expression, with isolated case reports of T-LGL with a CD4 phenotype [56]. A subset of cases with CD4−/CD8− T-LGL have a higher prevalence of anemia [57]. Diminished expression of CD5 and CD7 is common. Coexpression of NK cell associated antigens is universal to some extent, and in particular for CD16 and CD57 (one or both are found in >80 % of cases). CD56 is not commonly seen, as opposed to hepatosplenic T-cell lymphoma (HTCL). CD57 expression by

immunohistochemistry is only seen in <20 % of bone marrow specimens [58, 59]. Strong homogeneous expression of CD16 appears to be more useful when compared to CD57. Abnormal expression of Killer-cell immunoglobulin-like receptor antigens (KIR) can be seen in 50 % of T-LGL [59, 60]. CD335 is also positive in T-LGL [61].

The vast majority of cases of T-LGL show no cytogenetic aberrations. Deletion of 6q, translocations and inversions involving the T-cell receptor loci on chromosomes 7 and 14 has been rarely described [62]. At a molecular level, most cases show clonality by PCR or flow cytometric analysis. However, as opposed to other T-cell lymphoproliferative disorders, T-LGL lacks the homogeneity of the clones. Most cases appear to be actually oligoclonal [63]. Molecular pathways that appear to be altered in T-LGL include resistance to *FAS* and activation of STAT3 pathway [41–43, 45, 46]. The *STAT3* mutations are present in a 1/3 of T-LGL cases. STAT3 is involved in cell proliferation, apoptosis, angiogenesis, and immune responses. STAT3, a latent transcription factor, has been shown to play a central role in conferring cell survival [46].

1.1.3 Adult T-Cell Leukemia/Lymphoma (ATLL)

Clinical and Epidemiologic Features

Adult T-Cell Leukemia/Lymphoma (ATLL) is a mature T-cell leukemia, which its pathogenesis is linked to the infection with human T-cell leukemia virus type-1 (HTLV-1) [64]. It is more commonly seen in areas where the infection with the virus is endemic, particularly in Japan, the Caribbean, parts of central Africa, South America, and Iran [65, 66]. The majority of the individuals who develop the disease do so after a very long latency period. In some areas of Japan, the prevalence ranges from 0.3 to 13 % of the population [67]. The cumulative incidence of ATLL is estimated to be 2.5 % of those individuals infected by HTLV-1. ATLL is only present in adults, at a mean age of 58, with a slightly higher prevalence in male.

Most cases of ATLL present with widespread disease including lymph node and peripheral blood involvement. The degree of bone marrow involvement does not correlate with the burden of disease in the blood, suggesting that the tumor cells are recruited from other sites. In fact, the skin is a very common site of involvement (>50 %) [65, 68–70]. The cutaneous manifestations are diverse including erythema, papules, nodules, and rarely erythroderma. Other extranodal sites of involvement include the lung, liver, spleen, GI tract, and CNS [65, 71, 72]. Different variants have been described according to the Japanese lymphoma study group [73]: Those include smoldering, chronic, acute (leukemic), and lymphomatous. The most common form is the leukemic (60 %), typically accompanied by significant leukocytosis, rash, generalized lymphadenopathy, hypercalcemia, and lytic bone lesions. Even in the setting of marked leukocytosis, bone marrow involvement may be absent. Additional opportunistic infections are also frequent. The lymphomatous (20 %) variant does not show peripheral blood involvement, and hypercalcemia is infrequent. The survival in the acute and lymphomatous variants is very poor

(2 weeks–1 year). The chronic variant (15 %) presents with a rash, leukocytosis, fewer numbers of circulating tumor cells and prolonged survival. The smoldering variant (5 %) has a normal WBC, skin lesions, and no systemic disease. This form can evolve into the more advanced presentations in 25 % of cases [65, 68, 73].

Histopathology, Immunophenotypic, and Molecular Features

ATLL (Fig. 2) shows a protean morphology [64, 65, 68, 74–76]. The classic features associated with the disease include neoplastic lymphoid cells, which are polylobulated, hyperchromatic, with nuclear convolutions, and have been termed as 'flower cells.' The cells do not have a prominent nucleolus and have a classic basophilic cytoplasm (useful features to distinguish from Sézary cells). Numerous morphologic variants have been described: pleomorphic, anaplastic, and a rare variant resembling angioimmunoblastic T-cell lymphoma (AITL) [77].

The architecture of the lymph nodes is completely effaced, and some cases can show a sinusoidal pattern of involvement. Eosinophilia in the background can be prominent. Blast-like cells with transformed nuclei and dispersed chromatin are also present. Giant cells with bizarre and cerebriform nuclei can be seen. Some cases have features indistinguishable from ALCL (but are ALK) [53, 65, 68, 74]. Hodgkin-like histology has also been described [78, 79]. The cutaneous histopathologic findings are equivalent to those seen in cases of MF [70], including the presence of Pautrier microabscesses [74, 80]. Some unusual variants in a cutaneous site include folliculotropic (follicular mucinosis) [81], pagetoid reticulosis-like [82], and vesiculobullous [83]. Within the bone marrow, the infiltrate might be subtle, typically interstitial, and osteoclastic activity might be prominent. In the liver, the infiltrates are portocentric, and interface hepatitis can be present.

Immunophenotypically, the tumor cells express T-cell markers and typically have marked decreased expression of CD7. Most cases are CD4+/CD8−, some are CD4−/CD8+ and also CD4+/CD8+. CD25 and IL-2R are expressed in nearly all cases. CD30 can also be positive. ALK and cytotoxic molecules are negative [84]. CCR4 and FOXP3 are expressed in 68 % of cases [85, 86]. The combination of these markers suggests T regulatory phenotype. Targeted therapy for anti-CCR4 is currently in clinical trials [87–89].

At a molecular level, there is a clonal population of T cells with integration of the HTLV-1 virus into the neoplastic cells. Oligoclonal populations of T cells can be seen in HTLV-1 carriers. The viral protein Tax has been shown to be critical for leukemogenesis [90]. More recently, the viral basic leucine zipper factor (HBZ) has been shown overexpressed in all leukemic cases [91–96], and it appears to be the most important gene in the pathway to malignant transformation. HBZ is linked to cell proliferation, but also reacts with Smad2/3 and p300 proteins and enhancing the transcription of the *HBZ* gene. More than 80 % of cases show aneuploidy, and there is no distinctive cytogenetic alteration [97]. Overexpression of BIRC5 can be seen, and it is linked to resistance to chemotherapy [98].

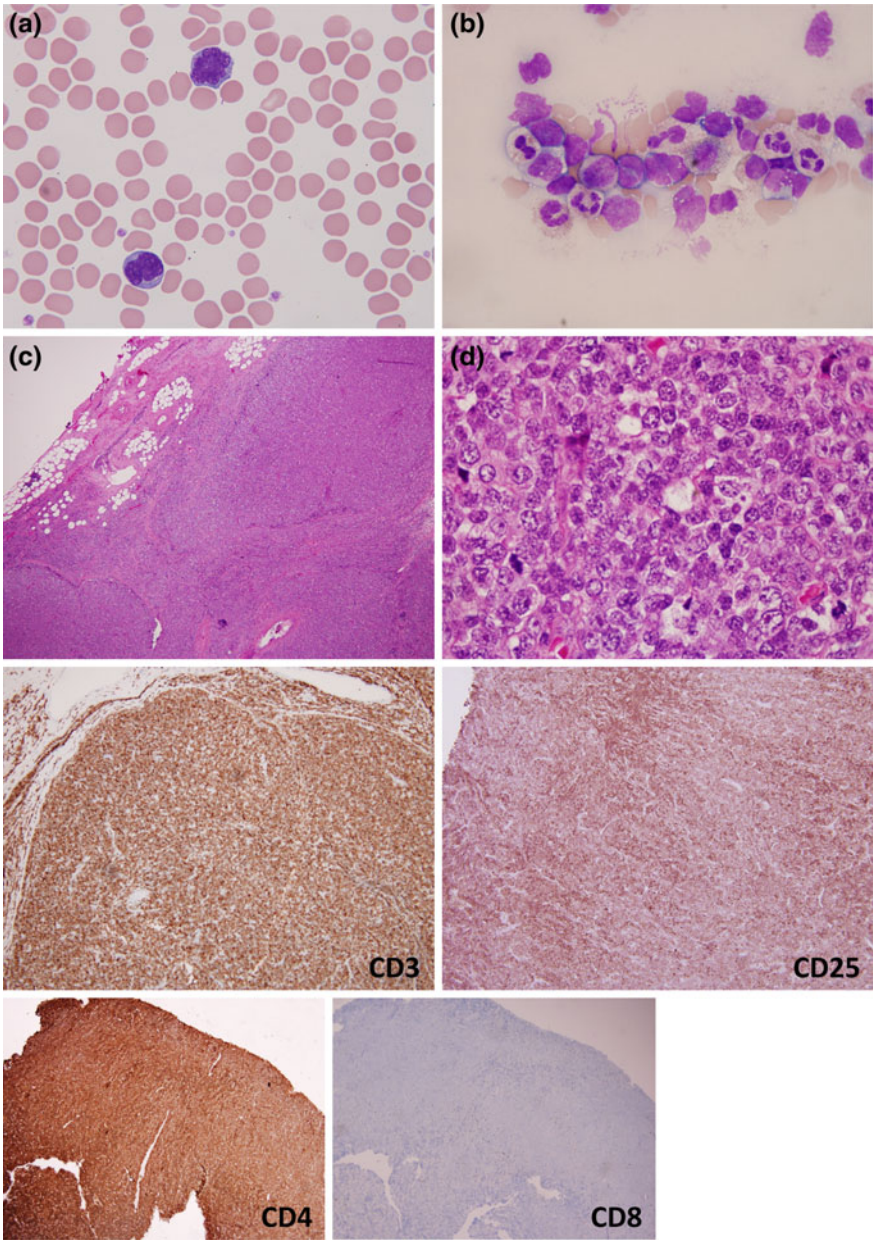


Fig. 2 **a** (PBL) and **b** (aspirate): malignant lymphoid cells in ATLL. The cells show a 'flower-like' appearance. **c–d** lymph node involvement by ATLL. The tumor cells efface the architecture and are medium to large in this pleomorphic variant. The malignant cells are positive for CD3, CD25 and CD4, but negative for CD8

1.1.4 Sézary Syndrome (SS)

Clinical and Epidemiologic Features

SS is defined as a separate entity by the WHO/EORTC classification and is an aggressive disease [99, 100]. SS is characterized by circulating atypical T cells (Sézary cells), erythroderma, and pruritus with or without lymphadenopathy [101]. Most cases present de novo, over a short period of time, but some can have a prodrome of pruritus and non-specific dermatitis. It may follow classic MF. It has been proposed that these should be classified as 'SS preceded by MF' to separate from classic MF [102]. Erythrodermic MF usually follows MF and is distinguished from SS by absent or minimal blood involvement. The clinical features of SS range from mild erythema, to generalized diffuse erythroderma with involvement of the palms and soles. Males are affected more commonly. Other common clinical features include alopecia, ectropion, and onychodystrophy.

Histopathology, Immunophenotypic, and Molecular Features

The histologic features of SS are variable and often subtle in the skin. The epidermotropic T cells, Pautrier microabscesses, and haloed lymphocytes are less prominent. Some cases can just present with a perivascular lymphoid infiltrate (Fig. 6) [103, 104]. The detection and quantification of Sézary cells for the diagnosis of SS and MF with leukemic involvement have traditionally been determined by their morphologic identification on peripheral blood smears. Sézary cells have characteristic cerebriform nuclei. This has largely been replaced by flow cytometry because of high interobserver variability in cell counts. In addition, atypical lymphocytes with cerebriform nuclei can be found in the blood of healthy individuals and those with benign inflammatory skin diseases.

Most cases show expression of T-cell antigens (CD2, CD3, CD5) and CD4. Rare cases can be CD8 positive or even CD4+/CD8+ [105]. Most typically the abnormal cells lack CD7 and CD26 [106–109]. Some inflammatory dermatoses unfortunately can show this abnormal phenotype.

At a molecular level, in advanced stage MF and SS, there is a switch from Th1 cytokines to Th2. Th2 cytokines, such as IL-4, IL-5, IL-10, and IL-13, correlate with eosinophilia, erythroderma, high levels of IgE, immunosuppression, and increased susceptibility to bacterial infections. Additional studies have also revealed that MF and SS arise from different memory T-cell subsets. The malignant T cells in SS are of central memory and capable of circulating between skin, lymph nodes, and blood [107, 110–112]. Expression of tissue addressins such as CLA and the lack of L-selectin/CCR7 coexpression are characteristics of effector memory T (Tem) cells [113]. Amplification of the *JUNB* gene has been identified in SS [114–116]. *JUNB* is involved in cell differentiation, proliferation, and apoptosis. In SS, the Th2 phenotype is linked to overexpression of GATA3 and CTLA-4. Impaired proteasome function, with altered degradation of GATA3, has been shown in SS patients [117]. The immunologic disturbances seen in SS impair the function of plasmacytoid dendritic cells, features that constitute a rational explanation for the

immune suppression associated with SS [118, 119]. By aCGH, the most common abnormalities are gains in 8q and 17q and losses at 17p and 10q [120, 121]. Using the same methodology and hierarchical clustering, SS appears to be a distinctive group separated from transformed MF and cutaneous ALCL [122]. A membrane molecule that belongs to the CD28/CTLA-1 receptor family, programmed death-1 (PD-1), may have utility in the identification of SS. Engagement of PD-1 on T cells with its ligand has been shown to inhibit T-cell activation and proliferation. Recent studies have shown a high expression of PD-1 by neoplastic T cells in SS but not MF [123].

1.2 Nodal and Extranodal Mature T-Cell Lymphomas

1.2.1 Anaplastic Large Cell Lymphoma, ALK Positive (ALK+ ALCL)

Clinical and Epidemiologic Features

ALK+ ALCL is a mature T-cell lymphoma with expression of CD30 and ALK1, and a rearrangement of the *ALK1* gene. It is the second most common subtype of PTCL (25 %) and accounts for 5 % of NHL. It is the most common subtype of PTCL in children and accounts for 10–30 % of all pediatric lymphomas [124–126]. Most cases present as lymphadenopathy, and the most common extranodal site is the skin. Other affected sites include bone, lung, liver, and soft tissues [127]. A leukemic presentation is rare and more frequent in the small cell variant [128]. The bone marrow is affected in a small percentage (10–30 %) of cases [129].

Histopathology, Immunophenotypic, and Molecular Features

The classic morphologic picture (Fig. 3) of ALCL includes the so-called hallmark cell: This is a large cell with a pleomorphic, horseshoe-shaped nuclei, a prominent central golgi zone, and abundant cytoplasm [53]. A wreath-like appearance of the cells can also be seen. The tumor cells are usually located around vessels. The typical pattern of lymph node involvement in ALK+ ALCL is sinusoidal, where the tumor cells surround residual lymphoid follicles. Certain histologic variants are recognized in the WHO: (a) lymphohistiocytic (10 % cases) [130, 131] with abundant histiocytes sometimes obscuring the malignant cells and rarely erythrophagocytosis; (b) small cell variant (5–10 % of cases) [132] with a worse prognosis and more frequent leukemic forms [133]; (c) Hodgkin-like variant (3 % of cases) that mimics classical HL [134] and a nodular sclerosing variant. The tumor cells in this variant lack the very large, prominent cherry red nucleoli seen in RS cells. In 15 % of cases, there is a combination of patterns. More uncommon variants include multinucleated giant cells, sarcomatoid, and myxoid forms [124–127]. A novel cutaneous-only form of ALK+ ALCL has been proposed [135].

The immunophenotype must demonstrate the presence of CD30 and ALK expression. In the majority of the cases, there is loss of multiple T-cell antigens

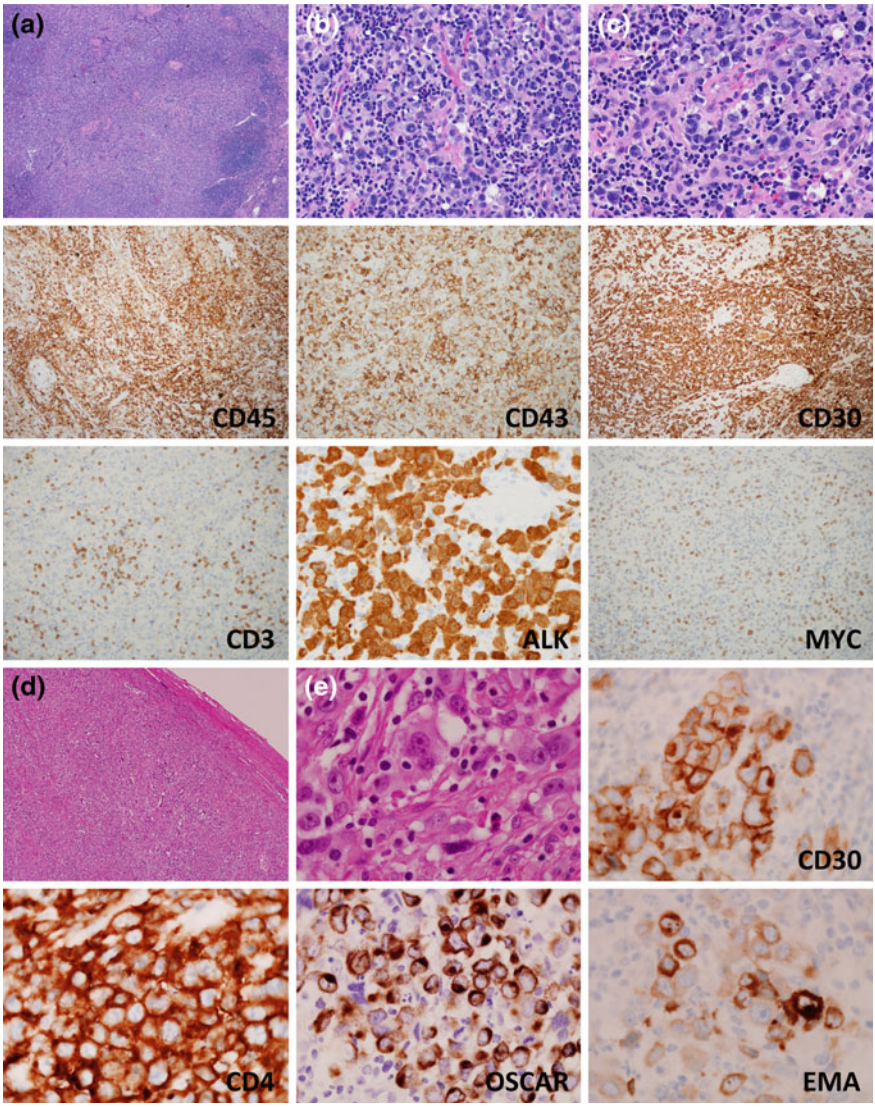


Fig. 3 **a–c** ALK+ ALCL with a typical sinusoidal pattern. The classic cells in this tumor are the so-called hallmark cells. The tumor cells are positive for CD45, CD43, CD30, ALK (nuclear and cytoplasmic patterns), and MYC. The tumor cells have loss of CD3. **d–e** ALK− ALCL, with a similar type of histology to ALK+. In this particular case, there is coexpression of CD30, CD4, EMA, and aberrant expression of keratins (OSCAR)

[136]. CD2 and CD4 are most frequently preserved. Some cases can be positive for TCR receptors. The cytotoxic markers (perforin, TIA-1, and granzyme B) are expressed [137]. The majority of ALCL is positive for EMA. Rare cases can be

CD15+ [138]. Other aberrant markers than can be expressed are cytokeratins, PAX5, CD13, and CD33.

By cytogenetics and FISH, ALK+ ALCL has rearrangements of the *ALK* gene on 2p23 with various partner genes, most typically the nucleophosmin (*NPM*) on 5q35 [139, 140]. Some translocations can be cryptic and not picked up on conventional cytogenetic studies. The cellular localization of the ALK expression correlates with the pattern of translocation: The *NPM/ALK* fusion leads to both nuclear and cytoplasmic staining for ALK; less common partners lead to diffuse cytoplasmic only staining (e.g., *TPM3, ATIC, TFG, TPM4, MYH9, ALO17*), granular cytoplasmic (*CLTC*) or membranous (*MSN*). The translocation partner is not required for a diagnosis, and using a break-apart FISH probe for *ALK* is the most common modality for diagnosis. EBV is not present in ALK+ ALCL, and invariably there is clonality by PCR methods. Inhibitors of the *ALK* gene are currently under investigation for the treatment of relapsed disease [141]. Minimal residual disease assessment using PCR is also being used. Rare cases with rearrangements of the *MYC* gene have also been described [142].

1.2.2 Anaplastic Large Cell Lymphoma, ALK Negative (ALK− ALCL)

Clinical and Epidemiologic Features

In the current WHO, this neoplasm remains under a provisional category. ALK− ALCL is a mature T-cell lymphoma with CD30 expression, morphologically identical to ALK+ ALCL but lacks the expression of ALK. It represents 40–50 % of all ALCLs, but occurs in the older population, predominantly in the sixth decade [143]. The affected individuals present with lymphadenopathy, and extranodal involvement is very rare [144, 145]. Most patients have advanced disease (stage III or IV) and B-symptoms. Because cutaneous ALCL (C-ALCL) and systemic forms are morphologically and immunophenotypically identical (and C-ALCL can extend locoregionally to lymph nodes), the clinical information is imperative [146] to distinguish between the two.

Histopathology, Immunophenotypic, and Molecular Features

The cytology of the tumor cells is identical to ALK+ ALCL but, in general, the tumor cells tend to be larger and more pleomorphic than its ALK+ counterpart (Fig. 3). Multinucleated wreath-like forms are frequent. Histologic variants are not strictly defined, but some cases resemble the lymphohistiocytic variant and others the Hodgkin-like forms [53]. The background cells can include histiocytes, plasma cells, eosinophils, and small lymphocytes [143, 144]. A small cell variant is not typical and, when present, should be classified as peripheral T-cell lymphoma (PTCL), not otherwise specified (NOS). Emphasis in distinguishing ALK− ALCL from PTCL should be placed, as most cases of ALCL will potentially benefit from bone marrow transplant and appear to have a relative better prognosis [8, 143, 144, 147, 148]. A recently described form of ALCL, occurring in association with breast

implants, has a much more indolent behavior and potentially does not require further treatment other than removal of the implants [10]: To this respect, two clinical presentations have been noted: one with an effusion, no mass effect and ± capsule contracture; and a second one with mass effect and ± effusion [149].

The immunophenotype of ALK− ALCL shows strong and diffuse expression of CD30. The pattern can be membranous, Golgi and/or cytoplasmic. The strong and diffuse character is often a helpful clue to distinguish it from PTCL. It often lacks multiple T-cell antigens, and cytotoxic markers are often expressed. EMA and clusterin can be positive, but to a lesser extent than in ALK+ ALCL [145]. Cytokeratin expression can sometimes lead to a misdiagnosis of metastatic carcinoma [150]. At the molecular level, rearrangements of the *DUSP22-IRF4* locus have been described [151–153]. The most common partner is *FRA7H* at 7q32. 6p25.3 rearrangements are mutually exclusive of ALK translocations. They are classically seen in C-ALCL, but can be seen in ALK− ALCL in 18 % of cases.

1.2.3 Angioimmunoblastic T-Cell Lymphoma (AITL)

Clinical and Epidemiologic Features

AITL was originally described as an abnormal immune reaction or form of atypical hyperplasia [154–158]. It is now regarded as a mature T-cell lymphoma. This lymphoma subtype occurs in adults and has not been described in children. Clinically, most patients present with generalized adenopathy, hepatosplenomegaly, skin rash, and prominent constitutional symptoms [159, 160]. Other extranodal sites, such as lung and bone marrow, are also frequently involved. Patients can have polyclonal hypergammaglobulinemia (>50 % of cases) and Coombs-positive autoimmune hemolytic anemia. LDH levels are usually high. In addition, recurrent opportunistic infections may occur.

Histopathology, Immunophenotypic, and Molecular Features

The nodal architecture is typically effaced with open and sometimes dilated sinuses. There is a very striking proliferation of postcapillary or high endothelial venules with prominent arborization (Fig. 4). A dendritic cell proliferation (FDC) around the high endothelial venules is typical. The neoplastic lymphoid cells have clear cytoplasm, and are associated with small lymphocytes, immunoblasts, plasma cells, and histiocytes [53]. Three patterns are now recognized: In pattern 1 (15 % of cases), partial preservation of the architecture with hyperplastic B-cell follicles and a paracortical expansion of T cells and prominent vascularity is seen. In pattern 2 (25 % of cases), there is loss of the architecture with atrophic follicles and concentrically arranged FDC. In pattern 3 (60 %), there is total loss of the architecture with no residual follicles [6, 34, 160–162]. The histologic features in extranodal sites can be relatively non-specific: In the skin, the infiltrate is superficial and deep, and usually spares the epidermis [163–165]. But occasionally, epidermotropic cases mimicking MF have been seen [166]. Cases resembling marginal zone B-cell lymphomas have been reported [167].

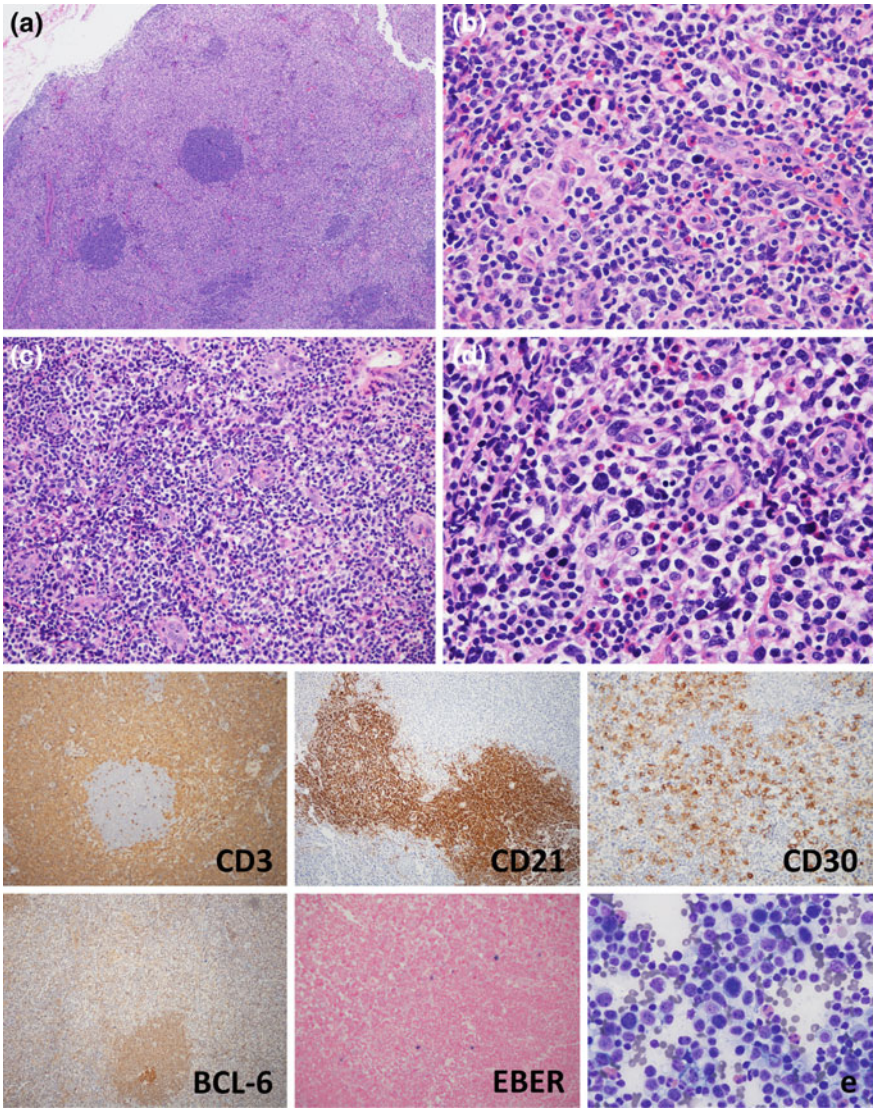


Fig. 4 **a**–**d** AITL. There is preservation of residual follicles, and the neoplastic cells have a clear abundant cytoplasm, and there are associated high endothelial venules and focal RS-like cells (**d**). The tumor cells are positive for CD3 and BCL-6. CD21 shows disrupted FDC. CD30 stains numerous immunoblasts (B cells). EBER is positive in scattered lymphoid cells. The touch preparation **e** also shows eosinophilia

The abnormal cells are usually positive for CD3, CD4, CD10, and CD279 (PD-1), a phenotype characteristic of TFH [159, 160, 162]. Strong expression of CD10 and PD-1 in perifollicular lymphocytes can be helpful in the differential

diagnosis with reactive hyperplasia [168, 169]. However, PD-1 is more weakly expressed normally in paracortical T cells, and therefore, only strong intense staining is diagnostically useful. CXCL13 [170], a chemokine involved in B-cell trafficking into the germinal centers, is also expressed in AITL. B-cell markers are positive in the residual follicles and also stain many immunoblasts in the interfollicular areas. The population of immmunoblasts, when extraordinary numerous, can mimic a DLBCL [171]. Expansion of the FDC is better seen with CD21. EBV-positive B cells comprise the most frequently identified atypical B-cell component in AITL and are nearly always present in the background. The EBV+ cells can resemble Hodgkin lymphoma morphologically and immunophenotypically, and a wrong diagnosis could sometimes be made [172, 173]. Some cases can transform into a DLBCL [174]. Rarely, proliferations of TdT+ cells can be seen [175]. TCR gene rearrangement and clonal IGH can also be present.

At a molecular level, *TET2, DNMT3A,* and *IDH2* mutations have been recently reported in AITL [176, 177]. These mutations, however, are not specific and can also be seen in other PTCLs. Lately, a mutation of *RHOA*, which encodes a GTPase, was reported in 68 % of AITL and is associated with TET2 mutations [178, 179]. CGH studies have shown non-specific gains of chr X, 3, 5, 11q13, 13q, and 22q [180].

1.2.4 Peripheral T-Cell Lymphoma, Not Otherwise Specified (PCTL, NOS)

Clinical and Epidemiologic Features

PTCL, NOS encompasses a heterogeneous group of lymphomas, which essentially do not meet the criteria for any of the other subtypes of mature T-cell lymphomas. Using this definition PTCL, unfortunately, represents a diagnosis of exclusion [181]. PCTL can present in nodal and extranodal sites and has a variety of morphologic patterns, some of which are shared by other subtypes of NHLs. In this sense, the use of PTCL, NOS as a 'waste basket' category not only represents a histologic dilemma, but also affects substantially the way the patients are treated and the overall prognosis. Novel approaches, using gene expression arrays, are a promising tool to better define this subgroup of T-cell lymphoproliferative disorders [2, 140, 161, 182]. Interestingly, the WHO has preserved its use as a 'permanent' and not 'provisional' category. Comparison of interobserver agreement has shown a high rate of discordant results for its diagnosis [34, 183, 184].

PTCL, NOS is the most common subtype of PTCL (30–60 % of cases). It is less common in Asia, where EBV-associated PTCLs are more common [185]. It affects primarily adults, with a mean age of 60, and a higher prevalence in males (2:1). Most patients present with lymphadenopathy with or without extranodal extension. Between 40 and 60 % of patients present with stage IV disease [186–188]. Some can have eosinophilia, pruritus, erythroderma, and rarely hemophagocytosis [163, 189, 190].

Histopathology, Immunophenotypic, and Molecular Features

By definition, the morphology is heterogeneous [53]. In most circumstances, the neoplastic cells are medium to large, and some cases can have a small cell component [2, 5]. In those cases with resemblance to AITL (Follicular variant, see below), cells with clear cytoplasm are present. The presence of Hodgkin-like cells (RS-like) has been documented (particularly in the lymphoepithelial variant) [173, 191]. The background cells include eosinophils, plasma cells, histiocytes, and prominent vessels (Fig. 5). The nodal involvement shows effacement of the architecture with a follicular or parafollicular patterns. Prominent sinusoidal involvement can be seen in those with features resembling ALCL. The distinction between ALCL and PTCL, NOS is important, as the former appears to have a better prognosis and a better outcome after bone marrow transplantation [144, 192]. The WHO recognizes three specific variants: lymphoepithelial, follicular, and T-zone [181]. Lennert lymphoma (lymphoepithelial variant, LE) [191, 193, 194] usually shows scattered neoplastic cells (small to medium in size) with an abundance of epithelioid histiocytes with a granulomatous appearance. Admixed RS-like cells are present [173, 191]. Eosinophils and plasma cells in the background are seen. In the follicular variant [2, 4, 170, 172], there are expanded follicles with a population of neoplastic interfollicular cells of medium size and with clear cytoplasm. FDCs can be present. The T-zone variant [186] shows residual non-neoplastic follicles and an extensive perifollicular neoplastic population of cells.

The immunophenotype shows aberrant loss of 1 or more T-cell antigens, most commonly CD3, CD5, or CD7. The majority of the cases are CD4+, but some can be CD8+, particularly the LE variant [191, 193]. In addition, some cases can be double positive or double negative for CD4 and CD8. The majority of cases have a TCRαβ phenotype, but some are negative, and a small subset is TCRγδ [195]. Cytotoxic markers can be expressed in PTCL, NOS. Some cases express CD30, and a minority CD15 [196]. Cases with significant expression of CD30 should be distinguished from ALK− ALCL. Gene expression profiles have proven beneficial in this situation [197]. Aberrant B-cell antigenic expression can be seen, including multiple B-cell markers (CD20, CD79a, and CD19) [198–200]. The follicular variant shows markers of follicle center T-cell phenotype (T_{FH}): CD10, BCL-6, CXCL13, and/or PD1. The distinction between AITL and the PCTL with T-helper phenotype could be very difficult, and overlap between the two, by gene expression analysis, has been seen [148]. However, this PTCL subtype appears to have a better prognosis and is of extraordinary importance to recognize. It has been proposed more recently the use of at least 3 T-helper markers (e.g., CD10, BCL-6, PD-1, CXCL13) on the evaluation of a new diagnosis of T-cell lymphoma [2]. EBV positivity can be seen in some cases of PTCL, NOS, but diffuse positivity is certainly uncommon [201, 202].

Molecular studies have shown non-specific alterations with some exceptions: Some cases of the follicular variant show a t(5;9) translocation fusing the tyrosine kinase genes *ITK* and *SYK* [2, 4, 6, 203, 204]. Rare cases have shown a t(6;14) translocation with involvement of the *IRF4* and *TRA* genes [152]. Those cases show

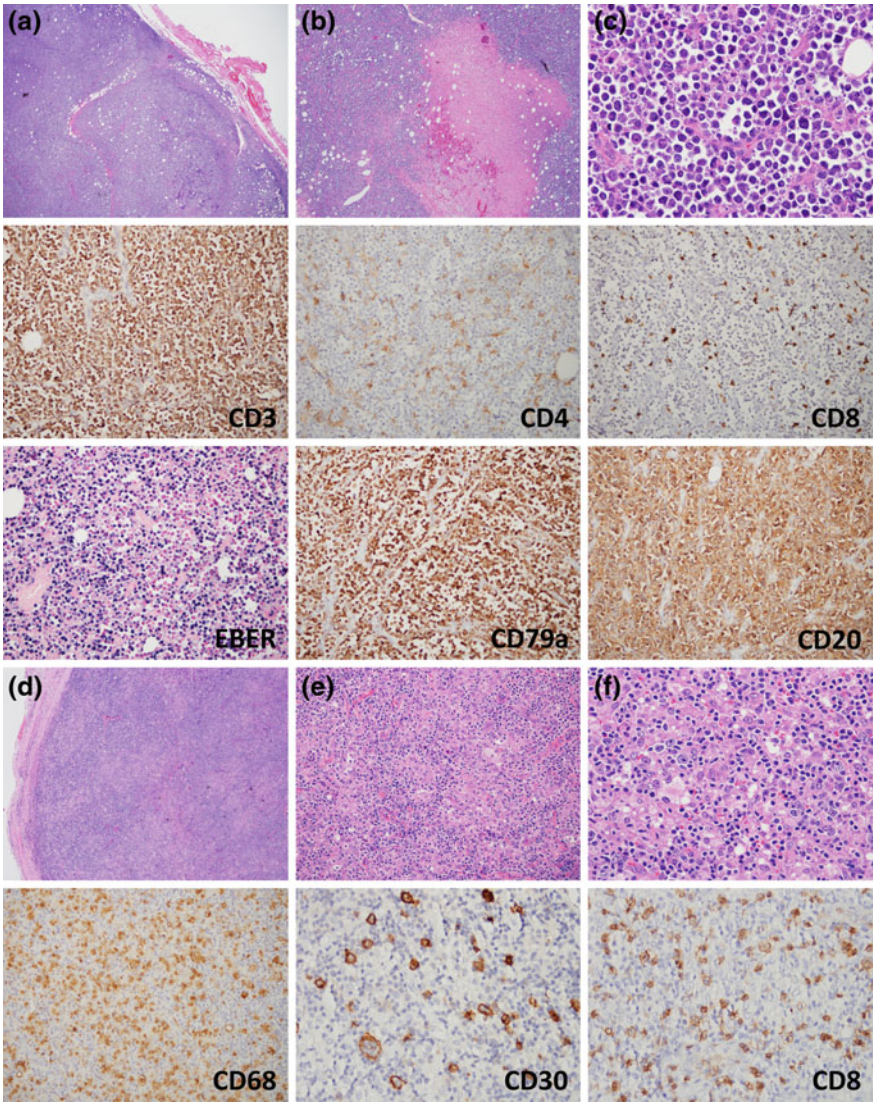


Fig. 5 PTCL, NOS. **a–c** there is a diffuse proliferation of medium-to-large cells which form vague alveolar structures. In this case, the tumor shows diffuse expression of CD3 and EBER, with aberrant coexpression of CD79a and CD20. Both CD4 and CD8 are negative in the tumor cells. **d–f** lymphoepithelial variant (Lennert lymphoma). A rich histiocytic background and granulomatous-like areas are seen. Frequent RS-like cells are present. The immunophenotype of this variant is typically CD8 positive, and CD30 stains the RS-like cells. CD68 shows numerous histiocytes in the background

MUM1 expression and a cytotoxic phenotype in association with diffuse bone marrow infiltration. Amplification of the *CDK6* gene is seen in 23 % of cases [205]. *TET2* mutations, typical or AITL, are present in 20 % of cases and cannot be used to distinguish between the two [6, 177, 206, 207]. Similarly, more recently mutations of the RHOA GTPase appear to be very frequent among them [208]. *BCL-3* rearrangements are also rare [209]. Certain micro-RNA (miRNA) profiles can also help to distinguish among certain subtypes of PCTLs [210]. Rearrangements of the *TP63* gene, present in only 5 % of cases of PTCL, NOS, predict a worse prognosis [211]. Cases with overlap features with AITL can show *IDH2* mutations [197].

1.3 Extranodal T-Cell Lymphomas, Non-Cutaneous

1.3.1 Hepatosplenic T-Cell Lymphoma (HTCL)

Clinical and Epidemiologic Features

HTCL accounts for only 3 % of all T-cell lymphomas in the United States. It affects the liver and spleen and is characterized by a γδ phenotype [212]. Normally, this population of lymphocytes accounts for only 1–3 % of the peripheral blood lymphoid cells. The γδ cells represent part of a repertoire linked to innate or non-specific immune system. The neoplastic T cells in HTCL are similar to the functionally immature cytotoxic γδ T cells observed in the setting of solid organ transplantation [213–216]. The potential role of an immunosuppressed state in the pathophysiology of the disease is also supported by an increased incidence of HTCL in patients with inflammatory bowel disease and rheumatoid arthritis who have undergone treatment with tumor necrosis factor (TNF) blockers and thiopurine [214, 217–219]. HTCL usually presents in young individuals (mean age of 34) with fever, weight loss, splenomegaly and, in some cases, jaundice. Hemophagocytosis can also be seen [220, 221]. Hepatomegaly is seen in 50 % of cases. Laboratory findings include elevation of liver enzymes, LDH, anemia, and thrombocytopenia [57, 212, 214, 222, 223].

Histopathology, Immunophenotypic, and Molecular Features

The neoplastic cells infiltrate the red pulp of the spleen, with a reduction or complete atrophy of the white pulp [224]. In the liver, sinusoidal infiltration by neoplastic cells can be observed in virtually all cases [53]. The portal triads are relatively spared [225]. Perisinusoidal fibrosis without hepatocyte involvement has been described. Lymphadenopathy is almost never present, at least at the time of first presentation, with only a few cases that demonstrated infiltrated lymph nodes by medium-sized lymphoma cells. Bone marrow infiltration is virtually always present (Fig. 6). The infiltration is better appreciated with the use of immunostains. Throughout the course of the disease, a change in the pattern of bone marrow infiltration as well as cell morphology can be observed. With progression, the distribution changes from sinusoidal to increasingly interstitial, and the neoplastic cells become larger and blast-like [226, 227].

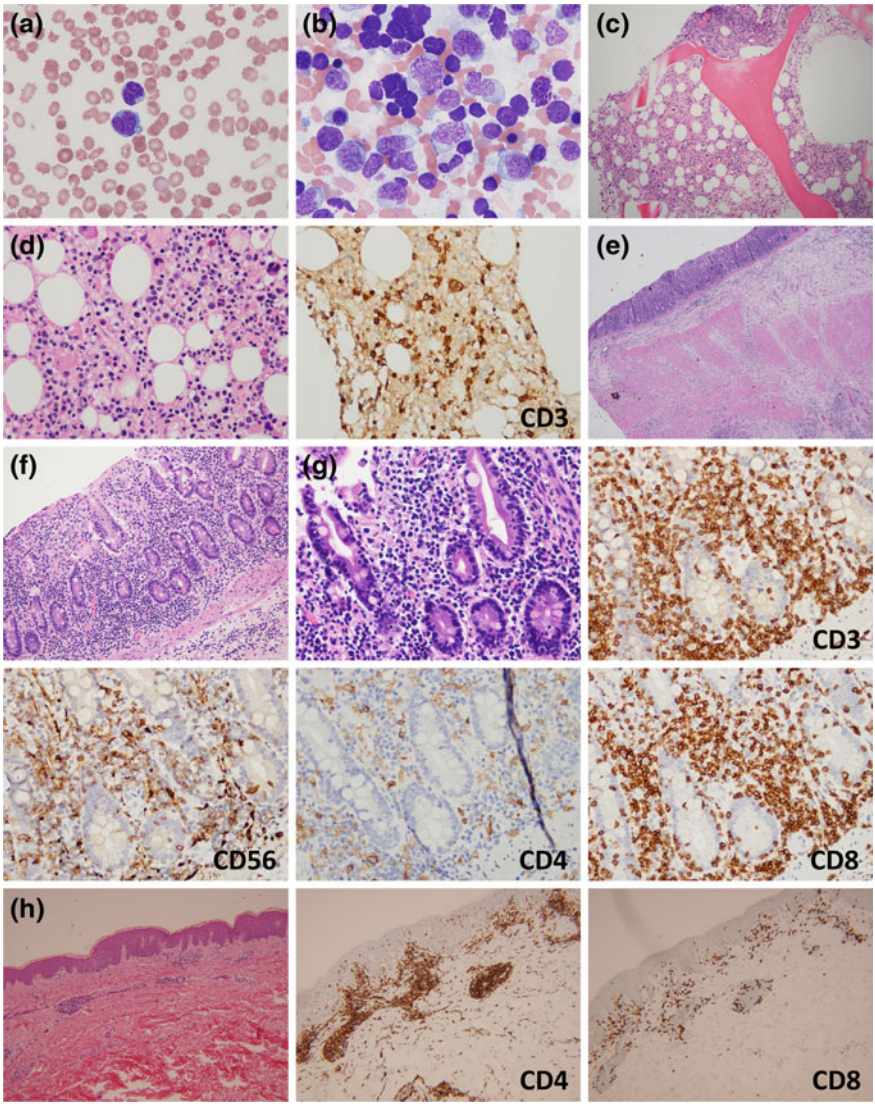


Fig. 6 **a** (PBL)–**b** (aspirate) of HSTCL. In the evolution of the disease, blast-like forms are appreciated. **c**, **d** show bone marrow involvement with a sinusoidal pattern, shown better with the CD3 immunostain. **e** and **f** EATL-2. There is a monotonous infiltrate of medium-to-large cells in the jejunum. The malignant cells are positive for CD3, CD56, and CD8, while negative for CD4. 6 h—SS with cutaneous involvement. Often the histologic findings are very deceptive, with only a mild perivascular lymphocytic infiltrate. The CD4:CD8 ratio is markedly increased

Immunophenotypic studies reveal a γδ phenotype (which can be demonstrated by flow cytometry or the use of BF1 or γδ-antibodies). Rare cases show an αβ-phenotype [228, 229]. Most cases express CD3 and are positive for CD8 and CD56.

The malignant cells express TIA-1 and granzyme M, but negative for perforin and granzyme B. These features are indicative of a non-activated cytotoxic phenotype [2, 6, 57, 212, 214, 225]. KIRs are often expressed [230]. Isochromosome 7q seems to be the primary and consistent chromosomal abnormality detected in most patients with the disease [231–234]. The presence of i(7)(q10) in HTCL seems to be independent of the immunophenotype of the malignant cells. One study revealed the tendency of HTCL to multiply the i(7)(q10) chromosome during evolution and progression of the disease [234]. Clonal ring chromosome 7 formation has been described in HTCL as well, in which the ring chromosome often exhibits 7q amplification with 7p deletion, which results in a i(7)(q10)- equivalent genetic situation [235, 236]. Trisomy 8 is another frequently observed genetic abnormality in HTCL. Other, less common, genetic mutations are loss of chromosome 21, Y and deletion of chromosome 11q14, t(7;14)(q34;q13), and deletion of chromosome 2q23;q37 [226, 227].

1.3.2 Enteropathy-Associated T-Cell Lymphoma (EATL)

Clinical and Epidemiologic Features

Enteropathy-Associated T-cell Lymphoma (EATL) is an intestinal T-cell lymphoma that occurs most commonly in the jejunum or ileum and is particularly frequent in Northern Europe, where the prevalence of celiac disease (CD) is high [237–241]. Rare cases present in the duodenum, stomach, or colon [242, 243]. It has seen in approximately 2–3 % of patients with CD [239]. There are two separate subtypes: EATL-1 and EATL-2. EATL-1 is associated with CD and EATL-2 is not. Clinically, EATL presents at a mean age of 57. In 32 % of cases, EATL was diagnosed during an emergency surgery for small bowel obstruction or peritonitis, due to a perforated tumor. The remaining is diagnosed due to pain, weight loss, or fever [240]. Rare cases present with a hemophagocytic syndrome [244, 245]. In 27 % of cases, CD and EATL are diagnosed simultaneously. The interval between the development of CD and EATL is approximately 50 months. The lymphoma is localized (57 %) or diffuse (43 %). Grossly, the typical features include ulcers (29.7 %), or infiltration and induration of the intestinal wall with out without nodules (48.6 %) [240]. Involvement of extra-intestinal sites such as lymph nodes [246], skin [247], and CNS [248] has been described.

Histopathology, Immunophenotypic, and Molecular Features

In EATL-1, there is an infiltration of lymphoma cells with pleomorphic appearance (medium-to-large lymphoid cells), mixed with reactive small lymphocytes, plasma cells, histiocytes, and eosinophils. The mucosa adjacent to the tumor shows enteropathic changes of CD, including an increase in intraepithelial lymphocytes and villous blunting [237, 238, 240, 249]. EATL-2 shows a monomorphous population of large cells with hyperchromatic nuclei and pale cytoplasm (Fig. 6). It often lacks the inflammatory background seen in EATL-1 and frequently is accompanied by necrosis [237, 241, 250]. Some very early cases may not present with a mass and

show an in situ (intraepithelial component) only ('in situ' EATL). A histologic variant of EATL with anaplastic morphology has also been described.

In EATL-1, the neoplastic T cells are positive for CD3, CD7, and CD103, but are typically negative for CD4, CD8, and CD5, and show variable reactivity with CD30 and TCRαβ [237, 239, 240, 251]. They may also co-express cytotoxic markers such as granzyme B, perforin, and/or TIA1. The adjacent intraepithelial lymphocytes may also express abnormal immunophenotype with loss of CD5, CD4, and CD8 expression. In EATL-2, the malignant cells express CD3, CD56, CD8, and TCRαβ. Molecular studies show (in both EATL-1 and EATL-2) 9q31.3-qter amplifications or 16q12.1 deletions [5, 238, 249, 252]. Gains in 1q32.2-q41 and 5q34-q35.2 occur in EATL-1, while amplifications of *MYC* are present in EATL-2 [250, 253, 254]. More recently, Perry et al. [255] have proposed the term indolent T-cell lymphoproliferative disease of the gastrointestinal tract, to define a rare group of lesions with an immunophenotype similar to EATL. This group of patients had no history of enteropathy and had a very indolent clinical course, without the need for chemotherapy.

1.4 Extranodal T-Cell Lymphomas, Cutaneous

1.4.1 Mycosis Fungoides

Clinical and Epidemiologic Features

MF is the most common type of cutaneous T-cell lymphoma, accounting for 50 % of all primary cutaneous lymphomas [99, 256]. Its annual incidence has been estimated to 6 or 7 cases per 1 million persons. MF occurs more commonly in adults, although any age group can be affected. The male-to-female ratio is 2:1 [257]. Classic MF is a disease that progresses slowly over years and sometimes decades, presenting with well defined, often pruritic erythematous patches distributed in non-sun-exposed 'bathing suit' areas, including the breasts, buttocks, lower trunk, and groin. These patches may evolve to infiltrative plaques and tumors, and all 3 lesion types can be seen concomitantly. Hypopigmented lesions are a rare presentation of MF, most often seen in children, adolescents, and dark-skinned individuals [258–261]. Approximately 30 % of patients present with skin tumors or erythroderma at disease onset [101, 262]. Extracutaneous dissemination occurs in advanced stages and may involve lymph nodes [263], liver [264, 265], spleen [266, 267], lung [268], and blood. However, bone marrow involvement is rare [269].

Histopathology, Immunophenotypic, and Molecular Features

MF is an epidermotropic T-cell lymphoproliferative disorder composed of small-to-intermediate atypical lymphocytes with enlarged hyperchromatic, cerebriform nuclei, and clear cytoplasm. The morphology varies with the clinical stage of disease (Fig. 7) [99, 101, 104, 120, 257, 262]. The early patch of skin shows lichenoid atypical lymphoid infiltrate colonizing the basal layer in a singly or linear

fashion, whereas plaque stage shows more prominent epidermotropism. Pautrier microabscesses, consisting of small intraepidermal aggregates of atypical lymphocytes and Langerhans cells, are seen in only 25 % of cases. With progression from patch/plaque stages to tumor stage, more diffuse involvement of papillary dermis and increase in number and size of neoplastic T-cell lymphoid infiltrates occurs. Epidermotropism may be lost. The following features are not typically seen in MF: marked spongiosis, vacuolar change, keratinocyte necrosis, or numerous eosinophils and/or neutrophils. Histologic transformation, defined as the presence of large T cells in more than 25 % of the total lymphoid infiltrate or forming microscopic nodules, is an adverse finding. Some histologic variants are summarized in Table 2 and illustrated in Fig. 7 [99, 265, 270–278].

Immunophenotypically, the neoplastic T cells in MF are usually CD4 mature T cells with expression of CD2, CD3, TCRB, and CD5. Loss of one or more T-cell antigens (CD7, most common) is frequent in all stages [99, 101, 104, 120, 257, 262, 272]. CD8− positive MF has been seen more commonly in pediatric MF [258, 261] and with pagetoid reticulosis [274]. The clinical behavior of CD8 phenotype MF is similar to that of CD4 phenotype. These large cells may be CD30− or CD30+. Prognosis appears to be better in LCT with CD30 expression [279]. MF may coexist with CD30+ T-cell lymphoproliferative disorders such as cutaneous ALCL and lymphomatoid papulosis (LyP) [280, 281]. It is important to distinguish concurrent MF with cutaneous ALCL with transformed MF because of their prognosis and therapeutic implications are different. MF is a clonal disorder of memory T cells. Clonal T-cell gene rearrangement has been seen in variable proportion of cases by molecular studies [282]. However, clonal T cells are frequently found in non-neoplastic and inflammatory skin conditions [283–287], thus diagnosis of MF cannot be made on the basis of molecular study alone and requires clinical–pathological correlation. The diagnosis of early MF can be challenging because not all pathologic findings are present or because of overlapping findings with other reactive dermatoses. At a molecular level, MF lacks CCR7/L-selectin and CD27 but strongly expresses CCR4 and CLA, a phenotype suggestive of skin resident effector memory T cells [288]. This phenotype is different from SS where the T cells show a central memory pattern. Expression with CCR7 also correlates with loss of epidermotropism and subcutaneous extension [289]. aCGH studies have shown some numerical alterations including recurrent loss of 19, 7p22.1-p22.3, 7q11.1-q11.23, 9q34.12, 12q24.31, and 16q22.3-q23.1, and gain of 8q22.3-q23.1 and 21q22.12 [290]. Limited micro-RNA (miRNA) studies have shown a signature of specific miRNA when comparing MF against inflammatory skin disorders [291, 292].

1.4.2 Subcutaneous Panniculitis-like T-Cell Lymphoma (SPTCL)

Clinical and Epidemiologic Features

SPTCL is a primary cutaneous T-cell lymphoma with preferential involvement of the subcutaneous tissue and is now a distinct category in the WHO. The cases, originally included under this category with a TCRγδ phenotype, are now included

Table 2 Histological variants of mycosis fungoides

	Morphology	Histology	Immunophenotype	Prognosis
Mycosis fungoides	Clinical three stages: patches, plaques and tumor stages	Superficial band such as atypical small-to-medium-sized, indented nuclei (cerebriform) lymphoid infiltrate, at dermoepidermal junction. Epidermotropism with little spongiosis and Pautrier microabscesses	CD3+, CD4+, CD8 −, with loss of pan-T-cell antigen (CD2, CD7, CD5)	Depends on clinical stage; type and extent of cutaneous lesion, presence of extracutaneous disease and/or lymph node involvement
Folliculotropic MF	Follicular papules, acneiform lesions and indurated plaques	Atypical lymphocytic infiltrate around and within the follicular epithelium and relative sparing of surface epithelium	CD3+, CD4+, CD8 −, CD30+ (variable blast cells)	Worse prognosis than that of patients with classical plaque stage MF
Pagetoid reticulosis (PR)	Solitary psoriasiform or hyperkeratotic patch or plaque	Hyperplastic epidermis with marked infiltration by atypical pagetoid medium-to-large-sized lymphocytes	CD3+, CD4+, CD8 − or CD3+, CD4−, CD8+	Indolent course with potential to frank malignant behavior
Granulomatous slack skin (GSS)	Circumscribed erythematous masses of lax skin (body folds)	Dense granulomatous dermal infiltrate containing atypical T cells, macrophages and multinucleated giant cells	CD3+, CD4+, CD8 −	Indolent clinical course

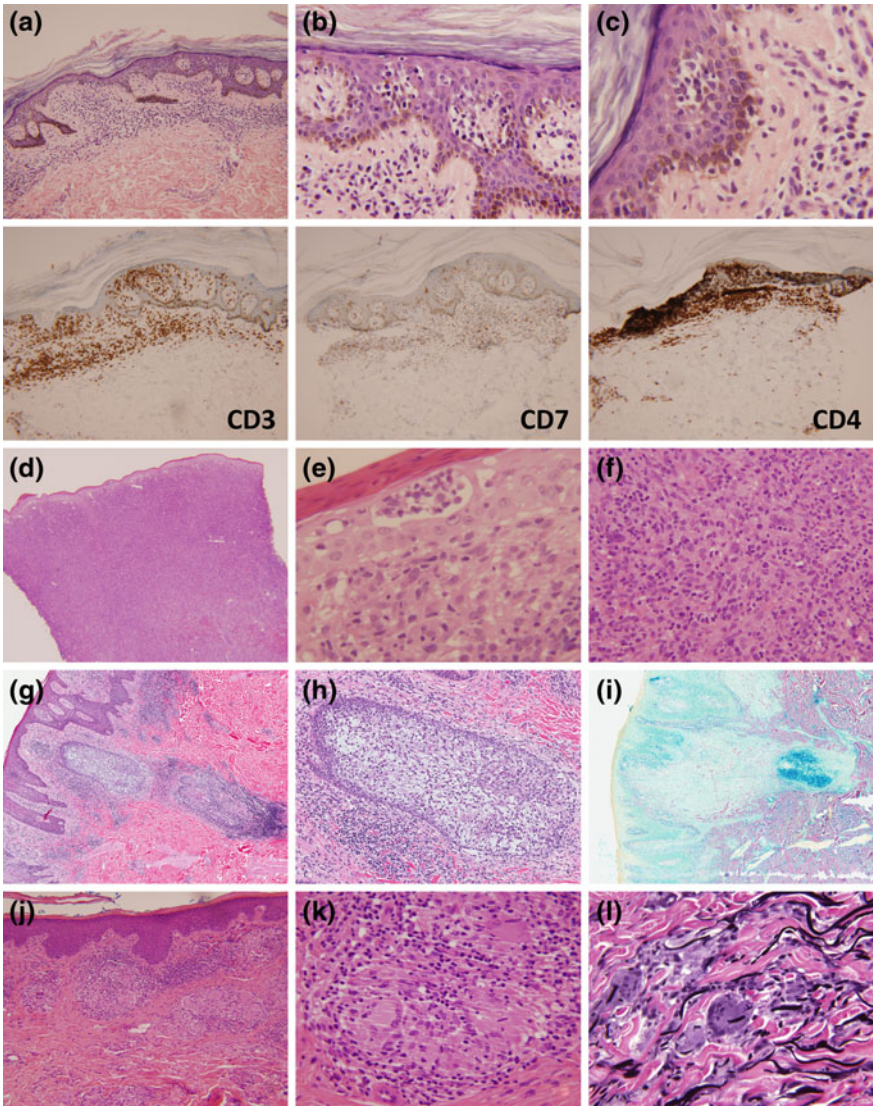


Fig. 7 MF and variants. **a**–**c** Classic variant with epidermotropism, and tagging of lymphocytes along the dermal–epidermal junction. The abnormal T cells are CD3+ , have loss of CD7, and have a CD4+ phenotype. **d**–**f**, Large cell transformation. This is defined by >25 % large cells. The presence of Pautrier microabscesses is seen in **e**. **g** and **h**, folliculotropic variant with associated follicular mucinosis (**i**, colloidal iron). **j**– **k**, Granulomatous variant (granulomatous slack skin) with elastophagocytosis (**l**, elastic stain)

under the provisional category of primary cutaneous γδ-T-cell lymphoma [101, 163, 165, 293–296]. It is more common in younger individuals, and 20 % of cases occur in individuals of <20 years of age [297]. The clinical presentation includes skin

nodules in the extremities and the trunk, but some cases can present in the head and neck region. Ulceration is usually rare. Extracutaneous involvement is also uncommon [298]. Those cases with hemophagocytosis (17 %) have a worse prognosis [294, 296, 299].

Histopathology, Immunophenotypic, and Molecular Features

There is a dense lymphoid infiltrate of small-, medium-, and large-sized lymphocytes, preferentially in the subcutaneous tissue, within the fat lobule. Extension in the dermis is usually seen. The epidermis must be spared. The atypical lymphocytes are hyperchromatic, have angulated nuclei, and clear cytoplasm. Admixed inflammatory cells, such as histiocytes, plasma cells, and neutrophils, can be seen [295, 296]. The classic pattern of infiltration shows rimming of the adipocytes by the atypical lymphoid cells (Fig. 8). The immunophenotype shows that the abnormal T cells are CD3+, CD4−, CD8+, and CD56−. In some cases, the T cells are CD4−/CD8−. Invariably they express TCRβ. The tumor cells express the cytotoxic markers TIA-1, perforin and granzyme. Cases with overlap features with lupus panniculitis have been described [300–302]. In those, CD123 has been proposed to be useful (positive in plasmacytoid dendritic cells in lupus) [303]. At a molecular level, CGH revealed large numbers of DNA copy number changes, the most common of which were losses of chromosomes 1pter, 2pter, 10qter, 11qter, 12qter, 16, 19, 20, and 22 and gains of chromosomes 2q and 4q [294, 304].

1.4.3 Cutaneous Anaplastic Large Cell Lymphoma (C-ALCL)

Clinical and Epidemiologic Features

C-ALCL usually has an indolent clinical course and overlaps with the clinicopathologic features with LyP. Most patients are adults, and usually 50–60 years of age [305, 306]. It is rare in children, but occasionally reported [307]. Clinically presents as a solitary tumor or nodule, often ulcerated and located on the face, extremities, or less frequently the trunk. Multifocality is seen in 20 % of cases. Spontaneous regression occurs in 25 % of cases [308, 309]. Mucosal CD30 + lymphoid proliferations with significant overlap features to C-ALCL have been reported recently [310].

Histopathology, Immunophenotypic, and Molecular Features

The histology of the lesion includes cohesive sheets of large anaplastic cells, with indented and irregular nuclei, and abundant cytoplasm, similar to other systemic ALCLs. In 20 % of cases, a variant morphologic appearance is seen (a rare small cell variant has been reported). The infiltrate extends from the dermis into the subcutis [280, 308, 311, 312]. Admixed acute inflammatory cells can be very prominent (giving rise to pyogenic- and eosinophilic-rich cell variants). In 25–30 % of cases, there is pseudoepitheliomatous hyperplasia of the skin. The pathologic overlap between C-CLCL, LyP and large cell transformation in MF heralds the absolute need of a good clinical history to distinguish between the different lesions

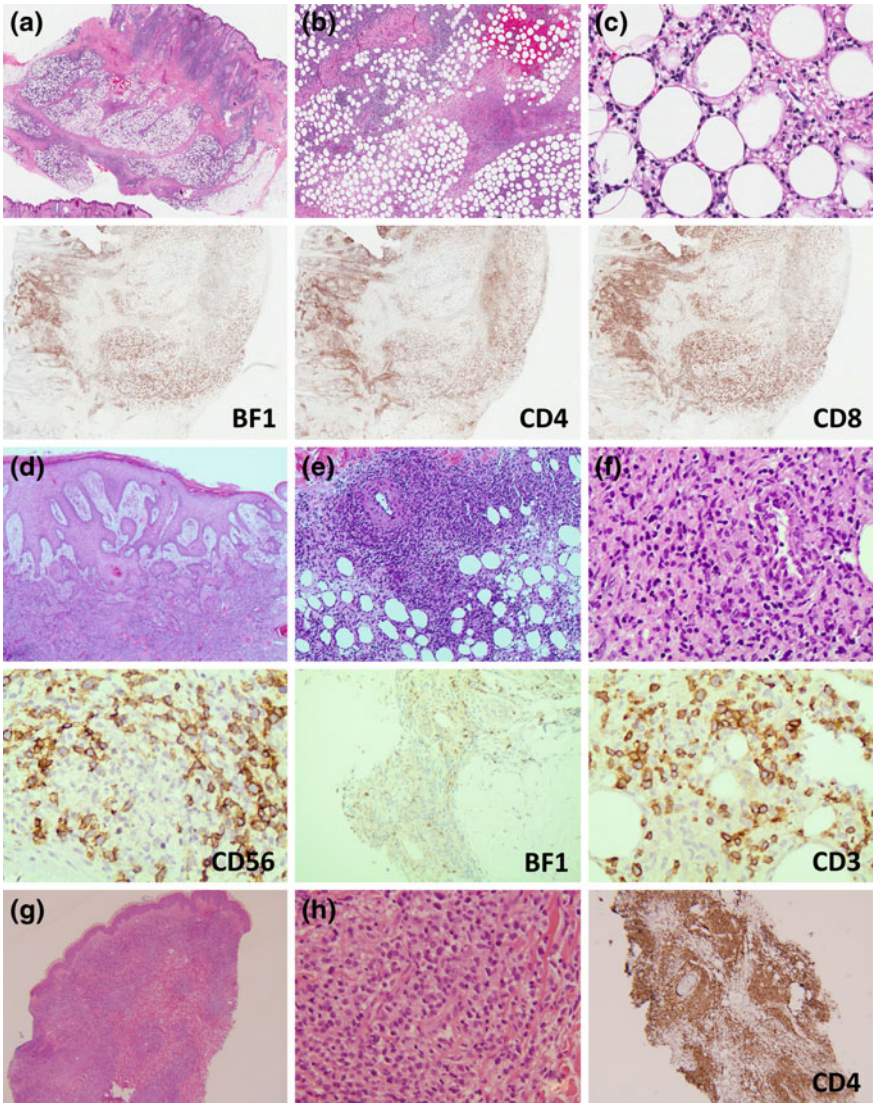


Fig. 8 Cutaneous T-cell lymphomas, rare forms. **a–c** SPTCL. There is a classic lobular panniculitis with necrosis and rimming of adipocytes by the abnormal cells. BF1 is positive indicating a TCR-αβ receptor. The CD4:CD8 ratio is inverted as the tumor cells are CD8 positive. **d–f** PCGDTCL. Pseudoepitheliomatous hyperplasia is frequently observed (**d**). The tumor also infiltrates the fat, and angioinvasion is frequent (**f**). The tumor cells are CD56+ and CD3+. BF1 is predominantly negative, indicating a γδ-receptor. **g–h**, SMPTCL. There is sparing of the epidermis and a CD4 phenotype

[313]. Some cases can have a marked predominant intravascular distribution [314–316]. All cases show diffuse and strong CD30 expression, variable loss of pan-T-cell antigens and some expression of cytotoxic markers. EMA is expressed in 20–30 % of cases. ALK expression is associated with systemic disease, but rare cases of C-ALK+ ALCL have been published [135]. Variable expression of clusterin and CD56 (12–75 %) is seen. Like LyP, MUM1 is frequently positive.

Loss of TGF-β–induced lymphocyte growth inhibition has been demonstrated in C-ALCL, due to a dominant negative mutation of the TGF-β type II receptor or deletion of the initiating sequence for translation of the type I receptor transcripts; this observation suggests that altered TGF-β/SMAD signaling may play a role in the progression of LyP to ALCL. Recurrent translocations involving multiple myeloma oncogene 1/interferon regulatory factor 4 (*MUM1/IRF4*) (located on 6p25) have been detected by FISH in C-ALCL (57 % of cases tested), rare PTCL, NOS (5 %), and systemic ALK2 ALCL (4 %) [152, 153, 317]. Amplification of *JUNB* (19p13) is reported in 70 % of C- ALCLs, and JUNB protein expression is present in virtually all cases, which might promote tumor cell survival via its upregulation of CD30 and thereby NF-kB activation [114, 115]. Numerous other alterations have been described on aCGH. Differences in miRNA have been shown to distinguish it from MF [318]. CALCL exhibits gain of 7q31 and loss on 6q16-6q21 and 13q34, each affecting 45 % of the patients, and has a distinct signature that distinguishes it from MF and SS [122, 319].

2 Concluding Remarks

Our understanding of the diversity of PTCLs has evolved substantially over the past 20 years and is reflected in the evolution of classification schemes. Numerous categories in the WHO still remain at a provisional stage, and some include very complex and heterogeneous disorders, for which we expect subclassifications to arise. The accuracy in the diagnosis of PTCL among different experts has been previously studied: The International T-cell Lymphoma Project showed an overall rate of 81 %. The reproducibility for the diagnosis of ALK+ ALCL was 91 %, but was much lower for PTCL, NOS (81 %) and AITL (75 %) [145]. More recently, the accuracy has increased to 92 % on a study from Hsi et al. [184]. Gene expression profiling (GEP) has been performed to improve the diagnosis of PTCL and to better understand its pathobiology. To this respect, a study by Iqbal et al. [197] showed that by using this methodology 14 % of cases previously diagnosed as PCTL were reclassified as AITL. In the same study, 11 % of PTCL were reclassified as ALK− ALCL. Interestingly, those cases that were categorized as ALK− ALCL had a worse prognosis compared to PTCL, NOS. A study by Piccaluga et al. [320] has also shown that GEP improves classifications of AITL and ALCL: In their study, the traditional morphologic and immunophenotypic approach failed to provide significance in survival between ALCL and PTCL, NOS but, with the use of GEP, a statistical significance in survival was achieved. Two genetic subgroups emerged in

the PTCL, NOS groups: a *GATA3* high subgroup with enrichment of proliferation signatures (*MYC,* mTOR, β-catenin) and a *TBX21* subgroup, with activation of pathways linked to IFN-γ and NFkB, and better prognosis. Ultimately, not only will molecular classification improve diagnostic accuracy, but it may lead to the development of a targeted therapy approach with drugs directed to specific pathways: For example, AITL may benefit from FDA-approved drugs that target the NF-κβ pathway including bortezomib or carfilzomib or specific inhibitors against *IDH2* mutation currently in development. ALK− ALCL patients may benefit from drugs that target mitotic cells (e.g., aurora kinase inhibitors) in combination with drugs that target the PI3-kinase/AKT pathway. Similarly, a subset of patients in the GATA3 subgroup may benefit from drugs that target the mTOR (e.g., rapamycin, temsirolimus) pathway [182]. It is more than likely that the next generation of classifications will incorporate these types of approaches for diagnostic purposes and will allow for a better understanding of the pathobiology of these diseases.

References

1. Jaffe ES (2008) Introduction and overview of the classification of lymphoid neoplasms, 4th edn. International Agency for Research on Cancer, Lyon
2. Jaffe ES, Nicolae A, Pittaluga S (2013) Peripheral T-cell and NK-cell lymphomas in the WHO classification: pearls and pitfalls. Mod Pathol 26(1):S71–S87 (an official journal of the United States and Canadian Academy of Pathology, Inc.)
3. William BM, Armitage JO (2013) International analysis of the frequency and outcomes of NK/T-cell lymphomas. Best Practice Res. Clin Haematol 26(1):23–32
4. Hu S, Young KH, Konoplev SN, Medeiros LJ (2012) Follicular T-cell lymphoma: a member of an emerging family of follicular helper T-cell derived T-cell lymphomas. Hum Pathol 43 (11):1789–1798
5. Bajor-Dattilo EB, Pittaluga S, Jaffe ES (2013) Pathobiology of T-cell and NK-cell lymphomas. Best Practice Res. Clin Haematol 26(1):75–87
6. Gaulard P, de Leval L (2013) VIII. New markers in peripheral T-cell lymphomas: more entities or more confusion? Hematol Oncol 31(1):51–56
7. Gaulard P, de Leval L (2014) Pathology of Peripheral T-Cell Lymphomas: where do we stand? Semin Hematol 51(1):5–16
8. Shustov A (2013) Controversies in autologous and allogeneic hematopoietic cell transplantation in peripheral T/NK-cell lymphomas. Best Practice Res Clin Haematol 26 (1):89–99
9. Miranda RN, Aladily TN, Prince HM et al (2014) Breast implant-associated anaplastic large-cell lymphoma: long-term follow-up of 60 patients. J Clin Oncol 32(2):114–120 (official journal of the American Society of Clinical Oncology)
10. Taylor CR, Siddiqi IN, Brody GS (2013) Anaplastic large cell lymphoma occurring in association with breast implants: review of pathologic and immunohistochemical features in 103 cases. Appl Immunohistochem Mol Morphol (AIMM) 21(1):13–20 (official publication of the Society for Applied Immunohistochemistry)
11. Jaffe ES, Chaganti RSK, Nanjangud G (2011) Memorial Sloan-Kettering Cancer Center (MSKCC). In: Hematopathology, 1st edn. Saunders/Elsevier, Philadelphia
12. Chen X, Cherian S (2013) Immunophenotypic characterization of T-cell prolymphocytic leukemia. Am J Clin Pathol 140(5):727–735
13. Dearden CE (2006) T-cell prolymphocytic leukemia. Med Oncol 23(1):17–22

14. Dearden CE (2009) T-cell prolymphocytic leukemia. Clin Lymphoma Myeloma 9(3): S239–S243
15. Dungarwalla M, Matutes E, Dearden CE (2008) Prolymphocytic leukaemia of B-and T-cell subtype: a state-of-the-art paper. Eur J Haematol 80(6):469–476
16. Khot A, Dearden C (2009) T-cell prolymphocytic leukemia. Expert Rev Anticancer Ther 9 (3):365–371
17. Kaminska EC, Yu Z, Kress J, Petronic-Rosic V, Soltani K (2012) Erythematous eruption with marked conjunctival injection–quiz case. Diagnosis: leukemia cutis with conjunctival involvement in the setting of T-cell prolymphocytic leukemia (T-PLL). Arch Dermatol 148 (10):1199
18. Ventre MO, Bacelieri RE, Lazarchick J, Pollack RB, Metcalf JS (2013) Cutaneous presentation of T-cell prolymphocytic leukemia. Cutis 91(2):87–91
19. Kojima K, Hara M, Sawada T et al (2000) Human T-lymphotropic virus type I provirus and T-cell prolymphocytic leukemia. Leuk Lymphoma 38(3–4):381–386
20. Kameoka J, Takahashi N, Noji H et al (2012) T-cell prolymphocytic leukemia in Japan: is it a variant? Int J Hematol 95(6):660–667
21. Jevremovic D, Morice WG (2014) Leukemias of mature T-cells and natural killer cells, 3rd edn.
22. Catovsky D, Muller-Hermelink HK, Ralfkiaer E (2008) T-cell prolymphocytic leukaemia, 4th edn. International Agency for Research on Cancer, Lyon
23. Herling M, Valbuena JR, Jones D, Medeiros LJ (2007) Skin involvement in T-cell prolymphocytic leukemia. J Am Acad Dermatol 57(3):533–534
24. Tse E, So CC, Cheung WW, Kwong YL (2011) T-cell prolymphocytic leukaemia: spontaneous immunophenotypical switch from CD4 to CD8 expression. Ann Hematol 90 (4):479–481
25. Dearden C (2006) The role of alemtuzumab in the management of T-cell malignancies. Semin Oncol 33(2 Suppl 5):S44–S52
26. Dearden C (2012) B- and T-cell prolymphocytic leukemia: antibody approaches. Hematology 2012:645–651 (the Education Program of the American Society of Hematology. American Society of Hematology. Education Program)
27. Dearden C (2012) How I treat prolymphocytic leukemia. Blood 120(3):538–551
28. Lu L, Peters J, Roome C, Stein K (2012) Cost-effectiveness of alemtuzumab for T-cell prolymphocytic leukemia. Int J Technol Assess Health Care 28(3):241–248
29. Boultwood J (2001) Ataxia telangiectasia gene mutations in leukaemia and lymphoma. J Clin Pathol 54(7):512–516
30. Bellanger D, Jacquemin V, Chopin M et al (2014) Recurrent JAK1 and JAK3 somatic mutations in T-cell prolymphocytic leukemia. Leukemia 28(2):417–419
31. Bergmann AK, Schneppenheim S, Seifert M et al (2014) Recurrent mutation of JAK3 in T-cell prolymphocytic leukemia. Genes Chromosom Cancer 53(4):309–316
32. Urbankova H, Holzerova M, Balcarkova J et al (2010) Array comparative genomic hybridization in the detection of chromosomal abnormalities in T-cell prolymphocytic leukemia. Cancer Genet Cytogenet 202(1):58–62
33. CHan WC, Foucar K, Morice WG, Catovsky D (2008) T-cell large granular lymphocytic leukaemia, 4th ed. International Agency for Research on Cancer, Lyon
34. Attygalle AD, Cabecadas J, Gaulard P et al (2014) Peripheral T-cell and NK-cell lymphomas and their mimics; taking a step forward—report on the lymphoma workshop of the XVIth meeting of the European Association for haematopathology and the society for hematopathology. Histopathology 64(2):171–199
35. Watters RJ, Liu X, Loughran TP Jr (2011) T-cell and natural killer-cell large granular lymphocyte leukemia neoplasias. Leuk Lymphoma 52(12):2217–2225
36. Kwong YL, Wong KF, Chan LC et al (1994) The spectrum of chronic lymphoproliferative disorders in Chinese people. An analysis of 64 cases. Cancer 74(1):174–181
37. Lamy T, Loughran TP Jr (2011) How I treat LGL leukemia. Blood 117(10):2764–2774

38. Lamy T, Loughran TP Jr (2003) Clinical features of large granular lymphocyte leukemia. Semin Hematol 40(3):185–195
39. Liu X, Loughran TP Jr (2011) The spectrum of large granular lymphocyte leukemia and Felty's syndrome. Curr Opin Hematol 18(4):254–259
40. Bockorny B, Codreanu I, Dasanu CA (2013) Prevalence of autoimmune hematologic and non-hematologic conditions in large granular lymphocytic leukemia: exploratory analysis of a series of consecutive patients. Leuk Lymphoma
41. Ishida F, Matsuda K, Sekiguchi N et al (2014) STAT3 gene mutations and their association with pure red cell aplasia in large granular lymphocyte leukemia. Cancer Sci 105(3):342–346
42. Jerez A, Clemente MJ, Makishima H et al (2012) STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. Blood 120(15):3048–3057
43. Koskela HL, Eldfors S, Ellonen P et al (2012) Somatic STAT3 mutations in large granular lymphocytic leukemia. New Engl J Med 366(20):1905–1913
44. Kristensen T, Larsen M, Rewes A, Frederiksen H, Thomassen M, Moller MB (2014) Clinical relevance of sensitive and quantitative STAT3 mutation analysis using next-generation sequencing in T-cell large granular lymphocytic leukemia. J Mol Diagn (JMD)
45. Schultz KR (2013) STAT3 mutations and persistence of autoimmunity. Blood 122 (14):2295–2296
46. Zhang D, Loughran TP Jr (2012) Large granular lymphocytic leukemia: molecular pathogenesis, clinical manifestations, and treatment. Hematology 2012:652–659 (the Education Program of the American Society of Hematology. American Society of Hematology. Education Program)
47. Gill H, Ip AH, Leung R et al (2012) Indolent T-cell large granular lymphocyte leukaemia after haematopoietic SCT: a clinicopathologic and molecular analysis. Bone Marrow Transplant 47(7):952–956
48. Huh YO, Medeiros LJ, Ravandi F, Konoplev S, Jorgensen JL, Miranda RN (2009) T-cell large granular lymphocyte leukemia associated with myelodysplastic syndrome: a clinicopathologic study of nine cases. Am J Clin Pathol 131(3):347–356
49. Loughran TP Jr (1993) Clonal diseases of large granular lymphocytes. Blood 82(1):1–14
50. Semenzato G, Zambello R, Starkebaum G, Oshimi K, Loughran TP Jr (1997) The lymphoproliferative disease of granular lymphocytes: updated criteria for diagnosis. Blood 89(1):256–260
51. Chen X, Bai F, Sokol L et al (2009) A critical role for DAP10 and DAP12 in CD8+T cell-mediated tissue damage in large granular lymphocyte leukemia. Blood 113(14):3226–3234
52. Go RS, Lust JA, Phyliky RL (2003) Aplastic anemia and pure red cell aplasia associated with large granular lymphocyte leukemia. Semin Hematol 40(3):196–200
53. Feldman AL, Dogan A (2014) Peripheral T-cell Lymphomas, 3rd edn.
54. Osuji N, Beiske K, Randen U et al (2007) Characteristic appearances of the bone marrow in T-cell large granular lymphocyte leukaemia. Histopathology 50(5):547–554
55. Osuji N, Matutes E, Catovsky D, Lampert I, Wotherspoon A (2005) Histopathology of the spleen in T-cell large granular lymphocyte leukemia and T-cell prolymphocytic leukemia: a comparative review. Am J Surg Pathol 29(7):935–941
56. Olteanu H, Karandikar NJ, Eshoa C, Kroft SH (2010) Laboratory findings in CD4(+) large granular lymphocytoses. Int J Lab Hematol 32(1 Pt 1):e9–e16
57. Chen YH, Chadburn A, Evens AM et al (2011) Clinical, morphologic, immunophenotypic, and molecular cytogenetic assessment of CD4−/CD8− gammadelta T-cell large granular lymphocytic leukemia. Am J Clin Pathol 136(2):289–299
58. Evans HL, Burks E, Viswanatha D, Larson RS (2000) Utility of immunohistochemistry in bone marrow evaluation of T-lineage large granular lymphocyte leukemia. Hum Pathol 31 (10):1266–1273

59. Morice WG, Kurtin PJ, Leibson PJ, Tefferi A, Hanson CA (2003) Demonstration of aberrant T-cell and natural killer-cell antigen expression in all cases of granular lymphocytic leukaemia. Br J Haematol 120(6):1026–1036
60. Lundell R, Hartung L, Hill S, Perkins SL, Bahler DW (2005) T-cell large granular lymphocyte leukemias have multiple phenotypic abnormalities involving pan-T-cell antigens and receptors for MHC molecules. Am J Clin Pathol 124(6):937–946
61. Freud AG, Zhao S, Wei S et al (2013) Expression of the activating receptor, NKp46 (CD335), in human natural killer and T-cell neoplasia. Am J Clin Pathol 140(6):853–866
62. Man C, Au WY, Pang A, Kwong YL (2002) Deletion 6q as a recurrent chromosomal aberration in T-cell large granular lymphocyte leukemia. Cancer Genet Cytogenet 139 (1):71–74
63. Wlodarski MW, O'Keefe C, Howe EC et al (2005) Pathologic clonal cytotoxic T-cell responses: nonrandom nature of the T-cell-receptor restriction in large granular lymphocyte leukemia. Blood 106(8):2769–2780
64. Ohshima K, Jaffe ES, Kikuchi M (2008) Adult T-cell leukaemia/lymphoma, 4th edn. International Agency for Research on Cancer, Lyon
65. Hsi AC, Kreisel FH, Frater JL, Nguyen TT (2014) Clinicopathologic features of adult T-cell leukemias/lymphomas at a North American tertiary care medical center: infrequent involvement of the central nervous system. Am J Surg Pathol 38(2):245–256
66. Yoshida M (2005) Discovery of HTLV-1, the first human retrovirus, its unique regulatory mechanisms, and insights into pathogenesis. Oncogene 24(39):5931–5937
67. Tajima K, Ito S (1992) Epidemiology of HTLV-I. Uirusu 42(1):49–57
68. Qayyum S, Choi JK (2014) Adult T-cell leukemia/lymphoma. Arch Pathol Lab Med 138 (2):282–286
69. Setoyama M, Katahira Y, Kanzaki T (1999) Clinicopathologic analysis of 124 cases of adult T-cell leukemia/lymphoma with cutaneous manifestations: the smouldering type with skin manifestations has a poorer prognosis than previously thought. J Dermatol 26(12):785–790
70. Tokura Y, Sawada Y, Shimauchi T (2014) Skin manifestations of adult T-cell leukemia/ lymphoma: clinical, cytological and immunological features. J Dermatol 41(1):19–25
71. Blayney DW, Blattner WA, Robert-Guroff M et al (1983) The human T-cell leukemia-lymphoma virus in the southeastern United States. J Am Med Assoc (JAMA) 250 (8):1048–1052
72. Blayney DW, Jaffe ES, Fisher RI et al (1983) The human T-cell leukemia/lymphoma virus, lymphoma, lytic bone lesions, and hypercalcemia. Ann Intern Med 98(2):144–151
73. Shimoyama M (1991) Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma. A report from the Lymphoma study group (1984–1987). Br J Haematol 79(3):428–437
74. Jaffe ES, Blattner WA, Blayney DW et al (1984) The pathologic spectrum of adult T-cell leukemia/lymphoma in the United States. Human T-cell leukemia/lymphoma virus-associated lymphoid malignancies. Am J Surg Pathol 8(4):263–275
75. Ohshima K (2007) Pathological features of diseases associated with human T-cell leukemia virus type I. Cancer Sci 98(6):772–778
76. Ohshima K, Nakamura N (2014) Adult T-cell leukemia/lymphoma, 3rd edn.
77. Karube K, Suzumiya J, Okamoto M et al (2007) Adult T-cell lymphoma/leukemia with angioimmunoblastic T-cell lymphomalike features: Report of 11 cases. Am J Surg Pathol 31 (2):216–223
78. Duggan DB, Ehrlich GD, Davey FP et al (1988) HTLV-I-induced lymphoma mimicking Hodgkin's disease. Diagnosis by polymerase chain reaction amplification of specific HTLV-I sequences in tumor DNA. Blood 71(4):1027–1032
79. Ohshima K, Suzumiya J, Kato A, Tashiro K, Kikuchi M (1997) Clonal HTLV-I-infected CD4+ T-lymphocytes and non-clonal non-HTLV-I-infected giant cells in incipient ATLL with Hodgkin-like histologic features. Int J Cancer 72(4):592–598

80. Sugaya M, Hamada T, Kawai K, et al (2013) Guidelines for the management of cutaneous lymphomas (2011): a consensus statement by the Japanese Skin Cancer Society—lymphoma study group. J Dermatol 40(1):2–14
81. Ballester LY, Cowen EW, Richard Lee CC (2014) Adult T-cell leukemia-lymphoma associated with follicular mucinosis. Am J Dermatopathol
82. Mouzakis J, Black W, Messina J, Cherpelis B (2011) Vesiculobullous variant of adult T-cell leukemia/lymphoma in a Caribbean Emigre. J Drugs Dermatol (JDD) 10(12):1469–1471
83. Grubb B, Henderson DB, Pandya AG (2011) Adult T-cell lymphoma/leukemia presenting as pagetoid reticulosis of the palms and soles. J Am Acad Dermatol 65(5):1063–1064
84. Takeshita M, Akamatsu M, Ohshima K et al (1995) CD30 (Ki-1) expression in adult T-cell leukaemia/lymphoma is associated with distinctive immunohistological and clinical characteristics. Histopathology 26(6):539–546
85. Roncador G, Brown PJ, Maestre L et al (2005) Analysis of FOXP3 protein expression in human CD4+ CD25+ regulatory T cells at the single-cell level. Eur J Immunol 35 (6):1681–1691
86. Roncador G, Garcia JF, Garcia JF et al (2005) FOXP3, a selective marker for a subset of adult T-cell leukaemia/lymphoma. Leukemia 19(12):2247–2253
87. Ishida T (2013) Leukemia: recent progress in diagnosis and treatment. Topics: IV. Recent topics; 1. Novel antibody therapy targeting CCR4 for adult T-cell leukemia/lymphoma. Nihon Naika Gakkai zasshi 102(7):1744–1750 (The Journal of the Japanese Society of Internal Medicine)
88. Ishida T, Ueda R (2011) Antibody therapy for Adult T-cell leukemia-lymphoma. Int J Hematol 94(5):443–452
89. Yano H, Ishida T, Inagaki A et al (2009) Anti-CCR4 monoclonal antibody as a novel therapy for advanced mycosis fungoides and Sezary syndrome. Rinsho ketsueki 50(12):1671–1678 (The Japanese journal of clinical hematology)
90. Grassmann R, Aboud M, Jeang KT (2005) Molecular mechanisms of cellular transformation by HTLV-1 Tax. Oncogene 24(39):5976–5985
91. Satou Y, Matsuoka M (2012) Molecular and cellular mechanism of leukemogenesis of ATL: emergent evidence of a significant role for HBZ in HTLV-1-induced pathogenesis. Leuk Res Treat 2012:213653
92. Satou Y, Yasunaga J, Yoshida M, Matsuoka M (2006) HTLV-I basic leucine zipper factor gene mRNA supports proliferation of adult T cell leukemia cells. Proc Natl Acad Sci USA 103(3):720–725
93. Satou Y, Yasunaga J, Zhao T et al (2011) HTLV-1 bZIP factor induces T-cell lymphoma and systemic inflammation in vivo. PLoS Pathog 7(2):e1001274
94. Shimizu-Kohno K, Satou Y, Arakawa F et al (2011) Detection of HTLV-1 by means of HBZ gene in situ hybridization in formalin-fixed and paraffin-embedded tissues. Cancer Sci 102 (7):1432–1436
95. Sugata K, Satou Y, Yasunaga J et al (2012) HTLV-1 bZIP factor impairs cell-mediated immunity by suppressing production of Th1 cytokines. Blood 119(2):434–444
96. Zhao T, Satou Y, Sugata K et al (2011) HTLV-1 bZIP factor enhances TGF-beta signaling through p300 coactivator. Blood 118(7):1865–1876
97. Oshiro A, Tagawa H, Ohshima K et al (2006) Identification of subtype-specific genomic alterations in aggressive adult T-cell leukemia/lymphoma. Blood 107(11):4500–4507
98. Pise-Masison CA, Radonovich M, Dohoney K et al (2009) Gene expression profiling of ATL patients: compilation of disease-related genes and evidence for TCF4 involvement in BIRC5 gene expression and cell viability. Blood 113(17):4016–4026
99. Song SX, Willemze R, Swerdlow SH, Kinney MC, Said JW (2013) Mycosis fungoides: report of the 2011 Society for Hematopathology/European association for haematopathology workshop. Am J Clin Pathol 139(4):466–490
100. Ralfkiaer E, Willemze R, Whittaker SJ (2008) Sezary syndrome, 4th edn. International Agency for Research on Cancer, Lyon

101. Jawed SI, Myskowski PL, Horwitz S, Moskowitz A, Querfeld C (2014) Primary cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome): part II. Prognosis, management, and future directions. J Am Acad Dermatol. 70(2):223 e221–217 (quiz 240-222)
102. Vonderheid EC, Bernengo MG, Burg G et al (2002) Update on erythrodermic cutaneous T-cell lymphoma: report of the International Society for Cutaneous Lymphomas. J Am Acad Dermatol 46(1):95–106
103. Kohler S, Kim YH, Smoller BR (1997) Histologic criteria for the diagnosis of erythrodermic mycosis fungoides and Sezary syndrome: a critical reappraisal. J Cutan Pathol 24 (5):292–297
104. Willemze R, Jaffe ES, Burg G et al (2005) WHO-EORTC classification for cutaneous lymphomas. Blood 105(10):3768–3785
105. Wu R, Zippin JH, Magro C (2014) Double-positive CD4(+) CD8(+) Sezary syndrome: an unusual phenotype with an aggressive clinical course. Cutis 93(2):E18–E25
106. Bernengo MG, Novelli M, Quaglino P et al (2001) The relevance of the CD4+ CD26-subset in the identification of circulating Sezary cells. Br J Dermatol 144(1):125–135
107. Narducci MG, Scala E, Bresin A et al (2006) Skin homing of Sezary cells involves SDF-1-CXCR4 signaling and down-regulation of CD26/dipeptidylpeptidase IV. Blood 107 (3):1108–1115
108. Harmon CB, Witzig TE, Katzmann JA, Pittelkow MR (1996) Detection of circulating T cells with CD4+ CD7-immunophenotype in patients with benign and malignant lymphoproliferative dermatoses. J Am Acad Dermatol 35(3 Pt 1):404–410
109. Jones D, Dang NH, Duvic M, Washington LT, Huh YO (2001) Absence of CD26 expression is a useful marker for diagnosis of T-cell lymphoma in peripheral blood. Am J Clin Pathol 115(6):885–892
110. Caudron A, Marie-Cardine A, Bensussan A, Bagot M (2012) New developments in Sezary syndrome. Ann Dermatol Venereol 139(1):31–40
111. Chong BF, Wilson AJ, Gibson HM et al (2008) Immune function abnormalities in peripheral blood mononuclear cell cytokine expression differentiates stages of cutaneous T-cell lymphoma/mycosis fungoides. Clin Cancer Res 14(3):646–653 (an official journal of the American Association for Cancer Research)
112. Clark RA, Watanabe R, Teague JE et al (2012) Skin effector memory T cells do not recirculate and provide immune protection in alemtuzumab-treated CTCL patients. Sci Transl Med 4(117):117ra117
113. Campbell JJ, Murphy KE, Kunkel EJ et al (2001) CCR7 expression and memory T cell diversity in humans. J Immunol 166(2):877–884
114. Mao X, Orchard G, Lillington DM et al (2004) BCL2 and JUNB abnormalities in primary cutaneous lymphomas. Br J Dermatol 151(3):546–556
115. Mao X, Orchard G, Lillington DM, Russell-Jones R, Young BD, Whittaker SJ (2003) Amplification and overexpression of JUNB is associated with primary cutaneous T-cell lymphomas. Blood 101(4):1513–1519
116. Mao X, Orchard G, Mitchell TJ et al (2008) A genomic and expression study of AP-1 in primary cutaneous T-cell lymphoma: evidence for dysregulated expression of JUNB and JUND in MF and SS. J Cutan Pathol 35(10):899–910
117. Gibson HM, Mishra A, Chan DV, Hake TS, Porcu P, Wong HK (2013) Impaired proteasome function activates GATA3 in T cells and upregulates CTLA-4: relevance for Sezary syndrome. J Invest Dermatol 133(1):249–257
118. Olsen EA, Rook AH, Zic J et al (2011) Sezary syndrome: immunopathogenesis, literature review of therapeutic options, and recommendations for therapy by the United States Cutaneous Lymphoma Consortium (USCLC). J Am Acad Dermatol 64(2):352–404
119. Olsen EA, Whittaker S, Kim YH et al (2011) Clinical end points and response criteria in mycosis fungoides and sezary syndrome: a consensus statement of the International Society for Cutaneous Lymphomas, the United States cutaneous lymphoma consortium, and the cutaneous lymphoma task force of the European Organisation for Research and Treatment of

Cancer. J Clin Oncol 29(18):2598–2607 (official journal of the American Society of Clinical Oncology)
120. Espinet B, Salgado R (2013) Mycosis fungoides and Sezary syndrome. Methods Mol Biol 973:175–188
121. Salgado R, Servitje O, Gallardo F et al (2010) Oligonucleotide array-CGH identifies genomic subgroups and prognostic markers for tumor stage mycosis fungoides. J Invest Dermatol 130 (4):1126–1135
122. Laharanne E, Oumouhou N, Bonnet F et al (2010) Genome-wide analysis of cutaneous T-cell lymphomas identifies three clinically relevant classes. J Invest Dermatol 130(6):1707–1718
123. Cetinozman F, Jansen PM, Willemze R (2012) Expression of programmed death-1 in primary cutaneous CD4−positive small/medium-sized pleomorphic T-cell lymphoma, cutaneous pseudo-T-cell lymphoma, and other types of cutaneous T-cell lymphoma. Am J Surg Pathol 36(1):109–116
124. Benharroch D, Meguerian-Bedoyan Z, Lamant L et al (1998) ALK-positive lymphoma: a single disease with a broad spectrum of morphology. Blood 91(6):2076–2084
125. Falini B, Pileri S, Zinzani PL et al (1999) ALK+ lymphoma: clinico-pathological findings and outcome. Blood 93(8):2697–2706
126. Ferreri AJ, Govi S, Pileri SA, Savage KJ (2012) Anaplastic large cell lymphoma. ALK-positive. Crit Rev Oncol/Hematol 83(2):293–302
127. Delsol G, Falini B, Muller-Hermelink HK et al (2008) Anaplastic large cell lymphoma (ALCL), ALK-positive, 4th edn. International Agency for Research on Cancer, Lyon
128. Bayle C, Charpentier A, Duchayne E et al (1999) Leukaemic presentation of small cell variant anaplastic large cell lymphoma: report of four cases. Br J Haematol 104(4):680–688
129. Fraga M, Brousset P, Schlaifer D et al (1995) Bone marrow involvement in anaplastic large cell lymphoma. Immunohistochemical detection of minimal disease and its prognostic significance. Am J Clin Pathol 103(1):82–89
130. Pileri S, Falini B, Delsol G et al (1990) Lymphohistiocytic T-cell lymphoma (anaplastic large cell lymphoma CD30+/Ki-1+ with a high content of reactive histiocytes). Histopathology 16 (4):383–391
131. Pileri S, Sabattini E, Poggi S, Amini M, Falini B, Stein H (1994) Lymphohistiocytic T-cell lymphoma. Histopathology 25(2):191–193
132. Kinney MC, Collins RD, Greer JP, Whitlock JA, Sioutos N, Kadin ME (1993) A small-cell-predominant variant of primary Ki-1 (CD30)+ T-cell lymphoma. Am J Surg Pathol 17 (9):859–868
133. Lamant L, McCarthy K, d'Amore E et al (2011) Prognostic impact of morphologic and phenotypic features of childhood ALK-positive anaplastic large-cell lymphoma: results of the ALCL99 study. J Clin Oncol 29(35):4669–4676 (official journal of the American Society of Clinical Oncology)
134. Vassallo J, Lamant L, Brugieres L et al (2006) ALK-positive anaplastic large cell lymphoma mimicking nodular sclerosis Hodgkin's lymphoma: report of 10 cases. Am J Surg Pathol 30 (2):223–229
135. Oschlies I, Lisfeld J, Lamant L et al (2013) ALK-positive anaplastic large cell lymphoma limited to the skin: clinical, histopathological and molecular analysis of 6 pediatric cases. A report from the ALCL99 study. Haematologica 98(1):50–56
136. Foss HD, Anagnostopoulos I, Araujo I et al (1996) Anaplastic large-cell lymphomas of T-cell and null-cell phenotype express cytotoxic molecules. Blood 88(10):4005–4011
137. Foss HD, Demel G, Anagnostopoulos I, Araujo I, Hummel M, Stein H (1997) Uniform expression of cytotoxic molecules in anaplastic large cell lymphoma of null/T cell phenotype and in cell lines derived from anaplastic large cell lymphoma. Pathobiol 65(2):83–90 (journal of immunopathology, molecular and cellular biology)
138. Rosso R, Paulli M, Magrini U et al (1990) Anaplastic large cell lymphoma, CD30/Ki-1 positive, expressing the CD15/Leu-M1 antigen. Immunohistochemical and morphological

relationships to Hodgkin's disease. Virchows Arch A 416(3):229–235 (Pathological anatomy and histopathology)
139. de Leval L (2010) Molecular classification of ganglionic T cell lymphomas. Pathological and diagnostic implications. Bull Mem Acad R Med Belg 165(1–2):99
140. de Leval L, Bisig B, Thielen C, Boniver J, Gaulard P (2009) Molecular classification of T-cell lymphomas. Crit Rev Oncol/Hematol 72(2):125–143
141. Ceccon M, Mologni L, Bisson W, Scapozza L, Gambacorti-Passerini C (2013) Crizotinib-resistant NPM-ALK mutants confer differential sensitivity to unrelated Alk inhibitors. Mol Cancer Res (MCR) 11(2):122–132
142. Moritake H, Shimonodan H, Marutsuka K, Kamimura S, Kojima H, Nunoi H (2011) C-MYC rearrangement may induce an aggressive phenotype in anaplastic lymphoma kinase positive anaplastic large cell lymphoma: Identification of a novel fusion gene ALO17/C-MYC. Am J Hematol 86(1):75–78
143. Mason DY, Harris NL, Delsol G et al (2008) Anaplastic large cell lymphoma, ALK-negative, 4th edn. International Agency for Research on Cancer, Lyon
144. Ferreri AJ, Govi S, Pileri SA, Savage KJ (2013) Anaplastic large cell lymphoma ALK-negative. Crit Rev Oncol/Hematol 85(2):206–215
145. Savage KJ, Harris NL, Vose JM et al (2008) ALK− anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK+ ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International peripheral T-cell lymphoma project. Blood 111(12):5496–5504
146. Eberle FC, Song JY, Xi L et al (2012) Nodal involvement by cutaneous CD30-positive T-cell lymphoma mimicking classical Hodgkin lymphoma. Am J Surg Pathol 36(5):716–725
147. Moskowitz AJ, Lunning MA, Horwitz SM (2014) How I treat the peripheral T-cell lymphomas. Blood 123(17):2636–2644
148. Piccaluga PP, Fuligni F, De Leo A et al (2013) Molecular profiling improves classification and prognostication of nodal peripheral T-cell lymphomas: results of a phase III diagnostic accuracy study. J Clin Oncol 31(24):3019–3025 (official journal of the American Society of Clinical Oncology)
149. Thompson PA, Prince HM (2013) Breast implant-associated anaplastic large cell lymphoma: a systematic review of the literature and mini-meta analysis. Current Hematol Malignancy Rep 8(3):196–210
150. Nguyen TT, Kreisel FH, Frater JL, Bartlett NL (2013) Anaplastic large-cell lymphoma with aberrant expression of multiple cytokeratins masquerading as metastatic carcinoma of unknown primary. J Clin Oncol 31(33):e443–e445 (official journal of the American Society of Clinical Oncology)
151. Feldman AL, Dogan A, Smith DI et al (2011) Discovery of recurrent t(6;7)(p25.3;q32.3) translocations in ALK-negative anaplastic large cell lymphomas by massively parallel genomic sequencing. Blood 117(3):915–919
152. Feldman AL, Law M, Remstein ED et al (2009) Recurrent translocations involving the IRF4 oncogene locus in peripheral T-cell lymphomas. Leukemia 23(3):574–580
153. Wada DA, Law ME, Hsi ED et al (2011) Specificity of IRF4 translocations for primary cutaneous anaplastic large cell lymphoma: a multicenter study of 204 skin biopsies. Mod Pathol 24(4):596–605 (an official journal of the United States and Canadian Academy of Pathology, Inc.)
154. Frizzera G, Long JC, Berard CW (1977) Evolution of angio-immunoblastic lymphadenopathy. New Engl J Med 297(1):59–60
155. Frizzera G, Moran EM, Rappaport H (1974) Angio-immunoblastic lymphadenopathy with dysproteinaemia. Lancet 1(7866):1070–1073
156. Frizzera G, Moran EM, Rappaport H (1975) Angio-immunoblastic lymphadenopathy. Diagnosis and clinical course. Am J Med 59(6):803–818

157. Lennert K (1979) Nature, prognosis and nomenclature of angioimmunoblastic (lymphadenopathy (lymphogranulomatosis X or T-zone lymphoma). Dtsch Med Wochenschr 104(35):1246–1247
158. Lukes RJ, Tindle BH (1975) Immunoblastic lymphadenopathy. A hyperimmune entity resembling Hodgkin's disease. New Engl J Med 292(1):1–8
159. Federico M, Rudiger T, Bellei M et al (2013) Clinicopathologic characteristics of angioimmunoblastic T-cell lymphoma: analysis of the international peripheral T-cell lymphoma project. J Clin Oncol 31(2):240–246 (official journal of the American Society of Clinical Oncology)
160. Grogg KL, Attygalle AD, Macon WR, Remstein ED, Kurtin PJ, Dogan A (2005) Angioimmunoblastic T-cell lymphoma: a neoplasm of germinal-center T-helper cells? Blood 106(4):1501–1502
161. Attygalle AD, Chuang SS, Diss TC, Du MQ, Isaacson PG, Dogan A (2007) Distinguishing angioimmunoblastic T-cell lymphoma from peripheral T-cell lymphoma, unspecified, using morphology, immunophenotype and molecular genetics. Histopathology 50(4):498–508
162. Mourad N, Mounier N, Briere J et al (2008) Clinical, biologic, and pathologic features in 157 patients with angioimmunoblastic T-cell lymphoma treated within the Groupe d'Etude des Lymphomes de l'Adulte (GELA) trials. Blood 111(9):4463–4470
163. Choi MY, Lechowicz MJ (2012) Management of the cutaneous peripheral T-cell lymphomas: when subtypes matter. Cancer J 18(5):439–444
164. Jacobson RK, Lee KC, Muglia JJ, Robinson-Bostom L (2013) Cutaneous manifestations of angioimmunoblastic T-cell lymphoma. J Am Acad Dermatol 69(1):e25–e26
165. Quintanilla-Martinez L, Jansen PM, Kinney MC, Swerdlow SH, Willemze R (2013) Non-mycosis fungoides cutaneous T-cell lymphomas: report of the 2011 Society for Hematopathology/European Association for Haematopathology workshop. Am J Clin Pathol 139(4):491–514
166. Ponciano A, de Muret A, Machet L et al (2012) Epidermotropic secondary cutaneous involvement by relapsed angioimmunoblastic T-cell lymphoma mimicking mycosis fungoides: a case report. J Cutan Pathol 39(12):1119–1124
167. Balaraman B, Conley JA, Sheinbein DM (2011) Evaluation of cutaneous angioimmunoblastic T-cell lymphoma. J Am Acad Dermatol 65(4):855–862
168. Dorfman DM, Brown JA, Shahsafaei A, Freeman GJ (2006) Programmed death-1 (PD-1) is a marker of germinal center-associated T cells and angioimmunoblastic T-cell lymphoma. Am J Surg Pathol 30(7):802–810
169. Attygalle A, Al-Jehani R, Diss TC et al (2002) Neoplastic T cells in angioimmunoblastic T-cell lymphoma express CD10. Blood 99(2):627–633
170. Grogg KL, Attygalle AD, Macon WR, Remstein ED, Kurtin PJ, Dogan A (2006) Expression of CXCL13, a chemokine highly upregulated in germinal center T-helper cells, distinguishes angioimmunoblastic T-cell lymphoma from peripheral T-cell lymphoma, unspecified. Mod Pathol 19(8):1101–1107 (an official journal of the United States and Canadian Academy of Pathology, Inc.)
171. Zettl A, Lee SS, Rudiger T et al (2002) Epstein-Barr virus-associated B-cell lymphoproliferative disorders in angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, unspecified. Am J Clin Pathol 117(3):368–379
172. Moroch J, Copie-Bergman C, de Leval L et al (2012) Follicular peripheral T-cell lymphoma expands the spectrum of classical Hodgkin lymphoma mimics. Am J Surg Pathol 36 (11):1636–1646
173. Nicolae A, Pittaluga S, Venkataraman G et al (2013) Peripheral T-cell lymphomas of follicular T-helper cell derivation with Hodgkin/Reed-Sternberg cells of B-cell lineage: both EBV-positive and EBV-negative variants exist. Am J Surg Pathol 37(6):816–826
174. Willenbrock K, Brauninger A, Hansmann ML (2007) Frequent occurrence of B-cell lymphomas in angioimmunoblastic T-cell lymphoma and proliferation of Epstein-Barr virus-infected cells in early cases. Br J Haematol 138(6):733–739

175. Ohgami RS, Zhao S, Ohgami JK et al (2012) TdT+ T-lymphoblastic populations are increased in Castleman disease, in Castleman disease in association with follicular dendritic cell tumors, and in angioimmunoblastic T-cell lymphoma. Am J Surg Pathol 36 (11):1619–1628
176. Cairns RA, Iqbal J, Lemonnier F et al (2012) IDH2 mutations are frequent in angioimmunoblastic T-cell lymphoma. Blood 119(8):1901–1903
177. Lemonnier F, Couronne L, Parrens M et al (2012) Recurrent TET2 mutations in peripheral T-cell lymphomas correlate with TFH-like features and adverse clinical parameters. Blood 120(7):1466–1469
178. Sakata-Yanagimoto M, Enami T, Yoshida K et al (2014) Somatic RHOA mutation in angioimmunoblastic T cell lymphoma. Nat Genet 46(2):171–175
179. Yoo HY, Sung MK, Lee SH et al (2014) A recurrent inactivating mutation in RHOA GTPase in angioimmunoblastic T cell lymphoma. Nat Genet 46(4):371–375
180. Thorns C, Bastian B, Pinkel D et al (2007) Chromosomal aberrations in angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma unspecified: A matrix-based CGH approach. Genes Chromosom Cancer 46(1):37–44
181. Pileri S, Weisenburger D, Sng I et al (2008) Peripheral T-cell lymphoma, not otherwise specified, 4th edn. International Agency for Research on Cancer, Lyon
182. Karlin L, Coiffier B (2014) The changing landscape of peripheral T-cell lymphoma in the era of novel therapies. Semin Hematol 51(1):25–34
183. Herrera AF, Crosby-Thompson A, Friedberg JW et al (2014) Comparison of referring and final pathology for patients with T-cell lymphoma in the national comprehensive cancer network. Cancer
184. Hsi ED, Said J, Macon WR et al (2014) Diagnostic accuracy of a defined immunophenotypic and molecular genetic approach for peripheral T/NK-cell lymphomas: a North American PTCL study group project. Am J Surg Pathol 38(6):768–775
185. Vose J, Armitage J, Weisenburger D (2008) International TCLP: international peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol 26(25):4124–4130 (official journal of the American Society of Clinical Oncology)
186. Rudiger T, Ichinohasama R, Ott MM et al (2000) Peripheral T-cell lymphoma with distinct perifollicular growth pattern: a distinct subtype of T-cell lymphoma? Am J Surg Pathol 24 (1):117–122
187. Rudiger T, Weisenburger DD, Anderson JR et al (2002) Peripheral T-cell lymphoma (excluding anaplastic large-cell lymphoma): results from the Non-Hodgkin's lymphoma classification project. Ann Oncol 13(1):140–149 (official journal of the European Society for Medical Oncology/ESMO)
188. Savage KJ, Chhanabhai M, Gascoyne RD, Connors JM (2004) Characterization of peripheral T-cell lymphomas in a single North American institution by the WHO classification. Ann Oncol 15(10):1467–1475 (official journal of the European Society for Medical Oncology/ ESMO)
189. Falini B, Pileri S, De Solas I et al (1990) Peripheral T-cell lymphoma associated with hemophagocytic syndrome. Blood 75(2):434–444
190. Saragoni A, Falini B, Medri L et al (1990) Peripheral T-cell lymphoma associated with hemophagocytic syndrome: a recently identified entity. Clinico-pathologic and immunohistochemical study of 2 cases. Pathologica 82(1080):359–369
191. Yamashita Y, Nakamura S, Kagami Y et al (2000) Lennert's lymphoma: a variant of cytotoxic T-cell lymphoma? Am J Surg Pathol 24(12):1627–1633
192. Schmitz N, Trumper L, Ziepert M et al (2010) Treatment and prognosis of mature T-cell and NK-cell lymphoma: an analysis of patients with T-cell lymphoma treated in studies of the German high-grade Non-Hodgkin lymphoma study group. Blood 116(18):3418–3425

193. Geissinger E, Odenwald T, Lee SS et al (2004) Nodal peripheral T-cell lymphomas and, in particular, their lymphoepithelioid (Lennert's) variant are often derived from CD8(+) cytotoxic T-cells. Virchows Arch 445(4):334–343 (an international journal of pathology)
194. Patsouris E, Noel H, Lennert K (1988) Histological and immunohistological findings in lymphoepithelioid cell lymphoma (Lennert's lymphoma). Am J Surg Pathol 12(5):341–350
195. Zhou J, Kang N, Cui L, Ba D, He W (2012) Anti-gammadelta TCR antibody-expanded gammadelta T cells: a better choice for the adoptive immunotherapy of lymphoid malignancies. Cell Mol Immunol 9(1):34–44
196. Barry TS, Jaffe ES, Sorbara L, Raffeld M, Pittaluga S (2003) Peripheral T-cell lymphomas expressing CD30 and CD15. Am J Surg Pathol 27(12):1513–1522
197. Iqbal J, Wright G, Wang C et al (2014) Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphoma. Blood 123(19):2915–2923
198. Matnani RG, Stewart RL, Pulliam J, Jennings CD, Kesler M (2013) Peripheral T-cell lymphoma with aberrant expression of CD19, CD20, and CD79a: case report and literature review. Case Rep Hematol 2013:183134
199. Makita M, Murakami I, Yoshioka T et al (2009) Extranodal CD20-positive peripheral T-cell lymphoma presenting with adrenal and testicular masses. Rinsho Ketsueki 50(5):413–418 (The Japanese journal of clinical hematology)
200. Song JY, Strom T, Raffeld M, Pittaluga S, Jaffe ES (2011) Peripheral T-cell lymphoma with aberrant expression of CD30, CD15, and CD20. J Clin Oncol 29(32):e789–e791 (official journal of the American Society of Clinical Oncology)
201. Fox CP, Bishton MJ, O'Connor S, Hughes S, Byrne JL, Russell N (2014) EBV-driven relapse of peripheral T-cell lymphoma, masquerading as a post transplant lymphoproliferative disorder following allo-SCT. Bone Marrow Transpl 49(1):150–152
202. Ha SY, Sung J, Ju H et al (2013) Epstein-Barr virus-positive nodal peripheral T cell lymphomas: clinicopathologic and gene expression profiling study. Pathol Res Pract 209 (7):448–454
203. Pileri SA (2012) Piccaluga PP. New molecular insights into peripheral T cell lymphomas. J Clin Investig 122(10):3448–3455
204. Streubel B, Vinatzer U, Willheim M, Raderer M, Chott A (2006) Novel t(5;9)(q33;q22) fuses ITK to SYK in unspecified peripheral T-cell lymphoma. Leukemia 20(2):313–318
205. Nagel S, Leich E, Quentmeier H et al (2008) Amplification at 7q22 targets cyclin-dependent kinase 6 in T-cell lymphoma. Leukemia 22(2):387–392
206. Ahearne MJ, Allchin RL, Fox CP, Wagner SD (2014) Follicular helper T-cells: expanding roles in T-cell lymphoma and targets for treatment. Br J Haematol
207. Palomero T, Couronne L, Khiabanian H et al (2014) Recurrent mutations in epigenetic regulators, RHOA and FYN kinase in peripheral T cell lymphomas. Nat Genet 46 (2):166–170
208. Manso R, Sanchez-Beato M, Monsalvo S et al (2014) The RHOA G17 V gene mutation occurs frequently in peripheral T-cell lymphoma and is associated with a characteristic molecular signature. Blood 123(18):2893–2894
209. Martin-Subero JI, Wlodarska I, Bastard C et al (2006) Chromosomal rearrangements involving the BCL3 locus are recurrent in classical Hodgkin and peripheral T-cell lymphoma. Blood 108(1):401–402 (author reply 402–403)
210. Suzuki HI, Matsuyama H, Noguchi M et al (2013) Computational dissection of distinct microRNA activity signatures associated with peripheral T cell lymphoma subtypes. Leukemia 27(10):2107–2111
211. Vasmatzis G, Johnson SH, Knudson RA et al (2012) Genome-wide analysis reveals recurrent structural abnormalities of TP63 and other p53-related genes in peripheral T-cell lymphomas. Blood 120(11):2280–2289
212. Visnyei K, Grossbard ML, Shapira I (2013) Hepatosplenic gammadelta T-cell lymphoma: an overview. Clin Lymphoma Myeloma Leuk 13(4):360–369

213. Clarke CA, Morton LM, Lynch C et al (2013) Risk of lymphoma subtypes after solid organ transplantation in the United States. Br J Cancer 109(1):280–288
214. Ferreri AJ, Govi S, Pileri SA (2012) Hepatosplenic gamma-delta T-cell lymphoma. Crit Rev Oncol/Hematol 83(2):283–292
215. Roelandt PR, Maertens J, Vandenberghe P et al (2009) Hepatosplenic gammadelta T-cell lymphoma after liver transplantation: report of the first 2 cases and review of the literature. Liver Transplant 15(7):686–692 (official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society)
216. Tey SK, Marlton PV, Hawley CM, Norris D, Gill DS (2008) Post-transplant hepatosplenic T-cell lymphoma successfully treated with HyperCVAD regimen. Am J Hematol 83 (4):330–333
217. Basic Kinda S, Durakovic N, Dotlic S et al (2013) Hepatosplenic alphabeta T-cell lymphoma arising after long-term azathioprine therapy successfully treated with allogeneic bone marrow transplant. Leuk Lymphoma 54(6):1334–1335
218. Beaugerie L (2012) Immunosuppression-related lymphomas and cancers in IBD: how can they be prevented? Dig Dis 30(4):415–419
219. Mason M, Siegel CA (2013) Do inflammatory bowel disease therapies cause cancer? Inflamm Bowel Dis 19(6):1306–1321
220. Iyama S, Matsunaga T, Sato T et al (2008) A case of hepatosplenic gammadelta T-cell lymphoma associated hemophagocytic syndrome. Gan to kagaku ryoho. Cancer Chemother 35(9):1623–1627
221. Nosari A, Oreste PL, Biondi A et al (1999) Hepato-splenic gammadelta T-cell lymphoma: a rare entity mimicking the hemophagocytic syndrome. Am J Hematol 60(1):61–65
222. Calvaruso M, Gulino A, Buffa S et al (2014) Challenges and new prospects in hepatosplenic gammadelta T-cell lymphoma. Leuk Lymphoma 0:1–9
223. te Boekhorst PA, van Lom K (2013) Hepatosplenic T-cell lymphoma. Br J Haematol 163 (4):422
224. Suarez F, Wlodarska I, Rigal-Huguet F et al (2000) Hepatosplenic alphabeta T-cell lymphoma: an unusual case with clinical, histologic, and cytogenetic features of gammadelta hepatosplenic T-cell lymphoma. Am J Surg Pathol 24(7):1027–1032
225. Cooke CB, Krenacs L, Stetler-Stevenson M et al (1996) Hepatosplenic T-cell lymphoma: a distinct clinicopathologic entity of cytotoxic gamma delta T-cell origin. Blood 88 (11):4265–4274
226. Vega F, Medeiros LJ, Bueso-Ramos C et al (2001) Hepatosplenic gamma/delta T-cell lymphoma in bone marrow. A sinusoidal neoplasm with blastic cytologic features. Am J Clin Pathol 116(3):410–419
227. Vega F, Medeiros LJ, Gaulard P (2007) Hepatosplenic and other gammadelta T-cell lymphomas. Am J Clin Pathol 127(6):869–880
228. Cohen J, Hariton E, Kothari D, Pihan GA, Robson SC (2013) Hepatosplenic alpha/beta T-cell lymphoma masquerading as cirrhosis. J Gastrointest Oncol 4(2):131–136
229. Macon WR, Levy NB, Kurtin PJ et al (2001) Hepatosplenic alphabeta T-cell lymphomas: a report of 14 cases and comparison with hepatosplenic gammadelta T-cell lymphomas. Am J Surg Pathol 25(3):285–296
230. Morice WG, Macon WR, Dogan A, Hanson CA, Kurtin PJ (2006) NK-cell-associated receptor expression in hepatosplenic T-cell lymphoma, insights into pathogenesis. Leukemia 20(5):883–886
231. Alonsozana EL, Stamberg J, Kumar D et al (1997) Isochromosome 7q: the primary cytogenetic abnormality in hepatosplenic gammadelta T cell lymphoma. Leukemia 11 (8):1367–1372
232. Belhadj K, Reyes F, Farcet JP et al (2003) Hepatosplenic gammadelta T-cell lymphoma is a rare clinicopathologic entity with poor outcome: report on a series of 21 patients. Blood 102 (13):4261–4269

233. Travert M, Huang Y, de Leval L et al (2012) Molecular features of hepatosplenic T-cell lymphoma unravels potential novel therapeutic targets. Blood 119(24):5795–5806
234. Wlodarska I, Martin-Garcia N, Achten R et al (2002) Fluorescence in situ hybridization study of chromosome 7 aberrations in hepatosplenic T-cell lymphoma: isochromosome 7q as a common abnormality accumulating in forms with features of cytologic progression. Genes Chromosom Cancer 33(3):243–251
235. Shetty S, Mansoor A, Roland B (2006) Ring chromosome 7 with amplification of 7q sequences in a pediatric case of hepatosplenic T-cell lymphoma. Cancer Genet Cytogenet 167(2):161–163
236. Tamaska J, Adam E, Kozma A et al (2006) Hepatosplenic gammadelta T-cell lymphoma with ring chromosome 7, an isochromosome 7q equivalent clonal chromosomal aberration. Virchows Arch 449(4):479–483 (an international journal of pathology)
237. Arps DP, Smith LB (2013) Classic versus type II enteropathy-associated T-cell lymphoma: diagnostic considerations. Arch Pathol Lab Med 137(9):1227–1231
238. Bautista-Quach MA, Ake CD, Chen M, Wang J (2012) Gastrointestinal lymphomas: Morphology, immunophenotype and molecular features. J Gastrointest Oncol 3(3):209–225
239. Ferreri AJ, Zinzani PL, Govi S, Pileri SA (2011) Enteropathy-associated T-cell lymphoma. Crit Rev Oncol/Hematol 79(1):84–90
240. Malamut G, Chandesris O, Verkarre V et al (2013) Enteropathy associated T cell lymphoma in celiac disease: a large retrospective study. Dig Liver Dis 45(5):377–384 (official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver)
241. Raza A, Zu Y (2014) Enteropathy-associated T-cell lymphoma, type II (monomorphic variant). Blood 123(14):2136
242. Kim JB, Kim SH, Cho YK et al (2013) A case of colon perforation due to enteropathy-associated T-cell lymphoma. World J Gastroenterol (WJG) 19(11):1841–1844
243. Wang L, Liu Y, Lin XY et al (2012) A case of enteropathy-associated T-cell lymphoma (type I) arising in stomach without refractory celiac disease. Diagn Pathol 7:172
244. Amiot A, Allez M, Treton X et al (2012) High frequency of fatal haemophagocytic lymphohistiocytosis syndrome in enteropathy-associated T cell lymphoma. Dig Liver Dis 44 (4):343–349 (official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver)
245. Varghese D, Koya HH, Cherian SV et al (2013) Hemophagocytic lymphohistiocytosis: an uncommon presentation of enteropathy-associated T-cell lymphoma. J Clin Oncol 31(13): e226–230 (official journal of the American Society of Clinical Oncology)
246. Schwock J, Hyjek EM, Torlakovic EE, Geddie WR (2014) Enteropathy-associated intestinal T-cell lymphoma in cavitating mesenteric lymph node syndrome: fine-needle aspiration contributes to the diagnosis. Diagn Cytopathol
247. Garcia-Herrera A, Song JY, Chuang SS et al (2011) Nonhepatosplenic gammadelta T-cell lymphomas represent a spectrum of aggressive cytotoxic T-cell lymphomas with a mainly extranodal presentation. Am J Surg Pathol 35(8):1214–1225
248. Berman EL, Zauber NP, Rickert RR, Diss TC, Isaacson PG (1998) Enteropathy-associated T cell lymphoma with brain involvement. J Clin Gastroenterol 26(4):337–341
249. Takeshita M, Nakamura S, Kikuma K et al (2011) Pathological and immunohistological findings and genetic aberrations of intestinal enteropathy-associated T cell lymphoma in Japan. Histopathology 58(3):395–407
250. Tan SY, Chuang SS, Tang T et al (2013) Type II EATL (epitheliotropic intestinal T-cell lymphoma): a neoplasm of intra-epithelial T-cells with predominant CD8 alpha alpha phenotype. Leukemia 27(8):1688–1696
251. O'Malley DP, Goldstein NS, Banks PM (2014) The recognition and classification of lympho proliferative disorders of the gut. Hum Pathol 45(5):899–916
252. Sun J, Lu Z, Yang D, Chen J (2011) Primary intestinal T-cell and NK-cell lymphomas: a clinicopathological and molecular study from China focused on type II enteropathy-associated T-cell lymphoma and primary intestinal NK-cell lymphoma. Mod Pathol 24

(7):983–992 (an official journal of the United States and Canadian Academy of Pathology, Inc.)
253. Kikuma K, Yamada K, Nakamura S et al (2014) Detailed clinicopathological characteristics and possible lymphomagenesis of type II intestinal enteropathy-associated T-cell lymphoma in Japan. Hum Pathol
254. Okumura K, Ikebe M, Shimokama T et al (2012) An unusual enteropathy-associated T-cell lymphoma with MYC translocation arising in a Japanese patient: a case report. World J Gastroenterol (WJG) 18(19):2434–2437
255. Perry AM, Warnke RA, Hu Q et al (2013) Indolent T-cell lympho proliferative disease of the gastrointestinal tract. Blood 122(22):3599–3606
256. Criscione VD, Weinstock MA (2007) Incidence of cutaneous T-cell lymphoma in the United States, 1973–2002. Arch Dermatol 143(7):854–859
257. Cho-Vega JH, Tschen JA, Duvic M, Vega F (2010) Early-stage mycosis fungoides variants: case-based review. Ann Diagn Pathol 14(5):369–385
258. Ben-Amitai D, Michael D, Feinmesser M, Hodak E (2003) Juvenile mycosis fungoides diagnosed before 18 years of age. Acta Dermato-Venereol 83(6):451–456
259. Castano E, Glick S, Wolgast L et al (2013) Hypopigmented mycosis fungoides in childhood and adolescence: a long-term retrospective study. J Cutan Pathol 40(11):924–934
260. Cooper D, Jacobson M, Bart RS (1992) Hypopigmented macules. Hypopigmented mycosis fungoides (MF). Arch Dermatol 128(9):1266–1267, 1269–1270
261. Hodak E, Amitay-Laish I, Feinmesser M et al (2014) Juvenile mycosis fungoides: Cutaneous T-cell lymphoma with frequent follicular involvement. J Am Acad Dermatol 70(6):993–1001
262. Massone C, Kodama K, Kerl H, Cerroni L (2005) Histopathologic features of early (patch) lesions of mycosis fungoides: a morphologic study on 745 biopsy specimens from 427 patients. Am J Surg Pathol 29(4):550–560
263. Breneman DL, Raju US, Breneman JC et al (2003) Lymph node grading for staging of mycosis fungoides may benefit from examination of multiple excised lymph nodes. J Am Acad Dermatol 48(5):702–706
264. Brugiere C, Karanian-Philippe M, Comoz F, Dompmartin A, Galateau-Salle F, Verneuil L (2014) Two cases of aggressive non-tumoral folliculotropic mycosis fungoides with visceral involvement. Br J Dermatol
265. Shibata S, Sugaya M, Minatani Y et al (2009) Folliculotropic mycosis fungoides with severe hepatic failure due to hepatic involvement. Acta Dermato-Venereol 89(4):423–424
266. Bennett SR, Greer JP, Stein RS, Glick AD, Cousar JB, Collins RD (1984) Death due to splenic rupture in suppressor cell mycosis fungoides: a case report. Am J Clin Pathol 82 (1):104–109
267. Schiodt I, Duun E, Fischer TK, Christiansen AP, Ralfkiaer E (2000) Fatal rupture of the spleen caused by infiltration of T-cell lymphoma. Ann Hematol 79(3):158–160
268. Ueda T, Hosoki N, Isobe K et al (2002) Diffuse pulmonary involvement by mycosis fungoides: high-resolution computed tomography and pathologic findings. J Thorac Imaging 17(2):157–159
269. Graham SJ, Sharpe RW, Steinberg SM, Cotelingam JD, Sausville EA, Foss FM (1993) Prognostic implications of a bone marrow histopathologic classification system in mycosis fungoides and the Sezary syndrome. Cancer 72(3):726–734
270. van Doorn R, Scheffer E, Willemze R (2002) Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis: a clinicopathologic and follow-up study of 51 patients. Arch Dermatol 138(2):191–198
271. van Haselen CW, Toonstra J, van der Putte SJ, van Dongen JJ, van Hees CL, van Vloten WA (1998) Granulomatous slack skin. Report of three patients with an updated review of the literature. Dermatology 196(4):382–391
272. Ally MS, Pawade J, Tanaka M et al (2012) Solitary mycosis fungoides: a distinct clinicopathologic entity with a good prognosis: a series of 15 cases and literature review. J Am Acad Dermatol 67(4):736–744

273. Gallardo F, Garcia-Muret MP, Servitje O et al (2009) Cutaneous lymphomas showing prominent granulomatous component: clinicopathological features in a series of 16 cases. J Eur Acad Dermatol Venereol (JEADV) 23(6):639–647
274. Haghighi B, Smoller BR, LeBoit PE, Warnke RA, Sander CA, Kohler S (2000) Pagetoid reticulosis (Woringer-Kolopp disease): an immunophenotypic, molecular, and clinicopathologic study. Mod Pathol 13(5):502–510 (an official journal of the United States and Canadian Academy of Pathology, Inc.)
275. Mantaka P, Helsing P, Gjersvik P, Bassarova A, Clausen OP, Delabie J (2013) Clinical and histopathological features of folliculotropic mycosis fungoides: a Norwegian patient series. Acta Dermato-Venereol 93(3):325–329
276. Mattox AR, Missall TA, Guo A (2014) Granulomatous mycosis fungoides with clinical features of granulomatous slack skin. Cutis 93(3):E4–E5
277. Muniesa C, Estrach T, Pujol RM et al (2010) Folliculotropic mycosis fungoides: clinicopathological features and outcome in a series of 20 cases. J Am Acad Dermatol 62 (3):418–426
278. Shah A, Safaya A (2012) Granulomatous slack skin disease: a review, in comparison with mycosis fungoides. J Eur Acad Dermatol Venereol (JEADV) 26(12):1472–1478
279. Benner MF, Jansen PM, Vermeer MH, Willemze R (2012) Prognostic factors in transformed mycosis fungoides: a retrospective analysis of 100 cases. Blood 119(7):1643–1649
280. Kunishige JH, McDonald H, Alvarez G, Johnson M, Prieto V, Duvic M (2009) Lymphomatoid papulosis and associated lymphomas: a retrospective case series of 84 patients. Clin Exp Dermatol 34(5):576–581
281. Zackheim HS, Jones C, Leboit PE, Kashani-Sabet M, McCalmont TH, Zehnder J (2003) Lymphomatoid papulosis associated with mycosis fungoides: a study of 21 patients including analyses for clonality. J Am Acad Dermatol 49(4):620–623
282. Thurber SE, Zhang B, Kim YH, Schrijver I, Zehnder J, Kohler S (2007) T-cell clonality analysis in biopsy specimens from two different skin sites shows high specificity in the diagnosis of patients with suggested mycosis fungoides. J Am Acad Dermatol 57(5):782–790
283. Clark RA, Shackelton JB, Watanabe R et al (2011) High-scatter T cells: a reliable biomarker for malignant T cells in cutaneous T-cell lymphoma. Blood 117(6):1966–1976
284. Dabiri S, Morales A, Ma L et al (2011) The frequency of dual TCR-PCR clonality in granulomatous disorders. J Cutan Pathol 38(9):704–709
285. Dereure O, Levi E, Kadin ME (2000) T-Cell clonality in pityriasis lichenoides et varioliformis acuta: a heteroduplex analysis of 20 cases. Arch Dermatol 136(12):1483–1486
286. Lor P, Krueger U, Kempf W, Burg G, Nestle FO (2002) Monoclonal rearrangement of the T cell receptor gamma-chain in lichenoid pigmented purpuric dermatitis of gougerot-blum responding to topical corticosteroid therapy. Dermatology 205(2):191–193
287. Lukowsky A, Muche JM, Sterry W, Audring H (2000) Detection of expanded T cell clones in skin biopsy samples of patients with lichen sclerosus et atrophicus by T cell receptor-gamma polymerase chain reaction assays. J Invest Dermatol 115(2):254–259
288. Campbell JJ, Clark RA, Watanabe R, Kupper TS (2010) Sezary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. Blood 116(5):767–771
289. Hu SC, Lin CL, Hong CH, Yu HS, Chen GS, Lee CH (2014) CCR7 expression correlates with subcutaneous involvement in mycosis fungoides skin lesions and promotes migration of mycosis fungoides cells (MyLa) through mTOR activation. J Dermatol Sci 74(1):31–38
290. Carbone A, Bernardini L, Valenzano F et al (2008) Array-based comparative genomic hybridization in early-stage mycosis fungoides: recurrent deletion of tumor suppressor genes BCL7A, SMAC/DIABLO, and RHOF. Genes Chromosom Cancer 47(12):1067–1075
291. Ralfkiaer U, Hagedorn PH, Bangsgaard N et al (2011) Diagnostic microRNA profiling in cutaneous T-cell lymphoma (CTCL). Blood 118(22):5891–5900
292. van Kester MS, Ballabio E, Benner MF et al (2011) miRNA expression profiling of mycosis fungoides. Mol Oncol 5(3):273–280

293. Jaffe ES (2009) The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. Hematology/the Education Program of the American Society of Hematology. Am Soc Hematol. Educ Prog 2009:523–531
294. Kong YY, Dai B, Kong JC et al (2008) Subcutaneous panniculitis-like T-cell lymphoma: a clinicopathologic, immunophenotypic, and molecular study of 22 Asian cases according to WHO-EORTC classification. Am J Surg Pathol 32(10):1495–1502
295. Parveen Z, Thompson K (2009) Subcutaneous panniculitis-like T-cell lymphoma: redefinition of diagnostic criteria in the recent World Health Organization-European Organization for Research and Treatment of Cancer classification for cutaneous lymphomas. Arch Pathol Lab Med 133(2):303–308
296. Willemze R, Jansen PM, Cerroni L et al (2008) Subcutaneous panniculitis-like T-cell lymphoma: definition, classification, and prognostic factors: an EORTC cutaneous lymphoma group study of 83 cases. Blood 111(2):838–845
297. Acree SC, Tovar JP, Pattengale PK et al (2013) Subcutaneous Panniculitis-like T-cell lymphoma in two pediatric patients: an HIV-positive adolescent and a 4-month-old infant. Fetal Pediatr Pathol 32(3):175–183
298. Gao J, Gauerke SJ, Martinez-Escala ME et al (2013) Bone marrow involvement by subcutaneous Panniculitis-like T-cell lymphoma: a report of three cases. Mod Pathol (an official journal of the United States and Canadian Academy of Pathology, Inc.)
299. Voelkl A, Then C, Schmidmaier R, Seibold C, Reincke M, Oduncu F (2011) Subcutaneous panniculitis-like T-cell lymphoma-associated hemophagocytic syndrome with fulminant relapse during corticosteroid treatment and fatal outcome. Leuk Res 35(9):e154–e156
300. Arps DP, Patel RM (2013) Lupus profundus (panniculitis): a potential mimic of subcutaneous panniculitis-like T-cell lymphoma. Arch Pathol Lab Med 137(9):1211–1215
301. Li JY, Liu HJ, Wang L (2013) Subcutaneous panniculitis-like T-cell lymphoma accompanied with discoid lupus erythematosus. Chin Med J 126(18):3590
302. Pincus LB, LeBoit PE, McCalmont TH et al (2009) Subcutaneous panniculitis-like T-cell lymphoma with overlapping clinicopathologic features of lupus erythematosus: coexistence of 2 entities? Am J Dermatopathol 31(6):520–526
303. Liau JY, Chuang SS, Chu CY, Ku WH, Tsai JH, Shih TF (2013) The presence of clusters of plasmacytoid dendritic cells is a helpful feature for differentiating lupus panniculitis from subcutaneous panniculitis-like T-cell lymphoma. Histopathology 62(7):1057–1066
304. Hahtola S, Burghart E, Jeskanen L et al (2008) Clinicopathological characterization and genomic aberrations in subcutaneous panniculitis-like T-cell lymphoma. J Invest Dermatol 128(9):2304–2309
305. Benner MF, Willemze R (2009) Applicability and prognostic value of the new TNM classification system in 135 patients with primary cutaneous anaplastic large cell lymphoma. Arch Dermatol 145(12):1399–1404
306. Booken N, Goerdt S, Klemke CD (2012) Clinical spectrum of primary cutaneous CD30-positive anaplastic large cell lymphoma: an analysis of the Mannheim Cutaneous Lymphoma Registry. J Ger Soc Dermatol (JDDG) 10(5):331–339
307. Kacerovska D, Michal M, Kazakov DV (2014) Pediatric case of primary cutaneous eosinophil-rich CD30 + anaplastic large-cell lymphoma with follicular mucinosis. Am J Dermatopathol 36(3):e78–e80
308. Kinney MC, Higgins RA, Medina EA (2011) Anaplastic large cell lymphoma: twenty-five years of discovery. Arch Pathol Lab Med 135(1):19–43
309. Gencoglan G, Ozturk F, Inanir I, Miskioglu M, Temiz P, Gunduz K (2011) Primary cutaneous anaplastic CD30+ large-cell lymphoma that completely regressed after incisional skin biopsy. Cutan Ocular Toxicol 30(2):163–166
310. Sciallis AP, Law ME, Inwards DJ et al (2012) Mucosal CD30-positive T-cell lymphoproliferations of the head and neck show a clinicopathologic spectrum similar to cutaneous CD30-positive T-cell lympho proliferative disorders. Mod Pathol 25(7):983–992 (an official journal of the United States and Canadian Academy of Pathology, Inc.)

311. Plaza JA, Feldman AL, Magro C (2013) Cutaneous CD30-positive lympho proliferative disorders with CD8 expression: a clinicopathologic study of 21 cases. J Cutan Pathol 40 (2):236–247
312. Pulitzer M, Brady MS, Blochin E, Amin B, Teruya-Feldstein J (2013) Anaplastic large cell lymphoma: a potential pitfall in the differential diagnosis of melanoma. Arch Pathol Lab Med 137(2):280–283
313. Benner MF, Jansen PM, Meijer CJ, Willemze R (2009) Diagnostic and prognostic evaluation of phenotypic markers TRAF1, MUM1, BCL2 and CD15 in cutaneous CD30-positive lympho proliferative disorders. Br J Dermatol 161(1):121–127
314. Foss HD, Marafioti T, Stein H (2000) The many faces of anaplastic large cell lymphoma. Der Pathologe 21(2):124–136
315. Metcalf RA, Bashey S, Wysong A, Kim J, Kim YH, Gratzinger D (2013) Intravascular ALK-negative anaplastic large cell lymphoma with localized cutaneous involvement and an indolent clinical course: toward recognition of a distinct clinicopathologic entity. Am J Surg Pathol 37(4):617–623
316. Samols MA, Su A, Ra S et al (2014) Intralymphatic cutaneous anaplastic large cell lymphoma/lymphomatoid papulosis: expanding the spectrum of CD30-positive lympho proliferative disorders. Am J Surg Pathol
317. Kiran T, Demirkesen C, Eker C, Kumusoglu H, Tuzuner N (2013) The significance of MUM1/IRF4 protein expression and IRF4 translocation of CD30(+) cutaneous T-cell lympho proliferative disorders: a study of 53 cases. Leuk Res 37(4):396–400
318. Benner MF, Ballabio E, van Kester MS et al (2012) Primary cutaneous anaplastic large cell lymphoma shows a distinct miRNA expression profile and reveals differences from tumor-stage mycosis fungoides. Exp Dermatol 21(8):632–634
319. Szuhai K, van Doorn R, Tensen CP, Van K (2013) Array-CGH analysis of cutaneous anaplastic large cell lymphoma. Methods Mol Biol 973:197–212
320. Piccaluga PP, Tabanelli V, Pileri SA (2014) Molecular genetics of peripheral T-cell lymphomas. Int J Hematol 99(3):219–226

Gene Expression Profiling in Non-Hodgkin Lymphomas

Joo Y. Song, Jianbo Yu and Wing C. Chan

Abstract

Although the current WHO classification (Swerdlow et al. WHO classification of tumours of haematopoietic and lymphoid tissues. International Agency for Research on Cancer, Lyon, 2008 [1]) for hematolymphoid neoplasms has delineated lymphomas based on the combined morphologic, immunophenotypic, and genotypic findings, further refinement is necessary especially in regard to therapeutics and prognostic implications. High-throughput gene expression profiling (GEP) using microarray technology (Schena et al. Science 270:467–470, 1995 [2]; Augenlicht et al. Proc Natl Acad Sci USA 88:3286-3289, 1991 [3]) was developed about 20 years ago, and further refinement of the technology and analytical approaches has enabled us to routinely evaluate practically the entire transcriptome at a time. GEP has helped to improve the classification and prognostication of non-Hodgkin lymphomas (NHL) as well as improved our understanding of their pathophysiology and response to new therapeutics. In this

J.Y. Song · J. Yu · W.C. Chan (✉)
Department of Pathology, City of Hope Medical Center, 1500 East Duarte Road, Duarte, CA 91010, USA
e-mail: jochan@coh.org

J.Y. Song
e-mail: josong@coh.org

J. Yu
Department of Pathology and Key Laboratory of Cancer Prevention and Treatment of Heilongjiang Province, Mudanjiang Medical University, Mudanjiang, China
e-mail: jianboyu2013@gmail.com

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_4

paper, we will briefly review how this revolutionary tool has transformed our understanding of lymphomas and given us insight into targeted therapeutics. We will also discuss the current efforts in adapting the findings to routine clinical practice, the evolution of the research technology and directions in the future.

Keywords
Gene expression profiling · lymphoma

Contents

1 Concept and Technology

1.1 DNA Microarray

Current commercially available arrays are extremely close to whole transcriptome coverage. This technology relies on nucleic acid hybridization of labeled targets from the tissue sample to probes of complementary DNA (cDNA) fragments/oligonucleotides that represent genes of interest that are immobilized on a solid surface. Analysis of the messenger RNA (mRNA) creates a molecular fingerprint for the tumor, and in general, tumors with a closely related fingerprint will have similar biological and clinical features. There are two main platforms, one using cDNA probes and the other using oligonucleotide probes in the microarrays. Briefly, cDNA probes consist of polymerase chain reaction (PCR) products from cDNA libraries representing genes of interest which are then systematically spotted on a nitrocellulose filter or slide. Oligonucleotide arrays are constructed with probes that are directly synthesized in situ or oligonucleotides synthesized off-line and robotically spotted on glass slides. Oligonucleotides offer greater specificity compared to cDNA because they can be tailored to minimize cross-hybridization and provide

more uniform melting temperature. Oligonucleotide probes may consistent of long (50–70 bps) probes or short (25 bps) probe sets as in arrays manufactured by Affymetrix. Each array can generate an extensive amount of data that requires bioinformatic tools many of which are now publicly available [4]. Analysis of the array data can be supervised and unsupervised. Unsupervised analysis uses algorithms such as hierarchical clustering or self-organizing maps and analyzes the data without external information such as clinical data. This approach is particularly useful for discovery and identifying unknown relationships. Supervised learning utilizes external information to guide the analysis. It divides samples using known parameters such as survival data or certain genetic characteristics into groups for comparative analysis. This type of learning requires accurate sample characterization, which can be the issue when based on parameters that are not accurately quantifiable.

1.2 Limitations

Gene expression profiling assays initially were questioned in regard to their accuracy and reproducibility, their clinical relevance, and possible utility in diagnosis. These questions have been largely answered, and GEP has been determined to be a powerful and useful tool in research and potentially also in the clinical arena. With the advent of microarrays commercially manufactured under strictly controlled conditions and experiments performed according to established procedures, array experiments are highly reproducible when performed on the same platform and even results on different platforms at multiple large centers have given highly comparable results [5, 6]. It is true that the expression of single genes may not be entirely reproducible, particularly for cross-platform comparisons, but current analysis places the emphasis more on expression signatures rather than single genes, and signatures containing an aggregate of tens to several hundreds of transcripts are very reproducible.

However, like all tests, there are limitations that we need to be aware of. Traditionally, GEP has been performed on fresh or frozen tissue, which may not be readily available. Progress in RNA extraction and processing from paraffin-embedded tissue has allowed the implementation of this platform for tissues with reasonably well-preserved mRNA [7, 8]. Other considerations are the sampling of the tumor. Delay in sample processing may produce significant artifact. Samples from frozen tissue need to be examined to ensure that they contain representative tumor tissues. Tumor contains not only the neoplastic cells but also varying amounts of stromal elements and inflammatory cells. While the stromal components may provide highly useful information, there has to be sufficient amounts of tumor to study the characteristics of the tumor component. There is also heterogeneity in the tumor cells of a particular lesion that may complicate the interpretation of gene expression studies even further. Careful sampling is required, and dissecting tumor heterogeneity may even require microdissection to enrich and isolate specific cells.

From the analytical standpoint, the importance of validation cannot be stressed more. Validation may be computational as in using separate training and validation set or using the leave-one-out cross-validation (LOOCV) approach. Significant findings can be validated by quantitative reverse transcription polymerase chain reaction (qRT-PCR) or immunohistochemistry or even further functional analysis. Validation may also include the correlation with certain clinical factors or genetic alterations. Since GEP is evaluating thousands of parameters on a single sample, the number of cases included should be as large as possible to reach meaningful conclusions even though the numbers of samples are frequently not ideal for statistical confidence.

Alternative splicing is an important mechanism for modulating the function of the gene and can change the way the transcript is translated or regulated in different tissues or different conditions [9]. Earlier microarrays may not be designed to measure these variations. With currently available arrays with near whole exome coverage, and proper sample preparation, one can determine the expression of alternatively spliced forms in addition to estimating total transcripts from a gene.

2 Progress in B-cell Lymphomas

2.1 Diffuse Large B-cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma in adults and a heterogeneous group of B-cell lymphomas that has been further subdivided into specific entities in the WHO classification [1]. Prior to gene expression profiling, grouping was largely based on morphologic findings. In a seminal study by Alizadeh et al., GEP was used to define DLBCL with a germinal center B-cell-like (GCB) or the activated B-cell-like (ABC) [10] signature and the former showed a better overall survival when treated with CHOP. This study was validated by a subsequent larger study [11], and a recent study confirmed the relevance of this classification in the Rituximab (R)-(cyclophosphamide, doxorubicin, vincristine, and prednisone) CHOP era [12]. GCB and ABC DLBCL also differ in the profile of genetic alterations including copy number abnormalities (CNAs) and mutational landscape [13]. t(14; 18) has been noticed to be almost exclusive to GCB-DLBCL, while BCL6 rearrangement is present in both GCB and ABC types but more common in the latter. Many CNAs are present differentially in the different DLBCL subtypes [14]. With the combination of CNA and GEP data, it is possible to identify the likely candidate genes in the minimal common regions of gains or loss. For example, *SPIB*, which encodes an ETS family transcription factor that is normally expressed in lymphocytes and plasmacytoid dendritic cells and is required for normal germinal center reactions, was found to be amplified mostly in ABC DLBCL. Trisomy 3 or 3q+ was also found to be frequent in ABC DLBCL (26 %) compared to GCB-DLBCL (1 %). *FOXP1* copy number was also found to be increased in 38 % ABC compared to 3 % GCB-DLBCL [13]. In GCB-DLBCL,

gain or amplification of the c-rel locus (chromosome 2p) and the microRNA cluster 17–92 at 13q are often observed. Loss of *p14/16* is more common for the ABC subtype, while *PTEN* loss is related to the GCB subtype.

There is frequent constitutive activation of NF-κB and IRF4 [15] in ABC DLBCL. NF-κB activation in DLBCL may be mediated by B-cell receptor (BCR) signaling, which can be blocked by certain drugs such as ibrutinib that synergized with lenalidomide to block IRF4 and kill tumor cells in ABC DLBCL [16]. Chronic active BCR signaling is believed to be required for cell survival particularly in ABC DLBCL [17, 18], and this may be mediated through mutations in CD79B and rarely CD79A. Alternatively, NFκB signaling may be mediated by more downstream mutations such as *CARD11*, *A20*, *RANK*, *TRAF2* and *TRAF3*, and *BCL10*. *MYD88* (myeloid differentiation factor 88), which mediates TOLL and interleukin-1 receptor signaling, has also been implicated in the pathogenesis of ABC DLBCL, has been seen in 29 % [19] of this subtype as well as 90 % of lymphoplasmacytic lymphomas [20], and is likely involved in the activation of NF-κB through IRAK1 and IRAK4.

Rosenwald et al. [11] used 17 genes to create a multivariate model divided patients with DLBCL into distinct quartiles with 5-year survival rates ranging from 73 to 15 %. Lenz et al. [21] also evaluated the stromal environment of pre-treatment DLBCL and how this related to prognosis after R-CHOP therapy. They found two main stromal signatures: (1) fibrotic state with associated tumor macrophages and myeloid cells and (2) angiogenic-rich environment with neovascular signature. High expression of the latter (stromal-2 signature) was noted to have an adverse outcome compared to stromal-1 signature. Therapy that alters the tumor microenvironment may be promising in the treatment of DLBCL.

Multiple immunohistochemical (IHC) algorithms have been created by Hans et al. [22], Colomo et al. [23], Muris et al. [24], Meyer et al. [25], Visco et al. [12], and Choi et al. [26] (using various combinations of GCET1, LMO2, BCL2, BCL6, CD10, FOXP1, and MUM1) to help classify DLBCL in routine clinical practice as GCB or non-GCB subtype. In general, studies that correlated the algorithm with their corresponding GEP classification have reported 80–90 % correlation but studies that tried to use survival as the standard usually failed. Variability inherent in the IHC procedure when performed in different laboratories without strict adherence to protocol as well as inter-observer variability accounts for some of the discrepancies reported. However, survival should not be used as the indicator of validity of a classification algorithm as survival is influenced by many other factors such as the number of patients enrolled, the composition of the patient population, and the uniformity of treatment. Overall, GEP has had excellent correlation with prognosis and some novel therapies have preferential activities against one specific subtype [16, 27–30]; therefore, there is added incentive to accurately subclassify the DLBCL in the clinical setting. Recently, a robust method of cell-of-origin assignment has been demonstrated using the Lymph2Cx assay (a digital gene expression-based test utilizing NanoString technology) and FFPE tissue [31] (Fig. 1a, b). This method has demonstrated high accuracy when compared with the Affymetrix-based

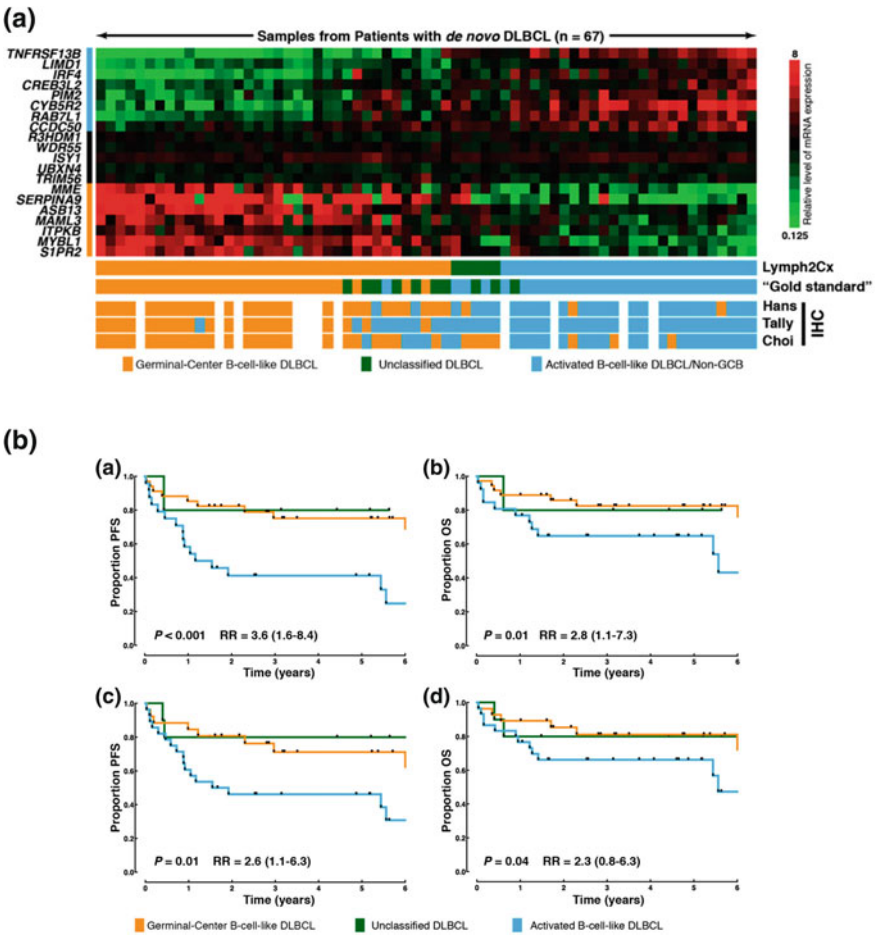


Fig. 1 **A** Performance of the Lymph2Cx assay using an abbreviated gene set (20 genes) compared to the "gold standard method" of previously reported GEP as well as three IHC-based algorithms [31]. **B** *a*, *b* show the progression-free survival (*PFS*) and overall survival (*OS*), respectively, determined by the Lymph2Cx assay. *c*, *d* show the PFS and OS determined by the "gold standard method" [31]

gold standard using a small selected set of genes with fast turnaround times, which will likely guide appropriate therapy and management in the future.

GEP has also proven to be useful in distinguishing a particular subgroup of DLBCL, primary mediastinal large B-cell lymphoma (PMBL). It has been found to have a unique signature that can distinguish it from ABC and GCB-DLBCL [32, 33]. PMBL showed a favorable clinical outcome comparable to GCB-DLBCL and an overlap with the gene expression signature of classical Hodgkin lymphoma cell lines. Interestingly, PMBL and Hodgkin lymphoma also share some common genetic changes such as gain or amplification of 9p24 (*JAK2*, *PDL1,* and *PDL2*)

[32] and genomic *CIITA* (MHC class II transactivator) breaks [34]. Eberle et al. [35] evaluated mediastinal gray zone lymphomas (B-cell lymphoma unclassifiable intermediate between DLBCL and classical Hodgkin lymphoma) by DNA methylation analysis and also found a close relationship between gray zone lymphomas and Hodgkin lymphoma as well as PMBL; however, there were important differences seen to justify the intermediate category in the current WHO classification.

For primary cutaneous large B-cell lymphomas, GEP has been particularly helpful in supporting the distinction of primary cutaneous follicle center lymphoma (PCFCL) from primary cutaneous DLBCL, leg type (PCLBL-leg type). Both diseases can have numerous large atypical cells, but the latter has a preference for the lower extremities. PCFCL also differs from PCLBL-leg type with the latter seen in older age, frequent extracutaneous involvement, and poorer prognosis [36]. The study by Hoefnagel et al. [37] further supported the distinction of these two entities by showing PCFCL and PCLBL-leg type have a GCB- and ABC-like expression profile, respectively. The PCLBL-leg type showed deregulation of several oncogenes regulating cell cycle function, such as *CMYC*, *PIM1*, *PIM2*, *MUM1*, and *OCT2*. The findings of an ABC-like phenotype in PCLBL-leg type and associated poor prognosis indicate that this DLBCL is similar to that of the ABC-like DLBCL, NOS.

Primary CNS lymphoma (PCNSL) has also recently been evaluated by GEP showing downregulation of 8 genes associated with extracellular matrix, cell adhesion (*FNDC1*, *EMCN*, *COL12A1*), and other genes involved in mediation of cell signaling (*OSMR*, *C4orf7*, *NPFFR2*, *TPO*, and *MSC*) [38] when compared to peripheral blood of healthy males. This study by Sung et al. found that PCNSL shows a different expression profile compared to DLBCL, NOS with genes associated with extracellular matrix, and cell adhesion (*FNDC1*, *EMCN*, *COL12A1*) contributes to the pathogenesis of PCNSL. They also were able to identify increased expression of JAK-1 [38, 39], suggesting the JAK-STAT pathway may have a significant role in the pathogenesis of PCNSL.

2.2 Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) is typically an aggressive disease with poor prognosis. The hallmark translocation for MCL is the t(11;14) involving the *CCND1* gene. The prognosis of this disease is correlated with the deregulation of cellular proliferation [40], and more aggressive disease may demonstrate high chromosomal instability secondary to disruption to the DNA damage response [41]. Hartmann et al. [42] showed deregulation of the Hippo signaling pathway, which appears to be important in the regulation of proliferation and mitotic checkpoints. Although the majority of MCL is cyclin D1 positive, negative variants [43] have been controversial in the past, but recent studies have supported the existence of this variant, which appears to behave similarly to cyclin D1-positive forms [42] (Fig. 2). SOX11 has been found to be highly expressed in MCL and is a component of the diagnostic signature. Recently, immunostaining for SOX11 has been found to be a useful adjunct in the diagnosis of MCL, particularly for the cyclin D1-negative cases [44].

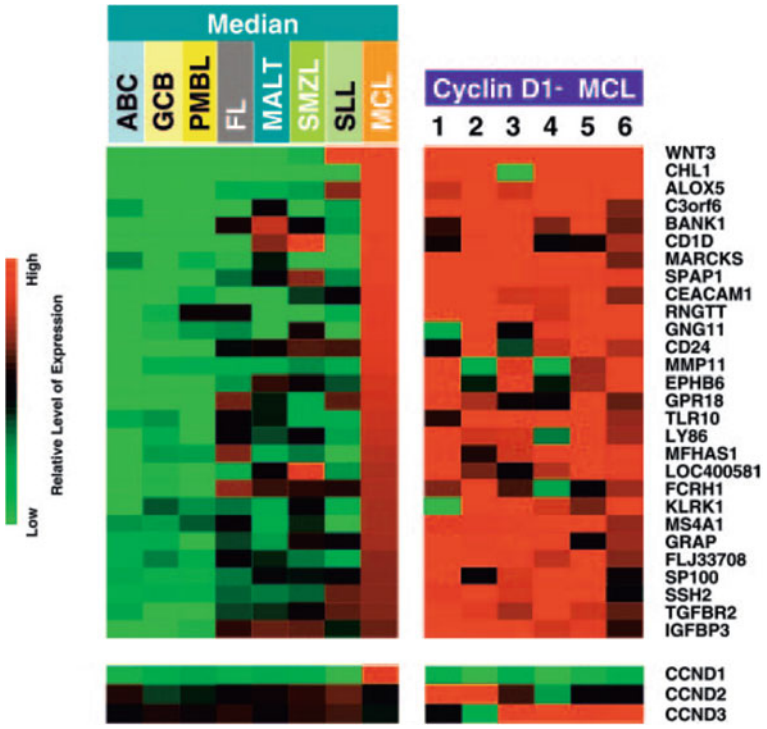


Fig. 2 Expression profiles of various B-cell lymphomas and cyclin D1-positive mantle cell lymphoma (22 cases) compared with 6 cases of cyclin D1-negative MCL. The cyclin D1-negative MCL cases showed gene expression signatures consistent with cyclin D1-positive cases [43]

Not all MCLs have aggressive disease and there are indolent forms seen as well. These patients typically have no nodal disease and present with a leukemic form, having hypermutated *IGVH* without complex karyotypes [41]. SOX11 protein is expressed in nearly all conventional MCLs, even cyclin D1-negative MCLs [44]. Fernandez et al. [45] suggest from their study that the lack of SOX11 protein expression in MCLs may identify a subset of patients with a more indolent behavior. These cases typically lacked *p53* mutations, and *ATM*, or *CDKN2a* deletions, which are frequently seen in conventional MCLs. Recently, Liu et al. [46] looked at the tumor microenvironment of MCL and chronic lymphocytic leukemia (CLL) by GEP focusing particularly on three gene signatures (BCR, NF-κB, and MCL proliferation) and found a twofold increase in the expression of these genes in the lymph node samples compared to the peripheral blood, suggesting that MCL relies on the lymph node microenvironment for BCR engagement, cell activation, and proliferation. BCR signaling may be highly important in MCL, and this is reflected in the excellent response in patients with MCL treated with the BTK inhibitor ibrutinib.

2.3 Follicular Lymphoma

Follicular lymphoma (FL) is the second most common type of lymphoma, typically an indolent disease, and 90 % of the cases carry the classical translocation t(14;18) involving *IGH*/*BCL2* gene. The current classification divides the tumor into grades 1–2, 3A, and 3B; however, the latter behaves more like DLBCL. GEP also supports the distinct grouping of the grades [47]. A GEP study in 2004 showed two signatures in the microenvironment, (1) immune response 1, which included T-cell-associated transcripts as well as those that were expressed in macrophages and (2) immune response 2, which were enriched in genes that were expressed in macrophages, dendritic cells, or both. Immune response 1 profile was found to be associated with a favorable survival prediction compared to the second immune response profile [48]. Recent analyses of copy number alterations (CNA) and gene mutation have provided greatly increased information on the pathogenesis and progression of FL [49–51]. This includes frequent abnormalities of genes involved in chromatin modification. One prominent example is *EZH2* mutations that have been described in DLBCL as well as FL (7–22 %). Normally, EZH2 is a methyltransferase involved in repressing gene expression through trimethylation of histone 3 lysine 27 (H3K27). *EZH2* mutants in lymphoma are gain-of-function mutations leading to increased trimethylation of H3K27 [52], which contributes to lymphomagenesis in germinal center B cells [53]. *EZH2* mutated cases are more frequently

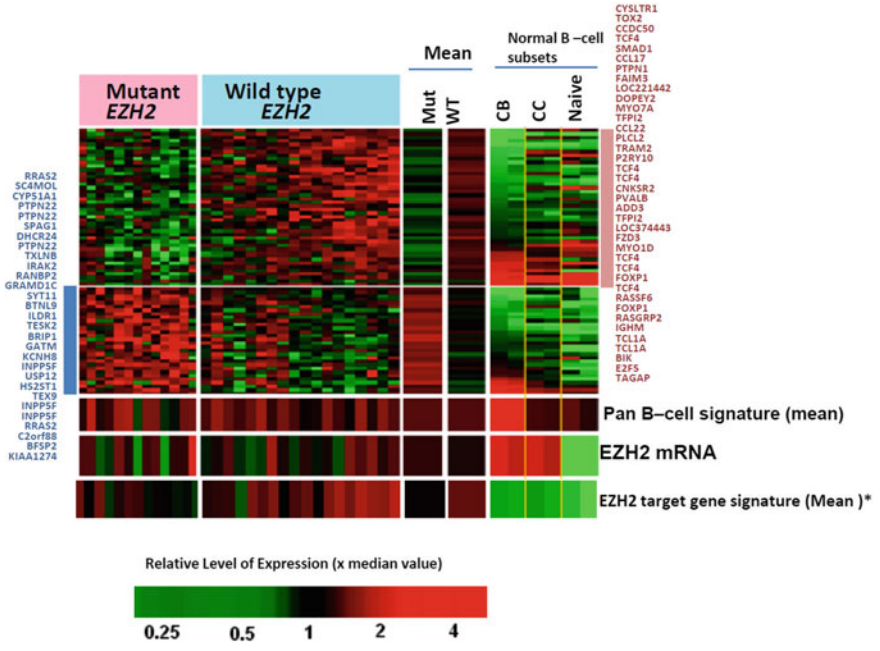


Fig. 3 Differential gene expression between EZH2 mutant versus wild-type FL cases ($p < 0.01$; >1.5-fold changes) [128]

seen in the *BCL2* rearranged group and show downregulation of *several genes important for differentiation beyond the GCB cell stage* and *TCL1A* (confirmed by IHC) and upregulation of *PTPN22* (Fig. 3). Inhibitors of EZH2 mutants have been developed and could have potential impacts on the management of DLBCL and FL with the mutation. A detailed discussion of all these genetic abnormalities is beyond the scope of this review, but it would be interesting to correlate GEP findings with the various genetic changes to gain a better understanding of the functional consequences associated with the alterations in future studies.

Approximately 30–40 % of FLs undergo transformation to DLBCL. Although clonal relationship can be seen in paired samples of FL and transformed follicular lymphoma (tFL), currently no unifying genetic abnormalities are identified for transformation to DLBCL although some abnormalities are more common in transformed FL [49, 51]. Recurrent alterations were noted mainly in cell cycle control (*MYC*, *CDKN2A/B*), DNA damage response (*TP53*), immunosurveillance, and the NF-κB pathway supporting these changes are involved in various combinations in the transformation of FL [49]. Interestingly, while the majority of cases of tFL had a phenotype similar to GCB-DLBCL, transformation may be associated with a change to a more ABC-like profile. The exact mechanism of transformation requires further studies.

2.4 Burkitt Lymphoma

Burkitt lymphoma (BL) typically has a translocation of the *MYC* gene with one of the immunoglobulin loci, but MYC translocation is not specific for BL, and it can also be seen in DLBCL (5–10 % of cases) and other lymphomas. DLBCL typically does not have an immunoglobulin gene partner for *MYC* and has complex chromosomal abnormalities. The distinction between BL and DLBCL is important as the treatment is significantly different. DLBCL typically receives standard chemotherapy (R-CHOP), while BL requires intensive therapy with good response. GEP has improved the distinction between DLBCL and BL [54, 55] but not entirely as there are still cases that cannot be definitively diagnosed even with GEP. Currently, the diagnosis is based on the morphologic, immunophenotypic, and genetic findings, but there are cases that cannot be placed in one or the other category and have been designated as gray zone lymphomas (B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL). Cases that were molecularly defined BL based on high-level MYC, low-level MHC I, unique GCB signature, and low NF-κB signature, which were treated with a very intensive regimen (for BL), had an 80 % 5-year survival as compared to those with a CHOP-like regimen (20 % 5-year survival) [54]. These findings support the necessity of the distinction between these two entities for choosing the appropriate therapy. Recently, BL has been found to have a distinctive mutation landscape compared with DLBCL such as the marked prevalence of *ID3* (inhibitor of DNA binding) [56, 57] and transcription factor 3 (*TCF3*) [58], mutations that promote cell cycle progression and

proliferation. This finding may also be exploited in the diagnosis and treatment of BL. BL may arise in different settings such as endemic versus sporadic cases, HIV+ background, and EBV status that may have different genetic abnormalities and GEP.

2.5 Other B-cell Lymphomas

Marginal zone lymphoma (MZL) is a challenging diagnosis due to the lack of specific markers in many cases, heterogeneity in the morphologic features, and it is typically considered after other lymphomas (e.g., follicular, CLL) have been excluded. The distinction and interrelationship of nodal marginal zone lymphoma (NMZL), extranodal marginal zone lymphoma, MALT type (EMZL), and splenic marginal zone lymphoma (SMZL) is not entirely clear. EMZL is characterized by the *API2-MALT1*, *BCL10-IGH,* and *IGH-MALT1* translocation, which commonly activates the NF-κB pathway. When gastric EMZL develops these translocations, they typically do not respond to *H. pylori* eradication. Increased gene expression in translocation-positive EMZLs of Toll-Like Receptors (*TLR6*, *TLR7*) and chemokine receptors (*CCR2*, *CXCR4*, *CCR6*, *CCR7*) may contribute to the persistent activation of NF-κB, leading to prolonged survival even after the obliteration of the microbe [59]. Arribas et al. [60] used GEP and miRNA expression pattern to compare SMZL with disseminated NMZL and found distinguishing patterns. Similar to a prior study [61], they found that NF-κB and CD40 pathways appear to be important in the pathogenesis of SMZL. In another recent study, Arribas et al. [62] used gene set enrichment analysis (GSEA) on 15 NMZL cases and further validated their findings on 61 paraffin-embedded NMZLs. Their study confirmed similarity of the lymphoma cells to memory B cells, as well as upregulation of genes that are important in immune response. They also found a large number of genes that are associated with NF-κB signaling pathway that are upregulated (*CD74*, *CD81*, *CD82*, *RELA*, and *TRAF4*). Also, the authors note that TACI (TNFRSF13B), a transmembrane activator that has multiple functions involved in apoptosis regulation and NF-κB activation, could be a new candidate gene for NMZL. However, unlike LPL and ABC DLBCL, MYD88 mutations are not commonly seen in NMZLs [63].

2.6 Peripheral T-cell Lymphomas

Peripheral T-cell lymphomas (PTCL) are relatively uncommon (10 % of all lymphomas) and often difficult to diagnose. Although there are many histologic subtypes, which include anaplastic large cell lymphoma (ALCL), angioimmunoblastic T-cell lymphoma (AITL), adult T-cell lymphoma/leukemia (ATLL), and extranodal NK/T-cell lymphoma of the nasal type, a large number of cases are placed in the not otherwise specified category (PTCL, NOS). Genetic abnormalities have been

reported in PTCLs, but until recently, none have been recurrent with the exception of ALK-positive ALCLs, which is characterized by the classic t(2;5) and its variants. GEP has recently improved [64] our diagnosis and understanding of the pathobiology of PTCLs and justification of the subtypes.

Extranodal NK/T-cell lymphomas (ENKTCL) and ATLL have shown highly specific classifiers by GEP with little overlap with other entities. ENKTCLs share similar signatures with normal activated NK cells. NK and gamma–delta T-cell lymphomas within ENKTCL also have very similar gene expression profile reflecting their origin from the innate immune system [65]. ENKTCL has the classic findings of angioinvasion/angiocentricity, which can be highlighted by the gene expressed (*VCAM1*, *CXCL9*, and *CXCL10*) that are associated with endothelium interaction or in the pathogenesis of the vascular damage associated with EBV-positive lymphoid proliferations [66]. In a study by Huang et al. [67], they found the receptor tyrosine kinase PDGFR α was expressed at a higher level compared to normal NK cells and in vitro the ENKTCL cell line was sensitive to imatinib. Although ENKTCL may share some similarities with cytotoxic PTCLs, the prior shows greater transcript levels of cytotoxic markers, particularly granzyme H. Another study on FFPE samples showed increased expression of antiapoptotic genes as well as activation of *MYC*, *NF-κB*, and deregulation of *TP53* in ENKTCLs [68]. Deletion of chromosome 6q21 appears to be characteristic of ENKTCL (40 % of the cases as well as cell lines), which may be associated with loss of function mutations and more commonly methylation of PRDM1, a tumor suppressor gene [67, 69]. Methylation of a few other genes (*SHP1*, *p73*) [70, 71], of interest has also been reported, and global methylation studies with correlation to GEP may lead to the discovery of additional tumor suppressor genes.

ATLL has a specific geographic distribution, being endemic in southwest Japan, Caribbean, Middle-East, Papua New Guinea, and South America. The disease is associated with the retrovirus, HTLV-1. HTLV-1 encodes the oncogenic protein, Tax, which dysregulates the cell cycle checkpoints as well as suppresses DNA damage repair [72]. GEP has shown a robust classifier that includes the expression of TAX and its target genes (*IL2*, *IL2RA, IL15R*, *GMCSF*, and *TNF-α*) [72]. There is also increased expression of TSLC1 (tumor suppressor in lung cancer-1) which may enhance aggregation and adhesion to endothelial cells [73]. The increase in BIRC5 (survivin) expression in ATLL may play a role in cell survival by inhibiting apoptosis and is associated with resistance to chemotherapy and poor prognosis. CD25 (TAC) tends to be highly expressed in ATLL, and attempts have been made to target this using monoclonal antibody–toxin conjugates [74–76]. More recently, CCR4 (chemokine receptor 4) expression has been reported to be frequent in ATLL [77] and may account for the frequent infiltration of the lymphoma in skin and lymph nodes. Mogamulizumab (anti-CCR4) has exhibited antitumor effect in patients with relapsed PTCL and cutaneous T-cell lymphomas [78, 79]. BIRC5 can be a potential target for therapeutics and be used in conjunction with conventional therapy for ATLL [80].

AITL has shown a prominent signature of T follicular helper cells (TFH) as well as B cell, follicular dendritic cell, and angiogenesis-related microenvironment

genes. Interestingly, the prognosis of AITL patients is highly related to the stromal signature expressed [81]. Isocitrate dehydrogenase 2 (IDH2) mutations can be seen in gliomas and acute myeloid leukemia but has not been detected in other PTCLs or subtypes and appears to be fairly specific for AITL and seen in 30–40 % cases [82]. IDH enzymes normally convert isocitrate to α-ketoglutarate (α-KG); however, cancer-associated mutations result in the acquisition of new enzyme activity that converts α-KG to 2-hydroxyglutarate (2HG) which leads to DNA and histone hypermethylation through the inhibition of 2 oxoglutarate dependent dioxygenases including histone demethylases and the TET family of DNA demethylase enzymes [83]. It appears there is no prognostic implication between IDH2 mutated and non-mutated forms; however, these findings may have therapeutic implications as these tumors may be sensitive to reversing DNA hypermethylation with drugs such as 5-azacytidine or drugs specifically inhibiting IDH2 mutant activity.

ALK-negative ALCL has been included in the current 2008 WHO classification as a provisional entity although some have felt that these tumors are better classified under PTCL, NOS. IHC and morphologic findings suggest that this entity is distinct from PTCL-NOS with more similarities with the ALK+ ALCL. Earlier studies [84] by GEP have shown that ALK-negative ALCLs are closely related to ALK+ ALCLs but separated from PTCL, NOS, which has been confirmed with more recent studies [64, 84, 85]. Salaverria et al. [86] evaluated ALCLs by comparative genomic hybridization (aCGH) and found gains in 17p and 17q24-qter with losses in 4q13-q21 and 11q14 were associated more with ALK+ tumors, while 1q and 6p21 gains were more common in the ALK-negative group. Iqbal et al. [81] was able to develop a diagnostic signature for ALK-negative ALCLs that included the three genes (*TNSFR8*, *BATF3*, and *TMOD1*) signature reported previously. They further showed significantly increased expression of MYC and IRF4 target signatures, as well as proliferation and mTOR pathway signatures compared to PTCL, NOS [64]. ALK-negative ALCLs may benefit from aurora kinase inhibitors in conjunction with drugs that target PI3-kinase/AKT pathway [87]. The STAT3 signaling pathway [84] is activated in ALK+ ALCL and appears to be a major mechanism which promotes oncogenesis and suppresses antitumor immunity [84]. It could be a promising additional target for therapy aside from the inhibition of ALK+ [88–90]. As both ALK+ and ALK-negative ALCL highly express CD30, they are both good candidates for therapy with brentuximab vedotin [91, 92].

With GEP, many of the cases from the PTCL, NOS group can be reclassified into other established categories, but there is still a large group of cases that remain in this group. Recently, two major distinct subgroups with significantly different outcomes can be separated by GEP [64] (Fig. 4). The first group has high expression of GATA3, which appears to be associated with a poor survival compared to the other group that is TBX21 high. Within the latter group, two additional groups were identified, one with a high cytotoxic T-cell transcript expression and the other with a low transcript expression. The cytotoxic-high subgroup showed a poorer outcome compared with the cytotoxic-low subgroup. Poor clinical outcome of cases with high expression of GATA3 was confirmed by an independent study using IHC assays [93]. Patients within the GATA3 and TBX21 subgroups

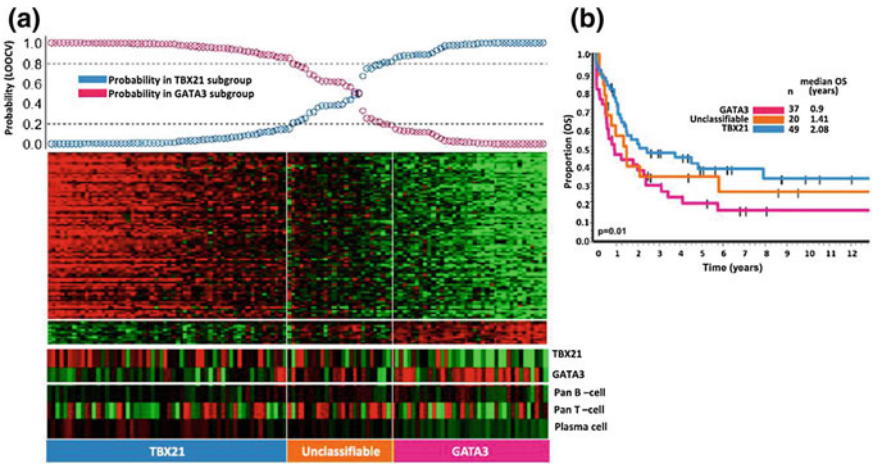


Fig. 4 Two major molecular subgroups within PTCL, NOS with biological and overall survival difference. The overall survival between the TBX21 subgroup and GATA3 subgroup was significantly different [64]

preferentially express different pathways and may benefit from drugs targeting some of these pathways such as mTOR inhibitors (e.g., rapamycin, temsirolimus).

Although there has been a significant progress in the further characterization and classification of mature T-cell and NK cell lymphomas, there are still rare subtypes (e.g., many of the extranodal T-cell lymphomas) that require additional studies and collaboration from multiple large institutions will be necessary to obtain sufficient cases for meaningful studies of these rare entities.

3 Future Directions in GEP

Evaluating lymphomas by GEP has expanded our understanding of the biology and pathogenesis of these tumors, but the power of these studies will be much enhanced from the combination of GEP with additional genetic analysis, e.g., chromosome CNAs and somatic mutations as well as DNA and histone methylation status.

Both single nucleotide polymorphism arrays (SNP-A) and array comparative genomic hybridization (aCGH) have been extremely powerful in karyotypic analysis and have markedly improved resolution over conventional metaphase cytogenetics. SNP-A have advantages over aCGH and conventional cytogenetics in that it is able to detect copy number neutral loss of heterozygosity or uniparental disomy. These technologies are objective, can be automated and systematically analyzed using biostatistical algorithms [94], and have proven to be powerful discovery platforms in multiple hematologic malignancies from plasma cell myeloma [95], ALL [96], to myeloid disorders including AML [97] and MDS [98]. Aberrant DNA methylation is common in hematologic malignancies, and abnormal hypermethylation may lead to inactivation of tumor suppressor genes, while hypomethylation

may lead to activation of oncogenes. Correlation of the corresponding data from GEP can be very helpful when interpreting results from these studies. GEP not only provides the expression levels of the genes of interest but also functional data of pathway activation. This information will help to identify likely candidate genes among the genes in different loci of interest [99]. It may also help to identify driver mutations, and putting mutated genes into functional pathways from a list of genes found to harbor somatic mutations. Correlative studies may not yield the desired information because of the low expression of the gene in tumor cells or expression in multiple cell types in the tumor or low tumor content. It may also be due to incomplete information in studies such as the lack of a complete picture of the methylation status of a gene [100].

While the study of protein coding transcripts has yielded highly useful information, it is now clear that noncoding RNAs are also a highly significant and important component of the transcriptome. MicroRNAs (miRNAs) are small regulatory RNAs that post-transcriptionally regulate the expression of large groups of genes that play a critical role in a broad range of biological processes [101, 102]. MiRNAs can be found in blood as well as tissue and are generally well preserved and can be extracted from FFPE tissue, which makes them useful biomarkers for investigation from archival materials including serum or plasma. MiRNAs have been found to be important in the pathogenesis of B lymphoblastic leukemias (miR-155) [103], MCL (miR-17-92) [104], CLL (miR-15a/16-1 cluster) [105], ALCL (miR-17-92), and BL (miR-28) [106]. MiR-155 has also been found to be dramatically elevated in a number of B-cell lymphomas (Hodgkin lymphoma, ABC DLBCL, PMBL, BL) [107, 108]. Overexpression of miR-155 activates the PI3K-AKT signaling pathway likely through the inhibition of p85α [109] and thus increases cell viability. Interestingly, while miR-17-92 is a MYC target, many miRNAs have been shown to be downregulated by the MYC oncogene and their repression may contribute to lymphomagenesis. A more comprehensive review is beyond the scope of this article, and the readers are referred to excellent reviews on this topic [110–112]. The role of miRNAs in lymphomas is an exciting and important discovery that will complement the data obtained through GEP of protein coding genes.

To further add to the vast information in the transcriptome is the recent discovery of long noncoding RNAs (lncRNA), which may participate in almost every step of a gene's life cycle from transcription, splicing, decay to translation [113]. Therefore, one could imagine alterations in lncRNA expression could be highly correlated with disease. LncRNAs have been implicated in the prognosis of breast, lung, and liver cancers, but the exact role of these noncoding RNAs is yet to be defined. Preliminary data have been recently presented at national meetings and have shown exciting implications of lncRNAs in lymphomas such as MCL and Hodgkin lymphoma [114]. Coding RNA may on occasion affect the function of ncRNA by acting as a competitive inhibitor through multiple binding sites as the case of HMGA2 and the Let7 family of miRNAs [115]. With the recent advances in the area of RNA biology, GEP will be expanded to gain a better understanding of the role of noncoding RNA in lymphomagenesis and disease progression.

4 Evolution of the Platform in GEP

With the advent of next-generation sequencing (NGS), it is now possible to obtain the whole transcriptome of a tumor sample at sufficient depth for gene expression profiling and at a reasonable cost comparable to array-based GEP. Technical problems that may be associated with probe hybridization would be eliminated, but transcripts could still contain regions that are difficult to sequence. Microarrays generally lack sensitive in the range of a low copy number transcript, but with sufficient coverage, sequencing is much better in quantitating these low-level transcripts. Alternatively, spliced forms can be readily quantitated by transcriptome sequencing, and in addition, fusion transcripts and somatic mutations can be detected through sequencing technology but not by the microarray platform. For example, gene fusion involving MHC class II transactivator (*CIITA*) was identified in Hodgkin lymphoma and primary mediastinal B-cell lymphoma [34]. *TP63* rearrangements were identified in peripheral T-cell lymphoma using RNA-seq, and further studies confirmed that the rearrangement occurred in 11 (5.8 %) of 190 cases, which was associated with inferior overall survival [116]. The expression of immunoglobulin genes, T-cell receptor genes or killer inhibitory receptors genes (*KIR*), and C-type lectin molecules in NK cells can also be readily detected and quantitated through transcriptome sequencing. This is useful in clonality analysis as well as in determining the repertoire of immune receptor expression in particular individuals or conditions. However, whole transcriptome sequence analysis involves a much larger data set, sequence alignment, and greater complexity in their analysis. With the development of robust and user friendly analytical programs, it is likely that more and more routine molecular biology laboratories would be able to employ this platform for their studies.

GEP relies on RNA extracted from cells or tissue samples with a varying degree of cellular heterogeneity. There is substantial interest in obtaining GEP from specific cellular populations or even single cells [117] to unravel the complexities inherent in the study of mixed populations. This can be achieved by tissue microdissection or flow sorting of disaggregated cells [118, 119]. Recently, Fluidigm has introduced a platform (BioMark HD) that uses microfluidic technology to extract mRNA from single cells and synthesizing cDNA on the same device allowing large-scale single cell GEP [120].

5 Translating Signatures to Routine Clinical Practice

One can argue that obtaining global gene expression profiling data through microarray or transcriptome sequencing would be highly valuable for personalized medicine. In a clinical trial setting, the effectiveness of pathway-directed therapy can be evaluated by correlating between pathway activation and response to the drug administered. For example, one can evaluate whether lymphomas with STAT3 pathway activation are more likely to respond to JAK1 and 2 inhibitors. If that is

proven, then STAT3 activation can guide the selection of specific inhibitors in the future. In another scenario, there may be strong preliminary evidence to suggest that a drug will be effective for a certain type of lymphoma and it is desirable to perform additional validation studies, and if it is validated, then subsequent therapy can be administered according to the type of tumor present. For example, there is evidence that ibrutinib is specifically effective against the ABC type of DLBCL but not the GCB type of DLBCL. It would be important to validate this finding, and if validated, subsequent treatment should be administered to patient with the ABC but not GCB type of tumors. Accurate classification of DLBCL becomes important in the validation studies and possibly subsequent therapeutic intervention. The gold standard for the ABC versus GCB classification is the reported microarray-based GEP signature [10, 11]. There have been a number of reported IHC algorithms attempting to reproduce the GEP classification through the combination of a number of immunostains [22, 23]. Due to the difficulties in the standardization of IHC assays, it may not be an ideal assay for use across numerous institutions. It is possible to reduce the original diagnostic signature to a small set of transcripts and yet retaining the diagnostic power. Traditionally, assays such as quantitative RT-PCR have been used to quantitate these transcripts. There are several new technologies available that are highly sensitive and quantitative such as the RNA protection-based assay by high-throughput genomics or the NanoString nCounter technology.

NanoString nCounter technology has been developed to capture and count specific nucleic acid molecules using color-coded probes [121]. Briefly, the technology employs a capture probe and a reporter probe per target mRNA. The former is coupled to a tag such as biotin for binding to a surface, and the latter provides the detection signal. The probes are first hybridized to the mRNA and then captured on a surface and oriented, and each color code generated from the reporter is counted (Fig. 5). The sensitivity and accuracy of nCounter are similar to that of real-time PCR but does not need any enzymatic reactions [121]. The current NanoString nCounter platform is able to measure up to 800 transcripts on one setting [122]. Payton et al. [123] studied a subtype of acute myeloid leukemia using NanoString nCounter, and the results were highly concordant with their microarray data. Several studies have evaluated whether formalin-fixed and paraffin-embedded (FFPE) samples are suitable for the NanoString nCounter technology. A study evaluated 20 genes and found a higher correlation coefficient between fresh-frozen and FFPE samples was obtained compared to quantitative real-time PCR [124]. Other studies also indicated that archival FFPE tissues can be used for retrospective transcriptome-based class prediction analysis [125]. As mentioned above, a recent report has demonstrated that a microarray-derived classifier for ABC versus GCB-DLBCL can be transferred to the NanoString platform and provide robust classification results [31]. Nuclease protection assay (NPA) is a highly reproducible and sensitive method for detection and quantitation of mRNA levels. This assay utilizes antisense probes to capture specific mRNA from the tissue, and unhybridized RNAs are digested using S1 nuclease. The captured RNA will hybridize to immobilize complementary probe at one end and linked to probes for signal detection at the other end (Fig. 6).

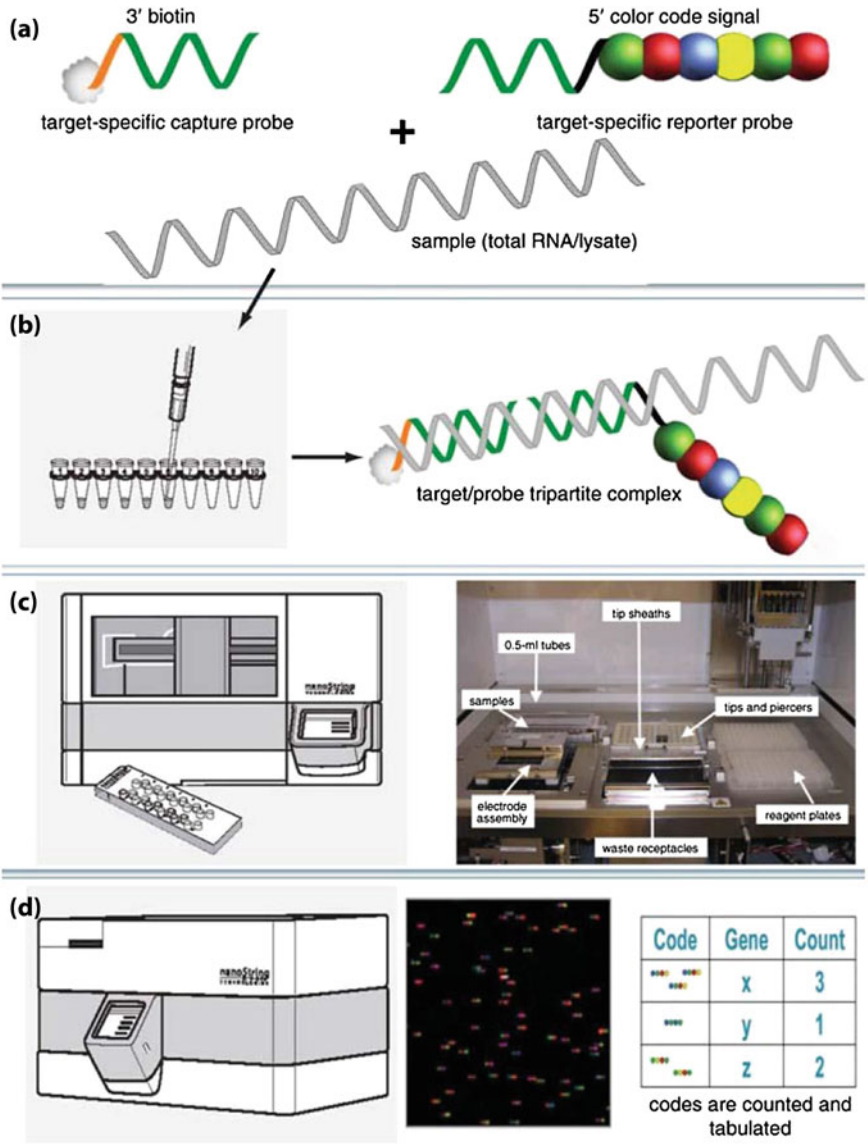


Fig. 5 nCounter gene expression assay: **a** the *orange* segment in the capture probe represents a 30-base sequence common to all capture probes, and the *black* segment in the reporter probe represents a 60-base sequence common to all reporter probes, **b** hybridization results in the formation of tripartite structures comprising target mRNA bound to specific capture and reporter probes, **c** strip tubes containing tripartite complexes are placed on the prep station (*left*) for automated post-hybridization processing. The deck layout is shown on the *right*, **d** the loaded sample cartridge is transferred to the digital analyzer (*left*) for imaging and data processing. A raw image of *color-coded* reporter probes bound to complementary target mRNA is shown (*middle*) along with a schematic of the tabulated counts for target genes (*right*) [122]

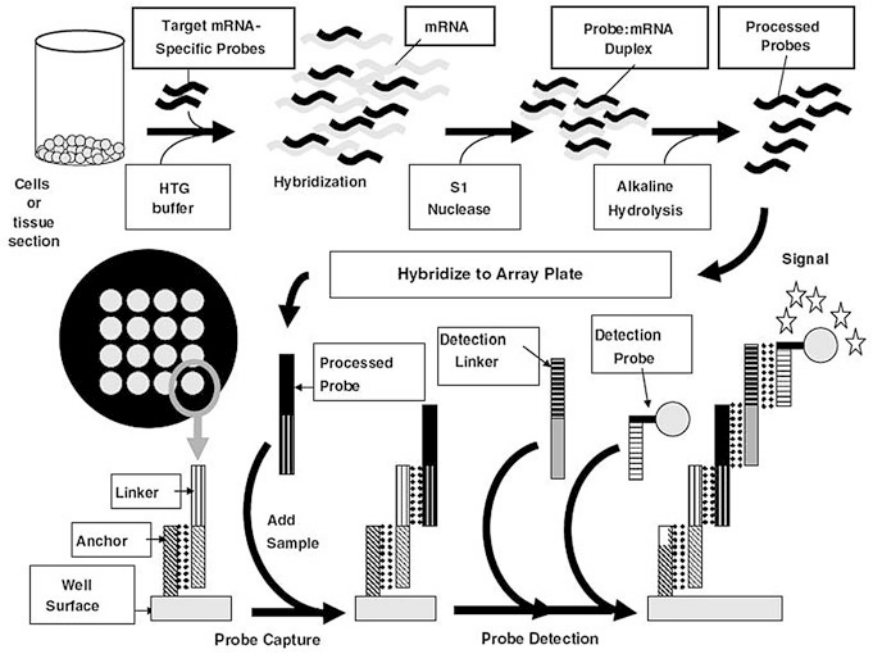


Fig. 6 Quantitative nuclease protection assays: A mixture of custom programming linkers is designed to hybridize to a specific anchor and capture a gene-specific nuclease protection probe (*shown for one linker*). The array now captures probes for a custom set of genes. The probes hybridize to all mRNA, are soluble, and are cross-linked. S1 nuclease destroys nonspecific RNA producing a stoichiometric amount of target mRNA/probe duplexes. Probes are transferred to a programmed array plate well and detection linker added, and both probes and detection linkers captured onto the array [126]

This method also analyzes mRNA without the necessity of extraction followed by qRT-PCR [126]. Recently, several studies have used quantitative NPA to detect specific gene expression profiles in DLBCL, and the results were highly correlated with the data acquired from microarray studies [14, 127].

These new platforms are promising in translating defined signatures to clinical practice. However, the instrument costs could be substantial, but it is likely that many useful diagnostic, prognostic, or treatment relevant signatures will come up in the future and can be specifically translated to one of these platforms for more ready application in the clinical setting.

6 Summary

Although we are seeing an explosion in the area of sequencing, microarray studies have been shown to provide important insight in the biology and improvement of the classification of lymphomas. Molecular studies will provide important

information that will factor into the management and treatment of a particular patient's lymphoma. Obtaining fresh or frozen tissue is often challenging in the clinical setting, progress has been made to translate the findings to a platform or format that is applicable to FFPE tissues.

It is clear that one platform will not yield all the pertinent information; therefore, it is important to evaluate data from GEP in the context of other genomic data (e.g., methylation, aCGH, noncoding RNAs). The information from multiple platforms can further specify the tumor type and likely identify novel targets and specific drugs for these targets. Having all the transcriptome information on every sample is likely not an efficient method in the clinical setting; thus, focused transcriptome platforms that yield important information regarding the prognostic and treatment implication will likely be paramount in this advent of personalized medicine.

References

1. Swerdlow S, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (2008) WHO classification of tumours of haematopoietic and lymphoid tissues. International Agency for Research on Cancer, Lyon
2. Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270(5235):467–470
3. Augenlicht LH, Taylor J, Anderson L, Lipkin M (1991) Patterns of gene expression that characterize the colonic mucosa in patients at genetic risk for colonic cancer. Proc Natl Acad Sci USA 88(8):3286–3289
4. Sherlock G (2000) Analysis of large-scale gene expression data. Curr Opin Immunol 12 (2):201–205
5. Shi L, Reid LH, Jones WD et al (2006) The microarray quality control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol 24(9):1151–1161
6. Shi L, Campbell G, Jones WD et al (2010) The microarray quality control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models. Nat Biotechnol 28(8):827–838
7. Godfrey TE, Kim SH, Chavira M et al (2000) Quantitative mRNA expression analysis from formalin-fixed, paraffin-embedded tissues using 5′ nuclease quantitative reverse transcription-polymerase chain reaction. J Mol Diagn 2(2):84–91
8. Bibikova M, Talantov D, Chudin E et al (2004) Quantitative gene expression profiling in formalin-fixed, paraffin-embedded tissues using universal bead arrays. Am J Pathol 165 (5):1799–1807
9. Johnson JM, Castle J, Garrett-Engele P et al (2003) Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. Science 302(5653):2141–2144
10. Alizadeh AA, Eisen MB, Davis RE et al (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403(6769):503–511
11. Rosenwald A, Wright G, Chan WC et al (2002) The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 346 (25):1937–1947
12. Visco C, Li Y, Xu-Monette ZY et al (2012) Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the international DLBCL Rituximab-CHOP consortium program study. Leukemia 26 (9):2103–2113

13. Lenz G, Wright GW, Emre NC et al (2008) Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. Proc Natl Acad Sci USA 105(36):13520–13525
14. Takeuchi I, Tagawa H, Tsujikawa A et al (2009) The potential of copy number gains and losses, detected by array-based comparative genomic hybridization, for computational differential diagnosis of B-cell lymphomas and genetic regions involved in lymphomagenesis. Haematologica 94(1):61–69
15. Shaffer AL, Emre NC, Romesser PB, Staudt LM (2009) IRF4: immunity. Malignancy! Therapy? Clin Cancer Res 15(9):2954–2961
16. Yang Y, Shaffer AL 3rd, Emre NC et al (2012) Exploiting synthetic lethality for the therapy of ABC diffuse large B cell lymphoma. Cancer Cell 21(6):723–737
17. Lenz G, Davis RE, Ngo VN et al (2008) Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science 319(5870):1676–1679
18. Davis RE, Ngo VN, Lenz G et al (2010) Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 463(7277):88–92
19. Ngo VN, Young RM, Schmitz R et al (2011) Oncogenically active MYD88 mutations in human lymphoma. Nature 470(7332):115–119
20. Yang G, Zhou Y, Liu X et al (2013) A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in waldenstrom macroglobulinemia. Blood 122(7):1222–1232
21. Lenz G, Wright G, Dave SS et al (2008) Stromal gene signatures in large-B-cell lymphomas. N Engl J Med 359(22):2313–2323
22. Hans CP, Weisenburger DD, Greiner TC et al (2004) Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103(1):275–282
23. Colomo L, Lopez-Guillermo A, Perales M et al (2003) Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. Blood 101(1):78–84
24. Muris JJ, Meijer CJ, Vos W et al (2006) Immunohistochemical profiling based on Bcl-2, CD10 and MUM1 expression improves risk stratification in patients with primary nodal diffuse large B cell lymphoma. J Pathol 208(5):714–723
25. Meyer PN, Fu K, Greiner TC et al (2011) Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. J Clin Oncol 29(2):200–207
26. Choi WW, Weisenburger DD, Greiner TC et al (2009) A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res 15(17):5494–5502
27. Dunleavy K, Pittaluga S, Czuczman MS et al (2009) Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood 113 (24):6069–6076
28. Brown JR (2013) Ibrutinib (PCI-32765), the first BTK (Bruton's tyrosine kinase) inhibitor in clinical trials. Curr Hematol Malig Rep 8(1):1–6
29. Cerchietti LC, Yang SN, Shaknovich R et al (2009) A peptomimetic inhibitor of BCL6 with potent antilymphoma effects in vitro and in vivo. Blood 113(15):3397–3405
30. Cerchietti LC, Ghetu AF, Zhu X et al (2010) A small-molecule inhibitor of BCL6 kills DLBCL cells in vitro and in vivo. Cancer Cell 17(4):400–411
31. Scott DW, Wright GW, Williams PM et al (2014) Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin embedded tissue. Blood 123:1214–1217

32. Rosenwald A, Wright G, Leroy K et al (2003) Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to hodgkin lymphoma. J Exp Med 198(6):851–862
33. Savage KJ, Monti S, Kutok JL et al (2003) The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. Blood 102(12):3871–3879
34. Steidl C, Shah SP, Woolcock BW et al (2011) MHC class II transactivator CIITA is a recurrent gene fusion partner in lymphoid cancers. Nature 471(7338):377–381
35. Eberle FC, Rodriguez-Canales J, Wei L et al (2011) Methylation profiling of mediastinal gray zone lymphoma reveals a distinctive signature with elements shared by classical Hodgkin's lymphoma and primary mediastinal large B-cell lymphoma. Haematologica 96 (4):558–566
36. Grange F, Bekkenk MW, Wechsler J et al (2001) Prognostic factors in primary cutaneous large B-cell lymphomas: a European multicenter study. J Clin Oncol 19(16):3602–3610
37. Hoefnagel JJ, Dijkman R, Basso K et al (2005) Distinct types of primary cutaneous large B-cell lymphoma identified by gene expression profiling. Blood 105(9):3671–3678
38. Sung CO, Kim SC, Karnan S et al (2011) Genomic profiling combined with gene expression profiling in primary central nervous system lymphoma. Blood 117(4):1291–1300
39. Rubenstein JL, Fridlyand J, Shen A et al (2006) Gene expression and angiotropism in primary CNS lymphoma. Blood 107(9):3716–3723
40. Rosenwald A, Wright G, Wiestner A et al (2003) The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. Cancer Cell 3(2):185–197
41. Jares P, Colomer D, Campo E (2007) Genetic and molecular pathogenesis of mantle cell lymphoma: perspectives for new targeted therapeutics. Nat Rev Cancer 7(10):750–762
42. Hartmann EM, Campo E, Wright G et al (2010) Pathway discovery in mantle cell lymphoma by integrated analysis of high-resolution gene expression and copy number profiling. Blood 116(6):953–961
43. Fu K, Weisenburger DD, Greiner TC et al (2005) Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling. Blood 106(13):4315–4321
44. Mozos A, Royo C, Hartmann E et al (2009) SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. Haematologica 94(11):1555–1562
45. Fernandez V, Salamero O, Espinet B et al (2010) Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res 70(4):1408–1418
46. Liu D, Herman SEM, Grant C et al (2013) Gene expression profiling reveals the lymph node microenvironment as a niche for BCR engagement, NFκB pathway activation, and tumor proliferation in mantle cell lymphoma. Blood 122(21):82
47. Piccaluga PP, Califano A, Klein U et al (2008) Gene expression analysis provides a potential rationale for revising the histological grading of follicular lymphomas. Haematologica 93 (7):1033–1038
48. Dave SS, Wright G, Tan B et al (2004) Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med 351(21):2159–2169
49. Pasqualucci L, Khiabanian H, Fangazio M et al (2014) Genetics of follicular lymphoma transformation. Cell Rep 6(1):130–140
50. Brodtkorb M, Lingjaerde OC, Huse K et al (2014) Whole-genome integrative analysis reveals expression signatures predicting transformation in follicular lymphoma. Blood 123 (7):1051–1054
51. Bouska A, McKeithan TW, Deffenbacher KE et al (2014) Genome-wide copy-number analyses reveal genomic abnormalities involved in transformation of follicular lymphoma. Blood 123(11):1681–1690

52. Yap DB, Chu J, Berg T et al (2011) Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. Blood 117(8):2451–2459
53. Velichutina I, Shaknovich R, Geng H et al (2010) EZH2-mediated epigenetic silencing in germinal center B cells contributes to proliferation and lymphomagenesis. Blood 116 (24):5247–5255
54. Dave SS, Fu K, Wright GW et al (2006) Molecular diagnosis of Burkitt's lymphoma. N Engl J Med 354(23):2431–2442
55. Hummel M, Bentink S, Berger H et al (2006) A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med 354(23):2419–2430
56. Richter J, Schlesner M, Hoffmann S et al (2012) Recurrent mutation of the ID3 gene in Burkitt lymphoma identified by integrated genome, exome and transcriptome sequencing. Nat Genet 44(12):1316–1320
57. Love C, Sun Z, Jima D et al (2012) The genetic landscape of mutations in Burkitt lymphoma. Nat Genet 44(12):1321–1325
58. Schmitz R, Young RM, Ceribelli M et al (2012) Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. Nature 490(7418):116–120
59. Hamoudi RA, Appert A, Ye H et al (2010) Differential expression of NF-kappaB target genes in MALT lymphoma with and without chromosome translocation: insights into molecular mechanism. Leukemia 24(8):1487–1497
60. Arribas AJ, Gomez-Abad C, Sanchez-Beato M et al (2013) Splenic marginal zone lymphoma: comprehensive analysis of gene expression and miRNA profiling. Mod Pathol 26 (7):889–901
61. Ruiz-Ballesteros E, Mollejo M, Rodriguez A et al (2005) Splenic marginal zone lymphoma: proposal of new diagnostic and prognostic markers identified after tissue and cDNA microarray analysis. Blood 106(5):1831–1838
62. Arribas AJ, Campos-Martin Y, Gomez-Abad C et al (2012) Nodal marginal zone lymphoma: gene expression and miRNA profiling identify diagnostic markers and potential therapeutic targets. Blood 119(3):e9–e21
63. Gachard N, Parrens M, Soubeyran I et al (2013) IGHV gene features and MYD88 L265P mutation separate the three marginal zone lymphoma entities and Waldenstrom macroglobulinemia/lymphoplasmacytic lymphomas. Leukemia 27(1):183–189
64. Iqbal J, Wright G, Wang C et al (2014) Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphoma. Blood 123(19):2915–2923
65. Iqbal J, Weisenburger DD, Chowdhury A et al (2011) Natural killer cell lymphoma shares strikingly similar molecular features with a group of non-hepatosplenic gammadelta T-cell lymphoma and is highly sensitive to a novel aurora kinase A inhibitor in vitro. Leukemia 25 (2):348–358
66. Teruya-Feldstein J, Jaffe ES, Burd PR et al (1997) The role of Mig, the monokine induced by interferon-gamma, and IP-10, the interferon-gamma-inducible protein-10, in tissue necrosis and vascular damage associated with Epstein-Barr virus-positive lymphoproliferative disease. Blood 90(10):4099–4105
67. Huang Y, de Reynies A, de Leval L et al (2010) Gene expression profiling identifies emerging oncogenic pathways operating in extranodal NK/T-cell lymphoma, nasal type. Blood 115(6):1226–1237
68. Ng SB, Selvarajan V, Huang G et al (2011) Activated oncogenic pathways and therapeutic targets in extranodal nasal-type NK/T cell lymphoma revealed by gene expression profiling. J Pathol 223(4):496–510
69. Iqbal J, Kucuk C, Deleeuw RJ et al (2009) Genomic analyses reveal global functional alterations that promote tumor growth and novel tumor suppressor genes in natural killer-cell malignancies. Leukemia 23(6):1139–1151

70. Oka T, Yoshino T, Hayashi K et al (2001) Reduction of hematopoietic cell-specific tyrosine phosphatase SHP-1 gene expression in natural killer cell lymphoma and various types of lymphomas/leukemias: combination analysis with cDNA expression array and tissue microarray. Am J Pathol 159(4):1495–1505
71. Siu LL, Chan JK, Wong KF, Kwong YL (2002) Specific patterns of gene methylation in natural killer cell lymphomas : p73 is consistently involved. Am J Pathol 160(1):59–66
72. Gatza ML, Watt JC, Marriott SJ (2003) Cellular transformation by the HTLV-I Tax protein, a jack-of-all-trades. Oncogene 22(33):5141–5149
73. Sasaki H, Nishikata I, Shiraga T et al (2005) Overexpression of a cell adhesion molecule, TSLC1, as a possible molecular marker for acute-type adult T-cell leukemia. Blood 105 (3):1204–1213
74. Kreitman RJ, Chaudhary VK, Waldmann T, Willingham MC, FitzGerald DJ, Pastan I (1990) The recombinant immunotoxin anti-Tac(Fv)-Pseudomonas exotoxin 40 is cytotoxic toward peripheral blood malignant cells from patients with adult T-cell leukemia. Proc Natl Acad Sci USA 87(21):8291–8295
75. Zhang Z, Zhang M, Garmestani K et al (2006) Effective treatment of a murine model of adult T-cell leukemia using 211At-7G7/B6 and its combination with unmodified anti-Tac (daclizumab) directed toward CD25. Blood 108(3):1007–1012
76. Hattori T, Uchiyama T, Toibana T, Takatsuki K, Uchino H (1981) Surface phenotype of Japanese adult T-cell leukemia cells characterized by monoclonal antibodies. Blood 58 (3):645–647
77. Yoshie O, Fujisawa R, Nakayama T et al (2002) Frequent expression of CCR4 in adult T-cell leukemia and human T-cell leukemia virus type 1-transformed T cells. Blood 99(5):1505–1511
78. Ogura M, Ishida T, Hatake K et al (2014) Multicenter phase II study of mogamulizumab (KW-0761), a defucosylated anti-cc chemokine receptor 4 antibody, in patients with relapsed peripheral T-cell lymphoma and cutaneous T-cell lymphoma. J Clin Oncol 32(11):1157–1163
79. Ishida T, Joh T, Uike N et al (2012) Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase II study. J Clin Oncol 30(8):837–842
80. Pise-Masison CA, Radonovich M, Dohoney K et al (2009) Gene expression profiling of ATL patients: compilation of disease-related genes and evidence for TCF4 involvement in BIRC5 gene expression and cell viability. Blood 113(17):4016–4026
81. Iqbal J, Weisenburger DD, Greiner TC et al (2010) Molecular signatures to improve diagnosis in peripheral T-cell lymphoma and prognostication in angioimmunoblastic T-cell lymphoma. Blood 115(5):1026–1036
82. Cairns RA, Iqbal J, Lemonnier F et al (2012) IDH2 mutations are frequent in angioimmunoblastic T-cell lymphoma. Blood 119(8):1901–1903
83. Figueroa ME, Abdel-Wahab O, Lu C et al (2010) Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell 18(6):553–567
84. Piva R, Agnelli L, Pellegrino E et al (2010) Gene expression profiling uncovers molecular classifiers for the recognition of anaplastic large-cell lymphoma within peripheral T-cell neoplasms. J Clin Oncol 28(9):1583–1590
85. Savage KJ, Harris NL, Vose JM et al (2008) ALK—anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK+ ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the international peripheral T-cell lymphoma project. Blood 111(12):5496–5504

86. Salaverria I, Bea S, Lopez-Guillermo A et al (2008) Genomic profiling reveals different genetic aberrations in systemic ALK-positive and ALK-negative anaplastic large cell lymphomas. Br J Haematol 140(5):516–526
87. Qi W, Spier C, Liu X et al (2013) Alisertib (MLN8237) an investigational agent suppresses Aurora A and B activity, inhibits proliferation, promotes endo-reduplication and induces apoptosis in T-NHL cell lines supporting its importance in PTCL treatment. Leuk Res 37 (4):434–439
88. McDermott U, Iafrate AJ, Gray NS et al (2008) Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. Cancer Res 68 (9):3389–3395
89. Christensen JG, Zou HY, Arango ME et al (2007) Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. Mol Cancer Ther 6(12 Pt 1):3314–3322
90. Gambacorti-Passerini C, Messa C, Pogliani EM (2011) Crizotinib in anaplastic large-cell lymphoma. N Engl J Med 364(8):775–776
91. Younes A, Bartlett NL, Leonard JP et al (2010) Brentuximab Vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med 363(19):1812–1821
92. Pro B, Advani R, Brice P et al (2012) Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. J Clin Oncol 30(18):2190–2196
93. Wang T, Feldman AL, Wada DA et al (2014) GATA-3 expression identifies a high-risk subset of PTCL, NOS with distinct molecular and clinical features. Blood 123(19):3007–3015
94. Maciejewski JP, Tiu RV, O'Keefe C (2009) Application of array-based whole genome scanning technologies as a cytogenetic tool in haematological malignancies. Br J Haematol 146(5):479–488
95. Gutierrez NC, Garcia JL, Hernandez JM et al (2004) Prognostic and biologic significance of chromosomal imbalances assessed by comparative genomic hybridization in multiple myeloma. Blood 104(9):2661–2666
96. Mullighan CG, Goorha S, Radtke I et al (2007) Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 446(7137):758–764
97. Fitzgibbon J, Smith LL, Raghavan M et al (2005) Association between acquired uniparental disomy and homozygous gene mutation in acute myeloid leukemias. Cancer Res 65 (20):9152–9154
98. Gondek LP, Haddad AS, O'Keefe CL et al (2007) Detection of cryptic chromosomal lesions including acquired segmental uniparental disomy in advanced and low-risk myelodysplastic syndromes. Exp Hematol 35(11):1728–1738
99. Martin-Subero JI, Kreuz M, Bibikova M et al (2009) New insights into the biology and origin of mature aggressive B-cell lymphomas by combined epigenomic, genomic, and transcriptional profiling. Blood 113(11):2488–2497
100. Pike BL, Greiner TC, Wang X et al (2008) DNA methylation profiles in diffuse large B-cell lymphoma and their relationship to gene expression status. Leukemia 22(5):1035–1043
101. Gaur A, Jewell DA, Liang Y et al (2007) Characterization of microRNA expression levels and their biological correlates in human cancer cell lines. Cancer Res 67(6):2456–2468
102. Krutzfeldt J, Stoffel M (2006) MicroRNAs: a new class of regulatory genes affecting metabolism. Cell Metab 4(1):9–12
103. Costinean S, Zanesi N, Pekarsky Y et al (2006) Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. Proc Natl Acad Sci USA 103(18):7024–7029
104. Rao E, Jiang C, Ji M et al (2012) The miRNA-17 approximately 92 cluster mediates chemoresistance and enhances tumor growth in mantle cell lymphoma via PI3K/AKT pathway activation. Leukemia 26(5):1064–1072

105. Calin GA, Dumitru CD, Shimizu M et al (2002) Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 99(24):15524–15529
106. Schneider C, Setty M, Holmes AB et al (2014) MicroRNA 28 controls cell proliferation and is down-regulated in B-cell lymphomas. Proc Natl Acad Sci USA 111:8185–8190
107. Metzler M, Wilda M, Busch K, Viehmann S, Borkhardt A (2004) High expression of precursor microRNA-155/BIC RNA in children with Burkitt lymphoma. Genes Chromosomes Cancer 39(2):167–169
108. Kluiver J, Poppema S, de Jong D et al (2005) BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. J Pathol 207(2):243–249
109. Huang X, Shen Y, Liu M et al (2012) Quantitative proteomics reveals that miR-155 regulates the PI3K-AKT pathway in diffuse large B-cell lymphoma. Am J Pathol 181(1):26–33
110. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6 (11):857–866
111. Croce CM (2009) Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 10(10):704–714
112. Farazi TA, Spitzer JI, Morozov P, Tuschl T (2011) miRNAs in human cancer. J Pathol 223 (2):102–115
113. Wapinski O, Chang HY (2011) Long noncoding RNAs and human disease. Trends Cell Biol 21(6):354–361
114. Sehgal L, Mathur R, Braun FK et al. (2014) FAS-antisense 1 lncRNA and production of soluble versus membrane Fas in B-cell lymphoma Leukemia 28(12):2376–2387
115. Paz EA, LaFleur B, Gerner EW (2014) Polyamines are oncometabolites that regulate the LIN28/let-7 pathway in colorectal cancer cells. Mol Carcinog 53(1):E96–E106
116. Vasmatzis G, Johnson SH, Knudson RA et al (2012) Genome-wide analysis reveals recurrent structural abnormalities of TP63 and other p53-related genes in peripheral T-cell lymphomas. Blood 120(11):2280–2289
117. Knecht H, Righolt C, Mai S (2013) Genomic instability: the driving force behind refractory/ relapsing Hodgkin's lymphoma. Cancers (Basel) 5(2):714–725
118. Cossman J (2001) Gene expression analysis of single neoplastic cells and the pathogenesis of Hodgkin's lymphoma. J Histochem Cytochem 49(6):799–800
119. Powell AA, Talasaz AH, Zhang H et al (2012) Single cell profiling of circulating tumor cells: transcriptional heterogeneity and diversity from breast cancer cell lines. PLoS One 7(5): e33788
120. Zhong JF, Chen Y, Marcus JS et al (2008) A microfluidic processor for gene expression profiling of single human embryonic stem cells. Lab Chip 8(1):68–74
121. Geiss GK, Bumgarner RE, Birditt B et al (2008) Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol 26(3):317–325
122. Kulkarni MM (2011) Digital multiplexed gene expression analysis using the NanoString nCounter system. Curr Protoc Mol Biol (Chapter 25:Unit25B.10)
123. Payton JE, Grieselhuber NR, Chang LW et al (2009) High throughput digital quantification of mRNA abundance in primary human acute myeloid leukemia samples. J Clin Invest 119 (6):1714–1726
124. Reis PP, Waldron L, Goswami RS et al (2011) mRNA transcript quantification in archival samples using multiplexed, color-coded probes. BMC Biotechnol 11:46
125. Kojima K, April C, Canasto-Chibuque C et al (2014) Transcriptome profiling of archived sectioned formalin-fixed paraffin-embedded (AS-FFPE) tissue for disease classification. PLoS One 9(1):e86961
126. Roberts RA, Sabalos CM, LeBlanc ML et al (2007) Quantitative nuclease protection assay in paraffin-embedded tissue replicates prognostic microarray gene expression in diffuse large-B-cell lymphoma. Lab Invest 87(10):979–997

127. Rimsza LM, Wright G, Schwartz M et al (2011) Accurate classification of diffuse large B-cell lymphoma into germinal center and activated B-cell subtypes using a nuclease protection assay on formalin-fixed, paraffin-embedded tissues. Clin Cancer Res 17(11):3727–3732
128. Guo S, Chan JK, Iqbal J, et al (2014) EZH2 mutations in follicular lymphoma from different ethnic groups and associated gene expression alterations. Clin Cancer Res 20(12):3078–3086.

Imaging of Non-Hodgkin Lymphomas: Diagnosis and Response-Adapted Strategies

Tarec Christoffer El-Galaly and Martin Hutchings

Abstract

Optimal lymphoma management requires accurate pretreatment staging and reliable assessment of response, both during and after therapy. Positron emission tomography with computerized tomography (PET/CT) combines functional and anatomical imaging and provides the most sensitive and accurate methods for lymphoma imaging. New guidelines for lymphoma imaging and recently revised criteria for lymphoma staging and response assessment recommend PET/CT staging, treatment monitoring, and response evaluation in all FDG-avid lymphomas, while CT remains the method of choice for non-FDG-avid histologies. Since interim PET imaging has high prognostic value in lymphoma, a number of trials investigate PET-based, response-adapted therapy for non-Hodgkin lymphomas (NHL). PET response is the main determinant of response according to the new response criteria, but PET/CT has little or no role in routine surveillance imaging, the value which is itself questionable. This review presents from a clinical point of view the evidence for the use of imaging and primarily PET/CT in NHL before, during, and after therapy. The reader is given an overview of the current PET-based interventional NHL trials and an insight into possible future developments in the field, including new PET tracers.

Keywords

Imaging · PET/CT · Lymphoma · Non-Hodgkin · Staging · Response

T.C. El-Galaly
Department of Haematology, Aalborg University Hospital, Aalborg, Denmark

M. Hutchings (✉)
Department of Haematology, Rigshospitalet, 9 Blegdamsvej, 2100 Copenhagen, Denmark
e-mail: martin.hutchings@gmail.com

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_5

Contents

1 Introduction

Over the last 2–3 decades, we have seen substantial improvements in the prognosis of patients with non-Hodgkin lymphomas (NHL) [1]. These improvements can be attributed to the following factors:

1. Better understanding of the biology of the diseases and advances in molecular pathology, leading to better disease characterization and identification of valuable prognostic markers as well as therapeutic targets.
2. Better imaging methods, most importantly computerized tomography (CT) with cross-sectional images providing more accurate staging, and the more recent introduction of functional imaging, notably positron emission tomography (PET) and PET/CT, which is now the cornerstone of lymphoma imaging.
3. Last and not least; improved treatment regimens, including the introduction of monoclonal anti-CD20 antibodies and high-dose chemotherapy with autologous stem cell support.

The introduction of CT in the 1980s resulted in improved staging accuracy, and CT replaced cumbersome staging procedures such as lymphangiography and staging laparotomy. The introduction of CT was not based on much evidence, as opposed to the more than 1,000 studies investigating PET and PET/CT in lymphoma. During the last 15 years, a large number of studies have shown superior accuracy and sensitivity of PET/CT over CT. Along with patient symptoms and clinical examination, CT and PET/CT are now the cornerstone of all lymphoma

imaging. The recent international guidelines for imaging in the staging and response assessment of lymphoma recommend PET/CT for staging and response assessment of all most NHL subtypes [2, 3]. The only PET tracer in widespread clinical use for lymphoma is still 2-[^{18}F]fluor-2-deoxyglucose (FDG). In the following and if not otherwise specified, PET refers to FDG-PET and PET/CT refers to FDG-PET/CT.

2 Staging

2.1 PET/CT for Staging in Non-Hodgkin Lymphomas

Accurate disease staging in NHL is crucial for pretherapy risk assessment and selection of optimal therapy [4, 5]. The main purpose of the staging process, however, depends on the type of the non-Hodgkin lymphoma. Indolent NHL are often considered incurable unless localized at the time of diagnosis. Accordingly, the main question in the staging of indolent NHL is: localized or non-localized disease? Most aggressive NHL, including both B- and T-cell subtypes, are potentially curable when patients are fit for first-line treatment. However, the actual prognosis depends on the specific lymphoma subtype and the presence of high-risk features. The most widely used prognostic scores for NHL are derived from simple clinicopathological features and include disease stage or measures related to disease burden [6–10]. Thus, staging of aggressive non-Hodgkin lymphoma contributes to prognostication and eventually selection of therapy intensity.

The modified Ann Arbor classification originally developed for Hodgkin lymphoma still provides the backbone of disease staging in NHL [3, 11]. The staging workup generally includes clinical examination, blood tests, a routine bone marrow biopsy, and imaging. Contrast-enhanced CT (ceCT) of the neck, chest, and abdomen is required as a minimum, but PET/CT imaging is recommended for all FDG-avid NHL. FDG-PET is routinely used for lymphomas and measures tissue glucose metabolism by use of a short-lived radioactive glucose analog tracer. An advantage of PET-based imaging over stand-alone CT is higher sensitivity and a better discrimination between lymphoma and non-malignant lesions. When PET and CT are combined in a single procedure (PET/CT), the abnormal glucose metabolism is correlated with detailed anatomy, and the increased sensitivity comes not at the expense of decreased specificity; hence, the overall accuracy is increased [12].

2.2 PET/CT Staging in Indolent Non-Hodgkin Lymphomas

Follicular lymphoma (***FL***): FL is the most common subtype of indolent lymphoma, and the vast majority of FL cases are FDG-avid [13]. The main purpose of FL staging is to identify patients with true localized disease, potentially curable with radiotherapy, but also to risk stratify patients according to the follicular lymphoma international prognostic index (FLIPI) [8]. Compared to conventional

stand-alone CT staging, PET/CT detects abnormal activity in normal sized lymph nodes as well as additional involvement of extranodal sites, all resulting in upstaging of 18–32 % of patients and leading to a treatment change in 11–28 % of patients [14–16]. In particular, a large number of patients considered to have limited-stage disease by conventional staging are upstaged by PET/CT to advanced-stage disease (31–62 % according to recent studies) [17, 18]. Upstaging in these cases will change therapy and therefore be clinically relevant. Use of PET/CT also assigns 18 % of the patients to poorer FLIPI risk group, whereas only 6 % are reallocated to lower-risk groups by PET/CT [17]. A replacement of the original FLIPI by a new PET/CT-based risk score has been proposed, as PET/CT-specific findings such as osteomedullar uptake and extranodal disease may be associated with a worse outcome [14].

Mantle cell lymphoma: Mantle cell lymphomas are FDG-avid, and staging with PET/CT results in change of the CT-assessed stage in almost half of the patients, most of whom are upstaged [13, 19]. Therefore, it is possible that patients with truly localized mantle cell lymphoma are more accurately identified with PET/CT. Patients with high FDG-avidity at baseline have a worse outcome than patients with low-grade uptake, which suggests that metabolic activity correlates with tumor aggressiveness [19].

Other indolent lymphomas: The value of PET/CT-based staging in MALT lymphoma and small lymphocytic lymphoma is controversial, and the reported FDG-avidity in these lymphomas is inconsistent [13, 20]. In FDG-avid cases, the uptake is usually discrete, and as for CLL, PET/CT is useful for selecting biopsy site in suspected Richter's transformation [21, 22]. Nodal marginal zone lymphomas are usually FDG-avid, while this is rarely the case for extranodal marginal zone [13].

Summary: PET/CT for staging of indolent NHL is not well investigated, and the present evidence is derived from few retrospective series. FL, mantle cell lymphoma, and nodal marginal zone lymphoma are FDG-avid, and PET/CT is likely to discriminate between localized and non-localized diseases more accurately than CT. For other indolent lymphomas, PET/CT can guide selection of biopsy site in case of suspected Richter's transformation. For all indolent lymphomas, the impact on patient outcome of the changes in risk stratification and treatment approach caused by PET/CT is yet largely unclear. Nevertheless, PET/CT is recommended for staging of all FDG-avid indolent lymphomas, while CT remains the recommended imaging tool for non-FDG-avid lymphomas [2].

2.3 PET/CT Staging in Staging of Aggressive Non-Hodgkin Lymphomas

Diffuse large B-cell lymphoma (**DLBCL**): Even though DLBCL is the most common of all lymphomas, the value of PET/CT for staging of DLBCL has mainly been studied in groups of mixed lymphoma histologies. When compared to CT, PET/CT provides more sensitive and accurate staging and results in upstaging of

10–20 % of patients, some of whom will be allocated to a more advanced treatment [23–25]. The reason for more upstaging is the high sensitivity of PET/CT for extranodal DLBCL. PET/CT accurately identifies bone marrow involvement by DLBCL, and the reported sensitivity and specificity for bone marrow involvement was 94 and 100 % in a recent study [26]. Although most cases of bone marrow involvement by DLBCL are correctly identified by focal bone marrow lesions on PET/CT, a low-grade discordant bone marrow infiltration can be missed [26, 27]. PET/CT staging impacts on pretherapy risk assessment since the number of extranodal sites and that of disease stage are both factors in the widely used IPI risk score [6], and since >1 extranodal site is a risk factor for CNS relapse which often leads to additional CNS prophylactic therapy [28]. The validity of IPI in PET/CT-staged patients is currently investigated. A number of groups have looked at different quantitative applications of PET for staging in DLBCL. While the role of the standardized uptake value (SUV) is unclear, the metabolic tumor volume (MTV) seems to be prognostic of long-term outcome [29, 30]. Furthermore, recent studies demonstrate a good prognostic value of total lesion glycolysis (TLG) which is a combined assessment of volume and metabolism [31, 32].

Burkitt: Burkitt lymphoma is the most aggressive type of non-Hodgkin lymphoma. All cases of Burkitt lymphoma are FDG-avid, and PET/CT staging detects more extranodal disease than conventional staging. Of interest, occult bone marrow involvement is found and occasional clinically important upstagings occur [33].

Peripheral T-cell lymphomas (**PTCLs**): PTCLs are a heterogeneous group of diseases, and FDG uptake is variable. While the cutaneous T-cell lymphomas have low or no FDG uptake, most nodal PTCLs are FDG-avid albeit with lower FDG uptake than the corresponding B-cell lymphomas [13, 34, 35]. PET/CT identifies additional disease sites in non-cutaneous PTCLs in almost 50 % of the patients, but only changes disease stage in 5 % [35]. Since most nodal PTCLs are disseminated at the time of diagnosis, the additional diagnostic information by PET/CT is less likely to impact overall stage. PET has low sensitivity for detection of bone marrow involvement in PTCLs (20 %) and cannot replace the iliac crest bone marrow biopsy [34]. Nasal-type NK/T-cell lymphoma is most often a disease localized to the head-and-neck region, but PET/CT staging will change stage in one fifth of these patients, and in few patients, disseminated disease is found by PET/CT. Change of treatment by PET/CT in nasal-type NK/T-cell lymphomas is even more common as PET/CT [often accompanied by magnetic resonance imaging (MRI)] modifies radiotherapy planning in many cases [36].

Summary: PET/CT is recommended and widely used for staging of aggressive NHL. Disease stage and pretherapy risk assessment is modified in a large proportion of patients, and for some patients, the additional diagnostic information by PET/CT will change treatment intensity. The impact of PET/CT staging on patient outcome, however, is unknown.

3 PET and PET/CT for Early Treatment Monitoring and Response-Adapted Therapy

A number of well-established pretreatment prognostic factors have been shown to predict survival in large cohort studies of lymphoma patients [6–9]. These prognostic factors are used along with the clinical stage to determine the initial treatment strategy. However, in both indolent and aggressive lymphomas, the tumor response to induction treatment is an important surrogate for other measures of clinical benefit from the treatment, including progression-free survival and overall survival. A reliable and early prediction of response to therapy can possibly separate good-risk patients who will be cured with conventional therapy or even less intensive and less toxic regimens from poor-risk patients for whom an early switch to alternative, more aggressive treatment strategies could improve the likelihood of remission and cure. This concept called risk-adapted therapy is widely recognized as a potential way to achieve higher cure rates with lower or equal risk of treatment-related morbidity and mortality.

Conventional methods for treatment response monitoring are based on morphological criteria, and a reduction in tumor size on CT is the most important determinant [37–39]. However, size reduction is not necessarily an accurate predictor of outcome. But even more importantly, tumor shrinkage takes time and depends on a number of factors in the host. So the rate of structural regression cannot form the basis for therapy response assessment until rather late during treatment, at which point a treatment modification might be less useful.

As opposed to the morphological changes of the lymphoma occurring later during therapy, functional imaging with FDG-PET enables early evaluation of the metabolic changes that take place very early during the treatment induction. Several studies of FDG-PET after 1–4 cycles of chemotherapy in aggressive NHL [40–48] and FL have shown that these early metabolic changes are highly predictive of final treatment response and progression-free survival (PFS). However, treatment-induced inflammation and concomitant infections may lead to false-positive interim scans, and this risk is particularly pertinent in patients treated with intensive regimens [49]. Most evidence is available for performing early interim FDG-PET after 2–4 cycles of chemotherapy. However, reports from Kostakoglu et al. [44, 48] strongly suggest that the predictive value may be as high after only one cycle of chemotherapy. This is of importance for early PET response-adapted treatment regimens, since the treatment adaptation is best performed as early as possible in order to increase the chance of satisfactory remission and to avoid unnecessary ineffective chemotherapy.

Interim treatment monitoring is more a tradition than an evidence-based practice. Even though it is well established that continued treatment in patients with progression or no response during first-line therapy will invariably lead to treatment failure, the widespread use of CT for treatment monitoring has never been proven to improve the outcome of lymphoma patients. This is true for PET/CT imaging as well, and until we have data from the studies of PET response-adapted therapy,

Table 1 The Deauville five-point scale for PET response in lymphoma

1	No uptake
2	Uptake ≤ mediastinum
3	Uptake > mediastinum but ≤ liver
4	Uptake moderately higher than liver
5	Uptake markedly higher than liver and/or new lesions
X	New areas of uptake unlikely to be related to lymphoma

interim imaging should only be performed if clinically justified. The recently revised lymphoma imaging recommendations state that if PET-CT is superior to CT alone to assess early response, if such an assessment is needed, but changing therapy solely on the basis of interim PET-CT is not recommended, unless there is clear evidence of progression. It is recommended that both interim and post-treatment PET scans are interpreted and reported according to the Deauville five-point scale (Table 1) [2]. Figure 1 shows examples of PET images with response scored using the five-point scale.

3.1 Interim PET in Indolent NHL

There is a relative paucity of data on the value of interim FDG-PET in indolent lymphomas. Bishu et al. reported a retrospective series of 31 patients with advanced-stage FL, of whom 11 had FDG-PET scans midway through four cycles of induction chemotherapy. The number of patients was too small for any firm conclusions. Still, the results showed interesting differences: Four patients with some persistent FDG uptake had a mean PFS of 17 months and seven interim PET-negative patients had a mean PFS of 30 months [50]. There are only casuistic reports on the value of interim FDG-PET in localized FL or the more uncommon subtypes of indolent NHL, such as marginal zone lymphoma and small lymphocytic lymphoma.

Even if a clear correlation between interim FDG-PET and treatment outcome is shown in the future, the clinical implications are uncertain, since advanced-stage indolent lymphoma is very different from aggressive NHL. First of all, current first-line therapies for advanced-stage FL are not curative, and it is unclear whether early treatment intensification with autologous stem cell transplant (ASCT) or allogeneic bone marrow transplantation is of any benefit to poor-risk patients. Secondly, the long natural history of FL and the relative success of subsequent treatment lines in inducing lasting remissions mean that a longer time to first progression does not necessarily translate into longer survival.

3.2 Interim PET in Aggressive NHL

In aggressive NHL, PFS ranges from 10 to 50 % at 1 year for early PET-positive patients and from 79 to 100 % at 1 year for early PET-negative patients. The high

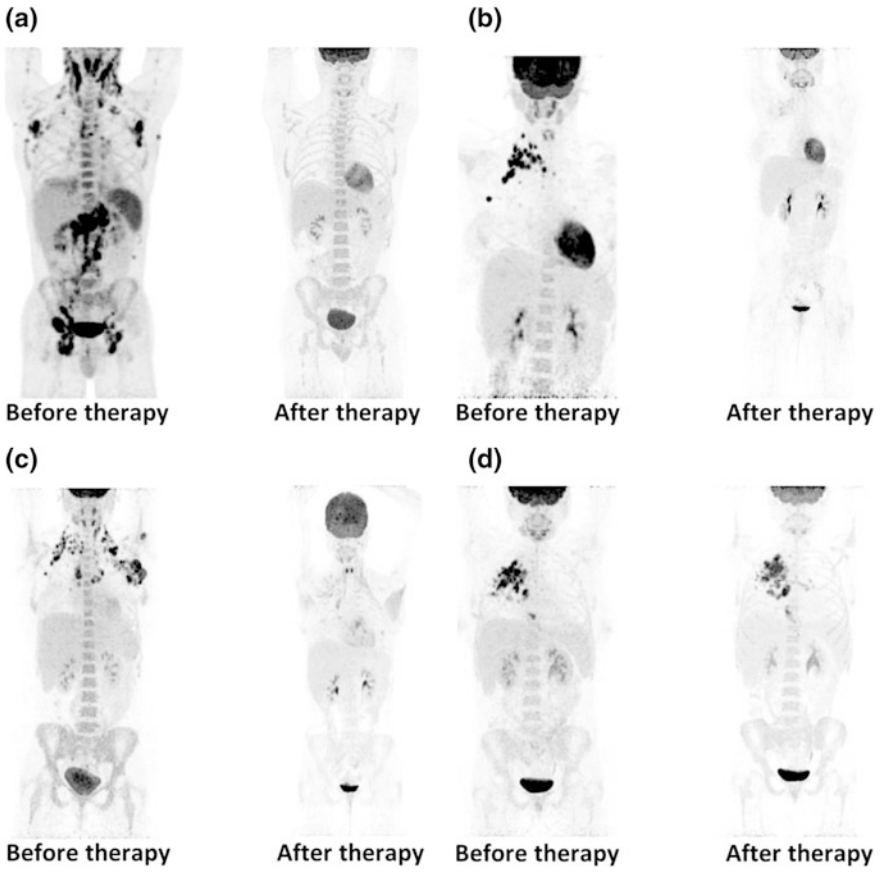


Fig. 1 *Legend* Examples of baseline and interim PET images with response assessed according to the Deauville five-point scale. **a** Deauville score 1. **b** Deauville score 1. **c** Deauville score 1. **d** Deauville score 1

risk of relapse seen among PET-positive patients is consistently shown in both early and advanced stages. Mikhaeel et al. studied a large cohort of 121 high-grade NHL patients, retrospectively and with a median follow-up of 28.5 months. They confirmed that response on FDG-PET after two or three cycles strongly predicts PFS and OS. Estimated 5-year PFS was 89 % for the PET-negative group, 59 % for patients with minimal residual uptake (MRU) on FDG-PET, and 16 % for the PET-positive group. Survival analyses showed strong associations between early FDG-PET results and PFS and OS [46]. Haioun et al. prospectively studied 90 patients with aggressive NHL, performing FDG-PET after two cycles of chemotherapy. They similarly found 2-year PFS rates of 82 and 43 % in early PET-negative and early PET-positive patients, respectively, and 2-year OS rates of 90 and 60 % [47]. Spaepen et al. compared interim FDG-PET after two cycles of chemotherapy with

the International Prognostic Index (IPI). In multivariate analysis, FDG-PET at mid-treatment was a stronger prognostic factor for PFS and OS than was the IPI ($P < 0.58$ and $P < 0.03$, respectively) [42]. A number of other more recent studies have investigated the value of interim PET specifically in DLBCL cohorts and found negative predictive values similar to those found in the above-mentioned studies of mixed aggressive NHL populations. But the positive predictive value is variable and generally poor [51, 52]. A possible explanation for the low positive predictive value in aggressive NHL cohorts is the higher risk of inflammation and infections among patients who are treated with the more dose-dense and dose-intensive NHL regimens, as mentioned above. In order to overcome the low positive predictive value of interim PET, some investigators have proposed a semiquantitative approach (relative or absolute change in SUV from baseline to interim scan) as superior to visual PET assessment in patients with DLBCL [53, 54].

Aggressive NHL covers a number of lymphoma subtypes where first-line treatment of both localized and advanced disease stages is given with a curative intent. Aggressive NHL patients who respond poorly to first-line treatment or relapse soon afterward generally have a very poor prognosis even with high-dose salvage regimens. Such poor-risk patients could benefit from having their treatment failure recognized early during first-line therapy in order to switch a more intensive regimen as soon as possible. Much inspired by a similar and more widespread trend in Hodgkin lymphoma, a number of trials are currently investigating early PET response-adapted treatment strategies (Table 2). Most studies test whether early or mid-treatment PET-positive patients with aggressive NHL (mainly DLBCL) will benefit from early escalation to more intensive regimens or even high-dose therapy with autologous stem cell support (ASCT) [55–60]. One de-escalation study compares standard treatment with six cycles R-CHOP to good-risk DLBCL patients to abbreviated therapy with four cycles R-CHOP in patients with a negative PET after two cycles of therapy [61].

4 PET/CT for Post-therapy Response Evaluation of Lymphomas

Response to treatment serves as an important surrogate for other measures of clinical benefit such as progression-free survival and overall survival. Response is also an important guide in decisions regarding continuation or change of therapy. Until recently, response evaluation in NHL was done according to the International Workshop Criteria [62], based mainly on morphological evaluation with a reduction in tumor size on CT being the most important factor. But after completion of therapy, CT scans will often reveal residual masses, and the presence of such masses is not highly predictive of outcome. By conventional methods, it is very difficult to assess whether this represents viable lymphoma or fibrotic scar tissue. An extensive number of studies have shown that FDG-PET performed post-treatment is highly predictive of PFS and OS with and without residual masses on CT.

Table 2 PET response-adapted trials in aggressive NHL

Study title/description	Study group	Ref	Patients	Main PET-driven intervention	Study type
Risk-adapted dose-dense immunochemotherapy determined by interim FDG-PET in advanced DLBCL	MSKCC	52	DLBCL	Salvage with HD + ASCT if PET positive after 4 × R-CHOP	Phase II
FDG-PET in predicting relapse in NHL patients undergoing chemotherapy with or without ASCT	Johns Hopkins	55	Aggressive NHL	Salvage with HD + ASCT if PET positive after 2–3 × (R-)CHOP	Phase II
Positron emission tomography-guided therapy of aggressive non-Hodgkin's lymphomas (PETAL)	University Hospital, Essen	57	Aggressive NHL	(R-)CHOP versus Burkitt regimen if PET positive after 2 × (R-)CHOP	Phase III
Tailoring treatment for B-cell non-Hodgkin's lymphoma based on PET scan results mid-treatment	British Columbia Cancer Agency	58	Advanced DLBCL	4 cycles R-ICE if PET positive after 4 × R-CHOP	Phase II
FDG-PET-stratified R-DICEP and R-Beam/ASCT for diffuse large B-cell lymphoma (PET CHOP)	Alberta Cancer Board	59	DLBCL	Salvage with HD + ASCT if PET positive after 2 × R-CHOP	Phase II
A study of associations between rituximab and chemotherapy, with PET-driven strategy in lymphoma (LNH2007-3B)	LYSA	60	DLBCL	Salvage with HD + ASCT if PET positive after 2 × R-CHOP	Phase III[a]
Therapy for aaIPI intermediate or high-risk DLBCL	MSKCC	56	DLBCL	High dose if PET positive and biopsy positive after 3 cycles of immunochemotherapy	Phase II
Treatment adapted to the early PET compared to a standard treatment in low risk (aaIPI = 0) DLBCL	LYSA	61	DLBCL	4 × R-CHOP versus 6 × R-CHOP for early PET-negative patients	Phase III

[a]No randomization regarding PET response-adapted therapy

DLBCL diffuse large B-cell lymphoma; *HD + ASCT* high-dose chemotherapy with autologous stem cell transplantation; *R-CHOP* rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; *NHL* non-Hodgkin lymphoma; *R-ICE* rituximab, ifosfamide, carboplatin, etoposide; *LYSA* the Lymphoma Study Association; *MSKCC* Memorial Sloan-Kettering Cancer Center

The vast number of studies in aggressive NHL has been summarized in a systematic review [63], while the data on indolent NHL are based on fewer studies [64–66].

Based on these findings, the International Harmonization Project developed new recommendations for response criteria for aggressive malignant lymphomas, incorporating FDG-PET into the definitions of end-of-treatment response in FDG-avid lymphomas [67, 68]. Subsequent retrospective analyses confirmed the superiority of the new PET/CT-based response criteria [69]. The recent revision of the international lymphoma staging and response criteria still uses PET response (according to the Deauville five-point scale) as the main determinant for post-treatment assessment, while measurements of the sum of the product of perpendicular diameters of measureable lesions are mainly important for the definition of progressive disease [3]. The negative predictive value of post-treatment PET/CT is high in most NHL subtypes, but due to a relatively low positive predictive value, treatment failure suspected due to a positive PET should be confirmed by biopsy before additional therapy is initiated. Alternatively, it is often preferable to wait and repeat the PET/CT after 1–2 months, provided the patient is not in clinical progression.

Unlike in Hodgkin lymphoma, the role of post-treatment PET/CT for selection of NHL patients for consolidation radiotherapy is unclear. Based on promising data showing a very high negative predictive value of PET/CT in primary mediastinal B-cell lymphoma [70], the International Extranodal Lymphoma Study Group conducts an experimental approach where consolidation radiotherapy is given only to patients with a PET-positive residual mass [71]. Also, the British Columbia Cancer Agency has since 2005 routinely used post-chemotherapy PET for all patients with residual masses larger than 2 cm. Only PET-positive patients receive consolidation radiotherapy. With 262 patients included in the series and a median follow-up of 45 months, only one of 179 PET-negative patients has received radiotherapy. The 4-year PFS and OS are 74 and 83 %, respectively. Since the data have still only been presented in abstract form, it is unfortunately unclear whether the relapses were predominantly located within or outside the sites of residual disease. If most relapses occur outside the sites of residual disease, there seems to be little value of consolidation radiotherapy in PET-negative patients, but this is not quite so clear whether a substantial number of relapses are seen within the sites of residual disease after first-line treatment [72].

5 Disease Surveillance After First Remission

5.1 Aggressive Non-Hodgkin Lymphomas

The value of routine surveillance imaging in aggressive NHL in first remission is controversial. Despite the lack of evidence, surveillance imaging is often performed with regular intervals during the first years in remission. The rationale behind this may be a presumed better outcome in patients with early and asymptomatic relapse as compared to those patients presenting with overt lymphoma symptoms.

Early detection of relapse could in theory be advantageous for the following reasons: (1) lower tumor burden at relapse (and maybe reduced tumor heterogeneity and prevention of treatment resistant clones) and (2) less disease-related impairment of function and organs with better tolerability of treatment. Low disease stage and ECOG performance status 0–1 at relapse are associated with a better outcome in DLBCL [73]. With less than a quarter of the relapses being imaging detected, routine imaging did not contribute significantly to relapse detection in studies based on imaging modalities less sensitive than PET/CT [74–78]. Furthermore, the number of scans per relapse exceeded 100 per preclinical relapse for both DLBCL and PTCLs in first complete remission, and out of all patients in complete remission entering intensive follow-up protocols, only a very small minority (2–3 %) will experience imaging-detected relapse [78]. Few studies have investigated the value of PET/CT in the surveillance of aggressive NHL, mainly reporting on DLBCL. Generally, the rate of imaging-detected relapse seems to be higher when using regular PET/CT surveillance in first remission, and depending on the surveillance protocol, 31–100 % of relapses were detected by imaging [79–81]. However, with the number of PET/CT scans per relapse ranging between 35 and 120 [81, 82] and an estimated cost of up to US$85,550 per preclinical PET/CT-detected relapse, an indiscriminate use of PET/CT surveillance is not likely to be cost-effective. Restricting the use of PET/CT to high-risk patients (IPI > 2) decreases the number of PET/CT scans per relapse to 22 and may tip the balance toward better cost-effectiveness [81]. In patients with transformed indolent lymphomas undergoing routine imaging with PET/CT at least one time during follow-up, the PET/CT-detected relapses were all indolent histologies and less important from a clinical perspective. On the contrary, cases of relapsed DLBCL were symptomatic [83]. Since FDG uptake is unspecific and not restricted to lymphoma recurrence, the positive predictive value (PPV) of PET/CT surveillance in aggressive NHL has been reported low to moderate with values between 21 and 60 % [79, 81, 84]. The increasing use of PET/CT for lymphoma has probably led to safer recognition of uptake patterns characteristic for lymphoma and hence less false-positive reporting. Still, a confirmatory biopsy of lymphoma suspicious findings on PET/CT is mandatory whenever possible. As opposed to the problematic PPV of PET/CT, the negative predictive value has been reported uniformly high and often 100 % [79, 81, 84]. This means that a negative PET/CT study virtually rules out recurrent disease and the need for further investigations in patients with symptoms suggesting lymphoma relapse. Finally, the use of routine surveillance imaging in aggressive NHL can only be justified if early relapse detection is associated with improved survival. Some studies have suggested that imaging-detected relapse of aggressive non-Hodgkin lymphoma could be associated with improved outcome, whereas others found no difference in outcome [76, 78, 81]. However, the retrospective design of these studies does not allow firm conclusions about the causal role of imaging due the presence of important bias (lead time, length time, and guarantee time). Interestingly, a recent randomized trial of surveillance

strategies in Hodgkin lymphoma showed that the use of chest X-ray and ultrasound was just as effective as PET/CT in early relapse detection, but at a much lower cost and with higher PPV [85].

5.2 Indolent Non-Hodgkin Lymphomas

While use of routine imaging in the follow-up of aggressive NHL is still widespread, the use of routine imaging in the follow-up of indolent NHL is not recommended. An observational approach without initial treatment is often chosen for patients with an asymptomatic relapse of an indolent lymphoma. An exception could be specific cases where an asymptomatic relapse could lead to unrecognized compression of vital organs such as vascular structures. In any case, PET/CT has no role in this setting.

6 Response Prediction with FDG-PET Before High-Dose Salvage Therapy

Duration of remission prior to relapse and the response to induction therapy are important prognostic factors that predict a good outcome after high-dose chemotherapy with autologous stem cell support (HD + ASCT) [86, 87]. A number of studies have shown that PET performed after induction therapy and before HD + ASCT is predictive of long-term remission. These studies all report short PFS in patients with persistent disease on pretransplant PET [88–93]. In relapsed Hodgkin lymphoma, Moskowitz and his group demonstrated that patients could benefit from changing to another non-cross-resistant regimen before HD + ASCT rather than proceeding straight to high-dose therapy if the PET was still positive after the initial induction therapy [94]. Even though no such data are yet available in NHL, it seems clear that achieving a negative PET before HD + ASCT should be a goal for all patients.

7 Other Imaging Methods than FDG-PET and CT

7.1 Newer PET Tracers

Like other cancers, lymphoma is characterized by deregulated cell cycle progression and most anticancer drugs are designed to inhibit cell proliferation. So a tracer enabling imaging of cell proliferation could be useful for both initial characterization and treatment monitoring of the disease. FDG uptake is somewhat correlated with cell proliferation, but this correlation is weakened by a number of factors, including FDG uptake in non-malignant lesions [95, 96]. The nucleoside [^{11}C]thymidine was the first PET tracer to specifically address cell proliferation. Early studies showed

that [^{11}C]thymidine could determine both disease extent and early response to chemotherapy in aggressive NHL patients [97, 98]. However, the short 20-min half-life of ^{11}C along with rapid in vivo metabolism has limited the clinical application of [^{11}C]thymidine. The thymidine analogue 3′-deoxy-3′-[^{18}F]fluorothymidine (FLT) offers a more suitable half-life of 110 min and is stable in vivo [99]. More recent studies have shown that FLT-PET can sensitively identify lymphoma sites [100]. FLT uptake is highly correlated with proliferation rate and may thus be able to distinguish between high- and low-grade lymphomas [101, 102]. And furthermore, recent studies have showed a potential of FLT for imaging early response to treatment in NHL [103–106]. Amino acid metabolism of cancer cells is influenced by catabolic processes favoring tumor growth [107]. It has been shown that increased uptake of amino acids reflects the increased transport and protein synthesis of malignant tissue [108, 109]. This is the background for PET imaging of amino acid metabolism with the labeled amino acids L-[methyl-^{11}C]methionine (MET) and O-2-[^{18}F]fluoroethyl)-L-tyrosine (FET) [110]. Nuutinen et al. [111] studied 32 lymphoma patients and found MET-PET highly sensitive for the detection of disease sites although there was no correlation between MET uptake and patient outcome. Also, MET-PET has an advantage over FDG-PET in imaging of CNS lymphoma [112]. While these results are encouraging, it should be noted that no studies have shown the usefulness or cost-effectiveness of amino acid or nucleoside tracers in large patient cohorts. Furthermore, high physiological tracer uptake in the abdomen limits the usefulness of these tracers for imaging of abdominal and pelvic lymphomas.

With CD20 expressed as a cell surface antigen in most cases of B-NHL, and with CD20 as an important therapeutic target, it is logical to also target CD20 for the purpose of lymphoma imaging. However, most of the available so-called *immunoPET* tracers are based on full-size, intact antibodies, and as a result of this, it takes a long time for the background activity levels to drop sufficiently to provide acceptable target-to-background ratios. Therefore, Olafsen and colleagues generated a recombinant anti-CD20 fragment which, labeled with a PET tracer, produced high-contrast images in a small-animal model [113]. This and similar targeted PET methods may in the future provide very lymphoma-specific images and prove useful in the staging and treatment monitoring of NHL.

7.2 Magnetic Resonance Imaging

MRI has long been the main structural imaging methods in pediatric lymphomas, due to the reduction in radiation exposure. MRI is used in adult patients to evaluate central nervous system disease and for disease sites in the head-and-neck region (e.g., NK/T-cell lymphomas of nasal type). Recent studies show that diffusion-weighted MRI early during treatment may identify responders before a reduction in tumor size occurs [114].

8 Conclusions and Recommendations

Supported by a large number of studies and in line with the recent consensus recommendations for lymphoma imaging as well as the revised criteria for lymphoma staging and response evaluation, PET/CT is recommended for staging and post-treatment response assessment of all FDG-avid NHLs [2, 3]. There are sufficient data to support the use of interim PET for treatment monitoring in aggressive B-NHL when interim imaging is appropriate. CT is still the preferred imaging method for non-FDG-avid lymphomas, as well as for selected cases where routine surveillance imaging is considered necessary. For interim and post-treatment imaging, the Deauville five-point scale is recommended for interpretation and reporting of PET results. The use of interim PET imaging to guide therapeutic decisions is still investigational and should preferably be done in the setting of clinical trials. Semiquantitative and quantitative PET assessments (SUV, MTV, TLG) are promising, but their role is still to be defined. Currently, much effort is put into the standardization of PET/CT methodology and interpretation, making the results of trials more comparable and ensuring a better translation into clinical practice. Upcoming results of interim PET response-adapted therapy trials are likely to impact on the management of de novo and relapsed NHL patients in the near future. The dissemination of novel and more disease-specific PET tracers may improve the basis for risk- and response-adapted therapy, hopefully in combination with better pretreatment predictive molecular pathology markers.

References

1. Flowers CR, Armitage JO (2010) A decade of progress in lymphoma: advances and continuing challenges. Clin Lymphoma Myeloma Leuk 10(6):414–423
2. Barrington SF, Mikhaeel NG, Kostakoglu L, Meignan M, Hutchings M, Mueller SP et al (2014) Role of imaging in the staging and response assessment of lymphoma: consensus of the international conference on malignant lymphomas imaging working group. J Clin Oncol 32(27):3048–3058
3. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E et al (2014) Recommendations for initial evaluation, staging, and response assessment of hodgkin and non-Hodgkin lymphoma: the lugano classification. J Clin Oncol 32(27):3059–3068
4. Dreyling M, Ghielmini M, Marcus R, Salles G, Vitolo U, Ladetto M (2014) Newly diagnosed and relapsed follicular lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 25(suppl 3):iii76–82
5. Tilly H, Vitolo U, Walewski J, da Silva MG, Shpilberg O, Andre M et al (2012) Diffuse large B-cell lymphoma (DLBCL): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 23(suppl 7):vii78–vii82
6. A predictive model for aggressive non-Hodgkin's lymphoma. The international non-Hodgkin's lymphoma prognostic factors project. N Engl J Med 1993; 329(14):987-994
7. Gallamini A, Stelitano C, Calvi R, Bellei M, Mattei D, Vitolo U et al (2004) Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. Blood 103(7):2474–2479
8. Solal-Celigny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R et al (2004) Follicular lymphoma international prognostic index. Blood 104(5):1258–1265

9. Hoster E, Dreyling M, Klapper W, Gisselbrecht C, van Hoof A, Kluin-Nelemans HC et al (2008) A new prognostic index (MIPI) for patients with advanced-stage mantle cell lymphoma. Blood 111(2):558–565
10. Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P et al (2007) The revised international prognostic index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood 109(5):1857–1861
11. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M (1971) Report of the committee on Hodgkin's disease staging classification. Cancer Res 31(11):1860–1861
12. Kwee TC, Kwee RM, Nievelstein RA (2008) Imaging in staging of malignant lymphoma: a systematic review. Blood 111(2):504–516
13. Weiler-Sagie M, Bushelev O, Epelbaum R, Dann EJ, Haim N, Avivi I et al (2010) 18F-FDG avidity in lymphoma readdressed: a study of 766 patients. J Nucl Med 51(1):25–30
14. Le Dortz L, de Guibert S, Bayat S, Devillers A, Houot R, Rolland Y et al (2010) Diagnostic and prognostic impact of 18F-FDG PET/CT in follicular lymphoma. Eur J Nucl Med Mol Imaging 37(12):2307–2314
15. Janikova A, Bolcak K, Pavlik T, Mayer J, Kral Z (2008) Value of [18F]fluorodeoxyglucose positron emission tomography in the management of follicular lymphoma: the end of a dilemma? Clin Lymphoma Myeloma 8(5):287–293
16. Scott AM, Gunawardana DH, Wong J, Kirkwood I, Hicks RJ, Ho SI et al (2009) Positron emission tomography changes management, improves prognostic stratification and is superior to gallium scintigraphy in patients with low-grade lymphoma: results of a multicentre prospective study. Eur J Nucl Med Mol Imaging 36(3):347–353
17. Luminari S, Biasoli I, Arcaini L, Versari A, Rusconi C, Merli F et al (2013) The use of FDG-PET in the initial staging of 142 patients with follicular lymphoma: a retrospective study from the FOLL05 randomized trial of the Fondazione Italiana Linfomi. Ann Oncol 24 (8):2108–2112
18. Wirth A, Foo M, Seymour JF, MacManus MP, Hicks RJ (2008) Impact of [^{18}F] fluorodeoxyglucose positron emission tomography on staging and management of early-stage follicular non-Hodgkin lymphoma. Int J Radiat Oncol Biol Phys 71(1):213–219
19. Karam M, Ata A, Irish K, Feustel PJ, Mottaghy FM, Stroobants SG et al (2009) FDG positron emission tomography/computed tomography scan may identify mantle cell lymphoma patients with unusually favorable outcome. Nucl Med Commun 30(10):770–778
20. Perry C, Herishanu Y, Metzer U, Bairey O, Ruchlemer R, Trejo L et al (2007) Diagnostic accuracy of PET/CT in patients with extranodal marginal zone MALT lymphoma. Eur J Haematol 79(3):205–209
21. Falchi LK (2014) Correlation between FDG/PET, histology, characteristics, and survival in 332 patients with chronic lymphoid leukemia. Blood 123(18):2783–2790
22. Papajik T, Myslivecek M, Urbanova R, Buriankova E, Kapitanova Z, Prochazka V et al (2013) 2-[^{18}F]fluoro-2-deoxy-d-glucose positron emission tomography/computed tomography examination in patients with chronic lymphocytic leukemia may reveal Richter transformation. Leuk Lymphoma 55(2):314–319
23. Raanani P, Shasha Y, Perry C, Metser U, Naparstek E, Apter S et al (2006) Is CT scan still necessary for staging in Hodgkin and non-Hodgkin lymphoma patients in the PET/CT era? Ann Oncol 17(1):117–122
24. Elstrom RL, Leonard JP, Coleman M, Brown RK (2008) Combined PET and low-dose, noncontrast CT scanning obviates the need for additional diagnostic contrast-enhanced CT scans in patients undergoing staging or restaging for lymphoma. Ann Oncol 19(10):1770–1773
25. Pelosi E, Pregno P, Penna D, Deandreis D, Chiappella A, Limerutti G et al (2008) Role of whole-body [^{18}F] fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) and conventional techniques in the staging of patients with Hodgkin and aggressive non Hodgkin lymphoma. Radiol Med 113(4):578–590

26. Khan AB, Barrington SF, Mikhaeel NG, Hunt AA, Cameron L, Morris T et al (2013) PET-CT staging of DLBCL accurately identifies and provides new insight into the clinical significance of bone marrow involvement. Blood 122(1):61–67
27. Paone G, Itti E, Haioun C, Gaulard P, Dupuis J, Lin C et al (2009) Bone marrow involvement in diffuse large B-cell lymphoma: correlation between FDG-PET uptake and type of cellular infiltrate. Eur J Nucl Med Mol Imaging 36(5):745–750
28. Boehme V, Zeynalova S, Kloess M, Loeffler M, Kaiser U, Pfreundschuh M et al (2007) Incidence and risk factors of central nervous system recurrence in aggressive lymphoma—a survey of 1693 patients treated in protocols of the German high-grade non-Hodgkin's lymphoma study group (DSHNHL). Ann Oncol 18(1):149–157
29. Song MK, Chung JS, Shin HJ, Lee SM, Lee SE, Lee HS et al (2012) Clinical significance of metabolic tumor volume by PET/CT in stages II and III of diffuse large B cell lymphoma without extranodal site involvement. Ann Hematol 91(5):697–703
30. Gallicchio R, Mansueto G, Simeon V, Nardelli A, Guariglia R, Capacchione D et al (2014) F-18 FDG PET/CT quantization parameters as predictors of outcome in patients with diffuse large B-cell lymphoma. Eur J Haematol 92(5):382–389
31. Esfahani SA, Heidari P, Halpern EF, Hochberg EP, Palmer EL, Mahmood U (2013) Baseline total lesion glycolysis measured with (18)F-FDG PET/CT as a predictor of progression-free survival in diffuse large B-cell lymphoma: a pilot study. Am J Nucl Med Mol Imaging 3 (3):272–281
32. Kim TM, Paeng JC, Chun IK, Keam B, Jeon YK, Lee SH et al (2013) Total lesion glycolysis in positron emission tomography is a better predictor of outcome than the international prognostic index for patients with diffuse large B cell lymphoma. Cancer 119(6):1195–1202
33. Carrillo-Cruz E, Marin-Oyaga VA, Rodriguez M, Borrego-Dorado I, de la Cruz Vicente F, Cantero EQ et al (2014) Role of 18F-FDG-PET/CT in the management of burkitt lymphoma. Eur J Haematol
34. Kako S, Izutsu K, Ota Y, Minatani Y, Sugaya M, Momose T et al (2007) FDG-PET in T-cell and NK-cell neoplasms. Ann Oncol 18(10):1685–1690
35. Casulo C, Schoder H, Feeney J, Lim R, Maragulia J, Zelenetz AD et al (2013) 18F-fluorodeoxyglucose positron emission tomography in the staging and prognosis of T cell lymphoma. Leuk Lymphoma 54(10):2163–2167
36. Moon SH, Cho SK, Kim WS, Kim SJ, Chan Ahn Y, Choe YS et al (2003) The role of 18F-FDG PET/CT for initial staging of nasal type natural killer/t-cell lymphoma: a comparison with conventional staging methods. J Nucl Med 54(7):1039–1044
37. Rankin SC (2003) Assessment of response to therapy using conventional imaging. Eur J Nucl Med Mol Imaging 30(1):S56–S64
38. Armitage JO, Weisenburger DD, Hutchins M, Moravec DF, Dowling M, Sorensen S et al (1986) Chemotherapy for diffuse large-cell lymphoma–rapidly responding patients have more durable remissions. J Clin Oncol 4(2):160–164
39. Gupta RK, Gospodarowicz MK, Lister TA (1999) Clinical evaluation and staging. In: Mauch P, Armitage JO, Diehl V, Hoppe R, Weiss LM (eds) Hodgkin's disease. Lippincott Williams and Wilkins, Philadelphia, pp 223–240
40. Jerusalem G, Beguin Y, Fassotte MF, Najjar F, Paulus P, Rigo P et al (2000) Persistent tumor 18F-FDG uptake after a few cycles of polychemotherapy is predictive of treatment failure in non-Hodgkin's lymphoma. Haematologica 85(6):613–618
41. Mikhaeel NG, Timothy AR, O'Doherty MJ, Hain S, Maisey MN (2000) 18-FDG-PET as a prognostic indicator in the treatment of aggressive non-Hodgkin's lymphoma-comparison with CT. Leuk Lymphoma 39(5–6):543–553
42. Spaepen K, Stroobants S, Dupont P, Vandenberghe P, Thomas J, de Groot T et al (2002) Early restaging positron emission tomography with (18)F-fluorodeoxyglucose predicts outcome in patients with aggressive non-Hodgkin's lymphoma. Ann Oncol 13(9):1356–1363

43. Hoekstra OS, Ossenkoppele GJ, Golding R, van Lingen A, Visser GW, Teule GJ et al (1993) Early treatment response in malignant lymphoma, as determined by planar fluorine-18-fluorodeoxyglucose scintigraphy. J Nucl Med 34(10):1706–1710
44. Kostakoglu L, Coleman M, Leonard JP, Kuji I, Zoe H, Goldsmith SJ (2002) PET predicts prognosis after 1 cycle of chemotherapy in aggressive lymphoma and Hodgkin's disease. J Nucl Med 43(8):1018–1027
45. Torizuka T, Nakamura F, Kanno T, Futatsubashi M, Yoshikawa E, Okada H et al (2004) Early therapy monitoring with FDG-PET in aggressive non-Hodgkin's lymphoma and Hodgkin's lymphoma. Eur J Nucl Med Mol Imaging 31(1):22–28
46. Mikhaeel NG, Hutchings M, Fields PA, O'Doherty MJ, Timothy AR (2005) FDG-PET after two to three cycles of chemotherapy predicts progression-free and overall survival in high-grade non-Hodgkin lymphoma. Ann Oncol 16(9):1514–1523
47. Haioun C, Itti E, Rahmouni A, Brice P, Rain JD, Belhadj K et al (2005) [18F]fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) in aggressive lymphoma: an early prognostic tool for predicting patient outcome. Blood 106(4):1376–1381
48. Kostakoglu L, Goldsmith SJ, Leonard JP, Christos P, Furman RR, Atasever T et al (2006) FDG-PET after 1 cycle of therapy predicts outcome in diffuse large cell lymphoma and classic Hodgkin disease. Cancer 107(11):2678–2687
49. Han HS, Escalon MP, Hsiao B, Serafini A, Lossos IS (2009) High incidence of false-positive PET scans in patients with aggressive non-Hodgkin's lymphoma treated with rituximab-containing regimens. Ann Oncol 20(2):309–318
50. Bishu S, Quigley JM, Bishu SR, Olsasky SM, Stem RA, Shostrom VK et al (2007) Predictive value and diagnostic accuracy of F-18-fluoro-deoxy-glucose positron emission tomography treated grade 1 and 2 follicular lymphoma. Leuk Lymphoma 48(8):1548–1555
51. Cashen AF, Dehdashti F, Luo J, Homb A, Siegel BA, Bartlett NL (2011) 18F-FDG PET/CT for early response assessment in diffuse large b-cell lymphoma: poor predictive value of international harmonization project interpretation. J Nucl Med 52(3):386–392
52. Moskowitz CH, Schoder H, Teruya-Feldstein J, Sima C, Iasonos A, Portlock CS et al (2010) Risk-adapted dose-dense immunochemotherapy determined by interim FDG-PET in advanced-stage diffuse large B-cell lymphoma. J Clin Oncol 28(11):1896–1903
53. Itti E, Lin C, Dupuis J, Paone G, Capacchione D, Rahmouni A et al (2009) Prognostic value of interim 18F-FDG PET in patients with diffuse large B-cell lymphoma: SUV-based assessment at 4 cycles of chemotherapy. J Nucl Med 50(4):527–533
54. Casasnovas RO, Meignan M, Berriolo-Riedinger A, Bardet S, Julian A, Thieblemont C et al (2011) SUVmax reduction improves early prognosis value of interim positron emission tomography scans in diffuse large B-cell lymphoma. Blood 118(1):37–43
55. Fludeoxyglucose F 18 positron emission tomography in predicting risk of relapse in patients with non-Hodgkin's lymphoma who are undergoing combination chemotherapy with or without autologous stem cell or bone marrow transplant. http://www.clinicaltrials.gov/ct2/show/NCT00238368 Accessed Dec 17 2014
56. Therapy for patients with untreated age-adjusted international prognostic index low-intermediate risk, high-intermediate risk, or high risk diffuse large B cell lymphoma. http://www.clinicaltrials.gov/ct2/show/NCT00712582 Accessed Dec 17 2014
57. Positron emission tomography guided therapy of aggressive non-Hodgkin's lymphomas (PETAL). http://www.clinicaltrials.gov/ct2/show/NCT00554164 Accessed Dec 17 2014
58. Tailoring treatment for B cell non-Hodgkin's lymphoma based on pet scan results mid treatment. http://www.clinicaltrials.gov/ct2/show/NCT00324467 Accessed Dec 17 2014
59. FDG-PET-stratified R-DICEP and R-beam/ASCT for diffuse large B-cell lymphoma (PET chop). http://www.clinicaltrials.gov/ct2/show/NCT00530179 Accessed Dec 17 2014
60. A study of two associations of rituximab and chemotherapy, with a PET-driven strategy, in lymphoma (LNH2007-3B). http://www.clinicaltrials.gov/ct2/show/NCT00498043 Accessed Dec 17 2014

61. Study evaluating the non-inferiority of a treatment adapted to the early response evaluated with 18f-fdg pet compared to a standard treatment, for patients aged from 18 to 80 years with low risk (aa IPI = 0) diffuse large B-cells non Hodgkin's lymphoma CD 20+. http://www.clinicaltrials.gov/ct2/show/NCT01285765 Accessed Dec 17 2014
62. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM et al (1999) Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI sponsored international working group. J Clin Oncol 17(4):1244
63. Zijlstra JM, Lindauer-van der Werf G, Hoekstra OS, Hooft L, Riphagen II, Huijgens PC (2006) 18F-fluoro-deoxyglucose positron emission tomography for post-treatment evaluation of malignant lymphoma: a systematic review. Haematologica 91(4):522–529
64. Trotman J, Fournier M, Lamy T, Seymour JF, Sonet A, Janikova A et al (2011) Positron emission tomography–computed tomography (PET-CT) after induction therapy is highly predictive of patient outcome in follicular lymphoma: analysis of PET-CT in a subset of PRIMA trial participants. J Clin Oncol 29(23):3194–3200
65. Zinzani PL, Musuraca G, Alinari L, Fanti S, Tani M, Stefoni V et al (2007) Predictive role of positron emission tomography in the outcome of patients with follicular lymphoma. Clin Lymphoma Myeloma 7(4):291–295
66. Tychyj-Pinel C, Ricard F, Fulham M, Fournier M, Meignan M, Lamy T et al (2014) PET/CT assessment in follicular lymphoma using standardized criteria: central review in the PRIMA study. Eur J Nucl Med Mol Imaging 41(3):408–415
67. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ et al (2007) Revised response criteria for malignant lymphoma. J Clin Oncol 25(5):579–586
68. Juweid ME, Stroobants S, Hoekstra OS, Mottaghy FM, Dietlein M, Guermazi A et al (2007) Use of positron emission tomography for response assessment of lymphoma: consensus of the imaging subcommittee of international harmonization project in lymphoma. J Clin Oncol 25(5):571–578
69. Brepoels L, Stroobants S, De Wever W, Spaepen K, Vandenberghe P, Thomas J et al (2007) Aggressive and indolent non-Hodgkin's lymphoma: response assessment by integrated international workshop criteria. Leuk Lymphoma 48(8):1522–1530
70. Martelli M, Ceriani L, Zucca E, Zinzani PL, Ferreri AsJM, Vitolo U et al (2014) [18F] Fluorodeoxyglucose positron emission tomography predicts survival after chemoimmunotherapy for primary mediastinal large B-cell lymphoma: results of the international extranodal lymphoma study group IELSG-26 study. J Clin Oncol 32(17):1769–1775
71. International extranodal lymphoma study group (IELSG): a randomized, open-label two-arm phase iii comparative study assessing the role of involved mediastinal radiotherapy after rituximab containing chemotherapy regimens to patients with newly diagnosed primary mediastinal large B-cell lymphoma. http://clinicaltrials.gov/ct2/show/NCT01599559 Accessed Dec 17 2014
72. Sehn LH, Klasa R, Shenkier T, Villa D, Slack GW, Gascoyne RD et al (2013) Long-term experience with PET-guided consolidative radiation therapy (XRT) in patients with advanced stage diffuse large B-cell lymphoma (DLBCL) treated with R-CHOP. Hematol Oncol 31 (S1):137 1-6-2013
73. Hamlin PAZ (2003) Age-adjusted International Prognostic Index predicts autologous stem cell transplantation outcome for patients with relapsed or primary refractory diffuse large B-cell lymphoma. Blood 102(6):1989–1996
74. Weeks JC, Yeap BY, Canellos GP, Shipp MA (1991) Value of follow-up procedures in patients with large-cell lymphoma who achieve a complete remission. J Clin Oncol 9 (7):1196–1203
75. Guppy AE, Tebbutt NC, Norman A, Cunningham D (2003) The role of surveillance CT scans in patients with diffuse large B-cell non-Hodgkin's lymphoma. Leuk Lymphoma 44 (1):123–125

76. Liedtke M, Hamlin PA, Moskowitz CH, Zelenetz AD (2006) Surveillance imaging during remission identifies a group of patients with more favorable aggressive NHL at time of relapse: a retrospective analysis of a uniformly-treated patient population. Ann Oncol 17 (6):909–913
77. Lin TL, Kuo MC, Shih LY, Dunn P, Wang PN, Wu JH et al (2012) Value of surveillance computed tomography in the follow-up of diffuse large B-cell and follicular lymphomas. Ann Hematol 91(11):1741–1745
78. El Galaly TC, Mylam KJ, Boegsted M, Brown P, Rossing M, Gang AO et al (2014) Role of routine imaging in detecting recurrent lymphoma: a review of 258 patients with relapsed aggressive non-Hodgkin and Hodgkin lymphoma. Am J Hematol 89(6):575–580
79. El Galaly T, Prakash V, Christiansen I, Madsen J, Johansen P, Boegsted M et al (2011) Efficacy of routine surveillance with positron emission tomography/computed tomography in aggressive non-Hodgkin lymphoma in complete remission: status in a single center. Leuk Lymphoma 52(4):597–603
80. Zinzani PL, Stefoni V, Tani M, Fanti S, Musuraca G, Castellucci P et al (2009) Role of [18F] fluorodeoxyglucose positron emission tomography scan in the follow-up of lymphoma. J Clin Oncol 27(11):1781–1787
81. Cheah CY, Hofman MS, Dickinson M, Wirth A, Westerman D, Harrison SJ et al (2013) Limited role for surveillance PET-CT scanning in patients with diffuse large B-cell lymphoma in complete metabolic remission following primary therapy. Br J Cancer 109 (2):312–317
82. Abel GA, Vanderplas A, Rodriguez MA, Crosby AL, Czuczman MS, Niland JC et al (2011) High rates of surveillance imaging for treated diffuse large B-cell lymphoma: findings from a large national database. Leuk Lymphoma, 1–4
83. Cheah C, Dickinson M, Hofman M, George A, Ritchie D, Prince HM et al (2014) Limited clinical benefit for surveillance PET-CT scanning in patients with histologically transformed lymphoma in complete metabolic remission following primary therapy. Ann Hematol 93 (7):1193–1200
84. Avivi I, Zilberlicht A, Dann EJ, Leiba R, Faibish T, Rowe JM et al (2013) Strikingly high false positivity of surveillance FDG-PET/CT scanning among patients with diffuse large cell lymphoma in the rituximab era. Am J Hematol 88(5):400–405
85. Picardi M, Pugliese N, Cirillo M, Zeppa P, Cozzolino I, Ciancia G et al (2014) Advanced-stage Hodgkin lymphoma: US/chest radiography for detection of relapse in patients in first complete remission—randomized trial of routine surveillance imaging procedures. Radiology 272(1):262–274
86. Haioun C, Lepage E, Gisselbrecht C, Salles G, Coiffier B, Brice P et al (2000) Survival benefit of high-dose therapy in poor-risk aggressive non-Hodgkin's lymphoma: final analysis of the prospective LNH87-2 protocol–a groupe d'Etude des lymphomes de l'Adulte study. J Clin Oncol 18(16):3025–3030
87. Stiff PJ, Dahlberg S, Forman SJ, McCall AR, Horning SJ, Nademanee AP et al (1998) Autologous bone marrow transplantation for patients with relapsed or refractory diffuse aggressive non-Hodgkin's lymphoma: value of augmented preparative regimens–a southwest oncology group trial. J Clin Oncol 16(1):48–55
88. Becherer A, Mitterbauer M, Jaeger U, Kalhs P, Greinix HT, Karanikas G et al (2002) Positron emission tomography with [18F]2-fluoro-D-2-deoxyglucose (FDG-PET) predicts relapse of malignant lymphoma after high-dose therapy with stem cell transplantation. Leukemia 16(2):260–267
89. Terasawa T, Dahabreh IJ, Nihashi T (2010) Fluorine-18-fluorodeoxyglucose positron emission tomography in response assessment before high-dose chemotherapy for lymphoma: a systematic review and meta-analysis. Oncologist 15(7):750–759
90. Dickinson M, Hoyt R, Roberts AW, Grigg A, Seymour JF, Prince HM et al (2010) Improved survival for relapsed diffuse large B cell lymphoma is predicted by a negative pre-transplant FDG-PET scan following salvage chemotherapy. Br J Haematol 150(1):39–45

91. Cremerius U, Fabry U, Wildberger JE, Zimny M, Reinartz P, Nowak B et al (2002) Pre-transplant positron emission tomography (PET) using fluorine-18-fluoro-deoxyglucose (FDG) predicts outcome in patients treated with high-dose chemotherapy and autologous stem cell transplantation for non-Hodgkin's lymphoma. Bone Marrow Transplant 30(2):103–111
92. Spaepen K, Stroobants S, Dupont P, Vandenberghe P, Maertens J, Bormans G et al (2003) Prognostic value of pretransplantation positron emission tomography using fluorine 18-fluorodeoxyglucose in patients with aggressive lymphoma treated with high-dose chemotherapy and stem cell transplantation. Blood 102(1):53–59
93. Filmont JE, Czernin J, Yap C, Silverman DH, Quon A, Phelps ME et al (2003) Value of F-18 fluorodeoxyglucose positron emission tomography for predicting the clinical outcome of patients with aggressive lymphoma prior to and after autologous stem-cell transplantation. Chest 124(2):608–613
94. Moskowitz CH, Matasar MJ, Zelenetz AD, Nimer SD, Gerecitano J, Hamlin P et al (2012) Normalization of pre-ASCT, FDG-PET imaging with second-line, non–cross-resistant, chemotherapy programs improves event-free survival in patients with Hodgkin lymphoma. Blood 119(7):1665–1670
95. Buck AK, Halter G, Schirrmeister H, Kotzerke J, Wurziger I, Glatting G et al (2003) Imaging proliferation in lung tumors with PET: 18F-FLT versus 18F-FDG. J Nucl Med 44(9):1426–1431
96. Sandherr M, von Schilling C, Link T, Stock K, von Bubnoff N, Peschel C et al (2001) Pitfalls in imaging Hodgkin's disease with computed tomography and positron emission tomography using fluorine-18-fluorodeoxyglucose. Ann Oncol 12(5):719–722
97. Martiat P, Ferrant A, Labar D, Cogneau M, Bol A, Michel C et al (1988) In vivo measurement of carbon-11 thymidine uptake in non-Hodgkin's lymphoma using positron emission tomography. J Nucl Med 29(10):1633–1637
98. Shields AF, Mankoff DA, Link JM, Graham MM, Eary JF, Kozawa SM et al (1998) Carbon-11-thymidine and FDG to measure therapy response. J Nucl Med 39(10):1757–1762
99. Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM et al (1998) Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. Nat Med 4(11):1334–1336
100. Buchmann I, Neumaier B, Schreckenberger M, Reske S (2004) [18F]3′-deoxy-3′-fluorothymidine-PET in NHL patients: whole-body biodistribution and imaging of lymphoma manifestations–a pilot study. Cancer Biother Radiopharm 19(4):436–442
101. Buck AK, Bommer M, Stilgenbauer S, Juweid M, Glatting G, Schirrmeister H et al (2006) Molecular imaging of proliferation in malignant lymphoma. Cancer Res 66(22):11055–11061
102. Kasper B, Egerer G, Gronkowski M, Haufe S, Lehnert T, Eisenhut M et al (2007) Functional diagnosis of residual lymphomas after radiochemotherapy with positron emission tomography comparing FDG- and FLT-PET. Leuk Lymphoma 48(4):746–753
103. Buck AK, Kratochwil C, Glatting G, Juweid M, Bommer M, Tepsic D et al (2007) Early assessment of therapy response in malignant lymphoma with the thymidine analogue [18F] FLT. Eur J Nucl Med Mol Imaging 34(11):1775–1782
104. Herrmann K, Buck AK, Schuster T, Rudelius M, Wester HJ, Graf N et al (2011) A pilot study to evaluate 3′-Deoxy-3′-18F-Fluorothymidine PET for initial and early response imaging in mantle cell lymphoma. J Nucl Med 52(12):1898–1902
105. Herrmann K, Buck AK, Schuster T, Junger A, Wieder H, Graf N et al (2011) Predictive value of initial 18F-FLT uptake in patients with aggressive non-Hodgkin lymphoma receiving R-CHOP treatment. J Nucl Med 52(5):690–696
106. Graf N, Herrmann K, den Hollander J, Fend F, Schuster T, Wester HJ et al (2008) Imaging proliferation to monitor early response of lymphoma to cytotoxic treatment. Mol Imaging Biol 10(6):349–355

107. Hoffman RM (1984) Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. A review and synthesis. Biochim Biophys Acta 738(1–2):49–87
108. Stern PH, Wallace CD, Hoffman RM (1984) Altered methionine metabolism occurs in all members of a set of diverse human tumor cell lines. J Cell Physiol 119(1):29–34
109. Wheatley DN (1982) On the problem of linear incorporation of amino acids into cell protein. Experientia 38(7):818–820
110. Leskinen-Kallio S, Ruotsalainen U, Nagren K, Teras M, Joensuu H (1991) Uptake of carbon-11-methionine and fluorodeoxyglucose in non-Hodgkin's lymphoma: a PET study. J Nucl Med 32(6):1211–1218
111. Nuutinen J, Leskinen S, Lindholm P, Soderstrom KO, Nagren K, Huhtala S et al (1998) Use of carbon-11 methionine positron emission tomography to assess malignancy grade and predict survival in patients with lymphomas. Eur J Nucl Med 25(7):729–735
112. Kawase Y, Yamamoto Y, Kameyama R, Kawai N, Kudomi N, Nishiyama Y (2011) Comparison of 11C-Methionine PET and 18F-FDG PET in patients with primary central nervous system lymphoma. Mol Imaging Biol 13(6):1284–1289
113. Olafsen T, Betting D, Kenanova VE, Salazar FB, Clarke P, Said J et al (2009) Recombinant anti-CD20 antibody fragments for small-animal PET imaging of B-cell lymphomas. J Nucl Med 50(9):1500–1508
114. van Ufford HMEQ, Kwee TC, Beek FJ, van Leeuwen MS, Takahara T, Fijnheer R et al (2011) Newly diagnosed lymphoma: initial results with whole-body T1-weighted, STIR, and diffusion-weighted MRI compared with 18F-FDG PET/CT. Am J Roentgenol 196(3):662–669

Prognosis and Therapy of Chronic Lymphocytic Leukemia and Small Lymphocytic Lymphoma

Clare Sun and Adrian Wiestner

Abstract

Chronic lymphocytic leukemia (CLL) is characterized by a highly variable clinical course that has guided treatment principles in as much as anti-leukemic therapy is reserved for patients with active disease. This heterogeneity is somewhat dissected by prognostic markers, many of which represent pathogenic mechanisms. Recently, the introduction of highly active targeted agents and maturing data on predictive markers may lead to more individualized therapeutic approaches. In this chapter, we review key prognostic markers, current and emerging therapy, and will attempt to outline a future "where the two may connect".

Keywords

Leukemia · Lymphocytic · Chronic · B-Cell · Biological markers · Therapy · Prognosis

AW is named as an inventor on a US patent held by NIH on the use of ZAP-70 as a prognostic marker in CLL and has received royalty payments. AW has received research support from Pharmacyclics and served on advisory boards (without compensation) for Pharmacyclics and Janssen.

C. Sun · A. Wiestner (✉)
Hematology Branch, National Heart, Lung, and Blood Institute,
National Institutes of Health, Bld 10, CRC 3-5140, 10 Center Drive,
Bethesda, MD 20892-1202, USA
e-mail: wiestnera@mail.nih.gov

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_6

Contents

1 Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of mature, clonal B cells in the blood, bone marrow, and lymphoid organs [62]. Diagnosis requires the presence of at least 5×10^9 clonal B cells/L of blood having a characteristic immunophenotype (CD19+, CD5+, CD23+, dim CD20) and comprising ≤55 % prolymphocytes [62]. Small lymphocytic lymphoma (SLL) is considered biologically equivalent to CLL except for the preferential distribution of malignant lymphocytes to lymphoid organs rather than the blood [62]. Patients with $\leq 5 \times 10^9$ clonal B cells/L of blood and do not meet criteria for SLL have monoclonal B-lymphocytosis (MBL) [62]. MBL may be considered a precursor to CLL [111].

CLL has a highly variable clinical course [71]. Although the median overall survival (OS) of all comers at a large referral center was 11 years [145], it can be considerably shorter for patients with high-risk features or as long as that of age-matched controls for those with indolent disease [9, 39, 105]. Despite the apparent heterogeneity, current treatment guidelines are generic and directed primarily by the presence of active or symptomatic disease and the fitness of the patient [62].

Whole genome and exome sequencing of a large number of CLL cases has revealed numerous somatic mutations [82, 104, 143]. However, there is still no clear-cut genetic lesion that is necessary or sufficient for the development of CLL. In contrast, extensive functional and cell biology studies have revealed a dependence of CLL cells on supportive factors in the tissue microenvironment for proliferation and survival [15, 66]. Among the many different pathways that appear to play a role in tumor–host interactions, B-cell receptor signaling has emerged as a pivotal pathway in the pathogenesis of CLL and has become the target of novel treatment approaches [16, 149].

2 Prognostic Markers

2.1 Clinical Stage

The course and outcome of CLL is highly heterogeneous [71]. Early efforts to stratify patients relied on clinical stage for prognosis (Table 1). Two widely adopted staging systems, Rai et al. [105] and Binet et al. [9], define low to high-risk patients based on the extent of organ involvement and cytopenias. CLL at the earliest stage

Table 1 Abbreviated list of prognostic markers in CLL

Clinical	Stage
	Absolute lymphocyte count
	Lymphocyte doubling time
	Bone marrow infiltration pattern
	Treatment response
	Age
	Performance status
Genetic	*IGHV* mutational status
	FISH
	Low risk: del(13q)
	Intermediate risk: trisomy 12
	High risk: del(17p), del(11q), complex karyotype
	Mutations
	Favorable: *MYD88*
	Unfavorable: *TP53*, *NOTCH1*, *SF3B1*, *BIRC3*, *ATM*
Flow cytometry	ZAP-70
	CD49d
	CD38
Serum	Lactate dehydrogenase
	β2-microglobulin
	Thymidine kinase
	CCL3 and CLL4 serum levels

is manifested by lymphocytosis with or without lymphadenopathy. More advanced stages are characterized by liver or spleen involvement and ultimately by anemia or thrombocytopenia. In this manner, the disease is staged from 0 to 4 [105] or from A to C [9]. In the original cohorts from which these staging systems were derived, early stage patients had a life expectancy similar to the general population, while those with advanced disease had a median survival not exceeding 2 years [9, 105].

3 IGHV Mutational Status

B-cell development begins in the bone marrow when hematopoietic progenitor cells (HPCs) differentiate into pro-B cells [54]. These cells undergo sequential rearrangement of the B-cell receptor (BCR) immunoglobulin heavy and light chains, then exit into the peripheral blood as naïve B cells [54]. Upon migration into lymphoid tissue, naïve B cells are activated by antigen-presenting cells and may differentiate into plasma cells or memory B cells [54]. The variable regions of the immunoglobulins, which encode the BCR antigen-binding domains, can acquire somatic mutations that increase the affinity to a specific antigen [54]. The mutational status of clonal immunoglobulin heavy chain variable (*IGHV*) genes is determined by comparing the *IGHV* sequence of malignant cells to known germ line sequences [56]. An *IGHV* is considered mutated if it shares less than 98 % homology to the corresponding germ line sequence [56].

As recognized in the early 1990s, CLL patients can be divided into two groups based on the *IGHV* mutational status [122]. In about half of the patients, CLL cells express an *IGHV* gene in germ line configuration, while in the other half, >2 % of the bases are mutated [122]. In 1999, these two groups of patients were reported to have significantly disparate clinical outcomes [34, 64]. Patients with *IGHV*-unmutated CLL (U-CLL) have a median OS of approximately 9 years compared to 24 years for those with *IGHV*-mutated CLL (M-CLL) [34, 64]. Furthermore, patients with U-CLL more often have advanced stage disease at diagnosis, progress faster, and require earlier and more frequent treatment [34, 64, 109]. Most cases of transformation to high-grade lymphoma (Richter's transformation) also occur in U-CLL [86, 126]. Although the incidence of hypogammaglobulinemia is similar in M-CLL and U-CLL, the latter is associated with more infection and infection-related mortality [51]. Unfavorable genetic aberrations, including 17p and 11q deletions, are common in U-CLL, while 13q14 deletion or translocation occur more often in M-CLL [64, 79, 98].

In a subset of patients, the clonal cells express a restricted repertoire of *IGHV* genes and in some cases, carry BCRs that are structurally virtually identical, referred to as stereotypic BCRs [1, 130]. This suggests that in these cases, CLL originates from a B cell with distinct antigen specificity. In particular, the sequence and length of the complementarity-determining region 3 (CDR3) appear to contribute to stereotypic BCRs and differ between U-CLL and M-CLL [87, 153]. U-CLL cells commonly employ longer, stereotyped CDR3s [130], which are

thought to enhance BCR polyreactivity [87]. Indeed, the BCRs of U-CLL tend to be more polyreactive and of lower affinity than those found in M-CLL or normal B cells [69]. Consequently, it has been hypothesized that U-CLL may respond to multiple trophic signals or antigens, leading to more rapid expansion of the malignant clone [131]. In contrast, M-CLL cells appear to be anergic, that is, antigen-hyporesponsive, likely as a result of prior high affinity or repetitive antigen-binding [131]. This may, at least in part, explain the differences in disease pace between the two subtypes. Thus, *IGHV* mutation status may be viewed as a surrogate of BCR signaling strength (Fig. 1). In addition, chemokines, CCL3 and CLL4, are secreted by activated CLL cells, facilitate crosstalk with the microenvironment, and may serve as markers for BCR signaling [125]. As is the case in an increasing number of B-cell malignancies, antigenic stimulation has emerged as a driver pathway in the pathogenesis of CLL [93, 154].

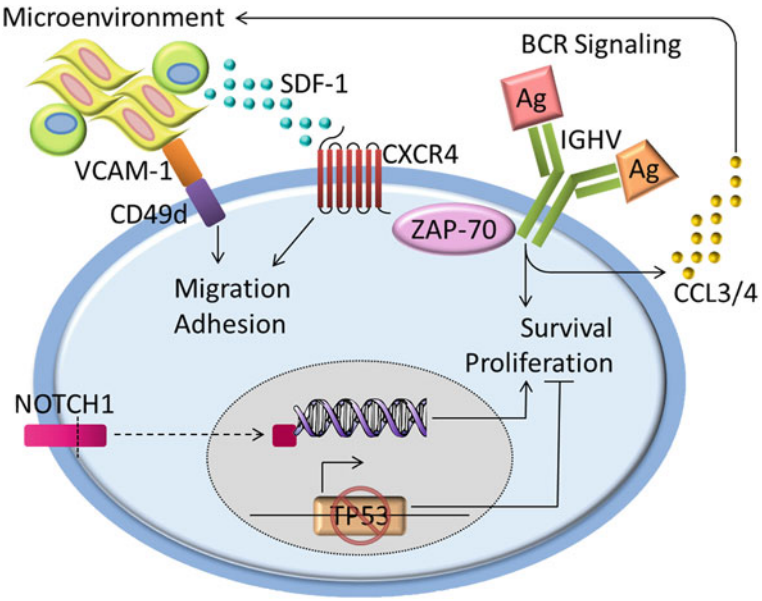


Fig. 1 Key prognostic markers in CLL reflect underlying pathogenic pathways. BCR signaling is a central pathway in the pathogenesis of CLL. The BCR appears to be polyreactive in U-CLL, leading to increased receptor activation and downstream signal transduction. Activated CLL cells secrete chemokines, CCL3 and CLL4, which attract accessory cells to the microenvironment. Migration and adhesion to the microenvironment is further facilitated by ligation of CXCR4 and CD49d on the surface of CLL cells. ZAP-70 appears to directly associate with the BCR to enhance signaling and modulate CXCR4-mediated chemotaxis. Specific genetic lesions promote CLL cell survival and proliferation. Abnormalities in *TP53* impair the cellular response to DNA damage and avert apoptosis and growth arrest. Activating mutations in *NOTCH1* produce a more stable intracellular signaling protein that regulates the transcription of multiple survival pathways

3.1 Zap-70

Zeta-chain-associated protein of 70 kD (ZAP-70) is a cytoplasmic kinase expressed in T and NK cells [22]. Upon T-cell receptor (TCR) stimulation, ZAP-70 associates with the zeta-chain of TCR and undergoes phosphorylation [22]. ZAP-70 is structurally homologous to spleen tyrosine kinase, which plays an analogous role in BCR signal transduction [22]. Although ZAP-70 is not found in mature peripheral blood B cells, it is expressed in pro- and pre-B cells [32] as well as activated tonsillar and splenic B cells [95]. ZAP-70 is one of the most differentially expressed genes between U-CLL and M-CLL [116, 148]. Because ZAP-70 is highly concordant with *IGHV* mutational status [31, 97, 108, 116, 148, 155], it has been suggested as a surrogate. ZAP-70 expression appears to be regulated by methylation of the 5′ region of the ZAP-70 gene [28, 30] and heat shock protein 90 chaperoning [21]. The level of expression follows a continuum, remains stable over time and is similar across peripheral blood and bone marrow specimens [38, 108]. Cutoffs ranging from 10 to 20 % of ZAP-70+ CLL cells have been shown to best correlate with *IGHV* mutational status [31, 97, 108]. ZAP-70 is most commonly detected by flow cytometry, but can also be evaluated by RT-PCR, Western blotting, and immunohistochemistry with comparable results [31, 97, 148]. Nevertheless, it has been difficult to standardize ZAP-70 testing in clinical practice, and the absence of a bimodal distribution results in indeterminate or borderline results for many patients. Recent studies suggest that analysis of ZAP-70 promoter methylation may yield more robust results [29].

Patients with ZAP-70+ CLL have a median survival of 9 years versus 24 years [97], more rapid disease progression [31], and require treatment earlier [38, 108]. ZAP-70+ CLL is associated with advanced clinical stage, higher lymphocyte count, and higher tumor proliferation [38, 113, 127]. Interestingly, patients with ZAP-70+ CLL are more likely to develop autoimmune cytopenias [155]. While ZAP-70 is more often found in U-CLL than M-CLL, it maintains prognostic value even in discordant cases [38, 108].

CLL cells expressing ZAP-70 have an augmented response to BCR activation. ZAP-70 may directly associate with the BCR and enhance downstream signal transduction [24, 25]. Interestingly, this effect is independent of its kinase activity [58]. ZAP-70 also enhances CLL cell migration in response to the chemokines CCL19 and CCL21 [37, 113]. The effect of PI3K inhibition, which impairs survival and adhesion of CLL cells in culture, is attenuated by ZAP-70 expression [80]. In the microenvironment, ZAP-70+ CLL is associated with effector T cells and a cytokine profile that is skewed toward IL-4 and IL-10 [91]. These changes, characteristically seen in advanced stages of disease, are observed even in early stage ZAP-70+ CLL and may contribute to a more aggressive disease course [91].

3.2 Cd49d

CD49d is the α4 subunit of the integrin heterodimers α4β1 and α4β7. α4β1, present on B and T cells, mediates leukocyte development and trafficking through binding to its major ligands, vascular cell adhesion molecule-1 (VCAM-1), and fibronectin [65]. In the B-cell compartment, α4β1 facilitates localization to the bone marrow and germinal centers and promotes survival by up-regulation of anti-apoptotic Bcl-XL [115]. The role of α4β7 is more restricted, directing lymphocytes into intestinal lymphoid tissue via ligation to mucosal addressin cell adhesion molecule-1 [8].

The expression of CD49d is measured by flow cytometry [55, 118, 124]. Tumor cells in approximately 40 % of CLL cases and in a larger proportion of other chronic lymphoproliferative disorders express CD49d [33]. Proposed cutoff values to predict clinical outcome in CLL range from 30 to 45 % [14, 55, 124]. Because the rate of CD49d expression follows a U-shaped distribution across a group of patients, CD49d can be clearly read out as positive or negative in most cases [14, 55].

Patients with CD49d+ CLL have more advanced stages [45], more rapid disease progression, and worse OS [14, 55]. CD49d is associated with a number of unfavorable prognostic factors, including CD38, ZAP-70, and possibly *IGHV* mutation status [55, 124]. The CD49d gene is hypomethylated in CLL with trisomy 12, leading to increased expression compared to CLL without trisomy 12 [158]. CD49d is superior to Rai stage, ZAP-70, CD38, β2-microglobulin, and del(11q) in prognosticating outcome [14, 124]. In a recent multicenter analysis, CD49d was identified as the only independent flow cytometry-based prognostic factor for OS, along with age, gender, *IGHV* mutation status, del(17p), and absolute lymphocyte count [14].

In vitro studies have demonstrated the role of CD49d in transendothelial migration of CLL cells [137] and recruitment of monocytes, endothelial, and stromal cells to the bone marrow microenvironment [157]. Furthermore, α4β1, together with CD44v, anchors matrix metalloproteinase-9 to the surface of CLL cells [112]. As a result, malignant cells are arrested in the bone marrow and lymph node, thereby presumably enhancing the nurturing effect of the microenvironment on the tumor cells [112]. CD49d may also competitively engage ligands in the bone marrow and displace HPCs, leading to increased numbers of HPCs in the circulation [118]. In addition, CD49d ligation leads to up-regulation of anti-apoptotic Bcl-2 and Bcl-XL and down-regulation of pro-apoptotic Bax, thus preventing spontaneous [35, 81] and fludarabine-induced apoptosis [36] of CLL cells in culture.

3.3 Genetic Aberrations

Interphase cytogenetic analysis using fluorescent DNA probes for in situ hybridization (FISH) allows detection of chromosomal abnormalities in over 80 % of non-dividing CLL cells [39]. Previously, CLL cells required mitogen stimulation for traditional chromosome analysis in metaphase, which yielded an abnormal karyotype in only half of patients [73]. Using FISH, the most commonly observed

abnormality is 13q deletion in over half of patients, followed in order by 11q deletion, 12q trisomy, and 17p deletion [39]. These can present as a sole abnormality or in combination with one another [39].

Survival of early stage patients ranges from a median of 133 months with 13q deletion to 79 months with 11q deletion and 32 months with 17p deletion [39]. Deletions in 11q and 17p occur almost exclusively in U-CLL and have independent prognostic value [79]. These deletions are also associated with advanced disease and rapid disease progression requiring early treatment [39]. In contrast, one-third of patients with 13q deletion do not require any therapy [39]. The prognosis of del (17p) CLL has been correlated with the percentage of affected CLL cells [136]. In the largest series reported, patients with del(17p) in ≤25 % of CLL cells had a relatively indolent disease course and a 3-year OS of ≥90 % [136]. Del(17p) occurring in M-CLL also appears to be compatible with relatively slower disease progression [57].

Genetic mutations and chromosomal abnormalities have significant overlap in CLL. Loss of tumor suppressor genes contributes to the aggressive nature of CLL with unfavorable karyotype. Mutation in *TP53*, located on chromosome 17p, is found in approximately 80 % of del(17p) CLL [60, 156]. The combination of *TP53* mutation and del(17p) predicts shorter progression-free survival (PFS) than del (17p) alone [60]. In addition, sole *TP53* mutation, without del(17p), portends a similar prognosis to del(17p) CLL [156]. The ataxia-telangiectasia (*ATM*) gene on chromosome 11q is mutated in 30 % of del(11q) CLL [4]. Mutation in *ATM*, which coordinates the cellular response to DNA damage, is associated with shorter OS and PFS [4]. Conversely, the favorable outcome observed in del(13q) CLL, which is superior to that of normal karyotype, has not been well elucidated. Down-regulation of miR15 and miR16 appears to play a role [20], leading to an increase in target proteins, including anti-apoptotic Bcl-2 [27].

Whole genome and exome sequencing has identified a number of recurrent genetic mutations with prognostic relevance. *NOTCH1* is a commonly mutated gene in CLL [104] and partially correlates with trisomy 12 [7, 83]. Patients with *NOTCH1*-mutated CLL have shorter survival and increased risk of Richter's transformation [104]. Mutation in the spliceosomal gene, *SF3B1*, is associated with more rapid disease progression. In contrast, mutations in *MYD88*, which encodes an adaptor protein in interleukin-1/toll-like receptor signaling, tend to be more common in younger patients [104] with mutated *IGHV* genes [143] and have relatively favorable disease course [88]. Importantly, it appears that specific genetic abnormalities, including trisomy 12, del(13q), and *MYD88* mutation, appear to represent driver events in CLL, while other aberrations, such as del(17p) and mutations in *TP53*, *SF3B1*, and *ATM*, occur later with disease progression and increase in frequency in patients with relapsed and refractory disease [82].

4 First-Line Treatment

4.1 Standard Chemoimmunotherapy

Treatment of CLL is generally reserved for patients with active disease or constitutional symptoms fulfilling specific treatment criteria as outlined by the International Workshop on Chronic Lymphocytic Leukemia (Table 2) [62]. The purine analogue, fludarabine, and its combinations have been the mainstay of therapy for many years. In a landmark trial led by the Cancer and Leukemia Group B (CALGB), initial therapy with fludarabine, compared to chlorambucil, improved overall response rate (ORR) from 37 to 63 % [106]. However, perhaps owing to its crossover design, no difference in OS was initially found [106]. Subsequently, a long-term follow-up analysis suggested an eventual benefit in OS for patients treated with fludarabine [107]. However, the benefits of fludarabine appear to be limited to younger patients. The German Chronic Lymphocytic Leukemia Study Group (GCLLSG) randomized older patients with a median age of 70 years to first-line therapy with fludarabine or chlorambucil [44]. While fludarabine improved ORR, it did not change OS or PFS [44]. Rather, patients treated with chlorambucil had a median OS of 64 months compared to 46 months with fludarabine, although this difference was not statistically significant [44]. A retrospective analysis of consecutive trials from CALGB also found similar OS and PFS between fludarabine and chlorambucil treatment in the elderly [151].

Table 2 Treatment criteria for CLL [62]

Indications for treatment	Criteria (at least one of the following)
Marrow failure	Anemia (hemoglobin <10 g/dL)
	Thrombocytopenia (platelet count <100 K/μL)
Splenomegaly	Spleen ≥6 cm below left costal margin
	Progressive/symptomatic
Lymphadenopathy	Lymph node ≥10 cm in diameter
	Progressive/symptomatic
Lymphocytosis	Lymphocyte doubling time <6 months[1]
	Lymphocyte count increase of >50 % in 2 months[1]
	[1]Should not be used as only criteria if initial lymphocyte count <30 K/μL
	[1]Exclude infection as cause for worsening lymphocytosis
Autoimmune anemia or thrombocytopenia	Poor response to corticosteroids or other standard therapies
Constitutional symptoms	Weight loss ≥10 % in 6 months
	Fatigue (ECOG PS ≥2)
	Fever >38 °C for ≥2 weeks[2]
	Night sweats >1 month[2]
	[2]Exclude infection as cause for fever or night sweats

For younger, physically fit patients, the development of fludarabine-based combination regimens has led to a new standard in CLL treatment. The addition of cyclophosphamide to fludarabine (FC) increased complete response (CR) rate from 5 to 20 % and median PFS from approximately 20–40 months in two prospective, randomized trials of 375 patients from Germany [43] and 278 patients from the United States [49]. However, neither trial found a difference in OS [43, 49]. Meanwhile, rituximab monotherapy was demonstrated to have moderate activity against CLL [61, 96]. In addition, several phase II trials supported the combined use of rituximab and fludarabine, with or without cyclophosphamide [18, 76, 123, 135, 144]. Ultimately, the GCLLSG conducted a prospective, randomized, multicenter study (CLL8) to examine the benefit of adding rituximab to FC (FCR) in 817 treatment-naïve CLL patients [63]. Chemoimmunotherapy improved OS at 3 years from 83 to 87 %, ORR from 80 to 90 %, CR rate from 22 to 44 %, and median PFS from 32.8 to 51.8 months [63].

4.2 Options for Older Patients

Bendamustine with rituximab (BR) may be better tolerated in older patients with comorbidities. A phase II study of 117 previously untreated patients demonstrated an ORR of 88 %, a CR rate of 23 %, median event-free survival of 33.9 months, and OS of 90.5 % at a median follow-up of 27 months [48]. A quarter of these patients were older than 70 years and 35 % had impaired renal function [48]. Toxicities were primarily hematologic and included grade 3 or 4 neutropenia in 19.7 % of patients [48], compared to 34 % with FCR [63]. Subsequently, the GCLLSG led an international phase III trial randomizing 688 patients to FCR or BR [47]. Results of the interim analysis were presented at the American Society of Hematology 2013 annual meeting [47]. Although there was no difference in OS, FCR produced a higher CR rate (47.4 % vs. 38.1 %) and longer PFS (85 % vs. 78.2 %) at 2 years, but only in patients younger than 65 years [47]. These benefits were offset by an increased risk of severe neutropenia and infection [47].

Obinutuzumab, a humanized, type 2, glycoengineered antibody against CD20, was recently demonstrated to improve response and survival when added to chlorambucil [59]. The GCLLSG randomized 781 patients with a median age of 73 years and multiple co-morbidities to chlorambucil with or without obinutuzumab (G-Cbl) or rituximab (R-Cbl) [59]. At a median follow-up of 14 months, G-Cbl reduced the risk of death from 20 to 9 % compared to patients treated with chlorambucil alone [59]. G-Cbl was also superior to R-Cbl with respect to response (ORR 65.1 % vs. 78.4 %; CR rate 7 % vs. 20.7 %), disease progression (median PFS 26.7 vs. 15.2 months), and survival (risk of death 8 % vs. 12 %) [59]. However, the dosing of obinutuzumab was reasonably different from that of rituximab [59] so as to make a direct comparison between the two antibodies difficult.

5 Relapsed/Refractory CLL

Definitions of relapsed/refractory disease facilitate clinical decision making and scientific study [62]. Relapse occurs in patients previously in complete or partial remission who progress after 6 or more months [62]. Refractory disease is defined by treatment failure or disease progression within 6 months of treatment [62]. Patients refractory to purine analogue-based regimens are considered particularly high risk [62]. Although the biology of non-responders is conceivably different from those who relapse after a period of disease control, most clinical trials consider relapsed or refractory disease as one entity.

Treatment options for relapsed/refractory CLL are growing despite the fact that a standard treatment has yet to be established (Table 3). Consequently, participation in clinical trials should be considered for the majority of patients in this setting. For those who previously achieved a durable remission, repeating the same regimen may be efficacious at relapse. In particular for patients with early relapse or refractory disease, novel agents (discussed below) have demonstrated significant activity.

5.1 Chemoimmunotherapy

FCR induced an ORR of 74 % and a CR rate of 30 % in 284 patients with relapsed/refractory CLL enrolled on an open-label, phase II trial [5]. Response was preserved in patients previously treated with fludarabine or rituximab, but not in those who received a prior alkylating agent [5]. As expected, patients who responded to fludarabine in the past had a better ORR with FCR salvage [144]. Median PFS and OS were 21 and 47 months, respectively [5].

Table 3 FDA-approved drugs for CLL

Drug	Mechanism	Approval	Indication
Chlorambucil	Alkylating agent	1957	Unspecified
Cyclophosphamide	Alkylating agent	1959	Unspecified
Fludarabine	Nucleotide analogue	1991	Relapsed/refractory
Alemtuzumab	Monoclonal antibody	2001	Relapsed/refractory
		2007	First-line
Bendamustine	Alkylating agent	2008	Unspecified
Ofatumumab	Monoclonal antibody	2009	Relapsed/refractory
		2014	First-line in combination with chlorambucil
Rituximab	Monoclonal antibody	2010	First-line in combination with fludarabine and cyclophosphamide for CD20+ CLL
Obinutuzumab	Monoclonal antibody	2013	First-line in combination with chlorambucil
Ibrutinib	Kinase inhibitor	2014	Relapsed/refractory

5.2 Monoclonal Antibodies

Approximately half of patients respond to ofatumumab in the salvage setting [146], including those previously treated with rituximab [147]. Median PFS and OS are 5.5 and 13–16 months, respectively [146, 147].

Alemtuzumab, a humanized monoclonal antibody against CD52, has been studied both as first-line and salvage treatment in CLL. Relapsed/refractory patients treated with alemtuzumab have an ORR of 33–42 %, median PFS of 8–12 months, and median OS of 16–19 months [75, 100, 132]. Response is significantly higher in treatment-naïve patients [85]. However, alemtuzumab also causes prolonged lymphopenia, increasing the risk of opportunistic infections (OI) [75, 85, 100, 132]. CMV reactivation is the most common OI, occurring in up to 30 % of patients receiving alemtuzumab [75, 85, 132]. Grade 3 or 4 CMV infections have been reported, but respond to ganciclovir [132]. Accordingly, regular monitoring and prompt treatment of CMV viremia are strongly encouraged. In 2012, alemtuzumab or Campath® was withdrawn from the US and EU markets in anticipation of a relaunch under a different trade name Lemtrada® for the treatment of multiple sclerosis. Although Lemtrada® has not been approved in the USA, alemtuzumab remains available through the Campath® distribution program.

5.3 Kinase Inhibitors

Much interest has been focused on targets of the BCR signaling pathway. Ibrutinib, an inhibitor of Bruton tyrosine kinase, was recently approved for use in previously treated CLL [19]. In a landmark trial, patients treated with ibrutinib had an ORR of 71 %, PFS of 74 %, and OS of 83 % at 26 months, as well as a lower risk of severe hematologic toxicity and infection than conventional salvage regimens [19]. An international, phase 3 trial comparing ibrutinib to ofatumumab in 391 patients found an ORR of 43 % versus 4 % and a 78 % reduction in risk of progression or death with ibrutinib [17]. Notably, one-third of these patients had deletion of 17p [17].

Idelalisib inhibits PI3Kδ and results in an ORR of 81 % when combined with rituximab in the salvage setting [53]. Compared to rituximab monotherapy, the addition of idelalisib improves PFS from 46 to 93 % and OS from 80 to 92 % at 12 months [53]. These kinase inhibitors cause transient lymphocytosis [19, 53] comprised of biologically inert cells [67, 152], that egress from lymph nodes and spleen [68].

Fostamatinib and dasatinib, which inhibit Syk and Lyn/BTK, respectively, have been studied in early phase I/II trials, but produced less impressive responses and more myelosuppression [2, 52].

5.4 BH3 Mimetics

BH3 mimetics are small molecule inhibitors of the Bcl-2 family of proteins that regulate cell survival and death. Navitoclax, a non-specific inhibitor of Bcl-2, Bcl-$_{XL}$, and Bcl-$_{W}$, was studied in 29 patients with relapsed/refractory CLL with an ORR of 31 %, including cases with 17p deletion. However, it also uniformly causes dose-limiting thrombocytopenia as a result of Bcl-$_{XL}$ inhibition. Consequently, ABT-199, a BH3 mimetic specifically targeting Bcl-2, was developed and shown to have preclinical activity against CLL [134, 142], multiple myeloma [138], AML [10, 94], and breast cancer [141]. Several phase I trials evaluating ABT-199, as monotherapy or in combination with monoclonal antibody or bendamustine, are currently underway.

5.5 Lenalidomide

Immunomodulating agent, lenalidomide, was initially studied as salvage monotherapy with an ORR of 32–47 % [23, 46]. Response is improved to 66 % when combined with rituximab and is associated with an OS of 71 % at 3 years [6]. Lenalidomide may cause grade 3 or higher neutropenia in up to 70 % of patients [6, 23] and is often associated with a cytokine release syndrome and tumor flare reactions [3, 23, 46]. In 2013, a phase 3 trial comparing lenalidomide to chlorambucil in previously untreated patients older than 65 years was terminated by the US Food and Drug Administration due to increased deaths in the lenalidomide arm [140]. Thirty-four of 210 patients treated with lenalidomide and 18 of 211 patients treated with chlorambucil died [140]. The Chronic Lymphocytic Leukemia Research Consortium subsequently reported the results of a phase 2 trial of initial therapy with lenalidomide and rituximab [72]. Among 29 patients older than 65 years, the ORR was 79 %, median PFS was 20 months, and 4 had died over a median follow-up of 20 months [72].

5.6 Cellular Therapy

Allogeneic hematopoietic stem cell transplantation (HSCT) provides graft-versus-leukemia effect and a potential for cure to patients with high-risk CLL [40]. Myeloablative conditioning followed by matched related [90] or unrelated [102] HSCT can lead to durable remissions, but carries a 38–46 % risk of treatment-related mortality (TRM). With reduced-intensity conditioning, TRM is reduced to 15–22 %, improving OS without sacrificing response [120, 128, 129]. However, graft-versus-host disease (GVHD) remains a significant problem, with grade 2–4 acute and chronic GVHD in 55 and 50–75 % of patients, respectively [120, 128, 129].

Genetically modified T cells expressing chimeric antigen receptor (CAR) have specific activity against their ligands. The receptor is engineered to directly activate

T cells upon ligation without major histocompatibility complex restriction. In phase I trials, patients infused with autologous anti-CD19 CAR T cells were able to achieve stabilization of disease, partial remission (PR), or in some instances, sustained CR [13, 74, 103]. A subset of CAR T cells acquires a central memory phenotype in vivo, allowing for persistent activity against CLL cells [74]. Allogeneic CAR T cells have been studied in allogeneic HSCT recipients at relapse [78]. Infusion of CAR T cells from the original donor led to an ORR of 20 % and stable disease in 8 of 10 patients [78].

6 Predictive Markers

Whereas prognostic markers inform the overall clinical course of a disease, predictive markers identify patients who are susceptible to specific therapies. Ideally, predictive markers can help physicians tailor treatment for individual patients, thereby avoiding wasted time and toxicities from less-effective options. Despite the apparent wealth of prognostic markers in CLL, the literature on predictive markers is limited.

6.1 Deletion of 17p

Deletion of 17p or mutation in *TP53*, one of the best established predictive markers, is typified by poor response to standard chemoimmunotherapy. Among 51 patients with del(17p) enrolled in the CLL8 trial comparing FC to FCR, only 1 patient achieved CR compared with a CR rate of 44 % for all patients following treatment with FCR [63]. At 3 years, 19 patients with del(17p) were alive and all had progressive disease [63]. A follow-up report showed comparably poor outcomes in CLL with mutated *TP53*; the majority of which were associated with loss of 17p [133]. As expected, responses are worse when FCR is given as salvage therapy [5]. Furthermore, patients with del(17p) or TP53 mutations also appear resistant to combinations of bendamustine or chlorambucil with rituximab [48, 50]. In contrast, small molecule inhibitors of BCR signaling, such as ibrutinib [19] or idelalisib [53], have shown promising activity against this high-risk subgroup. Alemtuzumab is also effective for both treatment-naïve [26, 70] and relapsed/refractory patients [84, 101, 132] irrespective of 17p or *TP53* status.

The European Group for Blood and Marrow Transplantation (EBMT) considers 17p deletion as an indication for allogeneic HSCT [40]. A retrospective analysis of del(17p) CLL from the EBMT database found an ORR of 84 % in 19 patients with stable or progressive disease and an ORR of 94 % in 17 patients in PR at the time of transplantation [121]. Importantly, 16 patients remained in CR after a median follow-up of 39 months [121]. In a phase 2 trial of reduced-intensity conditioning with allogeneic HSCT (CLL3X), event-free survival and OS were similar between patients with and without 17p deletion [41]. Furthermore, long-term remission with MRD negativity was achieved in 6 of 13 cases of del(17p) CLL [41, 42].

6.2 Deletion of 11q

As previously mentioned, 11q deletion is another adverse prognostic marker associated with a median OS of 49 months [39]. Unlike del(17p), patients with del (11q) CLL respond well to FCR, achieving an ORR of 93 %, CR rate of 51 %, 3-year PFS of 64 %, and 3-year OS of 94 % in the CLL8 trial [63]. These response and survival rates were similar to those observed in patients without del(17p) or del (11q) [63]. A retrospective analysis of 69 patients with del(11q) CLL corroborated the benefits of FCR-based regimens in this genetic subgroup [139]. Without an alkylating agent, however, outcomes are significantly worse for del(11q) CLL, with a median PFS and OS of 25 and 63 months, respectively [150]. Therefore, alkylating agents appear to be particularly valuable in reversing the poor prognosis of del (11q) CLL.

6.3 Novel Mutations

The role of novel genetic mutations as predictive markers has recently been explored by several groups. Methods include targeted and whole-exome sequencing (WES) of cases drawn from both retrospective series and prospective cohorts. WES of fludarabine-refractory patients has identified mutations in *TP53*, *NOTCH1*, *SF3B1*, or *BIRC3* in 70 % of cases [89].

Mutations in *NOTCH1* occur in 20 % of fludarabine-refractory disease [117]. *NOTCH1*-mutated patients treated with chlorambucil or a fludarabine-based regimen have shorter survival and more rapid disease progression compared to wild-type cases [99]. Median OS after treatment may approach that of *TP53*-mutated CLL as demonstrated by Rossi et al. [117]. Interestingly, *NOTCH1* mutation identifies a unique subgroup of patients who appear to have no benefit from the addition of rituximab to FC [133].

6.4 Minimal Residual Disease

Minimal residual disease (MRD), a sensitive measure of tumor burden post-treatment, predicts shorter PFS [11, 41, 48, 92, 114] and OS [110, 119] for most types of therapy, including standard chemoimmunotherapy and HSCT. Flow cytometry or molecular assays reliably detect residual tumor at a frequency of >1 in 10,000 cells [62]. In addition to evaluating for treatment response, MRD status can be serially monitored to track disease activity [92, 110, 114]. For the most part, conversion from a negative to positive MRD state heralds clinical relapse [92, 110, 114]. Although a powerful predictor of duration of response, MRD status can only be determined after therapy. A practical application may be to stratify patients for

additional therapy based on MRD status. For example, patients achieving MRD-negative CR during induction therapy may be adequately treated with an abbreviated course, while those with MRD positivity at the completion of treatment may benefit from consolidation or maintenance. A comprehensive review on this topic was recently published by Böttcher et al. [12].

6.5 Low-Risk CLL

For select patients with favorable prognostic markers, treatment with FCR may offer a potential cure. In a long-term follow-up of 300 patients treated with FCR, median time to progression was 85 months in patients with CR [135]. Age <70 years, absence of 17p abnormalities, and lower β2-microglobulin level and leukocyte count were independently associated with CR [135]. A subgroup analysis of 222 patients demonstrated that 35 % remained relapse-free at 10 years [77]. In addition, some patients have achieved durable remissions without an alkylating agent. Of 104 patients treated with fludarabine and rituximab on the CALGB study 9712, 14 remained progression-free at a median follow-up of 114 months [150]. A majority of these patients had *IGHV*-mutated CLL [150].

7 Personalized Medicine

The need for an individualized approach in CLL is highlighted by inferior outcomes with conventional treatment in defined high-risk groups. However, limited appreciation of predictive biomarkers and lack of effective therapies for non-responders previously hampered progress toward personalized medicine. As a result, treatment of CLL has been roughly guided by clinical indication rather than consideration for the underlying biology of disease. An exception to this rule is deletion of 17p, for which most experts recommend enrolment on a clinical trial, consideration of early allogeneic transplantation, or alternative agents.

The recent introduction of highly active novel agents and emerging data on predictive markers promise exciting therapeutic advances in CLL. Ideally, biologic characteristics that predict the response to specific therapies will be used to optimize treatment. High-risk groups have the most to gain from receiving novel agents upfront in place of less-effective conventional options and these patients should be referred for clinical trials. Hopefully soon, decisions on treatment strategies may be based on the biology of the disease, age, and general health of the patient, and whenever possible on individual preferences.

References

1. Agathangelidis A, Darzentas N, Hadzidimitriou A, Brochet X, Murray F, Yan X-J, Davis Z, van Gastel-Mol EJ, Tresoldi C, Chu CC, Cahill N, Giudicelli V, Tichy B, Pedersen LB, Foroni L, Bonello L, Janus A, Smedby K, Anagnostopoulos A, Merle-Beral H, Laoutaris N, Juliusson G, di Celle PF, Pospisilova S, Jurlander J, Geisler C, Tsaftaris A, Lefranc M-P, Langerak AW, Oscier DG, Chiorazzi N, Belessi C, Davi F, Rosenquist R, Ghia P, Stamatopoulos K (2012) Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. Blood 119 (19):4467–4475
2. Amrein PC, Attar EC, Takvorian T, Hochberg EP, Ballen KK, Leahy KM, Fisher DC, LaCasce AS, Jacobsen ED, Armand P, Hasserjian RP, Werner L, Neuberg D, Brown JR (2011) Phase II study of dasatinib in relapsed or refractory chronic lymphocytic leukemia. Clin Cancer Res 17(9):2977–2986
3. Aue G, Njuguna N, Tian X, Soto S, Hughes T, Vire B, Keyvanfar K, Gibellini F, Valdez J, Boss C, Samsel L, McCoy JP, Wilson WH, Pittaluga S, Wiestner A (2009) Lenalidomide-induced upregulation of CD80 on tumor cells correlates with T-cell activation, the rapid onset of a cytokine release syndrome and leukemic cell clearance in chronic lymphocytic leukemia. Haematologica 94(9):1266–1273
4. Austen B, Powell JE, Alvi A, Edwards I, Hooper L, Starczynski J, Taylor AMR, Fegan C, Moss P, Stankovic T (2005) Mutations in the ATM gene lead to impaired overall and treatment-free survival that is independent of IGVH mutation status in patients with B-CLL. Blood 106(9):3175–3182
5. Badoux XC, Keating MJ, Wang X, O'Brien SM, Ferrajoli A, Faderl S, Burger J, Koller C, Lerner S, Kantarjian H, Wierda WG (2011) Fludarabine, cyclophosphamide, and rituximab chemoimmunotherapy is highly effective treatment for relapsed patients with CLL. Blood 117(11):3016–3024
6. Badoux XC, Keating MJ, Wen S, Wierda WG, O'Brien SM, Faderl S, Sargent R, Burger JA, Ferrajoli A (2013) Phase II study of lenalidomide and rituximab as salvage therapy for patients with relapsed or refractory chronic lymphocytic leukemia. J Clin Oncol 31(5):584–591
7. Balatti V, Bottoni A, Palamarchuk A, Alder H, Rassenti LZ, Kipps TJ, Pekarsky Y, Croce CM (2012) NOTCH1 mutations in CLL associated with trisomy 12. Blood 119(2):329–331
8. Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, Weissman IL, Hamann A, Butcher EC (1993) Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. Cell 74(1):185–195
9. Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, Vaugier G, Potron G, Colona P, Oberling F, Thomas M, Tchernia G, Jacquillat C, Boivin P, Lesty C, Duault MT, Monconduit M, Belabbes S, Gremy F (1981) A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 48(1):198–206
10. Bogenberger JM, Delman D, Hansen N, Valdez R, Fauble V, Mesa RA, Tibes R (2014) Ex vivo activity of BCL-2 family inhibitors ABT-199 and ABT-737 combined with 5-Azacytidine in myeloid malignancies. Leuk Lymphoma 0:1–10
11. Böttcher S, Ritgen M, Fischer K, Stilgenbauer S, Busch RM, Fingerle-Rowson G, Fink AM, Bühler A, Zenz T, Wenger MK, Mendila M, Wendtner C-M, Eichhorst BF, Döhner H, Hallek MJ, Kneba M (2012) Minimal residual disease quantification is an independent predictor of progression-free and overall survival in chronic lymphocytic leukemia: a multivariate analysis from the randomized GCLLSG CLL8 trial. J Clin Oncol 30(9):980–988
12. Böttcher S, Hallek M, Ritgen M, Kneba M (2013) The role of minimal residual disease measurements in the therapy for CLL: is it ready for prime time? Hematol/Oncol Clin N Am 27(2):267–288

13. Brentjens RJ, Rivière I, Park JH, Davila ML, Wang X, Stefanski J, Taylor C, Yeh R, Bartido S, Borquez-Ojeda O, Olszewska M, Bernal Y, Pegram H, Przybylowski M, Hollyman D, Usachenko Y, Pirraglia D, Hosey J, Santos E, Halton E, Maslak P, Scheinberg D, Jurcic J, Heaney M, Heller G, Frattini M, Sadelain M (2011) Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 118(18):4817–4828
14. Bulian P, Shanafelt TD, Fegan C, Zucchetto A, Cro L, Nuckel H, Baldini L, Kurtova AV, Ferrajoli A, Burger JA, Gaidano G, Del Poeta G, Pepper C, Rossi D, Gattei V (2014) CD49d is the strongest flow cytometry-based predictor of overall survival in chronic lymphocytic leukemia. J Clin Oncol 32(9):897–904
15. Burger JA (2011) Nurture versus nature: the microenvironment in chronic lymphocytic leukemia. ASH Educ Program Book 2011(1):96–103
16. Burger JA, Chiorazzi N (2013) B cell receptor signaling in chronic lymphocytic leukemia. Trends Immunol 34(12):592–601
17. Byrd JC, Brown JR, O'Brien S, Barrientos JC, Kay NE, Reddy NM, Coutre S, Tam CS, Mulligan SP, Jaeger U, Devereux S, Barr PM, Furman RR, Kipps TJ, Cymbalista F, Pocock C, Thornton P, Caligaris-Cappio F, Robak T, Delgado J, Schuster SJ, Montillo M, Schuh A, de Vos S, Gill D, Bloor A, Dearden C, Moreno C, Jones JJ, Chu AD, Fardis M, McGreivy J, Clow F, James DF, Hillmen P (2014) Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. N Engl J Med 371(3):213–223
18. Byrd JC, Rai K, Peterson BL, Appelbaum FR, Morrison VA, Kolitz JE, Shepherd L, Hines JD, Schiffer CA, Larson RA (2005) Addition of rituximab to fludarabine may prolong progression-free survival and overall survival in patients with previously untreated chronic lymphocytic leukemia: an updated retrospective comparative analysis of CALGB 9712 and CALGB 9011. Blood 105(1):49–53
19. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, Grant B, Sharman JP, Coleman M, Wierda WG, Jones JA, Zhao W, Heerema NA, Johnson AJ, Sukbuntherng J, Chang BY, Clow F, Hedrick E, Buggy JJ, James DF, O'Brien S (2013) Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med 369(1):32–42
20. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM (2002) Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci 99(24):15524–15529
21. Castro JE, Prada CE, Loria O, Kamal A, Chen L, Burrows FJ, Kipps TJ (2005) ZAP-70 is a novel conditional heat shock protein 90 (Hsp90) client: inhibition of Hsp90 leads to ZAP-70 degradation, apoptosis, and impaired signaling in chronic lymphocytic leukemia. Blood 106 (7):2506–2512
22. Chan AC, Iwashima M, Turck CW, Weiss A (1992) ZAP-70: a 70 kd protein-tyrosine kinase that associates with the TCR zeta chain. Cell 71(4):649–662
23. Chanan-Khan A, Miller KC, Musial L, Lawrence D, Padmanabhan S, Takeshita K, Porter CW, Goodrich DW, Bernstein ZP, Wallace P, Spaner D, Mohr A, Byrne C, Hernandez-Ilizaliturri F, Chrystal C, Starostik P, Czuczman MS (2006) Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study. J Clin Oncol 24(34):5343–5349
24. Chen L, Widhopf G, Huynh L, Rassenti L, Rai KR, Weiss A, Kipps TJ (2002) Expression of ZAP-70 is associated with increased B-cell receptor signaling in chronic lymphocytic leukemia. Blood 100(13):4609–4614
25. Chen L, Huynh L, Apgar J, Tang L, Rassenti L, Weiss A, Kipps TJ (2008) ZAP-70 enhances IgM signaling independent of its kinase activity in chronic lymphocytic leukemia. Blood 111 (5):2685–2692
26. Chiaretti S, Marinelli M, Del Giudice I, Bonina S, Piciocchi A, Messina M, Vignetti M, Rossi D, Maio VD, Mauro FR, Guarini A, Gaidano G, Foà R (2014) NOTCH1, SF3B1, BIRC3 AND TP53 mutations in chronic lymphocytic leukemia patients undergoing first-line

treatment: correlation with biological parameters and response to treatment. Leuk Lymphoma 0:1–17
27. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, C-G L, Kipps TJ, Negrini M, Croce CM (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA 102(39):13944–13949
28. Claus R, Lucas DM, Stilgenbauer S, Ruppert AS, Yu L, Zucknick M, Mertens D, Bühler A, Oakes CC, Larson RA, Kay NE, Jelinek DF, Kipps TJ, Rassenti LZ, Gribben JG, Döhner H, Heerema NA, Marcucci G, Plass C, Byrd JC (2012) Quantitative DNA methylation analysis identifies a single CpG dinucleotide important for ZAP-70 expression and predictive of prognosis in chronic lymphocytic leukemia. J Clin Oncol 30(20):2483–2491
29. Claus R, Lucas DM, Ruppert AS, Williams KE, Weng D, Patterson K, Zucknick M, Oakes CC, Rassenti LZ, Greaves AW, Geyer S, Wierda WG, Brown JR, Gribben JG, Barrientos JC, Rai KR, Kay NE, Kipps TJ, Shields P, Zhao W, Grever MR, Plass C, Byrd JC (2014) Validation of ZAP-70 methylation and its relative significance in predicting outcome in chronic lymphocytic leukemia. Blood 124(1):42–48
30. Corcoran M, Parker A, Orchard J, Davis Z, Wirtz M, Schmitz O, Oscier D (2005) ZAP-70 methylation status is associated with ZAP-70 expression status in chronic lymphocytic leukemia. Haematologica 90(8):1078–1088
31. Crespo M, Bosch F, Villamor N, Bellosillo B, Colomer D, Rozman M, Marcé S, López-Guillermo A, Campo E, Montserrat E (2003) ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. N Engl J Med 348(18):1764–1775
32. Crespo M, Villamor N, Giné E, Muntañola A, Colomer D, Marafioti T, Jones M, Camós M, Campo E, Montserrat E, Bosch F (2006) ZAP-70 expression in normal pro/pre B cells, mature B cells, and in B-cell acute lymphoblastic leukemia. Clin Cancer Res 12(3):726–734
33. Csanaky G, Matutes E, Vass JA, Morilla R, Catovsky D (1997) Adhesion receptors on peripheral blood leukemic B cells. A comparative study on B cell chronic lymphocytic leukemia and related lymphoma/leukemias. Leukemia 11(3):408–415
34. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, Lichtman SM, Schulman P, Vinciguerra VP, Rai KR, Ferrarini M, Chiorazzi N (1999) Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 94(6):1840–1847
35. de la Fuente MT, Casanova B, Garcia-Gila M, Silva A, Garcia-Pardo A (1999) Fibronectin interaction with alpha4beta1 integrin prevents apoptosis in B cell chronic lymphocytic leukemia: correlation with Bcl-2 and Bax. Leukemia 13(2):266–274
36. de la Fuente MT, Casanova B, Moyano JV, Garcia-Gila M, Sanz L, Garcia-Marco J, Silva A, Garcia-Pardo A (2002) Engagement of alpha4beta1 integrin by fibronectin induces in vitro resistance of B chronic lymphocytic leukemia cells to fludarabine. J Leukoc Biol 71(3):495–502
37. Deaglio S, Vaisitti T, Aydin S, Bergui L, D'Arena G, Bonello L, Omedé P, Scatolini M, Jaksic O, Chiorino G, Efremov D, Malavasi F (2007) CD38 and ZAP-70 are functionally linked and mark CLL cells with high migratory potential. Blood 110(12):4012–4021
38. Del Principe MI, Del Poeta G, Buccisano F, Maurillo L, Venditti A, Zucchetto A, Marini R, Niscola P, Consalvo MAI, Mazzone C, Ottaviani L, Panetta P, Bruno A, Bomben R, Suppo G, Degan M, Gattei V, de Fabritiis P, Cantonetti M, Lo Coco F, Del Principe D, Amadori S (2006) Clinical significance of ZAP-70 protein expression in B-cell chronic lymphocytic leukemia. Blood 108(3):853–861
39. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P (2000) Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 343(26):1910–1916
40. Dreger P, Corradini P, Kimby E, Michallet M, Milligan D, Schetelig J, Wiktor-Jedrzejczak W, Niederwieser D, Hallek M, Montserrat E (2006) Indications for allogeneic stem cell

transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus. Leukemia 21(1):12–17
41. Dreger P, Döhner H, Ritgen M, Böttcher S, Busch R, Dietrich S, Bunjes D, Cohen S, Schubert J, Hegenbart U, Beelen D, Zeis M, Stadler M, Hasenkamp J, Uharek L, Scheid C, Humpe A, Zenz T, Winkler D, Hallek M, Kneba M, Schmitz N, Stilgenbauer S (2010) Allogeneic stem cell transplantation provides durable disease control in poor-risk chronic lymphocytic leukemia: long-term clinical and MRD results of the German CLL study group CLL3X trial. Blood 116(14):2438–2447
42. Dreger P, Schnaiter A, Zenz T, Böttcher S, Rossi M, Paschka P, Bühler A, Dietrich S, Busch R, Ritgen M, Bunjes D, Zeis M, Stadler M, Uharek L, Scheid C, Hegenbart U, Hallek M, Kneba M, Schmitz N, Döhner H, Stilgenbauer S (2013) TP53, SF3B1, and NOTCH1 mutations and outcome of allotransplantation for chronic lymphocytic leukemia: six-year follow-up of the GCLLSG CLL3X trial. Blood 121(16):3284–3288
43. Eichhorst BF, Busch R, Hopfinger G, Pasold R, Hensel M, Steinbrecher C, Siehl S, Jäger U, Bergmann M, Stilgenbauer S, Schweighofer C, Wendtner CM, Döhner H, Brittinger G, Emmerich B, Hallek M, Group tGCS (2006) Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood 107(3):885–891
44. Eichhorst BF, Busch R, Stilgenbauer S, Stauch M, Bergmann MA, Ritgen M, Kranzhöfer N, Rohrberg R, Söling U, Burkhard O, Westermann A, Goede V, Schweighofer CD, Fischer K, Fink A-M, Wendtner CM, Brittinger G, Döhner H, Emmerich B, Hallek M, Group tGCS (2009) First-line therapy with fludarabine compared with chlorambucil does not result in a major benefit for elderly patients with advanced chronic lymphocytic leukemia. Blood 114 (16):3382–3391
45. Eksioglu-Demiralp E, Alpdogan O, Aktan M, Firatli T, Ozturk A, Budak T, Bayik M, Akoglu T (1996) Variable expression of CD49d antigen in B cell chronic lymphocytic leukemia is related to disease stages. Leukemia 10(8):1331–1339
46. Ferrajoli A, Lee B-N, Schlette EJ, O'Brien SM, Gao H, Wen S, Wierda WG, Estrov Z, Faderl S, Cohen EN, Li C, Reuben JM, Keating MJ (2008) Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. Blood 111(11):5291–5297
47. Fink A-M, Busch R, Lange E, Köppler H, Kiehl M, Sökler M, Schlag R, Vehling-Kaiser U, Köchling G, Plöger C, Gregor M, Plesner T, Trneny M, Fischer K, Döhner H, Kneba M, Wendtner C, Klapper W, Kreuzer K-A, Stilgenbauer S, Böttcher S, Hallek M (2013) Chemoimmunotherapy with fludarabine (F), cyclophosphamide (C), and rituximab (R) (FCR) versus bendamustine and rituximab (BR) in previously untreated and physically fit patients (pts) with advanced chronic lymphocytic leukemia (CLL): results of a planned interim analysis of the CLL10 trial, an international, randomized study of the German CLL study group (GCLLSG). Blood 122(21):526
48. Fischer K, Cramer P, Busch R, Böttcher S, Bahlo J, Schubert J, Pflüger KH, Schott S, Goede V, Isfort S, von Tresckow J, Fink A-M, Bühler A, Winkler D, Kreuzer K-A, Staib P, Ritgen M, Kneba M, Döhner H, Eichhorst BF, Hallek M, Stilgenbauer S, Wendtner C-M (2012) Bendamustine in combination with rituximab for previously untreated patients with chronic lymphocytic leukemia: a multicenter phase II trial of the german chronic lymphocytic leukemia study group. J Clin Oncol 30(26):3209–3216
49. Flinn IW, Neuberg DS, Grever MR, Dewald GW, Bennett JM, Paietta EM, Hussein MA, Appelbaum FR, Larson RA, Moore DF, Tallman MS (2007) Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US intergroup trial E2997. J Clin Oncol 25(7):793–798
50. Foà R, Del Giudice I, Cuneo A, Del Poeta G, Ciolli S, Di Raimondo F, Lauria F, Cencini E, Rigolin GM, Cortelezzi A, Nobile F, Callea V, Brugiatelli M, Massaia M, Molica S, Trentin L, Rizzi R, Specchia G, Di Serio F, Orsucci L, Ambrosetti A, Montillo M, Zinzani PL, Ferrara F, Morabito F, Mura MA, Soriani S, Peragine N, Tavolaro S, Bonina S, Marinelli M,

De Propris MS, Starza ID, Piciocchi A, Alietti A, Runggaldier EJ, Gamba E, Mauro FR, Chiaretti S, Guarini A (2014) Chlorambucil plus rituximab with or without maintenance rituximab as first-line treatment for elderly chronic lymphocytic leukemia patients. Am J Hematol 89(5):480–486
51. Francis S, Karanth M, Pratt G, Starczynski J, Hooper L, Fegan C, Pepper C, Valcarcel D, Milligan DW, Delgado J (2006) The effect of immunoglobulin VH gene mutation status and other prognostic factors on the incidence of major infections in patients with chronic lymphocytic leukemia. Cancer 107(5):1023–1033
52. Friedberg JW, Sharman J, Sweetenham J, Johnston PB, Vose JM, LaCasce A, Schaefer-Cutillo J, De Vos S, Sinha R, Leonard JP, Cripe LD, Gregory SA, Sterba MP, Lowe AM, Levy R, Shipp MA (2010) Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. Blood 115 (13):2578–2585
53. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, Barrientos JC, Zelenetz AD, Kipps TJ, Flinn I, Ghia P, Eradat H, Ervin T, Lamanna N, Coiffier B, Pettitt AR, Ma S, Stilgenbauer S, Cramer P, Aiello M, Johnson DM, Miller LL, Li D, Jahn TM, Dansey RD, Hallek M, O'Brien SM (2014) Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med 370(11):997–1007
54. Garcia-Munoz R, Galiacho VR, Llorente L (2012) Immunological aspects in chronic lymphocytic leukemia (CLL) development. Ann Hematol 91(7):981–996
55. Gattei V, Bulian P, Del Principe MI, Zucchetto A, Maurillo L, Buccisano F, Bomben R, Dal-Bo M, Luciano F, Rossi FM, Degan M, Amadori S, Del Poeta G (2008) Relevance of CD49d protein expression as overall survival and progressive disease prognosticator in chronic lymphocytic leukemia. Blood 111(2):865–873
56. Ghia P, Stamatopoulos K, Belessi C, Moreno C, Stilgenbauer S, Stevenson F, Davi F, Rosenquist R (2007) ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. Leukemia 21(1):1–3
57. Gladstone DE, Blackford A, Cho E, Swinnen L, Kasamon Y, Gocke CD, Griffin CA, Bolanos-Meade J, Jones RJ (2012) The importance of IGHV mutational status in del(11q) and del(17p) chronic lymphocytic leukemia. Clin Lymphoma Myeloma Leuk 12(2):132–137
58. Gobessi S, Laurenti L, Longo PG, Sica S, Leone G, Efremov DG (2006) ZAP-70 enhances B-cell–receptor signaling despite absent or inefficient tyrosine kinase activation in chronic lymphocytic leukemia and lymphoma B cells. Blood 109(5):2032–2039
59. Goede V, Fischer K, Busch R, Engelke A, Eichhorst B, Wendtner CM, Chagorova T, de la Serna J, Dilhuydy M-S, Illmer T, Opat S, Owen CJ, Samoylova O, Kreuzer K-A, Stilgenbauer S, Döhner H, Langerak AW, Ritgen M, Kneba M, Asikanius E, Humphrey K, Wenger M, Hallek M (2014) Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. N Engl J Med 370(12):1101–1110
60. Gonzalez D, Martinez P, Wade R, Hockley S, Oscier D, Matutes E, Dearden CE, Richards SM, Catovsky D, Morgan GJ (2011) Mutational status of the TP53 gene as a predictor of response and survival in patients with chronic lymphocytic leukemia: results from the LRF CLL4 trial. J Clin Oncol 29(16):2223–2229
61. Hainsworth JD, Litchy S, Barton JH, Houston GA, Hermann RC, Bradof JE, Greco FA (2003) Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: a phase II trial of the minnie pearl cancer research network. J Clin Oncol 21(9):1746–1751
62. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, Hillmen P, Keating MJ, Montserrat E, Rai KR, Kipps TJ (2008) Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International workshop on chronic lymphocytic leukemia updating the National Cancer Institute-working group 1996 guidelines. Blood 111(12):5446–5456
63. Hallek M, Fischer K, Fingerle-Rowson G, Fink AM, Busch R, Mayer J, Hensel M, Hopfinger G, Hess G, von Grunhagen U, Bergmann M, Catalano J, Zinzani PL, Caligaris-

Cappio F, Seymour JF, Berrebi A, Jager U, Cazin B, Trneny M, Westermann A, Wendtner CM, Eichhorst BF, Staib P, Buhler A, Winkler D, Zenz T, Bottcher S, Ritgen M, Mendila M, Kneba M, Dohner H, Stilgenbauer S (2010) Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet 376(9747):1164–1174

64. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK (1999) Unmutated Ig VH genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 94 (6):1848–1854
65. Hemler ME, Elices MJ, Parker C, Takada Y (1990) Structure of the integrin VLA-4 and its cell-cell and cell-matrix adhesion functions. Immunol Rev 114:45–65
66. Herishanu Y, Katz B-Z, Lipsky A, Wiestner A (2013) Biology of chronic lymphocytic leukemia in different microenvironments: clinical and therapeutic implications. Hematol Oncol Clin North Am 27(2):173–206
67. Herman SEM, Mustafa RZ, Gyamfi JA, Pittaluga S, Chang S, Chang B, Farooqui M, Wiestner A (2014) Ibrutinib inhibits BCR and NF-κB signaling and reduces tumor proliferation in tissue-resident cells of patients with CLL. Blood 123(21):3286–3295
68. Herman SEM, Niemann CU, Farooqui M, Jones J, Mustafa RZ, Lipsky A, Saba N, Martyr S, Soto S, Valdez J, Gyamfi JA, Maric I, Calvo KR, Pedersen LB, Geisler CH, Liu D, Marti GE, Aue G, Wiestner A (2014) Ibrutinib-induced lymphocytosis in patients with chronic lymphocytic leukemia: correlative analyses from a phase II study. Leukemia 28(11):2188–2196
69. Herve M, Xu K, Ng YS, Wardemann H, Albesiano E, Messmer BT, Chiorazzi N, Meffre E (2005) Unmutated and mutated chronic lymphocytic leukemias derive from self-reactive B cell precursors despite expressing different antibody reactivity. J Clin Investig 115(6):1636–1643
70. Hillmen P, Skotnicki AB, Robak T, Jaksic B, Dmoszynska A, Wu J, Sirard C, Mayer J (2007) Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. J Clin Oncol 25(35):5616–5623
71. Howlader NNA, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (2014) SEER cancer statistics review, 1975–2011. National Cancer Institute, Bethesda (based on Nov 2013 SEER data submission, posted to the SEER web site, Apr 2014)
72. James DF, Werner L, Brown JR, Wierda WG, Barrientos JC, Castro JE, Greaves A, Johnson AJ, Rassenti LZ, Rai KR, Neuberg D, Kipps TJ (2014) Lenalidomide and rituximab for the initial treatment of patients with chronic lymphocytic leukemia: a multicenter clinical-translational study from the chronic lymphocytic leukemia research consortium. J Clin Oncol 32(19):2067–2073
73. Juliusson G, Oscier DG, Fitchett M, Ross FM, Stockdill G, Mackie MJ, Parker AC, Castoldi GL, Cuneo A, Knuutila S, Elonen E, Gahrton G (1990) Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. N Engl J Med 323(11):720–724
74. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, June CH (2011) T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Trans Med 3(95):95ra73
75. Keating MJ, Flinn I, Jain V, Binet J-L, Hillmen P, Byrd J, Albitar M, Brettman L, Santabarbara P, Wacker B, Rai KR (2002) Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood 99 (10):3554–3561
76. Keating MJ, O'Brien S, Albitar M, Lerner S, Plunkett W, Giles F, Andreeff M, Cortes J, Faderl S, Thomas D, Koller C, Wierda W, Detry MA, Lynn A, Kantarjian H (2005) Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. J Clin Oncol 23(18):4079–4088

77. Keating MJ, Tam CS, Wierda WG, O'Brien S, Thomas DA, Cortes JE, Lerner S, Plunkett W (2012) Is chronic lymphocytic leukemia still incurable? ASH Ann Meet Abs 120(21):3929
78. Kochenderfer JN, Dudley ME, Carpenter RO, Kassim SH, Rose JJ, Telford WG, Hakim FT, Halverson DC, Fowler DH, Hardy NM, Mato AR, Hickstein DD, Gea-Banacloche JC, Pavletic SZ, Sportes C, Maric I, Feldman SA, Hansen BG, Wilder JS, Blacklock-Schuver B, Jena B, Bishop MR, Gress RE, Rosenberg SA (2013) Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. Blood 122(25):4129–4139
79. Krober A, Seiler T, Benner A, Bullinger L, Bruckle E, Lichter P, Dohner H, Stilgenbauer S (2002) V(H) mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. Blood 100(4):1410–1416
80. Lafarge ST, Johnston JB, Gibson SB, Marshall AJ (2014) Adhesion of ZAP-70+ chronic lymphocytic leukemia cells to stromal cells is enhanced by cytokines and blocked by inhibitors of the PI3-kinase pathway. Leuk Res 38(1):109–115
81. Lagneaux L, Delforge A, De Bruyn C, Bernier M, Bron D (1999) Adhesion to bone marrow stroma inhibits apoptosis of chronic lymphocytic leukemia cells. Leuk Lymphoma 35(5–6):445–453
82. Landau DA, Carter SL, Stojanov P, McKenna A, Stevenson K, Lawrence MS, Sougnez C, Stewart C, Sivachenko A, Wang L, Wan Y, Zhang W, Shukla SA, Vartanov A, Fernandes SM, Saksena G, Cibulskis K, Tesar B, Gabriel S, Hacohen N, Meyerson M, Lander ES, Neuberg D, Brown JR, Getz G, Wu CJ (2013) Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. Cell 152(4):714–726
83. Lionetti M, Fabris S, Cutrona G, Agnelli L, Ciardullo C, Matis S, Ciceri G, Colombo M, Maura F, Mosca L, Gentile M, Recchia AG, Ilariucci F, Musolino C, Molica S, Di Raimondo F, Cortelezzi A, Rossi D, Gaidano G, Morabito F, Ferrarini M, Neri A (2014) High-throughput sequencing for the identification of NOTCH1 mutations in early stage chronic lymphocytic leukaemia: biological and clinical implications. Br J Haematol 165(5):629–639
84. Lozanski G, Heerema NA, Flinn IW, Smith L, Harbison J, Webb J, Moran M, Lucas M, Lin T, Hackbarth ML, Proffitt JH, Lucas D, Grever MR, Byrd JC (2004) Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood 103(9):3278–3281
85. Lundin J, Kimby E, Björkholm M, Broliden P-A, Celsing F, Hjalmar V, Möllgård L, Rebello P, Hale G, Waldmann H, Mellstedt H, Österborg A (2002) Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). Blood 100(3):768–773
86. Mao Z, Quintanilla-Martinez L, Raffeld M, Richter M, Krugmann J, Burek C, Hartmann E, Rudiger T, Jaffe ES, Muller-Hermelink HK, Ott G, Fend F, Rosenwald A (2007) IgVH mutational status and clonality analysis of Richter's transformation: diffuse large B-cell lymphoma and Hodgkin lymphoma in association with B-cell chronic lymphocytic leukemia (B-CLL) represent 2 different pathways of disease evolution. Am J Surg Pathol 31(10):1605–1614
87. Martin T, Crouzier R, Weber JC, Kipps TJ, Pasquali JL (1994) Structure-function studies on a polyreactive (natural) autoantibody. Polyreactivity is dependent on somatically generated sequences in the third complementarity-determining region of the antibody heavy chain. J Immunol 152(12):5988–5996
88. Martínez-Trillos A, Pinyol M, Navarro A, Aymerich M, Jares P, Juan M, Rozman M, Colomer D, Delgado J, Giné E, González-Díaz M, Hernández-Rivas JM, Colado E, Rayón C, Payer AR, Terol MJ, Navarro B, Quesada V, Puente XS, Rozman C, López-Otín C, Campo E, López-Guillermo A, Villamor N (2014) Mutations in TLR/MYD88 pathway identify a subset of young chronic lymphocytic leukemia patients with favorable outcome. Blood 123(24):3790–3796
89. Messina M, Del Giudice I, Khiabanian H, Rossi D, Chiaretti S, Rasi S, Spina V, Holmes AB, Marinelli M, Fabbri G, Piciocchi A, Mauro FR, Guarini A, Gaidano G, Dalla-Favera R,

Pasqualucci L, Rabadan R, Foà R (2014) Genetic lesions associated with chronic lymphocytic leukemia chemo-refractoriness. Blood 123(15):2378–2388
90. Michallet M, Archimbaud E, Bandini G, Rowlings PA, Deeg HJ, Gahrton G, Montserrat E, Rozman C, Gratwohl A, Gale RP (1996) HLA-identical sibling bone marrow transplantation in younger patients with chronic lymphocytic leukemia. Ann Intern Med 124(3):311–315
91. Monserrat J, Sánchez MÁ, de Paz R, Díaz D, Mur S, Reyes E, Prieto A, de la Hera A, Martínez-A C, Álvarez-Mon M (2014) Distinctive patterns of naïve/memory subset distribution and cytokine expression in CD4 T lymphocytes in ZAP-70 B-chronic lymphocytic patients. Cytometry Part B: Clin Cytometry 86(1):32–43
92. Moreno C, Villamor N, Colomer D, Esteve J, Giné E, Muntañola A, Campo E, Bosch F, Montserrat E (2006) Clinical significance of minimal residual disease, as assessed by different techniques, after stem cell transplantation for chronic lymphocytic leukemia. Blood 107(11):4563–4569
93. Niemann CU, Wiestner A (2013) B-cell receptor signaling as a driver of lymphoma development and evolution. Semin Cancer Biol 23(6):410–421
94. Niu X, Wang G, Wang Y, Caldwell JT, Edwards H, Xie C, Taub JW, Li C, Lin H, Ge Y (2014) Acute myeloid leukemia cells harboring MLL fusion genes or with the acute promyelocytic leukemia phenotype are sensitive to the Bcl-2-selective inhibitor ABT-199. Leukemia 28(7):1557–1560
95. Nolz JC, Tschumper RC, Pittner BT, Darce JR, Kay NE, Jelinek DF (2005) ZAP-70 is expressed by a subset of normal human B-lymphocytes displaying an activated phenotype. Leukemia 19(6):1018–1024
96. O'Brien SM, Kantarjian H, Thomas DA, Giles FJ, Freireich EJ, Cortes J, Lerner S, Keating MJ (2001) Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol 19 (8):2165–2170
97. Orchard JA, Ibbotson RE, Davis Z, Wiestner A, Rosenwald A, Thomas PW, Hamblin TJ, Staudt LM, Oscier DG (2004) ZAP-70 expression and prognosis in chronic lymphocytic leukaemia. Lancet 363(9403):105–111
98. Oscier DG, Gardiner AC, Mould SJ, Glide S, Davis ZA, Ibbotson RE, Corcoran MM, Chapman RM, Thomas PW, Copplestone JA, Orchard JA, Hamblin TJ (2002) Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood 100(4):1177–1184
99. Oscier DG, Rose-Zerilli MJJ, Winkelmann N, Gonzalez de Castro D, Gomez B, Forster J, Parker H, Parker A, Gardiner A, Collins A, Else M, Cross NCP, Catovsky D, Strefford JC (2013) The clinical significance of NOTCH1 and SF3B1 mutations in the UK LRF CLL4 trial. Blood 121(3):468–475
100. Osterborg A, Dyer MJ, Bunjes D, Pangalis GA, Bastion Y, Catovsky D, Mellstedt H (1997) Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European study group of CAMPATH-1H treatment in chronic lymphocytic leukemia. J Clin Oncol 15(4):1567–1574
101. Osuji N, Del Giudice I, Matutes E, Wotherspoon A, Dearden C, Catovsky D (2005) The efficacy of alemtuzumab for refractory chronic lymphocytic leukemia in relation to cytogenetic abnormalities of p53. Haematologica 90(10):1435–1436
102. Pavletic SZ, Khouri IF, Haagenson M, King RJ, Bierman PJ, Bishop MR, Carston M, Giralt S, Molina A, Copelan EA, Ringdén O, Roy V, Ballen K, Adkins DR, McCarthy P, Weisdorf D, Montserrat E, Anasetti C (2005) Unrelated donor marrow transplantation for B-Cell chronic lymphocytic leukemia after using myeloablative conditioning: results from the center for international blood and marrow transplant research. J Clin Oncol 23(24):5788–5794
103. Porter DL, Levine BL, Kalos M, Bagg A, June CH (2011) Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 365(8):725–733
104. Puente XS, Pinyol M, Quesada V, Conde L, Ordonez GR, Villamor N, Escaramis G, Jares P, Bea S, Gonzalez-Diaz M, Bassaganyas L, Baumann T, Juan M, Lopez-Guerra M, Colomer D, Tubio JMC, Lopez C, Navarro A, Tornador C, Aymerich M, Rozman M, Hernandez JM,

Puente DA, Freije JMP, Velasco G, Gutierrez-Fernandez A, Costa D, Carrio A, Guijarro S, Enjuanes A, Hernandez L, Yague J, Nicolas P, Romeo-Casabona CM, Himmelbauer H, Castillo E, Dohm JC, de Sanjose S, Piris MA, de Alava E, Miguel JS, Royo R, Gelpi JL, Torrents D, Orozco M, Pisano DG, Valencia A, Guigo R, Bayes M, Heath S, Gut M, Klatt P, Marshall J, Raine K, Stebbings LA, Futreal PA, Stratton MR, Campbell PJ, Gut I, Lopez-Guillermo A, Estivill X, Montserrat E, Lopez-Otin C, Campo E (2011) Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature 475 (7354):101–105

105. Rai K, Sawitsky A, Cronkite E, Chanana A, Levy R, Pasternack B (1975) Clinical staging of chronic lymphocytic leukemia. Blood 46(2):219–234
106. Rai KR, Peterson BL, Appelbaum FR, Kolitz J, Elias L, Shepherd L, Hines J, Threatte GA, Larson RA, Cheson BD, Schiffer CA (2000) Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med 343(24):1750–1757
107. Rai KR, Peterson BL, Appelbaum FR, Tallman MS, Belch A, Morrison VA, Larson RA (2009) Long-term survival analysis of the North American intergroup study C9011 comparing fludarabine (F) and chlorambucil (C) in previously untreated patients with chronic lymphocytic leukemia (CLL). ASH Ann Meet Abs 114(22):536
108. Rassenti LZ, Huynh L, Toy TL, Chen L, Keating MJ, Gribben JG, Neuberg DS, Flinn IW, Rai KR, Byrd JC, Kay NE, Greaves A, Weiss A, Kipps TJ (2004) ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N Engl J Med 351(9):893–901
109. Rassenti LZ, Jain S, Keating MJ, Wierda WG, Grever MR, Byrd JC, Kay NE, Brown JR, Gribben JG, Neuberg DS, He F, Greaves AW, Rai KR, Kipps TJ (2008) Relative value of ZAP-70, CD38, and immunoglobulin mutation status in predicting aggressive disease in chronic lymphocytic leukemia. Blood 112(5):1923–1930
110. Rawstron AC, Kennedy B, Evans PAS, Davies FE, Richards SJ, Haynes AP, Russell NH, Hale G, Morgan GJ, Jack AS, Hillmen P (2001) Quantitation of minimal disease levels in chronic lymphocytic leukemia using a sensitive flow cytometric assay improves the prediction of outcome and can be used to optimize therapy. Blood 98(1):29–35
111. Rawstron AC, Bennett FL, O'Connor SJM, Kwok M, Fenton JAL, Plummer M, de Tute R, Owen RG, Richards SJ, Jack AS, Hillmen P (2008) Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med 359(6):575–583
112. Redondo-Munoz J, Ugarte-Berzal E, Garcia-Marco JA, del Cerro MH, Van den Steen PE, Opdenakker G, Terol MJ, Garcia-Pardo A (2008) Alpha4beta1 integrin and 190-kDa CD44v constitute a cell surface docking complex for gelatinase B/MMP-9 in chronic leukemic but not in normal B cells. Blood 112(1):169–178
113. Richardson SJ, Matthews C, Catherwood MA, Alexander HD, Carey BS, Farrugia J, Gardiner A, Mould S, Oscier D, Copplestone JA, Prentice AG (2006) ZAP-70 expression is associated with enhanced ability to respond to migratory and survival signals in B-cell chronic lymphocytic leukemia (B-CLL). Blood 107(9):3584–3592
114. Ritgen M, Bottcher S, Stilgenbauer S, Bunjes D, Schubert J, Cohen S, Humpe A, Hallek M, Kneba M, Schmitz N, Dohner H, Dreger P (2008) Quantitative MRD monitoring identifies distinct GVL response patterns after allogeneic stem cell transplantation for chronic lymphocytic leukemia: results from the GCLLSG CLL3X trial. Leukemia 22(7):1377–1386
115. Rose DM, Han J, Ginsberg MH (2002) α4 integrins and the immune response. Immunol Rev 186(1):118–124
116. Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, Yang L, Pickeral OK, Rassenti LZ, Powell J, Botstein D, Byrd JC, Grever MR, Cheson BD, Chiorazzi N, Wilson WH, Kipps TJ, Brown PO, Staudt LM (2001) Relation of gene expression phenotype to immunoglobulin mutation genotype in b cell chronic lymphocytic leukemia. J Exp Med 194 (11):1639–1648
117. Rossi D, Rasi S, Fabbri G, Spina V, Fangazio M, Forconi F, Marasca R, Laurenti L, Bruscaggin A, Cerri M, Monti S, Cresta S, Famà R, De Paoli L, Bulian P, Gattei V, Guarini

A, Deaglio S, Capello D, Rabadan R, Pasqualucci L, Dalla-Favera R, Foà R, Gaidano G (2012) Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. Blood 119(2):521–529
118. Rossi FM, Zucchetto A, Tissino E, Dal Bo M, Bomben R, Caldana C, Pozzo F, Del Poeta G, Rossi D, Gaidano G, Gattei V (2013) CD49d expression identifies a chronic-lymphocytic leukemia subset with high levels of mobilized circulating CD34 hemopoietic progenitors cells. Leukemia 28(3):705–708
119. Santacruz R, Villamor N, Aymerich M, Martínez-Trillos A, López C, Navarro A, Rozman M, Beà S, Royo C, Cazorla M, Colomer D, Giné E, Pinyol M, Puente XS, López-Otín C, Campo E, López-Guillermo A, Delgado J (2014) The prognostic impact of minimal residual disease in patients with chronic lymphocytic leukemia requiring first-line therapy. Haematologica 99(5):873–880
120. Schetelig J, Thiede C, Bornhäuser M, Schwerdtfeger R, Kiehl M, Beyer J, Sayer HG, Kröger N, Hensel M, Scheffold C, Held TK, Höffken K, Ho AD, Kienast J, Neubauer A, Zander AR, Fauser AA, Ehninger G, Siegert W (2003) Evidence of a graft-versus-leukemia effect in chronic lymphocytic leukemia after reduced-intensity conditioning and allogeneic stem-cell transplantation: the cooperative German transplant study group. J Clin Oncol 21(14):2747–2753
121. Schetelig J, van Biezen A, Brand R, Caballero D, Martino R, Itala M, García-Marco JA, Volin L, Schmitz N, Schwerdtfeger R, Ganser A, Onida F, Mohr B, Stilgenbauer S, Bornhäuser M, de Witte T, Dreger P (2008) Allogeneic hematopoietic stem-cell transplantation for chronic lymphocytic leukemia with 17p deletion: a retrospective European group for blood and marrow transplantation analysis. J Clin Oncol 26(31):5094–5100
122. Schroeder HW Jr, Dighiero G (1994) The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire. Immunol Today 15(6):288–294
123. Schulz H, Klein SK, Rehwald U, Reiser M, Hinke A, Knauf W-U, Aulitzky W-E, Hensel M, Herold M, Huhn D, Hallek M, Diehl V, Engert A (2002) Phase 2 study of a combined immunochemotherapy using rituximab and fludarabine in patients with chronic lymphocytic leukemia. Blood 100(9):3115–3120
124. Shanafelt TD, Geyer SM, Bone ND, Tschumper RC, Witzig TE, Nowakowski GS, Zent CS, Call TG, Laplant B, Dewald GW, Jelinek DF, Kay NE (2008) CD49d expression is an independent predictor of overall survival in patients with chronic lymphocytic leukaemia: a prognostic parameter with therapeutic potential. Br J Haematol 140(5):537–546
125. Sivina M, Hartmann E, Kipps TJ, Rassenti L, Krupnik D, Lerner S, LaPushin R, Xiao L, Huang X, Werner L, Neuberg D, Kantarjian H, Brien S, Wierda WG, Keating MJ, Rosenwald A, Burger JA (2010) CCL3 (MIP-1α) plasma levels and the risk for disease progression in chronic lymphocytic leukemia. Blood 117(5):1662–1669
126. Smit LA, van Maldegem F, Langerak AW, van der Schoot CE, de Wit MJ, Bea S, Campo E, Bende RJ, van Noesel CJ (2006) Antigen receptors and somatic hypermutation in B-cell chronic lymphocytic leukemia with Richter's transformation. Haematologica 91(7):903–911
127. Šoljić V, Perak RB, Vukojević K, Saraga-Babić M, Bubalo P, Karan D, Todorović J, Batinić D (2013) ZAP-70 expression and proliferative activity in chronic lymphocytic leukemia. Leuk Lymphoma 54(6):1171–1176
128. Sorror ML, Maris MB, Sandmaier BM, Storer BE, Stuart MJ, Hegenbart U, Agura E, Chauncey TR, Leis J, Pulsipher M, McSweeney P, Radich JP, Bredeson C, Bruno B, Langston A, Loken MR, Al-Ali H, Blume KG, Storb R, Maloney DG (2005) Hematopoietic cell transplantation after nonmyeloablative conditioning for advanced chronic lymphocytic leukemia. J Clin Oncol 23(16):3819–3829
129. Sorror ML, Storer BE, Sandmaier BM, Maris M, Shizuru J, Maziarz R, Agura E, Chauncey TR, Pulsipher MA, McSweeney PA, Wade JC, Bruno B, Langston A, Radich J, Niederwieser D, Blume KG, Storb R, Maloney DG (2008) Five-year follow-up of patients with advanced

chronic lymphocytic leukemia treated with allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. J Clin Oncol 26(30):4912–4920
130. Stamatopoulos K, Belessi C, Moreno C, Boudjograh M, Guida G, Smilevska T, Belhoul L, Stella S, Stavroyianni N, Crespo M, Hadzidimitriou A, Sutton L, Bosch F, Laoutaris N, Anagnostopoulos A, Montserrat E, Fassas A, Dighiero G, Caligaris-Cappio F, Merle-Béral H, Ghia P, Davi F (2007) Over 20 % of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations. Blood 109(1):259–270
131. Stevenson FK, Caligaris-Cappio F (2004) Chronic lymphocytic leukemia: revelations from the B-cell receptor. Blood 103(12):4389–4395
132. Stilgenbauer S, Zenz T, Winkler D, Bühler A, Schlenk RF, Groner S, Busch R, Hensel M, Dührsen U, Finke J, Dreger P, Jäger U, Lengfelder E, Hohloch K, Söling U, Schlag R, Kneba M, Hallek M, Döhner H (2009) Subcutaneous alemtuzumab in fludarabine-refractory chronic lymphocytic leukemia: clinical results and prognostic marker analyses from the CLL2H study of the German chronic lymphocytic leukemia study group. J Clin Oncol 27(24):3994–4001
133. Stilgenbauer S, Schnaiter A, Paschka P, Zenz T, Rossi M, Döhner K, Bühler A, Böttcher S, Ritgen M, Kneba M, Winkler D, Tausch E, Hoth P, Edelmann J, Mertens D, Bullinger L, Bergmann M, Kless S, Mack S, Jäger U, Patten N, Wu L, Wenger MK, Fingerle-Rowson G, Lichter P, Cazzola M, Wendtner CM, Fink AM, Fischer K, Busch R, Hallek M, Döhner H (2014) Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial. Blood 123(21):3247–3254
134. Tam CCS, Seymour JF, Bell A, Westerman DA, Juneja S, Huang DCS, Roberts AW (2013) Selective Bcl-2 inhibition with ABT-199 is highly active against chronic lymphocytic leukemia (CLL) irrespective of TP53 mutation or dysfunction. Blood 122(21):1304
135. Tam CS, O'Brien S, Wierda W, Kantarjian H, Wen S, Do K-A, Thomas DA, Cortes J, Lerner S, Keating MJ (2008) Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. Blood 112(4):975–980
136. Tam CS, Shanafelt TD, Wierda WG, Abruzzo LV, Van Dyke DL, O'Brien S, Ferrajoli A, Lerner SA, Lynn A, Kay NE, Keating MJ (2009) De novo deletion 17p13.1 chronic lymphocytic leukemia shows significant clinical heterogeneity: the M. D. Anderson and Mayo clinic experience. Blood 114(5):957–964
137. Till KJ, Lin K, Zuzel M, Cawley JC (2002) The chemokine receptor CCR7 and alpha4 integrin are important for migration of chronic lymphocytic leukemia cells into lymph nodes. Blood 99(8):2977–2984
138. Touzeau C, Dousset C, Le Gouill S, Sampath D, Leverson JD, Souers AJ, Maiga S, Bene MC, Moreau P, Pellat-Deceunynck C, Amiot M (2014) The Bcl-2 specific BH3 mimetic ABT-199: a promising targeted therapy for t(11;14) multiple myeloma. Leukemia 28(1):210–212
139. Tsimberidou A-M, Tam C, Abruzzo LV, O'Brien S, Wierda WG, Lerner S, Kantarjian HM, Keating MJ (2009) Chemoimmunotherapy may overcome the adverse prognostic significance of 11q deletion in previously untreated patients with chronic lymphocytic leukemia. Cancer 115(2):373–380
140. USF (2013) FDA statement: FDA halts clinical trial of drug Revlimid (lenalidomide) for chronic lymphocytic leukemia due to safety concerns. http://www.fda.gov/Drugs/DrugSafety/ucm361444.htm
141. Vaillant F, Merino D, Lee L, Breslin K, Pal B, Ritchie ME, Smyth GK, Christie M, Phillipson LJ, Burns CJ, Mann GB, Visvader JE, Lindeman GJ (2013) Targeting BCL-2 with the BH3 mimetic ABT-199 in estrogen receptor-positive breast cancer. Cancer Cell 24 (1):120–129
142. Vogler M, Dinsdale D, Dyer MJS, Cohen GM (2013) ABT-199 selectively inhibits BCL2 but not BCL2L1 and efficiently induces apoptosis of chronic lymphocytic leukaemic cells but not platelets. Br J Haematol 163(1):139–142

143. Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K, Werner L, Sivachenko A, DeLuca DS, Zhang L, Zhang W, Vartanov AR, Fernandes SM, Goldstein NR, Folco EG, Cibulskis K, Tesar B, Sievers QL, Shefler E, Gabriel S, Hacohen N, Reed R, Meyerson M, Golub TR, Lander ES, Neuberg D, Brown JR, Getz G, Wu CJ (2011) SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. N Engl J Med 365(26):2497–2506
144. Wierda W, O'Brien S, Wen S, Faderl S, Garcia-Manero G, Thomas D, Do K-A, Cortes J, Koller C, Beran M, Ferrajoli A, Giles F, Lerner S, Albitar M, Kantarjian H, Keating M (2005) Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. J Clin Oncol 23(18):4070–4078
145. Wierda WG, O'Brien S, Wang X, Faderl S, Ferrajoli A, Do KA, Cortes J, Thomas D, Garcia-Manero G, Koller C, Beran M, Giles F, Ravandi F, Lerner S, Kantarjian H, Keating M (2007) Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. Blood 109(11):4679–4685
146. Wierda WG, Kipps TJ, Mayer J, Stilgenbauer S, Williams CD, Hellmann A, Robak T, Furman RR, Hillmen P, Trneny M, Dyer MJS, Padmanabhan S, Piotrowska M, Kozak T, Chan G, Davis R, Losic N, Wilms J, Russell CA, Österborg A (2010) Ofatumumab as single-agent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. J Clin Oncol 28(10):1749–1755
147. Wierda WG, Padmanabhan S, Chan GW, Gupta IV, Lisby S, Österborg A, Investigators ftH-C-S (2011) Ofatumumab is active in patients with fludarabine-refractory CLL irrespective of prior rituximab: results from the phase 2 international study. Blood 118(19):5126–5129
148. Wiestner A, Rosenwald A, Barry TS, Wright G, Davis RE, Henrickson SE, Zhao H, Ibbotson RE, Orchard JA, Davis Z, Stetler-Stevenson M, Raffeld M, Arthur DC, Marti GE, Wilson WH, Hamblin TJ, Oscier DG, Staudt LM (2003) ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood 101(12):4944–4951
149. Wiestner A (2012) Emerging role of kinase-targeted strategies in chronic lymphocytic leukemia. Blood 120(24):4684–4691
150. Woyach JA, Ruppert AS, Heerema NA, Peterson BL, Gribben JG, Morrison VA, Rai KR, Larson RA, Byrd JC (2011) Chemoimmunotherapy with fludarabine and rituximab produces extended overall survival and progression-free survival in chronic lymphocytic leukemia: long-term follow-up of CALGB study 9712. J Clin Oncol 29(10):1349–1355
151. Woyach JA, Ruppert AS, Rai K, Lin TS, Geyer S, Kolitz J, Appelbaum FR, Tallman MS, Belch AR, Morrison VA, Larson RA, Byrd JC (2013) Impact of age on outcomes after initial therapy with chemotherapy and different chemoimmunotherapy regimens in patients with chronic lymphocytic leukemia: results of sequential cancer and leukemia group B studies. J Clin Oncol 31(4):440–447
152. Woyach JA, Smucker K, Smith LL, Lozanski A, Zhong Y, Ruppert AS, Lucas D, Williams K, Zhao W, Rassenti L, Ghia E, Kipps TJ, Mantel R, Jones J, Flynn J, Maddocks K, O'Brien S, Furman RR, James DF, Clow F, Lozanski G, Johnson AJ, Byrd JC (2014) Prolonged lymphocytosis during ibrutinib therapy is associated with distinct molecular characteristics and does not indicate a suboptimal response to therapy. Blood 123(12):1810–1817
153. Xu JL, Davis MM (2000) Diversity in the CDR3 region of VH is sufficient for most antibody specificities. Immunity 13(1):37–45
154. Young RM, Staudt LM (2013) Targeting pathological B cell receptor signalling in lymphoid malignancies. Nat Rev Drug Discov 12(3):229–243
155. Zanotti R, Frattini F, Ghia P, Visco C, Zamò A, Perbellini O, Stella S, Facco M, Giaretta I, Chilosi M, Pizzolo G, Ambrosetti A (2010) ZAP-70 expression is associated with increased risk of autoimmune cytopenias in CLL patients. Am J Hematol 85(7):494–498
156. Zenz T, Eichhorst B, Busch R, Denzel T, Häbe S, Winkler D, Bühler A, Edelmann J, Bergmann M, Hopfinger G, Hensel M, Hallek M, Döhner H, Stilgenbauer S (2010) TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol 28(29):4473–4479

157. Zucchetto A, Benedetti D, Tripodo C, Bomben R, Dal Bo M, Marconi D, Bossi F, Lorenzon D, Degan M, Rossi FM, Rossi D, Bulian P, Franco V, Del Poeta G, Deaglio S, Gaidano G, Tedesco F, Malavasi F, Gattei V (2009) CD38/CD31, the CCL3 and CCL4 chemokines, and CD49d/vascular cell adhesion molecule-1 are interchained by sequential events sustaining chronic lymphocytic leukemia cell survival. Cancer Res 69(9):4001–4009
158. Zucchetto A, Caldana C, Benedetti D, Tissino E, Rossi FM, Hutterer E, Pozzo F, Bomben R, Dal Bo M, D'Arena G, Zaja F, Pozzato G, Di Raimondo F, Hartmann TN, Rossi D, Gaidano G, Del Poeta G, Gattei V (2013) CD49d is overexpressed by trisomy 12 chronic lymphocytic leukemia cells: evidence for a methylation-dependent regulation mechanism. Blood 122 (19):3317–3321

Biology, Prognosis, and Therapy of Waldenström Macroglobulinemia

Jorge J. Castillo, Irene M. Ghobrial and Steven P. Treon

Abstract

Waldenström Macroglobulinemia (WM) is a rare B-cell lymphoma characterized by the uncontrolled accumulation of malignant lymphoplasmacytic cells, mainly in the bone marrow, and monoclonal IgM production. Despite its rarity, our understanding of the biology of this disease has improved significantly in recent years with the identification of recurrent mutations in the MYD88 and CXCR4 genes. Based on the diversity of clinical features observed in WM patients, therapy should be highly personalized having into account several factors such as age, co-morbidities, IgM levels, and presence of hyperviscosity, coagulopathy, cryoglobulinemia, or cold agglutinin disease. In this chapter, we review the recent advances in the biology of WM and the current therapeutic options for untreated and relapsed WM patients. Finally, we discuss the role of prognostic factors and current evidence supporting an improvement in the survival of WM patients in the last decade.

Keywords

Waldenström Macroglobulinemia · MYD88 · CXCR4 · Biology · Therapy · Survival

J.J. Castillo (✉) · I.M. Ghobrial · S.P. Treon
Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Harvard Medical School, 450 Brookline Ave, M221, 02215 Boston, MA, USA
e-mail: jorgej_castillo@dfci.harvard.edu

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_7

Contents

1 Introduction

Waldenström Macroglobulinemia (WM) is a rare B-cell lymphoproliferative disorder characterized by the uncontrolled accumulation of malignant immunoglobulin M (IgM)-secreting lymphoplasmacytic cells. WM belongs to the lymphoplasmacytic lymphoma (LPL) category as defined by the 2008 World Health Organization classification [1]. However, over 95 % of the cases of LPL are WM with the remainder comprised by IgA, IgG, and non-secreting LPL. WM occurs in adults with a median age in the 60s with a slight male predominance. A familial predisposition has been described in approximately 25 % of the patients with WM, with familial patterns varying from presence of various B-cell malignancies, and families in which multiple cases of WM or IgM MGUS have been observed [2].

2 Clinical Features of WM

WM presents predominantly with bone marrow involvement and only a minority of patients (15–30 %) present with extramedullary disease such as lymphadenopathy or hepatosplenomegaly. The most common clinical features associate with anemia (i.e., fatigue, tiredness, and/or shortness of breath) due to overcrowding of the bone marrow space by LPL or iron deficiency [3]. However, given the physicochemical

Table 1 Diagnostic criteria for Waldenström macroglobulinemia

IgM monoclonal gammopathy of any concentration
Bone marrow infiltration by small lymphocytes showing plasmacytic differentiation
Intertrabecular pattern of bone marrow infiltration
Surface IgM+, CD19+, CD20+, CD22+, CD25+. CD27+, FMC7+, CD5±, CD10−, CD23−, CD103−

characteristics of IgM, patients can experience signs and symptoms associated with other mechanisms such as hyperviscosity, cryoglobulinemia, peripheral neuropathy, coagulopathy, cold agglutinins, and tissue deposition in the skin (Schnitzler syndrome), gastrointestinal tract, central nervous system (Bing-Neel syndrome), or kidneys. Patients can rarely present with amyloid deposition, which can cause edema, hepatomegaly, macroglossia, cardiac, liver and kidney dysfunction, and axonal peripheral neuropathy.

3 Diagnosis of WM

The diagnostic criteria for WM are shown in Table 1. A bone marrow aspiration and biopsy is a key component of the diagnostic work-up. The immunophenotypical profile of WM cells shows expression of surface IgM, CD19, CD20, CD22, CD38, and CD79. Up to 20 % of cases may express CD5, CD10, or CD23. An increased number of mast cells in the bone marrow may help differentiate WM from other indolent B-cell lymphomas. A great variety of cytogenetic abnormalities have been described in WM; however, chromosome 6q deletions have been observed in half of the patients [4]. More recently, a recurrent mutation in the MYD88 gene (MYD88 L265P) has been identified in over 90 % of cases with WM [5]. The occurrence of this mutation in WM has since been validated in several independent cohorts [6–9]. In contrast, the MYD88 L265P gene mutation was not detected in patients with IgM myeloma and was detected in less than 10 % of patients with marginal zone lymphoma. The high specificity and sensitivity of the MYD88 L265P gene mutation has obvious diagnostic implications in patients in whom a diagnosis of WM is suspected but uncertain.

4 Biology of WM

The MYD88 L265P gene mutation has shown to support growth and survival of WM cells in several studies. A knockdown model of MYD88 showed decreased survival of MYD88 L265P expressing WM cells, whereas survival was more enhanced by knock-in of mutant *versus* wild-type MYD88 [10]. MYD88 acts as an adaptor molecule in toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling [11]. Following stimulation of TLR or IL-1R, MYD88 is recruited to the activated receptor complex as a homodimer which complexes with IL-1R-associated

kinase 4 (IRAK4) and subsequently activates IRAK1 and IRAK2 [12]. IRAK1 activation then leads to NF-κB activation via IκBα phosphorylation [13]. Recently, a study has shown that MYD88 L265P also activates the Bruton's Tyrosine Kinase (BTK) pathway [10]. In this preclinical study, the activation of BTK by MYD88 could be abrogated by the use of BTK kinase inhibitors. A diagram of the activation of BTK and NF-κB via MYD88 is shown in Fig. 1.

A recent study from our group first ever reported the occurrence of recurrent somatic CXCR4 gene mutations in approximately 30 % of WM patients [14]. The somatic mutations occur in the C-terminal domain and are similar to those observed in patients with WHIM (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) syndrome. These mutations regulate signaling of CXCR4 by its ligand SDF-1a [15]. In WM patients, two classes of CXCR4 mutations occur: non-sense ($CXCR4^{WHIM/NS}$) and frameshift ($CXCR4^{WHIM/FS}$) mutations [14, 16]. Non-sense and frameshift mutations are almost equally divided among WM patients, and over 30 different types of CXCR4 mutations have been identified. Preclinical studies with the most common CXCR4 S338X mutation in WM have shown sustained signaling of AKT, ERK, and BTK following SDF-1a binding in comparison with wild-type CXCR4, as well increased cell growth and survival of WM cells [17]. Figure 2 shows (a) the protein sequence for the full-length transcript, (b) the crystal structure of CXCR4, and (c) the location of the WHIM-like mutations.

5 Criteria for Initiation of Therapy

Given the indolent and incurable nature of WM, a large proportion of patients would not need immediate therapy upon diagnosis and will be placed on watchful waiting. Current guidelines do not recommend initiation of therapy based on IgM levels alone since they might not correlate with clinical manifestations of the disease [18]. Initiation of therapy is, however, reasonable in patients demonstrating rising IgM levels along with signs or symptoms associated with disease progression. Criteria for initiation of therapy are shown in Table 2. In patients in whom an immediate control of the disease is warranted, such as symptomatic hyperviscosity, coagulopathy, cryoglobulinemia, or cold agglutinin disease, a rapid reduction of the IgM paraprotein should be achieved with plasmapheresis. Treatment directed at WM should follow as soon as possible since plasmapheresis does not constitute definitive therapy and IgM levels will rise and return to baseline levels within 4 weeks.

6 Frontline Therapy for WM

There are several options for the frontline therapy of patients with WM. These options include alkylating agents (i.e., chlorambucil, cyclophosphamide, and bendamustine), nucleoside analogs (i.e., fludarabine and cladribine), the immunomodulator

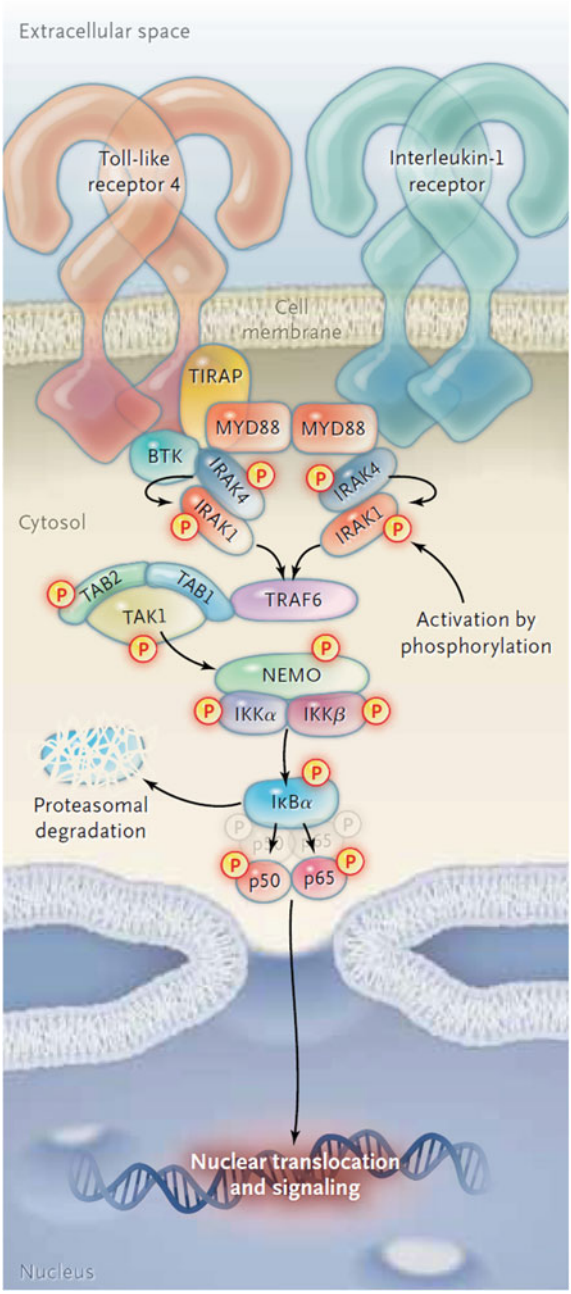


Fig. 1 MYD88-directed BTK and NF-κB signaling. Reproduced with permission from [5]

thalidomide, and the proteasome inhibitor bortezomib, with or without the anti-CD20 monoclonal antibody rituximab [19]. Individual patient considerations should be taken into account when making the choice of frontline treatment including the

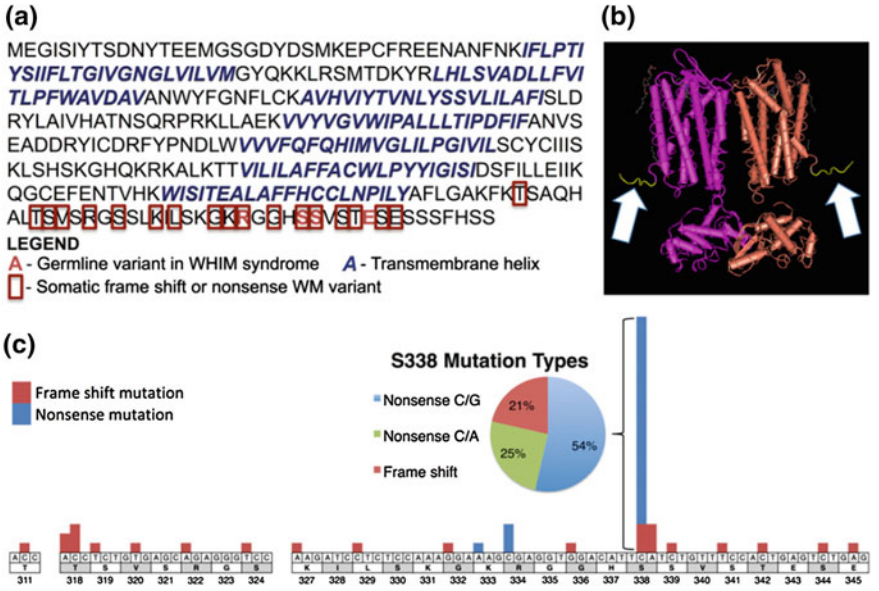


Fig. 2 CXCR4 full-length transcript and structure, and the location of WHIM-like mutations. Reproduced with permission from [14]

Table 2 Criteria for initiation of therapy in Waldenström macroglobulinemia

Constitutional symptoms (i.e., fever, night sweats, fatigue due to anemia or weight loss)
Progressive, symptomatic lymphadenopathy or splenomegaly
Anemia with a hemoglobin ≤10 g/dl
Platelet count <100 × 10^9/L
Hyperviscosity syndrome
Symptomatic sensorimotor peripheral neuropathy
Systemic amyloidosis
Symptomatic cryoglobulinemia

presence of cytopenias, need for rapid disease control, age, pre-therapy IgM levels, pre-existing neuropathy, and candidacy for autologous transplantation therapy. For candidates for autologous transplantation, exposure to alkylator therapy or nucleoside analogs should be limited because of the difficulties with stem cell collection. The use of nucleoside analogs should be approached cautiously given that increased risk of disease transformation, myelodysplasia, and acute myeloid leukemia have been reported.

6.1 Proteasome Inhibitor-Based Therapy

The WM Clinical Trial Group presented data on a study that included 23 patients using bortezomib, dexamethasone, and rituximab (BDR) for the primary therapy of symptomatic WM [20]. Bortezomib was administered at a dose of 1.3 mg/m^2 IV along with dexamethasone 40 mg on days 1, 4, 8, and 11 and rituximab 375 mg/m^2 IV on day 11 every 21 days for four induction cycles, followed by four maintenance cycles administered every 3 months. The overall response rate (ORR) was 96 % with three complete responses (CR), two near complete responses (nCR), and three very good partial responses (VGPR) for a VGPR or better of 35 %. The median time to response was 1.4 months, which makes BDR an excellent choice if a rapid control of disease is required or in young patients to decrease the risk of secondary myeloid malignancies. With a median follow-up of 23 months, 18 out of 23 patients (78 %) remained free of progression. The most common toxicity was peripheral neuropathy; 39 and 30 % of patients, respectively, experienced grade 2 and grade 3 peripheral neuropathy. Grade 2 and grade 3 neutropenia were also reported in 26 % and 26 % of patients, respectively. It is important to note that appropriate Herpes zoster prophylaxis should be instituted in all patients receiving proteasome inhibitor therapy.

Another study evaluated weekly bortezomib in combination with rituximab in 26 untreated patients with WM [21]. In this study, bortezomib was administered at a dose of 1.6 mg/m^2 IV weekly on days 1, 8, and 15 every 28 days for six cycles with rituximab 375 mg/m^2 weekly times four during cycles 1 and 4 only. The ORR was 88 % with a VGPR or better of 8 %. The median time to best response was 3.7 months. The 1-year PFS rate was 75 %. There was 54 % of peripheral neuropathy grade 2 or lower but no grade 3 or higher neuropathy was observed. Grade 3 or higher neutropenia was seen in 12 % of patients. More recently, a study by the European Myeloma Network evaluated the efficacy of weekly bortezomib, low-dose dexamethasone, and rituximab in 59 untreated WM patients [22]. Bortezomib was administered as a single agent at 1.3 mg/m^2 IV on days 1, 4, 8, and 11 (cycle 1) followed 3 weeks later by weekly bortezomib at 1.6 mg/m^2 IV and dexamethasone 40 mg IV on days 1, 8, 15, and 22 and rituximab 375 mg/m^2 IV every 35 days (cycles 2–5). The ORR was 85 % with VGPR or better of 10 %. After a 32-month follow-up, the median PFS was 42 months. Grade 3 or higher peripheral neuropathy occurred in 7 % of patients. The subcutaneous (SQ) administration of bortezomib has shown to be effective and induces less neuropathy than the IV route in patients with myeloma [23]; however, the safety and efficacy of bortezomib SQ has not been formally studied in patients with WM.

A recent study evaluated the combination of carfilzomib, dexamethasone, and rituximab (CaRD) in 31 patients with WM [24]. Carfilzomib was administered at a dose of 20 mg/m^2 IV during cycle 1 and at 36 mg/m^2 IV during cycles 2–6 with dexamethasone 20 mg IV on days 1, 2, 8, and 9 along with rituximab on days 2 and 9 every three weeks during induction therapy. Maintenance therapy consisted on carfilzomib 36 mg/m^2 IV and dexamethasone 20 mg IV on days 1 and 2 and rituximab 375 mg/m^2 on day 2 every 2 months for eight cycles. The ORR was 87 %

with one CR and ten VGPR for a VGPR or better of 35 %. The 15-month PFS rate was 65 %. No grade 3 neuropathy was observed. Grade 3 hyperglycemia and hyperlipasemia were observed in 23 and 16 % of patients, respectively. Of note, the rate of IgG hypogammaglobulinemia increased from 48 % at baseline to 90 % following therapy.

6.2 Alkylator-based Therapy

Cyclophosphamide-based therapy includes regimens in which cyclophosphamide is combined with rituximab and prednisone (CP-R), rituximab, prednisone and vincristine (CVP-R), and rituximab, prednisone, vincristine and doxorubicin (CHOP-R). These regimens would induce an ORR of 70–80 % with CR rate around 10 % [25]. It is unclear if the addition of vincristine or doxorubicin translates into clinical benefits in patients with WM. A retrospective study compared the activity and toxicity of CP-R, CVP-R, and CHOP-R in patients with WM [26]. In that study, CP-R was associated with similar response rates than CVP-R and CHOP-R. However, the rate of treatment complications such as febrile neutropenia, hospitalizations, and neuropathy was lower. Based on these data, CP-R might be preferred over CVP-R or CHOP-R in patients with WM.

The combination of bendamustine and rituximab (BR) has been compared with CHOP-R in a randomized controlled study by the Study Group for Lymphomas in a cohort that included 42 patients with WM [27]. Bendamustine was administered at a dose of 90 mg/m^2 IV on days 1 and 2 along with rituximab 375 mg/m^2 IV on day 1 at 3-week intervals for 6 cycles. The ORR for BR was 96 % and 94 % for CHOP-R. With a median follow-up of 26 months, the PFS rate for BR was 90 % compared with 59 % for CHOP-R. BR was associated with lower rates of grade 3 or higher neutropenia, infectious complications, and alopecia, but with higher rates of skin rash. BR might provide an excellent therapeutic option for patients with lymphadenopathy or patients with pre-existing neuropathy.

6.3 Nucleoside Analog-Based Therapy

In the large randomized controlled study to date, which enrolled 414 patients (339 WM, 37 MZL, and 38 LPL) from 101 centers in five countries, fludarabine was compared with chlorambucil in untreated WM patients [28]. In this study, the ORR for fludarabine was 48 % versus 39 % for chlorambucil. The median PFS was also longer for fludarabine (36 months) in comparison with chlorambucil (27 months). There was a significant improvement in median OS, which was not reached for fludarabine versus 70 months for chlorambucil. Finally, the rate of second malignancies was higher in the chlorambucil arm than in the fludarabine arm (21 % vs. 4 %).

The long-term outcomes of the combination of fludarabine and rituximab (FR) were evaluated in 43 patients with WM, from which 27 patients were untreated

[29]. Therapy consisted of 8 infusions of rituximab at 375 mg/m^2 IV per week administered at weeks 1–4, 17, 18, 30, and 31, along with 6 cycles of fludarabine 25 mg/m^2 IV daily given for 5 days at weeks 5, 9, 13, 19, 23, and 27. The ORR in untreated patients was 96 %. The median time to response was 3.9 months, and the median time to best response was 19 months. The median time to progression in untreated patients was 78 months. Grade 3 or higher adverse events included myelosuppression and infections. Other observed complications were aggressive transformation and secondary malignancies, although most of these events were seen in the previously treated group of patients.

It is unclear at this time whether the combination of an alkylating agent such as cyclophosphamide, a nucleoside analog such as fludarabine, and rituximab (FCR) provides any additional benefits in patients with WM. A study evaluated FCR in 43 patients with WM of which 28 were previously untreated [30]. The FCR regimen consisted of rituximab 375 mg/m^2 IV on day 1 and fludarabine 25 mg/m^2 and cyclophosphamide 250 mg/m^2 IV on days 2 through 4. Cycles were repeated every four weeks for a maximum of six cycles. The ORR was 79 % with no difference between untreated and previously treated patients. Grade 3 or higher neutropenia was seen in 88 % of the patients. After a median follow-up of 39 months, 11 patients (25 %) have died, including five from progressive disease, three from pneumonia, and one from acute myeloid leukemia.

6.4 Other Options

The combination of thalidomide and rituximab (TR) has been associated with an ORR of 70 % and a median PFS of 3 years [31]. TR could be useful in myelosuppressed patients in whom an immediate disease control is not required. The main adverse event associated with thalidomide is neuropathy, which can be seen in up to 40 % of patients treated with TR.

The use of rituximab as single agent can be considered in selected patients with low IgM levels or concurrent autoimmune processes such as cold agglutinin disease, autoimmune thrombocytopenia, or IgM-related neuropathy. The ORR to 4 weekly infusion of rituximab is 20–30 % [32] and to 4 weekly infusions followed by 4 weekly infusions 12 weeks later of 40–50 % [33]. The use of single-agent rituximab in patients with high IgM levels has been associated with IgM flares with an occurrence rate of 40–50 % [34]. These flares might lead to symptomatic hyperviscosity as well as worsening IgM-related neuropathy or cryoglobulinemia. Hence, rituximab monotherapy should be avoided in patients with IgM levels >4,000 mg/dl.

7 Salvage Therapy for Relapsed/Refractory WM

For those patients with relapsed/refractory disease, any of the frontline therapies mentioned above are appropriate options. Therapies in the relapsed setting should also be personalized to the individual patient considering age, performance status, pre-existing neuropathy, IgM levels, and/or need for immediate control of the disease. One must also have in mind to avoid the use of stem cell toxic therapy in patients in whom high-dose chemotherapy with autologous stem cell rescue is being considered.

7.1 Bortezomib

Bortezomib-based therapy has shown to be effective in relapsed or refractory WM patients in several studies, either alone or in combination with rituximab [35–38]. The ORR ranged between 30 and 80 % depending on the study with the most common adverse events being peripheral neuropathy, neutropenia, and thrombocytopenia. Once-weekly administration of bortezomib has been associated with lower rates of neuropathy when compared with the twice-weekly regimen. In one study, bortezomib seemed to be less effective on inducing lymph node responses [35]. As mentioned previously, patients undergoing therapy with proteasome inhibitors should receive prophylaxis against herpes zoster.

7.2 Nucleoside Analogs and Alkylating Agents

Fludarabine has single-agent activity in relapsed WM with an approximate 40 % ORR [39]. The combination FR showed high activity in relapsed/refractory WM patients with ORR of 93 % [29]. The most common adverse events were neutropenia and infections. There were also concerns about aggressive transformation and the development of secondary myeloid malignancies.

The combination BR showed an ORR of 83 % with a median PFS of 13 months [40]. The most common adverse events were prolonged myelosuppression, specifically in patients who had received prior nucleoside analog therapy.

7.3 Ofatumumab

The fully human anti-CD20 monoclonal antibody ofatumumab is also useful in the relapsed/refractory setting. Ofatumumab has shown to be effective and well tolerated in WM patients who develop rituximab intolerance [40]. A study published in abstract form showed an ORR of 57 % in patients with relapsed/refractory WM [41]. The occurrence rate of IgM flare to ofatumumab in this study was 5 %. The most common adverse events were infections.

7.4 Everolimus

Everolimus is an oral mammalian target of rapamycin inhibitor and has induced an ORR of 50 % in patients with relapsed/refractory WM [42]. The median time to response in patients who achieved a PR was 2 months with a median PFS of 21 months. The most common adverse events were anemia, leukopenia, thrombocytopenia, and stomatitis. Everolimus has also been studied in the frontline setting [43]. In this multicenter prospective study, 33 WM patients who were not previously treated received everolimus at a dose of 10 mg PO once daily. The best ORR was 72 % with a median time to best response of 6 months. Interestingly, the bone marrow burden of disease remained unchanged. This bone marrow–IgM discordance complicated response assessment. The most common adverse events were anemia, oral ulcerations, and pneumonitis.

7.5 Ibrutinib

The oral Bruton tyrosine kinase inhibitor ibrutinib has been evaluated in 63 patients with relapsed/refractory WM [44]. Ibrutinib was administered at a dose of 420 mg PO daily. The ORR was 81 % with a median time to response of 4 weeks. The most common adverse events were thrombocytopenia and neutropenia. Of note, WM patients with CXCR4 gene mutations experienced lower response rates to ibrutinib than patients with wild-type CXCR4.

8 Maintenance Therapy

A retrospective study examined the outcome of 248 WM rituximab-naïve patients who were treated with rituximab-containing regimen and then either observed or received maintenance rituximab [45]. In this study, further improved responses after induction therapy were seen in 10 % (16 out of 162) of observed patients and in 42 % (36 out of 86) of patients who received maintenance rituximab. Both PFS (56 vs. 29 months) and OS (>120 vs. 116 months) were longer in patients who received maintenance rituximab. Improved PFS was evident despite previous treatment status or type of induction therapy. Among patients receiving maintenance rituximab, an increased number of infectious events, predominantly grades 1 and 2 sinusitis and bronchitis, were observed. A prospective study examining the role of maintenance rituximab in patients with WM has been initiated by the German STiL group [46]. After undergoing induction therapy with BR, 100 out 162 patients responded to BR (ORR 86 %). From these patients, 43 were then randomized to observation and 47 to maintenance rituximab. Enrollment for this study is complete, and final results are awaited.

9 High-Dose Therapy and Stem Cell Transplantation

The use of stem cell transplantation (SCT) therapy has been explored in patients with WM. Several small series have been reported for autologous and allogeneic transplantation, with variable outcomes. Kyriakou and colleagues reported the largest experience using European Bone Marrow Transplant (EBMT) data for WM patients receiving autologous SCT [47]. Among 158 relapsed/refractory WM patients receiving an autologous SCT, the 5-year PFS and OS rates were 49 and 69 %, respectively. Non-relapse mortality at 1 year was 4 %. Chemorefractory disease and number of prior lines of therapy at time of autologous SCT were the most important prognostic factors for PFS and OS. When used as consolidation at first response, autologous SCT provided a PFS rate of 44 % at 5 years. Long-term outcomes of WM patients undergoing allogeneic SCT from EBMT have been reported [48]. A total of 86 patients received allograft by either myeloablative or reduced-intensity conditioning (RIC). The median age was 49 years, and 47 patients had 3 or more previous lines of therapy. Eight patients failed prior autologous SCT. Fifty-nine patients (69 %) had chemotherapy-sensitive disease at the time of allogeneic SCT. Non-relapse mortality rate at 3 years was 33 % for patients receiving myeloablative transplant and 23 % for those who received RIC. The ORR was 76 %. The relapse rates at 3 years were 11 % for myeloablative and 25 % for RIC recipients. Five-year PFS and OS rates for WM patients who received a myeloablative allogeneic SCT were 56 and 62 %, respectively, and for patients who received RIC were 49 and 64 %, respectively. The occurrence of chronic graft-versus-host disease was associated with improved PFS and suggested the existence of relevant graft-versus-WM effect in this study.

10 Prognostic Factors for Survival in WM

In 2003, the Consensus Panel Recommendation from the Second International Workshop on WM emitted a statement on prognostic markers for survival in patients with WM [18]. In this paper, prognostic factors such as age, hemoglobin level, white blood cell count, platelet count, weight loss, cryoglobulinemia, serum albumin level, IgM level, and beta-2-microglobulin level were identified based on three separate multivariate analyses. However, these studies reported on the survival of WM patients treated with chemotherapy alone 5–10 years before the actual report and, likely, are no longer representative of current outcomes. Since then, multiple studies have aimed at developing prognostic scores that can guide practitioners and WM patients.

Arguably, the most widely used prognostic score is the International Prognostic Scoring System for WM (IPSSWM) [49]. In this study, data on 597 previously untreated WM patients from seven institutions were analyzed. After univariate and multivariate evaluation, five adverse prognostic factors were identified: age >65 years,

hemoglobin ≤11.5 g/dl, platelet count ≤100 × 10^9/L, beta-2-microglobulin >3 mg/dl, and monoclonal IgM concentration >7 g/dl. The hazard ratio (HR) for OS of most of the factors ranged between 1.5 and 1.9, with the exception of age (HR 2.8). Patients were stratified in three risk groups: low (0 or 1 factor except for age), intermediate (age or 2 factors), and high risk (≥3 factors). The distribution of the groups was as follows: low (27 %), intermediate (38 %), and high risk (35 %). The median OS for the low, intermediate, and high-risk groups were 143, 99, and 44 months, respectively. One of the caveats of this study is that 63 % of patients were treated with alkylating agents and 32 % with fludarabine alone; only 4 % of patients received rituximab.

In several other recent studies, age, hemoglobin, and beta-2-microglobulin have been identified as prognostic factors. Selected studies and their results are shown in Table 3. However, most of the studies included patients diagnosed and/or treated before 2003, which implies that the large majority of the patients included in those analyses were not treated with anti-CD20 monoclonal antibodies or proteasome inhibitors. It is important to note that the VGPR and CR rates in WM patients appear higher with the combination of proteasome inhibitors and rituximab than with alkylating agents or nucleoside analogs. It is likely that deeper responses will be associated with prolonged survival times, which has been seen in chronic lymphocytic leukemia and multiple myeloma (references).

Table 3 Selected studies evaluating prognostic factors for overall survival in patients with Waldenström macroglobulinemia

Reference	Country	Accrual period	*N*	Prognostic factors
Dimopoulos et al. [22]	Greece	1985–2001	122	Age ≥65 years
				Hemoglobin <10 g/dl
Dimopoulos et al. [36]	Greece	1990–2003	83	Beta-2-microglobulin <3.5, 3.5–5.5, > 5.5 mg/dl
				Albumin <3.5 mg/dl
Ghobrial et al [37, 42]	USA	1960–2001	337	Age >65 years
				Organomegaly
[62]	USA	1992–1998	59	Age ≥70 years
				Previous non-protocol therapy
				Beta-2-microglobulin ≥3 mg/dl
				Elevated LDH level
Morel et al. [49]	Multinational	Before 2002	587	Age >65 years
				Hemoglobin ≤11.5 g/dl
				Platelets ≤100 × 10^9/L
				Beta-2-microglobulin >3 mg/dl
				Serum monoclonal protein >7 g/dl
Kastritis et al [51]	Greece	Unknown	232	IPSSWM
				Elevated LDH level

A recent study evaluated differences in PFS in WM patients who obtained a CR or VGPR with rituximab-containing therapy [50]. In this study, 159 rituximab-naïve WM patients were treated with rituximab-containing regimens that included rituximab alone or in combination with cyclophosphamide, fludarabine, immunomodulatory agents, or bortezomib. Patients who achieved a CR or VGPR had a median PFS over 75 months compared with 43 months and 31 months in patients who achieved a PR or a minor response to therapy, respectively. Neither age, IgM level, hematocrit, platelet count, beta-2-microglobulin, IPSSWM, or treatment group were predictors of the attainment of CR/VGPR.

11 Trends in Survival in WM

Previous epidemiologic studies suggested that the survival of WM patients could be prolonged and sometimes measured in decades. It is unclear, however, if the survival of patients with WM has improved with the advent of novel therapies.

A Greek study included 345 patients with WM, of who 130 initiated therapy before the year 2000 and 215 who started after 2000, and showed no evidence of overall survival improvement in the latter group. A survival benefit was expected based on the availability of the chimeric anti-CD20 antibody rituximab in Greece after 2000 [51]. However, the group treated after the year 2000 was older (70 vs. 65 years) and presented with higher-risk disease when compared with those treated before 2000. Also, the median follow-up for both groups was rather short, approximately 9 and 3 years for the groups before and after 2000, respectively. It is possible that given the small sample size and short follow-up, the study might have been underpowered to detect the expected benefit.

A Swedish study included 1,555 patients with WM diagnosed between 1980 and 2005 [52]. In this study, in which 1,187 patients were diagnosed before 2000 and 368 after 2000, a continuous RS benefit was identified with improvements seen in 1990s as well as the 2000s. Older patients and men had worse outcomes. The authors evaluated lead-time bias (i.e., longer survival in patients diagnosed earlier in the course of the disease) as one of the factors associated with the improvement seen in the outcomes of patients with WM and did not find differences in the proportion of asymptomatic patients diagnosed before or after 2000.

More recently, a large study based on data from the Surveillance, Epidemiology, and End Results database included over 6,000 patients and aimed at evaluating survival trends in US patients with WM [53]. In this study, the RS of patients with WM has improved over the last decade when compared with the 1980s and the 1990s. Figure 3 shows 5-year RS in WM patients over the last 3 decades in 5-year period. The survival benefits were observed regardless of age, sex, extramedullary disease, histological subtype (LPL vs. WM), and US region. Survival benefits were seen in whites but not in blacks. Blacks have had consistently worse outcomes than whites in previous epidemiological studies in various lymphoproliferative disorders [54–58]. This disparity in outcomes seen in blacks has been ascribed to differences

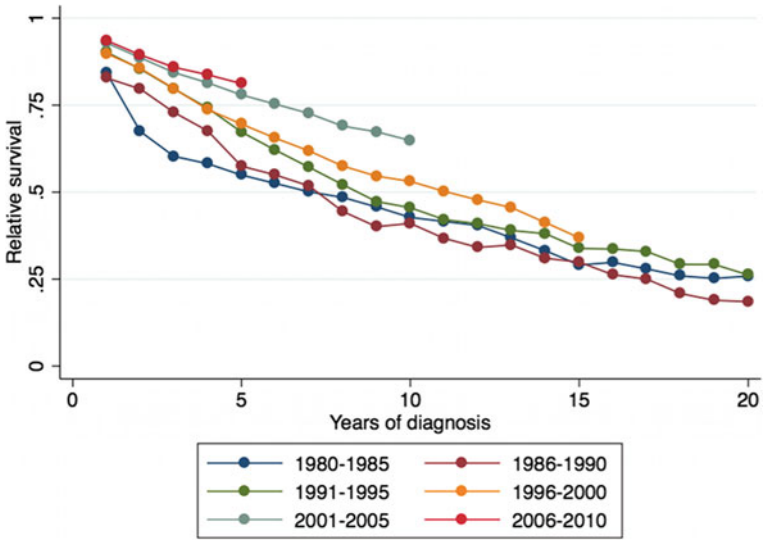


Fig. 3 5-year relative survival estimates in patients with Waldenström Macroglobulinemia from the SEER database (1980–2010), according to 5-year period of diagnosis. Reproduced with permission from [51]

in socioeconomical status, health insurance coverage, and patients' or providers' attitudes toward therapy. Purely biological factors may also play a role, considering that blacks have an increased incidence of IgG and IgA MGUS but a lower incidence of IgM MGUS. It is unclear, however, if this difference is responsible for the worse outcomes seen in black patients with WM.

12 Conclusion

There are multiple options for the treatment of patients with WM in the frontline as well as the relapsed setting. However, the treatment regimens would have to be thoughtfully selected depending on the patient's characteristics such as age, comorbidities, IgM levels, and genetic mutations. Age, beta-2-microglobulin level, and hematological parameters seemed to be the best prognostic factors associated with OS in patients with WM. However, studies evaluating these factors in large cohorts of patients treated with novel agents are lacking. Finally, the survival of patients with WM appears to be improving in the last decade, probably associated with the advent of novel agents and better supportive therapy. Novel agents such as the phosphatidylinositol 3-kinase inhibitor idelalisib (CAL-101, GS-1101), the bcl-2 inhibitor GDC-199 (ABT-199), the oral proteasome inhibitor ixazomib (MLN9708), and the glycoengineered anti-CD20 monoclonal antibody obinutuzumab (GA-101), to cite a few, will shortly enter clinical trials for WM. A recent phase II study studied idelalisib in 125 patients with relapsed/refractory indolent

lymphoma, of which 10 patients had LPL/WM [59]. From these, 2 patients achieved a PR and 6 a minor response for an ORR of 80 %. In a smaller phase I study on 64 patients with relapsed/refractory indolent lymphoma [60], the ORR in LPL/WM patients was 55 % (5/9 patients). In a phase I study on 44 patients with relapsed/refractory indolent lymphoma, GDC-199 induced a response in 3 out of 4 WM patients (ORR 75 %), including one CR [61]. Promising agents with greater efficacy, novel mechanisms of action, and better safety profiles are likely the future of WM therapy.

References

1. Swerdlow SH, Berger F, Pileri SA et al (2008) Lymphoplasmacytic lymphoma. In: Swerdlow SH, Campo E, Harris NL et al (eds) WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France, IARC, pp 194–195
2. Treon SP, Hunter ZR, Aggarwal A et al (2006) Characterization of familial waldenstrom's macroglobulinemia. Ann Oncol 17:488–494
3. Ciccarelli BT, Patterson CJ, Hunter ZR et al (2011) Hepcidin is produced by lymphoplasmacytic cells and is associated with anemia in waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma Leuk 11:160–163
4. Schop RF, Kuehl WM, Van Wier SA, Ahmann GJ, Price-Troska T, Bailey RJ, Jalal SM, Qi Y, Kyle RA, Greipp PR, Fonseca R (2002) Waldenström macroglobulinemia neoplastic cells lack immunoglobulin heavy chain locus translocations but have frequent 6q deletions. Blood 100 (8):2996–3001.
5. Treon SP, Xu L, Yang G et al (2012) MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. N Engl J Med 367:826–833
6. Jimenez C, Sebastian E, Chillon MC et al (2013) MYD88 L265P is a marker highly characteristic of, but not restricted to waldenstrom's macroglobulinemia. Leukemia 27:1722–1728
7. Mori N, Ohwashi M, Yoshinaga K et al (2013) L265P mutation of the MYD88 gene is frequent in waldenstrom's macroglobulinemia and its absence in myeloma. PLoS one 8:80088
8. Poulain S, Roumier C, Decambron A et al (2013) MYD88 L265P mutation in Waldenstrom macroglobulinemia. Blood 121:4504–4511
9. Varettoni M, Arcaini L, Zibellini S et al (2013) Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms. Blood 121:2522–2528
10. Yang G, Zhou Y, Liu X et al (2013) A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenstrom macroglobulinemia. Blood 122:1222–1232
11. Watters TM, Kenny EF, O'Neill LA (2007) Structure, function and regulation of the Toll/IL-1 receptor adaptor proteins. Immunol Cell Biol 85:411–419
12. Lin SC, Lo YC, Wu H (2010) Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. Nature 465:885–890
13. Kawagoe T, Sato S, Matsushita K et al (2008) Sequential control of Toll-like receptor-dependent responses by IRAK1 and IRAK2. Nat Immunol 9:684–691
14. Hunter ZR, Xu L, Yang G et al (2014) The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. Blood 123:1637–1646
15. Busillo JM, Benovic JL (2007) Regulation of CXCR4 signaling. Biochim Biophys Acta 1768:952–963

16. Treon SP, Cao Y, Xu L et al (2014) Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood 123:2791–2796
17. Roccaro AM, Sacco A, Jimenez C, Maiso P, Moschetta M, Mishima Y, Aljawai Y, Sahin I, Kuhne M, Cardarelli P, Cohen L, San Miguel JF, Garcia-Sanz R, Ghobrial IM (2014) C1013G/CXCR4 acts as a driver mutation of tumor progression and modulator of drug resistance in lymphoplasmacytic lymphoma. Blood 123(26):4120–4131
18. Kyle RA, Treon SP, Alexanian R et al (2003) Prognostic markers and criteria to initiate therapy in Waldenstrom's macroglobulinemia: consensus panel recommendations from the second international workshop on Waldenstrom's macroglobulinemia. Semin Oncol 30:116–120
19. NCCN Clinical Practices Guidelines in Oncology (NCCN Guidelines). Waldenstrom's macroglobulinemia/lymphoplasmacytic lymphoma. Available at http://www.nccn.org/professionals/physician_gls/pdf/waldenstroms.pdf . Accessed 1 June 2014
20. Treon SP, Ioakimidis L, Soumerai JD et al (2009) Primary therapy of Waldenstrom macroglobulinemia with bortezomib, dexamethasone, and rituximab: WMCTG clinical trial 05-180. J Clin Oncol 27:3830–3835
21. Ghobrial IM, Xie W, Padmanabhan S et al (2010) Phase II trial of weekly bortezomib in combination with rituximab in untreated patients with Waldenstrom macroglobulinemia. Am J Hematol 85:670–674
22. Dimopoulos MA, Garcia-Sanz R, Gavriatopoulou M et al (2013) Primary therapy of Waldenstrom macroglobulinemia (WM) with weekly bortezomib, low-dose dexamethasone, and rituximab (BDR): long-term results of a phase 2 study of the European Myeloma Network (EMN). Blood 122:3276–3282
23. Moreau P, Pylypenko H, Grosicki S et al (2011) Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: a randomised, phase 3, non-inferiority study. Lancet Oncol 12:431–440
24. Treon SP, Tripsas CK, Meid K, Kanan S, Sheehy P, Chuma S, Xu L, Cao Y, Yang G, Liu X, Patterson CJ, Warren D, Hunter ZR, Turnbull B, Ghobrial IM, Castillo JJ (2014) Carfilzomib, rituximab, and dexamethasone (CaRD) treatment offers a neuropathy-sparing approach for treating Waldenström's macroglobulinemia. Blood 124(4):503–510
25. Treon SP (2009) How I treat Waldenstrom macroglobulinemia. Blood 114:2375–2385
26. Ioakimidis L, Patterson CJ, Hunter ZR et al (2009) Comparative outcomes following CP-R, CVP-R, and CHOP-R in waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma 9:62–66
27. Rummel MJ, Niederle N, Maschmeyer G et al (2013) Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet 381:1203–1210
28. Leblond V, Johnson S, Chevret S et al (2013) Results of a randomized trial of chlorambucil versus fludarabine for patients with untreated Waldenstrom macroglobulinemia, marginal zone lymphoma, or lymphoplasmacytic lymphoma. J Clin Oncol 31:301–307
29. Treon SP, Branagan AR, Ioakimidis L et al (2009) Long-term outcomes to fludarabine and rituximab in Waldenstrom macroglobulinemia. Blood 113:3673–3678
30. Tedeschi A, Benevolo G, Varettoni M et al (2012) Fludarabine plus cyclophosphamide and rituximab in Waldenstrom macroglobulinemia: an effective but myelosuppressive regimen to be offered to patients with advanced disease. Cancer 118:434–443
31. Treon SP, Soumerai JD, Branagan AR et al (2008) Thalidomide and rituximab in Waldenstrom macroglobulinemia. Blood 112:4452–4457
32. Gertz MA, Rue M, Blood E et al (2004) Multicenter phase 2 trial of rituximab for Waldenstrom macroglobulinemia (WM): an Eastern Cooperative Oncology Group Study (E3A98). Leuk Lymphoma 45:2047–2055

33. Treon SP, Emmanouilides C, Kimby E et al (2005) Extended rituximab therapy in waldenstrom's macroglobulinemia. Ann Oncol 16:132–138
34. Treon SP, Branagan AR, Hunter Z et al (2004) Paradoxical increases in serum IgM and viscosity levels following rituximab in waldenstrom's macroglobulinemia. Ann Oncol 15:1481–1483
35. Chen C, Kouroukis CT, White D et al (2009) Bortezomib in relapsed or refractory waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma 9:74–76
36. Dimopoulos MA, Anagnostopoulos A, Kyrtsonis MC et al (2005) Treatment of relapsed or refractory waldenstrom's macroglobulinemia with bortezomib. Haematologica 90:1655–1658
37. Ghobrial IM, Hong F, Padmanabhan S et al (2010) Phase II trial of weekly bortezomib in combination with rituximab in relapsed or relapsed and refractory Waldenstrom macroglobulinemia. J Clin Oncol 28:1422–1428
38. Treon SP, Hunter ZR, Matous J et al (2007) Multicenter clinical trial of bortezomib in relapsed/refractory waldenstrom's macroglobulinemia: results of WMCTG Trial 03-248. Clin Cancer Res 13:3320–3325
39. Zinzani PL, Gherlinzoni F, Bendandi M et al (1995) Fludarabine treatment in resistant waldenstrom's macroglobulinemia. Eur J Haematol 54:120–123
40. Treon SP, Hanzis C, Tripsas C et al (2011) Bendamustine therapy in patients with relapsed or refractory waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma Leuk 11:133–135
41. Furman RR, Eradat H, DiRienzo CG et al (2011) A phase II trial of ofatumumab in subjects with Waldenstrom's macroglobulinemia. ASH Annu Meet Abstr 118:3701
42. Ghobrial IM, Gertz M, Laplant B et al (2010) Phase II trial of the oral mammalian target of rapamycin inhibitor everolimus in relapsed or refractory Waldenstrom macroglobulinemia. J Clin Oncol 28:1408–1414
43. Treon SP, Tripsas CK, Ioakimidis L et al (2011) Prospective, multicenter study of the MTOR inhibitor everolimus (RAD001) as primary therapy in Waldenstrom's macroglobulinemia. ASH Annu Meet Abstr 118:2951
44. Treon SP, Tripsas CK, Yang G et al (2013) A prospective multicenter study of the Bruton's tyrosine kinase inhibitor ibrutinib in patients with relapsed or refractory Waldenstrom's macroglobulinemia. Blood 122:251
45. Treon SP, Hanzis C, Manning RJ et al (2011) Maintenance rituximab is associated with improved clinical outcome in rituximab naive patients with Waldenstrom macroglobulinaemia who respond to a rituximab-containing regimen. Br J Haematol 154:357–362
46. Rummel MJ, Lerchenmuller C, Greil R et al (2012) Bendamustin-rituximab induction followed by observation or rituximab maintenance for newly diagnosed patients with waldenstrom's macroglobulinemia: results from a prospective, randomized, multicenter study (StiL NHL 7-2008 -MAINTAIN-; ClinicalTrials.gov Identifier: NCT00877214). ASH Ann Meet Abstr 120:2739
47. Kyriakou C, Canals C, Sibon D et al (2010) High-dose therapy and autologous stem-cell transplantation in Waldenstrom macroglobulinemia: the lymphoma working party of the european group for blood and marrow transplantation. J Clin Oncol 28:2227–2232
48. Kyriakou C, Canals C, Cornelissen JJ et al (2010) Allogeneic stem-cell transplantation in patients with Waldenstrom macroglobulinemia: report from the lymphoma working party of the european group for blood and marrow transplantation. J Clin Oncol 28:4926–4934
49. Morel P, Duhamel A, Gobbi P et al (2009) International prognostic scoring system for Waldenstrom macroglobulinemia. Blood 113:4163–4170
50. Treon SP, Yang G, Hanzis C et al (2011) Attainment of complete/very good partial response following rituximab-based therapy is an important determinant to progression-free survival, and is impacted by polymorphisms in FCGR3A in Waldenstrom macroglobulinaemia. Br J Haematol 154:223–228
51. Kastritis E, Kyrtsonis MC, Hatjiharissi E et al (2011) No significant improvement in the outcome of patients with waldenstrom's macroglobulinemia treated over the last 25 years. Am J Hematol 86:479–483

52. Kristinsson SY, Eloranta S, Dickman PW et al (2013) Patterns of survival in lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia: a population-based study of 1,555 patients diagnosed in Sweden from 1980 to 2005. Am J Hematol 88:60–65
53. Castillo JJ, Olszewski AJ, Cronin AM et al (2014) Survival trends in Waldenstrom macroglobulinemia: an analysis of the surveillance, epidemiology and end results database. Blood 123:3999–4000
54. Shenoy PJ, Malik N, Nooka A et al (2011) Racial differences in the presentation and outcomes of diffuse large B-cell lymphoma in the United States. Cancer 117:2530–2540
55. Shenoy P, Maggioncalda A, Malik N, Flowers CR (2011) Incidence patterns and outcomes for hodgkin lymphoma patients in the United States. Adv Hematol 2011:725219
56. Nabhan C, Aschebrook-Kilfoy B, Chiu BC et al. (2014) The impact of race, ethnicity, age, and sex on clinical outcome in chronic lymphocytic leukemia: a comprehensive SEER analysis in the modern era. Leuk Lymphoma 2014
57. Nabhan C, Aschebrook-Kilfoy B, Chiu BC et al. (2014) The impact of race, age, and sex in follicular lymphoma: a comprehensive SEER analysis across consecutive treatment eras. Am J Hematol, 89(6): 633–638
58. Castillo JJ, Winer ES, Olszewski AJ (2013) Population-based prognostic factors for survival in patients with burkitt lymphoma: an analysis from the surveillance, epidemiology, and end results database. Cancer 119:3672–3679
59. Gopal AK, Kahl BS, de Vos S et al (2014) PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med 370:1008–1018
60. Flinn IW, Kahl BS, Furman RR et al (2014) Idelalisib, a selective inhibitor of phosphatidylinositol 3-kinase-δ, as therapy for previously treated indolent non-Hodgkin lymphoma. Blood 123:3406–3413
61. Davids SM, Seymour JF, Gerecitano JF et al (2014) Phase I study of ABT-199 (GDC-199) in patients with relapsed/refractory non-Hodgkin lymphoma (NHL): responses observed in diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) at higher cohort doses. J Clin Oncol 32:8522
62. Dhodapkar MV, Hoering A, Gertz MA, Rivkin S, Szymonifka J, Crowley J, Barlogie B (2009) Long-term survival in Waldenstrom macroglobulinemia: 10-year follow-up of Southwest Oncology Group-directed intergroup trial S9003. Blood 113(4):793–796

Current Therapeutic Strategies and New Treatment Paradigms for Follicular Lymphoma

Athena Kritharis, Jaya Sharma and Andrew M. Evens

Abstract

Follicular lymphoma (FL) is an indolent non-Hodgkin's lymphoma that remains an incurable disease for most patients. It is responsive to a variety of different treatments, however it follows a pattern of relapsing and remitting disease. Traditional therapeutic options for patients with untreated FL include expectant observation for asymptomatic and low tumor burden and multiagent cytotoxic chemotherapy for symptomatic and/or high tumor burden. Biologics have become an integral part of therapy with agents that target B lymphocytes, including monoclonal anti-CD20 antibodies and radiolabeled anti-CD20 antibodies. Treatment response to cytotoxic and biologic therapy is high initially; however, with subsequent treatments, response rate and remission duration typically decline and cumulative toxicities increase. The identification of novel targeted agents, use of stem cell transplantation, and new treatment combinations provide the opportunity to enhance patient outcomes. In this review, we critically examine standard treatment strategies for patients with newly diagnosed and relapsed or refractory FL and discuss established and emerging novel therapeutic approaches.

Keywords

Non-hodgkin lymphoma · Cancer · Follicular · Rituximab · Antibody · Treatment

A. Kritharis · J. Sharma · A.M. Evens (✉)
Tufts Medical Center, 800 Washington Street, Boston, MA 02111, USA
e-mail: AEvens@tuftsmedicalcenter.org

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_8

Contents

1 Introduction

Follicular lymphoma (FL) is an indolent non-Hodgkin's lymphoma (NHL) that comprises more than 70 % of "low-grade" histologies and 22 % of all NHL cases, second only to diffuse large B-cell lymphoma [1, 2]. The clinical presentation may be nodal or extranodal, and bone marrow involvement occurs in the majority of cases. Extensive intra-abdominal adenopathy without peripheral node enlargement is not uncommon. Clinical behavior is variable, reflecting the heterogeneity of underlying biology; some patients survive for decades, whereas others progress rapidly to resistant disease or transform to a more aggressive histology. Spontaneous remissions occur, albeit rarely.

Although generally responsive to treatment, the clinical course of FL is characterized by a pattern of relapsing and remitting disease. Transformation to a higher grade NHL occurs in approximately 30 % of all FL patients (2–3 % of patients per year) [3] and is typically heralded by deterioration in the clinical condition of the patient. Overall survival (OS) in patients with FL was relatively unchanged from the 1950s through the late 1980s. Since the 1980s, however, there has been a definitive improvement in the median OS from 8 to 10 years to at least 12–14 years in the current era [4]. A recent US SEER analysis showed that the 5-year OS for FL

patients from 2001 to 2009 was 77 %, which was significantly improved compared with the prior era 1992–2000 at 67 % (Fig. 1) [5]. Furthermore, OS was significantly improved for all age groups <80 years and both genders across the two treatment eras.

Despite improvements in therapy, a number of challenges remain in the management of FL. In addition, it is a disease in which relatively long survival times elevate the importance of quality of life when treatments are considered.

2 Tumor Burden and Clinical Prognostication

The identification of prognostic factors and scoring systems, both clinical and tissue/host based, has helped to refine prognostication for patients with FL and may help guide therapeutic decisions. The Groupe D'Etude des Lymphomes Folliculaires (GELF) and The Follicular Lymphoma International Prognostic Index (FLIPI) scores have emerged as important prognostic models in FL [6, 7]. While the GELF criteria seek to stratify patients by tumor burden and determine when to start cytotoxic therapy, the FLIPI serves as a prognostic model for survival determination. Entry criteria for most clinical trials of untreated FL separate patient groups based on the GELF and/or FLIPI criteria.

The GELF criteria were established in the late 1990s to separate patients into low and high tumor burden based on prognostic factors as noted in Table 1 [7, 8]. In the original 1997 study, patients designated with high tumor burden had a shorter median survival of 71 months compared with the low-risk group whose survival endpoint had not been reached. These criteria have been used to define patient populations who warranted initiation of cytotoxic chemotherapy based on symptoms attributable to their disease/lymphoma or high-risk features (e.g., compression of vital organs) versus asymptomatic patients with low-risk features who were included in "watch-and-wait" (W&W) randomized trials. Over the past two decades, lymphoma groups around the world have used similar, but slightly modified criteria (Table 1) [7–18].

The FLIPI was originally published in 2004, as a prognostic score for newly diagnosed advanced-stage FL [19], then for early-stage disease [20], and also FL in first relapse [21]. An updated report by Federico et al. [6] included the prognostic significance of FLIPI among FL patients treated with rituximab-based treatment (i.e., FLIPI-2). This analysis yielded 3 distinct risk groups with significantly different outcomes (Table 2). The German Low-Grade Lymphoma Study Group (GLSG) also validated the FLIPI risk groups for newly diagnosed high tumor burden FL patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) [11]. An important distinction of the FLIPI is that it is a prognostic scoring system for survival; however, unlike GELF criteria, it is not necessarily a tool to dictate when treatment should or should not be initiated for FL. There is no published data showing that treatment initiation based on FLIPI is associated with enhanced outcomes, especially toward potential improvement in the

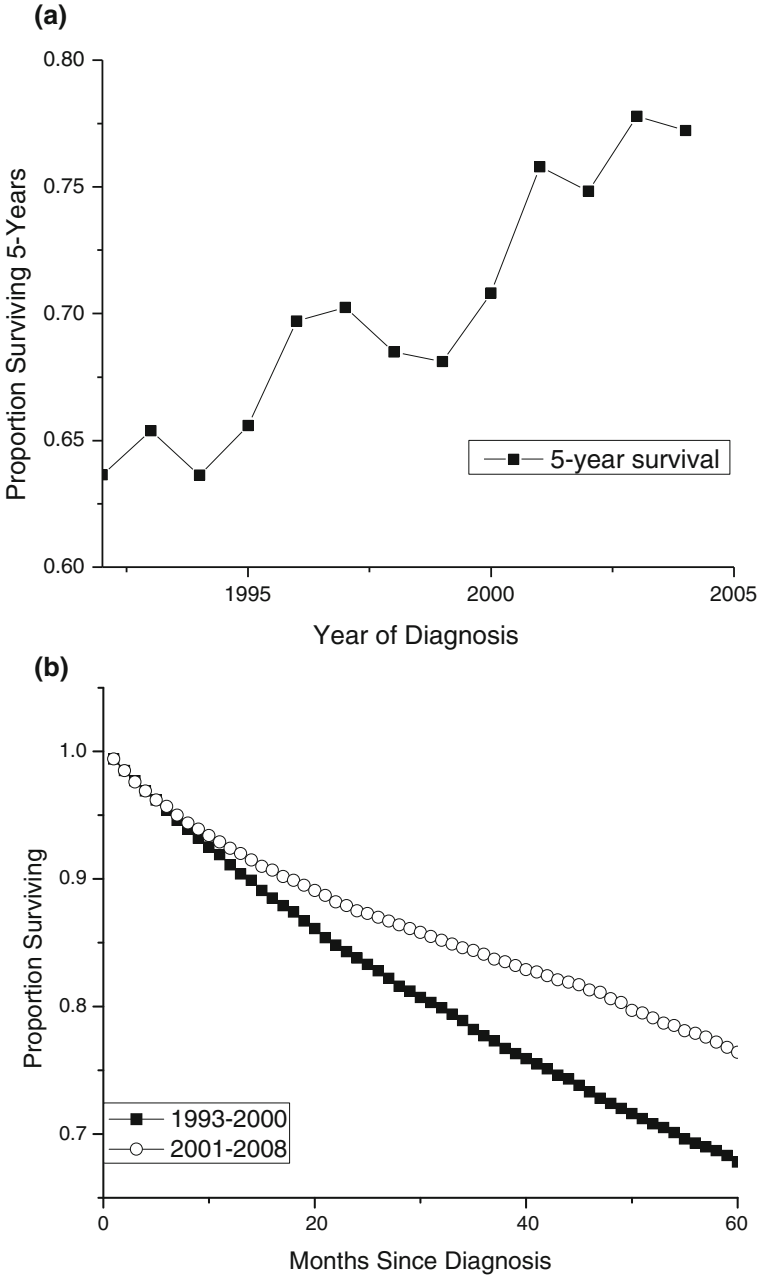


Fig. 1 **a** Kaplan–Meier curve showing progressive improvement in the overall survival of FL patients over time. **b** Patients diagnosed and treated in post-rituximab era-2 (*light color*) had a statistically significant improvement in overall survival compared with patients treated in pre-rituximab era-1 (*dark color*)

Table 1 Definition(s) of tumor burden in follicular lymphoma[a]

Original GELF [7, 8]	Modified GELF (PRIMA) [16]	BNLI [10]	GLSG [13]	East German Study Group [17]	ECOG (modified GELF) [18]
• Any nodal or extranodal tumor mass >7 cm	• Any nodal or extranodal tumor mass >7 cm	• Rapid generalized disease progression in preceding 3 months	• Bulky disease (mediastinal disease >7.5 cm or other mass >5 cm)	• Bulky disease (mediastinal tumor >one-third of thorax or other mass >7.5 cm)	• Nodal or extranodal mass ≥7 cm
• At least 3 nodal sites, each with a diameter of >3 cm	• At least 3 nodal sites, each with a diameter of >3 cm	• Life-threatening organ involvement	• Presence of B-symptoms	• B symptoms or extranodal manifestation	• At least 3 nodal sites, each with a diameter of >3 cm
• Any B symptom	• Systemic symptoms	• Renal or macroscopic liver infiltration	• Rapidly progressive disease	• Rapid tumor growth (doubling of the product of the end-to-end diameters measurable lymphoma within 6 months)	• Systemic symptoms or any B symptom
• Splenic enlargement with inferior margin below the umbilicus line	• Symptomatic splenic enlargement	• Bone lesions	• Cytopenia (hemoglobin level <10.0 g/L, ANC <1.5 × 10^9/L, and/or platelets <100 × 10^9/L)	• Cytopenia (granulocyte count <1.0 × 10^9/L and/or platelets <100 × 10^9/L)	• Splenomegaly >16 cm by CT scan
• Compression syndrome (ureteral, orbital, GI)	• Organ compression	• Systemic symptoms or pruritus		• Immunologic phenomena (e.g., hemolytic anemia or immune thrombocytopenia)	• Compression of a vital organ (e.g., ureteral, epidural)
• Pleural or peritoneal serous effusion	• Pleural or peritoneal serous effusion	• Cytopenia (Hgb < 10 g/L or WBC < 3.0 × 10^9/L or platelet counts < 100 × 10^9/L)			• Leukemic phase (>5.0 × 10^9/L circulative malignant cells)

(continued)

Table 1 (continued)

Original GELF [7, 8]	Modified GELF (PRIMA) [16]	BNLI [10]	GLSG [13]	East German Study Group [17]	ECOG (modified GELF) [18]
• Leukemic phase (>5.0 × 10^9/L circulative malignant cells)	• ECOG performance status 2–4				• Cytopenia (Hgb <10.0 g/L, granulocyte <1.5 × 10^9/L, and/or platelets <100 × 10^9/L)
• Cytopenia (ANC <1.0 × 10^9/L and/or platelets <100 × 10^9/L)	• Serum LDH or β2-microglobulin above normal				

[a]Patients must meet only 1 criterion in any group criteria to be considered "high" tumor burden

GI gastrointestinal; *ANC* absolute neutrophil count; *cm* centimeters; *LDH* lactate dehydrogenase; *Hgb* hemoglobin; *CT* computerized tomograph

Table 2 Survival by risk group as defined by the FLIPI-2 [91]

Risk group	No. factors	Distribution of patients (%)	3-year PFS (%)	5-year PFS (%)
Low	0	20	91	79
Intermediate	1–2	53	69	51
High	3–5	27	51	19

Factors adversely affecting outcome include (1) Age (>60 years vs. 60 years or less); (2) Hemoglobin level (<120 g/L vs. 120 g/L or higher); (3) β2-microglobulin (above normal vs. normal or below); (4) Largest involved lymph node (>6 cm vs. 6 cm or lower); and (5) Bone marrow (involved vs. not involved)

FLIPI follicular lymphoma international prognostic index; *LDH* lactate dehydrogenase; *PFS* progression-free survival

natural history of the disease. Nonetheless, an adverse FLIPI score (i.e., 3–5) has been integrated into several ongoing clinical trials as selection criteria for initiation of therapy in patients with untreated FL.

3 Treatment Approaches

Traditional treatment options for FL include expectant observation for asymptomatic and low tumor burden disease and multiagent cytotoxic chemotherapy for patients who are symptomatic and/or have high tumor burden. Biologic therapy has become an integral part of therapy with agents that target B lymphocytes, such as monoclonal anti-CD20 antibodies and radiolabeled anti-CD20 antibodies. Treatment response to cytotoxic and biologic therapies is high initially; however, with subsequent treatments, response rate and remission duration typically decline and cumulative toxicities increase. The identification of novel targeted agents and new combinations of therapeutics provide the opportunity to continue to improve outcomes for patients with FL.

3.1 Early-Stage Disease

The majority of FL patients present with advanced-stage disease, however, approximately 10–15 % will have stage I disease at initial presentation [22]. For early-stage disease outside of the abdomen and pelvis, definitive radiotherapy (RT) continues to be the recommended approach because of the potential for long-term disease-free survival and possible cure. MacManus and Hoppe [23] evaluated 177 patients with stage I and II NHL who received radiotherapy over median follow-up of 7.7 years. Compared with actuarial survival rates, relapse rates were lower in patients who received RT; 55, 44, 40, and 37 % were relapse free at 5, 10, 15, and 20 years. In addition, results from the Princess Margaret Hospital's series of involved-field RT (IFRT) for early-stage disease show cumulative relapse rates of 54 and 56 % at 15 and 25 years, with only a 2 % risk of relapse beyond 15 years

[24]. The recommended dose is approximately 30 Gray (Gy) for non-bulky disease showing prompt regression and 36 Gy for bulky or slowly regressive disease, in 1.75–2.0 Gy daily fractions. Combined modality therapy has also resulted in excellent disease control [25], and a randomized trial comparing involved-field RT with combined modality therapy is ongoing.

Despite the excellent outcomes associated with RT, the US Lymphocare Study revealed that the majority of early-stage FL patients are either observed or treated with rituximab alone or in combination with chemotherapy, foregoing the potential for cure, even in young patients [22]. In a prior study from Stanford, they had identified 43 patients with early-stage FL who had been observed without therapy [26]. With median follow-up of 7 years, the estimated 10-year survival rate was 85 %, while 63 % of patients had still not required treatment. Thus, there may be select patients with early-stage FL where observation without therapy may be a rationale approach, especially for patients where toxicity concerns of RT outweigh potential benefit.

3.2 Advanced-Stage: Low Tumor Burden

3.2.1 Watch and Wait

A long-standing management of asymptomatic patients with low tumor burden FL has been a W&W approach. Treatment is delayed until the development of symptoms due to lymphoma, the presence of significant cytopenias, and/or if there is compromise of vital organs. In the pre-rituximab era, multiple phase III randomized trials compared immediate chemotherapy with observation for asymptomatic patients with advanced-stage FL showed no difference in OS. In one study, the actuarial chance of patients ages >70 years not needing chemotherapy was 40 % at 10 years [10]. A recently reported European-led registry study studied outcomes of a cohort of 107 low tumor burden FL patients who were managed with a W&W strategy [27]. After an approximate 5-year median follow-up, 50 % of patients had not started on treatment. On multivariate analysis, only involvement of >4 nodal sites correlated with time to lymphoma therapy, while the FLIPI and FLIPI-2 did not. Additionally, the investigators compared freedom from treatment failure (FFTF) and OS among the W&W cohort versus 242 low tumor burden FL patients who received rituximab-containing therapy (initiation of first treatment was not considered an event in the W&W group in order to compare a similar clinical end point), there were no differences in FFTF or OS.

3.2.2 Single-Agent Rituximab

In patients where W&W might hinder quality of life, single-agent rituximab may be considered. In previously untreated FL patients with low tumor burden, the overall response rate (ORR) with rituximab is 47–74 % [14–16], with a median PFS of approximately 2 years without maintenance therapy [14, 16] or 3 years with abbreviated post-induction rituximab [15]. A phase III trial of patients with

untreated low tumor burden FL randomized patients to W&W versus immediate induction rituximab therapy (with or without maintenance therapy) was recently reported [9]. At 3 years, 46 % versus 88 % ($p < 0.0001$) of patients in the W&W group versus maintenance rituximab group did not need treatment. There were no differences in OS noted, although follow-up may be premature to rule out a difference. Interestingly, 78 % of patients in the rituximab induction group did not need treatment at 3 years, which was the same as the maintenance rituximab group.

This latter finding was substantiated in the E4402 Rituximab Extended Schedule or Retreatment (RESORT) study [18]. In RESORT, all 384 low tumor burden FL patients were treated with 4 weeks of induction rituximab and then subsequently randomized to observation or indefinite maintenance rituximab. In terms of the primary end point of time to treatment failure (TTF), rituximab re-treatment was as effective as continual rituximab maintenance. There were no differences in quality of life or OS, while the two arms differed in time to first cytotoxic chemotherapy at 3 years (95 % for maintenance rituximab vs. 86 %, $P = 0.03$). Altogether, these results suggest that if low tumor burden FL patients are treated with rituximab, 4 weeks of induction therapy are indicated with re-treatment at time of disease progression.

3.3 Advanced-Stage: High Tumor Burden

Several treatment options are available, including single- or multiple-agent chemotherapy with concurrent CD20 antibody therapy; CD20 antibody therapy alone in selected cases; radioimmunoconjugates; and therapy with new targeted therapeutic agents, such as bortezomib (Velcade, Millennium), lenalidomide (Revlimid, Celgene), and idelalisib (Zydelig, Gilead). Autologous and allogeneic hematopoietic stem cell transplantations (SCTs) traditionally are reserved for patients with recurrent or refractory disease, but they may be used earlier in the disease course for patients with a poorer prognosis.

3.3.1 Induction Therapy

The most common treatment for untreated, advanced FL with high tumor burden includes immunotherapy-based treatment with concurrent rituximab and chemotherapy [28]. Several randomized phase III trials evaluating various chemotherapy combinations plus rituximab versus chemotherapy alone have been reported and updated (Table 3) [11, 13, 15, 17, 29–32]. The response rates and either median TTF or median event-free survival (EFS) were superior in all chemoimmunotherapy arms (vs. chemotherapy alone) for chemotherapy-naïve patients and for those who were previously treated. Moreover, OS improvements for the chemoimmunotherapy arms have also been documented.

Bendamustine plus rituximab (BR) and R-CHOP are the most common first-line regimens, with BR becoming widely used regimen in North America and Europe. Bendamustine was approved in the USA for lymphoid malignancies in 2008, but

Table 3 Selected randomized studies of chemoimmunotherapy for patients with FL

Series/author	Year	No. patients	Arms	Conclusions
Hochster [31]	2005	401	CVP versus CVP with rituximab post-induction maintenance	Improved PFS (median with rituximab 5.6 years versus 1.8 without maintenance, $P < 0.0001$) and OS (88 % vs. 72 %, $p = 0.03$) with post-induction rituximab
Hiddeman and Buske [11, 13]	2005 and 2006	428	8 × CHOP versus R-CHOP (all patients then randomized to IFN maintenance versus autoSCT)	4-year PFS: CHOP 28 % versus R-CHOP 62 % ($p < 0.0001$) 4-year OS: CHOP 81 % versus R-CHOP 90 % versus ($p = 0.039$)
Marcus [32]	2006	321	8 × CVP versus R-CVP	Median TTF: CVP 15 months versus R-CVP 34 months; OS improved for R-CVP ($p = 0.029$)
Foussard and Salles [15, 30]	2006	358	CHVP × 12 over 18 months versus 6 cycles R-CHVP over 6 months (both arms: interferon-alpha × 18 months)	EFS: CHVP 37 % versus R-CHVP 53 % ($p < 0.0001$); Median OS: CHVP 79 % versus R-CHVP 84 % (p = NS)
Herold [17]	2007	358	8 × MCP versus R-MCP (both followed by interferon-alpha)	Median EFS ($p = 0.001$) and median OS ($p = 0.0096$) improved with R-MCP
Forstpointner [29]	2006	125 (relapsed)	4 × FCM versus R-FCM with second randomization of rituximab maintenance (4 weekly doses at 3 and 9 months) versus observation	Response duration significantly prolonged by R-maintenance after R-FCM
Van Oers [84]	2006	465 (relapsed)	6 × CHOP versus R-CHOP induction followed by 2nd randomization to rituximab maintenance (one dose q3 months × 24 months) versus observation	Improved PFS with R-maintenance after CHOP induction (HR, 0.30; $P < .001$) and R-CHOP induction (HR, 0.54; P 0.004)

CHVP cyclophosphamide, adriamycin, etoposide, prednisone; *CHOP* cyclophosphamide, doxorubicin, vincristine, prednisone; *CI* confidence interval; *IFN* interferon; *CVP* cyclophosphamide, vincristine, prednisone; *EFS* event-free survival; *FCM* fludarabine, cyclophosphamide, mitoxantrone; *FL* follicular lymphoma; *HSCT* hematopoietic stem cell transplantation; *MCP* mitoxantrone, chlorambucil, prednisone; *ns* not significant; *OS* overall survival; *pts* patients; *PFS* progression-free survival; *R* rituximab; *TTF* time to treatment failure; *TTP* time to disease progression

was first only available in East Germany until 1990. In 2005, Rummel et al. [33] showed BR to be highly active in indolent and mantle-cell lymphoma with ORR of 90 and 75 %, respectively, and complete remission (CR) of 60 and 50 %, respectively. Rummel et al. [34] then showed that BR can be used as first-line therapy in FL due to increased PFS and lower toxicity in their non-inferiority trial. In total, 549 patients were enrolled comparing BR versus CHOP in newly diagnosed, untreated stage III or IV high tumor burden indolent NHL or mantle-cell lymphoma. Superior median PFS was seen with BR versus R-CHOP (69 months vs. 31 months), while there was no difference in OS at follow-up of 45 months. Lower rates of grade 3 and 4 neutropenia, infection, paresthesia, and stomatitis were seen, and there was no alopecia with BR compared with R-CHOP.

Flinn et al. [35] also compared BR to R-CHOP and R-CVP as first-line treatment in the BRIGHT study. BR was found to be non-inferior to R-CHOP and R-CVP in patients with indolent NHL or mantle-cell lymphoma. Although BR had slightly better response rates than R-CHOP/R-CVP (97 % vs. 91 %, $p = 0.01$), R-CHOP/R-CVP appeared to be acceptable alternatives with similar PFS ands OS rates. Further, there were several toxicities that occurred at a higher rate with BR (i.e., allergic reactions, nausea/vomiting, and skin reactions). R-CHOP may be preferred over BR or R-CVP in situations where patients have a more aggressive histology including patients with FL grade 3b [35]. In the FOLL05 trial, R-CHOP was shown to have better risk–benefit ratio and superior 3 year TTF and PFS compared to R-CVP in the FOLL05 trial [6]. This suggests that the role of an anthracycline in patients with high-risk disease, such as bone marrow invasion and high β2-microglobulin concentration, may be important. R-CVP though is a well-tolerated induction regimen and may be considered especially in patients with concern of cardiac toxicity with anthracycline therapy.

3.3.2 Maintenance Therapy

The concept of maintenance chemotherapy has become a recurrent theme in hematology, which was initially pioneered in pediatric ALL with the POMP regimen that contributed to durable remissions [36]. As FL is an incurable disease, there have been attempts to improve OS and PFS. Initial studies with interferon, chlorambucil, and multiagent chemotherapy showed mixed results and overall unacceptable toxicity. With the integration of anti-CD20 antibodies into clinical practice, there was a renewed opportunity to explore the potential benefit of maintenance therapy following induction treatment for newly diagnosed FL patients.

In a randomized phase III trial known as the Primary Rituximab and Maintenance (PRIMA) study, Salles et al. [16] evaluated the benefit of 2 years of rituximab maintenance in FL after upfront R-chemotherapy (R-CHOP, R-CVP, and R-FCM). With median follow-up of 36 months, rituximab maintenance improved PFS (75 % vs. 58 %), with higher EFS and a higher percentage of patients in CR or unconfirmed CR at 24 months (72 % vs. 52 %). There were higher rates of severe (grade 3/4) adverse events (24 % vs. 17 %) and infection (39 % vs. 24 %) [16].

There was no significant difference in quality of life or OS. In January 2011, the FDA approved use of rituximab maintenance therapy for patients with previously untreated follicular CD-20 positive B-cell NHL who achieve a response to rituximab in combination with chemotherapy.

It is not clear whether these results can be extrapolated to patients treated with other initial chemotherapy regimens. Despite the lack of data, many physicians may administer maintenance rituximab after BR in the hopes of better outcomes than BR alone [37]. In addition, further follow-up is needed to measure long-term toxicities and determine whether the improvement in PFS translates into an OS benefit. If maintenance rituximab is given in a patient with newly diagnosed FL with response to induction therapy, we recommend rituximab to be given every 2 months for a total of 2 years.

4 Radioimmunotherapy

The anti-CD20 radioimmunoconjugates ^{131}I-tositumomab (Bexxar, GlaxoSmithKline) and ^{90}Y-ibritumomab tiuxetan (Zevalin, Spectrum Pharmaceuticals) deliver ionizing radiation to target tumor cells and their neighbors. They are relatively easy to administer, safe, and effective in relapsed/refractory FL [33, 34], including rituximab-refractory disease [35]. Contraindications to their use include thrombocytopenia (i.e., $<100 \times 10^9$/L) and/or >20–25 % bone marrow involvement with lymphoma. Radioimmunotherapy (RIT) has also been incorporated into the frontline management of patients with untreated indolent lymphoma as a single agent [38] and also through the paradigm of sequential therapy giving one dose of RIT after multiagent induction chemotherapy. Morschhauser et al. reported results of phase III FIT (first-line indolent trial) examining consolidative RIT versus no consolidation in patients who achieved CR or partial remission (PR) following chemotherapy induction [30]. Patients were randomized to one dose of ^{90}Y-ibritumomab tiuxetan or no consolidation. RIT significantly improved median PFS in all patients (36.5 months vs. 13.3 months with no RIT; $P < 0.0001$). This gain was observed regardless of the depth of initial response to induction chemotherapy, although the benefit appeared to be greater for patients in PR versus CR. A limitation of the FIT trial was that the majority of patients did not receive rituximab-based induction chemotherapy. The extent of benefit of consolidation RIT after combined rituximab/chemotherapy therapy is not clear.

^{131}I-tositumomab also has been incorporated sequentially following chemotherapy [16, 37]. Further information regarding the role of up-front, sequential RIT in indolent NHL should be available soon from the large US intergroup study led by the Southwest Oncology Group (S0016). In that phase III trial, untreated FL patients were randomly assigned to 6 cycles of R-CHOP versus 6 cycles of CHOP followed by ^{131}I-tositumomab.

4.1 Autologous Stem Cell Transplantation (SCT) for First-Line Treatment/Consolidation

Randomized studies that have examined the role of autologous SCT versus non-transplant strategies in the front line setting for FL have shown an improvement in PFS, but without improvement in OS (Table 4). The American Society of Blood and Marrow Transplantation (ASBMT) society does not recommend autologous SCT in the first-line setting for FL based on review of these trials and given the risk

Table 4 Autologous as a first-line consolidative therapy

Reference	Treatment	Number	PFS/ EFS	OS
Horning et al. [85]	Induction: CVP or other Mobil: G-CSF HDT: Cy + VP − 16 + TBI	37	Not given	92 %
Prospective, nonrandomized	Non-ASCT: not known	188 (historical)		88 %
Lenz et al. [86]	Induction: CHOP or MCP Mobil: Dexa-BEAM HDT: Cy + TBI	114	65 % (5 year)	Not given
Prospective	Non-ASCT: CHOP or MCP +IFN maintenance	126	33 %	
Lenz et al. [87]	Induction: CHOP, R-CHOP Mob: Dexa-BEAM HDT: CY + TBI	142 (73 %)	60 % (5 year)	Not given
Prospective	Non-SCT: CHOP-like and IFN maintenance	180 (76 %)	32 %	
Sebban et al. [88]	ASCT: Induction: CHOP × 4 Mob: CY, VP – 16 HDT: Cy + VP – 16 + TBI	172 (95 %)	40 % (7 year)	76 %
Prospective	Non-ASCT: CHVP × 6 and IFN maintenance	167	29 %	71 %
Ladetto et al. [89]	ASCT: Induction: APO Purging: Rituximab HDT: Mito/Mel	68	68 %	81 % (4 year)
Prospective	Non-ASCT: R-CHOP × 6	66	31 %	80 %
Gyan et al. [90]	ASCT: Induction: VCAP (DHAP for salvage) HDT: Cy + f – TBI	86	64 % (9 year)	76 % (9 year)
Prospective	Non-ASCT: CHVP and IFN maintenance	80	39 %	80 %

CHOP cyclophosphamide, doxorubicin, vincristine, prednisone; *CVP* cyclophosphamide, vincristine, prednisone; *EFS* event-free survival; *PFS* progression-free survival; *ASCT* autologous stem cell transplantation

of secondary hematological malignancies associated with autologous SCT [39]. A 2012 Cochrane review came to a similar conclusion regarding a consistent improvement in PFS without improved OS [40].

5 Relapsed or Refractory FL

Most patients with FL will develop progressive disease including after an initial durable response to immunochemotherapy. Patients with asymptomatic recurrent FL do not require immediate treatment, but need close monitoring for the development of symptomatic disease. There are a number of treatment options for patients with relapsed or refractory FL (Table 5). A number of factors will impact the treatment choice for relapsed disease. These include duration of previous remission(s), type and amount prior therapy used, current tumor burden (at progression), and patient age and comorbidities. Treatment options include immunotherapy alone, immunotherapy combined with chemotherapy, chemotherapy alone, and radioimmunotherapy. Additionally, there should be consideration for consolidation with stem cell transplantation (autologous vs. allogeneic) in select patients.

5.1 Immunotherapy

Immunotherapy with rituximab as a single agent or as part of a chemotherapy–immunotherapy regimen has efficacy in relapsed/refractory FL [41]. Single-agent rituximab has shown a response rate of around 40 % in relapsed patients [42]. One

Table 5 Therapeutic strategies/options for relapsed or refractory FL[a]

Re-challenge with original therapy (with disease-free remission of >18–24 months)
Single-agent rituximab or rituximab plus chemotherapy (e.g., CVP, CHOP, bendamustine, gemcitabine, fludarabine, etc.)
Radioimmunotherapy
Radiation therapy (select cases: e.g., localized disease)
Idelalisib (PI3 K inhibitor)
[b]FDA approved agents with efficacy in FL: lenalidomide, ibrutinib, bortezomib, obinutuzumab, and temsirolimus
Non-FDA approved agents with promising activity in FL: HDAC inhibitors (e.g., abexinostat); pro-apoptotic agents (BH3 mimetics: ABT-199; TRAIL-R1: mapatumumab; anti-PD1 antibody: pidilizumab); monoclonal antibodies (anti-CD22: epratuzumab, anti-CD74 milatuzumab; bispecific single-chain BiTE antibody: blinatumomab); and antibody drug conjugates (anti-CD22: inotuzumab ozogamicin; anti-CD19: SAR3419)

[a]A clinical trial is always the preferred therapeutic strategy
[b]These agents are currently FDA approved for non-FL lymphoid malignancies

of the strongest predictors of rituximab for relapsed/refractory patients is disease-free interval from prior rituximab use. Rituximab has also been combined with several chemotherapeutic agents in the relapsed/refractory setting. An increasing common clinical scenario in FL being encountered is the occurrence of rituximab-refractory disease. The definition of rituximab-refractory disease (i.e., lack of response or disease progression within 6 months of rituximab or rituximab-based therapy) is arbitrary; however, it connotes a clinical scenario whereby the expected remission duration is shorter than expected in most cases.

5.2 Chemotherapy

As discussed before, there are a number of different chemotherapy regimens that have been examined in FL. Interestingly, relatively few have been tested in patients with rituximab-refractory disease. Bendamustine given as a single agent at the dose of 120 mg/m^2 intravenously on days 1 and 2 of each 21-day cycle for 6 cycles was associated with an ORR of 77 % with CR rate of 15 % [43]. The duration of response was somewhat modest at 6.7 months. In terms of other chemotherapeutic agents or combinations (Table 5), there should be examination of benefits and risks in FL in terms of infectious complications [44, 45], risk of myelodysplastic syndrome [46–48], and the adverse impact on autologous stem cell collection/bone marrow harvest [48–50].

5.3 Radioimmunotherapy (RIT)

The beta-emitter, Y-90 ibritumomab, yields an ORR of 83 % with 37 % CR in relapsed or refractory FL and transformed CD20+ lymphomas with an associated approximate median duration of response of 14 months [51]. For FL patients refractory to rituximab, response rates with Y-90 ibritumomab remain high (i.e., 74 %), but the median duration of response (DOR) is shorter (i.e., 6.4 months). In an integrated efficacy analysis of 5 clinical trials of patients with heavily pretreated low-grade or transformed NHL treated with ^{131}I-tositumomab, ORRs ranged from 47 to 68 % (CR rates, 20–38 %), with median DOR of approximately 13 months [52]. It should be noted that recent analyses have shown that use of RIT does not appear to impair collection of autologous bone marrow/stem cells [53]. Further, the risk of secondary MDS after RIT appears modest and should not dissuade use of this therapy [54].

Additionally, there are several novel RIT-based therapeutic approaches including concurrent use of radiation-enhancing agents, fractionation of RIT, pretargeting, and use of new humanized antibodies that are being explored to increase efficacy and potentially mitigate toxicity [38]. Novel combinations include potentially synergistic radiation-sensitizing agents—e.g., bortezomib [39], the synthetic 24-mer oligodeoxynucleotide CpG 7909 [40], and the expanded porphyrin agent

motexafin gadolinium (MGd; Xcytrin, Pharmacyclics) [41], which targets redox-dependent pathways and enhances the sensitivity of tumor cells to ionizing radiation [42]. In a phase I clinical trial in which MGd was given concurrently with ^{90}Y-ibritumomab tiuxetan, the ORR was 57 % (CR 43 %), with median TTF of 10 months and median duration of 17 months [41]. Moreover, in rituximab-refractory FL, the ORR was 86 % (CR 64 %), with a median TTF of 14 months, which compares favorably with previous data [35]. These results are encouraging, but randomized trials will be needed to prove the superiority of this combination over conventional RIT.

5.4 Radiation Therapy

Irradiation for clinical stages III and IV, low-grade, extensive-stage NHL is used primarily for palliation of symptomatic sites of disease using abbreviated fractionated schedules (25–30 Gy in 2.5–3 Gy daily fractions). A low-dose regimen of 4 Gy in 2 fractions has also been shown to be effective, with an ORR of approximately 80 % in the palliation of symptoms, and is overall well tolerated [55].

5.5 SCT for Relapsed/Refractory FL

5.5.1 Autologous SCT

Several studies have examined the role of autologous SCT versus chemotherapy in the salvage setting, though most analyses have been conducted in the pre-rituximab, retrospective, or single-arm phase II studies [56, 57]. Given limited data involving the use of rituximab, ASBMT made no recommendations on the use of autologous SCT versus chemotherapy including rituximab in the salvage setting [57]. Nevertheless, many patients benefit from this strategy and a number of centers use autologous SCT in the salvage setting for FL [58]. A recent analysis examined potential prognostic factors that predict outcomes for relapsed/refractory FL patients who receive autologous SCT [59]. Clinical factors that most closely impacted survival were ages >60 years and >3 prior therapies. Further, a CART survival model was formed for patients incorporating these prognostic covariates. An increasing number of these two independent variables (i.e., age ≥60 years and >3 prior therapies) was associated with divergent survival rates (Figs. 2 and 3).

5.5.2 Allogeneic SCT

Allogeneic HSCT is a potential curative treatment option for patients with FL. With use of reduced-intensity conditioning (RIC) and the greater availability of matched unrelated donors and other donor options (e.g., cord and haploidentical), it is an option for an increasing number of patients [60]. Patients should still be carefully selected, however, as the treatment-related morbidity and mortality remain problematic.

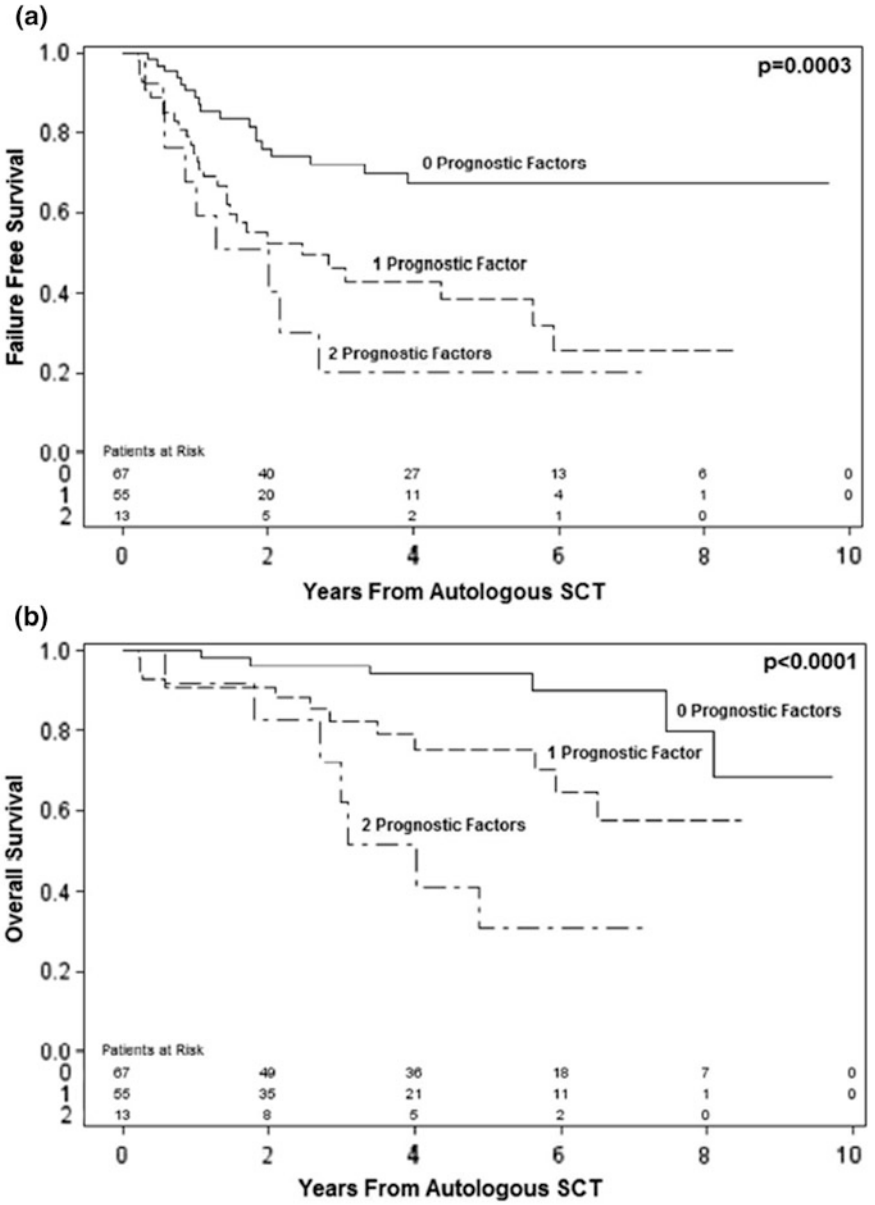


Fig. 2 Survival model with autologous SCT for relapsed/refractory FL. CART survival model for FL patients who underwent autologous SCT. 3-year FFS (**a**) and OS (**b**) based on the number of the adverse prognostic factors present (i.e., 0, 1, or 2 factors of age >60 years and >3 prior therapies): 72, 47, and 20 %, respectively (FFS $P = 0.0003$); and 96, 82, and 62 %, respectively (OS $P < 0.0001$)

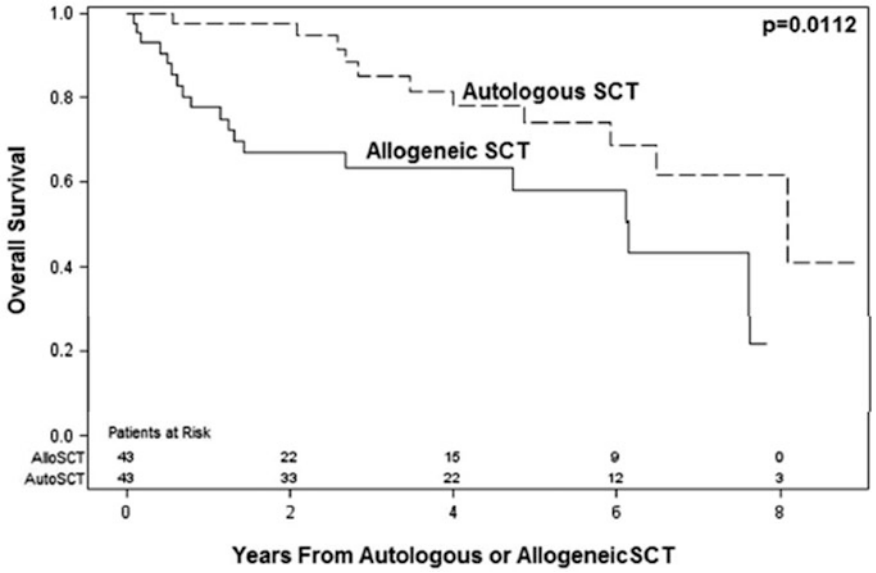


Fig. 3 Comparison of autologous versus allogeneic SCT for relapsed/refractory FL patients in the post-rituximab era. Kaplan–Meier overall survival for an exact matched cohort with propensity matching analysis (for age, number of prior treatments, and disease status at time of SCT) comparing autologous SCT with allogeneic SCT. The 3-year OS on this matched analysis was 85 % (95 % CI 73–97 %) for autologous SCT versus 63 % (95 % CI 47–79 %) for allogeneic SCT ($P = 0.011$)

Choosing between an autologous HSCT and allogeneic HSCT is a difficult decision and depends on many factors, including donor availability, donor age, patient age, and comorbidities. Many centers will perform an autologous SCT first, reserving an allogeneic SCT for patients who later relapse. Today, the vast majority of allogeneic SCTs are based on reduced-intensity conditioning, which is applicable to a wider range of patients and appears to result in survival rates that are similar to that of myeloablative transplantation [45]. The addition of rituximab to conditioning for both allogeneic and autologous SCT has been associated with excellent outcomes in some series, but its role in transplantation for FL has to be better defined [43, 46].

5.5.3 Autologous Versus Allogeneic SCT

Comparative cohort studies have evaluated the efficacy of autologous SCT versus allogeneic SCT in relapsed/refractory FL. Most studies have shown an increased incidence of transplant-related mortality (TRM) with allogeneic SCT but lower relapse rate. It should be noted that the patient population in most studies are different with the allogeneic group frequently having younger patients and a higher incidence of bone marrow involvement. Evens et al. [59] compared 136 autologous SCT patients with 48 allogeneic SCT patients with relapsed/refractory FL who had

been treated with prior rituximab. The cumulative 100-day non-relapse mortality (NRM) for was 1 % for autologous SCT versus 6 % for allogeneic SCT ($P < 0.0001$), while 3-year NRM rates were 3 % versus 24 %, respectively ($P < 0.0001$). For autologous SCT and allogeneic SCT, cumulative rates of relapse, progression, and/or transformation were 32 % versus 16 %, respectively ($P = 0.03$), while 3-year OS rates were 87 % versus 61 % ($P < 0.0001$); there were no differences in failure-free survival (FFS). Interestingly, allogeneic SCT was associated with increased death on multivariate analysis, which persisted on propensity scoring (exact matching).

6 Novel Therapeutic Agents

There are multiple new and exciting targeted therapeutic agents that are being evaluated for the treatment of patients with FL. Furthermore, an improved understanding of the biology of FL has led to the development of novel targeted therapeutic agents for the treatment of this disease (Table 5). This is critically important given the significant relapse risk and toward the hope of avoiding the toxicity associated with repeated cytotoxic therapy.

6.1 Monoclonal Antibodies

Some malignant B cells are resistant to rituximab straightaway, whereas others develop resistance over time after exposure to the antibody. New targeted antibodies are being developed to potentially overcome resistance mechanisms. Obinutuzumab (Gazyva, Genentech) is a novel anti-CD20 that is the first humanized and "glyco-engineered" anti-CD20 to be investigated clinically. "Glyco-engineering" enhances antibody-dependent cellular cytotoxicity (ADCC) by increasing affinity to the ADCC receptor FCgRIIIA. Obinutuzumab binds a type II epitope on CD20 with high affinity and has greater direct cell cytotoxicity compared with type I antibodies. Complement-dependent cytotoxicity, however, is reduced. Toxicities mostly are related to the infusion, and response rates have been high among patients with FL. Single-agent obinutuzumab has shown efficacy in relapsed/refractory indolent NHL with an overall response rate of 55 % in a phase I/II study with a median progression-free survival of 11.9 months [61]. Obinituzumab has also been evaluated in combination with CHOP or FC followed by maintenance therapy. ORR was 93–96 % with evidence of a 25–30 % response in rituximab refractory patients [62]. Phase III studies are currently being conducted to confirm the efficacy of this agent in combination with chemotherapeutic agents.

Ofatumumab (Azerra, GlaxoSmithKline), a fully human antibody that targets a novel epitope of the CD20 molecule that was approved in October 2009 for the treatment of chronic lymphocytic leukemia (CLL), is reported to have stronger complement-dependent cytotoxicity than rituximab. Although this antibody is very

effective in CLL, the ORR in rituximab-resistant FL was only 10–13 % [63]. Trials are ongoing to evaluate combination therapy with bendamustine with or without ofatumumab.

Veltuzumab (Immunomedics) is a second-generation humanized anti-CD20 that differs from rituximab by one amino acid in one complementarity-determining region, but it has completely different framework regions. Among patients with FL, ORRs have been high (47 %), with 24 % achieving CR/CRu [64]. Other anti-CD20s under development include ocrelizumab (Roche), a humanized antibody with fewer infusion-related side effects and increased binding affinity to the low-affinity FcγRIIIa receptor. Some studies have shown that patients who are homozygous for high-affinity variants have superior disease outcome when treated with rituximab compared with those carrying low-affinity variants [65]. In a phase I/II trial of ocrelizumab in patients with relapsed/refractory FL, an ORR of 38 % was observed, with similar response rates in patients with low-affinity and high-affinity variants [66]. RO13192 (Genentech), a third-generation humanized anti-CD20 with enhanced ADCC and complement-dependent cytotoxicity in in vitro models, also is under investigation [67].

CD22 is a B cell-associated antigen expressed on the surface of mature B cells. The humanized anti-CD22 epratuzumab (Immunomedics) acts predominantly by ADCC, but some studies suggest that it acts, at least in part, by mechanisms different from rituximab. Whereas epratuzumab and rituximab target different antigens and likely effect cell kill through different signaling pathways, the combination has been studied in relapsed or refractory low-grade, CD20-positive NHL [68]. A high ORR (85 %) was reported for FL patients with low-risk FLIPI scores, whereas those with intermediate- or high-risk scores had an ORR of 39 %. This combination with extended dosing recently has been evaluated, and results are undergoing analysis. CD74, the invariant chain of the major histocompatibility complex class II molecule, is another attractive target for the treatment of FL. Milatuzumab (Immunomedics), a humanized anti-CD74 monoclonal antibody, is rapidly internalized and consequently has very limited capacity for ADCC or CDC, but it may prove to be an ideal agent for conjugation with radioisotopes or cytotoxic agents [63]. A fully human agonistic monoclonal antibody to the tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1), mapatumumab, has also shown activity in FL [69].

6.2 Antibody Drug Conjugates

Similar to the anti-leukemia agent gemtuzumab ozogamicin (Mylotarg, Wyeth), inotuzumab ozogamicin (CMC-544; Pfizer) is composed of a humanized antibody conjugated to calicheamicin, a potent cytotoxic antitumor agent. This chemoimmunoconjugate targets the B-cell antigen CD22. Responses have been seen in both FL and diffuse large B-cell lymphoma [63]. Toxicity primarily has been self-limited thrombocytopenia. FL treated with inotuzumab ozogamicin in combination with

rituximab in a phase I/II found an ORR of 87 %, with a median PFS of 68 % [70]. A new immunoconjugate, SAR3419 (ImmunoGen), consisting of the humanized anti-CD19 antibody huB4 conjugated to a potent tubulin inhibitor, has shown tumor shrinkage in 68 % of patients studied [69]. Corneal changes resulting in blurred vision proved to be the dose-limiting toxicity.

6.3 PI3K Inhibitors

Phosphatidylinositol-3-kinase delta (PI3Kδ) mediates B-cell receptor signaling and microenvironmental support signals that promote the growth and survival of malignant B lymphocytes. In a phase 1 study, idelalisib, an orally active selective PI3Kδ inhibitor, showed significant antitumor activity in patients with previously treated indolent NHLs, including 38 FL patients [71]. A pivotal phase II study studied the efficacy of idelalisib in 72 refractory FL patients; this study confirmed the clinical activity of this novel agent with a reported ORR of 57 % and associated median PFS of 11 months [72]. The most common adverse events of grade 3 or higher are neutropenia, transaminitis, diarrhea, and pneumonia. On July 23, 2014, the FDA approved idelalisib for the treatment of relapsed FL, relapsed SLL, and relapsed CLL patients (latter in combination with rituximab). Further, four black box warnings were included as part of this approval (i.e., hepatotoxicity, severe diarrhea or colitis, pneumonitis, and intestinal perforation). The recommended starting dose is 150 mg administered orally twice daily.

6.4 Proteasome Inhibitors

The ubiquitin–proteasome pathway plays a critical role in regulating cell cycle progression, transcription-factor activation, apoptosis, and cell trafficking. Bortezomib in combination with bendamustine and rituximab has shown activity in phase II studies of relapsed or refractory lymphoma, with ORR ranging of 83–86 % [73, 74]. Neutropenia and thrombocytopenia are the dose-limiting toxicities with grade 3/4 neuropathy seen in 11 % patients in the above trial.

Bortezomib showed encouraging activity in relapsed or refractory indolent NHL [68, 69]. O'Connor and colleagues reported an ORR of 50 % (22 % CR) using bortezomib 1.5 mg/m^2 on days 1, 4, 8, and 11 [69] whereas Di Bella et al. showed an ORR of 13 % using bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 [68]. The discrepancy between these trials is not known, but may in part be related to dosing as well as length of bortezomib therapy. O'Connor et al. showed that the median time to response was 12 weeks [69]. A large randomized phase III study in patients with relapsed/refractory FL compared rituximab alone versus rituximab and bortezomib [75]. The bortezomib/rituximab arm was associated with statistically significant longer PFS; however, the absolute clinical improvement was modest. There were two subsequent correlative analyses (clinical and scientific) performed as part of this study.

Zinzani et al. [76] showed that the benefit of bortezomib may primarily be in FL patients with higher risk disease. In relapsed/refractory FL patients with both FLIPI score ≥ 3 and high tumor burden (by modified GELF criteria), the median PFS was 9.5 months in the bortezomib-rituximab arm versus 6.7 months in the rituximab arm (HR 0.667, $p = 0.01$). Similar benefits were seen when analyzing high FLIPI score or GELF tumor burden cohorts separately. Additionally, correlative tumor and host studies identified several strongly predictive biomarkers in this study [75]. Patients who had a particular host single nucleotide polymorphism genotype (i.e., PSMB1 P11A C/G heterozygote) in combination with low CD68 tissue expression had significantly improved PFS (HR 0.47, $P < 0.0001$) and moreover superior OS (HR 0.49, $P = 0.04$). Further prospective examination and validation of these clinical and scientific biomarkers are needed.

6.5 Immunomodulatory Drugs

Immunomodulatory drugs (IMiDs) such as thalidomide (Thalomid, Celgene) and lenalidomide (Revlimid, Celgene) have been investigated in a number of lymphoma subtypes. IMiDs directly induce cell cycle arrest and also possess potent anti-angiogenic activity in vitro. Elegant preclinical studies by Gribben et al. showed that lenalidomide repaired several T-cell defects in FL cells and ultimately significantly enhanced the dysfunctional immune synapse function between tumor and host T cells [77]. Clinically, Wang et al. [73] reported an ORR of 33 % with lenalidomide and rituximab in relapsed/refractory follicular grade 3 lymphoma. Furthermore, Fowler et al. [78] reported encouraging data using lenalidomide combined with rituximab for the treatment of untreated FL patients. In 110 untreated FL patients (52 % with high tumor burden), patients received lenalidomide 20 mg Days 1–21 of 28-day cycles along with rituximab. The ORR on intent-to-treat analysis was 85 % with a CR rate of 60 %; response did not appear to differ based on presenting tumor burden and the 3-year PFS rate for all patients was 78 %. Therapy was overall well tolerated with main toxicities being hematologic.

Both bortezomib and lenalidomide are being incorporated earlier in the treatment paradigm of FL studies. Evens et al. [79] recently reported results of a multicenter phase II clinical study using frontline bortezomib and rituximab therapy for indolent NHL patients with high tumor burden. Among 42 patients, 33 had FL; therapy was overall well tolerated including minimal neurotoxicity. On intent to treat, the ORR at end of therapy for FL patients was ORR 76 % with 44 % CR rate. Further, with median follow-up of 50 months, the 4-year PFS was 44 % and OS 97 %, which approximates long-term survival rates of several rituximab/cytotoxic chemotherapy series for untreated HTB FL. There is an ongoing large randomized phase II study being conducted by ECOG comparing the addition of bortezomib to BR induction and also analyzing the use of lenalidomide consolidation (×1 year) following BR induction therapy (E2408 or BIONIC study: bortezomib-based Induction Or novel IMID-based continuation; NCT01216683) (Fig. 4). In addition,

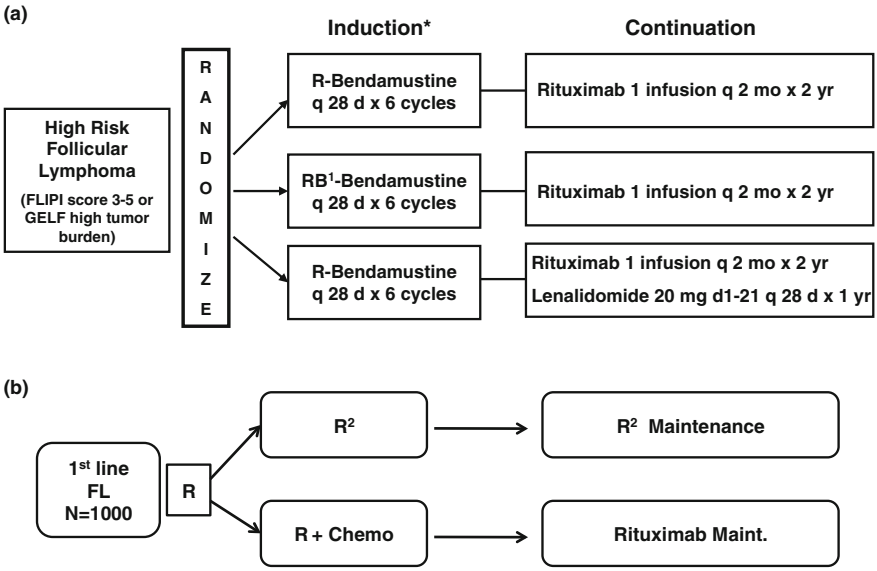


Fig. 4 Study schemas of two ongoing frontline FL clinical trials. **a** Shows the study schema for the E2408 Bortezomib Induction or Novel Imid Continuation (BIONIC) study for patients with untreated high-risk FL. Accrual goal for this study is 300 patients (*1:2:2 randomization vertically top to bottom; [1]bortezomib (1.3 mg/m^2 days 1, 4, 8, and 11). **b** Shows the study schema for the Rituximab Lenalidomide versus any Chemotherapy (RELEVANCE) study for patients with untreated FL. Accrual goal is 1,000 patients. The rituximab and chemotherapy is investigators choice among R-CHOP, R-CVP, or bendamustine/rituximab. Lenalidomide dosing during induction therapy is 20 mg for 6 cycles and then 10 mg for maintenance therapy. Abbreviations: FLIPI, follicular lymphoma international prognostic index; GELF, Groupe D'Etude des Lymphomes Folliculaires; R, rituximab; RB, rituximab/bortezomib; d, days; mo, months, year, years; FL, follicular lymphoma; R(2), rituximab and revlimid; maint, maintenance

an ongoing worldwide, randomized phase III study is comparing a non-chemotherapy induction strategy using lenalidomide and rituximab versus rituximab-chemotherapy induction (RELEVANCE study: Rituximab lenalidomide vs. any chemotherapy, NCT01650701) (Fig. 4).

6.6 Other Novel Agents

Other novel agents being studied in FL include histone deacetylase (HDAC) inhibitors, mammalian target of rapamycin (mTOR) inhibitors, and B-cell receptor (BCR)-modulating agents. HDACs have been shown to regulate cellular functions, such as cell cycle progression, proliferation, survival, transcription factors, and signal transduction, and are a target of interest in lymphoma [77]. Preclinical data support the use of these agents in the treatment of lymphoma, and data from early phase clinical trials have shown encouraging activity in FL [80, 81]. A downstream

target of the PI3K/AKT pathway is the kinase, mTOR. The mTOR pathway also is important for cellular functions such as initiation of transplant, protein stability, and transcription of ribosome and stress response genes. Smith et al. showed that the rapamycin ester derivative, temsirolimus (Torisel, Wyeth) resulted in an ORR of 54 % in patients with FL histology [82]. Finally, immune checkpoints in the tumor microenvironment are important in tumor growth. Westin et al. reported encouraging results on pidilizumab, a humanized anti-programmed cell death 1 (PD1) monoclonal antibody [83]. In combination with rituximab, the ORR was 66 % with an associated CR rate of 52 %. Other classes of novel targeted agents being studied in relapsed/refractory FL as single agents or in combination with other therapeutics include HDAC inhibitors, mTOR inhibitors, Syk inhibitors, Bcl-2 antagonists, BTK inhibitors, and chimeric antigen receptor (CAR) T cells (Table 5).

7 Conclusion

The treatment of FL has been significantly enhanced over the past several years, in part due to the development of antibody-based and other innovative, targeted approaches as well as through the refinement of SCT. These therapies have translated into improvements in the natural history of FL and increases in OS. Despite this optimism, FL remains an incurable disease for most patients. It is important for oncologists and patients to be involved in clinical trials in order to facilitate advances in the field so that patient outcomes can continue to be improved. In addition, it will be critical for future studies that incorporate novel therapeutic agents into treatment paradigms to investigate tissue and host-based predictive biomarker as well as imaging studies so that response and survival rates may be maximally enriched.

References

1. Chan WC, Armitage JO, Gascoyne R et al (1997) A clinical evaluation of the international lymphoma study group classification of non-Hodgkin's lymphoma. The non-Hodgkin's lymphoma classification project. Blood 89(11):3909–3918
2. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS (2006) Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. Blood 107(1):265–276
3. Link BK, Maurer MJ, Nowakowski GS et al (2013) Rates and outcomes of follicular lymphoma transformation in the immunochemotherapy era: a report from the University of Iowa/MayoClinic specialized program of research excellence molecular epidemiology resource. J Clin Oncol 31(26):3272–3278
4. Tan D, Horning SJ, Hoppe RT et al (2013) Improvements in observed and relative survival in follicular grade 1-2 lymphoma during 4 decades: the Stanford University experience. Blood 122(6):981–987
5. Nabhan C, Aschebrook-Kilfoy B, Chiu BC, Kruczek K, Smith SM, Evens AM (2014) The impact of race, age, and sex in follicular lymphoma: a comprehensive SEER analysis across consecutive treatment eras. Am J Hematol 89(6):633–638

6. Federico M, Luminari S, Dondi A et al (2013) R-CVP versus R-CHOP versus R-FM for the initial treatment of patients with advanced-stage follicular lymphoma: results of the FOLL05 trial conducted by the Fondazione Italiana Linfomi. J Clin Oncol 31(12):1506–1513
7. Brice P, Bastion Y, Lepage E et al (1997) Comparison in low-tumor-burden follicular lymphomas between an initial no-treatment policy, prednimustine, or interferon alfa: a randomized study from the Groupe d'Etude des Lymphomes Folliculaires. Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol 15(3):1110–1117
8. Solal-Celigny P, Lepage E, Brousse N et al (1998) Doxorubicin-containing regimen with or without interferon alfa-2b for advanced follicular lymphomas: final analysis of survival and toxicity in the Groupe d'Etude des Lymphomes Folliculaires 86 Trial. J Clin Oncol 16 (7):2332–2338
9. Ardeshna KM, Qian W, Smith P et al (2014) Rituximab versus a watch-and-wait approach in patients with advanced-stage, asymptomatic, non-bulky follicular lymphoma: an open-label randomised phase 3 trial. Lancet Oncol 15(4):424–435
10. Ardeshna KM, Smith P, Norton A et al (2003) Long-term effect of a watch and wait policy versus immediate systemic treatment for asymptomatic advanced-stage non-Hodgkin lymphoma: a randomised controlled trial. Lancet 362(9383):516–522
11. Buske C, Hoster E, Dreyling M, Hasford J, Unterhalt M, Hiddemann W (2006) The follicular lymphoma international prognostic index (FLIPI) separates high-risk from intermediate- or low-risk patients with advanced-stage follicular lymphoma treated front-line with rituximab and the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) with respect to treatment outcome. Blood 108(5):1504–1508
12. Colombat P, Salles G, Brousse N et al (2001) Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: clinical and molecular evaluation. Blood 97(1):101–106
13. Hiddemann W, Kneba M, Dreyling M et al (2005) Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 106(12):3725–3732
14. Romaguera JE, McLaughlin P, North L et al (1991) Multivariate analysis of prognostic factors in stage IV follicular low-grade lymphoma: a risk model. J Clin Oncol 9(5):762–769
15. Salles G, Mounier N, de Guibert S et al (2008) Rituximab combined with chemotherapy and interferon in follicular lymphoma patients: results of the GELA-GOELAMS FL2000 study. Blood 112(13):4824–4831
16. Salles G, Seymour JF, Offner F et al (2011) Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet 377(9759):42–51
17. Herold M, Haas A, Srock S et al (2007) Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: an East German Study Group Hematology and Oncology Study. J Clin Oncol 25(15):1986–1992
18. Kahl BS (2006) Eastern Cooperative Oncology Group 4402: rituximab extended schedule or retreatment trial (RESORT). Clin Lymphoma Myeloma. 6(5):423–426
19. Solal-Celigny P, Roy P, Colombat P et al (2004) Follicular lymphoma international prognostic index. Blood 104(5):1258–1265
20. Plancarte F, Lopez-Guillermo A, Arenillas L et al (2006) Follicular lymphoma in early stages: high risk of relapse and usefulness of the follicular lymphoma international prognostic index to predict the outcome of patients. Eur J Haematol 76(1):58–63
21. Montoto S, Lopez-Guillermo A, Altes A et al (2004) Predictive value of follicular lymphoma international prognostic index (FLIPI) in patients with follicular lymphoma at first progression. Ann Oncol 15(10):1484–1489

22. Friedberg JW, Taylor MD, Cerhan JR et al (2009) Follicular lymphoma in the United States: first report of the national LymphoCare study. J Clin Oncol 27(8):1202–1208
23. Mac Manus MP, Hoppe RT (1996) Is radiotherapy curative for stage I and II low-grade follicular lymphoma? Results of a long-term follow-up study of patients treated at Stanford University. J Clin Oncol 14(4):1282–1290
24. Tsang RW, Gospodarowicz MK (2005) Radiation therapy for localized low-grade non-Hodgkin's lymphomas. Hematol Oncol 23(1):10–17
25. Seymour JF, Pro B, Fuller LM et al (2003) Long-term follow-up of a prospective study of combined modality therapy for stage I-II indolent non-Hodgkin's lymphoma. J Clin Oncol 21 (11):2115–2122
26. Advani R, Rosenberg SA, Horning SJ (2004) Stage I and II follicular non-Hodgkin's lymphoma: long-term follow-up of no initial therapy. J Clin Oncol 22(8):1454–1459
27. Solal-Celigny P, Bellei M, Marcheselli L et al (2012) Watchful waiting in low-tumor burden follicular lymphoma in the rituximab era: results of an F2-study database. J Clin Oncol 30 (31):3848–3853
28. Li ZM, Ghielmini M, Moccia AA (2013) Managing newly diagnosed follicular lymphoma: state of the art and future perspectives. Expert Rev Anticancer Ther 13(3):313–325
29. Forstpointner R, Dreyling M, Repp R et al (2004) The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 104(10):3064–3071
30. Foussard C, Colombat P, Maisonneuve H et al (2005) Long-term follow-up of a randomized trial of fludarabine-mitoxantrone, compared with cyclophosphamide, doxorubicin, vindesine, prednisone (CHVP), as first-line treatment of elderly patients with advanced, low-grade non-Hodgkin's lymphoma before the era of monoclonal antibodies. Ann Oncol 16(3):466–472
31. Hochster H, Weller E, Gascoyne RD et al (2009) Maintenance rituximab after cyclophosphamide, vincristine, and prednisone prolongs progression-free survival in advanced indolent lymphoma: results of the randomized phase III ECOG1496 study. J Clin Oncol 27(10):1607–1614
32. Marcus R, Imrie K, Belch A et al (2005) CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. Blood 105(4):1417–1423
33. Rummel MJ, Al-Batran SE, Kim SZ et al (2005) Bendamustine plus rituximab is effective and has a favorable toxicity profile in the treatment of mantle cell and low-grade non-Hodgkin's lymphoma. J Clin Oncol 23(15):3383–3389
34. Rummel MJ, Niederle N, Maschmeyer G et al (2013) Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet 381 (9873):1203–1210
35. Flinn IW, van der Jagt R, Kahl BS et al (2014) Randomized trial of bendamustine-rituximab or R-CHOP/R-CVP in first-line treatment of indolent NHL or MCL: the BRIGHT study. Blood 123(19):2944–2952
36. Baum E, Sather H, Nachman J et al (1979) Relapse rates following cessation of chemotherapy during complete remission of acute lymphocytic leukemia. Med Pediatr Oncol 7(1):25–34
37. Jacobson CA, Freedman AS (2013) One size does not fit all in follicular lymphoma. J Clin Oncol 31(27):3307–3308
38. Kaminski MS, Tuck M, Estes J et al (2005) 131I-tositumomab therapy as initial treatment for follicular lymphoma. N Engl J Med 352(5):441–449
39. Oliansky DM, Gordon LI, King J et al (2010) The role of cytotoxic therapy with hematopoietic stem cell transplantation in the treatment of follicular lymphoma: an evidence-based review. Biol Blood Marrow Transplant 16(4):443–468

40. Schaaf M, Reiser M, Borchmann P, Engert A, Skoetz N (2012) High-dose therapy with autologous stem cell transplantation versus chemotherapy or immuno-chemotherapy for follicular lymphoma in adults. Cochrane Database Syst Rev 1:CD007678
41. Cheson BD, Leonard JP (2008) Monoclonal antibody therapy for B-cell non-Hodgkin's lymphoma. N Engl J Med 359(6):613–626
42. Davis TA, Grillo-Lopez AJ, White CA et al (2000) Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: safety and efficacy of re-treatment. J Clin Oncol 18(17):3135–3143
43. Friedberg JW, Cohen P, Chen L et al (2008) Bendamustine in patients with rituximab-refractory indolent and transformed non-Hodgkin's lymphoma: results from a phase II multicenter, single-agent study. J Clin Oncol 26(2):204–210
44. Sacchi S, Pozzi S, Marcheselli R et al (2007) Rituximab in combination with fludarabine and cyclophosphamide in the treatment of patients with recurrent follicular lymphoma. Cancer 110 (1):121–128
45. Tam CS, Wolf M, Prince HM et al (2006) Fludarabine, cyclophosphamide, and rituximab for the treatment of patients with chronic lymphocytic leukemia or indolent non-Hodgkin lymphoma. Cancer 106(11):2412–2420
46. Al-Tourah AJ, Gill KK, Chhanabhai M et al (2008) Population-based analysis of incidence and outcome of transformed non-Hodgkin's lymphoma. J Clin Oncol 26(32):5165–5169
47. Carney DA, Westerman DA, Tam CS et al (2010) Therapy-related myelodysplastic syndrome and acute myeloid leukemia following fludarabine combination chemotherapy. Leukemia 24 (12):2056–2062
48. Waterman J, Rybicki L, Bolwell B et al (2012) Fludarabine as a risk factor for poor stem cell harvest, treatment-related MDS and AML in follicular lymphoma patients after autologous hematopoietic cell transplantation. Bone Marrow Transplant 47(4):488–493
49. Janikova A, Koristek Z, Vinklarkova J et al (2009) Efficacious but insidious: a retrospective analysis of fludarabine-induced myelotoxicity using long-term culture-initiating cells in 100 follicular lymphoma patients. Exp Hematol 37(11):1266–1273
50. Lanza F, Lemoli RM, Olivieri A et al (2014) Factors affecting successful mobilization with plerixafor: an Italian prospective survey in 215 patients with multiple myeloma and lymphoma. Transfusion. 54(2):331–339
51. Wiseman GA, Gordon LI, Multani PS et al (2002) Ibritumomab tiuxetan radioimmunotherapy for patients with relapsed or refractory non-Hodgkin lymphoma and mild thrombocytopenia: a phase II multicenter trial. Blood 99(12):4336–4342
52. Fisher RI, Kaminski MS, Wahl RL et al (2005) Tositumomab and iodine-131 tositumomab produces durable complete remissions in a subset of heavily pretreated patients with low-grade and transformed non-Hodgkin's lymphomas. J Clin Oncol 23(30):7565–7573
53. Derenzini E, Stefoni V, Maglie R et al (2013) Collection of hematopoietic stem cells after previous radioimmunotherapy is feasible and does not impair engraftment after autologous stem cell transplantation in follicular lymphoma. Biol Blood Marrow Transplant 19(12):1695–1701
54. Czuczman MS, Emmanouilides C, Darif M et al (2007) Treatment-related myelodysplastic syndrome and acute myelogenous leukemia in patients treated with ibritumomab tiuxetan radioimmunotherapy. J Clin Oncol 25(27):4285–4292
55. Chan EK, Fung S, Gospodarowicz M et al (2011) Palliation by low-dose local radiation therapy for indolent non-Hodgkin lymphoma. Int J Radiat Oncol Biol Phys 81(5):e781–786
56. Montoto S, Corradini P, Dreyling M et al (2013) Indications for hematopoietic stem cell transplantation in patients with follicular lymphoma: a consensus project of the EBMT-lymphoma working party. Haematologica 98(7):1014–1021
57. Oliansky DM, Gordon LI, King J et al (2010) The role of cytotoxic therapy with hematopoietic stem cell transplantation in the treatment of follicular lymphoma: an evidence-based review. Biol Blood Marrow Transplant 16(4):443–468

58. Rohatiner AZ, Nadler L, Davies AJ et al (2007) Myeloablative therapy with autologous bone marrow transplantation for follicular lymphoma at the time of second or subsequent remission: long-term follow-up. J Clin Oncol 25(18):2554–2559
59. Evens AM, Vanderplas A, LaCasce AS et al (2013) Stem cell transplantation for follicular lymphoma relapsed/refractory after prior rituximab: a comprehensive analysis from the NCCN lymphoma outcomes project. Cancer 119(20):3662–3671
60. Khouri IF, McLaughlin P, Saliba RM et al (2008) Eight-year experience with allogeneic stem cell transplantation for relapsed follicular lymphoma after nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab. Blood 111(12):5530–5536
61. Morschhauser FA, Cartron G, Thieblemont C et al (2013) Obinutuzumab (GA101) monotherapy in relapsed/refractory diffuse large b-cell lymphoma or mantle-cell lymphoma: results from the phase II GAUGUIN study. J Clin Oncol 31(23):2912–2919
62. Radford J, Davies A, Cartron G, et al (2013) Obinutuzumab (GA101) plus CHOP or FC in relapsed/refractory follicular lymphoma: results of the GAUDI study (BO21000). Blood 122 (7):1137–1143
63. Advani A, Coiffier B, Czuczman MS et al (2010) Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: results of a phase I study. J Clin Oncol 28(12):2085–2093
64. Negrea GO, Elstrom R, Allen SL et al (2011) Subcutaneous injections of low-dose veltuzumab (humanized anti-CD20 antibody) are safe and active in patients with indolent non-Hodgkin's lymphoma. Haematologica 96(4):567–573
65. Genovese MC, Kaine JL, Lowenstein MB et al (2008) Ocrelizumab, a humanized anti-CD20 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: a phase I/II randomized, blinded, placebo-controlled, dose-ranging study. Arthritis Rheum 58(9):2652–2661
66. Morschhauser F, Marlton P, Vitolo U et al (2010) Results of a phase I/II study of ocrelizumab, a fully humanized anti-CD20 mAb, in patients with relapsed/refractory follicular lymphoma. Ann Oncol 21(9):1870–1876
67. Casulo C, Vose JM, Ho WY et al (2014) A phase I study of PRO131921, a novel anti-CD20 monoclonal antibody in patients with relapsed/refractory CD20 + indolent NHL: correlation between clinical responses and AUC pharmacokinetics. Clin Immunol 154(1):37–46
68. Leonard JP, Schuster SJ, Emmanouilides C et al (2008) Durable complete responses from therapy with combined epratuzumab and rituximab: final results from an international multicenter, phase 2 study in recurrent, indolent, non-Hodgkin lymphoma. Cancer 113 (10):2714–2723
69. Younes A, Kim S, Romaguera J et al (2012) Phase I multidose-escalation study of the anti-CD19 maytansinoid immunoconjugate SAR3419 administered by intravenous infusion every 3 weeks to patients with relapsed/refractory B-cell lymphoma. J Clin Oncol 30(22):2776–2782
70. Fayad L, Offner F, Smith MR et al (2013) Safety and clinical activity of a combination therapy comprising two antibody-based targeting agents for the treatment of non-Hodgkin lymphoma: results of a phase I/II study evaluating the immunoconjugate inotuzumab ozogamicin with rituximab. J Clin Oncol 31(5):573–583
71. Flinn IW, Kahl BS, Leonard JP et al (2014) Idelalisib, a selective inhibitor of phosphatidylinositol 3-kinase-delta, as therapy for previously treated indolent non-Hodgkin lymphoma. Blood 123(22):3406–3413
72. Gopal AK, Kahl BS, de Vos S et al (2014) PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med 370(11):1008-1018
73. Wang M, Fowler N, Wagner-Bartak N et al (2013) Oral lenalidomide with rituximab in relapsed or refractory diffuse large cell, follicular and transformed lymphoma: a phase II clinical trial. Leukemia 27(9):1902–1909
74. Friedberg JW, Vose JM, Kelly JL et al (2011) The combination of bendamustine, bortezomib, and rituximab for patients with relapsed/refractory indolent and mantle cell non-Hodgkin lymphoma. Blood 117(10):2807–2812

75. Coiffier B, Li W, Henitz ED et al (2013) Prespecified candidate biomarkers identify follicular lymphoma patients who achieved longer progression-free survival with bortezomib-rituximab versus rituximab. Clin Cancer Res 19(9):2551–2561
76. Zinzani PL, Khuageva NK, Wang H et al (2012) Bortezomib plus rituximab versus rituximab in patients with high-risk, relapsed, rituximab-naive or rituximab-sensitive follicular lymphoma: subgroup analysis of a randomized phase 3 trial. J Hematol Oncol 5:67
77. Ramsay AG, Clear AJ, Kelly G et al (2009) Follicular lymphoma cells induce T-cell immunologic synapse dysfunction that can be repaired with lenalidomide: implications for the tumor microenvironment and immunotherapy. Blood 114(21):4713–4720
78. Fowler NH, Davis RE, Rawal S et al (2014) Safety and activity of lenalidomide and rituximab in untreated indolent lymphoma: an open-label, phase 2 trial. Lancet Oncol 15(12):1311–1318
79. Evens AM, Smith MR, Lossos IS et al (2014) Frontline bortezomib and rituximab for the treatment of newly diagnosed high tumour burden indolent non-Hodgkin lymphoma: a multicentre phase II study. Br J Haematol 166(4):514–520
80. Witzig TE, Wiernik PH, Moore T et al (2009) Lenalidomide oral monotherapy produces durable responses in relapsed or refractory indolent non-Hodgkin's lymphoma. J Clin Oncol 27(32):5404–5409
81. Kirschbaum M, Frankel P, Popplewell L et al (2011) Phase II study of vorinostat for treatment of relapsed or refractory indolent non-Hodgkin's lymphoma and mantle cell lymphoma. J Clin Oncol 29(9):1198–1203
82. Smith SM, van Besien K, Karrison T et al (2010) Temsirolimus has activity in non-mantle cell non-Hodgkin's lymphoma subtypes: the University of Chicago phase II consortium. J Clin Oncol 28(31):4740–4746
83. Westin JR, Chu F, Zhang M et al (2014) Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial. Lancet Oncol 15(1):69–77
84. van Oers MH, Klasa R, Marcus RE et al (2006) Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: results of a prospective randomized phase 3 intergroup trial. Blood 108(10):3295–3301
85. Horning SJ, Negrin RS, Hoppe RT et al (2001) High-dose therapy and autologous bone marrow transplantation for follicular lymphoma in first complete or partial remission: results of a phase II clinical trial. Blood 97(2):404–409
86. Lenz G, Dreyling M, Schiegnitz E et al (2004) Myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission prolongs progression-free survival in follicular lymphoma: results of a prospective, randomized trial of the German Low-Grade Lymphoma Study Group. Blood 104(9):2667–2674
87. Lenz G, Dreyling M, Schiegnitz E et al (2004) Moderate increase of secondary hematologic malignancies after myeloablative radiochemotherapy and autologous stem-cell transplantation in patients with indolent lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group. J Clin Oncol 22(24):4926–4933
88. Sebban C, Mounier N, Brousse N et al (2006) Standard chemotherapy with interferon compared with CHOP followed by high-dose therapy with autologous stem cell transplantation in untreated patients with advanced follicular lymphoma: the GELF-94 randomized study from the Groupe d'Etude des Lymphomes de l'Adulte (GELA). Blood 108 (8):2540–2544
89. Ladetto M, De Marco F, Benedetti F et al (2008) Prospective, multicenter randomized GITMO/IIL trial comparing intensive (R-HDS) versus conventional (CHOP-R) chemoimmunotherapy in high-risk follicular lymphoma at diagnosis: the superior disease control of R-HDS does not translate into an overall survival advantage. Blood 111(8):4004–4013

90. Gyan E, Foussard C, Bertrand P et al (2009) High-dose therapy followed by autologous purged stem cell transplantation and doxorubicin-based chemotherapy in patients with advanced follicular lymphoma: a randomized multicenter study by the GOELAMS with final results after a median follow-up of 9 years. Blood 113(5):995–1001
91. Federico M et al (2009) Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol 27:1–9

Management of the Marginal Zone Lymphomas

Barbara Vannata, Anastasios Stathis and Emanuele Zucca

Abstract

Marginal zone lymphomas (MZL) represent around 8 % of all non-Hodgkin lymphomas. During the last decades a number of studies have addressed the mechanisms underlying the disease development. Extranodal MZL lymphoma usually arises in mucosal sites where lymphocytes are not normally present from a background of either autoimmune processes, such as Hashimoto thyroiditis or Sjögren syndrome or chronic infectious conditions. In the context of a persistent antigenic stimulation, successive genetic abnormalities can progressively hit a B-cell clone among the reactive B-cells of the chronic inflammatory tissue and give rise to a MALT lymphoma. The best evidence of an etiopathogenetic link is available for the association between Helicobacter pylori-positive gastritis and gastric MALT lymphoma. Indeed, a successful eradication of this micro-organism with antibiotics can be followed by gastric MALT lymphoma regression in more than 2/3 of cases. Other microbial agents have been implicated in the pathogenesis of MZL arising in the skin (Borrelia burgdorferi), in the ocular adnexa (Chlamydophila psittaci), and in the small intestine (Campylobacter jejuni). The prevalence of hepatitis C virus (HCV) has also been reported higher in MZL patients (particularly of the splenic type) than in the control population, suggesting a possible causative role of the virus. In non-gastric MALT lymphoma and in splenic MZL the role of the antimicrobial therapy is, however, less clear.This review summarizes the recent advances in Marginal Zone Lymphomas, addressing the critical points in their diagnosis, staging and clinical management.

B. Vannata · A. Stathis · E. Zucca (✉)
Lymphoma Unit, Oncology Institute of Southern Switzerland,
Ospedale San Giovanni, 6500 Bellinzona, Switzerland
e-mail: ielsg@ticino.com

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_9

Keywords
Marginal zone B-cell lymphoma · Mucosa-Associated Lymphoid Tissue · Extranodal lymphomas

Contents

1 Definition and Classification

Marginal zone lymphomas (MZL) represent a group of lymphomas that originate from B lymphocytes normally present in the "marginal zone," which is the external part of the secondary lymphoid follicles. MZLs develop in spleen, mucosa-associated lymphoid tissue (MALT), and/or in lymph nodes. In the most recent WHO classification, the MZL category comprises three different subtypes with specific diagnostic criteria, different behavior, and therapeutic implications: the extranodal MZL of mucosa-associated lymphoid tissue type (MALT lymphoma), the splenic MZL with or without villous lymphocytes (SMZL), and nodal MZL (NMZL) [1].

2 Epidemiology and Pathogenesis

Primary splenic and nodal MZLs are rare, each accounting for approximately less than 2 % of the non-Hodgkin lymphomas (NHLs). The extranodal MZLs of MALT type represent around 7–8 % of the NHL, including both the common gastrointestinal and the less usual non-gastrointestinal localizations [1].

MALT lymphoma B cells present somatically mutated IGHV genes in all cases with a pattern of somatic hypermutation and intraclonal variations suggesting that the tumor cells have undergone antigen selection and that their expansion may remain antigen-driven [2, 3]. Indeed, MALT lymphoma usually arises in mucosal sites where lymphocytes are not normally present and where MALT is acquired in response to either autoimmune processes, such as Hashimoto thyroiditis, Sjögren syndrome, or chronic infectious conditions. It is believed that, in the context of a persistent antigenic stimulation, additional genetic abnormalities may progressively affect a B-cell clone among the reactive B cells of the chronic inflammatory tissue and give rise to a MALT lymphoma.

The stomach is the most common site of localization, and the remission of most gastric MALT lymphomas after eradication of *Helicobacter pylori* (HP) strongly indicates a pathogenetic link between tumor cell proliferation and chronic *H. pylori*-induced inflammation [2]. Moreover, other infectious agents may have a putative role in non-gastric MZL pathogenesis at different sites: *Borrelia burgdorferi* in cutaneous lymphomas [4], *Chlamydophila psittaci* in the lymphoma of the ocular adnexa [5–7], and *Campylobacter jejuni* in small intestine lymphoma [8]. The prevalence of hepatitis C virus (HCV) infection has also been reported higher in patients with MZLs (particularly of splenic and nodal type) than in the control population [9, 10], suggesting a possible causative role of the viral agent [11]. In extranodal MZL, HCV seems more often present in non-gastric lymphomas, particularly in patients with subcutaneous [12] or salivary gland [13] involvement.

At least four recurrent chromosomal aberrations—the apparently mutually exclusive translocations t(11; 18)(q21; q21), t(1;14)(p22; q32), t(14; 18)(q32; q21), and the 6q23.3 deletion—have been reported in MALT lymphomas to affect the same signaling pathway, resulting in the activation of nuclear factor kappa B (NF-κB), a transcription factor with a central role in immunity, inflammation, and apoptosis [14–20]. The occurrence of the translocations varies according to the anatomical site, and their incidence and distribution may also have geographical differences, possibly reflecting different genetic backgrounds of either the patients or the infectious agents [21].

These site-specific biological differences might influence outcome and therapeutic approaches. Indeed, while antibiotic therapy is nowadays well established as the standard of care in patients with *H. pylori*-associated gastric MALT lymphoma, much less is known about the value of anti-infectious therapy in non-gastric MALT lymphomas and in non-MALT cases, where the standard treatment is less well defined.

3 Diagnosis

The diagnosis of MZL should be made in accordance with the current WHO classification [1].

It is advisable that the diagnosis is confirmed by an expert hematopathologist since differentiation from other lymphomas that can mimic MZLs is not always straightforward. A minimum immunohistochemistry panel is therefore recommended and it should include CD20, CD10, CD5, and cyclin D1 [22]. The presence of lymphoepithelial lesions, despite being very typical of MALT lymphoma, is not essential for the diagnosis as they can be seen both in some reactive conditions and in other indolent lymphomas. For extranodal MZL, assessment of a potential-associated histologic transformation is essential and, since the term "high-grade MALT lymphomas" is no longer accepted in the current WHO classification, the cases with solid or sheet-like proliferation of transformed large cells have to be diagnosed as diffuse large B-cell lymphomas [23].

At present, the diagnosis of SMZL does not strictly require a splenectomy [24]. In fact, following the analysis of cases in which the diagnosis has been confirmed by review of splenic histology, characteristic features to allow a diagnosis based on bone marrow examination and peripheral blood flow cytometry have been established, [25, 26]. Cytoplasmic villi may not be present in all cases and not all cases with villous lymphocytes will necessarily correspond to a SMZL; sometimes a definitive diagnosis may not be possible without splenectomy (which may not be required as treatment) [24].

4 Clinical Characteristics

4.1 Extranodal MZL (MALT Lymphoma)

MALT lymphoma is a neoplasm of adults with a median age at presentation of 60 years and a slight predominance in females. This type of lymphoma usually remains localized for a prolonged period within the tissue of origin, but involvement of regional lymph nodes and dissemination to multiple sites may occur. The stomach is the most common location, but MALT lymphoma has been described in several non-gastric sites, such as salivary gland, thyroid, skin, conjunctiva, larynx, lung, breast, kidney, liver, prostate, and also intracranial dura. Within the stomach, the disease is usually multifocal and concomitant gastrointestinal (GI) or non-GI involvement can be detected in 20 % of the cases. Disseminated disease is more common in non-GI MALT lymphomas, in which it is reported in up to one-quarter of the cases [27–30]. Bone marrow infiltration is described in up to 20 % of the cases [2, 31], while constitutional B-symptoms are rarely seen at diagnosis. Patients with lymph node or bone marrow involvement at presentation, but not those with involvement of multiple mucosal sites, are associated with a worse prognosis [27].

4.2 Splenic MZL

Splenic MZL (SMZL) is a disseminated disease at diagnosis in around 95 % of the cases. SMZL comprise around 20 % of MZLs, and it has usually an indolent course with overall survival ranging from 5 to 10 years. In around one-third of the cases, median survival is less than 4 years [32]. Histological transformation to DLBCL is rare (10–20 % of the patients) and is associated with a worse outcome. SMZL mainly affects elderly or middle-aged patients, with a median age of 65 years. SMZL is characterized by massive splenomegaly with minimal or absent lymphadenopathy, other than in the splenic hilum, and no other extranodal involvement, except bone marrow and liver. Cytopenias and lymphocytosis are frequently observed. When circulating villous lymphocytes are prominent, the term "splenic lymphoma with villous lymphocyte" has often been used in the past. Several reports have shown an epidemiological association between hepatitis C chronic infection and splenic and nodal MZLs. The strength of the relation between HCV and lymphomas has been found to be highly variable among countries, although HCV seems involved in the lymphomagenesis at least in a portion of cases. The best evidence of a causal link between HCV and lymphomas came from the observation of SMZL regression after antiviral therapy, first reported in 2002 by Hermine et al. [9], who described nine patients with splenic marginal zone lymphoma who had a lymphoma remission following interferon-α and ribavirin treatment. In the same report, six patients with SMZL, but without HCV infection, had no hematologic response to the antiviral therapy, thus ruling out a direct antitumor effect of interferon-α. These results were reproduced by other groups [33], showing that antiviral treatment with interferon-α with or without ribavirin can be an effective treatment for the majority of HCV-associated marginal zone lymphomas.

Around one-third of patients present a small serum monoclonal protein, mainly of IgM subtype. Sometimes, in HCV cases, the presence of cryoglobulins is detected. Autoimmune phenomena are seen in 15 % of the cases and comprise autoimmune hemolytic anemia, immune thrombocytopenia, cold agglutinin, antiphospholipid antibodies, acquired von Willebrand disease, and angioedema due to acquired C1-esterase inhibitor deficiency [32].

An Italian lymphoma cooperative group (Intergruppo Italiano Linfomi, IIL) developed a score model in 309 patients based on three risk factors (Hb less than 12 g/dl; albumin less than 3.5 g/dl; and LDH greater than normal). Patients were divided into three risk group with a 5-year OS of 88, 73, and 50 % for low-intermediate- and high-risk group, respectively [34]. Recently, a newer prognostic model (named HPLL on the basis of determinant factors, hemoglobin concentration, platelet count, high lactate dehydrogenase level, and extrahilar lymphadenopathy) has been developed by the SMZL Study Group from an international retrospective survey of 593 patients [35]. The HPLL score allowed to identify three risk groups with significantly different 5-year lymphoma-specific survival (94, 78 and 69 %, respectively) and appeared to have a better discriminative power than the

IIL score. Despite the fact that their clinical utility has not yet been confirmed in prospective studies, these indices are expected to improve the selection of patients for risk-tailored treatment approaches.

4.3 Nodal MZL

This lymphoma is rather uncommon, it comprises 10 % of MZLs and occurs in adults with a median age of 60 years, but it has also been reported in children. NMZL shares morphologic and immunophenotypic similarities with the other MZLs, and its differential diagnosis from other indolent lymphoma, in particular from lymphoplasmacytic lymphoma, is often very difficult [36–40]. Mutations of the MYD88 gene have been recently reported to occur in the large majority of lymphoplasmacytic lymphomas, and it is not usually present in MZL, thus making this finding very useful for the differential diagnosis [41].

NMZL presents with disseminated lymphadenopathy (mostly cervical and abdominal), with or without bone marrow and blood involvement at diagnosis in the vast majority of the cases [38]. The disease is often advanced at diagnosis, but usually patients do not have B-symptoms [42, 43]. A primary extranodal marginal zone lymphoma has to be ruled out, since around one-third of the cases represent nodal dissemination of a MALT lymphoma. In 10 % of the patients, a small monoclonal component, mainly of IgM-type, is detected [38].

OS of patients with NMZL is around 60 % at 5 years in most studies [38]. In a retrospective series of 93 patients analyzed by the International Lymphoma Study Group for the Non-Hodgkin's Lymphoma Classification Project, the OS for NMZL has been reported to be lower than that of patients with MALT lymphoma (56 % vs. 81 %, at 5 years, respectively) [44].

5 Staging

Extensive staging assessment is indicated in all MZL types, regardless of their presentation site [24]. It should include a history and physical examination; complete blood cell counts and basic biochemical studies, including evaluation of renal and liver function; LDH and β2-microglobulin levels; serum protein immunofixation; human immunodeficiency virus (HIV); hepatitis B (HBV) and C virus (HCV) serologies; CT scan of the chest, abdomen, and pelvis; and bone marrow aspirate and biopsy. Site-specific recommended procedures for MALT lymphoma staging are reported in Table 1. Gastroduodenal endoscopy (EGD) with multiple biopsies is also recommended to exclude gastric involvement in all cases of disseminated MZL. Localized MALT lymphoma is often multifocal within the involved organ (i.e., stomach and skin); nevertheless, this may not reflect a truly disseminated disease.

Table 1 Site-specific work-up procedures for extranodal MZL of MALT type

Site	Procedure
Gastric MALT lymphoma	
Stomach	• Endoscopic ultrasound
	• *H. pylori* status (by IHC)[a]
	• FISH or molecular assay for the t(11;18) translocation
Non-gastric MALT lymphoma	
Breast	• Mammography and MRI (or CT scan)
Intestine, small	• Endoscopy
	• Small bowel series (double contrast X-ray examination of the small intestine)
	• *Campylobacter Jejuni* search in the tumor biopsy by PCR, IHC or in situ hybridization
Intestine, large	• Colonoscopy
Lung	• Bronchoscopy + Broncho-alveolar lavage
Ocular adnexa	• MRI (or CT scan)
	• Ophthalmologic examination
	• *Chlamydophila psittaci* in the tumor biopsy and PBMNCs by PCR
Salivary glands	• ENT examination and echography
Skin	• *Borrelia Burgdorferi* in the tumor biopsy by PCR
Thyroid	• Echography ± CT scan of the neck thyroid function tests

IHC immune histochemistry, *MRI* magnetic resonance imaging, *CT* compute tomography, *PBMNCs* peripheral blood mononuclear cells, *PCR* polymerase chain reaction, *ENT* ear nose throat

[a]Breath test and serology studies for HP detection are recommended when the results of histology are negative

The value of the positron emission tomography (PET) scan is still unclear. In general, the use of PET-CT scan in the routine staging of MZL is not recommended [22, 24], except for selected cases (i.e., when a transformation to high-grade lymphoma is suspected). However, there is some evidence that many non-gastric sites are usually PET-positive [31]. In a meta-analysis of the published literature, the pooled detection rate of 18F-FDG PET or PET/CT in MALT lymphoma was 71 % and appeared particularly high in the pulmonary (94 %) and head and neck (90 %) localizations, showing that this type of lymphoma can often be FDG-avid and suggesting a potential clinical role of PET/CT in the initial evaluation of these patients, especially when the disease is apparently localized and radiotherapy is planned [45].

6 Treatment

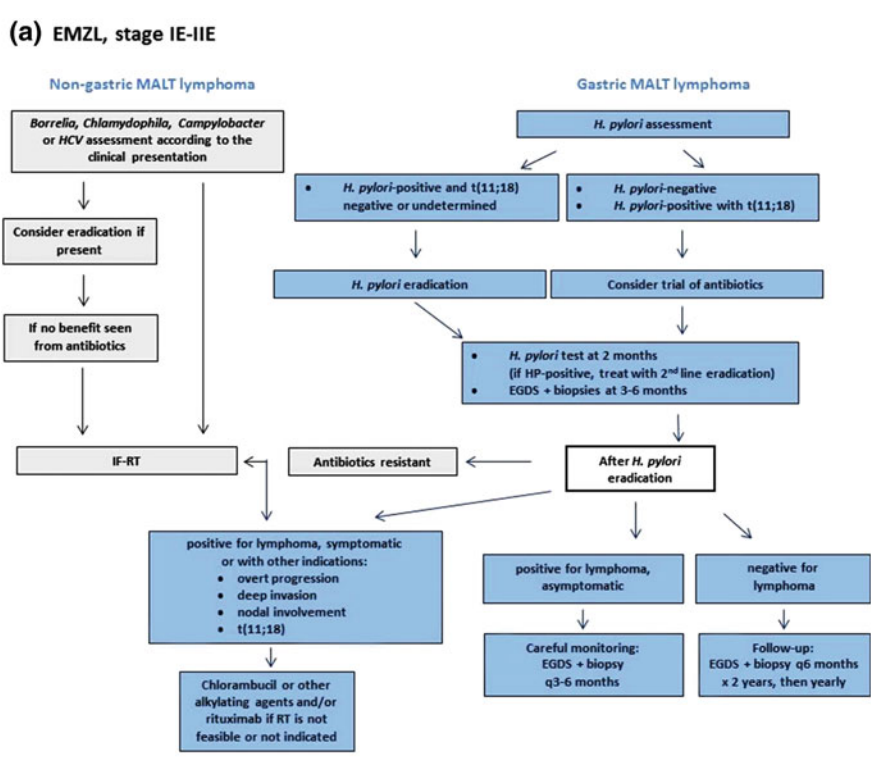
6.1 Extranodal MZL of MALT Type

The aforementioned etiologic association with some chronic infections has therapeutic implications in patients with MZL. Indeed, *H. pylori* eradication with antibiotics can lead to gastric MALT lymphoma regression in 60–100 % of the cases [46–51]. Anti-infectious treatments for non-gastric MZL are, however, largely investigational.

6.2 Gastric Marginal Zone Lymphoma of MALT Type, HP Positive

Figure 1 reports the algorithm of our treatment recommendation for limited and advanced-stage HP-positive gastric MALT lymphoma. Eradication of *H. pylori* with antibiotics should be the sole initial therapy for localized *H. pylori*-positive gastric MALT lymphoma, where this treatment can induce lymphoma regression and long-term clinical disease control in most patients [2]. Several effective anti-HP treatments are available. The choice should be based on the epidemiology of the infection and on the expected antibiotic resistance in different countries. The most commonly used regimen comprises a proton pump inhibitor (PPI) associated with clarithromycin and amoxicillin, administered for 10–14 days. Metronidazole can be used instead of amoxicillin for penicillin-allergic patients. Other regimens comprising H2-blockers or bismuth can be also effective. Breath test for *H. pylori* assessment should be repeated at least six weeks after the eradication therapy and at least two weeks after withdrawal of the PPI. In case of HP-eradication failure, a second-line therapy should be tried with a different triple- or a quadruple-therapy regimen [22] (Table 2). Histological remission is usually achieved within six months from *H. pylori* eradication. However, the length of time necessary to obtain a lymphoma regression ranges from very few months to more than 12 months. Hence, it is reasonable to wait for at least 12 months before starting another treatment in patients who achieve a clinical and endoscopic remission together with eradication of *H. pylori*, although histological residual lymphoma persists [2, 52]. The interpretation of the residual lymphoid infiltrate post-treatment in gastric biopsies can be very difficult. Differences in the response criteria adopted in the individual studies may explain the wide range of reported remission rates. Indeed, there are no uniform criteria for the definition of histological remission [2, 52]. Comparison with previous biopsies should be carried out to assess response, and we recommend the Group d'Etude des Lymphomes de l'Adult (GELA) scoring system as a reproducible method [53] (Table 3).

In fact, histological evaluation of gastric biopsies repeated on a fixed schedule is crucial for the follow-up in order to rule out either the disease persistence or the appearance of early epithelial changes, which may indicate an incoming gastric


(a) EMZL, stage IE-IIE
Non-gastric MALT lymphoma
Gastric MALT lymphoma
Borrelia, Chlamydophila, Campylobacter or HCV assessment according to the clinical presentation
H. pylori assessment
Consider eradication if present
H. pylori-positive and t(11;18) negative or undetermined
H. pylori-negative
H. pylori-positive with t(11;18)
If no benefit seen from antibiotics
H. pylori eradication
Consider trial of antibiotics
H. pylori test at 2 months (if HP-positive, treat with 2nd line eradication)
EGDS + biopsies at 3-6 months
IF-RT
Antibiotics resistant
After H. pylori eradication
positive for lymphoma, symptomatic or with other indications:
overt progression
deep invasion
nodal involvement
t(11;18)
positive for lymphoma, asymptomatic
negative for lymphoma
Careful monitoring: EGDS + biopsy q3-6 months
Follow-up: EGDS + biopsy q6 months x 2 years, then yearly
Chlorambucil or other alkylating agents and/or rituximab if RT is not feasible or not indicated
(b) EMZL, stage III-IV
Non-gastric MALT lymphoma
Gastric MALT lymphoma
Borrelia, Chlamydophila, Campylobacter or HCV assessment according to the clinical presentation
H. pylori assessment
consider eradication if the infection is present
eradication if H. pylori is present
Asymptomatic
Symptomatic
or with other indications to treat:
overt progression
bulky disease
organ damage
patient wish
Asymptomatic
Wait and see
Wait and see EGDS and biopsies every 3-6 months
Chemotherapy and/or rituximab, or consider including in clinical trials

◀ **Fig. 1** Algorithm for the treatment of patients with localized (Panel **a**) or disseminated (Panel **b**) MALT lymphomas. In principle, to remove an antigenic stimulation that may favor a relapse, eradication of putative driver chronic infections (*H. pylori* for gastric lymphomas, *B. burgdorferi* for cutaneous lymphoma, *C. psittaci* for lymphoma of the ocular adnexa, *C. jejuni* for IPSID, and *HCV* for salivary glands or subcutaneous lymphomas) should be given also to patients with disseminated disease together with the required lymphoma treatment. This may reduce the risk of gastric cancer in patients with *H. pylori* gastritis and liver cancer in those with chronic *HCV* infection

Table 2 Schedules for *H. pylori* eradication

Scheme	Drugs	Recommendation
Triple therapy	• PPI (standard dose mg twice daily)	• First line (low prevalence of clarithromycin resistance)
	• Clarithromycin (500 mg twice daily)[a]	
	• Amoxicillin (1,000 mg twice daily) for 10–14 days	
Triple therapy	• PPI (standard dose mg twice daily)	• First line (low prevalence of clarithromycin resistance)
	• Clarithromycin (500 mg twice daily)[a]	
	• metronidazole (400 or 500 mg twice daily)[b] for 10–14 days	
Quadruple therapy	• PPI (standard dose mg twice daily)	• First line (high prevalence of clarithromycin resistance)
	• Metronidazole (500 mg three times a day)	
	• Tetracycline 500 mg four times a day	
	• Bismuth sub citrate 120 mg four times a day for 10–14 days	• Second-line treatment[c]

[a]Triple therapy is recommended in areas where the prevalence of clarithromycin resistance is less than 10–15 %

[b]The use of metronidazole instead of amoxicillin is suggested in area with less than 40 % metronidazole resistance or in penicillin-allergic patients

[c]PPI plus amoxicillin, tetracycline, and metronidazole are recommended when bismuth is not available. When a third choice in needed, it should be based on the antimicrobial susceptibility test

carcinoma development, especially when *H. pylori* infection persists. Once *H. pylori* eradication has been demonstrated (by breath test or by a monoclonal stool antigen test), it is recommended to repeat EGD with multiple biopsies 2–3 months after treatment to exclude lymphoma progression, and subsequently (every 6 months for 2 years) to monitor the histological lymphoma regression [22].

Gastric MALT lymphoma less commonly spreads to distant organs, and the frequency of the histological transformation into diffuse large B-cell lymphoma (DLBCL) is low. Transient histological relapses can be observed in endoscopic biopsies during long-term follow-up, but they tend to be self-limiting, and especially without the stimulus from *H. pylori* reinfection, they do not implicate a true clinical relapse. Hence, when persistent but not progressive residual disease or histological relapse is documented, a "wait and see" policy seems safe [46, 49, 54, 55]. Nevertheless, a long-term careful endoscopic and systemic follow-up (clinical examination, blood counts and minimal adequate radiological or ultrasound examinations

Table 3 GELA criteria for post-treatment histological evaluation of gastric endoscopic biopsies in gastric—MALT lymphoma [53]

Gela grading score	
CR, complete histological remission	• Normal or empty LP and/or
	• Fibrosis with absent or scattered plasma cells and small lymphoid cells in the LP
	• No LEL
pMRD, probable minimal residual disease	• Normal or empty LP and/or
	• Fibrosis with aggregates of lymphoid cells or lymphoid nodules in the LP/MM
	• Focal or absent LEL
rRD, responding residual disease	• Focal empty LP and/or
	• Fibrosis with dense, diffuse, or nodular lymphoid infiltrate extending around glands in the LP
	• Focal or absent LEL
NC, no change	• Dense, diffuse, or nodular lymphoid infiltrate
	• LEL usually present

LP lamina propria, *LEL* lymphoepithelial lesions, *MM* muscolaris mucosa, *SM* submucosa

every 12–18 months) is strongly advisable for all patients. Furthermore, the risk of gastric adenocarcinoma among individuals with gastric MALT lymphoma has been reported to be six fold higher, while the risk of other non-Hodgkin lymphomas should be considered higher than in the general population [56, 57].

An obvious prerequisite for a response to antibiotics is the presence of a *H. pylori* infection. However, there are reports of lymphoma regression following antibiotics also in *H. pylori*-negative patients, and first-line therapy with antibiotics has to be considered at least in those patients without the t(11;18) translocation, possibly due to a false-negative test or to infection by other Helicobacter species [52]. The response rates of lymphomas are around 70-90 % for the mucosa-confined lymphomas and decrease for the tumors infiltrating the submucosa, the muscularis propria, and the serosa [58–62]. At the same time, the involvement of perigastric lymph nodes, detected by either CT scan or endoscopic ultrasound, rarely respond to antibiotics [60–62]. Moreover, the presence of a high-grade lymphoma component and a history of autoimmune disease were associated with antibiotic therapy resistance [63]. Nearly all gastric lymphomas with t(11;18) translocation will not respond to *H. pylori* eradication therapy [62, 64]. The t(11;18) is also associated with the resistance to chlorambucil or thalidomide as single agents [65, 66]. Notably, data on a very small series of 13 patients have suggested that the combination of chlorambucil and rituximab is active in t(11;18)-positive cases [67].

H. pylori eradication therapy should be considered in all gastric MALT lymphomas, independent of the stage [22], but patients with symptomatic disseminated disease should also be considered for systemic treatment, e.g., the combination of rituximab and chemotherapy. The role of immunochemotherapy will be further discussed in the following sections which present the management of non-gastric presentations.

6.3 Gastric HP-Negative or Antibiotic-Resistant MALT Lymphoma and Non-gastric MALT Lymphoma

No definite guidelines exist for the management of patients with *H. pylori*-negative gastric-lymphoma, for patients failing anti-HP treatment, or for the non-gastric localizations. In *H. pylori*-negative cases, a regression of the lymphoma after antibiotic treatment is unlikely and the immediate start of oncological treatments should be considered. Nevertheless, a course of anti-HP treatment may be considered since occasional lymphoma responses have been reported [2, 52]. An oncological treatment should, eventually, be considered when no signs of lymphoma regression are seen at a repeated endoscopy assessment 2–3 months after antibiotics administration. No significant survival difference between patients who received different initial treatments (including chemotherapy alone, surgery alone, surgery with additional chemotherapy, and radiation therapy) has been shown [68, 69]. Radiotherapy may be the favored choice for patients with localized disease HP-negative or for patients who do not achieve a lymphoma regression following antibiotic therapy [70]. Indeed, involved-field radiotherapy to the stomach and perigastric lymph nodes has results in excellent disease control and most reports support the use of a moderate dose (24–30 Gy given in 3–4 weeks) [71–74].

Radiotherapy has become a standard treatment, at least in North America, also for most non-gastric localized presentations. Literature reports a high rate of local control in MALT lymphoma, with a high proportion of patients likely to be cured [75–84]. The use of radiotherapy in this setting has been recommended by the NCCN Clinical Practice Guidelines in Oncology [85]. The modern radiotherapy techniques, such as three-dimensional conformal radiotherapy and intensity modulated radiotherapy, allow an accurate determination of the clinical target volume, thus reducing the toxicity to surrounding organs [73, 86]. The curative doses required (25–35 Gy) are generally associated with mild and reversible acute toxicity and a low risk of long-term side effects, although special caution should be given for specific localizations such as the ocular adnexa or the lung [72, 73, 74, 86].

In patients with disseminated non-gastric MALT lymphoma, observation with a careful monitoring can be often an adequate initial approach. When treatment is required, there is no consensus for the choice of treatment, but rituximab alone or chemotherapy with or without rituximab appear the most appropriate choice. The treatment approach of disseminated MALT lymphomas is the same in patients with primary gastric and non-gastric origin, and the enrollment in controlled clinical trials is advisable. Indeed, there is no standard recommendation, as only a limited number of drugs and regimens have been specifically tested in MALT lymphomas [2]. Oral alkylating agents (either cyclophosphamide or chlorambucil) or purine nucleoside analogues (fludarabine, cladribine) are active as single agents [87, 88]. Rituximab monotherapy has also been tested in phase II studies [89, 90]. However, there is not yet a widely accepted standard immunochemotherapy regimen. Recently, the efficacy and safety of the combination of rituximab plus chlorambucil has been proven in a phase III study of the International Extranodal Lymphoma

Study Group (IELSG) in gastric (failing antibiotics) or non-gastric MALT lymphomas. In comparison with either rituximab or chlorambucil given as single agent, chlorambucil plus rituximab resulted in significantly superior complete remission, progression-free and event-free survival rates; however, no overall survival benefit was shown [91, 92]. The combination of rituximab and bendamustine [93] as well as the combination of fludarabine and rituximab has also shown high rates of disease control in smaller non-randomized studies [93]. However, the hematological and infectious toxicity observed with the latter regimen, both during and after therapy, was significant in this patient population [94]. Aggressive anthracycline-containing chemotherapy regimen should be reserved for patients with high tumor burden (bulky masses, unfavorable International Prognostic Index) or for those with histological transformation [95].

6.4 Antibiotic Treatment in Non-gastric MALT Lymphoma

The role of antibiotic therapy in patients with non-gastric MALT lymphoma is unclear [96]. Some anecdotal reports described regressions of non-gastric MALT lymphomas in *H. pylori*-infected patients after *H. pylori* eradication [97–99], but this approach is not effective in the majority of patients with non-gastric localization [100]. Antibiotic therapy appears nowadays a reasonable first-line therapy for patients with ocular adnexa MALT lymphoma and may be considered for some cutaneous marginal zone lymphoma; however, it remains experimental in other non-GI localization of MALT lymphomas.

6.5 Antibiotic Therapy for Ocular Adnexa MALT Lymphoma (OAMZL)

The finding that *Chlamydophila psittaci* has been detected in up to 80 % of Italian patients with OAMZL provided the rationale for the antibiotic treatment of localized lesions [5, 7], and a pivotal Italian experience showed that the eradication of *C. psittaci* infection using doxycycline for patients with OAMZL may result in lymphoma regression in approximately 50 % of patients, including pre-treated patients and patients with regional lymph node involvement [101, 102]. Following this first demonstration, a prospective international phase II study was later conducted by the IELSG. In this study, *C. psittaci* DNA was detected in nearly 90 % of the lymphoma biopsy specimens. Thirty-four patients were treated front line with doxycycline and assessed for chlamydia eradication and lymphoma response. Chlamydia eradication was achieved in 14/34 patients (48 %), and six patients obtained a complete lymphoma regression (overall response rate, ORR 65 %). At a median follow-up of 37 months, the 5-year progression-free survival was 55 %. Moreover, in this study, a consistent concordance between *C. psittaci* detected in tumor tissue and *C. psittaci* on conjunctival swab indicated that conjunctival swab may be a

simple, noninvasive tool for monitoring the infection [6]. Globally, doxycycline has been tested in 120 patients with OAMZL, with an ORR of around 50 % [96]. The median time for response after antibiotic therapy is 6 months. Analogous to *H. pylori* eradication in gastric MALT lymphoma [52], in some patients, responses are slow and may require up to 36 months [102]. Further investigations are warranted to clarify the appropriate time for starting a different treatment after doxycycline failure, the optimal schedule of doxycycline as well as to identify other potential infective agents, and improve the eradicative antibiotic efficacy [6, 103, 104].

6.6 Antibiotic Therapy for Primary Cutaneous Marginal Zone Lymphoma (PCMZL)

The term "pseudolymphoma" refers to a typical cutaneous B-cell infiltration known to be induced by *Borrelia burgdorferi* chronic infection. *B. burgdorferi* has also been reported to have a potential pathogenetic role in marginal zone lymphomas of the skin. Some case reports have shown that the eradication of *B. burgdorferi* following ceftriaxone therapy resulted in regression of an associated cutaneous marginal zone lymphoma [4]. However, the evidence is based on a limited number of patients [96], and therefore, no recommendations can be made.

6.7 Immunoproliferative Small Intestinal Disease (IPSID)

IPSID has a long natural history, often over many years, including a potentially reversible early phase. If left untreated, however, the lymphoma can undergo a histologic transformation to a DLBCL. In its early phases, IPSID can be treated with prolonged antibiotic therapy (i.e., tetracycline or metronidazole and ampicillin for at least 6 months), which can lead to lymphoma regression. These results may suggest a role for an infectious agent, *Campylobacter jejuni* seeming the best candidate [8]. Anthracycline-containing regimens, combined with nutritional support plus antibiotics to control diarrhea and malabsorption, represent the best chance of cure for patients with advanced-stage disease. Surgery has no therapeutic role, since the lymphoma usually diffusely involves the intestine.

6.8 Splenic MZL

Most patients with SMZL can initially be managed with a "wait and see" strategy. Cytopenia or symptomatic massive splenomegaly is the main indication for treatment start. When treatment is needed, therapeutic options are splenectomy, chemotherapy, and rituximab alone or in combination with chemotherapy [24].

Splenectomy has been for a long time the therapy of choice. It allows prolonged remissions with rapid alleviation of splenomegaly-related symptoms, accompanied

by resolution of cytopenia and disappearance of circulating lymphocytes in around 90 % of the cases. Even when bone marrow involvement and lymphocytosis persist, the time to next treatment may be longer than 5 years [32]. However, SMZL is a systemic disease, and splenectomy cannot be considered a curative option. Moreover, it is a major surgical procedure with significant morbidity and non-negligible mortality, especially in older patients [105].

There is no evidence for a survival benefit when chemotherapy is added to splenectomy. Chemotherapy alone has been used for patients unsuitable for splenectomy or relapsing after surgery. Alkylating agents (i.e., chlorambucil or cyclophosphamide) alone or in combination (i.e., CHOP) with fludarabine monotherapy have shown to be effective. More recently, since most patients are elderly and at high risk for surgery, frontline immunotherapy with rituximab alone or in combination with chemotherapy has become more and more widely used [105–109]. In a Greek study, 58 patients were treated with rituximab at a dose of 375 mg/m^2 per week for 6 weeks as induction, followed by rituximab maintenance in 43 patients (375 mg/m^2 every 2 months for 1–2 years). The overall response rate in this study was 95 %, with almost half of responses being complete, while the 5-year progression-free survival was 77 %. Maintenance therapy with rituximab appeared to induce a significantly longer response duration (the 5-year progression-free survival was 84 % for patients receiving maintenance and 36 % for patients without maintenance) [108].

A survey on the survival outcomes after surgery or rituximab-based systemic therapy in the Surveillance Epidemiology and End Results-Medicare database (SEER) included 227 patients, diagnosed between 2000 and 2007, and treated with splenectomy (68 %), rituximab alone (23 %) or in combination with chemotherapy (9 %) within 2 years from diagnosis. It showed higher rates of hospitalizations, infections, transfusions, and cardiovascular or thromboembolic events after chemoimmunotherapy than after splenectomy. Conversely, there was no significant difference in the complication rates between groups treated with splenectomy or rituximab alone [109].

Other retrospective series of rituximab monotherapy, including both chemotherapy-naive and refractory patients, showed overall responses of 88–100 % with marked and prompt regression of splenomegaly and improvement of cytopenias with overall survival comparable to that reported after splenectomy. Sustained responses occurred both with and without rituximab maintenance and relapsed patients responded to second courses of rituximab monotherapy [106]. The remission rates and durations after rituximab, given either alone or with chemotherapy, were significantly better than after chemotherapy without rituximab in the same patients, with manageable toxicity [107]. The combination of rituximab with chemotherapy may further improve progression-free survival, but further evaluation with confirmatory prospective trials is warranted [105–107]. The available evidence, despite being mainly based on retrospective studies, seems to suggest that rituximab could replace splenectomy as first-line treatment, particularly in SMZL patients over the age of 65 years. In these patients, the risk of lymphoma-related death and overall survival were similar to rituximab or splenectomy as initial

therapy, indicating that single-agent rituximab may carry the most favorable risk/benefit ratio in this population [109], but optimal schedule and long-term outcome have not been fully defined yet.

For patients with SMZL HCV-associated, there is evidence that antiviral treatment may be an acceptable option. As already mentioned, the use of antiviral therapy to treat HCV-associated lymphomas was established after the demonstration by Hermine et al. [9] of splenic marginal zone lymphoma regression following HCV eradication. Later, other reports confirmed that antiviral therapy can be an efficacious frontline therapy for HCV-associated SMZL and nodal MZL [10, 12, 110, 111]. HCV-RNA clearance is needed to attain lymphoma response [111], and the achievement of a virologic response can be followed by a lymphoma regression in up to 75 % of the cases. At present, in HCV-positive patients with marginal zone lymphoma who do not need immediate conventional treatment for lymphoma, antiviral treatment with pegylated-interferon-α and ribavirin should be considered as first-line treatment [24, 111]. However, novel and better tolerated antiviral agents are under development and will hopefully make easier the treatment of both lymphoma and hepatitis. Indeed, less toxic and shorter interferon-free regimens will very soon become a suitable option, and this may be particularly advantageous for the treatment of lymphoma patients with interferon-resistant HCV genotypes [112, 113].

6.9 Nodal MZL

At present, no large prospective trials have been published and there are no definite guidelines for the management of NMZL. It is generally accepted that treatment should be planned according to the therapeutic principles adopted for follicular lymphomas [24]. Therefore, patients affected by NMZL are often treated with a combination of immunotherapy with anti-CD20 monoclonal antibody alone or in combination with chemotherapy. Good responses are in general reported across studies. However, relapses are frequent [114, 115]. In young patients with early relapse, high-dose chemotherapy with autologous stem cell transplantation may be an option, while radiotherapy, using low doses of radiation, may be considered in localized cases or as palliative treatment of symptomatic lesions [36, 38]. However, none of these approaches has been prospectively tested.

Analogous to SMZL, in patients with HCV-associated chronic infection, elimination of the infection may induce a subsequent lymphoma regression [111]; therefore, an attempt to achieve HCV eradication with interferon and ribavirin should be considered before any other treatment in patients who do not require immediate immunochemotherapy. The role of new antiviral agents remains to be evaluated.

References

1. Swerdlow S, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al (eds) (2008) WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC, Lyon
2. Bertoni F, Coiffier B, Salles G, Stathis A, Traverse-Glehen A, Thieblemont C et al (2011) MALT lymphomas: pathogenesis can drive treatment. Oncology 25(12):1134–1142 47
3. Craig VJ, Arnold I, Gerke C, Huynh MQ, Wundisch T, Neubauer A et al (2010) Gastric MALT lymphoma B cells express polyreactive, somatically mutated immunoglobulins. Blood 115(3):581–591. doi:10.1182/blood-2009-06-228015
4. Roggero E, Zucca E, Mainetti C, Bertoni F, Valsangiacomo C, Pedrinis E et al (2000) Eradication of Borrelia burgdorferi infection in primary marginal zone B-cell lymphoma of the skin. Hum Pathol 31(2):263–268
5. Ferreri AJ, Guidoboni M, Ponzoni M, De Conciliis C, Dell'Oro S, Fleischhauer K et al (2004) Evidence for an association between chlamydia psittaci and ocular adnexal lymphomas. J Natl Cancer Inst 96(8):586–594
6. Ferreri AJ, Govi S, Pasini E, Mappa S, Bertoni F, Zaja F et al (2012) Chlamydophila psittaci eradication with doxycycline as first-line targeted therapy for ocular adnexae lymphoma: final results of an international phase II trial. J Clin Oncol 30(24):2988–2994. doi:10.1200/jco.2011.41.4466
7. Ferreri AJM, Dolcetti R, Du MQ, Doglioni C, Giordano Resti A, Politi LS et al (2008) Ocular adnexal MALT lymphoma: an intriguing model for antigen-driven lymphomagenesis and microbial-targeted therapy. Ann Oncol 19(5):835–846. doi:10.1093/annonc/mdm513
8. Lecuit M, Abachin E, Martin A, Poyart C, Pochart P, Suarez F et al (2004) Immunoproliferative small intestinal disease associated with *Campylobacter jejuni*. N Engl J Med 350(3):239–248
9. Hermine O, Lefrere F, Bronowicki JP, Mariette X, Jondeau K, Eclache-Saudreau V et al (2002) Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med 347(2):89–94
10. Arcaini L, Paulli M, Boveri E, Vallisa D, Bernuzzi P, Orlandi E et al (2004) Splenic and nodal marginal zone lymphomas are indolent disorders at high hepatitis C virus seroprevalence with distinct presenting features but similar morphologic and phenotypic profiles. Cancer 100(1):107–115
11. Arcaini L, Burcheri S, Rossi A, Paulli M, Bruno R, Passamonti F et al (2007) Prevalence of HCV infection in nongastric marginal zone B-cell lymphoma of MALT. Ann Oncol 18 (2):346–350. doi:10.1093/annonc/mdl388
12. Paulli M, Arcaini L, Lucioni M, Boveri E, Capello D, Passamonti F et al (2010) Subcutaneous 'lipoma-like' B-cell lymphoma associated with HCV infection: a new presentation of primary extranodal marginal zone B-cell lymphoma of MALT. Ann Oncol 21 (6):1189–1195. doi:10.1093/annonc/mdp454
13. Ambrosetti A, Zanotti R, Pattaro C, Lenzi L, Chilosi M, Caramaschi P et al (2004) Most cases of primary salivary mucosa-associated lymphoid tissue lymphoma are associated either with Sjoegren syndrome or hepatitis C virus infection. Br J Haematol 126(1):43–49
14. Isaacson PG, Du MQ (2004) MALT lymphoma: from morphology to molecules. Nat Rev Cancer 4(8):644–653
15. Jost PJ, Ruland J (2007) Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences and therapeutic implications. Blood 109(7):2700–2707
16. Bertoni F, Zucca E (2006) Delving deeper into MALT lymphoma biology. J Clin Investig 116(1):22–26
17. Langel FD, Jain NA, Rossman JS, Kingeter LM, Kashyap AK, Schaefer BC (2008) Multiple protein domains mediate interaction between Bcl10 and MALT1. J Biol Chem. doi:10.1074/jbc.M800670200
18. Lucas PC, McAllister-Lucas LM, Nunez G (2004) NF-kappaB signaling in lymphocytes: a new cast of characters. J Cell Sci 117(Pt 1):31–39

19. Baens M, Fevery S, Sagaert X, Noels H, Hagens S, Broeckx V et al (2006) Selective expansion of marginal zone B cells in Emicro-API2-MALT1 mice is linked to enhanced IkappaB kinase gamma polyubiquitination. Cancer Res 66(10):5270–5277
20. Novak U, Rinaldi A, Kwee I, Murty VV, Nandula SV, Rancoita PMV et al. (2008) The NF-kB pathway component A20 (TNFAIP3) is commonly inactivated by DNA mutations and deletions in extranodal marginal zone B-cell lymphoma (EMZL). Ann Oncol abstract at the 10-ICML
21. Kwee I, Rancoita PM, Rinaldi A, Ferreri AJ, Bhagat G, Gascoyne RD et al (2011) Genomic profiles of MALT lymphomas: variability across anatomical sites. Haematologica 96 (7):1064–1066. doi:10.3324/haematol.2011.040402
22. Zucca E, Copie-Bergman C, Ricardi U, Thieblemont C, Raderer M, Ladetto M (2013) Gastric marginal zone lymphoma of MALT type: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 24(6):vi144–vi148. doi:10.1093/annonc/mdt343
23. Isaacson PG, Chott A, Nakamura S, Muller-Hermelink HK, Harris NL, Swerdlow S (2008) Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In: Swerdlow S, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al (eds) WHO classification of tumours of haematopoietic and lymphoid tissues. IARC, Lyon, pp 214–217
24. Dreyling M, Thieblemont C, Gallamini A, Arcaini L, Campo E, Hermine O et al (2013) ESMO Consensus conferences: guidelines on malignant lymphoma. part 2: marginal zone lymphoma, mantle cell lymphoma, peripheral T-cell lymphoma. Ann Oncol 24(4):857–877. doi:10.1093/annonc/mds643
25. Franco V, Florena AM, Iannitto E (2003) Splenic marginal zone lymphoma. Blood 101 (7):2464–2472
26. Matutes E, Oscier D, Montalban C, Berger F, Callet-Bauchu E, Dogan A et al (2008) Splenic marginal zone lymphoma proposals for a revision of diagnostic, staging and therapeutic criteria. Leukemia 22(3):487–495
27. Zucca E, Conconi A, Pedrinis E, Cortelazzo S, Motta T, Gospodarowicz MK et al (2003) Nongastric marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Blood 101(7):2489–2495
28. de Boer JP, Hiddink RF, Raderer M, Antonini N, Aleman BMP, Boot H et al (2008) Dissemination patterns in non-gastric MALT lymphoma. Haematologica 93(2):201–206. doi:10.3324/haematol.11835
29. Thieblemont C, Coiffier B (2004) MALT lymphoma: sites of presentations, clinical features and staging procedures. In: Zucca E, Bertoni F (eds) MALT lymphomas. Landes Bioscience/ Kluwer Academic, Georgetown, pp 60–80
30. Raderer M, Wohrer S, Streubel B, Troch M, Turetschek K, Jager U et al (2006) Assessment of disease dissemination in gastric compared with extragastric mucosa-associated lymphoid tissue lymphoma using extensive staging: a single-center experience. J Clin Oncol 24 (19):3136–3141
31. Zucca E, Stathis A, Bertoni F (2014) The management of nongastric MALT lymphomas. Oncology 28(1):86–93
32. Thieblemont C, Davi F, Noguera ME, Briere J, Bertoni F, Zucca E et al (2012) Splenic marginal zone lymphoma: current knowledge and future directions. Oncology 26(2):194–202
33. Arcaini L, Merli M, Volpetti S, Rattotti S, Gotti M, Zaja F (2012) Indolent B-cell lymphomas associated with HCV infection: clinical and virological features and role of antiviral therapy. Clin dev immunol 2012:638185. doi:10.1155/2012/638185
34. Arcaini L, Lazzarino M, Colombo N, Burcheri S, Boveri E, Paulli M et al (2006) Splenic marginal zone lymphoma: a prognostic model for clinical use. Blood 107(12):4643–4649. doi:10.1182/blood-2005-11-4659
35. Montalban C, Abraira V, Arcaini L, Domingo-Domenech E, Guisado-Vasco P, Iannitto E et al (2012) Risk stratification for Splenic Marginal Zone Lymphoma based on haemoglobin

concentration, platelet count, high lactate dehydrogenase level and extrahilar lymphadenopathy: development and validation on 593 cases. Br J Haematol 159(2):164–171. doi:10.1111/bjh.12011
36. Angelopoulou MK, Kalpadakis C, Pangalis GA, Kyrtsonis MC, Vassilakopoulos TP (2014) Nodal marginal zone lymphoma. Leuk Lymphoma 55(6):1240–1250. doi:10.3109/10428194.2013.840888
37. van den Brand M, van Krieken JH (2013) Recognizing nodal marginal zone lymphoma: recent advances and pitfalls. A systematic review. Haematologica 98(7):1003–1013. doi:10.3324/haematol.2012.083386
38. Traverse-Glehen A, Bertoni F, Thieblemont C, Zucca E, Coiffier B, Berger F et al (2012) Nodal marginal zone B-cell lymphoma: a diagnostic and therapeutic dilemma. Oncology 26 (1):92–99 103-4
39. Kojima M, Inagaki H, Motoori T, Itoh H, Shimizu K, Tamaki Y et al (2007) Clinical implications of nodal marginal zone B-cell lymphoma among Japanese: study of 65 cases. Cancer Sci 98(1):44–49. doi:10.1111/j.1349-7006.2006.00345.x
40. Camacho FI, Algara P, Mollejo M, Garcia JF, Montalban C, Martinez N et al (2003) Nodal marginal zone lymphoma: a heterogeneous tumor: a comprehensive analysis of a series of 27 cases. Am J Surg Pathol 27(6):762–771
41. Gachard N, Parrens M, Soubeyran I, Petit B, Marfak A, Rizzo D et al (2013) IGHV gene features and MYD88 L265P mutation separate the three marginal zone lymphoma entities and Waldenstrom macroglobulinemia/lymphoplasmacytic lymphomas. Leukemia 27(1):183–189. doi:10.1038/leu.2012.257
42. Oh SY, Ryoo BY, Kim WS, Kim K, Lee J, Kim HJ et al (2006) Nodal marginal zone B-cell lymphoma: analysis of 36 cases. Clinical presentation and treatment outcomes of nodal marginal zone B-cell lymphoma. Ann Hematol 85(11):781–786. doi:10.1007/s00277-006-0160-y
43. Berger F, Felman P, Thieblemont C, Pradier T, Baseggio L, Bryon PA et al (2000) Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. Blood 95(6):1950–1956
44. Nathwani BN, Anderson JR, Armitage JO, Cavalli F, Diebold J, Drachenberg MR et al (1999) Marginal zone B-cell lymphoma: a clinical comparison of nodal and mucosa-associated lymphoid tissue types. Non-Hodgkin's lymphoma classification project. J Clin Oncol 17(8):2486–2492
45. Treglia G, Zucca E, Sadeghi R, Cavalli F, Giovanella L, Ceriani L (2014) Detection rate of fluorine-18-fluorodeoxyglucose positron emission tomography in patients with marginal zone lymphoma of MALT type: a meta-analysis. Hematological oncology doi: 10.1002/hon.2152. [Epub ahead of print, July 22, 2014]
46. Wundisch T, Thiede C, Morgner A, Dempfle A, Gunther A, Liu H et al (2005) Long-term follow-up of gastric MALT lymphoma after Helicobacter pylori eradication. J Clin Oncol 23 (31):8018–8024
47. Montalban C, Santon A, Redondo C, Garcia-Cosio M, Boixeda D, Vazquez-Sequeiros E et al (2005) Long-term persistence of molecular disease after histological remission in low-grade gastric MALT lymphoma treated with *H. pylori* eradication. Lack of association with translocation t(11;18): a 10-year updated follow-up of a prospective study. Ann Oncol 16 (9):1539–1544
48. Kim JS, Chung SJ, Choi YS, Cheon JH, Kim CW, Kim SG et al (2007) Helicobacter pylori eradication for low-grade gastric mucosa-associated lymphoid tissue lymphoma is more successful in inducing remission in distal compared to proximal disease. Br J Cancer 96 (9):1324–1328
49. Stathis A, Chini C, Bertoni F, Proserpio I, Capella C, Mazzucchelli L et al (2009) Long-term outcome following Helicobacter pylori eradication in a retrospective study of 105 patients with localized gastric marginal zone B-cell lymphoma of MALT type. Ann Oncol 20 (6):1086–1093. doi:10.1093/annonc/mdn760

50. Terai S, Iijima K, Kato K, Dairaku N, Suzuki T, Yoshida M et al (2008) Long-term outcomes of gastric mucosa-associated lymphoid tissue lymphomas after Helicobacter pylori eradication therapy. Tohoku J Exp Med 214(1):79–87
51. Andriani A, Miedico A, Tedeschi L, Patti C, Di Raimondo F, Leone M et al (2009) Management and long-term follow-up of early stage *H. pylori*-associated gastric MALT-lymphoma in clinical practice: an Italian, multicentre study. Dig Liver Dis 41(7):467–473. doi:10.1016/j.dld.2008.09.009 (official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver)
52. Ruskone-Fourmestraux A, Fischbach W, Aleman BM, Boot H, Du MQ, Megraud F et al (2011) EGILS consensus report. Gastric extranodal marginal zone B-cell lymphoma of MALT. Gut 60(6):747–58. doi:10.1136/gut.2010.224949
53. Copie-Bergman C, Wotherspoon AC, Capella C, Motta T, Pedrinis E, Pileri SA et al (2013) Gela histological scoring system for post-treatment biopsies of patients with gastric MALT lymphoma is feasible and reliable in routine practice. Br J Haematol 160(1):47–52. doi:10.1111/bjh.12078
54. Nakamura S, Sugiyama T, Matsumoto T, Iijima K, Ono S, Tajika M et al (2012) Long-term clinical outcome of gastric MALT lymphoma after eradication of Helicobacter pylori: a multicentre cohort follow-up study of 420 patients in Japan. Gut 61(4):507–513. doi:10.1136/gutjnl-2011-300495
55. Fischbach W, Goebeler ME, Ruskone-Fourmestraux A, Wundisch T, Neubauer A, Raderer M et al (2007) Most patients with minimal histological residuals of gastric MALT lymphoma after successful eradication of Helicobacter pylori can be managed safely by a watch and wait strategy: experience from a large international series. Gut 56(12):1685–1687
56. Capelle LG, de Vries AC, Looman CW, Casparie MK, Boot H, Meijer GA et al (2008) Gastric MALT lymphoma: Epidemiology and high adenocarcinoma risk in a nation-wide study. Eur J Cancer 44(16):2470–2476. doi:10.1016/j.ejca.2008.07.005
57. Wundisch T, Dieckhoff P, Greene B, Thiede C, Wilhelm C, Stolte M et al (2012) Second cancers and residual disease in patients treated for gastric mucosa-associated lymphoid tissue lymphoma by Helicobacter pylori eradication and followed for 10 years. Gastroenterology 143(4):936–942. doi:10.1053/j.gastro.2012.06.035 (quiz e13–e14)
58. Sackmann M, Morgner A, Rudolph B, Neubauer A, Thiede C, Schulz H et al (1997) Regression of gastric MALT lymphoma after eradication of Helicobacter pylori is predicted by endosonographic staging MALT Lymphoma study group. Gastroenterology 113(4):1087–1090
59. Nakamura S, Matsumoto T, Suekane H, Takeshita M, Hizawa K, Kawasaki M et al (2001) Predictive value of endoscopic ultrasonography for regression of gastric low grade and high grade MALT lymphomas after eradication of Helicobacter pylori. Gut 48(4):454–460
60. Ruskone-Fourmestraux A, Lavergne A, Aegerter PH, Megraud F, Palazzo L, de Mascarel A et al (2001) Predictive factors for regression of gastric MALT lymphoma after anti-Helicobacter pylori treatment. Gut 48(3):297–303
61. Steinbach G, Ford R, Glober G, Sample D, Hagemeister FB, Lynch PM et al (1999) Antibiotic treatment of gastric lymphoma of mucosa-associated lymphoid tissue. An uncontrolled trial. Ann Intern Med 131(2):88–95
62. Liu H, Ye H, Ruskone-Fourmestraux A, De Jong D, Pileri S, Thiede C et al (2002) T(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to *H. pylori* eradication. Gastroenterology 122(5):1286–1294
63. Neubauer A, Thiede C, Morgner A, Alpen B, Ritter M, Neubauer B et al (1997) Cure of Helicobacter pylori infection and duration of remission of low- grade gastric mucosa-associated lymphoid tissue lymphoma. J Natl Cancer Inst 89(18):1350–1355
64. Alpen B, Neubauer A, Dierlamm J, Marynen P, Thiede C, Bayerdorfer E et al (2000) Translocation t(11;18) absent in early gastric marginal zone B-cell lymphoma of MALT type responding to eradication of Helicobacter pylori infection. Blood 95(12):4014–4015

65. Kuo SH, Cheng AL, Lin CW, Hsu CH, Wu MS, Yeh KH et al (2011) t(11;18)(q21;q21) translocation as predictive marker for non-responsiveness to salvage thalidomide therapy in patients with marginal zone B-cell lymphoma with gastric involvement. Cancer Chemother Pharmacol 68(6):1387–1395. doi:10.1007/s00280-011-1631-y
66. Levy M, Copie-Bergman C, Gameiro C, Chaumette MT, Delfau-Larue MH, Haioun C et al (2005) Prognostic value of translocation t(11;18) in tumoral response of low-grade gastric lymphoma of mucosa-associated lymphoid tissue type to oral chemotherapy. J Clin Oncol 23 (22):5061–5066
67. Levy M, Copie-Bergman C, Molinier-Frenkel V, Riou A, Haioun C, Gaulard P et al (2010) Treatment of t(11;18)-positive gastric mucosa-associated lymphoid tissue lymphoma with rituximab and chlorambucil: clinical, histological, and molecular follow-up. Leuk Lymphoma 51(2):284–290. doi:10.3109/10428190903431820
68. Thieblemont C, Dumontet C, Bouafia F, Hequet O, Arnaud P, Espinouse D et al (2003) Outcome in relation to treatment modalities in 48 patients with localized gastric MALT lymphoma: a retrospective study of patients treated during 1976-2001. Leuk Lymphoma 44 (2):257–262
69. Pinotti G, Zucca E, Roggero E, Pascarella A, Bertoni F, Savio A et al (1997) Clinical features, treatment and outcome in a series of 93 patients with low-grade gastric MALT lymphoma. Leuk Lymphoma 26(5–6):527–537
70. Yahalom J (2011) Patients with H pylori-independent MALT lymphoma are curable with radiotherapy. Oncology 25(12):1147–1149
71. Wirth A, Gospodarowicz M, Aleman BM, Bressel M, Ng A, Chao M et al (2013) Long-term outcome for gastric marginal zone lymphoma treated with radiotherapy: a retrospective, multi-centre, international extranodal Lymphoma study group study. Ann Oncol. doi:10.1093/annonc/mds623
72. Goda JS, Gospodarowicz M, Pintilie M, Wells W, Hodgson DC, Sun A et al (2010) Long-term outcome in localized extranodal mucosa-associated lymphoid tissue lymphomas treated with radiotherapy. Cancer 116(16):3815–3824. doi:10.1002/cncr.25226
73. Tsang RW, Gospodarowicz MK (2005) Radiation therapy for localized low-grade non-Hodgkin's lymphomas. Hematol Oncol 23(1):10–17
74. Koch P, Probst A, Berdel WE, Willich NA, Reinartz G, Brockmann J et al (2005) Treatment results in localized primary gastric lymphoma: data of patients registered within the German multicenter study (GIT NHL 02/96). J Clin Oncol 23(28):7050–7059
75. Tsang RW, Gospodarowicz MK, Pintilie M, Wells W, Hodgson DC, Sun A et al (2003) Localized mucosa-associated lymphoid tissue lymphoma treated with radiation therapy has excellent clinical outcome. J Clin Oncol 21(22):4157–4164
76. Hitchcock S, Ng AK, Fisher DC, Silver B, Bernardo MP, Dorfman DM et al (2002) Treatment outcome of mucosa-associated lymphoid tissue/marginal zone non-Hodgkin's lymphoma. Int J Radiat Oncol Biol Phys 52(4):1058–1066
77. Le QT, Eulau SM, George TI, Hildebrand R, Warnke RA, Donaldson SS et al (2002) Primary radiotherapy for localized orbital MALT lymphoma. Int J Radiat Oncol Biol Phys 52 (3):657–663
78. Ansell SM, Grant CS, Habermann TM (1999) Primary thyroid lymphoma. Semin Oncol 26 (3):316–323
79. Brogi E, Harris NL (1999) Lymphomas of the breast: pathology and clinical behavior. Semin Oncol 26(3):357–364
80. Agulnik M, Tsang R, Baker MA, Kazdan MS, Fernandes B (2001) Malignant lymphoma of mucosa-associated lymphoid tissue of the lacrimal gland: case report and review of literature. Am J Clin Oncol 24(1):67–70
81. Jhavar S, Agarwal JP, Naresh KN, Shrivastava SK, Borges AM, Dinshaw KA (2001) Primary extranodal mucosa associated lymphoid tissue (MALT) lymphoma of the prostate. Leuk Lymphoma 41(3–4):445–449

82. Suchy BH, Wolf SR (2000) Bilateral mucosa-associated lymphoid tissue lymphoma of the parotid gland. Arch Otolaryngol Head Neck Surg 126(2):224–226
83. Puri DR, Tereffe W, Yahalom J (2008) Low-dose and limited-volume radiotherapy alone for primary dural marginal zone lymphoma: treatment approach and review of published data. Int J Radiat Oncol Biol Phys 71(5):1425–1435. doi:10.1016/j.ijrobp.2007.12.007
84. Milgrom SA, Yahalom J (2013) Indolent non-Hodgkin lymphoma primarily involving the hard palate: outcome following radiotherapy. Leuk Lymphoma 54(6):1208–1211. doi:10.3109/10428194.2012.741232
85. NCCN National Comprehensive Cancer Network Guidelines (2013) V.2.2013—Non-Hodgkin's lymphoma. http://www.nccn.org/professionals/physician_gls/PDF/nhl.pdf. Accessed 11 Nov 2013
86. Yahalom J (2001) MALT lymphomas: a radiation oncology viewpoint. Ann Hematol 80(3): B100–B105
87. Jager G, Hofler G, Linkesch W, Neumeister P (2004) Occurrence of a myelodysplastic syndrome (MDS) during first-line 2-chloro-deoxyadenosine (2-CDA) treatment of a low-grade gastrointestinal MALT lymphoma. Case report and review of the literature. Haematologica 89(4):ECR01
88. Zinzani PL, Stefoni V, Musuraca G, Tani M, Alinari L, Gabriele A et al (2004) Fludarabine-containing chemotherapy as frontline treatment of nongastrointestinal mucosa-associated lymphoid tissue lymphoma. Cancer 100(10):2190–2194
89. Conconi A, Martinelli G, Thieblemont C, Ferreri AJ, Devizzi L, Peccatori F et al (2003) Clinical activity of rituximab in extranodal marginal zone B-cell lymphoma of MALT type. Blood 102(8):2741–2745
90. Martinelli G, Laszlo D, Ferreri AJ, Pruneri G, Ponzoni M, Conconi A et al (2005) Clinical activity of rituximab in gastric marginal zone non-Hodgkin's Lymphoma resistant to or not eligible for anti-helicobacter pylori therapy. J Clin Oncol 23(9):1979–1983
91. Zucca E, Conconi A, Martinelli G, Tucci A, Vitolo U, Russo E et al (2013) Chlorambucil plus rituximab produces better event-free and progression-free survival in comparison with chlorambucil or rituximab alone in extranodal marginal zone B-cell lymphoma (MALT lymphoma): results of the IELSG-19 study. Hematol Oncol 31(S1):97–98
92. Zucca E, Conconi A, Laszlo D, Lopez-Guillermo A, Bouabdallah R, Coiffier B et al (2013) Addition of rituximab to chlorambucil produces superior event-free survival in the treatment of patients with extranodal marginal-zone B-cell lymphoma: 5-year analysis of the IELSG-19 Randomized Study. J Clin Oncol 31(5):565–572. doi:10.1200/JCO.2011.40.6272
93. Salar A, Domingo-Domenech E, Panizo C, Nicolas C, Bargay J, A. M et al. (2013) Bendamustine plus rituximab in first line systemic treatment for extranodal MALT lymphoma: final results of phase II trial of the Spanish lymphoma study group (GELTAMO). Hematol oncol 31(S1):129–130
94. Brown JR, Friedberg JW, Feng Y, Scofield S, Phillips K, Dal Cin P et al (2009) A phase 2 study of concurrent fludarabine and rituximab for the treatment of marginal zone lymphomas. Br J Haematol 145(6):741–748. doi:10.1111/j.1365-2141.2009.07677.x
95. Thieblemont C (2005) Clinical presentation and management of marginal zone lymphomas. Hematology (Am Soc Hematol Educ Program) 2005:307–313
96. Kiesewetter B, Raderer M (2013) Antibiotic therapy in nongastrointestinal MALT lymphoma: a review of the literature. Blood 122(8):1350–1357. doi:10.1182/blood-2013-02-486522
97. van den Bosch J, Kropman RF, Blok P, Wijermans PW (2002) Disappearance of a mucosa-associated lymphoid tissue (MALT) lymphoma of the urinary bladder after treatment for Helicobacter pylori. Eur J Haematol 68(3):187–188
98. Raderer M, Pfeffel F, Pohl G, Mannhalter C, Valencak J, Chott A (2000) Regression of colonic low grade B cell lymphoma of the mucosa associated lymphoid tissue type after eradication of Helicobacter pylori. Gut 46(1):133–135

99. Arima N, Tsudo M (2003) Extragastric mucosa-associated lymphoid tissue lymphoma showing the regression by Helicobacter pylori eradication therapy. Br J Haematol 120 (5):790–792
100. Grunberger B, Wohrer S, Streubel B, Formanek M, Petkov V, Puespoek A et al (2006) Antibiotic treatment is not effective in patients infected with Helicobacter pylori suffering from extragastric MALT lymphoma. J Clin Oncol 24(9):1370–1375. doi:10.1200/JCO.2005.02.9025
101. Ferreri AJ, Ponzoni M, Guidoboni M, De Conciliis C, Giordano Resti A, Mazzi B et al. (2005) Regression of ocular adnexal lymphoma following Chlamydia psittaci-eradicating antibiotic therapy. J Clin Oncol 23:5067–5073
102. Ferreri AJ, Ponzoni M, Guidoboni M, Resti AG, Politi LS, Cortelazzo S et al (2006) Bacteria-eradicating therapy with doxycycline in ocular adnexal MALT lymphoma: a multicenter prospective trial. J Natl Cancer Inst 98(19):1375–1382
103. Kim TM, Kim KH, Lee MJ, Jeon YK, Lee SH, Kim DW et al (2010) First-line therapy with doxycycline in ocular adnexal mucosa-associated lymphoid tissue lymphoma: a retrospective analysis of clinical predictors. Cancer Sci 101(5):1199–1203. doi:10.1111/j.1349-7006.2010.01502.x
104. Troch M, Kiesewetter B, Raderer M (2011) Recent developments in nongastric mucosa-associated Lymphoid Tissue Lymphoma. Curr Hematol Malignancy Rep. doi:10.1007/s11899-011-0095-9
105. Kalpadakis C, Pangalis GA, Vassilakopoulos TP, Sachanas S, Angelopoulou MK (2014) Treatment of splenic marginal zone lymphoma: should splenectomy be abandoned? Leuk Lymphoma 55(7):1463–1470. doi:10.3109/10428194.2013.845884
106. Bennett M, Schechter GP (2010) Treatment of splenic marginal zone lymphoma: splenectomy versus rituximab. Semin Hematol 47(2):143–147. doi:10.1053/j.seminhematol.2010.01.004
107. Else M, Marin-Niebla A, de la Cruz F, Batty P, Rios E, Dearden CE et al (2012) Rituximab, used alone or in combination, is superior to other treatment modalities in splenic marginal zone lymphoma. Br J Haematol 159(3):322–328. doi:10.1111/bjh.12036
108. Kalpadakis C, Pangalis GA, Angelopoulou MK, Sachanas S, Kontopidou FN, Yiakoumis X et al (2013) Treatment of splenic marginal zone lymphoma with rituximab monotherapy: progress report and comparison with splenectomy. Oncologist 18(2):190–197. doi:10.1634/theoncologist.2012-0251
109. Olszewski AJ, Ali S (2014) Comparative outcomes of rituximab-based systemic therapy and splenectomy in splenic marginal zone lymphoma. Ann Hematol 93(3):449–458. doi:10.1007/s00277-013-1900-4
110. Vallisa D, Bernuzzi P, Arcaini L, Sacchi S, Callea V, Marasca R et al (2005) Role of Anti-Hepatitis C Virus (HCV) treatment in HCV-related, low-grade, B-Cell, Non-Hodgkin's Lymphoma: a multicenter Italian experience. J Clin Oncol 23(3):468–473
111. Arcaini L, Vallisa D, Rattotti S, Ferretti VV, Ferreri AJ, Bernuzzi P et al. (2014) Antiviral treatment in patients with indolent B-cell lymphomas associated with HCV infection: a study of the Fondazione Italiana Linfomi. Ann Oncol mdu166. doi:10.1093/annonc/mdu166
112. Liang TJ, Ghany MG (2013) Current and future therapies for hepatitis C virus infection. N Engl J Med 368(20):1907–1917. doi:10.1056/NEJMra1213651
113. Liang TJ, Ghany MG (2014) Therapy of hepatitis C–back to the future. N Engl J Med 370 (21):2043–2047. doi:10.1056/NEJMe1403619
114. Arcaini L, Paulli M, Burcheri S, Rossi A, Spina M, Passamonti F et al (2007) Primary nodal marginal zone B-cell lymphoma: clinical features and prognostic assessment of a rare disease. Br J Haematol 136(2):301–304. doi:10.1111/j.1365-2141.2006.06437.x
115. Traverse-Glehen A, Felman P, Callet-Bauchu E, Gazzo S, Baseggio L, Bryon PA et al (2006) A clinicopathological study of nodal marginal zone B-cell lymphoma. A report on 21 cases. Histopathology 48(2):162–173. doi:10.1111/j.1365-2559.2005.02309.x

Treatment Strategies in Mantle Cell Lymphoma

Kami Maddocks and Kristie A. Blum

Abstract

Mantle cell lymphoma (MCL) is a distinct B-cell non-Hodgkin's lymphoma (NHL) defined by the translocation t(11;14). MCL combines characteristics of both indolent and aggressive lymphomas, and it is incurable with conventional chemoimmunotherapy but has a more aggressive disease course. Minimal data exist on treatment of patients diagnosed with early-stage disease (stage I–II non-bulky), as this represents only a small portion of the patients diagnosed with MCL, but therapeutic options evaluated in retrospective studies include radiation or combination radiation and chemotherapy. There is a subset of patients with newly diagnosed MCL that can be observed without treatment, but the majority of patients will require treatment at diagnosis. Treatment is often based on age (≤65–70 years of age), comorbidities, and risk factors for disease. The majority of patients who are younger and without significant comorbidities are treated with intensive induction using combination chemoimmunotherapy regimens, many which include consolidation with autologous stem cell transplant (ASCT). Several regimens have been studied that show improved median progression-free survival (PFS) to 3–6 years in this population of patients. The majority of older patients (≥65–70 years of age) are treated with combination chemoimmunotherapy regimens with consideration of rituximab maintenance, with enrollment on a clinical trial encouraged. Therapy for relapsed disease is dependent on prior treatment, age, comorbidities, and toxicities but includes targeted therapies such as the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib, the immunomodulatory agent lenalidomide, the proteasome inhibitor bortezomib, combination

K. Maddocks (✉) · K.A. Blum
Arthur G James Comprehensive Cancer Center, The Ohio State University Comprehensive Cancer Center, 320 W 10th Street A350C Starling Loving Hall, Columbus, OH 43210, USA
e-mail: kami.maddocks@osumc.edu

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_10

chemoimmunotherapy, ASCT, and allogeneic stem cell transplant in selected cases. Several novel agents and targeted therapies alone or in combination are currently being studied and developed in both the upfront and relapsed setting.

Keywords

Mantle cell lymphoma · Dose intensified · Autologous stem cell transplant · Rituximab maintenance · Ibrutinib

Contents

1 Introduction

Mantle cell lymphoma (MCL) is a distinct B-cell lymphoma defined by the translocation t(11;14), resulting in the constitutive overexpression of cycle D1 [1]. MCL comprises 3–10 % of all non-Hodgkin lymphomas (NHL) [1]. The median age at diagnosis is 60–65 years with at least a 2:1 male predominance [2, 3]. The majority of patients will present with Ann Arbor stage III or IV disease (advanced stage) with the most common sites of disease involvement including the lymph nodes, bone marrow, spleen and peripheral blood, and the most common sites of extranodal disease involvement including the gastrointestinal tract and Waldeyer's ring [1]. MCL is thought to combine the unfavorable features of both indolent and aggressive NHL subtypes; it is incurable with conventional chemoimmunotherapy but has a more aggressive disease course.

Different clinical and biologic features have been identified as prognostic factors in MCL. Both the International Prognostic Index (IPI) for lymphoma [4] and the Mantle Cell International Prognostic Index (MIPI) [5] identify clinical risk factors for shorter survival including increased age, stage III/IV disease, high lactate dehydrogenase (LDH), poor performance status, and extranodal disease sites for the IPI and increased age, poor performance status, high LDH, and higher white blood cell count at diagnosis for the MIPI. Other clinical factors that have been suggestive of worse prognosis include occurrence of B symptoms, bulky disease, splenomegaly, anemia, and high β2-microglobulin.

In addition to clinical risk factors, certain biologic risk factors have been associated with prognosis. The presence of p53 mutations has been associated with shorter overall survival [6, 7]. Higher proliferation rate, determined by number of mitoses or Ki-67, has also been associated with shorter survival where patients with tumors demonstrating Ki-67 levels of less than 30 % have been shown to have significantly longer median overall survival (OS) times than patients with tumors demonstrating Ki-67 levels of greater than 10 % [8, 9]. Complex karyotype, as defined by ≥3 unrelated chromosomal abnormalities [including t(11;14)], has been associated with shortened progression-free survival (PFS) and OS in newly diagnosed patients, regardless of initial therapy [10, 11]. Mutated IgVH genes and the absence of the transcription factor SOX11 have been associated with more indolent disease [12, 13]. Tumors with high levels of mutation are associated with less genomic complexity, SOX11 negativity, and improved overall survival.

2 Treatment

The optimal upfront treatment regimen in MCL is not clearly defined. While several acceptable treatment regimens have been shown to prolong response durations, none of these regimens have proven curative and it remains unclear that any provides a survival advantage over another. Due to the relatively small numbers of patients diagnosed with MCL, there is a lack of large randomized controlled trials of these regimens, and therefore, most of the data come from single-arm phase II studies.

2.1 Limited Stage (Stage I–II Non-bulky)

There is a paucity of data that include only retrospective analysis on appropriate treatment for patients who present with limited bulk, early-stage (stage IA or IIA) disease. Radiation can be considered as a treatment option in selected cases. In a retrospective analysis of 26 patients with limited-stage disease, inclusion of radiation therapy with or without chemotherapy was associated with significantly improved PFS at 5 years (68 % vs. 11 %, $p = 0.0002$) and a trend toward improvement in overall survival [14]. This presentation represents only a small fraction of the population of patients diagnosed with MCL.

2.2 Advanced Stage (Stage II Bulky, Stage III–IV)

While there is a population of patients with MCL who can be observed without treatment similar to an indolent lymphoma, the majority of patients will require treatment at diagnosis. While there are several acceptable frontline regimens for patients with newly diagnosed stage II–IV MCL, treatment is often based on age (≤65–70 years of age), comorbidities, and risk factors for disease.

2.2.1 Observation

Although MCL tends to more rapidly progress in the majority of patients, there may be a role for observation in selected patients including elderly, asymptomatic, or low-risk patients by MIPI score. A study at Weill Cornell Medical Center identified a group of patients who did well with deferring initial therapy [15]. Out of 97 patients evaluated, they identified thirty-one asymptomatic patients without cytopenias, bulky disease, or organ compromise, who were observed for more than 3 months after their initial diagnosis. The median time to treatment in these patients was 12 months, although the time to initiation of treatment ranged from 4 months to 128 months with 14 patients being observed for 12 months or longer, 3 of whom were observed for more than 5 years. Survival of the observed patients was superior to survival of the patients treated within 3 months of diagnosis (OS not reached vs. 64 months) with performance status and lower-risk IPI associated with an increased likelihood of observation. However, it is important to recognize that this is a small, retrospective, non-randomized trial.

While certain characteristics of disease have been associated with more indolent disease course including non-nodal disease, IPI, IgVH mutational status, and SOX11 expression [11, 12, 15], these are not well described or validated and additional trials are required to identify which patients will benefit from observation in the initial treatment of MCL prior to it being routinely recommended.

2.3 Frontline Therapy for Younger Patients (≤65–70 Years of Age)

The majority of patients who are younger and without significant comorbidities are treated with intensive induction using combination chemoimmunotherapy regimens or intensive induction using combination chemoimmunotherapy regimens typically followed by consolidation with autologous stem cell transplantation (ASCT) as conventional therapy does not provide long-term control of MCL. Several single-arm phase II studies have shown that these intensive treatment options improve median PFS to 3–6 years in this population of patients.

2.3.1 Dose-Intensified Chemoimmunotherapy Regimens

The addition of rituximab to more intensive combination chemotherapy regimens has shown to translate into improved outcomes in this population of patients. Three separate phase II studies using the combination of rituximab and hyper-CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) alternating with rituximab, high-dose methotrexate, and cytarabine (R-MTX/Ara-C) have been shown to prolong PFS in younger patients. M.D. Anderson conducted a

study of 97 patients aged 41–81 years using 6–8 cycles (the 29 patients in CR after only 2 cycles received 6 total cycles, all others received 8 cycles) of R-Hyper-CVAD alternating with R-MTX/Ara-C, with 97 % of patients responding to therapy and 87 % achieving a complete response (CR) [16]. In all patients, the 3-year failure-free survival (FFS) was 64 % with 40 months of follow-up and was 73 % in patients less than 65 years ($n = 3$). At 7 years, FFS was 43 % with 60 % OS. A total of 29 % of patients were unable to finish their intended cycles of therapy (80 % scheduled to receive 8 cycles). The hematologic toxicity was significant with this regimen, including 4 patients who developed treatment-related myelodysplastic syndrome/acute myelogenous leukemia (MDS/AML). Additionally, 5 patients died from acute toxicity which included 3 with neutropenic sepsis, one with pulmonary hemorrhage, and one of unknown causes, and 3 patients died while in remission, all from MDS, for a total of eight (8 %) treatment-related deaths. In a multicenter phase II study by the Southwest Oncology Group (SWOG) in patients with previously untreated MCL less than 70 years of age, 49 patients were treated with R-Hyper-CVAD alternating with R-MTX/Ara-C for eight cycles [17, 18]. The overall response rate (ORR) was 86 % with a CR rate of 58 %. The 2-year PFS and OS were 65 % and 76 %, respectively, and with a median follow-up at 4.8 years, the median PFS was 4.8 years, increasing to 5.5 years for those patients ≤65 years, and median OS was 6.8 years. This study reported significant toxicity, with 19 (39 %) patients not completing the full course of treatment due to toxicity. Hematologic and infectious toxicities were high with 10 episodes of grade 3 febrile neutropenia and 20 episodes of grade 3–4 infection including respiratory, urinary, and catheter-related infections. There were two cases of treatment-related MDS and one treatment-related death from clostridium difficile (C. diff) colitis with toxic megacolon and sepsis. A multicenter Italian study alternating R-Hyper-CVAD with R-MTX/Ara-C for eight cycles treated 60 patients ≤70 years [19]. The ORR was 83 %, with a CR of 72 %, and 5-year PFS and OS were 61 % and 73 %, respectively. This regimen was also associated with significant toxicity in this study, with the incidence of grade 3–4 infections being 50 %: only 22 patients (37 %) actually completing the planned therapy and 3 patients with treatment-related deaths due to sepsis, cardiac failure, and pulmonary aspergillosis. Due to the shortened FFS and significant toxicity in patients over 65, R-Hyper-CVAD is not routinely recommended for patients >65 years of age.

2.3.2 Autologous Stem Cell Transplantation

For those patients who are candidates for ASCT, first-line consolidation therapy with autologous transplant has demonstrated improved PFS in a number of different studies using several different chemoimmunotherapy regimens for induction. In a randomized study by the European MCL Network, 122 patients ≤65 years with advanced-stage MCL who had received a CHOP-like regimen (majority receiving CHOP or R-CHOP) and achieved a CR or PR with no MCL cells detectable in the peripheral blood and <20 % detectable in the bone marrow were randomized to

ASCT versus interferon-α maintenance (IFN-α) [20]. ASCT resulted in a significantly longer median PFS compared IFN-α maintenance, 39 months versus 17 months (p = 0.011), respectively, with 3-year OS 83 % following ASCT compared to 77 % with IFN-α. Infectious and hematologic toxicities were high, with 5 % mortality due to infectious complications in the ASCT arm. The Cancer and Leukemia Group B (CALGB 59909) trial treated 78 patients <70 years with two or three cycles of rituximab combined with methotrexate and augmented CHOP, one cycle of rituximab, etoposide and cytarabine (EAR) and filgrastim to mobilize autologous peripheral blood stem cells, and ASCT utilizing high-dose carmustine, etoposide, and cyclophosphamide as conditioning regimen [21]. This regimen yielded an ORR of 88 % with 69 % CR, 5-year PFS of 56 %, and 5-year OS of 64 %. Eight of the first 20 patients treated experienced unexpected acute renal failure, which prompted a reduction in the methotrexate dose. Renal failure did not occur after this change in dosing, and the 2-year PFS in the 58 patients treated at the lower methotrexate dose was 73 %. There were two treatment-related mortalities, a cardiovascular collapse from severe anemia and sepsis. The event rate (early progression plus non-relapse mortality) was only 5.1 % at day + 100 following transplant. The CALGB group followed this trial with CALGB 50403 in which patients <70 years were treated with the exact same induction and transplant regimen as 59909, with treatment following transplant to include 2 doses of post-transplant rituximab followed by randomization between bortezomib consolidation (BC—1.3 mg/m^2 days 1, 4, 8, 11 of a 3-week cycle for 4 cycles) or bortezomib maintenance (BM—1.6 mg/m^2 weekly 4 of 8 weeks for 18 months) beginning 90 days post-transplant. A total of 147 patients were treated, and 102 were randomized with 34 (65 %) patients completing BM and 33 (66 %) completing BC. The 3-year PFS from time of transplant was 67 % compared to 59 % on CALGB 59909 (p = 0.0086). There were 4 treatment-related deaths. Although both were feasible, the BC arm had 14 (28 %) patients withdraw due to toxicity, with only 7 (13 %) patients in the BM withdraw due to toxicity (p = 0.088), most of which were attributed to cytopenias or peripheral neuropathy [22].

Several additional studies suggest that inclusion of cytarabine in the pretransplant induction regimen improves outcomes. The Nordic Lymphoma Group (NLG) conducted a phase II trial (MCL-2) in 160 patients ≤66 years with untreated advanced-stage MCL [23, 24]. Induction therapy consisted of rituximab in combination with alternating cycles of maxi-CHOP and high-dose cytarabine for a total of 6 cycles with the sixth cycle of cytarabine plus filgrastim serving as the stem cell mobilization followed by ASCT utilizing carmustine, etoposide cytarabine and cyclophosphamide or melphalan (BEAM or BEAC). This resulted in an ORR of 96 % with a 54 % CR, 6-year PFS of 66 %, and 6-year OS of 70 % which was a significant improvement from the NLG MCL-1 trial which treated patients with 4 cycles of intensified CHOP followed by ASCT with a 4-year failure-free survival of 15 % and OS 51 % [25]. This demonstrated the contribution of the high-dose cytarabine to improved patient outcomes. Five patients failed to proceed to ASCT due to toxicity which included aortic stenosis (n = 1), gastric bleeding (n = 1), and severe infection (n = 3). A total of 8 (5 %) non-relapse deaths occurred, four during

high-dose therapy due to graft failure ($n = 1$) and infections ($n = 3$) and four due to heart failure ($n = 3$) (8, 20, and 28 months post-ASCT) and pulmonary embolism ($n = 1$) (45 months after ASCT). There was 1 case of MDS. Further follow-up from this study showed a median event-free survival (EFS) of 7.4 years and median OS to exceed 10 years. While initially no relapses were reported after 5 years, with further follow-up, late relapses were reported in 6 patients more than 5 years after the end of therapy. Ki-67 was found to be the only independent factor predictive of survival.

In a phase II multicenter trial conducted by the French cooperative group GELA, 60 patients ≤66 years were treated with two cycles of CHOP, 1 cycle of R-CHOP, and 3 cycles of R-DHAP followed by ASCT with TBI, high-dose cytarabine, and high-dose melphalan [26]. The ORR was 95 % with 57 % of patients achieving a CR after R-DHAP but only 12 % of those achieving a CR after R-CHOP, similar to the improvement seen with the addition of cytarabine in the NLG MCL-2 trial. Forty-nine patients received ASCT, and at a median follow-up of 67 months, median EFS was 83 months and median OS not reached, with 5-year OS 75 %. One patient experienced sudden death within 2 weeks of the first treatment, 3 patients were unable to proceed with ASCT due to therapy-related toxicity (renal in $n = 3$ and neurologic in $n = 1$), and seven patients progressed prior to ASCT. No patients died during the ASCT, and there were no cases of MDS reported at a median follow-up of 67 months. A phase III randomized trial by the European MCL Network confirmed the benefits for cytarabine prior to ASCT [27]. The group enrolled 455 evaluable patients with Stage II-IV MCL up to 65 years of age who were randomized to recieve either a standard arm wtih 6 cycles of R-CHOP therapy followed by stem cell mobilization with DexaBEAM and myeloablative treatment with TBI and high-dose cyclophosphamide and ASCT or an experimental arm wtih 6 cycles of alternating R-CHOP/R-DHAP followed by a high-dose cytarabine containing myeloablative therapy with TBI, high-dose cytrabine and melphalan and ASCT. ORRs were similar (90 % R-CHOP vs. 95 % R-CHOP/R-DHAP, $p = 0.19$) prior to ASCT and after ASCT (98 % R-CHOP vs. 97 %); however, CR was significantly higher in the arm alternating R-CHOP with R-DHAP as compared to the R-CHOP arm (54 % vs. 40 %, $p = 0.0003$) prior to ASCT although similar after ASCT (63 % vs. 61 %). After a median follow-up of 51 months, time to treatment failure (TTF) and OS were longer following R-CHOP/R-DHAP induction with TTF 88 months with R-CHOP/R-DHAP vs. 46 months with R-CHOP, $p = 0.0382$, and OS not reached with R-CHOP/R-DHAP versus 82 months R-CHOP, $p = 0.045$. Toxicity was comparable in both arms.

It still remains unclear that all younger patients will benefit most from intensive therapy and ASCT. In patients treated in both the NLG MCL-2 and the CALGB 59909 trials, it was shown that the MIPI was predictive of PFS and OS in patients treated uniformly with intensified therapy and ASCT [21–24]. In patients treated on the CALGB trial, 66.7 % of high-risk MIPI patients progressed compared with 32.3 % of low-intermediate risk ($p < 0.02$) with 67 % high-risk patients dying compared with 25 % of the low-intermediate risk ($p < 0.03$) [21]. In patients treated on the NLG trial, 70 % of patients with low-intermediate risk MIPI were alive at

10-year follow-up in comparison with only 23 % of the patients with high MIPI [24]. In a study of 118 patients with MCL who received HDT and ASCT with 85 of those having ASCT as first consolidation, the MIPI was found to be independently associated with OS and PFS, regardless of induction regimen or timing of transplant [28]. OS at 2.5 years was 93, 60, and 32 % for low-, intermediate-, and high-risk MIPI. When adjusting for age, the MIPI remained associated with improved OS, whereas induction regimen did not.

In addition to the MIPI, achievement of molecular remission has also been associated with outcome in patients undergoing intensive induction therapy followed by ASCT. In the CALGB 59909 study, 39 patients had evaluable peripheral blood and bone marrow for minimal residual disease (MRD) by real-time polymerase chain reaction (PCR) for identification of rearranged immunoglobulin heavy-chain genes (IgH) or BCL-1/JH gene rearrangements (translocation 11;14) [29]. Of these 39 patients, 18 (46 %) had achieved molecular remission following induction (two cycles of rituximab, methotrexate, and augmented CHOP as above) with 21 patients having detectable MRD. Twelve (57 %) of the MRD-positive patients progressed within 3 years of follow-up, compared to four (22 %) of the MRD-negative patients. The 3-year probability of progression was 82 % for those with MRD-positive disease versus 48 % with MRD-negative disease. The NLG also evaluated MRD by PCR for t(11;14). They found that a larger number of patients were able to collect PCR-negative stem cells in the MCL-2 trial compared with the MCL-1 trial (88 % vs. 12 %, $p < 0.001$) and patients obtained a longer duration of molecular remission supporting the importance of rituximab and cytarabine for in vivo stem cell purging [23, 24]. They also noted that PFS differed between patients with PCR-positive samples at different time points with patients having detectable MRD within the first year of follow-up having a median PFS of 1.5 years, those detected later a median PFS of 5 years, and those that still remained without detectable disease median survival was not reached ($p < 0.001$). The rate of molecular remission was also shown to be predictive of response in the European MCL trial where MRD was detected by PCR for t(11;14). In the arm alternating therapy with R-CHOP and R-DHAP, achievement of MRD after induction was higher than in the arm receiving therapy with R-CHOP (73 % vs. 32 %), and achievement of molecular remission was associated with significantly improved 2-year PFS (94 % vs. 74 %, $p = 0.022$) [27, 30].

It is therefore possible that the benefit of transplant is greatest in patients with low-risk disease and those that are able to achieve MRD-negative disease with induction therapy prior to transplant. There remains the possibility that similar outcomes could be obtained by using novel therapies and maintenance strategies, though for the time, ASCT remains the most utilized therapy in this population of patients.

2.4 Frontline Therapy for Older Patients (≥65–70 Years of Age)

2.4.1 Chemoimmunotherapy

As noted above, older patients are not usually considered candidates for ASCT and have increased toxicities associated with more intensive chemoimmunotherapy regimens such as R-Hyper-CVAD. Options for treatment include single-agent rituximab, rituximab in combination with chemotherapy, or enrollment onto a clinical trial.

Single-agent rituximab consisting of 4-weekly infusions of 375 mg/m^2 has only moderate single-agent activity in MCL, with two separate trials and one retrospective analysis showing response rates of 27–38 %, median time to progression (TTP) of 6–7 months, and median duration of response of 12–14 months [31–33]. Therefore, this is not typically recommended as first-line therapy alone, but instead in combination therapy.

The European MCL Network conducted a trial randomizing 455 evaluable patients >60 years with untreated MCL who were not eligible for high-dose therapy to R-CHOP versus R-FC (rituximab, fludarabine, and cyclophosphamide) [34]. The median age of patients was 70 years. The OR was similar between the two arms (40 % R-FC vs. 34 % R-CHOP, $p = 0.10$), but the overall survival was significantly shorter with R-FC than with R-CHOP (4-year survival rate 47 % R-FC vs. 62 % R-CHOP, $p = 0.005$).

In addition to R-CHOP, bendamustine and rituximab (BR) have proven effective in untreated MCL. In a randomized European phase III trial comparing BR to R-CHOP in untreated indolent and MCL with 513 evaluable patients including 94 patients (20 %) with MCL, response rates were similar with BR and R-CHOP (93 % BR vs. 91 % R-CHOP). However, the BR regimen resulted in improved complete response rates (40 % BR vs. 30 % R-CHOP, $p = 0.021$) and prolonged PFS (69.5 months BR vs. 31.2 months R-CHOP, $p = 0.0002$, in all patients, and 35.4 months BR vs. 22.1 months R-CHOP, $p = 0.0044$, in the MCL patients) [35]. There was no difference in overall survival. Of additional importance was the less frequent serious adverse events seen in the patients treated with BR including less grade 3–4 hematologic toxicity, lower number of infectious complications, and lower incidence of peripheral neuropathy. Additionally, BR is without the risk of cardiac toxicity. These results were recently confirmed in the BRIGHT study, a non-inferiority study of BR versus R-CHOP/R-CVP in initial therapy of indolent NHL or MCL [36]. This study evaluated 224 patients assigned to BR and 223 patients to R-CHOP/R-CVP including 77 with MCL. The ORR was 94 % with BR versus 85 % with R-CHOP, with a CR rate of 50 % with BR versus 27 % with R-CHOP, with the most common toxicities of BR being vomiting and drug hypersensitivity and of R-CHOP being peripheral neuropathy and alopecia.

Purine analog therapies, including cladribine and fludarabine, do have activity alone or in combination with rituximab in MCL. In a study of 29 patients treated with cladribine and rituximab as initial therapy, the OR was 66 % with median PFS

of only 12 months [37]. As noted above, treatment with R-FC was shown to be associated with lower OS compared to R-CHOP. With inferior response durations and survival, chemoimmunotherapy with purine analog therapy would not be recommended over R-CHOP as initial treatment.

2.4.2 Maintenance Therapy

Post-induction maintenance has proven beneficial in prolonging PFS in MCL patients >60 years of age. The European MCL Network trial in patients >60 years with untreated MCL mentioned above performed included a second randomization in those patients achieving a PR or CR to R-FC or R-CHOP to receive maintenance rituximab (R) every 2 months until progression versus interferon alpha (IFN-α) maintenance until progression [34]. Among the 316 patients who underwent the second randomization, median remission duration was significantly improved with R maintenance as compared with IFN-α (75 months vs. 27 months, $p < 0.001$). Although the OS was not statistically different in those patients receiving R maintenance (4-year OS 79 % R vs. 67 % IFN, $p = 0.13$), in those patients randomized to R-CHOP induction, the median duration of response exceeded 6 years and the 4-year OS was 87 % with maintenance R compared to 63 % with interferon ($p = 0.005$). Therefore, maintenance R should be offered to patients >60 receiving R-CHOP without consolidative ASCT. However, additional data are needed utilizing R maintenance following other chemotherapy combinations such as R-bendamustine. In addition, the optimal duration of maintenance R is not yet known as no studies have compared maintenance until progression with shorter-duration maintenance therapy or even treatment with rituximab upon evidence of MRD.

2.5 Relapsed Disease

Therapy for relapsed disease is dependent on prior treatment utilized, age, performance status, and toxicities. Treatment options include treatment with molecular targeted therapies, other chemotherapeutic agents, ASCT if not utilized in the frontline setting, and allogeneic stem cell transplantation in selected cases.

2.5.1 Molecular Targeted Therapies

The most recently FDA-approved therapy and preferred treatment for most patients at the time of first relapse is the targeted agent ibrutinib. Ibrutinib is a small-molecule inhibitor of Bruton's tyrosine kinase (BTK), an essential component in B-cell receptor (BCR) signaling. BCR signaling plays a key role in the survival and proliferation of malignant B cells. A phase I study in B-cell malignancies included nine patients with MCL, with seven out of nine (78 %) of these patients responding

to therapy, including a CR in 3 (33 %) patients [38]. No dose-limiting toxicities occurred, and the drug did not have significant myelosuppression. On the basis of this, a phase II study at a dose of 560 mg orally daily was conducted in MCL. In this multicenter phase II study of oral ibrutinib, 111 patients with relapsed/refractory MCL were enrolled into two groups, those that had received prior treatment with bortezomib, and those that had no prior treatment with bortezomib [39]. A 68 % OR was observed with a CR of 21 % and estimated median PFS of 13.9 months, with no difference in those with prior bortezomib treatment versus those without. Additionally, responses increased as time on treatment increased. Ibrutinib was well tolerated with grade 3 or higher hematologic adverse events being infrequent. Grade 3 and 4 events included neutropenia (16 %), thrombocytopenia (11 %), anemia (10 %), diarrhea (6 %), fatigue (5 %), abdominal pain (5 %), dyspnea (5 %), peripheral edema (2 %), anorexia (2 %), rash (2 %), and pyrexia (1 %). There were 5 events of grade 3 bleeding including two subdural hematomas. The most common non-hematologic adverse treatment-related events occurring in more than 20 % of patients included diarrhea, fatigue, nausea, peripheral edema, constipation, upper respiratory tract infection, vomiting, and decreased appetite. Adverse events did lead to discontinuation of therapy in 8 patients (7 %), and 4 deaths during therapy were attributable to adverse events (2 pneumonia, 1 sepsis, and 1 cardiac arrest). This trial did exclude patients on anticoagulation with warfarin after the four patients with subdural hematomas and required patients to have platelet count of 50×10^9/L and absolute neutrophil count of 0.75×10^9/L. Although the package label does not advise against in patients who are taking anticoagulation, ibrutinib should be used in caution with these patients.

Lenalidomide is an immunomodulatory agent with activity in indolent and aggressive lymphomas, approved by the FDA in June 2013 for the treatment of relapsed/refractory MCL based on the results of 3 multicenter phase II studies [40–42]. In the subset of patients treated in the first phase II study (NHL-002) that included 15 patients with MCL, the OR was 53 % with a 20 % CR, median duration of response of 14 months, and PFS of 6 months [41]. A larger study that included 57 patients with MCL showed similar results with an OR of 42 % and 17 % CR with a median PFS of 6 months [42, 43]. The most common toxicities were myelosuppression with neutropenia in 43–46 % of patients and thrombocytopenia in 28–30 %. A phase I/II study of lenalidomide combined with rituximab in relapsed/refractory MCL patients included 52 patients with the maximum-tolerated dose of lenalidomide being 20 mg [44]. The OR was 57 % with a 36 % CR in the patients treated at the recommended phase II dose. Median duration of response was 19 months, and PFS was 11 months. The most common toxicity was grade 3–4 hematologic toxicity including neutropenia in 66 % and thrombocytopenia in 23 % of these patients.

Bortezomib is a proteasome inhibitor with FDA approval based on relapsed/refractory MCL with one prior therapy based on the results of the PINNACLE trial [45, 46]. In this trial, 141 evaluable patients were treated with single-agent bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 of a 21-day cycle for up to 17 cycles. The OR was 33 % with 8 % CR and median duration of response of 9 months. At a

median follow-up of 26 months, the median OS was 23.5 months in all patients and 35 months in those responding. The most common averse events were fatigue (61 %), peripheral neuropathy (55 %), constipation (50 %), and nausea (44 %). Grade ≥3 peripheral neuropathy occurred in 13 % of patients and was the most common adverse event leading to treatment discontinuation (10 %). While different approaches to treatment with bortezomib have been utilized to decrease the incidence of neuropathy (such as weekly dosing and subcutaneous administration), these are not well studied in MCL.

2.5.2 Chemoimmunotherapy

Combination of rituximab and bendamustine is an effective chemoimmunotherapy approach in relapsed disease. Two phase II studies evaluating the combination in relapsed/refractory indolent and MCLs showed high response rates. A study of 67 patients included 12 patients with MCL with an ORR of 92 % and CRR of 42 % with median duration of response being 19 months [47]. Another phase II trial included 16 patients with MCL with an ORR of 75 % and CRR of 50 % [48]. A small multicenter phase II study looking at the combination of rituximab, bendamustine, and bortezomib included 29 evaluable patients, 7 with MCL. The OR in the MCL patients was 71 % with 2-year PFS in the entire group being 47 % [49].

2.5.3 Autologous Stem Cell Transplantation

For those patients who are candidates for ASCT but did not undergo transplant with initial therapy, there is a potential role for transplant at relapse although the outcomes are less favorable. In a study of 56 patients receiving ASCT, 36 received ASCT in first CR or partial remission (PR), while 20 received ASCT at disease progression. The 3-year PFS of the group transplanted in first CR/PR was 93 % compared to 46 % in those transplanted at relapse, while the OS was 63 % compared to 36 %, suggesting that the benefits of ASCT are of greatest at the time of initial therapy as opposed to at time of relapse [50]. However, based on median 3-year PFS of 46 % at first relapse which is superior to the 6–14-month PFS with targeted agents such as ibrutinib, bortezomib, or lenalidomide, ASCT should be considered in suitable patients who did not receive this in first remission.

2.5.4 Allogeneic Stem Cell Transplantation

Allogeneic stem cell transplantation is considered the only potentially curative therapy for advance-stage MCL. Small studies using reduced-intensity conditioning (RIC) regimens have reported benefit in select patients with available donors [51, 52], although complicated by a high risk of treatment-related morbidity and mortality from infectious complications and graft-versus-host disease. The benefits

of RIC allogeneic SCT seem to be greatest in those patients with chemosensitive disease who are transplanted in remission. The MD Anderson Cancer Center reported on 18 patients with relapsed MCL, 5 of who had failed prior ASCT, treated with RIC allogeneic transplant. At a median follow-up of 26 months, the estimated 3-year PFS was 82 % and OS 85 %, with the majority of patients (89 %) having chemosensitive disease [52]. In another study evaluating 33 patients receiving RIC allogeneic transplant, including 42 % who had failed prior ASCT, the 2-year disease-free survival was 60 % and OS 65 %, with none of the patients transplanted in CR (n = 13) experiencing disease relapse at a median follow-up of 25 months [53].

2.6 Studies in Development

2.6.1 Novel Frontline Approaches

A number of frontline studies are being pursued incorporating the use of different molecular targeted agents and maintenance strategies.

A phase II study of lenalidomide and rituximab in patients with previously untreated MCL is currently ongoing at Weill Cornell Medical University (NCT01472562) [54]. This trial is open to patients older than 18 years of age in patients that the investigator feels is not a candidate for chemotherapy. Treatment consists of an induction phase with lenalidomide 20 mg/day for days 1–21 of 28-day cycles for 12 cycles and rituximab 375 mg/m^2 per dose for a total of 9 doses with 4-weekly doses (days 1, 8, 15, and 22) and subsequent dosing weeks 12, 20, 28, 36, and 44. The maintenance phase includes treatment with lenalidomide 15 mg/day for days 1–21 of a 28-day cycle with rituximab at 375 mg/m^2 per dose one dose every 8 weeks starting at week 52 with both drugs continued until progression. A total of 31 patients were enrolled at 4 centers. Median age was 65 (range 42–86). At a median follow-up of 12 months, 27 (87 %) patients remain on study without disease progression with 18 of those having completed induction therapy and in the maintenance phase. With 30 patients evaluable for response, the ORR was 77 % with 40 % CR. Median PFS or duration of response had not been reached. Toxicity was as expected with grade 3–4 hematologic toxicity including neutropenia (39 %) and thrombocytopenia (13 %) and grade 3–4 non-hematologic toxicity including rash (23 %). One patient did go off study for tumor flare. An expansion study with this regimen is currently ongoing.

The Eastern Cooperative Oncology Group (ECOG) has an ongoing trial to evaluate BR alone and in combination with bortezomib followed by consolidation with rituximab or rituximab and lenalidomide (NCT01415752) in patients $\geq$18 years of age. A SWOG trial that was recently closed to accrual evaluated R-Hyper-CVAD/MTX/Ara-C induction followed by ASCT versus BR induction followed by ASCT in patients $\leq$65 years of age (NCT01412879). The R-Hyper-CVAD/MTX/Ara-C induction followed by ASCT arm closed early due to inability to collect stem cells in a significant number of patients, and the BR induction followed by ASCT has

recently met accrual goal and results are awaited. Lastly, there is an ongoing pharmaceutical-sponsored phase III trial randomizing untreated MCL patients to BR versus BR and ibrutinib (NCT01776840) in patients ≥65 years of age.

2.6.2 Novel Agents and Combinations at Relapse

A number of ibrutinib combination regimens are under evaluation for patients with relapsed MCL. In a phase I/IB dose-escalation study of BR and ibrutinib in relapsed or refractory B-cell malignancies with expansion cohorts in DLBCL, MCL, and FL (NCT01479842), no dose-limiting toxicities were observed utilizing 560 mg ibrutinib days 1–28 with rituximab 375 mg/m^2 day 1 and bendamustine 90 mg/m^2 days 1 and 2 of a 28-day cycle [55]. The most common toxicity serious adverse events reported were hematologic. Accrual is completed, with 46 patients accrued including 17 patients with MCL. ORR is 74 % in 38 evaluable patients, with 100 % ORR (81 % CR) in 16 evaluable patients with MCL (personal communication Dr. Blum). There is also an ongoing phase I study of ibrutinib in combination with lenalidomide for all B-cell malignancies (NCT01955499) and a phase II study of ibrutinib in combination with rituximab in MCL (NCT01880567).

2.6.3 Novel Agents

Several other novel therapies are in development including a number of oral therapies targeting inhibition of the B-cell receptor signaling pathway.

Idelalisib is a selective inhibitor of PI3 kinase delta, FDA-approved for the treatment of follicular lymphoma in January 2014, which is critical for survival of B cells and overactive in many B-cell malignancies. Idelalisib was evaluated in MCL in a phase I study that enrolled 40 patients [56]. The ORR was 40 %, with a 5 % CR, median duration of response of 2.7 months, and median PFS of 3.7 months. Another 47.5 % of patients had stable disease for a total of 85 % of patients that had some lymph node response. The most common toxicities were diarrhea, nausea, pyrexia, fatigue, rash, decreased appetite, URI, pneumonia, and transaminitis. Overall, the drug was well tolerated with acceptable toxicities and efficacious in this population of patients. There is ongoing Alliance trial evaluating the combination of idelalisib with lenalidomide in relapsed/refractory MCL (NCT01838434).

ABT-199/GDC-0199 is a bcl-2 inhibitor in development. Bcl-2 proteins are expressed at high levels in NHL and impact malignant cell growth. ABT-199 works by blocking the function of these proteins and promoting cell death. A phase I study of 32 patients with relapsed or refractory NHL showed a 53 % ORR with 9 % CR [57]. In the six patients treated with MCL, the ORR was 100 %. Most common toxicities were nausea, diarrhea, vomiting, fatigue, fever, and cough.

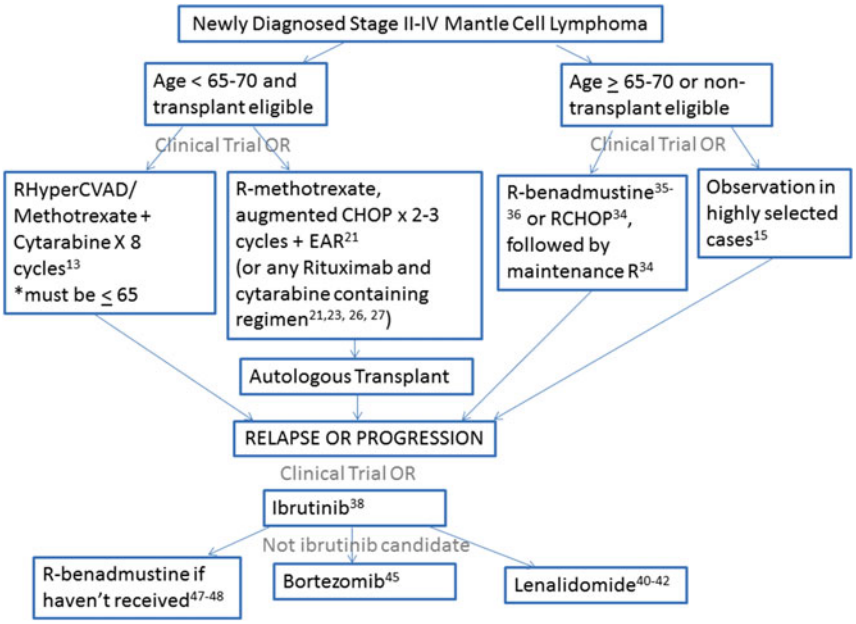


Fig. 1 Our approach to MCL treatment

Table 1 Frontline regimens in patients ≤70 years

Frontline regimen	Age (years)	ORR (%)	PFS/FFS	OS
R-Hyper-CVAD [16]	≤81	97	3 years 64 % (≤65 3 years 73 %)	3 years 82 %
R-Hyper-CVAD [17]	<70	86	5 years 49 %	5 years 63 %
R-Hyper-CVAD [19]	≤70	83	5 years 61 %	5 years 73 %
CALGB 59909 [21]	<70	88	5 years 56 %	5 years 64 %
CALGB 50403 [22]	<70	81	3 years 67 %	
NLG MCL-2 [23]	≤66	96	6 years 66 %	6 years 70 %
GELA [26]	≤66	95	5 years 64 %	5 years 75 %
European MCL [27]	≤65	95	Median TTF 88 months	Median OS not reached

Table 2 Frontline regimens in patients ≥70 years

Frontline regimen	Age (years)	ORR (%)	PFS/FFS/TTF/DOR	OS
R-CHOP [34]	>60	78	TTF 28 months, median DOR 36 months	4 years 62 %
R-FC [34]	>60	86	TTF 26 months, median DOR 37 months	4 years 47 %
R-CHOP [35]	70 (median)	91	22.1 months (median)	Median not reached
BR [35]	70 (median)	93	35.4 months (median)	Median not reached
European older MCL [34] (R-CHOP + maintenance R)	>60	95	Median DOR >6 years	4 years 87 %

3 Conclusions

MCL represents a B-cell lymphoma associated with an overall worse prognosis due to more aggressive behavior but incurability with standard treatment regimens. The treatment strategy for MCL is currently based on age and comorbidities, with the younger (≤65–70 years), fit patients being treated with more aggressive frontline therapy including R-Hyper-CVAD or intensified chemoimmunotherapy followed by ASCT. These regimens have led to improved median PFS in this population of patients, although it remains unclear the extent to which characteristics of disease and treatment (IPI, MIPI, complex cytogenetics, Ki-67, MRD) dictate these responses. These intensive regimens are associated with greater risk of toxicity and treatment-related mortality and therefore not considered as options in all patients. Patients who are elderly (≥65–70 years) or not considered candidates for intensive therapy are treated with R-CHOP or BR followed by rituximab maintenance. Despite the improved PFS with frontline regimens, these patients will eventually relapse with their disease requiring salvage therapy. The BTK inhibitor, ibrutinib, is recommended for use as initial relapsed therapy if there is not a contraindication to treatment with this agent. Other available therapies at relapse need to take into consideration age, comorbidities, prior therapy, and toxicities and include clinical trials with targeted agents, lenalidomide, bortezomib, bendamustine, ASCT, and allogeneic transplantation in select cases. Figure 1 details our approach to the treatment of MCL (Tables 1 and 2).

References

1. Swerdlow S, Campo E, Harris N et al (2008) WHO classification of tumors of hematopoietic and lymphoid tissues. World Health Organization, Lyon, pp 229–232
2. Anderson JR, Armitage JO et al (1998) Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's lymphoma classification project. Ann Oncol 9(7):717–720
3. Zhou Y, Wang H et al (2008) Incidence trends of mantle cell lymphoma in the United States between 1992 and 2004. Cancerb 113(4):791–798
4. International Non-Hodgkin's Lymphoma Prognostic Factors Project (1993) A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 329:987–994
5. Hoster E, Dreyling M, Klapper W et al (2008) A new prognostic index (MIPI) for patients with advanced mantle cell lymphoma. Blood 111:558–565
6. Hernandez L, Fest T, Cazorla M, Teruya-Feldstein J, Bosch F, Peinado MA et al (1996) p53 gene mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas. Blood 87(8):3351–3359
7. Greiner TC, Moynihan MJ, Chan WC, Lytle DM, Pedersen A, Anderson JR et al (1996) p53 mutations in mantle cell lymphoma are associated with variant cytology and predict a poor prognosis. Blood 87(10):4302–4310
8. Martinez A, Bellosillo B, Bosch F, Ferrer A, Marce S, Villamor N et al (2004) Nuclear surviving expression in mantle cell lymphoma is associated with cell proliferation and survival. Am J Pathol 164(2):501–510
9. Raty R, Franssila K, Joensuu H, Teerenhovi L, Elonen E (2002) Ki-67 expression level, histological subtype, and the International Prognostic Index as outcome predictors in mantle cell lymphoma. Eur J Haematol 69(1):11–20
10. Cohen JB, Ruppert AS, Heerema NA et al (2012) Complex Karyotype (CK) is associated with a shortened progression-free survival (PFS) in patients with newly diagnosed mantle cell lymphoma. Blood A:2691
11. Sarkozy C, Terré C, Jardin F et al (2014) Complex karyotype in mantle cell lymphoma is a strong prognostic factor for the time to treatment and overall survival, independent of the MCL international prognostic index. Genes Chromo Cancer 53(1):106–116. doi:10.1002/gcc.22123
12. Nodit L, Bahler D, Jacobs S et al (2003) Indolent mantle cell lymphoma with nodal involvement and mutated immunoglobulin heavy chain genes. Hum Pathol 34:1030–1034
13. Navarro A, Clot G, Royo C et al (2012) Molecular subsets of mantle cell lymphoma defined by the IGHV mutational status and SOX22 expression have distinct biologic and clinical features. Cancer Res 72(20):5307–5316
14. Leitch HA, GAscoyne RD, Chhanabhai M et al (2003) Limited-stage mantle-cell lymphoma. Ann Oncol 14:1555–1561
15. Martin P, Chadburn A, Christos P et al (2009) Outcome of deferred initial therapy in mantle cell lymphoma. J Clin Oncol 27:1209–1213
16. Romaguera J, Fayad L, Rodriquez MA et al (2005) High rate of durable remissions after treatment of newly diagnosed aggressive mantle-cell lymphoma with rituximab plus hyper-CVAD alternating with rituximab plus high-dose methotrexate and cytarabine. J Clin Oncol 23:7013–7023
17. Epner EM, Unger J, Miller T, Romzsa L, Spier C, Leblanc M, Fisher R (2007) A multi-center trial of hyperCVAD + rituxan in patients with newly diagnosed mantle cell lymphoma. Blood 110:A387
18. Bernstein SH, Epner E, Unger JM et al (2013) A phase II multicenter trial of hyperCVAD MTX/Ara-C and rituximab in patients with previously untreated mantle cell lymphoma; SWOG 0213. Ann Oncol 24:1587–1593
19. Merli F, Luminari S, Ilariucci F, et al (2012) Rituximab plus hyper CVAD alternating with high dose cytarabine and methotrexate for the initial treatment of patients with mantle cell lymphoma. A multicenter trial from GISL. Brit J Haematol 156:346–353

20. Dreyling M, Lenz G, Hoster E et al (2005) Early consolidation by myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission significantly prolongs progress-free survival in mantle-cell lymphoma: results of a prospective randomized trial of the European MCL network. Blood 105:2677–2684
21. Damon LE, Johnson JL, Niedzwiecki D et al (2009) Immunochemotherapy and autologous stem-cell transplantation for untreated patients with mantle-cell lymphoma: CALGB 59909. J Clin Oncol 27:6101–6108
22. Kaplan LD, Jung S, Bartelet N et al (2013) Bortezomib maintenance (BM) versus consolidation (BC) following aggressive immunochemotherapy and autologous stem cell transplant (ASCT) for untreated mantle cell lymphoma (MCL): CALGB 50403. Hematol Oncol 31:A89
23. Geisler CH, Kolstad A, Laurell A, Andersen NS, Pedersen LB, Jerkeman M, Eriksson M, Nordström M, Kimby E, Boesen AM, Kuittinen O, Lauritzsen GF, Nilsson-Ehle H, Ralfkiaer E, Akerman M, Ehinger M, Sundström C, Langholm R, Delabie J, Karjalainen-Lindsberg ML, Brown P, Elonen E, Nordic Lymphoma Group (2008) Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: a nonrandomized phase II multicenter study by the Nordic Lymphoma Group. Blood 112:2687–2693
24. Geisler CH, Kolstad A, Laurell A et al (2012) Nordic MCL2 trial update: six-year follow-up after intensive immunochemotherapy for untreated mantle cell lymphoma followed by BEAM or BEAC + autologous stem- cell support: still very long survival but late relapses do occur. Br J Haematol 158:355–362
25. Andersen NS, Pedersen L, Elonen E et al. (2003) Primary treatment with autologous stem cell transplantation in mantle cell lymphoma: outcome related to remission pretransplant. Eur J Haematol 71:73–80
26. Delarue R, Haioun C, Ribrag V et al (2013) CHOP and DHAP plus rituximab followed by autologous stem cell transplantation (ASCT) in mantle cell lymphoma (MCL): a phase II study from the GELA. Blood 121:48–53
27. Pott C, Hoster E, Beldjord K, et al (2010) R-CHOP/R-DHAP compared to R-CHOP induction followed by high dose therapy with autologous stem cell transplantation induces higher rates of molecular remission in MCL: results of the MCL younger intergroup trial of the European MCL Network [abstract]. Blood 116:965
28. Budde LE, Guthric KA, Til BG et al (2011) Mantle Cell lymphoma international prognostic index but not pretransplantation induction regimen predicts survival for mantle-cell lymphoma receiving high-dose therapy and autologous stem-cell transplantation. J Clin Oncol 29:3023
29. Liu H, Johnson JL, Loval G et al (2012) Detection of minimal residual disease following induction chemoimmunotherapy predicts progression free survival in mantle cell lymphoma: final results of CALGB 59909. Hematologica 97(4):579–585
30. Pott C, Hoster E, Delfau-Larue MH et al (2010) Molecular remission is an independent predictor of clinical outcome in patients with mantle cell lymphoma after combined immunochemotherapy: a European MCL intergroup study. Blood 115:3215–3223
31. Foran JM, Cunningham D, Coiffier B (2000) Treatment of mantle-cell lymphoma with rituximab (chimeric anti-CD20 antibody): analysis of factors associated with response. Ann Oncol 11(1):117–121
32. Foran JM, Rohatiner AZ, Cunningham D et al (2000) European phase II study of rituximab (chimeric anti-CD20 monoclonal antibody) for patients with newly diagnosed mantle-cell lymphoma and previously treated mantle-cell lymphoma, immunocytoma, and small B-cell lymphocytic lymphoma. J Clin Oncol 18(2):317–324
33. Ghielmini M, Schmitz SF, Cogliatti S et al (2005) Effect of single-agent rituximab given at the standard schedule or as prolonged treatment in patients with mantle cell lymphoma: a study of the Swiss Group for Clinical Cancer Research (SAKK). J Clin Oncol 23(4):705–711
34. Kluin-Nelemans JC, Hoster E, Walewski J, et al (2011) R-CHOP versus R-FC followed by maintenance with Rituximab versus interferon-Alfa: outcome of the first randomized trial for elderly patients with mantle cell lymphoma [abstract]. Blood 118:439

35. Rummel MJ, Niederle N, Maschmeyer G et al (2013) Bendamustine plus rituximab (B-R) versus CHOP plus rituximab (CHOP-R) as first-line treatment in patients with indolent and mantle cell lymphomas (MCL): an open-label, multicentre, randomized, phase 3 non-inferiority trial. Lancet 381:1203–1210
36. Flinn IW, van der Jagt R, Kahl BS et al (2014) Open-label, randomized, noninferiority study of bendamustine-rituximab or R-CHOP/R-CVP in first-line treatment of advanced indolent NHL or MCL: the BRIGHT study. Blood, Epub
37. Inward DJ, Fishkin PA, Hillman DW et al (2008) Long-term results of the treatment of patients with mantle cell lymphoma with cladribine (2-CDA) alone or 2-CDA and rituximab in the North Central Cancer Treatment Group. Cancer 113:108–116
38. Advani RH, Buggy JJ, Sharman JP et al (2013) Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. J Clin Oncol 31:88–94
39. Wang ML, Rule S, Martin P et al (2013) Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. N Engl J Med 369:507
40. Budde LE, Guthric KA, Til BG et al (2011) Mantle cell lymphoma international prognostic index but not pretransplantation induction regimen predicts survival for mantle-cell lymphoma receiving high-dose therapy and autologous stem-cell transplantation. J Clin Oncol 29:3023
41. Habermann TM, Lossos IS, Justice G, Vose JM, Wiernik PH, McBride K, Wride K, Ervin-Haynes A, Takeshita K, Pietronigro D, Zeldis JB, Tuscano JM (2009) Lenalidomide oral monotherapy produces a high response rate in patients with relapsed or refractory mantle-cell lymphoma. Brit J Haem 145:344–349
42. Reeder CB, Witzig TE, Zinzani PL et al (2009) Efficacy and safety of lenalidomide oral monotherapy in patients with relapsed or refractory mantle-cell lymphoma: results from an international study (NHL-003) [abstract]. J Clin Oncol 27:8569
43. Zinzani PL, Vose JM, Czuczman MS, et al (2012) Phase II multicenter study of the safety and efficacy of single-agent lenalidomide in subjects with relapsed/refractory mantle cell lymphoma: long-term follow-up analysis of the nhl-003 study [abstract]. Blood 120:2738
44. Wang M, Fayad L, Wagner-Bartak N et al (2012) Lenalidomide in combination with rituximab for patients with relapsed or refractory mantle-cell lymphoma: a phase 1/2 clinical trial. Lancet Oncol 13:716–723
45. Fisher RI, Bernstein SH, Kahl BS et al (2006) Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle cell lymphoma. J Clin Oncol 24:4867–4874
46. Goy A, Bernstein SH, Kahl BS et al (2009) Bortezomib in patients with relapsed or refractory mantle cell lymphoma: updated time-to-event analysis of the multicenter phase 2 PINNACLE study. Ann Oncol 20:520–525
47. Rummel MJ, Al-Batran SE, Kim SZ et al (2005) Bendamustine plus rituximab is effective and has a favorable toxicity profile in the treatment of mantle cell and low-grade non-Hodgkin's lymphoma. J Clin Oncol 23:3383–3389
48. Robinson KS, Williams ME, van der Jagt RH, Cohen P, Herst JA, Tulpule A, Schwartzberg LS, Lemieux B, Cheson BD (2008) Phase II multicenter study of bendamustine plus rituximab in patients with relapsed indolent B-cell and mantle cell non-Hodgkin's lymphoma. J Clin Oncol 26:4473–4479
49. Friedberg JW, Vose JM, Kelly JL et al (2011) The combination of bendamustine, bortezomib, and rituximab for patients with relapsed/refractory indolent and mantle cell lymphoma. Blood 117:2807–2812
50. Till BG, Gooley TA, Crawford N et al (2008) Effect of remission status and induction chemotherapy regimen on outcome of autologous stem cell transplantation for mantle cell lymphoma. Leuk Lymphoma 49(6):1062–1073
51. Tam CS, Bassett R, Ledesma C et al (2009) Mature results of the M.D. Anderson Cancer Center risk-adapted transplantation strategy in mantle cell lymphoma. Blood 113:444–4152
52. Khouri IF, Lee MS, Saliba RM et al (2003) Nonablative allogeneic stem-cell transplantation for advanced/recurrent mantle-cell lymphoma. J Clin Oncol 21:4407–4412

53. Mari MB, Sandmaier BM, Storer BE et al (2004) Allogeneic hematopoietic cell transplantation after fludarabine and 2 Gy total body irradiation for relapsed and refractory mantle cell lymphoma. Blood 104:3535–3542
54. Ruan J, Martin P, Shah B et al (2013) Combination biologic therapy without chemotherapy as initial treatment for mantel cell lymphoma: multi-center phase II study of lenalidomide plus rituximab. Blood 21:247
55. Blum K (2012) A phase i trial of the Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib (PCI-32765), in combination with rituximab (R) and bendamustine in patients with relapsed/refractory non-Hodgkin's lymphoma (NHL). Blood, p 1643
56. Kahl BS, Spurgeon, SE, Furman RR et al (2013) Results of a phase I study of idelalisb, a PI3κδ inhibitor with relapsed or refractory mantle cell lymphoma (MCL). Blood Abstract85
57. Davids MS, Roberts AW, Anderson MA et al (2012) The BCL-2-specific BH3-mimetic ABT-199 (GDC-0199) is active and well-tolerated in patients with relapsed non-hodgkin lymphoma: interim results of a phase I study [abstract]. Blood 120:404

Management of Diffuse Large B-Cell Lymphoma (DLBCL)

Boris Kubuschok, Gerhard Held and Michael Pfreundschuh

Abstract

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma. While CHOP was the standard combination chemotherapy for 25 years, the incorporation of the CD20 antibody rituximab at the beginning of this century has considerably improved the outcome of all patients with DLBCL: Depending on the prognostic subgroup, only half to one-third of the patients die of their DLBCL compared to pre-rituximab era. Treatment is usually tailored according to the individual risk profile of a DLBCL patient according to the International Prognostic Index (IPI). Assignment of DLBCL according to the gene expression profile into DLBLC originating from a germinal center B cell (GC type) or from an activated B cell (ABC type) has provided novel insights into the pathogenesis of the respective DLBCL, identified molecules which are indispensable for the survival of the lymphoma cells and provided targets for novel "targeted therapies" drugs. Incorporating these new drugs into combination immunochemotherapy or substituting single drugs in the R-CHOP combination will result in even higher cure rates of and/or less toxicity for patients with DLBCL in the decade to come.

Keywords

Diffuse large B-cell lymphoma · Risk assessment · Chemotherapy · Immunotherapy · Review

B. Kubuschok · G. Held · M. Pfreundschuh (✉)
German High-Grade Non-Hodgkin-Lymphoma Study Group (DSHNHL), Department Internal Medicine I, Saarland University Medical School, 66421 Homburg, Saarland, Germany
e-mail: michael.pfreundschuh@uks.eu

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_11

Contents

1 Introduction

Diffuse large B-cell non-Hodgkin lymphoma (DLBCL) is a malignant proliferation of B lymphocytes and constitutes, depending on the geographic area, 30–60 % of non-Hodgkin lymphomas (NHL). The incidence increases with age and is 3–4/100,000/year [1] in Europe and approximately 4.68 cases per 100,000 per year in the USA. An estimated 10,000 deaths result from DLBCL annually in the USA. While there was a steadily increasing incidence at the end of the last century, the incidence appears to have plateau since [2]. DLBCL is usually aggressive, marked by rapidly growing tumors in lymph nodes, spleen, liver, bone marrow, or other organs [3]. According to the cell of origin (COO), the germinal center (GC) B-cell-like DLBCL subtype has a better prognosis than the activated B-cell (ABC)-like DLBCL [4].

2 Diagnosis

Histopathological diagnosis of DLBCL should only be made by an experienced hematopathologist based on a biopsy sample of sufficient size, i.e., a surgical specimen, excision lymph node, or extranodal tissue biopsy. Core biopsies are acceptable only in cases requiring immediate treatment, while fine-needle aspirates are not acceptable. Mandatory immunohistochemistry includes CD45, CD20, and CD3. Staining for Ki-67, BCL-2, and MYC should only be done in case of therapeutic consequences. While the demonstration of MYC and BCL-2 at the protein level has been shown to be associated with a bad outcome [5–7], no specific treatment options are generally available for these patients [8]. The same applies to DLBCL cases with breaks of the MYC gene [9–12], whereas the role of double-hit lymphomas as independent prognostic factor has recently been questioned [13–16] and might depend on the fusion partner of the MYC gene [17]. Fresh-frozen material for gene expression profiling and assignment to the ABC and GC subtype is recommended for clinical trials. The value of various immunohistochemical

algorithms for the determination of the COO continues to be debated, and there is only poor concordance between the different immunohistochemical algorithms commonly used [18]. The Lymphoma/Leukemia Molecular Profiling Project's Lymph2Cx (20 gene) assay, a parsimonious digital gene expression (NanoString)-based test for COO assignment in formalin-fixed paraffin-embedded tissue (FFPET) holds promise as a reliable FFPE-derived surrogate for gene expression profiling (GEP) with $a > 95$ % concordance of COO assignment between 2 independent laboratories [19]. The histological report should give the diagnosis according to the current World Health Organization classification [20].

3 Staging and Risk Assessment

Staging procedures consist of a complete blood count, routine blood chemistry including lactate dehydrogenase (LDH) and ß2 microglobulin. Screening for human immunodeficiency virus and hepatitis B and C is required. Imaging must include CT scans of the neck, chest abdomen, and pelvis. Pre-treatment [18F]desoxyglucose positron emission tomography (PET) scanning is recommended because it facilitates the evaluation of response to treatment by a post-treatment PET. The latter is mandatory for the evaluation of the response to treatment according to the revised criteria [21] and will be obligatory in an update of the latter [22, 23]. Based on the imaging results, patients are assigned to stages I–IV according to the Ann Arbor system. For the assignment to one of the four (low, low-intermediate, high-intermediate, and high) risk groups according to the IPI [24], age (>60 years), LDH (elevated), the performance status (ECOG ≥ 2 vs. 0, 1), and the number of extralymphatic sites (≥2) of involvement must be known (Table 1). Recently, an "enhanced" IPI (NCCN-IPI) was suggested, with statistical efforts to further refine the categorization of age and normalized LDH. The same 5 predictors (age, LDH, sites of involvement, Ann Arbor stage, and ECOG performance status) as in the IPI were identified and a maximum of 8 points assigned [25] (Table 2); however, only extranodal involvement of bone marrow, CNS, gastrointestinal tract/liver, and lung is considered as a risk factor. Similar to the IPI, four prognostic groups were suggested (low risk = 0, 1; low-intermediate risk = 2–3; high intermediate = 4–5; high = 6–8 points). Compared with the IPI, the NCCN-IPI better discriminates low- and high-risk subgroups, in particular between the high-intermediate and high-risk groups (Table 3). Whether the NCCN-IPI will substitute the classical IPI remains to be seen and will depend on whether it can be confirmed by the datasets of prospective trials [25].

Table 1 Risk assignment according to the International Prognostic Index (IPI) [24]

Prognostic group	# risk factors[a]
Low risk	0, 1
Low intermediate	2
High intermediate	3
High	4, 5

[a]elevated LDH, advanced stage (Ann Arbor III/IV), age > 60, ≥ 2 extralymphatic sites of involvement

Table 2 Scoring system according to the NCCN-IPI [25]

NCCN-IPI	Score
Age (years)	
41–60	1
61–75	2
>75	3
LDH, normalized	
>1 to ≤3	1
>3	2
Extranodal disease[a]	1
Performance status ≥2	1

[a]Bone marrow, CNS, GI tract/liver, and lung

Table 3 Comparison of NCCN-IPI and IPI (adapted from [25])

	Score		5-y OS		5-y PFS	
	NCCN-IPI	IPI	NCCN-IPI (%)	IPI (%)	NCCN-IPI (%)	IPI (%)
Low	0–1 (19 %)	0–1 (38 %)	96	90	91	95
Low intermed.	2–3 (42 %)	2 (26 %)	82	77	74	66
High intermed.	4–5 (31 %)	3 (22 %)	64	62	51	52
High	>6 (8 %)	4–5 (14 %)	33	54	30	39

4 Treatment

Most cooperative groups tailor treatment strategies according to age and age-adapted IPI. In patients with high tumor loads, a so-called pre-phase treatment with prednisone (100 mg/d over several days up to 1 week) [26] is recommended to prevent tumor lysis syndrome and ameliorates the so-called first cycle effect, i.e., the phenomenon that side effects, in particular myelosuppression, are most pronounced after the first chemotherapy cycle. G-CSF should be given to all elderly patients and younger patients at risk for febrile neutropenias, treatment delays, and/or dose reductions.

4.1 Young Low-Risk Patients (aaIPI = 0) Without Bulky Disease

Six cycles of chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone combined with six doses of rituximab given every 21 days represent the current standard treatment for these patients [27, 28]. Results achieved with

6 × R-CHOP-21 combination in the MInT study [29] are so excellent (6-year PFS: 93 %, 6-year OS: 100 %) that there is no room for additive radiotherapy or any other treatment intensification (Fig. 1). Whether 4 cycles of R-CHOP-21 are sufficient in patients with a negative PET after 3 R-CHOP-21, as suggested by a retrospective register study of 50 patients, which was published in abstract form only already several years ago [30], remains to be confirmed. The DSHNHL is currently conducting the FLYER study which randomizes young patients with favorable prognosis (no risk factor, no bulky disease) into the MInT standard of 6 × CHOP-21 versus 4 × CHOP-21 each in combination with 6 administrations of rituximab every 3 weeks, to determine whether the number of CHOP-21 cycles can indeed be reduced in this favorable subgroup.

Based on the results of a phase II study with only 60 patients, a different approach to these patients is popular in North America, consisting of 3 × CHOP-21 in combination with 4 applications of rituximab followed by involved-field radiotherapy [31]. However, in contrast to the MInT results, no plateau has been observed for patients treated according to this strategy, indicating that this abbreviated chemoimmunotherapy is insufficient to eradicate the malignant clone. Interestingly, the addition of only 4 administrations of rituximab resulted in only a small improvement compared to a historical control, suggesting that this short exposure to the CD20 antibody does not exploit the full potential of this drug [31].

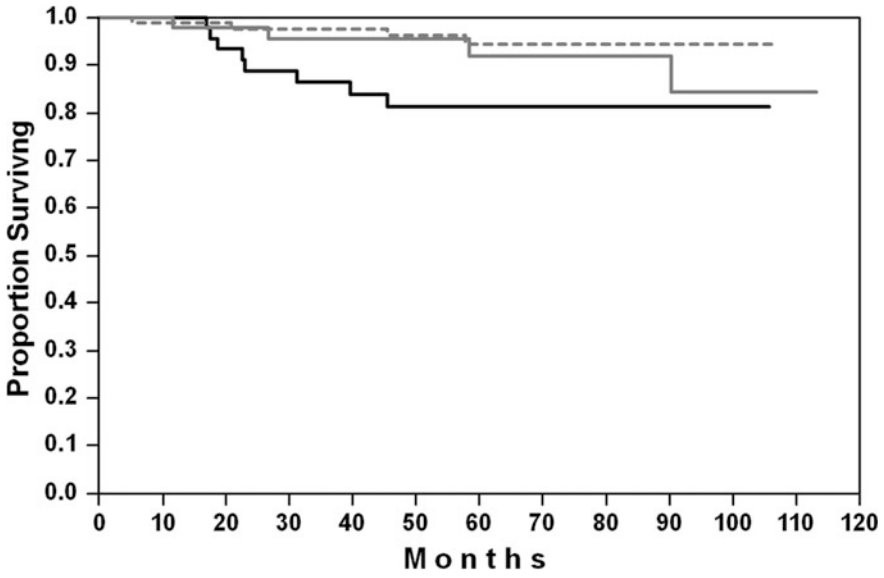


Fig. 1 Overall survival of "very favorable young patients" (no bulky disease, aaIPI = 0) in the MInT study [29]. *Black solid curve* CHOP-21; *gray solid curve* CHOEP-21; *dotted line* R-CHO(E)P-21

4.2 Young Low-Risk Patients (aaIPI = 0) with Bulky Disease and Low-Intermediate Risk (aaIPI = 1)

Bulky disease was a strong prognosticator in the MInT study [27, 29], despite the fact that patients in that study received additive radiotherapy to areas of primary bulky disease after immunochemotherapy. For CHOP-like treatment and rituximab plus radiotherapy to bulky disease, a cutoff point of 10 cm maximum tumor diameter separated two populations with a significant EFS difference, but any cutoff point of 6 cm or more separated two populations with a significant OS difference. Even though, a bulk definition as a mass with >10 cm maximal tumor diameter is applied by many cooperative groups, it must be assumed that the cutoff points for bulky disease not treated with additive radiotherapy will be even smaller than the 10 and 6 cm for EFS and OS, respectively.

Young low-intermediate risk patients (aaIPI = 1) or IPI low-risk (aaIPI = 0) patients with bulky disease have a less favorable outcome. While the overall survival of this group was ≈90 % in the MInT study, the EFS was roughly 75 % (Fig. 2), necessitating salvage treatment for about a quarter of this young population which usually consists of salvage chemotherapy followed by ASCT, with all its

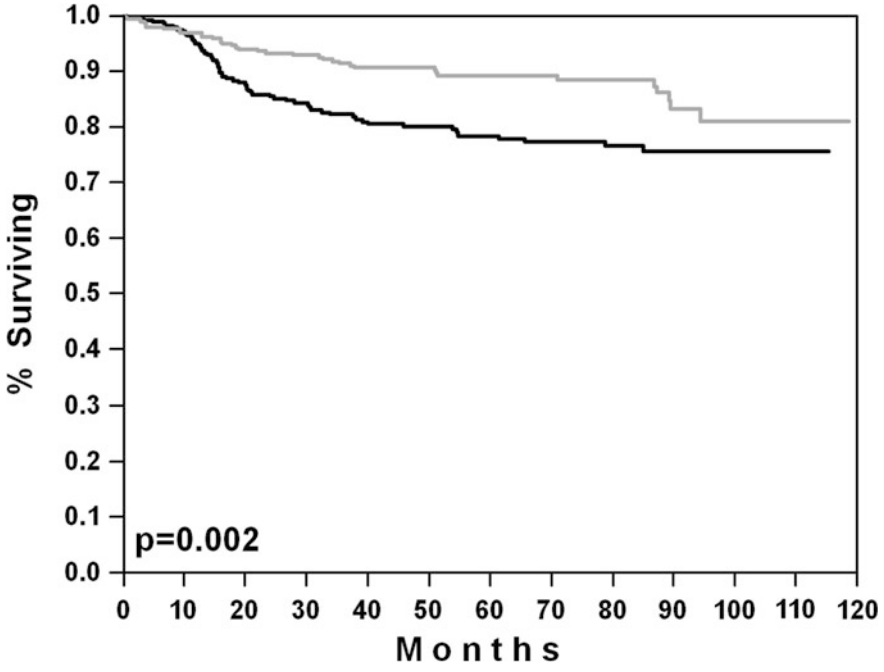


Fig. 2 Overall survival of "less favorable young patients" (aaIPI = 1; aaIPI = 0 with bulky disease) in the MInT study [29]. *Black curve* CHOP-like chemotherapy (n = 302); *gray curve* CHOP-like chemotherapy plus rituximab (n = 312)

well-known acute and long-term toxicity. In order to improve outcome of this subgroup, the GELA NHL 03-2B study compared 8 cycles of R-CHOP-21 with the R-ACVBP-14 program (an R-CHOP-14 variant which includes consolidation with high-dose methotrexate, ifosfamide, and high-dose cytarabine). The R-ACVBP-14 program was significantly better with respect to 3-year PFS (87 % vs. 73 %; $p = 0.0015$) and OS (92 % vs. 84 %; $p = 0.0071$) in this population [32]. However, the R-ACVBP-14 program was also significantly more toxic than R-CHOP-21, with 38 % of the R-ACVBP-14 patients experiencing neutropenic fever compared to only 9 % with R-CHOP-21. Interestingly, in a (historical) comparison of this well-defined subgroup of young patients with one risk factor according to the age-adjusted IPI in the MInT and NHL 03-2B studies, 6 × R-CHOP-21 in MInT yielded considerably better results than 8 × R-CHOP-21 in the French trial and indeed appears to be as good as the more toxic R-ACVBP-14. The only plausible explanation for this paradox is that the French abandoned radiotherapy from their studies after the advent of rituximab, while the patients with bulky disease and/or extralymphatic involvement in the MInT study had received radiotherapy to the respective areas.

While the comparison between MInT and NHL 03-2B was the first evidence that radiotherapy to bulky disease has still a value in the rituximab era, this assumption has recently been supported by the interim results of the randomized UNFOLDER study of the DSHNHL, which compares 6 × R-CHOP-21 with 6 × R-CHOP-14 and radiotherapy to areas of bulky and extralymphatic disease with observation in a 2 × 2 factorial design. A planned interim analysis with 285 patients revealed a highly significantly better 3-year EFS of patients randomized to radiotherapy (81 % vs. 64 %; $p = 0.004$); this difference was above the preset stopping rule ($p = 0.008$) and made the DSMC to order the early closure of the two arms of the UNFOLDER study without radiotherapy. Accordingly, the current ESMO guidelines recommend either the R-ACVBP-14 program or 6 cycles of R-CHOP-21 with radiotherapy to bulky disease for this subgroup of young DLBCL patients [28].

4.3 Primary Mediastinal B-Cell Lymphoma (PMBCL)

Most patients with PMBCL also fall into the subgroup of young patients with one aaIPI risk factor and/or bulky disease. A small study of only 48 PMBCL patients treated with dose-adjusted infusional EPOCH-R without radiotherapy made it into the New England Journal of Medicine reporting a 5-year EFS of 93 % and 5-year OS of 97 % (without showing any confidence intervals) [33]. Since PMBCL patients are recruited for the UNFOLDER study, the DSMC ordered an interim analysis of these patients. It showed a 3-year overall survival rate of 100 % in the 69 PMBCL patients evaluated thus far in the UNFOLDER study, of whom half had received either R-CHOP-21 or R-CHOP-14, and half radiotherapy to bulky disease or not. This demonstrates that excellent results can be luckily achieved if the number of patients included in a study is only small enough. Because of the

excellent outcome of PMBCL patients with any one of four different strategies in UNFOLDER study, they keep on being recruited to this randomized trial and we see no reason for an intensified chemotherapy regimen such as DA-EPOCH-R for these patients.

4.4 Young High and High-Intermediate Risk Patients (aaIPI ≥ 2)

The first formal proof that rituximab improves also the outcome of this DLBCL subgroup came from the Mega-CHOEP study of the DSHNHL [34]. The Mega-CHOEP study randomized young poor-prognosis (aaIPI = 2–3) patients into 8 cycles of dose-DENSE-R-CHOP-14 (CHOP plus 100 mg/m^2 etoposide d1–3) or one cycle of dose-escalated "midi-R-CHOEP" followed by 3 cycles of high-dose ("mega") R-CHOEP, each necessitating autologous stem cell support. Originally, the Mega-CHOEP study had a second randomization with and without rituximab, but this randomization was stopped when the MInT study demonstrated the efficacy of rituximab in young DLBCL patients. By that time, 31 patients had been randomized not to receive rituximab. Despite of this small number of patients, the difference in 3-year EFS of nearly 30 % (37 % vs. 66 %) was highly significant ($p < 0.001$) in favor of patients who had received rituximab and the two arms without rituximab were closed (Fig. 3). In Mega-CHOEP patients receiving rituximab, there was no significant difference with respect to 3-year EFS (69.5 % vs. 61.4 %; $p = 0.14$), PFS (73.7 % vs. 69.8 %; $p = 0.48$), or OS (84.6 % vs. 77.0 %; $p = 0.08$) between 130 patients randomized to R-CHOEP-14 and 132 patients randomized to the triple-transplant R-Mega-CHOEP program. Patients with age-adjusted IPI = 2 had a significantly better event-free survival (75.5 % vs. 63.5 %; $p = 0.0509$) and overall survival (91.0 % vs. 77.1 %; $p = 0.01$) if treated with R-CHOEP-14, demonstrating that aaIPI = 2 patients have a high cure rate and do not really belong to a "poor-prognosis" subgroup anymore. In contrast, in patients with aaIPI = 3, where no differences were observed between R-CHOEP-14 and the triple-transplant Mega-CHOEP with a 3-year OS of around 75 %, there is still room for improvement.

Two other studies, so far published only in abstract form, also addressed the role of high-dose chemotherapy and stem cell transplantation in the rituximab era. The French study [35] did not find any advantage of an intensified strategy including high-dose BEAM and stem cell support compared to R-CHOP-14, while R-CHOP-14 followed by a consolidation with 2 cycles of MAD (mitoxantrone, high-dose cytarabine, and dexamethasone) and myeloablative therapy with BEAM was superior to R-CHOP-14 in an Italian study [36] with respect to PFS, but not to OS. This led the authors conclude that high-dose chemotherapy and stem cell transplantation should not be part of a first-line therapy for young poor-prognosis patients in the rituximab era.

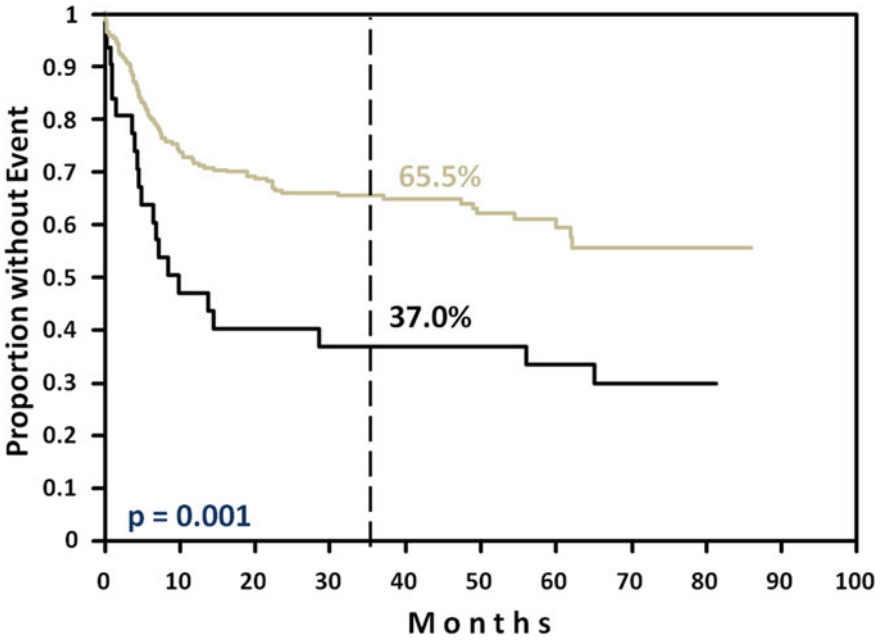


Fig. 3 Event-free survival of young poor-prognosis patients (aaIPI = 2, 3) in the Mega-CHOEP trial [34]. *Black curve* CHOEP-14/Mega-CHOEP (n = 31); *gray curve* R-CHOEP-14/R-Mega-CHOEP (n = 262)

The SWOG-9704 study [37] also addressed the role of ASCT in the first-line treatment of young poor-prognosis patients. Patients responding to 5 cycles of (R)-CHOP-21 were randomized to receive either 3 additional CHOP-21 cycles or 1 CHOP-21 followed by ASCT after induction with high-dose BEAM or total body irradiation. One-third of the patients were treated before 2005 and did not receive rituximab. Of 370 induction-eligible patients, only 125 were randomly assigned to the transplantation group and 128 to the control group. 2-year PFS rates were 69 and 55 % ($p = 0.005$), and 2-year OS rates were 74 and 71 %, respectively ($p = 0.30$). The 2-year PFS and OS results in both arms of the SWOG study are considerably worse than the 3-year results of any of the two arms of the Mega-CHOEP [34] or the Italian study [36], despite the fact that the survival curves of only those 253/370 patients from the SWOG trial who made it into the randomization are reported, and no results for the overall population recruited to the trial are shown. This means that the respective PFS and OS rates of all 370 patients (including those progressing during the first CHOP-21 or not achieving a response) in the SWOG trial are even considerably worse. The only merit of the SWOG study is that it is the first to evaluate R-CHOP-21 for young poor-prognosis patients in a prospective fashion, and the only lesson that can be learnt from this trial is that R-CHOP-21 is unacceptably ineffective for these patients. Having selected an unacceptably inefficacious

comparator arm, all conclusions made by the authors in their publication with respect to the role of ASCT in the rituximab era must be declined.

In summary, while there is no generally accepted standard for young poor-prognosis patients, the results obtained with 8 × R-CHOEP-14 are the best reported to date for this population. This is also supported by a comparison of R-CHOP-14 in the Italian [36] and R-CHOEP-14 in the DSHNHL trial [34] presented at ASH 2013 [38]: Patients treated with R-CHOP-14 had a 3-year PFS of 63 % compared to 74 % of those treated with R-CHOEP-14. In a Cox model, for PFS including treatment arm, age, gender, aaIPI, extranodal sites, bulky disease, and BM involvement, the adjusted HR was 0.68 in favor of R-CHOEP-14, but due to the limited number of patients, this difference was not significant ($p = 0.128$). Moreover, a retrospective register study from Scandinavia [39] also found a significantly better outcome of young poor-prognosis patients treated with R-CHOEP-14 compared to R-CHOP-14.

For aaIPI = 3 patients, treatment within prospective trials is recommended. R-CHOEP-14 toxicity leaves room for additional drugs for these patients. Since aaIPI = 3 patients make up only roughly 15 % of all young patients, international cooperative efforts are necessary to evaluate innovative concepts within acceptable time frames.

4.5 Patients Aged 60–80 Years

Eight cycles of combination chemotherapy with R-CHOP-21 is the most widely used standard for these patients. Six cycles of R-CHOP-14 plus 2 additional administrations of rituximab or eight cycles of R-CHOP-14 were not superior to 8 cycles of R-CHOP-21 in two prospective randomized studies [16, 40], of which the British trial included all DLBCL patients up to 80 years, while the French included only patients 61–80 years of age. In particular, the French study did not stick to the supportive measures recommended for R-CHOP-14 in elderly patients with DLBCL [26, 41]. This resulted in an unacceptably high therapy-associated death rate of 9 % in the first 100 patients receiving R-CHOP-14 which went down to 2.5 % in the last 200 patients treated with R-CHOP-14 in this trial, indicating a steep learning curve for R-CHOP-14 among the participants of this trial. It can only be speculated on how the results of this randomized study would have looked like if the first 100 R-CHOP-14 had been treated with state-of-the-art supportive measures. With respect to toxicity, no clinically relevant differences were observed between R-CHOP-14 and R-CHOP-21; this was also the case in the British study [16], which included DLBCL patients of any age and IPI. Based on the confirmed equal efficacy and toxicity of R-CHOP-21 and R-CHOP-14, respectively, the 2012 ESMO guidelines recommend either 8 × R-CHOP-21 or 6 × R-CHOP-14 + 2R for DLBCL patients between 61 and 80 years of age. It should be emphasized here that there are no prospective data on 6 × R-CHOP-21 in elderly patients, and in the absence of the latter, we discourage the use of 6 × R-CHOP-21 for elderly DLBCL

patients outside clinical trials. R-CHOP-14 has the advantage of a shorter time under chemotherapy (10 weeks compared to 21 weeks with 8 × R-CHOP-21) and therefore is standard for elderly patients in several countries worldwide. 6 × R-CHOP-14 has also the advantage of giving 25 % less doses of doxorubicin and the other cytotoxic drugs, which should have an impact on the rates of cardiotoxicity and second neoplasms with longer follow-up.

Radiotherapy to bulky disease is also recommended for this elderly population of DLBCL patients, based on the results of a prospective trial with 164 patients [42], the RICOVER-NoRTH study. A historical comparison with the RICOVER-60 study revealed in a multivariable analysis of patients treated per protocol a hazard ratio of 2.7 ($p = 0.011$) for EFS, 4.4 ($p = 0.001$) for PFS, and 4.3 ($p = 0.002$) for OS for patients not receiving RT to bulky disease. As long as appropriately designed prospective studies do not demonstrate that radiotherapy can be abandoned in cases with a negative PET after immunochemotherapy, elderly patients should receive radiotherapy to sites of bulky disease outside clinical trials. Radiotherapy is also recommended to sites of bone involvement by DLBCL, because in contrast to radiotherapy rituximab did not improve outcome of patients with skeletal involvement [43].

4.6 Patients >80 Years of Age

Incidence and severity of frank pathologic dysfunction or comorbidity increase with age and the association of comorbidity and survival has been demonstrated by Charlson et al. [44] who showed that comorbidities are independent predictors of survival. Comorbidities and polymedications for the treatment thereof can further compromise the tolerability of the lymphoma therapy. Therefore, in order to objectify the individual patient's risk, a geriatric assessment is mandatory before making any treatment decisions. Functional scales, such as the Eastern Cooperative Oncology Performance Scale, may underestimate or miss problems that are perceived using geriatric-specific assessments. Useful scores based on self-reported measures are the ability to complete activities of daily living (ADLs), instrumental ADLs, and basic performance tests (e.g., gait speed and the "get-up and go" test). Comorbidities and their functional consequence can be measured by the Cumulative Illness Rating Scale (CIRS) [45].

The published literature on the treatment of very old patients is scarce, despite the fact that this is the fastest growing subgroup of patients with DLBCL. Full-dose R-CHOP treatment can usually be given only to selected fit patients >80 years of age. Rituximab in combination with dose-reduced CHOP ("R-miniCHOP") achieved encouraging results in this population [46], even though the population included in this trial was not really representative for everyday octogenarians. The fact that myelotoxicity and therapy-associated deaths, in particular after the first cycle, were considerable despite the significant dose reductions in the "R-miniCHOP" protocol underlines the importance of optimized supportive measures in

this population, first and foremost the so-called pre-phase treatment with oral prednisone as discussed above and anti-infective prophylaxis including aciclovir and cotrimoxazole [47].

If the patient's cardiac function prohibits the use of doxorubicin, doxorubicin can be substituted by gemcitabine [48]; alternatively, the well-tolerated combination of rituximab with gemcitabine and oxaliplatin (R-GemOx), originally designed for elderly patients with relapsed DLBCL [49], can be given with good tolerability and results, while the combination of rituximab with bendamustine appears to be less effective [50].

Because of the limited available data on very old patients and hence the lack of a commonly accepted standard treatments, these patients should be treated within prospective trials whenever possible and cooperative study groups are encouraged to design studies that appropriately address the specific problems of this population.

5 Perspectives

Besides numerous new drugs for DLBCL that have entered early clinical trials, further improvement of outcome of patients with DLBCL appears to be possible with a more intelligent use of the drugs available for the treatment of DLBCL. Recent studies provide evidence that similar to elderly male patients, both young male and female patients have an unfavorable pharmacokinetics compared to elderly female patients due to a faster clearance and shorter half-life of rituximab, strongly suggesting that the majority of DLBCL patients are underdosed when rituximab is given at 375 mg/m^2 synchronously with CHOP every three and even more so when given every 2 weeks, due to an even shorter rituximab exposure time [51, 52]. Indeed, the unfavorable rituximab pharmacokinetics of elderly males compared to elderly females appears to be responsible for the significantly increased outcome hazard of the former when treated with rituximab, which was not observed in elderly patients treated without rituximab [51, 52]. In the phase II DENSE-R-CHOP-14 trial, four additional rituximab applications were given during the first 3 weeks of 6 × R-CHOP-14. This resulted in significantly higher rituximab serum levels compared to eight 2-week application; however, these higher serum levels were associated with an increased toxicity (infections, in particular interstitial pneumonitis), while the outcome of the patients was not significantly improved [47].

The SEXIE-R-CHOP-14 study of the DSHNHL was a prospective phase II trial where female patients received the standard of eight doses of 375 mg/m^2 in combination with 6 × R-CHOP-14, while male patients received the increased dose 500 mg/m^2. With this increased dose, the outcome of elderly male patients improved considerably: 3-year PFS was 74 % in males and 68 % in females (p = 0.396); 3-year OS was 80 % in males and 72 % in females (p = 0.111), demonstrating that the outcome of elderly males can be improved by increasing the rituximab dose, thus eliminating male sex as a risk factor in elderly DLBCL patients [53]. That an increased rituximab dose significantly improves outcome not only of

elderly male patients, but also of young male and female patients who have a rituximab pharmacokinetics similar to elderly male patients should be confirmed in a larger randomized study.

Another approach to improve the efficacy of rituximab was pursued in the SMARTE-R-CHOP-14 trial, a phase II study of the DSHNHL with 189 elderly DLBCL patients. In this study, 8 applications of rituximab (375 mg/m^2) were given on days −4, 0, 10, 29, 57, 99, 155, and 239 in combination with 6 cycles of CHOP-14. This extended rituximab exposure time resulted in a significant improved outcome for high-risk patients (3-year OS 80 % compared to 67 % in RICOVER-60), which was most pronounced in elderly poor-prognosis males with their fast rituximab clearance who benefited most from the prolonged rituximab exposure in the SMARTE-R protocol [54] with a 20 % better 3-year OS (80 % vs. 60 %) compared to the same population in RICOVER-60. The results observed in the SMARTE-R-CHOP-14 study are by far the best reported to date for elderly patients with poor-prognosis DLBCL (Fig. 4). SMARTE-R-CHOP-14 also suggests that a minimum exposure time to rituximab is more important than peak and trough serum level and can serve as an explanation (besides others) why R-CHOP-14 was not superior to R-CHOP-21 in two previously discussed French and British randomized trials [16, 40], despite the fact that without rituximab CHOP-14 had been shown to be superior to CHOP-21 [26]: Obviously, CHOP-14, the more effective

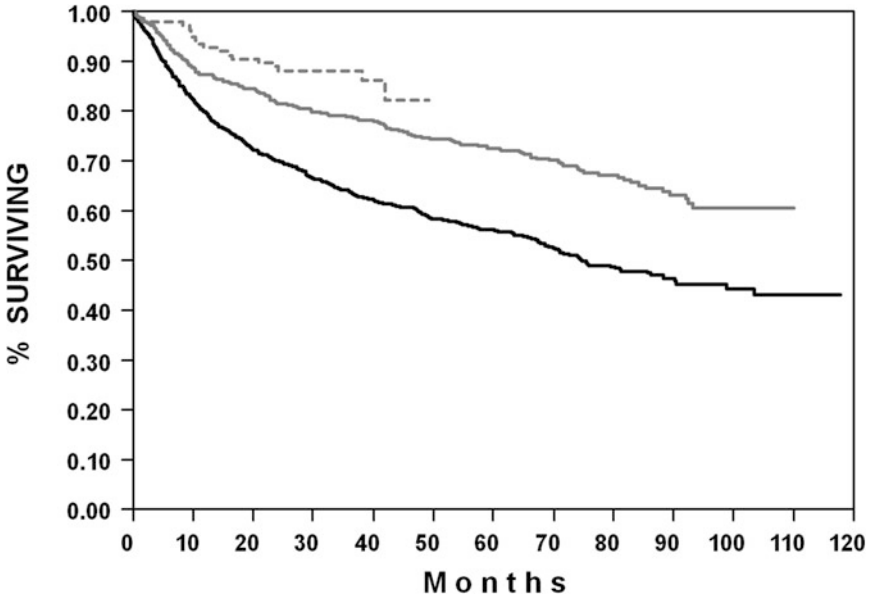


Fig. 4 Overall survival of elderly (61–80 year old) DLBCL patients. *Black solid curve* CHOP-14/CHOP-21 (n = 943) from the NHL-B2 [26] and the RICOVER-60 study [41]; *gray solid curve* R-CHOP-14 (n = 546) from the RICOVER [41], RICOVER-NoRTH [42], and Pegfilgrastim [63] studies; *dotted curve* SMARTE-R-CHOP-14 (n = 134) [54] study

chemotherapy, is compromised by too short an antibody exposure time when combined and synchronized with rituximab (last application of 8 rituximab every 2 weeks: day 99, every 3 weeks: day 148).

Another promising strategy to improve the efficacy of rituximab might be vitamin D substitution. Vitamin D deficiency (<8 ng/ml) was associated with a significantly worse outcome in the RICOVER-60 trial in patients treated with, but not in patients treated without rituximab, and the improvement (3-year EFS) achieved by the addition of rituximab was considerably greater in patients with higher vitamin D levels (31 %) compared to patients with vitamin D levels <8 ng/ml (16 %) [55]. This can be explained by the observation that vitamin D deficiency impairs NK-cell activity and rituximab-dependent cellular cytotoxicity (RDCC) against the $CD20^+$ B-cell lymphoma line Daudi. Notably, RDCC is the major mechanism of action of rituximab. That vitamin D substitution significantly increased RDCC in vitamin D-deficient individuals in vitro indicates that vitamin D substitution might increase rituximab efficacy in vivo and thus improve outcome of DLBCL patients with vitamin D deficiency. This hypothesis is prospectively addressed in the ongoing OPTIMAL >60 trial for elderly DLBCL patients.

Besides optimizing rituximab, many more approaches hold the promise of improving outcome of DLBCL patients in the foreseeable future. Most promising appears ibrutinib [56], which was reported to have a preferential effect on the ABC type of DLBCL, while the BCL-2 inhibitor ABT199 might be more effective in the GC type of DLBCL [57]. The phosphoinositol-3 kinase-δ inhibitor idelalisib which has recently been licensed for the treatment of relapsed CLL, indolent, and mantle cell lymphomas [58–60] also showed encouraging results in early clinical studies with DLBLC [61, 62]. Indeed, so many new "small molecules" are available for clinical testing that it is difficult to find enough appropriate patients for the respective clinical studies. Therefore, as many patients with (relapsing) DLBCL as possible should be treated within adequately designed trials with these new drugs, in order to define the role of these new drugs for the treatment of DLBCL in the future.

Acknowledgments This work was supported in part by Deutsche Krebshilfe, a charity organization.

References

1. Morgan G, Vornanen M, Puitinen J et al (1997) Changing trends in the incidence of non-Hodgkin's lymphoma in Europe. Ann Oncol 8(2):49–54
2. Shiels MS, Engels EA, Linet MS et al (2013) The epidemic of non-Hodgkin lymphoma in the United States: disentangling the effect of HIV, 1992-2009. Cancer Epidemiol Biomarkers Prev 22:1069–1078
3. Coiffier B (2001) Diffuse large cell lymphoma. Curr Opin Oncol 13:325–334
4. Lossos IS (2005) Molecular pathogenesis of diffuse large B-cell lymphoma. J Clin Oncol 23:6351–6357

5. Green TM, Young KH, Visco C et al (2012) Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 30:3460–3467
6. Johnson NA, Slack GW, Savage KJ et al (2012) Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 30:3452–3459
7. Horn H, Ziepert M, Becher C et al (2013) MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood 121:2253–2263
8. Dunleavy K, Wayne AS, Shovlin M et al (2011) MYC+aggressive B-cell lymphomas: novel therapy for untreated Burktit lymphoma and MYC+diffuse large B-cell lymphoma with DA-EPOCH-R. Ann Oncol 22:iv106
9. Barrans S, Crouch S, Smith A et al (2010) Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. J Clin Oncol 28:3360–3365
10. Cuccuini W, Briere J, Mounier N et al (2012) MYC+diffuse large B-cell lymphoma is not salvaged by classical R-ICE or R-DHAP followed by BEAM plus autologous stem cell transplantation. Blood 119:4619–4624
11. Dave SS, Fu K, Wright GW et al (2006) Molecular diagnosis of Burkitt's lymphoma. N Engl J Med 354:2431–2442
12. Klapper W, Stoecklein H, Zeynalova S et al (2008) Structural aberrations affecting the MYC locus indicate a poor prognosis independent of clinical risk factors in diffuse large B-cell lymphomas treated within randomized trials of the German high-grade non-hodgkin's lymphoma study group (DSHNHL). Leukemia 22:2226–2229
13. Aukema SM, Siebert R, Schuuring E et al (2011) Double-hit B-cell lymphomas. Blood 117:2319–2331
14. Copie-Bergman C, Culliere-Dartigue P, Baia M et al (2012) MYC gene simple hit is a strong independent predictive factor of survival in diffuse large B-Cell lymphomas in contrast to MYC double-hit gene alterations: A study by the Groupe d'Etude Des Lymphomes De l'Adulte. Blood 120:541
15. Kobayashi T, Tsutsumi Y, Sakamoto N et al (2012) Double-hit lymphomas constitute a highly aggressive subgroup in diffuse large B-cell lymphomas in the era of rituximab. Jpn J Clin Oncol 42:1035–1042
16. Cunningham D, Hawkes EA, Jack A et al (2013) Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: a phase 3 comparison of dose intensification with 14-day versus 21-day cycles. Lancet 381:1817–1826
17. Ølgod Pedersen M, Ortved Gang A, Svenstrup Poulsen T et al (2012) MYC translocation partner gene determines survival in large B-cell lymphoma with MYC- or double hit MYC/BCL2 translocation. Blood 120:542
18. Coutinho R, Clear AJ, Owen A et al (2013) Poor concordance among nine immunohistochemistry classifiers of cell-of-origin for diffuse large B-cell lymphoma: implications for therapeutic strategies. Clin Cancer Res 19:6686–6695
19. Scott DW, Wright GW, Williams PM et al (2014) Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue. Blood 123:1214–1217
20. WHO (2008) WHO classification of tumours of haematopoietic and lymphoid tissues. WHO Press, Geneva
21. Cheson BD, Pfistner B, Juweid ME et al (2007) Revised response criteria for malignant lymphoma. J Clin Oncol 25:579–586
22. Cheson BD, Fisher RI, Barrington SF et al (2014) Recommendations for initial evaluation, staging, and response assessment of hodgkin and non-hodgkin lymphoma: the lugano classification. J Clin Oncol 32:3059–3068

23. Barrington SF, Mikhaeel NG, Kostakoglu L et al (2014) Role of imaging in the staging and response assessment of lymphoma: consensus of the international conference on malignant lymphomas imaging working group. J Clin Oncol 32:3048–3058
24. The International Non-Hodgkin's Lymphoma Prognostic Factors Project (1993) A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 329:987–994
25. Zhou Z, Sehn LH, Rademaker AW et al (2014) An enhanced international prognostic index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood 123:837–842
26. Pfreundschuh M, Trumper L, Kloess M et al (2004) Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of elderly patients with aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. Blood 104:634–641
27. Pfreundschuh M, Truemper L, Osterborg A et al (2006) CHOP-like chemotherapy plus rituximab compared with CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large B-cell lymphoma: a randomized controlled trial by the mabthera international trial (MInT) group. Lancet Oncol 7:379–391
28. Ghielmini M, Vitolo U, Kimby E et al (2013) ESMO guidelines consensus conference on malignant lymphoma 2011 part 1: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL). Ann Oncol 24:561–576
29. Pfreundschuh M, Kuhnt E, Trumper L et al (2011) CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the mabthera international trial (MInT) group. Lancet Oncol 12:1013–1022
30. Sehn LH, Savage KJ, Hoskins P et al (2007) Limited-stage diffuse large B-cell lymphoma patients with a negative PET scan following three cycles of R-CHOP can be effectively treated with abbreviated chemoimmunotherapy alone. Blood 110:242 abstract
31. Persky DO, Unger JM, Spier CM et al (2008) Phase II study of rituximab plus three cycles of CHOP and involved-field radiotherapy for patients with limited-stage aggressive B-cell lymphoma: Southwest Oncology Group study 0014. J Clin Oncol 26:2258–2263
32. Recher C, Coiffier B, Haioun C et al (2011) Intensified chemotherapy with ACVBP plus rituximab versus standard CHOP plus rituximab for the treatment of diffuse large B-cell lymphoma (LNH03-2B): an open-label randomised phase 3 trial. Lancet 378:1858–1867
33. Dunleavy K, Pittaluga S, Maeda LS et al (2013) Dose-adjusted EPOCH-rituximab therapy in primary mediastinal B-cell lymphoma. N Engl J Med 368:1408–1416
34. Schmitz N, Nickelsen M, Ziepert M et al (2012) Conventional chemotherapy (CHOEP-14) with rituximab or high-dose chemotherapy (MegaCHOEP) with rituximab for young, high-risk patients with aggressive B-cell lymphoma: an open-label, randomised, phase 3 trial (DSHNHL 2002-1). Lancet Oncol 13:1250–1259
35. Le Gouill S, Milpied N, Lamy T et al (2011) First-line rituximab (R) high-dose therapy (R-HDT) versus R-CHOP-14 for young adults with diffuse large B-cell lymphoma: preliminary results of the GOELAMS 075 prospective multicenter randomized trial. J Cliln Oncol 29:540 S
36. Vitolo U, Chiapella A, Brusamolino E et al (2012) Rituximab dose-dense chemotherapy followed by intensified high-dose chemotherapy and autologous stem cell transplantation (HDC+ASCT) significantly reduces the risk of progression compared to standard rituximab dose-dense chemotherapy as first line treatment in young patients with high-risk (aaIPI 2-3) diffuse large B-cell lymphoma: final results of phase III randomized trial DLCL04 of the fondazione Italiana linfomi. Blood 120:688
37. Stiff PJ, Unger JM, Cook JR et al (2013) Autologous transplantation as consolidation for aggressive non-Hodgkin's lymphoma. N Engl J Med 369:1681–1690
38. Vitolo U, Ziepert M, Glass B et al (2013) Comparison of R-CHOP-14 and R-CHOEP-14 as first line treatment in young patients with high-risk (aaIPI 2-3) diffuse large B-cell lymphoma (DLBCL): a joint analysis of 2 prospective phase III randomized trials conducted by the Fondazione Italiana Linfomi (FIL) and the german high-grade non-hodgkin lymphoma study group (DSHNHL). Blood 122:4341 (abstract)

39. Gang AO, Strom C, Pedersen M et al (2012) R-CHOEP-14 improves overall survival in young high-risk patients with diffuse large B-cell lymphoma compared with R-CHOP-14. A population-based investigation from the Danish lymphoma group. Ann Oncol 23:147–153
40. Delarue R, Tilly H, Mounier N et al (2013) Dose-dense rituximab-CHOP compared with standard rituximab-CHOP in elderly patients with diffuse large B-cell lymphoma (the LNH03-6B study): a randomised phase 3 trial. Lancet Oncol 14:525–533
41. Pfreundschuh M, Schubert J, Ziepert M et al (2008) Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+B-cell lymphomas: a randomised controlled trial (RICOVER-60). Lancet Oncol 9:105–116
42. Held G, Murawski N, Ziepert M et al (2014) Role of radiotherapy to bulky disease in elderly patients with aggressive B-cell lymphoma. J Clin Oncol 32:1112–1118
43. Held G, Zeynalova S, Murawski N et al (2013) Impact of rituximab and radiotherapy on outcome of patients with aggressive B-cell lymphoma and skeletal involvement. J Clin Oncol 31:4115–4122
44. Charlson ME, Pompei P, Ales KL, MacKenzie CR (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 40:373–383
45. Gosney MA (2005) Clinical assessment of elderly people with cancer. Lancet Oncol 6:790–797
46. Peyrade F, Jardin F, Thieblemont C et al (2011) Attenuated immunochemotherapy regimen (R-miniCHOP) in elderly patients older than 80 years with diffuse large B-cell lymphoma: a multicentre, single-arm, phase 2 trial. Lancet Oncol 12:460–468
47. Pfreundschuh M, Zeynalova S, Poeschel V et al (2008) Improved outcome of elderly patients with poor-prognosis diffuse large B-cell lymphoma (DLBCL) after dose-dense Rituximab: results of the DENSE-R-CHOP-14 trial of the German high-grade non-hodgkin lymphoma study group (DSHNHL). Ann Oncol 19:iv99
48. Fields PA, Townsend W, Webb A et al (2014) De novo treatment of diffuse large B-cell lymphoma with rituximab, cyclophosphamide, vincristine, gemcitabine, and prednisolone in patients with cardiac comorbidity: a United Kingdom national cancer research institute trial. J Clin Oncol 32:282–287
49. Mounier N, El GT, Tilly H et al (2013) Rituximab plus gemcitabine and oxaliplatin in patients with refractory/relapsed diffuse large B-cell lymphoma who are not candidates for high-dose therapy. A phase II Lymphoma Study Association trial. Haematol 98:1726–1731
50. Walter E, Schmitt T, Dietrich S, Ho A, Witzens-Harig M (2012) Rituximab and bendamustine in patients with CD20+diffuse large B-cell lymphoma not eligible for cyclophosphamide, doxorubicin, vincristine and prednisone-like chemotherapy. Leuk Lymphoma 53:2290–2292
51. Muller C, Murawski N, Wiesen MH et al (2012) The role of sex and weight on rituximab clearance and serum elimination half-life in elderly patients with DLBCL. Blood 119:3276–3284
52. Pfreundschuh M, Muller C, Zeynalova S et al (2014) Suboptimal dosing of rituximab in male and female patients with DLBCL. Blood 123:640–646
53. Pfreundschuh M, Poeschel V, Zeynalova S et al (2014) Increased rituximab doses eliminate increased risk of elderly male patients with aggressive $CD20^+$ B-cell lymphomas: results from the SEXIE-R-CHOP-14 trial of the DSHNHL. J Clin Oncol 32:8501 (abstract)
54. Pfreundschuh M, Poeschel V, Zeynalova S et al (2014) Optimization of rituximab for the treatment of DLBCL (II): extended rituximab exposure time in the SMARTE-R-CHOP-14 trial of the German High-Grade Non-Hodgkin Lymphoma Study Group. J Clin Oncol pii: JCO.2013.54.6861. [Epub ahead of print]
55. Bittenbring J, Neumann F, Altmann B et al (2014) Vitamin D deficiency impairs rituximab-mediated cellular cytotoxicity and outcome of DLBCL patients treated with, but not without rituximab. J Clin Oncol 32:3242–3248
56. Wilson WH, Gerecitano JF, Goy A et al (2012) The Bruton's tyrosine kinase inhibitor ibrutinib (PCI-32765) has preferential activity in the ABS subtype of relapsed/refractory de novo diffuse large B-cell lymphoma: interim results of a multicenter, open-label phase 2 study. Blood 120:686 abstract

57. Divids SM, Seymour JF, Gerecitano JF et al (2013) The single-agent Bcl-2 inhibitor ABT-199 (GDC-0199) in patients with relapsed/refractory (R/R) non-Hodgkin lymphoma (NHL): responses observed in all mantle cell lymphoma (MCL) patients. Blood 122:1789
58. Furman RR, Sharman JP, Coutre SE et al (2014) Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med 370:997–1007
59. Gopal AK, Kahl BS, de Vos S et al (2014) PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med 370:1008–1018
60. Lopez JP, Jimeno A (2014) Idelalisib for the treatment of indolent non-Hodgkin's lymphoma. Drugs Today (Barc) 50:113–120
61. Burger JA, Okkenhaug K (2014) Haematological cancer: Idelalisib-targeting PI3K delta in patients with B-cell malignancies. Nat Rev Clin Oncol 11:184–186
62. Fruman DA, Cantley LC (2014) Idelalisib–a PI3K delta inhibitor for B-cell cancers. N Engl J Med 370:1061–1062
63. Zwick C, Hartmann F, Zeynalova S et al (2011) Randomized comparison of pegfilgrastim day 4 versus day 2 for the prevention of chemotherapy-induced leukocytopenia. Ann Oncol 22:1872–1877

Current Management of Peripheral T-Cell Lymphomas

M. Gooptu, R. Rhoades and B. Pro

Abstract

Peripheral T-cell lymphomas (PTCLs) are an uncommon group of lymphoproliferative disorders accounting for approximately 10–15 % of all non-Hodgkin lymphomas (NHL) in Western countries. Although PTCLs are associated with poor prognosis, outcomes vary with disease subtype. The standard of care has been anthracycline-based induction combination chemotherapy, however, with the exception of low-risk ALK-positive anaplastic large cell lymphoma, relapse rates are high. Therefore, consolidation with autologous stem cell transplantation is usually recommended for patients deemed candidates, and with aggressive subtypes. In recent years, a number of novel agents including pralatrexate, histone deacetylase inhibitors, immunotoxins, proteasome inhibitors, aurora kinase inhibitors and the CD30 antibody-drug conjugate brentuximab vedotin, have shown promise in the treatment of PTCLs. Studies are underway to explore the activity of these newer agents used in the frontline setting.

Keywords

Lymphoma · T-cell lymphoma · Diagnosis · Treatment · Novel therapies

Contents

M. Gooptu · R. Rhoades · B. Pro (✉)
Thomas Jefferson University, 834 Chestnut Street, Suite 320,
Philadelphia, PA 19107, USA
e-mail: Barbara.Pro@jefferson.edu

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_12

1 Introduction

T-cell lymphomas may be broadly divided into those arising from precursor T cells and those arising from mature T cells, which are referred to as peripheral T-cell lymphomas or PTCLs [1]. PTCLs are a heterogenous group of lymphoproliferative disorders characterized by clonal expansion of a population of post-thymic T cells and NK cells [2], and in general, this group is marked by a poorer prognosis than its B-cell counterparts. The PTCLs are less common than B-cell non-Hodgkin's lymphomas (NHL), accounting for approximately 10–15 % of lymphoid malignancies in North America [1]. Consequently, large randomized clinical trials have been difficult, and treatment is often adapted from studies in B-cell neoplasms. However, these lymphomas demonstrate distinctive biology and molecular characteristics and, with the exception of the more indolent cutaneous T-cell lymphomas, are usually aggressive [1]. Prognosis remains poor due to poor response rates to frontline therapy, leading to a dismal overall and failure-free survival [3]. More recently, however, the landscape has begun to change, with a number of important new therapies developed in the last few years and a better understanding of the biology of the various subtypes of PTCL.

2 Definition and Classification

The classification of mature T-cell lymphomas has undergone a number of revisions. With the development of the Revised European-American Lymphoma (REAL) classification, T-cell lymphomas were recognized as a distinct entity for the first time [4]. In 2008, the fourth edition of the World Health Organization (WHO) *Classification of Tumors of Hematopoietic and Lymphoid Tissues* subdivided PTCLs into four categories: nodal, extra-nodal, disseminated/leukemic, and cutaneous [5]. As noted earlier, the last subtype is distinguished, with few exceptions, by a more

indolent behavior. Within the nodal subgroup, the most prevalent disease is PTCL not otherwise specified (NOS), accounting for approximately 26 % of cases, followed by angioimmunoblastic lymphoma (AITL), which constitutes 18 % of cases. The anaplastic large cell lymphomas (ALCL) are subdivided by the presence of a mutation in the anaplastic lymphoma kinase (ALK); ALK-positive (ALK+) lymphomas account for 6.6 % of cases, while ALK− cases represent 5.5 % [3].

The extra-nodal subgroup of PTCLs comprises different entities usually named after the tissue in which they manifest, e.g., hepatosplenic T-cell lymphoma. The leukemic subtypes include adult T-cell leukemia/lymphoma (ATLL, HTLV-1 associated), chronic large granular lymphocytic leukemia (LGL), prolymphocytic T-cell leukemia, and aggressive NK cell leukemia (Table 1).

Epidemiologically, the PTCLs are geographically more common in Asia than in North America, which is due to both a true increase in PTCLs, including the HTLV-driven adult T-cell leukemia/lymphoma, and a relative decrease in the B-cell NHLs [6]. The International T-cell and natural killer/T-cell lymphoma study [7] provided further insight into the epidemiology of this group of diseases. While PTCL-NOS

Table 1 Classification of nature T-/NK-cell neoplasms (2008)

T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cell
Aggressive NK leukemia
Systemic EBV-positive T-cell lymphoproliferative disorder of childhood
Hydroa vaccineforme-like lymphoma
Adult T-cell leukemia/lymphoma
Extra-nodal NK/T-cell lymphoma, nasal type
Enteropathy associated T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis Fungoides
Sezary Syndrome
Primary cutaneous CD30-positive T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous γδ T-cell lymphoma
Primary cutaneous CD-8 positive aggressive epidermotropic cytotoxic T-cell lymphoma
Primary cutaneous CD-4 positive small/medium T-cell lymphoma
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma, ALK positive
Anaplastic large-cell lymphoma, ALK negative

predominates in the United States and Europe (accounting for 34 % of cases), ATLL and NK/T-cell lymphomas are the most common types in Asia (25 and 22 % of cases, respectively). Other geographic trends include a higher prevalence of ALK+ALCL in the United States (16 % of cases) and high rates of AITL in Europe (29 % of cases).

This review will focus on the most common nodal entities, as the management of less common PTCLs can be quite different.

Prognosis of PTCLs differs by subtype. Based on data from the international T-cell lymphoma project (IPTCL) and the British Columbia Cancer Agency (BCCA), with most patients receiving an upfront anthracycline-containing chemotherapy regimen, the 5-year overall survival (OS) of PTCL-NOS was 32–35 % and the 5-year failure-free survival (FFS) was 20–29 %. AITL has a similar 5-year OS, but FFS was worse at 13–18 %. ALCL fared better, particularly the ALK + subtype with a 5-year OS of 58–70 % and FFS of 28–60 % [8].

3 Clinical Presentation and Diagnosis

Among patients with PTCL, 38 % present with nodal disease alone, 49 % with both nodal and extra-nodal disease, and 13 % with extra-nodal disease only (commonly skin and gastrointestinal tract). The bone marrow is involved in one-fifth of patients. Approximately half of patients present with stage IV disease, and one-third have B-symptoms. Eosinophilia and pruritus is often seen, and hemophagocytic syndrome may be present [7, 9, 10].

Although pathologists can accurately distinguish B cell from T-cell lymphomas, elucidation of subtypes has been difficult due to molecular heterogeneity. T-cell antigens (CD2, CD3, CD4, CD5, CD7, and CD52) are variably expressed, and clonal T-cell receptor (TCR) gene rearrangements are characteristic but not always seen due to relative genomic stability of the TCR genes [11]. Gene expression profiling (GEP) has helped in the differential diagnosis of some subtypes; for example, AITL has been shown to have follicular T-lymphocyte derivation, and PTCL-NOS seems to be derived from activated rather than resting T cells [12, 13].

4 Management of Peripheral T-Cell Lymphomas

4.1 Frontline Therapy of Aggressive PTCL

Despite advances in the understanding of the biology of PTCLs, the standard first-line or induction chemotherapy regimens for these disorders have been derived from the treatment of B-cell neoplasms. Cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP), mainstay backbone chemotherapy for aggressive B-cell neoplasms, is the regimen typically used.

In a single-institution study with 208 PTCL patients who received frontline CHOP, complete remission (CR) was obtained in 57 %, 5-year OS was 28.5 % (22.3–36.3), and 5-year event-free survival (EFS) was 18.4 % (13.4–25.3) [14]. In

general, outcomes with CHOP are inferior in PTCL compared to aggressive B-cell lymphomas [15], with the exception of ALK + ALCL. In a study by Akagi et al. [16], CR rate was 39 % in PTCL versus 67 % in DLBCL ($P < 0.008$), and 3-year OS was 26 % versus 50 %, respectively ($P = 0.005$). However, patients with ALK + ALCL patients have acceptable overall survival rates (58–70 %) with a frontline anthracycline-based regimen like CHOP, compared to ALK-ALCL (49 %), AITL or, PTCL-NOS (32–35 %) [17]. An attempt to improve these outcomes was made by adding etoposide to CHOP, with encouraging results. In an important analysis of a series of studies by the German high-grade non-Hodgkin's Lymphoma study group, a total of 343 patients were analyzed with 289 belonging to one of the four major subtypes of PTCL [18], (ALCL ALK+ = 78, ALCL ALK− = 113, PTCL-NOS = 70, and AITL = 28). These patients were given either conventional CHOP or CHOP plus etoposide (CHOEP). Three-year event-free survival (EFS) and OS were 75.8 and 89.8 % (ALK + ALCL), 45.7 and 62.1 % (ALK-ALCL), 50.0 and 67.5 % (AITL), and 41.1 and 53.9 % (PTCL-NOS), respectively, between the two groups. Of note, the ALK + ALCL patients did particularly well with CHOEP, with a significant improvement in both EFS and OS ($P <0.001$ for both endpoints). Patients with AITL did better than those with ALK- ALCL, but the difference was not statistically significant. Across both treatment groups, patients with international prognostic index (IPI) >1 did worse, and those with ALK + ALCL fared better. In younger patients, with normal LDH, CHOEP significantly improved 3-year EFS (75.4 % vs. 51.0 %); however, OS was not significantly affected ($P = 0.176$). When ALK + ALCL was excluded, the difference in EFS between CHOP and CHOEP was not significant in younger patients but showed a trend toward improvement. Older patients (>60 years of age) did worse with the addition of etoposide. More intensive regimens have been used in an attempt to improve the response rates and survival over CHOP/CHOEP, without much success. In a single-institution study from the MD Anderson Cancer Center, 135 patients with PTCL other than mycosis fungoides were treated with either CHOP (37 %) or more intensive regimens, such as hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone (HyperCVAD) or hyperCVAD/ESHAP (etoposide, methylprednisolone, cytarabine, and cisplatin) [19]. The 3-year OS was 62 % for CHOP versus 56 % for more intensive regimens. After excluding the ALK+ ALCL patients who traditionally do better, the 3-year OS was 43 % for CHOP versus 49 % for more intensive therapies. Another prospective trial comparing CHOP/ESHAP to CHOP alone with a plan for autologous transplant in first CR did not show any advantage for the more intensive regimen [20]. Etoposide, ifosfamide, cisplatin alternating with doxorubicin, bleomycin, vinblastine, and dacarbazine (VIP-reinforced ABVD; VIP-rABVD) were compared to the CHOP/21 regimen in 88 patients with PTCL by The Groupe Ouest Est d'Etude des Leucemies et Autres Maladies du Sang (GOELAMS). Two-year EFS in the two groups was nearly identical (41 % vs. 45 %) with a median overall survival of 42 months for each arm [21]. Finally, gemcitabine-based regimens have been evaluated for PTCL and have shown good response rates but no improvement in OS when compared to CHOP/CHOEP; they also appear to be associated with more toxicity [22–24].

CD52 is expressed in up to 42 % of patients with PTCL, and attempts have been made to exploit this possible therapeutic target by adding alemtuzumab, an anti-CD52 monoclonal antibody, to CHOP (CHOP-C) [25]. In a phase I/II trial, 24 patients received 8 cycles of CHOP with alemtuzumab. The CR rate was 71 %, and at a median follow-up of 16 months, median duration of response (DOR) was 11 months [26]. Serious infectious complications were observed, including febrile neutropenia, CMV and JC virus reactivation, pulmonary aspergillosis, and staphylococcal sepsis. In a larger phase II trial (41 patients), patients received CHOP or CHOEP followed by alemtuzumab consolidation (29 patients) if a clinical response was attained following CHO(E)P. CR rate was 58.5 %; EFS and OS at 3 years in the whole intent-to-treat population were 32.3 and 62.5 %, respectively, and 42.4 and 75.1 % in the patients who received alemtuzumab [27]. Two randomized phase III trials are underway (ACT-1 and ACT-2) comparing CHOP to CHOP-C.

Bortezomib, a proteasome inhibitor, has activity in relapsed PTCL. Early, small phases I and II studies with PTCL found an ORR of 62–67 % [28]. A large multicenter phase II trial with 46 patients evaluated bortezomib plus CHOP in the frontline setting; although ORR was 76 % (CR = 65 %), 3-year OS was 47 %, and PFS was 35 %, thus similar to the results achieved with CHOP alone [29].

Other studies are underway, attempting to improve response rates combining other novel agents to CHOP, or using maintenance strategies.

5 The Role of Transplantation in Frontline Therapy

5.1 Autologous Stem Cell Transplant

High-dose chemotherapy with autologous stem cell rescue (HDT/ASCR) has been investigated as consolidative therapy in PTCL given the poor outcomes with available frontline chemotherapy, particularly in non-ALK + ALCL subtypes. However, there are no prospective randomized trials comparing chemotherapy alone with chemotherapy followed by HDT/ASCR. A meta-analysis of 21 phase I/II trials showed a trend toward survival advantage for HDT/ASCR in comparison with historical controls (HR 0.81, 95 % CI 0.31–2.13). The presence of CR at transplant and good IPI scores significantly affected survival [30]. Various other retrospective and non-randomized prospective studies report PFS and OS in the range of 35–58 % and 44–65 % with HDT/ASCR following induction chemotherapy. Again, CR at transplant, favorable-risk IPI scores, and ALK + ALCL subtype were good prognostic factors [20, 31–38].

In a phase II study by the Nordic Lymphoma Group [39], 160 patients with biopsy proven PTCL (treatment-naïve) underwent induction with 6 cycles of bi-weekly CHOEP (etoposide omitted for age >60 years) followed by HDT/ASCR in responders. Most patients presented with advanced stage disease, and 72 % had IPI scores of 2 or more. One hundred and fifteen patients underwent ASCT. The 5-year overall and progression-free survival (PFS) were 51 and 44 %, respectively.

Patients with ALK-ALCL fared the best, but not much better than historical controls treated with induction chemotherapy alone.

Reimer and colleagues conducted another large prospective study evaluating ASCT in first remission after induction chemotherapy with CHOP. Eighty-three patients were enrolled with 55 going on to consolidation with transplant. The 3-year OS rate was 48 % but was more favorable (71 %) in patients who underwent ASCT [37].

Randomized trials are still needed to establish HDT/ASCR as a standard frontline consolidative approach. It remains an option for patients with significant responses to induction chemotherapy.

5.2 Allogeneic Transplant

In principle, allogeneic transplant for PTCL would utilize a graft-versus-lymphoma effect (as well as infusing a lymphoma-free graft) and might perform better than HDT/ASCR [40]. However, there are no randomized, phase III trials evaluating allogeneic transplant in the frontline setting, and most of the retrospective analyses have been conducted in the relapsed/refractory setting.

A study by Kanakry et al. reviewed the outcomes of 44 consecutive, related-donor allogeneic transplants for PTCL, 24 with reduced-intensity conditioning (RIC) and 20 with myeloablative conditioning (MAC), and included 18 reduced-intensity/haploidentical transplants. The estimated 2-year PFS was 40 % and OS was 43 %, with a tendency toward superior outcomes for those undergoing transplant in first remission ($P = 0.08$). Of note, approximately 50 % of the patients presented with poor-risk or chemorefractory disease, and 25 % received transplant with active disease. Relapse in RIC and MAC regimens were comparable, with less treatment-related mortality (TRM) with RIC [41]. A recent phase II study evaluated frontline autologous and allogeneic transplants in different age groups with CHOP-alemtuzumab conditioning ($n = 86$) and found that auto/allotransplants improved DFS in younger patients only [42].

In summary, allogeneic transplant is usually recommended in the relapsed setting but can be an option in first remission in select poor-risk patients.

6 Treatment of Relapsed/Refractory Disease

Traditionally, refractory or relapsed PTCL has been treated with second-line chemotherapy regimens often followed by autologous or allogeneic transplant. However, the recent development of therapies specific to T-cell lymphomas is rapidly changing this paradigm. We will briefly discuss the role of transplant and then discuss chemotherapy and other novel therapies in this setting.

There are no large randomized trials evaluating transplant in relapsed and refractory disease, and our practices are derived mainly from retrospective data. A retrospective study by Le Gouill et al. reported on 77 patients with PTCLs (84 %

ALCL, AITL, or PTCL-NOS) treated with allotransplant. PFS and OS were 53 and 57 %, respectively, with a 43 months follow-up following allogeneic transplant [40]. Many of these patients had received at least two prior lines of therapy including autologous transplant (25 %). Of note, AITL patients fared the best (OS rate 80 %) followed by PTCL-NOS and ALCL (63 and 55 %, respectively). TRM was 21 % at 100 days and 34 % at 5 years. A study by Kyriakou et al. [43] found similar PFS/OS for AITL patients, with chemotherapy-sensitive disease faring better. Similar outcomes have been seen in other retrospective analyses; however, early TRM remains a significant problem. It has been difficult to compare autologous and allogeneic transplant in the relapsed/refractory setting, but in general, results appear to be better with allogeneic transplant. However, a recent retrospective study by Smith et al. [44] suggested a possible benefit in favor of autologous transplant. Of 241 patients with PTCL, 115 underwent autologous and 126 allogeneic transplant. Patients receiving autologous versus allogeneic transplant had significantly greater adjusted 3-year OS (59 % vs. 47 %, $P = 0.046$) and lower NRM or non-relapse mortality ($P < 0.001$). In the absence of mature data from trials of novel agents, transplant following chemotherapy remains the recommended option for patients considered good candidates.

7 Non-transplant Therapies for Relapsed/Refractory PTCLS

Transplant may not always be an option for PTCL patients with relapsed/refractory disease due to presence of co-morbidities or unavailability of donors, and even when it is possible, it does not have robust outcomes. There is a clear need for the development of therapies directed specifically toward T-cell neoplasms, and the field has advanced significantly in the last few years. Once again, there is a paucity of large randomized phase III studies, so we will review mainly data from phase II studies, as well as ongoing trials.

7.1 Chemotherapy

Among the various chemotherapeutic agents that have been investigated in the relapsed setting, the nucleoside analog gemcitabine has shown significant activity. In a small study, 13 patients with PTCL-NOS and mycosis fungoides (MF) received single agent gemcitabine, and 9 of 13 patients responded [45]. The long-term outcome data for gemcitabine monotherapy in relapsed PTCL (PTCL-NOS and MF) were published in 2010 by the same group [46]. Five out of 20 patients with PTCL-NOS had continuous CR at the time of final follow-up, with a median duration of CR of 34 months (range 15–60 months).

In a small trial, 24 elderly patients received treatment with gemcitabine in combination with oxaliplatin and dexamethasone; the ORR was 25 % and the median OS was 14 months [47].

The other chemotherapeutic agent that has shown promise in the treatment of PTCLs is bendamustine. Bendamustine is a "dual-structure" drug consisting of an alkylating portion, derived from nitrogen mustard, and a purine analog portion. An open-label phase II trial of bendamustine was conducted in patients with relapsed PTCL (primarily AITL and PTCL-NOS). Patients had received a median of one previous line of therapy, thus not heavily pretreated [48]. In the intention to treat (ITT) analysis, the ORR was 50 % (CR 28 %), and the PFS and OS were 3.6 and 6.2 months, respectively. However, only 25 % of patients completed the planned six cycles of bendamustine. Common reasons for early discontinuation included toxicities (cytopenia and infections) and disease progression. Future studies will likely incorporate bendamustine into multidrug regimens.

Forodesine is a potent inhibitor of the enzyme purine nucleoside phosphorylase, which leads to intracellular accumulation of deoxyguanosine triphosphate (dGTP), causing reduced proliferation of lymphocytes and apoptosis [49]. In a phase I–II study, patients received oral forodesine. A maximum tolerated dose (MTD) was not reached, and the optimal biologic dose was determined as being 80 mg/m^2 daily. Thirty-six patients received this dose and the ORR was 39 %. Long-term data are awaited.

8 Antibody-Directed Therapy

8.1 Brentuximab Vedotin

Systemic ALCL is characterized by strong CD30 positivity, and the search for an effective monoclonal antibody has been an active area of research in the field of PTCL. Brentuximab vedotin (BV) is an antibody–drug conjugate, comprising an anti-CD30 monoclonal antibody conjugated to the potent antimicrotubule agent monomethyl auristatin E (MMAE). The monoclonal antibody binds to CD30-positive neoplastic T cells and is internalized; MMAE is then cleaved from the molecule exerting its action through inhibition of microtubule formation. Hence, a potent chemotherapeutic agent can be delivered in a targeted manner [50]. A phase II multicenter study by Pro et al. evaluated the activity of BV in 58 patients with systemic ALCL who had relapsed after at least one prior line of therapy. The ORR was 86 % (CR = 57 %, PR = 29 %) [51] with a median overall response duration of 12.6 months, and CR duration of 13.2 months. Grade 3–4 adverse events included neutropenia, thrombocytopenia, and peripheral sensory neuropathy. On the basis of this study, BV was approved by the FDA for use in relapsed systemic ALCL.

A recent phase II multicenter study evaluated the efficacy and safety of brentuximab vedotin in relapsed/refractory PTCL other than ALCL. Of 34 evaluable patients (PTCL-NOS n = 22, AITL n = 13), ORR was 41 % with a median PFS of 6.7 months. Interestingly, there was no correlation between CD30 expression and response [52, 53]. A retrospective analysis of brentuximab vedotin in CD30-positive relapsed lymphomas in older patients showed a 100 % RR in systemic ALCL, with tolerable toxicities [54]. A strategy of combining standard frontline therapy

(CHP) with BV was shown to be safe and effective in a phase I trial and is now being tested in an international randomized phase III trial comparing CHOP to CHP-BV as upfront therapy for CD30-positive PTCL. The results are eagerly awaited at present [55].

8.2 Denileukin Diftitox

Denileukin Diftitox (DD) is a unique genetically engineered chimeric protein, which combines IL-2 with certain domains of the diphtheria toxin (membrane translocating and cytotoxic domains). A phase II study in PTCL (excluding cutaneous) demonstrated an ORR of 48 % and median duration of response was 6 months. In CD25-positive (i.e., IL-2-receptor-positive) PTCL, a greater ORR (61.5 %) was observed [56]. As a follow-up to this, DD was evaluated as frontline therapy for PTCL in combination with CHOP in a phase II trial. In the ITT analysis, the overall response rate was 65 % and median progression-free survival was 12 months [57].

8.3 Folate Analogs: Pralatrexate

Pralatrexate is a novel folate analog with improved membrane transport and polyglutamylation within tumor cells [58]. Early phase I/II studies of pralatrexate in patients with relapsed B- or T-cell lymphomas established a significant activity in PTCL, with an ORR of 54 % [59]. In an important prospective phase II trial, 115 PTCL patients (109 evaluable) received pralatrexate 30 mg/m^2 weekly for 6 out of 7 weeks. Overall response rate was 29 % [60], and median PFS and OS were 3.5 months and 14.5 months, respectively. The most common grade 3/4 adverse events were thrombocytopenia (32 %), mucositis (22 %), neutropenia (22 %), and anemia (18 %).

Pralatrexate was the first FDA-approved agent for the treatment of patients with relapsed/refractory PTCL.

8.4 Histone Deacetylase Inhibitors

The histone deacetylase (HDAC) inhibition is an important therapeutic strategy based on the principle that increased histone acetylation leads to enhanced tumor-suppressor gene transcription, cell cycle regulation, apoptosis induction, DNA repair, protein acetylation, and induction of autophagy [61]. Romidepsin, a potent selective HDAC-I inhibitor, was evaluated in a single-arm, phase II, international prospective trial which enrolled 131 patients. Patients with relapsed/refractory disease received romidepsin at a dose of 14 mg/m^2 [2]. The ORR was 25 %, including 15 % complete response/unconfirmed complete response (CR/CRu). The median duration of response was 17 months, and among patients who achieved CR/

CRu, 89 % had not progressed at 13.4 months. Toxicities (cytopenias and infections) were tolerable. Consequently, this drug was FDA approved for relapsed/refractory PTCL [62]. Long-term follow-up data for this study was recently reported [63], and 10 of the 19 patients who achieved CR/CRu had durable responses. Median progression-free survival was 29 months, with significantly longer survival in patients achieved CR/CRu for ≥12 months.

The success of romidepsin led to the exploration of other HDAC inhibitors. Belinostat or PXD 101, a hydroxyamate HDAC I and II inhibitor, was evaluated in a phase I and then phase II multicenter clinical trials. In 19 patients with relapsed/refractory T-cell lymphoma, the ORR was 32 % and median duration of response 268+ days [64]. A larger phase II trial was recently reported, and enrolled 129 patients with relapsed/refractory PTCL following at least one prior therapy [65]. ORR was 26–28 %, with median duration of response 8.3 months. Additionally, belinostat was very well tolerated, suggesting that it can be used in combination with other agents. Other HDAC inhibitors now under investigation include panobinostat and vorinostat.

8.5 Aurora Kinase Inhibitors

Aurora A is a mitotic kinase implicated in oncogenesis and has been found to be upregulated in PTCL, most strongly in ALK+ ALCL, followed by ALK-ALCL and PTCL-NOS [66]. The Aurora A kinase inhibitor alisertib was shown to have preclinical activity against PTCL cell lines [67]. A phase II trial evaluated its efficacy against a variety of B- and T-cell lymphomas. In this trial, patients received alisertib at a dose of 50 mg twice daily for 7 days on 21-day cycles, until PD or unacceptable toxicities. ORR in 48 enrolled patients was 27 and 50 % in the subset of patients with PTCL ($n = 8$). On the basis of these results, a phase III study of alisertib versus investigator's choice of treatment is underway for patients with relapsed PTCL [68, 69].

9 Summary

In summary, PTCL represents a unique group of neoplasms characterized by marked molecular heterogeneity and poor response to conventional chemotherapy regimens. The addition of etoposide to CHOP (CHOEP) appears to be an effective up-front therapy in select patients, and alternative regimens to CHOP are currently being explored. Various approaches to consolidation have been studied, including stem cell transplant, chemotherapy, and novel immunotherapies. However, consolidation with autologous transplant is still considered the standard approach with the exception of low-risk ALK+ALCL, even in the absence of *randomized phase* III *trials.* Allogeneic transplant is usually reserved for relapsed disease and has variable outcomes. The novel agents romidepsin, pralatrexate, and brentuximab vedotin (for systemic ALCL), are currently FDA approved in the relapsed or refractory

setting. The future of treating PTCL will likely involve incorporation of these and other novel agents in frontline regimens, and a number of studies are already exploring newer combinations.

References

1. Armitage JO (2013) The aggressive peripheral T-cell lymphomas: 2013. Am J Hematol 88:910–918
2. Savage KJ (2007) Peripheral T-cell lymphomas. Blood Rev 21:201–216
3. Foss FM et al (2011) Peripheral T-cell lymphoma. Blood 117:6756–6767
4. Harris NL et al (1994) A revised European-American classification of lymphoid neoplasms: a proposal from the International lymphoma study group. Blood 84:1361–1392
5. Campo E et al (2011) The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood 117:5019–5032
6. Anderson JR, Armitage JO, Weisenburger DD (1998) Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. non-Hodgkin's lymphoma classification project. Ann Oncol Off J Eur Soc Med Oncol ESMO 9:717–720
7. Vose J, Armitage J, Weisenburger D (2008) International T-cell lymphoma project. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol Off J Am Soc Clin Oncol 26:4124–4130
8. Moskowitz AJ, Lunning M, Horwitz SM (2014) How I treat the peripheral T cell lymphomas. Blood 123: 2636. doi:10.1182/blood-2013-12-516245
9. Weisenburger DD et al (2011) Peripheral T-cell lymphoma, not otherwise specified: a report of 340 cases from the International peripheral T-cell Lymphoma project. Blood 117:3402–3408
10. Falini B et al (1990) Peripheral T-cell lymphoma associated with hemophagocytic syndrome. Blood 75:434–444
11. Weiss LM, Picker LJ, Grogan TM, Warnke RA, Sklar J (1988) Absence of clonal beta and gamma T-cell receptor gene rearrangements in a subset of peripheral T-cell lymphomas. Am J Pathol 130:436–442
12. Iqbal J et al (2010) Molecular signatures to improve diagnosis in peripheral T-cell lymphoma and prognostication in angioimmunoblastic T-cell lymphoma. Blood 115:1026–1036
13. Agostinelli C et al (2008) Peripheral T cell lymphoma, not otherwise specified: the stuff of genes, dreams and therapies. J Clin Pathol 61:1160–1167
14. Broussais-Guillaumot F et al (2013) Peripheral T-cell lymphomas: analysis of histology, staging and response to treatment of 208 cases at a single institution. Leuk Lymphoma 54:2392–2398
15. Coiffier B et al (2002) CHOP chemotherapy plus Rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 346:235–242
16. Akagi T et al (2011) Comparison of long-term clinical outcomes of CHOP chemotherapy between Japanese patients with nodal peripheral T-cell lymphomas and those with diffuse large B-cell lymphoma in the study group of the Tohoku Hematology Forum. J Clin Exp Hematop (JCEH) 51:29–35
17. Savage KJ et al (2008) ALK-anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK+ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the international peripheral T-cell lymphoma project. Blood 111:5496–5504
18. Schmitz N et al (2010) Treatment and prognosis of mature T-cell and NK-cell lymphoma: an analysis of patients with T-cell lymphoma treated in studies of the German High-Grade Non-Hodgkin Lymphoma Study Group. Blood 116:3418–3425
19. Escalón MP et al (2005) Prognostic factors and treatment of patients with T-cell non-Hodgkin lymphoma: the M. D. Anderson Cancer Center experience. Cancer 103:2091–2098

20. Mercadal S et al (2008) Intensive chemotherapy (high-dose CHOP/ESHAP regimen) followed by autologous stem-cell transplantation in previously untreated patients with peripheral T-cell lymphoma. Ann Oncol Off J Eur Soc Med Oncol ESMO 19:958–963
21. Simon A et al (2010) Upfront VIP-reinforced-ABVD (VIP-rABVD) is not superior to CHOP/21 in newly diagnosed peripheral T cell lymphoma. Results of the randomized phase III trial GOELAMS-LTP95. Br J Haematol 151:159–166
22. Arkenau H-T et al (2007) Gemcitabine, cisplatin and methylprednisolone for the treatment of patients with peripheral T-cell lymphoma: the Royal Marsden Hospital experience. Haematologica 92:271–272
23. Kim JG et al (2006) CHOP plus etoposide and gemcitabine (CHOP-EG) as front-line chemotherapy for patients with peripheral T cell lymphomas. Cancer Chemother Pharmacol 58:35–39
24. Mahadevan D et al (2013) Phase 2 trial of combined cisplatin, etoposide, gemcitabine, and methylprednisolone (PEGS) in peripheral T-cell non-Hodgkin lymphoma: Southwest Oncology Group Study S0350. Cancer 119:371–379
25. Piccaluga PP, Agostinelli C, Righi S, Zinzani PL, Pileri SA (2007) Expression of CD52 in peripheral T-cell lymphoma. Haematologica 92:566–567
26. Gallamini A et al (2007) Alemtuzumab (Campath-1H) and CHOP chemotherapy as first-line treatment of peripheral T-cell lymphoma: results of a GITIL (Gruppo Italiano Terapie Innovative nei Linfomi) prospective multicenter trial. Blood 110:2316–2323
27. Binder C et al (2013) CHO(E)P-14 followed by alemtuzumab consolidation in untreated peripheral T cell lymphomas: final analysis of a prospective phase II trial. Ann Hematol 92:1521–1528
28. Zinzani PL et al (2007) Phase II trial of proteasome inhibitor bortezomib in patients with relapsed or refractory cutaneous T-cell lymphoma. J Clin Oncol Off J Am Soc Clin Oncol 25:4293–4297
29. Kim SJ et al (2012) Bortezomib in combination with CHOP as first-line treatment for patients with stage III/IV peripheral T-cell lymphomas: a multicentre, single-arm, phase 2 trial. Eur J Cancer Oxf Engl 1990(48):3223–3231
30. Yin J, Wei J, Xu JH, Xiao Y, Zhang YC (2014) Autologous stem cell transplantation as the first-line treatment for peripheral T cell lymphoma: results of a comprehensive meta-analysis. Acta Haematol 131:114–125
31. Ahn J-S et al (2013) Autologous stem cell transplantation with busulfan, cyclophosphamide, and etoposide as an intensifying frontline treatment in patients with peripheral T cell lymphomas: a multicenter retrospective trial. Ann Hematol 92:789–797
32. Schetelig J et al (2003) Long-term disease-free survival in patients with angioimmunoblastic T-cell lymphoma after high-dose chemotherapy and autologous stem cell transplantation. Haematologica 88:1272–1278
33. Jantunen E et al (2004) Autologous stem cell transplantation in adult patients with peripheral T-cell lymphoma: a nation-wide survey. Bone Marrow Transplant 33:405–410
34. Feyler S et al (2007) The role of high-dose therapy and stem cell rescue in the management of T-cell malignant lymphomas: a BSBMT and ABMTRR study. Bone Marrow Transplant 40:443–450
35. Kyriakou C et al (2008) High-dose therapy and autologous stem-cell transplantation in angioimmunoblastic lymphoma: complete remission at transplantation is the major determinant of outcome-lymphoma working party of the European group for blood and marrow transplantation. J Clin Oncol Off J Am Soc Clin Oncol 26:218–224
36. Yang D-H et al (2009) Prognostic factors and clinical outcomes of high-dose chemotherapy followed by autologous stem cell transplantation in patients with peripheral T cell lymphoma, unspecified: complete remission at transplantation and the prognostic index of peripheral T cell lymphoma are the major factors predictive of outcome. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant 15:118–125

37. Reimer P et al (2009) Autologous stem-cell transplantation as first-line therapy in peripheral T-cell lymphomas: results of a prospective multicenter study. J Clin Oncol Off J Am Soc Clin Oncol 27:106–113
38. Corradini P et al (2006) Long-term follow-up of patients with peripheral T-cell lymphomas treated up-front with high-dose chemotherapy followed by autologous stem cell transplantation. Leukemia 20:1533–1538
39. D' Amore F et al (2012) Up-front autologous stem-cell transplantation in peripheral T-cell lymphoma: NLG-T-01. J Clin Oncol Off J Am Soc Clin Oncol 30:3093–3099
40. Le Gouill S et al (2008) Graft-versus-lymphoma effect for aggressive T-cell lymphomas in adults: a study by the Société Francaise de Greffe de Moëlle et de Thérapie Cellulaire. J Clin Oncol Off J Am Soc Clin Oncol 26:2264–2271
41. Kanakry JA et al (2013) Outcomes of related donor HLA-identical or HLA-haploidentical allogeneic blood or marrow transplantation for peripheral T cell lymphoma. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant 19:602–606
42. Corradini P et al (2014) Intensified chemo-immunotherapy with or without stem cell transplantation in newly diagnosed patients with peripheral T-cell lymphoma. *Leukemia*. doi:10.1038/leu.2014.79
43. Kyriakou C et al (2009) Allogeneic stem cell transplantation is able to induce long-term remissions in angioimmunoblastic T-cell lymphoma: a retrospective study from the lymphoma working party of the European group for blood and marrow transplantation. J Clin Oncol Off J Am Soc Clin Oncol 27:3951–3958
44. Smith SM et al (2013) Hematopoietic cell transplantation for systemic mature T-cell non-Hodgkin lymphoma. J Clin Oncol Off J Am Soc Clin Oncol 31:3100–3109
45. Zinzani PL et al (1998) Therapy with gemcitabine in pretreated peripheral T-cell lymphoma patients. Ann Oncol Off J Eur Soc Med Oncol ESMO 9:1351–1353
46. Zinzani PL et al (2010) Gemcitabine as single agent in pretreated T-cell lymphoma patients: evaluation of the long-term outcome. Ann Oncol Off J Eur Soc Med Oncol (ESMO) 21: 860–863
47. Yao Y et al (2013) Gemcitabine, oxaliplatin and dexamethasone as salvage treatment for elderly patients with refractory and relapsed peripheral T-cell lymphoma. Leuk Lymphoma 54:1194–1200
48. Damaj G et al (2013) Results from a prospective, open-label, phase II trial of bendamustine in refractory or relapsed T-cell lymphomas: the BENTLY trial. J Clin Oncol Off J Am Soc Clin Oncol 31:104–110
49. Forero-Torres A, Foss F, Olsen E, Pinter-Brown L, Kim Y (2009) Long-term treatment of CTCL with the oral PNP inhibitor, forodesine. J Clin Oncol 27:15s
50. Fanale MA et al (2012) A phase I weekly dosing study of brentuximab vedotin in patients with relapsed/refractory CD30-positive hematologic malignancies. Clin Cancer Res 18:248–255
51. Pro B et al (2012) Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. J Clin Oncol Off J Am Soc Clin Oncol 30:2190–2196
52. Oki Y (2013) Safety and efficacy of brentuximab vedotin for treatment of relapsed or refractory mature T-/NK-cell lymphomas. http://onlinelibrary.wiley.com/doi/10.1002/hon.2055/full
53. Horwitz SM et al (2014) Objective responses in relapsed T-cell lymphomas with single-agent brentuximab vedotin. Blood 123:3095–3100
54. Gopal AK et al (2014) Brentuximab vedotin in patients aged 60 years or older with relapsed or refractory CD30-positive lymphomas: a retrospective evaluation of safety and efficacy. Leuk Lymphoma. doi:10.3109/10428194.2013.876496
55. A Phase III Study of Brentuximab Vedotin plus CHP versus CHOP Therapy for Patients with Previously Untreated CD30-Positive Peripheral T-Cell Lymphoma. Meml Sloan Kettering Cancer Cent http://www.mskcc.org/cancer-care/trial/13-055

56. Dang NH et al (2007) Phase II trial of denileukin diftitox for relapsed/refractory T-cell non-Hodgkin lymphoma. Br J Haematol 136:439–447
57. Foss FM et al (2013) A multicenter phase II trial to determine the safety and efficacy of combination therapy with denileukin diftitox and cyclophosphamide, doxorubicin, vincristine and prednisone in untreated peripheral T-cell lymphoma: the CONCEPT study. Leuk Lymphoma 54:1373–1379
58. Foss FM (2011) Evaluation of the pharmacokinetics, preclinical and clinical efficacy of pralatrexate for the treatment of T-cell lymphoma. Expert Opin. Drug Metab. Toxicol. 7: 1141–1152
59. O'Connor OA et al (2009) Phase II-I-II study of two different doses and schedules of pralatrexate, a high-affinity substrate for the reduced folate carrier, in patients with relapsed or refractory lymphoma reveals marked activity in T-cell malignancies. J Clin Oncol Off J Am Soc Clin Oncol 27:4357–4364
60. O'Connor OA et al (2011) Pralatrexate in patients with relapsed or refractory peripheral T-cell lymphoma: results from the pivotal PROPEL study. J Clin Oncol Off J Am Soc Clin Oncol 29:1182–1189
61. Harrison SJ et al (2012) A focus on the preclinical development and clinical status of the histone deacetylase inhibitor, romidepsin (depsipeptide, Istodax(®)). Epigenomics 4:571–589
62. Coiffier B et al (2012) Results from a pivotal, open-label, phase II study of romidepsin in relapsed or refractory peripheral T-cell lymphoma after prior systemic therapy. J Clin Oncol Off J Am Soc Clin Oncol 30:631–636
63. Coiffier B et al (2014) Romidepsin for the treatment of relapsed/refractory peripheral T-cell lymphoma: pivotal study update demonstrates durable responses. J Hematol Oncol J Hematol Oncol 7:11
64. Zain JM, O'Connor O, Zinzani PL, Norman A, Brown de NP (2010) Multicenter, open-label trial of PXD 101 in patients with relapsed/refractory peripheral T-cell lymphoma. J Clin Oncol 28
65. O'Connor OA et al (2013) Belinostat, a novel pan-histone deacetylase inhibitor (HDACi), in relapsed or refractory peripheral T-cell lymphoma (R/R PTCL): results from the BELIEF trial. J Clin Oncol 31:8507
66. Kanagal-Shamanna R et al (2013) Differential expression of aurora-A kinase in T-cell lymphomas. Mod. Pathol. Off. J. US. Can. Acad. Pathol. Inc 26:640–647
67. Qi W et al (2013) Alisertib (MLN8237) an investigational agent suppresses Aurora A and B activity, inhibits proliferation, promotes endo-reduplication and induces apoptosis in T-NHL cell lines supporting its importance in PTCL treatment. Leuk Res 37:434–439
68. Friedberg JW et al (2014) Phase II study of alisertib, a selective aurora a kinase inhibitor, in relapsed and refractory aggressive B- and T-cell non-Hodgkin lymphomas. J Clin Oncol Off J Am Soc Clin Oncol 32:44–50
69. National Cancer Institute (2000) In Clin Internet (National Library of Medicine). http://clinicaltrials.gov/ct2/show/NCT01466881

Post-transplant Lymphoproliferative Disorders

Arun K. Singavi, Alexandra M. Harrington and Timothy S. Fenske

Abstract

Post-transplant lymphoproliferative disorders (PTLD) are a serious complication after solid organ or allogeneic hematopoietic stem cell transplantation and include a range of diseases from benign proliferations to malignant lymphomas. Risk factors for developing PTLD include Epstein-Barr virus (EBV) infection, recipient age, transplanted organ, type of immunosuppression, and genetics. Uncontrolled proliferation of EBV-infected B cells is implicated in EBV-positive PTLD, whereas the pathogenesis of EBV-negative PTLD may be similar to non-Hodgkin's lymphoma in the general population. The World Health Organization (WHO) classifies PTLD into four categories: early lesions, polymorphic PTLD, monomorphic PTLD, and classical Hodgkin's lymphoma (cHL). Treatment is aimed at cure of PTLD, while maintaining transplanted organ function. However, there are no established guidelines for the treatment of PTLD. Immune suppression reduction (ISR) is the first line of treatment in most cases, with more recent data suggesting early use of rituximab. In more aggressive forms of PTLD, upfront chemotherapy may offer a better and more durable response. Sequential therapy using rituximab followed by chemotherapy has demonstrated promising results and may establish a standard of care. Novel therapies including anti-viral agents, adoptive immunotherapy, and monoclonal

A.K. Singavi
Department of Internal Medicine, Medical College of Wisconsin, Milwaukee, WI, USA

A.M. Harrington
Department of Pathology, Medical College of Wisconsin, Milwaukee, WI, USA

T.S. Fenske (✉)
Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI, USA
e-mail: tfenske@mcw.edu

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_13

antibodies targeting cytokines require further study in the prevention and treatment of PTLD.

Keywords

Post-transplant lymphoproliferative disorder · PTLD · Lymphoma · Non-Hodgkin's lymphoma · Epstein-Barr virus (EBV) · Rituximab · Chemotherapy · Anti-viral therapy · Adoptive immunotherapy

Contents

1 Introduction

Post-transplant lymphoproliferative disorders (PTLD) include a wide range of diseases from benign hyperplasia to malignant lymphomas that occur after solid organ transplantation (SOT) and allogeneic hematopoietic cell transplantation (allo-HCT) in the setting of immunosuppression. PTLD are the most common post-SOT malignancy in children. In adults, PTLD are the second most common post-SOT malignancy, after non-melanoma skin cancer [1, 2]. PTLD were first described in the late 1960s in patients following renal transplantation [3, 4]. More than 40 years later, PTLD remain a serious and, at times, fatal complication of transplantation. In SOT, it is the most common cause of cancer-related mortality [5–7].

2 Epidemiology

Though there are many commonalities between PTLD in SOT and allo-HCT, distinct differences exist in the epidemiology and pathophysiology of the disease. In both SOT and allo-HCT, many cases are associated with Epstein-Barr virus (EBV) [8].

For adult SOT recipients, PTLD are seen in up to 10–15 % of all recipients and the highest incidence is after small bowel transplantation (20 %), followed by lung (10 %), heart (6 %), liver (2.8 %), and renal (2.3 %) transplantation [8–11]. The incidence of PTLD is significantly higher in children compared to adults, owing to a high rate of primary EBV infection after transplantation [8]. While early studies found the highest incidence of PTLD to be in the first year post-transplant, more recent data suggest a median onset of PTLD after SOT to be 30–40 months [12, 13].

For allo-HCT patients, the incidence of PTLD is significantly lower. In several large retrospective studies, the incidence of PTLD in patients following allo-HCT has been 0.5–2.5 %, with peak incidence between 2 to 6 months post-transplant [9, 14–16].

3 PTLD Risk Factors

3.1 Solid Organ Transplantation

Various risk factors for post-SOT PTLD have been identified, including recipient age, transplanted organ, characteristics of immunosuppressive therapy, and EBV status [8, 9, 17]. First recognized in 1985, EBV infection plays an integral role in PTLD [18]. The risk of PTLD after SOT is highest in those who develop a primary EBV infection after transplantation, specifically when an EBV-seronegative recipient receives an allograft from an EBV-seropositive donor [19]. Over 95 % of the world's population has been exposed to EBV by adulthood [8]; as such, primary EBV infections are more worrisome in the pediatric population [20]. EBV-seronegative recipients have a 10–76 times greater incidence of PTLD [17, 19, 21–25].

The type of SOT and the type of immunosuppression used contribute to the risk of PTLD. Early PTLD are likely due to the combined intensity of immunosuppression, while late PTLD are related to the duration of immunosuppression [9]. Specific immunosuppressive agents have also been associated with increasing risks of PTLD, such as cyclosporine [26, 27], tacrolimus [8], OKT3 (a T cell depleting anti-CD3 monoclonal antibody used to prevent and treat acute rejections) [6, 9], and anti-thymocyte globulin (ATG) [6].

Other risk factors implicated in PTLD include hepatitis C [28], cytomegalovirus (CMV) [9, 29], or HHV-8 [30] and age younger than 10 or older than 60 years [6]. The combination of multiple risk factors (CMV mismatch, OKT3 exposure, and pre-transplant EBV-seronegative recipient) can increase the risk of PTLD up to 500-fold compared to patients with no risk factors [17].

3.2 Allogeneic Hematopoietic Cell Transplantation

The most important risk factor in the development of PTLD after allo-HCT is T-cell depletion of the donor marrow or peripheral blood stem cell product [15]. Other factors include the degree of HLA mismatching, EBV serology, degree and severity of graft versus host disease (GVHD), and age older than 50 at time of transplant [14, 15, 31, 32].

As in SOT, EBV-seronegative recipients who are grafted from EBV-seropositive donors are at significantly higher risk for PTLD. Although rare, PTLD are still possible in patients with EBV-seronegative donors [14]. CMV seropositive status for donors or recipients has also been associated with increased risk for PTLD after allo-HCT [14]. The degree of HLA mismatch can increase the relative risk (RR) of developing PTLD by up to 8.9 times the general population [31, 32]. Patients who undergo myeloablative conditioning regimens and receive T-cell depleting antibodies are at higher risk for EBV-associated PTLD [33]. Agents that selectively target T cells and/or NK cells are associated with a higher risk of PTLD than those that deplete both T and B cells, such as alemtuzumab [15, 34–36]. If multiple risk factors are combined, patients at a particularly high risk for PTLD can be identified.

3.3 Genetics

Host and donor genetic variation in human leukocyte antigen (HLA) loci and genes for several cytokines have been implicated in PTLD. In one study, donor and recipient HLA-A26 and B38 haplotypes were independent risk factors for developing PTLD, while donor HLA-A1, B8, and DR3 haplotypes were protective against PTLD [37]. A separate study found HLA-B donor–recipient mismatching alone to be associated with PTLD in renal transplant patients [38].

Cytokine polymorphisms have been also implicated as risk factors for PTLD. These include the genes encoding interleukin (IL)-10, IL-6, interferon gamma (IFN-γ), transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α) promoter, and TNF-α receptor [9, 39–43].

4 Pathogenesis

In Europe and USA, the majority (approximately 85 %) of PTLD cases arise from B cells and of these, more than 80 % are associated with EBV infection [8]. Over 90 % of the world population is exposed to EBV by adulthood [44]. Though usually acquired in infancy, EBV can cause infectious mononucleosis (IM) in up to 50 % of adolescents [10].

The virus gains entry into hosts via salivary exchange and infects B cells by binding to CD21. It then replicates by lysis and proliferation of infected B cells. EBV-infected latent B cells then begin to express multiple latent membrane proteins (LMP) and EB nuclear antigens (EBNA). Recognizing the viral antigens, the host

mounts a primary CD8+ cytotoxic T lymphocyte (CTL) response affecting both lytic and latent cells. This response leads to a decrease in EBV-infected B cells; however, EBV establishes itself in memory B cells for the duration of the host's life. LMP1 upregulates anti-apoptotic genes and moves the infected cells into the latent phase [8]. Though infected memory B cells express a restricted range of viral antigens, these limited antigens produce a secondary CTL response, which in turn creates a balance of proliferation and destruction of infected B cells which persists throughout life [45]. Thereafter, EBV can be present in up to 1 in 10^6 circulating B cells [10].

In addition to EBV infection, other contributing factors are likely necessary, such as allo-antigens and cytokines such as interleukin (IL)-10 and IFN-α, for the development of PTLD [45].

Most of the studied pathogenesis in PTLD is linked to EBV. However, there is limited literature on the pathogenesis of non-EBV-related PTLD, which may be similar to non-Hodgkin's lymphoma in the general population [8]. In fact, some authors have suggested that late occurring PTLD should be considered a distinct entity from early PTLD [46]. EBV-negative PTLD typically present as a late complication of transplantation, with a median time of 50–60 months, and has more aggressive features [47, 48]. Given the increased survival of patients following SOT and allo-HCT, the incidence of EBV-negative PTLD may be on the rise [47, 49].

5 Clinical Presentation

Patients with PTLD may have an array of clinical signs and symptoms, depending on the organ system and degree of organ involvement. As previously discussed, PTLD may present at any time after transplantation. Some patients can present with clinical emergencies such as intestinal perforations or fulminant PTLD with disseminated disease mimicking septic shock [8, 50]. Lymphadenopathy alone is less commonly seen as a presenting sign, when compared to the non-transplant population. Commonly, extra-nodal organ involvement is seen [51]. Symptoms are often due to dysfunction of the organ involved, but patients can also develop constitutional (or "B") symptoms [11]. Extra-nodal sites may include the central nervous system (CNS), skin, gastrointestinal tract, lungs, renal, skin, and bone marrow [12, 47, 52, 53]. Among these, gastrointestinal involvement is most commonly reported (22–25 %) [52, 54]. Allografts themselves are less frequently involved, with exception of lung transplants [8].

6 Pathology, Diagnosis, and Staging

Current diagnosis and classification of PTLD is based on the 2008 World Health Organization (WHO) system [55]. Although it is sometimes difficult to clearly distinguish between these lesions, the WHO divides PTLD into 4 main histologic categories as detailed below (see also Table 1).

1. Early lesions (Fig. 1a). These are typically seen within a year of transplantation. In this type, lymphoid tissues maintain normal architecture by definition and present with one of two distinct histological patterns—plasmacytic hyperplasia or IM-like form. In the former, scattered EBV-positive immunoblasts are seen in the background of sheets of polytypic, mature appearing plasma cells. The latter histology resembles IM, demonstrating paracortical expansion by variable numbers of immunoblasts and unremarkable lymphocytes. The immunoblasts include EBV-infected B cells [11].
2. Polymorphic PTLD (Fig. 1c). In this type, the lymphoid tissue architecture is effaced or a destructive extra-nodal mass is observed. As the name implies, the lymphoid cells are polymorphic and include small- to medium-sized lymphocytes with variable nuclear atypia, immunoblasts, and mature plasma cells. Necrosis may be observed [11]. These can be monoclonal or polyclonal, as establishing clonality is dependent on the analysis technique and clonal burden.

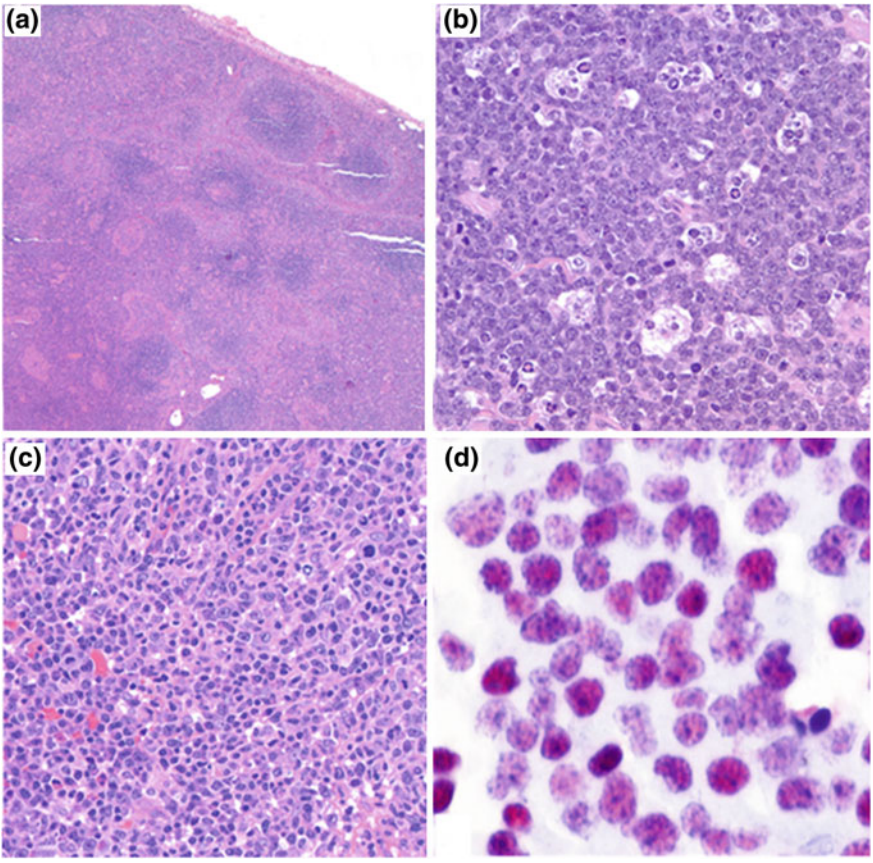


Fig. 1 The histopathology of PTLD. **a** Infectious mononucleosis-like "early" PTLD lesion. **b** Monomorphic PTLD, Burkitt lymphoma type. **c** Polymorphic PTLD. **d** Positive EBV in situ hybridization stain in a PTLD

Table 1 WHO classification of post-transplant lymphoproliferative disorders

Early lesions (5 %)	Polymorphic PTLD (15–20 %)	Monomorphic PTLD		Classical Hodgkin's lymphoma (<5 %)
		B-cell subtypes (>70 %)	*T-cell subtypes* (<5 %)	
Plasmacytic hyperplasia		Diffuse large B-cell lymphoma	Peripheral T-cell lymphoma, not otherwise specified	
Infectious mononucleosis-like		Burkitt lymphoma	Other	
		Plasmacytoma-like		
		Other		

Importantly, these do not meet diagnostic criteria for specific WHO-defined B-cell or T- /NK-cell lymphoma categories [56].

3. Monomorphic PTLD (Fig. 1b). This is the most common type of PTLD. These are monoclonal proliferations that can be separated into specific B-cell and T-cell lymphomas, using the same WHO criteria/classification as in non-transplant patients. B-cell PTLD are more common and include diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL) (Fig. 1b), plasma cell myeloma, and plasmacytoma-like PTLD. DLBCL accounts for the majority of monomorphic PTLD cases [11].
   T-cell PTLD are rare and include peripheral T-cell lymphoma, hepatosplenic T-cell lymphoma, and anaplastic large cell lymphoma (ALCL). Up to 90 % of T-cell PTLD are EBV negative [57], while the majority of NK cell PTLD are EBV positive [58]. Compared to B-cell PTLD, T-cell PTLD usually occur later and carry a poorer prognosis. T-cell PTLD cases have been associated with human T-lymphotrophic virus type 1 (HTLV-1) and may be a factor in the rising incidence of T-cell lymphomas in Japan [59].
4. Classical Hodgkin's Lymphoma (cHL)—This is the rarest form of PTLD and usually presents late after transplantation. It is histopathologically identical to cHL, showing Reed–Sternberg cells in a variable inflammatory cell milieu.

An excisional tissue biopsy is the preferred specimen for PTLD diagnosis. If an excisional biopsy is not feasible, for example, in case of suspected extra-nodal involvement, core needle biopsy and needle aspiration may be diagnostic [28]. Tissue should be examined for histology, immunophenotyping, EBER-ISH (EBV-encoded small nuclear RNA—in situ hybridization), and cytogenetic studies for classification.

Basic laboratory tests should include complete blood count with differential, comprehensive metabolic panel, lactate dehydrogenase, and uric acid. Patients should also be checked for EBV viral load, HIV, and hepatitis serologies. There is insufficient data at this time to recommend following serial EBV viral loads to assess response to therapy. Modern imaging, usually computed tomography (CT) scanning with or without positron emission tomography (PET) scanning, is an

essential tool in diagnosis and staging and should include neck, chest, abdomen, and pelvis. If cytopenias are present, bone marrow biopsy may be warranted [11].

Similar to Hodgkin's and non-Hodgkin's lymphoma in the non-transplant setting, the Ann Arbor classification is typically used for staging.

7 Prognosis

Due to the large variability of disorders encompassed in PTLD, no reliable prognostic scoring system exists. In a French study of 500 patients with PTLD post-renal transplant, the authors constructed a 5-point prognostic score based on the following: age older than 55 years, serum creatinine greater than 1.5 g/dl, elevated LDH, disseminated PTLD, and monomorphic histology [60]. Patients were risk stratified into low risk (0 risk factors), moderate risk (1 risk factor), high risk (2–3 risk factors), or very high risk (4–5 risk factors). Five-year OS was 92, 83, 59, and 25 %, respectively. Another study of 80 PTLD patients after SOT noted 3 prognostic factors: CNS involvement, bone marrow involvement, and hypoalbuminemia [61]. Three-year survival was 93 % with 0 risk factors, 68 % with 1 risk factor, and 11 % with 2–3 risk factors. CNS involvement has been associated with poor prognosis in more than one study, though this may be improving with the use of rituximab and high-dose methotrexate [11, 61].

8 Treatment

There are no uniformly applicable guidelines for the treatment of PTLD, due to the wide spectrum of disease and scarcity of prospective phase II and III studies. The goals of treatment in PTLD are twofold: first, to eliminate the PTLD and second, to preserve the transplanted graft [56]. The majority of evidence available for the treatment of PTLD has been seen in SOT (particularly CD20-positive B-cell PTLD), with limited data available regarding PTLD after allo-HCT or T-cell PTLD.

In general, the initial therapeutic intervention for PTLD consists of immune suppression reduction (ISR) [62, 63]. Unfortunately, only about half of patients respond to ISR. In addition, it can take several weeks before a response is evident after ISR [13, 64]. Many other treatment options have been studied and are used either following, or in conjunction with ISR (see Table 2 and Fig. 2). These include the use of rituximab (a monoclonal antibody directed against CD20), chemotherapy regimens, local therapy with radiation or surgery, EBV-specific CTL infusions, and more recent novel therapies.

8.1 Immune Suppression Reduction (ISR)

In most cases of PTLD, ISR is the first step in treatment. ISR should partially restore the ability of CTLs to eliminate EBV-infected lymphocytes [8]. There are, however, several potential drawbacks associated with ISR—the most significant

Table 2 Phase II/III studies of PTLD treatment and outcomes

Authors	Year	N	Treatment		ORR, % (95 % CI, if available)	Median OS (mo)
			Initial therapy (prior to study enrollment)	Subsequent (protocol) therapy		
Oertel et al. [116]	2005	17	ISR (100 %)	Rituximab × 4 cycles	59 (36–78)	37
Blaes et al. [117]	2005	11	ISR + CHOP (100 %)	Rituximab × 4 cycles, repeated every 6 months until progression	64 (35–85)	14
Choquet et al. [74]	2006	43	ISR (100 %)	Rituximab × 4 cycles	44 (30–59)	15
Gonzales-Barca et al. [118]	2007	38	ISR (100 %)	Rituximab × 4–8 cycles	66 (50–79)	42
Haque et al. [97]	2007	33	ISR (100 %) + other	Allogenic EBV-specific CTL	64 (47–78)	Not reached
Swinnen et al. [119]	2008	16	ISR + Acyclovir (100 %)	If no CR, IFN-α daily × 3 cycles (28 days)	N/A	19
				If no CR after IFN, ProMACE-CytaBOM × 6 cycles		
Gross et al. [120]	2012	55	ISR (100 %)	Rituximab with CP	69 (57–84)	Not reached
Trappe et al. [81]	2012	74	ISR (100 %)	Rituximab × 4 cycles	90 %	79
				With CR, Rituximab × 4 cycles (40 %)		
				Without CR, R-CHOP × 4 cycles (60 %)		

ProMACE-CytaBOM cyclophosphamide 650 mg/m^2 IV on day 1, doxorubicin 25 mg/m^2 IV on day 1, etoposide 120 mg/m^2 infused IV over 60 min on day 1, prednisone 60 mg/m^2 orally (PO) on days 1–14, cytosine arabinoside 300 mg/m^2 IV on day 8, bleomycin 5 mg/m^2 IV on day 8, vincristine 1.4 mg/m^2 IV on day 8, methotrexate 120 mg/m^2 IV on day 8, and leucovorin 25 mg/m^2 PO or IV every 6 h for four doses beginning 24 h after methotrexate

Rituximab with CP Six total cycles given every 3 weeks. Each cycle included cyclophosphamide (600 mg/m^2 intravenous) on day 1, prednisone (1 mg/kg orally twice a day) or methylprednisolone (0.8 mg/kg intravenous every 12 h) on days 1–5. For last four cycles, rituximab (375 mg/m^2 intravenous) on days 1, 8, and 15

R-CHOP: Rituximab, cyclophosphamide (750 mg/m^2) on day 1, doxorubicin (50 mg/m^2) on day 1, vincristine (1.4 mg/m^2) on day 1, prednisone (50 mg/m^2) on days 1–5

*Table adapted from [62, 81]

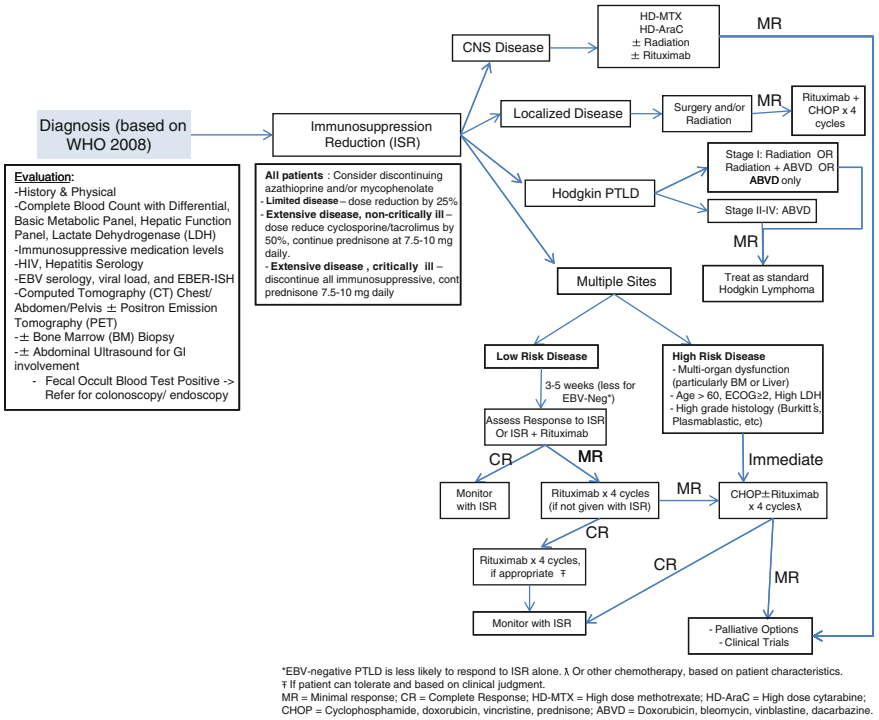


Fig. 2 Treatment algorithm for PTLD

being graft rejection. In addition, ISR as monotherapy has a relatively slow time to response (on average>2–4 weeks) [65] and lower efficacy compared to ISR combined with rituximab and/or chemotherapy. Reported overall response rates (ORR) to ISR vary from 0 to 74 % [64, 66–68], with durable responses of less than 30 % of patients in several studies [13, 64, 66, 67].

In older studies, patients with early lesions and polyclonal PTLD appeared to respond better to ISR, while those with monoclonal tumors [46], in particular with *bcl-6* expression, were less likely to respond [69, 70]. However, in a more recent study, monomorphic versus polymorphic histology did not predict for response to ISR [66].

In theory, EBV-positive PTLD should have a higher likelihood to respond to ISR; however, recent evidence suggests this may not necessarily be the case. In a recent study of SOT patients, EBV positivity was not a predictor of response—with only non-bulky disease (<7 cm) and age <50 at diagnosis being predictive [66]. Some factors such as high LDH and multi-organ dysfunction or involvement have inconsistently been associated with poor response to ISR [65, 66]. Multiple factors can be combined to help predict response to ISR. For example, using LDH elevation, hepatitis C infection, bone marrow or liver involvement, and B symptoms, 3-year overall survival (OS) was 100 % in patients with none of these factors, 79 % with one, and 8 % with two or more factors [66].

The specific dose modifications for ISR in an individual patient are based on multiple factors, such as the transplanted organ, extent of PTLD, perceived risk of rejection, symptoms, physical exam, and laboratory data. Decisions regarding ISR should be pursued in close collaboration with the transplant team. Anti-proliferative agents such as azathioprine and mycophenolate should be dose-reduced or discontinued, if possible [11]. Guidelines suggest dose reduction of 25 % in limited disease. With extensive disease in non-critically ill patients, cyclosporine/tacrolimus is typically reduced to 50 %, with discontinuation of azathioprine/mycophenolate and continuation of prednisone at 7.5–10 mg daily [71]. In critically ill patients, it may be necessary to discontinue all immune suppressing agents except prednisone 7.5–10 mg daily. Patients need to be followed closely to assess disease response and to monitor for graft rejection. Though there are risks with ISR, the majority of evidence supports the use of ISR as the first step in management of PTLD in most cases. In patients with aggressive disease, low predicted response to ISR alone or a contraindication to ISR may necessitate consideration of alternative or augmented therapies.

8.2 Rituximab

Rituximab, a chimeric monoclonal antibody targeting the B-cell surface protein CD20, has improved outcomes in many B-cell lymphomas. Data showing rituximab to be superior to other treatment approaches started to emerge around 2005 [72]. Prior to the rituximab era, the 3-year OS for PTLD ranged from 30 to 50 % but has now improved to >60 % [62, 72–75].

In the first trial of rituximab monotherapy use after ISR failure in PTLD, an ORR of 44 % was seen, with a median OS of 15 months [74]. Since then, several other studies have shown ORR ranging from 44 to 68 % and median OS up to 42 months (Table 2).

More recently, in a large, multicenter retrospective analysis, Evens et al. reviewed 80 SOT PTLD patients to establish the impact of the introduction of rituximab on outcomes [61]. All patients in the study had ISR, with 74 % of patients receiving rituximab with or without chemotherapy. With regard to first-line therapy, patients had a 73 % OS when rituximab was a part of the regimen compared to 33 % without rituximab. The 3-year progression-free survival (PFS) was 70 % with rituximab and 21 % without.

8.3 Chemotherapy

Prior to rituximab, chemotherapy was the primary treatment in patients who failed ISR. Several regimens have been described for use in PTLD. Anthracycline-based chemotherapy with rituximab, such as R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) is the most widely used regimen [71, 76–78]. It is preferred in patients for whom a rapid clinical response is required, such as those with multi-organ involvement or rapidly declining clinical status. Although the risk of toxicity and treatment-related mortality (TRM) can be

as high as 25–50 % [79], the ORR is higher than rituximab monotherapy, ranging between 65 and 100 % [71]. Given the increased risk of TRM, chemotherapy should be reserved for those who fail to achieve appropriate response with ISR or ISR and rituximab, who have highly aggressive histology, or who require a rapid clinical response such as patients presenting with severe organ dysfunction or rapidly declining clinical status [71]. In some scenarios, it may be prudent to avoid certain chemotherapy drugs if possible, such as anthracyclines in heart transplant patients and high-dose methotrexate in renal transplant patients.

8.4 Sequential Therapy

Many experts recommend a sequential or staged approach to the treatment of PTLD, in which patients generally receive ISR or ISR + rituximab as a starting point. An assessment of response is made 4–8 weeks later and, if the response is insufficient, additional therapy is then administered [63, 71, 80, 81]. This strategy was recently tested in a large prospective study (the "PTLD-1" trial) and may provide the foundation for a standard approach in the future [81]. In this study, 70 patients who failed ISR were then treated with 4 weekly doses of rituximab followed by 4 cycles of CHOP. Following rituximab, there was a 60 % ORR (with 20 % CR). After completion of rituximab × 4 and CHOP × 4, the ORR increased to 90 % (with 68 % CR) [81]. In this study, the planned therapy was rituximab × 4 followed by CHOP × 4, so it is unclear whether the patients who achieved CR with rituximab alone might have maintained their responses without chemotherapy.

In practice, a commonly utilized approach is to initially implement ISR or ISR + rituximab and then, for those who have not achieved CR, to subsequently apply chemotherapy. The "sequential therapy" approach of the PTLD-1 trial was modified to test this "risk-adapted" approach, after an interim analysis demonstrated that response to rituximab correlated with OS [82]. In the "risk-adapted" approach, patients achieving CR with 4 weeks of rituximab received 4 additional doses of rituximab, whereas those who did not achieve CR were then treated with 4 cycles of R-CHOP. In a preliminary analysis ($n = 90$), an ORR of 93 % and CR rate of 78 % were seen. The CR rate to rituximab alone was 27 %, with an additional 51 % going on to achieve CR after CHOP. Only 13 % of patients achieving CR with rituximab alone went on to relapse. Treatment-related mortality was low at 8 %. Comparing to previous data from the "sequential therapy" part of the PTLD-1 trial, it appears that (1) omitting chemotherapy from those achieving CR to rituximab is safe, and (2) for those who have progressive disease with rituximab, R-CHOP is more effective than CHOP. Those who failed to achieve CR after rituximab × 4 ± R-CHOP × 4 (22 %) did not benefit from additional R-CHOP and had nearly 80 % risk of progressive lymphoma in the following 1–2 years. Therefore, with this approach, most patients were able to achieve CR, while the relatively high treatment-related toxicity seen with CHOP was avoided except for patients who truly required chemotherapy [82]. Given these promising results, further study may help establish this "response-adapted" approach as a standard of care in CD20-positive PTLD [63].

8.5 Localized Therapy

Localized therapy using either radiation therapy (RT) or surgery can be utilized in carefully selected PTLD patients. When combined with ISR, this may be curative in patients with PTLD localized to a single site (Ann Arbor Stage I disease) [63]. RT may also have a significant role in the treatment of CNS or limited-stage PTLD, with some studies demonstrating complete responses [83, 84]. Incorporation of RT may also allow for an abbreviated course of chemotherapy for those with limited-stage disease or avoidance of chemotherapy for those who are unlikely to tolerate chemotherapy.

8.6 Primary CNS PTLD

Primary CNS PTLD is unique in both its presentation and treatment compared to other forms of PTLD. CNS involvement occurs in up to 15–22 % of PTLD, most of which are primary CNS PTLD, and has consistently been associated with poor outcomes across multiple studies [6, 12, 61, 75, 84–87].

In a recent multicenter international retrospective analysis of 84 cases of primary CNS PTLD over a 14-year period, it was found that although 83 % of cases occurred late (>1 year post-transplant), over 90 % were EBV positive [87]. The large majority (79 %) were associated with renal transplantation, presented with a median time to onset of 54 months, and 79 % had diffuse large B-cell lymphoma (DLBCL) histology. Median PFS and OS for the cohort were 8 months and 17 months, respectively.

There are no clear guidelines for the treatment of primary CNS PTLD. Options include the use of ISR, rituximab, high-dose methotrexate (HD-MTX), high-dose cytarabine, and whole brain radiation [63]. The role of rituximab is poorly defined in this setting, given its poor penetration across the blood brain barrier [71]. Response to first-line therapy is the most important prognostic factor [85, 87]. In a review of 289 patients with primary CNS PTLD after SOT, it was reported that 32 of the 39 cases of CR occurred in patients who received RT [84]. Overall, however, the outcomes were poor, with <20 % of patients achieving long-term remission. In general, treatment with ISR alone is rarely effective for primary CNS PTLD. For now, treatment should be approached in a manner akin to the primary CNS lymphoma, including whole brain RT, HD-MTX, and high-dose cytarabine, bearing in mind that HD-MTX is likely to have increased toxicity in renal transplant patients [71, 87, 88].

8.7 Burkitt PTLD

Burkitt PTLD accounts for less than 5 % of PTLD cases [63]. It is a highly aggressive malignancy with frequent extra-nodal manifestations, high proliferative index, high latent EBV infection, and rapid tumor growth. Translocations involving

c-myc and the heavy or light chain immunoglobulin loci underlie the aggressive biology. Burkitt Lymphoma, in the non-transplant population, has been successfully treated with short-duration intensive combination chemotherapy, along with aggressive CNS prophylaxis [89].

Unfortunately, there are no consensus guidelines or treatment protocols for the treatment of Burkitt PTLD. Due to the aggressive nature of the disease, ISR alone or ISR + rituximab are not recommended. Instead, most clinicians proceed directly to a combined approach involving ISR, rituximab, and chemotherapy. In a small study, CR was achieved in 5/5 patients prospectively treated with ISR and R-CHOP [90]. The role of intrathecal chemoprophylaxis is unclear in Burkitt PTLD. However, extrapolating from the non-transplant setting, we recommend CNS prophylaxis in cases of Burkitt PTLD as well. Highly aggressive regimens typically used for BL in the non-transplant setting should be used with extreme caution in PTLD patients due to the increased risk for toxicity [91].

8.8 Hodgkin's and Hodgkin-like PTLD

Hodgkin PTLD is the rarest form of PTLD; as a result, treatment data are limited to a few case reports and series [92, 93]. The approach to treatment for Hodgkin PTLD is similar to that employed in Hodgkin's lymphoma (HL). This typically involves chemotherapy or combined modality treatment using chemotherapy and RT [94]. The most commonly used chemotherapy regimen for HL in the USA is ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine). In one study of 7 patients with Hodgkin PTLD treated with standard HL therapy along with ISR, 4 patients achieved CR [93]. In another series, 4 out of 4 patients treated with standard regimens achieved CR [92].

8.9 Anti-viral Therapy

Anti-viral agents, such as acyclovir and ganciclovir, have been studied as either prophylaxis or treatment for PTLD. These agents require activation by EBV thymidine kinase (EBV-TK), an enzyme expressed in infected replicating cells, and are therefore ineffective against latent EBV-infected B cells [95, 96]. Arginine butyrate can be used to induce EBV-TK in infected latent B cells, with subsequent administration of ganciclovir to eliminate these cells. Results utilizing arginine butyrate and ganciclovir are encouraging [95], but further trials are required to prove clear clinical benefit. Prophylactic use of these agents has had mixed results [71]. Outside of clinical trials, anti-viral agents are not currently recommended for routine prophylaxis or treatment of PTLD.

8.10 Adoptive Immunotherapy

Adoptive T-cell therapy with EBV-specific cytotoxic T lymphocytes (CTL) has also been used with success in treating PTLD. Since the pathogenesis of PTLD can be from EBV infection or reactivation due to an impaired CTL response, EBV-specific CTLs are thought to possibly restore this response [97]. However, one study reported favorable response even in EBV-negative PTLD [98].

In SOT patients, PTLD originate from recipient origin (i.e. EBV reactivation), and therefore, EBV-specific CTL from the recipient are required to effectively target infected B cells [8]. In patients unresponsive to ISR, autologous EBV CTL, in combination with rituximab and/or chemotherapy, have shown some therapeutic success [99, 100]. In contrast to SOT, post-allo-HCT PTLD are typically of donor origin and effective immunotherapy against PTLD would require donor CTL for tumor targeting. In one recent study, 49 allo-HCT patients with biopsy-proven EBV-positive PTLD were treated with EBV CTL infusions (many with ISR or prior rituximab) and resulted in a sustained CR rate of 68 %. In a phase II study, 33 patients with both SOT and allo-HCT PTLD who failed conventional therapy were infused with HLA-matched donor EBV-specific CTL, resulting in an ORR of 64 % [97]. Due to a lack of consensus on the use of EBV CTL in PTLD treatment, this approach is currently recommended for use only in the context of clinical trials [71].

8.11 Novel Therapies

Multiple newer therapies are being investigated for the treatment and prevention of PTLD. These include monoclonal antibodies against cytokines and mammalian target of rapamycin (mTOR) inhibitors (such as sirolimus). IL-6 is elevated in most PTLD patients, and monoclonal antibodies targeting IL-6 are under investigation [101]. Other targets with promise include IL-10 and interferon alpha (INF-α) [102–106].

Activation of the mTOR signaling pathway has been implicated in all PTLD subtypes [8], and sirolimus has shown anti-proliferative properties in PTLD [107]. However, confounding this, use of mTOR inhibitors post-transplant has been associated with an increased risk of PTLD when compared to non-mTOR inhibitors [108]. Further study is required to understand the clinical relevance of this pathway and its inhibitors in PTLD.

9 Prevention

Many studies have looked at approaches to identify patients at risk for PTLD so that measures can be taken to prevent its development. These include monitoring for primary EBV infection and monitoring for reactivation of EBV with PCR post-transplant. Though a rising EBV viral load is concerning, it does not consistently predict the development of PTLD [109]. Monitoring EBV viral loads and pre-emptive modulation of immunosuppressive regimens with or without rituximab

therapy can decrease the incidence of PTLD and related mortality [110–112]. In a recent prospective study, 299 heart transplant patients were monitored for EBV reactivation (viral loads $>10^5$) or primary infection in which case immunosuppression was tapered and response assessed in 1 month. Patients that continued to have high viral loads received one dose of rituximab. In this cohort, only one patient developed PTLD, responsive to ISR, and one patient died due to respiratory complications from PTLD. Mean follow-up time for the entire group of patients was 2.11 years. None of the patients had evidence of transplant rejection by biopsy [110]. Anti-viral therapies, aimed to decrease lytic replication of EBV-infected B cells, have also been investigated to reduce viral loads. Patients receiving acyclovir and ganciclovir have been shown to have decreased risk of PTLD when compared to those without anti-viral therapy in small studies [113–115]. However, this has not been proven in larger studies. Though promising, this approach is only applicable to EBV-positive PTLD, and further study is needed before routine implementation can be recommended.

10 Conclusions

More than 40 years since recognition, PTLD remain a serious complication for patients undergoing allo-HCT and SOT. The understanding of the pathophysiology of PTLD continues to expand. With the identification of multiple risk factors, timely intervention, and more effective treatment, the morbidity and mortality from PTLD have decreased. However, there are no clearly established consensus guidelines for the treatment of PTLD, due in part to the scarcity of large phase 3 prospective trials and heterogeneity of the disease. For patients with minimal disease and good prognostic markers, PTLD may resolve with ISR or ISR+ rituximab. The results of the PTLD-1 trial using sequential therapy are very promising and may establish a standard of care for PTLD treatment.

Continued research on new and novel therapies for PTLD, ideally in the form of multicenter prospective trials, is needed to further improve outcomes for PTLD .

References

1. Feng S, Buell JF, Chari RS, DiMaio JM, Hanto DW (2003) Tumors and transplantation: the 2003 third annual ASTS state-of-the-art winter symposium. Am J Transpl 3(12):1481–1487
2. Boubenider S, Hiesse C, Goupy C, Kriaa F, Marchand S, Charpentier B (1997) Incidence and consequences of post-transplantation lymphoproliferative disorders. J Nephrol 10(3):136–145
3. Doak PB, Montgomerie JZ, North JD, Smith F (1968) Reticulum cell sarcoma after renal homotransplantation and azathioprine and prednisone therapy. Br Med J 4(5633):746–748
4. Penn I, Hammond W, Brettschneider L, Starzl TE (1969) Malignant lymphomas in transplantation patients. Transpl Proc 1(1):106–112
5. Leblond V, Sutton L, Dorent R, Davi F, Bitker MO, Gabarre J et al (1995) Lymphoproliferative disorders after organ transplantation: a report of 24 cases observed in a single center. J Clin Oncol 13(4):961–968

6. Opelz G, Dohler B (2004) Lymphomas after solid organ transplantation: a collaborative transplant study report. Am J Transpl 4(2):222–230
7. Newell KA, Alonso EM, Whitington PF, Bruce DS, Millis JM, Piper JB et al (1996) Posttransplant lymphoproliferative disease in pediatric liver transplantation. Interplay between primary epstein-barr virus infection and immunosuppression. Transplantation 62 (3):370–375
8. Taylor AL, Marcus R, Bradley JA (2005) Post-transplant lymphoproliferative disorders (PTLD) after solid organ transplantation. Crit Rev Oncol Hematol 56(1):155–167
9. Cockfield SM (2001) Identifying the patient at risk for post-transplant lymphoproliferative disorder. Transpl Infect Dis 3(2):70–78
10. Burns D, Crawford DH (2004) Epstein–Barr virus-specific cytotoxic T-lymphocytes for adoptive immunotherapy of post-transplant lymphoproliferative disease. Blood Rev 18 (3):193–209
11. Parker A, Bowles K, Bradley JA, Emery V, Featherstone C, Gupte G et al (2010) Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients—BCSH and BTS guidelines. Br J Haematol 149(5):675–692
12. Leblond V, Dhedin N, Mamzer Bruneel MF, Choquet S, Hermine O, Porcher R et al (2001) Identification of prognostic factors in 61 patients with posttransplantation lymphoproliferative disorders. J Clin Oncol 19(3):772–778
13. Ghobrial IM, Habermann TM, Maurer MJ, Geyer SM, Ristow KM, Larson TS et al (2005) Prognostic analysis for survival in adult solid organ transplant recipients with post-transplantation lymphoproliferative disorders. J Clin Oncol 23(30):7574–7582
14. Sundin M, Le Blanc K, Ringden O, Barkholt L, Omazic B, Lergin C et al (2006) The role of HLA mismatch, splenectomy and recipient epstein-barr virus seronegativity as risk factors in post-transplant lymphoproliferative disorder following allogeneic hematopoietic stem cell transplantation. Haematologica 91(8):1059–1067
15. Landgren O, Gilbert ES, Rizzo JD, Socie G, Banks PM, Sobocinski KA et al (2009) Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. Blood 113(20):4992–5001
16. Juvonen E, Aalto SM, Tarkkanen J, Volin L, Mattila PS, Knuutila S et al (2003) High incidence of PTLD after non-T-cell-depleted allogeneic haematopoietic stem cell transplantation as a consequence of intensive immunosuppressive treatment. Bone Marrow Transpl 32(1):97–102
17. Walker RC, Marshall WF, Strickler JG, Wiesner RH, Velosa JA, Habermann TM et al (1995) Pretransplantation assessment of the risk of lymphoproliferative disorder. Clin Infect Dis 20 (5):1346–1353
18. Ho M, Miller G, Atchison RW, Breinig MK, Dummer JS, Andiman W et al (1985) Epstein-barr virus infections and DNA hybridization studies in posttransplantation lymphoma and lymphoproliferative lesions: the role of primary infection. J Infect Dis 152(5):876–886
19. Walker RC, Paya CV, Marshall WF, Strickler JG, Wiesner RH, Velosa JA et al (1995) Pretransplantation seronegative epstein-barr virus status is the primary risk factor for posttransplantation lymphoproliferative disorder in adult heart, lung, and other solid organ transplantations. J Heart Lung Transpl 14(2):214–221
20. Dharnidharka VR, Sullivan EK, Stablein DM, Tejani AH, Harmon WE (2001) North American pediatric renal transplant cooperative study (NAPRTCS). Risk factors for posttransplant lymphoproliferative disorder (PTLD) in pediatric kidney transplantation: a report of the North American pediatric renal transplant cooperative study (NAPRTCS). Transplantation 71(8):1065–1068
21. Younes BS, McDiarmid SV, Martin MG, Vargas JH, Goss JA, Busuttil RW et al (2000) The effect of immunosuppression on posttransplant lymphoproliferative disease in pediatric liver transplant patients. Transplantation 70(1):94–99

22. Cockfield SM, Preiksaitis JK, Jewell LD, Parfrey NA (1993) Post-transplant lymphoproliferative disorder in renal allograft recipients. Clinical experience and risk factor analysis in a single center. Transplantation 56(1):88–96
23. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, O'Sullivan EJ, Johnson MR, Heroux AL et al (1990) Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med 323 (25):1723–1728
24. Ellis D, Jaffe R, Green M, Janosky JJ, Lombardozzi-Lane S, Shapiro R et al (1999) Epstein-barr virus-related disorders in children undergoing renal transplantation with tacrolimus-based immunosuppression. Transplantation 68(7):997–1003
25. Swerdlow AJ, Higgins CD, Hunt BJ, Thomas JA, Burke MM, Crawford DH et al (2000) Risk of lymphoid neoplasia after cardiothoracic transplantation. A cohort study of the relation to epstein-barr virus. Transplantation 69(5):897–904
26. Gao S, Chaparro SV, Perlroth M, Montoya JG, Miller JL, DiMiceli S et al (2003) Post-transplantation lymphoproliferative disease in heart and heart–lung transplant recipients: 30-year experience at Stanford university. J Heart Lung Transpl 22(5):505–514
27. Libertiny G, Watson CJE, Gray DWR, Welsh KI, Morris PJ (2001) Rising incidence of post-transplant lymphoproliferative disease in kidney transplant recipients. Br J Surg 88 (10):1330–1334
28. Buda A, Caforio A, Calabrese F, Fagiuoli S, Pevere S, Livi U et al (2000) Lymphoproliferative disorders in heart transplant recipients: role of hepatitis C virus (HCV) and epstein-barr virus (EBV) infection. Transpl Int 13(1):S402–S405
29. Manez R, Breinig MC, Linden P, Wilson J, Torre-Cisneros J, Kusne S et al (1997) Posttransplant lymphoproliferative disease in primary epstein-barr virus infection after liver transplantation: the role of cytomegalovirus disease. J Infect Dis 176(6):1462–1467
30. Kapelushnik J, Ariad S, Benharroch D, Landau D, Moser A, Delsol G et al (2001) Post renal transplantation human herpesvirus 8-associated lymphoproliferative disorder and kaposi's sarcoma. Br J Haematol 113(2):425–428
31. Bhatia S, Ramsay NK, Steinbuch M, Dusenbery KE, Shapiro RS, Weisdorf DJ et al (1996) Malignant neoplasms following bone marrow transplantation. Blood 87(9):3633–3639
32. Witherspoon RP, Fisher LD, Schoch G, Martin P, Sullivan KM, Sanders J et al (1989) Secondary cancers after bone marrow transplantation for leukemia or aplastic anemia. N Engl J Med 321(12):784–789
33. Gerritsen EJ, Stam ED, Hermans J, van den Berg H, Haraldsson A, van Tol MJ et al (1996) Risk factors for developing EBV-related B cell lymphoproliferative disorders (BLPD) after non-HLA-identical BMT in children. Bone Marrow Transpl 18(2):377–382
34. Hale G, Waldmann H (1998) Risks of developing epstein-barr virus-related lymphoproliferative disorders after T-cell-depleted marrow transplants. CAMPATH users. Blood 91(8):3079–3083
35. Weinstock DM, Ambrossi GG, Brennan C, Kiehn TE, Jakubowski A (2006) Preemptive diagnosis and treatment of epstein-barr virus-associated post transplant lymphoproliferative disorder after hematopoietic stem cell transplant: an approach in development. Bone Marrow Transpl 37(6):539–546
36. Fox CP, Burns D, Parker AN, Peggs KS, Harvey CM, Natarajan S et al (2013) EBV-associated post-transplant lymphoproliferative disorder following in vivo T-cell-depleted allogeneic transplantation: clinical features, viral load correlates and prognostic factors in the rituximab era. Bone Marrow Transpl 49:280–286
37. Reshef R, Luskin MR, Kamoun M, Vardhanabhuti S, Tomaszewski JE, Stadtmauer EA et al (2011) Association of HLA polymorphisms with post-transplant lymphoproliferative disorder in solid-organ transplant recipients. Am J Transpl 11(4):817–825
38. Bakker NA, van Imhoff GW, Verschuuren EA, van Son WJ, van der Heide JJ, Lems SP et al (2005) HLA antigens and post renal transplant lymphoproliferative disease: HLA-B matching is critical. Transplantation 80(5):595–599

39. Van Buskirk AM, Malik V, Xia D, Pelletier RP (2001) A gene polymorphism associated with posttransplant lymphoproliferative disorder. Transpl Proc 33(1-2):1834
40. Lee TC, Savoldo B, Barshes NR, Rooney CM, Heslop HE, Gee AP et al (2006) Use of cytokine polymorphisms and epstein-barr virus viral load to predict development of post-transplant lymphoproliferative disorder in paediatric liver transplant recipients. Clin Transpl 20(3):389–393
41. Babel N, Vergopoulos A, Trappe RU, Oertel S, Hammer MH, Karaivanov S et al (2007) Evidence for genetic susceptibility towards development of posttransplant lymphoproliferative disorder in solid organ recipients. Transplantation 84(3):387–391
42. Thomas RV, McAulay K, Higgins C, Wilkie G, Crawford DH (2005) Interferon gamma (IFN-gamma) polymorphism in posttransplantation lymphoproliferative disease. Blood. 106 (4):1502, 3; author reply 1503
43. McAulay KA, Haque T, Crawford DH (2009) Tumour necrosis factor gene polymorphism: a predictive factor for the development of post-transplant lymphoproliferative disease. Br J Cancer 101(6):1019–1027
44. Nourse JP, Jones K, Gandhi MK (2011) Epstein-barr virus-related post-transplant lymphoproliferative disorders: pathogenetic insights for targeted therapy. Am J Transpl 11 (5):888–895
45. Lim WH, Russ GR, Coates PT (2006) Review of epstein-barr virus and post-transplant lymphoproliferative disorder post-solid organ transplantation. Nephrology (Carlton) 11 (4):355–366
46. Dotti G, Fiocchi R, Motta T, Gamba A, Gotti E, Gridelli B et al (2000) Epstein-barr virus-negative lymphoproliferate disorders in long-term survivors after heart, kidney, and liver transplant. Transplantation 69(5):827–833
47. Leblond V, Davi F, Charlotte F, Dorent R, Bitker MO, Sutton L et al (1998) Posttransplant lymphoproliferative disorders not associated with epstein-barr virus: a distinct entity? J Clin Oncol 16(6):2052–2059
48. Nelson BP, Nalesnik MA, Bahler DW, Locker J, Fung JJ, Swerdlow SH (2000) Epstein-barr virus-negative post-transplant lymphoproliferative disorders: a distinct entity? Am J Surg Pathol 24(3):375–385
49. Duvoux C, Pageaux GP, Vanlemmens C, Roudot-Thoraval F, Vincens-Rolland AL, Hezode C et al (2002) Risk factors for lymphoproliferative disorders after liver transplantation in adults: an analysis of 480 patients. Transplantation 74(8):1103–1109
50. Orjuela M, Gross TG, Cheung YK, Alobeid B, Morris E, Cairo MS (2003) A pilot study of chemoimmunotherapy (cyclophosphamide, prednisone, and rituximab) in patients with post-transplant lymphoproliferative disorder following solid organ transplantation. Clin Cancer Res 9(10 Pt 2):3945S–3952S
51. Bates WD, Gray DW, Dada MA, Chetty R, Gatter KC, Davies DR et al (2003) Lymphoproliferative disorders in oxford renal transplant recipients. J Clin Pathol 56(6): 439–446
52. Caillard S, Lelong C, Pessione F, Moulin B, French PTLD (2006) Working group. Post-transplant lymphoproliferative disorders occurring after renal transplantation in adults: report of 230 cases from the French registry. Am J Transpl 6(11):2735–2742
53. Oton AB, Wang H, Leleu X, Melhem MF, George D, Lacasce A et al (2008) Clinical and pathological prognostic markers for survival in adult patients with post-transplant lymphoproliferative disorders in solid transplant. Leuk Lymphoma 49(9):1738–1744
54. Subklewe M, Marquis R, Choquet S, Leblond V, Garnier JL, Hetzer R et al (2006) Association of human leukocyte antigen haplotypes with posttransplant lymphoproliferative disease after solid organ transplantation. Transplantation 82(8):1093–1100
55. Swerdlow S, Campo E, Harris NL, Jaffe E, Pileri S, Stein H (2008) WHO classification of tumours of haematopoietic and lymphoid tissues (international agency for research on cancer, lyon, France)

56. Jagadeesh D, Woda BA, Draper J, Evens AM (2012) Post transplant lymphoproliferative disorders: risk, classification, and therapeutic recommendations. Curr Treat Options Oncol 13 (1):122–136
57. Dockrell DH, Strickler JG, Paya CV (1998) Epstein-barr virus-induced T cell lymphoma in solid organ transplant recipients. Clin Infect Dis 26(1):180–182
58. Kwong YL, Lam CC, Chan TM (2000) Post-transplantation lymphoproliferative disease of natural killer cell lineage: a clinicopathological and molecular analysis. Br J Haematol 110 (1):197–202
59. Rajakariar R, Bhattacharyya M, Norton A, Sheaff M, Cavenagh J, Raftery MJ et al (2004) Post transplant T-cell lymphoma: a case series of four patients from a single unit and review of the literature. Am J Transpl 4(9):1534–1538
60. Caillard S, Porcher R, Provot F, Dantal J, Choquet S, Durrbach A et al (2013) Post-transplantation lymphoproliferative disorder after kidney transplantation: report of a nationwide French registry and the development of a new prognostic score. J Clin Oncol 31(10):1302–1309
61. Evens AM, David KA, Helenowski I, Nelson B, Kaufman D, Kircher SM et al (2010) Multicenter analysis of 80 solid organ transplantation recipients with post-transplantation lymphoproliferative disease: outcomes and prognostic factors in the modern era. J Clin Oncol 28(6):1038–1046
62. Al-Mansour Z, Nelson BP, Evens AM (2013) Post-transplant lymphoproliferative disease (PTLD): risk factors, diagnosis, and current treatment strategies. Curr Hematol Malig Rep 8 (3):173–183
63. Zimmermann H, Trappe RU (2013) EBV and posttransplantation lymphoproliferative disease: what to do? Hematol Am Soc Hematol Educ Program 2013:95–102
64. Knight JS, Tsodikov A, Cibrik DM, Ross CW, Kaminski MS, Blayney DW (2009) Lymphoma after solid organ transplantation: risk, response to therapy, and survival at a transplantation center. J Clin Oncol 27(20):3354–3362
65. Tsai DE, Hardy CL, Tomaszewski JE, Kotloff RM, Oltoff KM, Somer BG et al (2001) Reduction in immunosuppression as initial therapy for posttransplant lymphoproliferative disorder: analysis of prognostic variables and long-term follow-up of 42 adult patients. Transplantation 71(8):1076–1088
66. Reshef R, Vardhanabhuti S, Luskin MR, Heitjan DF, Hadjiliadis D, Goral S et al (2011) Reduction of immunosuppression as initial therapy for posttransplantation lymphoproliferative disorder (bigstar). Am J Transpl 11(2):336–347
67. Swinnen LJ, Mullen GM, Carr TJ, Costanzo MR, Fisher RI (1995) Aggressive treatment for postcardiac transplant lymphoproliferation. Blood 86(9):3333–3340
68. Caillard S, Dharnidharka V, Agodoa L, Bohen E, Abbott K (2005) Posttransplant lymphoproliferative disorders after renal transplantation in the united states in era of modern immunosuppression. Transplantation 80(9):1233–1243
69. Cesarman E, Chadburn A, Liu YF, Migliazza A, Dalla-Favera R, Knowles DM (1998) BCL-6 gene mutations in posttransplantation lymphoproliferative disorders predict response to therapy and clinical outcome. Blood 92(7):2294–2302
70. Penn I (1998) The role of immunosuppression in lymphoma formation. Springer Semin Immunopathol 20(3–4):343–355
71. Parker A, Bowles K, Bradley JA, Emery V, Featherstone C, Gupte G et al (2010) Management of post-transplant lymphoproliferative disorder in adult solid organ transplant recipients—BCSH and BTS guidelines. Br J Haematol 149(5):693–705
72. Ghobrial I, Habermann T, Ristow K, Ansell S, Macon W, Geyer S et al (2005) Prognostic factors in patients with post-transplant lymphoproliferative disorders (PTLD) in the rituximab era. Leuk Lymphoma 46(2):191–196
73. Jain AB, Marcos A, Pokharna R, Shapiro R, Fontes PA, Marsh W et al (2005) Rituximab (chimeric anti-CD20 antibody) for posttransplant lymphoproliferative disorder after solid

organ transplantation in adults: long-term experience from a single center. Transplantation 80 (12):1692–1698
74. Choquet S, Leblond V, Herbrecht R, Socie G, Stoppa AM, Vandenberghe P et al (2006) Efficacy and safety of rituximab in B-cell post-transplantation lymphoproliferative disorders: results of a prospective multicenter phase 2 study. Blood 107(8):3053–3057
75. Maecker B, Jack T, Zimmermann M, Abdul-Khaliq H, Burdelski M, Fuchs A et al (2007) CNS or bone marrow involvement as risk factors for poor survival in post-transplantation lymphoproliferative disorders in children after solid organ transplantation. J Clin Oncol 25 (31):4902–4908
76. Fohrer C, Caillard S, Koumarianou A, Ellero B, Woehl-Jaegle ML, Meyer C et al (2006) Long-term survival in post-transplant lymphoproliferative disorders with a dose-adjusted ACVBP regimen. Br J Haematol 134(6):602–612
77. Mamzer-Bruneel MF, Lome C, Morelon E, Levy V, Bourquelot P, Jacobs F et al (2000) Durable remission after aggressive chemotherapy for very late post-kidney transplant lymphoproliferation: a report of 16 cases observed in a single center. J Clin Oncol 18 (21):3622–3632
78. Giraldi E, Provenzi M, Fiocchi R, Colledan M, Cornelli P, Torre G et al (2011) Fludarabine, cyclophosphamide, doxorubicin (FCD), and rituximab: a remission induction therapy for aggressive pediatric post-transplant lymphoproliferative disease (PTLD). Pediatr Blood Cancer 57(2):324–328
79. Elstrom RL, Andreadis C, Aqui NA, Ahya VN, Bloom RD, Brozena SC et al (2006) Treatment of PTLD with rituximab or chemotherapy. Am J Transpl 6(3):569–576
80. Shah NM, Fenske TS (2013) Post-transplant lymphoproliferative disorders. Hematology 8 (Part 2):1
81. Trappe R, Oertel S, Leblond V, Mollee P, Sender M, Reinke P et al (2012) Sequential treatment with rituximab followed by CHOP chemotherapy in adult B-cell post-transplant lymphoproliferative disorder (PTLD): the prospective international multicentre phase 2 PTLD-1 trial. Lancet Oncol 13(2):196–206
82. Trappe RU, Dierickx D, Reinke P, Neuhaus R, Morschhauser F, Zaucha JM et al (2012) Interim analysis of the largest prospective trial to date in adult CD20-positive post-transplant lymphoproliferative disorder (PTLD): introducing risk-stratified sequential treatment (RSST). J Clin Oncol 30 (suppl; abstract 8030)
83. Dotti G, Fiocchi R, Motta T, Mammana C, Gotti E, Riva S et al (2002) Lymphomas occurring late after solid-organ transplantation: influence of treatment on the clinical outcome. Transplantation 74(8):1095–1102
84. Penn I, Porat G (1995) Central nervous system lymphomas in organ allograft recipients. Transplantation 59(2):240–244
85. Buell JF, Gross TG, Hanaway MJ, Trofe J, Roy-Chaudhury P, First MR et al (2005) Posttransplant lymphoproliferative disorder: significance of central nervous system involvement. Transpl Proc 37(2):954–955
86. Cavaliere R, Petroni G, Lopes MB, Schiff D (2010) International primary central nervous system lymphoma collaborative group. Primary central nervous system post-transplantation lymphoproliferative disorder: an international primary central nervous system lymphoma collaborative group report. Cancer 116(4):863–870
87. Evens AM, Choquet S, Kroll-Desrosiers AR, Jagadeesh D, Smith SM, Morschhauser F et al (2013) Primary CNS posttransplant lymphoproliferative disease (PTLD): an international report of 84 cases in the modern era. Am J Transpl 13(6):1512–1522
88. Taj MM, Messahel B, Mycroft J, Pritchard-Jones K, Baker A, Height S et al (2008) Efficacy and tolerability of high-dose methotrexate in central nervous system positive or relapsed lymphoproliferative disease following liver transplant in children. Br J Haematol 140 (2):191–196
89. Blum KA, Lozanski G, Byrd JC (2004) Adult burkitt leukemia and lymphoma. Blood 104 (10):3009–3020

90. Zimmermann H, Reinke P, Neuhaus R, Lehmkuhl H, Oertel S, Atta J et al (2012) Burkitt post-transplantation lymphoma in adult solid organ transplant recipients: sequential immunochemotherapy with rituximab (R) followed by cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or R-CHOP is safe and effective in an analysis of 8 patients. Cancer 118(19):4715–4724
91. Xicoy B, Ribera J, Esteve J, Brunet S, Sanz M, Fernández-Abellán P et al (2003) Post-transplant burkitt's leukemia or lymphoma. Study of five cases treated with specific intensive therapy (PETHEMA ALL-3/97 trial). Leuk Lymphoma 44(9):1541–1543
92. Bierman PJ, Vose JM, Langnas AN, Rifkin RM, Hauke RJ, Smir BN et al (1996) Hodgkin's disease following solid organ transplantation. Ann Oncol 7(3):265–270
93. Garnier JL, Lebranchu Y, Dantal J, Bedrossian J, Cahen R, Assouline D et al (1996) Hodgkin's disease after transplantation. Transplantation 61(1):71–76
94. Diehl V, Stein H, Hummel M, Zollinger R, Connors JM (2003) Hodgkin's lymphoma: biology and treatment strategies for primary, refractory, and relapsed disease. Hematology Am Soc Hematol Educ Program 2003(1):225–247
95. Perrine SP, Hermine O, Small T, Suarez F, O'Reilly R, Boulad F et al (2007) A phase 1/2 trial of arginine butyrate and ganciclovir in patients with epstein-barr virus-associated lymphoid malignancies. Blood 109(6):2571–2578
96. Mentzer SJ, Perrine SP, Faller DV (2001) Epstein–barr virus post-transplant lymphoproliferative disease and virus-specific therapy: pharmacological re-activation of viral target genes with arginine butyrate. Transpl Infect Dis 3(3):177–185
97. Haque T, Wilkie GM, Jones MM, Higgins CD, Urquhart G, Wingate P et al (2007) Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. Blood 110(4):1123–1131
98. Nalesnik MA, Rao AS, Furukawa H, Pham S, Zeevi A, Fung JJ et al (1997) Autologous lymphokine-activated killer cell therapy of epstein-barr virus-positive and -negative lymphoproliferative disorders arising in organ transplant recipients. Transplantation 63 (9):1200–1205
99. Comoli P, Labirio M, Basso S, Baldanti F, Grossi P, Furione M et al (2002) Infusion of autologous epstein-barr virus (EBV)-specific cytotoxic T cells for prevention of EBV-related lymphoproliferative disorder in solid organ transplant recipients with evidence of active virus replication. Blood 99(7):2592–2598
100. Heslop HE, Slobod KS, Pule MA, Hale GA, Rousseau A, Smith CA et al (2010) Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood 115(5):925–935
101. Haddad E, Paczesny S, Leblond V, Seigneurin JM, Stern M, Achkar A et al (2001) Treatment of B-lymphoproliferative disorder with a monoclonal anti-interleukin-6 antibody in 12 patients: a multicenter phase 1–2 clinical trial. Blood 97(6):1590–1597
102. Davis CL, Wood BL, Sabath DE, Joseph JS, Stehman-Breen C, Broudy VC (1998) Interferon-alpha treatment of posttransplant lymphoproliferative disorder in recipients of solid organ transplants. Transplantation 66(12):1770–1779
103. Komrokji RS, Oliva JL, Zand M, Felgar R, Abboud CN (2005) Mini-BEAM and autologous hematopoietic stem-cell transplant for treatment of post-transplant lymphoproliferative disorders. Am J Hematol 79(3):211–215
104. Williams KM, Higman MA, Chen AR, Schwartz CL, Wharam M, Colombani P et al (2008) Successful treatment of a child with late-onset T-cell post-transplant lymphoproliferative disorder/lymphoma. Pediatr Blood Cancer 50(3):667–670
105. Bobey NA, Stewart DA, Woodman RC (2002) Successful treatment of posttransplant lymphoproliferative disorder in a renal transplant patient by autologous peripheral blood stem cell transplantation. Leuk Lymphoma 43(12):2421–2423
106. Mahe B, Moreau P, Le Tortorec S, Hourmant M, Harousseau JL, Milpied N (1994) Autologous bone marrow transplantation for cyclosporin-related lymphoma in a renal transplant patient. Bone Marrow Transpl 14(4):645–646

107. Majewski M, Korecka M, Kossev P, Li S, Goldman J, Moore J et al (2000) The immunosuppressive macrolide RAD inhibits growth of human epstein-barr virus-transformed B lymphocytes in vitro and in vivo: a potential approach to prevention and treatment of posttransplant lymphoproliferative disorders. Proc Natl Acad Sci USA 97(8):4285–4290
108. Kirk AD, Cherikh WS, Ring M, Burke G, Kaufman D, Knechtle SJ et al (2007) Dissociation of depletional induction and posttransplant lymphoproliferative disease in kidney recipients treated with alemtuzumab. Am J Transpl 7(11):2619–2625
109. Green M, Soltys K, Rowe DT, Webber SA, Mazareigos G (2009) Chronic high epstein-barr viral load carriage in pediatric liver transplant recipients. Pediatr Transpl 13(3):319–323
110. Choquet S, Varnous S, Deback C, Golmard JL, Leblond V (2014) Adapted treatment of epstein-barr virus infection to prevent posttransplant lymphoproliferative disorder after heart transplantation. Am J Transpl 14(4):857–866
111. Lee TC, Savoldo B, Rooney CM, Heslop HE, Gee AP, Caldwell Y et al (2005) Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. Am J Transpl 5(9):2222–2228
112. Jang JY, Kim KM, Lee YJ, Lee SG, Chi HS (2008) Quantitative epstein-barr virus viral load monitoring in pediatric liver transplantation. Transpl Proc 40(8):2546–2548
113. Darenkov IA, Marcarelli MA, Basadonna GP, Friedman AL, Lorber KM, Howe JG et al (1997) Reduced incidence of epstein-barr virus-associated posttransplant lymphoproliferative disorder using preemptive antiviral therapy. Transplantation 64(6):848–852
114. Davis CL, Harrison KL, McVicar JP, Forg PJ, Bronner MP, Marsh CL (1995) Antiviral prophylaxis and the epstein barr virus-related post-transplant lymphoproliferative disorder. Clin Transpl 9(1):53–59
115. Keay S, Oldach D, Wiland A, Klassen D, Schweitzer E, Abruzzo LV et al (1998) Posttransplantation lymphoproliferative disorder associated with OKT3 and decreased antiviral prophylaxis in pancreas transplant recipients. Clin Infect Dis 26(3):596–600
116. Oertel SH, Verschuuren E, Reinke P, Zeidler K, Papp-Vary M, Babel N et al (2005) Effect of anti-CD 20 antibody rituximab in patients with post-transplant lymphoproliferative disorder (PTLD). Am J Transpl 5(12):2901–2906
117. Blaes AH, Peterson BA, Bartlett N, Dunn DL, Morrison VA (2005) Rituximab therapy is effective for posttransplant lymphoproliferative disorders after solid organ transplantation: results of a phase II trial. Cancer 104(8):1661–1667
118. Gonzalez-Barca E, Domingo-Domenech E, Capote FJ, Gomez-Codina J, Salar A, Bailen A et al (2007) Prospective phase II trial of extended treatment with rituximab in patients with B-cell post-transplant lymphoproliferative disease. Haematologica 92(11):1489–1494
119. Swinnen LJ, LeBlanc M, Grogan TM, Gordon LI, Stiff PJ, Miller AM et al (2008) Prospective study of sequential reduction in immunosuppression, interferon alpha-2B, and chemotherapy for posttransplantation lymphoproliferative disorder. Transplantation 86 (2):215–222
120. Gross TG, Orjuela MA, Perkins SL, Park JR, Lynch JC, Cairo MS et al (2012) Low-dose chemotherapy and rituximab for posttransplant lymphoproliferative disease (PTLD): a children's oncology group report. Am J Transpl 12(11):3069–3075

Allogeneic Hematopoietic Cell Transplantation in Non-Hodgkin's Lymphomas

Ibrahim Aldoss and Auayporn Nademanee

Abstract

Allogeneic hematopoietic cell transplant (alloHCT) has emerged as a potential curative treatment for advanced non-Hodgkin's lymphoma (NHL), especially for patients with chemorefractory disease, relapsed after prior autologous HCT and those with relapsed lymphoma who failed to collect adequate stem cells for autologous HCT. There are several phase II studies supported the role of alloHCT in low-grade lymphomas, but the data is scarce on the other subtypes of lymphomas. However, retrospective registries studies highlighted the inferior outcomes of alloHCT in aggressive lymphomas, with unacceptable higher relapse rate and non-relapse mortality when compared to low-grade lymphomas. Patients with chemorefractory disease and those with active disease at alloHCT had poor outcome. Therefore, incorporation of new target therapies to induce remission prior to transplant or as a bridge to alloHCT may lead to better outcome of alloHCT in NHL. Furthermore, well design prospective studies of alloHCT in NHL and employment of novel transplant approaches tailored toward specific histological subtype are urgently needed.

Keywords

Allogeneic HCT for lymphoma · Transplantation for NHL · Reduced-intensity allo-HCT for NHL

I. Aldoss · A. Nademanee (✉)
City of Hope National Medical Center, Hematology and Hematopoietic Cell Transplantation, 1500 East Duarte Road, Duarte, CA 91010, USA
e-mail: anademanee@coh.org

A.M. Evens and K.A. Blum (eds.), *Non-Hodgkin Lymphoma*,
Cancer Treatment and Research 165, DOI 10.1007/978-3-319-13150-4_14

Contents

1 Introduction

Non-Hodgkin's lymphoma (NHL) includes heterogeneous entities of mature lymphoid malignancies that vary in biology, clinical behavior, and treatment. Chemotherapy alone remains the most utilized upfront therapy for the majority of NHL cases. Chemotherapy alone is capable of curing roughly half of aggressive NHL cases and attaining long-term control in the majority of indolent subtypes. Nevertheless, there is a well-established role for autologous (auto) hematopoietic cell transplant (HCT) in relapsed chemosensitive aggressive NHL, and occasionally, it has a role in the upfront setting such as consolidation modality for mantle cell lymphoma (MCL). Although auto-HCT is a safe procedure, and it is associated with low morbidity and mortality, it has a limited role in heavily pretreated or relapsed patients that had failed to mobilize adequate stem cells, chemorefractory relapsed lymphomas, and those who relapsed/progressed after prior auto-HCT. In those situations, allogeneic HCT (allo-HCT) may be the only curative option, and it has been shown to be effective but is also associated with high transplant-related morbidity and mortality (TRM). In this review, we will discuss the current available evidence for the role of allo-HCT in the different subtypes of NHL.

2 Allogeneic HCT in Indolent B-Cell NHL

Follicular lymphoma (FL) is the prototype of low-grade lymphoma. It is characterized by indolent clinical behavior and sensitivity to chemotherapy. However, the natural history of FL involves frequent disease recurrences and shorter response durations with each relapse. Eventually, FL progresses to become chemoresistant, and portion of cases transforms to more aggressive lymphoma subtypes. Despite long survival that exceeds a decade in more recent studies, FL is considered an incurable disease with conventional chemotherapy, and there is an increasing interest in using allo-HCT as a curative modality in advanced disease and younger patients.

In relapsed/refractory (R/R) FL, phase II prospective studies from the MD Anderson Cancer Center (MDACC), the Cancer and Leukemia Group B (CALGB 10901), the Spanish group (GELTAMO), and the Canadian transplant group have

established the feasibility of reduced-intensity conditioning (RIC)/non-myeloablative conditioning (NMA) allo-HCT and have documented low relapsed/progression risk (RR) as well as low non-relapse mortality (NRM) [1–4]. Investigators from MDACC prospectively treated 47 chemosensitive relapsed FL with allo-HCT following NMA conditioning with FCR (fludarabine, cyclophosphamide, and rituximab). The majority of patients transplanted from matched–related donors (MRD), and 19 % had already failed prior autograft. Long-term follow-up demonstrated low RR and NRM of 6 and 13 %, respectively, in addition to high 11-year progression-free survival (PFS) and overall survival (OS) of 72 and 78 %, respectively. Subsequently, rituximab was replaced with ^{90}Y-ibritumomab (YFC), and another 26 relapsed FL patients were treated prospectively. Among the YFC cohort, more patients were chemorefractory compared to the FCR cohort (38 vs. 0 %) and have undergone transplant from matched unrelated donor (MUD) (42 vs. 4 %). Relapse risk and NRM were low, and both were 8 %. Three-year PFS and OS were 85 and 88 %, respectively. Although pretransplant PET activity did not predict outcomes in either cohort, there was a trend toward decreased 3-year OS in the FCR cohort for patients with positive PET activity (57 % vs. 90 %. $P = 0.06$). Complete remission (CR) was achieved in the majority of patients after transplant, including 9 of 10 chemorefractory patients, and there was no significant difference in PFS or OS based on chemosensitivity. The 6-year OS for chemorefractory disease was 80 %, which is very promising in this high-risk cohort [1].

The CALGB 109901 is a prospective phase II study that enrolled 44 patients with relapsed chemosensitive indolent NHL and chronic lymphocytic leukemia (CLL), including 16 patients with FL. All patients underwent allo-HCT from MRD using RIC with FC. The RR and NRM rates were 19 and 9 %, respectively. The 3-year event-free survival (EFS) and OS rates for the FL cohort were 75 and 81 %, respectively, which were higher than their counterpart patients with CLL and other indolent lymphomas in the study [2].

The Spanish group (GELTAMO) analyzed the outcomes of 2 prospective protocols including 37 patients with FL who had undergone allo-HCT from MRD using RIC with fludarabine and melphalan (Flu/Mel). Among enrolled patients, 18 % were chemorefractory and 46 % had already failed prior autograft. The 4-year RR and NRM rates were 8 and 37 %, respectively. Chemorefractory lymphoma prior to transplant was a risk factor for increasing NRM ($P = 0.04$). The 4-year PFS and OS rates for the whole group were 55 and 57 %, respectively. Disease status prior to transplant was associated with a trend toward improved PFS and OS after transplant, but it did not reach statistical significance. The 4-year OS in patients with CR, PR, and refractory disease were 71, 48, and 29 %, respectively ($p = 0.09$) [3].

The Canadian study used a different approach to treat R/R FL by applying tandem auto-HCT followed by NMA allo-HCT from MRD. Among 27 enrolled patients, 19 % were considered chemorefractory at the time of auto-HCT and 19 % had transformed histology. The conditioning regimen was BEAM (carmustine, etoposide, cytarabine, melphalan) or BEAC (carmustine, etoposide, cytarabine, cyclophosphamide) for auto-HCT and Flu/Cy (Fludarabine, cyclophosphamide) for allo-HCT, and the median time between the 2 transplants was 133 days. The Canadian approach was

proven to be active and safe. There was no reported relapse during the follow-up period, and the NRM was as low as 4 %. The 3-year PFS and OS rates were 96 % for both. No cases of therapy-related MDS/AML was observed during follow-up [4].

However, retrospective analysis of transplant registries reported the outcome of allo-HCT in R/R FL were inferior to single center experiences, but these results provide more insights regarding the role of allo-HCT in FL. The largest analysis was reported by the Center for International Blood and Marrow Transplant Research (CIBMTR), in which myeloablative (MAC) (n = 120) was compared to RIC (n = 88) in patients who had undergone allo-HCT from MRD for R/R FL. Although 3-year OS (62 % vs. 71 %, p = 0.15) and PFS (55 % vs. 67 %, p = 0.07) were not different between RIC and MAC, respectively, there was an increased risk for lymphoma relapse after RIC in multivariate analysis (Relative risk = 2.97, p = 0.04). The 3-year probability for lymphoma progression was 8 % after MAC and 17 % after RIC in univariate analysis. Poor performance status (PS) and chemorefractory disease were associated with lower PFS and OS, and higher TRM [5].

In contrast to the CIBMTR analysis, the European Group for Blood and Marrow transplantation (EBMT) analysis compared the outcome of allo-HCT in 131 patients who had undergone allo-HCT MUD for R/R FL (RIC = 87, MAC = 44). The RIC cohort was older, more frequently had undergone prior autograft, and less frequently used ATG as part of conditioning compared to the MAC cohort. The 3-year NRM, RR, PFS, and OS rates were 33, 30, 47, and 51 %, respectively, for the whole cohort. No significant difference was noticed between RIC and MAC in the risk of acute or chronic graft versus host disease (GVHD); however, the occurrence of acute GVHD (aGVHD) was associated with higher risk of NRM, and shorter PFS and OS. In multivariate analysis, lower NRM and higher PFS and OS were seen for patients who had undergone RIC, and lower PFS and OS for patients who had prior autograft. Of 61 patients with prior autograft, 3-year NRM, RR, PFS, and OS rates were 42, 19, 39, and 42 %, respectively [6].

The role of T cell-depleted RIC allo-HCT in FL was the focus of the United Kingdom (UK) analysis. Among 82 patients who were conditioned with Flu/Mel/Alemtuzumab, 9 % had chemorefractory disease, 26 % had failed prior autograft, and 54 % received transplant from MUD. The study reported low NRM (15 %), aGVHD (13 %), and chronic GVHD (cGVHD) (18 %). The 4-year PFS and OS rates were 76 % for the whole cohort. Post-transplant, 52 % of 64 evaluable patients with chimerism study had mixed chimerism (MC). Of 28 patients who received DLI for MC, 61 % achieved full chimerism (FC). The 4-year RR was higher for patients with persistent MC compared to FC cohort (36 % vs. 10 %; p = 0.03). There was a trend toward higher NRM in MUD recipients compared to MRD (22 % vs. 8 %, p = 0.08). The 4-year PFS was lower in MUD compared to MRD (64 % vs. 90 %; p = 0.012), and in patients who had prior autograft compared for those without (57 % vs. 83 %; p = 0.016), but there was no difference based on disease chemosensitivity at the time of transplant (p = 0.133). Nevertheless, in multivariate analysis, MRD was the only variable associated with improved PFS (p = 0.035) and OS (p = 0.037). Salvage DLI was effective for relapse after transplant as 10 of 13 patients (77 %) achieved CR, and this including 9 of whom had a durable response [7].

The Fukuoka BMT Group from Japan analyzed the outcome of 30 R/R FL who had undergone allo-HCT (MAC = 43 %, RIC = 57 %), including 50 % with chemorefractory disease and 7 % with prior autograft. There was no difference in outcomes between RIC and MAC. The 2-year RR, NRM, PFS, and OS rates were 20, 33, 47, and 47 %, respectively, for the whole cohort [8].

3 Allogeneic HCT in Aggressive B-Cell NHL

3.1 Diffuse Large B-Cell Lymphoma (DLBCL)

Combination chemotherapy/immunotherapy is a curative treatment in over half of newly diagnosed *diffuse large B-cell lymphoma* (*DLBCL*); however, some patients will be refractory to upfront chemotherapy and some will relapse after initial CR. Auto-HCT is considered the standard practice for relapsed chemosensitive DLBCL, with a 5-year EFS of 46 % reported in those transplanted in the pre-rituximab era [9]. Nowadays, rituximab is given routinely upfront and the outcome for those with relapsed DLBCL seems inferior. In the CAROL randomized study comparing salvage R-ICE versus R-DHAP in R/R DLBCL, and in which the treatment was followed by auto-HCT in responders, the 3-year EFS was only 20 % in those with prior rituximab exposure, high IPI score and/or short prior duration of remission (less than one year) [10].Therefore, auto-HCT is not justified in these cases, neither in those who relapsed/progressed after prior autograft, those with relapsed DLBCL who fail to collect enough cells to undergo auto-HCT, nor those who are chemorefractory to salvage therapy. Allogeneic HCT is considered a potential curative therapy in the formerly mentioned situations; however, the lack of prospective data precludes deriving a definitive role for the procedure. Furthermore, in contrast to FL, DLBCL seems to be associated with higher NRM and post-transplant RR, and therefore lower long-term outcomes.

Retrospective analysis from the CIBMTR included 396 patients who had undergone allo-HCT for R/R DLBCL [(MAC = 165 (42 %); RIC = 143 (36 %); NMA = 88(22 %)]. Recipients of MAC were younger, had more advanced stage and B-symptoms at diagnosis, had more chemorefractory disease at the time of transplant, and less frequently had undergone prior autograft compared to RIC/NMA. The 5-year NRM was higher in MAC recipients compared to RIC and NMA recipients (56 % vs. 47 % vs. 36 %, p = 0.007), while 5-year RR rate was lower in MAC compared to RIC/NMA (26 % vs. 38 % vs. 40 %, p = 0.031). There was no significant difference in 5-year PFS (15–25 %) and OS (18–26 %) according to conditioning regimen. Acute GVHD and chronic GVHD were similar among the different groups. In multivariate analysis, NRM was greater in patients with poor PS, MUD, and chemorefractory disease. Relapse rate was higher in patients with chemorefractory disease and those who had not undergone prior rituximab therapy. DLBCL progression/relapse was uncommon after 1 year post-transplant [11]. Another analysis from the CIBMTR database was restricted only on patients who had their first transplant for DLBCL, and compared the outcomes for auto-HCT (n = 837) vs. MAC

allo-HCT from MRD (n = 79). Allo-HCT recipients had higher risk features, including more chemorefractory disease (42 % vs. 15 %, p = <0.001). Relapse/progression rate was similar in both cohorts. Compared to auto-HCT, allo-HCT was associated with higher TRM, lower PFS, and lower survival. The significant difference in TRM, PFS, and OS was only seen in the first year after transplant; however, no difference in outcome was observed in one-year survivors [12].

In contrast to the CIBMTR, the EBMT (n = 101) and the GITMO (n = 165) analyses focused on the significance of salvage allo-HCT for those who had prior autograft in R/R DLBCL. The EBMT analysis compared the outcome of MAC (n = 37) and RIC (n = 64). RIC recipients were significantly older and had longer interval from diagnosis to transplant as well as longer interval from autograft to allo-HCT. There was no significant difference in PFS and OS between RIC and MAC, but there was a trend toward a higher NRM and lower RR in the MAC recipients. The 3-year NRM, RR, PFS, and OS rates were 28, 30, 42, and 54 %, respectively, for the whole cohort. In multivariate analysis, NRM was higher in those older than 45, had early relapse after autograft, and recipients of marrow graft, while RR was higher in the chemorefractory cohort [13]. In contrast, the GITMO analysis (RIC = 116; MAC = 49) reported 5-year NRM, PFS, and OS rates of 28, 31, and 39 %, respectively. Despite more chemorefractory patients treated with MAC compared to RIC (49 % vs. 27 %, p = 0.003), there was no significant difference in PFS or OS. In multivariate analysis, disease status at the time of transplant influenced PFS and OS, and donor type influenced PFS only. Twenty-three percent of 26 relapsed patients responded to DLI, but data regarding additional treatment and the durability of responses were missing [14].

The Seattle group examined the role NMA allo-HCT in 31 patients with R/R DLBCL. Seventy-five percent of the cohort had failed prior autograft, and 28 % were considered chemorefractory. The 3-year NRM, RR, PFS, and OS rates were 25, 41, 35, and 45 %, respectively. In multivariate analysis, disease sensitivity was associated with improved survival (3-year OS rate of 56 %) [15]. On the other hand, John Hopkins group examined the role of T cell-depleted MRD allo-HCT with R/R DLBCL and compared it to their experience with autograft in the same setting (allo = 45; auto = 138). Although allo-HCT recipients were more chemorefractory and heavily pretreated, they were younger and had earlier disease compared to auto-HCT recipients. Allo-HCT was associated with higher TRM (51 % vs. 24 %, p < 0.001), but the 3-year OS was not significantly different between transplant cohorts (auto = 33 %, allo = 24 %, p = 0.7). There was no difference in OS between chemosensitive (allo = 52 %; auto = 46 %) and chemorefractory disease (allo = 12 %; auto = 19 %). Continuous lymphoma relapse was seen overtime in auto-recipients, even after 4 years from transplant, but no relapse was seen in allo-recipients beyond 13 months of transplant, suggesting the graft versus lymphoma effect in allo-HCT [16].

Other retrospective analysis from United Kingdom (n = 48, including 18 transformed follicular) and France (n = 68) examined the role of RIC allo-HCT in R/R DLBCL. The UK cohort received alemtuzumab as part of conditioning regimen. Among 12 patients who received DLI ± chemoimmunotherapy for relapsed

disease after transplant, 42 % achieved durable remission. The 4-year NRM, RR, PFS, and OS rates were 32, 33, 48, and 47 %, respectively. Donor type, prior autograft, and de novo versus transformed disease did not affect PFS or OS. Chemorefractory recipients ($n = 8$) had a dismal outcome as half relapsed and half died prior to day 100 [17]. The French analysis reported 1-year NRM of 23 %, and 2-year RR, PFS, and OS rates of 44, 49, and 41 %, respectively. Multivariate analysis showed CR at the time of transplant was associated with longer PFS and lower RR. The median time for relapse/progression was 3 months from the time of transplant, and only 3 patients relapsed beyond 1 year [18].

3.2 Other Aggressive B-Cell NHL

Burkitt's lymphoma (BL) is a very aggressive subtype of NHL, and R/R BL is associated with dismal outcomes. Nevertheless, the role of transplant is not well defined in BL. Analysis using the CIBMTR database that included 128 patients with BL who had undergone allo-HCT between 1985 and 2007 showed 5-year RR, PFS, and OS of 27, 50, and 53 % for those transplanted in CR1, and 51, 19, and 20 % for those transplanted in non-CR1, respectively. For those who were not in CR at the time of transplant, the 5-year RR, PFS, and OS rates were 54, 11, and 12 %, respectively [19].

Richter's syndrome (RS) is defined as transformed CLL into aggressive NHL, most commonly DLBCL. RS is more aggressive than de novo DLBCL, and autograft is recommended in CR1 for eligible patients. Analysis from the EBMT database identified 25 patients with RS who had undergone allo-HCT (RIC = 18; MAC = 7). The cohort included 36 % who were considered chemorefractory and 16 % with prior failed autograft. The 3-year NRM, RR, RFS, and OS rates were 26, 47, 27, and 36 %, respectively. Chemosensitive disease and RIC were associated with better EFS. Outcomes were compared to patients with RS who had undergone autograft, in which the majority had chemosensitive disease. The 3-year NRM, RR, RFS, and OS rates for autograft recipients were 12, 43, 45, and 59 %, respectively [20].

Transformed lymphoma (TL) is another aggressive form of NHL that develops in about a third of FL cases. The Canadian Blood and Marrow Transplant Group reviewed retrospectively the outcome of 22 recipients of allo-HCT and compared to 97 of autograft recipients for TL, and 53 who had received rituximab-containing regimen without transplant. TRM was higher in allo-recipients compared to auto-recipients (23 % vs. 5 %). There was no difference in post-transplant survival between allo-HCT and auto-HCT (45 % vs. 57 %, $p = 0.12$). Auto- but not allo-HCT was associated with superior survival in multivariate analysis when compared to rituximab-containing regimen. The 5-year PFS and OS rates from the time of transformation were 46 and 46 % for allo-recipients, 55 and 65 % for auto-recipients, and 40 and 61 % for non-transplant cohort [21]. Analysis from United Kingdom showed no difference in outcomes between de novo and transformed lymphoma for patients who had undergone allo-HCT [17].

4 Allogeneic HCT in Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) is a unique form of NHL that shares the incurability feature of low-grade lymphoma and the clinical behavior of aggressive subtypes. Auto-HCT is part of MCL treatment in the upfront and the relapsed setting. Although allo-HCT was used in those with chemorefractory disease and those relapsed after prior autograft, there are no prospective studies that define the exact role.

Data from the CIBMTR compared the outcomes of auto (n = 381)- versus RIC allo-HCT (n = 138) in chemosensitive MCL and analyzed outcomes based on the timing of transplantation (early = 299, late 220). Early transplantation was defined as those who received transplant in PR1/CR1 with no more than two lines of prior chemotherapy. Allo-HCT was associated with higher NRM but lower RR in both early and late cohorts. There was no significant difference in 5-year OS for early (61 % vs. 62 %, $p = 0.95$) and late (44 % vs. 31 %, $p = 0.2$) transplantation for auto-HCT and RIC allo-HCT cohorts, respectively. Early transplant was associated with survival benefit for both transplant cohorts in multivariate analysis [22]. Another analysis from the CIBMT focusing on the role of allo-HCT in chemorefractory MCL identified 202 patients (MAC = 74, RIC/NMA = 128). More patients in the RIC/NMA cohort had history of prior autograft, received prior rituximab, and had undergone MUD transplant. Despite this, there was no significant difference between MAC and RIC/NMA cohorts in terms of NRM (47 % vs. 43 %, $p = 0.68$), RR (33 % vs. 32 %, 0 = 0.89), PFS (20 % vs. 25 %, $p = 0.53$), and OS (25 % vs. 30 %, $p = 0.45$) at 3 years. This study showed that about 25–30 % of refractory MCL can be cured with allo-HCT [23].

The EBMT study focused on analyzing allo-HCT outcomes for MCL relapsed after prior autograft. The analysis identified 80 patients (22 %) who had relapsed and undergone allo-HCT among 360 patients who had undergone auto-HCT for MCL. The majority of patients (91 %) were in PR/CR at the time of allo-HCT after salvage chemotherapy. The 2-year NRM, RR, and OS rates were 30, 33, and 46 %, respectively. Prolonged duration between autograft and subsequent relapse was the only factor that improved outcomes. The 5-year OS for patients with progression-free interval more than a year after autograft and chemosensitive disease was 60 %. Survival was very uncommon for patients who relapsed after autograft and did not undergo allo-HCT [24].

A retrospective analysis from UK reviewed the outcome of RIC allo-HCT in 70 patients with R/R MCL, with the majority (81 %) had alemtuzumab as part of their conditioning regimen. Thirty-four percent of patients had a prior autograft and 17 % were chemorefractory. The 5-year NRM, RR, PFS, and OS rates were 21, 65, 14, and 37 %, respectively. Thirteen patients with MC received DLI, and all achieved FC, and another 14 relapsed after transplant and received DLI, in which 11 remained in CR at the time of the publication. Occurrence of post DLI GVHD was associated with improved survival. The use of alemtuzumab in conditioning was associated with decreased NRM (p = 0.002), increased RR (p = 0.04), and

improved survival, while chemosensitivity was associated with decreased relapse (p = 0.036) [25]. Another retrospective analysis from France and Germany reviewed RIC allo-HCT in 70 patients with MCL. The 2-year NRM, EFS, and OS rates were 32, 50, and 53 %, respectively. Only disease status directly affected EFS and OS in multivariate analysis. The 2-year EFS and OS rates for chemorefractory disease were 11 and 31 %. In contrast to the British study, the use of ATG during conditioning was associated with a trend toward lower survival in univariate analysis (p = 0.11) [26].

Investigators at MDACC compared the outcomes between NMA allo-HCT (n = 35) and auto-HCT (n = 86) in 121 patients with MCL enrolled in sequential transplantation protocols. Allo-HCT recipients had higher risk features compared to auto-HCT recipients. The NMA cohort had initially lower survival compared to the auto-cohort; however, the picture inverted at 8 years because of plateau in survival curves and absent of late deaths among the NMA recipients. For the NMA cohort, 1-year NRM was 9 %, and the 6-year PFS and OS rates were 46 and 53 %, respectively [27]. Investigators at FHCRC reviewed their experience with NMA allo-HCT in R/R MCL. Among 33 patients conditioned with single-dose TBI and Flu, none of 13 enrolled patients who received transplant in CR relapsed. The 2-year NRM, RR, PFS, and OS rates were 24, 16, 60, and 65 %, respectively. The only predictor for PFS was the number of prior therapies [28].

5 Allogeneic HCT in T-Cell NHL

Peripheral T-cell lymphoma (PTCL) encompasses heterogeneous subtypes of NHL characterized by aggressive behavior and poor long-term outcomes. The role of transplant is not well defined in PTCL.

The only prospective study of allo-HCT in PTCL was conducted in Italy. The study enrolled 17 patients with PTCL, including 47 % who had failed prior autograft. Patients were conditioned with thiotepa/Flu/Cy. The 2-year NRM was 6 %. The 3-year PFS and OS rates were 64 and 81 %, respectively. [29]. A retrospective analysis from Italy reviewed the outcomes of 52 patients with T-cell NHL who had undergone RIC allo-HCT with the same conditioning regimen. The 5-year NRM, RR, PFS, and OS rates were 12, 49, 50, and 40 %, respectively. In multivariate analysis, older age and chemorefractory disease adversely influenced outcomes. Of 12 patients who had received DLI for relapsed disease post-transplant, 8 (66 %) attained response [30].

The CIBMTR database identified 126 patients with T-cell NHL who had undergone allo-HCT (MAC = 74, RIC/NMA = 45), and their outcomes were compared with 241 patients who had undergone auto-HCT. The allo-HCT cohort had higher risk features, including more chemorefractory disease, patients not in CR, higher number of prior therapies, and more non-anaplastic large cell (ALCL) histology. The 3-year NRM, RR, PFS, and OS rates were 34, 38, 37, and 46 %, respectively. Excluding patients in CR1 at the time of transplant, allo-recipients had higher NRM (34 % vs. 6 %) and lower RR (38 % vs. 53 %, p = 0.044) when compared to auto-recipients, but there was no difference in PFS or OS. Among allo-

HCT recipients, number of prior lines of therapy and CR status at transplant was associated mortality and treatment failure [31].

Investigators at John Hopkins reviewed the outcomes of 44 patients with PTCL who had undergone allo-HCT (MAC = 20, RIC = 24). RIC recipients were older than MAC. The 2-year PFS and OS rates were 38 and 45 %, respectively. The 1-year NRM and RR were 10 and 38 % for MAC and 8 and 34 % for RIC, respectively. Grade II–IV acute or chronic GVHD was associated with decreased RR compared to patients without (17 % vs. 66 %, $p = 0.04$) [32]. The French analysis included 77 patients with PTCL who had undergone allo-HCT, including 57 who were conditioned with MAC. The 5-year NRM, EFS, and OS rates were 33, 53, and 57 %, respectively. Disease status at the time of transplant influenced OS and EFS, and occurrence of grade III/IV acute GVHD was associated with worse survival [33].

The EBMT analysis was restricted on allo-HCT outcome for **angioimmunoblastic T lymphoma (AITL)**. Among 45 patients included in the study (MAC = 25, RIC = 20), 18 had chemorefractory disease and 11 had prior autograft. The 3-year NRM, RR, PFS, and OS were 27, 20, 53, and 64 %, respectively. NRM was higher in patients with poor PS, while RR was lower in those who developed cGVHD. Chemosensitivity was a predictor for improved PFS and OS [34].

Adult T-cell leukemia/lymphoma (ATLL) is a unique subtype of PTCL associated with HTLV1 infection. ATLL has different presentation and is usually resistant to conventional chemotherapy. Investigators from Japan reviewed the outcomes of 40 patients (Lymphoma type = 10) with ATLL who had undergone MAC allo-HCT. The 3-year RR, PFS, and OS rates were 39, 34, and 45 %, respectively. No difference in outcomes was observed between lymphoma and acute type. There was a suggestion of graft versus ATLL effect as 3 out of 10 relapsed patients after transplant achieved CR with reduction or cessation of immunosuppressive agents (IS) [35]. Another retrospective analysis from Japan Marrow Donor Program (JMDP) focused on the role of MUD allo-HCT in ATLL. Among 33 included patients, the 1-year NRM, RR, PFS, and OS rates were 32, 19, 49, and 50 %, respectively. Only age was a significant prognostic factor in multivariate analysis [36]. A prospective study of RIC allo-HCT in older patients (>50 years) with ATLL from Japan showed promising results. Patients received ATG as part of the conditioning regimen. Among 15 evaluable patients, 75 % achieved CR (pretransplant CR was 20 %), only 1 patient had early TRM, and 9 patients relapsed subsequently (6 within 100 days). Three relapsed patients achieved response with tapering down IS. The estimated 5-year OS was 33 %, and there was a trend toward improved survival in patients who developed aGVHD (50 % vs. 0 %, $p = 0.06$). What was interesting in the study is that HTLV1 viral load became undetectable in 8 of 15 patients, which indicates a potential role of allo-HCT as anti-viral therapy [37].

NK/T-cell lymphoma is another aggressive subtype of T-cell NHL associated with poor outcomes. A retrospective analysis from Japan identified 28 patients with NK/T-cell lymphoma/leukemia (extranodal = 22, blastic = 3, NK-cell leukemia = 3), including 16 with chemorefractory disease. The 2-year TRM, PFS, and OS rates were 28, 34, and 40 %, respectively. No disease relapse occurred after

10 months from the time of transplant. Only older age, marrow source, and diagnosis of extra-nodal lymphoma were associated with shorter PFS in multivariate analysis [38].

Primary cutaneous T-cell lymphoma (CTCL) includes several subtypes of lymphomas that are usually incurable with conventional chemotherapy. MDACC reports their outcomes of 19 patients with CTCL who had undergone total skin radiation followed by RIC allo-HCT. The ORR and CR rates were 68 and 58 %, respectively. Five of 8 relapsed patients after transplant achieved CR with tapering IS or DLI. The 2-year PFS and OS rates were 53 and 79 %, respectively [39]. City of Hope performed allo-HCT in 8 patients with advanced heavily pretreated CTCL. All patients achieved CR after transplant, and 2 patients had TRM. With a median follow-up of 56 months, 6 patients were alive and disease free [40]. The EBMT reviewed the outcomes of 60 patients with CTCL (RIC = 44, MAC = 16), including 47 % with chemorefractory disease. The 2-year NRM was 22 %. The 3-year RR, PFS, and OS rates were 47, 34, and 53 %, respectively. RIC cohort had decreased NRM (relative risk = 4.7, $p = 0.008$) without increased RR compared to MAC, and therefore, RIC had improved survival ($p = 0.03$). Advanced disease and MUD recipients were associated with shorter PFS and OS [41]. Meta-analysis identified 29 cases from the literature with CTCL who had undergone HCT (allo = 20, auto = 19) and compared the outcomes based on the type of HCT. Despite the fact that allo-recipients had more prior lines of therapies, EFS and OS were superior compared to auto-HCT [42].

6 Summary and Conclusion

Allo-HCT is an understudied area in NHL management. Among NHL subtypes, R/R FL has the best and mature data for allo-HCT. Several prospective studies confirmed the feasibility, safety, and activity of RIC/NMA allo-HCT in R/R FL. The longest follow-up was provided by MDACC study, which showed 11-year PFS and OS rates of 72 and 78 %, respectively [1]. Other prospective and retrospective studies consistently showed potential cure in half or even more of R/R FL patients [1–8]. RR was impressively low post-allo-transplant, and it was reported as less than 10 % in the MDACC, the Spanish, and the Canadian studies [1, 3, 4]. On the other hand, TRM was acceptable and it was as low as less than 15 %, with the exception of the GELTAMO study, which included more patients with prior autograft [1, 2, 4]. Although disease status adversely influenced lymphoma outcomes after allo-HCT in the CIMBTR analysis [5], other studies showed no effect [1, 4, 7] or only trend toward adverse effect [1, 3]. Replacing rituximab with Yttium-90 ibritumomab tiuxetan has been shown to overcome chemorefractory diseases in the MDACC study [1]. The GVL effect was demonstrated by the response to DLI in relapsed patients post-transplant [8].The Canadian approach was different and used tandem auto- followed by allo-HCT, and it showed safety and high activity [4]. Although the CIBMTR and Fukuoka BMT Group analyses failed to show difference in PFS or OS based on conditioning regimen [5, 8], multivariate

analysis from the EBMT showed improved PFS and survival in RIC recipients [6]. The inclusion of alemtuzumab in the conditioning regimen was examined by the British group, and despite higher RR, the long-term PFS and survival was comparable to other studies [7].

In contrast, the role of allo-HCT data is less convincing in DLBCL histology. There are no prospective studies exclusively focusing on allo-HCT in DLBCL. However, retrospective data have shown long-term survival in the range of 18–54 %, depending on the study inclusion criteria. RR for DLBCL seems to be higher after transplant in comparison with FL (26–41 %) as well as NRM (23–51 %). However, lymphoma relapse after one year of transplant was uncommon [11, 16, 18]. Chemorefractory disease and early relapse after prior autograft were most consistent factors adversely influencing outcomes [11, 13–18]. The intensity of conditioning regimen (MAC vs. RIC/NMA) did not influence PFS or survival data despite some disparities in RR and NRM [11, 13, 14]. DLI ± chemoimmunotherapy achieved responses in 20–40 % for post-transplant relapsed patients [14, 17]. In other aggressive, less common, B-cell NHL (BL, RS, TL), allo-HCT showed feasibility in small retrospective studies for R/R cases; however, for those with BL beyond CR1, allo-HCT cures only minority of patients.

For MCL, the controversy regarding the role of all-HCT is not solved yet due to the lack of prospective data. However, retrospective analysis demonstrated a potential long-term control of allo-HCT in 14–60 % of R/R MCL, including possible cure in quarter of chemorefractory cases [22–25, 27, 28]. When auto-HCT was compared to RIC allo-HCT for MCL retrospectively, no difference in long-term outcomes was noticed in early and late transplants [22]. No difference in outcome was observed based on conditioning intensity [23]. The role of in vivo T-cell depletion has not been answered yet. Although alemtuzumab in the conditioning regimen was associated with reduced NRM, increased RR, and improved survival in the British analysis [25], the retrospective analysis from France and Germany showed a trend toward worsened survival with the use of anti-thymocye globulin [26]. DLI showed activity in converting MC to FC and in achieving durable response for relapse after transplant [26].

PTCL data are more complicated area due to the heterogeneity of subtypes and the rarity of each subtype. Nevertheless, one small prospective study ($n = 17$) from Italy showed encouraging outcomes, with low NRM (6 %) and high 3-year PFS and OS (64 and 81 %, respectively) [29]. However, retrospective analysis of the outcome of allo-HCT in R/R PTCL showed long-term PFS and survival of 37–53 % and 43–64 %, respectively. The RR post-transplant was significant (20–49 %). When allo-HCT was compared to auto-HCT after excluding cases in CR1 in the CIBMTR analysis, no difference in PFS or survival was reached between the 2 cohorts, despite higher NRM and lower RR in the allo-cohort [31]. Studies restricted to specific subtypes of PTCL (AITL, ATLL, NK/T-cell, and CTCL) showed feasibility and activity [36, 37, 39–42]. An interesting graft versus HTLV1 effect was observed in the Japanese prospective study of allo-HCT in ATLL, as the viral load became undetectable in over half of enrolled patients after transplant [39].

MAC was the predominant conditioning used in allo-HCT until recently when RIC/NMA regimens utilization increased to reduce regimen-related toxicity and widen the indication of transplant in older patients. The outcome of MAC vs. RIC vs. NMA conditioning in NHL was compared in retrospective manners. Single institution analysis from COH reviewed the outcome of MAC (n = 48) and RIC (n = 40) in NHL patients who had undergone allo-HCT. RIC recipients had higher risk features. The study concluded that there was no significant difference in NRM, PFS, or OS between MAC and RIC despite that RIC and prior autograft were predictors of increased RR in multivariate analysis [43]. Other retrospective analyses reached the same conclusion [5, 8, 11, 13, 14, 23, 31]. The majority of studies of allo-HCT in NHL showed no influence of donor source (MRD vs. MUD) on long-term outcomes [1, 8, 15, 17, 18, 25, 33]; however, other studies showed negative effect on NRM [11], PFS [7, 14], and OS [7] for MUD recipients. The use of alternative donors with umbilical cord was shown to be viable in NHL, and outcomes were comparable to MUD [44].

Graft versus host disease is the leading cause of NRM in allo-HCT. The incidence of grade II–IV aGVHD in allo-HCT for NHL is in the range of 13–50 %, but it is remarkably lower for T cell-depleted studies (<20 %) [7, 17, 25, 27]. The occurrence of aGVHD was associated with adverse outcomes in some studies [6, 33, 35, 40, 43] The incidence of extensive cGVHD was reported in 18–64 % of NHL patients, and the occurrence of extensive cGVHD was also adversely affected outcomes in some studies [21, 43] despite decreased RR in one study [34]. Furthermore, the occurrence of GVHD after DLI improved survival in another study [25].

Response prior to transplant was the most important predictor of transplant outcome in most studies. The majority of studies showed worse outcomes for chemorefractory disease, especially in RIC recipients [45]. With the availability of newer agents for treatment of NHL, more patients may be able to achieve a complete or partial remission before transplant, thus leading to improved survival post-allo-HCT. The use of pretransplant FDG-PET scan in chemosensitive disease based on CT scan did not influence EFS or survival in one retrospective study of patients who had undergone NMA transplant for NHL [44]. Future studies on the role of FDG-PET in predicting the outcome of allo-HCT are required.

References

1. Khouri IF, Saliba RM, Erwin WD et al (2012) Nonmyeloablative allogeneic transplantation with or without 90yttrium ibritumomab tiuxetan is potentially curative for relapsed follicular lymphoma: 12-year results. Blood 119:6373–6378
2. Shea T, Johnson J, Westervelt P et al (2011) Reduced-intensity allogeneic transplantation provides high event-free and overall survival in patients with advanced indolent B cell malignancies: CALGB 109901. Biol Blood Marrow Transplant 17:1395–1403
3. Piñana JL, Martino R, Gayoso J et al (2010) Reduced intensity conditioning HLA identical sibling donor allogeneic stem cell transplantation for patients with follicular lymphoma: long-term follow-up from two prospective multicenter trials. Haematologica 95:1176–1182

4. S Cohen, Kiss T, Lachance S et al (2012) Tandem autologous-allogeneic nonmyeloablative sibling transplantation in relapsed follicular lymphoma leads to impressive progression-free survival with minimal toxicity. Biol Blood Marrow Transplant 18:951–957
5. Hari P, Carreras J, Zhang MJ et al (2008) Allogeneic transplants in follicular lymphoma: higher risk of disease progression after reduced-intensity compared to myeloablative conditioning. Biol Blood Marrow Transplant. 14:236–245
6. Avivi I, Montoto S, Canals C et al (2009) Matched unrelated donor stem cell transplant in 131 patients with follicular lymphoma: an analysis from the lymphoma working party of the European group for blood and marrow transplantation. Br J Haematol 147:719–728
7. Thomson KJ, Morris EC, Milligan D et al (2010) T-cell-depleted reduced-intensity transplantation followed by donor leukocyte infusions to promote graft-versus-lymphoma activity results in excellent long-term survival in patients with multiply relapsed follicular lymphoma. J Clin Oncol 28:3695–3700
8. Ito Y, Miyamoto T, Kamimura T et al (2013) Clinical outcomes of allogeneic stem cell transplantation for relapsed or refractory follicular lymphoma: a retrospective analysis by the fukuoka blood and marrow transplantation group. Int J Hematol 98:463–471
9. Philip T, Guglielmi C, Hagenbeek A et al (1995) Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med 333:1540–1545
10. Gisselbrecht C, Glass B, Mounier N et al (2010) Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol 28:4184–4190
11. Bacher U, Klyuchnikov E, Le-Rademacher J et al (2012) Conditioning regimens for allotransplants for diffuse large B-cell lymphoma: myeloablative or reduced intensity? Blood 120:4256–4262
12. Lazarus HM, Zhang MJ, Carreras J et al (2010) A comparison of HLA-identical sibling allogeneic versus autologous transplantation for diffuse large B cell lymphoma: a report from the CIBMTR. Biol Blood Marrow Transplant. 16:35–45
13. van Kampen RJ, Canals C et al (2011) Allogeneic stem-cell transplantation as salvage therapy for patients with diffuse large B-cell non-Hodgkin's lymphoma relapsing after an autologous stem-cell transplantation: an analysis of the European group for blood and marrow transplantation registry. J Clin Oncol 29:1342–1348
14. Rigacci L, Puccini B, Dodero A et al (2012) Allogeneic hematopoietic stem cell transplantation in patients with diffuse large B cell lymphoma relapsed after autologous stem cell transplantation: a GITMO study. Ann Hematol 91:931–939
15. Rezvani AR, Norasetthada L, Gooley T et al (2008) Non-myeloablative allogeneic haematopoietic cell transplantation for relapsed diffuse large B-cell lymphoma: a multicentre experience. Br J Haematol 143:395–403
16. Aksentijevich I, Jones RJ, Ambinder RF et al (2006) Clinical outcome following autologous and allogeneic blood and marrow transplantation for relapsed diffuse large-cell non-Hodgkin's lymphoma. Biol Blood Marrow Transplant. 12:965–972
17. Thomson KJ, Morris EC, Bloor A et al (2009) Favorable long-term survival after reduced-intensity allogeneic transplantation for multiple-relapse aggressive non-Hodgkin's lymphoma. J Clin Oncol 20(27):426–432
18. Sirvent A, Dhedin N, Michallet M et al (2010) Low nonrelapse mortality and prolonged long-term survival after reduced-intensity allogeneic stem cell transplantation for relapsed or refractory diffuse large B cell lymphoma: report of the société française de greffe de moelle et de thérapie cellulaire. Biol Blood Marrow Transplant 16:78–85
19. Maramattom LV, Hari PN, Burns LJ et al (2013) Autologous and allogeneic transplantation for Burkitt lymphoma outcomes and changes in utilization: a report from the center for international blood and marrow transplant research. Biol Blood Marrow Transplant 19: 173–179

20. Cwynarski K, van Biezen A, de Wreede L et al (2012) Autologous and allogeneic stem-cell transplantation for transformed chronic lymphocytic leukemia (Richter's syndrome): a retrospective analysis from the chronic lymphocytic leukemia subcommittee of the chronic leukemia working party and lymphoma working party. J Clin Oncol 30:2211–2217
21. Villa D, Crump M, Panzarella T et al (2013) Autologous and allogeneic stem-cell transplantation for transformed follicular lymphoma: a report of the Canadian blood and marrow transplant group. J Clin Oncol 31:1164–1171
22. Fenske TS, Zhang MJ, Carreras J et al (2014) Autologous or reduced-intensity conditioning allogeneic hematopoietic cell transplantation for chemotherapy-sensitive mantle-cell lymphoma: analysis of transplantation timing and modality. J Clin Oncol 32:273–281
23. Hamadani M, Saber W, Ahn KW et al (2013) Allogeneic hematopoietic cell transplantation for chemotherapy-unresponsive mantle cell lymphoma: a cohort analysis from the center for international blood and marrow transplant research. Biol Blood Marrow Transplant 19: 625–631
24. Dietrich S, Boumendil A, Finel H et al (2014) Outcome and prognostic factors in patients with mantle-cell lymphoma relapsing after autologous stem cell transplantation: a retrospective study of the European group for blood and marrow transplantation (EBMT). Ann Oncol 25:1053–1058
25. Cook G, Smith GM, Kirkland K et al (2010) Outcome following reduced-intensity allogeneic stem cell transplantation (RIC AlloSCT) for relapsed and refractory mantle cell lymphoma (MCL): a study of the British society for blood and marrow transplantation. Biol Blood Marrow Transplant 16:1419–1427
26. Le Gouill S, Kröger N, Dhedin N et al (2012) Reduced-intensity conditioning allogeneic stem cell transplantation for relapsed/refractory mantle cell lymphoma: a multicenter experience. Ann Oncol 23:2695–2703
27. Tam CS, Bassett R, Ledesma C et al (2009) Mature results of the M. D. Anderson Cancer Center risk-adapted transplantation strategy in mantle cell lymphoma. Blood 113:4144–4152
28. Maris MB, Sandmaier BM, Storer BE et al (2004) Allogeneic hematopoietic cell transplantation after fludarabine and 2 Gy total body irradiation for relapsed and refractory mantle cell lymphoma. Blood 104:3535–3542
29. Corradini P, Dodero A, Zallio F et al (2004) Graft-versus-lymphoma effect in relapsed peripheral T-cell non-Hodgkin's lymphomas after reduced-intensity conditioning followed by allogeneic transplantation of hematopoietic cells. J Clin Oncol 22:2172–2176
30. Dodero A, Spina F, Narni F et al (2012) Allogeneic transplantation following a reduced-intensity conditioning regimen in relapsed/refractory peripheral T-cell lymphomas: long-term remissions and response to donor lymphocyte infusions support the role of a graft-versus-lymphoma effect. Leukemia 26:520–526
31. Smith SM, Burns LJ, van Besien K et al (2013) Hematopoietic cell transplantation for systemic mature T-cell non-Hodgkin lymphoma. J Clin Oncol 31:3100–3109
32. Kanakry JA, Kasamon YL, Gocke CD et al (2013) Outcomes of related donor HLA-identical or HLA-haploidentical allogeneic blood or marrow transplantation for peripheral T cell lymphoma. Biol Blood Marrow Transplant 19:602–606
33. Le Gouill S, Milpied N, Buzyn A et al (2008) Graft-versus-lymphoma effect for aggressive T-cell lymphomas in adults: a study by the Société Francaise de Greffe de Moëlle et de Thérapie Cellulaire. J Clin Oncol 26:2264–2271
34. Kyriakou C, Canals C, Finke J et al (2009) Allogeneic stem cell transplantation is able to induce long-term remissions in angioimmunoblastic T-cell lymphoma: a retrospective study from the lymphoma working party of the European group for blood and marrow transplantation. J Clin Oncol 27:3951–3958
35. Fukushima T, Miyazaki Y, Honda S et al (2005) Allogeneic hematopoietic stem cell transplantation provides sustained long-term survival for patients with adult T-cell leukemia/lymphoma. Leukemia 19:829–834

36. Kato K, Kanda Y, Eto T, Muta T et al (2007) Allogeneic bone marrow transplantation from unrelated human T-cell leukemia virus-I-negative donors for adult T-cell leukemia/lymphoma: retrospective analysis of data from the Japan marrow donor program. Biol Blood Marrow Transplant 13:90–99
37. Okamura J, Uike N, Utsunomiya A, Tanosaki R (2007) Allogeneic stem cell transplantation for adult T-cell leukemia/lymphoma. Int J Hematol 86:118–125
38. Murashige N, Kami M, Kishi Y et al (2005) Allogeneic haematopoietic stem cell transplantation as a promising treatment for natural killer-cell neoplasms. Br J Haematol 130:561–567
39. Duvic M, Donato M, Dabaja B et al (2010) Total skin electron beam and non-myeloablative allogeneic hematopoietic stem-cell transplantation in advanced mycosis fungoides and Sezary syndrome. J Clin Oncol 28:2365–2372
40. Molina A, Zain J, Arber DA et al (2005) Durable clinical, cytogenetic, and molecular remissions after allogeneic hematopoietic cell transplantation for refractory Sezary syndrome and mycosis fungoides. J Clin Oncol 23:6163–6171
41. Duarte RF, Canals C, Onida F et al (2010) Allogeneic hematopoietic cell transplantation for patients with mycosis fungoides and Sézary syndrome: a retrospective analysis of the lymphoma working party of the European group for blood and marrow transplantation. J Clin Oncol 28:4492–4499
42. Wu PA, Kim YH, Lavori PW et al (2009) A meta-analysis of patients receiving allogeneic or autologous hematopoietic stem cell transplant in mycosis fungoides and Sezary syndrome. Biol Blood Marrow Transplant 15:982–990
43. Rodriguez R, Nademanee A, Ruel N et al (2006) Comparison of reduced-intensity and conventional myeloablative regimens for allogeneic transplantation in non-Hodgkin's lymphoma. Biol Blood Marrow Transplant 12:1326–1334
44. Rodrigues CA, Rocha V, Dreger P et al (2014) Alternative donor hematopoietic stem cell transplantation for mature lymphoid malignancies after reduced-intensity conditioning regimen: similar outcomes with umbilical cord blood and unrelated donor peripheral blood. Haematologica 99:370–377
45. Castagna L, Boubdallah R, Furst S et al (2013) Disease status is a more reliable predictive factor than histology in lymphoma patients after reduced-intensity conditioning regimen and allo-SCT. Bone Marrow Transplant 48:794–798