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Introduction

Although the use of antibodies as tumor-targeting agents dates back to 1953 when Pressman and Korngold [1] showed that radiolabeled rabbit antisera could specifically target mouse osteogenic sarcoma cells, not until 1975, with the development of the hybridoma technology [2], adequate quantities of murine monoclonal antibodies (mAbs) became available for clinical use. These early trials demonstrated that murine mAbs had some measure of antitumor activity in hematological malignancies but also highlighted the limiting effect of the human-anti-mouse humoral immune responses on multiple mAb administrations. Despite the simplicity of the concept (antibody–antigen interaction), researchers had to confront a number of technical difficulties, including the selection of a proper and stable target, efficient delivery, and immunogenicity due to the murine components of the mAbs [3].

In 1997 the anti-CD20 antibody rituximab became the first recombinant chimeric mAb approved by the US Food and Drug Administration (FDA) for use in the treatment of cancer, demonstrating very limited immunogenicity and leading to the explosion of the field on mAb-based therapeutics. The methodology for the manufacturing of mAbs for clinical use has evolved substantially over the past 15 years, allowing the creation of “designer” antibody biopharmaceuticals that have fully human sequence and combine the desired antigen-binding specificity with unique biodistribution and pharmacodynamic properties. The nomenclature of mAb reflects this diversity. While the suffix *mab* defines the product as a monoclonal antibody, the letter or syllable in front of the *mab* reveals the antibody’s source as murine (*-omab*), chimeric (*-ximab*), humanized (*-zumab*), or fully human (*-umab*) [4]. Thus edrocolomab is a murine anti-EpCAM antibody, galiximab is a chimeric anti-CD80 antibody, alemtuzumab is a humanized anti-CD52 antibody, and ofatumumab is a fully human anti-CD20 antibody.

One of the key goals in the clinical development of mAbs for clinical use is the identification of specific surface antigens—defined according to standardized nomenclature as clusters of differentiation (CD)—whose expression is ideally restricted to the malignant cells. Unfortunately, such degree of specificity is rarely achieved and many clinically relevant antigens represent essential components of vital activation, survival, and trafficking pathways for both malignant and

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normal cells. Therefore, the fact that cell surface antigens expressed on lymphoma cells are virtually always present on their normal counterparts continues to represent a significant limit for the clinical application of these agents. mAbs induce tumor cell death by blocking survival pathways (direct apoptosis) along with other immunologic mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). mAbs have been used as single agent therapy or, in the attempt to enhance their ability to destroy tumor cells, in combination with chemotherapy, other antibodies or conjugated either to radioisotopes or to immunotoxins. Based on the activity in hematologic malignancies and their safety profile, mAbs are now among the most frequently used biopharmaceutical agents.

The development of mAbs for the treatment of T-cell lymphomas (TCLs) is rooted in the early functional characterization of the T-cell receptor and other surface antigens in human T-cells [3–6], and in subsequent studies aimed at the discovery of novel immune suppressive drug for use in human transplantation. The first mAb approved for clinical use was the anti-CD3 murine antibody OKT3 (muromonab), approved in 1986 for the prevention of kidney transplant rejection. Clinical experience with OKT3 showed that the drug was potentially immune suppressive, achieving the desired effect against organ rejection, but also revealed a significantly higher risk of development of post-transplant lymphoproliferative disorder (PTLD), compared to conventional anti-rejection drugs such as antimetabolites and calcineurin inhibitors. The progressive discovery and characterization of surface markers such as CD2, CD4, CD5, CD6, CD25, CD30, and CD52 that are expressed on various functional subsets of T-cells, led to the development of corresponding murine or chimeric mAbs for clinical use. Although early investigations of these mAbs were focused on prevention of graft versus host disease (GVHD) and solid organ allograft rejection, as well as the treatment of severe autoimmune diseases, pilot studies in select subtypes of TCL we also conducted, with encouraging results. Both early and later studies of mAb-based therapy

in TCL, however, have so far failed to achieve the efficacy, flexibility of use in combination, and safety profile of rituximab. Most of the anti-T-cell mAbs in clinical development or approved for TCL interfere with T-cell activation and trafficking and result in severe and prolonged T-cell depletion. Many have been associated with reactivation of latent herpesviruses, such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV), and a broad spectrum of bacterial and fungal opportunistic infections. Thus, in the absence of truly tumor-specific markers, the difficulty of balancing an efficient kill of malignant T-cells with an adequate preservation of normal cell-mediated immunity remains the most challenging obstacle to the development of active and safe mAbs for the treatment of TCL. Presently a number of mAbs targeting T-cell surface antigens are being evaluated in clinical trials (Table 14.1) and the purpose of this chapter is to summarize the present and potentials roles for mAbs in the treatment of TCL.

CD2 Antigen

CD2 is an approximately 50 KD transmembrane glycoprotein expressed on dendritic cells, natural killer (NK) cells, thymocytes, and mature T-cells [6–9] with much higher expression on activated T-cells than on resting T-cells [10]. Although the specificity for T-cell activation resides in the TCR/CD3 complex, the interaction of additional accessory molecules such as CD2, CD28, and lymphocyte functional antigen-1 (LFA-1) with ligands on the antigen presenting cells (APCs), is required for complete T-cell activation to occur [11]. CD2 acts as an adhesion molecule [12] through its extracellular region which has two Ig superfamily domains, the first one binds CD58 (LFA-3) [13] and the second one binds CD59 [14], helping to localize the T-cells in proximity of APCs [11, 15–17]. CD2 acts also as a signaling molecule through its large intracytoplasmic domain: as a result of CD2–CD58 interaction, the CD2 intra-cytoplasmic domain physically associates with the Wiskott-Aldrich syndrome protein [18] and the tyrosine kinase

Table 14.1 mAbs therapy for T-cell leukemia and lymphoma

Target	Description	Targeting agent	References
CD2	LFA-3 (CD58) receptor	Alefacept (Amevive) Siplizumab (MEDI-507)	[24–30, 171, 172]
CD3	Signaling chain (CD3 ζ) of the T-cell receptor (TCR)	Muromonab-CD3 (Orthoclone, OKT3)	[34–39, 173]
CD4	TCR co-receptor Anti-Leu3a M-T412	Zanolimumab (HuMax-CD4)	[43, 47–49]
CD5	Scavenger receptor family member	Anti-Leu1 T101 Anti-CD5-ricin A (CD5-Plus)	[4, 174]
CD25	IL-2 receptor α -subunit	Murine anti-Tac Daclizumab (Zenapax) Denileukin diftitox (Ontak)	[57, 63, 69, 72–76, 175]
CD122	IL-2 and IL-15 receptor β -subunit	Murine and humanized Mik- β 1	[81]
CD30	TNF receptor family member	Ber-H2 Hefi-1 M67 MDX-060 SGN-30 SGN-35	[103–105, 119–122]
CD52	GPI-anchored glycoprotein	Alemtuzumab (Campath)	[124]

p56^{lck} [19, 20] resulting in the initiation of the phospholipase C pathway [21, 22], in actin polymerization with further strengthening of intercellular adhesion, in the production of IL-2 and, ultimately, into T-cell activation and proliferation [23].

CD2-Targeted Therapy

The limited expression of CD2 restricted to mature T-cells, the expression of CD2 on malignant T-cells, combined with preliminary data showing that engagement of CD2 by mAbs can directly trigger apoptosis [24], provided the scientific rationale for their use in immunotherapy for TCL. A number of mAbs directed against CD2 have demonstrated the ability to inhibit T-cell activation. In particular, the rat mAb BTI-322 (MedImmune, Inc.) has been used in the prevention of allograft rejection [25] and in the therapy of GVHD [26]. Given the immunosuppressive properties of BTI-322, a humanized genetically engineered version of this molecule was developed for clinical application (Siplizumab, BioTransplant, Inc.). Siplizumab (MEDI-507) is a humanized IgG1 κ monoclonal

antibody initially studied in murine models of adult T-cell leukemia (ATL) [27]: severe combined immune deficiency (SCID) mice engrafted with human HTLV-1 positive T-cell leukemia cells treated with MEDI-507 survived significantly longer when compared either with mice treated with a humanized mAb direct toward CD25 or with phosphate-buffered saline (PBS) (control group). Based on this preclinical data and on the data obtained with its use in the prevention in the therapy of transplant rejection and autoimmune disease [28], phase I trials were conducted in patients with CD2⁺ lymphomas/leukemias [29, 30]. In the 2006 report, 16 patients diagnosed with peripheral T-cell lymphoma (PTCL) ($n=9$), cutaneous T-cell lymphoma (CTCL) ($n=6$), and natural killer (NK)-large granular lymphocytic (NK-LGL) leukemia ($n=1$) were treated with biweekly infusion of escalating doses of intravenous (IV) siplizumab (range 0.7–4.8 mg/kg). Two responses were observed, one partial response (PR) in the NK-LGL patient and one complete response (CR) in a PTCL patient, both treated at 3.4 mg/kg. Dose-limiting toxicities (DLT) included one case of erythematous confluent dermatitis and one case of pulmonary edema, no maximum tolerated dose (MTD) was

established. In the 2009 report, 29 patients with T-cell malignancies received escalating doses of IV sipilizumab (range 0.2–4.8 mg/kg), either with a weekly schedule or biweekly schedule. Although the initial responses were encouraging, 4 patients (13.7%) (3 of 7 patients treated with the weekly schedule and 1 of 22 treated with biweekly schedule) developed an EBV-lymphoproliferative disease (LPD) and the trial was stopped. The weekly administration of sipilizumab decreased the CD2 expressing cells to a greater extent than the biweekly schedule highlighting the importance of NK/T cells depletion in conjunction with no effect on the B cell compartment in the pathogenesis of EBV-LPD.

CD3 Antigen

The TCR heterodimer is associated with the polypeptide dimers of the CD3 complex which comprises five invariant polypeptide chains: γ , δ , ϵ , ζ , and η [31]. The CD3 complex stabilizes the TCR and participates in signal transduction through its extracellular (γ , δ , ϵ), transmembrane and intra-cytoplasmic domain [32, 33]. Engagement of the TCR by antigen or mAbs anti-CD3 induces phosphorylation of CD3 γ , δ , ϵ , and ζ chains and internalization of the TCR. The outcome of these interactions, including activation and apoptosis, is influenced by the dynamic interactions between TCR and peptide-MHC complexes and, specifically, depends on slight differences in terms of affinity and dissociation rates of the antigen/TCR complex.

CD3-Targeted Therapy

The clinical utility of the first generation of mAbs specific for CD3 (such as muromonab-CD3, also called Orthoclone OKT3, Ortho Biotech, Inc.) has been limited by several drawbacks related to their composition. The first was the induction of neutralizing anti-mouse Ab response which reduces the re-treatment efficacy [34–36]. The second is a syndrome of adverse events which includes flu-like symptoms, respiratory distress,

hypotension, and hypoglycemia [36]. This first generation of mAbs can induce T-cell activation through cross-linking of the CD3/TCR complex (via the F(ab')₂ arms) and Fc γ receptor (Fc γ R) on the surface of accessory cells (via the Fc region), with consequent release of cytokine such as tumor necrosis factor α (TNF α), interleukin-2 (IL-2), and interferon γ (IFN- γ) [37, 38]. Recently, a humanized, non-Fc γ R-binding, anti-CD3 mAb (visilizumab, PDL BioPharma Inc.) directed against the ϵ invariant chain of the CD3/TCR complex, has been evaluated for the treatment of steroid refractory acute GVHD [39]. This mAb retains the specificity for CD3 but has an engineered and modified Fc portion that fails to trigger T-cell activation and therefore is associated with significantly less cytokine release. Despite this significant structural improvement, to date, there are no published reports of the use of mAbs specific for CD3 in TCL. A phase I, multiple dose escalation trial of IV humanized anti-CD3 mAb (HuM291) in patients with CD3⁺ TCL has been recently completed at Stanford University (clinical trial number NCT00006009); however, the results are not yet available.

CD4 Antigen

CD4 is a 55 kD single chain glycoprotein containing four immunoglobulin-like domains that project from the T-cell surface, a hydrophobic transmembrane domain and a long cytoplasmic tail [40]. In association with the TCR, CD4 acts as a co-receptor for the major histocompatibility complex (MHC) class II molecules [41]. The main physiological importance of this surface glycoprotein is signaling which takes place through its cytoplasmic tail. The intracytoplasmic region of CD4 is constitutively and non-covalently associated with a src-like tyrosine kinase (p56^{lck}) that initiates the transduction cascade following antigen binding [42]. CD4 is normally expressed on helper/regulatory T-cells, and, at a lower level, on monocytes, macrophages, and dendritic cells [40]. CD4 is also expressed on the malignant cells of PTCL and CTCL patients [43]. The fact that CD4 is expressed only on a

subset of normal T-cells, it is brightly expressed on malignant cells from CTCL patients, it is present in all stages of disease with no down-modulation during disease progression and it is involved in cell signaling, has provided the rationale for targeting CD4 with mAbs.

CD4-Targeted Therapy

The therapeutic activity of mAbs specific for CD4, in their murine and humanized variant, has been evaluated in human inflammatory diseases such as rheumatoid arthritis and psoriasis [44–46] as well as in CTCL [43, 47, 48]. In an early study [47], seven previously treated mycosis fungoides (MF) patients were treated with a chimeric murine antibody composed of the IgG1 κ human constant regions and the mouse variable regions directed against CD4 (SK3/anti-Leu3a; Becton-Dickinson, Inc.). Patients received IV doses of 10, 20, 40, or 80 mg twice a week for 3 consecutive weeks. At the 80 mg dose, the antibody was detected in skin lesions and also coating circulating CD4⁺ T-cells in the peripheral blood, however, no significant depletion of CD4⁺ cells was observed. In a second phase I study [48], 8 previously treated MF patients received a single IV dose (50, 100 or 200 mg) of a different chimeric murine anti-CD4 mAb directed to a distinct epitope on CD4 (cM-T412; Centocor, Inc.). Following the antibody infusion there was a depletion of peripheral blood CD4⁺ cells in 7 of the 8 patients. The overall response rate (ORR) was 88% with 5 patients achieving a PR and 2 patients achieving a minor response (MR). The time to progression (TTP) ranged from 6 to 52 weeks, with an average of 25 weeks. More recently, HuMax-CD4 (zanolimimumab, Genmab, Inc.), a fully human anti-CD4 mAb, isolated from transgenic mice as a hybridoma clone and subsequently expressed in Chinese hamster ovary cells, was able to induce significant T-cell depletion in repeated dosing and was found to be safe and well tolerated in human inflammatory diseases [45, 46]. Based on its safe profile and the preliminary encouraging results obtained with murine anti-CD4 mAbs, the therapeutic activity of

zanolimimumab was also evaluated in two separate but otherwise identical phase 2 clinical trials in refractory CTCL [43]. In the first of the two studies, 27 patients with early-stage MF received weekly IV infusion of zanolimimumab at either 280 mg (13 patients) or 560 mg (14 patients) for a total of 16 weeks. In the second study, 11 patients with advanced-stage treatment refractory MF and 9 patients with Sezary syndrome (SS) were treated with weekly IV infusion of zanolimimumab at either 280 mg (7 and 4 patients, respectively) or 960 mg (4 and 5 patients, respectively). Overall, 13 of 38 patients (34.2%) with MF and 2 of 9 patients (22.2%) with SS obtained an objective response to zanolimimumab (MF: 1 CR, 3 CCRs, and 9 PRs; SS: 2 PRs). 56% of the MF patients (7 of 14 at 560 mg and 3 of 4 at 980 mg) treated at the high-dose levels achieved objective response compared with only 15% at the 280 mg dose (3 of 20). In patients with SS, the response rate was 1 in 4 at the 280 mg dose and 1 in 5 at the 980 mg dose. In patients with MF, high-dose treatment resulted in an earlier time to response with 9 of 10 responses achieved within 8 weeks (median time to response 8 weeks, range 2–12 weeks) and more durable responses (median duration of response 81 weeks, range 8–91 weeks) compared with low dose treatment (three responses obtained at 4, 8, and 12 weeks; duration of response 12, 13, and 24 weeks). In patients with SS, the two responses lasted 8 weeks (280 mg) and 61 weeks (980 mg). Overall, treatment was well tolerated with infections and eczematous dermatitis being the most frequent adverse events observed. This antibody was designed to prevent the interaction between the CD4 receptor and the MHC class II molecule, thereby interfering with T-cell activation, and, recently, the mechanism through which zanolimimumab may inactivate or delete T-cells has been described [49]. Ligation of CD4 by zanolimimumab inhibits the necessary p56^{lck}-mediated co-stimulation by uncoupling this kinase from the TCR and allowing this enzyme to transmit an inhibitory signal via Dok-1 and SHIP-1 inhibitory molecules that may also reduce AKT activity. Secondly, zanolimimumab induces potent ADCC with no role played by CDC and, lastly,

it may act by down-modulating CD4 itself. Despite its promising profile in terms of therapeutic activity and safety, future developments for zanolimumab and its clinical applications are on hold due to pharmaceutical company's decision.

CD25 Antigen

Interleukin-2 (IL-2) is a potent immunomodulatory cytokine whose function is the activation of T- and B-lymphocytes, NK cells, and macrophages [50]. Three polypeptide chains (α , β , and γ) are non-covalently associated to form the high affinity interleukin-2 receptor (IL-2R) [51–53]. The α (CD25 or p55) and β (CD122 or p75) chains are involved in binding IL-2, while signal transduction which involves Janus kinase-1 (Jak-1), Jak-3, and STAT-5 pathways, is carried out by the γ (CD132 or p64) chain, along with the β subunit [54–56]. CD25 is a 55 kD, heavily glycosylated, integral membrane protein which, by itself, functions as a low affinity IL-2R. CD25, initially defined using the murine mAb anti-Tac [57], is a polypeptide composed of 272 aminoacids with an extracellular, transmembrane, and a very short intracellular domain. CD25 is dimly expressed on normal, resting peripheral blood T-cells, however is rapidly upregulated following antigen or cytokines stimulation [55]. Among T-cell malignancies, CD25 is strongly expressed by ATL and ALCL [55], highly variable expressed on CTCL [58]. It has been shown that CD25 expression varies depending on tissue site from CTCL patients: CD25 has been found to be highly expressed in epidermis, down-regulated in dermis, and extensively down-regulated in lymph nodes, suggesting that the microenvironment may play a role in affecting CD25 expression [58]. It has also been shown that CD25 expression correlates with advanced stage (T status) and histological grade (large cell transformation) of CTCL, suggesting a role as prognostic marker for this antigen [59]. Recently, soluble IL2-R has been identified as a reliable marker of disease activity in patients with ATL [60].

CD25-Targeted Therapy

Several studies have established the importance of CD25 as a valuable target for immunotherapy [61–63]. A number of mAbs specific for CD25 have been evaluated in several T-cell mediated disorders including prevention of rejection of transplanted organs [64], noninfectious uveitis [65], multiple sclerosis [66], and HTLV-1-associated disorders [67]. In 1997, daclizumab, a therapeutical humanized mAb specific for CD25 (zenapax, Hoffmann-La Roche) has been approved by the FDA for use in the prevention of renal allograft rejection [68]. Also among T-cell malignancies, various forms of CD25-specific mAbs have been tested in a preclinical and clinical setting. These include the murine mAb anti-Tac [57], daclizumab [69, 70], and 7G7/B6 mAb that targets CD25 at an epitope other than the IL-2 and anti-Tac binding sites [71]. These mAbs significantly prolonged the survival of MET-1 mice (murine model of ATL), however cure was not achieved [72]. For this reason, daclizumab has been combined with flavopiridol in the MET-1 murine model in which a marked synergy between these two drugs has been observed [73]. The clinical use of the murine mAbs has been limited due to their immunogenicity associated with rapid generation of neutralizing anti-mouse Ab, short in vivo survival and modest ADCC-mediated cell death due to the reduced binding to human Fc receptors [74]. However also daclizumab, as many other unmodified mAbs, has shown only modest activity as single cytotoxic agent in ATL patients. To overcome these limitations, daclizumab has been employed as a carrier of cytotoxic agents such as radionuclides or toxins. In a phase I/II trial, 18 patients with ATL were treated with 55 doses of ^{90}Y -labeled anti-Tac mAb involving 5–15 Ci per patient [75]. Ten of the evaluable patients responded to treatment, with 8 patients achieving a PR and 2a CR. Hematologic toxicity was the limiting side effect. In an alternative approach, the antitumor activity of the anti-Tac mAb conjugated with a truncated *Pseudomonas* exotoxin (anti-Tac (Fv)-PE38 (LMB-2)) was evaluated in a phase I/II trial [76] in which 35 patients with a CD25⁺ lymphoma/

leukemia were enrolled. Among these patients, 1 had CTCL/SS, 3 had PTCL unspecified and 2 ATL. Patients received escalating dose of LMB-2 on alternate days for 3 doses (1 cycle), with a starting dose of 2 µg/kg administered IV, every 3 weeks. Of the 6 patients with T-cell malignancies, 3 achieved a PR (1 with CTCL/SS, 1 with PTCL, 1 with ATL). Responding patients experienced rapid reductions of circulating malignant cells, improvement in skin lesions, and regression of lymphomatous masses and splenomegaly; however, all responses were of short duration.

CD122 Antigen

CD122 or p75 represents the β subunit shared by the heterotrimeric IL-2 and IL-15 receptors [77]. IL-15 is produced by macrophages, dendritic cells, and other non-lymphoid cells, it mainly works inducing T-cells and NK-cells maturation, enhancing the survival of CD8⁺ cytotoxic lymphocytes and stimulating the production of TNFα, IL-1β, and other cytokines [78]. Dysregulation of IL-15 and its receptor have been reported in CTCL, acute lymphoblastic leukemia (ALL), ATL, and T-LGL leukemia [79–81].

CD122-Targeted Therapy

In a phase I trial, 12 patients with T-LGL leukemia received escalating dose of Mik-β1 murine mAb, every 3 days, with a starting dose of 0.5 mg/kg, administered IV [81]. Greater than 95% saturation of CD122 was achieved in all patients; however, no responses in terms of decreases in T-LGL cell count were observed. A phase I safety and pharmacokinetic study of the humanized form of Mik-β1 in T-LGL leukemia is now underway.

CD26 Antigen

CD26 is a 110 kD surface glycoprotein expressed by a variety of cells including epithelial cells, T-lymphocytes, and NK cells [82]. CD26 possesses an intrinsic dipeptidyl peptidase IV

(DPPIV) activity in its extracellular domain [83] and plays an important role in T-cell activation [82]. CD26 expression is tightly regulated on T-cells and it is significantly enhanced after activation [84]. CD26 is capable of providing a potent costimulatory signal which can induce several activation pathways leading to proliferation and cytokines production [82]. However the mechanism of costimulation remains unclear since the cytoplasmic domain consists of only six amino-acids and lacks a phosphorylation site, leading to the conclusion that CD26 must interact with other molecules to exert this function. CD26, through its peptidase activity, can also cleave certain chemokines involved in T-cell function and monocytes function, including RANTES and LD78β [85, 86]; it has also been shown that CD26 regulates adenosine deaminase surface expression with this complex perhaps playing a role in regulating immune function [87].

CD26-Targeted Therapy

Among TCL, CD26 is absent or dimly expressed in MF/SS [88], variably expressed in T-LGL [89], and expressed at high levels in γδ hepatosplenic and ALCL [90]. An mAb specific for CD26 (1F7) has shown to have in vitro and in vivo antitumor effect in human CD30⁺ ALCL cell lines by inducing G1–S cell cycle arrest and enhancement of p21^{Cip1} expression [91]. It has also been shown that CD26 is a marker of aggressive clinical behavior and poor prognosis for T-LGL [89] and T-cell lymphoblastic leukemia/lymphoma [92]. A phase I trial, involving a humanized anti-CD26 Mab, will be conducted in relapsed/refractory patients with CD26 positive tumors, including TCL, in the near future (Dr. NH Dang, Nevada Cancer Institute).

CD30 Antigen

CD30 is a 105–120 kD type I transmembrane glycoprotein and a member of the tumor necrosis factor (TNF) receptor superfamily [93]. The mature CD30 comprises 577 amino acids, with a

365 amino acids extracytoplasmatic domain physiologically bound by CD30 ligand (CD153), a 24 amino acids transmembrane domain and a 188 amino acids intracytoplasmatic domain which regulates signaling transduction following receptor binding through its serine/threonine phosphorylation sites [94]. In normal tissues, CD30 expression is restricted to activated B- and T-lymphocytes [95]. In the immune system, CD30 functions as a costimulatory molecule in the presence of TCR stimulation, it activates T-cells production of cytokines such as IL-2, TNF, and IFN γ and it appears to be involved in the negative selection of autoreactive lymphocytes [96]. In tumor cells, CD30 was originally identified on the surface of Reed-Sternberg cells [97]; however, it has been shown to be highly expressed also on ALCL tumor cells [98] and can be expressed at variable levels on CTCL cells [99]. A cleaved form of CD30 (sCD30) can be found in the plasma of CD30⁺ lymphoma patients as a result of cleavage by TNF α -converting enzyme, a zinc metalloproteinase, with serum levels of sCD30 correlating with neoplastic activity, tumor burden, response to treatment, and time to treatment failure in ALCL [100].

CD30-Targeted Therapy

Although its function has not been completely clarified, CD30-mediated signaling has been implicated in both lymphocytes death and proliferation, therefore a number of different mAbs targeting different epitopes of CD30 have been developed [101–107]. It is even more interesting that the same antibody (M67) has showed opposite effect in ALCL cell lines and Hodgkin's disease (HD) cell lines [108]. Specifically, M67 was able to induce enhanced apoptosis in ALCL cell lines; however, it had no effects on HD cell lines. CD30 binding has been associated with NF- κ B activation [109] and the difference in outcome between ALCL and HD cells following anti-CD30 treatment was attributed to the inability of ALCL cells to activate NF- κ B [110], in contrast with HD cells which constitutively express NF- κ B [111]. Chimeric anti-CD30 mAbs have

also been tested in murine xenograft model of ALCL in which treatment with these mAbs was able to significantly prolong survival of mice bearing chemotherapy resistant human ALCL [112, 113]. In the attempt to improve its in vitro and in vivo antitumor activity, Ber-H2, an mAb specific for CD30, has been conjugated with the plant ribosome-inactivating protein saporin and showed promising initial results [114]. Based on these preclinical data, mAbs targeting CD30 have been evaluated in phase I/II clinical trials. In a phase I/II trial, MDX-060 (Medarex, Inc.), a fully human anti-CD30 mAb, was administered at doses up to 15 mg/kg to 21 patients (16 with HD, 3 with ALCL, and 2 with CD30⁺ TCL unspecified) [115]. Adverse events were common but primarily mild or moderate in intensity and an MTD was not defined. In phase II, an additional 51 patients were treated, 4 of which with ALCL. Of the 7 patients with ALCL, 2 (28%), with a predominantly skin disease, achieved a CR. Both CR lasted more than 1 year with both patients receiving additional cycles of therapy. In another phase I trial, SGN-30 (Seattle Genetics, Inc.), a chimeric anti-CD30 mAb, was administered at four dose levels (2, 4, 8, or 12 mg/kg) on a weekly schedule for 6 consecutive weeks, to 24 patients with refractory lymphoma (21 with HD and 3 with CD30⁺ NHL) [116]. Antibody treatment was well tolerated and, although antitumor activity was not an objective of this trial, of the 3 patients with CD30⁺ NHL, 1 patient (33%) with a history of cutaneous ALCL achieved a CR and 2 patients achieved stable disease (SD). Forero-Torres et al. [117] recently reported on 79 patients, 38 with refractory/relapsed HD and 41 with systemic ALCL (35 with ALK⁻ ALCL), treated in a multicenter phase II trial with SGN-30 at a dose of 6 mg/kg for 6 weeks. After the first 24 patients were enrolled, the dose was escalated to 12 mg/kg per week. Overall the treatment was well tolerated, adverse events were common but primary mild or moderate. In the ALCL group, 2 patients achieved a CR and 6 patients a PR for an ORR of 21% and with a response duration ranging from 27 to 1,460+ days. Duvic et al. [118] recently reported the results from another multicenter phase II trial in which SGN-30 was administered

to patients with CD30⁺ lymphomas. Specifically, 5 patients with primary cutaneous ALCL (pc-ALCL) and 1 patient with multiple clinical diagnosis (mixed CD30⁺ lesions), received 1 dose of IV SGN-30 at 4 mg/kg every 3 weeks for up to six doses. Six patients with pc-ALCL, 3 with lymphomatoid papulosis (LyP), 3 with transformed MF, and 5 with multiple clinical diagnosis (mixed CD30⁺ lesions), received 1 dose of IV SGN-30 at 12 mg/kg every 3 weeks for up to six doses. Eligible patients could receive two additional courses. Overall the treatment was well tolerated but adverse events were common, 15 patients (65%) experienced at least 1 adverse event with fatigue being the most common. 10 patients (43%) achieved a CR (6 with pc-ALCL, 1 with LyP, and 3 with mixed CD30⁺ lesions) and 6 patients (26%) achieved a PR (3 with pc-ALCL, 1 with LyP, 1 with MF, and 1 with mixed CD30⁺ lesions) for an ORR of 87%. However the responses were of short duration (the overall median duration of objective response, CR+PR, was 84 days, range 1–238). Overall, anti-CD30 mAbs have shown an acceptable safety profile, however, in the majority of studies, anti-CD30 mAbs as single agents showed only modest anti-tumor activity in most CD30⁺ TCL, suggesting combination strategies as an attractive alternative option. Phase I/II trials with SGN-30 either in combination with chemotherapy or conjugated with drugs/toxins are now ongoing in pretreated patients with CD30⁺ hematologic malignancies.

Better efficacy with CD30-targeting agents has been observed with brentuximab vedotin (SGN-35, Adcetris, Seattle Genetics, Inc), an antibody-drug conjugate which consists of the anti-CD30 monoclonal antibody cAC10 (SGN-30) conjugated with the cytotoxic agent monomethylauristatinE(MMAE) via a protease-cleavable linker [119, 120]. Brentuximab vedotin was recently approved by the US FDA in August 2011 for the treatment of relapsed or refractory Hodgkin's lymphoma (HL) and ALCL. In the initial phase I dose-escalation study [121], brentuximab vedotin was administered intravenously on days 1, 8, and 15, of each 28-day cycle at doses ranging from 0.4 to 1.4 mg/kg. Forty-four patients were enrolled: 38 with Hodgkin's

lymphoma, 5 with systemic ALCL, and 1 with PTCL. Doses were escalated in increments of 0.2 mg/kg until DLT was observed. Antitumor assessments were carried out every two cycles. The MTD was 1.2 mg/kg. The most common adverse events were peripheral sensory neuropathy, fatigue, nausea, diarrhea, arthralgia, and fever. The majority of events were mild to moderate in severity. Tumor regression occurred in 85% of patients and the overall objective response rate was 59% ($n=24$), with 34% ($n=14$) complete remissions. The median duration of response was not reached at a median follow-up of 45 weeks on study. FDA approval was based on data from two pivotal, open-label clinical trials independent review of response rate. Brentuximab vedotin was administered intravenously at a dose of 1.8 mg/kg over the course of 30 min once every 3 weeks. Results from the Hodgkin study ($n=102$) showed an ORR of 73% (95% confidence interval [CI], 65–83%), including 32% with complete remission (95% CI, 23–42%). Response duration averaged 6.7 months (range, 1.3–21.9+ months). Data from the ALCL study ($n=58$) revealed an 86% response rate (95% CI, 77–95%), with 57% complete remission rate (95% CI, 44–70%). Median response duration was 12.6 months (range, 0.1–15.9+ months) [122]. Based on these data, brentuximab vedotin represents a welcome addition to the therapeutic tool box for CD30⁺ lymphomas, including ALCL and HL, with high response rates and manageable toxicity. While the drug appears to be safe on early follow up, more recently the FDA issued a warning to health care professionals due to three cases of progressive multifocal leukoencephalopathy (PML), and a new boxed warning highlighting this risk has been added to the drug label [123]. In addition, a contraindication was added, warning against the use of brentuximab with the cancer drug bleomycin because of the increased risk for pulmonary toxicity.

CD52 Antigen

CD52 is a 21–28 kD, glycosylphosphatidylinositol (GPI)-anchored, heavily glycosylated, protein,

encoded by a gene on human chromosome 1 [124]. The peptide component of CD52 is represented by a 12 aminoacids scaffold, which undergoes significant posttranslational modification [125]. Attempts to identify a ligand have not been successful and the physiologic function of CD52 remains to be clarified: it may mediate a variety of biological effects such as signal transduction including promotion of cell–cell adhesion and protection of the cell from environmental insult, however, CD52 knock-outs or congenitally CD52-deficient animals have not been described. Relatively recently, it has also been suggested that CD52 may contribute to the activation of CD4⁺ regulatory T-cells [126]. CD52 is highly expressed on membrane lipids raft of all B and T lymphocytes at most stages of differentiation (except plasma cells), as well as on monocytes, macrophages, eosinophils, NK cells, and dendritic cells [124, 127–130]. The antigen is also found in the male reproductive tract, where it is strongly expressed on epithelial cells lining the epididymis, vas deferens, and seminal vesicle [131]. Notably, CD52 is not expressed on hematopoietic stem cells, erythrocytes, and platelets [132]; however, it has been found on a subpopulation of CD34⁺CD38⁺ cells which are believed to represent lymphocyte-committed progenitors [133]. CD52 is also variably expressed on subsets of tumor cells, at a particular high density on T-prolymphocytic leukemia (T-PLL) cells followed by B-CLL, hairy cell leukemia (HCL), ALL, and NHL, including TCL [128, 134, 135]. However, in contrast with B-cell malignancies, there is a great variability in terms of CD52 expression in TCL. Currently the expression of CD52 is best assessed by flow cytometry [136], in fact, a reliable assay for the detection of CD52 on paraffin-embedded, formalin-fixed tissue is not available and data on CD52 expression in various subtypes of TCL are conflicting, due to inconsistent reproducibility [134, 135, 137, 138]. Alemtuzumab has shown encouraging activity in T-cell malignancies [139], however, the variable expression of CD52 among TCL and the profound lymphopenia associated with the use of this mAb, have partially limited its use in TCL.

CD52-Targeted Therapy

Campath-1H (alemtuzumab [U.S.], mabCampath [E.U.], Genzyme, and Bayer HealthCare Pharmaceuticals Inc.) is a humanized IgG1κ mAb directed against the human CD52 antigen that is FDA approved for the management of patients with pretreated [140] and untreated B-CLL [141]. The CD52 epitope recognized by alemtuzumab corresponds to the last three aminoacids of the peptide scaffold and part of the GPI anchor [139]. The mechanisms underlying the therapeutic effect of alemtuzumab in lymphoid malignancies have not been well characterized. The spectrum of biologic effects on tumor cells is heterogeneous and the dominant mechanism of antitumor activity may differ from disease to disease. In vitro and in vivo evidence show that alemtuzumab induces CDC, ADCC, and direct apoptosis in B- and T-cell malignancies [125, 142–147]. The pharmacokinetics (PK) of alemtuzumab has been studied in B-CLL, using the approved schedule consisting of a 2 h IV infusion at a starting dose of 3 mg on day 1, 10 mg on day 2 up to the target dose of 30 mg, three times per week for up to 12 weeks [148]. Considering the fact that remarkable “first-dose” reactions consisting in fever, vomiting, rigors, skin rash, dyspnea, and hypotension have been noted in the majority of patients treated with the IV route [149], Hale et al. [148] compared blood concentrations of alemtuzumab in B-CLL patients after IV and subcutaneous (SC) dosing. SC administration was not associated with “first-dose” reactions and similar peak drug concentrations were achieved; however, a higher cumulative dose of SC alemtuzumab was required to reach concentrations similar to the IV administration. PK data with alemtuzumab (IV or SC) in T-cell malignancies are not available. Multiple studies have shown single-agent alemtuzumab to be active in T-cell malignancies (*T-PLL*: [150]; *CTCL*: [151–155]; *PTCL*: [156]); however, clinical experience with alemtuzumab treatment in T-cell malignancies has been limited to small series and approval for this indication has not been granted yet. The first suggestion of activity in T-PLL came from a phase II trial [150] in which 39 patients with

previously treated T-PLL received IV alemtuzumab (30 mg, three times a week until maximum response). The ORR was 76% (60% complete response, CR, and 16% partial response, PR). The TTP was 10 months (range 3–45) and median OS was 13 months (24 months for patients in CR). The response rate and survival were significantly longer than those reported for conventional therapies [157]. In a retrospective study of 76 patients with heavily pretreated T-PLL, alemtuzumab was administered at the standard schedule for up to 12 weeks. The ORR was 50% with 28 patients (37.5%) achieving a CR. The TTF was 4.5 months, the median OS was 7.5 months (14.8 months for patients in CR) [158]. In an early, preliminary, European experience, 50 B- and T-cell NHL patients received single-agent alemtuzumab [151]. Eight of these patients had advanced refractory MF: an ORR of 50% with 2 CRs and 2 PRs was seen. These promising results led to a multicenter phase II trial with the largest reported CTCL series [152] in which 22 patients with heavily pretreated, CD52-positive, advanced stage MF/SS, were treated with single-agent alemtuzumab. Alemtuzumab was administered IV at the standard dose of 30 mg, three times per week, for up to 12 weeks. The ORR rate was 55% (32% CR, 23% PR), with better efficacy in patients with erythroderma (69% ORR) than those with plaques/skin tumors (40% ORR). Median TTF for all responders was 12 months (range 5–32). Infectious complications were observed, including asymptomatic CMV reactivation (4 patients), generalized HSV (1 patient), 1 case of fatal aspergillosis, and 1 case of fatal *Mycobacterium pneumonia*. In another phase II trial [153], 8 MF/SS patients (7 of whom with refractory disease), received IV alemtuzumab according to the standard schedule; however, the ORR was only 38%, with no CR observed and a shorter TTF (4 months). In the attempt to reduce the incidence of hematologic toxicities and the risk of infectious complications associated with the standard schedule, Zinzani et al. [154] assessed the impact of a reduced-dose schedule of IV alemtuzumab (10 mg, three times per week, for 4 weeks) in 10 pretreated patients (6 PTCL and 6 MF). Despite the dose reduction, an ORR

of 75% (with no CRs) among the MF patients and an ORR of 50% (with 2 CRs) among the PTCL patients were observed. More importantly, no grade 3–4 hematologic toxicities were noticed and CMV reactivation occurred in only 1 patient. More recently Bernengo et al. [155] investigated the association of a reduced-dose schedule of alemtuzumab with the SC administration route in the attempt to also eliminate the systemic reactions associated with the IV route. Eleven relapsed/refractory MF patients and 3 untreated SS patients were enrolled, 4 of them received alemtuzumab 3 mg on day 1, 10 mg on day 3, then 15 mg on alternating days, for a total of four doses. A reduced dosage (3 mg on day 1, then 10 mg on alternating days) was administered to the remaining patients. The ORR was 85.7%, with 3 CRs (21.4%). After a median follow-up of 16 months, the median TTF was 12 months. Infectious complications occurred in 4 patients. Lenihan et al. [159] described the activity of alemtuzumab, at the standard IV 12-week dosing schedule, in 5 patients with SS. Activity was modest with only 3 PR achieved, and a variety of cardiovascular adverse events were observed, including chronic heart failure, hypotension, pulmonary thromboembolism, and atrial fibrillation, even in the absence of known cardiac risk factors. However evidence of cardiac toxicity with alemtuzumab treatment has not been confirmed [160]. A few additional small studies reporting on the activity of alemtuzumab in SS have been published [161–163]. Data on PTCL patients and single-agent alemtuzumab are even scarcer than those available for T-PLL and CTCL. In the only prospective trial in PTCL patients treated with single-agent alemtuzumab published so far [156], 14 patients with advanced relapsed/refractory disease received IV alemtuzumab following the standard schedule. The ORR was 36% with 3 patients achieving a CR and 2 patients achieving a PR. The duration of response was 2, 6, and 12 months for each of the CR patients. However, in these heavily pretreated patients, alemtuzumab therapy was associated with significant toxicity, including CMV reactivation in 6 patients, pulmonary aspergillosis in 2 patients, EBV-related hemophagocytosis in 2 patients.

In the attempt to enhance the response rate and prolong the TTF, alemtuzumab has also been administered in association with chemotherapy in the treatment of TCL patients.

In the first phase II trial, 20 patients with previously untreated PTCL (10 patients with PTCL-*unspecified*, 3 with *angioimmunoblastic lymphoma*, 3 with *extranodal NK/TCL, nasal type*, 2 with *ALK⁻ ALCL*, and 2 with *subcutaneous panniculitis-like TCL*) received CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) plus alemtuzumab (10 mg IV on day 1, 20 mg on day 2 of the first cycle, then 30 mg IV on day 1 in the subsequent cycles), based on 3 week interval [164]. An ORR of 80% with a 65% CR rate was observed however the trial was prematurely closed due to two treatment-related deaths and high incidence of infectious complications as well as hematologic adverse events. An Italian prospective multicenter phase II trial evaluated the activity of alemtuzumab in combination with CHOP in 24 patients with previously untreated PTCL (8 patients with PTCL-*unspecified*, 7 with *angioimmunoblastic lymphoma*, 3 with *ALK⁻ ALCL*, and 1 with *enteropathy-associated TCL*) [165]. SC alemtuzumab was administered at 30 mg at day 1 to 8 courses of CHOP-28. An ORR of 75% with a 71% CR rate was observed. Thirteen patients were disease free with an overall median duration of response of 11 months. Neutropenia and CMV reactivation were the most common hematologic and non-hematologic complications, occurring in 59 of 176 (34%) and 15 of 176 (9%) of CHOP chemotherapy courses, respectively. Two cases of invasive aspergillosis and 1 case of PML were also noticed. Alemtuzumab was also combined with CHOP and ESHAP (ethopside, cytarabin, cisplatin, and methylprednisolone) in 13 patients with untreated PTCL (3 patients with PTCL-*unspecified*, 5 with *extranodal NK/TCL, nasal type*, 4 with *subcutaneous panniculitis-like TCL*, and 1 with *enteropathy-associated TCL*) [166]. SC alemtuzumab was administered at 30 mg at day 1–3 of cycle 1–5 plus CHOP (day 1 of cycle 1, 3, 5) and ESHAP (day 1 of cycle 2, 4, 6) at 28-day intervals. In the 10 evaluable patients, an ORR of 90% with a 80% CR rate was observed.

Infection was the major adverse event with CMV reactivation occurring in 54% of the patients.

Recently, two phase III trials (ACT1 and ACT2) combining CHOP with alemtuzumab as primary treatment for patients with PTCL, have been opened by the European Intergroup.

In a German phase II trial, alemtuzumab was combined with fludarabine, cyclophosphamide, and doxorubicin (alemtuzumab 3, 10, 30, 30 mg, days 1–4, fludarabine 25 mg/m² days 2–4, cyclophosphamide 600 mg/m² day 3, and doxorubicin 50 mg/m² day 4, every 28 days) [167] to treat 19 newly diagnosed and 11 relapsed/refractory PTCL patients (13 patients with PTCL-*unspecified*, 9 with *angioimmunoblastic lymphoma*, 2 with *ALK⁻ ALCL*, 2 with *enteropathy-associated TCL*, 2 with *nasal-type NK-/TCL*, 1 with an *NK-cell lymphoma* and 1 with a *T-PLL*). An ORR of 63% with a 58% CR rate and with ten ongoing remissions at the time of publication, were observed in the previously untreated patients' group. An ORR of 45% with a 28% CR rate was observed in the relapsed/refractory group. The toxicity was significant with 65% of the patients experiencing a sustained grade III/IV neutropenia, 40% of the patients had a CMV reactivation which was symptomatic in 2.

As emerged from many clinical trials, most of the TCL patients experience variable grades of cytopenia during treatment with alemtuzumab, especially if heavily pretreated and with advanced stage of disease. Thrombocytopenia is generally most common during the first 2 weeks and neutropenia is most common during weeks 5 and 6 [152]. Importantly, grade 4 neutropenia could be an indication to postpone alemtuzumab therapy but prematurely terminating treatment should be avoided especially in SS patients because, although resolution of peripheral blood lymphocytosis occurs early, bone marrow is unlikely to be clear of disease after 4 weeks of therapy [152, 155]. Monocytes, NK cells and peripheral blood but not tissue antigen-presenting dendritic cells are also profoundly depleted but this reduction is not as pronounced as for lymphocytes. Profound and sustained T and B lymphopenia is the most significant and consistent side-effect of

alemtuzumab, according to the high expression of CD52 antigen on these cells' surface [152]. Granulocytes population usually returns to baseline levels early during the unmaintained follow-up while monocytes remain at around 50% of their baseline value for a prolonged period of time. B lymphocytes normalize relatively quickly, CD8⁺ T lymphocytes recover slower, and CD4⁺ T lymphocytes may remain subnormal for over a year [152]. The stage of the disease, the disease itself, the number of prior treatments and the myelo/immunosuppression alemtuzumab-induced, increase the susceptibility to opportunistic and other severe infections, primarily bacterial [152, 168]. The most frequent opportunistic infection is caused by CMV. The overall incidence of CMV reactivation ranges between 5 and 43% in patients treated with alemtuzumab [150–153, 155, 156] and it is usually observed shortly after the nadir in T-cell counts, between 4 and 6 weeks of therapy. Antiviral prophylaxis (acyclovir, famciclovir, valaciclovir) is a mandatory procedure in these patients but these antiviral agents do not prevent CMV reactivation, although most patients respond rapidly to IV ganciclovir. At the present, no data are available to recommend routine prophylaxis for CMV but routine weekly monitoring with CMV antigenemia is indicated. Antibacterial and anti-Pneumocystis Jiroveci prophylaxis is recommended as well. CMV monitoring and prophylaxis should be continued for 2–4 months after alemtuzumab discontinuation [168]. Recently, cases of EBV-associated B and TCL after treatment with alemtuzumab in combination with chemotherapy for TCL have been reported [169, 170]. Alemtuzumab administered with proper antimicrobial prophylaxis has shown to be active in TCL; however, hematologic toxicities and infection complications are a major concern. The infusion related events are drastically reduced by the SC administration without affecting the efficacy. Alemtuzumab as single agent does not appear to be curative, therefore combination strategies with chemotherapeutic, biological agents and emerging class of new compounds such as histone deacetylase inhibitors should be further explored.

Conclusions

TCL represent a heterogeneous group of lymphoid neoplasms, and, with the exception of ALK⁺ ALCL, early stage MF and T-LGL leukemia, TCL have a suboptimal response to conventional chemotherapy and a poor prognosis. Few patients achieve CR, relapse is common and usually associated with chemo-resistance. Malignant T-cells express a number of potential targets for immunotherapy. mAbs such as alemtuzumab have shown significant clinical activity in a variety of T-cell malignancies and represent a significant improvement over previous therapy considering that durable remissions have been seen in heavily pretreated patients with TCL. However, mAbs as single agents do not appear to be curative in TCL. Therefore, although active as single agents, mAbs may have a greater role in alternative strategies such as combinations with other biological or chemotherapeutic agents, either simultaneously or sequentially, with the hope to translate durable remissions into improved survival. Maintenance therapy or retreatment based on the monitoring of malignant T-cells should also be considered although antigen mutation or modulation may limit repeated administration activity. Another insight derives from studies involving mAbs covalently bound to radioisotope or toxins to enhance their ability to destroy tumor cells. This multimodality strategy together with a personalized, tailored, stage-specific approach may represent the future management of TCL patients. Based on the number of investigational mAbs now available and the activity shown by some mAbs alone or as a part of a multimodality strategy, there is no doubt that mAbs therapy of TCL will continue to be an area of active interest and expanding clinical trials.

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