Management of Diffuse Large B-Cell Lymphoma (DLBCL)

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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

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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].

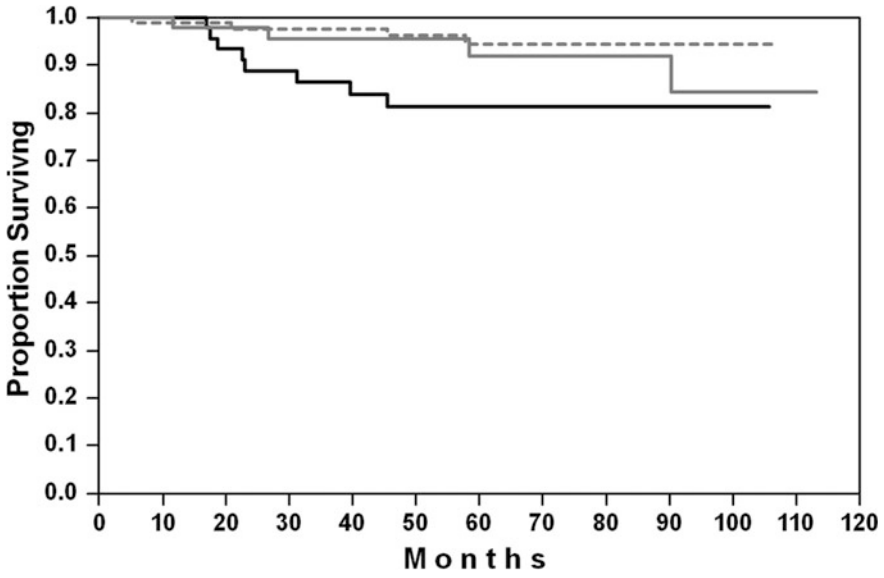

Fig. 1 Overall survival of "very favorable young patients" (no bulky disease, aaIPI = 0) in the MInT study [29]. *Black solid curve* CHOP-21; *gray solid curve* CHOEP-21; *dotted line* R-CHO(E)P-21

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

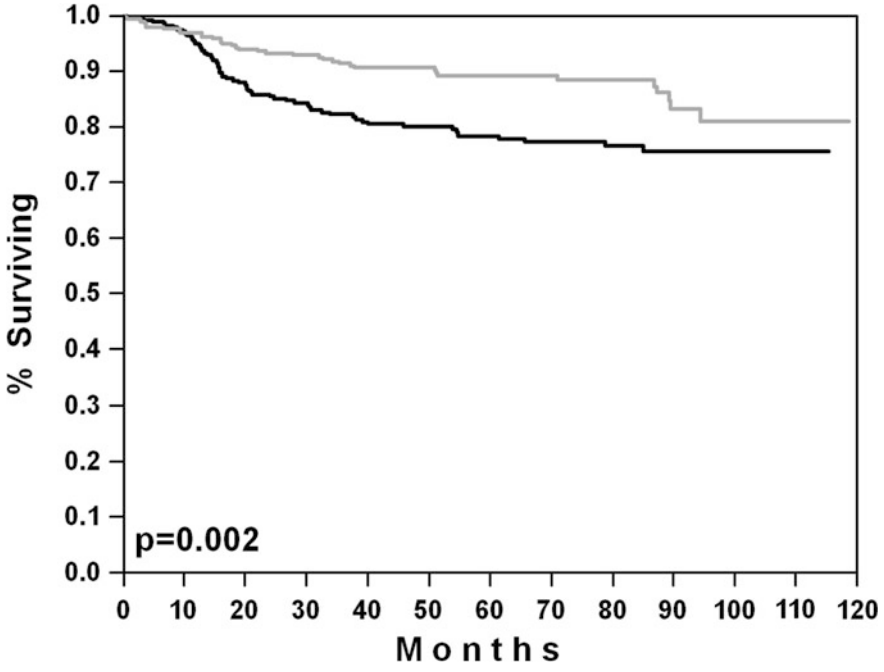

Fig. 2 Overall survival of "less favorable young patients" (aaIPI = 1; aaIPI = 0 with bulky disease) in the MInT study [29]. *Black curve* CHOP-like chemotherapy (n = 302); *gray curve* CHOP-like chemotherapy plus rituximab (n = 312)

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.

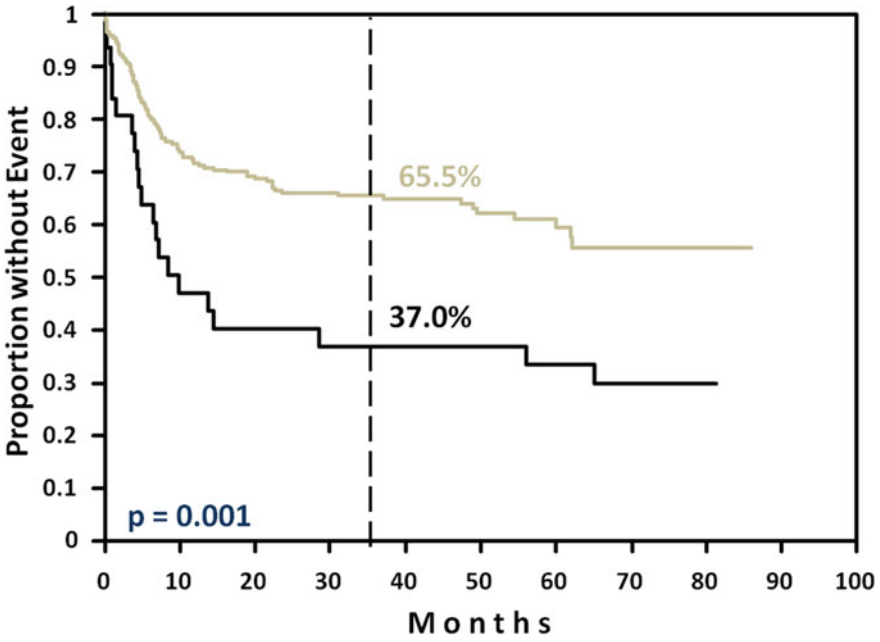

Fig. 3 Event-free survival of young poor-prognosis patients (aaIPI = 2, 3) in the Mega-CHOEP trial [34]. *Black curve* CHOEP-14/Mega-CHOEP (n = 31); *gray curve* R-CHOEP-14/R-Mega-CHOEP (n = 262)

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

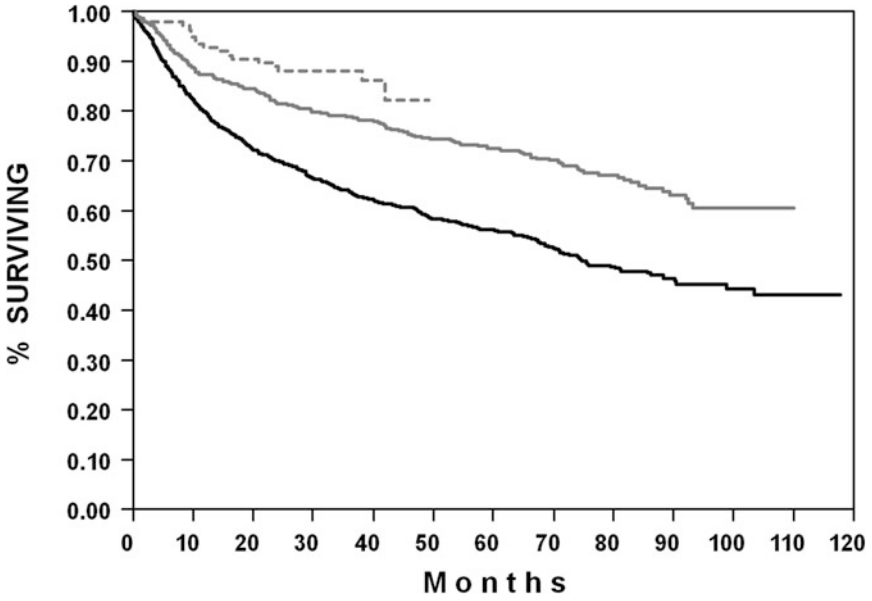

Fig. 4 Overall survival of elderly (61–80 year old) DLBCL patients. *Black solid curve* CHOP-14/CHOP-21 (n = 943) from the NHL-B2 [26] and the RICOVER-60 study [41]; *gray solid curve* R-CHOP-14 (n = 546) from the RICOVER [41], RICOVER-NoRTH [42], and Pegfilgrastim [63] studies; *dotted curve* SMARTE-R-CHOP-14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

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