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Dose-intensified treatment of diffuse large B-cell lymphomas

*Carsten Zwick, Gerhard Held, Michael Pfreundschuh *, for the German High-Grade Non-Hodgkin-Lymphoma Study Group*

Innere Medizin I, Saarland University Medical School, Homburg, Germany

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ABSTRACT

About 50% of all patients treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) achieve complete remission, and about one third experience long-term disease-free survival and cure. Attempts to improve results by modifications of CHOP using escalated doses, additional drugs or the alternative use of putatively non-cross resistant chemotherapy regimens failed in randomised trials. With the availability of granulocyte colony stimulating factor (G-CSF) and the tool of autologous stem-cell support, dose escalation, dose densification (by interval reduction) or combinations thereof were pursued to increase dose intensity. While dose escalation strategies including high-dose approaches necessitating stem cell support have not yet unequivocally been demonstrated to be superior to a baseline CHOP-21, dose dense (bi-weekly) modifications improved the outcome of young and elderly patients with aggressive lymphomas compared to baseline CHOP-21. Major goals in the rituximab era are the identification of the ideal chemotherapy partner for rituximab and the determination of the role of intensified rituximab within such approaches.

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1. Introduction

Risk factor profile and age are the main determinants for the primary therapeutic strategy in aggressive lymphomas. According to the number of risk factors present, the International Prognostic Index (IPI) distinguishes four prognostic subgroups; however, for practical reasons, low-risk and low-intermediate risk patients are often grouped together as 'good-prognosis', and patients with high-intermediate and high risk as 'poor-prognosis' patients.

In the era of chemo-immunotherapy with rituximab, two novel subgroups amongst the young good-prognosis patients have evolved after a combination of CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone)-like chemotherapy and rituximab:[1] a very favourable and a less favourable group. While results of the very favourable subgroup (no age-adjusted IPI [aaIPI] risk factor, no bulky disease) with an estimated 3-year event-free survival (EFS) rate of 97% and an overall survival (OS) rate of 100% after six cycles of CHOP-21 with rituximab can hardly be improved any more, the results in all other subgroups of patients with diffuse large B-cell lymphoma (DLBCL) need further improvement.

The most important development in the treatment of advanced aggressive lymphomas before the advent of rituximab was the demonstration that CHOP was shown to induce complete remissions and long-term disease-free survival in a considerable proportion of patients.[2] After its publication in 1976, many attempts to improve results further failed when compared to CHOP in randomised trials. In 1993, the results of the US South Western Oncology Group/Eastern Cooperative Oncology Group (SWOG/ECOG) Intergroup trial, which had randomised 899 eligible patients into CHOP, m-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone),

* *Corresponding author:* Michael Pfreundschuh, Klinik für Innere Medizin I, Saarland University Medical School, D-66421 Homburg (Saar), Germany. Tel: +49 6841 162 3002; fax: +49 6841 162 3002.

E-mail address: michael.pfreundschuh@uniklinikum-saarland.de.

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ProMACE-CytaBOM (bleomycin, cyclophosphamide, cytarabine, doxorubicin, etoposide, leucovorin, methotrexate, prednisone, vincristine) and MACOP-B (methotrexate, leucovorin, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin) showed that the so-called third-generation regimens had a median disease-free survival after 3 years of 41% (MACOP-B) and 46% (m-BACOD and ProMACE-CytaBOM), which was not different from that achieved with CHOP (41%; $P = 0.35$).[3] In contrast to efficacy, there was a significant difference between the rate of therapy-associated deaths among patients in the CHOP treatment arm (1%) compared to the other three treatment arms (5% after m-BACOD, 3% after ProMACE-CytaBOM, and 6% after MACOP-B [$P = 0.09$]).

With the availability of granulocyte colony stimulating factor (G-CSF) and the tool of autologous stem-cell support in the 90s of the last century, different strategies for increasing dose-intensity[4] were pursued: dose escalation, dose densification (by interval reduction), or a combination thereof.

2. Conventional dose escalation without stem cell support

The NHL-B1 trial of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL) tested whether the addition of etoposide to the CHOP regimen (CHOEP-21)[5] and/or the reduction of treatment intervals from 3 to 2 weeks (CHOP-14, CHOEP-14) improve the outcome of young good-prognosis patients (defined as those with normal pretreatment serum LDH (lactate dehydrogenase). Patients receiving CHOEP (CHOP plus etoposide 100 mg/m^2 day 1 to 3) had higher rates of complete remission (87.6% versus 79.4%; $P = 0.03$) and 5-year EFS (69.2% versus 57.6%; $P = 0.004$) than those receiving CHOP, suggesting CHOEP as the standard chemotherapy combination for young good-prognosis patients in the pre-rituximab era.[6] The superiority of CHOEP over CHOP in young good-prognosis patients was recently confirmed in the Mabthera International Trial Group (MInT) trial.[1]

In order to improve results further, the DSHNHL compared a maximal dose-escalated version of CHOEP-21 tolerable without stem cell support (High-CHOEP-21: cyclophosphamide 1200 mg/m^2, doxorubicin 65 mg/m^2, vincristine 2 mg, etoposide 175 mg/m^2 × 3, prednisone 100 mg) with baseline CHOEP-21 in a subsequent randomised trial of 389 subjects. Despite excellent adherence to the protocol (median relative dose CHOEP-21: 96%; High-CHOEP: 93%), there was no significant difference in either time to treatment failure (68% versus 64%; $P = 0.639$) or OS (86% versus 83%; $P = 0.849$) between High-CHOEP-21 and baseline CHOEP-21 treatment arms, respectively, after 37 months median observation, in low-risk or low-intermediate risk patients.[7] This indicates that the window of opportunity for conventional dose-escalation without stem cell support is small, even in young patients. In elderly patients who are more sensitive to the additional toxicities of dose-escalated regimens, this window might be even smaller, as demonstrated by the experience with CHOEP in elderly compared to young patients: in contrast to young patients, the addition of etoposide to CHOP was too toxic for elderly patients, required frequent treatment delays and dose reductions, resulting in a reduced delivered dose intensity and loss of superiority over CHOP.[8]

In summary, the results from randomised trials suggest a small window for conventional dose-escalated modifications of CHOP. This window is smaller in elderly patients due to the higher toxicity of dose-escalated regimens in this population.

3. High-dose chemotherapy with stem-cell support

In relapsing aggressive lymphomas high-dose chemotherapy with stem cell support was superior to conventional chemotherapy[9] and is widely accepted as the standard approach for patients with relapsing aggressive lymphomas, who are fit enough to tolerate the considerable toxicity and morbidity associated with this approach. In contrast to the relapse situation, the results of high-dose chemotherapy in the primary treatment of aggressive lymphomas are contradictory. In a small, but pivotal trial, B-cell type aggressive lymphomas were randomly assigned to receive either MACOP-B (50 patients) or a so-called 'high-dose sequential therapy' (48 patients), comprising six chemotherapeutic agents administered sequentially at high doses followed by myeloablative treatment and bone marrow transplantation. After a median follow-up of 55 months, the patients given high-dose sequential therapy, as compared with those treated with MACOP-B, had statistically significantly higher rates of complete response (96% versus 70%; $P = 0.001$), freedom from disease progression (84% versus 49%; $P < 0.001$), freedom from relapse (88% versus 70%; $P = 0.055$), and EFS (76% versus 49%; $P = 0.004$). The difference in OS at 7 years, which also favoured the group assigned to high-dose sequential therapy, was marginally statistically significant (81% versus 55%; $P = 0.09$).[10]

Several other randomised trials which evaluated the role of high-dose chemotherapy in the primary treatment of aggressive lymphomas yielded conflicting results.[11–21] As shown in Table 1, most trials did not demonstrate statistically significant differences between the conventionally dosed and high-dosed treatment strategies, some favoured the high-dose approach and some did so only in retrospective subgroup analyses. One randomised trial showed even inferior results for the high-dose approach compared to the conventional chemotherapy.[16]

In general, the results of these trials are difficult to interpret because the conventional and high-dose arms in these trials differed in more than the dose of chemotherapy. For example, Gianni's approach of 'sequential high-dose chemotherapy'[10] uses high-dose methotrexate and the repeated application of single maximally-dosed cytotoxic drugs. In contrast to the superiority of the high-dose sequential approach observed in the original study,[10] three subsequent trials comparing the high-dose sequential approach with dose-dense Mega-CEOP (escalated doses of cyclophosphamide, epirubicin, vincristine, prednisone), MACOP-B and CHOP-21, respectively, observed similar efficacy to the conventionally dosed regimens in young poor-prognosis patients. The GOELAMS Groupe Ouest Est d'étude

Table 1 – Randomised comparisons between conventional and high-dose chemotherapy with stem cell support in aggressive lymphoma

Author *	Number of patients randomised	Population	Randomised population	EFS	OS
Haioun et al. 2000[11]	236	≥1 RF, Bulk	CR	NS	NS
		≥2 RF	CR **	0.02 **	0.04 **
Verdonck et al. 1995[12]	69	I-IV	<CR	NS	NS
Gianni et al. 1997[10]	*89*	*I/II$_{bulky}$,III/IV*	*All*	*0.004*	*NS*
Santini et al. 1998[13]	61	II$_{bulky}$,III/IV	All	NS	NS
			≥2 RF**	0.008**	NS
Kluin N et al. 2001[14]	194	All	CR	NS	NS
Kaiser et al. 2002[15]	312	LDH > UNL	CR, PR	NS	NS
Gisselbrecht et al. 2002[16]	**370**	**≥1 RF**	**All**	**–0.01**	**–0.009**
Martelli et al. 2003[17]	150	≥2 RF	CR, PR	NS	NS
Milpied et al. 2004[18]	197	*1 & 2 RF*	*All*	*0.04*	*NS*
			>1 RF **	*0.01* **	*0.001* **
Betticher et al. 2005[19]	129	>1 RF	All	NS	NS
Olivieri et al. 2005[20]	223	II$_{bulky}$,III/IV	All	NS	NS
Vitolo et al. 2005[21]	126	>1 RF	All	NS	NS

Abbreviations: EFS, event-free survival; OS, overall survival; RF, risk factor according to aaIPI; CR, complete remission; NS, not significant; LDH, lactate dehydrogenase; UNL, upper limit of normal; PR, partial remission.
* Highlighted studies showed an advantage of high-dose over conventional chemotherapy. In the study in bold (Gisselbrecht et al. 2002), high dose chemotherapy yielded inferior results, and all other studies showed no difference between conventional and high-dose chemotherapy.
** Retrospective subgroup analysis.

des Leucémies at Autres Maladies du Sang) trial[18] compared eight cycles of CHOP-21 with two cycles of a variant of a bi-weekly CHOEP (CEEP [cyclophosphamide etoposide. prednisone, procarbazine]-15) regimen, followed by high-dose methotrexate plus cytarabin and a myeloablative BEAM (carmustine etoposide cytarabine and melphalan) regimen with autologous stem cell support in patients of low-intermediate and high-intermediate risk according to the aaIPI. Authors reported a statistically significant advantage for the high-dose sequential regimen compared to conventional CHOP-21 with respect to EFS for the entire study population (55% versus 37%; $P = 0.037$) and with respect to OS after 5 years for the subpopulation with intermediate-high risk only (74% versus 44%; $P = 0.001$). However, it is not clear, whether the high-dose component (BEAM) or the dose-dense chemotherapy given early in the experimental arm (two cycles of CEEP-15) are responsible for the superiority of this approach over eight cycles of 3-weekly CHOP. The fact that patients in the experimental arm of the GOELAMS study received all their therapy (including high-dose BEAM) by day 64, while patients in the CHOP-21 arm had received only 50% of their chemotherapy by day 64, demonstrates that the experimental arm has a much higher dose density than the control arm. Hence, the overall dose-density of the experimental arm in the GOELAMS study rather than its single high-dose component BEAM is likely to be responsible for its superiority over classical CHOP-21.

Similar to the GOELAMS study, the remainder randomised trials listed in Table 1 are difficult to interpret with respect to the role of high-dose chemotherapy. All these studies used different drugs in the conventional and the high-dose arm making it impossible to ascribe the results obtained in the high-dose arm to dose escalation or the different combination of cytotoxic drugs.

In summary, the statement of the Lyon 1997 consensus conference is still valid in 2007: there is no justification of high-dose chemotherapy in the primary treatment of aggressive lymphomas outside clinical trials.[22] Novel approaches of high-dose chemotherapy are evaluating high-dose regimens that use the cytotoxic drugs with the highest efficacy in aggressive lymphomas, in particular alkylating agents and anthracyclines, at the maximal tolerated doses and/or try to escalate the total dose of the regimen by applying the high-dose regimen in addition to a fully-dose conventional chemotherapy. In the so-called Mega-CHOEP trials of the DSHNHL patients receive a first cycle of conventionally escalated CHOEP followed by three cycles of high-dose CHOEP each necessitating stem-cell support.[23] The DSHNHL is currently comparing Mega-CHOEP with eight cycles of CHOEP-14 in a randomised fashion, both arms with rituximab.

4. Dose densification

Dose intensity can not only be increased by dose escalation, but also by dose densification, i.e. reduction of treatment intervals. A reduction of treatment intervals between two cycles of CHOP from 3 to 2 weeks equals an increase of dose intensity, i.e. the dose per week[4] by 50%. This compares favourably with strategies that try to increase dose intensity by dose escalation, because increases in excess of 25% compared to classical CHOP-21 can hardly be achieved without stem cell support.

Based on these considerations, we designed a dose-dense bi-weekly version of CHOP plus etoposide (CHOEP-14). After demonstrating its feasibility in a phase II study, the dose-dense bi-weekly CHOP and CHOEP regimens (CHOP-14 and CHOEP-14) were compared with their conventional 3-weekly counterparts (CHOP-21 and CHOEP-21) in two randomised trials in young good-prognosis patients (NHL-B1)[6] and elderly patients (NHL-B2)[8] in a 2×2 factorial design. In young patients, the addition of etoposide improved the results with

respect to the primary endpoint, EFS, while the reduction of treatment intervals from 3 to 2 weeks resulted in a statistically significant improvement in OS.[6] When the three intensified regimens CHOP-14, CHOEP-21 and CHOEP-14 were compared with the standard CHOP-21 regimen, CHOEP-21 improved EFS, while CHOEP-14 improved EFS, complete remission rates and OS over baseline CHOP-21. The advantage of the 2-weekly over the 3-weekly CHOEP was most pronounced in patients with bulky disease. Therefore, CHOEP-14 appears to be the preferred chemotherapy regimen for young good-prognosis patients, as long as rituximab is not incorporated into the therapeutic strategy.

The results of the elderly patients in the NHL-B2 trial were quite similar to those observed in young patients with the exception that double-intensified CHOEP-14 was too toxic, caused many treatment delays and dose reductions and was associated with a higher therapy-associated death rate in the elderly patients, while CHOP-14 was not more toxic than CHOP-21, and was significantly better than CHOP-21 with respect to CR (complete response) rate, EFS and OS. The advantage of the bi-weekly CHOP-14 over 3-weekly CHOP-21 was most pronounced in patients with elevated LDH and – similar to the situation with younger patients in the NHL-B1 trial (where patients with elevated LDH had been excluded) – in patients with bulky disease.[8]

Another advantage of dose densification is that in contrast to dose-escalation, dose densification or interval reduction from 3-weekly to 2-weekly intervals has not only become feasible using G-CSF, it also has no clinically relevant increase of toxicity,[6] allowing for high received relative dose-intensities (>90%) even in elderly patients in nationwide trials if certain rules are obeyed, which include: first, a so-called pre-phase treatment consisting of a single shot of 1 mg vincristine and 100 mg prednisone orally per day over 7 days, which results in a significant improvement of patients with poor performance status and ameliorates toxicity of the first chemotherapy cycle; second, a consequent dose reduction scheme which allows for treatment delay of 3 days increments, but no dose reductions[8] and hydrocortisone substitutions (20 mg in the morning, 10 mg after lunch) in patients who complain about fatigue after tapering the prednisone. Besides different risk profiles of the DLBCL population in different trials, non-adherence to these recommendations and lack of therapeutic discipline might be responsible for big differences in outcome after CHOP-14 in different studies: while the 2.5-year EFS after CHOP-14 in elderly patients in the German RICOVER-60 (rituximab with CHOP over 60) trial was 75%,[24] it was only 25% in a Dutch-Scandinavian trial.[25]

5. Combined dose escalation and dose densification

The most prominent example of this hybrid, chimeric or double-intensive strategy, which combines both dose escalation and dose densification is ACVBP (doxorubicin, cyclophosphamide, vindesine, bleomycin and prednisone), which was designed by the GELA (Groupe d' Etude des Lymphomes des Adultes). The ACVBP regimen consists of 4 bi-weekly courses of doxorubicin (75 mg/m^2), cyclophosphamide (1200 mg/m^2), vindesine (2 mg/m^2) on days 1 and 5, bleomycin (10 mg) on days 1 and 5, prednisone (60 mg/m^2) orally from day 1 to day 5, and intrathecal methotrexate (15 mg) on day 2. This is followed by a sequential consolidation therapy with high-dose methotrexate (3 g/m^2), etoposide (300 mg/m^2), ifosfamide (1500 mg/m^2), and cytosine-arabinoside (100 mg/m^2). Thus, ACVBP has a higher dose intensity than CHOP-14 (Fig. 1). ACVBP achieved a better EFS and OS rate than CHOP in patients 61–69 years of age; however, the therapy-associated death rate was significantly higher (13% versus 7%), and the authors do not recommend ACVBP in patients >65 years of age.[26]

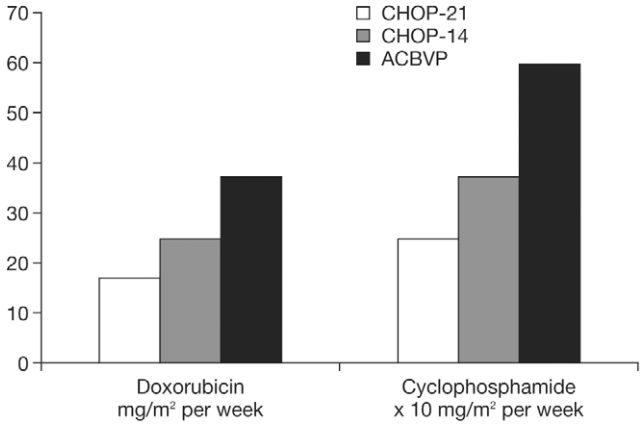

Fig. 1 – Dose intensities of doxorubicin and cyclophosphamide in dose-dense modifications of CHOP. □ = CHOP-21; ▒ = CHOP-14[6]; ■ = ACBVP[11]. *Abbreviations:* CHOP, cyclophosphamide, vincristine, doxorubicin and prednisone; ACBVP, doxorubicin, cyclophosphamide, vindesine, bleomycin and prednisone.

The already discussed GOELAMS[18] trial which compared CHOP-21 with an experimental approach consisting of both dose-dense components and a high-dose BEAM regimen in young patients with aaIPI=1,2 represents another example of a so-called hybrid approach. Recently, the Dutch-Belgian Hemato-Oncology Cooperative Group presented results with six cycles of a dose-escalated I-CHOP-14 which has the same total dose as eight cycles of CHOP-21, but the double intensity of cyclophosphamide and doxorubicin. The dose-intense I-CHOP-14 appeared to improve outcome for young patients with low-intermediate risk.[27]

6. Role of rituximab

The introduction of rituximab and its combination with CHOP represents the major milestone in the treatment of DLBCL for the last 30 years. Combinations of chemotherapy with rituximab are hardly more toxic than the respective chemotherapy alone, except for viral infections.[24,28,29]

The first randomised trial with rituximab in DLBCL was performed by the French cooperative group GELA and compared standard eight cycles of 3-weekly CHOP-21 with and without rituximab in previously untreated elderly patients

(61–80 years) in stages 2–4. After a median follow-up of 5 years, EFS, progression-free survival, disease-free survival, and OS were statistically significant in favour of the combination of R-CHOP.[28] Patients with low-risk, but not with high-risk lymphoma according to the IPI had a significantly longer survival if treated with the combination.[29]

A second randomised trial[30] in elderly patients which compared CHOP-21 with and without rituximab yielded similar results. Patients achieving a partial or complete remission in this trial had a second randomisation to either rituximab maintenance or observation. Interestingly, patients who received rituximab maintenance had a significantly prolonged EFS, but this advantage was only observed in patients who had not received rituximab as part of their induction therapy.

A third randomised trial compared six and eight cycles of dose-dense bi-weekly CHOP-14 with and without rituximab in elderly patients (61–80 years). EFS after eight cycles of CHOP-14 without rituximab was better than six cycles, but this advantage of two additional chemotherapy cycles was not observed when rituximab was added. Indeed, there was a trend for a better OS after six × R-CHOP-14 compared to eight × R-CHOP-14, and only six × R-CHOP-14, but not eight × R-CHOP-14 had a significantly better OS compared to six × CHOP-14 and eight × CHOP-14 without rituximab, respectively.[24]

The MInT study, which included young (18–60 years of age) patients with good-prognosis (aaIPI=0,1) DLBCL, showed a significantly better 3-year EFS (59% after chemotherapy and 79% after chemotherapy plus rituximab) and 3-year OS (93% versus 84%) after chemotherapy plus rituximab compared to chemotherapy alone, establishing the combination of CHOP and rituximab as the reference standard also in this population of DLBCL patients.[1]

In contrast, in young high-risk patients, there is no data from randomised trials formally proving the role of rituximab for these patients, even though such a role can be expected from the results of the randomised trials in young low-risk and elderly patients discussed above.

An important issue with respect to integrating rituximab into intensified chemotherapy regimens is the lack of knowledge on the most important effector mechanism of rituximab operative *in vivo*. If this is indeed ADCC (antibody-dependent cell mediated cytotoxicity), then natural killer cells are essential for the efficacy of rituximab and combinations of rituximab and more myelosuppressive chemotherapies would carry an increased risk of compromising its efficacy. In this respect, it is of interest that CHOEP was confirmed to be superior to CHOP in the MInT study,[1] but in combination with rituximab CHOP was of similar efficacy to CHOEP and there was even a trend that R-CHOP was better than R-CHOEP for some subpopulations in the MInT trial. The lack of superiority of R-CHOEP over R-CHOP might be explained by a 'chemotherapy-regimen-equalising effect' of rituximab; alternatively, however, this could be due to the more pronounced myelosuppression after CHOEP, which annihilates its greater cytotoxicity in combination with the antibody. The efficacy of rituximab might be even more compromised if rituximab is combined with even more myelosuppressive or even myeloablative regimens.[31] In summary, these considerations and the experience with combining rituximab with CHOP or CHOEP in the MInT trial caution against combining rituximab with chemotherapy combinations other than CHOP outside controlled trials.

7. Intensifying rituximab

Another problem is the lack of pharmacokinetic data of rituximab in DLBCL and the lack of knowledge on minimal serum levels and areas under the curve necessary to achieve tissue levels that are sufficient to kill the malignant DLBCL clone *in situ*. The commonly used dosage per application (375 mg/m^2) and the schedule of giving rituximab with each cycle of chemotherapy are mostly based on practicability and economic considerations.

Rituximab has been shown to improve results compared to chemotherapy alone both in combination with CHOP-21[1,28–30] and with dose-dense CHOP-14.[24] However, while the benefit of adding rituximab was much smaller for poor-prognosis patients in the 3-weekly schedule in the GELA 95.8 trial (where it even failed to reach significance with respect to survival), poor-prognosis and good-prognosis patients profited from the bi-weekly application of rituximab to an at least equal extent in the German trial.[24] Pharmacokinetic studies of bi-weekly rituximab in combination with CHOP-14[32,33] showed an increase of pre-infusion rituximab serum concentrations with each additional application for the first four cycles, and rituximab serum levels did not reach a plateau until between cycles 5 and 8 and then decreased continuously after the end of treatment with detectable levels even after 9 months in some patients.[34] The lower serum levels in the first four cycles are probably due to the destruction of normal B-cells together with maximal reduction of tumour load after the first treatment cycle.[34] It must be assumed that rituximab serum levels fall even deeper and possibly below a critical level in a 3-weekly schedule of rituximab, in particular in patients with a high tumour load and hence poor prognosis explaining the reduced efficacy of 3-weekly rituximab in high-risk patients in the GELA trial.[34] In this respect, it is noteworthy that the therapeutic gain achieved by the addition of rituximab with eight applications in the GELA LNH 98.5 trial[28,29] was very similar to the one achieved in the ECOG trial[30], where only five applications were given, but with a different schedule. Taking into consideration that the first application of rituximab must eliminate circulating B-cells first before it can access the tumour site, the rituximab schedule with a loading dose in the ECOG trial, where rituximab was given twice (on days –7 and –3) before the first CHOP cycle, might have been more adequate to cope with this situation (Fig. 2) than the once-every-3-weeks schedule in the GELA trial.

The pharmacokinetics of bi-weekly CHOP, where a plateau is not achieved until cycle 4, also suggests that bi-weekly rituximab may not be dose-dense enough and that smaller intervals are indicated, in particular during the early treatment phase in order to achieve high serum levels with the first chemotherapy cycle. A phase II study evaluating the pharmacokinetics and efficacy of dose-dense rituximab (five

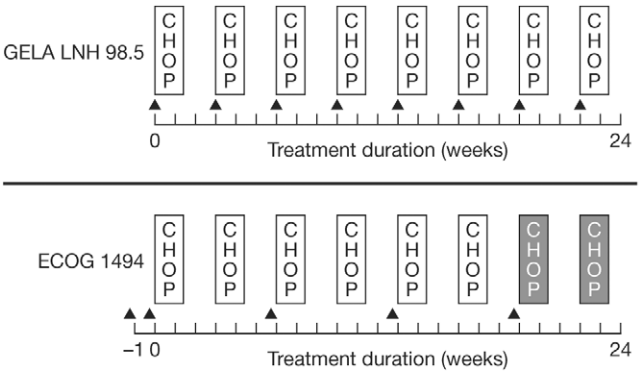

Fig. 2 – Schedule of rituximab application (▲) in combination with CHOP-21 in randomised trials demonstrating superiority of CHOP plus rituximab over CHOP alone. A similar improvement was observed in the Groupe d' Etude des Lymphomes des Adultes (GELA)[29] and the Eastern Cooperative Oncology Group (ECOG)[36] trial, even though the latter used only five applications of rituximab, but had two dose-dense applications before the first chemotherapy cycle. In the ECOG trial, CHOP was given for two cycles beyond CR (total 6–8 cycles). *Abbreviations:* CHOP, cyclophosphamide, vincristine, doxorubicin and prednisone; CR, complete response.

applications within the first 2 weeks) in combination with CHOP-14 is currently ongoing in Germany.[35]

8. Conclusions

Reducing treatment intervals of CHOP from 3 to 2 weeks (CHOP-14) together with the addition of rituximab represent the major milestone in the treatment of aggressive lymphomas, where results had stagnated for over 25 years. The RICOVER-60 trial shows that the combination of both approaches (interval reduction and the addition of rituximab) is a further step forward. The observation that bi-weekly rituximab in combination with CHOP-14 in contrast to the 3-weekly application of the antibody in combination with CHOP-21 improves outcome in poor-prognosis as much as in good-prognosis patients suggests that not only densification of the cytotoxic drugs, but also dose-dense application of the antibody contribute to the high efficacy of R-CHOP-14. The key issues with respect to the use of dose-dense chemo-immunotherapy refer to the lack of pharmacokinetic data available for rituximab in DLBCL and the lack of knowledge on minimal serum levels and areas under the curve necessary to achieve tissue levels that are sufficient to kill the malignant DLBCL clone in situ. The chances that current protocols give *too much* rituximab for DLBCL are as good as the chances that *not enough* rituximab is given, or that enough rituximab is given, but with a suboptimal schedule. Well-designed prospective trials incorporating pharmacokinetic studies are necessary to exploit the full potential of dose-intensified treatment both with respect to its chemo- and immunotherapy component in the era of modern chemo-immunotherapy for DLBCL.

9. Conflict of interest statement

Carsten Zwick has no potential conflict of interest to declare. Gerhard Held has no potential conflict of interest to declare. Michael Pfreundschuh receives support for a research project from Chugai Germany, and is a member of a Roche and Genentech advisory board.

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