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Original article

Dose-densified chemoimmunotherapy followed by systemic central nervous system prophylaxis for younger high-risk diffuse large B-cell/follicular grade 3 lymphoma patients: Results of a phase II Nordic Lymphoma Group study

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Key words: Age adjusted IPI; Central Nervous System; Diffuse large B-cell Lymphoma; Intensive chemotherapy; prophylaxis.

Background

Many patients with aggressive B-cell lymphomas and high clinical risk score still die from lymphoma after conventional R-CHOP chemoimmunotherapy. We hypothesized that intensified chemoimmunotherapy including systemic central nervous system (CNS) prophylaxis improves outcome and reduces the incidence of CNS related events.

Patients and Methods

Inclusion criteria were age 18-65 years, primary diffuse large B–cell lymphoma or grade III follicular lymphoma without clinical signs of CNS disease and negative cerebrospinal fluid cytology, age-adjusted International Prognostic Index 2-3 and WHO performance score 0-3. Treatment consisted of six courses of R-CHOEP-14 followed by a course of high-dose cytarabine and a course of high-dose methotrexate. Primary endpoint was failure-free survival (FFS) at three years.

Results

156 eligible patients with a median age of 54 years (range 20-64) were included. Three toxic deaths were observed. Three-year overall survival (OS) and FFS rates (median observation time 52 months for survivors) were 81% and 65%, respectively. Seven patients experienced CNS relapse, all within 6 months.

Conclusions

The results are promising with favorable three-year OS and FFS rates, a low toxic death rate and a lower than expected number of CNS events. CNS progression might be further reduced by earlier CNS prophylaxis. (CinicalTrials.gov. Identifier: NCT01502982).

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a curable disease with combination chemotherapy. The outcome is variable but can to some extent be predicted from clinical risk factors included in the International Prognostic Index (IPI) score [1]. Of all patients, less than 50% are cured with a CHOP-like (cyclophosphamide, doxorubicin, vincristine and prednisone) therapy [2], while the corresponding cure rates for low and high risk patients are 80% and 30% (1). Introduction of rituximab, a monoclonal antibody targeting CD20, in combination with CHOP has led to a marked improvement of survival [3-7]. Survival benefit was also obtained in the German NHL-B2 trial for elderly patients by reducing intervals between CHOP cycles from 21 to 14 days [8]. An additional attempt to improve the CHOP-14 regimen was tested by combining etoposide with CHOP-14 (CHOEP) in the German NHL-B1 trial for patients less than 60 years [9], resulting in improved progression-free survival (PFS), but not overall survival (OS). Similar results were reported in a subgroup analysis of the MInT trial [6] but not when rituximab was added to CHOEP-14 [4]. In the German RICOVER-60 study [10], the patients received six or eight cycles of CHOP-14 with or without eight rituximab infusions. Six cycles of CHOP-14 in combination with eight rituximab infusions yielded the best results.

For young high-risk DLBCL patients the optimal therapy has not been established. At the time this study was initiated, the benefit of rituximab and/or dose densification from 21 to 14 days had not been specifically investigated in the young high-risk subgroup. Randomized studies comparing conventional doses of chemotherapy with high-dose treatment (HDT) followed by stem cell transplantation have not convincingly shown an advantage for HDT [11, 12]. It is argued that patients with risk factors have a disease with high proliferation rate, and that these patients may benefit from dose densification (13). Thus, for younger patients

with risk factors, we hypothesized that R-CHOEP-14 is a treatment with three favorable modifications from the conventional CHOP-21.

In addition to the risk of a systemic relapse, patients with DLBCL are at risk for relapse in the central nervous system (CNS). Several studies have shown that the risk of CNS relapse in the pre-rituximab era to be in the range of 4-6% [14-17]. Patients with high tumor load including high IPI score and extra nodal involvement in more than one site carry the highest risk to develop this ominous complication. In one retrospective analysis, patients with four or five of out of five defined risk factors had a five year CNS recurrence risk of more than 25% [16].

In the present prospective Nordic phase II study, the efficacy and safety of an intensive, dose-dense regimen with systemic CNS prophylaxis for high-risk patients [1] younger than 65 years with DLBCL or follicular lymphoma (FL) grade 3 were investigated.

Patients and Methods

Eligibility, staging and response assessment

Eligible patients were 18-65 years old with a biopsy-confirmed CD20-positive DLBCL or FL grade 3, age adjusted (aa) IPI 2-3 (1) and a WHO performance status < 4. Post-transplantation lymphoma, DLBCL with features intermediate between DLBCL and Burkitt lymphoma, primary CNS lymphoma, discordant FL grade 1-2 – DLBCL, transformed DLBCL from FL, and cases with leptomeningeal or parenchymal CNS lymphoma involvement were ineligible. Patients had to present adequate organ function, allowing the planned treatment schedule.

Standard staging procedure was performed in all patients with the addition of electrocardiogram and evaluation of the left ventricular ejection fraction (echo-cardiography or MUGA scintigraphy) and cerebrospinal fluid (CSF) cytology examination for CNS involvement. The protocol was approved by the Medical Agencies and Ethics Committees in

Finland, Denmark, Norway and Sweden, and the trial registered at ClinicalTrials.gov, number NCT01502982. All patients signed informed consent before study participation.

Response to therapy was evaluated after three courses and approximately four weeks after end of treatment according to Cheson 1999 criteria [18]. In 49 patients a FDG-PET was performed at the end of chemotherapy, and the Cheson 2007 criteria also applied for these patients [19]. Biopsy was recommended in PET positive cases. Patients with complete remission unconfirmed (CRu) and not experiencing relapse within 6 months were reclassified as a complete remission (CR), and if a relapse occurred, as a partial remission (PR).

Patients went off the protocol if lymphoma progressed or relapsed during treatment, if the patient declined to continue with the protocol, or at the discretion of the treating physician in case of intercurrent diseases or excessive toxicity, which prohibited further protocol treatment.

All hematological and non-hematological toxicities, except hair loss, were assessed and graded according to the WHO Common Toxicity Criteria (www://ctep.cancer.gov, version 3.0).

Pathology

Patients were included in the study based on a histological diagnosis from the local pathologists. After inclusion, samples were forwarded to the National Pathology Review representatives (JD, CS, M-LKS) for confirmation of the diagnosis and further sub-classification. The sub-classification was expanded after the WHO report of 2008 [20] to include the relevant subgroups, and also to perform a prognostic sub-classification of the DLBCL NOS into the two immunohistochemically (IHC) defined subgroups of germinal centre B-cell type (GCB) and non-germinal centre B-cell type (non-GCB) according to Hans

algorithm [21]. Assessment of Ki67-positivity was performed semi-quantitatively by the central pathology reviewer.

Treatment schedule

R-CHOEP-14 consisted of rituximab 375 mg/m2, cyclophosphamide 750 mg/m^2 i.v., doxorubicin 50 mg/m^2 i.v., and vincristine 1.4 mg/m^2 (max. 2.0 mg) i.v. on day 1, etoposide 100 mg/m^2 i.v. on days 1-3, and prednisone 100 mg daily p.o. for days 1-5. G-CSF support was given as filgrastim 5 µg/kg from day 4 or pegfilgrastim 6 mg on day 4. Six R-CHOEP-14 courses were followed by systemic CNS prophylaxis: high dose cytarabine (H-cytarabine; course number 7) 3 g/m^2 i.v. twice daily for two days (in total four times) and three weeks later high-dose methotrexate (H-MTX; course number 8) 3 g/m^2 i.v. as a 24-hour infusion. Folic acid rescue (calciumfolinate) was given after 36 hours. H-cytarabine was reduced from 3 to 2 g/m^2 and H-MTX from 3 to 1.5 g/m^2 for patients aged 60-65 years.

Intrathecal (i.t.) administration of cytostatic drugs was not part of the CNS prophylaxis, except that methotrexate 15 mg i.t. was allowed once after the diagnostic lumbar puncture before start of systemic treatment.

Radiotherapy was given at the discretion of the individual centers (36-45 Gy). Indications for giving radiotherapy after the completion of chemotherapy included bulky disease (≥10 cm) at diagnosis, localized PET positive residual lesions, and residual disease, not eligible for biopsy at a localized site, and potentially curable by radiotherapy.

Relative dose intensity. The relative dose (RD) for each drug of R-CHOEP was the ratio of dose received to protocol dose. The relative dose intensity (RDI) was the RD times stipulated protocol time divided by elapsed time for a given patient. For H-cytarabine and H-MTX, the RD was calculated.

Statistical analysis

The primary endpoint was failure free survival (FFS) defined as the interval between the registration date and the date of documented progression or lack of response, first relapse, death for any reason, or discontinuation/change of therapy because of toxicity, whichever occurred first. Otherwise, patients were censored at the last date they were known to be alive. For patients not responding at any time point on study treatment, FFS was defined as one day. The secondary endpoint, overall survival (OS) was defined as time from the registration date to the date of death. Patients still alive or lost to follow-up were censored at the last date they were known to be alive. Other secondary endpoints were response rates and toxicity.

All patients included according to protocol were analyzed. OS and FFS curves were estimated by means of the Kaplan-Meier method. Clinical and tumor related factors were analyzed by Chi square tests or non-parametric trend tests for response rates and log-rank tests and the Cox proportional hazards multivariate analysis for survival..

Results

Clinical characteristics

Between November 2004 and June 2008 160 patients were registered. Four patients were excluded after pathology review; three due to discordant or transformed lymphoma and one due to mantle cell lymphoma. 145 cases were centrally reviewed. One patient withdrew her informed consent during therapy, and her follow-up was censored from the date of withdrawal. Patient characteristics are summarized in Table 1.

Pathology

The pathology subgroups at inclusion and at review are summarized in Table 2. At review, 16 patients (10%) had FL grade 3, either with simultaneous DLBCL (n=3) or without (n=13). Of

the DLBCL NOS (74%), 46% were classified as GC- and 21% as non-GC subtypes, while 7% could not be sub classified. There was no significant difference in age between the patients with GC and non-GC subtypes. The Ki67 staining showed a relatively high median value of 70 % positive cells. The non-GC group had a significantly higher Ki67 score than the GC group; $p = 0.025$).

Toxicity and relative dose intensity (RDI)

135 patients (92%) received full treatment according to protocol, while 21 patients received 1-7 courses (Table 3). Reasons for reduced number of courses were treatment related deaths (*n=3*), severe toxicities (*n=7*; 4 septicemias, 1 mucositis, 1 cardiac insufficiency, 1 renal insufficiency), progressive disease (PD; *n=8*), consent withdrawn (*n=1*), no reason given (*n=1*), and suspected CNS involvement (*n=1*; turned out not to be the case).

The fraction of patients with reported grade 3-4 toxicities are shown in Table 3. After 40 patients had been treated, an interim analysis was performed. At the time, five patients had documented pneumocystis jirovecii pneumonia. Accordingly, the protocol was amended to include prophylaxis with trimethoprim-sulfamethoxazole.

The RDI based on all courses given was high (Table 3), and the RDI reductions for cyclophosphamide, doxorubicin and etoposide were mainly due to a mean prolonged treatment duration of approximately one week (112 days instead of 105 days for patients given all 8 courses). The RDI for vincristine was somewhat lower (0.94); 0.92 and 0.95 for patients ≥ 60 years and < 60 years, respectively. The RD for H-MTX for patients above and below 60 years was 1.0 and 0.97, respectively, and for H-cytarabine the corresponding numbers were 0.92 and 0.97.

Radiotherapy

Radiotherapy was given to 26 patients (17%) for the following indications (more than one reason may have been given): Bulky disease at the start of therapy, (*n = 13*), residual disease (*n= 21*), epidural lesion (*n = 1*), and bony lesion (*n = 3*).

Responses and CNS relapses

Response to therapy is shown in Table 4. Eleven of 19 treatment failures were not evaluable due to toxic deaths (*n=3*), toxicity and protocol treatment modifications (*n=6*), consent withdrawn (*n=1*) and unknown reason (*n=1*). Among remaining failures one patient had stable disease and seven PD at end of study treatment. Of the 49 patients with PET / CT performed, 5 of 12 patients with CT based PR converted to a CR, otherwise the remission status was unchanged.

Seven patients had CNS relapse, detailed in Table 5; all occurred within six months after study inclusion. Only three of the cases had both involvement of more than one extra nodal site and elevated LDH at registration.

Survival

After a median follow up of 52 months for surviving patients, 33 out of 156 patients (21%) had died; 22 due to lymphoma. Other causes of deaths are given in Table 4. The primary end point, three-year FFS rate, including patients not treated according to protocol, was 65% (confidence interval (CI) 59 - 73%) (Figure 1A). The secondary endpoint, three-year OS rate was 81% (CI 75% - 87%) (Figure 1B). There was better OS (*p = 0.011*), but only a weak trend for better FFS in patients with aaIPI 2 score as compared to those with aaIPI 3 (*p= 0.107*). FFS was not affected by sex, age below or above 60 years (*p=0.75*), by DLBCL IHC subtype (GCB versus non-GCB; *p = 0.82*, Figure 1C), nor number of extra nodal sites less than versus at least two (*p = 0.69*). However, patients with a high Ki67 score (≥ 70%) had a

better FFS than those with a low score (*p=0.035*; Figure 1D). When Ki67 score (low versus high), aaIPI 2 versus 3, number of extra nodal sites and DLBCL subtype were entered into a Cox multivariate analysis, no significant prognostic factors were identified for FFS (*p = 0.07* for Ki67).

When eight cases with follicular lymphoma grade 3A were excluded, three-year FFS and OS rates were still 65% and 82%.

Discussion

This phase II study in patients less than 65 years with DLBCL / FL grade 3 with aaIPI score 2-3 treated with a dose-dense chemoimmunotherapy regimen and systemic CNS prophylaxis demonstrates favourable three-year FFS (65%) and OS (81%). A CNS relapse rate of 4.5% is also lower than expected from previous studies. Furthermore, the low toxic death rate of 2% shows that the intensive regimen can be performed safely for most patients.

Our aim was to provide our Nordic younger high-risk DLBCL patients with what we considered to be the optimal therapy within a phase II study. At the time the protocol was initiated, the treatment schedule was based on data showing a benefit from the addition of rituximab [3-5] and dose intensification of the CHOP regimen for patients with DLBCL [8, 9], although not specifically for the young high-risk subgroup. Furthermore, data from several retrospective analyses of patients receiving CHOP-based chemotherapy showed a CNS relapse rate of more than 10% for a selected group of high-risk patients represented by those with high tumor load including high IPI score [13-16]. Results from a French study, including consolidation with high-dose methotrexate, showed a low rate of CNS relapses [22]. In addition to H-MTX, we included H-cytarabine in our regimen - a drug included in effective regimen for various aggressive lymphomas (Hyper CVAD, BFM, GMALL, CODOX-M/IVAC, Nordic regimen for mantle cell lymphoma) and with CNS prophylactic efficacy.

Recently, a report from the German High Grade Lymphoma Group showed superior outcome in younger high-risk DLBCL patients receiving R-CHOEP-14 regimen in comparison to the Mega R-CHOEP regimen with OS rate comparable to our study [23]. Furthermore, a recent, retrospective population-based Danish study provides additional support for a survival benefit of R-CHOEP-14 compared to R-CHOP-14 [24]. The survival benefit of adding etoposide to a R-CHOP-21 regimen could, however, not be confirmed in a subgroup analysis of patients with aaIPI 0-1 in the MiNT study [4]. Another argument for the inclusion of etoposide is that the drug can to some extent penetrate through the blood-brain barrier (25) and that inclusion of etoposide in a compilation of randomized German studies showed a statistically reduced incidence of CNS relapses [26].

When pooling several recent German prospective studies on aggressive B-cell lymphomas in which younger high-risk patients have received intensified regimen [26], both with and without rituximab, the risk for the CNS relapses was 4% for aaIPI 2 and 11% for aaIPI 3 patients while 4.5% of the patients in our study had a relapse in the CNS. Reports from the four – armed SWOG study (27) and from a population – based US study (28) show even lower CNS relapse rates, but whether the great majority of the CNS reølated events are identified may be questioned (28). It is plausible to suggest that more effective, dose dense therapy resulting in fewer systemic relapses leads to less frequent CNS relapses. Inclusion of rituximab to CHOP like therapy improves survival, but whether rituximab reduces the risk of CNS relapse is however, controversial [29-32]. Whether or to what extent the H-MTX and H-cytarabine courses used in our study prevent CNS relapses needs to be confirmed in a randomized study.

There is currently no consensus whether, how and to which patient group CNS prophylaxis should be given [32]. Patients at risk are generally considered to have high tumor

burden [13-16] - as the main criterion in our study -, CNS near lesions, bone marrow infiltration or testicular involvement. While some patients have been given combined i.t. and systemic i.v. H-MTX prophylaxis [22, 33], others have been given MTX either i.t. [16, 26-27] or i.v. [2, 36]. Only few of these studies support the use of CNS prophylaxis [22, 33, 35-36]. In the present study, in six of the seven patients with CNS relapse this was isolated to the CNS, and in four out of them in the brain parenchyma. In previous studies with CHOP chemotherapy, more than half of the cases had a combined systemic and CNS relapse, the median time to CNS relapse was longer than in this study, and the majority of the cases were meningeal [14-17]. Of note, all seven relapses occurred within 6 months after registration, implying that the CNS involvement may have been present, but undetected at diagnosis. For further reduction of CNS relapse without compromising a low systemic relapse rate, a combination of better CNS detection analysis and earlier CNS prophylaxis may be indicated. For parenchymal lymphoma involvement, MRI or a CT scan may be applicable, and for a more sensitive analysis of CSF, flow cytometry, which has been shown to be more sensitive than conventional cytological analysis of CSF (37), may be indicated.

In most studies a high fraction of cycling cells in the lymphoma tissue, as determined by Ki67 staining, is a factor predicting an unfavorable outcome [38-40]. In the present study, the results were opposite, presumably due to the intensity of the R-CHOEP-14 regimen. Interestingly, the non-GC group showed a significantly higher Ki67 score compared to the GC group, while there was no difference in age distribution or patients with more than one extra nodal site between these two groups. Furthermore, no survival difference between GC and non-GC subgroups was found. These data are in accordance with other datasets [41-42] indicating that the Hans´ algorithm is inadequate in discriminating survival difference between the GC and non-GC groups as defined by gene expression profiling [43]. We are presently examining the more recent IHC algorithms, showing better concordance with gene

expression profiling (44-45Tally??), with respect to prognostic value of the GC versus non-GC subtyping.

In conclusion, we found highly satisfactory OS and FFS for high-risk patients with aggressive B-cell lymphomas. Furthermore, a CNS relapse rate of 4.5% was lower than expected, all events occurring within 6 months after study inclusion. In an ongoing Nordic phase II study for younger high-risk DLBCL patients, CSF flow cytometry and CT or MRI of the brain are required as CNS directed staging procedures. In addition, both systemic and local (liposomal cytarabine) CNS prophylaxis is administered earlier, with the hope to further reduce lymphoma CNS events.

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References

1. The International non-Hodgkin's lymphoma prognostic factor project: A predictive model for aggressive non-Hodgkin's lymphoma New Engl J Med 1993; 329: 987–994.
2. Fisher RI, Gaynor E, Dahlberg H, et al: A phase III comparison of CHOP versus m-BACOD versus ProMACE- CytaBOM versus MACOP-B in patients with intermediate or high grade non- Hodgkin's lymphoma. New Engl J Med 1995; 328: 1002–1006.
3. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. New Engl J Med 2002; 346: 235-242.
4. Pfreundshuh M, Trümper L, Österborg A, et al: CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large B-cell lymphoma: a randomized controlled trial by the MabThera International Trial (MInT) group. Lancet Oncol 2006; 7: 379-391,
5. Vose JM, Link BK, Grossbard ML, et al: Phase II study of rituximab in combination with CHOP chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. J Clin Oncol 2001; 19: 389-397.
6. Sehn LH, Donaldson J, Chhanabhai M, et al: Introduction of Combined CHOP Plus Rituximab Therapy Dramatically Improved Outcome of Diffuse Large B-Cell Lymphoma in British Columbia. J Clin Oncol 2005; 23: 5027-5033.
7. Feugier P, Van Hoof A, Sebban C, et al: Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol 2005; 20: 4117-4126.
8. Pfreundschuh M, Trumper L, Kloess M, et al: Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of elderly patients with

aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. Blood 2001; 104: 634-641.

19. Pfreundschuh M, Trumper L, Kloess M, et al: Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of young patients with good-prognosis (normal LDH) aggressive lymphomas: results of the NHL-B1 trial of the DSHNHL. Blood 2004; 104: 626-633.
10. Pfreundschuh M, Schubert J, Ziepert M, et al: Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+ B-cell lymphomas: a randomised controlled trial (RICOVER-60). L ancet Oncol 92008; 9: 105-116.
11. Strehl J, Mey U, Glasmacher A, et al: High-dose chemotherapy followed by autologous stem cell transplantation as first-line therapy in aggressive non-Hodgkin's lymphoma: a meta-analysis. Haematologica 2003; 88: 1304-1315.
12. Greb A, Bohlius J, Schiefer D et al: High-dose chemotherapy with autologous stem cell transplantation in the first line treatment of aggressive non-Hodgkin lymphoma (NHL) in adults. Cochrane Database Syst Rev 2008; Jan 23: CD004024, 2008
13. Lymana RL. Barron JL. Natoli et al. Systematic review of efficacy of dose-dense versus non-dose-dense chemotherapy in breast cancer, non-Hodgkin lymphoma, and non-small cell lung cancer. Crit Rev Oncol Hematol 2012; 81: 296-308.
14. van Besien K, Ha CS, Murphy S et al: Risk factors, treatment and outcome of central nervous system recurrence in adults with intermediate-grade and immunoblastic lymphoma. Blood 1998; 91: 1178-1184.
15. Bos GMJ, van Putten WLJ, van der Holt B, et al: For which patients with aggressive non-Hodgkin's lymphoma is prophylaxis for central nervous system disease mandatory? Ann Oncol 1998; 9: 191-194.

16. Hollender A, Kvaloy S, Nome O, et al:. Central nervous system involvement following diagnsois of non-Hodgkin's lymphoma: a risk model. Ann Oncol 2002; 13: 1099-1107.
17. Björkholm M, Hagberg H, Holte H, et al: for the Nordic Lymphoma Group (NLG). Central nervous system occurrence in elderly patients with aggressive non-Hodgkin lymphoma and a long term follow-up. Ann Oncol 2007; 18: 1085-1089.
18. Cheson BD, Horning SJ, Coiffier B, et al: Report of an international workshop to standardize response criteria for non-Hodgkin's lymphoma. J Clin Oncol 199; 19: 1244-1253.
19. Cheson BD, Pfistner B, Juweid ME, et al: Revised response criteria for malignant lymphoma. J Clin Oncol 2007; 25:579-586.
20. Swerdlow SH, Campo E, Harris NL, et al: WHO Classification of tumours of hematopoietic and lymphoid tissues. Lyon: IARCH Press, 2008 (4th Edition).
21. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103: 275-282.
22. Haioun C, Besson C, Lepage E, et al : Incidence and risk factors of central nervous system relapse in histologically aggressive non-Hodgkin's lymphoma uniformly treated and receiving intrathecal central nervous system prophylaxis: a GELA study on 974 patients Ann Oncol 2000; 11: 685-690.
23. Schmitz N, Nickelsen M, Ziepert M, et al: M..Conventional chemoimmunotherapy (R-CHOEP-14) or high-dose therapy (R-Mega-CHOEP) for young, high-risk patients with aggressive B-cell lymphoma: Final results of the randomized Mega-CHOEP trial of the German High-Grade Non- Hodgkin Lymphona Study Group (DSHNHL). J Clin Oncol 2011; 29: abstr 8002.

24. Gang AO, Strøm C, Pedersen M, et al: R-CHOEP-14 improves overall survival in young high-risk patients with diffuse large B-cell lymphoma compared with R- CHOP-14. A population-based investigation from the Danish Lymphoma Group. Ann Oncol 2012; 23: 147-153.

25. Relling MV, Mahmoud HH, Pui C-H, et al: Etoposide achieves potentially cytotoxic concentrations in CSF of children with acute lymphoblastic leukemia. J Clin Oncol 1996; 14: 399-404.

26. Boehme V, Zeynalova S, Kloess M, et al: Incidence and risk factors of central nervous system recurrence in aggressive lymphoma--a survey of 1693 patients treated in protocols of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL). Ann Oncol 2007; 18: 149-157.

27. Kumar A, Vanderplas A, LaCasce AS et al.Lack of benefit of central nervous system prophylaxis for diffuse large b-cell lymphoma in the rituximab era. Cancer 2012; 118:2944-51.

28. Bernstein SH, Unger JM, LeBlanc M et al. Natural history of CNS relapse in patients with aggressive non-Hodgkin’s lymphoma: a 20-year follow-up analysis of SWOG 8516-the South Westy Oncology Group.

29. Tai WM, Chung J, Tang PL, et al: Central nervous system (CNS) relapse in diffuse large B cell lymphoma (DLBCL): pre- and post-rituximab. Ann Hematol 2011; 90: 809-818.

30. Yamamoto W, Tomita N, Watanabe R, et al: Central nervous system involvement in diffuse large B-cell lymphoma. Eur J Haematol 2010; 85: 6-10.

31 Feugier P, Virion JM, Tilly H et al. Incidence and risk factors for central nervous rituximab. Annals of Oncology 2004; 15: 129-133.

32 Siegal T and Goldschmidt N: CNS prophylaxis in diffuse large B-cell lymphoma. If, when, how and for whom? Blood Rev 2012 ; 26: 97-106.

33. Tilly H, Lepage E, Coiffier B, et al: Intensive conventional chemotherapy (ACVBP regimen) compared with standard CHOP for poor-prognosis aggressive non-Hodgkin lymphoma. Blood 2003; 102: 4284-4289.

34. Schmitz N, Zeynalova S, Loeffler M, et al: Response: Intrathecal methotrexate and central nervous system events. Blood 2009; 114: 1999-2000.

35. Arkenau HT, Chong G, Cunningham D, et al: The role of intrathecal chemotherapy prophylaxis in patients with diffuse large B-cell lymphoma. Ann Oncol 2007; 18: 541-545.

36. Abramson JS and Hochberg EP: Intravenous methotrexate as central nervous system (CNS) prophylaxis is associated with a low risk of CNS recurrence in high-risk patients with diffuse large B-cell lymphoma. Cancer 2010; 116: 4283-4290.

37. Hegde U, Filie A, Little RF, et al: High incidence of occult leptomeningeal disease detected by flow cytometry in newly diagnosed aggressive B-cell lymphomas at risk for central nervous system involvement: the role of flow cytometry versus cytology. Blood 2005; 105: 496-502.

38. Salles G, de Jong D, Xie W, et al: Prognostic significance of immunohistochemical biomarkers in diffuse large B-cell lymphoma: a study from the Lunenburg Lymphoma Biomarker Consortium. Blood 2011; 117: 7070-7078.

39. van Imhoff GW, Boerma EJ, van der Holt B, et al: Prognostic impact of germinal center-associated proteins and chromosomal breakpoints in poor-risk diffuse large B-cell lymphoma. J Clin Oncol 2006; 24: 4135-4142.

40. Gaudio F, Giordano A, Perrone T, et al: High Ki67 index and bulky disease remain significant adverse prognostic factors in patients with diffuse large B cell lymphoma before and after the introduction of rituximab. Acta Haematol 2011; 126: 44-51.

41. Ott G, Ziepert M, Klapper W, et al: Immunoblastic morphology but not the immunohistochemical GCB/nonGCB classifier predicts outcome in diffuse large B-cell lymphoma in the RICOVER-60 trial of the DSHNHL. Blood 2010; 116: 4916-25.

42. Nyman H, Adde M, Karjalainen-Lindsberg ML, et al: Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy. Blood 2007; 109: 4930-4935.

43. Lenz G, Wright G, Dave SS, et al. for the Lymphoma/Leukemia Molecular Profiling Project: A Gene Expression-based Survival Predictor For Diffuse Large B Cell Lymphoma Treated With Chemotherapy Plus Rituximab. New Engl J Med 2008; 359: 2313-2323.

44. Choi WWL, Weisenburger DD, Greiner TC et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res 2009; 15: 5494-502.

45. ??

Table 1. Clinical characteristics

	n = 156 (%)
Sex	
Male	97 (62)
Female	59 (38)
Age, years	
Median (range)	54 (18-65)
WHO performance status (%)	
0	35 (22)
1	70 (45)
2	36 (23)
3	15 (10)
Stage (%)	
II	6 (4)
III	62 (40)
IV	88 (56)
B symptoms (%)	
Yes	94 (60)
No	62 (40)
LDH elevated > 1 x UNV	
Yes	151 (97)
No	5 (3)
Bulky disease (> 10 cm) (%)	
Yes	68 (43)
No	88 (56)
BM involvement (%)	
Yes	30 (19)
No	126 (81)
aaIPI (%)	
2	117 (75)
3	39 (25)
Number of extranodal sites (%)	
0	67 (43)
1	48 (31)
2	20 (13)
3	14 (9)
4 or more	7 (4.5)

Hollender score* (15)	
1	4 (3)
2	18 (12)
3	3 (44)
4	4 (28)
5	7 (5)
Mean	3.2
ND	15 (10)

WHO, World Health Organization; LDH, lactate dehydrogenase; UNV, upper normal value; BM, bone marrow; aaIPI, age-adjusted International Prognostic Index score, ND?
*Including the following variables: Albumin <35, retroperitoneal lymph node involvement, elevated LDH, age < 60 years, number of extra nodal sites > 1.

Table 2. Histological findings

	No (%)
Reviewed cases	145 (93)
Diffuse large B-cell lymphoma NOS	115 (74)
Germinal center B-cell type (GC)*	72 (46)
Non-GC type	32 (21)
Not specified or unclassified	11 (7)
Intravascular	1 (1)
Primary mediastinal	7 (5)
T-cell / histiocyte rich	8 (5)
Plasmablastic	1 (1)
Follicular lymphoma grade 3B (FL3B)	5 (3)
Follicular lymphoma grade 3A	8 (5)
*2 cases had concomitant FL3B, 1 case FL3A)	
Not reviewed	11 (7)
Diagnosis at inclusion	
Diffuse large B-cell lymphoma	145 (93)
Follicular lymphoma grade 3	11 (7)
Ki67 (% positive), median 70%	
< 50	15 (10)
50-69	29 (19)
70-89	37 (25)
90-100	26 (18)
not done	40 (28)

Table 3. Number of treatment cycles given. RDI of CHOEP14 and RD for H-MTX and H-cytarabine. Toxicity grade 3-4. Explain RDI and RD?

Age (years)	**All**		**18-59**		**60-64**	
No of patients	**156**		**115**		**41**	
Number of cycles given according to protocol						
1	3		1		2	
3	6		5		1	
5	2		2		0	
6	3		1		2	
7	7		2		5	
8 (completed according to protocol)	135		104		31	
Treatment duration, % of planned days (mean)	109		105		113	
RD / RDI (mean)						
Cyclophosphamide	0.99 / 0.91					
Doxorubicin	0.99 / 0.91					
Vincristine	0.94 / 0.86		0.95 / 0.90		0.92 / 0.81	
Etoposide	0.98 / 0.90					
Methotrexate			0.97/-		1.0/-.	
Cytarabine			0.97/-		0.92/-	
Toxicity grade	3	4	3	4	3	4
	%	%	&	%	%	%
Hematological	14	79	15	77	13	85
Mucositis	7	3	7	3	5	3
Gastrointestinal	13	3	11	2	18	5.
Infection	32	7	31	8	335	8

Table 4. Outcomes do you need abbreviations?

	n =156 (%)
Response	137 (87.8)
Complete response (CR)	102 (65.4)
Partial response (PR)	35 (22.4)
Failure, end of study	19 (12.2)
No change (NC)	1 (0.6)
Progressive disease (PD)	7 (4.5)
Not evaluable, toxicity (NE)	11 (7.1)
Dead	33 (21.1)
Cause of death	
NHL	22 (14.1)
Toxicity protocol treatment	3 (1.9)
Toxicity second line treatment	3 (1.9)
Suicide	2 (1.2)
Secondary malignancy	2 (1.2)
Pulmonary embolus	1 (0.6)
CNS relapses	7 (4.5)
Survival	
3-year Time To Treatment Failure*	65% (95% CI 59-73)
3-year Overall Survival	81% (95% CI 75-87)

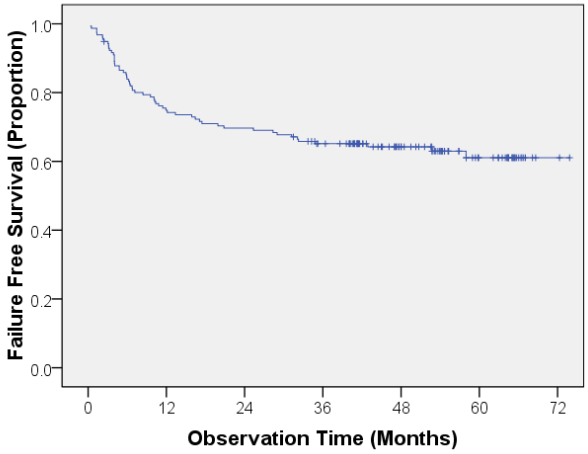
*including non-adherence to protocol treatment due to toxicity

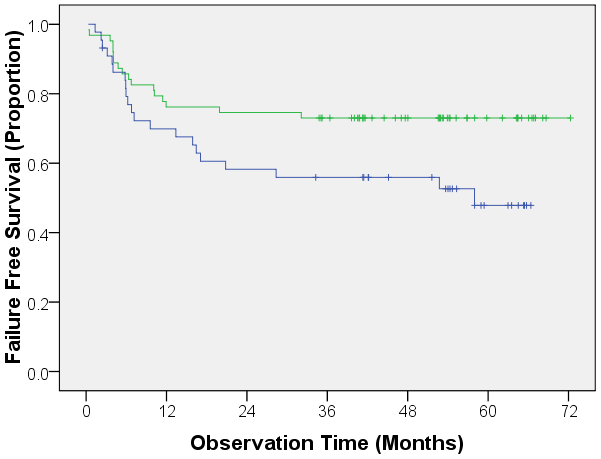
Table 5. Cases with CNS progression/relapse: CSF and pathology at diagnosis, treatment of CNS relapse and outcome

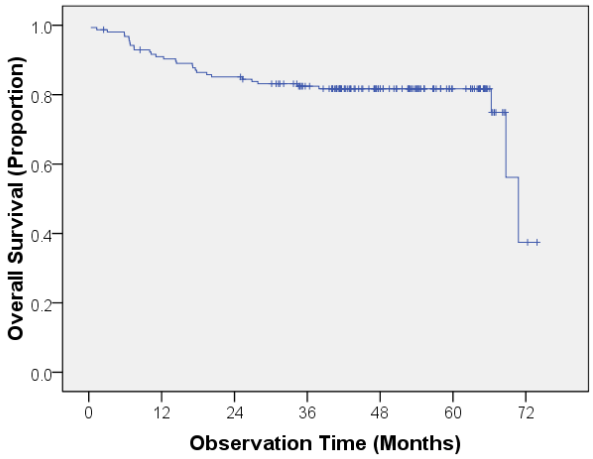
CSF neg cytology	CSF flow cytometry	Initial pathology: DLBCL subtype	CNS relapse: Intracerebral / Meningeal / Both	Treatment after CNS relapse	Outcome after CNS relapse
yes	negative	Germinal center	Intracerebral	Radiotherapy	PD, Mors
yes	nd	Germinal center	intracerebral	HD-MTX HD-Ara-C it Depocyte it MTX +RT	CCR*
nd	nd	DLBCL NUD	Meningeal and abdominal (combined)	HD MTX x 3 HD Ara C x 1 i.t.triple x 1 R-ICE x 1	PD mors
yes	negative	Germinal center	Meningeal	Primary CNS protocol +BEAM + RT	CCR
yes	nd	Non-germinal center	meningeal	HD-MTX It Depocyte	PD, death lymphoma
Yes	negative	Mediastinal	Intracerebral	Primary CNS protocol with RT	CCR
yes	negative	Germinal center	Intracerebral	Primary CNS protocol + BEAM	Toxic death after BEAM

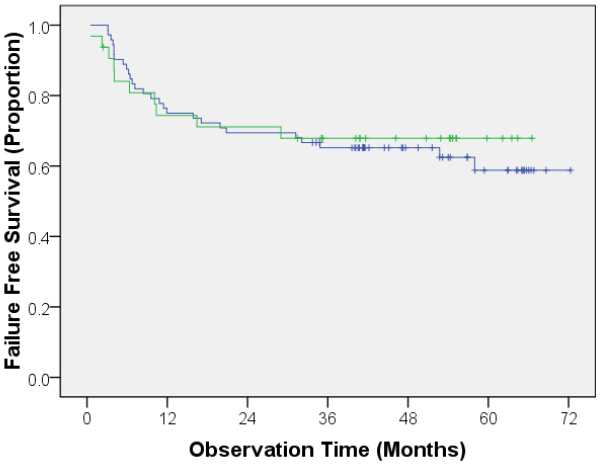
*continuous complete remission

Figure 1A. Failure Free Survival, N = 156. Figure 1B. Overall survival, N = 156. Figure 1C. Effect of % positive Ki67 tumor cells on Failure Free Survival. Green line: Ki67 like or above median, n = 63, blue line: Ki67 below median value, n = 44. P = 0.035. Figure 1D. Effect of immunohistochemically defined DLBCL subgroup on Failure Free Survival. Green line: non-GC phenotype, n = 32, blue line: GC phenotype, n = 72. P = 0.753


Failure Free Survival (Proportion)
1.0
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
Observation Time (Months)


Failure Free Survival (Proportion)
1.0
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
Observation Time (Months)


Overall Survival (Proportion)
1.0
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
Observation Time (Months)


Failure Free Survival (Proportion)
1.0
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
Observation Time (Months)