Hanne Kuitunen

DLBCL, PRIMARY AND SECONDARY CENTRAL NERVOUS SYSTEM INVOLVEMENT, TREATMENT AND PROPHYLAXIS
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DLBCL, PRIMARY AND SECONDARY CENTRAL NERVOUS SYSTEM INVOLVEMENT, TREATMENT AND PROPHYLAXIS

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Abstract
Diffuse large B-cell lymphoma (DLBCL) is the most common type of Non-Hodgkin’s Lymphoma (NHL). The standard treatment for DLBCL is R-CHOP chemoimmunotherapy (rituximab, cyclophosphamide, vincristine, doxorubicin and prednisone). About one-third of patients have refractory disease or the lymphoma relapses. Prognosis after relapse of refractory disease is poor. Fitter and younger patients are recommended new intensive salvage chemotherapy followed by autologous stem cell transplantation. Central nervous system (CNS) relapse is the most feared complication with dismal prognosis in DLBCL. High dose methotrexate intravenously administered concurrently with R-CHOP treatment has shown to be most promising to prevent CNS relapses.

Primary CNS lymphoma (PCNSL) is a rare aggressive lymphoma limited to the CNS and eyes. PCNSL is a chemo-and radiosensitive disease, but long-term response is rare since the blood brain barrier (BBB) limits access of many drugs to the CNS. BBB disruption (BBBD) is a treatment modality where the BBB is opened by hypertonic mannitol infusion. Administration of chemotherapeutics will achieve over ten-fold concentrations in the CNS and eradicate microscopic disease involvement.

This study retrospectively analyses patients who treated as first line with Bonn/Bonn-like treatment (study I), with BBBD treatment followed by high-dose treatment/autologous stem cell transplantation (HDT/ASCT) in first- or second-line (study II) or those treated with primary R-CHOP or its derivatives with or without concurrent CNS-targeted treatment (study III).

HD-MTX-based multichemotherapy is an effective induction treatment in CNS lymphoma, but long-lasting responses are rare. BBBD-treatment is well-tolerated and a promising method to attain high drug concentrations in the CNS to eradicate microscopic disease involvement in first- and second-line. CNS-prophylaxis with HD-MTX prevents CNS events in high risk DLBCL. PCNSL is aggressive disease despite excellent primary response with HD-MTX based multichemotherapy. BBBD-treatment is a promising method to eradicate microscopic disease in the CNS and achieve a long-term response and cure rate. Fatal CNS relapses can be avoided using CNS-targeted treatment.

Keywords: BBBD-treatment, CNS-relapse, CNS-targeted treatment, COO, DE-HGBL, HD-MTX, HGBL-DH, PCNSL
Kuitunen, Hanne, DLBCL, primaari ja sekundaari keskushermostoilmentymä, hoito ja ennaltaehkäisy.
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Medical Research Center Oulu; Oulun yliopistollinen sairaala
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Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

**Tiivistelmä**


PCNSL on agressiivinen tauti huolimatta erinomaisista metotreksaatipohjaisilla hoidoilla saavutetuista ensilinjan vastoista. BBBD-hoito on lupaavaa keinoa eradikoida mikroskooppinen tauti keskushermostosta ja saavuttaa pitkäaikaisia hoitovasteita, sekä pysyvää paranemista aivolymfoomassa. Suurannosmetotreksaatista sisältävällä sytostaattihoidolla voitetaan tämän valinna laaja aivorelappejä DLBCL:ssä.

**Asiakirjat:** COO, DE-HGBL, HGBL-DH, keskushermostoon suunnattu hoito, keskushermostouusiutuma, primaari keskushermostoilmentymä, suurannosmetotreksaatihoidoito, veriaivoesteen aukaisuhoito
“Siksi laulan ‘Eläköön päivät, jotka juoksi iltaan -
niiden riemut ja työt, rohkeus mennä vastavirtaan -
eläköön hellät yö ja rakkaus arpinenkin - suru, myös
sirpaleet, sillä tarvitsin mä senkin - eläköön kyyneleet’”
Juha Tapio
Acknowledgements

This thesis was undertaken in the Cancer and Translational Medicine Research Unit and in the Department of Oncology and Radiotherapy as part of the lymphoma research unit starting in 2014. The purpose of these studies was to analyse treatment outcomes and survival of PCNSL patients and patients with DLBCL and CNS relapses in our clinics.

I want to express my greatest appreciation to the thesis supervisors Docent Outi Kuittinen, M.D., Ph.D. and Professor Taina Turpeenniemi-Hujanen, M.D., Ph.D. for their expertise, support and guidance during this process. Thank you Outi for your endless optimism, positivity and friendliness these years. You are my role model and the way and attitude with which you treat your patients and guide doctoral students is admirable. Thank you Taina for your support and comments during the writing process. In the beginning of my thesis there were no conclusions as to how to treat PCNSL and the harmful preventable CNS event in DLBCL. During these years many studies have been published with different settings in PCNSL and DLBCL and most of those unsolvable questions have been clarified. Those questions mentioned above remain challenging, however. During this thesis we managed to extend the knowledge of the clinical behaviour and treatment possibilities of PCNSL and to estimate the impact of HD-MTX on incidence to CNS relapses and identify a really high-risk patient group in DLBCL.

I would like to thank my doctoral training follow-up members, Docent Arja Jukkola, M.D., Ph.D., Docent Jussi Koivunen, M.D., Ph.D. and Docent Peeter Karihtala, M.D., Ph.D. I am grateful to my reviewers Docent Maija Itälä-Remes, M.D., Ph.D. and Professor Antti Jekunen M.D., Ph.D. for your comments and respectable expertise.

I would like to thank my friends and colleagues who have encouraged and supported me during this project especially at difficult moments when I was ready to give up. I especially praise my little sister Heidi. I have sat for hours on end writing and healing the world with her.

Finally, I would like to thank my family; my parents, siblings, my sons Joonas, Julius and Magnus and my husband Jani. You are my life. Without your support this thesis would never have reached a successful conclusion.

Oulu September 2017

Hanne Kuitunen
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>aa-IPI</td>
<td>age adjusted International Prognostic Index</td>
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<tr>
<td>ABC</td>
<td>activated b-cell like</td>
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<tr>
<td>ACVBP</td>
<td>doxorubicin, cyclophosphamide, vindecine, bleomycin, prednisone</td>
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<tr>
<td>AIDS</td>
<td>acquired immuno deficiency syndrome</td>
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<tr>
<td>ASCT</td>
<td>autologous stem cell transplantation</td>
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<tr>
<td>BAK</td>
<td>BCL2 antagonist/killer1</td>
</tr>
<tr>
<td>BAX</td>
<td>BCL2 associated X</td>
</tr>
<tr>
<td>BBB</td>
<td>blood brain barrier</td>
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<td>BBBD</td>
<td>blood brain barrier disruption</td>
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<tr>
<td>Bcl-2</td>
<td>B-cell lymphoma-2 protein</td>
</tr>
<tr>
<td>Bcl-6</td>
<td>B-cell lymphoma-6 protein</td>
</tr>
<tr>
<td>BCNU</td>
<td>carmustine</td>
</tr>
<tr>
<td>BCR</td>
<td>b-cell receptor signalling</td>
</tr>
<tr>
<td>BEAM</td>
<td>carmustine, etoposide, cytarabine, melphalan</td>
</tr>
<tr>
<td>BIM</td>
<td>Bcl-2-like protein 11</td>
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<tr>
<td>BL</td>
<td>Burkitt’s lymphoma</td>
</tr>
<tr>
<td>BTK</td>
<td>bruton tyrosinekinase</td>
</tr>
<tr>
<td>CALGB</td>
<td>the Cancer and Leukaemia Group B</td>
</tr>
<tr>
<td>cART</td>
<td>combined antiretroviral therapy</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
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<tr>
<td>CSF</td>
<td>cerebro spinal fluid</td>
</tr>
<tr>
<td>CIOP</td>
<td>cyclophosphamide, idarubicin, vincristine, prednisone</td>
</tr>
<tr>
<td>CHOP</td>
<td>cyclophosphamide, doxorubicin, vincristine, prednisone</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CNS-IPI</td>
<td>central nervous system-IPI</td>
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<tr>
<td>COO</td>
<td>cell of origin</td>
</tr>
<tr>
<td>CORAL</td>
<td>collaborative trial in relapsed aggressive lymphomas</td>
</tr>
<tr>
<td>CR</td>
<td>complete response</td>
</tr>
<tr>
<td>CT</td>
<td>computerized tomography</td>
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<tr>
<td>DE-DLBCL</td>
<td>double expressor diffuse large B-cell lymphoma</td>
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<td>DE-HGBL</td>
<td>double expressor high grade B-cell lymphoma</td>
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<tr>
<td>DFS</td>
<td>disease free survival</td>
</tr>
<tr>
<td>DLBCL</td>
<td>diffuse large B-cell lymphoma</td>
</tr>
<tr>
<td>DLBCL-DH</td>
<td>diffuse large B-cell lymphoma double hit</td>
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</table>
DNA  deoxyribonucleic acid
DSHNHL  German High-Grade Lymphoma Study Group
EBV  Epstein Barr virus
ECOG  Eastern Cooperative Oncology Group
EFS  event-free survival
EP300  histone acetyltransferase p300
FDG  fluorodeoxyglucose
FISH  fluorescence in situ hybridization
GC  germinal centre
GCB  germinal centre B-cell
GELA  Group d’Etudes des lymphomes de l’Adulte (Adult Lymphoma Study Group)
GEP  gene expression profiling
GY  grey
HD-MTX  high dose methotrexate
HDT  high dose treatment
HDT/ASCT  high-dose treatment/autologous stem cell transplantation
HGBL  high grade b-cell lymphoma
HGBL-DH  high grade b-cell lymphoma double hit
HIV  human immunodeficiency virus
HLA  human leukocyte antigen
ICA  internal carotid artery
ICH  immunohistochemistry
IELSG  The International Extranodal Lymphoma Study Group
IFRT  involved field radiotherapy
IA  intra-arterial
IG  immunoglobulin
IOL  intra-ocular lymphoma
IPI  International Prognostic Index
IT  intrathecal
IV  intravenous
LDH  lactate dehydrogenase
LP  lumbar puncture
MAG  myelin associated glycoprotein gene
MATRix  rituximab, sytarabine, methotrexate, thiotepa
MRI  magnetic resonance imaging
mRNA  messenger ribonucleic acid
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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>MSK</td>
<td>Memorial Sloan-Kettering</td>
</tr>
<tr>
<td>MTX</td>
<td>methotrexate</td>
</tr>
<tr>
<td>NF-kappa-B</td>
<td>nuclear factor kappa-B</td>
</tr>
<tr>
<td>NHL</td>
<td>Non-Hodgkin Lymphoma</td>
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<tr>
<td>ORR</td>
<td>overall response rate</td>
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<tr>
<td>OS</td>
<td>overall survival</td>
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<tr>
<td>PET-CT</td>
<td>fluorodeoxyglucose computerised tomography</td>
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<tr>
<td>PCNSL</td>
<td>primary central nervous system lymphoma</td>
</tr>
<tr>
<td>PCV</td>
<td>procarbazine, CCNU (lomustine), vincristine</td>
</tr>
<tr>
<td>PFS</td>
<td>progression-free survival</td>
</tr>
<tr>
<td>PS</td>
<td>performance status</td>
</tr>
<tr>
<td>PTLD</td>
<td>post-transplant lymphoproliferative disease</td>
</tr>
<tr>
<td>R-CEOP</td>
<td>rituximab, cyclophosphamide, epirubicin, vincristine, prednisone</td>
</tr>
<tr>
<td>R-CHOEP</td>
<td>rituximab, cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone</td>
</tr>
<tr>
<td>R-CHOP</td>
<td>rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone</td>
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<tr>
<td>R-DHAP</td>
<td>rituximab, cisplatin, cytarabine, dexamethasone</td>
</tr>
<tr>
<td>R-EPOCH</td>
<td>rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin</td>
</tr>
<tr>
<td>RT</td>
<td>radiotherapy</td>
</tr>
<tr>
<td>SEER</td>
<td>The Surveillance, Epidemiology and End Results</td>
</tr>
<tr>
<td>SSP1</td>
<td>secreted phosphoprotein 1</td>
</tr>
<tr>
<td>SWOG</td>
<td>Southwest Oncology Group</td>
</tr>
<tr>
<td>TBC</td>
<td>thiothepa, busulfan, cyclophosphamide</td>
</tr>
<tr>
<td>TMZ</td>
<td>temozolomide</td>
</tr>
<tr>
<td>TTH</td>
<td>time to treatment failure</td>
</tr>
<tr>
<td>UNL</td>
<td>upper normal limit</td>
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<tr>
<td>VA</td>
<td>vertebral artery</td>
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<tr>
<td>WBRT</td>
<td>whole brain radiotherapy</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


Contributions in the publications: I participated in planning and designing the study, treated the study patients, collated and analysed the data and drafted the manuscript in publications I, II and III.
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1 Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of Non-Hodgkin lymphoma (NHL) (Campo et al., 2011; Martelli et al., 2013). Insidence is rising especially among those aged over 65 (Cummin & Johnson, 2016). The annual incidence of DLBCL varies between 500-600 cases in Finland (Finnish Cancer Registry-Institute for Statistical and Epidemiological Cancer Research).

DLBCLs include a heterogenous group of lymphomas with separate clinical and biological features. The disease can involve the lymph nodes, but approximately 40% of patients have extranodal involvement (Campo et al., 2011). Standard treatment of DLBCL is R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) with 60% of long terminal response and cure rate (Coiffier et al., 2002; Feugier et al., 2005; Pfreundschuh et al., 2006).

About one-third of patients have refractory disease or lymphoma relapse during follow-up, usually within two years from diagnosis (Cummin & Johnson, 2016). Prognosis after relapse of refractory disease is poor. The worst outcome is among patients with central nervous system (CNS) relapse (Bernstein et al., 2009; Boehme, Schmitz, Zeynalova, Loeffler, & Pfreundschuh, 2009; Feugier et al., 2004). If these patients are fit with adequate renal, heart and lung function without notable comorbidities, they recommended new intensive salvage chemotherapy. For good responders, high dose treatment (HDT) followed by autologous stem cell transplantation (ASCT) is the treatment of choice. Taking into account the aforementioned limitations a high dose treatment followed by ASCT is an option for only one-fifth of cases (Cummin & Johnson, 2016).

CNS relapse denominated secondary brain lymphoma is the most feared complication in DLBCL, because prognosis is mainly dismal regardless of the choice of treatment (Kridel & Dietrich, 2011; Martelli et al., 2013; Tai et al., 2011). CNS-targeted treatment as prophylaxis has been revealed to be effective to prevent this fatal event. In many published studies, clinical and biological risk factors and prophylactic treatments for CNS recurrence have been determined. Most of these studies are retrospective with a heterogenous patient population, low frequency of CNS events and variety of CNS prophylaxis methods. High dose methotrexate (HD-MTX) intravenously administered concurrently with R-CHOP treatment at the early stage of treatment has been shown to be most promising to prevent CNS relapses although there are no randomised trials to confirm this.
Primary CNS lymphoma (PCNSL) is a very rare aggressive lymphoma limited to the central nervous system and eyes. It accounts for 2% to 4% of intracranial malignancies and 4% to 6% of NHLs (Dolecek, Propp, Stroup, & Kruchko, 2012; J. Rubenstein, Ferreri, & Pittaluga, 2008). The annual incidence of PCNSL is 4.7 per 1,000,000 in industrialised countries (Villano, Koshy, Shaikh, Dolecek, & McVarthy, 2011) and based on SEER-data, incidence is increasing among those aged over 75 (Villano, Koshy, Shaikh, Dolecek, & McCarthy, 2011). The incidence rate varies between 4.4-9.7 per million in Finland (unpublished data from clinical patient registry).

Approximately 95% of PCNSLs are histologically diffuse large B-cell lymphoma, but PCNSL presents with different clinical behaviour and biology (Camilleri-Broet et al., 2006). Gene expression profile (GEP) studies have detected some differences in active genes between DLBCL and PCNSL such as SSP1 and MAG (Lim, Kim, Kim, Yoo, & Ko, 2015). PCNSL is sensitive for chemo-and radiotherapy, but unfortunately long-lasting responses are rare mainly since the blood brain barrier (BBB) limits penetration of many drugs to CNS. During the last decade, the treatment of PCNSL has been a moot point. Radiotherapy (RT) as consolidation treatment after chemotherapy has been criticised because of its serious neurotoxicity. Currently, high-dose treatment followed by autologous stem cell transplantation and nonmyeloablative high-dose therapy are studied instead of radiotherapy as a consolidation treatment (Citterio, Reni, Gatta, & Ferreri, 2017). For young and fit patients, induction treatment with MATRix regimen (Rituximab, Methotrexate, Cytarabine, Thiopeta) is the most recommendable treatment based on very promising results published by Ferreri et al (Ferreri et al., 2016).

Blood brain barrier disruption (BBBD) -treatment is an interesting treatment modality where the blood brain barrier is opened by hypertonic mannitol infusion (Neuwelt et al., 1991). Administration of chemotherapeutics will lead to attaining over ten-fold concentrations in the CNS and is considered as a cytotoxic dose to eradicate microscopic disease involvement. BBBD treatment may be one option to manage this aggressive disease (Angelov et al., 2009). BBBD treatment is technically challenging and requires a multiprofessional treatment team. In the Oulu University Hospital BBBD treatment has been offered for PCNSL patients since 2007.
2 Literature review

2.1 Diffuse large b-cell lymphoma: incidence, clinical features and principles of treatment

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin’s lymphoma accounting for approximately 25% to 58% of non-Hodgkin lymphoma cases (Campo et al., 2011; Martelli et al., 2013). The incidence of DLBCL is increasing among patients over 60 years. DLBCL includes a heterogeneous group of lymphomas with distinct pathophysiological, genetic and clinical features. The disease can present with nodal areas and approximately 40% of patients have extranodal involvement (Campo et al., 2011). Except for certain subtypes and disease with particular extranodal involvement such as the testis or brain, the standard regimen for DLBCL is R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone).

The cure rate for DLBCL has improved significantly within the last two decades following the addition of rituximab to chemotherapy regimens. In the prerituximab era this was 30-40% compared to 60% of cases who have received R-CHOP-like treatment (Coiffier et al., 2002; Feugier et al., 2005; Pfreundschuh et al., 2006). Patients alive without recurrence 24 months after diagnosis have prognosis with similar life expectancy to age and sexmatched controls (Gisselbrecht et al., 2010).

About one third of patients have refractory disease or lymphoma relapse during the follow-up period and these patients have a poor prognosis (Cummin & Johnson, 2016). The worst outcome is among patients with central nervous system relapse. If these patients are fit, with adequate renal, lung and heart functions without notable co-morbidities they are offered intensive salvage chemotherapy followed by autologous transplantation. However, unfortunately this is only an option for one fifth of cases. Among elderly patients, frailty and co-morbidities restrict the use of more intensive chemotherapy regimens. Chemorefractory disease and relapse within one year is a predictor of a poor prognosis and high dose chemotherapy/autologous stem cell transplantation may not be an option for those patients (J. Blay et al., 1998; Guglielmi et al., 1998).
2.2 Conventional treatment of diffuse large B-cell lymphoma

2.2.1 Risk classification

Treatment of DLBCL is based on stage, age, co-morbidities, presence of bulky disease and the International Prognostic Index (IPI) (International Non-Hodgkin's Lymphoma Prognostic Factors Project, 1993). IPI is the most widely used prognostic model for evaluating outcome in aggressive lymphoma. Patients can be split into four risk groups with different prognostic values; low risk IPI 0-1, moderate low risk IPI 2, moderate high risk IPI 3, high risk IPI 4-5 based on age, lactate dehydrogenase (LDH), Eastern Cooperative Oncology Group (ECOG) performance status (PS), stage and number of extranodal sites. Three-year overall survival rates are 91%, 81%, 65% and 59%, respectively.

2.2.2 Early stage disease

For localised DLBCL (stage I-II) the disease presents on the same side of the diaphragm. Representative evidence first-line treatment of localised DLBCL is based on two clinical trials by the Southwest Oncology Group (SWOG) and Group d’Etudes des lymphomes de l’Adulte (GEL). In the first study CHOPx3 plus involved-field radiotherapy (IFRT) with doses of 40-55 grey (Gy) were compared to CHOPx8 (Miller et al., 1998). Progression-free survival (PFS) and overall survival (OS) were both significantly better among patients in the group with IFRT. Estimated 5-year PFS rates were 74% and 64%. Estimated 5-year overall survival rates were 82% and 72%. In the second study CHOP followed by IFRT did not offer any advantage over the CHOP regimen for low-risk elderly patients (Bonnet et al., 2007).

In the rituximab era, there are no large clinical chemotherapy trials for localised DLBCL patients. In a randomised trial of 824 patients with DLBCL with an age-adjusted international prognostic index (aa-IPI) score of 0-1, patients were randomised into 6 cycles of CHOP(-like) or R-CHOP followed by IFRT of 30-40 Gy for diseases with bulky tumours over 5cm in diameter. Overall 72% of patients had limited stage. The rituximab arm was associated with a significantly better 6-year event free survival (EFS) of 55.8% versus 74.3%. Along these lines, 5-year OS rates were found to be 80% and 90.1%. For all these reasons, standard therapy for localised DLBCL is considered to be 3 cycles of R-CHOP followed by IFRT or 6-8 cycles of R-CHOP (Pfreundschuh et al., 2006; Pfreundschuh et al., 2011).
2.2.3 Advanced stage disease

The cornerstone for treatment of advanced stage DLBCL is R-CHOP based on the findings that the addition of rituximab to CHOP improved the outcome compared to the CHOP regimen. In the phase III study by GELA st II-IV, age 60-80 and PS 0-2, patients were randomised to receive R-CHOP21x8 or CHOP21x8 (Coiffier et al., 2002). In the R-CHOP arm the complete response rate (CR), 2-year event-free survival (EFS) and 2-year overall survival (OS) were more favourable compared to the CHOP arm. The difference remained in 10-year follow-up. Ten-year OS was 43.5% in the R-CHOP arm compared to 27.6% in the CHOP arm. Earlier in the prerituximab era the German High-Grade Lymphoma Study Group (DSHNHL) revealed that 6 cycles of CHOP14 was better than 6 cycles of CHOP21 for aggressive lymphoma among patients aged over 60 (Pfreundschuh et al., 2004). In the rituximab era DSHNHL reported the results of 2x2 factorial RICOVER-trial where patients aged 61-80 years and st I-IV were treated with 6 vs. 8 cycles of CHOP14 with or without 8 cycles of rituximab (Pfreundschuh et al., 2008). Outcome was better with patients who received rituximab. There was no difference in outcome between 8 cycles of R-CHOP14 and 6 cycles of R-CHOP-2R. In conclusion 6 cycles of chemotherapy is considered a standard of choice for older patients treated with R-CHOP14.

In two randomised phase III studies the efficacy of R-CHOP14 and R-CHOP21 were compared. In the first study 1080 untreated DLBCL patients aged 18 and older with stage I-IV and age-adjusted-IPI (aa-IPI) 1-3 diseases were treated either with 8 cycles of R-CHOP14 or 8 cycles of R-CHOP21 (Cunningham et al., 2013). Significant differences between randomised arms were not observed in the CR rate which were 63% in the R-CHOP21 group and 58% in the group of R-CHOP14. Two-year PFS rates were 74.8% and 75.4%, respectively two-year OS rates were reported to be 80.8% versus 82.7%. The LNH03-6B-trial by GELA in which DLBCL patients aged 60-80 and with aa-IPI-score 1-3 were randomised to 8 cycles of R-CHOP14 or 8 cycles of R-CHOP 21, confirmed similar results (Delarue et al., 2013). Overall, based on these findings, R-CHOP21 was considered a standard chemotherapy, but based on these studies, the fact of whether the optimal number of R-CHOP cycles is 6 or 8 is still unclear and undetermined.
2.3 Impact of new genetic and biological approaches to diffuse large B-cell lymphoma

2.3.1 The cell of origin model (COO); germinal centre B-cell (GCB) and activated B-cell (ABC) subtypes of DLBCL

DLBCL has historically been classified based on standard morphology and immunohistochemistry. This conventional classification cannot clarify the differences in clinical behaviour and treatment outcomes. Molecular phenotyping using gene expression profiles (GEP), and new deep sequencing technologies detecting different patterns of somatic mutations has offered the possibility of new classification.

GEP relies on microarray technology using hybridisation of nucleic acid of labelled targets from tissue samples to probes of complementary DNA (cDNA) oligonucleotides that represent potentially expressed genes of interest. GEP detects the relative expression of genes within a cell at the level of messenger ribonucleic acid (mRN). GEP arrays can evaluate thousands of genes, even close to whole transcriptome coverage, or target the most informative transcripts (Cummin & Johnson, 2016).

Based on GEP, two basic subtypes of DLBCL has been introduced: germinal centre B-cell (GCB), and non-germinal centre B-cell, also called activated B-cell (ABC), large B-cell lymphomas. Apart from these two subtypes, there is a third subtype of DLBCL, which has characteristics of both GC and ABC DLBCLs (Alizadeh et al., 2000; Klein et al., 1998; Rosenwald et al., 2002). These subtypes refer to the different points in B-cell ontogeny at which transformation to malignancy has occurred, “cell of origin” (COO). In clinical routine, detecting expression levels of coded protein by immunohistochemistry (ICH) has replaced GEP for practical reasons. The most widely adopted classification method based on ICH, Hans algorithm, includes expression of CD10, Bcl-6 and MUM-1 proteins; it has 80% similarity with GEP (Choi et al., 2009).

2.3.2 ABC and GCB phenotypes

The ABC-DLBCL subtype is associated with gene expression pattern involved in B-cell receptor (BCR) signalling and genes involved in NF-kappa-B pathway. GEP of GCB-DLBCL subtype resembles the normal germinal-centre derived B-cell
There is also a minority of DLBCL which cannot be classified into any of the three types reported above. The molecular distinction of subtypes also reflects clinical heterogeneity. ABC-DLBCL is the least curable subtype, and is also associated with a higher risk of central nervous system relapse. This subtype is more common in elderly patients. In large retrospective studies, worse prognosis has been observed with overall survival of 40% in ABC-DLBCL (Roschewski, Staudt, & Wilson, 2014). Findings cannot be shown in prospective settings and other factors e.g. chromosomal rearrangements or expression of genes such as MYC and BCL2 may have more influence (Hu et al., 2013).

### 2.3.3 Impact of ABC/GCP phenotypes on treatment

Understanding the biology behind DLBCL COO-subtypes may be used to select targeted treatments. In ABC-DLBCL, constitutionally activated NF-kappa-B pathway can be targeted by specific kinase inhibitors. Bruton’s tyrosine kinase (BTK) lies upstream of NF-kappa-B in the BCR signalling pathway, and is a suitable target for inhibition. Ibrutinib is the first agent that inhibits this kinase and a licence has been issued for B-cell malignancies. In a phase II trial of ibrutinib, patients with relapsed or refractory disease ORR was 37% among the ABC-DLBCL arm compared to 5% in the arm with GCB patients (Wilson et al., 2015). Bortezomib is a proteasome inhibitor that prevents degradation of I-kappa-B, which is a NF-kappa-B inhibitor. In a small pilot study, higher response rates were observed in ABC- than GCP relapsed/refractory DLBCL (Dunleavy et al., 2009). A large phase III trial involving bortezomib is ongoing and results are expected. Lenalidomide is an immune-modulating drug that may downregulate NF-kappa-B. Adding lenalidomide to R-CHOP results in similar outcomes in ABC-DLBCL to those in GCB-type disease (Nowakowski et al., 2015).

The GCB subtype appears to be less dependent on BCR-signalling. There are, however, other alternative mechanisms and potential targets for specific therapy. The most common mutations exist in the genes involved in epigenetic regulation and the cell cycle pathway. EZH2, CREBBP and EP300 are epigenetics regulators commonly detected to be mutated in the GCB-subtype and could be targeted (Dubois et al., 2016).
2.3.4 Double-hit and double-expressor lymphomas

DLBCL with dual rearrangements of MYC in chromosome 8;14 translocation and BCL2 in 14;18 translocation, has been reclassified as “high-grade B-cell lymphoma (HGBL), with MYC and BCL2 and/or BCL6 gene rearrangements”, and is denominated double-hit (or triple-hit with BCL6) HGBL. These aggressive lymphomas have been presented as a separate provisional entity in the 2016 revised World Health Organization (WHO) Classification of Lymphoid Tumours (Swerdlow et al., 2016).

MYC and BCL2 genes are located on chromosomes 8q24 and 18g21, respectively, and the most common partner of these genes in the translocation is a powerful promoter of immunoglobulin gene in chromosome 14. Translocation (14;18) including BCL2 rearrangement (first hit) usually leads to overexpression of bcl2 protein, and t(14;18) including MYC gene rearrangement (second hit) to overexpression of myc protein (Aukema et al., 2011; Gauwerky, Haluska, Tsujimoto, Nowell, & Croce, 1988). Fluorescence in situ hybridisation (FISH) is mandatory to identify HGBL-DH with these gene rearrangements.

Patients with HGBL-DH have concurrent MYC and BCL2 rearrangements in 60% of cases, 20% express MYC and BCL6 rearrangement, and the remaining patients (20%) have breakpoints in all 3 of these oncogenes, known as triple-hit lymphoma (Aukema et al., 2011; Ye et al., 2016).

Approximately 20% of cases having MYC and BCL2 translocations do not show expression of Myc and Bcl-2 proteins (Aukema et al., 2011; Herrera et al., 2017; Johnson et al., 2009; S. Li, Desai, Lin, Yin, Tang, Wang, Konoplev, Khoury, Bueso-Ramos, & Medeiros, 2016; Ye et al., 2016). The prognosis of patients with HGBL-DH but without Bcl-2 and Myc protein overexpression appears to be more favourable compared to patients having HGBL-DH with protein overexpression.

Thus, more than 80% of patients with HGBL-DH harbour coincidental translocations in MYC and BCL2, including those that also have BCL6 (Aukema et al., 2011). The last 20% harbour MYC and BCL6 translocations, and frequently express Bcl-2 despite an absence of BCL2 translocation (S. Li, Desai, Lin, Yin, Tang, Wang, Konoplev, Khoury, Bueso-Ramos, & Medeiros, 2016).

In case of Myc and Bcl-2 protein overexpression detected by ICH, DLBCL is called double-expressor (DE-DLBCL). DE-DLBCL has also been shown to have an inferior prognosis, although the outcome is superior than with HGBL-DL (Green et al., 2012; Hu et al., 2013; Johnson et al., 2012; Yan et al., 2014).
Co-expression of Myc and Bcl-2 proteins in DLBCL should be considered as a prognostic marker of a poor outcome (Green et al., 2012; Horn et al., 2013; Hu et al., 2013; Johnson et al., 2012; Perry et al., 2013; Yan et al., 2014; Ye et al., 2016). Co-expression after R-CHOP treatment predicts a poor response to a more intensive chemotherapy regimen, salvage chemotherapy, autologous stem cell transplant and resistance to panobinostat (Assouline et al., 2016; Herrera et al., 2017; Takahashi et al., 2016).

**MYC**

Myc protein is overexpressed in 30% to 40% of DLBCLs, 60% of HGBLs, and even 70% to 100% of Burkitt’s lymphomas (BLs). It is rare in the GCB phenotype, detected in only 5% of cases (Agarwal et al., 2016; Chisholm et al., 2015; Johnson et al., 2012; Perry et al., 2013). Overexpression of Myc in aggressive lymphomas controls cell cycle progression by interfering transcription of cyclin-dependent kinases, and promotes cell proliferation. It also causes genomic instability. The significance of isolated MYC translocation is still under consideration because most of those patients also express Bcl-2 protein. In a large cohort among DLBCL patients, treatment results of those cases with MYC translocation without Bcl-2 expression were excellent (Copie-Bergman et al., 2015).

**BCL2**

Bcl-2 protein is overexpressed in over 50% of DLBCLs and about 75% of HGBLs (Johnson et al., 2012; Perry et al., 2013). The primary function of Bcl-2 is to promote cell survival by inhibiting apoptosis (Vaux, Cory, & Adams, 1988). Induction of MYC increases the expression of BCL2-family member BIM, which, in the absence of anti-apoptotic Bcl-2 can bind and further activate pro-apoptotic BAX and BAK family members, leading to cell death. However, in the presence of Bcl-2, BIM induces Bcl-2, leading to prevention of cell death (Hemann et al., 2005). Bcl-2 potentiates other oncogenes, and is also associated with chemoresistance (Schmitt & Lowe, 2001).
BCL6

BCL6 is a transcription factor that induces the germinal centre reaction and suppresses Myc and Bcl-2 expression in normal GCB cells. Bcl-6 protein and BCL6 translocation take part in the pathogenesis of DLBCL.

2.3.5 Clinical significance of DE-DLBCL and DLBCL-DH and optimal treatment

Approximately 90% of HGBL DH patients have at least one of the following factors; leucocytosis, CNS involvement, lactase dehydrogenase > 3 times greater than upper limit, and advance stage disease (Johnson et al., 2009; Petrich et al., 2014). Among this patient group, in the absence of risk features, prognosis seems to be more favourable and there is a risk that those patients are overtreated with more intensive therapies. DE-GHBL patients can also present clinical risk features and it is notable that they have a 10% risk to develop CNS recurrence at 2 years (Savage et al., 2016).

Optimal treatments of DE-HGBL and HGBL-DH have not been determined. The outcome of GHBL-DH is poor and more intensive treatment regimens have been used especially among young and fit patients. In the retrospective study of Petrich et al., 311 patients with HGBL-DH were analysed (Petrich et al., 2014). Patients treated with more intensive regimens achieved better PFS, 22 months versus 8 months compared to patients who received R-CHOP. No overall difference in survival was observed. Dose-adjusted R-EPOCH (rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) had a superior complete response rate compared to R-CHOP. Treatment was well-tolerated and has been taken into clinical practice for HGBL-DH in many centres (Howlett et al., 2015; Petrich et al., 2014).

No study has demonstrated any advantage in using a more intensive treatment protocol among patients with DE-HGBL. CNS-prophylaxis could be recommended, but there is no consensus as to how to administer methotrexate; intravenous or is intrathecal administration as effective as IV dosing (Petrich et al., 2014).

Autologous stem cell transplantation as a consolidation treatment is an interesting issue for DE-HGBL and HGBL-DH. In the above-mentioned study by Petrich et al., ASCT as a consolidation improved overall survival among HGBL-DH patients (Petrich et al., 2014). In the Southwest Oncology Group (SWOG)
S9704 study, PFS was shown to be better, but not significantly with patients who received ASCT (Puvvada et al., 2016).

For relapsed or refractory DE-HGBL/HGBL-DH, prognosis is extremely poor. In the Collaborative trial in Relapsed Aggressive Lymphomas (CORAL) trial MYC translocation predicts a poor response for salvage treatment in patients with relapsed or refractory DLBCL. In this trial, 3-year PFS were only 17% to 19% respectively (Cuccuini et al., 2012). Most of these refractory cases had co-expression of Bcl-2. Even worse results were detected in the Canadian LY12 trial with DE-HGBL where none of the refractory patients achieved long-term response with effective induction treatment and ASCT and 3-year event-free-survival (EFS) was 0% (Crump et al., 2014). Dual expression together with HGBL-DH predicts a dismal prognosis with early relapse after ASCT (Herrera et al., 2017b).

A total of 20 to 30% of HGBL-DH are primary refractory diseases and there is an unmet need to find an effective induction treatment regimen for this entity. Promising results have been published with targeted agents such as the BCL2-inhibitor venetoclax (Souers et al., 2013; Vandenberg & Cory, 2013), immunomodulating agent lenalidomide (Salati, Tarantino, Maiorana, Bettelli, & Luminari, 2016), BTK inhibitor ibrutinib (Wilson et al., 2015) and proteasome inhibitor bortezomib (Vandenberg & Cory, 2013; Yang et al., 2012).

### 2.4 DLBCL and central nervous system relapse

Diffuse large B-cell lymphoma patients have a 5% overall risk of developing central nervous system involvement that complicates the clinical course of the disease (Zhang, Chen, & Xu, 2014). Median time from initial diagnosis to CNS relapse is commonly less than one year (da Rocha et al., 2013; Guirguis et al., 2012). Prognosis after CNS relapse is dismal with median survival under 6 months (Fletcher & Kahl, 2014). Preventing this life-threatening complication with CNS-targeted therapy is an important part of the treatment strategy for high-risk patients.

Defining risk patients and risk factors has been challenging. Several studies have attempted to identify patients who would benefit from CNS-targeted therapy. Most were retrospective in nature with a, relatively low frequency of CNS events and the heterogeneity of CNS prophylaxis methods. Based on these observations there are several risk models for predicting CNS relapse in the era of pre-and post-rituximab.

In many studies it has been revealed that CNS relapse rates have decreased in the rituximab era (Boehme et al., 2009; Mitrovic et al., 2012; Schmitz et al., 2012;
Wilson et al., 2014). This is associated with better systemic disease control. The pattern of CNS relapse has also changed during rituximab treatment. In the pre-rituximab era, CNS relapses were localised to the leptomeningeal structure but currently, isolated parenchymal lesions are more common.

2.4.1 Risk factors

Many studies have tried to determine risk factors for CNS relapse. Several large retrospective studies in the pre-rituximab era reported a higher incidence of CNS relapse in DLBCL patients with increased serum lactate dehydrogenase (LDH) levels, and/or involvement of more than one extranodal site (Boehme et al., 2007; Haioun et al., 2000; Hollender et al., 2002; van Besien et al., 1998). High/intermediate risk points in the International prognostic index (IPI score) ≥ 3 were associated with higher CNS relapse risk (Bernstein et al., 2009; Haioun et al., 2000).

In the rituximab era, many studies have tried to find predictive factors for CNS relapse. In the study by Feugier et al., 399 DLBCL patients were randomised to CHOP or R-CHOP treatment (Feugier et al., 2004). The authors identified an age-adjusted IPI (aa-IPI) > 1 as the only risk factor for CNS involvement. When this factor was excluded from the analysis, weakened performance status (PS) and elevated LDH were identified as predicting CNS involvement. In the RICOVER-60 study the elevated LDH levels together with impairment PS > 1 and more than one extranodal site were associated with risk of CNS recurrence (Boehme et al., 2009). The combination of increased LDH levels, involvement of more than one extranodal site and an intermediate or high international prognostic index has been shown to increase the risk for CNS events in many retrospective studies, reviews and meta-analyses in the rituximab era (Fletcher & Kahl, 2014; Kumar et al., 2012; Schmitz et al., 2012; Shimazu, Notohara, & Ueda, 2009; Zhang et al., 2014).

Certain specific extranodal involvements have been associated with higher sensitivity to CNS risk. Testicular or breast involvement in patients with DLBCL has been shown to correlate with higher risk for CNS involvement (Hosein et al., 2014; Tomita et al., 2012; Villa et al., 2010; Zucca et al., 2003). Renal involvement has been shown to increase the risk of CNS events (Villa, Connors, Sehn, Gascoyne, & Savage, 2011). Association of other extranodal sites with increased CNS relapse is somewhat controversial. Epidural space involvement located anatomically close to the central nervous system has previously been suggested to increase the risk for CNS events (MacKintosh et al., 1982). Disease localisation in the craniofacial area...
has earlier been assumed to elevate the rate of CNS relapse. However, the large-scale review by the German High-Grade Non-Hodgkin Lymphoma Study Group could not detect any difference in the 2-year cumulative incidence of CNS relapse between patients with or without craniofacial involvement (Murawski et al., 2014).

The most common risk models for CNS relapse were published by Hollender et al in the prerituximab era (Hollender et al., 2002). In this study, they observed that elevated LDH level, retroperitoneal lymph node involvement, more than one extranodal site, serum albumin level < 35g/L and age < 60 years predict a risk of CNS relapse. Among high-risk patients (risk factor 4-5) the risk for CNS recurrence was 25% at 5 years. A recently published study by Schmitz et al. created a new risk model and added to the classical IPI-score one to two points from possible renal and/or adrenal gland involvement (Schmitz et al., 2016). This new model identifies three risk groups; low 0-1 factor, intermediate 2-3 factors and high-risk 4-6 factors. Correspondingly 2-year CNS relapse risks were 0.65%, 3.4% and 10.2%.

The influence of the biology on CNS relapse is under discussion. There is still insufficient evidence to confirm any influence of B-cell origin on CNS relapses although in some studies the germinal centre phenotype has been associated with higher incidence of CNS recurrence (Kridel & Dietrich, 2011; Montesinos-Rongen et al., 2008). Many retrospective and recently published studies have shown a high incidence of CNS involvement in DLBCL cases with MYC translocation especially with BCL2-or BCL6 translocations. Frequency rates have been reported to vary between 9% and 45%. Dual expression of Myc (≥ 40%) and Bcl-2 (≥ 50%) determined by immunohistochemistry was associated with a statistically significant risk of CNS events (Savage et al., 2016b).

**LDH**

Serum lactate dehydrogenase (LDH) is included as one of the risk factors for IPI and CNS-IPI determination and reflects the tumour burden (Fletcher & Kahl, 2014; Shimazu et al., 2009). Many earlier studies have shown its role as a risk factor for CNS relapse. In a recently published prospective cohort study by Kim et al. the authors analysed 595 patients treated with R-CHOP (Kim et al., 2016). They tried to identify predictive risk factors for CNS events. In the multivariate analyses serum LDH levels > x3 UNL showed a notable risk for CNS relapse. This finding was in line with previous studies reporting its association with CNS relapses. The cut-off value of LDH > x 1 UNL did not show a higher risk for CNS recurrence,
whereas patients with LDH > x2 UNL had a clear association with CNS events (Tomita et al., 2000; Villa et al., 2010). The explanation for this association is that serum LDH values also reflect a growth tendency. Thus, patients with high tumour burden and a very rapidly proliferating tumour have an apparently high risk of treatment failure and CNS relapse, because the inadequate systemic treatment response increases the risk of CNS relapse.

**Ki-67**

Ki-67 is a nuclear antigen expressed in proliferating cells (Gerdes et al., 1984). It is a useful prognostic index for several malignancies including non-Hodgkin lymphoma (Dziegieł, Salwa-Zurawska, Zurawski, Wojnar, & Zabel, 2005; Szczuraszek et al., 2008). In the pre-rituximab era its prognostic value was controversial, but in recently published studies it seems to be an adverse predictor of prognosis among patients who have been treated with a rituximab-containing regimen (Szczuraszek et al., 2008; Yoon et al., 2010). In the study by Li et al., 118 DLBCL patients who received R-CHOP treatment were analysed (Z. M. Li et al., 2012). Overall and progression-free survival were lower in patients with high Ki-67 expression than in patients with low Ki-67 expression. Based on Ki-67 status by immunophenotype subgroups, patients with high Ki-67 expression in the non-GCB subgroup had the most dismal prognosis. These findings are in line with previous studies reported by Broyde et al. and Salle et al. (Broyde et al., 2009; Salles et al., 2011). In the primary central nervous system lymphomas, the high Ki-67 value has been shown to be associated with an adverse outcome in non-GCB phenotype patients (Cho, Oh, Hong, & Lee, 2016). There are no published studies about correlation with Ki-67 and secondary CNS lymphomas.

### 2.4.2 Prophylactic treatment of CNS relapse

**Intrathecal chemotherapy as CNS prophylaxis**

Intrathecal (IT) chemotherapy is the most useful method of delivering CNS prophylaxis. Cytarabine, methotrexate (MTX) and corticosteroids can be administered intrathecally. All these agents have been used as CNS prophylaxis for patients with high risk aggressive lymphomas, but intrathecal MTX in combination
Published data concerning the effect of MTX IT are controversial. In the retrospective study by Arkenau et al. 259 DLBCL patients were analysed (Arkenau et al., 2007). A total of 51 of these 259 patients received MTX IT prophylaxis and only 3 patients developed CNS relapse. Another large study by Haioun et al. reported promising results in 974 patients treated concurrently with MTX IT and ACVBP (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone) regimen (Haioun et al., 2000). The CNS relapse rate among study patients was very low at, only 2.2%, but it is difficult to estimate the effectiveness of MTX IT, because all these patients also received HD-MTX IV and only one -third of patients were high-intermediate or high-risk patients based on the IPI classification. MTX IT combined with hydrocortisone IT was found to be an effective CNS-targeted treatment (Tomita, Kodama, Kanamori, Motomura, & Ishigatsubo, 2002). In this study, there were 68 patients who achieved a complete response with systemic chemotherapy. After CR was attained, 29 of 68 patients received 4 doses of IT treatment with a good response; none of those study patients developed CNS recurrence whereas 6 patients in the follow-up group developed CNS relapse.

Many other studies have, however, reported no benefit of MTX IT in the prevention of CNS relapse. The addition of 4 doses of MTX 12mg IT to R-CHOP or -CHOP-treatment for at risk patients did not show any significant benefit in preventing CNS events (Tai et al., 2011). In the RICOVER-60 trial as well there was no significant advantage of using MTX IT in preventing CNS disease (Boehme et al., 2009).

In some studies, cytarabine and liposomal formulation especially have showed promising results as CNS prophylaxis in the treatment of aggressive lymphomas and leukaemia. Cytarabine IT has shown a similar anti-tumour effect and long-term therapeutic levels compared to intravenous cytarabine (Glantz et al., 1999; Gonzalez-Barca et al., 2016).

It is impossible to draw any conclusions regarding IT chemotherapy in CNS prophylaxis, as there are no randomised clinical trials in prospective settings. The available data is retrospective with a limited number of patients, heterogenous patient populations, varying dosing and agents and different combinations of systemic chemotherapy. The role of IT chemoprophylaxis is more unclear in the rituximab era, as many studies have observed that CNS relapses localise to brain parenchyma instead of leptomeningeal involvement. Due to the poor penetration of
IT chemotherapy, it is unlikely to achieve a protective effect on parenchymal relapses.

**Systemic chemotherapy as a CNS prophylaxis**

Data on the effectiveness of systemic chemotherapy for CNS prophylaxis in patients with aggressive non-Hodgkin lymphomas are based on studies from acute lymphoblastic lymphoma and Burkitt’s lymphoma. Most of these studies have combined CNS-penetrating agents, such as high-dose methotrexate (HD-MTX) or HD cytarabine in the treatment regimen.

HD-MTX is the most commonly used systemic CNS-targeted agent that can penetrate the blood brain barrier (BBB) and attain effective concentrations in the CNS. Although there are several studies that have shown the efficacy of systemic MTX in the rituximab era, the optimal dose, infusion time, number of cycles and the role of concomitant MTX IT is undetermined (Abramson et al., 2010; Cheah et al., 2014; Ferreri et al., 2015; Holte et al., 2013). Data on its effectiveness are based on primary central nervous system lymphoma studies, where HD-MTX was observed to reach superior outcomes when compared to chemotherapy regimens without HD-MTX or radiotherapy or MTX IT (T. Batchelor, Carson, O'Neill, Grossman, Alavi, New, Hochberg, & Priet, 2003; Khan, Shi, Thaler, DeAngelis, & Abrey, 2002). For inexplicable biological reasons, large-cell lymphomas present in the central nervous system have been observed to have two-fold greater sensitivity to HD-MTX-based treatment when compared to systemic lymphoma (Bokstein, Lossos, Lossos, & Siegal, 2002).

The appropriate dose of HD-MTX is controversial. Intravenously administered methotrexate (MTX IV) doses ≥ 3g/m² have been detected to produce therapeutic concentrations in CSF and brain parenchyma (Abramson et al., 2010; Fletcher & Kahl, 2014; Holte et al., 2013). HD-MTX treatment as a prophylaxis is not a suitable choice for all high-risk patients, due to its risk of toxicity, so patients should have adequate renal function and good general condition. The most common side-effects of HD-MTX-treatment are mucositis, delayed MTX elimination and nephrotoxicity. Pre-treatment urine alkalinisation, adequate hydration and post-treatment leucovorin rescue prevent these toxic effects.

In one retrospective study by Abrahamson et al., HD-MTX was used together with R-CHOP as CNS prophylaxis (Abramson et al., 2010). Patients received HD-MTX at a dose of 3.5g/m² 3 times on d15 with cycles 2, 4 and 6. The total incidence of CNS events was 3%, which was lower than suspected. In another study, HD-
MTX at a dose of 3g/m² 3-4 courses after completion of chemoimmunotherapy with or without IT treatment showed a notably decreased CNS relapse rate among patients who received prophylaxis (Ferreri et al., 2015). Taking into account the fact that CNS relapse can derive from occult CNS involvement at initial presentation, it is relevant to administer HD-MTX at an early stage of treatment (Bernstein et al., 2009; Villa et al., 2011).

Cytarabine is an agent which achieve high concentrations in the CNS. HD-cytarabine usually reaches active concentrations at a dose of 3g/m². Unfortunately, its neuro- and myelotoxicity has restricted its use as CNS prophylaxis. In the Nordic Lymphoma Group phase II study, HD-cytarabine was combined in an MTX-based chemotherapy regimen in high-risk DLBCL patients (Holte et al., 2013). Chemotherapy with R-CHOEP (rituximab-cyclophosphamide-doxorubicin-etoposide, vincristine, prednisone) regimen was followed by HD-MTX and HD-cytarabine as a CNS prophylaxis. The outcome was promising and showed a CNS recurrence rate of 4.5%.

Other chemotherapeutic agents have also been studied in high-risk lymphoma patients to prevent CNS events. The effectiveness of ifosfamide is difficult to define, as it has been used in combination with other CNS-penetrating drugs. Idarubicin is an anthracycline, which has an active metabolite that penetrates BBB. Despite this, in a study by Burton et al., CIOP (cyclophosphamide, idarubicin, vincristine, prednisone) was showed to be inferior when compared to a CHOP regimen (Burton et al., 2005).

The addition (Boehme et al., 2007) of etoposide to a CHOP regimen has been observed to be associated with a significantly lower CNS relapse rate in aggressive non-Hodgkin lymphoma. In the study by the Nordic Lymphoma Group, etoposide had a potential role in preventing CNS disease, although patients in this study also received HD-MTX and HD-cytarabine for CNS prophylaxis (Holte et al., 2013). Similar findings could have been identified earlier in a large 2007 DSHNHL study. However, a large retrospective study with 2,196 patients concluded that the addition of etoposide to a chemotherapy regimen did not decrease the incidence of CNS relapses (Schmitz et al., 2012).

### 2.4.3 CNS relapse

Prognosis after CNS relapse is dismal (Kridel & Dietrich, 2011; Martelli et al., 2013; Tai et al., 2011). High-dose chemotherapy followed by autologous stem cell
transplantation is a possibility for chemosensitive recurrent patients and the only available curative option (Bromberg et al., 2013).

For MTX-sensitive patients, new induction treatment with HD-MTX can achieve a good response and if achieved ASCT with carmustine-thiotepa is the most recommended choice of chemotherapy (J. L. Rubenstein, Gupta, Mannis, Lamarre, & Treseler, 2013). Patients with MTX-resistant lymphoma or relapsed within 6 months following treatment may not be candidates for intensive treatment and the purpose of the treatment is mainly palliative.

2.5 Primary central nervous system lymphomas

Primary central nervous system lymphomas are rare extranodal, malignant non-Hodgkin lymphomas arising inside the central nervous system (including brain, eyes, leptomeninges or spinal cord) in the absence of systemic lymphoma. Primary CNS lymphomas are estimated to account for up to 2% to 4% of intracranial neoplasms and 4% to 6% of extranodal lymphomas (Dolecek, Propp, Stroup, & Kruchko, 2012; J. Rubenstein et al., 2008). Very few population studies have been published about PCNSL. According to SEER data, the annual incidence in industrial countries is about 4.7 per million, incidence increases with age and especially among the oldest patients (> 75 years) (Villano, Koshy, Shaikh, Dolecek, & McCarthy, 2011).

PCNSL has long been associated with an inferior prognosis compared to other aggressive non-Hodgkin lymphomas. The 1, 2 and 5-year overall survival estimates for US patients diagnosed between 2000 and 2008 were 51.4%, 42.6% and 31.2% respectively (Villano et al., 2011). A trend toward better survival rates was published by Shields et al in an immunocompetent US population (Shiels et al., 2016), but there is some evidence, that advantageous survival rates are observed in patients recruited to clinical trials (Zeremski, Koehler, Fischer, & Schalk, 2016).

2.5.1 Risk factors for PCNSL

Risk factors for PCNSL include acquired and/or congenital immunodeficiency states, although most cases of PCNSL occur sporadically. PCNSL is an AIDS-defining illness associated with low CD4 cell count (< 50 cells/L) and Epstein Barr virus (EBV) while in systemic AIDS-related lymphomas, EBV infection of the tumour may be predictive of increased risk for secondary CNS involvement (Cingolani et al., 2000).
In the era of effective combined antiretroviral therapy (cART), the frequency of HIV-associated PCNSL has diminished (Shiels et al., 2011). Congenital immunodeficiency states such as ataxia-telangiectasia and Wiscott-Aldrich syndrome are associated with a 4% risk of developing PCNSL. Post-transplant lymphoproliferative disorder (PTLD) involving the CNS develops in 1% to 2% of renal transplant recipients and 2% to 7% of recipients of cardiac, lung and liver transplants. CNS PTLD is strongly associated with EBV in the setting of iatrogenic T-cell immunodeficiency by agents such as mycophenolate mofetil (Cell Cept) (Schabet, 1999). In post-transplant patients, the incidence is highest during the first year in patients with heart and lung transplants and in children younger than 10 years (Opelz & Dohler, 2004). Among PCNSL patients without evidence of immune suppression, EBV-associated lymphomas are rare (Camilleri-Broet et al., 2006).

2.5.2 Pathogenesis

PCNSL is a highly infiltrative neoplasm that has been characterised as a “whole brain disease” (Lai, Rosenblum, & DeAngelis, 2002). The radiographic appearance of the tumour underestimates disease extent and burden and is not amenable to curative resection (Lai et al., 2002). The pattern of growth is angiocentric, where the tumour cells gather around small and medium-sized vessels and contribute to disruption of the blood-brain barrier (Fine & Mayer, 1993).

Approximately 20% of PCNSL patients present involvement of the retina, vitreous, or uveal tract. Intraocular lymphoma (IOL) can be the first manifestation of brain lymphoma. As many as 90% of patients with isolated IOL will develop a delayed brain manifestation over the course of the disease (Chan et al., 2011; J. L. Rubenstein, Hsi, Johnson, Jung, Nakashima, Grant, Cheson, & Kaplan, 2013). Detection of IOL mandates staging of neuroaxis and evaluation of CSF and brain MRI in addition to therapies considering the risk for brain involvement.

Approximately 95% of PCNSL are diffuse large B-cell lymphomas. Despite the same histology as in systemic disease it has distinct clinical behaviour and biology (Camilleri-Broet et al., 2006). PCNSLs are more commonly classified as a non-germinal centre subtype, which carries an overall inferior prognosis among DLBCL. Other histologies that present as PCNSL include Burkitt’s lymphoma, low grade B-cell lymphoma and T-cell lymphoma (Partovi et al., 2014; Shenkier et al., 2005; Tang, Booth, Bhogal, Malhotra, & Wilhelm, 2011). The proliferative activity
index Ki67 is usually very high varying between 70% to 90% apart from low grade lymphomas such as dural-based marginal zone lymphoma.

To concentrate on immunohistochemistry, between 50% to 80% of PCNSL express BCL-6 and BCL-2 and at least 95% stain positive for MUM-1. Therefore, most PCNSL cases are of an activated B-cell immunophenotype of large cell lymphoma (Camilleri-Broet et al., 2006). It has been presented that due to its specific features, this classification cannot be directly applied to brain DLBCL. In the Cancer and Leukaemia Group B (CALGB) study, the authors evaluated immunohistochemical analyses of tumours and observed that high expression of BCL-6 by PCNSL tumours may correlate with refractory disease and shorter progression-free and overall survival (J. L. Rubenstein et al., 2013).

Common genomic aberrations in PCNSL include losses on chromosome 6p21 involving the HLA locus (56 -79%) (Gonzalez-Aguilar et al., 2012) found commonly in DLBCL arising in immunoprivileged sites and which represents a potential mechanism for immune escape (Booman et al., 2008). Deletions on chromosome 6q22-23 region (34 -50%) which contains several tumour suppressor genes (Cady et al., 2008; Gonzalez-Aguilar et al., 2012; McPhail, Law, Decker, & O'Neill, 2011) and silencing /deletion of the cell cycle regulator CDKN2A (45 -64%) have obvious adverse prognostic significance (Gonzalez-Aguilar et al., 2012; Hayashi et al., 2001)

PCNSL molecular studies have recognised potential mediators of disease pathogenesis including mutations in PAX5, TTF, PIM-1, MYC and somatic hypermutations in the immunoglobulin variable heavy chain genes, VH genes (Deckert et al., 2011). Deregulation by somatic mutations in genes involved in essential pathways such as B-cell receptor (CD79A), toll-like receptor (MYD88) and NF-kappaB (CARD11) are part of the driving mechanism in PCNSL tumorigenesis (Bruno et al., 2014; Gonzalez-Aguilar et al., 2012; Montesinos-Rongen et al., 2010; Montesinos-Rongen, Schafer, Siebert, & Deckert, 2012). Tumour suppressor gene PRDM1 is a regulator of B-cell differentiation (Courts et al., 2008), PTPRK participates in cell adhesion signalling events (Nakamura et al., 2003) and A20 (TNFAIP3) is a regulator of NFkappaB signalling (Braggio et al., 2011). CD79B is part of the B-cell receptor signalling pathway and is mutated in 20% of cases, which verifies dysregulation of the B-cell receptor and NFkappaB to pathogenesis of PCNSL (Montesinos-Rongen, Schafer, Siebert, & Deckert, 2012).

Despite the similar histology, gene-expression profile studies detected some genomic differences between DLBCL and PCNSL such as SSP1 and MAG (Lim et al., 2015). The variations of the SSP1 gene were associated with aggressive
Clinical behaviour, as the SSP1 gene expression in PCNSL increased CNS tropism, B-cell migration and tumour cell proliferation. Activation of MAG has been suggested to be an adhesion molecule with a pivotal role in perineural cancer invasion. However, it is sometimes difficult to differentiate between the impact of mutation and microenvironment on the PCNSL phenotype.

CNS tropism of lymphoma cells has been demonstrated in PCNSL. Expression of chemokines CXCL-12 and CXCL-13 has been detected to transact as neurotropic factors (Fischer et al., 2009; Smith et al., 2003; Smith et al., 2007). CXCL-13 also exists as a pro-survival factor in PCNLS. The high CXCL-13 concentration in tumour-associated CSF correlates with an adverse prognosis.

2.5.3 Clinical presentation

The median age of PCNSL patients at diagnosis is 56 years with a male-to-female ratio of 1.2:1 -1.7:1. Most immunocompetent patients present in the fifth to seventh decades of life, with immunocompromised patients presenting much earlier, in the third to fourth decades (Phan, O'Neill, & Kurtin, 2000; Remick et al., 1990; Ruiz, Post, Bundschu, Ganz, & Georgiou, 1997).

Clinical presentation usually reflects the neuroanatomical location of the lesion/lesions. The most typical symptoms are cognitive decline, personality changes and neurological deficits and 10% to 20% with seizures (Bataille et al., 2000; Josephson et al., 2007). Approximately 30% of patients have non-specific binocular visual symptoms such as blurred vision, decreased acuity, floaters, eye pain, and photophobia. Some patients suffer chronic visual symptoms even some years before the diagnosis (Josephson et al., 2007) and in other patients neurological decline is very rapid, reflecting the aggressiveness of the disease (Josephson et al., 2007).

2.5.4 Diagnosis

The diagnosis of PCNSL and/or IOL is challenging. Neurological decline can be variable and intermittent, and in some patients very rapid. The interval from the first onset of disease signs to diagnosis can vary between weeks to years. The cornerstone of diagnostic testing is contrast-enhanced MRI of the brain.
Neuroimaging

In 95% of PCNSL cases, there is pathological enhancement that homogeneously localizes to tumour masses. Radiographic evidence of necrosis is rare. In immunocompetent patients, the commonest sites of involvement are cerebral hemispheres and frontal lobes (20% to 43%). Deep grey matter nuclei (13% to 20%), corpus callosum (14%) and periventricular regions (12%) are less commonly involved (Yap et al., 2012). The occurrence of solitary and multiple lesions varies correspondingly between 40% to 80% and 20% to 40% (Coulon et al., 2002; Haldorsen, Espeland, & Larsson, 2011; Kuker et al., 2005; Yap et al., 2012). Over 95% of cases demonstrate at least one intra-axial lesion contacting a cerebrospinal fluid (CFS) surface (Tang, Booth, Bhogal, Malhotra, & Wilhelm, 2011).

In immunocompromised patients most tumours are located in supratentorial (75%) and basal ganglia (40-53%) areas (Haldorsen et al., 2011; Phan et al., 2000; Ruiz et al., 1997; Thurnher et al., 2001). Compared to immunocompetent patients, lesions are often multifocal, necrotic and exhibit heterogeneous enhancement (Haldorsen et al., 2011).

Brain biopsy

Diagnosis must be confirmed histologically by stereotactic brain biopsy. It is well-known that PCNSL is highly sensitive to corticosteroids and corticosteroids typically induce a rapid improvement in symptoms and radiographic response in at least 40% of patients (Porter et al., 2008; Smith et al., 2007). Corticosteroids restore the impaired blood-brain barrier, and have cytotoxic activity on lymphoma cells and destroy typical histological features of the disease. Corticosteroid-induced diagnostic delays may extend for several months and even for years (Pirotte et al., 1997). It is, therefore, important that glucocorticoids should not be administered until a diagnosis is confirmed and after that only with a minimal dose if there is symptomatic and/or life-threatening tumour-associated mass.

Cerebro-spinal fluid analysis

Flow-cytometric or cytological analysis of meningeal lymphoma cells isolated from CSF or vitrectomy may yield a diagnosis and in this case is sufficient to make the diagnosis. CSF is usually characterised by raised protein levels and pleocytosis. Lymphoma cells are detected in the CSF in 10% to 30% of cases (Balmaceda,
Gaynor, Sun, Gluck, & DeAngelis, 1995; Korfel et al., 2012). The low level of tumour cells in the CFS may hinder performing flow cytometric analyses.

Various molecular genetic markers and proteins have the potential to enhance diagnostic accuracy, such as soluble CD19, free immunoglobulin light chains, interleukin-10 and CXCL-13, but require further validation before being applied in clinical practice (Baraniskin et al., 2011; Muniz et al., 2014; Roy et al., 2008; J. L. Rubenstein, Wong et al., 2013; Schroers et al., 2010).

When the eyes are the only sites of lymphoma, ophthalmological involvement must be confirmed by vitreous biopsy.

### 2.5.5 Staging

Staging evaluation for the patient with possible PCNSL includes whole body CT and bone marrow biopsy to exclude extra-CNS disease as 4% to 12% of patients manifest extracranial disease (Ferreri, Reni, Zoldan, Terreni, & Villa, 1996). Ophthalmological examination with a slit lamp should be included in the primary diagnostic tools. Because of the high incidence of CNS manifestation in testicular lymphoma, scrotal ultrasound should be performed in elderly men (Abrey et al., 2005). FDG body PET-CT is more sensitive than contrast-enhanced CT, but is not used in routine diagnostic examination (Mohile, Deangelis, & Abrey, 2008). Serological testing for HIV, hepatitis C and B, in addition to measurement of serum LDH are standard baseline tests (Abrey et al., 2005).

The prognosis of PCNSL is inferior compared to other non-Hodgkin lymphomas. PCNSL is chemosensitive and radiosensitive, but remissions are commonly short-lasting (Citterio et al., 2017), mainly because of the blood-brain barrier that prevents/limits the access of many potential drugs to the CNS.

The International Extranodal Lymphoma Study Group (IELSG) identified five clinical variables that correlate with prognosis in PCNSL; elevated LDH, age over 60, Eastern Cooperative Group performance status greater than 1 and elevated CSF protein and tumour location within the deep regions of the brain. Two-year survival rates with risk factors 0-1, 2-3 and 4-5 were 80%, 48% and 15% respectively (Ferreri et al., 2003). Age has been considered a reliable prognostic factor, but among the existing risk scores, the cut-off value varies. In the IELSG study (Ferreri et al., 2003), the cut-off point is 60 years; this is 50 years in the MSK (Memorial Sloan-Kettering) score (Abrey et al., 2006). In the GALGB 50202 study in which patients were treated with intensive chemotherapy followed by high-dose consolidation without WBRT, outcomes in patients aged older 60 were similar to
those of younger patients. This finding suggests that the ideal cut-off point for age as a prognostic factor is linked to the delayed side-effects of the treatment (J. L. Rubenstein et al., 2013; Wieduwilt et al., 2012).

### 2.5.6 Prognosis

During the last two decades, treatment of PCNSL has changed with effective chemoimmunotherapy. The aim of research on PCNSL is to identify an effective and well-tolerated induction treatment followed by intensive consolidation treatment without whole brain radiotherapy.

### 2.5.7 Treatment

#### Surgery

Surgery has traditionally been considered to have no role in PCNSL, based upon evidence that surgical cytoreduction provides no survival benefit compared to biopsy, increases the risk of neurological deficit and causes possible delays in management of the PCNSL (Bellinzona et al., 2005). However, the recommendation to restrict surgical intervention is not based on randomised data and current modern neurosurgical techniques.

In a phase III trial published by the German Primary CNS Lymphoma Study Group-1, patients with subtotal or total resections had significantly longer progression-free survival and overall survival than patients who received biopsies. When adjusted for number of lesions, the difference in outcomes was restricted to progression free-survival only (Weller et al., 2012).

The only indication for surgical resection may be considered in patients with space-occupying lesions with acute steroid refractory intracranial pressure or brain herniation to reduce acute symptoms and enable chemotherapy to proceed.

#### Systemic chemotherapy

The CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) used for widespread non-Hodgkin lymphomas only induces a short-lasting response in PCNSL. The ineffectiveness is probably due to the blood-brain barrier limiting access of these drugs to the central nervous system. The addition of radiotherapy
to chemotherapy has not led to an improvement in patient survival in prospective trials (Mead et al., 2000; O’Neill et al., 1995; Schultz et al., 1996).

Various prospective and retrospective studies have suggested that high-dose methotrexate administered is regarded as the most effective and important single medicinal product (Deckert et al., 2011; Glass, Gruber, Cher, & Hochberg, 1994). Penetration of methotrexate into the CNS depends on total dose and rate of infusion. Optimal dose has not been established, but it has been estimated, that the IV MTX dose should be between 1g/m² to 8g/m² to cross the blood-brain barrier (Hiraga et al., 1999). Rapid infusion of methotrexate for 3h, at a dose of 3g/m² or more attains cytotoxic levels in the CSF and because the efficacy of methotrexate depends on duration of exposure, the optimal administration interval is between 10 days and 3 weeks (Glass, Gruber, Cher, & Hochberg, 1994).

The optimal number of methotrexate infusions to deliver is unknown. A minimum of 4 -6 infusions is delivered in most chemotherapy regimens without any consolidation treatment. Infusions of methotrexate require pre-and post-hydration, urine alkalinisation, leucovorin rescue and MTX concentration monitoring. Common factors limiting the use of high-dose methotrexate, are the patient’s age, comorbidities and renal failure.

To improve treatment outcome, most treatment protocols combine HD-MTX with a variety of other chemotherapeutic agents. The best evidence for this combination treatment method was achieved in a phase II randomised study performed by IELSG (International Extranodal Lymphoma Study Group) where HD-MTX 3g/m² administered every 21 days was compared to HD-MTX with cytarabine 2g/m² twice per day on days 2 -3. Both chemotherapy arms were followed by WBRT. Significantly higher CR rate, PFS and a trend towards better overall survival was observed in the HD-MTX-cytarabine arm (Ferreri et al., 2009).

The combination of methotrexate with an alkylating agents and rituximab has been tested in a few phase II single-arm studies. A combination of rituximab, methotrexate, procarbazine, and vincristine followed by low-dose whole-brain radiotherapy achieved an ORR of 79% and 2-year PFS of 57% among the 52 patients with newly diagnosed PCNSL (Morris et al., 2013). The same combination was used in a study by Omuro et al. followed by consolidative autologous stem cell transplantation. Outcomes were very promising, with an ORR of 94% and 2-year PFS of 79% although the patient population was small; only 33 patients were aged under 65 (A. Omuro et al., 2015).

A combination of methotrexate, temozolomide, and rituximab followed by non-myeloablative consolidation with cytarabine and etoposide was tested in 44
patients with an ORR of 77% and 2-year PFS of 59% (J. L. Rubenstein et al., 2013). Any conclusion about the effects of single agents cannot be drawn because of the small patient population, single-arm study and the fact that similar outcomes have been published with HD-MTX monotherapy.

During a recently published phase II multicentre study (IELSG#32 study), in the first randomisation, patients were randomised to three induction treatment arms; methotrexate 3.5g/m² on day 1 plus cytarabine 2g/m² twice-daily on day 2-3 (group 1) or the same combination plus 2 doses of rituximab on day -5 and 0 (group 2) or the combination of methotrexate, cytarabine and rituximab plus thiotepa 30mg/m² on day 4 (group 3) (Ferreri et al., 2016). At a median follow-up time of 30 months, complete remission rates in groups 1 to 3 were 23%, 30% and 49%, respectively. The combination of 4 drugs has been associated with significantly improved outcome, with a 5-year OS of 69%. A second randomisation in this promising study compared the effect of autologous stem cell transplantation to whole-brain radiotherapy among those patients who had a response or stable disease after induction.

Blood-brain barrier disruption by intra-arterial infusion of hypertonic mannitol has been shown to increase the drug concentrations in the CNS. BBBD with IA methotrexate administered in newly diagnosed PCNSL demonstrated a good safety profile and neurocognitive tolerance and achieved comparable outcomes to those detected with HD-intravenous MTX based chemotherapy regimens (Angelov et al., 2009; Doolittle et al., 2013; Neuwelt et al., 1991). This procedure requires careful patient selection and is limited to patients with no contraindication to general anaesthesia, no allergy to iodine-based contrast agents and no tumour mass effect obstructing the flow of spinal fluid. This approach enables the use of agents that do not penetrate the CNS across the intact BBB.

### 2.5.8 Rituximab

Rituximab is an anti-CD20 antibody that has dramatically improved the prognosis of B-cell malignancies (Cote et al., 2012; Gregory et al., 2013). Due to its large molecular size, its penetration into the CNS is poor (J. L. Rubenstein, Combs, Rosenberg, Levy, McDermott, Damon, Ignoffo, Aldape, Shen, Lee, Grillo-Lopez, & Shuman, 2003). The highest concentration and efficacy of rituximab in the CNS probably occurs during the early treatment phase when the integrity of the BBB is reduced at the location of the contrast-enhancing tumours.
The efficacy of rituximab was observed in a rituximab monotherapy study with 12 refractory or relapsed patients who received rituximab 375mg/m² intravenously weekly until 8 injections had been administered (T. T. Batchelor et al., 2011). ORR was 36% among these patients. This result provides evidence of rituximab monotherapy activity in PCNSL and promoted the use of rituximab as part of chemotherapy combinations (Birnbaum, Stadler, von Baumgarten, & Straube, 2012; Gregory et al., 2013; Holdhoff et al., 2014; Morris et al., 2013; J. L. Rubenstein et al., 2013).

The addition of rituximab to systemic chemotherapy has not notably increased treatment toxicity. Intrathecal rituximab administration has been evaluated in two phase I studies for refractory or relapsed PCNSL patients (J. L. Rubenstein et al., 2007; J. L. Rubenstein, Li et al., 2013). Favorable safety profile and objective responses were documented.

2.5.9 **Intrathecal chemotherapy**

Leptomeningeal disease is detectable in 15% of newly diagnosed PCNSL patients. However, this does not appear to confer any prognostic impact (Fischer et al., 2008). As HD-MTX achieves high concentrations in CSF, there is no clear role for intrathecal treatment. Although this approach has not been evaluated in a randomised trial, various studies have not found any difference in outcomes with the omission of intrathecal therapy and therefore, the use of IT therapy is no longer routine (Khan, Shi, Thaler, DeAngelis, & Abrey, 2002).

2.5.10 **Radiotherapy**

**Radiotherapy alone**

PCNSL is a radiosensitive disease with a high response rate of 50%. Median overall survival and 5-year overall survival are 10-18 months and 5% respectively. Considering that PCNSL is microscopically diffuse and multifocal, the target volume of radiotherapy involves the whole brain and eyes. The role of radiotherapy alone can be recommended for patients with a contraindication for systemic chemotherapy or relapsed or refractory disease when effective drugs have possibly been used without a response (Citterio, Ferreri, & Reni, 2013).
Radiotherapy as a consolidation treatment

Consolidation therapy after CR with HD-MTX-based chemotherapy is the most debated issue for RT in PCNSL. To compare to systemic NHL, the optimal dose and fractionation of post-chemotherapy radiotherapy has never been tested in a prospective setting. Doses of 23 -50 Gy and fractionation of 1.8 -2.0 Gy have generally been used.

One randomised trial by Thiel et al. clarifying radiotherapy versus a watch and wait approach after chemotherapy for PCNSL has been published to date (Thiel et al., 2010). In this trial, patients (n = 551) received HD-MTX 4g/m² IV 6 cycles with/without ifosfamide. Patients who achieved CR were randomised to observation or the consolidation WBRT arm (45Gy/30fr). OS was similar in both arms, but the WBRT arm was associated with a non-significant trend toward better PFS. This trial was criticised in the scientific community (DeAngelis, 2014; Ferreri et al., 2011; Weller, 2014; Zacher, Kasenda, Engert, & Skoetz, 2014). Some experts believe that the unmet primary endpoint for non-inferiority and the high rates of protocol violations prevent drawing any conclusions from this trial. Other experts advocate that consolidation WBRT after HD-MTX needs to remain as a standard of care.

The main reason to avoid radiotherapy is delayed treatment-related neurotoxicity that counteracts the beneficial effect of treatment on disease control. The combination of HD MTX and WBRT is associated with long-term progressive neurological side-effects with a cumulative 5-year incidence of 25% to 35% and mortality of 30% (Abrey, DeAngelis, & Yahalom, 1998; J. Y. Blay et al., 1998). Complications from treatment typically occur within a few years and causes death in less than 1-2 years. During neuropsychological examinations, psychomotor speed, executive function, attention and memory can be detected to be impaired (Correa et al., 2007; A. M. Omuro et al., 2005).

Cortical or subcortical atrophy and leukoencephalopathy are observed in radiographic examinations (Correa et al., 2007; A. M. Omuro et al., 2005). The risk for development of neurotoxicity is clearly higher in patients aged over 60 (A. M. Omuro et al., 2005). Neurotoxicity is associated with WBRT in many studies and by reducing radiation doses the incidence of treatment-related neurotoxicity has been observed to decrease (Morris et al., 2013). However, given that PCNSL is a highly-infiltrative brain tumour associated with varying neurological symptoms, the determination of whether impairments of neurological function are caused by lymphoma or are the consequence of delayed neurotoxicity of radiotherapy or
chemotherapies remains a major challenge. These circumstances emphasise the importance of neuropsychological testing in clinical trials on PCNSL and routine clinical practice. For all these above-mentioned reasons, it is necessary to avoid RT in front-line treatment of PCNSL. Currently, there is significant interest in determining the role of high-dose chemotherapeutic consolidation including autologous stem cell rescue in the treatment of PCNSL and avoiding the use of WBRT.

2.5.11 High-dose chemotherapy, myeloablative treatment and autologous transplant

HDC/ASCT is the standard treatment for chemosensitive relapsing DLBCL. For patients with relapsed or refractory PCNSL CR was 60% and median PFS and OS were 41 and 58 months, respectively, with a TBC (thiotepa, busulfan, cyclophosphamide) -containing regimen (Soussain et al., 2008). Data on alternative high-dose chemotherapy regimens for relapsed or refractory disease is rare and any conclusions regarding the optimal regimen are impossible to draw in this setting.

The specific role of HDC-ASCT as a consolidation treatment without first-line WBRT is a very interesting issue. The first study that used a BEAM regimen as a conditioning treatment reported 9.3 months of PFS (Abrey et al., 2003). Encouraging studies for which WBRT had been omitted in patients with CR after induction treatment were published subsequently (Alimohamed, Daly, Owen, Duggan, & Stewart, 2012; Cote et al., 2012; Illerhaus et al., 2009; Schorb et al., 2013). In these studies patients received HD thiotepa-based conditioning regimens or a combination with busulfan, cyclophosphamide and etoposide. Although there are no comparable studies between conditioning regimens, HD thiotepa regimens have been suggested to be as effective, but less toxic than a busulfan-based regimen (Ferreri et al., 2016; Illerhaus et al., 2016).

The superiority of HDC/ASCT treatment compared to conventional combined chemoradiotherapy as first-line treatment is under investigation in two ongoing trials (NCT01011920 and NCT00863460). Even more interesting are the IELSG-32 and the PRECIS trials, which are evaluating HDC/ASCT versus WBRT as a consolidation treatment after first-line HD-MTX-based induction chemotherapy.

There are two ongoing trials comparing the outcomes with myeloablative consolidation treatment to non-myeloablative treatment. In the first study (#NCT01511562) patients were randomised to the BCNU-thiotepa arm followed by ASCT or to the non-myeloablative HD-treatment arm with etoposide plus
cytarabine. In the second study (#02531841), after MATRix -induction patients were randomised to the BCNU-thiotepa -conditioning regimen arm followed by ASCT or the ifosfamide-based arm.

2.5.12 Maintenance treatment

Maintenance therapy is a way to achieve maximum disease control and long-term remission. This issue has been evaluated in two studies by Glass et al. and Pulczynski et al. In the first study, patients were treated with a combination of methotrexate, temozolomide (TMZ) and rituximab followed by hyperfractionated WBRT and subsequent TMZ (Glass et al., 2016). In this phase II study, 53 patients were treated and the 2-year OS rate was 80.8%.

In the second study, the treatment regimen included age-adjusted methotrexate-cytarabine treatment in which elderly patients who achieved a response received temozolomide maintenance treatment d 1 -5 at an interval of 28 days for one year or until progression/relapse (Pulczynski et al., 2015). The difference observed in results between younger and older (> 60 years) patients was interesting, as ORR among older patients was 80.8% compared to 69.9% for younger patients.

2.5.13 PCNSL and elderly patients

The incidence of PCNSL is increasing among elderly patients. This poses challenge for the treatment of PCNSL. In the literature, the definition of an elderly population is age over 60 years in most studies.

Three prospective studies (Fritsch et al., 2011; Laack, Ballman, Brown, O’Neill, & North Central Cancer Treatment Group, 2006; Illerhaus et al., 2009) have been published on treatment of elderly patients with PCNSL. Subgroup analyses on elderly patients have been reported in seven prospective trials and retrospective trials. A conclusion that can be drawn from these studies is that HD-MTX therapy is feasible and effective in elderly patients with adequate performance status and renal function and outcomes are better compared to WBRT (Bessell, Dickinson, Dickinson, & Salmon, 2011).

The risk of delayed leukoencephalopathy is unacceptably high in patients older than 60 years who have received chemoradiotherapy. HD-MTX-based chemotherapy without radiotherapy showed only minor or no cognitive treatment-related side-effects (Correa et al., 2012). When considering all these facts, the treatment of choice in elderly patients is HD-MTX-based chemotherapy without
WBRT. In patients aged older 80 with poor performance status and comorbidities, prognosis is poor (Welch, Omuro, & Deangelis, 2012). The best supportive care with dexamethasone and/or palliative radiotherapy is commonly the best choice of treatment.

2.5.14 Treatment of intraocular lymphoma

Intraocular lymphoma can be the first manifestation of PCNSL and can precede brain involvement. As many as 90% of IOL patients develop brain involvement over the follow-up period (Chan et al., 2011). Approximately 20% of new patients have concomitant intraocular and intracranial manifestations (J. L. Rubenstein et al., 2013).

Optimal treatment for IOL is not known as data on therapy and results are scarce and limited to small retrospective and heterogeneous patient series. Treatment can be focal including radiotherapy (Berenbom, Davila, Lin, & Harbour, 2007) or intravitreous methotrexate and/or rituximab (Frenkel, Hendler, Siegal, Shalom, & Pe'er, 2008; Hashida, Ohguro, & Nishida, 2012) or systemic chemotherapy (T. Batchelor, Carson, O'Neill, Grossman, Alavi, New, Hochberg, & Priet, 2003; Jahnke et al., 2009; Soussain et al., 2001). Intravitreal methotrexate is effective with an ORR of 100% in treated eyes, but does not affect OS. Side-effects of intravitreal methotrexate were observed in 73% of patients. Intravitreal rituximab is effective and has not been associated with such high side-effect rates, but data is still quite limited.

Studies with IOL have failed to confirm reliable predictors of brain dissemination. Some experts advocate local therapies for IOL and other experts recommend that the treatment should not differ from the PCNSL treatment regimen. The treatment decision should consider the individual risk for side-effects and local expertise (Chan et al., 2011). Unfortunately, the tools treatment regimen for individual patients remains ineffective.

2.5.15 Salvage treatment

Prognosis of relapsed or refractory CNS lymphoma is poor. About half of patients with PCNSL will relapse and one-third of patients do not respond to treatment. Salvage treatment depends on age, comorbidities, renal function, performance status, site of relapse, front-line treatment and duration of response.
In two retrospective studies with WBRT, the ORR was 75% and median survival was 11-16 months (Hottinger, DeAngelis, Yahalom, & Abrey, 2007; Nguyen et al., 2005). However, the WBRT does not improve survival compared to non-WBRT-based therapies (Thiel et al., 2010). HDC/ASCT could be an efficient option, but it is not suitable for older or frail patients. It is clear that tumour is assumed to be sensitive to second-line treatment (Soussain et al., 2008; Soussain et al., 2012). If the patient is not suitable for WBRT or HDC/ASCT, conventional and experimental treatment can be proposed. MTX rechallenge administered as a single agent or in combination may yield a good response in MTX-sensitive diseases (Pentsova, Deangelis, & Omuro, 2014).

Temozolomide (Nayak et al., 2013), topotecan (Fischer et al., 2006), IA carboplatin (Tyson et al., 2003), pemetrexed (Raizer et al., 2012), bendamustine (Chamberlain, 2014), PCV regimen (Vincristine, CCNU, Procarbazine) (Herrlinger et al., 2000), ifosfamide-etoposide (Mappa et al., 2013), cisplatin-cytarabine (del Rio et al., 2011) and temsirolimus (Korfel et al., 2016) have been demonstrated to achieve a response in relapsed or refractory PCNSL. The most promising results were obtained in two single-arm studies with ibrutinib and lenalidomide (Chamoun et al., 2017; Houillier et al., 2015). ORR values were 67% for lenalidomide and 75% for ibrutinib.
3 Aims of the study

1. To evaluate the long-term efficacy and outcomes of Bonn type treatment in PCNSL in real life.
2. To analyse the efficacy and side effects of BBBD treatment in first and second line treatment in PCNSL patients.
3. To evaluate the impact of different chemotherapy regimens in the efficacy on BBBD-therapy.
4. To evaluate the efficacy of high-dose i.v. mtx in preventing secondary CNS lymphoma.
5. To evaluate biological and histological features that predict CNS relapse.
4 Materials and methods

All these studies I-III were conducted in the Department of Oncology and Radiotherapy in Oulu University Hospital.

4.1 Patients (I-III)

Studies I-III were conducted according to the Declaration of Helsinki and approved by the Ethic Committee of Oulu University Hospital. Written informed consent was obtained from all participants. The number of patients recruited was 182.

4.1.1 Patients with primary central nervous system lymphoma treated with systemic chemotherapy (Study I)

A total of 54 newly-diagnosed primary central nervous system lymphoma patients (mean age 59.3±10.3 years) who were treated with Bonn or Bonn-like chemotherapy with curative intent during 2004-2007 at Oulu, Helsinki, Tampere or Kuopio University Hospital and Jyväskylä Central Hospital were selected to undertake this study. Gender distribution was 35 ♂ and 19 ♀. All patients were immunocompetent and fit for curative treatment attempt. Study data were collected retrospectively from clinical records.

4.1.2 Patients with primary central nervous system lymphoma treated with BBBD-treatment followed by HDT/ASCT as first- or second line (Study II)

A total of 25 primary central nervous system patients who received blood brain barrier disruption therapy as first (n = 9) or second line (n = 16) during 2007-2014 at Oulu University Hospital were retrospectively analysed in this study. Median age of patients was 57 years and gender distribution was 17 ♂ and 8 ♀. All patients were immunocompetent, fit to anaesthetise and as had adequate renal function. Study data were collected partly retrospectively and during follow-up time from clinical records.
4.1.3 Patients with aggressive lymphoma treated with CNS-targeted treatment, the impact of central nervous system prophylaxis on the incidence of CNS relapses (Study III)

A total of 103 high risk DLBCL or follicular lymphoma Grade 3b patients who received R-CHOP or its immunochemotherapy derivates with or without central nervous system -targeted treatment, were analysed retrospectively in this study. Patients were treated mainly in Oulu University Hospital except eight who were treated in Kuopio University Hospital during 2007-2012. Male/female ratio was 1.3 and median age was 62 yr±14. Study data were retrospectively collected from the clinical records.

4.2 Diagnosis and staging

4.2.1 Diagnosis

Diagnosis was based either on the histopathological evaluation of tumour biopsy (I-III) or positive cerebrospinal fluid (CSF) cytology (I-II) according to the World Health Organization (WHO) classification 2008.

4.2.2 Staging

Staging included whole-body computed tomography (CT), brain magnetic resonance imaging (MRI) and slit lamp examination. In study III, MRI was performed only if patients had neurological symptoms, and therefore, with none of those patients the slit lamp examination was conducted. CSF examination was mandatory for all patients in study I and II and performed for all patients in study III if there was suspicion of central nervous system involvement. Blood marrow aspiration and biopsy were included in routine diagnostic methods in study III and was conducted for all patients. Routine blood chemistry tests (full blood count, liver and renal function, electrolytes) were conducted as well as serological tests for HIV, hepatitis B and C and measurement of serum LDH for all patients. Detailed information on diagnostic tools used in studies I, II and III are shown in Table 1.
Table 1. Diagnostic tools.

<table>
<thead>
<tr>
<th>Study</th>
<th>CT</th>
<th>MRI</th>
<th>Slit lamp examination</th>
<th>CSF examination</th>
<th>Blood marrow test</th>
<th>Blood chemistry tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>II</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>IIIa</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

*Diagnostic tools applied if suspicion of central nervous system involvement, CT = computed tomography, CSF = cerebrospinal fluid, MRI = magnetic resonance imaging

Stage was defined in accordance with the Ann Arbor system (Table 2), which is the staging system for lymphomas, both in Hodgkin’s and Non-Hodgkin lymphoma (Carbone, Kaplan, Musshoff, Smithers, & Tubiana, 1971). It has the same function as TNM staging in solid tumours. Stage is based on location of the malignant tissue and on the systemic symptoms due to the lymphoma. Stage I indicates a tumour is located in a single region, commonly one lymph node and the surrounding area, stage II means the tumour is located in two separate regions, an affected lymph node or lymphatic organ and those affected regions are on one side of the diaphragm, stage III implies that tumour tissue has spread to both sides of the diaphragm and stage IV means tumour tissue has disseminated or diffuse involvement in one or more extralymphatic organs such as liver, bone marrow or as nodular involvement in the lungs.

World Health Organization (WHO) performance status (PS) is a commonly used classification in medicine based on determining the patient’s well-being and activities of daily living (Table 2). It can help to define whether patients are fit for chemotherapies and whether dose adjustment is necessary. We determined WHO grade for all patients (study I-III). WHO grade 0 is fully active and able to continue all pre-disease performance, grade 1 means restricted in strenuous activity but able to carry out light home- and office work, grade 2 is capable of all selfcare but unable to work in more than 50% of waking hours, grade 3 means capable of only limited selfcare, more than 50% of waking hours in the bed or chair, grade 4: cannot continue any selfcare, totally confined to bed or chair, grade 5 is dead. In studies I and II patients were suspected to be sufficiently fit to undergo intensive treatment. However, WHO score 3-4 patients were not excluded from these studies.

International Prognostic Index (IPI) (Table 2) is the most commonly used prognostic model for evaluating outcome in aggressive lymphoma (International Non-Hodgkin's Lymphoma Prognostic Factors Project, 1993). Based on age,
lactate dehydrogenase (LDH), Eastern Oncology Group (ECOG) performance status (PS), stage and number of extranodal sites can be determined for risk groups with different prognostic values, low risk IPI 0-1, moderate low risk IPI 2, moderate high risk IPI 4-5 and high risk IPI 4-5.

Central nervous system IPI, CNS-IPI (Table 2) by Schmitz et al. is a new prognostic model where one or two extra points according to possible renal and/or adrenal gland involvement were added to the conventional IPI classification (Schmitz et al., 2016). This new model identifies three risk groups, low 0-1 factor, intermediate 2-3 factors and high-risk 4-6 factors. Two-year CNS relapse risks were 0.65%, 3.4% and 10.2%, respectively.

The international Extranodal Lymphoma Study Group (IELSG) (Table 2) has identified five clinical variables that correlate with prognosis in PCNSL (Ferreri et al., 2003). Factors included are elevated LDH, age over 60, Eastern Cooperative Group Performance Status greater than 1, elevated CSF protein, and tumour location within the deep regions of the brain. Two-year survival rates according to risk factors 0-1, 2-3 or 4-5 were 80%, 48% and 15% respectively.
### Table 2. Staging tool.

<table>
<thead>
<tr>
<th>Staging tool</th>
<th>Ann Arbor</th>
<th>WHO</th>
<th>IPI</th>
<th>CNS-IPI</th>
<th>IELSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determination</td>
<td>Classification based on location of malignant tissue and systemic symptoms due to the lymphoma</td>
<td>Classification based on determining of patient’s well-being and activities of daily living</td>
<td>A prognostic model for evaluating outcome in aggressive lymphoma</td>
<td>A prognostic model for evaluating outcome in aggressive lymphoma and the risk for CNS events</td>
<td>A prognostic model for evaluating outcome in PCNSL</td>
</tr>
<tr>
<td>Classification</td>
<td>I-IV</td>
<td>0-4</td>
<td>0-5</td>
<td>0-6</td>
<td>1-5</td>
</tr>
<tr>
<td></td>
<td>I = tumour located in a single region, commonly one lymph node and the surrounding area</td>
<td>0 = fully active and able to carry on all pre-disease performance</td>
<td>0-1 = low risk</td>
<td>0-1 = low</td>
<td>0-1 = 2-year survival rate 80%</td>
</tr>
<tr>
<td></td>
<td>II = tumour located in two separate regions, an affected lymph node or lymphatic organ and those affected regions are on one side of the diaphragm</td>
<td>1 = restricted in strenuous activity, but able to carry out light home- and office work</td>
<td>2 = moderate low risk</td>
<td>2-3 = intermediate</td>
<td>2-3 = 2-year survival rate 48%</td>
</tr>
<tr>
<td></td>
<td>III = tumour tissue has spread to both sides of the diaphragm</td>
<td>2 = capable of all selfcare, but unable to work in more than 50% of waking hours</td>
<td>3 = moderate high risk</td>
<td>4-6 = high-risk</td>
<td>4-5 = 2-year survival rate 15%</td>
</tr>
<tr>
<td>Staging tool</td>
<td>Ann Arbor</td>
<td>WHO</td>
<td>IPI</td>
<td>CNS-IPI</td>
<td>IELSG</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>IV = tumour tissue has disseminated or diffuse involvement in one or more extralymphatic organs</td>
<td>3 = capable of only limited selfcare, more than 50% of waking hours in the bed or chair</td>
<td>4-5 = high risk</td>
<td>4 = cannot continue selfcare, totally confined to bed or chair</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CNS-IPI = Central nervous system-IPI, IELSG = International Extranodal Lymphoma Study Group, IPI = Internation Prognostic Index, WHO = World Health Organization
4.3 Treatments

The Bonn regimen is multiagent chemotherapy where high dose cytarabine had been added to high dose methotrexate based chemotherapy. In the original regimen IT methotrexate (MTX) administered d 2-4 in cycles A1, B1, A2 and B2. In cycles C1 and C2 MTX IT was conducted d 3-6. IT cytarabine was administered on d 5 in cycles A1, 2, and B1, 2 and in the cycles C1 and 2 on day 7. In a modified Bonn regimen, so-called Nordic regimen MTX infusion time was reduced to three hours instead of 24 hours and one rituximab infusion was added to the first treatment cycle. Conventional intrathecal treatment was also replaced with liposomal cytarabine injections in cycles 1, 2, 4 and 5 (Table 3).

In the blood brain barrier disruption treatment all patients received so called sytoreductive treatment with Bonn or Bonn-like treatment as a cytoreductive treatment. After three to four weeks the BBBD treatment was started. Patients received two BBBD treatments on two consecutive days every 3 to 4 weeks for 4–6 cycles or until disease progression. After a good partial or complete response to BBBD induction therapy, the stem cells were mobilised with a Bonn C regimen containing cytarabine or after switching to the five-drug combination after third, fourth, fifth or sixth treatment course. At the beginning of the treatment 4-drug treatment regimen that included rituximab, carboplatin, dexamethasone and cytarabine were used or alternatively the same regimen where methotrexate was replaced with cyclophosphamide and etoposide. After excellent tolerability of these chemotherapy regimes patients were treated with 5-drug regimen (rituximab, carboplatin, dexamethasone, cytarabine, cyclophosphamide and etoposide). The high dose protocol followed by autologous stem cell transplantation as a consolidation treatment included carmustine-thiotepa except for one patient who was treated with BEAM (Table 3).

In the treatment schedule prior to the first or second treatment course, a port-a-cath was inserted and patients were hydrated at 100–150 mL/h for a minimum of 6 h. Because of the risk of seizures patients were premedicated with an anticonvulsant. Atropine was administered intravenously immediately prior to BBBD to prevent bradycardia. BBB opening was conducted under general anaesthesia. Internal carotid artery (ICA) or vertebral artery (VA) was selectively catheterised via transfemoral access. In ICA, the catheter tip was placed at C1–C2 level and in the vertebral artery at C6 level. Warmed mannitol (25 %) was administered at 4–6 mL/s into the target. A non-ionic contrast agent was
administered intravenously after mannitol, and a CT brain scan was obtained. Documentation of degree of disruption (moderate, good, or excellent) was evaluated by visual grading of the CT scan. Contrast enhancement in the disrupted territories was compared with that in the nondisrupted territories. Following BBBD, patients remained in the post-anaesthesia care unit with frequent monitoring of vital signs, neurologic status, and fluid balance. Fluid balance was meticulously maintained with mannitol (15%) and fluid boluses. In patients treated with methotrexate, NaHCO3 was added to intravenous fluids and titrated to achieve urine pH greater than 7.5. In our study, the treatment cycle varied from 3 to 4 weeks depending on treatment tolerability. After complete response, patients received two additional treatments. The maximum number of treatments was determined to be six (Table 3).

R-CHOP or its derivates R-CEOP (rituximab, cyclofosfamide, epirubisin, vincristine, prednisone) and R-CHOEP (rituximab, cyclofosfamide, doxorubicin, etoposide, prednisone) were used as standard treatment in study III. CNS-targeted therapy for high risk patients was conducted with high dose methotrexate (HD-MTX) ± methotrexate IT (MTX-IT) simultaneously with R-CHOP or its derivates. The dose of MTX IT was 12–12.5 mg and high-dose methotrexate (HD-MTX) 1–5 g/m². CNS prophylaxis was administered on day 1 after R-CHOP or infusion of its derivates. The number of MTX cycles varied in accordance with tolerability from 1 to 3 cycles, and were typically administered with systemic immunochemotherapy cycles 1–3 or 2–4. Based on updated CNS-targeted treatment patients were split into three groups. In group 1 the HD-MTX iv dose was 3-5g/m² x 2-3 plus MTX IT 12.5mg x 2-3 and the minimum cumulative dose of HD-MTX was 9g/m². In group 2 the dose of HD-MTX was 1-3g/m² x 1-3 + MTX IT 12.5mg x 1-3 and the cumulative dose of MTX was < 9g/m². Group 3 did not receive CNS-targeted treatment (Table 3).
<table>
<thead>
<tr>
<th>Treatment regimen</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bonn</strong></td>
<td></td>
</tr>
<tr>
<td>A1, A2</td>
<td>Methotrexate 5000 mg/m² I.V., D1; Vincristine 2 mg I.V., D1; Iphosamide 800 mg/m² I.V., D2-5; Mesna 160 mg/m² x 3 I.V., D2-5; Dexamethasone 10 mg/m² P.O., D2-5; Prednisone 10 mg I.T., D1-4; Methotrexate 3 mg I.T., D1-4; Cytarabine 30 mg I.T., D5</td>
</tr>
<tr>
<td>B1, B2</td>
<td>Methotrexate 5000 mg/m² I.V., D1; Vincristine 2 mg I.V., D1; Cyclophosphamide 200 mg/m² I.V., D2-5; Dexamethasone 10 mg/m² P.O., D2-5; Prednisone 10 mg I.T., D1-4; Methotrexate 3 mg I.T., D1-4; Cytarabine 30 mg I.T., D5</td>
</tr>
<tr>
<td>C1, C2</td>
<td>Cytarabine 3000 mg/m² I.V., D1-2; Vindesine 5 mg I.V., D1; Dexamethasone 20 mg/m² P.O., D3-7; Prednisone 10 mg I.T., D3-6; Methotrexate 3 mg I.T., D3-6; Cytarabine 30 mg I.T., D7</td>
</tr>
<tr>
<td><strong>Nordic Bonn</strong>¹</td>
<td></td>
</tr>
<tr>
<td>A1, A2</td>
<td>Methotrexate 5000 mg/m² I.V., D1; Vincristine 2 mg I.V., D1; Iphosamide 800 mg/m² I.V., D2-5; Mesna 160 mg/m² x 3 I.V., D2-5; Dexamethasone 10 mg/m² P.O., D2-5; Prednisone 10 mg I.T., D1-4; Methotrexate 3 mg I.T., D1-4; Cytarabine 30 mg I.T., D5</td>
</tr>
<tr>
<td>B1, B2</td>
<td>Methotrexate 5000 mg/m² I.V., D1; Vincristine 2 mg I.V., D1; Cyclophosphamide 200 mg/m² I.V., D2-5; Dexamethasone 10 mg/m² P.O., D2-5; Prednisone 10 mg I.T., D1-4; Methotrexate 3 mg I.T., D1-4; Cytarabine 30 mg I.T., D5</td>
</tr>
<tr>
<td>C1, C2</td>
<td>Cytarabine 3000 mg/m² I.V., D1-2; Vindesine 5 mg I.V., D1; Dexamethasone 20 mg/m² P.O., D3-7; Prednisone 10 mg I.T., D3-6; Methotrexate 3 mg I.T., D3-6; Cytarabine 30 mg I.T., D7</td>
</tr>
<tr>
<td><strong>BBBD</strong></td>
<td></td>
</tr>
<tr>
<td>Four-drug²</td>
<td>Rituiximab 375 mg/m² I.V., D0; Methotrexate 2500 mg/m² I.A., D1-2; Carboplatin 200 mg/m² I.A., D1-2; Dexamethasone 6 mg x 4-6 P.O., D2-10; Cytarabine 40 mg I.T., D14</td>
</tr>
<tr>
<td>Five-drug</td>
<td>Rituiximab 375 mg/m² I.V., D0; Methotrexate 2500 mg/m² I.A., D1-2; Carboplatin 200 mg/m² I.A., D1-2; Dexamethasone 6 mg x 4-6 P.O., D2-10; Cytarabine 40 mg I.T., D14; Cyclophosphamide 330 mg/m² I.V., D1-2; Etoposide 200 mg/m² I.V., D1-2</td>
</tr>
<tr>
<td>Treatment regimen</td>
<td>Schedule</td>
</tr>
<tr>
<td>-------------------</td>
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</tr>
<tr>
<td>Carmustine-thiotepa</td>
<td>Carmustine 400 mg/m² I.V., D-6; Thiotepa 5 mg/kg x 2 I.V., D-5, 4</td>
</tr>
<tr>
<td>BEAM</td>
<td>Carmustine 300 mg/m² I.V., D-7; Etoposide 100 mg/m² x 2 I.V., D-6,-5,-4,-3; Cytarabine 200 mg/m² x 2 I.V., D-6,-5,-4,-3; Melphalan 140 mg/m² I.V., D-2</td>
</tr>
<tr>
<td>R-CHOP</td>
<td>Rituximab 375 mg/m² I.V., D1; Cyclophosphamide 750 mg/m² I.V., D1; Doxorubicin 50 mg/m² I.V., D1; Vincristine 1.4 mg/m² max 2 mg, D1; Prednisolone 100 mg P.O., D1-5</td>
</tr>
<tr>
<td>R-CEOP</td>
<td>Rituximab 375 mg/m² I.V., D1; Cyclophosphamide 750 mg/m² I.V., D1; Epirubicin 60 mg/m² I.V., D1; Vincristine 1.4 mg/m² max 2 mg, D1; Prednisone 100 mg P.O., D1-5</td>
</tr>
<tr>
<td>R-CHOEP</td>
<td>Rituximab 375 mg/m² I.V., D1; Cyclophosphamide 750 mg/m² I.V., D1; Doxorubicin 50 mg/m², I.V., D1; Vincristine 1.4 mg/m² max 2 mg, D1; Etoposide 100 mg/m² I.V., D1-3; Prednisone 100 mg P.O., D1-5</td>
</tr>
<tr>
<td>CNS Prophylaxis</td>
<td>HDMTX 1-5 g/m² I.V., D1; MTXIT 12.5 mg x 1-3 I.T., D1</td>
</tr>
</tbody>
</table>

1MTX infusion time reduced to 3-hour instead of 24 h and one Rituximab infusion added to the first treatment cycle and conventional intrathecal treatment replaced with liposomal Cytarabine injections in cycles 1, 2, 4 and 5.

2If Methotrexate contraindicated Etoposide 200 mg/m² I.V., D1-2 and Cyclophosphamide 330 mg/m² I.V., D1.2 were used.

3Added to R-CHOP, R-CEOP or R-CHOEP treatments starting from cycle 1 or 2.

I.A. = intra arterial, I.T. = intra thecal, I.V. = intravenous, BBBD = Blood brain barrier disruption, P.O. = oral administration
4.4 Response evaluation and follow up

The response in PCNSL was evaluated with MRI scans in accordance with the guidelines published by the international PCNSL collaborative group (Abrey et al., 2005) in BBBD study. Neuroimaging studies were obtained before each treatment course and thereafter every 3 months for 1 year, then every 6 months for 1 year and then annually. In the DLBCL patient, treatment response was evaluated in accordance with the International Working Group response criteria (Cheson et al., 1999) and after 2007 according to the revised International Working Group response criteria (Cheson et al., 2007). Response to therapy was evaluated after four, six and eight courses and thereafter every three months for two years and then every six months up until five years from treatment. Whole-body imaging was performed twice a year for two years and thereafter once a year until five years from diagnosis.

4.5 Statistics

Categorical variable tests were performed using two-sided Pearson chi-squared test or Fisher’s two-sided exact test, when possible. Continuous variables were analysed using the Mann-Whitney U-test or Kruskal-Wallis test. Survival analyses with corresponding p-values were calculated using the Kaplan-Meier method with log-rank test. Event-free and survival was calculated from the date of diagnosis to either disease progression, failure to achieve complete response, or change of therapy modality due to toxicity or poor response. Progression-free survival was calculated from the date of the first treatment to date of disease progression or from the date of pathological diagnosis to date of disease progression, or death or the last day of follow up. Overall survival was calculated from the date of pathological diagnosis to the date of death for any reason or the last date of follow up. CNS relapse-free survival was the time between the diagnosis date and CNS relapse. CNS survival was calculated from the date of CNS relapse to death due to disease progression. Patients dying because of systemic relapses or escaping from follow-up free of disease were censored on the date of death or the last day of follow-up. P-values < 0.05 were considered statistically significant.
5 Results

Primary CNS lymphomas diagnosed and treated in first and second line from 1995 to 2014 were analysed according to updated treatments (unpublished dat). Number of patients alive without disease relapsed after two-year follow-up are shown in Table 4.

Table 4. Updated PCNSL treatments 1995-2014.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>OUH</td>
<td>OUH</td>
<td>Other hospital district</td>
</tr>
<tr>
<td>Alive without relapse after 2 years</td>
<td>N years</td>
<td>N years</td>
<td>N years</td>
</tr>
<tr>
<td>I line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSC/DxM</td>
<td>10 0</td>
<td>3 0</td>
<td>0 0</td>
</tr>
<tr>
<td>RT</td>
<td>2 0</td>
<td>2 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Bonn</td>
<td>4 0</td>
<td>12 5</td>
<td>0 0</td>
</tr>
<tr>
<td>Bonn followed by ASCT</td>
<td>0 0</td>
<td>4 4</td>
<td>0 0</td>
</tr>
<tr>
<td>BBBD followed by ASCT</td>
<td>0 0</td>
<td>6 5</td>
<td>3 3</td>
</tr>
<tr>
<td>Other</td>
<td>4 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Total</td>
<td>20 0</td>
<td>27 14</td>
<td>3 3</td>
</tr>
<tr>
<td>II line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT</td>
<td>0 0</td>
<td>1 0</td>
<td>0 0</td>
</tr>
<tr>
<td>BBBD followed by ASCT</td>
<td>0 0</td>
<td>8 3</td>
<td>8 4</td>
</tr>
<tr>
<td>Total</td>
<td>0 0</td>
<td>9 3</td>
<td>8 4</td>
</tr>
</tbody>
</table>

ASCT = Autologous stem cell transplantation, BBBD = Blood brain barrier disruption, BSC/DxM = Best supportive care/Dexamethasone, OUH = Oulu University Hospital, RT = Radiotherapy

5.1 Constant pattern of relapse in primary central nervous system lymphoma (Study I)

In the study, the long-term treatment outcomes of the original or a modified Bonn regimen were evaluated retrospectively. Forty-one (76%) patients achieved CR and 12 (22%) of patients PR, with an overall response rate of 98%. After a median follow-up period of 54 months, median EFS for the whole-study population was 20 months (Fig. 1a). Two-year and five-year OS were 76% and 38%, respectively (Fig1b). There were no significant EFS or OS
rate differences according to therapy with the original or modified Bonn regimens. (Figure a. EFS, b. OS)

Fig. 1. a. Event-free survival (EFS) and b. Overall survival (OS).

5.2 Promising treatment result with blood brain barrier disruption based immunochemotherapy (Study II)

In Study II treatment results and toxicity with BBBD treatment were analysed. A total of 19 (76%) of 25 patients achieved CR. Six patients (24%) progressed before the treatment was completed. One patient (4%) died due to neutropenic sepsis. OS was 42 months for the whole-study after a median follow-up time of 15 months. Two-and five-year OS rates were 57% and 47%. In the patients treated with the five-drug regimen (n = 16), the CR rate was found to be 100% in first line (n = 6) and 60% in relapsed settings (n = 10) (Fig. 2b). Two-year OS in first-line and relapsed settings were 100% and 55%, respectively (Fig. 2a). All patients treated with BBBD in first line were in CR at the time of the last evaluation after a mean follow-up time of 25 months.
In unpublished updated data with 37 patients (20 patients in first-line and 17 patients in relapse) treated with 5-drug regimen. PFS was 93% and 43% in first-line and relapsed setting patients, respectively (Fig. 3).
This study evaluated periprocedural, chemotherapy-related toxicity and malignant condition-related complications. The most common periprocedural complication was the onset of focal seizures which occurred approximately in one-third of patients. Most of these occurred during blood–brain barrier disruption and methotrexate infusion. Toxicities were evaluated separately in the two groups of patients based on four-or five-drug treatment. In total, there were 43 and 74 cycles in the four-drug and five-drug treatments, respectively. Grade 3–4 thrombocytopenia was observed in 21 (28%) of the four-drug treatment cycles and in 21 (32%) of the five-drug treatment cycles. Grade 3–4 neutropenia was observed in ten (23%) and 21 (31%) of treatment cycles, respectively. One patient in complete response died due to neutropenic sepsis after the fourth BBBD treatment. The most common non-haematological complications were GI mucositis (14%) and superficial venous thrombophlebitis (10%). One patient with progressive disease had an ischaemic stroke with permanent neurological deficit.

5.3 Impact of central nervous system prophylaxis on the incidence of the CNS relapses (Study III)

In the Study III the impact of CNS-targeted treatment in preventing CNS relapses after primary treatment of aggressive lymphoma patients was evaluated. Median follow-up time was 51 months. 22 patients (21.4%) had a systemic relapse and this was in a median time of 9.5 months. Median survival after relapse was 11.5 months. Five-year OS and PFS in this high-risk patient population was 65% and 63%, respectively. Five-year overall survival based on cell of origin phenotype was 71% in the germinal centre B-cell type (GCB) group and 55% with activated B-cell type. Corresponding PFS was 67% and 51%.

CNS relapses were detected in thirteen patients (12.6%). A total of 11/13 (85%) of CNS relapses were isolated. Median time to isolated CNS relapse was 8 months (5-44 months) and median overall survival after isolated CNS relapse was 7 months. Five-year isolated CNS relapse rates in the treatment groups 1-3 were 5.2%, 10.0% and 23.5% (p = 0.063) (Fig. 4a). The Five-year isolated CNS relapse rates were 6.9% in the pooled prophylaxis group and 23.5% (p = 0.022) in the group without prophylaxis (Fig. 4b). Localisation of the relapses was analysed. A total of 6/13 of CNS relapse patients had meningeal disease. We focused on patients with CNS relapse and expanded data from pathological patient records. DE-HGBL and HGBL-DH were analysed in the CNS-relapse patients. One of them had HGBL-DH, but 9/13 of those patients had DE-HGBL. Ki-67 value over 80% was observed
in 9/13 CNS relapse patients (data was not available in four patients). The location of CNS relapses did not correlate to biological risk factors.

**Fig. 4.** a) Five-year isolated CNS relapse rates in treatment groups 1-3, b) Five-year isolated CNS relapses based on CNS-targeted treatment
6 Discussion

6.1 Primary Central Nervous System Lymphoma

6.1.1 PCNSL

PCNSL is a rare but very aggressive B-cell lymphoma compared to other lymphomas (Dolecek, Propp, Stroup, & Kruchko, 2012). Evolving diagnostic methods such as histological, immunohistological, molecular and gene expression profiling investigations have revealed its unique features compared to systemic disease. Despite new findings in the pathogenesis of PCNSL, several unexplained issues remain (Lim et al., 2015).

The incidence of PCNSL has been shown to increase among elderly patients (Villano et al., 2011). Prognosis of PCNSL has improved significantly over the past two decades because of more effective treatment.

To estimate the development of PCNSL treatments, we analysed PCNSL patients treated in our hospital in first or second line 1995-2014. We focused on examining patients in two-time periods; 1995-2006 and 2007-2014 after commencing BBBD treatment. Age over 75 years, comorbidities and contraindication to general anaesthesia were the most determining factors for patient selection to treatment groups. At the first period, the percentage of patients with continuous remission was notable low and only a few patients received curative treatment. Instead of a subsequent examination period, developed diagnostical methods, knowledge of the disease entity per se was observed. A total of 48% patients received effective chemotherapy with curative intention in the first-time period and the percentage was 88% in the later period. After establishing BBBD-therapy, patients were referred to OUH also from other hospital districts.

Conventional treatment of PCNSL

PCNSL is a chemo-and radiosensitive disease. However, the blood brain barrier limits penetration of many effective drugs into the central nervous system and therefore prevents achieving cytotoxic concentrations around the tumour involvement in the brain (Citterio et al., 2017). At the start of treatment, the blood brain barrier leaks, which enables many drugs to access the CNS, and consequently treatment responses are observed with several chemotherapeutics. After successful
treatment, macroscopic tumour involvement reduces and the tight junctions between endothelial cells close, which closes the BBB. Therefore, the microscopic disease behind the intact blood brain barrier is difficult to eradicate. Long-term remissions are rarely seen and relapses are mostly fatal.

HD-MTX is regarded as the most effective and important single drug in PCNSL (Deckert et al., 2011; Glass et al., 1994). The addition of high dose cytarabine to HD-MTX regimens has been shown to increase treatment response rate and the duration of response (Ferreri et al., 2009). In a Bonn and Bonn-like regimen both those drugs were included in treatment schema. In a Bonn regimen, patients were treated with four HD-MTX cycles (A1, A2, B1, B2) and two cycles of HD-Cytarabine (C1, C2) (Pels et al., 2003). In line with other earlier published studies (Haldorsen et al., 2007; Olson et al., 2002; Pels et al., 2003), it was shown in Study I that PCNSL is a chemosensitive disease but remissions are short-lived. Patients treated with Bonn-or Bonn-like regimen responded to treatment and overall response rate was as good as 98%. Unfortunately, after a median follow-up period of 54 months, median event-free survival (EFS) for the whole-study population was 20 months and only one patient stayed in primary remission for 60 months.

The duration of methotrexate infusion was different in Bonn and Bonn-like regimen, 24 hours versus 3 hours, which did not correlate with treatment response. Due to the retrospective nature of this study, toxicity data were not compared between treatment groups (Bonn versus modified Bonn).

Leptomeningeal disease is observed in approximately 15% of newly diagnosed PCNSL patients (Fischer et al., 2008). The role of intrathecal chemotherapy has not been confirmed. Albeit in the absence of randomised trials, many studies have not observed any difference in outcomes with the omission of intrathecal treatment (Khan et al., 2002). Both the Bonn and Bonn-like regimens contained intrathecal treatment and it is impossible to estimate the impact of omission of this.

The role of rituximab in the treatment of CNS lymphoma is quite complex. Because the blood-brain barrier excludes molecules over 400 Daltons, it is not surprising, that most studies have reported less than 1% of systemic rituximab access to the leptomeningeal space (J. L. Rubenstein, Combs, Rosenberg, Levy, McDermott, Damon, Ignoffo, Aldape, Shen, Lee, Grillo-Lopez, & Shuman, 2003). It has been suggested that the highest concentration and efficacy of rituximab is observed during the early treatment phase when the integrity of blood brain barrier is diminished (Jin et al., 2010). Intravenous rituximab has been used as a single treatment or combined with methotrexate based chemotherapy as an initial
treatment or in relapsed settings. This was based on retrospective comparisons with historical controls, the outcomes of three studies observed, the fact that addition of rituximab to HD-MTX-based chemotherapy increases treatment response and overall survival in newly diagnosed PCNSL (Birnbaum et al., 2012; Gregory et al., 2013; Holdhoff et al., 2014). In Study I, 23 patients received only one course of rituximab and 15 patients were treated with four courses of rituximab. There was no difference in survival between those groups. Most patients received only one course of rituximab, which may be too few.

In the original publication introducing the Bonn treatment there was a dramatic difference in time to treatment failure and overall survival according to patient age over or under 61 years. In our retrospective analyses, this could not be repeated. This finding may be linked to patient selection bias so that frailer elderly patients were excluded from treatment.

The International Extranodal Lymphoma Study Group (IELSG) has identified five clinical variables (elevated LDH, age over 60, EOG performance status > 1, elevated CSF protein and tumour location in deep areas of the brain) that correlate with prognosis in PCNSL. Prognosis declines as risk factors increase (Ferreri et al., 2003). In these analyses, there was no significant difference in survival according to IELSG.

**BBBD in PCNSL therapy**

The BBBD treatment schedule was developed by Neuwelt et al nearly three decades ago. The USA BBBD consortium has published treatments results considering patients treated with a three-drug regimen (1982-1993 methotrexate, cyclophosphamide/etoposide, procarbazine and 1994-2005 etoposide, cyclophosphamide, methotrexate) with four-week intervals in first line. In relapsed disease they have used a four-drug regimen at 4-week intervals (Angelov et al., 2009). Nowadays, rituximab has also been added to BBBD therapy in PCNSL in the BBBD consortium and the regimen used is R-methotrexate-carboplatin -cyclophosphamide combination at four-week intervals in first line. In relapsed setting, methotrexate is replaced with etoposide.

When establishing BBBD-treatment in Oulu University Hospital 2007, the most common regimen used was R-methotrexate-Carboplatin according to the original BBBD regimens used by the USA BBBD consortium. Patients not suitable for methotrexate treatment received R-carboplatin-etoposide-cyclophosphamide-regimen. After findings of excellent tolerability, but inadequate response in some
relapsed patients, it was decided to intensify treatment and shorten the treatment regimen intervals to three weeks and to use a combination of all five drugs (R-methotrexate-carboplatin-cyclophosphamide-etoposide).

In the whole patient population in Study II consisting mostly of patients with relapsed or refractory disease, overall response rate was 76% during mean and median follow up time at 15 and 25 months. Two-and 5-year overall survival rates were 57% and 47%, respectively.

The most notable difference was observed in long-term outcome rates and especially in patients treated with 5-drug regimen in first line. Overall response rate among those patients was 100%; 2-year PFS-and OS-rates were 100%. At the time of analyses none of those patients had relapsed after a median follow-up time of 15 months.

**Treatment of relapsed disease**

About one-third of patients with PCNSL do not respond to first-line treatment and approximately more than half of responders will relapse. Prognosis of relapsed and refractory patients is dismal. Overall response rate of 75% and median survival 11-16 months can be attained with WBRT (Hottinger, DeAngelis, Yahalom, & Abrey, 2007; Nguyen *et al.*, 2005b), but it does not seem to improve survival compared to non-WBRT-containing therapies (Thiel *et al.*, 2010). For younger and fitter patients, a new induction treatment followed by ASCT is an option. In a multicentre phase II trial relapsed or refractory PCNSL patients were treated with salvage treatment followed by HDC/ASCT (busulfan, cyclophosphamide, thiothepa) (Soussain *et al.*, 2008). For patients who completed the whole treatment plan, median PFS and OS rates were 11 and 18 months, respectively. In these analyses, 16 patients had relapsed or refractory disease. Ten of those 16 patients were treated with a 5-drug regimen and ORR was promising. Complete response rate was 60%; 2-year PFS was correspondingly 55%. These results show that in this poor prognosis group it is possible to achieve a long-term response with BBBD treatment. Unpublished updated data reveal that 5-year estimated OS seems to be 43%.

**BBBD-therapy followed by ASCT**

In this retrospective analysis of BBBD treatment, all except one patient, in good response proceeded to high dose treatment, with either BEAM or Carmustine-Thiotepa induction supported by autologous stem cell transplantation. Currently in
patients, who have achieved complete remission after first-line treatment, the high dose treatment followed by ASCT is replacing the status of radiotherapy. Because the long-lasting results of rituximab containing BBBD treatment and the data from the 5-drug regimen BBBD treatment alone have not been previously published, it is impossible to evaluate the impact of ASCT on these promising results. The role of ASCT as a consolidation treatment after successful induction treatment is to eradicate a possible microscopic disease. In the first line treatment of PCNSL, there are no prospective randomised trials where patients in CR after induction treatment are randomised either to ASCT or follow-up.

Toxicity

In the earlier studies BBBD with IA administered chemotherapy has been shown to be well-tolerated. We observed grade 3-4 neutropenia in 23% of 4-drug treatment patients and 31% of 5-drug treated patients. The corresponding values in grade 3-4 thrombocytopenia were observed to be 28% and 32%.

Unfortunately, in this retrospective analysis we did not have any neurocognitive surveillance and other data concerning non-haematological toxicity is not reliable and we are unable to compare with existing therapy models. However, we find serious long-term neurotoxicity is unlikely because Doolittle et al have shown the BBBD procedure to be safe (Doolittle et al., 2013). They analysed 26 patients with follow-up time varying between 2-26 years.

Future of PCNSL therapy

In this thesis, we have found that treatment results of PCNSL with Bonn or Bonn-like regimens are dismal. Currently MATRix regimen has replaced this in many centres. Results with BBBD therapy, especially with five-drugs regimen seems promising, but the low number of patients and limited follow-up time hinders drawing firm conclusions. Hopefully these results will be confirmed in our ongoing prospective phase II trial. A randomised comparison with intravenous regimens and BBBD therapy would be interesting, but challenging to establish.

New agents such as temozolomide, topotecan, pemetrexed, bendamustine and temsirolimus have shown activity in PCNSL with objective response rates of 26 to 50% and one year overall survival rates of 25% to 41% (Chamberlain, 2014; del Rio et al., 2011; Fischer et al., 2006; Herrlinger et al., 2000; Korfèl et al., 2016; Mappa et al., 2013; Nayak et al., 2013; Raizer et al., 2012; Tyson et al., 2003). The
most promising response rates have been seen in patients treated with lenalidomide or ibrutinib (Chamoun et al., 2017; Houillier et al., 2015). There are no published studies with new drugs delivered by the BBBD-method.

6.2 Diffuse Large B-cell Lymphoma

6.2.1 DLBCL

DLBCL is an aggressive malignancy, with a change to be cured with chemotherapy. Five-year PFS varies between 80% to 85% in limited stage disease and is about 50% in advanced stage disease (Martelli et al., 2013). Approximately in one-third of patients, disease course during first-line treatment or relapse after completed treatment (Cummin & Johnson, 2016). Patients who experience relapse or fail to attain a complete response after first-line treatment have dismal prognosis and less than 10% of failed patients achieve long-lasting remission (Martelli et al., 2013).

CNS relapse in DLBCL

Central nervous system relapse is mainly a fatal and early event in patients with DLBCL, after which median survival is 2-5 months (Kridel & Dietrich, 2011; Martelli et al., 2013; Tai et al., 2011). The incidence of CNS relapse in DLBCL is about 5% (Zhang et al., 2014). Data considering the effect of rituximab on the incidence of CNS disease is conflicting, but it seems that the number of isolated cases of CNS relapse is increasing during the rituximab era. Several prophylactic chemotherapeutics have been tested to prevent this complication. However, defining high-risk patients and finding optimal prophylactic treatment remains problematic. Available known parameters and score predictors of CNS recurrence show low sensitivity and this exposes patients to under- or overtreatment (Martelli et al., 2013). To consider the toxicity of CNS-targeted treatments, it would be essential to define risk-patients more accurately.

In study III 103 DLBCL patients with a high risk for CNS recurrence were analysed and treatment outcomes were compared according to updated CNS prophylaxis. This presented a population with a dismal disease presentation. All patients had either IPI $\geq 3$, elevated LDH or more than one extranodal site or specific high CNS relapse risk disease locations such as testis, breast, epidural or
sinus. A total of 65 out of 103 patients received CNS-targeted treatment concurrently with systemic chemotherapy.

**Treatment outcome in DLBCL**

In Study III 22 (21%) patients had a systemic relapse and two of them concurrently a CNS relapse: 11/13 (85%) of CNS relapses were isolated. Five-year OS was 65% in the whole study population.

5-year OS rates according to cell of origin were 71% in patients with GC phenotype group and 55% with activated b-cell type group. In line with our result in retrospective analyses ABC (activated b-cell type)-DLBCL has been associated with aggressive behaviour and a higher risk of CNS relapse and worse overall survival. These findings have not been confirmed in prospective settings. In line with these findings in Study III 11/13 (85%) of patients with CNS relapse had a non-GC phenotype, but because of the small sample size, it is not possible to draw any definitive conclusion.

**Limitations of the current study**

In line with current recommendations diagnostic lumbar puncture (LP) was not mandatory during diagnostic work-up and it was performed in 36/65 (55%) of those patients treated with CNS-targeted treatment. LP was not performed in any patient not receiving prophylactic therapy. Despite this, we consider that the risk for undiagnosed occult disease was low because in 11/13 of patients who developed CNS recurrence, LP and/or brain MRI had been performed, which did not demonstrate any signs of CNS involvement.

In this study population, the risk for CNS relapse was high even considering the disease baseline characteristics. We observed 13 (12.6%) CNS events in a mean follow up time of 49 months: 11/13 of those relapses (85%) were isolated. Five-year isolated CNS relapse rates were 6.9% and 23.5% in the pooled prophylaxis group and in the group without prophylaxis, respectively. Median time to isolated CNS relapse was 8 months and median survival after CNS was 7 months. This high number is in line with our clinical experience and the high incidence of PCNSL in our population. We do not have a solid explanation for this phenomenon. However, it may reflect the genetics of our population, because we have found that CNS tropism is associated with HLA genotype (unpublished data from our research group).
The standard treatment of DLBCL is R-CHOP (Coiffier et al., 2010). In Study III patients aged under 60 with IPI ≥ 3 were mainly treated with the R-CHOEP combination due to high risk disease. The impact of etoposide in preventing CNS events is controversial. In the 2007 DSHNHL study the addition of etoposide to CHOP reduced the incidence of CNS relapse in aggressive NHLs (Boehme et al., 2007) while in a large retrospective study by the DSHNHL the beneficial role of etoposide in preventing CNS relapse could not be detected (Schmitz et al., 2012). We strongly believe that etoposide has not had a major impact on our results.

*Location and timing of CNS disease*

The pattern of CNS relapses has been under discussion. Some studies have reported delayed CNS relapses in the era of rituximab (Guirguis et al., 2012; Shimazu et al., 2009; Villa et al., 2010). In Study III, median time to CNS event was 8 months which is in line with earlier published data. In a systematic review of prospective studies (Ghose et al., 2015) overall survival after CNS relapse was significantly longer among patients treated initially with a rituximab-containing regimen. Unfortunately, such lengthening was not observed here and median survival after CNS relapse was 7 months in the current study.

CNS lymphoma may involve brain parenchyma, the dura and leptomeningeal areas. In the prerituximab era leptomeningeal involvements have been observed to be more typical (Bernstein et al., 2009). However in the rituximab era some trials have reported brain parenchymal involvements to be more common (approximately 65% to 76%) (Shimazu et al., 2009; Villa et al., 2010) in line with the poor CNS penetration of rituximab into the CNS. The difference in location of CNS involvement was not detected in our small patient population of which 6/13 had leptomeningeal disease.

*Prevention of CNS relapse*

In the CNS prophylaxis study, CNS-targeted treatment was effective and reduced 77% of CNS events compared to the control group. In patients who received some CNS-targeted treatment, the incidence of isolated CNS recurrence was 6.9% compared to 23.5% of patients treated with a conventional treatment regimen and this difference was statistically significant. When analysing according to prophylactic treatment, there was a downward trend in all treatment groups.
However, the difference was greatest in patients receiving cumulative i.v. methotrexate dose over 9g/m². In this group only one CNS event was observed (incidence 4.7%). Lower methotrexate doses also revealed a trend towards decreased incidence of CNS events (10.2% vs 23.5%), but this was not statistically significant.

HD-MTX is the most commonly used systemic CNS-targeted agent. For uncertain biological reasons, the large-cell lymphomas present in the central nervous system are twice as sensitive for methotrexate-based chemotherapy in comparison to systemic large-cell lymphomas (Bokstein et al., 2002). There is no consensus about the optimal dose, infusion time and number of treatment cycles. However, MTX-doses ≥3g/m² are considered to lead to therapeutic concentrations in the CNS (Brugieres et al., 2009; Ferreri et al., 2004). In two retrospective studies where MTX was administered 3 or 4 times with dose 3-3.5g/m² a marked decrease in CNS events was observed when compared to treatment without MTX (Abramson et al., 2010; Ferreri et al., 2015) which is in line with our results.

Although some studies support using intrathecal MTX as CNS prophylaxis, several others have called into question its ability to prevent CNS dissemination (Boehme et al., 2009; Kumar et al., 2012; Schmitz et al., 2012; Tai et al., 2011). IT chemotherapy has been shown to be effective in preventing and treating leptomeningeal disease, but due to low penetration into brain tissue, its potency to prevent parenchymal relapses has been queried. Despite these reports, in this material the CNS relapse rate was lower in patients receiving i.t. prophylaxis alone compared to in patients without any prophylaxis: 12.5% vs. 23.5%, respectively.

**Predicting the risk of CNS lymphoma**

In addition to clinical risk factors, biological risk factors such as MYC and BCL-2 translocations (HGBL-DH) and overexpression of MYC and BCL-2 (DE-HGBL) in tumour tissue are considered to present risk factors for CNS recurrence in DLBCL. In some studies, the incidence of CNS relapse is even as high as 50% in HGBL-DH patients (S. Li et al., 2012). Correspondingly, in the literature the risk of CNS events was 10% among patients with DE-HGBL at two years (Savage et al., 2016). We retrospectively determined the HGBL-DH and DE-HGBL status among patients with CNS relapse. One patient with CNS recurrence had HGBL and as high as 9 out of 13 were observed to present DE-HGBL phenotype. The correlation between DE-HGBL phenotype and CNS events in this small sample was quite clear and even stronger than previously reported.
Ki-67 is a nuclear antigen expressed in proliferating cells. In the prerituximab era its value in predicting treatment response was controversial, but in recently published studies a high Ki-67 value seems to predict a worse outcome in systemic DLBCL and PCNSL among patients with non-GC-phenotype (Broyde et al., 2009; Z. M. Li et al., 2012; Salles et al., 2011). In our study, Ki-67 value was at least 80% with 9/13 CNS relapse patients. It is not known whether this presents as a predictive factor per se or is associated with more aggressive behaviour of a non-GC-phenotype. There are no published studies in the literature on the correlation with Ki-67 and secondary CNS lymphomas.
### 7 Conclusion

PCNSL is a rare and aggressive disease. Despite chemo-and radiosensitivity remission is mainly short-lasting because the blood brain barrier restricts penetration of chemotherapeutics into the central nervous system. The prognosis of PCNSL has improved over the last two decades because of better diagnostic tools and developing treatment strategies. Currently, the treatment of PCNSL involves effective HD-MTX-based polychemotherapy induction and a consolidation treatment. Under debate is the role of radiotherapy as a consolidation treatment after a full response with chemotherapy. Four ongoing studies about consolidation treatment will clarify this problematic issue. Two of them compare WBRT to ASCT and in the remaining two studies patients are randomised to myeloablative or non-myeloablative high-dose treatment. The role of high-dose treatment as a consolidation is under discussion.

Bonn-or Bonn-like treatment with HD-MTX-based multichemotherapy is an effective first-line induction treatment in PCNSL. Treatment responses attained are excellent, but unfortunately long-lasting remissions are rare. Thus, HD-MTX-based multichemotherapy per se is not able to eradicate microscopic disease and achieving a long-lasting response demands an even more aggressive method to assess the blood brain barrier and/or effective consolidation treatment.

Blood brain barrier disruption treatment by intra-arterial hypertonic infusion of mannitol followed by intra-arterial chemotherapy infusion is promising treatment method that enables achieving over ten-fold drug concentrations in the CNS and thereby eradicate the microscopic disease. The effectiveness of treatment has been confirmed in first-and second-line treatment in PCNSL. High dose treatment with carmustine-thiotepa combination regimen followed by autologous stem cell transplantation as part of treatment probably enhances the response achieved. The impact of HDT/ASCT to achieved promising results with BBBD treatment cannot be separated in the absence of published studies. Future ongoing consolidation studies will clarify the role of HDT/ASCT in PCNSL. The side-effects and tolerability of BBBD-treatment has been shown to be acceptable.

CNS relapse is mainly a fatal complication in DLBCL. The lack of consensus defining high-risk patients, and optimal prophylactic treatment is problematic. Available known parameters and score predictors of CNS recurrence show low sensitivity and this expose patients to under-or overtreatment. Approximately only half of risk patients can be identified by using screening methods of the known risk factors. HD-MTX administering i.v. as a CNS prophylaxis effectively prevents
CNS events. HD-MTX cumulative dose $\geq 9g/m^2$ at the earlier stage of treatment was shown to be effective in preventing CNS events. The difference between whether there was CNS relapse was also observed with lower HD-MTX doses and MTX-IT alone compared to patients who did not receive CNS-targeted treatment. However, differences were not statistically significant.

DE-HGBLs were overpresented among patients with CNS relapse and Ki-67 was high: 9/13 of patients (data from 4 patients was not useable). Based on these findings we recommendate that the cumulative dose of HD-MTX $\geq 9g/m^2$ at the earlier treatment stage concurrently with treatment cycles 1-3 or 2-4 is effective and could be considered for all high-risk patients. For unfit or frailer patients, a lower dose of HD-MTX or MTX IT alone could be an option. The addition of Ki-67 and HGBL-DH and/or DE-HGBL to clinical risk factor scoring might clarify the high-risk patient group and probably exclude some patients not at of developing CNS relapse. In future studies the correlation between Ki-67, DE-HGBL, HGBL-DH and CNS events should be indentified in a larger DLBCL patient population and whether this hypothesis exists to create a new prognostic model with IPI $\geq 3$, elevated LDH, more than one extranodal site or specific site such as the testis, kidney or adrenocorticoid glands, Ki-67 $\geq 80$ and DE-HGBL or HGBL-DH.
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Original publications


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1421. Lemma, Siria (2017) Migration and adhesion associated molecules in lymphoma biology and their potential roles as biomarkers


1426. Karhu, Toni (2017) Isolation of novel ligands for MAS-related G protein-coupled receptors X1 and X2, and their effect on mast cell degranulation

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