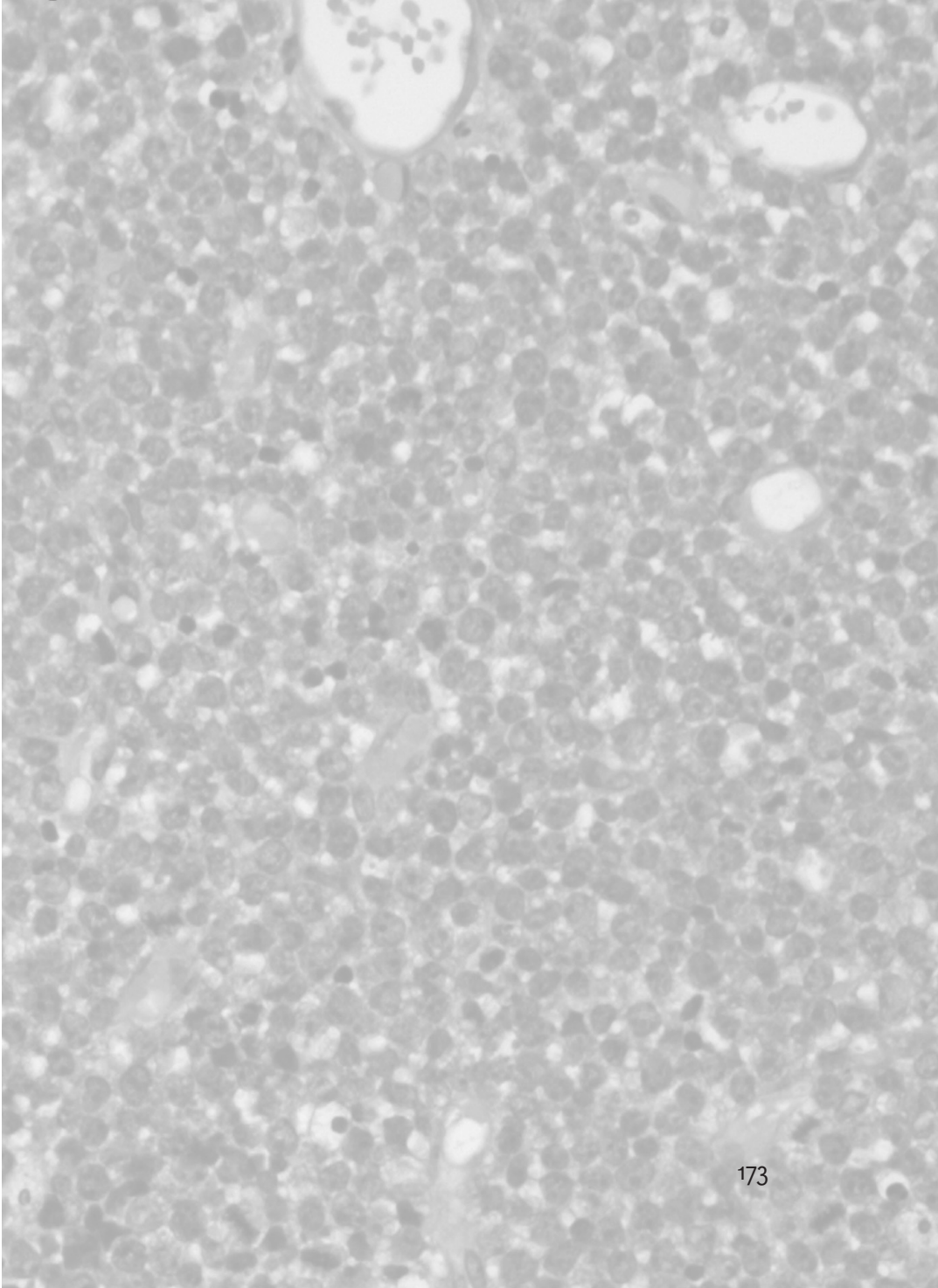


6 Summary and general discussion



In this thesis a number of individual studies are presented concerning treatment, and prognostic aspects of young patients with poor-risk aggressive lymphomas. Although these studies contain several overlapping and parallel issues, the disparities in clinical presentation, treatment, prognostic factors and outcome of distinct lymphoma entities are substantial. Therefore, the chapters in this thesis have been grouped according to the main lymphoma type addressed in the separate studies, i.e. diffuse large B-cell lymphoma, Burkitt lymphoma, and post-transplant lymphoproliferative disorder – also called post-transplant lymphoma –, respectively. In addition, prognostic factors related to outcome in relapsing aggressive lymphoma are presented. Accordingly, the summary and discussion follow this grouping.

Chapter 2 Diffuse large B-cell lymphoma

In **chapter 2.1 and 2.2** the results are presented of dose-intensified chemotherapy treatment in diffuse large B-cell lymphoma. When these HOVON (stichting Hemato-Oncologie voor Volwassenen Nederland) studies were initiated, 8 cycles of CHOP-21 (cyclophosphamide, hydroxydaunomycin, vincristine, prednisone) three-weekly was considered the standard of treatment.¹ Our main question was whether treatment results could be improved by intensifying chemotherapy. The rationale for these studies was based on the favorable results of high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) in patients with relapsed aggressive lymphoma (PARMA) and the importance of cyclophosphamide and doxorubicin dose for outcome in aggressive lymphoma.²

Short of autologous stem cell support, intensification of CHOP-21 had not been very successful.³ The introduction of G-CSF (granulocyte colony stimulating factor) provided the possibility to escalate the CHOP-21 regimen, without the use of autologous stem cell support. The IPI (International Prognostic Index) had not been published yet, therefore patients with aggressive lymphoma were stratified into low, intermediate or high-risk, according to the previously defined HOVON risk profile.⁴ Patients with high-risk, who according to this HOVON risk stratification had an expected survival of only 24% at 5 years, were enrolled into two consecutive phase II trials (HOVON-27 and -40) with front-line high-dose chemotherapy and ASCT, described in **chapter 2.1**. For patients with intermediate-risk, who had an expected 5 year survival of 49% according to HOVON risk criteria, a randomized phase III was initiated that compared intensified-CHOP to CHOP-21 (HOVON-26), described in **chapter 2.2**.

Chapter 2.1 Up-front ASCT in poor risk DLBCL, HOVON trials 27 and 40

The results of the first trial (HOVON-27) with up-front ASCT were disappointing. Outcome of this trial, which consisted of two high-dose induction courses of chemotherapy followed by ASCT, did not differ from a historical cohort of patients with the same risk factors treated with 8 cycles of CHOP-21. Based on the analysis of this HOVON-27 trial and the available literature that reported conflicting results of up-front ASCT at that time, it was hypothesized that a more robust induction treatment before ASCT, containing a higher cumulative dose of cyclophosphamide and doxorubicin might improve outcome. Our subsequent HOVON-40 trial tested this hypothesis by adding 3 courses of intensified CHOP to the induction treatment, which was otherwise equal to trial HOVON-27. The results of this strategy were remarkable, resulting in a marked improvement in event free, progression free and overall survival in the HOVON-40 trial compared with the HOVON-27 trial.⁵ We concluded from these consecutive studies that ASCT as front-line treatment in high-risk patients with diffuse large B-cell lymphoma is more successful after a robust induction treatment, i.e. – in the case of the HOVON-40 schedule – treatment with a sufficient amount of dose-dense cyclophosphamide and doxorubicin. Nevertheless, 25% of patients still failed to respond to induction treatment. Thus, reducing the number of primary refractory patients remains an important goal of current and future clinical studies in high-risk diffuse large B-cell lymphoma. This refractoriness will probably not be amenable to further chemotherapy manipulation, because the proportion of refractory patients in HOVON-27 and -40 was not different.

Overcoming primary resistance by adding (radio-)immunotherapy to chemotherapy, e.g. rituximab, will be the obvious next step in ameliorating treatment results. The addition of rituximab to chemotherapy schedules may substantially reduce the fraction of patients with primary refractory disease.^{6,7} Although rituximab was found to be active in combination with CHOP in all IPI risk classes in the elderly,⁶ and the IPI remained relevant as risk classifier in low risk young patients treated with rituximab,⁷ it remains to be determined whether rituximab will be equally effective in young patients with high-risk disease, such as those with advanced disease and highly elevated LDH levels included in our HOVON-27 and 40 trials. Moreover, highly elevated LDH levels may confer additional risk beyond the IPI for outcome.⁸ Thus, despite the expected strong effects of rituximab, there might still be a place for up-front ASCT in patients with poor-risk diffuse large B-cell lymphoma. Obviously, it remains to be determined whether, and if so, which patients will benefit from this strategy when rituximab is added to high-dose treatment schedules. Several prospective studies are currently comparing R-CHOP-like therapy to R-high-dose treatment followed by ASCT in poor-risk patients. Similarly, HOVON is conducting a prospective randomized study, comparing R-CHOP with an up-front ASCT regimen, based on

the HOVON-40 with the addition of rituximab in intermediate-high and high-risk age-adjusted IPI (aa-IPI) patients (HOVON-63).

Chapter 2.2 Intensified-CHOP-14 v. CHOP-21 in patients with intermediate-risk aggressive NHL (HOVON-26)

In chapter 2.2 the effect of doubling the dose intensity of cyclophosphamide and doxorubicin on outcome was investigated in patients with intermediate-risk aggressive lymphoma according to HOVON risk criteria. In this trial, the cumulative doses of the aforementioned drugs were the same, but the fractional dose of chemotherapy per cycle in the experimental, so called intensified-CHOP-14 arm was increased and delivered in 12 weeks instead of 24 weeks in the standard CHOP-21 arm. With G-CSF support the planned dose could be attained in 70% of the patients in both arms of the study and the median increment of actual delivered dose intensity, of doxorubicin and cyclophosphamide in the intensified CHOP-14 arm was almost twice that of the CHOP-21. However, no significant improvement in outcome of patients treated according to the intensified CHOP arm was observed.

Hypothesis-generating subgroup analysis indicated a possible improvement in FFS for patients with low or low-intermediate aa-IPI. However, these results must be interpreted with caution, because the trial was not powered for this subgroup analysis. Fortunately, the results of our subgroup analysis are supported by the German B1 study, in which CHOP-14 was better than CHOP-21 in patients with low, low-intermediate aa-IPI risk aggressive NHL.⁹ In addition, a 2 weekly CHOP schedule, although more toxic at least in case of intensified CHOP-14, will shorten the total duration of treatment substantially. Taken together, dose-dense CHOP either as CHOP-14 or intensified CHOP-14 may be the preferred chemotherapy in combination with rituximab in treatment for young patient with low or low-intermediate aa-IPI aggressive lymphoma. For patients with higher risk profiles chemotherapy intensification of this order of magnitude is apparently not sufficient. These patients might indeed be candidates for strategies incorporating up-front high-dose therapy and ASCT.^{5,10}

Both intensification of CHOP as well as the addition of rituximab may improve results in elderly patients of all IPI risks, as well as young patients with low, low-intermediate risk DLBCL.^{6,7,9,11} In elderly patients, preliminary results from HOVON indicate that R-CHOP-14 is also superior to CHOP-14.^{12,13} Thus, available evidence indicates that the additive effect of rituximab is independent of chemotherapy dose. Obviously, the question still to be answered is: will R-CHOP-14 or R-intensified-CHOP-14 be better than R-CHOP-21? This question will hopefully be answered by the French lymphoma group, which is currently comparing 8 cycles of R-CHOP-14 with R-CHOP-21 in elderly patients.

Furthermore, the addition of rituximab to dose-dense chemotherapy has also reopened the debate about the total amount of chemotherapy cycles necessary for cure. This debate has been fuelled by excellent results of 6 cycles of R-CHOP-21 combined with radiotherapy in young patients with low-risk disease in the MINT trial,⁷ and preliminary data from a recently completed DSGHL-trial (RICOVER) comparing 6 cycles of CHOP-14 with 8 cycles of CHOP-14 each combined with, or without rituximab, in elderly patients with diffuse large B-cell lymphoma. The latter trial contained a direct comparison between 6 or 8 cycles of CHOP-14. After a median observation time of 26 months, there was a trend for better freedom from treatment failure after 8 cycles of CHOP-14 compared to 6 cycles of CHOP-14 (58% v. 53%; $p=0.13$), but this trend was neutralized after the addition of rituximab: 70% for both 6 and 8 cycles R-CHOP-14.¹³ Although an impressive number of patients ($n=1330$) have been included in this trial, and it was powered to detect a 9% freedom from treatment failure at 3 years, follow-up is still short and these data need further maturation before more definite conclusions are possible. More importantly, the question whether 6 cycles of R-CHOP-14 *alone* will be sufficient for cure will not be answered by this trial, because additional radiotherapy was administered to patients with bulky and extranodal sites of disease, as has been the case in other trials of the DSGHL comparing six cycles of CHO(E)P-14 with CHO(E)P-21.^{9,11} Of note, the ongoing French study mentioned before is comparing 8 cycles of R-CHOP-14 with R-CHOP-21 without the additional use of radiotherapy. It will be very difficult to compare the efficacy and, moreover, potential late toxicity (such as in-field secondary tumors and vascular damage) introduced by the consolidation radiotherapy after 6 CHOP cycles, versus the addition of 2 extra CHOP cycles. Furthermore, chemotherapy seems to be less safe, as far as late toxicity is concerned, than previously assumed. A recent study by the EORTC based upon a large series of patients with aggressive NHL treated with 8 CHOP-like courses, showed impressive late cardiotoxicity, undoubtedly related to 'normal' (i.e. not exceeding the cumulative dose of 400 mg/m²) dose of adriamycin given.¹⁴

Chapter 2.3 Prognostic significance of germinal center associated proteins and chromosomal breakpoints in poor-risk diffuse large B-cell lymphoma

Apart from the IPI, intrinsic biological aspects of the tumor also play a role in prognosis. Although many studies have focused on the significance of intrinsic characteristics of the tumors, e.g. protein or gene expression and chromosomal breakpoints, the clinical relevance of many of these markers is inconsistent. Moreover, few markers retain sufficient prognostic significance independently when adjusted for the overriding prognostic impact of the IPI risk score. Gene expression analysis enables subclassification of diffuse large B-cell lymphoma

into tumors with an expression pattern resembling that of B-cells of the normal germinal center (GCB) or an expression pattern more resembling that of activated, post-germinal center B-cells, also called non-germinal B-cells (non-GCB). Patients with tumors exhibiting non-GCB gene expression have worse survival than those with tumors exhibiting GCB gene expression.¹⁵ Importantly, this effect is independent from IPI risk factors in patients treated with CHOP-like therapy.¹⁶ Moreover, similar grouping can be achieved by using a simple algorithm, combining the expression of CD10, bcl6 and MUM1 by immunohistochemistry.¹⁷

In chapter 2.3 we investigated whether the prognostic value of GCB versus non-GCB diffuse large B-cell lymphoma would also be present in a homogeneous group of patients with poor IPI risk, treated with high-dose therapy and ASCT as first-line treatment. In addition, we also analyzed the prognostic value of different chromosomal breakpoints. We could confirm the prognostic value of GCB v. non-GCB immunophenotype in our group of patients. In addition, based on analysis of the prognostic value of the individual markers, we argued that a higher cut-off value for MUM1/IRF4 might improve the negative predictive value of this marker and the performance of the algorithm.¹⁸

Chromosomal breakpoints did not seem to confer additional prognostic impact. However, given the number of patients analyzed, clinically relevant differences might still be present when analyzed in larger groups of patients. The adverse prognostic impact of bcl2 protein expression reported by others was confirmed. This effect was present both in GCB as well as in non-GCB tumors. Apparently, this adverse effect on prognosis associated with bcl2 was independent of the mechanism leading to over-expression, i.e. chromosomal translocation of the *BCL2* gene t(14;18), found in the majority of GCB tumors, or other mechanisms leading to bcl2 over-expression in non-GCB tumors.

Our findings confirm the prognostic value of the immunophenotype in DLBCL and extend these finding to patients with poor-risk disease treated with high-dose chemotherapy and ASCT. Future studies will have to determine, whether the prognostic value of this algorithm will keep its predictive value for prognosis in patients treated with rituximab. For instance, rituximab seems to have higher efficacy in tumors with bcl2 overexpression,¹⁹ a marker independently associated with worse prognosis in patients treated without rituximab. Thus, although the prognostic value of individual markers may be transient in the light of changing treatment paradigms, gene-expression studies in lymphoma are of great importance for unraveling histopathogenesis and guiding new treatment modalities in malignant lymphoma. Therefore, the retrieval of adequate (frozen) tumor material and construction of tissue microarrays in clinical studies is of utmost importance.

Chapter 3 Burkitt lymphoma

Chapter 3.1 Short intensive sequential therapy and ASCT in adult Burkitt lymphoma

In adults, this highly aggressive lymphoma is very rare and treatment not well defined. Treatment according to protocols usually employed for treatment of diffuse large B-cell lymphoma, such as CHOP chemotherapy, are not effective in Burkitt lymphoma. Its high growth fraction, presentation at extranodal sites and increased incidence of meningeal dissemination necessitate high-dose treatment regimens combined with central nervous system prophylaxis. These regimens, which are highly successful in children, are also effective in adults, but more toxic.

Based on promising results from of ASCT in patients with aggressive lymphoma in first remission, including Burkitt lymphoma, we decided to investigate the feasibility and efficacy of up-front ASCT in adult patients with advanced non-leukemic Burkitt lymphoma. The treatment protocol (HOVON-27BL) was similar to that of patients with high-risk diffuse large B-cell lymphoma included in the HOVON-27 protocol (see **chapter 2.1**). In contrast to patients with diffuse large B-cell lymphoma, in whom this very short high-dose treatment protocol was ineffective, the results in adult patients with Burkitt lymphoma were excellent and competitive to that of high-dose pediatric treatment regimens.²⁰ However, it must be stressed that we only included patients without meningeal disease or bone marrow dissemination. The efficacy of our treatment protocol in patients with extensive bone marrow involvement is unknown.

Comparison in a phase III setting of our ASCT-based regimen with a pediatric regimen in adult patients with Burkitt lymphoma would be the obvious next step. For patients with meningeal involvement we could combine our HOVON-27 protocol with more intensive intraventricular chemotherapy via an Ommaya reservoir.²¹ However, the rareness of Burkitt lymphoma in adults and the excellent results of our own, as well as other current treatment strategies would necessitate a large intergroup consortium to accrue sufficient patients for the detection of differences. In the mean time, the addition of rituximab to these regimens has even further increased cure rates, making the feasibility of conducting such a trial not very realistic.

Chapter 3.2 Gender and age related differences in Burkitt lymphoma in The Netherlands

Based on histological, immunophenotypical and genetic data Burkitt lymphoma is considered to be a single disease entity. However, the striking differences in absolute and relative incidence – as compared to other lymphoma types – of Burkitt lymphoma in children and adults, made us wonder whether Burkitt lymphoma in children and adults should not be considered different diseases.

We started with an analysis of differences in incidence and clinical characteristics of sporadic Burkitt lymphoma in adults and children in The Netherlands. For epidemiological reasons, incidence data of BL in The Netherlands, as classified by morphological data by the local pathologist, were retrieved from the National Cancer Registry. Unfortunately, this registry does not contain sufficient clinical data for analysis of disease extent at presentation. Therefore, differences in clinical characteristics between adults and children with Burkitt lymphoma were analyzed by comparing adult patients included in the HOVON-27 study with children treated in SKION/SNWLK protocols during the same time frame. In addition, in contrast to the epidemiological data, a very strict pathological definition of Burkitt lymphoma was required for patients included in the comparison of clinical characteristics from both the SKION and HOVON database, including the presence of a *MYC*/8q21 breakpoint in cases with atypical cytological features, or so-called atypical Burkitt lymphoma.

A striking male preponderance was seen at all ages for Burkitt lymphoma recorded in the national cancer registry, suggesting a possible difference in genetic background of the disease in male as opposed to female patients. In addition, in male patients a bimodal age incidence was found with a peak at pediatric age and a steady increase after 60 years, supporting the hypothesis of the existence of two disease entities for the different age groups. In contrast, the incidence in female patients was stable in all age categories. The major difference in clinical presentation of Burkitt lymphoma between children and adults was the frequency of extranodal disease, which was more frequently observed in childhood Burkitt lymphoma.

A major limitation of the epidemiological analysis is the absence of histological review of the registry data. This may have created bias especially in adults, because there may be a substantial gray zone between Burkitt lymphoma and diffuse large B-cell lymphoma in adults.²² As for the comparison of clinical characteristics at presentation, bias could have been created because patients were selected, based on the same criteria as used for entry in the HOVON-27 trial. Only patients younger than 66 years were included and patients with CNS disease, extensive bone marrow dissemination, or bad performance status were excluded. This led to the exclusion of 25% of childhood Burkitt lymphoma cases in the SKION database. The incidence of patients with these characteristics in the adult population is unknown and might be different. Nevertheless, despite the constraints of these analyses, the hypothesis that Burkitt lymphoma at least in male patients might consist of two different diseases is still tenable.

Because Burkitt lymphoma can also be classified based on specific gene expression profiles,^{23,24} analyzing differences in gene expression of Burkitt lymphoma in (male) children and adults might be an other way to further investigate our hypothesis.

Chapter 4 Post-transplant lymphoma

Post-transplant lymphomas (PTLD) are lymphomas developing in recipients of solid organ or bone marrow transplantation. Most of these lymphomas are of B-cell origin and contain EBV (Epstein Barr virus). Immunosuppressive treatment to prevent allograft rejection leading to diminished T-cell surveillance, may give rise to an unchecked EBV driven B-cell proliferation ultimately leading to lymphoma. These lymphomas often present at extranodal sites, including the allograft, are sometimes difficult to diagnose, and may behave very aggressively. Even with rituximab treatment mortality is high. We analyzed the clinical characteristics, treatment, outcome and prognostic factors of patients with these lymphomas observed in recipients of kidney or lung transplants between 1985 and 2002 in our hospital.

Chapter 4.1 Early PTLD is localized in the allograft

The incidence, clinical characteristics at presentation, treatment outcome and prognostic factors of PTLD were analyzed in a large cohort of kidney and lung transplant recipients in the UMCG. Forty patients with histological confirmed PTLD were observed in a cohort of 1354 kidney and 206 lung transplant recipients, who had been transplanted between 1985 and 2002. Lung transplant recipients had higher incidence rates of PTLD than did kidney transplant recipients (8.3% v. 1.7%), probably related to higher doses of immunosuppressive treatment in the lung transplant recipients. Extranodal presentation occurred in 73% of the patients including allograft localization. Half of the PTLD occurred within one year after transplantation. The allograft was significantly more frequently involved as primary site of PTLD presentation during the first year after transplantation. In multivariate analysis, predictive factors remaining significant for outcome of PTLD treatment were: performance status and advanced stage for response, and performance status for survival, respectively.

The results of this analysis are of course limited because they are not corrected for treatment modality, which has changed over time during the observation period. As soon as rituximab became available we have treated all patients with this monoclonal antibody. It may well be that prognostic factors of relevance for treatment outcome of PTLD before rituximab became available, will be less or no longer relevant after its introduction. Of the 14 consecutive patients treated with rituximab in our cohort, 12 patients (86%) had a complete response, which is higher than the 45% complete response rate reported recently in a prospective study of rituximab treatment in 43 PTLD patients.²⁵ Interestingly, in the latter cohort of patients only elevated LDH level remained as prognostic adverse factor for response.

The most interesting finding was our observation of the association between allograft localization and early PTLD. One could speculate that these lymphomas might be partially donor-derived, i.e. originated from donor lymphocytes transplanted with the allograft. However, this hypothesis will not suffice because most PTLD's, including those localized in the allograft, are recipient-derived. In contrast, we speculate that the microenvironment of the allograft may play a permissive role in the development of PTLD.²⁶ The intriguing association between, recipient derived EBV positive B-cells, decreased immune response, allograft microenvironment, HLA match and PTLD development needs further study.

Chapter 4.2 FDG-PET in PTLD

Given the excellent results of FDG-PET as tool in staging and treatment evaluation of malignant lymphoma, we also analyzed the applicability of FDG-PET (18-fluorodeoxyglucose positron emission tomography) in PTLD. Although the series of patients analyzed was small, all PTLD lesions studied were highly FDG avid. Extranodal localizations of PTLD, which are frequently present but often difficult to detect by conventional CT scanning, were easily detected by FDG-PET. Disappearance of PET positive lesions after treatment correlated well with freedom from PTLD recurrence. Thus, as is the case in diffuse large B-cell lymphoma, PET-FDG is also an excellent tool for staging and treatment evaluation in PTLD.

Chapter 5 Relapsed aggressive lymphoma

Young patients with aggressive lymphoma who relapse after-, or are primary refractory to first-line treatment can be salvaged by ASCT, provided their disease is still responsive to salvage chemotherapy.^{27,28} The latter is usually defined as: at least a partial response, based on conventional diagnostic methods, including a CT-scan. However, only half of the patients who have failed first-line treatment will be responsive and of those 50% will be cured by ASCT.²⁷ To improve upon these results, better parameters are required for the prediction of response and cure. To this end, we investigated the prognostic value of clinical factors and early FDG-PET defined response in two cohort studies of young patients with relapsed chemosensitive lymphoma who were treated with ASCT.

Chapter 5.1 Prognostic value of secondary age-adjusted IPI

We analyzed the prognostic value of the age-adjusted IPI at relapse in a cohort study of 88 young patients with NHL failing first-line CHOP-like treatment, and who were treated with ASCT in our hospital between 1989 and 2002. The secondary age-adjusted IPI (2nd-aa-IPI) proved to be highly predictive for outcome in these patients who were still chemosensitive, i.e. responded to sal-

vage chemotherapy based on CT findings, and were subsequently treated with ASCT.

Our study included patients with different duration of response to, and even patients with less than partial response to first-line treatment. The quality as well as duration of response to first-line chemotherapy are of predictive value for response to salvage chemotherapy.^{27,29} However, in keeping with the finding of others,³⁰ in patients who responded to salvage chemotherapy, quality and duration of response to first-line treatment were no longer of significance for outcome after ASCT in our analysis.

Our cohort contained not only patients with DLBCL, but also a substantial number of patients with other histologies. Although these subgroups were obviously too small for definite conclusions, our results indicate that the 2nd-aa-IPI might also have prognostic value in chemosensitive patients with other histologies.³¹

A limitation of this study is the fact that patients were selected based upon chemosensitivity, i.e. post-hoc determined response based on CT scanning after two courses of salvage induction treatment. Many patients received their second-line induction chemotherapy outside the UMCG, and were only referred for the transplant procedure if considered chemoresponsive. Therefore, we lacked data on the characteristics of those patients who had failed second-line treatment in this time frame. We estimate that this cohort contained approximately half of all young patients failing first-line chemotherapy, given the approximately 50% response rate to salvage chemotherapy usually observed in these patients in that time frame.²⁷ This means that the patient population will indeed have been selected and 2nd-aa-IPI might not necessarily have the same predictive value in those not responding to second-line treatment. However, our primary objective was to find prognostic factors in a population of patients selected on the basis of primary eligibility for ASCT, i.e. responding disease as proven by at least partial response after second-line chemotherapy, based on conventional diagnostic methods (CT scan). In addition, after the first report of our data (ASH 2002) the value of the 2nd-aa-IPI was confirmed in a large cohort of 150 DLBCL patients from New York,³⁰ both in all patients as well as in chemosensitive patients only.

Thus, it may be concluded that the 2nd-aa-IPI is of prognostic importance in all patients, as well as in chemosensitive patients only. Moreover, our results as well as those of others indicate that chemosensitivity and 2nd-aa-IPI are both important and independent prognostic factors for outcome of ASCT in patients with DLBCL who failed first-line treatment. Hence, in addition to determining the 2nd-aa-IPI, the early detection of patients with insufficient response to salvage treatment is important in order to determine which patients might need modified salvage treatment.

Chapter 5.2 Predictive value of early FDG-PET in chemosensitive relapsed lymphoma

In this chapter we addressed the question of better defining early response by analyzing the predictive value of midtreatment FDG-PET for outcome after ASCT in patients who had failed first-line treatment for aggressive non-Hodgkin's and Hodgkin lymphoma. Half of the patients with apparent responsive disease, again classified by conventional criteria based on CT scanning, ultimately relapsed after ASCT. From these patients, who all had complete or partial response based on conventional diagnostic methods, those with normalized FDG-PET scans after two courses of chemotherapy had excellent outcomes, whereas the failure rate after ASCT in patients with a positive midtreatment PET scan was 65%. In contrast to FDG-PET defined response, there was no correlation between the presence or absence of residual tumor mass on midtreatment CT scan and progression after ASCT. Our pilot study underscores the high performance of FDG-PET as compared with conventional CT scanning for defining responsive disease and shows promising results of midtreatment FDG-PET in the prediction of outcome of patients treated for relapsed malignant lymphoma.^{32,33} The predictive value of early FDG-PET for outcome has been further confirmed by us and others in relapsed³⁴ as well as in newly diagnosed aggressive lymphoma³⁵ and Hodgkin lymphoma.^{36,37} A prospective, blinded FDG-PET study in first-line (R)-CHOP treatment in aggressive lymphoma in The Netherlands (PALET study), has recently been completed and hopefully can validate the predictive value of midtreatment FDG-PET in front-line treatment of diffuse large B-cell lymphoma more precisely.

Based on these data, future treatment, at least for patients with relapsed DLBCL, will be based on FDG-PET-defined response criteria. For instance, patients with FDG-PET positive lesions after salvage treatment, and those with insufficient midtreatment FDG-PET-defined response, might be candidates for more intensive myeloablative treatment, or salvage therapy containing new treatment modalities, respectively. In addition, given the high predictive value of FDG-PET for residual lymphoma after treatment,³⁸ it can be envisaged that also primary endpoints in future clinical trials will be based on FDG-PET findings instead of time dependent survival endpoints. This will have important consequences for the design and statistics of new studies in aggressive lymphoma.

Concluding remarks

Considerable improvement in the outcome of patients with poor-risk aggressive lymphoma has been achieved in the past decade. Major steps forward have been the development of the IPI and the WHO lymphoma classification based on 'real' entities, enabling better comparison and stratification of patients according to distinct histologies and clinical risk. The use of G-CSF has enabled dose-dense

treatment strategies and harvesting of stem cells from the peripheral blood for myeloablative therapy with ASCT. The introduction of rituximab has already dramatically changed treatment of CD20 positive B-cell lymphoma, but has only heralded the arrival of a large array of new treatment modalities based on combinations of chemotherapy, immunotherapy, immunotherapy conjugates, and stem cell support. These modalities need careful clinical studies in close cooperation between the pharmaceutical companies that provide these new modalities and the clinical investigators. FDG-PET has substantially improved staging and treatment evaluation and will rapidly become an important tool to guide treatment early after the start of therapy. Biological studies on tumor material from uniformly treated patients with homogeneous clinical risk scores will enable the detection of clinically relevant (sub) groups of lymphoma with different prognosis, possibly in need of new treatment strategies. Future clinical studies will have to be coupled prospectively to biological side studies. Obtaining fresh tumor material and creating tissue microarrays for ancillary biological studies will become mandatory for future clinical studies in malignant lymphoma. This and future research in lymphoma has been, and will only be possible in the context of a multicenter cooperative structure, such as HOVON, enabling research by providing a forum for investigators with new ideas, serving as a robust research partner for pharmaceutical companies with investigational drugs, and last but not least obtaining sufficient financial support for conducting prospective clinical studies.

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