Chapter 9

From visualizing to vision: summary, discussion and future perspectives

Transformed follicular lymphoma is considered to have a poor outcome, however , that might not apply to all TFL patients. Because of the presumed rarity of transformation no studies are designed for patients with TFL and because of the therapy-resistance, patients with TFL are almost never allowed to participate in studies for patients with indolent or aggressive lymphoma. The purpose of this thesis was to generate data that will provide us with ideas for improvement of diagnosis and treatment and for future studies in this specific patient group.
Since different treatment paradigms are applicable to FL and TFL and diagnosis at an early stage improves outcome in TFL (1,2), we investigated PET as a means of improving diagnostic accuracy in the first part of this thesis.
After diagnosing TFL, treatment strategies vary in general practice, as there is no agreement on the optimal treatment. In order to obtain insight in treatment strategies and clinical outcome in TFL patients in the Netherlands, we performed an analysis on patients with TFL registered in the Dutch population based registration PHAROS.
Additionally we performed several retrospective studies on treatment of TFL, of which one in an international collaboration with a substantial patient number. We investigated the results and feasibility of the addition of radioimmunotherapy to ASCT in first line as well as in relapse of TFL and we reported results of allogeneic transplantation in TFL. Despite promising results in the abovementioned studies, 30%-40% of patient are still resistant to all available treatments, which necessitates finding new targets for therapy (3,4). This was the purpose of our final study investigating the expression of survivin in TFL and exploring its use as a possible target for therapy.

In **chapter 1** the biological background of transformation, diagnosis (histological and the role of PET scanning), incidence, prognosis and possible treatment of TFL were discussed.

In **chapter 2** positron emission tomography as a tool in detection of transformation was investigated. Two tracers were studied: 18F-FDG (thought to reflect proliferation and metabolism) and 18F-FLT (thought to reflect proliferation more specifically). 18F-FDG was very sensitive in detecting transformation when SUVmax in the lymph node with the highest uptake was above 14.5, or when the difference in uptake between the lymph node with the highest uptake versus the lymph node with the lowest uptake (SUVrange) was above 6. 18F-FLT could not detect TFL with sufficient accuracy. Obviously, these SUV-based thresholds need to be prospectively validated. 18F-FDG PET could be used when transformation is suspected, before biopsy, and also guide the biopsy to the lymph node with the highest uptake.

In Chapter 3 we hypothesized on an explanation for the observation in chapter 2 that FDG-PET outscored FLT-PET as a marker of transformation. We observed that uptake of 18F-FLT in FL was not related to the percentage of Ki67positive cells (a marker of proliferation). FL had a higher 18F-FLT uptake than would be expected by proliferation alone, complicating differentiation with transformed FL. We hypothesized that 18F-FLT is also being used in the process of DNA repair, a phenomenon that occurs continuously in FL during somatic hypermutation and class switch recombination, thereby leading to a high uptake in FL.

In chapter 4 the Dutch treatment strategies and the impact of different treatment strategies on survival of TFL were discussed. Two year OS for the all 161 included patients was 55%. The population based registry data revealed that when treatment with R-chemotherapy resulted in remission, subsequent 2 year OS was 83%. Patients > 65 had a 2 year OS of 60% after successful induction. Patients ≤ 65 years did significantly better, reaching a 2 year OS of 88% after successful induction with R-chemo only and 96% when R-chemo was followed by consolidation with an up-front ASCT. Our data suggested that up-front ASCT might overcome the negative effect of previous need for treatment of FL, although additional studies will be needed to investigate who will benefit most of up-front ASCT. Unfortunately, there was a high rate of refractoriness to induction therapy (34%) with a dismal outcome. For these patients salvage therapy with ASCT was the only chance for long term survival. Unfortunately this was possible in 27% of refractory patients only, indicating a high unmet need for novel therapies.

The subsequent part of the thesis described strategies to improve treatment results in TFL. In Chapter 5 a cohort of 32 patients with histologically confirmed TFL from the VU University Medical Center was described. These patients were uniformly treated in first line with rituximab containing induction therapy enabling 24 (of whom 15 were not R naïve at transformation) to reach consolidation with Zevalin-BEAM and up-front ASCT. After a median follow up of 20 months, 2 yr PFS was 80% and OS was 100% in these patients.

Chapter 6 reported the results of a multicenter retrospective trial evaluating ASCT after Zevalin-BEAM conditioning in patients with histologically confirmed TFL. Sixty-three patients were treated in four centers, median age 59.5 years, with a median of 2 prior treatments, 41 receiving ASCT in first line, 22 in second or third line (the 24 patients from chapter 5 were included in this analysis). Two year PFS and OS were encouraging at 68 and 90% and the regimen was well-tolerated (2 year NRM was 0%).

Chapter 7 described results of allogeneic transplantation after reduced-intensity conditioning with fludarabine/cyclophosphamide in low grade lymphomas, CLL, high grade lymphomas and TFL. Patients needed allogeneic transplantation for relapse after ASCT or multiple therapies. The TFL patients were heavily pretreated with a median of 3 treatment lines for FL, only 2 had received ASCT for their TFL, but all were chemosensitive preceding allogeneic SCT. A 4 year OS of 74% and EFS of 67% was reached in the 16 TFL patients included. Eleven of 16 developed chronic GvHD of whom 6 extensive.

In search for an alternative target for therapy for patients with refractory TFL, in Chapter 8 we showed that survivin expression in TFL is higher than in FL and comparable to DLBCL. Inhibition of survivin by YM155 induced apoptosis in TFL patient samples and TFL cell lines. Survivin expression levels in FL patients that developed TFL seemed to increase closer to the diagnosis of transformation. However, survivin expression levels did not increase over time in samples from FL patients that never transformed, suggesting a role for survivin in the process of transformation. Upregulation in FL might be predictive of subsequent transformation.

How to improve diagnosis of TFL?

"If you do not take a temperature, you cannot find a fever" (the Fat man in "the House of God" by S. Shem 1978). TFL has a better prognosis when diagnosed at an early stage (1,2).The most recent study showed a five year OS of 66% in patients with limited extent of disease versus 19% in patients with advanced extent of disease (advanced defined as stage III/IV or I/II with bulky disease (5)). To achieve this, awareness of the probability of transformation and its incidence (3% per year) is of utmost importance. When a patient with FL relapses, the possibility of transformation should always be considered. When suspicion of transformation arises, for example on clinical grounds like B symptoms, hypercalcemia, very large or extranodal disease, fast or discordant growth of lymph nodes or no response to FL therapy, this thesis provides evidence for performing a 18F-FDG PET scan. Although no study showed biopsies of all lymph nodes in a patient, we share the opinion that when the lymph node with the highest uptake is biopsied, it is most likely that it contains the transformed component (6,7). Thus 18F-FDG guided biopsy can improve diagnostic accuracy by minimizing sampling error. A high SUVmax or a high range in 18F-FDG uptake can support the suspicion of TFL and must even raise suspicion of transformation in a FL patient who does not have clinical signs pointing

towards transformation. Naturally we need to bear in mind that the cut off values found in chapter 2 have to be prospectively validated. 18F-FDG-PET is currently routinely used for staging and remission after therapy, using visual assessment, not SUV. However, for quantification of differences in uptake SUVs are more objective and reliable. Using SUVs requires a change in the way we (hematologists and nuclear physicians) interpret scans. That all nuclear medicine departments in the Netherlands strive to implement EANM guidelines, designed to optimize diagnostic quality and quantitative information (8), is a prerequisite for reliable quantification of uptake, making the use of SUV measurements in FL and TFL in everyday practice possible.

How to improve treatment of transformed lymphoma

To answer this the following questions are of importance:

1. How do we reach the highest PET negative complete remission rate?
2. If remission is reached, for whom do we need consolidation therapy to reach long term survival and if necessary which type of consolidation is superior?

Consolidation treatment

Starting with the second question, our goal must be to select the least toxic and most efficacious therapy. We have to identify patients who will be cured by immunochemotherapy only and patients that need consolidation with ASCT to reach long term survival. Currently the only data available are data from cohort and registry studies (including chapter 4). These data uniformly show previous treatment for FL as a negative prognostic factor for survival that might be overcome by up-front ASCT. In addition, it has been shown that treatment naïve patients do well after R-chemotherapy only (4,9,10). Based on literature and on our data, I would only consider to omit consolidation therapy in treatment-naïve patients, especially when they have other characteristics correlating with good prognosis like limited disease at diagnosis of TFL. All other patients I would advise consolidation with up-front ASCT. The only way to finally investigate first line therapy and the need for consolidation in a prospective and randomized manner, in a realistic time frame, is through (inter)national collaboration. Since studies randomizing between ASCT or no ASCT have been hampered by low accrual in the past, mostly due to a strong partiality of the treating physician for or against ASCT and a large difference in burden for the patient, this might prove impossible to carry out. The only alternatives are large population based registries or cohort studies,

carefully analyzing the influences of patient characteristics on survival. To create as much data as possible, transformation of FL should be registered when gathering data for population based registries and cooperation between countries is a prerequisite. Currently a European consortium is created to gather more data on FL and TFL patients.

If consolidation therapy has been decided on, the aim is to choose the most effective and least toxic therapy. Allogeneic SCT, although its efficacy is reasonable as described in chapter 7, infers too much toxicity to be used in first line, so autologous SCT (ASCT) is the better option for consolidation at this stage (11, chapter 7). The addition of radio-immunotherapy (RIT) to ASCT might prove beneficial. It has shown promising results (2 year OS 90-100%, PFS 68-80%) in TFL patients and is feasible (Chapter 5 and 6). Its benefit could come from the so called "crossfire" effect on CD20 negative tumor cells or CD20-positive cells that became resistant to anti-CD20 therapy, for example because of internalization of CD20 (12). These cells will be irradiated due to the tissue penetration of several millimeters by β particles attached to neighboring CD20-positive cells that were targeted. Hypothetically the cancer stem cell or common progenitor cells could be amongst those cells killed in the "crossfire". Long term follow up on the patients described in chapter 5 and 6 will show whether addition of RIT will be beneficial. Since in chapter 5 and 6 results using ^{90}Yttrium ibritumomab tiuxetan-BEAM and ASCT as consolidation were promising, especially in first line, currently we are conducting a prospective phase II trial with up-front ^{90}Yttrium ibritumomab tiuxetan -BEAM and ASCT in patients reaching at least PR after R-chemotherapy conditioning. In this protocol, the added benefit of ^{90}Yttrium ibritumomab tiuxetan consolidation in (mainly elderly) patients ineligible for ASCT is also investigated, providing insight in the efficacy of RIT only as consolidation.

Salvage after failed induction treatment

Before consolidation therapy is possible, reaching CR by induction therapy still remains an issue in TFL. Therefore, the first question the discussion was started with: "how to reach the highest PET negative remission rate", is still of utmost importance, even in the rituximab era. Currently the only possibility for long term survival in patients not in CR after induction therapy, is intensification of therapy and consolidation with an ASCT as described in chapter 4. However, in order to reach long term survival, sufficient remission after salvage therapy (preferably CR) is mandatory (13). In Chapter 4 we showed a 2 year OS of 86% in patients reaching CR before salvage ASCT versus 25% in patients reaching only PR or SD. Similar to relapsed/refractory DLBCL achieving this

CR is a major challenge. In general the combination of R combined with DHAP is used as salvage regimen. In both TFL and relapsed/refractory DLBCL 25-35% persistently refractory patients have been described and the regimen to improve on this remains to be found (13-16, Chapter 4). An alternative option might be to intensify the ASCT itself by adding RIT in patients with a suboptimal remission status before transplant, although higher doses than the conventional 0.4 mCi might be needed, depending on the depth of the remission reached, like has been used in DLBCL (17).
In addition, when CR has at last been reached following ASCT, consolidation with allogeneic SCT should be considered. For an allogeneic transplant to be successful chemo-sensitive disease is required as shown in chapter 7 (18,19). The remission reached by the ASCT allows time for the graft versus lymphoma effect to develop. Alternatively, allogeneic SCT could be reserved for a second relapse, where it might still lead to long term survival, as shown in chapter 7, although reaching sufficient remission again is a prerequisite and might prove impossible at this stage (18,19). This decision depends on the options that remain for re-induction and fitness of the patient.

What to do in persistent refractory disease?

For the 25-35% of patients completely refractory to all R-chemotherapy given, alternative treatments need to be found and explored. The motto here is: "to catch the bull, grab its horns". This implies selecting patients prone to resistance early in the course of their disease for different or more intensive therapy. To select patients with a less than optimal response to induction, interim PET might be useful. Patients not reaching PET negativity after 2-3 courses might be selected for studies with addition of more targeted treatments in order to reach a PET-negative CR before up-front ASCT. It has however to be taking into account that data on the predictive value of PET come from studies in DLBCL. It is clear that semi-quantitative analysis using SUV outperforms visual assessment, leading to less false positives. However, cut off values remain a difficult and controversial topic and defining these for TFL patients will require separate studies in these patients (20-22).
Lenalidomide has shown promising results in TFL, even as a single agent (23,24). These studies were conducted in heavily pretreated patients, reaching a 60% ORR. Promising results and feasibility have been shown with addition of lenalidomide to R-CHOP and R-ICE, in patients with DLBCL(25,26).
Other drugs inhibiting novel targets like Pidilizumab (anti PD-1monoclonal antibody) and alisertib (aurora A kinase inhibitor) were tested in patient groups containing 20 and 10% TFL patients respectively, with encouraging results (27,28). Alternative targets

worth studying in TFL are Bruton tyrosine kinase inhibitors (29), PI3Kinase inhibitors (30), demetylating agents and bcl-2 inhibitors (31). In chapter 8 we investigated survivin as a target for therapy, with promising effects of the small molecule survivin inhibitor YM155 in TFL patient samples and cell lines.

Idea(l)s for the future

The main contribution of 18F-FDG PET will be a fast and accurate diagnosis of TFL: supporting suspicion of transformation, guiding biopsy of the transformed lymph node, leading to a histological diagnosis.
18F-FDG PET will not abrogate the need for biopsy. When more experience with quantitative assessment has lead to prospectively validated cut off levels with a high sensitivity (preferably 100%) for transformation, quantifying uptake of 18F-FDG at each relapse of FL could provide us with additional evidence supporting the possibility or improbability of transformation. In FL patients with SUVs lower than the threshold with 100% sensitivity for transformation, TFL is very unlikely and biopsy will be unnecessary. When FL patients at relapse do have SUVs higher than the threshold for TFL, a biopsy will be necessary to rule out or diagnose transformation.
Another important reason for biopsy in the future is that biopsy material will most likely be needed not only for diagnosis but also to find druggable targets.
For the future, interim PET might be an interesting additional tool to select patients in complete remission in whom consolidation therapy might be omitted, since the negative predictive value of a negative interim PET-scan is high. It might also be used to select patients with a less than optimal response to induction with R-chemotherapy who need additional targeted therapy or intensified therapy (ASCT). However, data have been acquired in patients with DLBCL and cut off values remain a difficult and controversial topic. Applicability of interim PET scanning and definition of cut off values will require separate studies in TFL patients (20).
Due to the high rate of refractoriness to all currently available treatments, the future of TFL will require the identification of promising new targets for therapy, preferably individualised. The new techniques (for instance next generation sequencing) will enable implementation of genetic analysis in daily practice, identifying the pathways most essential to resistance in a patient. 18F-FDG PET could help locate the resistant lymph node (i.e. growing or with increasing or persistent uptake despite therapy) which could then be sampled and analyzed.

Currently not only registry of patients with TFL is important to identify the most effective treatment, but specifically molecular characterization (using biopsy materials) will be of importance, as transformation of FL is a complex process with many biological explanations. Therefore large RCTs will probably not answer the question: "what is the best treatment" for this heterogeneous disease. It might prove more fruitful to link clinical data from registries with biological data available from patients with TFL. This combined approach will hopefully enable individualized therapy in this heterogeneous disease.

References

1. Bastion Y, Sebban C, Berger F et al. Incidence, predictive factors, and outcome of lymphoma transformation in follicular lymphoma patients. J Clin Oncol. 1997;15(4):1587-1594
2. Yuen A, Kamel OW, Halpern J, Horning SJ. Long-term survival after histologic transformation of low-grade follicular lymphoma. J Clin Oncol 1995;13:1726-1733
3. Eide MB, Lauritzsen GF, Kvalheim G et al. High dose chemotherapy with autologous stem cell support for patients with histologically transformed B-cell non Hodgkin Lymphomas. A Norwegian multi center phase II study. Br J Haematol. 2011;152(5):600-610
4. Villa D, Crump M, Panzarella T et al. Autologous and allogeneic stem-cell transplantation for transformed follicular lymphoma: a report of the Canadian blood and marrow transplant group. J Clin Oncol. 2013;31(9):1164-1171
5. Al-tourah AJ, Gill KK, Chhanabhai M et al. Population based analysis of incidence and outcome of transformed non-Hodgkin's lymphoma. J Clin Oncol. 2008:26(32):3272-3278
6. Bodet-Millin C, Kraeber-Bodéré F, Moreau P, Campion L, Dupas B, Le Gouill S. Investigation of FDG-PET/CT imaging to guide biopsies in the detection of histological transformation of indolent lymphoma. Haematologica 2008;93: 471-472.
7. Casulo C, Burack WR, Friedberg JW. Transformed follicular non-Hodgkin lymphoma. Blood 2015;125:40-47.
8. Boellaard R, O'Doherty MJ, Weber WA et al. FDG PET and PET/CT: EANM procedure guidelines for tumour PET imaging: version 1.0. Eur J Nucl Med Molec Imaging. 2010;37:181-200
9. Ban-Hoeffen M, Vanderplas A, Crosby-Thompson AL et al. Transformed non-Hodgkin lymphoma in the rituximab era: analysis of the NCCN outcomes database. Br J Haematol. 2013;163(4):487-495
10. Lerch K, Meyer AH, Stroux A et al. Impact of prior treatment on outcome of transformed follicular lymphoma and relapsed diffuse large B cell lymphoma: a retrospective multicenter analysis. Ann Hematol 2015; 981-988
11. Pallua S, Giesinger J, Oberguggenberger A et al. Impact of GvHD on quality of life in long-term survivors of haematopoietic transplantation. Bone Marrow Transplant 2010:1-6
12. Beers SA, French RR, Chan HTC et al. Antigenic modulation limits the efficacy of anti CD20 antibodies: implications for antibody selection. Blood 2010;115(25):5191-5201
13. Williams CD, Harrison CN, Lister TA et al. European Bone Marrow Transplant Lymphoma Working Party. High-dose therapy and autologous stem-cell support for chemosensitive transformed low-grade follicular non-hodgkin's lymphoma: a case matched study from the European Bone Marow Transplant Registry. J Clin Oncol. 2001;19(3):727-735.
14. Foran JM, Apostolidis J, Papamichael D et al. High-dose therapy with autologous haematopoietic support in patients with transformed follicular lymphoma: a study of 27 patients from a single centre. Ann Oncol 1998;9(8):865-869
15. Da Villa D, Crump M, Keating A, Panzarella T, Feng B, Kuruvilla J. Outcome of patients with transformed indolent non-Hodgkin lymphoma referred for autologous stem-cell transplantation. Ann Oncol. 2013;24(6):1603-1609.
16. Gisselbrecht C, Glass B, Mounier N et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol 2010;28(27):4184-4190
17. Nademannee A, Forman S, Molina A et la. A phase ½ trial of high-dose Yttrium-90- ibritumomab tiuxetan in combination with high-dose etoposide and cyclophosphamide followed by autologous stem cell transplantation in patiens with poor risk or relapsed non-Hodgkin lymphoma. Blood 2005;106:2896-2902
18. Thomson KJ, Morris EC, Bloor A, Cook G, Milligan D, Parker A et al. Favourable long term survival after reduced-intensity alloSCT for multiple-relapse aggressive NHL. *J Clin Oncol* 2008;27:426-432
19. Clavert A, le Gouill S, Brissot E et al. Reduced-intensity conditioning allogeneic stem cell transplant for relapsed or transformed aggressive B-cell non-Hodgkin lymphoma. Leuk Lymphoma 2010;51(8):1502-1508
20. Moskowitz CH. Interim PET CT in the management of diffuse large B cell lymphoma. Hematology Am Soc Hematol Educ Program 2012;2012:397-401
21. Pardal E, Coronado M, Martin A et al. Intensification treatment based on early FDG PET in patients with high risk diffuse large B cell lymphoma: a phase II Geltamo study. Br J Haematol 2014;167:327-336
22. Carr R, Fanti S, Paez D et al. Prospective international cohort study demonstrates inability of interim PET to predict treatment failure in diffuse large B cell lymphoma. J Nucl Med 2014;55(12):1936-1944

23. Vose JM, Habermann TM, Czuczman MS et al. Single agent lenalidomide is active in patients with relapsed or refractory aggressive non Hodgkin lymphoma who received prior stem cell transplantation. Br J Haematol.2013;162(5): 639-647

24. Czuczman MS, Vose JM, Witzig TE et al. The differential effect of lenalidomide monotherapy in patients with relapsed or refractory transformed non-Hodgkin lymphoma of distinct histological origin. Br J Haematol. 2011:154(4):477-481

25. Feldman T, Mato AR, Chow KF et al. Addition of lenalidomide to rituximab, ifosfamide, carboplatin, etoposide (RICER) in first-relapse/primary refractory diffuse large B cell lymphoma. Br J Haematol 2014;166(1):77-83

26. Nowakowski GS, Laplant B, Macon WR et al. Lenalidomide combined with R-CHOP overcomes negative prognostic impact of non-germinal center B cell phenotype in newly diagnosed diffuse large B cell lymphoma: a phase II study. J Clin Oncol 2014;33: 251-257

27. Armand P, Nagler A, weller EA et al. Disabling immune tolerance by programmed death-1 blockade by pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B cell lymphoma: results of an international phase II trial. J Clin Oncol. 2013:31(33):4199-4206

28. Friedberg JW, Mahadevan D, Cebula E et al. Phase II study of alisertib, a selective Aurora A kinase inhibitor, in relapsed and refractory aggressive B- and T-cell non Hodgkin lymphomas. J Clin Oncol 2014;32(1):44-50

29. Advani RH, Buggy JJ, Sharman JP et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B cell malignancies. J Clin Oncol 2013;31(1):88-94

30. Gopal AK, Kahl BS, de Vos S et al. PI3K inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med 2014;370(11):1008-1018

31. Seymour JF, Davids MS, Pagel JM et al. Bcl-2 inhibitor ABT-199(GDC-0199) monotherapy shows anti tumor activity including complete remissions in high-risk relapsed/refractory chronic lymphocytic leukemia CLL) and small lymphocytic lymphoma (SLL) (abstract) Blood 2013;122(21):872