Chapter 1

Introduction and scope of the thesis
Chapter 1

Introduction
Transformation is the development of high-grade, aggressive non-Hodgkin lymphoma (mostly diffuse large B cell lymphoma: DLBCL) in patients with an underlying indolent lymphoma. Transformation has been described in all subtypes of indolent B cell lymphoma (1). However, since follicular lymphoma is the most common indolent lymphoma, the incidence of transformation of follicular lymphoma enables studies on risk factors and outcome. Therefore, the focus of this thesis is transformation of follicular lymphoma.

Follicular lymphoma (FL) is the most common indolent B cell lymphoma worldwide, comprising approximately 20% of all non-Hodgkin lymphomas. The clinical course of FL is characterized by a continuous pattern of relapses with repeated but transient responses to therapy, resulting in a median overall survival (OS) of more than 10 years. However, there is a pronounced variability in OS, ranging from months to decades (2). A high follicular lymphoma prognostic index (FLIPI) and several clinical factors such as bulky disease, B symptoms and short response to therapy predict an aggressive disease course, including transformation of FL (3). However, the discriminative power has not been found consistent enough yet to guide treatment decisions.

Incidence of transformation of follicular lymphoma
The incidence of transformation in patients with FL varies considerably due to the use of different diagnostic methods, definition of transformation and the duration of follow up. The most recent studies, in which in the majority of patients the diagnosis was biopsy proven, report an incidence of 2% to 3% per patient per year (4,5). While Al Tourah et al report an ongoing risk of transformation, most authors have reported that the frequency of transformation is highest during the first years after diagnosis of FL (5) or even reaches a plateau after approximately 15 years (4,6-8).

Although the incidence of transformation in the rituximab era appears not to be substantially different from the pre-rituximab era, recently it was described by the authors of the National LymphoCare study that the incidence was less in patients who received rituximab maintenance therapy, however, transformation still occurs (4,6,7,9-11). Most reports show that therapy early in the course of FL, or specific therapies (i.e. anthracyclines) do not meaningfully influence transformation risk (4,8,9,12). In conclusion, transformation of FL is an ongoing issue in the rituximab era.

Prediction of transformation of follicular lymphoma
Several clinical factors are associated with a higher risk of transformation, such as
advanced stage, high FLIPI, high LDH, older age, high β2 microglobulin, but none of these criteria, either alone or in risk models, can unequivocally predict transformation (4-7,9).

Additionally gene expression- and immunohistochemical studies of FL have identified characteristics of the micro-environment that predict FL progression and transformation. The presence, distribution pattern and molecular expression of the tumor infiltrating immune cells, such as T-lymphocytes, monocytes and dendritic cells, was found to be associated with outcome of FL (13-16) and transformation risk (17-19). However, results are not consistent enough to guide targeted treatment or actual treatment aimed at reducing transformation risk. This would require a more accurate prediction of transformation and further unraveling of molecular mechanisms responsible for transformation. Hopefully, integration of not only clinical data and biomarkers, but also other modalities like imaging will enable this in the future.

**Diagnosis of transformation of follicular lymphoma**

Transformation of FL is usually suspected on clinical and biochemical parameters, such as a sudden deterioration in performance status, development of B-symptoms, rapid discordant localized nodal growth or new extranodal disease, a rapid increase in LDH or hypercalcaemia. Biopsy of a lymph node (or other involved tissue) is imperative when suspicion of transformation arises (4,6,7), as histology is the gold standard for diagnosis of transformation. Histology will show aggressive NHL, mostly resembling DLBCL.

Development of TFL does not generally occur in all lymph nodes simultaneously. As a result lymph nodes containing FL coexist with lymph nodes containing DLBCL in the same patient. This creates the potential for sampling errors (biopsy of a non-transformed lymph node) which often creates a significant diagnostic delay or even leads to missing the diagnosis of transformation. Diagnosis early in the course of the disease improves outcome of TFL, probably because of better outcome of limited stage disease (4,6,20). Therefore, there is a need for a non-invasive technique indicating the lymph node of interest (i.e. the transformed lymph node), to minimize the chance of sampling errors and time to diagnosis.

**Positron emission tomography and diagnosis of transformation**

PET-CT is a non-invasive imaging technique of the whole body allowing quantitative assessment of biochemical and functional processes. It measures annihilated photons
that are emitted by positron emitter labelled radiotracers. Depending on the tracer used, areas with for instance high metabolic and/or proliferative activity can be identified.

The concurrent CT scan is used to anatomically identify the organs or tumors showing uptake of the tracer. Thus, a PET-CT scan can provide information on tumor localization and tumor activity. This activity can be quantified visually by comparing the uptake in the tumor to uptake in the liver, mediastinal blood pool or background (figure 1).

Figure 1 A: 18F-FDG PET scan. B 18F-FLT PET scan
Arrows: examples of enlarged lymph nodes with increased uptake. Uptake in the lymph nodes can be compared visually to uptake in the liver, mediastinal blood pool, or background.

or quantified using standardised uptake value (SUV). SUV is a measurement of tumor uptake normalised for distribution volume. It is calculated using the administered activity of the tracer (depending on dose and physical decay of the tracer) and the body weight of the patient and can be corrected for blood glucose as follows (21):

\[
SUV = \frac{\text{activity measured in the volume of interest (kBq/ml) \times glucose patient (mmol/l)}}{\text{administered activity (MBq)/ body weight (kg) \times 5.0 mmol/l}}
\]

SUV can quantify differences in uptake more objectively than visual analysis, and is therefore better for assessing response or for comparing uptake between lymph nodes (22). SUVmax is the SUV in the voxel with maximum uptake and is used in the studies described in this thesis. SUV measurements are most reliable within one patient, at a certain point in time using the same settings on one PET scanner. When SUV is measured in different patients, at different times in the same patient, or on different scanners,
numerous variables can influence SUV. To minimize variability, regular calibration of scanners, the use of standardised reconstruction and data analysis methods and measuring patient parameters like body weight and glucose are necessary (21,23).

The most commonly used tracer is 18-fluorodeoxyglucose (18F-FDG), which accumulates in cells with increased cellular uptake of glucose. After uptake in the cell, 18F-FDG is phosphorylated by hexokinase but cannot be glycolised. As a consequence 18F-FDG is essentially trapped in the cell (figure 2). Currently, 18F-FDG PET is used for staging and response evaluation both in aggressive and in more indolent types of lymphoma (24).

Proliferative activity as measured by Ki67 has been shown to correlate with 18F-FDG uptake (25). Accordingly, there is a clear trend towards higher 18F-FDG uptake in more aggressive histological subtypes (26-28). Therefore, a high uptake in an indolent lymphoma supports the suspicion of transformation. However, there is a considerable overlap in SUV of 18F-FDG between aggressive and indolent lymphomas potentially impairing its use in detecting transformation. Taking into consideration that values were obtained in multiple centers, consequently on different PET scanners and in patients with different indolent lymphoma histologies, proposed SUVs predicting for aggressive histology vary from 10-14 (26-29). In spite of the high sensitivities and specificities that were reported in these series, pronounced variability was found when uptake was compared between studies: in one report up to 37% of transformed patients had

**Figure 2: mechanism of tracer uptake:**

Tracer-P = phosphorylated tracer
- for 18F-FDG = tracer
  A: glucose transporter protein
  B: hexokinase
  C: glycolysis
- for 18F-FLT = tracer:
  A: facilitated diffusion via carrier proteins correlating with activity of B
  B: thymidine kinase-1
  C: DNA synthesis
a SUVmax below 10 (29), in another no transformed patient showed a SUVmax below 11.7 (28). This hampers the use of 18F-FDG and SUVmax for detection of transformation in clinical practice. In an attempt to improve on this, the use of alternative tracers can be explored. Conceptually, fluorothymidine (18F-FLT) reflects proliferation more closely than 18F-FDG (30,31). The presumed mechanism of 18F-FLT imaging is uptake in proliferating cells via the pyrimidine salvage pathway, mostly during S-phase (32). After phosphorylation by thymidine kinase 1 (TK-1), a principal enzyme in the DNA-salvage pathway, 18F-FLT is trapped in the cell (Figure 4). There is limited data about 18F-FLT uptake in both aggressive and indolent lymphoma, however hardly any data are available on the value of 18F-FLT-PET in patients with transformed FL (33,34).

In this thesis we compared the diagnostic accuracy of 18F-FDG and 18F-FLT to detect transformation (Chapter 2). As the uptake is known to vary for different indolent lymphoma types (26-29) we only included patients with FL and transformation of FL. We additionally explored the range in uptake defined as the difference between the SUVmax of the lymph node with the highest and the lowest uptake. This might better represent the intra-patient heterogeneity in TFL (lymph nodes with indolent and lymph nodes with aggressive histology co-existing in one patient) than only a SUVmax of the lymph node with the highest uptake. Moreover, it circumvents the problem of comparing values between different patients and scanners. In chapter 3 we further investigated why 18F-FLT uptake in FL does not seem to correlate with proliferation, which we concluded from the experiments described in chapter 2.

**Prognosis of transformed FL**

Historically, the prognosis of transformed follicular lymphoma is very poor, with a median OS ranging from 0.7 to 2.7 years only (1,4,6,8,9,20). The adverse effect of transformation on the survival of patients with FL is illustrated by the 10 year overall survival being 75% in FL patients versus 36% in transformed FL patients (4). Patients with limited disease, a complete response to treatment and those being chemotherapy naïve at the time of transformation fared better (4,9,20,35-37). Late transformation (>18 months after FL diagnosis) might also be associated with better survival, although, since it was only reported in one cohort of 60 TFL patients this finding will need to be confirmed (5).

The introduction of rituximab in the treatment of TFL has clearly improved survival, with a reported median survival up to 50 months (ref. 5,35,38, describing 60,118 and 172 patients with TFL respectively).

In chapter 4, survival of 161 TFL patients in the Netherlands in the rituximab era is described using data from the Population based Haematological Registry for
Observational Studies (PHAROS), covering 40% of the Dutch population. The data from PHAROS are sufficiently detailed to allow specific analyses on the prognostic value of patient- and disease related factors as well as the outcome reached with different treatment regimens. Moreover, by using data from a population based registry the selection bias that is present in observational or cohort studies is minimised (35,38).

Treatment

Treatment of FL
Because the choice of treatment for TFL is often dictated by previous treatment for FL, a brief outline of treatment guidelines for FL in the Netherlands is given here. Stage I FL is generally treated by local radiotherapy with curative intent (40Gy). Newly diagnosed FL stage II-IV is, when possible, initially managed with a watch and wait policy. If B symptoms, bulky disease, organomegaly or cytopenia because of bone marrow invasion arise, R-chemotherapy is given: R-CVP (rituximab, cyclophosphamide, vincristine and prednisone) for fit patients and R-chlorambucil for unfit patients. As second line treatment R-Fludarabine is generally used, however, in case of a late relapse (occurring >2 years after the end of previous treatment) the initial regimen is often being repeated. For subsequent relapses, there are no specific guidelines. At that stage anthracyclines can be used, especially as induction therapy for autologous or allogeneic transplant. Although the use of rituximab is implemented at every stage of the disease, the use of rituximab maintenance therapy varies. In some centers rituximab maintenance is given following first line, based on the data from the PRIMA study (39). However, because long term follow-up of the PRIMA study showed no OS advantage, which suggests that re-treatment at relapse is as effective as maintenance therapy (40), many Dutch centers now mainly use maintenance therapy with rituximab after second line treatment (www.hovon.nl). International guidelines are similar, although in many countries anthracyclines are incorporated more frequently in first line with the goal of prolonging progression free survival (41).

Overview of studies on treatment of transformed FL
Patients with TFL are often excluded both from first line DLBCL studies and from indolent lymphoma studies resulting in a scarcity of objective data upon which treatment decisions can be based. To check the number and type of studies that have been published on treatment of transformed follicular lymphoma we performed a search using Pubmed and Embase®. This search was done by two independent investigators (DE Issa and M)
Chapter 1

Wondergem) selecting all studies on therapeutic feasibility and/or efficacy including at least one adult patient with transformed B cell non-Hodgkin's lymphoma. Studies on transformed B-CLL patients (Richters' transformation), all reviews, case reports, guidelines and studies published as abstract only were excluded. This resulted in 107 articles, 62 with a separate analysis of outcome in TFL (table 1, ref 5,35,38, 42-100). There was a complete lack of randomized clinical trials. Studies were mainly retrospective (74%). As currently R is available for the treatment of TFL we first excluded all studies in which no R was given (n=30, grey in table 1), therefore 32 studies remained. Of these there were only 11 studies in which exclusively TFL patients were included, containing 660 patients in total (mean 60 per study, range 8-172). From these 11, we were the only studygroup exclusively describing the outcome of first line treatment, being described in chapter 5. There were 2 studies only investigating patients with relapsed disease, both about allogeneic SCT.

In these heavily pretreated patient population 5 year OS was 23 and 66% (ref 95 and 91 resp.). The remaining 8 studies described a heterogeneous patient population, both newly diagnosed and relapsed patients. This precludes cross trial comparisons and specific statements on PFS and OS. The details of these studies are described below. In conclusion no RCTs are published on first line therapy in TFL, consequently information guiding first line treatment must be deduced.

*: (‘Lymphoma, Non-Hodgkin’[Mesh] AND (transform* [tw])) AND
### Table 1: studies with separate analysis of outcome in TFL

<table>
<thead>
<tr>
<th>Therapy</th>
<th>author</th>
<th>year of publication</th>
<th>TFL patients</th>
<th>RS/P</th>
<th>U/R</th>
<th>Phase</th>
<th>therapy detail</th>
<th>ORR</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>chemotherapy</td>
<td>Churpek 2013</td>
<td>5(10)</td>
<td>P R 2</td>
<td>ixabepilone</td>
<td>40</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Czuczman 2011</td>
<td>23(70)</td>
<td>P R 2</td>
<td>lenalidomide</td>
<td>56,5</td>
<td>(26,1/30,4)</td>
<td>med</td>
<td>12.8 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>el Helw 2000</td>
<td>4(7)</td>
<td>P R 2</td>
<td>vincristine, epirubicine, dexamethasone</td>
<td>100 (25/75)</td>
<td>2 yr 75%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2006</td>
<td>15(20)</td>
<td>P R 2</td>
<td>bendamustine</td>
<td>66 (13/53)</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2013</td>
<td>5(10)</td>
<td>P R 2</td>
<td>alisertib</td>
<td>40%</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Czuczman 2011</td>
<td>23(70)</td>
<td>P R 2</td>
<td>lenalidomide</td>
<td>56,5</td>
<td>(26,1/30,4)</td>
<td>med</td>
<td>12.8 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>el Helw 2000</td>
<td>4(7)</td>
<td>P R 2</td>
<td>vincristine, epirubicine, dexamethasone</td>
<td>100 (25/75)</td>
<td>2 yr 75%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2006</td>
<td>15(20)</td>
<td>P R 2</td>
<td>bendamustine</td>
<td>66 (13/53)</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2013</td>
<td>5(10)</td>
<td>P R 2</td>
<td>alisertib</td>
<td>40%</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Czuczman 2011</td>
<td>23(70)</td>
<td>P R 2</td>
<td>lenalidomide</td>
<td>56,5</td>
<td>(26,1/30,4)</td>
<td>med</td>
<td>12.8 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>el Helw 2000</td>
<td>4(7)</td>
<td>P R 2</td>
<td>vincristine, epirubicine, dexamethasone</td>
<td>100 (25/75)</td>
<td>2 yr 75%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2006</td>
<td>15(20)</td>
<td>P R 2</td>
<td>bendamustine</td>
<td>66 (13/53)</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2013</td>
<td>5(10)</td>
<td>P R 2</td>
<td>alisertib</td>
<td>40%</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Czuczman 2011</td>
<td>23(70)</td>
<td>P R 2</td>
<td>lenalidomide</td>
<td>56,5</td>
<td>(26,1/30,4)</td>
<td>med</td>
<td>12.8 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>el Helw 2000</td>
<td>4(7)</td>
<td>P R 2</td>
<td>vincristine, epirubicine, dexamethasone</td>
<td>100 (25/75)</td>
<td>2 yr 75%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2006</td>
<td>15(20)</td>
<td>P R 2</td>
<td>bendamustine</td>
<td>66 (13/53)</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2013</td>
<td>5(10)</td>
<td>P R 2</td>
<td>alisertib</td>
<td>40%</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Czuczman 2011</td>
<td>23(70)</td>
<td>P R 2</td>
<td>lenalidomide</td>
<td>56,5</td>
<td>(26,1/30,4)</td>
<td>med</td>
<td>12.8 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>el Helw 2000</td>
<td>4(7)</td>
<td>P R 2</td>
<td>vincristine, epirubicine, dexamethasone</td>
<td>100 (25/75)</td>
<td>2 yr 75%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2006</td>
<td>15(20)</td>
<td>P R 2</td>
<td>bendamustine</td>
<td>66 (13/53)</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2013</td>
<td>5(10)</td>
<td>P R 2</td>
<td>alisertib</td>
<td>40%</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Czuczman 2011</td>
<td>23(70)</td>
<td>P R 2</td>
<td>lenalidomide</td>
<td>56,5</td>
<td>(26,1/30,4)</td>
<td>med</td>
<td>12.8 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>el Helw 2000</td>
<td>4(7)</td>
<td>P R 2</td>
<td>vincristine, epirubicine, dexamethasone</td>
<td>100 (25/75)</td>
<td>2 yr 75%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2006</td>
<td>15(20)</td>
<td>P R 2</td>
<td>bendamustine</td>
<td>66 (13/53)</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2013</td>
<td>5(10)</td>
<td>P R 2</td>
<td>alisertib</td>
<td>40%</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Czuczman 2011</td>
<td>23(70)</td>
<td>P R 2</td>
<td>lenalidomide</td>
<td>56,5</td>
<td>(26,1/30,4)</td>
<td>med</td>
<td>12.8 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>el Helw 2000</td>
<td>4(7)</td>
<td>P R 2</td>
<td>vincristine, epirubicine, dexamethasone</td>
<td>100 (25/75)</td>
<td>2 yr 75%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2006</td>
<td>15(20)</td>
<td>P R 2</td>
<td>bendamustine</td>
<td>66 (13/53)</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friedberg 2013</td>
<td>5(10)</td>
<td>P R 2</td>
<td>alisertib</td>
<td>40%</td>
<td>ng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Czuczman 2011</td>
<td>23(70)</td>
<td>P R 2</td>
<td>lenalidomide</td>
<td>56,5</td>
<td>(26,1/30,4)</td>
<td>med</td>
<td>12.8 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>el Helw 2000</td>
<td>4(7)</td>
<td>P R 2</td>
<td>vincristine, epirubicine, dexamethasone</td>
<td>100 (25/75)</td>
<td>2 yr 75%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: ORR = overall response rate; OS = overall survival; U = unclassified; R = randomized.
Table 1: continued

<table>
<thead>
<tr>
<th>Therapy</th>
<th>author</th>
<th>TFL patients</th>
<th>RS/P</th>
<th>U/R</th>
<th>Phase</th>
<th>therapy detail</th>
<th>ORR</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chen 2001</td>
<td>35(100)</td>
<td>RS</td>
<td>R</td>
<td>n/a</td>
<td>eto-mel-TBI</td>
<td>100(74/26)</td>
<td>5 yr 37%</td>
</tr>
<tr>
<td></td>
<td>Eide 2010</td>
<td>30(100)</td>
<td>P</td>
<td>R</td>
<td>2</td>
<td>BEAM</td>
<td>83 (60/23)</td>
<td>5 yr 47%</td>
</tr>
<tr>
<td></td>
<td>Foran 1998</td>
<td>27(100)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>Cy-TBI</td>
<td>100 (31/69)</td>
<td>5 yr 52%</td>
</tr>
<tr>
<td></td>
<td>Le Gouil 2002</td>
<td>5(33)</td>
<td>P</td>
<td>R</td>
<td>2</td>
<td>HDT cy-TBI</td>
<td>100</td>
<td>2 yr 82%</td>
</tr>
<tr>
<td></td>
<td>Hamadani 2008</td>
<td>24(100)</td>
<td>RS</td>
<td>R</td>
<td>n/a</td>
<td>Bu-Cy/BEAM/CBV</td>
<td>74 (66/8)</td>
<td>5 yr 52%</td>
</tr>
<tr>
<td></td>
<td>Holman 2012</td>
<td>2(15)</td>
<td>P</td>
<td>R</td>
<td>2</td>
<td>various</td>
<td>100</td>
<td>4 yr 50%</td>
</tr>
<tr>
<td></td>
<td>Kasamon 2011</td>
<td>12(15)</td>
<td>RS</td>
<td>R</td>
<td>n/a</td>
<td>bu-cy or cy-TBI purged</td>
<td>ng</td>
<td>4 yr 58%</td>
</tr>
<tr>
<td></td>
<td>Laudi 2004</td>
<td>9(14)</td>
<td>RS</td>
<td>R</td>
<td>n/a</td>
<td>various</td>
<td>ng</td>
<td>4 yr 44%</td>
</tr>
<tr>
<td></td>
<td>Sabloff 2007</td>
<td>23(17)</td>
<td>RS</td>
<td>R</td>
<td>n/a</td>
<td>BEAM or CBV</td>
<td>60 (40/20)</td>
<td>5 yr 56%</td>
</tr>
<tr>
<td></td>
<td>Schouten 1989</td>
<td>10(56)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>various</td>
<td>88 (88/0)</td>
<td>4 yr 66%</td>
</tr>
<tr>
<td></td>
<td>Smith 2009</td>
<td>25(45)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>bu-eto-cy</td>
<td>ng</td>
<td>4 yr 64%</td>
</tr>
<tr>
<td></td>
<td>Villa 2013</td>
<td>105(100)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>ASCT eto/mel/tbi</td>
<td>48 reaches AuSCT</td>
<td>3 yr 52%</td>
</tr>
<tr>
<td></td>
<td>Williams 2001</td>
<td>50(100)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>ASCT various</td>
<td>77 (62/15)</td>
<td>5 yr 51%</td>
</tr>
<tr>
<td></td>
<td>Clavert 2010</td>
<td>14(73)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>flu-bu-ATG</td>
<td>78 (71/7)</td>
<td>4 yr 71%</td>
</tr>
<tr>
<td></td>
<td>Doocy 2005</td>
<td>16(36)</td>
<td>RS</td>
<td>R</td>
<td>n/a</td>
<td>cy-TBI-eto</td>
<td>74 (37/37)</td>
<td>5 yr 35%</td>
</tr>
<tr>
<td></td>
<td>Hamadani 2008</td>
<td>8(100)</td>
<td>RS</td>
<td>R</td>
<td>n/a</td>
<td>various RIST</td>
<td>88 (88/0)</td>
<td>4 yr 66%</td>
</tr>
<tr>
<td></td>
<td>Khouri 1998</td>
<td>2(15)</td>
<td>RS</td>
<td>R</td>
<td>n/a</td>
<td>fludarabine based</td>
<td>100 (50/50)</td>
<td>2 yr 0%</td>
</tr>
<tr>
<td></td>
<td>Mortensen 2012</td>
<td>16(40)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>flu-TBI</td>
<td>ng</td>
<td>5 yr 78%</td>
</tr>
<tr>
<td></td>
<td>Novitzky 2007</td>
<td>11(28)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>various + alemtuzumab</td>
<td>ng</td>
<td>5 yr 68%</td>
</tr>
<tr>
<td></td>
<td>Ramadan 2008</td>
<td>40(100)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>various myeloablative</td>
<td>87 (32/57)</td>
<td>5 yr 23%</td>
</tr>
<tr>
<td></td>
<td>Rezvani 2008</td>
<td>16(26)</td>
<td>RS</td>
<td>R</td>
<td>n/a</td>
<td>flu-TBI</td>
<td>ng</td>
<td>3 yr 18%</td>
</tr>
<tr>
<td></td>
<td>Thomson 2008</td>
<td>18(38)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>flu-mel-alemtuzumab</td>
<td>ng</td>
<td>4 yr 61%</td>
</tr>
<tr>
<td></td>
<td>multiple therapies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R-CHOP-ASCT-alloSCT all together</td>
<td>2yr 68%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ban Hoef fen 2013</td>
<td>118(100)</td>
<td>RS</td>
<td>n/a</td>
<td></td>
<td>R-CHOP-ASCT-alloSCT all together</td>
<td>2yr 68%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Morley 2008</td>
<td>63(100)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>CHOP-ASCT-no treatment</td>
<td>ng</td>
<td>2 yr 59%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24(100)</td>
<td></td>
<td></td>
<td></td>
<td>CHOP group separate</td>
<td>63%</td>
<td>2 yr 74%</td>
</tr>
<tr>
<td></td>
<td>Reddy 2012</td>
<td>51(100)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>ASCT/alloSCT</td>
<td>100(69/31)</td>
<td>2 yr 65%</td>
</tr>
<tr>
<td></td>
<td>Villa 2013</td>
<td>172(100)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>RC vs auto vs allo</td>
<td>RC: ASCT alloSCT</td>
<td>5 yr 61%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RC vs auto vs allo</td>
<td>5 yr 65%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Link 2013</td>
<td>60(100)</td>
<td>RS</td>
<td>U+R</td>
<td>n/a</td>
<td>RC and ASCT</td>
<td>RC: ASCT alloSCT</td>
<td>5 yr 46%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RC and ASCT</td>
<td>RC: ASCT alloSCT</td>
<td>5 yr 66%</td>
</tr>
</tbody>
</table>
Introduction and scope of this thesis

RS=retrospective, P=prospective
U=up-front/1st line treatment for TFL,
R=relapse/2nd line treatment for TFL
R=rituximab RC=R-chemo
ng= not given for transformed lymphoma patients
n/a = not applicable

Treatment options for transformed lymphoma

Rituximab-chemotherapy
The introduction of rituximab to TFL therapy significantly improved OS, with median survival up to 50 months; a major improvement considering the historically poor prognosis of 0.7-2.7 years. The backbone of rituximab therapy in TFL is usually CHOP, or in patients already treated with anthracyclines, regimens containing either high dose cytarabine or cisplatinum or topoisomerase II inhibitors in combination with alkylating therapy. Rituximab appears especially effective if patients are rituximab naïve at transformation (101).

Consolidation therapy
Although the use of rituximab in combination with the drugs described above is generally accepted in TFL, the use of up-front autologous or even allogeneic SCT following the achievement of complete remission is currently not clear. In contrast, in patients not reaching CR after induction chemotherapy, being described in up to 30-40% of patients (77,87), the only chance of long term survival is when re-induction therapy results in a sufficient response to allow consolidation with a salvage SCT. The absence of long term survival of patients refractory to induction and salvage chemotherapy (9,20,chapter 4) demonstrates the urgent need for therapies other than rituximab and/or chemotherapy or radiotherapy (see “other treatments” below).

Autologous SCT
ASCT was introduced as a treatment for TFL in the pre-rituximab era to improve inferior outcome after chemotherapy only. Five year OS in TFL patients improved from 20-30% after chemotherapy only to 40-60% with up-front ASCT (9,20,76,84,88,101). This prompted many centers to adopt ASCT as first line therapy for TFL. However, the role of up-front ASCT in the rituximab era is subject of debate.
Two large comparative cohort studies reported a significantly better OS after ASCT, albeit only slightly better than the survival after R-chemo only. (Ban Hoeffen 2 yr OS 74% after ASCT vs 59% after R-chemo only, Da villa 5 yr 65% vs 61%, respectively, ref. 35,38). An important observation from these studies was that previous treatment of FL negatively influenced prognosis. This might be the group of patients benefitting from ASCT, since it has been reported that, when treated with R-chemotherapy only, treatment-naïve patients did much better than patients previously treated for FL (2 yr OS 81% vs 39%). This difference in survival was not observed when up-front ASCT was applied (35). In order to find additional evidence for this, the outcome of different treatment regimes in the Netherlands is described in chapter 4.

**Allogeneic SCT**

There is currently no role for allogeneic SCT as first line treatment, as Da Villa showed inferior survival with alloSCT in first line (38). Considering the increased morbidity, the negative impact on the quality of life in survivors of alloSCT (102,103) and the reasonable OS (5 year 60-75%) in patients reaching CR after induction with R-chemo only or with additional up-front ASCT, alloSCT will not be the preferred first line treatment. Additionally, the substantial TRM (around 20%) does not appear to outweigh any benefit conferred by a reduction in relapse rate (35,38,104).

Since relapse is still the major cause of death, even after autologous SCT, allogeneic SCT, with the potential benefit of a graft-vs-lymphoma effect can be considered for the treatment of relapsed TFL (89,97,105). The main problem with relapsed and refractory patients is that for an alloSCT to be successful, the disease has to be chemosensitive in order to reach preferably a complete remission allowing time for a GVL effect following allogeneic SCT (7,106). This is confirmed by our results of allogeneic SCT in patients with chemo-sensitive TFL, FL and aggressive lymphoma, as described in chapter 7.

**Radioimmunotherapy**

Radioimmunotherapy (RIT) is defined as therapy with monoclonal antibodies conjugated with a radioactive isotope to target radiation directly to tumor cells, thereby limiting radiation to non-target organs. Both ⁹⁰Yttrium ibritumomab tiuxetan (Zevalin®) and ¹³¹Iodine-tositumomab (Bexxar®), targeting the CD20 surface antigen, have been studied in TFL patients. RIT might be suitable to circumvent rituximab resistance or to eradicate the chemo-refractory cancer (stem-) cell by the so-called crossfire effect (irradiation of surrounding non-CD20-postive cells due to tissue penetration of several millimeters by β particles). RIT has been added to high dose therapy and ASCT in
relapsed and refractory DLBCL, with promising results (\(^{90}\)Yttrium ibritumomab tiuxetan: 68,107). Considering the effectiveness of RIT in FL, in terms of prolonging PFS (108,109), it might be a valuable addition to treatment of TFL patients. In chapter 5 (only first line treatment) and 6 (both first line and second line treatments) we demonstrate the effectiveness of consolidation with ASCT adding \(^{90}\)Yttrium ibritumomab tiuxetan to BEAM.

In relapse setting (mostly heavily pretreated) TFL patients have been treated with radioimmunotherapy as monotherapy. Response rates and duration of response are less pronounced as described in FL. Overall response rates (ORR) of up to 60% lasting 10-15 months have been found with \(^{90}\)Yttrium ibritumomab tiuxetan as monotherapy (65,110,111). A similar ORR of 60-80% with remission durations of 10 months to 1.3 years has been described with\(^{131}\)Iodine-tositumomab (59,60,64,112).

Other treatments and novel targets
Other treatments than the above-mentioned treatment regimes have been studied mostly in phase 1 or 2 studies, in patient groups with heterogeneous histology, and in heavily pretreated patients. Most data are available about the value of lenalidomide, with promising ORR of 45-60% inTFL patients (43,51,52 table 1).

There is a clear need for new targets to treat refractory patients with TFL. In chapter 8 we investigate survivin, a member of the family of inhibitor of apoptosis proteins as a possible target for therapy. We focused on survivin because we found a significantly higher survivin expression in TFL than in FL. This might be a cause for refractoriness of TFL as high expression in aggressive lymphoma has been found to be associated with resistance to therapy and poor outcome (113-115). The small molecule survivin inhibitor YM155 has shown promising results in refractory DLBCL patients in phase II studies (116,117) and we tested YM155 on TFL cell lines and patient samples.

Treatment of TFL: current practice in the Netherlands
Given that there is currently no standard treatment for TFL, as described above, we investigated Dutch practice of TFL treatment over the last decade as described in chapter 4 of this thesis. Additionally we sent an email questionnaire regarding treatment policy to all members of the HOVON lymphoma working group. Members from 17 hospitals, including all 8 academic medical centers responded (response rate 75%). Of the 8 academic medical centers 3 advised R-chemo only, 3 advised up-front consolidation with autologous stem cell transplantation (ASCT), in one center preceded by \(^{90}\)Yttrium ibritumomab tiuxetan, within a clinical study, in one center R-chemo was only followed
by up-front ASCT if the patient had received prior treatment for the FL and one center advised up-front consolidation with ASCT followed by allogeneic SCT. However, all responders mentioned that they regularly individualized treatment based on patient characteristics. The results obtained from the 9 non-academic hospitals were similar, as expected because of the consulting role of the academic medical centers. We conclude that there is no consensus on first line treatment of TFL, even in a small country like the Netherlands.

The data we present in this thesis add to existing information on diagnosis and treatment in TFL with the purpose to generate ideas for improvement of diagnosis and treatment and for future studies in this specific patient group.
Introduction and scope of this thesis

References

9. Ciné E, Montoto S, Bosch F et al. The Follicular Lymphoma International Prognostic Index (FLIPI) and the histological subtype are the most important factors to predict histological transformation in follicular lymphoma. Ann Oncol. 2006;17(10):1539-1545
Chapter 1


Introduction and scope of this thesis


73. Blystadt AK, Kvalheim G, Torlakovic E. High-dose therapy supported with immunomagnetic purged autologous bone marrow in high-grade B cell non Hodgkin's lymphoma. Bone Marrow Transplant 1999;24:862-872


Introduction and scope of this thesis


Chapter 1


112. Zelenetz A, Saleh M, Vose J et al. Patients with transformed low grade lymphoma attain durable responses following outpatient radioimmunotherapy with tositumomab and iodine 131 tositumomab (abstract) Blood 2002;100:357a


