Outline of thesis and future perspectives.

This thesis is divided into two different sections. The B-section involves reviews and studies on B-cell non-Hodgkin lymphoma [NHL] and radioimmunotherapy [RIT]. The T-section describes studies on T-cell NHL, focused on the clinical treatment of celiac patients with refractory celiac disease [RCD] and enteropathy associated T-cell NHL [EATL].

B.

Chapter B1

Chapter B1 shows a review of literature regarding the use of RIT in aggressive NHL. For about 10 years, standard treatment of aggressive NHL consists of chemotherapy [usually CHOP] combined with immunotherapy [antiCD20]. This combination treatment has made a significant contribution to improve survival. If this treatment induces no complete remission or NHL relapses, more intensive therapy may be given. Patients under the age of 66 can be treated with new, high-dose chemotherapy regimens. If NHL responds properly, consolidation therapy with high-dose chemotherapy followed by stem cell transplantation will be given. Studies on the addition of RIT [both 90Y-ibritumomab tiuxetan and 131I-tositumomab] to high dose chemotherapy have been published. For selected groups of patients, an advantage in overall survival has been demonstrated. New studies should try to solve this question.

Chapter B2

Chapter B2 is a literature review on studies involving the use of RIT in indolent NHL. These studies show the clinical applicability of RIT in patients with indolent NHL, even in heavily pretreated patients. There is no additional damage to healthy organs. Using a standard dose RIT, its inhibitory effects on the function of the bone marrow are temporarily. In some groups of patients, favorable long-term results have been demonstrated, even in patients with bulky disease. The key question remains at what stage of treatment RIT should be applied: during the first treatment, as consolidation of a first treatment or in second or third line after relapse. Because the natural course of
indolent NHL, with or without treatment, can be spread over many years, this question has no clear answer yet.

Chapters B3 - B7 focus on the use of 90Y-ibritumomab tiuxetan [Zevalin] in patients with relapsed, aggressive NHL. Since 2005, the VU university medical center initiated a clinical study in patients with relapsed B-cell NHL after previous treatment with chemotherapy and immunotherapy. The main goal of this study is to improve the survival of patients by adding Zevalin to high dose chemotherapy prior to stem cell transplantation. In Zevalin, due to the absence of $\gamma$-radiation, imaging and calculation of the amount of radiation delivered [dosimetry] is impossible. Therefore, in several other studies, the surrogate radionuclide indium-111 $[^{111}\text{In}]$ has been used, which shows approximately the same biodistribution compared to yttrium-90. The biodistribution of $^{111}\text{In}$ is detected by a so-called gamma camera. However, the sensitivity of a gamma camera is less compared to the sensitivity of a PET-CT scanner. A PET CT scan can visualize monoclonal antibodies labeled to a positron emitter. Instead of $^{111}\text{In}$, the positron emitter 89-zirconium $[^{89}\text{Zr}]$ was used for labeling ibritumomab. Biodistribution of Zevalin with PET CT was shown to be more efficiently and precisely. By implementing these new techniques, biodistribution can be better predicted and this may result in applying higher dose of Zevalin. Also, additional damage to healthy organs can be prevented.

Chapter B3
Chapter B3 describes how the technique of $^{89}\text{Zr}$ labeling to ibritumomab is performed. This labeling is similar to the labeling of yttrium to ibritumomab; the compound has the same properties and appears to be stable. In addition, in an animal-model [mice] is has been demonstrated that the biodistribution of Yttrium and Zirconium labeled to ibritumomab and visualized by PET scanning, is almost identical. This PET scan study obtained adequate images of all lymphomas after injection of $^{89}\text{Zr}$-Zevalin. This indicated that this new, so-called immuno-PET technique could be used to predict the biodistribution of 90 Y-Zevalin.

Chapter B4
The results of the study in chapter B3 were used in a clinical study in which patients have been treated with Zevalin prior to stem cell transplantation. In seven patients, extensive research has been performed, whether injection of $^{90}$Y-Zevalin influences biodistribution calculated with immuno-PET and $^{89}$Zr-Zevalin. Another goal of this research was, if and at what time prior to the actual treatment with $^{90}$Y-Zevalin, immuno-PET was able to predict its biodistribution and which specific organs are at risk of radiation damage.

In this study, patients were injected with of $^{89}$Zr-Zevalin, followed by PET scans at three different time points: immediately after injection, after three and six days. Thereafter, both $^{89}$Zr-Zevalin and $^{90}$Y-Zevalin were co-injected, and again, PET scans were performed at the same moments. Blood tests measuring the amount of radioactivity were performed during the whole study at different time points.

This study concluded that injection of $^{90}$Y-Zevalin has no influence on the biodistribution of $^{89}$Zr-Zevalin, and therefore $^{89}$Zr-Zevalin can be used for predicting biodistribution of $^{90}$Y-Zevalin. In this study group, the highest uptake of $^{89}$Zr-Zevalin was calculated in the liver.

Chapter B5
The impact of Zevalin to the microenvironment of the bone marrow was investigated in nine patients treated with Zevalin and high-dose chemotherapy. The microenvironment is important for adequate bone marrow function. In addition, several factors related to stem cell homing have been studied as well.

At three different time points during treatment, bone marrow and blood tests were performed. The results show no effect of Zevalin on a variety of factors that are involved in the microenvironment of bone marrow and homing of stem cells. The recovery of bone marrow function, measured as the recovery of white blood cells and platelets, was similar to patients without Zevalin added to the transplant regimen.

Chapter B6
Chapter 6 describes a retrospective study analyzing all patients who have been treated with high-dose chemotherapy [BEAM] and autologous stem cell transplantation for aggressive B-cell NHL, from 1984 up to January 2012. Medical records of all patients treated with BEAM [or a variant of BEAM] have been scored for survival, type and stage.
of NHL, relapse of NHL and short and long term side effects. A distinction has been made between three groups: patients who have never been treated with immunotherapy [BEAM, n = 106, treatment before 2002], patients treated at least once with immunotherapy [Rituximab; R-BEAM, n = 45, between 2002 and 2006] and patients treated with both, immunotherapy and RIT [Zevalin; Z-BEAM, n = 65, from 2006 up to 2012].

The main difference between the R-and Z-BEAM groups is that disease-free survival was significantly improved by addition of Zevalin to the conditioning regimen for stem cell transplantation [p = 0.028]. The overall survival between the two groups shows a clear trend in favor of the Z-BEAM group; this difference is not significant [p = 0.059]. An even longer follow up with a larger number of patients included, can hopefully show significant improvement of overall survival. Between the BEAM and the R-BEAM groups, no significant differences were found. This could imply that the group of patients with relapse after previous treatment with rituximab, experience a worse prognosis despite rescue therapy. This particular group of patients can benefit from intensifying conditioning for autologous stem cell transplantation with Zevalin. Between all groups, no significant differences in transplant related toxicity were observed.

This retrospective study demonstrates the potential and feasibility of the Zevalin-BEAM protocol, and preludes to a large, international randomized study, described in Chapter 7 [Appendix].

Chapter B7 [Appendix]

To establish the benefit of addition of Zevalin to the BEAM conditioning regimen in patients with aggressive B-cell NHL, a randomized trial is warranted. Colleagues from Israel have initiated this randomized study. Interim analysis of their results shows a survival advantage for a specific group of patients, but not for all patients. Inclusion of more patients is necessary. Therefore this study will be continued internationally, in collaboration with the Israeli group, the United States [City of Hope, Los Angeles, Mayo Clinic, Rochester] and the Netherlands [VUmc, Amsterdam]. In appendix B7, the protocol describes design, inclusion criteria and objectives of this study.
Chapter T1
In chapter T1, new insights in the treatment of refractory celiac disease [RCD] are discussed. Patients with RCDII have a significantly increased risk of developing EATL: 50-60% of patients develop this disease within 4-6 years. The main challenge in the care of these patients is to prevent the development of EATL. This review provides an overview of studies involving different strategies and treatments to patients with RCDII, such as treatment with cyclosporine, azathioprine, budesonide, cladribine and high-dose chemotherapy with autologous stem cell transplantation.

Chapter T2
In chapter T2, the first results of autologous stem cell transplantation in patients with RCDII have been described. Thirteen patients with RCDII were evaluated, 6 of them could not be transplanted [pre-transplant screening showed cardiac abnormalities [n = 2], patients had already developed EATL [n = 3] and one patient had a poor physical condition]. The remaining 7 patients [mean age 62 years] underwent a successful collection of stem cells [leukapheresis]. After conditioning with fludarabine and melphalan, an autologous stem cell transplant was performed. All patients showed a normal hematological recovery. During treatment, there were no significant or unexpected non-hematological adverse events. There was no transplantation-related mortality.

After transplant, checks of intestinal mucosa showed a significant decrease of the number of aberrant T cells. Also, patients showed a clinically important improvement of their physical wellbeing and normalization of hematologic and biochemical values [mean follow up: 16 months]. These first results indicated that high-dose chemotherapy and stem cell transplantation was feasible and safe in a selected group of patients with RCDII. Hopefully, development of EATL can be delayed or even avoided by this strategy.

Chapter T3
A follow-up study with the same strategy as described in chapter T2 is discussed in Chapter T3. Over 6 years, patients with RCDII were monitored and evaluated, because
treatment with cladribine showed incomplete or even lack of response. Of all 18 patients included, 13 were transplanted and follow up was at least 2 year. All transplanted patients showed a significant improvement of their clinical condition. Biopsies of the intestinal mucosa showed no abnormalities after transplant in 5/13 patients. One patient died as a result of the transplantation. The median overall survival was 66% at 4 years. This is in contrast to the 5 patients who, for various reasons, could not be transplanted: none of these survived [death, on average after 5 months]. In the group of transplanted patients, only 1 patient developed EATL, four years after treatment.

If the diagnosis EATL has been made, the prognosis of patients is poor. Treatment may consist of a laparotomy to confirm the diagnosis and, if possible, to debulk the tumor. In some cases, the diagnosis is only established after emergency laparotomy because of an acute perforation of the intestine. Thereafter, in most cases, treatment will be continued with CHOP chemotherapy. Given the poor prognosis of EATL, several strategies have been developed to consolidate the treatment outcome after CHOP with different types of stem cell transplantation. In chapter T4 autologous and in chapter T5 allogeneic stem cell transplantation will be discussed.

Chapter T4
Chapter T4 describes autologous stem cell transplantation in 4 patients diagnosed with EATL. Before chemotherapy treatment, three of the four included patients underwent surgery in order to determine the diagnosis after a perforation of the small intestine. Three patients were transplanted with BEAM conditioning directly after CHOP regimen; one patient was transplanted after relapse at eighteen months after the first line treatment. Despite this treatment, three of the four patients died within a few months after transplantation due to relapse. Induction treatment with CHOP alone and consolidation treatment with BEAM and autologous stem cell transplantation seems not effective. More intensive induction regimens consisting chemotherapy penetrating the central nervous system are necessary to improve the prognosis of patients with EATL.
Chapter T5

Chapter T5 illustrates two patients with EATL treated with allogeneic stem cell transplantation. After initial treatment with CHOP, both patients had no detectable disease. Meanwhile, both patients seemed to have suitable HLA identical sibling stem cell donor. Subsequently, after a reduced intensity-conditioning regimen, an allogeneic stem cell transplant was performed. Unfortunately, 6 to 8 weeks after transplantation, both patients had relapsed disease and died. It remains unclear whether early discontinuation of immunosuppressive medication, in order to introduce the so-called "graft-versus-lymphoma" effect, could be successful in other cases.

Future perspectives.

The randomized trial [appendix], which is now initiated, should hopefully give an answer to the benefit of Zevalin, added to BEAM conditioning, in relapsed or refractory B-cell NHL.

Nowadays, more and more is known about the biological properties of aggressive NHL, its different subtypes and/or subsequent mutations such as BCL2, BCL6 and MYC. Tailored medicine is the keyword but in “every day” clinical practice not implemented yet. If certain subtypes of aggressive NHL could be identified at diagnosis, such as the so-called “double hit” NHL, more specific treatments will be necessary to treat these various subtypes. Also, new chemotherapeutical regimens [i.e. DH-EPOCH] and new monoclonal antibodies [i.e. ofatumomab] have been implemented, sometimes, but not in all cases, specified to a certain subgroup of aggressive NHL. Practical issues such as standardization of different techniques, lack of prospective studies and financial aspects are major issues. First line treatment in aggressive NHL is moving; nevertheless, some patients will experience relapsed disease. For those cases, new consolidation treatments containing innovative strategies such as radioimmunotherapy will be worthwhile to explore.

Less is clear in the field of T-cell NHL, specifically EATL. Because it is a rare disease, no randomized trials have been published so far.
The best way to treat EATL is to prevent EATL. Patients with celiac disease, especially those with unsolved complaints such as sudden weight loss or abdominal pain should be investigated carefully. If refractory celiac disease is diagnosed, a stringent strategy should be implemented to diminish the amount of intra epithelial aberrant T-cells. Cladribine treatment seems the gentlest therapy, mostly without major side effects. So far, cladribine has been given intravenously. In hairy cell leukemia, cladribine subcutaneously injected seems as effective as IV therapy; this could also be explored in patients with RCD.

In EATL, several reports on different treatment strategies have been published, all with a limited number of patients included. Other, conventional agents such as etoposide, [high dose] methotrexate and ARA-C have been added to standard CHOP chemotherapy. These treatments are feasible in celiac patients and provide a better remission status before autologous stem cell transplantation. The CD52 antigen is expressed on aberrant T cells in RCD and EATL, but in most cases its expression is low. Little is known about effectiveness and feasibility of treatment with alemtuzumab [antiCD52] in these patients. Generally, effectiveness of alemtuzumab seems correlated with the dose used, but especially in higher doses, CMV reactivation or CMV disease is a major clinical issue. In vitro research on EATL cell lines shows promising results of the proteasome inhibitor bortezomib. The addition of bortezomib to conventional chemotherapy in first line treatment of EATL deserves further clinical investigation. As in other aggressive T cell NHL, remission should be consolidated with high dose chemotherapy and autologous stem cell transplantation in patients under the age of 66. Allogeneic stem cell transplantation seems not feasible due to severe immunosuppression and subsequently fast relapse of disease.