Efficacy and safety of a single dose of 14.8 MBq/kg (0.4 mCi/kg) ^{90}Y-ibritumomab tiuxetan ("Zevalin") in elderly patients with diffuse large B-cell lymphoma and FDG-PET positive partial remission following first-line R-CHOP therapy. A Phase II clinical trial (HOVON 77)

PROTOCOL

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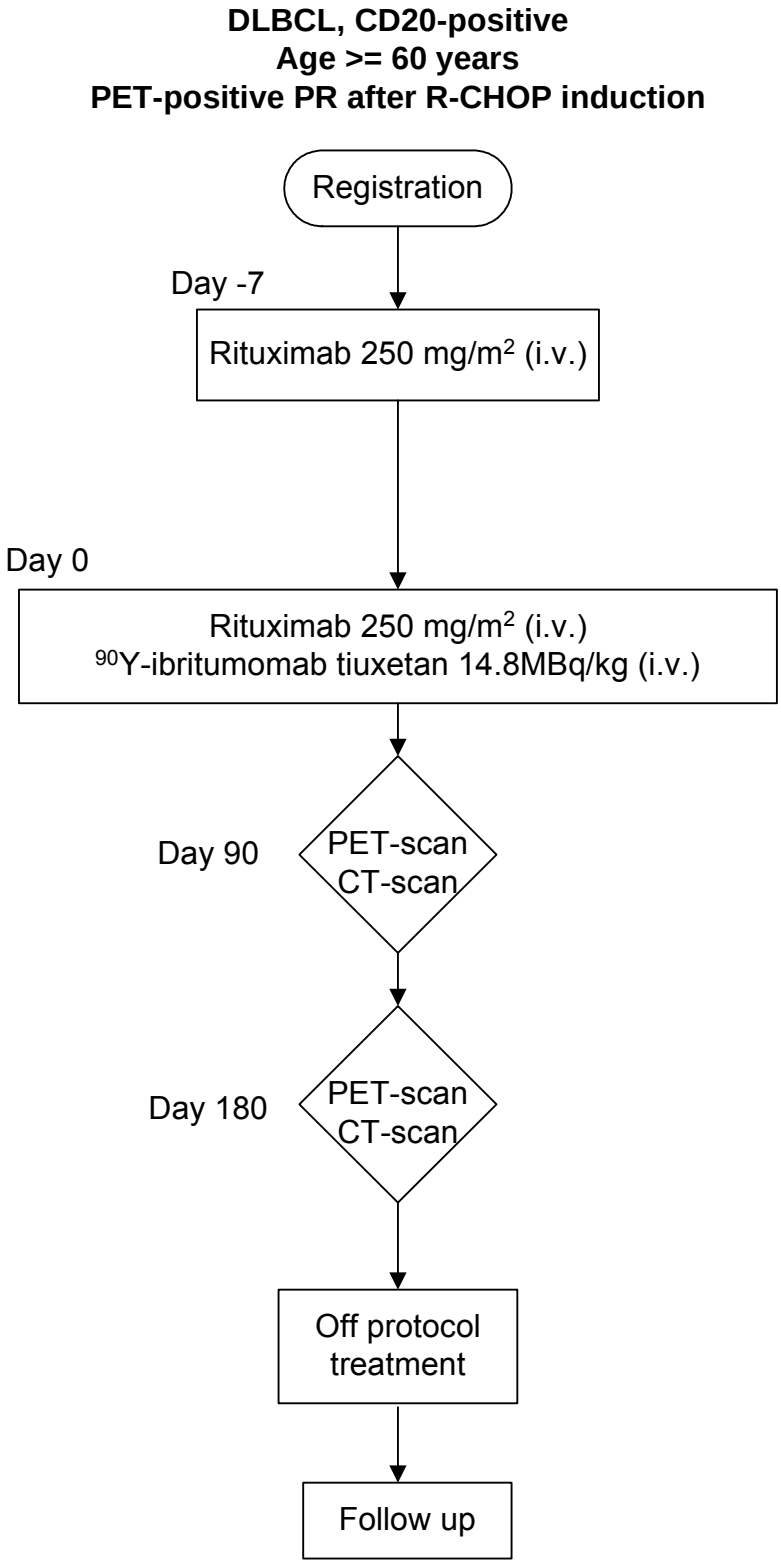
1 Scheme of study



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3 Synopsis

Study phase	Phase II
Investigational product	^{90}Y-ibritumomab tiuxetan ("Zevalin", SH no. L00749E), is composed of a murine IgG1 monoclonal antibody (ibritumomab) covalently bound to the chelating agent tiuxetan. The antibody is chelated with the ß-emitter yttrium-90 chloride immediately before intravenous administration to prepare [^{90}Y]Zevalin, the active therapeutic agent.
Indication	Treatment of aggressive lymphoma with PET-positive Partial Remission (PR) after first line chemotherapy
Study objectives	Evaluation of efficacy and safety of ^{90}Y-ibritumomab tiuxetan
Patient population	Patients with Diffuse Large B-Cell lymphoma, CD20-positive, age ≥ 60 years and good WHO performance status (0,1,2), with PET-positive PR after R-CHOP induction chemotherapy.
Study design	Prospective, multicenter, open label, non-randomized.
Duration of treatment	Infusion of rituximab at 250 mg/m^2 followed one week later by a second infusion of rituximab at 250 mg/m^2 and a single dose of ^{90}Y-ibritumomab tiuxetan of 14.8 MBq/kg (0.4 mCi/kg), at maximum dose of 1184 MBq (32 mCi)
Methodology	Efficacy parameters: conversion from PET-positive to PET-negative residual lesions, progression free survival time, overall survival time Safety parameters: vital signs, Adverse Events, safety lab
Number of study centres	Approximately 25
Total number of patients	37 evaluable patients
Planned start of recruitment	III, 2005
Planned end of recruitment	I, 2008
Manufacturer(s) of the investigational/ reference product(s)	Biogen-IDEC Pharmaceuticals Corporation, San Diego, USA

4 Investigators and study administrative structure

Responsibility	Name	Affiliation/Address
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	A. Hagenbeek	AMC, Amsterdam
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PET review	O.S. Hoekstra	VUMC, Amsterdam
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Pathology review	J.J. Oudejans	VUMC, Amsterdam
Statistician	W.L.J. van Putten	HOVON Data Center, Rotterdam
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4.1 Pathology review

A central review of the diagnosis is performed for each case by J.J. Oudejans to confirm the diagnosis of diffuse large B-cell lymphoma according to the WHO lymphoma classification and to assess CD20 expression. Classification of these lymphomas includes immunophenotypical characterization by a standard panel of markers including CD79a, CD20, Bcl-6, CD10, MUM1, Bcl-2, CD3, CD30 and granzyme B. If a definite diagnosis cannot be attained based on histological and immuno-histochemical findings alone, additional Ig rearrangement studies will be performed (department of pathology of the VU medical center) by multiplex PCR analysis using the Biomed-2 concerted action primers (van Dongen JJ et al. Leukemia 2003;17:2257-317). A review by a second

hematopathologist will be performed in case of discrepancy with the local pathologist or when judged appropriate.
The review analysis will be done without knowledge of patient outcome. Once a patient is registered in the study, the local pathologist as well as the central pathologist will be notified by the HOVON Data Center by e-mail. The local pathologist will be asked to send in 15 unstained slides (suitable for immunohistochemical stainings) or a paraffin embedded block of the primary biopsy (on which the B-cell lymphoma diagnosis is based) and the pathology report to the review pathologist. After review of diagnosis, including possible additional tests necessary for diagnosis, the material will be returned to the pathologist who has sent in the material. Review material will not be used for additional studies without consent of the local pathologist.
A copy of the results of the review will be sent to the local pathologist and to the HOVON Data Center.
All histological materials are to be sent to:

J.J. Oudejans
Department of Pathology
VU University Medical Center
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands

4.2 Central PET review

For central PET review, all FDG-PET scans and CT-scans have to be sent to:

Dr. E.F.I. Comans, nuclear medicine physician.
Department of Nuclear Medicine and PET research.
VU University Medical Center
PO box 7057
NL - 1007 MB AMSTERDAM
Tel : + 31 20 444 4214,
Fax: + 31 20 444 3090
Email: efi.comans@vumc.nl

The whole body scans will be displayed in both projection and volume views, the latter using coronal, sagittal and transaxial views. Two experienced readers from a group of three (consensus by the third) will independently interpret the images on an image display and score each lymph node region according to a three point visual scoring system: 0 =normal/benign; 1 = indeterminate; 2 = malignant. The PET scans are scored with knowledge of the CT data.
At study inclusion, PET is considered positive in case of clearly enhanced focal uptake (score 2) vs. background in a residual mass at CT. At follow-up, PET is also considered positive in case of new sites with focally enhanced uptake considered to represent lymphoma involvement.

5 Introduction

5.1 Non-Hodgkin's lymphoma

Non-Hodgkin's lymphomas (NHL) comprise more than a dozen neoplasms of the lymphoid system that, together, are sixth in incidence and mortality among all malignant neoplasms in the USA [1,2]. In the European Union, the incidence of NHL per 100,000 inhabitants is 5.9 in females and 9.5 in males [3], and is still increasing world-wide [4,5]. The majority of cases are observed in patients over 65 years of age [2].

NHL can be divided into two clinical groups: the indolent lymphomas and the aggressive lymphomas. Indolent NHL types have a relatively good prognosis, with median survival as long as 10 years, but they usually are not curable in advanced clinical stages.

The aggressive type of NHL has a shorter natural history, and a significant number of these patients can be cured with intensive combination chemotherapy regimens. The International Prognostic Index (IPI) for aggressive NHL identifies 5 significant risk factors prognostic of overall survival: age (< 60 years of age versus > 60 years of age), serum lactate dehydrogenase (normal versus elevated), performance status (0 or 1 versus 2-4), stage (I or II versus III or IV), and extranodal site involvement (0 or 1 versus 2-4) [6]. Index values of 0 and 1 are classified as low, 2 as low/intermediate, 3 as intermediate/high, and 4 together with 5 as high risk. Patients with 2 or more risk factors have less than a 50% chance of relapse-free and overall survival at 5 years.

5.2 Treatment of elderly patients with aggressive NHL

In a recent multicenter trial in the Netherlands 61% of elderly aggressive NHL patients were diagnosed with diffuse large B-Cell lymphoma (DLBCL) (HOVON- 25). The vast majority of DLBCL are phenotypically CD20-positive. Despite the high incidence of DLBCL in elderly patients, this age group has been poorly represented in large prospective trials of CHOP in NHL [9]. In the large prospective analysis of CHOP compared with other, more aggressive chemotherapy schedules, the response of the small subgroup of elderly patients was not significantly different from younger adults [9]. Four trials have specifically addressed the efficacy of CHOP chemotherapy in aggressive NHL in elderly patients [7,8,10]. In these studies the complete response rate was between 50 and 60%. However, the 5 years overall survival (25-30%) was significantly worse when compared with younger patients, a difference that was due to both toxicity and a higher proportion of relapsing patients. From the study from Coiffier et al it is clear that the addition of rituximab to CHOP leads to a prolongation of event-free survival and overall survival in elderly patients with DLBCL, so R-CHOP has become the standard treatment [10].

If patients relapse, the vast majority of relapses occur in the first 2 years after therapy [11]. The subsequent treatment of aggressive NHL depends mainly on the age of the patients. Stem cell transplantation is the treatment of choice for patients below the age of 65 years [12].

Elderly patients, however, are ineligible for stem cell transplantation. In general, these patients initially receive therapy with second-line chemotherapy. However, these patients have a poor prognosis and the available regimens are highly toxic to the patients. Attempts to improve the therapeutic outcome have focused on developing new relatively mild regimens using already existing cytostatic drugs that have shown efficacy in aggressive NHL [13,14]. At present, there is no evidence from randomized trials to indicate superiority of any specific second-line regimen. Overall, the results are disappointing.
The need for exploring new treatment modalities with substantial response rate and response duration in patients who cannot be treated with autologous stem cell transplantation is high. The patients' response to treatment is crucial: non-responders have a very poor prognosis.

5.3 Positron Emission Tomography

Apparently, conventional diagnostic methods like CT-scanning do not adequately differentiate patients who will relapse from those who are cured [15,16]. The main reason for this is that many NHL patients have post-treatment residual masses on CT-scans and CT-scanning cannot differentiate between fibrosis and active residual disease [17].

Evaluation of therapy response is one of the most promising applications of Positron Emission Tomography (PET). Since imaging with FDG-PET is based on functional characteristics of tissue, malignant lymphoma can be discriminated from scar tissue due to differences in metabolism. Various phase II studies in aggressive NHL have shown that PET can detect response post-treatment more adequately than CT-scan. In that respect, abnormal FDG uptake after first-line chemotherapy in NHL is highly predictive for active disease. In several studies, relapse rates were 100% for patients with a positive PET post-treatment [18,19]. A systematic review has been performed on the diagnostic accuracy of [^{18}F]-Fluorodeoxyglucose (FDG) PET in lymphoma patients after first line chemotherapy. In this review the sensitivity of PET in aggressive NHL was 72% (95% CI 61-81%) and the specificity was 100% (95% CI 97-100%)[35].

PET is rapidly gaining ground in the Netherlands, with nowadays 13 PET-scanners intramurally present and about 15 hospitals with access to mobile PET-scanners. While evaluation of therapy response is one of the most promising applications of PET, it is essential to investigate if PET is sensitive enough to detect the extent of residual lymphoma after first-line therapy to guide consolidation therapy.

In the current proposal for a clinical phase II study it is proposed to perform total body PET scanning in all elderly patients achieving a “conventional” PR after remission-induction treatment. PET-negative patients will not receive any further treatment. However, PET-positive patients will receive radio-immunotherapy with ^{90}Y-ibritumomab tiuxetan (^{90}Y-Zevalin). It will be investigated which

proportion of these PET-positive patients will turn PET-negative after Zevalin treatment. Toxicity, progression-free survival and overall survival are additional endpoints.
Evaluation of first-line chemotherapy with CT-scan has to be performed 3 weeks after the last cycle of R-CHOP. In case of partial remission, the FDG-PET scan has to be performed within 2 to 4 weeks after the post-treatment CT-scan. PET-scan procedures are described in 11.3.

5.3.1 FDG synthesis

FDG is produced according to EC-GMP guidelines. ^{18}F is produced by a (p,n) reaction on ^{18}O, originated from enriched water ($H_2{}^{18}O$). After irradiation ^{18}F is harvested. In an automated synthesizer (Nuclear Interface, BRD), ^{18}FDG is produced with triflate and tetra-butylammonium carbonate (TBA) reagents. The obtained ^{18}FDG is purified on ion-exchange, C18 Sep-Pak and Alumina Sep-Pak columns. The product is diluted to a isotonic solution (sodium chloride 0.9 %), containing 3 mCi/ml on ART (12:00) and sterilized by aseptic filtration. Characteristics are: A colorless, clear, isotonic and sterile solution with pH 4.5 to 8.5. Radiochemical purity > 97% (HPLC); > 95% (TLC). Chemical purity: ethanol < 1000 ppm; acetone < 50 ppm; acetonitril < 50 ppm; TBA < 50 ppm. Radionuclide purity > 99,9 %. Bacterial endotoxins < 25 EU/ml.
In Groningen, a fully automated production method is used, which does not require the presence of personnel since the synthesis is controlled by a home made PLC module. The procedure is as follows: ^{18}F is produced by a (p,n) reaction on ^{18}O in enriched water. After recovery from the ^{18}O enriched water, [^{18}F]-fluoride is dried by one coevaporation with acetonitrile (Kryptofix: 15 mg / K2CO3: 4 mg). Subsequently triflate precursor (20 mg) is added, followed by heating at 110 oC for 5 minutes. Acetonitrile is evaporated and the residue is dissolved in 0.3 M NaOH. After alkaline hydrolysis at room temperature for 1 min, the mixture is purified by commercially available cartridges: Maxi Clean IC H Plus, Chromafix 600 HR P and Sep Pak Light Alumina N. Sterile FDG is obtained by passing through a Cathivex GS 0.22 ìm filter (Millipore). Characteristics are: a colourless, clear, isotonic and sterile solution with pH 5 to 8. Radiochemical purity > 95 % (HPLC). Chemical purity: acetonitril < 10 ppm; kryptofix < 25 ìg/ml (DLC); iron < 5 mg/l; aluminium < 5 mg/l. Radionuclide purity > 99 %. Bacterial endotoxins < 2.3 IU/ml.

5.4 Radioimmunotherapy

Radiolabeled antibodies may be particularly effective in treating NHL for a variety of reasons: lymphocytes and lymphoma cells are inherently sensitive to radiotherapy; the local emission of ionising radiation by radiolabeled antibodies can kill cells with or without the target antigen in close proximity to the bound antibody, and penetrating radiation may obviate the problem of limited access in bulky or poorly vascularized tumours. A number of studies have been performed with iodinated antibodies [20,21,22]. However, the clinical usefulness of ^{131}Iodine (^{131}I) for radioimmunotherapy has been limited by several factors including the long eight-day half-life of the

isotope, dehalogenation of the iodinated antibody and the gamma (γ) component of the emission spectrum that leads to indiscriminate irradiation of non-tumor sites [23,24]. By attaching metal chelating groups to proteins, it is possible to study other radioisotopes, e.g. ^{90}Yttrium (^{90}Y). ^{90}Y has advantages over ^{131}I, since it delivers higher beta (ß) energy to the tumor, is a pure ß-emitter without a γ component, has a half-life of 64 hours, and has an optimal path length of 5–10 mm resulting in the ability to kill both targeted and neighbouring cells.
The anti-CD20 monoclonal antibody Ibritumomab is an IgG1 kappa antibody and the murine parent immunoglobulin to rituximab. Ibritumomab is covalently linked to the tiuxetan chelate and radiolabeled with ^{90}Y, producing ^{90}Y-ibritumomab tiuxetan. To optimise biodistribution, rituximab is given prior to the radiolabeled antibody.

5.5 ^{90}Y-ibritumomab tiuxetan ("Zevalin")

In the following, a brief summary of clinical data on ^{90}Y-ibritumomab tiuxetan ("Zevalin") is given.

- A Phase I dose-escalation study of ^{90}Y-ibritumomab tiuxetan was performed in 18 patients with refractory low-grade or intermediate-grade B-cell lymphoma. Following stem cell harvest, patients received the unlabeled antibody, ibritumomab, prior to imaging with ^{111}Indium (^{111}In)-ibritumomab tiuxetan and treatment with ^{90}Y-ibritumomab tiuxetan. Four single dose levels ranging from 740 to 1850 MBq (20 to 50 mCi) were used. Marrow ablation occurred in those patients receiving more than 1480 MBq (> 40 mCi, > 22.2 MBq/kg or > 0.6 mCi/kg) of ^{90}Y-ibritumomab tiuxetan. Dose limiting toxicity was hematological and correlated best with the administered ^{90}Y radioactivity per kg of body weight. Tumor response was seen at all dosing levels with an overall response rate (ORR) of 64% [25].
- 58 patients with low-grade or intermediate-grade NHL were enrolled in a Phase I/II clinical trial [26]. This study used rituximab as the unlabeled antibody infused prior to the radiolabeled antibody. No bone marrow or stem cell harvest was required. Two dose levels of rituximab (100 and 250 mg/m^2) were evaluated for ^{111}In-ibritumomab tiuxetan dosimetry and imaging efficiency. The 250 mg/m^2 rituximab dose was chosen for subsequent patients in Groups 2 and 3. The radiation doses to normal organs, bone marrow, and tumor from ^{90}Y-ibritumomab tiuxetan were estimated by dosimetry. A close fit of the data was demonstrated after comparing ^{90}Y activity in plasma and whole blood with ^{90}Y dosimetry estimates. Three dose levels of ^{90}Y-ibritumomab tiuxetan (7.4, 11.1 and 14.8 MBq/kg) were evaluated for efficacy and safety. Dose limiting hematologic toxicities in patients treated with ^{90}Y-ibritumomab tiuxetan were manageable and reversible. Baseline platelet counts and the degree of marrow involvement were better predictors of hematologic toxicity than bone marrow dosimetry from imaging or blood activity measurements.
An ORR of 67% was achieved for all patients at all doses. In the limited number of relapsed/refractory patients classified as having intermediate-grade lymphoma (n=14) an overall response rate of 43% with ^{90}Y-ibritumomab tiuxetan ("Zevalin") has been demonstrated. Four of 14 patients (29%) achieved a CR, and 2 patients (14%) achieved a PR.

- In a prospective randomised trial, in patients with refractory, low-grade, follicular, or transformed CD20 positive B-cell NHL, ^{90}Y-ibritumomab tiuxetan treatment was compared to a standard course of rituximab (4 weekly doses of 375 mg/m^2) [27]. The ^{90}Y-ibritumomab tiuxetan regimen in this trial consisted of Day 0 rituximab (250 mg/m^2) immediately followed by ^{111}In-ibritumomab tiuxetan for imaging and dosimetry, and Day 7 rituximab (250 mg/m^2) followed by 14.8 MBq/kg (0.4 mCi/kg) ^{90}Y-ibritumomab tiuxetan for therapy (maximal 1184 MBq or 32 mCi). ORR (overall response rate) in the ^{90}Y-ibritumomab tiuxetan group was significantly higher than ORR in the rituximab group (80% vs. 56% according to International Workshop Response criteria or 73% vs. 47% according to protocol-defined evaluation of response). In transformed NHL 1 of 9 patients (11 %) reached a CR, 4 of 9 (45 %) a PR. Time to response was for all patients between 29 and 129 days (median 35 days). Kaplan-Meier estimates of response duration (approx. 50% of patients censored) were not statistically different at 10.9+ and 11.5+ months. It should be mentioned that the study was not powered to detect response duration differences.
- Two further phase II trials were performed in specific populations: 1.) ^{90}Y-ibritumomab tiuxetan at a dose of 0.3 mCi/kg in a population similar to the patients described above, but with mild thrombocytopenia [28]; and 2.) ^{90}Y-ibritumomab tiuxetan therapy in patients with follicular NHL who were refractory to prior rituximab immunotherapy [29]. In both populations, ^{90}Y-ibritumomab tiuxetan could be safely administered and still achieved an excellent clinical response.
- Recently, the first results were presented of a Phase II study on ^{90}Y-ibritumomab tiuxetan (Zevalin) for patients with relapsed/refractory Diffuse Large B-Cell lymphoma not eligible for autologous stem cell transplantation. In this prospective, single-arm, open-label, non-randomized, multicenter phase II trial, the efficacy and safety of ^{90}Y-ibritumomab tiuxetan in elderly patients with histologically confirmed first relapsed or primary refractory DLBCL was evaluated. Pts were divided into 2 groups: those previously treated with chemotherapy alone [Group A, n=76], and those previously treated with chemotherapy and rituximab [Group B, n=28]. Primarily, this was a very bad prognosis subgroup with chemo refractory disease and with progression during first-line chemo-immunotherapy. Patients in Group A were further divided into 3 strata: patients with primary refractory disease (stratum 1, n= 33), patients relapsing within a year from presentation (stratum 2, n=10), and those relapsing more than 1 year from presentation (stratum 3, n=33). In this study, 103 patients were evaluable for efficacy and 104 for safety. An ORR of 44% was observed in the entire study population. In Group A, the ORR was 52% in stratum 1, 40% in stratum 2, 58% in stratum 3. In Group B, where 37% of pts were refractory to CHOP-rituximab, the ORR was 19%. The median PFS was 5.9, 2.3, and 6.2 months in strata 1, 2, 3 of Group A, respectively; the median PFS for Group B was 1.6 months. Median OS in Group A was 22.4 months in stratum 3 and has not yet been reached at a maximum follow-up time of 32 months in strata 1&2. Median OS was 4.5 months for Group B. [30]

In all studies, adverse events (AEs) were primarily hematologic, transient, and reversible with Grade 4 neutropenia, thrombocytopenia, and anemia occurring in 32%, 8.5%, and 4.3% of patients, respectively. Levels recovered in all patients, except when patients went on to other therapy or had

pre-existing cytopenias. The severity of hematologic toxicity was related to baseline platelet count and percent bone marrow involvement. Most frequent, related nonhematologic AEs (asthenia, chills, fever, nausea, headache) related to accompanying rituximab infusions. No major acute organ dysfunction was seen. B-cell depletion recovered by 6 to 9 months after therapy. Median serum immunoglobulins remained within the normal range and were relatively stable throughout the treatment period and during follow up. T-cells were not depleted. The incidence of severe infection was low, with only 7.6% of patients hospitalised with infection during the treatment period. HAMA/HACA occurred in < 2% of patients. No observable age-dependent differences were seen in the safety profile. Rare cases of myelodysplasia observed were within the expected rate for this heavily pre-treated patient population.

5.6 Rationale for the study

The aim of this study is to evaluate the efficacy and safety of ^{90}Y-ibritumomab tiuxetan in patients with diffuse large B-cell lymphoma (DLBCL) with PET-positive partial remission who are not eligible for stem cell transplantation. These patients would normally be treated with different types of polychemotherapy (see 5.2).

There is an obvious need to optimise the efficacy of the current treatment options; patients who do not respond to second-line therapy have a very poor prognosis.

Radioimmunotherapy with ^{90}Y-ibritumomab tiuxetan is thought to serve this need for the following reasons:

- Aggressive NHL is a very radiosensitive tumor and the disease is curable in the early stages I and II by radiation therapy only [31,32].
- Immunotherapy with the anti-CD20 monoclonal antibody rituximab can induce responses in patients with relapsed aggressive lymphoma in a substantial number of patients.
- In the relapsed and refractory DLBCL patients treated with ^{90}Y-ibritumomab tiuxetan (n = 103), an overall response rate (ORR) in the entire study population of 44% has been demonstrated, with an ORR of 58% for those patients relapsing more than one year from presentation.

Therefore it can be anticipated that mono-radioimmunotherapy with ^{90}Y-ibritumomab tiuxetan in DLBCL patients with PET-positive PR who are not eligible for stem cell transplantation could be beneficial in terms of an increase of the ORR and a reduction of side effects in comparison to standard polychemotherapy regimens alone, or in combination with rituximab.

However, due to the fact that only a limited number of patients with aggressive lymphoma have been treated with ^{90}Y-ibritumomab tiuxetan so far, a phase II trial is considered as the first approach in this disease.

5.6.1 Selection of doses in the study

The treatment regimen and doses recommended for this study are rationally based upon two dose-finding phase I/II studies performed in the US, numbered as 106-01 and 106-03. These studies were designed, in part, to determine the maximum tolerated dose (MTD) of ^{90}Y-ibritumomab tiuxetan under conditions of optimal biodistribution.
Available data only established a reliable correlation between the duration of thrombocytopenia and weight-adjusted doses of ^{90}Y-ibritumomab tiuxetan for body weight up to 80 kg. In view of concerns regarding received dosages of radiation, a maximum total dose of 32 mCi was adopted as the highest administered dose given during the trials. On the basis of these results, the recommended regimen and doses of ^{90}Y-ibritumomab tiuxetan consist of a preinfusion on day 1 with rituximab at 250 mg/m^2, a preinfusion one week later, on day 8, with rituximab at 250 mg/m^2 followed by a 10-minute injection of ^{90}Y-ibritumomab tiuxetan at a dose of 0.4 mCi/kg, up to a maximum of 32 mCi. This regimen and these doses were tested in all subsequent therapeutic trials performed in indolent NHL.
However, the rationale behind the recommended doses is applicable also for patients with aggressive NHL. This is supported by dosimetry and clinical results from patients with aggressive intermediate / high grade NHL and transformed NHL; these patients did not reveal differences in toxicity when compared with patients with indolent NHL. Furthermore, in indolent lymphoma advanced age was not predictive of response to or toxicity from the study treatment, the regimen was a safe and efficacious treatment for patients ≥ 60 years of age.

6 Study objective

6.1 Primary objective

The primary objective of this study is to evaluate the conversion rate from PET-positive to PET-negative residual masses after ^{90}Y-ibritumomab tiuxetan treatment in patients with PET-positive partial remission following first-line R-CHOP chemotherapy.

6.2 Secondary objectives

To evaluate the progression-free survival, the overall survival (OS) and the toxicity of DLBCL-patients with PET-positive partial remission treated with ^{90}Y-ibritumomab tiuxetan.

7 Study design

This is a phase II, prospective, open label, non-randomized, multicenter uncontrolled study to determine the efficacy and safety of a single dose of ^{90}Y-ibritumomab tiuxetan given at a dose of

14.8 MBq/kg (0.4 mCi/kg) in patients who reach a “conventional” PR with PET positive masses after R-CHOP chemotherapy.

Only patients who are in first partial remission according to the local radiologist can be considered for inclusion. For response assessment on CT-scan, the international workshop criteria according to Cheson et al [33] should be used (see Appendix A). If the patient gives informed consent to participate in the study, a PET-scan will be performed. PET-positivity of the residual masses on CT-scan is an inclusion criterion for participation in this study.
The study will be conducted with a total of 40 intent-to-treat patients in approximately 25 HOVON centres.

Patients who are eligible receive a treatment course that includes an infusion of rituximab at 250 mg/m^2 followed one week later by a second infusion of rituximab at 250 mg/m^2 and a single i.v. dose of 14.8 MBq/kg (0.4 mCi/kg) ^{90}Y-ibritumomab tiuxetan.
During the treatment period - which is scheduled to end 6 months after ^{90}Y-ibritumomab tiuxetan or at the time of disease progression, whichever comes first – patients will be evaluated for response by PET scanning at 3 and 6 months after ^{90}Y-ibritumomab tiuxetan. At the same time points, CT-scans will be performed for comparative purposes. These evaluation time points are based on data obtained from a study in patients suffering from low grade or follicular NHL (median time to response after ^{90}Y-ibritumomab tiuxetan 35 days, range 29 – 129 days). Criteria for defining tumor response are shown in Appendix A. CR/unconfirmed (CRu) will be categorized as CR.
The safety and tolerability of the study drug will be evaluated by assessing relevant laboratory parameters at weekly intervals during the first 3 months and at any re-staging visit during the study (for restaging procedures see also Appendix A). Any adverse event observed, mentioned upon questioning, or when spontaneously reported, will be documented.

After the end of the treatment period, all patients will be followed for disease progression and survival to collect additional data for progression-free survival time and overall survival. Time to next lymphoma treatment and type of treatment will be recorded. During this follow up period, a PET-scan and a CT-scan should be performed at 12 and 18 months.

8 Study population

The study population will consist of patients aged 60 years and above with histologically confirmed CD20-positive DLBCL who achieved a PET-positive partial remission to CHOP in combination with rituximab, and for whom autologous stem cell transplantation is not an appropriate option.

8.1 Eligibility for registration

8.1.1 Inclusion criteria

- Age ≥ 60 years old
- WHO performance status of 0-2 (see Appendix E)
- Life expectancy of at least 3 months
- Histologically confirmed CD20 positive Diffuse large B-cell lymphoma (DLBCL), according to the WHO classification (see Appendix B)
- First-line induction treatment with R-CHOP or R-CHOP-like chemotherapy (only CHOP in combination with rituximab; CHOP14 and CHOP21 are both allowed)
- Partial response on CT-scans after first-line treatment, with measurable disease
- PET-positive residual mass
- Patient is not eligible for high dose chemotherapy followed by autologous stem cell transplantation
- Less than 25% bone marrow involvement at the end of first-line treatment during PR analysis (measurement in a representative bone marrow biopsy)
- Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/l
- Hemoglobin (Hb) ≥ 6 mmol/l
- Platelets ≥ 150 x 10^9/l
- Written informed consent obtained according to local guidelines

8.1.2 Exclusion criteria

- Hypoplastic bone marrow at biopsy
- Prolonged pancytopenia during induction chemotherapy and delayed courses during R-CHOP induction (more than two weeks delay due to insufficient bone marrow reserve)
- Known hypersensitivity to murine antibodies or proteins
- Significant splenomegaly
- Patients with abnormal liver function (total bilirubin > 2.0 x ULN)
- Patients with abnormal renal function (serum creatinine > 2.0 x ULN)
- Presence of CNS involvement by NHL
- Presence of any other active neoplasms or history of prior malignancy, except non-melanoma skin tumours or stage 0 (in situ) cervical carcinoma during the past 5 years
- More than one prior R-CHOP or R-CHOP-like chemotherapy regimen for DLBCL
- Patients who have received prior external beam radiotherapy to > 25% of active bone marrow (involved field or regional)
- Patients who have received G-CSF or GM-CSF therapy within two weeks prior to study enrollment

- Concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes, congestive heart failure, myocardial infarction within 6 months of study, unstable and uncontrolled hypertension, chronic renal disease, or active uncontrolled infection) which could compromise participation in the study
- Patients who have received biologic therapy, immunotherapy, R-CHOP(-like) chemotherapy, surgery, or an investigational drugs less than 4 weeks prior to first day of study treatment or who have not recovered from the toxic effects of such therapy
- Patients who have received systemic corticosteroids at doses higher than 20 mg/day prednisolone or equivalent less than 2 weeks prior to ^{90}Y-ibritumomab tiuxetan administration
- Known diagnosis of HIV infection
- Patients unwilling or unable to comply with the protocol

9 Treatments

Agent	*Dose/day*	*Route*	*Days*
Rituximab (MabThera®)	250 mg/m^2	i.v.	-7, 0
^{90}Y-ibritumomab tiuxetan (Zevalin®)	14.8MBq/kg (0.4mCi/kg), max. dose 1184 MBq (32 mCi)	i.v.	0

All patients included in this study will receive two infusions of 250 mg/m^2 rituximab, given one week apart. The first rituximab infusion will be given alone. The second infusion of rituximab, administered one week later, will be followed immediately by an infusion of 14.8 MBq/kg (0.4 mCi/kg) of ^{90}Y-ibritumomab tiuxetan (maximal dose 1184 MBq or 32 mCi) given as a slow intravenous (i.v.) push over 10 minutes.

9.1 Rituximab administration

Hypersensitivity reactions may occur whenever protein solutions such as rituximab are administered. Premedication consisting of a pain-reliever and an antihistaminic, e.g. paracetamol and clemastine, should always be administered before each infusion of rituximab. Premedication with corticosteroids should also be considered.

9.2 ^{90}Y-ibritumomab tiuxetan administration

^{90}Y-ibritumomab tiuxetan will be administered 7 days after the first rituximab infusion. Each patient will receive one therapeutic dose of 14.8 MBq/kg (0.4 mCi/kg) total body weight of ^{90}Y-ibritumomab tiuxetan (maximal dose of 1184 MBq or 32 mCi). The exact dose of ^{90}Y-ibritumomab tiuxetan will be based on the patient's weight during the screening/baseline evaluation.

Immediately after the second rituximab infusion, ^{90}Y-ibritumomab tiuxetan should be administered intravenously as a slow IV push over 10 minutes. ^{90}Y-ibritumomab tiuxetan may be directly infused by stopping the flow from the IV bag and injecting the radiolabeled antibody directly into the line. A 0.22 micron filter must be on line between the patient and the infusion port. The line should be flushed with at least 10 ml of normal saline after the ^{90}Y-ibritumomab tiuxetan has been infused (see Appendix G). There is no provision for additional treatment courses for patients entered into this protocol.

Adverse events (AEs) associated with ^{90}Y-ibritumomab tiuxetan are primarily hematologic and dose dependent. Other serious adverse events (SAEs) reported as either related or unrelated to the study drug include: syncope, shortness of breath, cardiac arrhythmia, pulmonary embolus in a patient with pre-treatment deep vein thrombosis (DVT), clostridial sepsis in a patient with progressive NHL and second degree gastro-splenic fistula, transient pericarditis, and pneumonia.

9.2.1 Preparation of ^{90}Y-ibritumomab tiuxetan

Conjugated Ibritumomab tiuxetan will be radiolabeled with ^{90}Y using radiolabeling kits. The radiolabeling kits will be provided to the study center by Schering AG. All kit components will have been tested for sterility and pyrogenicity. The components of the radiolabeling kits are described in Appendix G.

^{90}Y-labeled ibritumomab tiuxetan should be prepared according to Appendix G.
After preparation of ^{90}Y-labeled ibritumomab tiuxetan, a radiochemical purity assay will be performed at the clinical site for release purposes. The assay ensures that an acceptable percentage of the radioisotope is chelated by the antibody conjugate. The release specification for radiochemical purity is ≥ 95% for ^{90}Y-ibritumomab tiuxetan. The radiopharmacist at the clinical site should record release test results. Based on the results, he/she will release or reject the ^{90}Y-ibritumomab tiuxetan dose for patient use.

9.3 Storage

Rituximab and the radiolabeling kits, should be stored in a secure refrigerator at 2 to 8°C. The ^{90}Yttrium-isotopes should be stored at room temperature. Rituximab solution for infusion is stable at 2 to 8°C for 24 hours and at room temperature for 12 hours. ^{90}Y-ibritumomab tiuxetan solutions are stable at 2 to 8°C for up to 8 hours following preparation. Due to the relatively short half-life of the isotopes, the actual dose will degrade and have to be recalculated if not used soon after the moment of calibration.

9.4 Prior and concomitant therapy

Any concomitant medication that is administered within 4 weeks prior to, and during and after the administration of the first rituximab infusion will be recorded on the appropriate source documentation at the clinical site and on the CRF until the end of the treatment phase. A description of the type of drug, the amount, duration and reason for administration should be recorded.
Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, anti-emetics, etc. where applicable, according to local guidelines. The reason(s) for treatment, dosage, and dates of treatment should be recorded on the CRF. If platelets drop below the level of 30 x 10^9/l a complete blood count (CBC) including platelet count has to be performed 3 times a week, until the unsupported platelet count recovers to above 30 x 10^9/l. A platelet perfusion should be performed according to local rules. It is also strongly recommended to use prophylactic platelet transfusions to maintain a minimum platelet count of 10 x 10^9/l to reduce the risk of haemorrhage.
Concomitant radiotherapy or treatment with other antilymphoma agents will result in the patient going off treatment.
The use of systemic corticosteroids should be reduced to a minimum, but is permitted.
All prior chemotherapy, biologic, immunologic, radiation therapy and surgery should be recorded on separate pages of the CRF.

10 End of protocol treatment

Reasons for going off protocol treatment are:

1. Normal completion of protocol treatment
2. Progression/relapse
3. Death due to protocol treatment toxicity
4. Radiotherapy or new lymphoma treatments
5. Intercurrent death
6. Withdrawal of consent by the patient
7. No compliance of patient
8. Major protocol violation

11 Required clinical evaluations

The efficacy of ^{90}Y-ibritumomab tiuxetan treatment will be assessed by evaluating patients' clinical response rate. Clinical response rate is assessed by serial examinations on CT-scan and PET-scan. Response on PET-scan will be reviewed (see 4.2) in order to determine the response of each patient.
After the end of the treatment period each individual patient will be followed for disease progression

and survival to collect additional data for progression-free-survival and overall survival.
The safety of ^{90}Y-ibritumomab tiuxetan treatment will be assessed by monitoring the incidence, severity, and type of adverse events. In addition, changes in physical examination findings and vital signs will be evaluated. Complete blood count (CBC), differential and platelet count will be assessed.

11.1 Required evaluations

	At entry	Treatment period *(At 3 months)*	End of treatment phase *(At 6 months or progression)*	FU
Informed consent	X			
Demographic data	X			
Inclusion/exclusion criteria	X			
Medical history	X			
Current medical condition	X			
Prior anti-neoplastic therapy	X			
Physical examination	X		X	X
Clinical laboratory examination				
Hematology	X	X[2)]	X	X
Blood chemistry	X	X[3)]	X	X[4)]
Immunochemistry	X		X	
Urine analysis	X		X	
Bone marrow biopsy				
BM Pathology	X		X	o.i.
BM Immunophenotyping	X			o.i.
BM NHL involvement	X		X	X
Specific investigations				
Chest X-ray	X			
ECG	X			
PET-scan	X	X	X	X[5)]
CT-scan	X	X	X	X[5)]
Lymph node biopsy	X[1)]			o.i.
Pathology review	X			
PET review	X	X	X	X[5)]
Adverse Events	X	X	X	

1) only if histology of NHL (presence of CD20+ lymphoma cells) from 1st diagnosis is not available

2) only CBC. Weekly during first 3 months. If platelets < $30x10^9$L: CBC and platelets 3 times a week, until unsupported platelets > $30x10^9$/l

3) weekly during first 3 months

4) only LDH

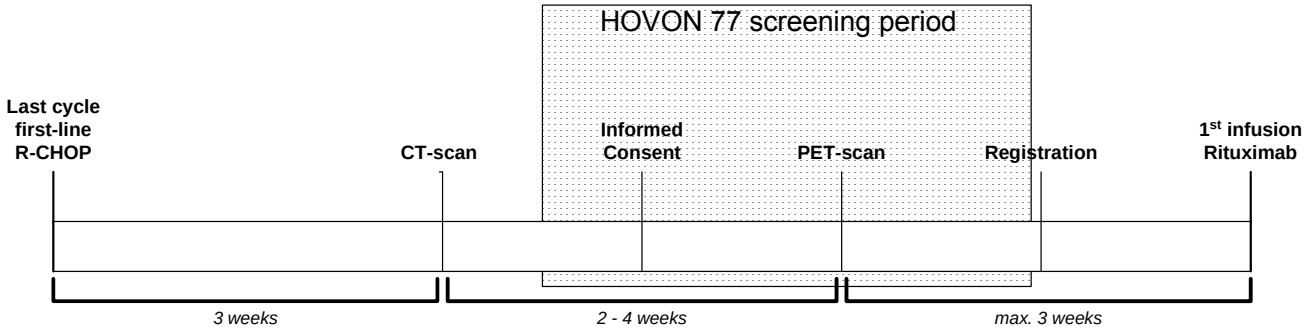
5) only if previous PET-scan result was negative: at 12 and 18 months after treatment

11.1.1 Evaluations at entry

The following evaluations must be performed for screening:

- Obtain patients written informed consent
- Check of inclusion/exclusion criteria
- Demographic data (including height and body weight)
- Medical history (incl. IPI risk classification at time of first diagnosis)
- CT-scan of cervical region (neck), chest, abdomen and pelvis (see 11.2)
- PET-scan (see 11.3), PET/CT-imaging is also acceptable

Screening evaluations should be performed according to the following time schedule:



The following baseline evaluations must be performed within three weeks prior to the first rituximab administration:

- Current medical condition
- Prior anti-neoplastic therapy (including best response status and duration of response)
- Physical examination: standard physical examination including general physical examination of major body systems, vital signs (temperature, blood pressure, heart rate), WHO performance status (see Appendix E) and measurement of spleen and liver size (in centimetres below left and right costal margins, respectively)
- ECG
- Chest X-ray (only if the last X-ray is older than 3 weeks)
- Clinical laboratory examinations :
    - Hematology: complete blood count (CBC), differential and platelet count
    - Serum chemistry: BUN, creatinine, uric acid, electrolytes, calcium, glucose, total bilirubin, alkaline phosphatase, LDH, AST (SGOT), ALT (SGPT), CRP, total protein and albumin
    - Quantitative serum immunoglobulins (IgG, IgA, IgM)
    - Urine analysis: pH-value, erythrocytes, total protein, glucose (dipstick)
- Bone marrow biopsy for pathology, immunophenotyping (CD20 staining) and NHL involvement (must be < 25%). Length of biopsy should be 2 cm at least.

- Only if histology of NHL (presence of CD20 positive lymphoma cells) from 1st diagnosis is not available: lymph node biopsy for confirmation and presence of CD20+ cells.
- Send in unstained paraffine embedded histological slides (from 1st diagnosis NHL, or if not available from lymph node biopsy) for pathology review (see 4.1).

11.1.2 Evaluations during the treatment period

The treatment period is defined as the time from the first rituximab infusion until either 6 months following the ^{90}Y-ibritumomab tiuxetan treatment or at the time of disease progression, whichever comes first.

The following evaluations must be performed during this period:

- Hematology: complete blood count (CBC) weekly during the first 3 months. If platelets drop below the level of 30 x 10^9/l, a CBC including platelet count has to be performed 3 times a week, until the unsupported platelet count recovers to above 30 x 10^9/l. A platelet perfusion should be performed according to local rules
- Blood chemistry: weekly during the first 3 months.
- CT-scan of cervical region (neck), chest, abdomen and pelvis (see 11.2): 3 months after ^{90}Y-ibritumomab tiuxetan infusion.
- PET-scan (see 11.3): 3 months after ^{90}Y-ibritumomab tiuxetan infusion
- Adverse Events

11.1.3 End of the treatment phase

The end of the treatment phase is 6 months after the ^{90}Y-ibritumomab tiuxetan infusion or at the time of disease progression, whichever comes first.

The following evaluations must be performed:

- Physical examination: standard physical examination including general physical examination of major body systems, vital signs (temperature, blood pressure, heart rate), WHO performance status (see Appendix E), disease related symptoms, and measurement of spleen and liver size (in centimetres below left and right costal margins respectively)
- PET-scan (see 11.3)
- CT-scan of cervical region (neck), chest abdomen and pelvis (see 11.2)
- Clinical laboratory tests
    - Hematology: CBC with differential and platelet count
    - Serum chemistry: BUN, creatinine, uric acid, electrolytes, calcium, glucose, total bilirubin, alkaline phosphatase, LDH, AST (SGOT), ALT (SGPT), CRP, total protein and albumin

- Quantitative serum immunoglobulins (IgG, IgA, IgM)
- Urine analysis (by dipstick): pH-value, erythrocytes, total protein, glucose

- Bone marrow biopsy, only if 'baseline' biopsy was positive for NHL or if clinically indicated. Length of biopsy should be at least 2 cm.
- Adverse Events

11.1.4 Follow up evaluations

After the end of the treatment phase each individual patient will be followed for disease progression and survival. Any decisions on further antilymphoma treatments will be made by the treating physician, and will be documented.
Follow up evaluations will be every 3 months during the first two years, every 6 months during the next two years and annually thereafter.

The following evaluations must be performed:

- Physical examination (including WHO performance status, see Appendix E)
- Blood count, LDH
- CT-scan at 12 and 18 months, if last PET-scan result was negative (see 11.2)
- PET-scan at 12 and 18 months, if previous PET-scan result was negative (see 11.3)

11.2 CT-scan

The measurements carried out during this study use standard imaging measurements which are routinely used for assessing NHL patients.
CT-scans, preferably spiral CT with 5 mm coupes, are the standard for evaluation of nodal disease. CT-scans of cervical region (neck), chest, abdomen and pelvis should be performed, even if those areas were not initially involved because of the unpredictable pattern of recurrence.
Copies of the CT-scans should be made available for central panel PET-review. (see 4.2)

11.3 PET-scan

PET-scans should be performed at baseline, 3 months after ^{90}Y-ibritumomab tiuxetan infusion, and 6 months after ^{90}Y-ibritumomab tiuxetan infusion to assess the response to ^{90}Y-ibritumomab tiuxetan treatment. If the '6 months' PET-scan result is negative, an extra PET-scan should be performed at 12 and 18 months after ^{90}Y-ibritumomab tiuxetan infusion to assess the duration of response.
All PET-scans have to be sent to the PET-reviewers for central analysis (see 4.2)

11.3.1 PET patient preparation

Patients should be fasting for at least 6 hours prior to scanning. Free access of water is allowed and the patients should be stimulated to drink at least 1 litre water prior to the injection of the FDG. Intravenously administered fluids should not contain glucose. Prior to injection of FDG a 1 ml blood sample (for serum glucose measurement with hexokinase – not bedside glucotouch methods) will be obtained from an intravenous catheter which also serves to inject the radioactive tracer. Patients should be weighed and their body length recorded.

11.3.2 PET acquisition

Acquisition should start about 60-90 minutes after injection of about 400 MBq in case of 2D imaging, and about 200 MBq for 3D – (these dosages are indicative, since local standards may very slightly). The interval between injection of FDG and starting of acquisition should be noted and must be constant in follow up scans in each individual patient. Scanning should be performed from the level of the perineum to the head (crown). Emission and transmission scans should be acquired alternating over the same positions. Total scanning time (emission and transmission scans) should not exceed 60 minutes.

11.3.3 PET reconstruction

All data should be corrected for dead time, scatter, decay and photon attenuation and an iterative reconstruction method should be used.

12 Toxicities

Rituximab

Side effects of rituximab may include fever, rigors, mucosal congestion or edema, and a drop in systolic blood pressure. These side effects are only observed during rapid infusion of rituximab. In patients who experience side effects, the infustion time has to be restricted to 100 mg/hr.

^{90}Y -ibritumomab tiuxetan

Toxicities that would be expected to occur in association with ^{90}Y -ibritumomab tiuxetan are summarized in this section, and investigators must be familiar with these. They must also be familiar with the Investigator's Brochure, where additional safety information can be obtained.
In previous clinical trials adverse events were reported as primarily hematologic, transient, and reversible, with Grade 4 neutropenia, thrombocytopenia, and anemia occurring in 32%, 8.5% and 4.3%, respectively. Blood count levels recovered, except in those cases where patients went on to other therapy, had pre-existing cytopenias, or died of rapidly progressive disease or concomitant illness. Platelets may fall to levels in which life-threatening hemorrhage occurs.

Until August 2002 two deaths of patients with profound thrombocytopenia were reported for this trial. In one case, the cause of death was disease progression and thrombocytopenia which possibly caused cerebral hemorrhage and neurologic symptoms. Regarding the second patient, a causal association between the study drug and gastrointestinal as well as fatal cerebral hemorrhage was assumed.

The most common related events (during treatment and follow-up) included asthenia, chills, fever, nausea, headache, throat irritation, tooth caries, hypoglycemia, increased cough, and rash. The most frequently reported AEs (during treatment) are arranged according to body system:

- Body as a whole: asthenia, chills, fever, headache, malaise, throat irritation, abdominal pain, flushing, pain, back pain, chest pain, neck pain, tumor pain, mucous membrane disorder and sepsis
- Cardiovascular system: hypotension, myocardial ischemia and deep thrombophlebitis
- Digestive system: nausea, vomiting, anorexia, diarrhea, dry mouth, hemorrhage gastrointestinal and melena
- Hemic and lymphatic system: ecchymosis, petechia, hypochromic anemia, easy bruisability, febrile neutropenia, granulocytosis (in one case only, neutropenia see above) and pancytopenia
- Metabolic and nutritional disorders: angioedema, peripheral edema, hyperglycemia
- Musculoskeletal system: arthralgia, myalgia
- Nervous system: dizziness, paresthesia and subdural hematoma
- Respiratory system: increased cough, dyspnea, rhinitis, bronchospasm, infection, hypoxia and pneumonia
- Skin and appendages: pruritus, rash, urticaria, sweats
- Urogenital system: urinary tract infection and vaginal hemorrhage
- Special senses: amblyopia

Toxicities should be reported on the CRF and scored according to the NCI Common Terminology Criteria for Adverse Events, CTCAE version 3.0, published Dec 12, 2003 (see Appendix F)

13 Safety evaluations and adverse events reporting

13.1 Definitions

Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject during protocol treatment. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Adverse reaction (AR)

Adverse reactions (AR) are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected.

Serious adverse event (SAE)

A serious adverse event is defined as any untoward medical occurrence that at any dose results in:

- death
- a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- hospitalization or prolongation of hospitalization
- significant / persistent disability
- a congenital anomaly / birth defect
- any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above)

Note that ANY death, whether due to side effects of the treatment or due to progressive disease or due to other causes is considered as a serious adverse event.

Unexpected SAE

Unexpected Serious Adverse Events are those SAE's of which the nature or severity is not consistent with information in the relevant source documents. For a medicinal product not yet approved for marketing in a country, a company's Investigator's Brochure will serve as a source document in that country.

Suspected unexpected serious adverse reaction (SUSAR)

All suspected ARs which occur in the trial and that are both unexpected and serious.

Protocol treatment period

The protocol treatment period is defined as the period from the first study-related procedure until 30 days following the last dose of protocol treatment or until the start of another systemic anti-cancer treatment off protocol, if earlier.

13.2 Reporting of (serious) adverse events

Adverse event

AEs will be reported on the CRF. All adverse events of Grade 2 or higher, with the exception of progression of disease, occurring during the protocol treatment period will be reported. Adverse

events occurring after that period should also be reported if considered related to protocol treatment.

SAE and Unexpected serious adverse event

All SAEs occurring during the protocol treatment period must be reported to the HOVON Data Center by fax **within 24 hours of the initial observation of the event**, except hospitalizations for:

- a standard procedure for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- the administration of blood or platelet transfusion. Hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable serious adverse event.
- a procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
- prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- a procedure that is planned (i.e., planned prior to starting of treatment on study; must be documented in the CRF). Prolonged hospitalization for a complication considered to be at least possibly related to the protocol treatment remains a reportable serious adverse event.

All details should be documented on the **Serious Adverse Event and Death Report**. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information. Initial reports must be followed-up by a complete report within a further 2 working days and sent to the HOVON Data Center. All SAE Reports must be dated and signed by the responsible investigator or one of his/her authorized staff members.

At any time after the protocol treatment period, *unexpected* Serious Adverse Events that are considered to be at least suspected to be related to protocol treatment must also be reported to the HOVON Data Center using the same procedure, **within 24 hours after the SAE was known to the investigator**.

The investigator will decide whether the serious adverse event is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the serious adverse event form. The assessment of causality is made by the investigator using the following:

RELATIONSHIP	DESCRIPTION
UNRELATED	There is no evidence of any causal relationship to the protocol treatment (also include pre-existing conditions)
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

13.3 Processing of serious adverse event reports

The HOVON Data Center will forward all reports within 24 hours of receipt to the study coordinator, the study central datamanager and to Schering AG. The report of an SAE will be the signal for the central datamanager to ask the investigator or the responsible local datamanager to complete and send as soon as possible all relevant CRF's for the involved patient with details of treatment and outcome.

14 Endpoints

Primary endpoint:

- Complete response on FDG-PET (i.e. PET-negative residual masses)

Secondary endpoints:

- Progression-free survival
- Overall survival
- Toxicity CTC AE grade 3-4

Progression-free survival and overall survival are measured from the day of infusion of ^{90}Y-ibritumomab tiuxetan until the date of progression and the date of death, respectively.

15 Data collection

Data will be collected on Case Report Forms (CRF) to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected on the CRF are derived from the protocol and will include at least:

- inclusion and exclusion criteria
- baseline status of patient including medical history and stage of disease
- timing and dosage of protocol treatment
- adverse events
- parameters for response evaluation
- any other parameters necessary to evaluate the study endpoints
- survival status of patient
- reason for end of protocol treatment

Each CRF page will be identified by a pre-printed trial number, and a unique combination of patient study number (assigned at registration), hospital and patient namecode (as documented at registration) to be filled out before completing the form.
The CRF will be completed on site by the local investigator or an authorised staff member. Each page must be dated and signed by the local investigator upon completion. All CRF entries must be based on source documents. The CRF and written instructions for completing the CRF will be provided by the HOVON Data Center.
Copies of the CRF will be kept on site. The original CRF pages must be sent to the HOVON Data Center at the requested timepoints. How and when to send in forms is described in detail in the CRF header and the CRF instructions.
All data from the CRF will be entered into the study database by the HOVON Data Center.

16 Registration

Eligible patients should be registered before start of treatment. Patients can be registered at the HOVON Data Center of the Erasmus MC - Daniel den Hoed by phone call: +31.10.4391568 or fax +31.10.4391028 Monday through Friday, from 09:00 to 17:00, or via the Internet through TOP (Trial Online Process; https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.

The following information will be requested at registration:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Patient’s initials or code

- Patient's hospital record number (optional)
- Sex
- Date of birth
- Eligibility criteria

All eligibility criteria will be checked with a checklist.
Each patient will be given a unique patient study number, which will be given immediately by TOP or phone and confirmed by fax or email.

17 Statistical considerations

This phase II trial follows a one-armed optimal two-stage Simon design, as described by Simon [34]. The design shields patients from an ineffective treatment by requiring early termination of the trial if the initial response rate is low.

17.1 Patient numbers and power considerations

The sample size calculation is based on the response endpoint with the following design criteria:

- A response rate larger than 40% is considered acceptable (i.e., worth further study).
- A response rate smaller than 20% is considered unacceptable.
- The probability of accepting a treatment as worth further study, while in fact its true response rate is unacceptable, is limited to 10% ($\alpha = 0.10$).
- The probability of rejecting a treatment for further study, while in fact it is acceptable with respect to response, is limited to 10% ($\beta = 0.10$).

These criteria imply a sample size of 37 patients to be included in the study, 17 patients in the first stage and 20 patients in the second stage. To account for an unexpected loss of 10%, the total number of patients to be included in the study is set to 40.

The accrual of the required 40 patients is expected to take place within two and a half years.
In the HOVON 46 study (patients ≥ 65 years) 90 patients were enrolled per year (50 from Dutch centres and 40 from the Nordic group in Norway, Sweden and Denmark). In a recent analysis (October 2004) the "conventional" PR rate was 40%. We estimate the percentage of PET-positive PR's at 60%. Taking into account patient refusals, medical contra-indications, etc, a conservative estimate would be that 10-15 patients could be enrolled per year from the total population of elderly with aggressive NHL.
All main analyses will be done in accordance with the intention-to-treat principle.

17.2 Efficacy analysis

Primary endpoint:

- The efficacy of the treatment of the main endpoint response will be evaluated as follows. If 10 or less out of the 37 patients obtain a response, the treatment is rejected due to inadequate response. Otherwise it is inferred that the treatment is worth further study.
- A point estimate and corresponding confidence interval of the response rate will be estimated.

Secondary endpoints:

- The progression-free survival will be analysed by drawing its Kaplan-Meier curve and the calculation of the point estimate and 95% confidence interval of the median progression-free survival.
- The overall survival will be analysed by drawing its Kaplan-Meier curve and the calculation of the point estimate and 95% confidence interval of the median overall survival.
- The toxicity will be analysed through tabulation. A point estimate and confidence interval of the toxicity rate will be calculated.

All analyses of secondary endpoints are of a non-inferential, i.e., of a hypotheses-generating, nature: no conclusions will be drawn from them.

17.3 Interim analysis and stopping rules

An interim analysis is planned when the response data of the first 17 patients are available. The trial will be terminated if 3 or less out of these 17 patients obtain a response. The treatment is rejected due to inadequate response. This stopping rule implies that the probability of early termination in the case of a poor response (a ‘true’ response rate smaller than 20%) is 55%. In the case of a good response (a ‘true’ response rate larger than 40%) this probability is less than 5%.

After inclusion of 17 patients the response data may not be available. The study will then be put on hold if at that time less than four responses have been reported. Otherwise new patients may be included.

18 Ethics

18.1 Independent ethics committee or Institutional review board

The study protocol and any amendment that is not solely of an administrative nature will be approved by an Independent Ethics Committee or Institutional Review Board.

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki (Edinburgh, Scotland, 2000) and the ICH-GCP Guidelines of 17 January 1997. The local investigator is responsible for ensuring that the study will be conducted in accordance with the protocol, the ethical principles of the Declaration of Helsinki, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory requirements.

18.3 Patient information and consent

Written informed consent of patients is required before PET-scan and registration.

19 Trial insurance

HOVON will ensure that insurance is in place for all participating sites.

HOVON will provide risk insurance to cover all patients from participating sites in the Netherlands according to Dutch law (WMO).
In case of an intergroup study, risk insurance of patients from centers participating within another cooperative group will be provided by that group, according to all applicable laws and regulations.
Individual participating centers from outside the Netherlands have to arrange risk insurance of their own patients according to all applicable laws and regulations.

20 Publication policy

The final publication of the trial results will be written by the Study Coordinators on the basis of the statistical analysis performed at the HOVON Data Center. A draft manuscript will be submitted to the Data Center and all co-authors for review. After revision by the Data Center, the other co-authors, the manuscript will be sent to a peer reviewed scientific journal.

Authors of the manuscript will include the study coordinators, the lead investigators of the major groups (in case of intergroup studies), investigators who have included more than 5% of the

evaluable patients in the trial (by order of number of patients included), the statistician(s) and the HOVON datamanager in charge of the trial, and others who have made significant scientific contributions.

Interim publications or presentations of the study may include demographic data, overall results and prognostic factor analyses.

Any publication, abstract or presentation based on patients included in this study must be approved by the study coordinator(s). This is applicable to any individual patient registered/randomized in the trial, or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study end-points unless the final results of the trial have already been published.

21 Glossary of abbreviations

(in alphabetical order)

AE	Adverse Event
ALT	Alanine Amino Transferase
ANC	Absolute Neutrophil Count
AST	Aspartate Amino Transferase
BM	Bone Marrow
CBC	Complete blood count
CHOP	Cyclophosphamide, Doxorubicin, Vincristine (Oncovin), Prednisone
CKTO	Commissie voor Klinisch Toegepast Onderzoek
CNS	Central nervous system
CR	Complete Remission
CRF	Case Report Form
CT	Computerized Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLBCL	Diffuse Large B-Cell Lymphoma
DFS	Disease free Survival
ECG	Electrocardiogram
EC-GMP	European Committee Good Medical Practise
EFS	Event Free Survival
EMD	Extra medullary disease
EORTC	European Organization for Research and Treatment of Cancer
FDG	[^{18}F]-Fluorodeoxyglucose
FU	Follow up
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HOVON	Dutch/Belgian Hemato-Oncology Cooperative Group
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IM	Intramuscular
IV	Intravenous
METC	Medical Ethical review committee
NHL	Non-Hogkin's Lymphoma
NR	No response
ORR	Overall Response Rate
OS	Overall Survival

PB	Peripheral Blood
PET	Positron Emission Tomography
PFS	Progression Free Survival
PR	Partial Response
SAE	Serious Adverse Event
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen
WBC	White Blood Count

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A. HOVON Staging and Response Criteria for Non Hodgkin's Lymphomas

This document describes the minimally required staging and evaluation procedures and response criteria that will be applied in all HOVON NHL studies. It is based on international working group recommendations (JCO, Vol.17, 1999, pp1244-1253 [Erratum, JCO, Vol.18, 2000, p2351]).

Response is currently assessed on the basis of clinical, radiologic, and pathologic (i.e., bone marrow) criteria. CT scans remain the standard for evaluation of nodal disease. Thoracic, abdominal, and pelvic CT scans are recommended even if those areas were not initially involved because of the unpredictable pattern of recurrence in NHL.

Immunophenotyping of blood or bonemarrow has not been included as standard minimum requirement for the staging and restaging of lymphoma, even though it may be done standard in some centers (Hanson, Blood, Vol 94, 1999, pp 3889-3896). It may be a requirement in specific studies involving monoclonal antibodies.

Staging and restaging procedures
Only minimal requirements are specified.

Staging at on study before start of treatment

- History (including B symptoms)
- WHO Performance status
- Physical examination
- Laboratory tests
- Hb, WBC. differential, platelet count, LDH
- Calcium, creatinine, uric acid, glucose, albumin, bilirubine, ALAT
- paraprotein by immuno-electrophoresis
- quantitative immunoglobulins only if immuno-electrophoresis abnormal
- Hepatitis-B in case of abnormal liver function tests
- HIV test
- Lymph node biopsy for morphology and immunopathology
- Bone marrow biopsy (≥ 20 mm biopsy core) for histopathology
- Bone marrow aspirate for cytology
- Peripheral blood for cytology
- Imaging
- CT thorax and abdomen including pelvis
- US cervical region strongly recommended (Br J Hem. 88 (3) 626-8, 1994); alternative: CT cervical region
- Consultation of ear-nose-throat specialist if indicated (i.e. complaints or gastro-intestinal lymphoma)
- Gastroscopy if indicated (i.e. localization ENT, thyroid)
- Lumbal punction if indicated (i.e. localization testis, nasopharynx or brain)

Restaging for the evaluation of treatment

Restaging for the evaluation of treatment should be performed within 2 months after the end of treatment to assess response.

- History (including B-symptoms)
- WHO Performance status
- Physical examination
- Laboratory tests
- Hb, WBC, platelet count, LDH
- Repeat previously abnormal tests
- Bone marrow biopsy (≥ 20 mm biopsy core) for histopathology if involved previously
- Bone marrow aspirate for cytology if involved previously
- Peripheral blood for cytology if involved previously
- Imaging
- CT thorax and abdomen including pelvis
- US of cervical region; alternative: CT cervical region
- Assessment of other localizations only if involved previously

Restaging during follow up to determine remission status (until progression)

In case of CRu (see below) repeat CT 2-4 months after last CT for response evaluation.
- Physical examination
- WHO Performance status
- Laboratory tests
- Hb, WBC, platelet count, LDH
- **Only if indicated**, i.e. LDH elevation or clinical signs of progression:
- Bone marrow biopsy (≥ 20 mm biopsy core) for histopathology (if indicated)
- Bone marrow aspirate for cytology (if indicated)
- Peripheral blood for cytology (if indicated)
- Imaging
    - CT thorax and abdomen including pelvis (if indicated)
    - US of cervical region; alternative CT of cervical region (if indicated)

Staging & Remission Status Evaluation

	On Study	Evaluation of Treatment	Follow up
• History	x	x	X
• WHO performance status	x	x	X
• Physical examination	x	x	X
• Laboratory tests			
▪ Hb	x	x	X
▪ WBC	x	x	X
▪ Differential	x	o.i.	
▪ Platelet count	x	x	X
▪ Calcium	x	o.i.	
▪ Creatinine	x	o.i.	
▪ Uric acid	x	o.i.	
▪ Glucose	x	o.i.	
▪ Bilirubine	x	o.i.	
▪ ALAT	x	o.i.	
▪ Albumin	x	o.i.	
▪ Immuno-electrophoresis	x	o.i.	
▪ Quantitative immunoglobulins	o.i.	o.i.	
▪ Hepatitis-B	x		
▪ HIV test	x		
• Lymph node biopsy	x	o.i.	o.i.
• BM biopsy	x	o.i.	o.i.
• BM aspirate	x	o.i.	o.i.
• PB for cytology	x	o.i.	o.i.
• Imaging			
▪ CT thorax	x	x	o.i.
▪ CT abdomen including pelvis	x	x	o.i.
▪ US/CT cervical region	r.	r.	o.i.
▪ ENT consultation	o.i.	o.i.	o.i.
▪ Gastroscopy	o.i.	o.i.	o.i.
▪ Lumbal punction	o.i.	o.i.	o.i.

o.i. on indication
r. strongly recommended

Bone marrow evaluation

Bone marrow biopsy must be adequate (≥ 20 mm biopsy core).
A bone marrow aspirate and biopsy should always be performed at diagnosis. If positive they should be repeated to determine response. They should also be performed in case of new abnormalities in the peripheral blood.

Bone marrow biopsies should be scored as
- positive unequivocal cytologic or architectural evidence of malignancy
- negative no aggregates or only a few well-circumscribed lymphoid aggregates
- indeterminate increased number or size of aggregates without cytologic or architectural atypia

The bone marrow report should be reported not only as positive or negative for lymphoma, but the percentage of invasion and the lymphoma subtype should be indicated, the latter to describe any discordance with the nodal disease.

Measurable disease and size of disease.

Response evaluation is primarily based on bi-dimensionally measurable nodes, nodal masses or nodules in liver or spleen.

Nodes with largest diameter ≤ 1 cm are considered normal and not pathologic. The size of a single node, nodal mass or nodule is defined as the product of the two largest perpendicular diameters (PPD). Nodes of which only one dimension is specified are considered as circular for the calculation of PPD size. If after treatment a nodal mass consisting of individual confluent nodes breaks up in separate nodes the sum of the PPD of the separate nodes must be compared with the size of the pretreatment nodal mass.
All nodules in liver and spleen are considered pathologic, irrespective of size.

The sum of the PPD (SPD) of a set of indicator lesions is used as a quantitative measure for response evaluation. The indicator lesions have to be chosen from the nodes and nodal masses in the following way. If the number of nodes or nodal masses before treatment is 6 or less, all these are considered as indicator lesions. If the number of nodes or nodal masses is more than 6, a minimum number of at least 6 indicator lesions have to be chosen. These nodes or nodal masses should be selected according to the following features:

a. they should be among the largest dominant sites
b. they should be clearly measurable in at least two perpendicular dimensions,
c. they should be from as disparate regions of the body as possible
d. they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.

The choice of the indicator lesions should be made before start of treatment. All indicator lesions must be numbered and measured bidimensionally before start of treatment and at the evaluation times specified in the protocol. The location and size must be documented and reported in the CRF.

Assessable disease

Assessable disease are considered all abnormalities that are not bidimensionally measurable, e.g. positive bone marrow or peripheral blood.

Response criteria

Complete response (CR) requires the following:
1. Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy
2. Normal LDH (i.e. <= ULN). An elevated LDH detracts from a CR unless it is attributable to causes not related to NHL, f.e. hemolysis.
3.
- All nodes and nodal masses must have reduced in size to ≤ 1.0 cm in greatest transverse diameter, **or**
- If some nodes have regressed to a size between 1.0 and 1.5 cm in greatest transverse diameter from a size over 1.5 cm, while none have a size over 1.5 cm, the SPD of the indicator lesions must have regressed by more than 75%.
4. The spleen, if considered to be enlarged before therapy on the basis of a CT scan, must have regressed in size and must not be palpable and/or no longer considered enlarged on physical examination. However, no normal size can be specified, because of the difficulties in accurately evaluating splenic size. Similarly, other organs considered to be enlarged before therapy due to involvement by lymphoma, such as liver and kidneys, must have decreased in size.
5. Any nodules in liver or spleen must have disappeared.
6. If the bone marrow was involved by lymphoma before treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy of the same site.

CR/unconfirmed (CRu) includes those patients who fulfill criteria 1, 2, 4 and 5 above, but with one or more of the following features/exceptions:
1. A residual lymph node mass greater than 1.5 cm in greatest transverse diameter that has regressed by more than 75% in the PPD size. Individual nodes that were previously confluent must have regressed by more than 75% in their SPD size compared with the size of the original mass. The SPD size of the indicator lesions must have regressed with more than 75%.
2. Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia).

In case of apparent CRu it is recommended to perform, if possible, a cytological punction or biopsy of a residual lymph node mass to determine the cytopathological status. It is also recommended in case of CRu to repeat CT or US of the residual lesion after 2-4 months.

Partial response (PR) requires the following:
1. ≥ 50% decrease in SPD of the indicator lesions.
2. ≥ 50% decrease in SPD of splenic and hepatic nodules if present and bi-dimensionally measurable at start of treatment.
3. No increase in the size of any single node, nodule, liver, or spleen by more than 25%.
4. No new sites of disease.
5. All patients who meet the criteria for CR or CRu except for an LDH >ULN that is not attributable to other causes than NHL or with remaining but decreased nodules in liver or spleen, or with remaining assessable disease are classified as PR.

Stable disease (SD) is defined as less than a PR (see above) but is not progressive disease (see below).

Progressive disease (PD) requires the following
1. ≥50% increase in the PPD-size of any at baseline identified abnormal node, nodal mass or nodule.
2. Appearance of any new lesion during or at the end of therapy.

Endpoints during follow up

Progression of disease is defined for all patients, irrespective of response on treatment. The following criteria apply:
1. ≥ 50% increase from nadir in the PPD-size of any previously identified abnormal node.
2. Appearance of any new lesion.

Relapse requires the following:
1. Previous achievement of CR or CRu.
2. Progression of disease as defined above.

Note:
1. *Relapse is the same as progression of disease after CR or CRu.*
2. *An abnormal or increasing abnormal LDH, not attributable to other causes than NHL, is not sufficient evidence for the determination of progression. Imaging studies must be performed in such a case.*
3. *Note the difference between PD as response category and Progression of disease as event during or after treatment. All patients whose best response on treatment is PD, per definition also have reached the endpoint Progression of disease. But also other patients with a better response may eventually show progression of disease.*

Definitions of End Points for Clinical Trials

End Point	Response Category	Definition	Point of Measurement
Overall survival	All patients	Death from any cause	Entry onto trial
Event-free survival	All patients	Disease progression, relapse or death from any cause	Entry onto trial
Progression-free survival	All patients	Disease progression or death from NHL	Entry onto trial
Disease-free survival	CR, CRu	Time to relapse	First documentation of response
Response duration	CR, CRu, PR	Time to relapse or progression	First documentation of response
Time to next treatment	All patients	Time when new treatment is needed	Entry onto trial
Cause-specific death	All patients	Death related to NHL	Entry onto trial

B. NHL WHO classification

B-cell neoplasms

WHO	
1	♦ Precursor B-cell lymphoblastic leukaemia / lymphoma
2	♦ B-cell chronic lymphocytic leukaemia / small lymphocytic lymphoma
3	♦ B-cell prolymphocytic leukaemia
4	♦ Lymphoplasmocytic lymphoma
5	♦ Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type
6	♦ Nodal marginal zone lymphoma (+/- monocytoid B cells)
7	♦ Splenic marginal zone B-cell lymphoma (+/- villous lymphocytes)
8	♦ Plasma cell myeloma / Plasmocytoma
9	♦ Follicular lymphoma; grade I, grade II, grade III
10	♦ Mantle-cell lymphoma
11	♦ Diffuse large B-cell lymphoma (subtypes: mediastinal, intravascular, primary effusion lymphoma)
12	♦ Burkitt's lymphoma
13	♦ Unclassifiable

T-cell neoplasms

WHO	
21	♦ Precursor T-cell lymphoblastic leukaemia / lymphoma
22	♦ T-cell prolymphocytic leukaemia
23	♦ T-cell granular lymphocytic leukaemia
24	♦ Aggressive NK-cell leukaemia
25	♦ Adult T-cell leukaemia / lymphoma (HTLV1+)
26	♦ Extranodal NK / T-cell lymphoma, nasal-type
27	♦ Enteropathy type T-cell lymphoma
28	♦ Hepatosplenic γ / δ T-cell lymphoma
29	♦ Subcutaneous panniculitis-like T-cell lymphoma
30	♦ Mycosis fungoides/Sézary syndrome
31	♦ Anaplastic large cell lymphoma, primary cutaneous type
32	♦ Peripheral T-cell lymphoma (not otherwise characterized)
33	♦ Angioimmunoblastic T-cell lymphoma
34	♦ Anaplastic large cell lymphoma (T- and null-cell types), primary systemic type
35	♦ Unclassifiable
	♦

C. Ann Arbor staging classification

Stage	Definition
I	Involvement of a single lymph node region (I) or of a single extralymphatic organ or site (I_E)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (II) or localized involvement of an extralymphatic organ or site and of one or more lymph node regions on the same side of the diaphragm (II_E)
III	Involvement of lymph node regions on both sides of the diaphragm (III), which may also be accompanied by involvement of the spleen (III_S) or by localized involvement of an extralymphatic organ or site (III_E) or both (III_{SE})
IV	Diffuse or disseminated involvement of one or more extralymphatic organs or tissues, with or without associated lymph node involvement

B symptoms
The absence or presence of fever, night sweats, and/or unexplained loss of 10% or more of body weight in the six months preceding admission are to be denoted in all cases by the suffix letter A or B, respectively.

Extranodal involvement
Involvement of extra lymphatic tissue on one side of the diaphragm by limited direct extension from an adjacent nodal site is classified as extranodal extension and denoted by suffix letter E. The E category may also include an apparently discrete single extranodal deposit consistent with the extension from a regionally involved node. More extensive extranodal disease, e.g. multiple extranodal deposits, is classified as stage IV. A single extralymphatic site as the only site of disease should be classified as I_E.

Notes
1. For the purpose of defining the number of anatomical lymph node regions the following areas are considered as one region:
- All nodes at one side of the neck are considered as in one region, i.e. consisting of the subregions supraclavicular, cervical, submandibular, occipital, preauricular and postauricular.
- The axillary region includes the infraclavicular nodes.
- The mediastinum is considered as one region, including the subcarinal and pericardial nodes.
2. The lung-hilus is considered as a separate region. Thus involvement of both the mediastinum and a hilar localisation implies stage II disease.
3. Hilar nodes should be considered lateralized and when involved on both sides constitute stage II disease.

D. International Prognostic Index

The age-adjusted international prognostic index (IPI) distinguishes 4 risk groups of patients according to their Ann Arbor stage, WHO performance status and LDH[19].

Risk factors are:

- Age >60 years
- Ann Arbor stage III or IV
- WHO performance status 2-4
- LDH > 1x Upper limit of normal (ULN)
- More than 1 extranodal site

The IPI:

- Low risk : 0 - 1 risk factors
- Low-intermediate risk : 2 risk factor
- High-intermediate risk : 3 risk factors
- High risk : 4 - 5 risk factors

E. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

2 Some bed time, but to be in bed less than 50% of normal daytime

3 Needs to be in bed more than 50% of normal daytime

4 Unable to get out of bed

F. Common Terminology Criteria for Adverse Events

The grading of toxicity and adverse events will be done using the NCI Common Terminology Criteria for Adverse Events, CTCAE version 3.0, published Dec 12, 2003. A complete document (72 pages) may be downloaded from the following sites:

http://ctep.info.nih.gov/reporting/ctc.html
http://www.hovon.nl (under Studies > Documents)

A hardcopy may be obtained from the HOVON Data Center on request.

G. Preparation and dispensing information on ^{90}Y- Ibritumomab tiuxetan

I. Components and preparation of ^{90}Y-ibritumomab tiuxetan

A **Radioisotopes**: Yttrium-[90] chloride used for preparing ^{90}Y-ibritumomab tiuxetan will be obtained from vendors identified by Schering AG. All lots of isotopes will be sterile, pyrogen-free and supplied in septum vials designed to maintain these conditions.

The following yttrium-90 characteristics are required:

Total extractable activity to deliver at time of use	:	≥ 1.48 GBq
Radioactivity concentration at time of use	:	1.67 to 3.34 GBq/ml
HCl concentration	:	0.035-0.045 M
Chloride identification	:	Positive
Yttrium identification	:	Positive
Radiochemical purity of the yttrium-90 chloride solution	:	≥ 95% of free ionic yttrium-90
Radiochemical purity of the radiolabeled	:	≥ 95% incorporation of yttrium-90 onto the monoclonal antibody preparation
Bacterial endotoxins	:	≤ 150 EU/ml
Sterility	:	No growth
Radionuclidic purity, strontium-90 content	:	≤ 0.74 MBq strontium-90 / 37 GBq yttrium-90
Metal impurities		
Total metals*	:	≤ 50 ppm
Individual metals*	:	≤ 10 ppm each

B **Ibritumomab tiuxetan:** The murine anti-CD20 monoclonal antibody ibritumomab is an IgG1 kappa antibody. It is produced in Chinese hamster ovary (CHO) cells in suspension culture. The purified antibody is then joined covalently to the chelating agent tiuxetan (conjugated antibody "Ibritumomab tiuxetan"). Ibritumomab tiuxetan is provided as part of the radiolabeling kit in the form of a 3 ml glass vial containing 2 ml (3.2 mg) of ibritumomab tiuxetan at a concentration of 1.6 mg/ml in low metal normal saline.

C **Radiolabeling kit components:** Conjugated ibritumomab tiuxetan will be radiolabeled with Yttrium-[90] using a radiolabeling kit. The radiolabeling kit will be provided by Schering AG to the clinical site. All kit components will be tested for sterility and pyrogenicity.

The kit should be stored at 2 - 8°C and consists of the following components:

1) Three ml glass septum vial (blue cap) containing 2 ml (3.2 mg) ibritumomab tiuxetan in low-metal normal saline at 1.6 mg/ml
2) Three ml glass septum vial (green cap) containing 2 ml low-metal 50 mM sodium acetate
3) Ten ml glass septum vial (red cap) containing 10 ml formulation buffer (1x PBS containing 7.5% human serum albumin and 1 mM DTPA, pH 7.2)
4) Ten ml glass septum reaction vial (yellow cap, empty)

D **Preparation of 90-ibritumomab tiuxetan:** Proper aseptic technique and precautions for handling radioactive materials should be employed. Waterproof gloves and ring dosimeters should be utilized in the preparation and determination of radiochemical purity assay of ^{90}Y-ibritumomab tiuxetan. The radiolabeling of ^{90}Y-ibritumomab tiuxetan should be done according to the following directions using the radiolabeling kit described above.

GENERAL INSTRUCTIONS:

- Perform ALL COMPOUNDING CALCULATIONS prior to labeling
- Perform labeling preferably in a laminar flow hood strictly adhering to sterile technique
- Arrange & set-up work station for RAPID addition of components to reaction vial

PREPARATION:

1) Before radiolabeling, bring refrigerated ibritumomab tiuxetan cold kit to room temperature (25°C); perform a visual inspection of all vials.
2) Clean the rubber stopper of all cold kit vials and the yttrium-[90] chloride vial with a suitable alcohol swab and allow to air dry.
3) Place the reaction vial in a suitable dispensing shield (plastic enclosed in lead, e.g. 1 cm lucite

surrounded by 1 cm of lead).

4) In a 1 ml syringe, draw sodium acetate buffer that is 1.2 times the starting volume of yttrium-[90] chloride (see below).
5) In a 2 - 3 ml syringe, draw 1.3 ml of ibritumomab tiuxetan.

[^{90}Y]-LABELING OF IBRITUMOMAB TIUXETAN:

Step 1: Transfer sodium acetate solution to the reaction vial

Using a 1-ml sterile syringe, transfer sodium acetate solution to reaction vial. The volume of sodium acetate solution added is equivalent to 1.2 times the volume of radioisotope to be transferred in step 2.

Step 2: Transfer yttrium-[90] chloride to the reaction vial.

Aseptically transfer 40 mCi (1480 MBq) of yttrium-[90] chloride with a 1-ml sterile syringe to the reaction vial containing the sodium acetate solution transferred in step 1. Mix completely by coating the entire inner surface of the reaction vial. Mix by inversion, rolling the container, avoid foaming or agitating the solution.

Step 3: Transfer ibritumomab tiuxetan solution to the reaction vial

Using a 2-3-ml sterile syringe, transfer 1.3 ml ibritumomab tiuxetan solution to the reaction vial. Mix completely by coating the entire inner surface of the reaction vial. Mix by inversion, rolling the container, avoid foaming or agitating the solution.

Incubate the yttrium-90 chloride/acetate/ibritumomab tiuxetan solution at room temperature for five minutes. Labeling time longer than six minutes, or shorter than four minutes will result in inadequate radioincorporation.

Step 4: Add the formulation buffer to the reaction vial

Using a 10-ml syringe with a large bore needle (18-20 G), draw formulation buffer that will result in a combined total volume of 10 ml.

Step 5: Assay the [^{90}Y]-labeled ibritumomab tiuxetan reaction vial for its specific radioactivity.

The percent radioincorporation of the prepared [^{90}Y]-labeled ibritumomab tiuxetan must be checked before administration to the patient according to the procedure outlined below.
Assay the reaction vial in a suitably calibrated dose calibrator.
The percent radiochemical purity of the prepared ^{90}Y-ibritumomab tiuxetan should be determined before administration to the patient.
If not immediately administered to the patient, store the reaction vial containing ^{90}Y-ibritumomab tiuxetan at 2° to 8° C and use within eight hours.
Calculate patient dose at 0.4 mCi/kg (max. 32 mCi/kg). Do not exceed the maximum allowed dose of 1184 MBq (32 mCi) of ^{90}Y-ibritumomab tiuxetan.

E <u>Initial determination of dose calibrator calibration, settings corrected for different geometries:</u>

1) <u>Calibration settings:</u> It is highly recommended that a range of different volumes from the 10 ml reaction vial and 10 ml syringe (Calibration Settings #B and #C) be checked. A calibration setting should be recorded for each volume frequently used.The establishment of calibration settings with ^{90}Y can be done with NIST traceable Radioactivity Standard Reference Materials (SRM's).
2) <u>Geometry changes:</u> It is highly recommended that every geometry configuration change be checked and a new calibration setting established. This would apply to a change in needle size, a change to a different syringe type or volume, or a change in the position of the container within the dose calibrator. Changing a container in the dose calibrator from a side reading, to an upright reading, or to an upside-down reading will all change the geometry and therefore the display readout with beta nuclides.
3) <u>Use of worksheet:</u> The worksheet represents only the determination of the initial calibration settings. Once initial calibration settings are established, steps can be eliminated. Using calibration setting #A should be eliminated after the initial set-up. Dial in settings determined in calibration settings #B and #C are applied to the 10-ml reaction vial, and 10-ml syringe respectively.
4) <u>Daily calibration check:</u> It is highly recommended that a daily channel check for each frequently used calibration setting be done with a long lived dose calibrator source. Suggested isotopes are ^{137}Cs, ^{60}Co, or ^{133}Ba. Consult your health physicist.
5) <u>Dose calibrator manufacturers:</u> It is highly recommended that the dose calibrator manufacturer be contacted for the initial isotope calibration number when working with ^{90}Y.

^{90}Y Decay Constant Tables

Hours Before Calibration										
	0	2	4	6	8	10	12	14	16	18
0	1.00	1.02	1.04	1.07	1.09	1.11	1.14	1.16	1.19	1.21
20	1.24	1.27	1.30	1.32	1.35	1.38	1.41	1.44	1.48	1.51
40	1.54	1.57	1.61	1.64	1.68	1.72	1.75	1.79	1.83	1.87
60	1.91	1.95	2.00	2.04	2.08	2.13	2.18	2.22	2.27	2.32
80	2.37	2.42	2.48	2.53	2.59	2.64	2.70	2.76	2.82	2.88
100	2.94	3.01	3.07	3.14	3.21	3.28	3.35	3.43	3.50	3.58
Hours After Calibration										
	0	2	4	6	8	10	12	14	16	18
0	1.00	0.979	0.958	0.937	0.917	0.898	0.787	0.860	0.841	0.823
20	0.806	0.788	0.771	0.755	0.739	0.723	0.707	0.692	0.678	0.663
40	0.649	0.635	0.621	0.608	0.595	0.582	0.570	0.558	0.546	0.534
60	0.523	0.511	0.501	0.490	0.479	0.469	0.459	0.449	0.440	0.430
80	0.421	0.412	0.403	0.395	0.386	0.378	0.370	0.362	0.354	0.347
100	0.399	0.332	0.325	0.318	0.311	0.304	0.298	0.291	0.285	0.279

To use the decay table, find the number of hours in the top and left-hand columns of the grids, then find the corresponding decay factor.

Worksheet

1. Consult concentration found on **Certificate of Analysis**. Use decay table for the determination of concentration, adjust for time zone changes (example: ^{90}Y at 22 hours pre-calibration [PST] = 1.27 x 1850 MBq/ml = 2349.5 MBq/ml). (Note: 1.27 = decay factor, 1850 MBq/ml = concentration on certificate of analysis)

   _____MBq/ml

2. Calculate the volume of activity for 1480 MBq (example: 1480 MBq ÷ 2349.5 MBq/ml = 0.63 ml) (Note: concentration is taken from calculation in Step 1)

   _____ml

3. In a 1 ml syringe, draw up the required volume (calculated in Step 2).

4. Place 1 ml syringe in dose calibrator. Change the "pot-setting/calibration number" to equal a 1480 MBq readout. **CAUTION: Do not leave ^{90}Y in syringe for extended period of time.**

   _____MBq

5. Record 1 ml syringe "pot-setting/calibration number."

   Calibration Setting (A): _____

6. Immediately transfer activity from 1 ml syringe to 10 ml reaction vial. Follow instructions for preparation of ^{90}Y-ibritumomab tiuxetan. While the five minute incubation time is taking place, assay residual activity in syringe (after *C Preparation of ^{90}Y- and ^{111}In-ibritumomab tiuxetan, Step* #6).

   _____MBq

7. Determine activity added to reaction vial (Step 4 – Step 6, example: 1480 MBq – 29.6 MBq = 1450.4 MBq).

   _____MBq

8. After incubation is complete, qs with **formulation buffer** in the 10 ml reaction vial (according to instructions in C, Step 9), place reaction vial in dose calibrator. Change the "pot-setting/ calibration number" to read the activity recorded in Step 7.

9. Record 10 ml reaction vial "pot-setting/calibration number."

   Calibration Setting (B): _____

10. Based on the activity determined in Step 7, draw up the "prescribed" activity by volume into a 10 ml syringe [example: ("prescribed" dose x 10 ml ÷ Step 7 MBq) = "prescribed" volume].

    _____MBq "prescribed"

    Place the 10 ml syringe in the dose calibrator. Change the "pot-setting/ calibration number" to reflect the "prescribed" activity in a 10 ml syringe in this step. (**Caution: the "prescribed" activity should not exceed 1184 MBq for ^{90}Y).** If ^{90}Y-/^{111}In-ibritumomab tiuxetan is not being used immediately, see instructions in C, Step 11 for storage.

    _____mL

11. Record the 10 ml syringe "pot-setting/calibration number."

    Calibration Setting (C): _____

CALIBRATION SETTINGS

***A* = 1 ml Syringe Geometry**

***B* = 10 ml Reaction Vial Geometry**

***C* = 10 ml Syringe Geometry**

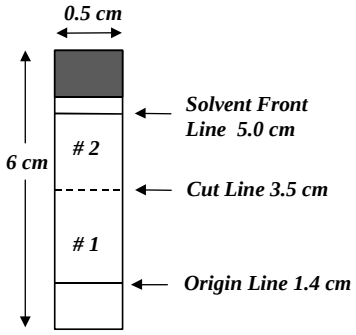
II. Clinical release testing for ^{90}Y-ibritumomab tiuxetan

Release assay is performed at the clinical site for 90Y-ibritumomab tiuxetan.

Radiochemical Purity: This assay insures that an acceptable percentage of the radioisotope is chelated by the antibody conjugate. An instant thin-layer chromatographic assay using a commercial kit (Biodex) is available for use. In this assay, conjugated antibody remains at the origin whereas tiuxetan or DTPA-chelated yttrium advances with the solvent front. The amount of radioactivity remaining at the origin bound to the antibody conjugate is expressed as a percentage of the total amount of radioactivity applied to the strip. See instructions below Steps #1 - #8.

The Radiochemical Purity by Instant Thin Layer Chromatography (ITLC) shall be done according to the following procedure at room temperature:

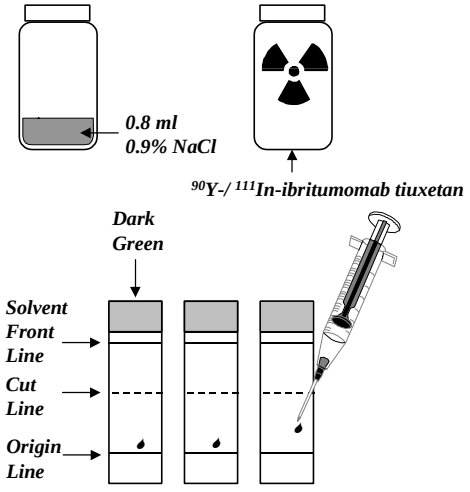
1. Required materials not supplied:
   - 1 ml insulin syringe with a 25 - 26 G needle
   - ITLC-SG, e.g., Biodex "Tec-Control" kit, part number 151-770
   - Single or multichannel analyzer



ITLC-SG Strip

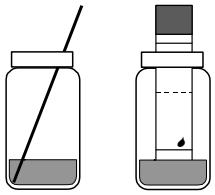
2. Set region of interest of single or multichannel analyzer to incorporate channels 140-1000 keV.

3. Using a 1 ml insulin syringe, place a hanging drop (7 - 10 µl) onto the ITLC-SG strip at its 1.4 cm mark "origin." Spot one strip at a time and run the procedure on three ITLC-SG strips. A 1:100 dilution may be necessary if the instrument deadtime is appreciable.

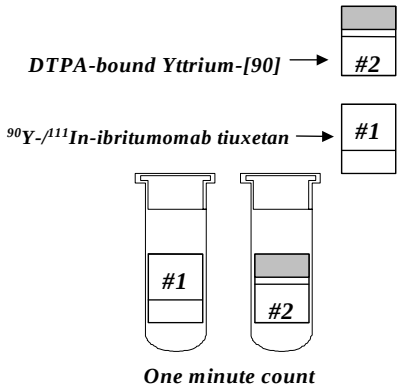


4. Fill developing chamber with 0.8 ml of bacteriostatic free 0.9% NaCl. The volume of 0.9% NaCl should not touch the 1.4 cm origin line.

5. Place ITLC-SG strip into developing chamber and allow the solution to migrate past the 5 cm "Solvent Front" line. Do not allow ITLC-SG strip to adhere to the side of the developing chamber. See illustration below.



6. Remove ITLC-SG strip and cut in half at the 3.5 cm "Cut-Line." Count each half of the ITLC-SG strip in a multi-channel or single channel analyzer counter for one-minute (cpm). Subtract background counts and use corrected counts.



7. Calculate the radiochemical purity % as follows:

$$\textit{(Radiochemical purity \%)} = \left(\frac{\textit{(cpm \#1)}}{\textit{(cpm \#1) + (cpm \#2)}} \right) \textit{x 100}$$

8. Repeat process three times and take the average percentage of the radiochemical purity (RCP).

The release specification for the average radiochemical purity is > 95% for ^{90}Y-ibritumomab tiuxetan.

III. Recommended handling and andministration of ^{90}Y- ibritumomab tiuxetan

1) Refer to the clinical study protocol for details about the dose and dose schedule. Patients will be treated with a 14.8 MBq/kg (0.4 mCi/kg) dose of yttrium-[90]. A 0.22 micron filter is required for the administration of the drug.

2) **Vials should be stored with proper shielding at 2 - 8°C**. Do not freeze or store at room temperature. The drug is a protein -- HANDLE GENTLY AND AVOID FOAMING. The avoidance of foaming during product handling, preparation and administration is important, as foaming may lead to the denaturing of the product proteins.
NOTE: Do not use evacuated glass containers which require vented administration sets because this causes foaming as air bubbles pass through the solution.

3) All transfer procedures require strict adherence to aseptic techniques, preferably in a laminar flow hood.

4) ^{90}Y-ibritumomab tiuxetan may be directly infused by stopping the flow from the IV bag and injecting the radiolabeled antibody directly into the infusion port. A 0.22 micron filter must be on line between the syringe and the infusion port.

5) The administration of the radiolabeled drug will be accomplished by a 10 minute slow IV push and should be completed within the time specified in the protocol.CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS BOLUS. Flush the line with at least 10 ml of normal saline after the radiolabeled product has been infused.

6) IV pumps may be used with the ^{90}Y-ibritumomab tiuxetan infusion. Do not infuse concomitantly with another IV solution or IV medications.

7) If a delay in administration occurs after the ^{90}Y-ibritumomab tiuxetan have been prepared, the radiolabeled product must be kept refrigerated after preparation at 2 - 8°C and may be used for up to 8 and 12 hours, respectively. If not used soon after the calibration time, the actual dose activity will have decayed and therefore requires recalculation.