Rituximab as adjuvant to high dose therapy and autologous hematopoietic cell transplantation for aggressive non-Hodgkin's lymphoma

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Submitted: 4/21/03

Grant support: Supported by grants from National Institutes of Health (CA 49605) and Genentech, Inc.

Presented in abstract form at the 43rd annual meeting of the American Society of Hematology, Orlando, FL, December 10th, 2001.46

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Abstract: Based upon the favorable safety profile and independent activity of rituximab in B-cell lymphoma, we evaluated its efficacy and toxicity after high dose therapy (HDT) and autologous hematopoietic cell transplantation (HCT). Thirty-five patients with diffuse large cell (25), mantle cell (3), transformed (3) or other (4) B-cell lymphoma subtypes received HDT followed by a purged autologous graft. The rituximab schedule was 4 weekly infusions (375mg/m²) starting at day +42 after HCT and, for patients 5-35, a second 4-week course 6 months after HCT. All planned therapy was completed in 29 patients. With 30 months' median follow-up, the two-year event-free survival (EFS) was 83% and overall survival (OS) was 88%. EFS was 81% and OS was 85% for 21 patients with relapsed or refractory large cell lymphoma. Grade 3-4 neutropenia occurred in 19 (54%) patients. Prospective study of immune reconstitution included measurements of lymphocyte subsets, immunoglobulins and response to vaccination. Serious infections were not observed despite delayed B-cell recovery in all patients and suppressed IgG levels and low pneumococcus antibody titers in a subset. Rituximab after HDT and HCT is feasible and these phase II data support the current U.S. Intergroup phase III trial in recurrent /refractory diffuse large cell lymphoma.

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Introduction

Whereas 40-50% of patients with diffuse aggressive non-Hodgkin’s lymphoma (NHL) will be cured of their disease by initial combination chemotherapy, standard dose chemotherapy is effective in salvaging no more than 10% of patients with recurrent or refractory disease. Based largely upon the randomized Parma trial, as well as other phase II trials, high dose therapy (HDT) followed by autologous hematopoietic cell transplantation (HCT) has become standard treatment for patients with chemosensitive, relapsed or refractory aggressive NHL. In this setting, HCT renders about 35-40% of patients free of disease on a long-term basis. Relapse is the primary cause of failure after HCT. In addition to residual disease after HCT, reinfusion of tumor cells in the autologous graft may contribute to relapse. A number of strategies, including in vivo and in vitro purging, have been employed to purify autologous grafts and minimize the risk of reinfusing tumor cells. Efforts to address resistant disease by increasing the intensity of HDT, double transplantation, or incorporating immunotherapy in the form of myeloablative allogeneic transplantation or post-transplant cytokines have been hampered by increased toxicity and, to date, have not demonstrated improvements in overall survival.

In the fall of 1997, rituximab, a chimeric anti-CD20 monoclonal antibody, was approved for use in the United States for patients with relapsed indolent lymphoma. In the pivotal study, single agent rituximab was tolerated well with minimal marrow toxicity, <1% incidence of grade 3-4 neutropenia and <1%
incidence of grade 3-4 thrombocytopenia. Responses were observed in 11 of 30 (37%) patients with relapsed or refractory diffuse large B-cell lymphoma (DLCL). There is evidence that rituximab can be added to combination chemotherapy regimens without significant increases in hematologic toxicity. Further, the addition of rituximab to cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) resulted in significantly longer overall and event free survival compared to CHOP alone in older patients with DLCL. On this basis, we initiated a prospective trial in 1998 with the hypothesis that rituximab would be tolerated by patients early in their recovery from HCT and would favorably impact on the minimal residual disease that leads to relapse.

**Methods**

Inclusion criteria included recurrent or refractory B-cell NHL, CD20 expression on the most recent biopsy specimen, age > 16 years, adequate marrow recovery (absolute neutrophil count ≥1500 cells/µL and platelet count of >50,000/µL on day 42 post-transplant), preserved kidney and liver function, and autologous peripheral blood HCT after carmustine (BCNU)-based preparation. During the course of the study, two modifications in eligibility took place to allow patients receiving a total-body irradiation (TBI) based HDT to participate as well as patients in first CR after induction chemotherapy with features that put them at high risk of relapse. Under these amendments, three patients with mantle cell lymphoma in first CR undergoing the TBI-based HDT and four patients with high-intermediate or high risk DLCL (age-adjusted international prognostic index) in
first CR were enrolled. In order to enrich the study for DLCL, the last ten places on the study were reserved for patients with that histology. Refractory disease was defined as failure to achieve a CR to the initial chemotherapy regimen.

Patients meeting inclusion criteria for histology and preparatory regimen (n=92) were screened for eligibility. Patients with ECOG performance status >2, active infection, active pneumonitis (n=3), concurrent steroid use, progressive disease after transplant (n=2), post-transplant radiotherapy (n=3), prior exposure to rituximab (n=4), marrow graft source (n=3), primary CNS (n=1), equivocal CD20 expression (n=1), failure to meet hematologic criteria (n=21) were ineligible per protocol. The major reasons that 19 eligible patients did not participate were concern for the investigational nature of the study or refusal to comply with the additional follow-up required. The treatment schema for the HCT and adjuvant rituximab is outlined in Figure 1. All patients were mobilized with a single dose of cyclophosphamide 4gm/m² and G-CSF. The peripheral blood progenitor cell product was enriched for CD34+ cells and purged with a panel of anti-B-cell antibodies as previously described. 7 Thirty-two patients received carmustine (BCNU), etoposide, and cyclophosphamide and 3 patients received TBI in place of BCNU as previously described. 2,3 At day 42 after HCT, all study patients were scheduled to receive 4 weekly infusions of rituximab 375 mg/m². All patients received premedication with acetaminophen and diphenhydramine 30 minutes prior to rituximab. As part of the feasibility evaluation of this trial, if relative safety of a 4-week course of rituximab was documented in the first 4 patients, the
remaining patients (5-35) were scheduled to receive a second course of rituximab 375 mg/m² weekly for 4 weeks at 6 months following HCT.

Patients were followed monthly for 6 months with complete blood counts (CBC), serum chemistries, and physical examination for evidence of toxicity or relapse. Patients receiving a second course of rituximab had a CBC monthly until 1 year following HCT. Patients were followed every 3 months with physical examination, CBC, and serum chemistries for the second year after HCT and then evaluated every 6-12 months. As part of routine post-transplant care and not prespecified by the study, computed tomography of the chest, abdomen, and pelvis and bone marrow biopsies were repeated at 6 and 12 months after HCT and then yearly unless clinically indicated.

Immune reconstitution was assessed with quantitative immunoglobulins and lymphocyte subsets at 6 and 12 months. Based on lack of B-cell recovery in patients at 12 months, unplanned assessment of lymphocyte subsets and quantitative immunoglobulins were collected on patients at 18 and 24 months from HCT when possible. Flow cytometry with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies were used to assess numbers of CD3+, CD4+, CD8+, CD16+, CD56+, CD45+, CD19+, and CD20+ cells. Vaccines against tetanus, Haemophilus influenzae (H. flu) and pneumococcus were administered to patients at 6 and 9 months from their last rituximab infusion (12 and 15 months from transplantation). Vaccines were selected to assess different mechanisms of action in eliciting an antibody response: pneumococcus for a T-cell independent pathway, tetanus for a T-cell dependent pathway, and H. flu for
a combined B-cell and T-cell pathway by the addition of a protein conjugate. Antibody titers were assessed immediately prior to the initial vaccinations (baseline) and 3 months after each set of vaccinations. Antibody titers were graded as protective based on absolute values or an observed $\geq 4$ fold rise over baseline.

The major study endpoint was toxicity with an emphasis on acute side effects, infections over a two-year period, and description of immune reconstitution. The treatment was considered to be feasible if the incidence of treatment-related mortality did not exceed 20\% and the incidence of non-hematologic grade III-IV toxicity did not exceed 30\%. The sample size was increased from 24 to 35 patients to increase the number of DLCL patients. Results are reported on an intent-to-treat basis for all enrolled patients, whether or not they received all planned therapy. Overall survival (OS) and event free survival (EFS) were calculated from the date of HCT. For the endpoint of EFS, the earlier event of relapse or death in remission was considered the date of failure. Survivals were estimated by the method of Kaplan and Meier.  

**Results**

From March 1998 to December 2000, 35 patients met the eligibility criteria and consented to participate in the study. Their characteristics are shown in Table 1. The median age was 51 years (range 28-70) and the majority had DLCL. Eighteen patients had relapsed after initial CR, 10 (8 with DLCL) were refractory to their first chemotherapy regimen and 7 patients (4 DLCL, 3 mantle
cell) were considered high-risk in first complete remission. The median number of prior chemotherapy regimens was two, with 7 patients receiving only one regimen and another 7 patients receiving ≥ 3 prior regimens. Prior regimens included CHOP (n=33); DHAP (n=17, dexamethasone, cytarabine, cisplatin); ESHAP (n=5, etoposide methylprednisolone, cytarabine, cisplatin) and CVP (n=3, cyclophosphamide, vincristine, prednisone). One or two patients each received a variety of other regimens. As described in Table 1, responses to the last regimen given prior to HCT were CR or minimal disease (n=25), defined as no nodal mass greater than 2 cm and/or a 75% reduction in disease, partial remission (n=9), and a minor response classified as stable disease in 1 patient.

Thirty-two patients received HDT with BCNU, etoposide, cyclophosphamide and 3 patients with mantle cell lymphoma received TBI with etoposide and cyclophosphamide. The median CD34+ cell dose of the purged autografts was 6.8 x 10^6 (range 1.9-11.7). Twenty-nine patients received all planned rituximab infusions. Reasons for the six who did not included progressive disease (2), death due to pneumonitis after the first rituximab course (1), neutropenia during rituximab administration (1), severe rituximab infusional reaction (1), and disseminated Herpes zoster at the time of the second rituximab course (1).

With a median follow-up of 30 months the estimated 2 year EFS was 83% (CI 70-95%) and the 2 year estimated OS was 88% (CI 78-99%) for all patients assessed on an intent-to-treat basis (Figure 2). Five patients died, four with progressive disease and one in remission from pneumonitis. Among seven
relapsed patients, two received a single course of rituximab. Relapses occurred in DLCL (3), transformed NHL (2) and mantle cell (2). Figure 3 shows the EFS and OS for the 21 patients with relapsed (13) or refractory (8) DLCL. At HCT, 13 had minimal disease, 7 were in PR and 1 had stable disease. The results are similar to that of the whole group. With a median follow-up of 28 months, the estimated 2 year EFS was 81% (CI 64-98) and the estimated 2 year OS was 85% (CI 70-100%). As described above, 3 of these patients relapsed and 2 died. A third death ascribed to BCNU pneumonitis occurred in a patient in CR.

Table 2 describes the hematologic toxicity observed in our study patients following the initial rituximab dose. Neutropenia (CTC criteria) was the most common toxicity with 21 patients developing at least one episode of grade 2-4 neutropenia. Grade 3-4 neutropenia was recorded in 19 (54%) patients who experienced a total of 46 episodes, including 13 patients with more than one episode. Neutropenia was noted as early as the fourth week of the initial rituximab infusion, but also occurred late as evidenced by episodes >300 days after HCT. There was no characteristic pattern of neutropenia as total WBC counts ranged from low-normal (3.2-4 x 10^6/µl) with isolated depression of neutrophils (6-8%) to low absolute WBC (0.7-0.9 x 10^6/µl) with normal percentages of neutrophils (57-62%). All episodes either resolved spontaneously within 7 days (n=14) or responded to 2-4 days of G-CSF (n=32). Administration of G-CSF was according to physician choice. Bone marrow biopsies performed in 8 patients while neutropenic were normocellular with granulocyte precursors present. A single episode of fever and no serious infections resulted from the
neutropenia. We were not able to identify an association of neutropenia with prior treatment, marrow involvement, CD34+ cell dose, or engraftment kinetics. Other hematologic toxicities were limited to 3 patients with grade 2-3 thrombocytopenia and 4 patients with grade 2-3 anemia. These events primarily occurred within the first 100 days from transplantation and in two cases were simultaneous with neutropenia.

Non-hematologic toxicities included BCNU-related pneumonitis in 8 patients (Table 3). This clinical syndrome was based on low grade fever, dyspnea, failure to thrive, or general malaise and a decrease in diffusing capacity of at least 20% from pre-transplant levels. Only two patients displayed classic pulmonary infiltrates, hypoxia, or respiratory compromise. All seven treated patients responded to corticosteroids whereas one patient died after a delayed diagnosis. Twenty patients participated in a double-blind phase III trial of a novel Herpes zoster vaccine. Six of these 20 patients developed zoster, disseminated in three, during follow-up. None of the 15 patients who did not participate in the study developed Herpes zoster. Other toxicities included community-acquired pneumonia in 4 patients, all occurring without neutropenia and resolving with oral outpatient antibiotics. Pleural effusion developed in one patient subsequently noted to have a decreased cardiac ejection fraction. A final unexpected adverse event, amyotrophic lateral sclerosis was diagnosed in a single patient more than three years after HCT. The diagnosis was made by neurologic evaluation on the basis of typical clinical findings, magnetic resonance imaging and nerve conduction studies.
Lymphocyte subsets and quantitative immunoglobulins were measured in follow-up, some of which are shown in Figure 4. Quantitative immunoglobulins appeared only modestly affected by the addition of rituximab in the post-transplant period. IgG levels were below the normal range in 14 of 35 patients prior to their first rituximab infusion. Mean values declined after rituximab infusions at day 42 and 6 months after HCT. IgG levels remained suppressed in 11 of 33 (33%) and 9 of 30 (30%) patients at 6 and 12 months post-transplantation, respectively. IgA and IgM levels appeared less affected as mean values for the group were in the normal range. Nonetheless, 10 and 9 patients had levels of IgA below normal at 6 and 12 months, respectively, while 11 and 9 patients showed similar modest deficiencies in IgM at the same time points. B-cells, measured by CD20+ and CD19+ cells were low in absolute number in 27 of 35 patients at study entry and declined to zero in all patients receiving rituximab as measured by flow cytometry at 6 months after HCT. The only patients with measurable B-cells (3) at 12 months received a single course of rituximab. By 18-24 months after HCT, B-cell numbers were recovering toward the normal range. As this was an unplanned timepoint for measuring lymphocyte subsets, the data are less complete, but 14 of 20 patients evaluated had recovered absolute numbers of CD19+ and CD20+ cells into the normal range.

Mean CD3+ numbers were only mildly depressed and remained so during the follow-up period. There was a wide interpatient variability in the absolute numbers of CD3+ cells with normal (n=12), decreased (n=16) or elevated (n=6) values at study entry. Rituximab had no consistent effect on total T-cell
numbers. Mean CD4+ cell numbers were decreased throughout the first year following HCT but recovered by 24 months. CD8+ cell numbers were largely normal at study entry and declined slightly after the first rituximab infusion. The CD4+:CD8+ ratio was 0.4 at study entry, 0.46 at 6 months, and 0.6 at 12 months. As with the total T-cell numbers, there was wide inter-patient variability in T-cell subsets. Less recovery was observed among the CD4+, CD45RA+ population of naïve T-cells. These cells were present in low numbers after HCT in contrast to the persistently higher numbers of CD4+CD45RO+ activated or memory T cells. Natural killer cells as identified by the CD56+/CD16+ subset of lymphocytes were present in normal quantities from study entry throughout the first year of follow-up.

Antibody titers against tetanus, H. flu and pneumococcus were measured at baseline, three months after the first vaccination set and three months after the second vaccination set. Complete data are available on 22 patients. At baseline 6 (27%) patients had protective antibodies against H.flu, 7 (32%) against pneumococcus, and 12 (55%) against tetanus. After the first and second vaccinations 16 (73%) and 17 (77%) showed protective antibody titers to H.flu, respectively. For tetanus, 14 (64%) and 15 (68%) had demonstrable protective antibody titers post first and second vaccinations. However, for pneumococcus, only 9 (41%) patients had protective titers after both vaccinations.
Discussion

HDT with autologous HCT is a standard treatment for patients with chemosensitive relapsed and refractory DLCL. Yet, 40-60% of patients successfully undergoing HCT will suffer a relapse of their disease. The objective of our study was to test the feasibility of adjuvant rituximab after HCT, a potential strategy to address the minimal residual disease that remains in many patients after transplantation and destines them to relapse. This strategy has been pursued with interferon and interleukin-2 but neither possesses the specificity, favorable toxicity profile or independent efficacy of rituximab.\textsuperscript{13,20,21} Subsequent to the initiation of our trial, other groups have reported their experiences with rituximab in combination with HCT.\textsuperscript{5,22-25} In these studies, rituximab was used as an \textit{in vivo} purging strategy prior to as well as after HCT. Treatment regimes for these trials are listed in Table 4 and their experiences are discussed below in relation to our trial.

The rates of EFS and OS of 83% and 88% at 2 years compare favorably to historical series, including our own.\textsuperscript{3} Our data are particularly noteworthy in the subgroup of 21 patients with relapsed or refractory DLCL for whom rates of relapse and survival are well characterized. The two- year EFS and OS were 58% and 62% respectively, in 203 patients with NHL transplanted at Stanford University between 1994-2000 undergoing similar preparatory regimens and \textit{in vitro} purging of the autologous grafts. However, our trial was primarily designed to test the feasibility of this approach and not intended as a comparison to standard high dose regimens. Further, as noted above, the study patients were
selected from a group of 92 patients transplanted within the time period, 57 of whom were not eligible or chose not to participate. Among these 57 patients who were not recruited to the study, 55 had chemosensitive disease compared with 34 of 35 study patients.

The excellent outcomes for the majority of our patients suggest value in testing this strategy further. It is of interest that a higher response rate of (78% vs. 43%, p< .01) was seen for patients treated with prior HCT compared to prior conventional therapy in the rituximab pivotal trial. Anecdotally, rituximab was surprisingly effective as a single agent in patients with DLCL relapsed after HCT. However, Rapaport et al. reported a 2 year EFS of 30% and OS of 35% in the only other trial of rituximab and HCT conducted primarily in DLCL. These differences emphasize the requirement for a phase III trial to determine if the addition of rituximab contributes meaningfully to the overall success of HCT.

As in the non-transplant setting, we found rituximab to be tolerated well after HCT. In the 35 patients treated, there was a single significant infusional toxicity. Despite the requirement for adequate engraftment, we observed grade 3-4 neutropenia in 54% of our patients with many experiencing multiple episodes occurring up to one year from transplantation. Flinn et al. reported transient neutropenia in 6 of 25 indolent NHL patients who received rituximab as part of an in vivo purging strategy prior to HCT and a single dose of rituximab after engraftment. Rapaport et al. described decreased white blood cell counts in only 4 of 26 patients while Mangel et al. and Ladetto et al. did not report neutropenia with rituximab after HCT in 13 and 32 patients, respectively.
retrospective review of 28 patients treated with the same HDT followed by HCT with a purged graft at our institution, we found no cases of documented delayed neutropenia. However, the patients on the current study were under closer surveillance with more opportunities to detect neutropenia. Other hematologic toxicities were rare in our trial.

Papadaki et al. reported on 2 cases of neutropenia in rituximab treated patients who showed evidence of large granular lymphocytes (LGL) as characterized by a predominance of CD3+ CD8+ CD57+ CD28-T-cells in blood and bone marrow.27 These authors postulated a mechanism whereby the LGLs induce neutropenia as has been described by CD95 triggered apoptosis from Fas and Fas-ligand secreted by LGLs as well as Fas/Fas-ligand independent mechanisms. 28,29 Although LGL infiltration was not noted in our bone marrow specimens or peripheral blood analyses, none of our samples were evaluated by flow cytometry. Another hypothesis regarding the etiology of neutropenia concerns the role of tumor necrosis factor (TNF). Voog et al. found that high circulating levels of the 75kd TNF receptor were associated with a greater incidence and longer duration of grade 4 neutropenia among 101 patients treated with chemotherapy alone. 30 Investigators described higher levels of TNF in a subset of patients receiving rituximab with CHOP as compared to patients treated with CHOP alone. 31 Further evaluation of these potential mechanisms is recommended for patients who develop neutropenia with rituximab in future studies.
The complete absence of B-cells at one year after HCT in all patients receiving two courses of rituximab in our study was notable. B-cell recovery was seen over the course of the next 12 months with return to normal values in most patients evaluated at 24 months post transplant. This delayed B-cell recovery is different from the experiences with HCT without rituximab where B-cell recovery occurs between 3-6 months. Early experience with a single course of rituximab in the non-transplant setting showed prompt B-cell depletion with recovery beginning at 6 months and recovery to the normal range by 9-12 months post therapy. T-cell recovery was not clearly influenced by the addition of rituximab to HCT. Consistent with the literature, T-cell recovery in our study demonstrated relatively normal numbers of CD8+ cells with CD4+ numbers remaining low for a year or longer, resulting in a persistently inverted CD4+:CD8+ ratio. Likewise, the CD45+RO+ memory cells far exceeded numbers of CD45+RA+ naïve T-cells during the first year. This phenomenon has been noted previously in patients recovering from HCT and suggests that T-cell recovery represents expansion of mature T-cells and a limited T-cell repertoire. We observed normal rather than elevated NK cell numbers but may have missed early elevations as our baseline measurements were 6 weeks after HCT.

Mean immunoglobulin levels were normal or near normal at study initiation and remained in this range during the follow-up period. However, despite normal mean values, many individual patients had suppressed immunoglobulin levels throughout the duration of follow-up. Our results are not different than experiences with HCT without rituximab. In the course of this study, we
evaluated the ability of patients to mount secondary humoral immune responses to recall antigens. Few patients were able to mount a new or show evidence of a boosted humoral response to the T-cell independent pathway required for pneumococcal vaccination. Better humoral responses were found for the conjugate H. flu vaccine as well as the T-cell dependent tetanus vaccine. Because Storek et al. found poor T-cell independent B-cell responses up to 2 years from bone marrow transplantation, it is not clear that rituximab adversely influenced humoral responses. However, van der Kolk et al. found 11 patients to have significantly decreased responses to recall antigens, tetanus and polio immunizations, and no response to primary antigens KLH (keyhole limpet hemocyanin) or hepatitis A vaccine after rituximab monotherapy. It is possible that elimination of B-cells for an extended period of time by rituximab could further blunt or even abrogate humoral immune responses after HCT. From a practical standpoint, we did not observe serious infections in our study. Other series employing rituximab in the setting of HCT described divergent results, ranging from limited infections seen by Flinn et al. and Mangel et al. to fatal pneumonia and CMV reactivation reported by Ladetto et al. to septic deaths and CMV infections in the series of Rapoport et al. and Goldberg et al. As described in Table 4, there are differences in rituximab scheduling in each of these studies but it is not obvious that these subtle differences would result in highly disparate toxicity.

The results from our study illustrate that rituximab is a feasible adjuvant to HCT. Although delayed and severe neutropenia was observed, this toxicity did
not result in significant adverse consequences and responded rapidly to granulocyte stimulating factors. The optimal dose and schedule for rituximab use in the peri-transplant setting has not been defined. The ability to effect an *in vivo* purge of circulating lymphoma cells with rituximab and the well-established ability of rituximab to achieve a molecular remission in follicular lymphoma recommends further study of rituximab during mobilization. Alternately, our study provides intriguing data regarding the use of rituximab after ACHT. It is important to establish if peri-transplant rituximab can improve the cure rate in relapsed or refractory DLCL and, to that end, we strongly support the U.S. Intergroup randomized controlled trial comparing a standard HCT with one that incorporates rituximab.

**References**


27. Papadaki T, Stamatopoulos K, Stavroyianni N, Paterakis G, Phisphis M, Stefanoudaki-Sofianatou K. Evidence for T-large granular lymphocyte-
mediated neutropenia in Rituximab-treated lymphoma patients: report of two cases. Leuk Res. 2002;26:597-600


### Table 1

**Patient Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>(%)</th>
</tr>
</thead>
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<tr>
<td>Male</td>
<td>23</td>
<td>66</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>34</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse large B-cell</td>
<td>25</td>
<td>71</td>
</tr>
<tr>
<td>Mantle cell</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Transformed</td>
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<td>9</td>
</tr>
<tr>
<td>Other B-cell(^1)</td>
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<td>11</td>
</tr>
<tr>
<td><strong>Disease status at AHCT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsed</td>
<td>18</td>
<td>51</td>
</tr>
<tr>
<td>Refractory</td>
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<td>29</td>
</tr>
<tr>
<td>First remission</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td><strong>Response to last chemotherapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete remission/minimal disease(^2)</td>
<td>25</td>
<td>71</td>
</tr>
<tr>
<td>Partial remission</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>Stable disease</td>
<td>1</td>
<td>3</td>
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</table>

\(^1\)Other histologies were follicular grade 2 (mixed) lymphoma, marginal zone lymphoma, CLL and a diffuse small cell lymphoma that could not be further classified.

\(^2\)Minimal disease is defined as 75% reduction in tumor volume and no mass greater than 2 cm.
### Table 2

**Hematologic Toxicity***

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N Patients</th>
<th>(%)</th>
<th>N Episodes by Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>21</td>
<td>60</td>
<td>26</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Anemia</td>
<td>4</td>
<td>11</td>
<td>4</td>
</tr>
</tbody>
</table>

* Graded according to the Common Toxicity Criteria
### Table 3

**Non-Hematologic Toxicity**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>N patients</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonitis&lt;sup&gt;¹&lt;/sup&gt;</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Herpetic infection</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Respiratory infection</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Infusional toxicity</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>¹</sup>Fatal in one patient
## Table 4
Rituximab and Autologous Hematopoietic Cell Transplantation

<table>
<thead>
<tr>
<th>Author/Reference</th>
<th>NHL Histology</th>
<th>N</th>
<th>Mobilization</th>
<th>Post-HCT</th>
<th>Median Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flinn(^{11})</td>
<td>Indolent</td>
<td>25</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Magni(^{25})</td>
<td>Indolent and mantle cell</td>
<td>15</td>
<td>4</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Ladetto(^{26})</td>
<td>Multiple</td>
<td>32</td>
<td>2-4</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Mangel(^{27})</td>
<td>Mantle cell</td>
<td>13</td>
<td>1</td>
<td>8(^1)</td>
<td>8</td>
</tr>
<tr>
<td>Rapoport(^{28})</td>
<td>Multiple (22 DLCL)</td>
<td>33</td>
<td>-</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>Horwitz</td>
<td>Multiple (25 DLCL)</td>
<td>35</td>
<td>-</td>
<td>8(^2)</td>
<td>28</td>
</tr>
</tbody>
</table>

1 Variable schedule post HCT  
2 Initial 4 patients received 4 doses only  

NHL=Non Hodgkin’s Lymphoma / DLCL=Diffuse large B-cell lymphoma
Figure Legends

**Figure 1.** Schema for rituximab after high dose therapy and autologous hematopoietic cell transplantation.

**Figure 2.** Event-free survival (top) and overall survival (bottom) for 35 patients enrolled in the study. Dotted lines represent 95% confidence intervals. Tic marks represent censored data.

**Figure 3.** Event-free survival (top) and overall survival (bottom) for 21 patients with relapsed or refractory diffuse large B-cell lymphoma. Dotted lines represent 95% confidence intervals. Tic marks represent censored data.

**Figure 4.** Serial assessment of IgG and lymphocyte subsets. Time is measured in months since transplantation. IgG levels are measured in mg/dl and CD20+, CD45 RA+ and CD45 RO+ numbers are expressed as number of cellsx10^6/µL. Each patient sample is represented by an X. Median values are represented by the line.
Cyclophosphamide 4gm/m², G-CSF 10µg/kg

↓

Leukapheresis
CD34⁺ enriched and purged

↓

BCNU (or TBI) /Etoposide/Cyclophosphamide

↓

HCT

↓

Day 42
Rituximab 375 mg/m² weekly x 4
(Patients 1-35)

↓

Day 180
Rituximab 375 mg/m² weekly x 4
(Patients 5-35)

Figure 1
Figure 2
Figure 3
Figure 4

- IgG
- CD20+
- CD45 RA+
- CD45RO+
Rituximab as adjuvant to high-dose therapy and autologous hematopoietic cell transplantation for aggressive non-Hodgkin's lymphoma

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