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Autologous transplant for relapsed follicular lymphoma: impact of pre-transplant rituximab sensitivity

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Abstract

Rituximab-refractory follicular lymphoma (FL) patients have limited options. Before the rituximab era, autologous stem cell transplantation (ASCT) was shown to improve outcomes in chemotherapy-sensitive, relapsed FL, but the impact of rituximab-sensitivity on these results is unknown. We analyzed 194 consecutive relapsed FL patients who underwent ASCT at our center and categorized them as rituximab-sensitive (RS, n=35), rituximab-refractory (RR, n=65), or no rituximab (NoR, n=94) if transplanted before rituximab was used. Progression-free survival at 3 years was 85% in RS and 35% in RR patients ($P = .0004$). Only rituximab-sensitivity was significant on multivariate analysis with improved OS (HR 0.24, $P = .01$) and PFS (HR 0.35, $P = .006$) in RS patients and increased relapse in RR patients (HR 2.11, $P = .01$). Pre-transplant rituximab-sensitivity is a strong independent predictor of post-transplant outcomes in relapsed FL, though one-third of RR patients achieved a PFS of over 3 years with ASCT.

Keywords

Follicular lymphoma; autologous transplant; rituximab-refractory

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This study or any of its parts have not been presented elsewhere.

Conflict of Interest Disclosure

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INTRODUCTION

Before the introduction of rituximab (R) into standard induction and maintenance regimens, high-dose therapy and autologous stem cell transplant (ASCT) had shown improved outcomes in chemotherapy-sensitive, relapsed follicular lymphoma (FL) [1,2]. Current day transplant practices have been influenced by these studies done in the pre-R era. While the addition of R to conventional chemotherapy is considered a standard of care in FL therapy, R-resistance is a common clinical occurrence, present in about a third [3] of treatment-naïve patients and increasing in frequency with repeated exposure. As evidenced in cell line models, R-resistant cells also acquire imbalances in pro-and anti- apoptotic mechanisms that can lead to chemotherapy cross-resistance. R-resistance may therefore not only affect subsequent responses to anti-CD20 monoclonal antibodies, but may also affect responses to high-dose chemotherapy and ASCT. We sought to evaluate the impact of R-sensitivity on ASCT outcomes for FL in the current R-era.

MATERIALS AND METHODS

We retrospectively analyzed 194 consecutive patients with relapsed grade 1, 2, or 3A FL who underwent ASCT at the Fred Hutchinson Cancer Research Center (FHCRC) from April 1993 to October 2011. Patients were categorized as R-sensitive (RS), R-refractory (RR), or no R (NoR) if transplanted prior to the R-era. Cases with aggressive transformation and grade 3B FL were excluded due to its closer relation to diffuse large B-cell lymphoma (DLBCL) [4]. To this end, patients with a diagnosis of centroblastic lymphoma, follicular and diffuse (Kiel classification) were also excluded [5].

The Institutional Review Board of FHCRC approved this minimal-risk study. Patients' data from our institutional database was analyzed independently by two authors (CPD and ENL). All patients had relapsed FL prior to the time of ASCT. Response to chemotherapy was defined as chemosensitive if the patient had achieved a complete remission (CR) or partial remission (PR) from their last chemotherapy immediately prior to ASCT and chemorefractory if they had not achieved at least a PR or had disease progression within 6 months of therapy. R-refractoriness was defined as failure to achieve at least a PR or documented disease progression within 6 months of 1). receiving the first dose of a full course of single-agent rituximab (4 doses of 375mg/m² weekly), 2). completion of rituximab maintenance (R-maintenance) or progression before the next scheduled rituximab dose, or 3). completion of 2 courses of rituximab combined with chemotherapy (R-chemotherapy). Disease status at the time of ASCT was defined as in remission or relapse (persistent disease or untreated relapse). Transplant conditioning regimens were categorized according as chemotherapy-only, radioimmunotherapy-based and TBI-based. The remission quotient (RQ) – calculated by dividing the time (in months) from diagnosis to ASCT by the number of prior treatments was assessed. We developed the RQ as a prognostic marker in relapsed mantle cell lymphoma patients undergoing ASCT [6], with a score of less than 6 being particularly unfavorable. Although its utility in FL has not yet been established, we found the RQ useful to demonstrate the number of treatments received as a function of disease duration prior to ASCT because the timing of and need for treatment/transplant in FL varies widely among referral practices, treating physicians, and individual disease states.

Primary endpoints were relapse rate, progression-free survival (PFS) and overall survival (OS). Response definitions are as per International Working Group criteria [7]. Relapse rate was calculated as a cumulative incidence in a competing risk framework considering non-relapse mortality as a competing event. As not all patients with post-ASCT relapse will need therapy, we analyzed the time-to-next treatment (TTNT), which was a time-dependent analysis starting from the time of post-transplant relapse and using treatment for that relapse as the endpoint. Group-wise comparisons of distributions of laboratory, treatment, and known pre-transplant prognostic markers were performed with Fisher's exact tests. Our Cox regression model included pre-ASCT factors that could affect post-ASCT outcomes, as follows: age 50, FL International Prognostic Index (FLIPI) 3, elevated LDH at time of transplant, prior chemotherapy regimens 3, chemoresistance, prior radiotherapy, stem cell source and $RQ < 6$. Pre-ASCT factors were assessed based on their value prior to initiation of the transplant-conditioning regimen. Survival curves were estimated using Kaplan-Meier plots.

RESULTS AND DISCUSSION

There were 35 RS, 65 RR, and 94 NoR patients. The median age at the time of ASCT, the median times to ASCT and baseline characteristics (Table I) were comparable except for high pre-ASCT FLIPI in 3%, 23%, and 26% of RS, RR, and NoR patients, respectively and receipt of post-transplant R-maintenance in 4, 8, and none of the RS, RR, and NoR patients, respectively.

Pre-transplant salvage chemotherapy included ICE (ifosfamide, carboplatin, etoposide) chemotherapy, high-dose anthracycline regimens, gemcitabine- or fludarabine- based therapy plus varying combinations of these with or without rituximab. The types of preparative regimen utilized were distributed with roughly similar frequencies between chemotherapy-only, radioimmunotherapy (RIT)-based and TBI-based regimens in the RR, RS, and NoR groups. Median follow-up from the time of ASCT to time of last contact in surviving patients was 65 months (range 10 – 169), 61 months (range 13 – 157), and 193 months (range 33 – 231) in the RS, RR, and NoR groups, respectively. Univariate analysis showed significantly fewer relapses in RS, compared with RR and NoR patients ($P = .004$) (Figure 1). Most of the relapses in RR patients occurred within 2 years of ASCT (32 of 37 patients, 86%) compared with 57% and 56% of relapses in RS and NoR patients, respectively. The median TTNT in these patients was 79, 40, and 52 days from the time of relapse in RS, RR, and NoR patients, respectively. Univariate analyses showed significantly better OS ($P = .003$) and PFS ($P = .0004$) in RS patients with a 3-year OS and PFS of 97% and 85% compared with 63% and 35% in RR and 73.4% and 49% in NoR patients, respectively (Figure 2 & 3). No difference in OS (Figure 4) or PFS (Figure 5) was appreciated when we limited our comparison to R versus NoR patients.

Multivariate adjustment showed OS to be affected only by rituximab sensitivity, with a lower risk of post-transplant death in RS patients (HR 0.24, $P = .01$). Multivariate analysis also showed increased risk of relapse in RR compared to RS and NoR patients (HR 2.11, $P = .01$) and better PFS in RS compared to RR and NoR patients (HR 0.35, $P = .006$). High FLIPI score and age ≥ 50 showed non-statistically significant increases in mortality risk (HR

1.69, $P = .07$ and HR 1.59, $P = .05$, respectively). The different outcomes between RS and RR patients were maintained independent of transplant conditioning regimen. We did not include R-maintenance in our multivariate model because only a small proportion received it and it had lacked significance on univariate analysis. There were no differences in outcomes whether RR patients were refractory to single-agent rituximab ($N=30$), R-maintenance ($N=5$), or R-chemotherapy ($N=30$). Subset analysis of patients who received RIT-based conditioning showed similar PFS and OS in the rituximab-treated and rituximab-naïve groups. Our objective was to analyze the impact of rituximab sensitivity on outcomes after ASCT. The mechanisms underlying rituximab refractoriness are not fully understood. Many patients with CD20-expressing tumors, including rituximab-naïve patients are refractory. In vitro studies with rituximab-resistant cell lines, apart from downregulation of CD20 antigen and mRNA, have also shown deregulation of the ubiquitin-proteasome system and complement-inhibitory proteins, as well as proapoptotic (Bax/Bak) and antiapoptotic (Mcl-1, Bcl-XL) proteins that eventually lead to cross-resistance to chemotherapeutic agents [8–11]. In patients with relapsed DLBCL, prior rituximab exposure has been shown to adversely affect response to salvage chemotherapy [12]. We speculate that the development of R-refractoriness reflects tumor biology evolution that confers more resistant disease which can only be partially overcome with high-dose therapy, making R-sensitivity an important pre-ASCT prognostic marker. Another pre-ASCT prognostic marker – high FLIPI score has limited use because it defines only a small proportion patients (15 to 36% in published studies) [13–15], and accounted for only 20% in our study, with heterogeneous outcomes seen in the remaining 80% of our patients.

Consistent with 2 other reports, we found no differences in OS between R and NoR patients. Le Gouill et al., as part of a larger retrospective analysis reported on 34 and 29 relapsed patients who were treated with rituximab and were rituximab-naïve, respectively. Post-transplant 3-year OS were similar in both groups [14]. Kang et al. also showed similar OS post-ASCT in patients who had ($N=35$) or had not ($N=71$) received rituximab prior to transplant [16]. However, in our study, the difference in OS (and PFS) became clear once we separated the RS from RR patients. This difference was retained on multivariate analysis correcting for known poor pre-transplant prognostic factors and differences between the groups. A recent retrospective series of 100 relapsed FL patients that included 38 R-naïve, 8 R-refractory, and 24 transformed lymphoma (TL) patients did not identify differences in outcome in their R-refractory group. This finding may be related to their small R-refractory population and the inclusion of TL patients that made up 24% of their cohort [17].

We also found that RR disease before ASCT predicted for increased post-transplant relapse with most (86%) occurring within 2 years after ASCT. This has implications on treatment decision-making – whether these patients should instead be considered for tandem autologous-mini-allogeneic transplant, or whether maintenance strategies would benefit this particular group of patients more. However, despite the early relapses seen after ASCT, some RR patients still derive benefit from high-dose therapy and are alive at more than 5-years follow up, although their OS and PFS curves for these patients do not plateau.

These results need to be considered in light of the limited treatment options available to heavily pre-treated RR patients. With single-agent radioimmunotherapy, overall response

rates (ORR) of 74% with yttrium-90 ibritumomab tiuxetan[18] and 62% with iodine-131 tositumomab[19] have been reported, but median time to progression was only 6.8 and 8.9 months, respectively. Bendamustine, another drug approved for R-refractory indolent lymphomas was shown to have an ORR of 74% but with median duration of response of only 10 months[20]. The similarity between the OS and PFS curves of RR and NoR patients provides support for the continuing relevance of ASCT as salvage treatment in a subset of RR FL patients in the current era.

We used the generally accepted criteria of rituximab resistance to categorize patients who had received rituximab before ASCT. Definitions were consistent with previously published studies[20,21], including the pivotal trial that led to approval of bendamustine in patients progressing during or after rituximab [22]. We acknowledge that these tumor-response criteria were developed for cytotoxic therapy and may not be an ideal gauge for antibody therapy. We also recognize that when combined with chemotherapy, the contribution of rituximab to the efficacy and resistance of a given regimen is unknown. However, no available laboratory test can define rituximab-resistance in vivo and therefore at present, clinical definitions prevail. Furthermore, analysis within the RR group according to whether patients were refractory to single-agent, R-maintenance or R-chemotherapy did not show any differences in OS, PFS or relapse rate.

This study has several limitations. Data were collected retrospectively from patients treated with different preparative regimens, subjected to different transplant selection criteria, follow up timings and changing patient care factors over time. However, multivariate adjustment for imbalances between the groups maintained rituximab sensitivity as highly prognostic. Improved PFS with post-ASCT R-maintenance, as demonstrated recently, was not seen in our study due to the small number of patients receiving maintenance. Despite R-maintenance, RR patients still suffered from more relapses than NoR patients. Furthermore, R-maintenance cannot account for the improved OS seen in the RS group[23].

In conclusion, we show for the first time that pre-transplant rituximab sensitivity and resistance are strong independent prognostic markers of post-transplant outcomes. Allogeneic donor options and novel post-ASCT maintenance strategies need to be considered, particularly in RR patients.

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References

1. Schouten HC, Qian W, Kvaloy S, et al. High-dose therapy improves progression-free survival and survival in relapsed follicular non-Hodgkin's lymphoma: results from the randomized European CUP trial. *J Clin Oncol.* 2003; 21(21):3918–3927. [PubMed: 14517188]
2. Rohatiner AZ, Nadler L, Davies AJ, et al. Myeloablative therapy with autologous bone marrow transplantation for follicular lymphoma at the time of second or subsequent remission: long-term follow-up. *J Clin Oncol.* 2007; 25(18):2554–2559. [PubMed: 17515573]

3. Ghielmini M, Schmitz SF, Cogliatti SB, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly x 4 schedule. *Blood*. 2004; 103(12):4416–4423. [PubMed: 14976046]
4. Bosga-Bouwer AG, van den Berg A, Haralambieva E, et al. Molecular, cytogenetic, and immunophenotypic characterization of follicular lymphoma grade 3B; a separate entity or part of the spectrum of diffuse large B-cell lymphoma or follicular lymphoma? *Hum Pathol*. 2006; 37(5): 528–533. [PubMed: 16647949]
5. Lennert, K.; Feller, AC. *Histopathology of non-Hodgkin Lymphomas : (based on the updated Kiel classification)*. ed 2nd completely rev.. Berlin ; New York: Springer-Verlag; 1992.
6. Cassaday RD, Guthrie KA, Budde EL, et al. Specific features identify patients with relapsed or refractory mantle cell lymphoma benefitting from autologous hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2013; 19(9):1403–1406. [PubMed: 23871782]
7. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol*. 1999; 17(4):1244. [PubMed: 10561185]
8. Takei K, Yamazaki T, Sawada U, Ishizuka H, Aizawa S. Analysis of changes in CD20, CD55, and CD59 expression on established rituximab-resistant B-lymphoma cell lines. *Leuk Res*. 2006; 30(5): 625–631. [PubMed: 16289746]
9. Bannerji R, Kitada S, Flinn IW, et al. Apoptotic-regulatory and complement-protecting protein expression in chronic lymphocytic leukemia: relationship to in vivo rituximab resistance. *J Clin Oncol*. 2003; 21(8):1466–1471. [PubMed: 12697868]
10. Jazirehi AR, Vega MI, Bonavida B. Development of rituximab-resistant lymphoma clones with altered cell signaling and cross-resistance to chemotherapy. *Cancer Res*. 2007; 67(3):1270–1281. [PubMed: 17283164]
11. Olejniczak SH, Hernandez-Ilizaliturri FJ, Clements JL, Czuczman MS. Acquired resistance to rituximab is associated with chemotherapy resistance resulting from decreased Bax and Bak expression. *Clin Cancer Res*. 2008; 14(5):1550–1560. [PubMed: 18316580]
12. Martin A, Conde E, Arnan M, et al. R-ESHAP as salvage therapy for patients with relapsed or refractory diffuse large B-cell lymphoma: the influence of prior exposure to rituximab on outcome. A GEL/TAMO study. *Haematologica*. 2008; 93(12):1829–1836. [PubMed: 18945747]
13. Vose JM, Bierman PJ, Loberiza FR, et al. Long-term outcomes of autologous stem cell transplantation for follicular non-Hodgkin lymphoma: effect of histological grade and Follicular International Prognostic Index. *Biol Blood Marrow Transplant*. 2008; 14(1):36–42. [PubMed: 18158959]
14. Le Gouill S, De Guibert S, Planche L, et al. Impact of the use of autologous stem cell transplantation at first relapse both in naive and previously rituximab exposed follicular lymphoma patients treated in the GELA/GOELAMS FL2000 study. *Haematologica*. 2011; 96(8):1128–1135. [PubMed: 21486862]
15. Keeney GE, Gooley TA, Pham RN, et al. The pretransplant Follicular Lymphoma International Prognostic Index is associated with survival of follicular lymphoma patients undergoing autologous hematopoietic stem cell transplantation. *Leuk Lymphoma*. 2007; 48(10):1961–1967. [PubMed: 17917964]
16. Kang TY, Rybicki LA, Bolwell BJ, et al. Effect of prior rituximab on high-dose therapy and autologous stem cell transplantation in follicular lymphoma. *Bone Marrow Transplant*. 2007; 40(10):973–978. [PubMed: 17873917]
17. Peters AC, Duan Q, Russell JA, Duggan P, Owen C, Stewart DA. Durable event-free survival following autologous stem cell transplant for relapsed or refractory follicular lymphoma: positive impact of recent rituximab exposure and low-risk Follicular Lymphoma International Prognostic Index score. *Leuk Lymphoma*. 2011; 52(11):2124–2129. [PubMed: 21740097]
18. Witzig TE, Flinn IW, Gordon LI, et al. Treatment with ibritumomab tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. *J Clin Oncol*. 2002; 20(15):3262–3269. [PubMed: 12149300]

19. Horning SJ, Younes A, Jain V, et al. Efficacy and safety of tositumomab and iodine-131 tositumomab (Bexxar) in B-cell lymphoma, progressive after rituximab. *J Clin Oncol.* 2005; 23(4): 712–719. [PubMed: 15613695]
20. Cheson BD, Friedberg JW, Kahl BS, Van der Jagt RH, Tremmel L. Bendamustine produces durable responses with an acceptable safety profile in patients with rituximab-refractory indolent non-Hodgkin lymphoma. *Clin Lymphoma Myeloma Leuk.* 2010; 10(6):452–457. [PubMed: 21189660]
21. Czuczman MS, Fayad L, Delwail V, et al. Ofatumumab monotherapy in rituximab-refractory follicular lymphoma: results from a multicenter study. *Blood.* 2012; 119(16):3698–3704. [PubMed: 22389254]
22. Kahl BS, Bartlett NL, Leonard JP, et al. Bendamustine is effective therapy in patients with rituximab-refractory, indolent B-cell non-Hodgkin lymphoma: results from a Multicenter Study. *Cancer.* 2010; 116(1):106–114. [PubMed: 19890959]
23. Pettengell R, Schmitz N, Gisselbrecht C, et al. Rituximab Purging and/or Maintenance in Patients Undergoing Autologous Transplantation for Relapsed Follicular Lymphoma: A Prospective Randomized Trial From the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. *J Clin Oncol.* 2013; 31(13):1624–1630. [PubMed: 23547078]

Figure 1.
Relapse rate according to whether patients were rituximab sensitive, rituximab refractory or rituximab naïve prior to autologous transplant

Figure 2.
Kaplan-Meier analysis of progression-free survival in rituximab sensitive, rituximab refractory or rituximab naïve patients

Figure 3.
Kaplan-Meier analysis of overall survival in rituximab sensitive, rituximab refractory or rituximab naïve patients

Figure 4.
Kaplan-Meier analysis of overall survival in rituximab versus rituximab naïve patients

Figure 5.
Kaplan-Meier analysis of progression-free survival in rituximab versus rituximab naïve patients

Table I

Characteristics of the 194 patients at the time of autologous transplant

	Rituximab sensitive	Rituximab refractory	No rituximab group	P-value
Number	35	65	94	
Male/Female	22/13	49/16	59/35	
Median age, y (range)	51.3 (18–69)	52.1 (32–69)	49.5 (30–64)	
FLIPI (missing = 8)				.009
0 to 2	34 (97%)	50 (77%)	62 (66%)	
3 to 5 (high)	1 (3%)	15 (23%)	24 (26%)	
LDH (missing = 5)				.07
Normal	30 (86%)	48 (74%)	58 (62%)	
Elevated	5 (14%)	17 (26%)	31 (33%)	
Previous radiotherapy	5 (14%)	6 (9%)	14 (15%)	.56
Chemoresistance (missing = 11)	2 (6%)	10 (15%)	5 (5%)	.108
Number of prior chemotherapy regimens (missing = 1)				.19
Less than 3	15 (43%)	18 (28%)	37 (39%)	
More than or equal to 3	20 (57%)	47 (72%)	56 (60%)	
Disease status at transplant (missing = 3)				.55
Responsive (CR or PR)	31 (89%)	52 (80%)	75 (80%)	
CR	4 (13%)	7 (13%)	18 (24%)	
PR	27 (87%)	45 (87%)	57 (76%)	
Relapse (refractory or untreated relapse)	4 (11%)	13 (20%)	16 (17%)	
Time to transplant in months (range)	33 (6–202)	39 (3–283)	31 (5–221)	
Remission quotient				.13
Less than 6	4 (11%)	16 (25%)	13 (14%)	
More than or equal to 6	31 (89%)	49 (75%)	81 (86%)	
⁺ Preparative regimens				.66
Chemotherapy only	9 (26%)	15 (23%)	20 (21%)	
Radiolabelled antibody-based	18 (51%)	29 (45%)	39 (42%)	
TBI-based	8 (23%)	21 (32%)	35 (37%)	
Rituximab maintenance after transplant (missing = 11)	4 (11%)	8 (12%)	0	.004

FLIPI indicates Follicular Lymphoma International Prognostic Index; LDH, lactate dehydrogenase; TBI, total body irradiation

⁺ chemotherapy-only regimens consisted of busulfan (Bu)(8 – 12mg/kg PO), melphalan (100mg/m² IV), and thiotepa 500mg/m² IV; carmustine (300mg/m² IV), etoposide (800mg/m² IV), cytarabine (800mg/m² IV), and melphalan (140mg/m² IV); high-dose radiolabelled antibody based regimens consisted of I-131-labelled anti-CD20 antibodies (maximum dose of 20 to 27 Gy to normal organs) either alone or in combination with cyclophosphamide (Cy)(60mg – 100mg/kg IV) and etoposide (VP16)(60mg/kg IV) or combined with fludarabine (180mg/m² IV); radiation-based regimens consisted of Cy (100mg/kg IV) and fractionated TBI (12 – 15 Gy) with or without VP16 (60mg/kg IV).