Rituximab with peripheral blood stem cell transplantation in CD20 lymphoproliferative disorders

Bone marrow involvement in follicular center and mantle cell lymphomas and chronic lymphocytic leukemia is difficult to eliminate. Rituximab, an anti-CD20 specific therapy has been demonstrated to be effective in marrow disease. We used rituximab in combination with peripheral blood stem cell (PBSC) transplantation following BEAM therapy in patients with CD20 positive marrow disease either in residual disease or as a purging approach. Our experience emphasizes that the purging modality is more effective than the treatment of residual disease.

It is difficult to eradicate low-grade non-Hodgkin's lymphomas with marrow involvement because pathologic remission is achievable in only a minority of patients. Despite the relatively good prognosis of these patients, there is growing interest in the possibility of inducing true remissions[1] and potentially curing patients. Innovative approaches based on high dose therapies and specific immunotherapy toward the CD20 antigen expressed in neoplastic B cells[2-4] are being tested and some studies have shown that anti-CD20 murine humanized monoclonal antibody (rituximab) is effective in most CD20 positive disorders.[2-5] We focus on the use of rituximab in low-intermediate grade B lymphoproliferative disorders as therapy for residual disease following an intensive program or as purging treatment *in vivo* in patients receiving autologous PBSC transplantation.

Since June 1999 patients younger than 60 years with follicular center cell lymphoma (FCCL) and mantle cell lymphoma (MCL) stage IV with marrow involvement and chronic lymphocytic leukemia (CLL) stage C have received rituximab at the end of initial therapy if residual marrow disease was detected. A second protocol, aimed at *in vivo* purging, was started more recently in patients with FCCL or MCL with leukemic involvement and advanced stage CLL. Rituximab is administered before the collection of CD34 positive cells with the aim of purging the blood

Table 1. Sequential aggressive therapy and rituximab in indolent lymphomas.

STEP I: 3 Monthly courses of CHOP
- Cyclophosphamide 800 mg/m^2
- Oncovin 1.4 mg/m^2
- Doxorubicin 50 mg/m^2
- 6-methylprednisolone 60 mg/m^2/day × 5d

STEP II: Collection OF CD34 cells
- Cyclophosphamide 7g/m^2

STEP III: PBSCT
- BCNU 400 mg/m^2
- Etoposide 400 mg/m^2/day × 4d
- Aracytin 400 mg/m^2/day × 4d
- Melphalan 120 mg/m^2

Rituximab 375 mg/m^2/week × 4 week

Purging

STEP I: 3 Monthly courses of CHOP
(as above)

First mobilization:
- Cyclophosphamide 7g/m^2

First rituximab 375 mg/m^2

Second mobilization
- Aracytin 8 g/m^2

Second rituximab 375 mg/m^2
- PBSCT
- BEAM therapy as above

Table 2.

Sequential aggressive therapy in indolent lymphomas

Age (yrs)	*Sex*	*Disease*	*CD20 at onset*	*Step I*	*Step II*	*Step III*	*Post rit*	*Status*
				residual % CD20 in the marrow				
55	Male	FCCL	73%	29%	20%	15%	0.1%	28M CR
59	Male	FCCL	83%	45%	33%	20%	2%	22M CR
58	Male	FCCL	95%	66%	47%	9%	1%	12M Dead
53	Male	FCCL	93%	36%	26%	9%	0	19M CR
48	Female	FCCL	73%	53%	38%	32%	0.1%	17M CR
37	Male	MCL	43%	24%	10%	6%	0	8M Dead
47	Male	FCCL	78%	43%	37%	55%	0	16M Rel
48	Male	CLL	82%	77%	60%	38%	5%	10M Prog

Purging sequential aggressive therapy in indolent lymphomas

Age (years)	*Sex*	*Disease*	*CD20 at onset*	*Pre rit*	*First rit*	*Second rit*	*Post PB*	*Status*
						% CD20 positive cells		
53	Male	FCCL	95%	77%	10%	0	Not done	11M Rel
48	Male	FCCL	98%	78%	8%	0	0	11MCR
49	Female	MCL	97%	57%	3%	0	0	8MCR
37	Male	SMCL	93%	65%	0	0	Not done	4M CR
54	Male	CLL	95%	85%	15%	0	0	8M CR

FCCL; follicular center cell lymphoma: CLL; chronic lymphocytic leukemia: MCL; mantle cell lymphoma: SMCL; splenic marginal cell lymphoma: Step I; CHOP therapy: Step II; mobilization by cyclophosphamide: Step III; PBSCT: Pre Rit; after CHOP: Post PB; after PBSCT.

from CD20 positive cells (Table 1).

A total of 13 patients are now evaluable; there are 2 females and 11 males. Eight patients entered the protocol for residual disease and 5 patients were admitted to the purging protocol. The mean age of the patients is 51 years (range 37 to 59). In the first group 6 patients had FCCL, 1 MCL and 1 CLL; all patients had bone marrow involvement and three of them had leukemic involvement. The second group of patients was formed of 2 patients with FCCL, 1 with MCL, 1 with CLL and 1 with splenic marginal cell lymphoma (SMCL); all patients had leukemic involvement of the blood. The immunophenotype showed a mean percentage of CD20 positive cells in the marrow of 77.5% (range:43 to 95%) in the first group of patients and 97% (range:93 to 98%) for the second group. The evaluation of marrow cells was done with a panel of monoclonal antibodies directed to CD19, CD22, CD5, CD23, CD25, CD11c, CD10.

At each step of therapy patients underwent a bone biopsy for the detection of residual disease with re-evaluation in the marrow cells of CD20 expression and those antigens positive at the onset of disease.

Patients enrolled in the study after the completion of the entire program had monthly clinical and hematologic evaluations; furthermore re-evaluation of marrow biopsy and residual disease was planned every two months.

The patients treated with rituximab for residual disease, before PBSC autotransplant, had between 10% to 60% (mean 34%) CD20 positive cells in the bone marrow. Following PBSC autotransplantation this percentage dropped to between 6% to 55% (mean 23%). Following four doses of rituximab CD20 positive cells almost completely disappeared in 5 patients (Table 2). However, three patients with a persistence of CD5 cells relapsed. Four patients are in complete remission at a follow-up of 17 to 28 months, mean 21.5 months.

Three patients of the second group completed the program, two patients stopped the procedure following the second mobilizing therapy and second rituximab because of the reactivation of HBV infection. A great reduction of CD20 positive cells was recorded after the first mobilizing therapy and first rituximab and CD20 positive cells were not detectable following the second mobilization and second rituximab in any patient. One of the patients who had reactivated HBV infection manifested hepatitis, the other only a viremia without hepatitis; both patients had 0% of CD20 positive cells following the second mobilizing therapy. One patient with residual CD5 cells (15%) and CD23 cells (5%) relapsed 11 months following the last dose of rituximab. All other patients are in complete remission.

Many reports emphasize the improvement of remission rate with rituximab, although there is no uniform modality of treatment or timing.[2-9] Our experience with rituximab in residual resistant disease after high dose therapy or as *in vivo* purging in patients with leukemic manifestations as part of program including high dose therapy and PBSCT transplantation shows that the remission rate is improved whatever the modality but the modality of purging exerts a major and rapid effect. Apparently, there is synergy with therapy for mobilization of CD34 cells[10] and, in these patients, the consolidation of remission with PBSC autotransplant could make the difference in long-lasting remission. Adjunctive investigations are in progress.

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