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ORIGINAL ARTICLE

Treatment of refractory chronic GVHD with rituximab: a GITMO study

F Zaja[1], A Bacigalupo[2], F Patriarca[1], M Stanzani[3], MT Van Lint[2], C Filì[1], R Scimè[4], G Milone[5], M Falda[6], C Vener[7], D Laszlo[8], PE Alessandrino[9], F Narni[10], S Sica[11], A Olivieri[12], A Sperotto[1], A Bosi[13], F Bonifazi[3] and R Fanin[1], for the GITMO (Gruppo Italiano Trapianto Midollo Osseo)

[1]*Clinica Ematologica DIRM, Azienda Ospedaliera Universitaria, Udine, Italy;* [2]*Divisione di Ematologia, Ospedale San Martino, Genova, Italy;* [3]*Istituto di Ematologia ed Oncologia Medica L. ed A. Seragnoli, Bologna, Italy;* [4]*Divisione di Ematologia e TMO, Azienda Cervello, Palermo, Italy;* [5]*Istituto di Ematologia, Ospedale Ferrarotto, Catania, Italy;* [6]*Servizio di Ematologia, Ospedale Maggiore, Torino, Italy;* [7]*IRCCS, Ospedale Maggiore Policlinico e Università degli Studi, Milano, Italy;* [8]*Istituto Europeo di Oncologia, Milano, Italy;* [9]*Divisione di Ematologia, Policlinico S. Matteo, Pavia, Italy;* [10]*Clinica Ematologica, Policlinico Universitario, Modena, Italy;* [11]*Istituto di Ematologia, Università Cattolica S. Cuore, Roma, Italy;* [12]*Clinica di Ematologia, Ospedale Regionale di Torrette, Ancona, Italy and* [13]*Divisione di Ematologia, Policlinico Careggi, Firenze, Italy*

The anti-CD20 chimaeric monoclonal antibody Rituximab has recently been shown to induce significant clinical response in a proportion of patients with refractory chronic graft-versus-host disease (cGVHD). We now report 38 patients, median age 48 years (22–61), receiving Rituximab for refractory cGVHD, assessed for clinical response and survival. Median duration of cGVHD before Rituximab was 23 months (range 2–116), the median number of failed treatment lines was 3 (range 1 to $\geqslant$6) and the median follow-up after Rituximab was 11 months (1–88). Overall response rate was 65%: skin 17/20 (63%), mouth 10/21 (48%), eyes 6/14 (43%), liver 3/12 (25%), lung 3/8 (37.5%), joints 4/5, gut 3/4, thrombocytopaenia 2/3, vagina 0/2, pure red cell aplasia 0/1 and, myasthenia gravis 1/1. During the study period 8/38 died: causes of death were cGVHD progression ($n=3$), disease relapse ($n=1$), infection ($n=3$), sudden death ($n=1$). The actuarial 2 year survival is currently 76%. We confirm that Rituximab is effective in over 50% of patients with refractory cGVHD and may have a beneficial impact on survival.

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Introduction

Refractory chronic graft-versus-host disease (cGVHD) is an intractable complication associated with considerable morbidity and mortality: the average mortality is 26% and there has been no improvement over the past two decades.[1–2] Intensification of immunosuppressive therapy has failed to show any beneficial effect on survival.[3] A new therapeutic approach is therefore greatly needed.

Recently, the Boston group has reported encouraging results with the anti-CD20 chimaeric monoclonal antibody Rituximab;[4] it is indeed possible that Rituximab may be acting on the B-cell component of the disease. In keeping with this observation is the increasing use of Rituximab in the treatment of several autoimmune disorders such as immune thrombocytopaenias,[5] haemolytic anemia,[6] rheumatoid arthritis,[7] mixed cryoglobulinemia,[8] systemic lupus erythematousus [9] and thrombotic thrombocytopenic purpura.[10]

To address this issue better, the GITMO (Italian Group Bone Marrow Transplant) performed a retrospective study concerning the use of Rituximab in patients with refractory cGHVD. The results of this analysis are reported here.

Patients, materials and methods

This study was conducted retrospectively to established GITMO transplant centre experience in the use of Rituximab for the treatment of refractory cGVHD, that is, cGHVD already treated and not responsive to one at least prior treatment and/or necessitating chronic administrations of medium to high-dose steroids. Patient details, time and type of transplant, time of cGVHD onset, organ involvement, previous treatments, therapeutic schedule, safety, response rate and response duration were investigated.

Because the study was retrospective and multicentre, no single study-specific consent form could be obtained. However, each GITMO centre was asked to report the data of only those patients with a documented consent in their medical records.

To optimize collection and standardization of the results, participating GITMO centres were required to report data concerning clinical, functional, biochemical and radiological appearances according to the different sites of organ

Correspondence: Dr F Zaja, Clinica Ematologica DIRM, Policlinico Universitario, P.zza S. Maria della Misericordia, 33100 Udine, Italy.
E-mail: francesco.zaja@med.uniud.it
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involvement on specific predefined data forms. One single individual with recognized experience in haematopoietic stem cell transplantation completed the response forms for each centre. Skin and mucosa were approached by mean of a clinical assessment, to establish the presence of rash, scleroderma, lichenoid changes, xerostomia and any modification after therapy. The lachrymal apparatus was investigated clinically and with the Shirmer test. Joint involvement was studied analyzing level of subjective discomfort and functional limitations. To assess the liver we analysed the laboratory parameters (that is, bilirubin, transaminases, cholestasis index). Data on thorax included X-rays and high-resolution computed tomography scans together with pulmonary function tests (forced expiratory volume, vital capacity and diffusion capacity of carbon monoxide), which were evaluated for patients with lung involvement. Finally, for the gastrointestinal system, we considered clinical symptoms (weight and diarrhoea, vomiting). Analyzing all these variables, we defined the following response criteria: complete response (CR) in cases of complete resolution of cGVHD manifestations in the organ involved; partial response (PR) in cases of 50% or more regression of cGVHD manifestations; no response (NR) less than 50% organ improvement, or exacerbation during or after therapy. Time to response (TTR) was considered as the time elapsed between Rituximab administration and start of cGVHD improvement. The safety of Rituximab was analyzed short and mid term.

Results

Data of 38 adult patients treated with Rituximab were supplied by 12 GITMO centres accredited for allogeneic stem cell transplantation. Main patient clinical characteristics are summarized in Table 1. Most cases (29 of 38) received a fully matched sibling transplant and only a minority (9 of 38) a matched unrelated transplant; eight patients received a nonmyeloablative conditioning regimen. In two cases cGVHD followed the administration of donor lymphocytes (DLI).

Thirty-one patients had an extensive form of cGVHD, according to the revised Seattle classification,[11] and the median time between cGVHD development and Rituximab treatment was 23 months (range 2–116). Skin and mouth were the two most frequent sites of cGVHD involvement, affecting 28 and 23 patients, respectively (Table 2). Other sites of organ involvement were eyes (16 patients), liver (14 patients), lung (nine patients), joints (six patients), gut (four patients), vagina (two patients); four patients had haematological manifestations (three immune thrombocytopaenia, one pure red cell aplasia (PRCA)) (Table 2); one patient, previously described,[12] had a very severe from of myasthenia gravis.

Rituximab was offered as a second or further line of therapy in 9 and 29 cases, respectively. The seven patients with limited cGVHD had previously failed therapy either with steroid and cyclosporine (five patients), steroid and azathioprine (two patients), steroid, azathioprine, mycophenolate mofetil and photopheresis (one patient). During the period of Rituximab administration, patients were continued on baseline immunosuppressive therapy. In all cases Rituximab was given intravenously at the conventional dose of 375 mg/m^2 weekly; the median number of administrations was 4 (range 1–20), with heterogeneity caused by different clinical situations or institutional therapeutic policies. In particular, eight patients received up to 16 other infusions aimed at consolidation or maintenance, besides the four weekly administrations. Six of seven patients with limited cGVHD received four infusions and one received three.

Rituximab resulted in active control of different cGVHD manifestations in 65% of patients; results are reported in Table 2. Briefly, as far as skin involvement is concerned, significant improvement was observed in 63% of cases, the majority (56%) reaching PR. Notably, treatment was effective for sclerodermatous manifestations in 64% of patients. Median TTR and response duration were 57 days and 10 months, respectively; 6 of 17 responding patients had skin cGVHD that recurred or worsened. A significant improvement was observed in 48% (19% CR and 29% PR) of patients with mouth involvement, with a median TTR and median response duration of 46 days and 11 months, respectively; 1 of 10 patients who responded had mouth cGVHD that worsened 7 months after Rituximab treatment. For patients with skin and mouth cGVHD, the therapeutic effect of Rituximab allowed the reduction of baseline immunosuppressive therapy in 11 of 17 and 6 of 10

Table 1 Patients' main clinical features

Patients	38
Median age, years (range)	48 (22–61)
Male/female	20/18
Diagnosis	
Chronic myeloid leukaemia	8
Acute myeloid leukaemia	13
Acute lymphoblastic leukaemia	3
Multiple myeloma	6
Chronic lymphocitc leukaemia	3
Follicular lymphoma	3
T-peripheral lymphoma	1
Hodgkin lymphoma	1
Donor: sibling/unrelated	29/9
Conditioning regimens: myeloablative/ nonmyeloablative	30/8
Stem cell source: bone marrow/peripheral blood	12/26
cGVHD: limited/extensive	7/31
Median duration of cGvHD before Rituximab, months (range)	23 (2–116)
Median number of failed treatment lines before Rituximab (range)	3 (1–$\geqslant$6)
Previous treatments of cGVHD	Patients
Prednisone	38
Cyclosporine or tacrolimus	34
Photopheresis	14
Mofetil mycophenolate	19
Other treatments	25
Rituximab administrations	
$\leqslant$4	30
5–8	5
$>$8	3
Median follow-up after Rituximab, months (range)	11 (1–88)

Abbreviation: cGVHD = chronic graft-versus-host disease.

Table 2 Results after treatment with Rituximab according to the different site of cGVHD involvement

Organ involvement	*Pts*	*NV*	*OR*	*CR*	*NR*	*DR-IS after Rituximab (patients)*	*Median DR-steroid after Rituximab (range)*	*TTR (days)*	*RD (months)*	*Follow-up (months)*	*cGVHD recurrence/ worsening*
Skin	28	1	17 (63%)	2 (7%)	10 (37%)	11/17	Evaluable pts: 10; DR: 82% (0–100)	57 (7–150)	10 (1–22)	12 (1–33)	6/17
Scleroderma	11	0	7 (64%)	0	4 (36%)						
Mouth	23	2	10 (48%)	4 (19%)	11 (52%)	6/10	Evaluable pts: 6; DR: 82% (25–100)	46 (7–210)	11 (1–84)	13 (2–84)	1/10
Eyes	16	2	6 (43%)	2 (14%)	8 (57%)	2/6	Evaluable pts: 3; DR: 100% (all pts)	138 (7–270)	12 (9–70)	14 (10–22)	0
Liver	14	2	3 (25%)	0	9 (75%)	0/3	Evaluable pts: 2; DR: 25% and 90%	49 (43–56)	13 (12–18)	16 (1–18)	0
Lung	9	1	3 (37.5%)	0	5 (63%)	3/3	Evaluable pts: 2; DR: 76% and 100%	60 (50–120)	12 (7–15)	15 (12–22)	0
Joints	6	1	4 (80%)	0	1 (20%)	2/4	Evaluable pts: 4; DR: 100% (76–100)	78 (22–210)	17 (12–22)	17 (12–22)	0
Gut	4	0	3 (75%)	0	1 (25%)	0/3	Evaluable pts: 2; DR: 0% and 100%	NA	NA	NA	NA
Vagina	2	0	0	0	2	/	/	/	/	/	/
Thrombo-cytopaenia	3	0	2 (66%)	1 (33%)	1 (33%)	2/2	NA	NA	NA	NA	NA
PRCA	1	0	0	0	1	/	/	/	/	/	/
Myasthenia gravis	1	0	1	1	0	1/1	Evaluable pts: 1 DR: 100%	30	84	84	0

Abbreviations: cGVHD = chronic graft-versus-host disease; CR = complete response; DR-IS = dose reduction of immunosuppressive therapy after Rituximab. The modification of previous baseline immunosuppressive therapy after Rituximab is here reported; DR-steroid = for patients in treatment with steroid, the percentage dose reduction is indicated; NA = data not available; NR = no response; NV = not valuable; OR = overall response; PRCA = pure red cell aplasia; Pts = patients; RD = response duration; follow-up = median follow-up from Rituximab treatment (range); TTR = time to response.

responding patients, respectively (Table 2). In particular, the median dose reduction of steroid therapy was 82% (range 0–100%; Table 2). With regard to the effect on liver, lung and gut manifestations, the small number of cases limited the analysis; however, the good results observed in some patients suggest a potential effect of Rituximab in controlling cGVHD in these organs too (Table 2). Similarly, a significant number of patients experienced clinical and functional improvement of eye and joint manifestations of cGVHD (Table 2). Neither of the two patients with vagina involvement, however, improved after Rituximab treatment. Two of the three patients with immune thrombocytopaenia responded (one CR and onc PR), whereas the patient with PRCA was refractory. One patient with refractory myasthenia gravis achieved complete regression of neurological symptoms and disappearance of the autoantibodies against acetyl-coline receptors,[12] still present after 84 months. Particularly for patients with skin, mouth and eyes involvement, response to therapy appeared to be related to grade of cGHVD (limited vs extensive) and the number of Rituximab administrations ($\leqslant 4$ vs 5–8 vs >8), but not with length of the interval between cGVHD onset and Rituximab ($\leqslant 23$ vs >23 months).

Short- and mid-term toxicity were characterized by the development of grade I–II infusion-related symptoms in four patients, renal failure, severe tremors and ischemic stroke in one patient each. As for infectious complications, we registered three cases of pneumonia and one case of Gram-negative sepsis. Because of the development of these adverse events, six patients interrupted the therapeutic program before the fourth administration.

During the study period 8/38 died: causes of death were cGVHD progression ($n=3$, all related to progressive lung cGVHD), disease relapse ($n=1$), infections ($n=3$; causative organisms not identified), sudden death ($n=1$). The actuarial 2 year survival is currently 76%.

Discussion

cGVHD is the most common nonrelapse complication after allogeneic stem cell transplantation and affects 40–70% of survivors beyond day 100. cGVHD is becoming a more frequent problem due to increasing age of recipients, use of alternative donors, use of peripheral blood stem cells as the source of the graft, and increasing use of donor lymphocyte infusions for the treatment of relapse. The pathophysiology of cGVHD is poorly understood; our current understanding starts with pathogenic donor T cell, that expands in response to allo- or autoantigens unchecked by normal thymus or other peripheral mechanisms of abrogation. These pathological T cells attack target tissues directly through cytotoxic attack, secretion of inflammatory cytokines, such as interleukin-β (IL-1β), tumour necrosis factor-α (TNF-α), IL-6 and interferon-γ (IFNγ), or promotion of B-cell activation and antibody production. Tissue damage may eventually lead to fibrosis and loss of function.

The mainstay of cGVHD treatment is a ciclosporin A and prednisone regimen. Approximately 40% of patients fail to respond to initial therapy. Patients with refractory cGVHD are usually treated with several salvage therapies mainly targeted at T-cells, such as mycophenolate mofetil, sirolimus, daclizumab, thalidomide and alemtuzumab or at cytokines, such as anti-TNF-alpha monoclonal antibodies.

There has recently been an emerging interest in the pathological effect of B cells in auto- and alloimmune

reactivity. B cells may have a primary direct and/or indirect role in the pathogenesis of several immune-mediated diseases by means of autoantibodies or cytokine production, interaction with T cells and acting as antigen-presenting cells. In cGVHD, the status of the B-cell compartment appears somewhat paradoxical. Patients with cGVHD may show severe B-lymphocytopaenia and B-cell hyperactivity at the same time, as documented by the production pathological antibodies.[13–14]

On the basis of such considerations and with the good results observed in the treatment of several autoimmune diseases,[5–10] Rituximab has been investigated in a small number of patients with refractory cGVHD using the standard regimen of 375 mg/m^2/week for 4 weeks.[4,15–17] Ratanatharathorn *et al*.[15] documented a sustained response in four of eight patients with steroid-refractory cGVHD with diffuse or localized sclerodermoid manifestations. Similarly, Canninga-vanDijk *et al*.[16] and Okamoto *et al*.[17] observed cases with clinical, laboratory and histological improvement after Rituximab treatment. Recently, Cutler *et al*.[4] reported the results of their phase I–II study with Rituximab in 21 patients with steroid-refractory cGVHD. Treatment was well tolerated, and toxicity limited to infectious events, without any haematological toxicities and only a significant reduction in circulating immunoglobulins documented after therapy. Objective responses were documented in 70% of patients (including 10% complete response) primarily for those with skin and musculoskeletal involvement, allowing tapering, and in some cases withdrawing, of previous immunosuppressant therapy. A correlation between clinical response and decrease in the titre of antibodies against Y chromosome-encoded minor HLA antigens was shown.

The results of these preliminary studies highlight the potential therapeutic activity of Rituximab on some cGVHD manifestations and a particularly high efficacy for skin involvement, including scleroderma. Systemic sclerosis, which shares some clinical aspects with sclerodermoid cGVHD, is characterized by altered B-cell homeostasis with hyper-gammaglobulinemia, polyclonal and memory B-cell hyperactivity and production of specific autoantibodies.[18–19] In this disease B-cell hyperactivity could be not only an epiphenomenon of T-activation but also the expression of a primary pathogenic activity in the development of fibrosis. This primary pathogenic role of B cells appears further emphasised by recent data showing the beneficial therapeutic effect of B-cell depletion in a preclinical mouse model.[20]

Our study confirms the activity of Rituximab in refractory cGVHD in a larger series of patients. Treatment was generally well tolerated and nearly 60 and 50% of patients had a clinical improvement of their skin and mouth manifestations, respectively. The median TTR was nearly 2 months and in some cases responses were durable. Responses were also detectable in some patients with eye, liver, lung, gut and joint involvement, allowing reduction and/or suspension of previous baseline immunosuppressive therapy in a significant number of patients. It should be noted that response appeared to be related to the duration of treatment and extent of cGVHD.

We are aware of the limitations of this retrospective multicentre study: the number of Rituximab administrations varied according to the individual institutional therapeutic policy; collection of objective clinical results may have had some limitations in some patients; furthermore, we are unable to collect sufficient biological data to evaluate immunological changes after treatment and the possible relationship between clinical and laboratory modifications. However, our results are in line with previously reported studies, and support a real therapeutic activity of Rituximab for the treatment of patients with cGVHD.

In conclusion, these data indicate that Rituximab may reduce multiorgan involvement of some cGVHD manifestations and stimulate confirmation through prospective controlled studies in more homogeneous groups of patients should be sought. Similar to that already observed in some autoimmune disorders, the beneficial effect of B-cell depletion highlights the potential primary pathologic role of B lymphocytes in the development of cGVHD. A better understanding of the immune-biologic mechanisms that regulate this disorder will be of assistance.

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