Fractionated subcutaneous rituximab is well-tolerated and preserves CD20 expression on tumor cells in patients with chronic lymphocytic leukemia

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ABSTRACT

A pilot study previously demonstrated that thrice-weekly, fractionated-dose intravenous rituximab (RTX) limits CD20 loss from chronic lymphocytic leukemia (CLL) B cells, thereby enhancing immunotherapeutic targeting. Here, we investigated the feasibility of giving 20 mg rituximab subcutaneously thrice weekly for up to 12 weeks in 4 previously treated CLL patients. Subcutaneous rituximab was well-tolerated with minimal injection site reactions; a variable degree of efficacy was observed, likely influenced by the size of the patients' B cell/CD20 burden. Subcutaneous RTX largely preserved CD20 expression on leukemic cells but the most effective therapeutic dosing regimen needs to be established *(ClinicalTrials.gov Identifier: NCT00366418).*

Key words: rituximab, subcutaneous, CD20 shaving, antigenic modulation, chronic lymphocytic leukemia.

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Introduction

The anti-CD20 monoclonal antibody rituximab (RTX) has shown remarkable efficacy in non-Hodgkin's lymphomas (NHL).[1,2] However, compared to therapy for NHL, RTX therapy in chronic lymphocytic leukemia (CLL) is associated with lower response rates.[3] Possible explanations include lower CD20 levels on CLL cells compared to NHL cells. Alternatively, due to high tumor burden or substantial leukemic disease, there can be exhaustion of effector mechanisms which kill RTX-targeted CLL B cells, such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).[4,5] Another potential limitation for standard dose, single-agent intravenous RTX therapy in CLL, manifested after saturation/exhaustion of clearance mechanisms, is the shaving reaction in which RTX/CD20 immune complexes on B cells are removed by effector cells expressing FcγR.[5,6] This process can reduce or completely abrogate the efficacy of subsequent RTX dosing. Fractionated dosing schedules that limit exhaustion of effector mechanisms may be more effective than current intravenous bolus schedules of 375 mg/m^2 RTX. A pilot trial suggested that low-dose RTX at 20 mg/m^2 intravenously thrice weekly promotes clearance of leukemic cells without inducing substantial loss of targeted CD20.[7] This trial was limited to a four-week treatment duration and required frequent clinic visits for patients. In principle, low RTX doses can be self-administered subcutaneously. If subcutaneous administration is safe and effective, it could be more convenient for patients than intravenous treatment and would make fractionated dosing over prolonged periods possible. Based on ease of use and tolerability considerations, subcutaneous injections in this pilot study were limited to 2 mL/day, thrice weekly, allowing for RTX doses of 20 mg due to its fixed formulation at 10 mg/mL.

Design and Methods

Patients

This pilot phase I study used 20 mg subcutaneous RTX doses thrice weekly for 6-12 weeks (*ClinicalTrials.gov Identifier: NCT00366418*). Criteria for inclusion were active CLL, previous fludarabine treatment, CD20 expression on leukemic

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cells, absolute neutrophil counts over 0.5×10^9/L, platelets over 30×10^9/L, and absence of bulky disease. Side effects were classified according to NCI toxicity criteria (version 3.0). All patients had prior RTX exposure, but not within six months prior to enrollment. The first 20 mg RTX dose was given intravenously, subsequent doses were given subcutaneously. Diphenhydramine and paracetamol were given before the first two doses only. Primary endpoints were safety and feasibility of subcutaneous RTX; secondary endpoints included efficacy, RTX pharmacokinetics and CD20 shaving.

Laboratory analysis

Blood counts were obtained before, and 2-24 hours after the first, second and third RTX doses and at three, six, and 12 weeks. Cell phenotyping for CD20 levels, complement C3dg deposition and assays for RTX in serum were performed as described;[7,8] phenotyping of blood samples from patient 3 were only performed out to four days.

Results and Discussion

Patient characteristics are given in Table 1. Of the 4 enrolled, 2 patients experienced grade 2 cytokine release syndrome during intravenous administration of 20 mg RTX on day 1. Subcutaneously injected RTX did not induce a cytokine release syndrome. Patient 1 had transient grade III thrombocytopenia (day 5) so that the subcutaneous RTX on day 5 was omitted. Occasional bruising, itching or erythema (all grade I) at the injection site were noted, but resolved rapidly. Patient 1, who had a history of deep venous thrombosis, developed grade III venous thrombosis on her contralateral leg (week 4). She required anticoagulation and continued RTX therapy. Patient 3, with a history of ventriculo-peritoneal shunt placement, developed grade III bacterial meningitis (week 7), which was treated with antibiotics with full recovery. Patient 1 received 12 weeks of therapy and achieved a partial response lasting four months; the other patients completed six weeks of therapy and elected to stop based on lack of reduction in ALC, although all had stable disease at that time.

The initial intravenous infusion of one 20 mg dose of RTX promoted rapid clearance of more than 2/3 of circulating CLL cells from the peripheral blood in all patients (Figure 1A). By six hours ALC counts started to rebound and by 24 hours almost reached baseline values. CD20 levels were acutely decreased on "surviving" circulating cells (Figure 1B). Based on previous work,[5,7] we suggest that due to saturation of clearance mechanisms these cells

Table 1. Patients' characteristics.

Patient N.	Sex/ Age (years)	Rai Stage	ALC ($\times10^9$/L)	Prior therapies N.	Cytogenetics FISH
Pat 1*	F/62	III	64	2	13q
Pat 2	M/64	II	65	1	13q, homozygote
Pat 3	M/53	II	112	3	13q
Pat 4**	F/63	III	66	1	trisomy12

**splenectomized; **atypical CLL with IgG paraprotein.*

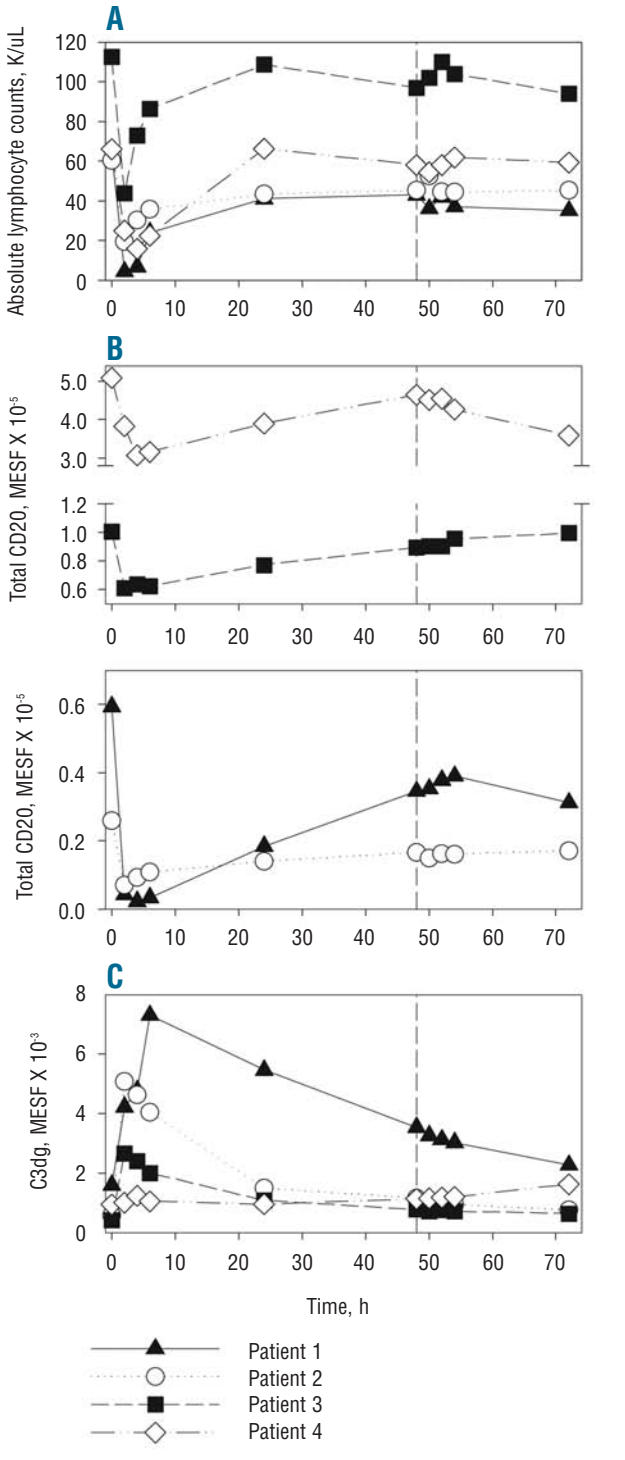

Figure 1. Absolute lymphocyte counts, CD20 expression and complement deposition on leukemic cells during the first 72 hours of treatment in chronic lymphocytic leukemia. The first 20 mg intravenous dose of RTX on day 1 was followed by a subcutaneous 20 mg dose of RTX on day 3. (A) Absolute lymphocyte counts (ALC) pretreatment, and 2, 4, 6, 24 hours after the first 20 mg intravenous infusion of RTX. The dashed line at the 48 hour timepoint indicates the first subcutaneous injection of 20 mg RTX. Measurements are taken 2, 4, 6, and 24 hours after the subcutaneous RTX administration. (B) Quantitative levels of CD20 on patient's B cells by flow cytometry (molecules of equivalent soluble fluorochome, MESF). (C) Cells were stained with an mAb specific for C3dg to measure deposition of complement fragments on cells after RTX treatment.

could not be cleared, and decreases in CD20 are likely due to shaving of CD20 from RTX-opsonized cells by macrophages. Indeed CD20 expression was rapidly reduced on leukemic cells after the start of the intravenous infusion but started to recover by six hours. In 3 patients cell-associated C3dg fragments became detectable immediately after starting RTX, indicating that circulating cells had fixed complement (Figure 1C).

The first subcutaneous infusion of 20 mg of RTX (Figure 1A-C) produced very modest changes in ALC and CD20, and did not activate complement on circulating cells. However, continued treatment of patient 1 with thrice-weekly 20 mg subcutaneous RTX doses promoted decreases in ALC (Figure 2A), and although CD20 levels on her circulating CLL B cells decreased (Figure 2B), enough CD20 remained on the cells for effective targeting. On day 84, the last day of RTX treatment, ALC was 3700 cells/μL and CD20 expression corresponded to 7800 MESF (pre-treatment values: 63,500 cells/μL and 59000 MESF); eight days later ALC increased to 5200 cells/μL and CD20 increased to 20,000 MESF. Patients 2-4 had stable disease with no decrease in ALC due to subcutaneous RTX injections. However, CD20 expression levels on leukemic cells remained partially reduced in patients 2 and 4 (Figure 2A-B). There was no evidence of C3dg deposition after the first week of treatment in any patient (*Data not shown*).

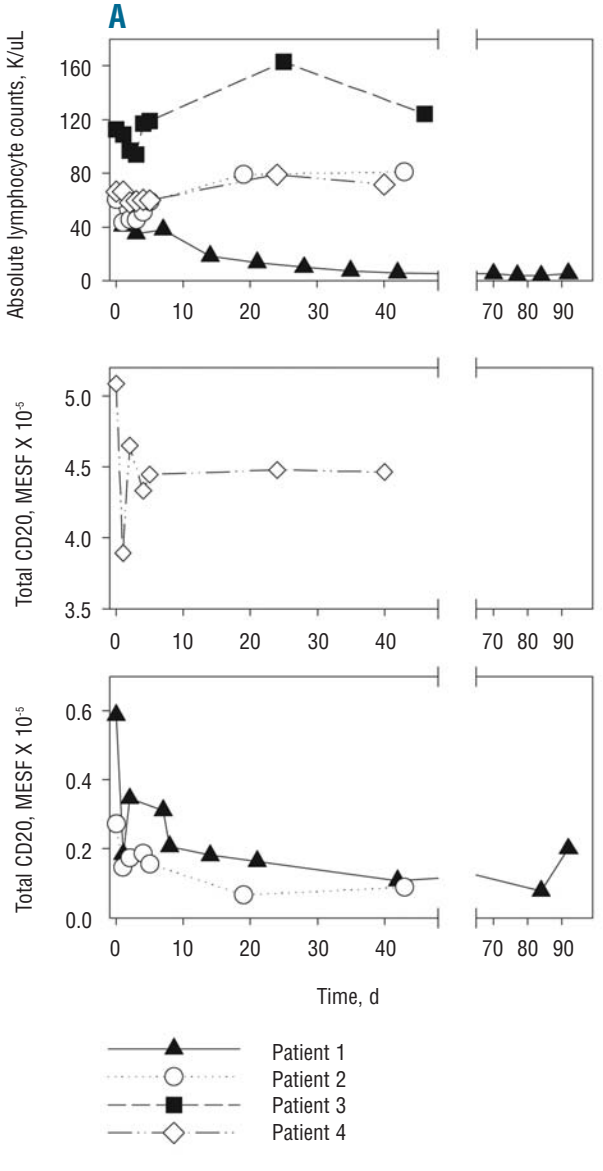

Figure 2. A single 20 mg intravenous dose of RTX on day 1 followed by thrice weekly subcutaneous 20 mg doses of RTX over 6-12 weeks. (A) ALC, and (B) quantitative levels of CD20 on patient's B cells are shown for the period of treatment. The last RTX injection for patient 1 was on day 84, and final measurements on day 92 are shown.

RTX serum concentrations 48 hours after subcutaneous dosing were 0.3 μg/mL, 0.1 μg/mL and undetectable for patients 1, 2 and 4 after three weeks and 0.5 μg/mL for patient 1 and undetectable for patients 2 and 4 after six weeks. Patient 1 had a RTX serum concentration of 1 μg/mL after 12 weeks.

Although for technical reasons RTX doses in this trial were limited to 20 mg; this dose, administered intravenously, promoted rapid clearance of the majority of circulating cells. This dose is approximately 60% of the previous dose (20 mg/m^2) we found to be effective for targeting circulating CLL cells.[7] Quantitatively, 20 mg corresponds to approximately 8×10^{16} antibody molecules, approximately equal to the number of cell-associated CD20 binding sites in 4 liters of blood, based on ALC of 100,000 cells/μL and 200,000 CD20 sites/cell. In most patients with CLL the CD20 burden should be considerably lower, and it is reasonable that 20 mg doses could be adequate for targeting. Indeed, in patient 1, continued administration of 20 mg RTX subcutaneously thrice weekly was effective in promoting slow but continuous decreases in ALC during the 12 week treatment period (Figure 2A).

Although CD20 levels on B cells of patient 1 decreased (Figure 2B), they remained sufficiently high for effective targeting. There was no evidence of C3dg deposition on circulating cells promoted by subcutaneous RTX; it is, therefore, likely that malignant cells were cleared based on recognition mechanism promoted by Fcγ receptors on effector cells, such as NK cell-meditated ADCC and phagocytosis by tissue macrophages.[9,10] Compared to requirements for CDC, far less IgG needs to be bound to target cells to promote these later reactions.[11,12] Interestingly, a recent study reported that complement deposition on target cells can inhibit interaction between RTX and NK-cell CD16, thereby antagonizing ADCC.[13] Therefore, modifying dosing regimens of RTX to avoid complement deposition on target cells could enhance it's efficacy through improved ADCC.

While results for patient 1 clearly indicate that subcutaneously injected RTX was biologically active, other patients failed to achieve decreases in ALC. As the ALC in these patients decreased at least transiently after 20 mg RTX administered intravenously, these results suggest that the total tumor burden in these patients was so high that the subcutaneous doses were inadequate to promote targeting of cells in the circulation and tissue compartments. Patient 1 had been splenectomized, and the burden of

CD20-positive target cells was likely considerably lower than in other patients, almost certainly contributing to her favorable response to subcutaneous RTX. Nevertheless, prolonged subcutaneous RTX therapy substantially preserved CD20 levels and was well-tolerated. Our findings suggest that dosing strategies of anti-CD20 mAbs that preserve CD20 cell-surface expression on CLL cells are worth investigating.

This trial was not designed for dose-escalation, because RTX is constituted in fixed formulation and maximum injectable doses were given. Higher subcutaneous doses of RTX or of newer anti-CD20 mAbs formulated for subcutaneous injection could be more effective. Also, in treatment settings where the burden of CD20 target cells is low, a subcutaneous dosing regimen could be attractive; these indications could include treatment of minimal residual disease, maintenance therapy after remission is achieved, and treatment of autoimmune diseases. Indeed, a recent report of high response rates to four weekly infusions of 100 mg rituximab in patients with autoimmune cytopenias supports the concept that the effective dose is influenced by the clinical setting.[14] Ours is a small study that, while showing feasibility of subcutaneous rituximab administration, should not be repeated outside of clinical trials. To confirm the safety of this approach, a larger cohort of patients and additional endpoints, especially the possible development of human anti-chimeric antibodies (HACA), would have to be studied.

Authorship and Disclosures

GA, TH, RPT and AW were investigators of the study. MAL, PVB, AWP and BV performed the laboratory work for this study. GA, RPT and AW participated in the statistical analysis. RPT and AW coordinated the research. GA, RPT and AW wrote the manuscript.

The authors report no potential conflicts of interest.

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