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Rituximab Is Potentiated by GM-CSF in Relapsed Follicular Lymphoma Patients: Results of a Phase II Study

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Abstract

Purpose

We hypothesized that granulocyte-macrophage colony-stimulating factor (GM-CSF) could potentiate the clinical activity of rituximab, given their individual and cooperative effects on Fc γ R11a- and Fc γ R11a-expressing cells. A phase II clinical study combining GM-CSF and rituximab was initiated in patients with relapsed follicular lymphoma (FL) to determine the clinical and biologic responses, as well as safety of the combination.

Patients and Methods

Thirty three patients with relapsed FL were treated with GM-CSF 5 μ g/kg/day on days 1 to 8 and rituximab 375 mg/m² on day 5 of each 21-day cycle for four cycles. Clinical response and tolerability were examined according to international criteria. Biologic monitoring included evaluation of immune cells involved in rituximab activity.

Results

Of 33 evaluated patients, a 70% overall response rate (CR + CRu: 45%) and median progression-free survival (PFS) of 16.5 months were achieved. Outcome was influenced by the quality of response and the FLIPI index, where low- and intermediate-risk FLIPI groups were associated with significantly better PFS. After treatment there was a significant increase in granulocyte and monocyte counts. Examination of dendritic cell response showed an overall increase in plasmacytoid dendritic cells, especially in non-CR patients, after treatment. Addition of GM-CSF did not impair tolerance to rituximab, and adverse events were rare and mild.

Discussion

GM-CSF plus rituximab results in high response rates, along with a tolerable safety profile in patients with relapsed or progressive FL. The improved efficacy over rituximab monotherapy may be due to increases seen in monocyte, granulocyte, and dendritic cell populations.

Introduction

Follicular lymphoma (FL) is the most frequently occurring low-grade non-Hodgkin's lymphoma (NHL).¹ Radiation and conventional chemotherapy have not significantly modified its natural history, as patient response is often characterized by remission followed by consecutive relapses until death. Recently, rituximab (a chimeric IgG1 monoclonal antibody directed against CD20) has considerably modified the therapeutic strategy for B lymphoproliferative malignancies.² As a single-agent treatment in relapsed indolent lymphoma, rituximab showed a 48% overall response rate (6% complete response [CR]) with a time to progression of 11.2 months.³ Randomized trials have demonstrated that rituximab combined with chemotherapy, as well as rituximab maintenance therapy, improves overall response and response duration over chemotherapy alone in patients with recurrent FL.^{4,5} Unfortunately, approximately 50% of patients with low grade NHL exhibit no clinical response to rituximab alone,^{3,6} demonstrating a need for improved knowledge of rituximab's mechanism of action and how that translates into a clinical response.

In vivo mechanisms of rituximab activity are not fully understood, although experimental data suggest that it induces apoptosis, complement-dependent cytotoxicity (CDC), and cellular mechanisms resulting in antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis.⁷ A more thorough understanding of its mechanisms would aid in improving its efficacy, as the respective contribution of each of these pathways *in vivo* is difficult to distinguish. However, it has been demonstrated that FL patients who are homozygous for the *FCGR3A*-158V allele (encoding the Fc γ RIIIa allotype of highest affinity for IgG1⁸) have a better response to rituximab.⁹ Fc γ RIIIa is expressed on monocytes and natural killer (NK) cells suggesting an involvement of ADCC in rituximab activity in humans. In contrast, there is a debate whether Fc γ RIIa-131HR polymorphism actually influences the response to rituximab,¹⁰ despite the fact that Fc γ RIIa has been

involved in the phagocytosis of rituximab-sensitized cells by macrophages. Fc γ RIIa is also expressed by human granulocytes, and murine models using rituximab have shown the role of neutrophils in the elimination of CD20-expressing tumors,¹¹ raising the question of their actual role in patients. Fc γ RIIa and Fc γ RIIIa are also expressed by dendritic cells¹², leading to the suggestion that such therapeutic effects could be explained in part by specific antilymphoma immunity.⁷

Because Fc γ RIIIa- and Fc γ RIIa-expressing cells (granulocytes, monocytes, dendritic cells [DCs]) are potentially involved in mechanisms of rituximab activity *in vivo*, we formulated the hypothesis that granulocyte-macrophage colony-stimulating factor (GM-CSF), which is known to act on these cells, could enhance the clinical activity of rituximab. GM-CSF belongs to the family of hematopoietic cytokines that can promote myeloid differentiation, as demonstrated by increased granulocyte proliferation and phagocytosis,^{13,14} promotion of macrophage/monocyte ADCC,^{15,16} and enhanced monocyte differentiation into DCs, also known as potent professional antigen-presenting cells.^{17,18} Because GM-CSF has demonstrated effects on the immune system, it is also being currently explored as a vaccine adjuvant in immunotherapy clinical trials.¹⁹

The combined effects of rituximab and GM-CSF were examined in a phase II clinical study in patients with relapsed or progressive FL to evaluate their coordinate clinical and biologic responses and the safety of the immunotherapy combination.

Patients and Methods

Patients

From January 2000 to October 2001, 33 patients with relapsed or progressive FL were included in this phase II trial. All patients were diagnosed and treated in the Hematology Department of the University Hospital of Montpellier, France. Patients were eligible for this study if they were older than 18 years of age, with grade 1, 2, or 3a CD20⁺ FL according to

World Health Organization (WHO) classification.²⁰ Patients were required to have the presence of at least one measurable site. Written informed consent was obtained from each patient before therapy.

Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of less than 3 and adequate hematologic, renal, and hepatic functions. They were excluded if they had infection with human immunodeficiency, hepatitis B, or hepatitis C virus, were pregnant, had active autoimmune disease, concomitant severe infection(s), cardiovascular disease, or prior or active malignancy. Patients also were excluded if they were taking high doses of systemic corticosteroids (≥ 1 mg/kg/day), were receiving immunosuppressive treatments, were enrolled in another clinical trial, or had known allergies to yeast-derived products.

Treatment

Subcutaneous (SC) GM-CSF (molgramostim, Leucomax[®], Schering-Plough Corporation, Kenilworth, NJ) was given at 5 μ g/kg/day on days 1 to 8 and intravenous (IV) rituximab (Mabthera[®], Produits Roche, Neuilly, France) was given at 375 mg/m² on day 5 (Figure 1). Each cycle was given every 21 days, for a total of four cycles. Comedication included antihistamines and corticosteroids as needed. An additional GM-CSF plus rituximab treatment cycle was administered to the last six patients who did not achieve CR at the end of the first cycle.

Clinical monitoring

Baseline evaluation included clinical examination; chest X-ray; computed tomography of the chest, abdomen, and pelvis; and unilateral bone marrow biopsy. Laboratory testing included routine hematology, serum chemistry, lactate dehydrogenase, and β_2 -microglobulin. Monitoring included clinical assessment, hematology, serum chemistry evaluations before each cycle and at day 8, and full tumor restaging at 30 days after the end of treatment, 3 months after that and every 3 months until progression.

Biologic monitoring

Immune cell counts (lymphocyte, monocyte, and granulocyte) were assessed from peripheral blood at baseline, before and at day 8 of each cycle, at day 30, and 3 months after treatment. At the same time, immunophenotypic analysis of DCs, CD14⁺, and CD19⁺ cell counts was performed. Peripheral blood mononuclear cells were freshly isolated from patients and labeled with the following monoclonal antibodies: PE-CD123; fluorescein isothiocyanate (FITC)–lineage cocktail 1 (Lin 1), which contains FITC-conjugated antibodies against CD3, CD14, CD16, CD19, CD20, and CD56, (BD Biosciences, Le Pont de Claix, France); PC5-HLA DR; and PE-CD11c (Beckman-Coulter, Villepinte, France). Negative controls were performed with corresponding conjugated isotypic control immunoglobulin. Cells were incubated at room temperature for 30 minutes with each antibody. After washing cells were analyzed by FACSCalibur™ and data were captured with CellQuest software. Myeloid DCs (mDCs) were identified as Lin⁻, DR^{bright}, CD11c⁺ and CD123⁻ cells, whereas plasmacytoid DCs (pDCs) were Lin⁻, DR^{bright}, CD11c⁻ and CD123⁺.

End points and statistics

The primary efficacy end point was the objective response (OR) rate, *ie*, the proportion of patients achieving either CR, unconfirmed CR (CRu) or partial response (PR).²¹ Clinical response was evaluated at 3 months, and all patients were evaluated for progression every 3 months until progression. Secondary end points were safety and progression-free survival (PFS). PFS was calculated for all patients according to the method of Kaplan and Meier and was measured from the start of treatment until progression, relapse, or death.^{21,22} For patients who were retreated, response was evaluated at the end of second cycle. Safety and tolerability were assessed using the National Cancer Institute's Common Toxicity Criteria (NCI-CTC).²³ Mann-Whitney nonparametric tests were used to compare cell counts. Patients who stopped treatment early due to toxicity were not included in the PFS analysis.

Results

Patient characteristics

Thirty-three patients were included in the study (Table 1). The median age was 59 years (range, 37 to 81), and there were 19 males and 14 females. The median number of previous lines of treatment was two (range, 1-7), including five patients who received autologous transplantation. There were 12 patients with stage I-II and 21 with stage III-IV. Only two patients had received rituximab prior to study initiation. According to the Follicular International Prognostic Index (FLIPI),²⁴ there were 15 patients (45%) in the low-risk group and 10 (30%) and eight (24%) in intermediate- and high-risk groups, respectively. A majority of patients were in relapse at study inclusion (25 patients), with seven patients showing progressive disease and one patient with stable disease.

Response and outcome

Of 33 patients included in this study, 23 (70%) achieved an OR, including 13 patients (39%) in CR, two (6%) in CRu, and eight patients (25%) in PR. Four patients (12%) stopped treatment early due to toxicity, although one was in CR and three were in PR. Six patients continued treatment for a second course of four cycles. Two patients had improved response from PR to CR, and one patient from SD to PR.

Response rates were 93% (CR + CRu = 60%), 57% (CR + CRu = 57%), and 72% (CR + CRu = 28%) for low-, intermediate- and high-risk groups, respectively, according to FLIPI (Table 2). Median PFS was 16.5 months (range, 1.0 to 57.3; Figure 2A). FLIPI at relapse influenced PFS, with a median of 16.4 months (range, 3.5 to 57.3), 33.2 (range, 1.0 to 52.0), and 7.6 months (range, 4.5 to 37.4) for low-, intermediate- and high-risk FLIPI, respectively ($P = .06$). This influence became statistically significant when comparing low- and intermediate-risk FLIPI groups (median 33.3 months; range, 1.0 to 57.3) to the high-risk FLIPI group (median 7.6 months; range, 4.5 to 37.4) ($P = .02$, Figure

2B). Patients with CR (CR + CRu) had a significantly higher PFS (median 34.0 months; range, 6.0 to 57.3) compared with patients with PR (median 7.6 months; range, 3.4 to 46.0) ($P < .01$, Figure 2C). The number of cycles did not influence PFS, with a median of 16.4 months (range, 1.0 to 51.9) and 8.3 months (range, 4.5 to 46.9) ($P = .8$, Figure 2D). Although most patients with relapsed FL had received one to three prior therapies, it was noted that a patient who had received seven prior therapies was in CR and achieved 24-month duration of response.

Safety

Four patients stopped treatment early because of a lack of tolerability to GM-CSF (bone pain and erythema at the injection site in two patients), GM-CSF plus rituximab (bone pain and fever in one patient), or rituximab (back pain and hypotension in one patient). Grade 2 to 3 adverse events included fever and pain (four patients), erythema at the injection site (four patients), and mild hypotension (two patients). In a majority of patients symptoms resolved with concomitant corticosteroid treatment.

Biologic follow-up

We first compared peripheral blood cell counts before and after each cycle (Figure 3). We found that rituximab combined with GM-CSF significantly increased white blood cell (WBC), neutrophil, eosinophil, and monocyte counts ($P < .001$), as well as total lymphocyte counts ($P = .03$). Immunophenotypic analysis showed that CD14⁺ cells and pDC counts were also statistically increased after treatment ($P < .01$ and $P = .02$, respectively), whereas there was a decrease in mDC counts ($P < .01$) and circulating B lymphocytes (ie, CD19⁺ cells) after treatment ($P < .01$).

When we analyzed mean cell counts according to response status we found that total lymphocyte counts ($P = .06$) and mDC counts ($P = .04$) were increased in CR patients before each cycle, whereas after each cycle we found a significant decrease in pDC counts ($P = .01$) in CR patients (data not shown). When associated with patient

responses, WBCs, neutrophils, eosinophils, monocytes, CD14⁺ cells, and CD19⁺ B lymphocytes showed no significant difference between CR and non-CR patients either before or after treatment.

Finally, when we analyzed the ratio of cell counts after versus before treatment according to response, we showed that patients in CR had significantly reduced CD19⁺ B lymphocyte ($P = .03$) and pDC ($P < .01$) cell counts compared with non-CR patients (Figure 4).

Discussion

The introduction of rituximab has led to improvements in the standard of care treatments for patients with FL. However, approximately 50% of patients do not respond to frontline rituximab alone,^{3,6} demonstrating a need to improve its efficacy. Given the expected role of Fc γ R11a- and Fc γ R11a-expressing cells (including monocytes, DCs and granulocytes) on *in vivo* rituximab activity and the impact of GM-CSF on these cells, we hypothesized that GM-CSF could potentiate the clinical activity of rituximab.

In this single-institution phase II study, the OR rate (70% with 39% CR) and PFS (median 16.5 months) seen in rituximab-naïve patients with relapsed or progressive FL shows improved efficacy of rituximab combined with GM-CSF. In the pivotal study, which also included rituximab-naïve patients with relapsed FL, McLaughlin et al reported a 60% OR rate for this histology subgroup with only 6% CR.³ Although no direct comparison is feasible, rituximab combined with GM-CSF appears to increase the CR rate. Moreover, our results are similar to those noted in interim reports by McLaughlin et al,²⁵ who cited a 79% OR rate (36% CR) with GM-CSF and rituximab for previously untreated and relapsed patients with indolent NHL. Our positive results are not explained by selection criteria, as the distribution of adverse prognostic factors assessed by the FLIPI index are similar to those found by Solal-Céligny et al.²⁴ We also found that FLIPI influenced PFS after

treatment with rituximab and GM-CSF. The prognostic index was first described for FL at diagnosis,²⁴ but recent data suggest that FLIPI could also be useful in predicting PFS at relapse.²⁶ In our study, the quality of response appeared to be significantly associated with outcome, a result also noted by other investigators.^{26,27}

In the past, extended courses of rituximab monotherapy to eight consecutive weekly cycles rather than the standard four have shown prolonged duration of response in patients with relapsed or refractory low-grade NHL.²⁸ In this study, improved responses were noted for three patients receiving a second cycle of four courses, suggesting that an additional cycle of therapy should have the potential to improve patient outcome. However, the impact of prolonged treatment on PFS remains to be demonstrated, since PFS for the six patients receiving two cycles was not different compared with patients receiving only one cycle. Even if these results have to be confirmed in a larger cohort, they suggest that patients likely do not benefit from prolonged therapy. Such results have also been seen in another study examining rituximab maintenance therapy compared with retreatment at progression for FL.²⁹

Examination of the potential biologic basis for this combined efficacy demonstrates that the immunotherapy combination of GM-CSF and rituximab activates numerous immune cells. The increase is notably significant for granulocytes and monocytes, which express Fc γ R11a and Fc γ R11a and are potentially involved in rituximab-mediated ADCC and phagocytosis. We have not evaluated ADCC or NK cell count in this study, but Liu and colleagues previously reported that the combination of rituximab and GM-CSF enhanced ADCC in patients with recurrent indolent lymphoma, especially for those in CR, without affecting NK cell activity.³⁰ Immunophenotypic analysis showed a significant increase in CD14⁺ cells, a marker typically found on monocytes, macrophages, granulocytes, and DC precursors. The increase in circulating DCs, which make up a very small proportion of peripheral blood, was confirmed by specific analysis. Of the two types of DCs, pDCs are

thought to originate from lymphoid precursor cells.³¹ We have found that GM-CSF combined with rituximab increased pDC counts. This was associated with a rise in pDC counts in non-CR patients after treatment. It is known that GM-CSF can enhance mDC cells less efficiently than pDC cells, thus affecting the mDC/pDC ratio.³² This pDC enhancement may favor an increase in regulatory T cells (T_{reg}), which are known to downregulate antitumor T cells.³³ Unfortunately, the T_{reg} subpopulation was not evaluated in this study because their functional and phenotypic characteristics were poorly known at the time of study initiation. Interestingly, an increase in pDCs was observed as early as following the first cycle of GM-CSF plus rituximab treatment. The increased pDC expression therefore may serve as an early marker of treatment failure, making it possible to prospectively monitor treatment efficacy in upcoming trials.

We found that total lymphocyte counts were significantly increased. Although myeloid effects of GM-CSF are well recognized, its activity on lymphocytes remains largely unknown. As typically observed after rituximab, immunophenotypic analysis showed that B lymphocyte counts were significantly decreased, demonstrating that the increase of lymphocyte counts involved mostly T lymphocytes and/or NK cells. This has been also reported with GM-CSF after conventional³⁴ or intensified chemotherapy³⁵ and could be related to an increase of memory T cells.³⁴ We have found that B lymphocyte counts were significantly less reduced after treatment in non-CR patients compared with CR patients, a result noted in a rituximab pivotal trial³ that could be due to intrinsic resistance to rituximab.

More than 540,000 patients have been treated with rituximab, and it is generally considered to have an excellent safety and tolerability profile.³⁶ Our results demonstrated that addition of GM-CSF does not impair tolerance to rituximab in patients with recurrent FL. The capacity of GM-CSF to stimulate immune cells involved in innate and adaptive immunity suggests that there is the potential for long-term benefit in terms of supporting

and/or regulating the immune system. Such a combination has also been tested in patients with chronic lymphocytic leukemia³⁷ or associated with chemotherapy in diffuse large B-cell lymphoma³⁸ and has demonstrated improved response rates. Rituximab alone could be considered in FL patients with a low tumor burden,³⁹ for control of minimal residual disease after autologous stem cell transplantation,^{40,41} or as maintenance therapy.^{4,5,42} In this setting, the addition of GM-CSF to rituximab could improved rituximab's response rate and/or residual disease control without increased toxicity. Furthermore, GM-CSF is a granulocyte growth factor and could therefore provide immune-stimulating benefits to dose-intensified immunochemotherapy regimens used in NHL patients.

This single-center study shows that GM-CSF enhances the efficacy of rituximab, with an increased CR rate over rituximab monotherapy (although not directly comparable to rituximab alone) for patients with relapsed or progressive FL. The safety profile of the combination treatment was mild. The increase in immune cells involved in rituximab's mechanism of action alludes to a role for GM-CSF in enhancing a patient's innate and adaptive immune response. Overall, these results provide a solid basis for future examination of the immunotherapy combination regimen of GM-CSF plus rituximab in a multicenter study of patients with relapsed/progressive FL, as well as support a potential benefit for frontline treatment of patients with FL. Six patients with FL were also treated in conjunction with this group of patients, but received frontline therapy, all of them responding to the immunotherapy combination (data not shown). In addition, this immunotherapy combination could be used in other low-grade B-cell malignancies, including Waldenström's disease, lymphocytic lymphoma, or mantle cell lymphoma.

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Table 1. Patient Demographics and Clinical Characteristics

Characteristic	Patients (N = 33)
Median age, y (range)	59 (37-81)
Male/female, n	19/14
Prior lines of therapy, n (%)	
1	8 (24)
2	14 (42)
≥ 3	11 (33)
Disease stage at inclusion, n (%)	
I-II	12 (36)
III-IV	21 (64)
FLIPI at inclusion, n (%)	
Low risk (0-1)	15 (45)
Intermediate risk (2)	10 (30)
High risk (≥ 3)	8 (24)
Disease status at inclusion, n (%)	
Relapsed	25 (76)
Stable disease	1 (3)
Progressive disease	7 (21)

Abbreviation: FLIPI, Follicular Lymphoma International Prognostic Index.

Table 2. Response According to FLIPI Groups ($P = .01$)

	Low Risk (n = 15)	Intermediate Risk (n = 7)*	High Risk (n = 7)*
OR, n (%)	14 (93)	4 (57)	5 (72)
CR	7 (47)	4 (57)	2 (28)
CRu	2 (13)	-	-
PR	5 (33)	-	3 (44)
SD or PD, n (%)	1 (7)	3 (43)	2 (28)
SD	-	1 (14)	1 (14)
PD	-	2 (29)	1 (14)

Abbreviations: CR, complete response; CRu, complete response unconfirmed; FLIPI, Follicular Lymphoma International Prognostic Index OR, objective response; PD, progressive disease; PR, partial response; SD, stable disease.

*Four patients (3 in intermediate risk group and 1 with high risk group) stopped treatment due to toxicity.

Figure Legends

Figure 1. Treatment regimen for GM-CSF plus rituximab. Patients were treated with subcutaneous GM-CSF (Leucomax[®]) 5 µg/kg/day from day 1 through day 8, with the addition of rituximab (MabThera[®]) intravenously at 375 mg/m² on day 5. Patients received this 8-day regimen every 21 days for a total of four courses. IV, intravenous; SC, subcutaneous.

Figure 2. Progression-free survival in patients with relapsed follicular lymphoma treated with rituximab and GM-CSF (n = 29). (A) In all patients. (B) Based on FLIPI risk at relapse. (C) Based on the quality of the clinical response. (D) Based on the number of cycles of treatment. One cycle was defined as four courses of rituximab and GM-CSF treatment.

Figure 3. Effect of GM-CSF plus rituximab treatment on mean cell counts (± SD) before and after treatment (**P* < .05). mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; SD, standard deviation; WBC, white blood cell.

Figure 4. Ratio of mean cell counts after versus before treatment according to response for CR patients and non-CR (PR + SD + PD) patients (**P* < .05). CR, complete response; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; PD, progressive disease; PR, partial response; SD, stable disease; WBC, white blood cell.

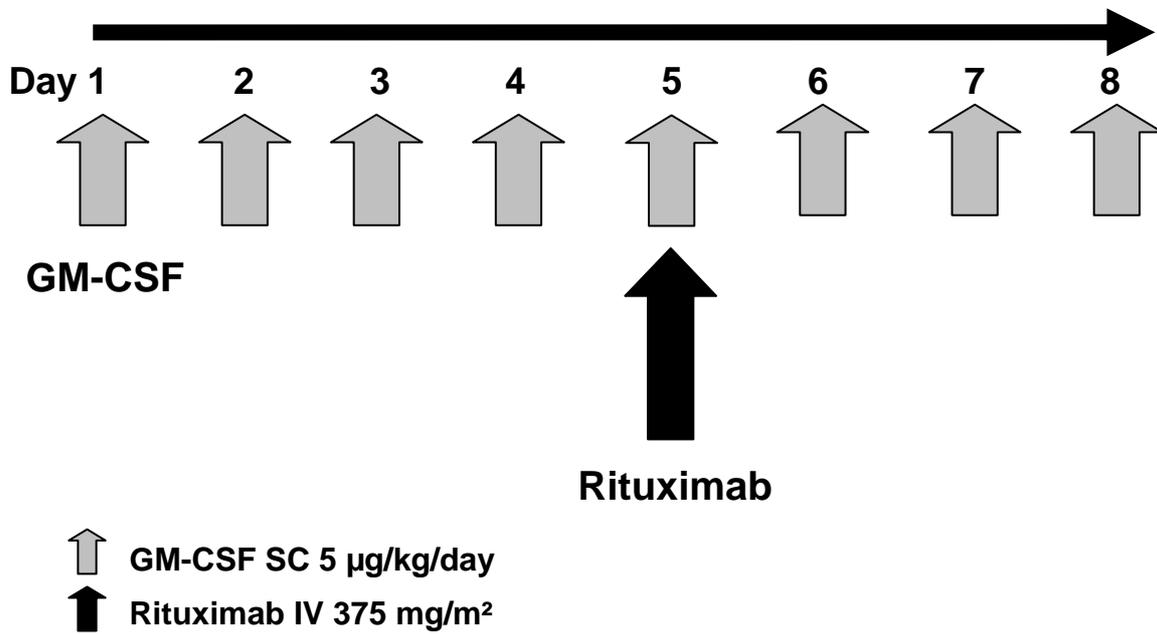


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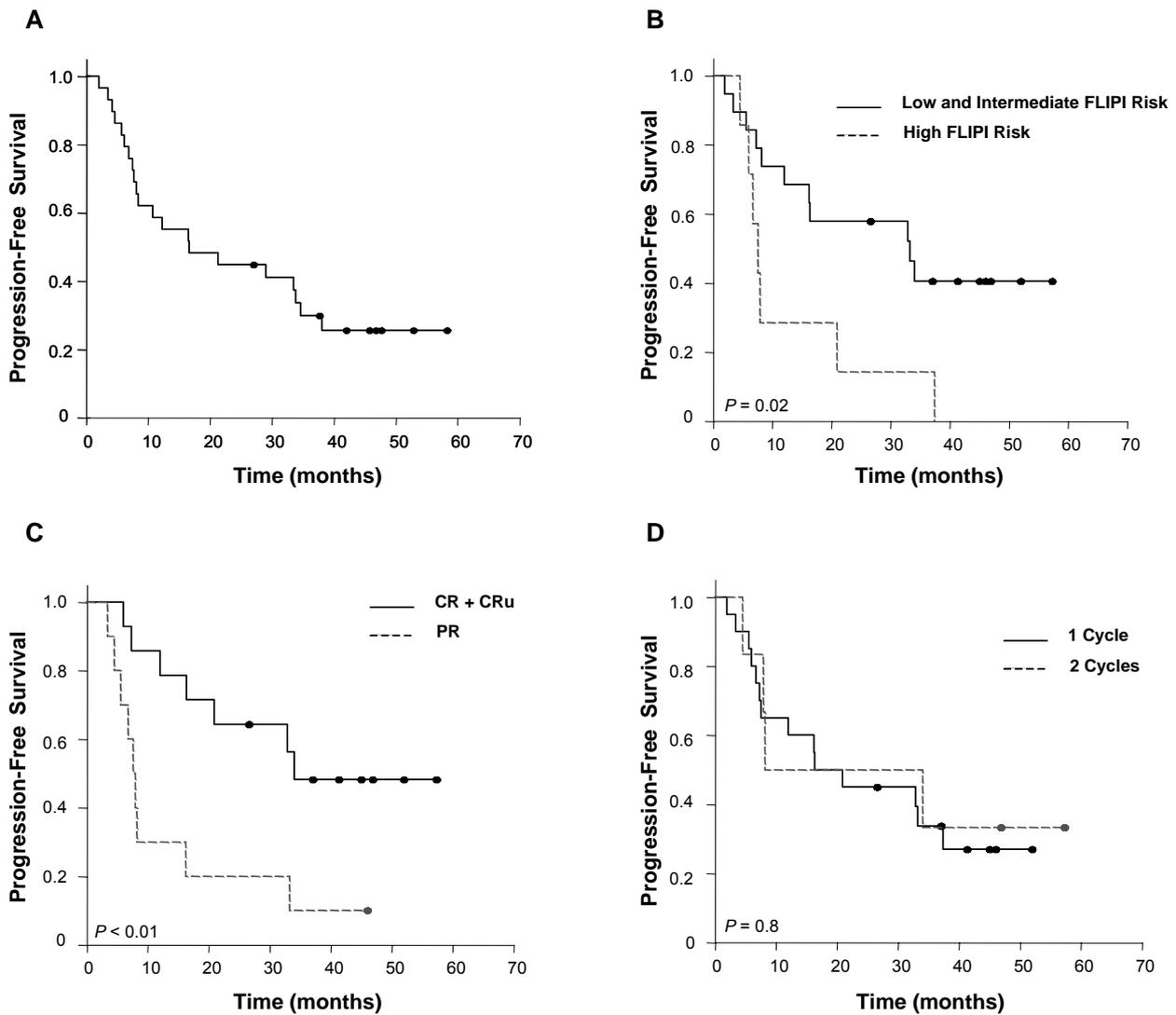


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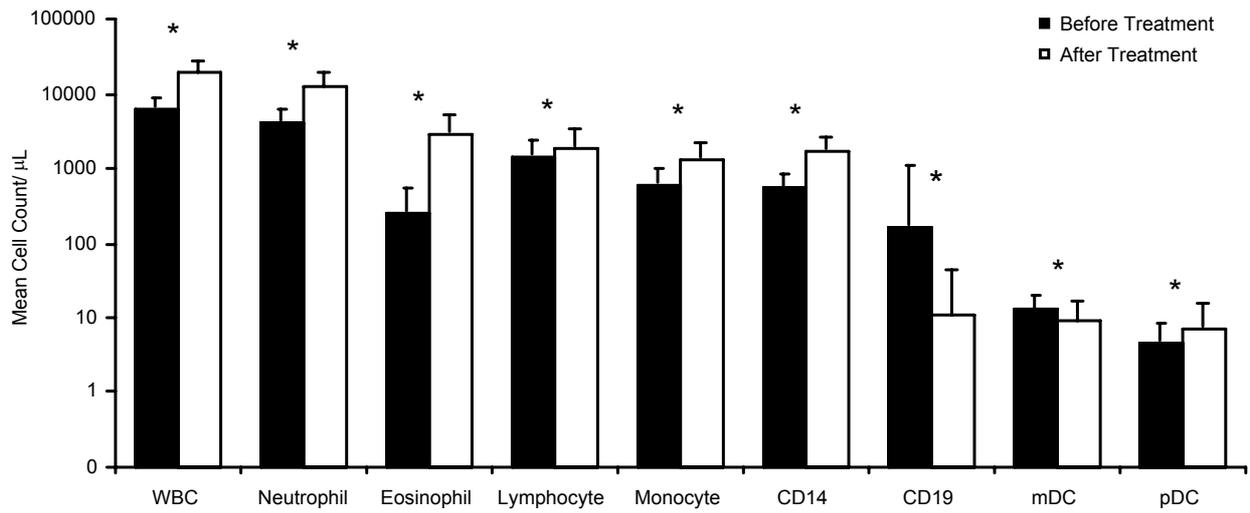


Figure 3. Effect of GM-CSF plus rituximab treatment on mean cell counts (± SD) before and after treatment (* $P < .05$).

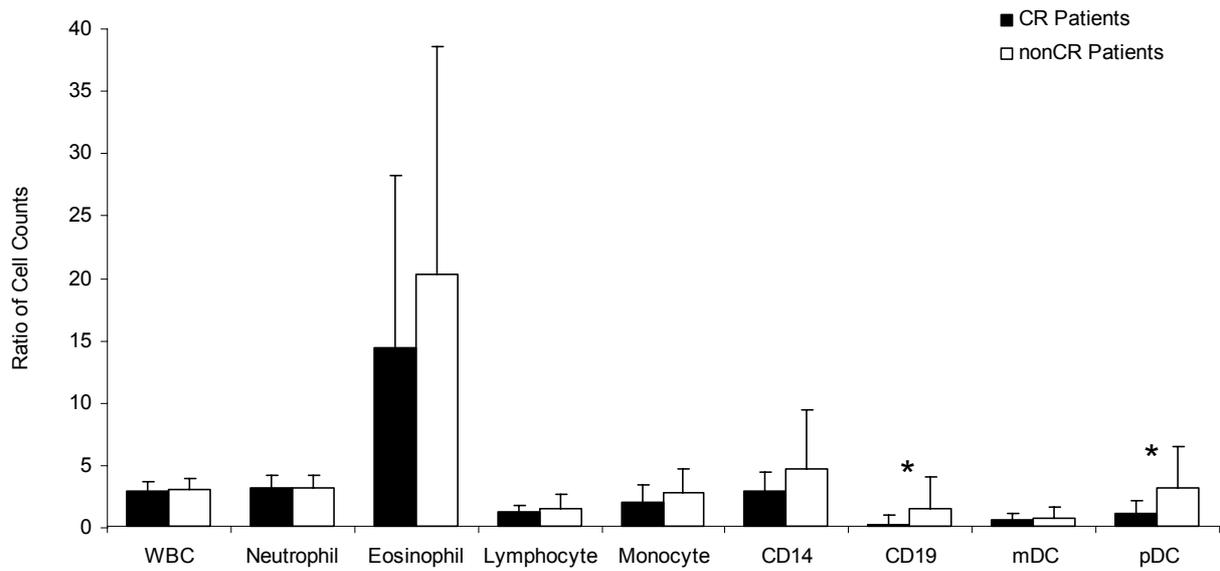


Figure 4. Ratio of mean cell counts after versus before treatment according to response for CR patients and non-CR (PR + SD + PD) patients (* $P < .05$).