To the editor:

Cytokine release in patients with CLL treated with obinutuzumab and possible relationship with infusion-related reactions

Infusion-related reactions (IRRs) occur commonly with rituximab administration, a type-I anti-CD20 monoclonal antibody that is routinely used in the treatment of chronic lymphocytic leukemia (CLL).[1,2] The pathophysiology of these reactions has been attributed to cytokine release with patients experiencing IRR found to release greater amounts of interleukin (IL) 8, IL-6, and tumor necrosis factor α (TNF-α) than those who did not and patients with baseline absolute lymphocyte count (ALC) ≥50 × 10⁹/L at greatest risk of developing a reaction.[2-4]

Obinutuzumab is a humanized glycoengineered immunoglobulin G1 type-II anti-CD20 monoclonal antibody, with enhanced antibody-dependent cell-mediated cytotoxicity and phagocytosis, increased direct cell death, and lower complement activation compared with rituximab in vitro.[5-7] These properties translated into improved clinical activity as demonstrated by the results of the CLL11 trial (#NCT01010061), and obinutuzumab plus chlorambucil has approval to treat patients with CLL and comorbidities as a result.[8,9] The incidence and severity of IRR in CLL patients treated with obinutuzumab appears to be greater than observed with rituximab, and we sought to investigate whether the underlying pathophysiology echoed that of rituximab.

We analyzed IRR frequency and severity in a subset of 38 patients, the entire complement with an underlying diagnosis of CLL pooled from 2 phase-1/2 trials, GAUSS (#NCT00576758),[10] and GAUGUIN (#NCT00517530).[11] Study methods were as previously published.[10,11] Patients were universally treated with obinutuzumab monotherapy and had frequent sequential blood samples taken. This enabled us to interrogate the association of IRR with patient baseline and tumor characteristics, peripheral blood leukocyte subsets, serum cytokine release, and complement activation.

Differences in baseline laboratory values and other variables between groups (severe IRRs vs nonsevere IRRs) were evaluated for statistical significance using the Student t test or Fisher's exact test where appropriate. In addition, patients were retrospectively stratified into 2 groups according to ALC at baseline, those considered as having "low" levels of circulating disease (<50 × 10⁹/L) and "high" levels of circulating disease (≥50 × 10⁹/L). All P values are 2-sided with a level of significance at <.05.

Of the 38 patients investigated, 35 (92%) developed symptoms of IRR (grade 1/2, n = 25; grade 3/4, n = 10) with the first infusion, and IRR resulted in permanent treatment discontinuation in 3 (8%) patients. The median time to onset was shorter in the group that had severe (grade 3/4) reactions (30 minutes, range 5-180 minutes) compared with those with less severe reactions (51 minutes, range 5-440 minutes). Even small doses of obinutuzumab (as little as 9 mg) had the potential to elicit symptoms. However, the majority of patients (27/35) completed their first infusion on day 1 despite developing IRR.

The initial obinutuzumab infusion was accompanied by a rapid decrease in circulating CD19⁺ B cells (−84% change from baseline to end of infusion), a decrease in the measurable circulating natural killer (NK) CD16⁺56⁺ cells (−97% change), as well as an increase of proinflammatory cytokines IL-6, IL-8, TNF-α, and interferon γ (Figure 1). Subsequent infusions of obinutuzumab did not induce significant cytokine release, nor were there any severe-grade IRRs observed beyond cycle 1.

Although the number of patients studied is small, there were statistically significant differences in baseline ALC (when analyzed as a continuous variable; P = .02) and baseline thrombocytopenia (P = .02), with those patients who had a severe-grade event more likely to have higher ALC and lower platelet count compared with those patients who had a nonsevere reaction. When patients were dichotomized into those with ALC <50 × 10⁹/L vs those with ALC ≥50 × 10⁹/L at baseline, the resultant loss of information meant that the increased risk of severe-grade IRR for those in the high ALC group did not achieve conventional statistical significance, with a P value of .06. Patients who had grade 3 or greater reactions also appeared to have had higher baseline values in parameters of tumor burden, nodal disease, thymidine kinase, β-2 microglobulin, and splenomegaly. However, none of these differences reached statistical significance.

At the midinfusion time point, patients with preinfusion ALC ≥50 × 10⁹/L released more proinflammatory cytokines, in particular IL-6 (mean IL-6 [log₁₀] 3.16 vs 2.41) and IL-8 (mean IL-8 [log₁₀] 3.57 vs 2.91) than those with ALC <50 × 10⁹/L. In addition, those patients with higher pretreatment ALC developed more pronounced decreases in their neutrophil and platelet counts at day 8 when compared with those with lower levels of circulating disease (Figure 2). Markers of complement activation, C5a and C3a, did not increase nor was there consumption of C3/C4 levels.

Do these results provide new insight into the pathophysiology and possible intervention strategies that could be exploited to prevent IRR? IL-8 is a proinflammatory chemokine that acts as a chemotactic factor for neutrophils, T cells, eosinophils, and NK cells.[12] Elevated serum concentrations have been noted in CLL[13] and correlate with advanced disease. However, IL-8 is required for effector cell migration and triggers several mechanisms required for phagocytosis[14]; thus, inhibiting this key cytokine may not be advantageous. In fact, CLL-secreted IL-8 appears to be required as a cofactor to maximize NK-mediated antibody-dependent cell-mediated cytotoxicity,[15] although this effect was more pronounced for those cells treated with rituximab than obinutuzumab.

Given the known biological properties of IL-6, with elevated levels causing hypotension, vascular leakage, tissue edema, and hypoxia,[16] it might prove a more appropriate target to inhibit or prevent severe IRR. Patients treated with recombinant IL-6 developed a clinical syndrome very similar to IRR, with pyrexia, headaches, flushing, and rigors.[17] Dexamethasone has been shown to inhibit both IL-6 and IL-6 receptor gene expression,[18] and this may explain the partial efficacy of steroid premedication in reducing the incidence and severity of IRR observed in the CLL11 trial.[8] Furthermore, blockade of IL-6 receptor with tocilizumab sensitized cells in vitro to the cytotoxic action of chlorambucil[19]; thus, the benefits of inhibiting this pathway in CLL may not be limited to the prevention of IRR.

First-cycle thrombocytopenia attributable to rituximab has been reported in up to 30% of patients for any grade and 20% for severe grades.[20] Risk factors include high levels of circulating disease, higher levels of CD20 expression, the presence of splenomegaly, and bone marrow infiltration.[4,20] Many of these were also recently identified as risk factors for the development of IRR with anti-CD20
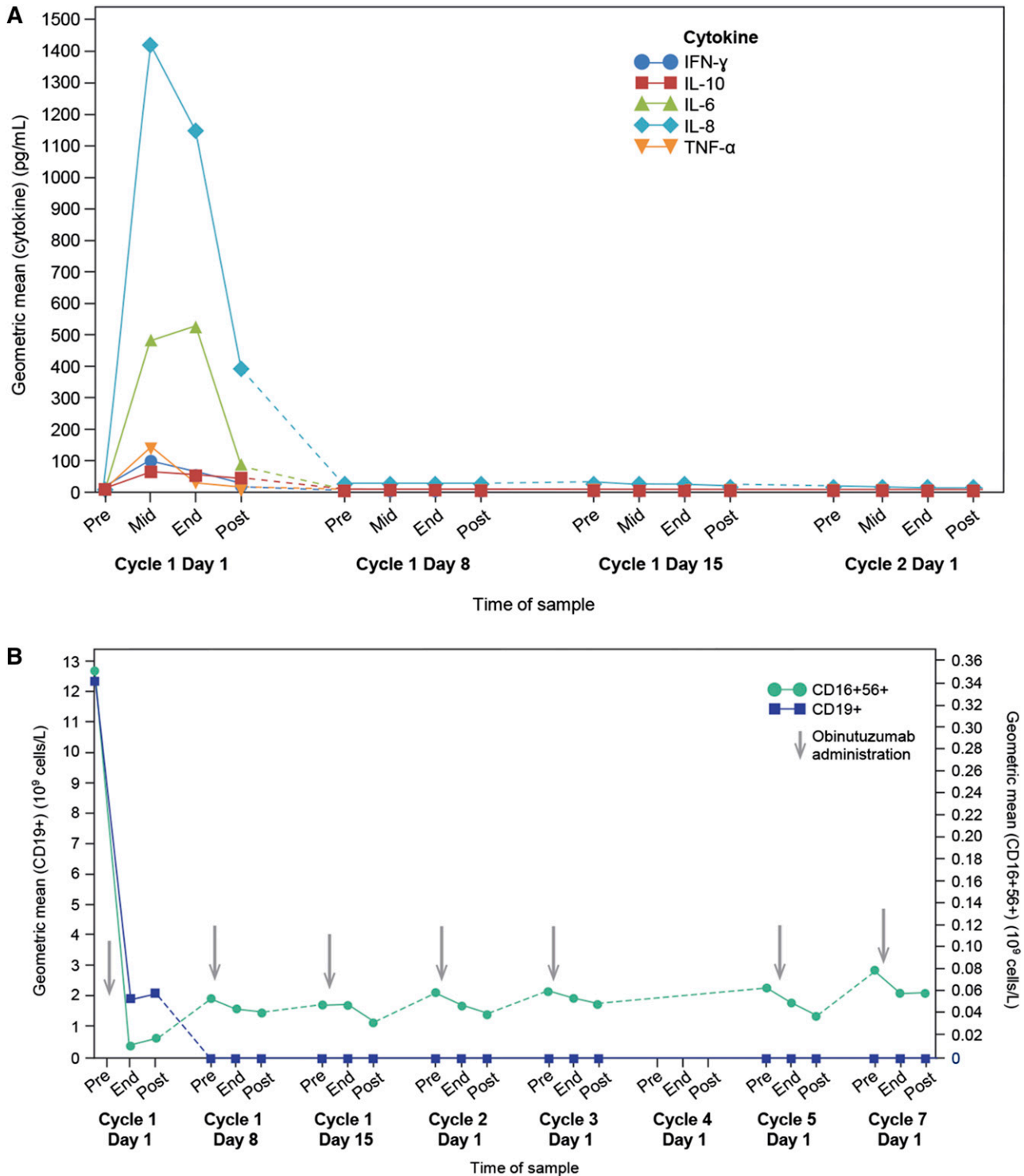
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Figure 1. Effects of obinutuzumab administration on cytokine release patterns, ALC, and circulating NK cells. (A) Cytokine release observed pre-, mid-, end-, and postadministration of obinutuzumab observed in n = 38 CLL patients. (B) Changes in circulating $CD19^+$ B cells (y-axis left) and $CD16^+56^+$ NK cells (y-axis right) pre-, end-, and postadministration of obinutuzumab observed in n = 38 CLL patients.

monoclonal administration in CLL.[21] In our pooled cohort, cycle-1 thrombocytopenia was observed in 25/38 patients, with severe grades in 8/38 (21%). This may result from an endothelial-mediated process or margination, as both TNF-α[22] and IL-6[23] in particular can induce expression of endothelial adhesion molecules. Acute leukopenia has also been described in response to rituximab administration, in particular in patients who experienced symptoms

of cytokine release.[24] Thus, the increased incidence of grade 3 or greater neutropenia (obinutuzumab 14% vs rituximab 9%) and thrombocytopenia (obinutuzumab 11% vs rituximab 3%) observed during the first cycle in the CLL11 trial may relate to the fact that the cytokine release induced by obinutuzumab appears to be quantitatively different from rituximab.[5,8] This too may explain the differences in IRRs observed between the 2 arms in this trial.
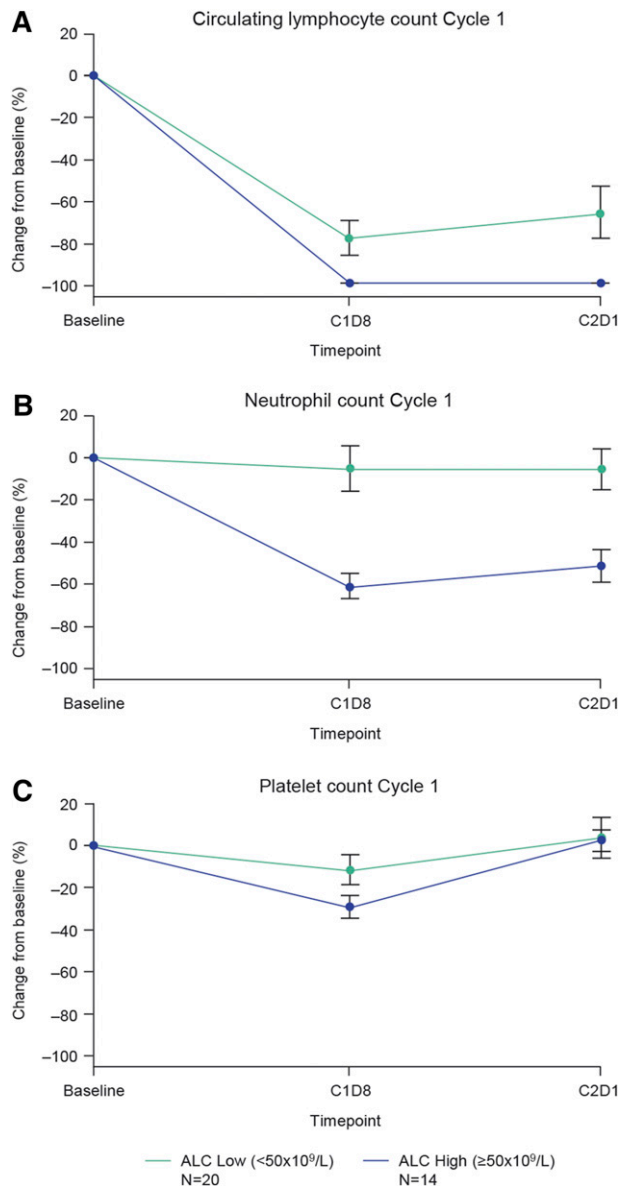
Figure 2. First cycle cytopenias observed in patients with high vs low ALC at baseline. Mean percentage change in circulating lymphocyte (A), neutrophil (B), and platelet (C) counts observed during cycle 1 in patients with high ($\geq$50 $\times$ 10^9/L) and low (<50 $\times$ 10^9/L) baseline ALC values.

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In conclusion, the administration of obinutuzumab to patients with CLL triggered immediate and marked release of cytokines, in particular IL-6 and IL-8, which was limited to the first infusion and accompanied by rapid destruction of circulating B cells, by decrease in circulating NK cells, and, in the majority of patients, by signs and symptoms suggestive of IRR. Patients with higher levels of circulating disease at baseline appear more likely to develop associated cytopenias with cycle 1. Intervention strategies that target these proinflammatory cytokines may be promising to reduce the incidence and severity of IRR with obinutuzumab and are currently being explored in clinical trials (#NCT01905943 and #NCT02336048).

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To the editor:

Dramatic transient improvement of metastatic BRAF^{V600E}-mutated Langerhans cell sarcoma under treatment with dabrafenib

Langerhans cell sarcoma (LCS) is a rare histiocytic neoplasm with overt malignant cytological features and an aggressive clinical course.[1] Disseminated LCS carries a poor prognosis.[1] We report a case of a metastatic BRAF^{V600E}-mutated LCS that dramatically improved after administration of the BRAF inhibitor (BRAFi) dabrafenib.

A 58-year-old man was referred in August 2014 with a diagnosis of progressive Langerhans cell histiocytosis (LCH). He was treated in July 2013 by surgery and radiotherapy for left humerus LCH diagnosed by bone biopsy. In February 2014, enlargement of the left axillary, pectoral, and supraclavicular lymph nodes was observed. Histologic examination of a lymph node biopsy indicated LCH

recurrence, although some atypical cells were described. CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy was initiated but was discontinued after 3 cycles because of disease progression. The patient's condition deteriorated. The lymph nodes increased in size and multiple lung nodules were observed on lung computed tomography (CT) scan. These lesions were highly hypermetabolic on fluorodeoxyglucose (^{18}FDG) positron emission tomography (PET)-CT (Figure 1). A new lymph node biopsy revealed massive infiltration by very large cells with irregular folded nuclei and necrotic areas. The mitotic rate was >50 per 10 high-power fields. The tumor cells expressed CD1a and langerin