Published in final edited form as:

J Immunother. 2013 ; 36(6): 331–341. doi:10.1097/CJI.0b013e31829d7e2e.

A Dose-Escalation Study of Recombinant Human Interleukin-18 in Combination with Rituximab in Patients with Non-Hodgkin's Lymphoma

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Summary

Interleukin-18 (IL-18) is an immunostimulatory cytokine with antitumor activity in preclinical models. Rituximab is a CD20 monoclonal antibody with activity against human B cell lymphomas. A phase I study of recombinant human (rh) IL-18 given with rituximab was performed in patients with CD20+ lymphoma. Cohorts of 3–4 patients were given infusions of rituximab 375 mg/m² weekly for 4 weeks with escalating doses of rhIL-18 as a 2-hour intravenous infusion weekly for 12 consecutive weeks. Toxicities were graded using standard criteria. Blood samples were obtained for safety, pharmacokinetic, and pharmacodynamic studies. Nineteen patients with CD20+ B cell non-Hodgkin's lymphoma were given rituximab in combination with rhIL-18 at doses of 1, 3, 10, 20, 30, and 100 µg/kg. Common side effects included chills, fever, headache, and nausea. Common laboratory abnormalities included transient, asymptomatic lymphopenia, hyperglycemia, anemia, hypoalbuminemia, and bilirubin and liver enzyme elevations. No dose-limiting toxicities were observed. Biologic effects of rhIL-18 included transient lymphopenia and increased expression of activation antigens on lymphocytes. Increases in serum concentrations of IFN- γ , GM-CSF, and chemokines were observed following dosing. Objective tumor responses were seen in 5 patients, including 2 complete and 3 partial responses. rhIL-18 can be given in biologically active doses by weekly infusions in combination with rituximab to patients with lymphoma. A maximum tolerated dose of rhIL-18 plus rituximab was

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CONFLICTS OF INTEREST / FINANCIAL DISCLOSURES H.S., K.M.K., J.W.B., O.S.G., S.C.M., F.G., Z.D., and J.F.T. are employees of and have ownership interest in GlaxoSmithKline. J.K. has served on an advisory board for Genentech. M.J.R. and J. W. have declared that there are no financial conflicts of interest in regard to this work.

Presented in abstract form at the 45th annual meeting of the American Society of Clinical Oncology, Orlando, FL, June 2009 and the 53rd annual meeting of the American Society of Hematology, San Diego, CA, December 2011.

Financial Disclosure: Herbert Struemper, Kevin M. Koch, John W. Bauman, Olivia S. Gardner, Sharon C. Murray, Fiona Germaschewski, Zdenka Jonak, and John F. Toso are employees of and have ownership interest in GlaxoSmithKline. Justin Kline has served on an advisory board for Genentech. Michael J. Robertson and Jill Weisenbach have declared that there are no financial conflicts of interest in regard to this work.

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not determined. Further studies of rhIL-18 and CD20 monoclonal antibodies in B cell malignancies are warranted.

Keywords

Rituximab; IL-18; IFN- γ ; lymphoma

Introduction

Lymphomas are cancers derived from mature lymphocytes or their precursors. More than 30 distinct lymphoma entities are recognized in the current World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues (1). The most common lymphoid neoplasms in the WHO Classification are diffuse large B cell lymphoma (DLBCL) and follicular lymphoma; together they comprise the majority of cases (1, 2). DLBCL is an aggressive neoplasm, but is potentially curable with conventional chemotherapy. In contrast, follicular lymphoma is usually associated with an indolent natural history but is incurable in advanced stages. Mantle cell lymphoma is a relatively uncommon subtype of lymphoma, comprising ~5–8% of cases. Unlike DLBCL, mantle cell lymphoma is not curable by conventional chemotherapy. Moreover, unlike follicular lymphoma, mantle cell lymphoma usually has an aggressive natural history (3). Management of mantle cell lymphoma is therefore particularly challenging and there is no consensus regarding its optimal treatment.

DLBCL, follicular lymphoma, and mantle cell lymphoma are mature B cell neoplasms and >90% of these tumors express the CD20 antigen. CD20 is a 33–35 kilodalton integral membrane glycoprotein that is expressed on the surface of virtually all mature B cells. Rituximab is a chimeric monoclonal antibody containing murine variable regions that recognize the human CD20 antigen fused to the human immunoglobulin G1 (IgG1) constant region (4). Randomized, prospective clinical trials have shown that addition of rituximab to chemotherapy improves the survival of patients with DLBCL, follicular lymphoma, and mantle cell lymphoma (5–10).

The mechanisms by which rituximab mediates antitumor activity in vivo may include antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity, and direct apoptotic effects on tumor cells (4, 11). Rituximab could also promote lymphoma-specific T cell immune responses by inducing presentation of tumor-associated antigens (12, 13). However, there is compelling evidence that signals mediated through Fc γ RIIIa (CD16) are required for optimal antitumor activity during rituximab-based therapy (14–18). CD16 is an Fc receptor for IgG that is expressed on natural killer (NK) cells as well as monocytes and macrophages (19, 20). One strategy to improve the efficacy of rituximab is administration of cytokines that enhance the effector function of NK cells and/or macrophages.

IL-18 is an immunostimulatory cytokine that regulates both innate and adaptive immune responses (21, 22). IL-18 has antitumor activity in animal models (23–26). Moreover, IL-18 augments ADCC mediated by human NK cells in vitro against rituximab-coated lymphoma cells (27). Administration of IL-18 to cancer patients causes the in vivo activation of human NK cells and monocytes (28, 29). Thus, it is rational to combine IL-18 with rituximab for treatment of patients with CD20+ lymphomas. We report the results of a phase I clinical trial of rhIL-18 given with a standard rituximab regimen to patients with B cell non-Hodgkin lymphoma.

PATIENTS AND METHODS

Patient Selection

Eligible patients included adults (age ≥ 18 years) with histologically confirmed CD20+ B cell non-Hodgkin's lymphoma that progressed after prior therapy or for which there was no standard curative therapy. Prior autologous peripheral blood stem cell transplantation (PBSCT) was allowed if transplantation had occurred at least 3 months prior to screening. Prior rituximab therapy was allowed provided that the last treatment with a rituximab-containing regimen occurred at least 6 months before enrollment. Patients were required to have measurable or evaluable disease, ECOG performance status less than 2, as well as adequate renal, hepatic, and hematologic function. Patients were excluded if they had a malignant cell count > 25,000 per μ L in peripheral blood, history of severe infusion-related reaction or tumor lysis syndrome following prior treatment with rituximab, were pregnant or breast-feeding, were seropositive for HIV or hepatitis B surface antigen, had known symptomatic or untreated leptomeningeal or brain metastases or major uncontrolled comorbid illnesses.

Study Design

This open-label, non-randomized, dose-escalation phase I clinical study (GSK Clinical Study 105618; [ClinicalTrials.gov](https://clinicaltrials.gov) identifier NCT00500058) was conducted at two centers. The protocol was approved by the Institutional Review Boards at Indiana University Medical Center (Indianapolis, IN) and the University of Chicago Medical Center (Chicago, IL). Written informed consent was obtained from each patient prior to enrollment on study. Study drug SB-485232 (iboctadekin), a rhIL-18 protein produced in *E. coli*, was supplied by GlaxoSmithKline (Research Triangle Park, NC). Rituximab 375 mg/m² was given by intravenous infusion once per week for four consecutive weeks (on day 1 of weeks 1–4). rhIL-18 was given by intravenous infusion over 2 hours once per week for 12 consecutive weeks (on day 2 of weeks 1–12). During weeks 1–4 of study, the rhIL-18 infusions were started at least 24 hours after initiation of the preceding rituximab infusion.

Successive cohorts of 3 patients received rhIL-18 in planned doses of 1, 3, 10, 20, 30, and 100 μ g/kg. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria Version 3.0. Dose-limiting toxicity was defined as any grade 3 or 4 toxicity observed during the first 6 weeks of treatment and assessed to be related to study drug, excluding grade 4 lymphopenia and grade 3 fever, nausea, vomiting, leukopenia, and neutropenia.

Pharmacokinetic Studies

Blood samples were collected for determination of rhIL-18 concentrations prior to and 1 hour following initiation of IL-18 infusion, immediately prior to termination of infusion and at 4, 6, 8, 48, and 168 hours after initiation of infusions on day 2 of weeks 1, 4, 8, and 12 of study. The total (bound plus unbound) concentration of rhIL-18 was measured using a specific fluoro-immunoassay method as described previously (28).

Pharmacodynamic Studies

Blood samples were collected for analysis of leukocyte markers by flow cytometry prior to initiation of rituximab infusion on day 1 of weeks 1 and 4 and prior to, and 4, 48, and 168 hours after initiation of the rhIL-18 infusion on day 2 of weeks 1, 4, 8, and 12 of study. Blood samples were collected for quantification of plasma cytokines and chemokines prior to initiation of rituximab infusion on day 1 of weeks 1–4 and prior to and 4 hours after initiation of rhIL-18 infusion on day 2 of weeks 1–12 of study. Blood samples were collected for quantification of IL-18 binding protein (BP) prior to initiation of rituximab

infusion on day 1 of week 1 and prior to initiation of rhIL-18 infusion on day 2 of weeks 1–12 of study.

Immunohistochemical Analysis of Tumor

Fine needle aspirates of a superficial lymphoma mass were obtained before and one month after the first infusion of rhIL-18 on study. Aspirated material was cytospun onto glass slides and fixed with acetone for 2 minutes at room temperature. Slides were incubated with 5 µg/mL murine monoclonal antibody to human CD69 (Caltag, Burlingame, CA) for 60 minutes at room temperature, followed by incubation with horse radish peroxidase-conjugated anti-mouse secondary antibody and diaminobenzidine chromogenic substrate (Dako, Carpinteria, CA). Slides were then counterstained with hematoxylin and coverslipped for microscopic imaging. Images (600×) were captured and acquired using an enclosed Olympus microscope/camera automated cellular imaging system and image software (Dako).

Evaluation of Clinical Characteristics and Response

Lymphoma subtypes were designated using the WHO Classification (1). Diagnostic biopsies from 2 patients could not be classified other than as low grade B cell lymphomas. These 2 patients as well as patients with follicular lymphoma of grade 1–2 were designated to have indolent lymphomas. Patients designated to have rituximab-refractory disease are those who failed to achieve complete or partial response or who developed objective disease progression 6 months after receiving a rituximab-containing regimen. Patients designated to have chemotherapy-refractory disease are those who failed to achieve complete or partial response or who developed objective disease progression 6 months after receiving a chemotherapy regimen. Number of prior therapies refers to the number of treatments given for an episode of disease progression (e.g., two cycles of salvage chemotherapy followed by high-dose chemotherapy and PBSCT were counted as one therapy). Combination chemotherapy regimens included cyclophosphamide/ vincristine/ prednisone (CVP), cyclophosphamide/ doxorubicin/ vincristine/ prednisone (CHOP), ifosfamide/ carboplatin/ etoposide (ICE), and high-dose carmustine/ etoposide/ cytarabine/ melphalan (BEAM) followed by PBSCT. Tumor measurements were obtained within 4 weeks of the first infusion of rhIL-18 and approximately one week after the last infusion of rhIL-18. Tumor responses were assessed using International Workshop Criteria (30). One patient had non-measurable bone lesions that were assessed by FDG-PET scan.

Statistical Analysis

Analysis of safety and efficacy data was descriptive in nature, with counts and percentages determined for categorical data and mean, median, standard deviation, minimum, and maximum for continuous data. Confidence interval for the overall response rate was calculated using the standard Wald asymptotic confidence limits based on the normal approximation to the binomial distribution.

RESULTS

Patient Characteristics

Nineteen patients were enrolled on study, including 14 women and 5 men. The median age was 68 years (range, 43 to 81 years). The histologic subtype of lymphoma was follicular lymphoma for 10 patients, mantle cell lymphoma for 5 patients, DLBCL for 2 patients, and low grade B cell lymphoma (not further classifiable) for 2 patients. All patients had relapsed and/or refractory lymphoma at the time of study enrollment. Patients had received a median of 2 (range, 1–7) prior therapies for lymphoma. All 19 patients had received prior treatment

with rituximab; 15 of these patients had received both rituximab and chemotherapy and 4 had received only rituximab.

Eleven patients had rituximab-refractory disease: 4 patients (1 with indolent follicular lymphoma, 3 with mantle cell lymphoma) experienced disease progression during maintenance rituximab therapy; 3 patients (1 each with DLBCL, follicular lymphoma, and mantle cell lymphoma) did not achieve objective complete or partial response after receiving single agent rituximab weekly for 4 consecutive weeks; 2 patients (1 with follicular lymphoma, 1 with indolent lymphoma not further classifiable) had objective disease progression 6 months after responding to a rituximab-based regimen; 2 patients (1 with DLBCL, 1 with indolent lymphoma not further classifiable) had objective disease progression 6 months after receiving single agent rituximab (with no documented assessment of response to rituximab).

Five patients had disease that had been refractory to both rituximab and chemotherapy and one patient had disease that was refractory to chemotherapy but not single agent rituximab. Among the 6 patients with chemotherapy-refractory disease, 3 patients (1 with follicular lymphoma, 2 with mantle cell lymphoma) were refractory to one prior chemotherapy regimen (2 CVP, 1 single agent bortezomib), one patient with follicular lymphoma was refractory to 2 prior regimens (R-CHOP, R-ICE followed by high-dose BEAM and PBSCT), one patient with DLBCL was refractory to 2 prior regimens (R-ICE, single agent gemcitabine), and one patient with DLBCL was refractory to 3 prior regimens (CHOP, CVP, single agent gemcitabine). Five of the 6 patients with chemotherapy-refractory disease were refractory to the last chemotherapy regimen given before they enrolled on study. Four patients (21%) had received prior radioimmunotherapy and 3 (16%) had previously undergone high-dose therapy and PBSCT.

Administration of Rituximab and rhIL-18 on Study

Fourteen patients completed all planned infusions of rituximab and rhIL-18. Four patients received the four planned rituximab infusions but withdrew from the study early due to disease progression: one patient after receiving all planned infusions of rhIL-18 but prior to the follow-up visit and three patients after receiving, respectively, 6, 8, and 9 infusions of rhIL-18. One patient (in the 1 µg/kg cohort) was taken off study (after 3 infusions of rituximab and rhIL-18) due to asymptomatic prolongation of the QTc interval on EKG. This patient had baseline grade 2 QTc prolongation prior to study drug administration and developed grade 3 QTc prolongation during the study. Although this patient had no symptoms or signs related to the QTc prolongation, it was decided that she should be taken off study to avoid any potential safety issues related to persistent grade 3 QTc prolongation. None of the enrolled patients was taken off study because of serious adverse events or failure to tolerate the treatment.

Toxicity and Laboratory Abnormalities during Administration of Rituximab and rhIL-18

Common side effects associated with weekly infusions of rhIL-18 included grade 1–2 chills, fever, headache, and nausea (Table 1). Adverse events attributed to study drug in weeks 1–4 of study (during concomitant weekly infusions of rituximab and rhIL-18) were not obviously different in frequency or severity compared to adverse events during weeks 4–12 of study. Five patients (26%) experienced grade 3 adverse events, including prolongation of QTc interval on EKG (1 patient; not attributed to study drug), confusional state (1 patient; not attributed to study drug), deep venous thrombosis (1 patient; not attributed to study drug), and anemia (2 patients; attributed to study drug and/or procedures in 1 patient).

Common laboratory abnormalities associated with rhIL-18 administration included grade 1–3 hyperglycemia, grade 1–2 bilirubin elevations, and grade 1–2 hypoalbuminemia (Table 2). The only grade 4 clinical chemistry abnormalities (grade 4 hypoglycemia in 1 subject, grade 4 hyperkalemia in another subject) seen in the study were assessed to be spurious and not clinically relevant. One subject in the 3 µg/kg cohort had a grade 4 low glucose value recorded in the study database for week 8, day 2 of study. The patient had no symptoms at this time and no other low glucose values (including one obtained earlier the same day) were observed for this subject during the study. After the study database had been locked, it was realized that the blood glucose value entered in the database (2.9 mg/dL) was an error. The actual blood glucose value (confirmed in source documents) for the subject at this time-point was 133 mg/dL. Another subject in the 3 µg/kg cohort had a grade 4 high potassium value that was deemed spurious as the blood specimen was grossly hemolyzed. Hematologic toxicity (Table 3) was similar to that seen in patients receiving rhIL-18 as monotherapy by the same schedule of administration (29). As described below, transient grade 3–4 lymphopenia was considered an expected biologic effect of rhIL-18 administration rather than an adverse event. No serious adverse events attributed to rhIL-18 occurred in this study and a maximum tolerated dose of rhIL-18 was not identified. Antibodies to rhIL-18 or rituximab were not detected in any patient treated on this study.

Pharmacokinetic Results

Plasma concentrations of rhIL-18 closely resembled those observed at corresponding doses in previous studies of rhIL-18 administered without rituximab (28, 29). rhIL-18 was eliminated slowly, with mean half-life values ranging from 51.4 to 88.3 hours. C_{max} and AUC values were approximately dose proportional in the 1, 3, 10, 20 µg/kg cohorts, but did not further increase in the 30 and 100 µg/kg cohorts (Fig. 1). A steep decline in plasma concentrations from peak levels was observed for rhIL-18 doses of 10 µg/kg and higher. These nonlinear features of the plasma pharmacokinetic profiles were also observed in other rhIL-18 studies and are probably the result of saturable binding of rhIL-18 to the IL-18BP as previously described (31).

Biological Effects of rhIL-18

Administration of rhIL-18 stimulated increases in plasma concentrations of pro-inflammatory cytokines (IFN- γ , GM-CSF, and TNF- α), CXC chemokines (MIG and IP-10), and the CC chemokine MCP-1 at 4 hours after rhIL-18 dosing with a normalization to baseline levels observed within a week (Fig. 2 and data not shown). MIG (CXCL9) and IP-10 (CXCL10) have been shown to contribute to antitumor responses during cytokine-based immunotherapy (32). Rituximab administration during the first 4 weeks had little impact on cytokine and chemokine levels. Strong rhIL-18 responses were already observed at doses of 1 µg/kg, however maximal stimulation measured in terms of fold-increase from baseline levels occurred for doses of 10–30 µg/kg; diminished responses were seen in the 100 µg/kg cohort (Fig. 3 and data not shown). Responses trended to be stronger during the first or first few rhIL-18 administrations; but this pattern varied among patients and strong stimulation was generally still observed during the last months of the 12 weekly rhIL-18 dosing cycles. There was a rise in mean plasma levels of free IL-18 BP in all cohorts following the first rituximab dose. This mean increase was driven by a strong (larger than 50% relative to baseline) transient increase in about half of the subjects. However, levels returned to baseline by the end of the first week and stayed close to baseline levels for the remainder of the study.

Circulating lymphocytes declined and recovered in a manner similar to that seen in previous studies of rhIL-18 monotherapy (28, 29). The mean reduction from pre-dose levels in the numbers of peripheral blood lymphocytes 4 hours after rhIL-18 dosing was 56%, 67%, 72%,

45%, 70%, and 66% for the 1, 3, 10, 20, 30, and 100 µg/kg cohorts, respectively. Thus, the maximal reduction in circulating lymphocytes was seen in the 10 µg/kg dose cohort. In contrast to rhIL-18 monotherapy studies, however, circulating B cells were generally undetectable at all time-points beyond week 1, presumably as a consequence of rituximab-mediated B cell depletion. The nadir in circulating lymphocyte counts reached a peak around 4 hours after exposure to rhIL-18 and was most evident in the NK cells (Fig. 4A) and to a lesser extent in the CD8+ and CD4+ T cells (data not shown). The depletion of circulating lymphocytes 4 hours post exposure to rhIL-18 was dose- and exposure-dependent with maximum % change from baseline observed at the higher doses and this was particularly evident for NK cells (Fig. 4B). The CD56dim and CD56bright NK cells remaining in circulation 4 hours post rhIL-18 dosing displayed an average increase of the CD69 activation antigen and for the CD69+ positive cells an increased proportion of cells expressing CD95 ligand (data not shown).

Lymphocyte counts returned to baseline levels around 48 hours after exposure to rhIL-18. At the 48 hour time-point there was a clear trend to activation of both the CD56dim and CD56bright NK cells as shown from the change from baseline of expression of CD69 (Fig. 4C). Thus there was an increase in the proportion of activated NK cells known to have strong cytolytic activity (CD56dim subset) and NK cells that produce inflammatory cytokines (CD56bright subset) (19, 33). Overall, there were no systematic changes observed in the percentage of CD69+ T cells between cycles. Moreover, the absolute numbers and percentages of NK cells and T cells did not change across cycles (data not shown).

The transient lymphopenia observed after rhIL-18 infusions is most likely due to in vivo activation of lymphocytes with their subsequent extravasation into normal tissues and/or tumors. Consistent with this hypothesis, infiltration of activated immune effector cells into tumor was seen in a patient with mantle cell lymphoma treated in the 3 µg/kg dose cohort (Fig. 5). An increase in the number of CD69+ cells was detected in a lymphoma mass one month after initiation of treatment with rhIL-18 and rituximab, compared to the pre-treatment biopsy (Fig. 5).

Tumor Response

Objective complete responses (CR) were seen in 2/19 (11%) patients and partial responses (PR) in 3/19 (16%) patients (Table 4). The overall objective response rate was 5/19 (26.3%; 95% confidence interval, 6.5% – 46.1%). Objective responses were seen in 3 patients with follicular lymphoma (1 CR, 2 PR) and 2 patients with low grade B cell lymphoma, not further classifiable (1 CR, 1 PR). For the 12 patients with indolent lymphoma the overall response rate was 5/12 (42%). Objective responses were seen in 2/5 (40%) indolent lymphoma patients with prior rituximab-refractory disease and 3/7 (43%) without prior rituximab-refractory disease. CR occurred in 1 indolent lymphoma patient with rituximab-refractory disease as well as 1 patient without rituximab-refractory disease. For the 8 subjects with indolent lymphoma who received rhIL-18 at doses of 10 µg/kg or higher, which were associated with the maximum rhIL-18 biologic responses based on peripheral blood markers, the overall response rate was 5/8 (62%). Only one patient with follicular lymphoma who received 10 µg/kg doses of rhIL-18 had progressive disease. In contrast, 2/4 (50%) patients with follicular lymphoma who received < 10 µg/kg doses of rhIL-18 had progressive disease, 1 patient had stable disease, and 1 patient was not evaluated for response due to early discontinuation for QTc prolongation.

DISCUSSION

Rituximab has dramatically improved therapeutic results for patients with both aggressive and indolent B cell non-Hodgkin's lymphomas. Despite the undisputed benefits provided by

rituximab, however, further improvement in treatment of lymphoma is needed. Improvements in immunotherapy for lymphoma should optimally be based on understanding the mechanisms by which rituximab provides its salutary effects. However, the mechanisms by which rituximab mediates antitumor activity are complex and have not been completely defined. Potential mechanisms include ADCC, complement-dependent cytotoxicity, and direct apoptotic effects on tumor cells (4, 11). Moreover, CD20 monoclonal antibodies can evoke tumor-specific T cell immune responses, possibly by enhancing the presentation of tumor-associated antigens by dendritic cells (12, 13, 34). Nevertheless, there is compelling evidence that signals mediated through CD16 on effector cells are required for optimal antitumor activity during rituximab-based therapy (14–17). A polymorphism in nucleotide 559 of the *FCGR3A* gene results in allotypes with either a phenylalanine (F) or valine (V) residue at amino acid position 158 of CD16 (35). This residue is directly involved in the binding of IgG1 to CD16 (36). Human NK cells expressing CD16 receptors with the V/V phenotype, as compared to the F/F phenotype, have been shown to bind to IgG with stronger avidity and to mediate higher levels of ADCC in vitro (37). Moreover, lymphoma patients with the *FCGR3A*-158V/V genotype have higher response rates and longer progression-free survival after rituximab-based therapy (15, 16). These data strongly support the hypothesis that effector cells expressing CD16, including NK cells, contribute to the antitumor activity of rituximab in vivo. Therefore, it is rational to combine rituximab with other agents that can enhance the function of Fc receptor-bearing effector cells.

We have previously shown that IL-18 can enhance NK cell ADCC against rituximab-coated lymphoma cells in vitro (27). We therefore investigated the safety and biological effects of rhIL-18 given together with rituximab in patients with relapsed and/or refractory CD20+ B cell lymphomas. Results of our phase I study clearly show that combined therapy with rituximab and rhIL-18 is feasible in this patient population. Indeed, the toxicity profiles of rituximab and rhIL-18 given together do not appear to differ significantly from those seen when either agent is given alone. Adverse effects were modest and tolerable, no dose-limiting toxicities were observed, and a maximum tolerated dose of rhIL-18 was not identified. Given the relatively mild toxicity of rhIL-18 seen in this and previous clinical trials, it seems likely that rhIL-18 could be safely administered in biologically active doses together with other monoclonal antibodies, additional immunostimulatory cytokines, and/or vaccines for cancer immunotherapy. Objective complete and partial responses were seen in five patients with indolent lymphoma treated on this study. The overall response rate was 62% (5/8) for the subjects with indolent lymphoma who received rhIL-18 at doses of 10 µg/kg or higher, which may be considered optimal biologic doses based on available pharmacodynamic data. The efficacy of rhIL-18 plus rituximab cannot be adequately assessed in a phase I clinical trial enrolling a relatively small number of heterogeneous patients. Nevertheless, observation of objective responses in patients who were previously refractory to rituximab-based therapies suggests that rhIL-18 may augment the antitumor activity of FcR-bearing effector cells against rituximab-sensitized lymphoma cells.

Negligible IFN- γ is secreted by human or murine monocytes and macrophages after in vitro stimulation with IL-18 alone (38, 39). In contrast, we have previously shown that purified human NK cells secrete IFN- γ in vitro after stimulation with IL-18 alone or through ligation of CD16 (27). Moreover, costimulation with IL-18 augments by ~3-fold the levels of IFN- γ produced by human NK cells activated via CD16 (27). Administration of rhIL-18 either alone (28, 29) or together with rituximab (Fig. 4) causes in vivo activation of human NK cells. Therefore, it is likely that IFN- γ detected in plasma of patients receiving rhIL-18 and rituximab is produced predominantly by activated NK cells. We are aware of no published data describing plasma IFN- γ levels in lymphoma patients after treatment with rituximab alone. However, IFN- γ was not detected in plasma of dialysis-dependent patients with

chronic renal failure after infusions of rituximab (40). Furthermore, we did not detect significantly increased plasma IFN- γ levels on day 2 of weeks 1–4 prior to rhIL-18 infusions (data not shown). NK cells stimulated during interactions with rituximab-coated lymphoma cells could secrete IFN- γ within the tumor microenvironment. However, if this indeed occurred during the study such local IFN- γ production did result in detectable increases in plasma IFN- γ levels after rituximab infusions. It is interesting to note that the levels of IFN- γ detected in plasma of patients receiving rhIL-18 in doses of 30 μ g/kg together with rituximab (Fig. 2A) are ~3-fold higher than those detected in plasma of patients treated with rhIL-18 alone at doses of 30–100 μ g/kg (28, 29). Although direct comparison is limited by the differences in patient populations involved, these data suggest that systemic rhIL-18 can augment IFN- γ production by NK cells that have been costimulated via CD16 by their interaction with rituximab-coated target cells in vivo.

IFN- γ can augment ADCC by monocyte/macrophages, enhance presentation of antigens by dendritic cells, and promote the differentiation of Th1 helper effector cells (41). IFN- γ secreted by activated NK cells also stimulates production of the CXC chemokines MIG (CXCL9) and IP-10 (CXCL10) (32). MIG and IP-10 can contribute to antitumor responses by inhibiting tumor angiogenesis and recruiting CXCR3-bearing effector cells (32, 42, 43). Thus, administration of rhIL-18 could promote antitumor immune responses by augmenting ADCC of NK cells and monocytes, stimulating production of IFN- γ , MIG, and IP-10, enhancing differentiation of Th1 cells, and facilitating recruitment of effector cells to tumor sites. Indeed, we detected significantly increased infiltration of activated effector cells into a tumor mass of a patient with mantle cell lymphoma after treatment with rhIL-18 plus rituximab.

Some published studies have suggested that pro-inflammatory cytokines, including IL-1 and IL-18, can enhance cancer invasiveness and progression (44, 45). In a murine model of melanoma, administration of lower doses of IL-18 promoted metastatic disease, whereas higher doses of IL-18 (resulting in serum levels greater than 1 ng/mL) inhibited tumor progression (46). Lower doses of IL-18 were found to promote tumor progression in part by suppressing mature NK cell numbers (46). In contrast, NK cells are not suppressed in human subjects treated with rhIL-18 (28, 29). Furthermore, plasma IL-18 levels were greater than 10 ng/mL for subjects in the lowest (1 μ g/kg) rhIL-18 dose cohort treated in our clinical trial of rhIL-18 plus rituximab (Fig. 1). Taken together with results of previous studies of rhIL-18 as monotherapy (28, 29), our current data strongly support the conclusion that rhIL-18 (given in clinically relevant doses) activates rather than suppresses immune effector cells that can mediate antitumor activity.

Other cytokines, including IL-2, IL-12, and GM-CSF, have been given in combination with rituximab to treat patients with lymphoma (47–51). However, rhIL-18 may be preferable to these cytokines for combined immunotherapy with CD20 monoclonal antibodies. IL-2 and IL-12 can augment ADCC mediated by NK cells, but do not appear to significantly enhance ADCC by monocytes or macrophages (52–55). In contrast, GM-CSF preferentially stimulates ADCC by monocyte/macrophages and has little effect on NK cell cytolytic activity (53, 55, 56). IL-18 can strongly activate both NK cells and monocyte/macrophages (21, 22, 57), and hence might be more potent than IL-2, IL-12, or GM-CSF in enhancing ADCC against rituximab-sensitized lymphoma cells. Furthermore, administration of IL-2 leads to the in vivo expansion of CD25⁺ CD4⁺ regulatory T cells, which can inhibit IFN- γ production and antitumor immune responses (58–60). Both standard dose and high dose chemotherapy cause an acquired STAT4 deficiency in lymphoma patients, which leads to impaired IL-12-induced IFN- γ production and Th1 immune responses (61–63). Therefore, IL-18 may prove more effective than IL-2 or IL-12 for cytokine-based immunotherapy of lymphoma.

Ofatumumab is a fully human IgG1 monoclonal antibody that binds to a different epitope of CD20 than that recognized by rituximab (64). Ofatumumab can be safely given to patients with relapsed and refractory lymphoma, with a toxicity profile that compares favorably to rituximab (65). Compared to rituximab, ofatumumab mediates more potent ADCC and complement-dependent cytotoxicity against CD20+ lymphoma cells in vitro (66–69). Moreover, SCID mice bearing disseminated human lymphoma xenografts have superior survival after treatment with rhIL-18 plus ofatumumab compared to treatment with rhIL-18 plus rituximab (GlaxoSmithKline, unpublished data). Therefore, rhIL-18 could be given in combination with ofatumumab or other novel CD20 monoclonal antibodies for treatment of B cell malignancies (70). Indeed, we are initiating a phase I clinical trial of rhIL-18 plus ofatumumab after autologous PBSCT for B cell lymphoma ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01768338) identifier NCT01768338). STAT4 is not known to participate in the signaling pathways required for IFN- γ production in response to IL-18 or after ligation of CD16. Therefore, administration of rhIL-18 in combination with ofatumumab may costimulate IFN- γ production by human NK cells in vivo and circumvent the profound STAT4 deficiency with consequent impaired IFN- γ -dependent immune responses seen after PBSCT (61–63).

Acknowledgments

The authors wish to thank the nurses in the Indiana Clinical Research Center for assistance and support in the completion of this clinical trial.

Financial Support: This study was supported in part by NIH grants UL RR025761 (Indiana Clinical Research Center) and P30 CA82709 (Indiana University Simon Cancer Center). Clinical trial design was based in part on preclinical studies supported by NIH grant RO1 CA118118 (MJR).

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FIGURE 1.

Pharmacokinetic analysis after rhIL-18 infusions. Median plasma concentrations (ng/mL) of rhIL-18 in each dose cohort averaged over available dosing cycles are shown.

FIGURE 2.

Production of IFN- γ and IP-10 in vivo after rhIL-18 administration. Plasma concentrations of IFN- γ (A; pg/mL) and IP-10 (B; ng/mL) from individual patients treated in 1, 10, or 30

µg/kg dose cohorts as indicated. Note differences in scales of plot on the ordinate for data from the different dose cohorts.

FIGURE 3.

Effects of rhIL-18 on plasma concentrations of IFN- (A) and IP-10 (B). Mean induction of cytokines (absolute change for IFN- and fold change for IP-10) at 4 hours after rhIL-18 infusion is shown on the ordinate as a function of rhIL-18 dose on the abscissa. Each filled circle is result from an individual patient averaged over available dosing cycles.

FIGURE 4.

Effects of rhIL-18 on number and activation status of peripheral blood NK cells. (A) Absolute numbers of NK cells in peripheral blood of individual patients in each dose cohort are shown on the ordinate and elapsed time after infusion of rhIL-18 on the abscissa. Mean values averaged over available dosing cycles are shown. (B) Mean percent change (from baseline) in number of NK cells 4 hours after rhIL-18 infusion is shown on the ordinate as a function of rhIL-18 exposure on the abscissa. Each filled circle is result from an individual patient averaged over available dosing cycles. (C) Mean percent change (from baseline) in number of activated (CD69+) NK cells 48 hours after rhIL-18 infusion is shown on the ordinate as a function of rhIL-18 dose on the abscissa. Data for gated CD56bright NK cells are shown in the right panel and for gated CD56dim NK cells in the left panel. Each filled circle is result from an individual patient.

FIGURE 5.

Infiltration of tumor by CD69+ cells after treatment with rituximab and rhIL-18. Fine needle aspiration of a tumor mass was performed before (left panel) and one month after (right panel) the first infusion of rhIL-18 for a patient with mantle cell lymphoma who had stable disease. CD69 expression was assessed by immunohistochemistry.

TABLE 1**Most Frequently Reported Adverse Events Regardless of Causality**

Preferred Term	rhIL-18 Doses + Rituximab 375 mg/m ²						
	1 µg/kg	3 µg/kg	10 µg/kg	20 µg/kg	30 µg/kg	100 µg/kg	Total
	N=4	N=3	N=3	N=3	N=3	N=3	N=19
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Any Adverse Event	4 (100) [4]	3 (100) [3]	3 (100) [3]	3 (100) [2]	3 (100) [3]	3 (100) [3]	19 (100) [18]
Pyrexia	4 (100) [3]	2 (67) [2]	2 (67) [2]	2 (67) [1]	2 (67) [2]	3 (100) [3]	15 (79) [13]
Chills	3 (75) [2]	2 (67) [2]	2 (67) [2]	2 (67) [2]	3 (100) [3]	2 (67) [2]	14 (74) [13]
Headache	2 (50) [0]	0 [0]	2 (67) [2]	2 (67) [2]	2 (67) [2]	1 (33) [1]	9 (47) [7]
Nausea	1 (25) [0]	1 (33) [0]	1 (33) [1]	2 (67) [1]	1 (33) [0]	0 [0]	6 (32) [2]
Cough	2 (50) [0]	1 (33) [0]	1 (33) [0]	0 [0]	0 [0]	1 (33) [0]	5 (26) [0]
Pain	1 (25) [0]	0 [0]	1 (33) [1]	2 (67) [2]	1 (33) [1]	0 [0]	5 (26) [4]
Back pain	1 (25) [0]	0 [0]	0 [0]	1 (33) [1]	2 (67) [2]	0 [0]	4 (21) [3]
Dyspnea	1 (25) [0]	0 [0]	1 (33) [0]	1 (33) [1]	1 (33) [1]	0 [0]	4 (21) [2]
Vomiting	0 [0]	1 (33) [0]	0 [0]	1 (33) [1]	1 (33) [0]	1 (33) [1]	4 (21) [2]

Adverse events occurring in at least four subjects are included.

Numbers in brackets are for events deemed possibly related to study drug.

TABLE 2Common Clinical Chemistry Abnormalities (Observed in at Least 4 Subjects)¹

Laboratory Abnormality:	rhIL-18 Doses + Rituximab 375 mg/m ²						
	Number of Patients with Grade 1–2 / 3–4 Laboratory Abnormality ¹						
	1 µg/kg	3 µg/kg	10 µg/kg	20 µg/kg	30 µg/kg	100 µg/kg	Total
	N=4	N=3	N=3	N=3	N=3	N=3	N=19
Elevated Total Bilirubin	4/0	3/0	2/0	1/1	2/0	2/0	15
Elevated Alkaline Phosphatase	2/0	0/0	0/0	0/0	1/0	1/0	4
Elevated ALT ²	1/0	0/0	2/0	0/0	0/0	1/0	4
Elevated AST ²	2/0	2/0	2/0	1/0	2/0	1/0	10
Elevated GGT ²	1/0	1/0	1/0	1/0	2/0	2/0	8
Elevated Creatine Kinase	1/0	1/0	2/0	1/0	1/0	0/0	6
Hyperglycemia	3/1	3/0	3/0	3/0	3/0	2/1	19
Hypoalbuminemia	4/0	3/0	3/0	2/0	3/0	3/0	18
Hypocalcemia	2/0	2/0	1/0	1/0	1/0	2/0	9
Hypokalemia	2/0	2/0	2/0	1/0	0/0	2/0	9
Hyponatremia	1/0	1/1	1/1	1/0	2/0	2/0	10

¹The only grade 4 abnormalities (grade 4 hypoglycemia in 1 subject and grade 4 hyperkalemia in 1 subject) seen in the study are not included in Table 2, as they were observed <4 subjects. Both of these grade 4 abnormalities were deemed to be spurious (see Results).

²ALT (Alanine Aminotransferase); AST (Aspartate Aminotransferase), GGT (Gamma Glutamyl Transferase)

TABLE 3

Common Hematologic Abnormalities (Observed in at Least 4 Subjects)

Reduction in Level of:	rhIL-18 Doses + Rituximab 375 mg/m ²						
	Number of Patients with Grade 1–2 / 3–4 Laboratory Abnormality						
	1 µg/kg	3 µg/kg	10 µg/kg	20 µg/kg	30 µg/kg	100 µg/kg	Total
	N=4	N=3	N=3	N=3	N=3	N=3	N=19
Hemoglobin	4/0	3/0	3/0	3/0	2/1	2/1	19
Platelet Count	2/0	0/0	0/0	2/0	1/0	2/0	7
White Blood Cell Count	3/0	3/0	3/0	3/0	1/0	3/0	16
Neutrophil Count	3/0	1/0	2/0	2/0	2/0	2/1	13
Lymphocyte Count	0/4 *	0/3 *	0/3	0/3 *	0/3 *	0/3 *	19

* Grade 4 lymphopenia occurred in 5 subjects in the indicated dose cohorts. Lymphopenia was considered to be an expected biologic effect of rhIL-18 rather than an adverse event (see Results and Discussion). No other grade 4 hematologic abnormalities were observed.

TABLE 4

Summary of Tumor Responses, Number of Subjects (%)

Best Response	rhIL-18 Doses + Rituximab 375 mg/m ²					
	1 µg/kg	3 µg/kg	10 µg/kg	20 µg/kg	30 µg/kg	100 µg/kg
Complete Response	0	0	1 (33)	1 (33)	0	0
Complete Response/unconfirmed	0	0	0	0	0	0
Partial Response	0	0	1 (33)	0	0	2 (67)
Stable Disease	1 (25)	2 (67)	0	0	2 (67)	1 (33)
Progressive Disease	2 (50)	1 (33)	1 (33)	2 (67)	1 (33)	0
Unknown	1 (25)	0	0	0	0	0

Best responses, as determined by investigators, are listed.