Clinical and Biological Effects of an Agonist Anti-CD40 Antibody: A Cancer Research UK Phase I Study

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Abstract

Purpose: This phase I study aimed to establish the biologic effects and MTD of the agonistic IgG1 chimeric anti-CD40 antibody ChiLob7/4 in patients (pts) with a range of CD40-expressing solid tumors and diffuse large B-cell lymphoma, resistant to conventional therapy. Potential mechanisms of action for agonistic anti-CD40 include direct cytotoxic effects on tumor cells and conditioning of antigen-presenting cells.

Experimental Design: ChiLob7/4 was given by IV infusion weekly for 4 doses at a range from 0.5 to 240 mg/dose. Validated ELISAs were used to quantify ChiLob7/4 in serum and test for anti-chimeric MAb (HACA) responses. Pharmacodynamic assessments included quantitation of T-cell, natural killer–cell, and B-cell numbers and activation in blood by flow cytometry and a panel of cytokines in plasma by Luminex technology. Planned dose escalation was in cohorts of 3 patients until MTD or biologic effect, defined as reduction of peripheral blood CD19+ B cells to 10% or less of baseline.

Results: Twenty-nine courses of treatment were given to 28 subjects. The MTD was 200 mg × 4, with dose-limiting toxicity of liver transaminase elevations at 240 mg. At 200 mg (range between 2.1 mg/kg and 3.3 mg/kg based on patient body weight), the trough level pretreatment was above 25 μg/mL. Grade 1–2 infusion reactions were seen above the dose of 16 mg, but could be prevented with single-dose corticosteroid premedication. HACA responses were seen after doses between 1.6 mg and 50 mg, but not above this. There were dose-dependent falls in blood B-cell numbers accompanied by reduced expression of CD21, and transient reductions in NK cell numbers with increased CD54 expression from 50 mg upward. MIP-1β and IL12 plasma concentrations rose after doses above 16 mg. Fifteen of 29 treatments were accompanied by disease stabilization for a median 6 months, the longest for 37 months.

Conclusions: ChiLob7/4 can activate B and NK cells at doses that can be administered safely, and should be tested in combination with other antibodies and chemotherapy agents. Clin Cancer Res; 21(6); 1321–8. ©2015 AACR.

Introduction

CD40 is a membrane protein belonging to the TNFR superfamily. It is expressed primarily on antigen-presenting cells (APC) such as dendritic cells, B lymphocytes, and monocytes, but has also been found on most B-cell lymphomas and a substantial number of epithelial malignancies. Bidirectional CD40–CD40L interactions are central to the generation of both T-cell–dependent, humoral immune responses and cytotoxic T-cell responses, licensing APC to present antigen to and activate responding CD8+ cytotoxic T-cell precursors (1). The expression pattern of CD40 on a broad range of malignancies and the important immunostimulatory role of CD40 in vivo make this an attractive target for agonistic antibody therapy, supported by data from mouse models which showed that effective and long-lasting immune responses could be evoked against a variety of tumor types (2). Potential mechanisms of action include recruitment of immune effectors such as complement-dependent cytotoxicity (CDC) and natural killer (NK) cells, direct signaling leading to apoptosis in malignant cells, and licensing of antigen presentation, bypassing the need for specific CD4+ T-cell help to activate CD8+ cytotoxic T-cell precursors (3, 4).

ChiLob7/4 is a chimeric IgG1 anti-human CD40 antibody which has shown growth-inhibitory effects in vitro against a variety of CD40 expressing human malignant lymphoma and epithelial cell lines (5). It was effective in assays of both CDC and ADCC, and the parent murine antibody showed agonistic activity in upregulation of costimulatory molecules in a dendritic cell culture system (6). A phase I trial was undertaken to define the effects of administration of ChiLob7/4 for the first time in humans.
Translational Relevance

CD40 is expressed on antigen-presenting cells (APC) and some malignancies. On APC, ligation results in upregulation of costimulatory molecules, potentially bypassing the need for T-cell help in an antitumor immune response. Anti-CD40 antibodies produce substantial responses and durable anti-cancer immunity in animal models, an effect mediated by cytotoxic T cells. The IgG1 chimeric antibody ChiLob7/4 was developed as an agonist for CD40, and this first-in-man dose-escalation study describes its pharmacokinetic and pharmacodynamic characteristics. An MTD of 200 mg weekly × 4 was established, yielding concentrations that showed physiologic activity including depletion and activation of peripheral blood B and NK cells, increases in MIP-1β and IL-12 levels, and disease stabilization in half of those treated. The treatment was well-tolerated, with infusion reactions readily controlled by single-dose corticosteroid premedication. Dose-limiting toxicity was a reversible hepatic transaminitis. This study provides the basis for exploring the activity of ChiLob7/4 in combination studies with chemotherapy, tumor antigen vaccines, and other immunomodulatory antibodies.

Materials and Methods

Patients and study design

Patients with tumors showing expression of CD40 on IHC, for which no curative treatment options existed and who gave written informed consent were enrolled in this National Research Ethics (NRES), UK-approved Phase I clinical trial (NCT01561911). Expression of CD40 was determined by staining with IgG2a anti-CD40 monoclonal antibody Lob 7/6, after proteinase K-based enzyme pretreatment and the horseradish peroxidase ChemMate EnVision Detection Kit. (Dako). Expression was scored as strong, weak or absent, focal or diffuse by a single observer (MA-K). Inclusion criteria included a good performance status (Eastern Cooperative Oncology Group 0-1; ECOG) and adequate hematologic and biochemical indices, including a total blood lymphocyte count of at least 0.5 × 10⁹/L, creatinine clearance of at least 40 mL/min, hepatitis transaminases no more than three times the upper limit of normal (ULN) and bilirubin up to 1.5 times ULN. Treatment was by intravenous infusion of the ChiLob7/4 antibody weekly for four doses, given over 30 minutes for the first three dose levels, and thereafter initially at 10 mg/hour, with planned acceleration in the absence of any infusion reaction after the first 30 minutes. The starting dose level was 0.5 mg per dose, chosen as being the equivalent human dose to one-hundredth of the highest rat anti-mouse CD40 monoclonal antibody given to mice without toxicity. The dose escalation scheme proceeded to 1.6, 5, 16, 50, 160, and 240 mg per dose, with three patients planned to receive treatment at each dose level, and expansion of the levels at which depletion of peripheral blood B cells confirmed a clear biologic effect. Escalation to the next dose level was only permitted when at least 3 patients had completed 4 weeks of treatment without dose-limiting toxicity. No routine premedication was mandated initially, but a protocol amendment introduced the prophylactic administration of 100 mg hydrocortisone, 1 g acetaminophen, and 10 mg chlorpheniramine before treatment with doses of 16 mg or more.

Evaluation of response

Patients underwent standard tumor assessments by clinical examination and cross-sectional imaging at baseline, and at 8 weeks from the first dose of antibody. Weekly clinical assessments were undertaken during the 4 weeks of treatment, together with routine hematology, biochemistry, measurement of immunoglobulin levels, and urinalysis, also repeated at the final study visit at week 8.

Pharmacokinetic analysis

Serum ChiLob7/4 antibody levels were measured by a validated ELISA technique as described previously (7) on samples taken predose and at 30 minutes, 1, 3, 6, 24, 48, and 72 hours after the start of each infusion. The lower level of quantification (LLOQ) for the assay was 2 ng/mL and the upper level of quantification was 20 ng/mL. Test samples were diluted 1:2,000 for each assay so the effective working range of the assay was 4 to 40 μg/mL. Pharmacokinetic parameters (half-life, AUC and Cmax) were estimated using best-fit regression analysis (one phase decay), assuming noncompartmental decay using GraphPad Prism 6 software.

Pharmacodynamic assessments

Human anti-chimeric antibody (HACA) serum responses were measured on weekly serum samples obtained before each dose using a validated semiquantitative ELISA, as previously described. (7) Pharmacodynamics were also assessed by flow cytometry where changes in peripheral blood leukocytes (PBL) were measured in whole blood samples taken preinfusion and at day 4 postinfusion of each cycle in all patients treated with doses up to and including 160 mg ChiLob7/4. At the two higher dose cohorts, a protocol amendment was incorporated, measuring PBL changes at 3 hours after first infusion. Follow-up samples were also taken at day 49 after first infusion. These whole blood samples were stained to assess changes in T-, B-, and NK-cell numbers using the BD Multitest CD3 FITC/CD4 PE, and CD56 PE/CD45 PerCP/CD19 APC Reagent (Becton Dickinson), and DC number and activation as described previously (8). Samples were acquired using FACSCanto flow cytometers (Becton Dickinson).

Cytokine levels in plasma samples were measured using a human custom multiplex-10 bead array assay kit for Luminex purchased from Life technologies to measure the following cytokines: IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IFNγ, TNFα, macrophage inflammatory protein (MIP)-1α and MIP-1β, as per the manufacturer’s instructions and as described previously (9) using a Luminex 100 instrument. Appropriate dilutions of the samples in assay diluent were made as required. Each sample was assayed in duplicate and cytokine standards supplied by the manufacturer were used to calculate the concentrations of the samples. Cytokine levels were measured in plasma or serum sample taken before each infusion, and at 3 hours, 6 hours, day 4, day 8, and week 8 postinfusion at each week during treatment.

Peripheral blood mononuclear cell isolation and cryopreservation

Peripheral blood mononuclear cells (PBMC) were isolated by Lymphoprep centrifugation (Axis-shield) at day 1 (preinfusion)
Flow cytometry from cryopreserved PBMC

Cryopreserved PBMC from a subset of patient cohorts and timepoints was stained in a three panels, B-cell subset and panel, NK-cell panel, and APC panel with the following monoclonal antibodies: B-cell subset and panel: V450-conjugated CD20, clone L27; FITC-conjugated IgD, clone IA6-2; phycoerythrin (PE)-conjugated CD27, clone M-T271; allophycocyanin (APC)-conjugated CD38, clone HIT2; PE-Cy7–conjugated CD24, clone ML5; APC-Cy7–conjugated CD19, clone S125C1 all from BD Pharmingen, and PerCP/Cy5.5-conjugated CD21, clone Bu32 from Biolegend. NK-cell panel: PE-conjugated CD54, clone LB-2; PerCP-conjugated CD3, clone SP34-2; PE-Cy7–conjugated CD56, clone B159; APC-Cy7–conjugated CD16, clone 3G8 all from BD Pharmingen. APC panel: V450-conjugated CD11c, clone B-ly6; V500-conjugated HLA-DR, clone G46-6; FITC-conjugated CD14, clone M5E2; PE-conjugated CD54, clone LB-2; PerCP-conjugated CD3, clone SP34-2; PerCP-conjugated CD19, clone S125C1; PE-Cy7–conjugated CD56, clone B159; APC-Cy7–conjugated CD16, clone 3G8 all from BD Pharmingen, and APC-conjugated CD303, clone 201A from Biolegend. Data acquisition was performed on a FACSCan II flow cytometer (Becton Dickinson) fitted with three lasers (488-nm 20-mW solid state; 633-nm 17-mW HeNe; and 405-nm 30-mW solid state). The data were analyzed using the FACSDiva software version 6.3.1 (Becton Dickinson) or FlowJo software version X (Treestar).

Table 1. Clinical characteristics of patients

<table>
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<th>Patient ID</th>
<th>Age at entry (CD40 staining)</th>
<th>Primary tumor (CD40 staining)</th>
<th>Performance status at entry (ECOG)</th>
<th>Time from diagnosis to treatment (months)</th>
<th>Prior therapy (S: surgery; C: chemotherapy; R: radiotherapy)</th>
<th>Time from most recent therapy (mo)</th>
<th>Antibody dose level (each dose: mg)</th>
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<td>200</td>
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Results

Twenty-eight patients received treatment in the study, and dose escalation continued up to 240 mg per dose. One patient who experienced prolonged disease stabilization after receiving treatment at 1.6 mg was re-treated after an interval of 3 years with 240 mg. The clinical characteristics of the patients are shown in Table 1. All patients had progressing tumors, and were treated at an interval of between 1 month and 12 months from their most recent prior therapy (median 3 months). CD40 was expressed on the malignant cells, with strong staining in 23 cases and weak staining in 6. All but four subjects completed 4 weeks of planned treatment. One patient was withdrawn from the study having developed neurologic symptoms from previously undiagnosed brain metastases shortly after the first infusion of 0.5 mg. Both patients treated at 240 mg developed grade 3 liver transaminase rises after the first dose and were withdrawn from treatment. Following this, the dose was reduced to 200 mg for expansion of the cohort, at which one patient out of 6 developed a grade 3 rise in γ-glutamyl transferase after two doses and also stopped treatment.

Adverse events and hematologic parameters

A total of 240 adverse events (AE) were reported (Table 2). Of these, 96 were considered possibly related to ChiLob7/4. No AEs related to the antibody were of grade 4 or 5. Only 5 AEs of grade 3 were considered possibly related to ChiLob7/4: 2 rises in alanine aminotransferase at 240 mg, 1 rise in γ-glutamyltransferase at 200 mg, and 1 episode of hypokalemia at 16 mg in a patient previously treated with cisplatin for mesothelioma, and 1 episode

and day 4 during weeks of treatment and cryopreserved at ≤150°C until use.
Infusion reaction 8 8 0 2
Raised AST 3 6 0 2
Pruritus 5 2 0 1
Nausea 3 1 0 1
Anorexia 3 1 0 2
Fever 5 3 0 1
Cough 7 1 0 2
Dyspnea 10 1 0 1
Diarrhea 8 7 0 1
Fatigue 17 13 0 2
Dyspepsia 10 1 0 1
Chest pain 11 3 0 2
Headache 11 4 0 2
Infusion reactions (grade 1 and 2 respectively) occurred in the patients treated at the 16 mg dose level. The reactions consist of flushing, low-grade pyrexia, and mild rigors, requiring interruption of the infusion and treatment with intravenous corticosteroids, after which the symptoms settled rapidly where the cei v e d or corticosteroids, after which the symptoms settled rapidly following interruption of the infusion and treatment with intravenous corticosteroids.

Pharmacokinetics

Serum Chilob7/4 was detected and quantifiable only at the 50 mg dose level and above (Fig. 1). Serum Chilob7/4 concentration peaked within the first 3 hours after the infusion. Half lives for the decay of Chilob7/4 were estimated where possible and are presented in Table 3 (half-life estimation after the first infusion was not possible for a proportion of patients since the plateau phases were not reached in these patients before 72 hours). There was a linear relationship between dose level and the calculated AUC. The only patient to develop grade 3 toxicity in the 200-mg cohort showed unusually slow clearance of the antibody after the first dose, with a half-life of over 80 hours. Repeat measurements during week 4 prolonged elimination, suggesting depletion of target antigen (data not shown). Patients treated at 160 mg had trough antibody levels of 10 to 25 μg/mL before administration of the fourth dose, and at 200 mg, the range was 27 to 52 μg/mL. No antibody could be detected at the 8 week visit in any cohort.

Pharmacodynamics

Infusion of Chilob7/4 was associated with dose-related and transient decreases in peripheral lymphocytes, including NK of raised C-reactive protein at 240 mg. There were few clinically significant AEs related to the antibody other than infusion reactions. Mild headache was reported by 11 patients, and considered possibly related to the drug in four cases. Chest pain was reported in 11 patients with chest wall involvement by tumor, and considered possibly related to the treatment in three cases. There were no significant changes in hematologic indices such as hemoglobin, platelet count or total white blood cell count. Immunoglobulin levels showed no significant changes during the period of study.

HACA responses were detected in 7 patients during the study: 1 of 3 patients at 1.6 mg (33%), 1 of 3 patients at 5 mg (33%), 3 of 4 patients at 16 mg (75%), and 2 of 3 patients at 50 mg (66%; data not shown). The positive responses were first detected in six cases before treatment at week 4, but in one case (at 5 mg), a reaction was detected at week 3. All positive responses remained detectable at week 8. None of the patients treated with 160 to 240 mg developed a positive HACA response, and in the 50-mg cohort, there was no apparent effect upon the pharmacokinetics of the antibody, with the HACA-negative patient showing similar levels to the two with positive results.

Infusion reactions and cytokine release

The antibody treatment was well tolerated, but infusion reactions (grade 1 and 2 respectively) occurred in the first two patients treated at the 16 mg dose level. The reactions consisted of flushing, low-grade pyrexia, and mild rigors, requiring interruption of the infusion and treatment with intravenous corticosteroids, after which the symptoms settled rapidly and the infusions could be completed without further symptoms. Following these findings, premedication was given routinely as described above. This prevented further infusion reactions at all doses up to the 200 mg dose level, at which three of the six subjects experienced grade 1-2 reactions, as did one of those treated at 240 mg. In all cases the infusion was successfully completed following a further dose of intravenous corticosteroids. These reactions were investigated by cytokine measurements in plasma or serum samples taken at baseline (preinfusion) 3, 6, 24 hours and day 4 and 8 after the start of antibody infusion. No rises in IL2, IFNγ, TNFα, or IL6 were detected (data not shown), but a consistent finding was of transiently raised levels of MIP-1β, maximal at 3 to 6 hours, and increases in IL12 most evident at the highest doses (Fig. 4A and B). The changes in these cytokines were seen in the patients treated at 16 mg without premedication who experienced infusion reactions, and in subsequent patients who received corticosteroid prophylaxis and who remained asymptomatic.

Pharmacokinetics

Serum Chilob7/4 was detected and quantifiable only at the 50 mg dose level and above (Fig. 1). Serum Chilob7/4 concentration peaked within the first 3 hours after the infusion. Half lives for the decay of Chilob7/4 were estimated where possible and are presented in Table 3 (half-life estimation after the first infusion was not possible for a proportion of patients since the plateau phases were not reached in these patients before 72 hours). There was a linear relationship between dose level and the calculated AUC. The only patient to develop grade 3 toxicity in the 200-mg cohort showed unusually slow clearance of the antibody after the first dose, with a half-life of over 80 hours. Repeat measurements during week 4 prolonged elimination, suggesting depletion of target antigen (data not shown). Patients treated at 160 mg had trough antibody levels of 10 to 25 μg/mL before administration of the fourth dose, and at 200 mg, the range was 27 to 52 μg/mL. No antibody could be detected at the 8 week visit in any cohort.

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Pharmacokinetics

Serum Chilob7/4 was detected and quantifiable only at the 50 mg dose level and above (Fig. 1). Serum Chilob7/4 concentration peaked within the first 3 hours after the infusion. Half lives for the decay of Chilob7/4 were estimated where possible and are presented in Table 3 (half-life estimation after the first infusion was not possible for a proportion of patients since the plateau phases were not reached in these patients before 72 hours). There was a linear relationship between dose level and the calculated AUC. The only patient to develop grade 3 toxicity in the 200-mg cohort showed unusually slow clearance of the antibody after the first dose, with a half-life of over 80 hours. Repeat measurements during week 4 prolonged elimination, suggesting depletion of target antigen (data not shown). Patients treated at 160 mg had trough antibody levels of 10 to 25 μg/mL before administration of the fourth dose, and at 200 mg, the range was 27 to 52 μg/mL. No antibody could be detected at the 8 week visit in any cohort.

Pharmacodynamics

Infusion of Chilob7/4 was associated with dose-related and transient decreases in peripheral lymphocytes, including NK
cells and monocytes, which were most evident at 3 hours after infusion (Fig. 2). Absolute numbers of CD19⁺ B cells in the peripheral blood were reduced, with partial depletion seen in one patient treated at 16 mg and further depletion at the higher doses (Fig. 3). In all cases where patients enrolled onto trial with normal absolute B-cell numbers (between 0.1 – 0.5 x 10⁹ cells/L), and received four drug infusions, B cells remained depleted (absolute number < 0.1 x 10⁹ cells/L) at 8 weeks after first infusion. No consistent alterations were observed in the phenotype of circulating dendritic cell populations (data not shown).

Elevated plasma concentrations of the cytokine MIP-1β were observed at 3 to 6 hour after infusion in most of the higher dose groups (Fig. 4A; range at 3 – 6 hours in 16-mg dose cohort 216 – 6,776 pg/mL MIP-1β; baseline levels range, 48 – 200 pg/mL). Similar rises were also seen in the higher dose cohorts in which premedication prevented infusion reactions; 50 mg (296 – 1,456 pg/mL); 160 mg (98 – 1,136 pg/mL), and 240 mg (342 – 416 pg/mL) cohorts. Figure 4B shows the plasma levels of IL12, which rose in the 16-mg cohort during the first 24 hours, between 1.32-fold and 2.8-fold from baseline (range, 260 – 1,068 pg/mL), with similar findings at the 50 mg dose, with levels 1.52- to 2.38-fold above baseline (424 – 660 pg/mL). The rises in IL12 were not sustained in the lower-dose cohorts, but at higher doses, they continued to rise, peaking during the second week for 160 mg doses at 1.74- to 3.14-fold (226 – 480 pg/mL), and at 200 mg at 1.84- to 12.4-fold (99 – 415 pg/mL).

Further flow-cytometric analysis was extended in a subset of patients using cryopreserved PBMC. Three panels of antibodies were used to identify modulation or activation of peripheral blood cell types which could contribute to the cytokine profile observed. From these analyses, no change in the proportions of naïve, memory, or antibody isotype class-switched B-cell subtypes was observed (data not shown), but a significant decrease in CD21 (complement receptor type II or CR2) was observed at all the doses tested (50, 160, 200, and 240 mg; Fig. 4C). The CD21 mean fluorescence intensity (MFI) relative to baseline was significantly decreased at day 4 and day 8 in the highest four dose groups, although the levels had returned to baseline by day 49.

We also evaluated NK cell activation using the cell surface marker CD54. We found an increase in CD54 expression in a subset of NK cells, (Fig. 4D), which was coincident with the transient falls in absolute numbers of CD3⁺ CD16⁺ CD56⁺ NK cells. The number of cells in the CD54 bright subset increased at the higher antibody doses, reaching statistical significance in the expanded 200-mg dose cohort (n = 6).

Antitumor activity
In fifteen treatment courses, patients had stable disease at the final study visit at week 8, while in 14 cases, the tumors showed...
evidence of continued progression during the study. Patients with stable disease had a median time to progression of 6 months (range, 5 to over 37), and the overall median survival for all patients was 11 months. There was no correlation between CD40 staining intensity and disease stabilization: 3 of 6 patients with weak CD40 expression showed stable disease. Similarly, there was no correlation between interval from last therapy and tumor stabilization.

**Discussion**

The aim of this study was to investigate the safety and tolerability of this anti-CD40 antibody, and to explore its biologic effects for the first time in man. We have shown that ChiLob7/4 can be given safely at a dose of up to 200 mg for four weekly doses, and that it is generally well tolerated, provided premedication is given with a single dose of corticosteroid. The infusion reactions seen when this premedication was not used occurred at a much lower dose (16 mg), but were not associated with a specific cytokine release syndrome. This is in contrast with the effects seen after treatment with a different agonistic anti-CD40 antibody, CP-870,893, which led to high levels of TNFα and IL6 production, with dose-limiting toxicity at 0.3 mg/kg (10). In our study, no induction of IL6 or TNFα plasma levels was observed, neither in the presence nor absence of corticosteroid premedication. As was seen with CP-870,893, dose-limiting toxicity was from raised transaminase levels. The two antibodies are of different isotype, with ChiLob 7/4 being an IgG1 whereas CP-870,893 is an IgG2 molecule. Recent evidence suggests that binding to inhibitory Fc receptors may be an important determinant of efficacy, at least in mouse models, implying that the isotype may have a strong influence upon the activity of agonistic antibodies (11). It is possible that the difference in cytokine production with ChiLob7/4 is due to lower agonistic potency than CP-870,893, and this is the subject of an ongoing comparative in vitro analysis.

The lack of toxicity allowed escalation of the dose of ChiLob7/4 to a much higher level than was achievable with CP-870,893, resulting in more durable B-cell depletion and prolonged elevation of antibody levels well above 10 ng/mL, the level at which maximal biologic effects were seen in vitro (12). At the lower doses, such depletion was transient and partial, in keeping with the findings with CP-870,893. Studies with a different anti-CD40, the humanized IgG1 dacetuzumab, have demonstrated that this too can evoke a cytokine release syndrome, with ocular toxicity and headaches the most prominent symptoms, although higher doses of up to 8 mg/kg could be given following intra-patient escalation and corticosteroid premedication in some cases (13).

We observed several effects in this study that suggest the recruitment of an active immune response. Transient falls in NK cell numbers in the blood at antibody doses as low as 16 mg were associated with rises in MIP-1β, and at higher doses with upregulation of CD54, suggestive of effector cells trafficking out of the circulation following activation. The development of HACA responses occurred at similarly low antibody doses and was seen at an unusually high frequency for a chimeric antibody. This suggests that the agonistic effects of ChiLob7/4 may be leading to enhanced antigen presentation, in keeping with the changes in production of IL12, although these were only detectable at higher antibody doses. The disappearance of HACA responses in the highest dose cohort reflects the durable B-cell depletion seen at this level, removing the population capable of mounting a humoral response.

Transient activation of B cells was also demonstrated in studies with CP-870,893 either when used as a single agent (10) or in combination with chemotherapy (14). In the present study, we demonstrated B-cell activation as evidenced by the shedding of CD21, also known as complement receptor II or
Further studies are required to examine the combination with other immunostimulatory antibodies or potential antitumor activity either as a single agent or in a CD16−CD54% bright positive population in relative to baseline. D, changes in (50–240 mg) measured from cryopreserved PBMC. C, changes in CD21 mean fluorescence intensity (MFI) in the CD19+CD20+ B cells relative to baseline. D, changes in CD54% bright positive population in CD16−NK cells relative to baseline. Data in the bar graphs are grouped for all patients in a given dose level cohort and reported as mean values ± SEM. *, P < 0.05; **, P < 0.01 paired two-tailed t tests.

The use of corticosteroid premedication is a debatable strategy for an immunostimulatory antibody. It might be expected that this could attenuate the effect, but we found no evidence of this upon the NK, T-, or B-cell subsets. HACA responses were seen in the routinely premedicated 50-mg cohort as well as at 16 mg, and progressive rises in IL12, B-cell, and NK cell activation markers were most evident at higher doses, suggesting that these effects at least were not suppressed. Studies of vaccination against viral antigens have previously shown that chronic concomitant administration of corticosteroids did not affect seroconversion rates (20), and the exposure in this study was of brief duration, and much less likely to affect cellular immunity. The use of corticosteroids has allowed progression of other cell types (15). Ligation of CD21 results in signals critical for normal B-cell responses (16, 17). Activation of B cells by stimulation of the BCR with anti-IgM and anti-CD40 has been shown to induce CD21 shedding (18), which then contributes to the plasma CD21 pool. Soluble CD21 has also been shown to act as a functional ligand for CD23-expressing monocytes, downregulating CD14, enhancing expression of HLA-DR and CD40, and eliciting production of IL6 and TNFα from stimulated monocytes (19).

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References
