An Integrative Approach to Inform Optimal Administration of OX40 Agonist Antibodies in Patients with Advanced Solid Tumors

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Abstract

Purpose: The success of checkpoint blockade has led to a significant increase in the development of a broad range of immunomodulatory molecules for the treatment of cancer, including agonists against T-cell costimulatory receptors, such as OX40. Unlike checkpoint blockade, where complete and sustained receptor saturation may be required for maximal activity, the optimal dosing regimen and receptor occupancy for agonist agents is less well understood and requires further study.

Experimental Design: We integrated both preclinical and clinical biomarker data sets centered on dose, exposure, receptor occupancy, receptor engagement, and downstream pharmacodynamic changes to model the optimal dose and schedule for the OX40 agonist antibody BMS-986178 alone and in combination with checkpoint blockade.

Results: Administration of the ligand-blocking anti-mouse surrogate antibody OX40.23 or BMS-986178 as monotherapy or in combination with checkpoint blockade led to increased peripheral CD4+ and CD8+ T-cell activation in tumor-bearing mice and patients with solid tumors, respectively. OX40 receptor occupancy between 20% and 50% both in vitro and in vivo was associated with maximal enhancement of T-cell effector function by anti-OX40 treatment, whereas a receptor occupancy > 40% led to a profound loss in OX40 receptor expression, with clear implications for availability for repeat dosing.

Conclusions: Our results highlight the value of an integrative translational approach applied during early clinical development to aggregate preclinical and clinical data in an effort to define the optimal dose and schedule for T-cell agonists in the clinic.

Introduction

Therapeutic blockade of T-cell inhibitory receptors, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 protein (PD-1), has provided meaningful clinical benefit for patients with a broad range of malignancies (1). This success has led to a significant increase in the efforts toward the preclinical and clinical development of an array of immunomodulatory molecules for the treatment of cancer. One class of molecules being developed to directly enhance antitumor T-cell responses is agonists of T-cell costimulatory receptors expressed on the surface of activated, tumor-infiltrating T cells belonging to the CD28 or TNF receptor superfamily (CD28SF and TNFRSF, respectively; ref. 2). OX40 (CD134) is a TNFRSF member expressed on activated CD4+ effector T cells and, to a greater extent, regulatory T cells (Tregs) in the tumor microenvironment (3, 4). OX40 has been shown to be an important costimulatory receptor for T-cell survival and function, as well as the establishment of T-cell memory in models of autoimmunity and cancer (4–7). Several anti-OX40 antibodies are currently in clinical development for the treatment of cancer, including the fully human agonist antibody BMS-986178, which is being evaluated as monotherapy and in combination with checkpoint blockade. A large body of preclinical data has demonstrated...
that the combination of anti-OX40 with other therapeutic agents enhances antitumor immunity over the individual agents alone, supporting the rationale for combination therapy (4, 8–10).

An often-overlooked aspect of T-cell agonist therapy, including anti-OX40 therapy, is the impact of dose and schedule on immunomodulatory pharmacology and/or efficacy, which may be particularly relevant in the therapeutic development of agonist agents (11). The complete saturation of checkpoint receptors or ligands by a therapeutic antibody may be required to reverse the immunosuppression elicited by the targeted pathway, whereas the optimal receptor occupancy and dosing regimen for agonists may be lower or more intermittent in nature, mirroring the physiologic context in which T cells receive costimulatory signals (12).

Preclinical models, such as human in vitro assays and subcutaneously implanted syngeneic immunocompetent mouse in vivo tumor models, are commonly used in the development of immunomodulatory therapies as they provide the ability to examine a dose range. Indeed, the initial starting dose for BMS-986178 was determined by examining the antitumor efficacy in preclinical mouse tumor models (13). However, these models are limited by a relatively short duration and fast growth kinetics, thereby limiting the window of opportunity for therapeutic intervention and reducing the ability to determine the impact of altering dosing schedule on the immunomodulatory effects of T-cell agonists. Herein, we describe an approach that circumvents this limitation through the integration of receptor occupancy, receptor regulation, and pharmacodynamic data from preclinical models and clinical biomarker data from patients treated with BMS-986178. This framework for investigating the relationships across preclinical and clinical data sets, performed in early clinical development, could be used to inform the optimal dose and schedule for anti-OX40 agonist antibody–containing therapeutic regimens for the treatment of patients with solid tumors.

Materials and Methods

Antibody generation

Transgenic mice with a germline configuration of human immunoglobulin (Ig) miniloci in an endogenous IgH and Igκ knockout background (14) were immunized with recombinant human OX40-Fc protein to generate anti-human OX40 mAbs. To generate a suitable anti-mouse OX40 surrogate antibody, Armenian hamsters were immunized with recombinant mouse OX40-huFc protein. Splenocytes from immunized mice were fused with SP2/0 myeloma cells, and antibodies specific for human (h) or mouse (m) OX40 were selected in a primary ELISA screen. A secondary screen by flow cytometric analysis was performed to identify antibodies that bind to Chinese hamster ovary (CHO) cells expressing hOX40 or mOX40, or activated primary human or mouse T cells. FACS-positive antibodies were further characterized for their ability to block the binding of human or mouse OX40 ligand (OX40L). Serial dilutions of the antibodies were preincubated with activated CD4+ T cells before addition of a fixed concentration of soluble His-tagged OX40L. Binding of OX40L-His was detected using an in-house allophycocyanin conjugated anti-His tagged secondary antibody and analyzed by flow cytometry. Selected clones were submitted for sequencing and cloned into CHO cells for recombinant expression with different IgG constant regions.

In vitro primary human T-cell assays

Irradiated CHO cells coexpressing single-chain anti-CD3 (clone OKT3) and human CD32a molecules (CHO-OKT3-CD32), or CHO cells expressing single-chain anti-CD3 only (CHO-OKT3) were seeded 1:8 with human CD4+ T cells isolated from peripheral blood (2.5 × 10^6 CHO cells:1 × 10^6 T cells) per well in a 96-well tissue culture–grade flat-bottom plate (Costar). Cells were incubated with a 9- or 10-point, 3-fold titration of BMS-986178 mAb or isotype control starting at 0.3 µg/mL (2 nmol/L). IFNγ secretion and T-cell proliferation were assessed as two measures of T-cell activation.

Patients and study design

During the dose-escalation phase of the first-in-human, open-label, dose-escalation, and expansion study (NCT02737475; Supplementary Table S1), patients with advanced solid tumors and ≥1 prior therapy received BMS-986178 monotherapy 20–320 mg i.v. every 2 weeks (n = 20), BMS-986178 20–320 mg in combination with nivolumab (anti–PD-1) 240 mg i.v. every 2 weeks (n = 38), or BMS-986178 20–320 mg in combination with ipilimumab (anti–CTLA-4) 1 mg/kg i.v. every 3 weeks (n = 32). Blood and serum samples from these cohorts were collected at baseline and multiple on-treatment timepoints (cycle 1 days 1, 2, and 8; cycle 2 day 1; and cycle 3 day 1). The protocol and any amendments were reviewed and approved by an institutional review board/independent ethics committee prior to initiation of the study. The study was conducted in accordance with the Declaration of Helsinki and in compliance with the protocol. All patients provided an informed consent form before enrollment.

Patient whole-blood immunophenotyping and receptor occupancy assessment

For immunophenotyping flow cytometric analysis, patient blood samples were collected in a Cytocube BCT tube, and samples for receptor occupancy flow cytometric analysis were...
collected in a Na heparin blood collection tube. After red blood cell lysis, cells were stained using fluorescently labeled antibodies specific for surface markers (Supplementary Table S2). After surface staining, samples were fixed, permeabilized, and then stained with anti-Ki67 antibody. For receptor occupancy assessment, patient blood samples were incubated for 30 minutes at 4°C with or without a saturating dose of BMS-986178 to measure total OX40 expression and drug binding, respectively. After incubation, samples were stained with fluorescently labeled antibodies specific to surface markers (Supplementary Fig. S1). Stained samples were acquired on a Beckman Coulter CytoFACS S flow cytometer or Becton Dickinson FACSCanto II flow cytometer, and the resulting data were analyzed using FlowJo v7 Software (FlowJo LLC). Receptor occupancy was calculated for each sample according to the following equation: % receptor occupancy = Δ mean fluorescence intensity (MFI) of test/Δ MFI of total × 100.

Mouse peripheral blood and tumor immunophenotyping and OX40 receptor occupancy assessment
Blood was obtained from mice via cardiac puncture into syringes containing ethylenediaminetetraacetic acid (EDTA). Viable white blood cells from whole blood were recovered by Histopaque-1083 (Sigma-Aldrich, catalog no. 10831) gradient separation following the manufacturer’s instructions. Tumors were removed, weighed, and processed on a gentleMACS Octo Dissociator (Miltenyi). The Mouse Tumor Dissociation Kit (Miltenyi, catalog no. 130-096-730) was used for tumor processing per the manufacturer’s instructions. After dissociation, the cell suspension was washed, filtered, and counted.

For immunophenotyping, single-cell suspensions were stained using the T-cell antibody panel (Supplementary Table S3). For receptor occupancy assessment, single-cell suspensions from blood and tumors from individual mice were duplicated into two plates to test for occupied and total receptors separately. To test for total receptors, excess anti–OX40-biotin antibody prepared in FACS buffer (2% FBS and 2 mmol/L EDTA in PBS) was added at a final concentration of 10 μg/mL and stained for 30 minutes at 4°C. Samples were washed three times with FACS buffer, followed by PE-streptavidin staining at 0.5 μg/mL for 30 minutes. To test for occupied receptors, only PE-streptavidin was added at a final concentration of 0.5 μg/mL for 30 minutes. Both total and occupied samples were then washed three times and stained for immune cell markers (Supplementary Fig. S2; Supplementary Table S3) using flow cytometry antibodies. DAPI was added at 1 μg/mL to distinguish between live and dead cells before running samples on the flow cytometer.

Antibody fluorescence was detected by flow cytometry on the Fortessa (BD Biosciences), and the results were analyzed using FlowJo software. See gating strategy in Supplementary Fig. S1. Receptor occupancy was calculated in the same way as patient receptor occupancy.

Patient serum cytokine/chemokine assay
Cytokines in patient serum were measured using Luminescence-based technology including a customized multianalyte profiling (MAP) panel by combining several multiplex human inflammatory MAP panels (Myriad RBM).

Mouse tumor models
Female BALB/c mice (Harlan Laboratories order no. RAY7341) were subcutaneously implanted with 1 × 10⁶ CT26 cells in 0.2 mL of PBS on day 0. Six days post-implantation, tumor-bearing mice were randomly divided into treatment groups (n = 10) and treated with anti–PD-1 (clone 4H12, mlgG1-D263A) at 10 mg/kg and/or anti-OX40 (clone 6E10, mlgG1) or biotin-labeled anti–OX40–mlgG1 at indicated doses (15, 16). All procedures were performed and approved in strict accordance with the Institutional Animal Care and Use Committee at Bristol-Myers Squibb, and with the NIH Guide for the Care and Use of Laboratory Animal guidelines.

IHC of patient biopsies
IHC for FoxP3 (clone 236A/E7) was performed on 4-μm thick formalin-fixed, paraffin-embedded sections using a Leica Bond RX (Leica Biosystems) by Mosaic Laboratories.

Statistical analysis and pharmacokinetic–pharmacodynamic analysis
For preclinical tumor efficacy and pharmacodynamic analysis in the murine model, pairwise statistical differences between various treatments and different controls were analyzed using the Wilcoxon rank sum test. P < 0.05 was considered statistically significant.

For clinical pharmacodynamic analysis in patients, longitudinal pharmacodynamic modulation was analyzed by a linear mixed-effects model using log₂ transformation on the readout from the clinical cytokine and flow cytometry assays. Dunnett post hoc testing was conducted at each measurement timepoint compared with baseline. P < 0.05 was considered statistically significant.

For in vitro data analysis, total OX40 expression (measured as MFI) and T-cell proliferation (measured by [3H]-thymidine uptake) were modeled against OX40 receptor occupancy (ROOX40) using the following equation: $E = \frac{E_{max} \times ROOX40}{EC_{50} + ROOX40} - E_{reduction} \times ROOX40$, where $E$ is the effect (total OX40 expression or T-cell proliferation). $E_{max}$ is the maximum effect, $EC_{50}$ is the ROOX40 value that corresponds to 50% of the maximum effect, and $E_{reduction}$ is the counter effect.

A population pharmacokinetic model was developed by nonlinear mixed-effects modeling using data from 90 participants from monotherapy and combination cohorts in the first-in-human clinical study of BMS-986178. The pharmacokinetics and pharmacodynamics of BMS-986178 was linear, and was described by a two-compartment, zero-order intravenous infusion with first-order elimination (17). Subsequently, a pharmacokinetic–pharmacodynamic analysis ($E_{max}$ model) was conducted to characterize the relationship between BMS-986178 concentrations and blood receptor occupancy using data from 19 patients treated with 20–320 mg BMS-986178 in the clinical study. The parameter estimate of $EC_{50}$ from the pharmacokinetic–pharmacodynamic analysis of blood receptor occupancy was 0.15 μg/mL.

Trial simulation ($N = 500$) was conducted to predict blood receptor occupancy levels at less frequent dosing regimens (e.g., every 4 weeks, every 6 weeks, and every 12 weeks). Both interpatient variability of pharmacokinetics and pharmacodynamics were accounted for in the trial simulation.

Results
Generation, characterization, and clinical development of BMS-986178
BMS-986178 is a fully human agonist anti–OX40 mAb of the human IgG1 subclass, which binds to primary human-activated...
CD4+ T cells with picomolar (pM) affinity and inhibits the OX40:OX40L receptor–ligand interaction (Supplementary Fig. S3A and S3B). BMS-986178 did not cross-react with murine OX40. Agonism of OX40 with BMS-986178 on primary human T cells led to a dose-dependent enhancement of anti-CD3-mediated induction of IFNγ (Supplementary Fig. S3C) and proliferation with pM potency (Supplementary Fig. S3D). The ability of BMS-986178 to stimulate T-cell responses in vitro was dependent on Fc–Fcγ receptor (FcγR) interactions; addition of the antibody in the absence of FcγR (CD32a) expression on antigen-presenting cells (APC) in the assay did not lead to enhancement of anti-CD3-mediated induction of IFNγ over the isotype control (Supplementary Fig. S3C). The requirement of cross-linking for agonist activity was observed in all in vitro assays used to characterize BMS–986178, including enhancement of IL2 secretion from human peripheral blood mononuclear cells stimulated with staphylococcal enterotoxin B (Supplementary Fig. S3E) and inhibition of TGFB3-mediated induction of primary human Tregs (Supplementary Fig. S3F).

Modulation of peripheral pharmacodynamic biomarkers in patients with advanced solid tumors treated with BMS-986178 as monotherapy or in combination with nivolumab or ipilimumab

To gain an understanding of the ability of BMS-986178 as monotherapy or in combination with nivolumab or ipilimumab to elicit immune activation in patients with solid tumors, T-cell immunophenotyping on whole-blood samples and cytokine analysis on serum was performed. We observed enhanced proliferation, as measured by an increase in the fold change of Ki67 positivity, in circulating populations of CD4+ effector memory and effector-like cells (CD4+CCR7−CD45RA− and CD4+CD38+HLA-DR+, respectively) as well as CD8+ effector memory cells (CD8+CCR7−CD45RA−) after treatment with BMS-986178 either as monotherapy or in combination with nivolumab or ipilimumab (Fig. 1A–C; Supplementary Fig. S4). In addition, the combination of BMS-986178 with nivolumab or ipilimumab led to significant increases in Ki67 expression within all populations examined and to a greater magnitude than BMS-986178 monotherapy (Supplementary Table S4). The ability of BMS-986178, either alone or in combination, to enhance peripheral T-cell proliferation was not dose dependent. In fact, a higher proportion of patients showed increased Ki67+CD4+ effector memory cells following treatment with a lower dose (20–80 mg) of BMS-986178 compared with patients receiving the higher 160- or 320-mg doses (Fig. 1A). Whereas the combination of BMS-986178 with either nivolumab or ipilimumab resulted in peak increases in Ki67 expression at 7 days after infusion (C1D8), peak Ki67 increases in patients treated with BMS-986178 monotherapy occurred 1 day after the first infusion (C1D2; Fig. 1A–C). This observation may indicate a more immediate impact of OX40 agonism on an effector T-cell response or a delayed peak of Ki67 induction from the combination treatments.

To determine whether peripheral T-cell activation was accompanied by an increase in cytokine production consistent with enhancement of a functional immune response, we evaluated a panel of pro- and anti-inflammatory cytokines in patient sera at baseline (C1D1) and posttreatment timepoints (C1D2, C1D8, C2D1, and C3D1). BMS-986178 significantly increased the levels of IFNγ (Supplementary Fig. S5A) and the IFNγ-induced cytokines IP-10 (Supplementary Fig. S5B) and CXCL9 (Supplementary Fig. S5C), with greater increases in IP-10 and CXCL9 when administered in combination with nivolumab or ipilimumab. Similar to the assessment of peripheral T-cell activation, there was no clear relationship between BMS-986178 dose and cytokine induction in serum. While there was a greater degree of variability associated with the kinetics of cytokine induction than with T-cell proliferation among patients treated with BMS-986178, the peak timing for cytokine enhancement was consistent between patients treated with monotherapy or combination therapy and occurred 24 hours after initial infusion, on C1D2 (Supplementary Fig. S5). These data suggest that engagement of OX40 by BMS-986178, when administered either alone or in combination with PD-1 or CTLA-4 blockade, leads to peripheral T-cell proliferation and induction of proinflammatory cytokines in patients with solid tumors.

Modulation of peripheral pharmacodynamic biomarkers in preclinical murine tumor models following treatment with anti-OX40 alone or in combination with murine PD-1 or CTLA-4 blockade

Despite clear evidence of peripheral pharmacodynamic modulation in patients by BMS-986178 alone or in combination with checkpoint blockade, the lack of a clear dose response in pharmacodynamic markers limits the ability to ascertain the optimal dose and schedule for the molecule. We used preclinical murine tumor models, which afford a more in-depth analysis of peripheral and intratumoral immune responses, to elucidate the relationship between dose and pharmacodynamic marker modulation following OX40 agonism alone or in combination with PD-1 or CTLA-4 blockade.

Many studies have demonstrated that agonist OX40 antibody therapy alone or in combination with other agents enhances the antitumor immune response and demonstrates efficacy in preclinical tumor models (18–20). However, all published reports have made use of the OX86 antibody clone, which, unlike BMS-986178, does not block the OX40–OX40L interaction (21). As BMS-986178 does not cross-react with mOX40, we generated a surrogate OX40L-blocking, anti-mouse OX40 agonist antibody (clone OX40.23) for evaluation in a CT26 syngeneic mouse tumor model. Mice bearing established CT26 tumors were administered OX40.23 across a dose range (0.1, 0.3, 1, and 3 mg/kg) either alone or in combination with anti-PD-1 or anti-CTLA-4. Treatment of CT26 tumor–bearing mice with OX40.23 monotherapy at ≥0.3 mg/kg delayed tumor growth, and 10%–30% of mice were tumor free at the end of the study (Supplementary Fig. S6A). Combination treatment with OX40.23 and either anti-PD-1 or anti-CTLA-4 significantly increased the number of tumor-free mice (80%–100%) over any single agent alone (Supplementary Fig. S6A–S6C; Supplementary Table S3). In particular, the combination of 0.1 mg/kg OX40.23 with anti-PD-1 demonstrated significant antitumor activity, whereas 0.1 mg/kg OX40.23 monotherapy showed no detectable tumor growth inhibition (Supplementary Fig. S6; Supplementary Table S5). Of note, we observed that concurrent administration of OX40.23 and anti-PD-1 was comparable, if not superior, to administering OX40.23 first followed by anti-PD-1 (Supplementary Fig. S7). These findings contrast with recent reports examining the impact of schedule on this particular combination in other preclinical tumor models (22, 23).
Figure 1.
BMS-986178 + nivolumab or ipilimumab increased levels of proliferating (Ki67⁺) CD4⁺ and CD8⁺ populations in patients with advanced solid tumors. Flow cytometric quantification of the frequency of Ki67⁺ CD4⁺ effector memory cells (A), Ki67⁺ CD38⁺ HLA-DR⁺ CD4⁺ effector cells (B), and Ki67⁺ CD8⁺ effector memory cells (C) in patients' peripheral blood at pretreatment (C1D1) and various posttreatment timepoints. See Supplementary Table S4 for summary of P values. IPI, ipilimumab; NIVO, nivolumab.
Having established in vivo agonist activity of the OX40.23 clone in the CT26 tumor model, we sought to determine whether administration of OX40.23 in the mouse model was accompanied by similar peripheral pharmacodynamic effects as observed in patients treated with BMS-986178. CT26 tumor-bearing mice were treated with two doses of OX40.23 alone or in combination with anti–PD-1 or anti–CTLA-4 on days 6 and 13 post-implantation, and Ki67 expression on peripheral CD4+ and CD8+ T cells was measured over time following treatment (Fig. 2A). Similar to what was observed clinically, OX40.23 monotherapy demonstrated a modest capacity to elicit peripheral T-cell proliferation. Unlike the clinical pharmacodynamic data, the impact was greatest on the peripheral CD8+ T-cell subset on day 12 following the intermediate 0.3 mg/kg dose (Fig. 2B and C). The combination of OX40.23 with anti–PD-1 or anti–CTLA-4 markedly increased the magnitude of both CD4+ and CD8+ peripheral T-cell proliferation compared with any of the single agents alone (Fig. 2B and C). The combination of OX40.23 with anti–PD-1 demonstrated a later onset of induction and perhaps a more sustained enhancement of peripheral T-cell activation in this model compared with the combination with anti–CTLA-4 (Fig. 2B and C). The percentage of Ki67+ CD8+ T cells elicited on day 12 by OX40.23 and anti–PD-1 corresponded with antitumor responses and reduction in tumor volume on day 20 in the model (Supplementary Fig. S8). With the exception of 0.1 mg/kg OX40.23 eliciting suboptimal peripheral T-cell proliferation, we did not observe a consistent dose response in the ability of OX40.23 to enhance T-cell proliferation either alone or in combination with PD-1 or CTLA-4 blockade. Doses of OX40.23 > 0.3 mg/kg demonstrated clear evidence of activity. Overall, these data show that peripheral pharmacodynamic markers modulated by agonist anti-OX40 treatment alone and in combination with PD-1 or CTLA-4 blockade are conserved between humans and mice. Furthermore, these data validate the use of the OX40.23 surrogate antibody in the CT26 tumor model to better understand the dose–pharmacodynamic relationship of OX40 agonist treatment.

Assessment of ROOX40 and receptor regulation in a preclinical mouse model and patients

The absence of a clear dose response of agonist OX40 antibody–mediated induction of peripheral pharmacodynamic markers of immune activation in patients treated with BMS-986178 and above a certain dose in the CT26 tumor model may be related to the degree of receptor engagement by the agonist antibody. Therefore, we evaluated the kinetics of ROOX40 on CD4+ T-cell subsets following antibody administration.

We first examined the degree of receptor occupancy on CD4+ Tregs in the periphery and tumor on days 8 and 13 following treatment in CT26 tumor–bearing mice with OX40.23 alone or in combination with anti–PD-1 via a flow cytometry–based assay (Supplementary Fig. S2). Mice were treated with three doses of OX40.23, spanning the dose range used in the pharmacodynamic biomarker experiments, including one in the suboptimal range (0.03, 0.3, and 3 mg/kg). The median ROOX40 by the OX40.23 antibody on peripheral and intratumoral CD4+ Tregs increased in

![Image](https://example.com/image.png)

**Figure 2.**
Peripheral blood pharmacodynamic profiling in preclinical murine model. **A,** Experimental timeline indicating the implantation of CT26 cell line, administration of therapeutic antibodies, and schedule of blood sampling. Pharmacodynamic assessments by flow cytometry of peripheral Ki67+ CD8+ and Ki67+ CD4+ T cells in response to anti-OX40 monotherapy, anti-OX40 in combination with anti–PD-1, and anti-OX40 in combination with anti–CTLA-4 on days 6, 12, 15, and 19. **N** = 10 mice per group. See Supplementary Table S4 for summary of P values.
a dose-dependent manner, with 3 mg/kg demonstrating similar receptor occupancy between the periphery and tumor (Fig. 3A). Similar intratumoral dose-dependent receptor occupancy changes were observed between Tregs and CD4\(^+\) effector T cells (Supplementary Fig. S9). Coadministration of anti–PD-1 did not affect OX40.23 receptor occupancy across the three doses studied (Fig. 3A). Compared with the day 8 timepoint, OX40.23 receptor occupancy on day 13 showed a reduction in peripheral blood but an increase in the tumor, suggesting clearance of the OX40.23 antibody from blood and accumulation in the tumor (Fig. 3A). To further investigate OX40.23 clearance in the tumor, receptor occupancy was assessed at later timepoints on intratumoral Tregs after single administration of OX40.23 (0.5 and 5 mg/kg) in combination with anti–PD-1. In the group treated with 0.5 mg/kg OX40.23, there was a rapid decrease in receptor occupancy between day 10 (4 days post-dose) and day 17 (11 days post-dose), from 60% to 20%, with complete absence of detectable receptor occupancy on day 22 (16 days post-dose; Fig. 3B). However, receptor occupancy in the group treated with 5 mg/kg OX40.23 was maintained at approximately 20% on day 22 (Fig. 3B), suggesting that RO\text{OX40}\text{a}\text{b} is dose dependent and is similar between peripheral blood and the tumor. These data, coupled with the pharmacodynamic biomarker data (Fig. 2B and C), indicate that an intratumoral receptor occupancy of approximately 40%, which is achieved with 0.3 mg/kg OX40.23 (alone or in combination with PD-1 or CTLA-4 blockade), is the minimal receptor occupancy required for relative maximal pharmacodynamic and antitumor activity in the CT26 tumor model (Supplementary Fig. S6). Interestingly, parallel assessment of total OX40 receptor expression on peripheral CD4\(^+\) T-cell subsets (Fig. 4B; Supplementary Fig. S10B). These data suggest that OX40 receptor regulation following treatment with an agonist antibody is conserved across species. We were not able to generate intratumoral receptor occupancy by BMS-986178 from biopsy material in the phase I clinical trial; however, the data from peripheral blood confirmed sustained peripheral receptor engagement by BMS-986178.

To extend the findings from the CT26 tumor model to the clinic, we examined RO\text{OX40}\text{a}\text{b} on peripheral CD4\(^+\) Tregs and effector T cells in patients treated with BMS-986178 monotherapy across all dose cohorts in the phase I trial (Supplementary Fig. S1; Supplementary Table S1). We observed that BMS-986178 receptor occupancy in peripheral blood of patients reached approximately 80% on both CD4\(^+\) Tregs (Fig. 4A) and effector T cells (Supplementary Fig. S10A) at the lowest dose in the study (20 mg) and approached saturation at doses \(\geq 40\) mg on C1D8. BMS-986178 showed a high degree of receptor occupancy even at the lowest dose in the study, and on the basis of the observed loss of OX40 receptor as receptor occupancy approached saturation in the CT26 tumor model, we examined the expression of total OX40 on peripheral CD4\(^+\) Tregs and effector T cells in patients treated with BMS-986178. Consistent with the mouse model, the majority of patients treated with BMS-986178 showed loss of total OX40 receptor from baseline on both peripheral CD4\(^+\) T-cell subsets (Fig. 4B; Supplementary Fig. S10B). These data suggest that OX40 receptor regulation following treatment with an agonist antibody is conserved across species. We were not able to generate intratumoral receptor occupancy by BMS-986178 from biopsy material in the phase I clinical trial; however, the data from peripheral blood confirmed sustained peripheral receptor engagement by BMS-986178.

Modeling clinical dose and schedule based on preclinical receptor occupancy, receptor regulation, and functional activity of agonist OX40 antibodies

To more carefully model the relationships between dose, receptor occupancy, receptor loss, and functional activity following treatment with BMS-986178, we turned to the artificial APC-primary human T-cell \textit{in vitro} system initially used to develop BMS-986178 (Supplementary Fig. S3). Using this
system, we found that BMS-986178 led to a dose-dependent increase in OX40 expression on activated primary human CD4\(^+\) T cells. OX40 expression reached a maximum between 20% and 40% receptor occupancy, followed by a profound loss in receptor expression at >40% receptor occupancy, and a return to near baseline expression as receptor occupancy approached 100% (Fig. 5A and B). The ability of BMS-986178 to increase the proliferation of human CD4\(^+\) T cells in relation to receptor occupancy was assessed. The maximum level of BMS-986178–induced CD4\(^+\) T-cell proliferation was also achieved between approximately 20% and 40% receptor occupancy (Fig. 5C and D).

Our results indicated that OX40.23 receptor occupancy of approximately 40% was sufficient to yield maximal antitumor activity in combination with anti–PD-1 or anti–CTLA-4 in the CT26 tumor model, and that maximal pharmacodynamic activity was achieved in human T cells between 20% and 40% receptor occupancy by BMS-986178. In addition, OX40 receptor expression was lost in mice and patients treated every 2 weeks with BMS-986178 at receptor occupancy >40%. Therefore, we integrated these findings to develop a model to predict the dose(s) and schedule(s) for administration of BMS-986178 that would result in receptor occupancy of 20%–50% (Fig. 6A).

Discussion

T-cell checkpoint blockade with antibodies targeting CTLA-4 and/or PD-1/programmed cell death 1 ligand 1 has revolutionized the management of a wide variety of malignancies, with complete regression and long-term survival achieved in some patients (24–26). Engagement of costimulatory molecules on T cells, such as OX40, may further promote T-cell responses that are restored by checkpoint blockade, potentially leading to enhanced clinical benefit. The preclinical and clinical experience with checkpoint blockade suggests that higher doses result in increased receptor occupancy and correlate with increased clinical response (27–29). However, it is unclear whether this linear correlation is true for T-cell agonists or agonist agents in general. Whether increased doses and/or more frequent dosing schedules will lead to increased activity of agonist agents in the clinic remains to be determined, which necessitates evaluation and optimization such that the minimal dose and schedule that yield maximal activity can be identified.

In patients receiving BMS-986178 alone or in combination with checkpoint blockade, we observed increases in proliferating CD4\(^+\) effector memory cells and activated HLA-DR\(^+\)CD38\(^+\) effector cells, with more patients demonstrating upregulation of CD4\(^+\) T-cell proliferation and activation in the combination cohorts. BMS-986178 monotherapy induced a maximum increase in proliferating CD4\(^+\) T cells on day 2, while combination therapy induced a peak increase on day 8, which may be due to the combinatorial effect. Further investigation is required to understand the difference in the timing of peak activation induced

![Figure 4.](image-url)
by BMS-986178 monotherapy versus combination therapy. OX40 has higher expression levels on activated CD4^+ T effector cells and Tregs compared with CD8^+ T cells in the periphery and tumor-infiltrating lymphocytes from patients with cancer (30). Therefore, the observed pharmacodynamic modulation of CD4^+ T-cell populations by BMS-986178 could be due to direct engagement of OX40 receptors. However, patients treated with BMS-986178 alone or in combination with checkpoint blockade also exhibited an increase in Ki67^+ CD8^+ T cells, perhaps suggesting that the agonistic effect of BMS-986178 on CD4^+ T cells strongly influences CD8^+ T-cell activity in vivo, which can contribute to optimal antitumor responses (31). The increase in circulating Th1 cytokines in patients treated with BMS-986178 alone or in combination with nivolumab or ipilimumab further confirmed the activation and functionality of the CD4^+ and CD8^+ populations.

We did not observe dose-dependent changes in pharmacodynamic biomarkers in patients treated with BMS-986178 monotherapy or combination therapy, making it difficult to identify the optimal dose of BMS-986178 to maximally potentiate patient T-cell responses. We observed similar induction of peripheral and intratumoral CD4^+ and CD8^+ T-cell proliferation using a surrogate ligand-blocking, anti-mouse OX40 agonist antibody in the murine syngeneic CT26 tumor model. Data from this model reinforce the value of the preclinical model to explore the relationships between dose, receptor occupancy, and pharmacodynamic marker induction by OX40 agonist antibodies.

Using the CT26 tumor model, we investigated the relationship between ROOX40 and the antitumor activity and pharmacodynamic biomarker modulations elicited by anti-OX40 alone or in combination with checkpoint blockade. Previous studies have shown that the murine antitumor efficacy of anti-OX40 relies on intratumoral Treg depletion in an FcγR-dependent manner (32, 33). In mouse studies, we also observed that the antitumor activity of anti-OX40 antibodies was related to their ability to preferentially bind to activating FcγR and deplete intratumoral Tregs (Supplementary Fig. S11). However, it is still unclear whether nonmodified human IgG1 would be competent for intratumoral Treg depletion via antibody-dependent cell-mediated cytotoxicity in patients. From limited number of paired biopsy samples in the nivolumab plus BMS-986178 cohort, we did observe a trend toward intratumoral Treg reduction posttreatment (Supplementary Fig. S12). To better understand and isolate the effect of OX40 agonism, we used OX40.23 with a mIgG1 isotype in all mouse studies, which was able to agonize OX40 receptors in the absence of FcγR-mediated cytotoxicity (34). Higher anti-OX40 doses increased both peripheral and intratumoral receptor occupancy, demonstrating that receptor activation.

![Figure 5. Relationships of receptor occupancy, OX40 receptor downregulation, and T-cell activation.](image-url)
occupancy is dose dependent and exposure related. However, the robust T-cell activation and proliferation in peripheral blood was observed at anti-OX40 0.3 mg/kg, the minimum efficacious dose when combined with checkpoint blockade, accompanied by only 20%–50% receptor occupancy in the tumor. In addition, in the artificial APC-primary human T-cell in vitro system, maximum enhancement of T-cell function by BMS-986178 was also achieved when receptor occupancy was between 20% and 50%. When receptor occupancy was >50%, T-cell activity plateaued or in some cases was slightly diminished (Fig. 6B). This may be due, in part, to the observed progressive loss of the OX40 receptor itself from the cell surface when receptor occupancy is >40% in both mice and humans. The mechanism of OX40 receptor loss is unknown, but these data suggest it is clearly related to anti-OX40 antibody exposure. Taken together, these data suggest that targeting receptor occupancy between
approximately 20% and 50% will result in maximal potentiation of T-cell responses by a therapeutic OX40 agonist antibody.

In patients treated with BMS-986176, approximately 85% receptor occupancy was observed at the lowest dose administered in the study (20 mg). Preclinical data suggest that ROXO40 in peripheral blood and tumors is similar. Extrapolating the consistency between peripheral and tumoral receptor occupancy to clinical data would suggest that the current doses and schedule for BMS-986178 in the early cohorts of the study may result in receptor occupancy within the tumor that is too high. Using the totality of the clinical exposure data along with preclinical and clinical pharmacodynamic and receptor occupancy data, we generated a mathematical model to identify a dose and schedule for BMS-986178 that would achieve receptor occupancy between 20% and 50%, and this resulted in an amendment to add new cohorts in the phase I study. On the basis of the findings from the pharmacodynamic/pharmacodynamic modeling of the relationship between receptor occupancy/pharmacodynamic modulation and efficacy, additional cohorts evaluating various BMS-986178 doses (20, 40, and 80 mg) given every 12 weeks in combination with nivolumab, as well as a nivolumab monotherapy cohort as a head-to-head comparison were added to the clinical study (NCT02737475).

In summary, we applied an integrated translational approach, wherein pharmacodynamic biomarkers identified in preclinical models were then tested in patients treated with BMS-986178 alone or in combination with checkpoint blockade, followed by further exploration in preclinical models to elucidate the impact of agonist OX40 antibody dose and schedule on antitumor activity. This represents an innovative method to identify the optimal dose and schedule for T-cell agonists during their clinical development as components of immunotherapeutic regimens for the treatment of patients with cancer.

Disclosure of Potential Conflicts of Interest

M. Gutierrez reports receiving speakers bureau honoraria from Bristol-Myers Squibb and Merck. I. Melero reports receiving speakers bureau honoraria from MSD, a consultant/advisory board member for Bristol-Myers Squibb, Roche, MDx, F-Stat, Gennab, Numab, Pieris, Catalym, Bayer, and Boehringer Ingelheim, and reports receiving commercial research grants from Alligator, Roche, Bristol-Myers Squibb, and Bioventech. A. Spreatico is a consultant/advisory board member for Merck, Bristol-Myers Squibb, Novartis, and Onconusa. R.D. Carvajal is a consultant/advisory board member for Bristol-Myers Squibb, Castle Biosciences, Compugen, Immunocore, I-Mab, Merck, PureTech Health, Sanofi Genzyme, Sorrento Therapeutics, Aura Biosciences, Chimeron, and Rgenix. M. Ong is a consultant/advisory board member for Bristol-Myers Squibb, Merck, AstraZeneca, Roche, and EMD Serono. A.J. Olszanski is a consultant/advisory board member for Merck, Pfizer, Array, EMD Serono, Immuno, Novartis, and Alkermes, and reports receiving commercial research support to Fox Chase Cancer Center from Alkermes, Amgen, Astellas, Bristol-Myers Squibb, Boston Biomedical, Checkmate Pharmaceuticals, Eli Lilly, EMD Serono, GlaxoSmithKline, Immunocore, Incyte, Intensity Therapeutics, Karlos, Nektar, and Oncoseics. C. Milburn is an employee of Bristol-Myers Squibb. K. Thudium has ownership interests (including patents) at Bristol-Myers Squibb. Y. Feng has ownership interests (including patents) at Bristol-Myers Squibb. P. M. Fraccasso is an employee of Adaptimmune LLC. A.J. Korman has ownership interests (including patents) at Bristol-Myers Squibb. S.-M.A. Huang is an employee of Bristol-Myers Squibb. M. Quigley has ownership interests (including patents) at Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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