Dual Roles for Regulatory T-cell Depletion and Costimulatory Signaling in Agonistic GITR Targeting for Tumor Immunotherapy

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Abstract

Agonistic monoclonal antibodies (mAb) targeting the T-cell receptor coregulatory molecule GITR exert potent therapeutic activities in preclinical tumor models. Although anti-GITR mAb are thought to act by depleting and destabilizing the intratumoral T regulatory cell (Treg) population, the precise mechanism of action is obscure. Here, we addressed this issue using a Treg fate-mapping approach, which revealed that Treg loss was primarily due to cell depletion, with minimal evidence of Treg conversion to a non–Foxp3-expressing population. Further characterization of persisting Tregs following anti-GITR mAb treatment showed that a highly activated subpopulation of CD44hiICOShi intratumoral Tregs were preferentially targeted for elimination, with the remaining Tregs exhibiting a less suppressive phenotype. With these changes in the Treg population, intratumoral CD8+ T cells acquired a more functional phenotype characterized by downregulation of the exhaustion markers PD-1 and LAG-3. This reversal of CD8+ T-cell exhaustion was dependent on both agonistic GITR signaling and Treg depletion, as neither mechanism by itself could fully rescue the exhaustion phenotype. Tests of anti-human GITR antibody MK-4166 in a humanized mouse model of cancer mimicked many of the effects of anti-mouse GITR mAb in syngeneic tumor models, decreasing both Treg numbers and immune suppressor phenotype while enhancing effector responsiveness. Overall, our results show how anti-GITR mAb shifts Treg populations to enable immune attack on tumors, with clinical implications for molecular markers to modify emerging treatments. Cancer Res; 77(5); 1108–18. ©2016 AACR.

Introduction

Glucocorticoid-induced TNFR family-related protein (GITR, TNFRSF18, CD357) is a costimulatory immune modulating receptor expressed on various immune cell subsets, including T cells, natural killer (NK) cells, and B cells, with particularly high expression on regulatory T cells (Tregs; refs. 1–3). A widely studied anti-GITR agonistic monoclonal antibody (mAb), clone DTA-1, has been shown to have costimulatory effects on T cells in vitro and in vivo (4) and potent antitumor efficacy in multiple mouse syngeneic tumor models (5, 6).

Previous studies investigating the mechanism of action behind the antitumor efficacy of DTA-1 have focused heavily on the impact on Tregs, a key population involved in the inhibition of antitumor immune responses in a range of solid tumor types (reviewed in ref. 7). These studies showed that Tregs are greatly reduced in number following DTA-1 admin-

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Materials and Methods

Mice and reagents
Wild-type C57BL/6, Foxp3-GDL, Foxp3-GFP-Cre, and ROSA26-loxP-Stop-loxP-Tomato mice were obtained from The Jackson Laboratory and housed and bred under specific pathogen-free conditions in the Merck Palo Alto animal facility. Hu-CD34⁺ NSG mice were purchased from The Jackson Laboratory after reconstitution and verification of human immune cell engraftment. All mouse experiments were performed according to Merck IACUC-approved protocols.

MC38 mouse colon carcinoma cell line was obtained from the Developmental Therapeutics Program Tumor Repository (Frederick National Laboratory), and SK-MEL-5 human melanoma cell line was purchased from ATCC in 2015. Both cell lines were authenticated using genomic profiling (IDEXX RADIL Cell Check) and tested to be Mycoplasma free (IMPACT I PCR Pro). MC38 was expanded and maintained in our laboratory in DMEM supplemented with 10% FBS and SK-MEL-5 in MEM supplemented with 10% FBS. Both cell lines were stored in liquid nitrogen to ensure that cells used for experiments were passaged for fewer than 6 weeks.

Rat anti-mouse DTA-1 GITR antibody (S. Sakaguchi, Kyoto University), was murinized as previously described (16). The N297A-mutant IgG2a DTA-1 was generated in-house by mutation of mIgG2a DTA-1. Murinized anti-GITR antibody (mDTA-1) is highly effective in the MC38 colon carcinoma model, where a single dose as low as 3 mg/kg provided complete tumor clearance in the majority of mice (Fig. 1A). GITR was expressed on peripheral blood mononuclear cells (PBMCs) by more than 30% of gated CD8⁺ T cells (Fig. 1B). Cytokine levels were measured with a Luminex kit following the manufacturer's instructions.

Statistical analysis

Statistical tests were run with the aid of Graph Pad Prism software. Two sample comparisons were made by unpaired t test. Comparisons of 3 or more samples were made by one-way ANOVA followed by Dunnett multiple comparisons test. Simultaneous comparison of treatment groups across multiple tissues was done by two-way ANOVA followed by the Sidak multiple comparisons test. For all tests, *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Results

mDTA-1 depletes rather than converts intratumoral Tregs
Murinized anti-GITR antibody (mDTA-1) is highly efficacious in the MC38 colon carcinoma model, where a single dose as low as 3 mg/kg provided complete tumor clearance in the majority of mice (Fig. 1A). GITR was expressed on peripheral Tregs and was upregulated on all T-cell subsets within the tumor, with intratumoral Tregs displaying the highest levels of GITR expression (Fig. 1B). Similar to previous observations (13), a rapid loss of intratumoral Tregs was observed upon treatment with mDTA-1 (Fig. 1C). This loss was highly specific to Tregs in the tumor microenvironment as the number and frequency of dLN Tregs and effector CD4⁺ and CD8⁺ T cells within the tumor were unchanged (Fig. 1D; Supplementary Fig. S1). This decline in intratumoral Tregs resulted in an increase in the ratio of CD8⁺ T cells to Tregs, an indicator of enhanced antitumor immune responses (Fig. 1E).

Discussion

Previous work has suggested that the loss of intratumoral Tregs following DTA-1 is due in part to Treg destabilization. Destabilized Tregs that lose their suppressive capacity could become potent antitumor effector cells due to their responsiveness to tumor antigens. To address the question of whether mDTA-1 treatment causes conversion of intratumoral Tregs into antitumor effector T cells, we used a previously described Treg lineage tracing system. For tumor-infiltrating lymphocyte (TIL) isolation, tumors were mechanically disrupted and digested for 45 minutes at 37 degrees in the presence of collagenase 1 (300 Collagenase Digestion Units/mL; Sigma), DNase I (400 Domains Units/mL; Calbiochem), and Dispase II (1 mg/mL; Roche). The digested tumor material was spun in 40% Percoll Plus (GE Healthcare) to further enrich leukocytes.
mouse, Foxp3-GFP-Cre × ROSA26-loxP-Stop-loxP-tdTomato (10). Foxp3-expressing Tregs in these mice express both GFP and Cre, the latter of which removes a stop codon downstream of the ROSA26 promoter to drive expression of the red fluorescent protein Tomato. In this way, cells stably expressing Foxp3 are marked by both GFP and Tomato (Fig. 2A). Cells that express Foxp3 long enough to allow Cre-mediated activation of the Tomato locus are permanently marked, even if Foxp3 expression is subsequently lost. Such Tomato⁺/GFP⁺ cells are referred to as “exFoxp3 cells.” These cells are present at low frequency in steady-state mice and can be expanded in settings of inflammation (10–12). Similar to a previous report in the B16F10 syngeneic tumor model (17), exFoxp3 cells were present at low frequency in MC38 tumors. These exFoxp3 cells were not enhanced in frequency in tumors as compared with other tissues, suggesting that the tumor microenvironment supports Treg stability (Fig. 2B). ExFoxp3 cells in the tumor microenvironment phenotypically resemble non-Tregs more so than Tregs, as shown by their low expression of the Treg markers CD25 and GITR (Fig. 2C).

When MC38 tumor-bearing Treg lineage tracing mice were treated with mDTA-1, no increase in the numbers of intratumoral exFoxp3 cells occurred, despite the reduction observed in intratumoral Tregs (Fig. 2D). Similar results were observed in the B16 melanoma tumor model (Supplementary Fig. S2). These data indicate that the loss of intratumoral Tregs following treatment with mDTA-1 is not due to conversion of these cells to an exFoxp3 lineage.

mDTA-1 preferentially reduces highly activated intratumoral Tregs

The lack of evidence for mDTA-1-mediated tumor Treg conversion to an alternate lineage, along with previous data linking FcR function to Treg reduction (13), suggested that the observed decrease in Treg numbers is primarily due to cell depletion. However, the specificity of this depletion and the potential impact on persisting Tregs remained unknown. GITR expression was elevated on intratumoral Tregs relative to dLN Tregs and GITRhi Tregs coexpressed elevated levels of Treg activation markers.
including CD44, ICOS, and TIGIT (Fig. 3A). This correlation led us to hypothesize that mDTA-1 treatment might preferentially affect highly activated Tregs. To address this, we performed extensive phenotypic profiling of persisting tumor Tregs at early time points following mDTA-1 treatment. Indeed, highly activated Tregs were the primary target of mDTA-1, as evidenced by the reduced expression of activation and functionality markers by the remaining Tregs (Fig. 3B). This change in the activation and functional status of tumor Tregs occurred within one day of treatment, corresponding with the rapid decline in Treg numbers.

To test whether this change in Treg phenotype was due to a preferential loss of highly activated Tregs or to a downregulation of suppressive markers induced by agonistic GITR signaling, we compared tumor Treg phenotype between mice treated with either mDTA-1 or with a variant containing an N297A mutation in the Fc domain that lacks FcR binding (18, 19). N297A-mutant
mDTA-1 showed agonist activity equivalent to mDTA-1 in activation of NF-kB signaling in splenic and tumor T cells and in T-cell proliferation assays (Supplementary Fig. S3). This suggested that the agonist signaling capacity of these two antibodies is equivalent and is not dependent on FcyR binding, unlike anti-CD40 antibodies that require FcyR binding for their agonist activity (20). When administered in vivo, N297A-mutant mDTA-1 showed similar binding characteristics on intratumoral T cells and had equivalent pharmacokinetics properties as non-mutant mDTA-1 (Supplementary Fig. S4). Using bioluminescence as a readout of Tregs in Fosp3-CDL mice, in which Fosp3 drives expression of GFP, human diphtheria toxin receptor, and luciferase (21), we observed no reduction in intratumoral Treg numbers in N297A-treated mice (Fig. 3C). Flow cytometry, however, did reveal a modest reduction in Treg percentage (Fig. 3D). Importantly, tumor Tregs of N297A-mutant–treated mice also showed little to no reduction in their expression of suppressive markers (Fig. 3E). This suggested that the reduced suppressive phenotype of remaining Tregs in mDTA-1–treated mice was due to a preferential depletion of the most highly activated Tregs rather than signaling-induced downregulation of suppressive markers on a subset of cells. Furthermore, pan-Treg depletion by diphtheria toxin administration in Fosp3-CDL mice reduced intratumoral Tregs to a similar extent as that seen with mDTA-1 (Fig. 3C and D). Unlike mDTA-1, however, persisting Tregs in DT-depleted mice did not show as large a reduction in their expression of suppressive markers (Fig. 3E). This further supported the hypothesis that by targeting GTRAg Tregs, mDTA-1 preferentially reduces a highly suppressive subpopulation. Taken together, these data suggest that in addition to the impact of mDTA-1 on tumor Treg numbers, diminished functionality of persisting Tregs could also contribute to the efficacy of mDTA-1.

Both GTR agonism and Treg depletion are required for reversal of CD8+ T-cell exhaustion and antitumor efficacy of mDTA-1

In the MC38 tumor model, treatment with the N297A variant of mDTA-1 showed intermediate efficacy (Fig. 4A). This was in contrast to previously published results in the CT26 tumor model where N297A showed no efficacy (13), and suggested that the MC38 model might be ideal for further dissecting the relative contributions of GTR agonism versus Treg depletion in the efficacy of mDTA-1. As above, we used Fosp3-CDL mice to test the relative impact of Treg depletion in the absence of GTR signaling. DT-mediated Treg depletion significantly inhibited tumor growth; however, unlike mDTA-1–treated animals, very few DT-treated mice showed a complete regression (CR; 9/10 CR for mDTA-1 vs. 1/10 CR for DT).

In addition to its impact on intratumoral Tregs, one effect of mDTA-1 treatment, which we and others have observed, is increased immune cell activation in the tumor dLN (22). This is noted most simply in the increased size of the dLN and was recapitulated by treatment with N297A mutant mDTA-1 and by DT-mediated systemic Treg depletion (Fig. 4B). Enhanced dLN priming was further observed specifically in CD8+ T cells, which exhibited increased IFNγ production upon stimulation following all treatments (Fig. 4C). Together, these data suggest that both GTR agonism and systemic Treg depletion alone are sufficient to enhance priming of CD8+ T cells in the tumor dLN.

In addition to its effect in the dLN, mDTA-1 treatment also affected the activation status of CD8+ T cells within the tumor. Compared with their dLN counterparts, CD8+ T cells in established MC38 tumors display an exhausted phenotype, as evident by their expression of high levels of inhibitory receptors, including PD-1 and LAG-3 (Fig. 4D; ref. 23). Upon treatment with mDTA-1, expression of these receptors decreased significantly. This change in CD8+ T-cell phenotype was apparent at day 5 following treatment but not at day 1 (data not shown), making it delayed relative to the rapid changes observed in tumor Tregs. Notably, neither DT-mediated Treg ablation nor treatment with N297A mutant mDTA-1 reproduced the reversal in intratumoral CD8+ T-cell exhaustion observed with mDTA-1 (Fig. 4D and E).

In further support of an antigen-experienced exhausted phenotype, intratumoral CD8+ T cells from control mice were competent producers of IFNγ and granzyme B when given a strong ex vivo stimulus (Fig. 4F). As a result, no further enhancement of these effector molecules was evident in treated mice under these ex vivo stimulation conditions (Fig. 4F). Taken together, these data indicate that the combination of both GTR agonism and a reduction in Treg numbers is required for reversal of intratumoral CD8+ T-cell exhaustion and concomitantly for full antitumor efficacy.

Anti-human GTR clinical candidate mimics the effects of mDTA-1 in a humanized mouse tumor model

Lastly, we wished to examine how our observations on the mechanism of action of the anti-mouse GTR antibody mDTA-1 might translate to human cancer patients. One way to circumvent the lack of compatibility of anti-human antibodies in mice is through the use of immunodeficient mice reconstituted with human immune cells, collectively known as humanized mice (reviewed in ref. 24). One such model involves the engraftment of human CD34+ hematopoietic stem cells into immunodeficient NOD.Cg-PrkdcscidIl2rgtm1Wjl (NSG) mice. Several weeks following engraftment, these mice possess human T cells, B cells, and some myeloid cells.

MK-4166 is a humanized anti-GTR agonist mAb that is currently being evaluated in a phase I clinical study in patients with cancer. In order to assess the effects of MK-4166 in an in vivo tumor model...
model, we tested it in hu-CD34+ NSG mice bearing SK-MEL-5 human melanoma tumors. Human CD25+ Foxp3+ Tregs were readily detectable in both spleens (NSG mice lack most tissue-draining LNs) and tumors of humanized mice, and were enriched in the tumor (Fig. 5A), similar to the syngeneic MC38 model (Fig. 1C). Furthermore, these human Tregs expressed high levels of GITR in both sites (Fig. 5B). In contrast to the tumor specificity of the Treg impact seen in the mDTA-1–treated syngeneic model, humanized mice treated with MK-4166 showed significantly reduced Treg frequency in the tumor (Fig. 5A). This reduced Treg frequency resulted in an increased CD8+ T cell to Treg ratio, especially in the spleen (Fig. 5C). Although not highly impacted numerically, intratumoral Tregs were affected at the functional level, as exhibited by reduced expression of the activation marker ICOS upon treatment with MK-4166 (Fig. 5D). These effects corresponded with increased production of antitumor effector cytokines within the tumor (Fig. 5E), which originated from both CD4+ and CD8+ tumor T cells (data not shown). Lastly, when followed over time, MK-4166–treated mice exhibited significantly
Figure 5.
Clinical candidate MK-4166 mimics the effects of mDTA-1 when tested in a humanized mouse tumor model. Hu-CD34^+ NSG mice bearing SK-MEL-5 human melanoma tumors were treated with either MK-4166 or an IgG1 isotype control at 10 mg/kg when tumors reached a volume of approximately 130 mm^3. A–E, 4 days later, spleens and TILs were harvested for analysis of the human immune compartment. A, Representative flow plots show gating of human CD4^+ and CD8^+ T cells among CD45^+ CD3^+ cells, and of Tregs among CD4^+ T cells in the spleen (top) and TILs (bottom). Graphs depict the summarized frequencies of Tregs among total CD3^+ T cells. B, Representative histograms showing GITR expression on human Tregs (blue line), non-Treg CD4^+ T cells (dashed line), and CD8^+ T cells (filled gray line) in the spleen (top) and TILs (bottom) of SKMEL-5 tumor-bearing humanized mice. C, Graph depicts CD8^+ T cell to Treg ratio in spleen and TILs as determined by flow cytometry. D, Representative histogram showing ICOS expression on intratumoral Tregs from isotype- (blue) and MK-4166-treated (red) mice. Graph summarizes median fluorescence intensity (MFI) on intratumoral Tregs for multiple mice. E, Following overnight culture of isolated TILs, levels of IL2 (left) and IFN-y (right) were measured in the culture supernatants. F, Tumor growth was monitored over time following treatment. Data represent the mean ± SEM of 7 mice per group. A and C–E, Each graph point represents an individual mouse and lines represent the mean.
attenuated tumor growth (Fig. 5F). In total, anti-human GITR mAb MK-4166 exhibited more systemic effects in SK-MEL-5 tumor–bearing hu-CD34+ NSG mice than the mouse surrogate mDTA-1 did in WT MC38 tumor–bearing mice with regard to Tregs; however, the overall effects were similar with increased immune activation, attenuated Treg frequency and suppressive phenotype, and overall tumor growth inhibition.

**Discussion**

In this study, we have demonstrated that mDTA-1 treatment in the MC38 tumor model leads to a tumor-specific reduction in not only Treg numbers but also in the functional status of those Tregs that persist. The observed reduction in intratumoral Tregs did not correspond with any observable increase in Treg conversion to an exFoxp3 lineage. Upon examination of intratumoral CD8+ T cells following mDTA-1 treatment, we observed a reversal of an exhaustion phenotype that could not be reproduced with either Treg depletion or agonistic GITR signaling alone. Finally, examination of an anti-human GITR clinical candidate antibody MK-4166 in a humanized mouse tumor model showed similarity to the mouse surrogate in terms of attenuating Treg frequency and suppressive phenotype.

While reduction in intratumoral Treg numbers appears to be a major contributing factor to the efficacy of mDTA-1, multiple lines of evidence suggest that this reduction alone is insufficient to account for efficacy. In support of a function of GITR signaling directly on CD8+ T cells, mice in which effector T cells alone lack GITR expression exhibited incomplete responses to DTA-1 (8). Additionally, previous studies comparing DTA-1 treatment to Treg depletion alone found that Treg depletion was insufficient to reproduce the efficacy of DTA-1 (6, 25, 26). A major caveat of previous studies, however, is that the methods used to deplete Tregs were imprecise and also depleted either effector T cells by using anti-CD25 antibody PC61 (6, 25), or total CD4+ T cells via anti-CD4 antibody (26). In our study, we compared mDTA-1 treatment with Treg depletion in Foxp3-CDL mice using DT administration for specific and systemic deletion of Tregs (21).

We have shown that while DT-mediated Treg depletion promotes a strong antitumor response, these responses are not as potent as those observed with mDTA-1 treatment. This was the case in spite of comparable levels of intratumoral Treg depletion. One possible explanation for the lesser efficacy of DT-mediated Treg depletion versus mDTA-1 is the selectivity of mDTA-1 in targeting the most highly activated Tregs. While nearly all intratumoral Tregs display a highly activated phenotype, the correlation of GITR expression with other functional and activation markers may play a role in the selective loss of the most highly activated Tregs. This was not the case with DT-mediated depletion, and the differences in the functional status of remaining Tregs following either treatment could contribute to the differential efficacy seen between the two. An additional explanation for the differential efficacy could be the role of costimulatory GITR signaling directly on effector T cells. The lack of complete tumor regression in DT-mediated Treg-depleted animals corresponded with a persistent exhaustion phenotype in intratumoral CD8+ T cells that was reversed by treatment with mDTA-1. We believe our approach more definitively demonstrates that Treg ablation alone does not account for the full effects of mDTA-1 and adds further support for the hypothesis that GITR agonism contributes to the efficacy of mDTA-1.

Although robust Treg depletion alone is insufficient to recapitulate the full antitumor effects of mDTA-1, it is necessary. N297A-mutant mDTA-1 is incapable of mediating FcR-dependent Treg depletion that has been shown to be the primary mechanism of Treg depletion for both DTA-1 and CTLA-4 IgG2a antibodies (13, 27, 28). Without this robust depletion, N297A-mutant mDTA-1 efficacy was greatly attenuated, and no reversal of the intratumoral CD8+ T-cell exhaustion phenotype was observed. This suggests that the reversal in CD8+ TIL exhaustion seen with mDTA-1 is due to a combination of intratumoral Treg removal as well as antibody-mediated GITR agonism. Interestingly, N297A-mutant mDTA-1 treatment did lead to a partial but significant reduction in intratumoral Tregs. This suggests that an additional mechanism other than FcγR-mediated cell removal could contribute to DTA-1–mediated Treg depletion, which has not been observed with Fc-mutant CTLA-4 antibody (27). Similarly to nonmutated mDTA-1, N297A mutant mDTA-1 treatment did not enhance Treg conversion in our lineage tracing system (data not shown). However, agonist GITR signaling mediated by N297A mutant mDTA-1 could lead to Treg loss via a signaling mechanism that warrants future study.

We believe that the partial reduction in tumor Tregs, along with the enhanced CD8+ T cell priming in the dLN, is the primary mechanism behind the tumor growth inhibition exhibited by N297A mutant mDTA-1; however, other mechanisms not examined in the current study may also contribute to the observed efficacy.

In this study, we have followed up on previous reports that DTA-1 may destabilize Treg lineage commitment. We felt this was an important undertaking as previous reports of Treg lineage instability have primarily arisen in the context of highly inflammatory microenvironments that are quite different from the immune-suppressive tumor microenvironment that is generally believed to promote Treg induction and function. Previous studies reporting Treg instability following DTA-1 treatment relied primarily on the adoptive transfer of Foxp3+ Tregs and found that these cells were more prone to loss of Foxp3 expression in DTA-1–treated recipients (9). This approach comes with the potential caveat that even a highly pure population of sorted Tregs may contain rare contaminating Foxp3− cells that can outgrow cotransferred Tregs and skew results. Indeed, the outgrowth of such a population would be predicted to be enhanced by the costimulatory effects of DTA-1. We have circumvented this caveat in the present study by using Foxp3-lineage tracing mice. By this approach, we have confirmed the findings of others that Tregs within the tumor microenvironment are relatively stable compared with those found in other peripheral tissues (17). However, we did not find any evidence for enhanced conversion of Tregs to exFoxp3 cells upon treatment with mDTA-1. Although this does not confirm previous reports of DTA-1–mediated intratumoral Treg instability (9), perhaps for the reasons outlined above, our data on the reduced functional phenotype of those Tregs that persist following mDTA-1 treatment strongly agree with previously published data on the emergence of a Treg population with reduced suppressive properties following DTA-1 treatment (9).

Despite a lack of full conversion of tumor Tregs to an alternate T-cell lineage, this weakening of tumor Treg suppressive capabilities, coupled with an overall reduction in their numbers, is likely critical to the antitumor efficacy of DTA-1.

As with nearly all novel disease therapeutics, it is difficult to predict with confidence how efficacy in mouse models will
translate to human patients. Previous work by Sanmamed and colleagues has shown efficacy of anti–PD-1 and anti–CD137 clinical candidate antibodies in a humanized mouse model where immunodeficient mice were reconstituted with either allogeneic or patient-derived PBMCs at the time of tumor transfer (15). In our experiments testing an anti-human GITR mAb in tumor-bearing humanized mice reconstituted with CD34+ hematopoietic stem cells, we observed both similarities and differences in terms of impact on Tregs as compared with mDTA-1 treatment of conventional tumor-bearing mice. The most obvious difference between the two was the difference in tissue specificity. While we and others have shown that mDTA-1 specifically affects tumor Tregs and leaves systemic Tregs relatively unaltered in syngeneic mouse models, MK-4166 greatly reduced peripheral Treg frequency in humanized mice. The exact reasons for this difference remain unknown. One hypothesis is the potential differential distribution of myeloid populations capable of carrying out FcγR-mediated cell depletion in the two models (13). Humanized mice contain both mouse and human derived myeloid populations. Detailed characterization of these populations in both the periphery and tumor remains to be done in order to more fully understand their likely interactions with a humanized IgG1 antibody. Although not highly impacted numerically, intratumoral Tregs were significantly affected at the functional level, as exhibited by reduced expression of the activation marker ICOS upon treatment. These effects directly correlated to increased production of antitumor effector cytokines within the tumor. These results suggest that MK-4166 depletes Tregs in settings where FcγR-effector functions are intact while the effects of GITR agonism on Tregs are more apparent in settings where they are deficient. While subcutaneous tumor engraftment in humanized mice provides a means to examine the impact of a humanized mAb on human immune cells in an in vivo setting, it remains far removed from a trial in human patients. Nonetheless, the observed similarities between mDTA-1 in conventional mice and anti-human GITR mAb in humanized mice are notable. Both resulted in increased ratios of CD8+ T cells to Tregs; be it in the tumor or in the periphery, and both resulted in a reduced functional phenotype of remaining intratumoral Tregs and enhanced immune activation that corresponded with inhibition of tumor growth.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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