Published in final edited form as:

Expert Opin Biol Ther. 2016 May ; 16(5): 655–674. doi:10.1517/14712598.2016.1152256.

Enhancing the safety of antibody-based immunomodulatory cancer therapy without compromising therapeutic benefit: Can we have our cake and eat it too?:

Expert Opinion on Biological Therapy

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Abstract

Introduction—Monoclonal antibodies (mAbs) targeting checkpoint inhibitors have demonstrated clinical benefit in treating patients with cancer and have paved the way for additional immune-modulating mAbs such as those targeting costimulatory receptors. The full clinical utility of these agents, however, is hampered by immune-related adverse events (irAEs) that can occur during therapy.

Areas covered—We first provide a general overview of tumor immunity, followed by a review of the two major classes of immunomodulatory mAbs being developed as cancer therapeutics: checkpoint inhibitors and costimulatory receptor agonists. We then discuss therapy-associated adverse events. Finally, we describe in detail the mechanisms driving their therapeutic activity, with an emphasis on interactions between antibody fragment crystallizable (Fc) domains and Fc receptors (FcR).

Expert Opinion—Given that Fc-FcR interactions appear critical in facilitating the ability of immunomodulatory mAbs to elicit both therapeutically useful as well as adverse effects, the engineering of mAbs that can effectively engage their targets while limiting interaction with FcRs might represent a promising future avenue for developing the next generation of immune-enhancing tumoricidal agents with increased safety and retention of efficacy.

Keywords

Accessory cell; adverse events; antibody; antitumor; biologic; cancer; checkpoint inhibitor; costimulatory agonist; cytotoxic; effector; efficacy; Fc; Fc receptor; FcR; immunomodulatory; immunosuppression; immunotherapy; irAE; mAb; monoclonal; side effects; tolerance; toxicity; tumor microenvironment; tumoricidal

Declaration of interests

A Vella and A Adler received grants from the NIH (AI094640 and CA109339). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

1. Introduction

There is an extensive list of drugs and treatment options at the disposal of clinical oncologists. Surgery, chemotherapy, and radiation have long been the standard of care therapies for most cancers. Nevertheless, surgery cannot eliminate highly metastatic disease, and chemotherapy and radiation cause significant side effects due to their inability to specifically target malignant cells. Furthermore, tumors that may initially respond to therapy often develop resistance, leading to disease recurrence.

The identification of genetic mutations common to particular types of cancer has facilitated the development of targeted therapies, such as the tyrosine kinase inhibitor imatinib, which is highly effective in chronic myelogenous leukemia (CML) patients harboring the corresponding Bcr-Abl fusion gene mutant ¹. Monoclonal antibodies (mAbs) are also used in cancer treatment. These mAbs can be categorized into two groups based on their function. Tumor-targeting mAbs function within the tumor microenvironment (TME), where they bind directly to tumor cells or stromal trophic factors ². The vascular endothelial growth factor (VEGF) mAb inhibitor bevacizumab, for example, interferes with survival-promoting signaling pathways important to tumor progression ^{3,4}. The anti-CD20 mAb rituximab works by a different mechanism, first binding to tumor cells (as well as to some healthy cells) expressing surface CD20, then engaging innate effector mechanisms for elimination ⁵⁻⁸. Tumor-targeting mAbs can also be chemically linked to radioactive or toxic entities so that the cytotoxic payload is selectively delivered to tumor cells without harming healthy tissue ^{9,10}.

The second group of mAbs modulate the immune system and work by promoting tumoricidal activity in a variety of immune effector cells by lowering their thresholds for initiating antitumor responses ¹¹. Tumors are generally considered to be non-immunogenic since they arise from the body's own cells ¹²; thus these mAbs are in essence "breaking" peripheral tolerance to self. These immunomodulatory mAbs can work by either antagonizing cell surface receptors that transmit inhibitory signals (i.e., checkpoint inhibitors) or, alternatively, activating stimulatory receptors (i.e., costimulatory agonists). The net effect in both cases is an enhancement of tumoricidal immune effector cell activity. This review describes the antitumor efficacies and the associated adverse events seen with antibody-based immunomodulatory cancer therapies and then considers if it might be possible to optimize their design in order to limit adverse events without compromising therapeutic benefit.

2. Tumor immunity

The immune system constantly surveys the body for potential threats, such as pathogens, with the goal of neutralizing them to maintain homeostasis. The T lymphocyte (T cell) compartment of the adaptive immune system is particularly effective at employing this "search and destroy" strategy. Individual T cells possess exquisite specificities endowed by the expression of a unique T cell receptor (TCR) capable of recognizing distinct peptides presented by major histocompatibility complex (MHC) molecules on the surface of cells. Due to recombinations between V, D, and J gene segments that comprise the TCR, the

diversity of specificities is immense, allowing for the recognition of virtually any antigen^{13, 14}. Given the potential for many of these TCRs to recognize self-antigens, a variety of tolerance mechanisms must be employed, operating both centrally during thymic development^{15, 16}, as well as in the periphery^{17–20}, to prevent autoimmunity. Consequently, tumor cells, are more difficult to detect since the majority of their antigens overlap with those expressed in their healthy progenitors to which T cells have already been tolerized. In some cases, vaccination against these non-mutated tumor-associated antigens (TAAs) can elicit effector T cells that simultaneously mediate tumor immunity and autoimmunity^{21–23}. Alternatively, the genetic instability characteristic of malignant cells^{24, 25} gives rise to mutations in protein-coding genes, resulting in the formation of tumor-specific (neo)-antigens (TSAs) that can be targeted by T cells without causing autoimmune disease^{26, 27}.

Naïve T cells are activated when they encounter cognate peptides complexed to MHC molecules on the surface of antigen-presenting cells (APCs). This event, referred to as “signal 1”, is the first of three sequential signals required to program proliferation, differentiation, and survival. Dendritic cells (DCs) represent the most potent APC population, in part owing to their efficiency in capturing and processing antigens into peptide fragments suitable for MHC presentation. Upon sensing pathogen-associated molecular patterns (PAMPs), DCs are also induced to express costimulatory ligands and pro-inflammatory cytokines, delivering signals 2 and 3, respectively^{28–30}. Costimulation occurs in two waves. First, the immunoglobulin (Ig) superfamily molecules CD80 (B7.1) and CD86 (B7.2) on DCs ligate CD28 on the antigen-responding T cells to facilitate the initial phase of activation^{31–33}. Tumor necrosis factor receptor (TNFR) superfamily members then deliver a second costimulatory wave. This process fine-tunes T cell responses by regulating the magnitude of clonal expansion, determining which particular cytokines the T cells become capable of secreting, and dictating whether long-lived memory is established^{34, 35}. The cytokines that constitute signal 3 further contribute to fine-tuning the T cell response³⁶.

In the absence of inflammation, however, steady-state DCs express only minimal amounts of costimulatory ligands and cytokines. Under these conditions, T cells encountering cognate antigens (e.g., self-antigens acquired from healthy tissues by DCs) undergo an abortive/tolerogenic response^{37–39}. This process not only serves to prevent autoimmunity mediated by self-reactive T cells that escape negative selection in the thymus⁴⁰, but also can dampen tumor immunity by tolerizing T cells specific for TAAs^{23, 41} and TSAs^{42, 43}. A critical factor in programming effective antitumor T cell responses is the amount and variety of costimulation and cytokines accompanying DC presentation of tumor epitopes. The PAMPs that help facilitate the potent activation of DCs during infection are generally absent from tumor sites, often resulting in a tolerogenic outcome (as described above). Nevertheless, productive T cell responses to tumor antigens do occur in some cases^{44–46}. DC activation can be achieved when tumor cells undergoing certain forms of programmed death⁴⁷ release damage-associated molecular patterns (DAMPs), such as heat shock proteins⁴⁸, uric acid⁴⁹ and HMGB1⁵⁰, which can act in a manner analogous to that of PAMPs.

Even when tumor-reactive T cells are successfully primed, with the capacity to migrate into tumors and kill target cells, the efficiency and overall impact of the response can be inadequate. In a process termed “immunoediting”, the most immunogenic tumor cell

variants are eliminated during the initial stages of tumorigenesis, while less immunogenic tumor cells are spared and gradually expand⁵¹. Whether T cells become tolerant of tumor antigens at the earliest stages of tumorigenesis due to a lack of DC activation, or alternatively following a period of immunoediting, clinically detectable tumors are inherently non-immunogenic. Furthermore, the resulting non-immunogenic tumor engages a variety of powerful immunosuppressive mechanisms that can thwart the activity of infiltrating effector T cells and foster a tumor-favorable microenvironment. For example, the recruitment of regulatory T cells (Tregs)^{52–55} and myeloid-derived suppressor cells (MDSCs)⁵⁶ establishes an immune barrier around tumor sites, effectively protecting malignant cells from immune intrusion. Additional mechanisms include the secretion of immune-regulating cytokines such as transforming growth factor beta (TGF- β)^{57, 58} and enzymes such as indoleamine 2,3-dioxygenase (IDO)⁵⁹, all of which are capable of altering the immune response within the TME.

An understanding of the mechanisms underlying tumor-induced immunosuppression is paramount to the development of effective cancer therapies, and as such has become a major focus of investigation; a comprehensive review on the subject is provided by Rabinovich and colleagues⁶⁰. To be effective, T cell-based immunotherapies will likely need to compensate for insufficiencies during the initial phase of T cell priming, as well as confer the capacity to overcome potent immunosuppression within the tumor microenvironment. Therefore, the most effective cancer therapies will likely consist of multiple agents targeting a variety of cancer-promoting pathways and mechanisms. Combination therapies have already begun to showcase their clinical potential, in some cases demonstrating enhanced efficacy and superiority over standard of care monotherapies. Although combining multiple agents can cause more frequent and severe adverse events, increases in efficacy may exceed increases in toxicity, suggesting combination approaches as one possibility for improving safety without sacrificing therapeutic benefit.

3. Checkpoint inhibitors

The immune system prevents excessive inflammation or autoimmunity via the engagement of inhibitory feedback networks. A commonly used analogy equates the immune system to an automotive vehicle with the antigen-stimulated TCR representing the transmission in drive, CD28 costimulatory receptor acting as the accelerator, the TNFR costimulators functioning as turbo boosters, and checkpoint inhibitory receptors serving as the brake.

Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) was the first checkpoint receptor identified and is the most extensively studied. Following the initial activation of naïve T cells, in which DCs provide signal 1 (MHC-peptide) along with signal 2 in the form of B7.1 and B7.2 ligating CD28, intracellularly stored CTLA-4 is relocated to the T cell surface where it competes with CD28 for binding to B7.1 and B7.2⁶¹, thereby helping to avoid excessive CD28-mediated stimulation. Upon ligation, CTLA-4 engages the protein phosphatases SHP2 and PP2A, which deactivate signaling proteins downstream from TCR engagement⁶². The net effect is the prevention of T cell hyper-responsiveness^{63–67}.

Mice harboring a null mutation in the gene encoding CTLA-4 develop fatal lymphoproliferative disease characterized by autoreactive T cell infiltration and extensive tissue destruction⁶⁸. This dramatic phenotype results not only from the predicted effect of releasing the brakes on self-reactive T cells, but also from impairment in the function of Tregs, which constitutively express surface CTLA-4 to maintain tolerance and suppress auto-reactivity^{19, 69–73}. That CTLA-4 functions on multiple cell types to maintain immune homeostasis raised the critical question of whether it could be targeted therapeutically without unleashing fatal adverse events. Nevertheless, preclinical studies using a variety of mouse tumor models demonstrated that a blocking mAb to CTLA-4 altering the activities of both tumor-reactive effector T cells and Tregs can produce therapeutic benefit, albeit with toxicity^{74–78}.

This work paved the way for the development of a humanized CTLA-4 antagonist, ipilimumab⁷⁹. A decade of clinical trials culminated in an international phase III trial that led to its 2011 approval by the FDA for treating melanoma, making it one of the first immune-based biologic therapies, and the first checkpoint inhibitor, to be used clinically⁸⁰. In this study, 676 patients with late-stage melanoma were given either ipilimumab, a vaccine targeting the melanoma TAA gp100, or ipilimumab plus vaccine. Objective response rates (ORR) for ipilimumab alone and gp100 alone were 10.9% and 1.5%, respectively. Importantly, ipilimumab (with or without vaccine) boosted median overall survival (OS) by 10 months, compared to 6.4 months for gp100 vaccination alone (Table 1). These encouraging results earned for ipilimumab its approval for the treatment of unresectable or metastatic melanoma.

Another checkpoint molecule, programmed cell death protein 1 (PD-1), is expressed primarily on already-activated effector T cells and regulates their activity in the periphery^{81, 82}. Upon binding of its ligands, PD-L1⁸³ or PD-L2⁸⁴, PD-1 engages phosphatases that inactivate signaling molecules downstream of the TCR to limit proliferation, survival, and cytokine production⁸⁵. PD-1 is highly expressed on CD8⁺ T cells residing within the TME⁸⁶. PD-L1 can be constitutively expressed by tumor cells^{87, 88} due to oncogenic signaling pathway aberrations^{89, 90} or induced in response to localized T cell secretion of cytokines (e.g., interferons)^{91–93} in a process referred to as adaptive immune resistance⁹⁴. Tumor-infiltrating myeloid cells and lymphocytes can express PD-L1, and their co-localization and interaction with PD-1-expressing cells further define PD-1-mediated activity within the TME^{95, 96}. PD-1 also plays a role in Treg function⁹⁷, and thus, similar to CTLA-4 blockade, targeting the PD-1/PD-L axis may enhance tumoricidal capacity through effects on both effector and regulatory T cells. Indeed, preclinical testing in mouse models demonstrated that mAb-mediated disruption of PD-1/PD-L1 interaction can boost tumor immunity^{98, 99}.

Two humanized antagonist mAbs targeting PD-1, pembrolizumab and nivolumab, have been approved after yielding significant therapeutic responses in phase III clinical trials^{100–102}. A double-blind phase III trial of 418 patients receiving either nivolumab or dacarbazine found ORR of 40.0% and 13.9%, respectively, and 1 year OS rates of 72.9% and 42.1%, respectively¹⁰³ (Table 1). Response to treatment has also been shown to correlate with tumor expression of PD-L1¹⁰⁰. Additional clinical data show similarly encouraging

therapeutic responses in patients treated with anti-PD-L1 mAb ¹⁰⁴, and provide preliminary evidence in support of using intratumoral PD-L1 expression as a biomarker to determine likelihood of therapeutic success on a patient-to-patient basis ^{95, 96, 105, 106}.

Checkpoint-inhibiting biologics are now being used routinely to treat patients with malignant melanoma, and numerous ongoing clinical studies raise the likelihood that they will soon be approved for treating patients with a variety of cancers including kidney, bladder, prostate, and lung ⁷⁹. Recently, nivolumab was approved for additional indications including non-small cell lung carcinoma (NSCLC) and renal cell carcinoma (RCC). Thus, in addition to the durability of treatment response, the potential for use in a number of different clinical conditions sets these mAbs apart from other oncologic agents. As shown in Table 1, checkpoint inhibitors and costimulatory agonists have demonstrated efficacy in both solid tumors and hematologic malignancies ¹⁰⁷. Particularly noteworthy is their efficacy in solid tumors, which can be especially difficult to treat due to the existence of localized immunosuppression in the TME ^{52–59}. Intriguingly, immunomodulatory mAbs enable antitumor activities that are relatively nonspecific with respect to cancer subsets, but relatively specific in terms of preferential targeting of cancerous cells while for the most part sparing healthy cells, in essence, approaching the “holy grail” of cancer therapy.

mAbs blocking both CTLA-4 and PD-1/PD-L1, enhance tumor immunity by disrupting T cell inhibitory signaling. However, they act during distinct early (CTLA-4) and late (PD-1) phases of the anti-tumor response. Thus, the possibility is raised that a combination therapy might be superior to either agent alone. Improved response has been confirmed in mice ¹⁰⁸, and human data obtained thus far have been encouraging. In one study the combination of nivolumab and ipilimumab given concurrently yielded objective clinical responses ¹⁰⁹ exceeding those reported previously for either monotherapy ^{80, 100}, although direct comparison was not made. While objective-response rates may not predict long-term benefit to patients, and survival data are needed, these early studies suggest a potential advantage of combination treatment strategies ¹⁰⁹.

More recently, a phase III study ¹⁰⁶ comparing nivolumab alone, ipilimumab alone, and combination of the two in previously-untreated metastatic melanoma (major findings highlighted in Table 2), demonstrated greater progression-free survival (PFS) for nivolumab, alone or in combination (both 14.0 months), compared with ipilimumab alone (3.9 months). Notably, in the context of PD-L1-positive tumors, the addition of ipilimumab to nivolumab did not improve PFS but did increase the overall rates of grades 3/4 adverse events, from 43.5% to 68.7%. In this case it would appear that combination therapy is not as safe as nivolumab monotherapy (Table 2). However, combination therapy proved superior efficacy to either monotherapy in the context of PD-L1-negative tumors (Median PFS of 11.2 months, compared to 5.3 months and 2.8 months for nivolumab and ipilimumab, respectively). Although the advantage over nivolumab monotherapy dissipated by 16 months of treatment (not shown in Table 2), these data suggest the possibility of value in combined therapy regimens ¹⁰⁶. Although this study showed no evidence of synergy, the additive effects of combination therapy seen with PD-L1-negative tumors provides some evidence that this multi-pronged approach may offer unique advantages over single-agent therapies in some tumor types. Nonetheless, definitive conclusions from this study regarding the value of

supplementing nivolumab with ipilimumab cannot be drawn until overall survival data is obtained.

4. Costimulatory agonists

Agonist mAbs targeting costimulatory receptors on antigen-primed T cells represent the second major category of immunomodulatory antibodies. Primarily targeting receptors of the TNFR superfamily that provide the second wave of signal 2, these antibodies are designed to mimic the activity of endogenous costimulatory ligands whose availability tends to be low in the context of tumor antigen presentation. In contrast to Ig superfamily costimulatory receptors such as CD28 that modulate activity of signaling proteins located proximally to the TCR, TNFR superfamily costimulatory receptors engage various combinations of TNFR-associated factors (TRAFs) 1–6, which control several downstream signaling pathways that include NF- κ B and MAP kinase^{34, 35}.

CD40 is a TNFR family member expressed on DCs that, when bound by its ligand CD40L (CD154) expressed on cognate CD4⁺ helper T cells, induces expression of MHC molecules, costimulatory ligands (B7.1 and B7.2) and cytokines such as IL-12, which collectively function to boost the response of CD8⁺ cytotoxic T lymphocytes (CTL)^{110–113}. A CD40 agonist mAb can thus activate DCs when CD4⁺ T cell help is unavailable and lead to robust effector T cell responsiveness and antitumor immunity^{114–116}.

Agonists can also directly target TNFR costimulatory receptors on T cells. For instance, CD134 (OX40) expressed on T cells following TCR stimulation^{117, 118} can be ligated by OX40L (CD252) expressed on activated, but not steady state, DCs¹¹⁹ to program T cell effector differentiation, survival, and memory^{118, 120–122}. CD134 agonist thus enables antigen-primed T cells to develop potent effector and tumoricidal capacity under otherwise tolerogenic (i.e., steady state) conditions^{123–130}. Similarly, T cell responsiveness and tumor immunity in mice can also be elicited with agonists to several other T cell TNFR family costimulatory receptors such as CD137 (4-1BB)¹³¹, glucocorticoid-induced TNFR-related protein (GITR)¹³² and CD27¹³³.

Extensive mouse studies characterizing the therapeutic potential of TNFR agonists have fueled ongoing efforts to develop humanized therapeutics. Although clinical testing of these agents has lagged behind that of the checkpoint inhibitors by several years, initial results for costimulatory agonists have been encouraging. For instance, a phase II trial evaluating the CD40 agonist dacetuzumab for use in the treatment of relapsed diffuse large B-cell lymphoma demonstrated clinical benefit in a subset of patients¹³⁴, complementing previous data from phase I testing of this agent in other cancers, which found only moderate toxicities associated with therapy^{135–137}. For example, one study demonstrated that out of 44 patients with multiple myeloma treated with dacetuzumab, adverse events of mild cytokine release syndrome, non-infectious ocular inflammation, and elevated liver enzymes were infrequent and well tolerated, with less than 10% experiencing serious toxicities¹³⁷. Additional phase I trials evaluating dacetuzumab in other cancers such as non-Hodgkin's lymphoma (NHL)¹³⁵ and chronic lymphocytic leukemia (CLL)¹³⁶ have generated similar results.

CP-870,893, a fully-human IgG2 mAb considered to be a more potent anti-CD40 agonist, has yielded relatively encouraging efficacy data, including a phase I ORR of 20% in combination with carboplatin and paclitaxel to treat various advanced solid tumors¹³⁸ (Table 1) and another phase I ORR of 19% with 4 partial responses (PRs) in combination with gemcitabine to treat advanced pancreatic ductal adenocarcinoma (PDA)¹³⁹, with generally tolerable side effects. CP-870,893 has since been licensed to Roche and is now, under the name RO7009789, undergoing phase I testing in patients with locally advanced and/or metastatic solid tumors and newly diagnosed resectable PDA.

Alternatively, a mouse mAb agonist specific for human CD134 given in a phase I trial to patients with various advanced cancers elicited objective clinical responses (regression of at least one tumor nodule) in 12 out of 30 patients¹⁴⁰ (Table 1). Although none of the patients demonstrated responses according to Response Evaluation Criteria in Solid Tumors (RECIST, tumor shrinkage of at least 30%), the fact that these results were achieved using a murine mAb that could only be given in a single course (due to the production of human anti-mouse antibodies (HAMA) in patients) is highly encouraging as it suggests that fully humanized CD134 agonists might elicit significant tumor regression, especially if given in multiple courses. A humanized CD137 agonist urelumab has also demonstrated therapeutic activity in phase I testing¹⁴¹, although liver toxicities led to the termination of phase II testing (Table 1). It was later determined that the toxicities that had occurred were dose-dependent, and careful dosing of the agent could avoid liver damage. Humanized agonists to other TNFR members are currently in various stages of clinical development^{142–144}.

Given the possibility that a variety of humanized TNFR agonists may gain FDA approval for the treatment of human cancer patients, it would be useful to consider if they might be combined to improve therapeutic efficacy, like the checkpoint inhibitors^{108, 109}. The rationale for combining agonists targeting different TNFR family members stems from their non-identical tissue expression patterns as well as the partial (but not complete) overlap in the signaling pathways they engage. These subtle differences likely reflect the potential of each member to uniquely contribute to the fine-tuning of T cell responses needed to optimally handle particular immunogenic challenges^{34, 35}. In a clinical setting, it might be possible to exploit this variation by combining particular TNFR agonists to tailor optimal antitumor responses¹⁴⁵. For instance, co-administration of agonists to CD134 and CD137^{146–150} or death receptor 5 (DR5, also known as TRAIL receptor 5) plus CD40 and CD137¹⁵¹ can be synergistic in programming T cell clonal expansion and effector function enabling the control of tumor growth in mice. Effective combination strategies might also involve the administration of a checkpoint inhibitor with a TNFR agonist, as has been demonstrated with anti-CTLA-4 plus anti-CD137¹⁵² and also with anti-PD-1 plus anti-CD134¹⁵³.

5. Adverse events

The elicitation of adverse events is a risk inherent to virtually any type of therapy. With immune-based therapies, adverse events can potentially involve several mechanisms, which vary depending on the particular nature of therapy. For example, therapeutically-administered cytokines can give rise to nonspecific, systemic immune reactivity due to their

widespread sites of action, whereas other immunotherapies such as adoptive cell therapies, tumor vaccines, and immunomodulatory antibodies tend to trigger more localized tissue destruction due to their more targeted and specific immune cell activation ¹⁵⁴. With immunomodulatory mAbs, tumoricidal effector T cells that recognize non-mutated TAAs can destroy healthy cells expressing that same TAA. The classic example of this type of immune-related adverse event (irAE) is vitiligo, in which T cells primed to non-mutated melanocytic antigens destroy both melanoma cells and healthy melanocytes ^{22, 155}. While vitiligo may be an acceptable trade-off for clearing aggressive melanomas (and other cancer types), autoimmune disease obviously would be more problematic when treating tumors derived from vital organs.

Another mechanism leading to irAEs is the disengagement of inhibitory pathways critical for maintaining tolerance to self and immune homeostasis. For instance, since part of the overall mechanism by which ipilimumab boosts tumor immunity involves neutralizing the function of Tregs ^{76, 78}, it might be expected that ipilimumab-treated patients would experience irAEs that would otherwise be kept in check by Treg oversight. Consistent with this prediction, in the landmark melanoma phase III clinical trial mentioned previously, 60% of ipilimumab-treated patients experienced irAEs, 10–15% of which were severe (grades 3/4) that included diarrhea, vitiligo, colitis, and endocrinopathies, as well as infusion reactions ⁸⁰ (Table 1). Careful examination of the specific mechanism(s) involved for individual antibodies (shown for a representative sample of mAbs in Table 4) can also shed light on how specific toxicities arise due to therapy. For example, because anti-CTLA-4 works, in part, by depleting Tregs and/or altering their function, the occurrence of diarrhea, colitis, and other gastrointestinal (GI) manifestations can be attributed to the abundant Treg populations located in the GI tract and their critical role in maintaining immune tolerance ¹⁵⁶. Tissue-specific and antibody-specific adverse events are discussed in greater depth elsewhere ¹¹.

In contrast, irAEs caused by targeting the PD-1 axis tend to be fewer in frequency and less severe ^{100, 101, 104}. Early human testing revealed tolerability of adverse events in addition to impressive efficacy, with response rates ranging from 18–27% when used to treat NSCLC, RCC, and melanoma, with grades 3/4 adverse events occurring in 14% of patients ¹⁰⁰. Adverse events observed during anti-PD-1 therapy have included hepatitis, colitis, hypophysitis, thyroiditis, pneumonitis, and vitiligo (Table 1). Antibodies targeting PD-L1 have demonstrated response rates and safety profiles comparable to those targeting PD-1 ¹⁰⁴. This difference in adverse event profiles, along with the fact that PD-1 blockade can be effective in ipilimumab-refractory melanoma ¹⁰⁹, suggest that these particular checkpoint inhibitors act via distinct cellular and molecular mechanisms. PD-1/PD-L1 interactions primarily take place peripherally at sites where activated T cells interact with PD-L1/2-expressing cells (e.g., within the TME), whereas B7/CD28/CTLA-4 interactions occur primarily in lymphoid organs ¹⁵⁷ harboring bountiful populations of T cells, and Tregs in particular (e.g., Peyer's patches in the lining of the small intestines). Thus, the action of mAbs targeting PD-1 are likely concentrated within the TME as opposed to anti-CTLA-4 mAbs, manifesting in a decreased likelihood of off-target effects and greater tolerability.

Combination therapies targeting CTLA-4 and PD-1 have generated positive data showing that combining immunomodulatory mAbs might have advantages over monotherapy with either agent, albeit with greater side effect frequency and severity. The phase 3 study discussed previously and outlined in Table 2 found that combination of nivolumab plus ipilimumab, compared with nivolumab alone, resulted in a greater incidence of grades 3/4 adverse events (68.7% versus 43.5%, respectively ¹⁰⁶). There were no drug-related deaths seen with combination therapy, and most adverse events (85–100%) could be managed successfully using standardized treatment guidelines, including steroids and infliximab ¹⁰⁶, but endocrine-related events were the most resistant to management. Thus, combined therapy targeting CTLA-4 and PD-1 might be advantageous in patient subsets determined by screening for the presence of predictive biomarkers, such as tumor PD-L1 expression.

Differences among checkpoint inhibitors are also reflected in their dosing regimens (outlined in their respective package inserts). For the treatment of melanoma, ipilimumab, for example, is given every 3 weeks for a total of four doses, at which point therapy must be stopped. On the other hand, nivolumab, with its milder side effect profile, is given every 2 weeks indefinitely, until the patient experiences either lack of clinical benefit or unacceptable toxicity. Nivolumab can also be combined with ipilimumab, with the two agents being co-administered every 3 weeks for 4 doses, after which point the normal dosing scheme of nivolumab is followed. Importantly, nivolumab is also indicated for NSCLC and RCC. Pembrolizumab, the other approved PD-1 antagonist, is currently indicated for melanoma and NSCLC, and is given every 3 weeks. For either anti-PD-1 therapy, treatment is maintained regardless of whether the tumor completely resolves or its size has stabilized. Thus a major advantage of anti-PD-1 mAbs is greater tolerability, making them more suitable for maintenance therapy in appropriate patient subsets.

Costimulatory agonists might be expected to elicit irAEs associated with the production of inflammatory mediators that drive T cell expansion and effector differentiation, but on the other hand also have potential toxic properties when present in large quantities systemically. For instance, CD40 ligation on DCs induces secretion of IL-12 ¹¹⁰, which facilitates beneficial tumoricidal effector T cell function ¹⁵⁸, but when present at high systemic levels can be toxic ¹⁵⁹. Perhaps the most dramatic example of this type of irAE occurred during a phase I clinical trial evaluating the safety of TGN1412, a superagonist to CD28 that had been designed to activate Tregs, but unfortunately ended in disaster when all six trial participants in the treatment group rapidly developed symptoms characteristic of cytokine release syndrome (cytokine storm involving rapid spikes in systemic TNF- α , IFN- γ , IL-6, IL-2, IL-1 β and others, as illustrated in Fig. 1), such as headache, nausea, diarrhea, vasodilation, hypotension, and eventually (non-fatal) multi-organ failure ¹⁶⁰.

Several mechanisms have been suggested for cytokine release induced by TGN1412. One viewpoint implicates a toxic inflammatory response due to T cell hyper-activation mediated by excessive costimulatory receptor stimulation (depicted in Fig. 1B, in this case using CD137 as a representative example) ^{161, 162}. Another school of thought points to excessive activation of innate effector cell populations (e.g., monocytes and NK cells) via antibody fragment crystallizable (Fc) engagement of Fc receptor (FcR) (discussed in the next section), thus triggering the massive release of pro-inflammatory cytokines ^{163, 164} (Fig. 1C).

Although the severe symptoms elicited by TGN1412 were impossible to predict, it was already known that infusion of immune-reactivity antibodies can trigger responses resembling cytokine release syndrome ^{165–167}. It is likely that both mechanisms were, at least to some extent, involved in mediating toxicity in these patients. Evidence supporting the latter assertion comes from recent work describing contributions of stimulation-induced FcγR signaling to the induction of pro-inflammatory cytokines ^{168, 169}, in addition to the suggested role of pro-inflammatory M1 macrophages in mediating immunotherapy-associated toxicity ¹⁷⁰.

In the case of the anti-CD137 agonist urelumab, phase II testing had to be terminated due to unanticipated severe toxicity in a substantial portion of patients. Grade 4 hepatitis was the most serious adverse event, although additional toxicities were commonly noted, including fatigue, rash, diarrhea, and neutropenia (Table 1). These toxicities may have been related in part to the production of pro-inflammatory cytokines, such as types I and II interferons (IFN) and tumor necrosis factor alpha (TNF-α) ^{107, 141}. These results were perhaps not entirely surprising since mouse studies have demonstrated that CD137 agonist administered chronically or at high dosage can induce immunologic dysfunction leading to organ-specific toxicities that correlate with increased CD8⁺ T cell infiltration into those tissues and organs ^{171, 172}. Subsequent clinical testing of CD137 agonists has utilized modified protocols with lower dosages, avoiding the dose-dependent hepatotoxicities observed previously ¹⁴².

During initial phase I clinical testing of a CD134 agonist for the treatment of various advanced cancers, including melanoma, RCC, squamous cell carcinoma (SCC) of the urethra, prostate cancer, and cholangiocarcinoma, fewer irAEs occurred and an acceptable toxicity profile was observed ¹⁴⁰. 12 out of 30 patients had regression of at least one tumor nodule, and in an additional 6 patients there were no changes in target lesion measurements (Table 1); however none of the patients demonstrated responses according to RECIST criteria, which mandate tumor shrinkage of at least 30% to be considered positive clinical responses ¹⁴⁰. Differences in adverse events seen with anti-CD134 versus anti-CD137 may in part be attributed to the more widespread expression pattern of CD137 that encompasses not only activated T cells and Tregs but also several innate cell types such as natural killer (NK) cells and DCs ^{173–175}, in contrast to CD134, whose expression appears to be more restricted to T cell subsets.

Management of irAEs should be guided by the severity of symptoms and typically involves early detection, temporary cessation of therapy, careful monitoring, supportive care such as oral hydration, and tailored symptomatic relief. For more severe irAEs, immunosuppressive drugs such as corticosteroids are often successful in mitigating symptoms, and infliximab (which neutralizes TNF-α) can be helpful in managing cytokine release syndrome. Additionally, there are clinical algorithms to aid in determining the appropriate strategy to best manage irAEs ^{107, 154, 176, 177}. As mentioned previously, symptomatic relief using these agents has been shown not to negatively impact treatment efficacy, which substantially enhances the clinical utility of immunomodulatory mAbs.

6. Mechanistic contributions of Fc-FcR interactions

An understanding of the structural features of immunomodulatory mAbs illuminates their efficacy and adverse event profiles. Such mAbs generally are IgG isotypes and thus share a common structure composed of two identical, variable fragment antigen-binding (Fab) domains that confer target specificity, as well as a single, non-variable Fc domain corresponding to the particular IgG subtype (1–4 in human; 1, 2a, 2b and 3 in mouse). The Fc domain can bind FcRs on the surface of innate immune effector cells (macrophages, DCs, NK cells, etc.) and B cells. Depending on cell type and environmental context, Fc binding to surface FcR will engage signaling pathways in the FcR-bearing cell and help shape functional responses. For example, interaction of macrophage FcR with the Fc domains present on an IgG-coated cell can induce that macrophage to initiate effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) ^{5–8, 178}. Fc-FcR interaction thus serves as a homing signal to direct immune effector mechanisms to a specific target, effectively bridging adaptive (e.g., B cell-generated IgG) and innate (e.g., phagocytosis) immunity and enabling a number of important biological activities ¹⁷⁹.

The canonical FcRs belong to the Ig superfamily and can either transmit an activating signal (FcγRI, IIA, IIC, IIIA and IIIB in human; FcγRI, III and IV in mouse) or alternatively an inhibitory signal (FcγRIIB in both human and mouse). The different IgG subtypes possess variable affinities for specific FcRs and thus differ in the balance of activating versus inhibitory signals they transmit into FcR-expressing cells ⁸. Table 3 provides a summary of IgG-FcR interactions relevant to immunomodulatory mAb-based therapy. Due to the fact that human FcγRIIC (expressed in only 20% of patients) and FcγRIIB (a GPI-linked receptor mainly restricted to neutrophils) have not been well characterized ¹⁸⁰, they have been left out of Table 3 and will not be considered further. Similarly, human IgG3, which is not used therapeutically due to relative instability and immunogenicity ¹⁸⁰, and mouse IgG3, which does not interact appreciably with FcγRs ^{180, 181}, are also excluded from Table 3 and from further discussion.

FcγRs can be either activating or inhibitory, as determined mainly by their intracellular signaling components. Activating receptors contain intracellular immunoreceptor tyrosine-based activation motifs (ITAMs) which transduce stimulatory signals to the receptor-bearing cell when the extracellular portion of the receptor is ligated. Conversely, inhibitory receptors contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which transduce suppressive signals when bound by ligand ¹⁷⁸ (Table 3). The opposing nature of the activating and inhibitory FcγRs allows for fine-tuning of FcR-mediated immune responses based on the summation of stimulatory and suppressive inputs ¹⁸² (see Fig. 1). The immune-regulating activities mediated by FcRs have been reviewed elsewhere ¹⁷⁸.

Recently, it was found that inhibitory ITAM (ITAMi) signaling, in the context of low-valency receptor binding ¹⁸³, such as that occurring with unbound, soluble IgG (Fig. 1F), represents another source of FcR-mediated inputs ¹⁸⁴. ITAMi signaling after FcγRI (an activating receptor) binding by immune complexes has been shown, for example, to attenuate IFN-γ signaling and induce the secretion of immunosuppressive IL-10 ¹⁶⁹. The

size of immune complexes seems to be an important factor in determining whether FcR signaling generates a pro- or anti-inflammatory response¹⁶⁸, with larger complexes generally triggering pro-inflammatory cytokine activity (Fig. 1E) and smaller immune complexes (and soluble, monomeric IgG) appearing to promote anti-inflammatory conditions (Fig. 1F). This phenomenon likely accounts for the clinical benefits of IVIG therapy in treating patients with rheumatoid arthritis¹⁸⁵, idiopathic thrombocytopenic purpura (ITP)¹⁸⁶, and other autoimmune disorders^{187, 188}. Our understanding of Fc-FcR interactions and their involvements in numerous biological processes, however, is far from complete, and their complexities are only now beginning to be unraveled. Overviews of Fc-FcR interactions and their role in mAb-based cancer therapy (specifically, their contributions to treatment efficacy) are available^{7, 8, 180, 189, 190}.

The role of FcR-mediated interactions in the function of cytotoxic mAbs such as rituximab arises predictably from the involvement of innate effector cells in their mechanisms of cellular elimination^{191–194}, but defies straightforward prediction in the case of immunomodulatory mAbs. Since checkpoint inhibitors disrupt inhibitory ligand-receptor interactions, the involvement of FcR would not seem necessary, for instance, to prevent CTLA-4 from disrupting B7.1/B7.2 interaction with CD28 during T cell priming in draining lymph nodes. However, the potency of ipilimumab was found to depend on intratumoral depletion of Tregs¹⁹⁵ due to their constitutive expression of the CTLA-4. Depletion was shown to depend on activating Fc γ RIV, suggesting macrophage-mediated ADCC as the driving mechanism^{78, 196, 197} (Table 4).

The story is further complicated by the fact that ADCC may not be the only mechanism of Treg depletion. There is evidence that Tregs possess a degree of plasticity and under certain circumstances can undergo a phenotypic conversion, in which they lose their suppressive properties and gain effector functionality¹⁹⁸. CD134 agonists were shown to inhibit de novo Treg induction, expansion, and suppressive function¹⁹⁹, in addition to their ability to promote effector CD4⁺ and CD8⁺ T cell proliferation^{125, 128}. Similarly, GITR agonism was shown to downregulate Treg-specific transcription factors and cytokines (with complete loss of Foxp3 expression) and upregulate T-bet and Eomesodermin (Eomes)²⁰⁰. More recent evidence suggests that costimulation with CD134 plus CD137 agonists can induce Tregs to adopt a cytotoxic CD4⁺ effector phenotype, expressing Eomes, Granzyme B (GzmB), and IFN- γ , while retaining Foxp3 expression²⁰¹. Additionally, CD137 costimulated Foxp3⁺ Tregs that express Eomes and GzmB can kill tumor target cells²⁰². Thus it appears that immunomodulatory mAb therapy can exploit Treg plasticity to reverse tumor-mediated immunosuppression and add to the overall effector T cell response (Table 4).

Costimulatory signaling by TNFR family members is initiated under physiological conditions when they undergo trimerization upon binding their natural ligands; however, mAbs can only bind a target bivalently (Fig. 1A). Thus, generally speaking, costimulatory agonists cannot on their own induce costimulation. Nevertheless, FcR-mediated, mAb-induced clustering of receptors facilitates the formation of higher order signaling complexes capable of initiating costimulation (Fig. 1B) and appears to be required for the efficacy of at least some agonists. Since engagement of activating FcR on DCs enhances their antigen presentation and costimulatory capacity^{203, 204}, one might predict that activating FcR would

be the most potent in facilitating therapeutic effects of costimulatory agonists. To the contrary, activity of the proapoptotic receptor agonists (PARA) targeting death receptor (DR) 4, DR5, and CD95 (Fas) was found to depend on either activating or inhibitory FcR, and in some contexts, inhibitory Fc γ RIIB was shown to be sufficient in mediating this activity²⁰⁵. Similar FcR requirements were later demonstrated for anti-CD40^{205–207}, thus extending the finding to non-apoptosis-inducing costimulatory TNFR agonists (Table 4). These observations suggested a mechanisms whereby Fc γ RIIB engagement on nearby FcR-expressing cells by TNFR-bound agonist mAbs induces clustering of TNFRs, thus enabling sufficient induction of costimulatory signaling (Fig. 1B). This proposed model was further supported by the discovery that efficacy did not depend on FcR-dependent signaling in the accessory cell that expresses it²⁰⁸. Thus, FcR appears to facilitate agonist-induced costimulation through receptor clustering.

CD40, CD28, and CD137 agonists containing a subdomain from the Fc hinge region of the B isoform of human IgG2 can function independently of Fc-FcR interactions, retaining biological effects even after enzymatic removal of antibody Fc domains. The authors propose that the constrained disulfide hinge configuration facilitates uniquely compact divalent receptor clustering and thereby the induction of costimulatory signaling²⁰⁹. On the other hand, activating FcR are required to enable other TNFR agonists, such as GITR¹⁹⁷, CD27²¹⁰ and CD134²¹¹, to deplete intratumoral Tregs (as with anti-CTLA-4 mAbs^{78, 196, 197}), implicating innate effector mechanisms (reviewed in¹⁸⁹ and shown in Table 4). Whether activating or inhibitory FcR are involved in the mechanism(s) underlying immunomodulatory agonist-induced boosting of initial antigen-priming in lymphoid organs, however, remains to be determined *in vivo*. Interestingly, the therapeutic activity of CD137 agonist in a mouse lymphoma model was actually enhanced in the absence of activating Fc γ RIII²¹². This is a surprising finding, particularly in light of earlier work demonstrating FcR-dependent CD137 induction on NK cells²¹³, whose tumoricidal function is well known to be engaged by CD137 agonist¹⁷³. As such, the outcome of FcR-mediated events occurring following administration of CD137 agonist can vary in a context-dependent manner.

Most recently, Dahan et. al., described the Fc-FcR interactions involved in the targeting of the PD-1/PD-L1 axis using a variety of antagonist mAbs²¹⁴. This study revealed that antagonistic anti-PD-1 mAbs mediate biological activity in a manner that is not dependent on FcR interaction. As with anti-CD137 agonist²¹², engagement of activating FcR leads to a decrease in efficacy (Table 4). This inhibition seems to be mediated by FcR-induced ADCC directed toward T cells expressing PD-1, depleting the cells intended to be activated. An additional proposed mechanism of FcR-related diminution of efficacy implicates Fc γ RIIB-induced clustering and consequent stimulation (as with TNFR agonists), in essence converting the intended blockade of PD-1 into agonistic PD-1 stimulation²¹⁴.

The same study uncovered entirely different FcR-dependent mechanisms at work in mAbs targeting PD-L1, which require the presence of activating FcR-expressing cells for optimal efficacy. These findings suggest that ADCC-mediated depletion of PD-L1-expressing myeloid cells within the TME is important for the efficacy of PD-L1-targeted immunomodulatory therapy (Table 4). Furthermore, this mechanism likely synergizes with

the FcR-independent disruption of PD-1/PD-L signaling facilitated by the antagonistic mAbs¹⁰⁶. These data are in agreement with previous work showing that immunosuppressive myeloid cell subsets, such as MDSCs⁵⁶, can foster a pro-cancer environment barricading the tumor from immune intervention.

The complexities of Fc-FcR interactions and the roles they play in mediating the activity of immunomodulatory antibodies is an area of active investigation and has been reviewed extensively elsewhere^{168, 178, 182, 215}. Additionally, Offringa and Glennie provide in a Cancer Cell preview²¹⁶ to Dahan et. al.'s report²¹⁴ a concise overview of Fc-FcR roles in immunomodulatory therapy that includes those just uncovered for anti-PD-1 and anti-PD-L1 antagonists.

7. Expert opinion: Is it possible to selectively increase safety while maintaining efficacy?

Modulation of the immune system with checkpoint inhibitors and costimulatory agonists has emerged as a highly promising approach to cancer therapy. Improved durability and rates of response, more tolerable side effect profiles and extensive clinical applicability (in both solid and liquid tumors) set immunomodulatory mAbs apart from previous generations of cancer therapeutics. A growing body of evidence supports the notion that immunomodulatory agents will soon become standard first-line therapies. Table 1 summarizes the current development status for representative mAbs and lists for each phase of clinical testing specific conditions/indications in which they are being evaluated. Data on PFS, OS, and best overall response (BOR) rates are essential to evaluate different treatments. Additionally, although rates of grades 3/4 side effects are included in clinical trials and can be used as an indirect measure of quality of life (QOL), direct assessment will be of great value moving forward.

Evidence has shown that the efficacy and adverse event profile of a given agent can vary significantly from patient to patient. The identification of biomarkers to predict patient likelihood of response and the potential of treatment toxicity is essential to the design of patient-specific treatment strategies, as well as to the proper clinical testing of these agents. Importantly, cancer patients must be screened to assess their eligibility, since inclusion in clinical trials of patients unable to respond can dilute overall efficacy determined by the study and potentially mask the true clinical potency of new agents being developed¹⁵⁶. One potential biomarker identified for ipilimumab response is the cancer/testis antigen NY-ESO-1, which is expressed normally in the testis and placenta, as well as aberrantly in a subset of advanced melanomas. It was found that NY-ESO-1 seropositivity, and to greater extent the existence of NY-ESO-1 specific CD8⁺ T cells in the periphery, correlates positively with clinical responsiveness to ipilimumab²¹⁷. It was also found that patients with lactate dehydrogenase levels exceeding twice the upper limit of normal were far less likely to benefit from ipilimumab therapy in the long-term²¹⁸. Moreover, recent evidence has demonstrated potential value in using PD-L1 expression as a biomarker to determine the likelihood of patient response to PD-1 antagonism and the likelihood of enhanced clinical

benefit from supplementing anti-PD-1 with additional therapeutics in combination¹⁰⁶ (Table 2).

Interestingly, it was recently shown that gut microbiota composition can influence the efficacy of anti-CTLA-4 therapy. Specifically, the gut microbiota is required for antitumor responses with the anti-CTLA-4 mAb 9D9 in multiple mouse models of melanoma, sarcoma, and colon cancer. Data also showed that the microbiota was necessary for mAb-induced activation of effector CD4⁺ T cells in the spleen²¹⁹. These findings align with previous knowledge that Tregs are abundant in the GI tract. The identification of individual bacterial species associated with anti-CTLA-4 efficacy suggested that their presence and relative abundance in a patient's gut microbiota could serve as a biomarker to predict likelihood of treatment response. Furthermore, feces harvested from ipilimumab-treated patients were found to differ in microbiota composition, which gradually shifted in favor of the efficacy-enhancing species. Intriguingly, fecal microbial transplants into tumor-inoculated mice with patient feces samples containing significant numbers of these microbes helped to generate effective antitumor responses, whereas transplantation of feces containing fewer of these specific microbes were unable to respond²¹⁹.

There also appears to be a genetic basis for the likelihood of beneficial response to immunomodulatory therapy. Genetic analysis of 64 ipilimumab-treated melanoma patients uncovered a correlation between mutational load and clinical benefit and identified a neo-antigen landscape specific to tumors that are highly responsive to treatment²²⁰. Some specific genetic mutations²²¹ and the presence of particular single-nucleotide polymorphisms²²² have also been shown to predict clinical response. Interestingly, the single-nucleotide polymorphisms (SNPs) that correlated with beneficial clinical response did not show any correlation with the occurrence or severity of adverse events²²², suggesting that the mechanisms underlying efficacy and toxicity do not overlap entirely and can thus be differentially targeted when designing future immunomodulatory mAbs for greater therapeutic index. This study also supports an earlier hypothesis that some mutations considered silent passengers should instead be classified as immune determinants²²³ that collectively define immunogenicity and susceptibility to antitumor effector mechanisms employed by the immune system. Accordingly, genetic screening could be employed to identify likely responders to therapy and help ensure that patients are offered only those treatments from which they are likely to benefit.

The process of selecting new immunomodulatory antibodies for clinical development also needs to be improved at the preclinical level in order to better predict their effects in humans. The use of *in vitro* screening assays is convenient, but results obtained must be interpreted with caution, because numerous factors such as cell culture density, microenvironment architecture, and stimulation timeframes often differ substantially from those in real patients²²⁴. The unanticipated consequences of the TGN1412 trial, for example, was later determined to result from failure to take into consideration differences in CD28 expression patterns between humans and cynomolgus macaques^{225, 226}. This unfortunate event has helped lead to the development of new screening techniques and safety measures, which are already being utilized in designing and testing new therapies²²⁶. Prevention of such incidents will be essential to continued progress in this field.

The difference in response kinetics when comparing immunomodulatory mAbs to traditional cancer therapeutics is also important to consider in future clinical testing protocols. Immunomodulatory antibodies, which function indirectly via immune system activation, can take longer to mount an attack and thus warrant adjustment to currently defined endpoints and other measures used to evaluate agents²²⁷. The importance of this last point is illustrated in the premature halting of a phase III trial with the anti-CTLA-4 agent tremelimumab due to apparent lack of improvement in survival. However, follow-up investigation of study participants eventually revealed improvements occurring after 2 years²²⁸, reflective of the delayed evidence of therapeutic response seen with these agents. Similarly, as discussed earlier, the absence of efficacy reported for an anti-OX40 agonist antibody despite initially promising data demonstrates the incompatibility of traditional RECIST criteria with immunomodulatory therapies¹⁴⁰. In fact, even with successful therapeutic outcomes tumor shrinkage would not be expected to occur for some time after treatment is initiated. This is because tumoricidal responses induced by immunomodulatory mAbs rely on effector cell infiltration into the TME; thus upon measurement, any tumor shrinkage would likely be difficult to detect due to lymphocytic influx localized to tumor sites. Therefore, appropriate additional criteria, such as immune cell phenotypes, frequencies, and overall abundances, both in circulation and within TMEs, need to be incorporated for improved evaluation of clinical trials²²⁹. Only then can we be confident that we are accurately characterizing future generations of immunomodulatory therapeutic candidates.

As mentioned earlier, the ability to selectively enhance efficacy and/or limit toxicity likely depends on the specific mechanism(s) responsible for each. There is considerable overlap in the immune effector mechanisms involved in beneficial therapeutic antitumor effects and harmful adverse events. For instance, TNF- α has potent tumoricidal activity, but can be a causative factor in cachexia, insulin resistance and cytokine release syndrome^{230–233}. Further, initial clinical trials testing ipilimumab found a positive correlation between therapeutic responses and the prevalence and severity of adverse events^{177, 234, 235}, raising the critical question as to what extent adverse events can be limited without compromising therapeutic efficacy. Fortunately, extensive clinical testing of ipilimumab has provided encouraging results in this regard, specifically that ipilimumab-responsive patients who develop irAEs can continue to benefit from an anti-tumor response even after pharmacologic reversal of associated symptoms with, for example, administration of corticosteroids^{106, 176}. Reversal of irAEs and cytokine release syndrome without loss of efficacy was also achieved in other T cell-mediated cancer therapies, in which inhibition of TNF- α with etanercept²³⁶ and inhibition of IL-6 signaling with tocilizumab^{236, 237} were effective. Alternative agents that might be useful in managing cytokine release syndrome during immunotherapy include inhibitors to MCP-1, MIP-1 β , IL-2R, and IL-1R²³⁸ (depicted in Fig. 1).

Combinations of immunotherapies¹⁰⁹, as well as immunotherapies used in conjunction with traditional treatment options such as chemotherapy²³⁹ and radiotherapy²⁴⁰ have been attempted in search of treatment approaches with enhanced efficacy and improved safety profiles. Combination therapy has opened the possibility of limiting the occurrence of irAEs due to additive (and for certain combinations perhaps even synergistic) effects of multiple agents, which could allow for administration of lower doses. Although the frequency and

severity of adverse events have been greater with combinations than with individual monotherapies thus far¹⁰⁶ (Table 2), the ability to titrate dosages could potentially avoid many adverse events directly. A detailed mechanistic understanding of how these therapeutics elicit their associated beneficial and toxic effects is crucial to designing strategies that selectively block the latter. Ideally, these approaches will facilitate an overall advantage in favor of enhanced efficacy over adverse events. Ultimately, the benefits and risks of specific combinations will need to be addressed in future trials. Melero and colleagues recently published a comprehensive review summarizing general concepts, specific examples under development, and important considerations in the design of combination immunotherapies²⁴¹.

It seems likely that Fc-FcR interactions also play a role in mediating at least some of the adverse events elicited by immunomodulatory mAbs. For instance, target expression on therapeutically-irrelevant cell types could potentially lead to collateral damage of these cells via ADCC or ADCP (Fig. 1D). Thus, while ADCC/ADCP might be therapeutically beneficial by depleting intratumoral Tregs with mAbs to CTLA-4, GITR and OX40^{78, 196, 197, 211} (Table 4), it might be problematic, for instance, with anti-CD137, since CD137 is expressed on a variety of cell types, some of which appear not to be involved in the antitumor response²⁴². Neurons, astrocytes and microglia²⁴³, for example, are among these therapeutically-irrelevant, CD137-expressing cell types. Additionally, endothelia can express CD137 in response to inflammatory mediators²⁴⁴ and in diseases such as atherosclerosis²⁴⁵.

Furthermore, expression of soluble forms of some targets (e.g., CD134 and CD137) has been found in a variety of pathological conditions^{246–248}, raising the potential for the formation of immune complexes which can trigger inflammation (Fig. 1E)¹⁶⁸ and may potentially be involved in mechanisms driving pathological immune hyper-reactivity, such as that occurring in IgG-mediated anaphylaxis²⁴⁹. Finally, surface coating of IgG on either therapeutically-relevant or -irrelevant cells could cause innate cells expressing activating FcγR to release pro-inflammatory cytokines contributing to adverse events^{168, 250}. In sum, although Fc-FcR interactions may be necessary for the full therapeutic efficacy of immunomodulatory mAbs, they might also be responsible for therapeutically-complicating adverse events through a variety of potential mechanisms. Further, these adverse events might be exacerbated by other pathologies (e.g., underlying infection or autoimmune predisposition) from which cancer patients might be suffering. Nevertheless, seemingly contradictory evidence showing both pro- and anti-inflammatory cytokine secretion in response to FcR engagement illustrates our incomplete understanding of FcR-mediated immune functions.

Despite these gaps in understanding, Fc-FcR interactions are likely responsible for at least some of the toxicities seen with immunomodulatory mAb therapy. In such cases, preventing these interactions may help limit adverse events to increase therapeutic index and enhance precision medicine. This notion is additionally supported by the heterogeneity of responses to FcR-dependent mAb activity. For instance, the absence of a human homolog to the high-avidity FcγRIIB-binding of mouse IgG1 may limit efficacy of some costimulatory agonists (Table 3). Furthermore, patient response heterogeneity can arise from relative levels of FcR

expression and functional status, which can vary widely between tissues and cell types. For example, although most innate cells express both activating and inhibitory Fc γ Rs, NK cells are known to express only activating receptors in mice and humans (except for a recently discovered subset of NK cells expressing Fc γ RIIB), whereas B cells only express inhibitory receptor¹⁷⁸. The different distributions of such cells likely explains the tissue- and organ-specific differences in overall response to therapy¹¹. Interestingly, it was recently suggested that in a murine model peritoneal and visceral adiposity can influence the magnitude of cytokine-mediated toxicity associated with the presence of resident M1-polarized macrophages¹⁷⁰. Other factors include current immune status (e.g., active responses to infection), and patient-specific differences in both FcR-mediated signaling summation patterns (i.e., activating vs. inhibitory FcR inputs) and FcR-specific avidities to various IgG isotypes (Table 3). Additionally, the existence of allotypic variants with different binding affinities contributes to patient response heterogeneity; so far, human Fc γ RIIa and Fc γ RIIIa each have had two variants characterized²⁵¹.

Establishing FcR-independent mechanisms of immunomodulatory mAb activity is therefore a highly desirable goal. Antibody multimerization has been shown to be an effective strategy to bypass FcR requirements, enabling the preservation of anti-CD40 agonist activity *in vitro* and *in vivo*, as well as in a model of lymphoma with mice completely lacking all Fc γ Rs²⁵² (Table 4). Immunomodulatory therapy can also be optimized using mAb engineering to alter FcR binding properties. Early studies focused on increasing binding avidity of IgG to activating FcR, which could be accomplished using protein engineering^{253, 254}, as well as modification to antibody glycosylation^{255, 256}. Additionally, selective enhancement of Fc γ RIIB binding was used to generate an anti-CD137 agonist with increased agonistic activity *in vitro*²⁵⁷. Individual FcRs have also been shown to exhibit differential contributions to cellular functions; for example, it has been demonstrated that Fc γ RI was largely responsible for secretion of both anti-inflammatory IL-10²⁵⁸ and pro-inflammatory cytokines²⁵⁹. A suggested implication of this findings was that macrophage-mediated ADCC and ADCP could potentially be uncoupled from antibody-dependent cytokine release (ADCR), a feat which was accomplished recently by Kinder et. al., using an Fc-engineering approach to modulate cytokine release while leaving cell-killing activity intact²⁵⁹. The ability to selectively fine-tune or prevent cytokine release thus opens the possibility of limiting cytokine-related adverse events without losing effector capacity and tumoricidal activity. Contrarily, in situations where the secretion of anti-inflammatory cytokines might be hindering efficacy, selective attenuation of ADCR would be advantageous.

Additionally, the human IgG2 isotype (specifically the B isoform) was shown to possess the unique ability to exhibit activity in an entirely FcR-independent manner, likely due to an especially compact geometry and constrained disulfide hinge configuration²⁰⁹. The authors propose that these properties enable sufficient clustering of TNFRs to reach a signaling threshold beyond which stimulation ensues. Notably, this phenomenon was shown for agonists targeting multiple costimulatory receptors, including CD40, CD28, and CD137, and it was further demonstrated that selective mutagenesis could generate a wide range of agonistic activities, without altering receptor binding avidity (Table 4). That human IgG2 is capable of exerting superior agonistic activity in an FcR-independent manner potentially

accounts for data from clinical trials demonstrating superior potency of the anti-CD40 agonist CP-870,893, an IgG2 isotype (Table 1), compared to those of other isotypes^{260, 261}.

A greater understanding of the mechanisms behind immunomodulatory mAb therapy will be critical for the future optimization of these therapeutic agents. Importantly, the mechanisms underlying efficacy and adverse events need to be further delineated. Recent characterization of PD-1/PD-L1-targeted therapies²¹⁴ further adds to the ever-expanding complexity of the mechanisms at work in immunomodulatory therapy, while also illustrating the importance of proper antibody isotype selection and the value of Fc domain modification in order to optimize efficacy. Much work is also needed to explain the context-dependent pro- and anti-inflammatory responses (Figs. 1E and 1F, respectively) induced by FcR binding to mAb Fc domain. While many data support the idea that avoiding FcR activity will help limit adverse events, the anti-inflammatory responses to FcR signaling in certain contexts now pose the possibility that FcR engagement actually contributes negatively to therapeutic efficacy, and that circumvention of FcR interaction could lead to an enhancement of activity (perhaps even concurrently with attenuation of adverse events). Conversely, it is possible that FcR-mediated pro-inflammatory cytokine secretion contributes to overall antitumor efficacy, representing a benefit that would be lost if FcR engagement were avoided. Only with continued investigation will we be able to accurately predict how adjustments to Fc-FcR interactions will impact efficacy and toxicity. Therapeutic approaches that do not engage FcR, yet retain the ability to effectively stimulate their target pathways, will potentially be capable of inducing beneficial therapeutic responses in cancer patients while sparing them from having to endure the dangerous adverse events of today's therapies. Hence, perhaps we can have our cake and eat it too.

Acknowledgments

Authors thank Dr. James Buturla (UConn Health) for his critical comments and editorial contributions.

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Article highlights box

- Immunomodulatory antibodies (mAbs), including checkpoint inhibitors and costimulatory agonists, represent a promising new approach to cancer therapy.
- These mAbs work by enhancing immunity, thereby increasing antitumor activities. In doing so, however, a variety of immune-related adverse events (irAEs) can occur.
- Successful translation of these agents depends on selectively limiting the frequency and severity of irAEs without losing therapeutic efficacy.
- Although the mechanisms involving efficacy and irAEs have much overlap, evidence supports the idea that these effects can be isolated and fine-tuned.
- Interactions between Fc and FcR are essential in mediating biological activity, but are also implicated in the eliciting of adverse events.
- Modifying the structures of these mAbs so that they avoid Fc-FcR interactions, while still retaining therapeutic efficacy, might be a viable strategy for the future development of immunomodulatory antibodies with more favorable therapeutic indices.

Figure 1. Role of Fc-FcR interactions in mediating therapeutic efficacy and adverse events of antibody-based immunomodulatory therapy (using anti-CD137 agonist as an example)

Monoclonal antibodies (mAbs) bind costimulatory tumor necrosis factor receptor (TNFR) superfamily members bivalently, which is insufficient to initiate costimulatory signaling in the T cell (A) unless fragment crystallizable receptor (FcR) is present on nearby innate accessory cells to facilitate costimulatory receptor clustering (B). Clustering of activating FcR can also signal to the FcR-bearing accessory cell (C), leading to the release of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, etc.), which can potentially contribute to cytokine release syndrome. Conversely, cytotoxic or phagocytic innate cells expressing activating FcR can kill therapeutically-irrelevant cells expressing the mAb target via antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP), respectively (D). It might also be directed against the T cells undergoing costimulation (not shown), which could account for recent findings that activating FcR engagement can negatively impact efficacy in certain scenarios. The precise nature of FcR-triggered responses by accessory cells depends ultimately on a variety of factors, such as the relative ratio of activating to inhibitory FcRs engaged (not depicted here), as well as the structural/geometric context of FcR ligation; for example, large immune

complexes comprised of IgG-coated soluble antigens have been shown to increase inflammatory cytokine production (E), whereas interaction with soluble, unbound IgG (i.e., under homeostatic conditions) or small immune complexes appears to suppress hyper-activation of immune cells by inducing the secretion of anti-inflammatory cytokines, such as IL-10, or by suppressing the secretion or signaling of inflammatory cytokine secretion (F). Although pro-inflammatory cytokines play a causative role in therapy-associated adverse events, their effects could also contribute to overall antitumor efficacy. This last point is an important consideration, since disruption of Fc-FcR interactions could impact both adverse events and efficacy, depending on the particular context. Also worth noting is that avoiding FcR-induced inflammatory responses would not completely prevent therapy-associated adverse events, which is thought to be mediated in part through the activation of specific effector T cells themselves.

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Table 1

Summary of clinical results for representative immunomodulatory mAbs used to treat cancer

Target	Checkpoint Inhibitors			Costimulatory Agonists		
	CTLA-4	PD-1	PD-L1	CD40	CD134 (OX40)	CD137 (4-1BB)
Agent *	Ipilimumab	Nivolumab	Atezolizumab	CP-870,893 ^{**}	MED16469 ^{****}	Urelumab
mAb Isotype	IgG1	IgG4	IgG1	IgG2	murine IgG1	IgG4
Company	Bristol-Myers Squibb	Bristol-Myers Squibb	Genentech/Roche	Pfizer/Roche	MedImmune/AstraZeneca	Bristol-Myers Squibb
Highest Phase of Testing	Approved/Marketed	Approved/Marketed	Phase III	Phase I	Phase I/II	Phase II
Marketed/registered Indications	Malignant melanoma	Malignant melanoma, NSCLC, RCC	-	-	-	-
Phase III Indications	Glioblastoma, NSCLC, RCC, SCLC, prostate	Glioblastoma, gastric, HNC, SCLC	Bladder, breast, NSCLC, RCC	-	-	-
Phase II Indications	Gastric, MDS, esophageal, ovarian, UGC, lymphoma, brain metastases	AML, CLL, breast, DLBCL, MDS, UGC, esophageal, ovarian, follicular and Hodgkin's lymphomas	CRC, solid tumors, soft tissue sarcoma	-	-	CLL, malignant melanoma
Phase I/II Indications	Solid tumors	CRC, HCC, NHL, solid tumors, hematological malignancies	DLBCL, follicular lymphoma	-	Breast, DLBCL, prostate cancer, solid tumors	NHL, solid tumors
Phase I Indications	-	CML, hepatitis C	Malignant melanoma, MM, MDS, hematological malignancies	PDA, solid tumors	CRC, HNC	MM, CRC, HNC
Efficacy	ORR 10.9%, phase III, stage III/IV melanoma vs 1.5% gp100 alone OS=10.1 months vs 6.4 (gp100 alone) ⁸⁰ ORR 19.0%, phase III, stage III/IV melanoma ¹⁰⁶	ORR 40.0%, phase III, stage III/IV melanoma, vs 13.9% for dacarbazine ¹⁰³ ORR 43.7%, phase III, stage III/IV melanoma ¹⁰⁶	ORR 38%, phase II, advanced NSCLC, PD-L1+; compared to 13% for docetaxel ²⁶²	ORR 20% (6 PR), phase I, with chemotherapy py, solid tumors ¹³⁸	No PR, but tumor shrinkage in 40% (12 of 30) following single dose; induction of HAMA prevented additional doses being given ^{140 ****}	ORR 5.6% (3 of 54), phase I, melanoma; phase II study terminated due to hepatotoxicity; newer trials using lower dosages ¹⁴¹
Adverse Events	Toxicities generally immune-related: rash, colitis, diarrhea, hepatotoxicity, endocrinopathies, neuropathies ^{80, 106, 263}	Generally more tolerable than ipilimumab: fatigue, rash, diarrhea, pruritus, pneumonitis,	Toxicities similar to those seen with anti-PD-1; grades 3/4 adverse events have been uncommon ²⁶²	Fatigue (81%) most common; does-limiting grade 3 CRS, grade 4 TIA ¹³⁸	Well-tolerated, brief grade 3 lymphopenia, fatigue, fevers, chills, mild rash observed ^{140 ****}	Cytopenias and liver inflammation (elevated ALT/AST) most concerning, dose dependent; also fatigue, rash, fever ¹⁴¹

Target	Checkpoint Inhibitors			Costimulatory Agonists		
	CTLA-4	PD-1	PD-L1	CD40	CD134 (OX40)	CD137 (4-1BB)
		fever can occur 100, 101, 106				

Information on clinical trials obtained from ClinicalTrials.gov.

* Particular agents listed here are only representative; there are a number of other agents to the same and/or other targets in various stages of development.

** Pfizer's CP-870,893 is now licensed to Roche and has been renamed RO7009789; ongoing clinical trials are listed under RO7009789.

*** Efficacy and adverse events based on phase I testing of a different OX40 agonist, 9B12.

**** MedImmune/AstraZeneca has recently begun recruiting for a phase I trial with a humanized anti-CD134 agonist (MEDI0562) in adults with selected advanced solid tumors (NCT02318394).

NSCLC, non-small cell lung carcinoma; RCC, renal cell carcinoma; SCLC, small cell lung carcinoma; MDS, myelodysplastic syndromes; UGC, urogenital carcinoma; HNC, head and neck cancers; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B cell lymphoma; NHL, non-Hodgkin's lymphoma; CML, chronic myeloid leukemia; CRC, colorectal cancer; MM, multiple myeloma; PDA, pancreatic ductal adenocarcinoma; CRS, cytokine release syndrome; TIA, transient ischemic attack; ORR, objective response rate; PR, partial response; HAMA, human anti-mouse antibodies.

Table 2

Combination immunotherapy with ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) in previously-untreated metastatic melanoma.

This table represents an abbreviated version of findings from the randomized, double-blind, phase 3 study ¹⁰⁶ deemed most important

	Treatment *	Ipilimumab	Nivolumab	Both
Efficacy	ORR **	19.0%	43.7%	57.6%
	Median PFS (Intention-to-treat)	2.9 months	6.9 months	11.5 months
	Median PFS (PD-L1-positive)	3.9 months	14.0 months	14.0 months
	Median PFS PD-L1-negative	2.8 months	5.3 months	11.2 months
Adverse events	Rate of any adverse event	99%	99.4%	99.7%
	Severe ***	55.6%	43.5%	68.7%

* Number of patients per group (n) ranged from 311–316.

** Response rates determined by the investigator using Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1.

*** Defined as grades 3/4 based on severity determined using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

ORR, objective response rate; PFS, progression-free survival.

Table 3

FcγR properties, expression patterns and IgG isotype binding affinities in mice and humans

Species	Mouse				Species		Human			
	FcγRI	FcγRIII	FcγRIV	FcγRII B	Receptor		FcγRI	FcγRIIa	FcγRIIIa	FcγRII B
Function	Activating	Activating	Activating	Inhibitory	Function		Activating	Activating	Activating	Inhibitory
Signaling	ITAM	ITAM	ITAM	ITIM	Signaling		ITAM	ITAM	ITAM	ITIM
Allotype	n/a	n/a	n/a	n/a	Allotype*		n/a	H ₁₃₁	F ₁₅₈	n/a
IgG1	–	++	–	+++	IgG1		++++	+++	+++	++
IgG2a	++++	++	+++	++	IgG2		–	++	–	–
IgG2b	++++	++	++	+++	IgG4		++++	++	++	++
Expression patterns**	DC	DC Mac PMN NK	DC Mac PMN NK	DC Mac PMN NK T ^{***}	Expression patterns**		DC Mac PMN	DC Mac PMN	Mac PMN NK	B DC Mac PMN NK ^{****}

* Only human allotypes that have been sufficiently characterized are included. For each receptor, the more common variant appears first.

** Low expression levels indicated by italics.

*** Expressed by mouse memory CD8⁺ T cells.

**** Expressed by a subset of human NK cells.

ITAM, immunoreceptor tyrosine-based activation motifs; ITIM, immunoreceptor tyrosine-based inhibitory motifs; B, B cell; DC, dendritic cell; Mac, macrophage/monocyte; PMN, polymorphonuclear cell (neutrophil); NK, NK cell; T, T cell.

Table 4

Characterizing potential Fc-FcR interactions required for efficacy and/or adverse events of representative immunomodulatory targets

FcR Requirements for Biological Functions		FcR Independent	FcR Dependent					
mAb Class	mAb Target	Block ligand binding	Counter-regulatory Signaling*	Activate Costim.	Deplete/ Alter Tregs in TME	Deplete/ Alter Myeloid Cells in TME	Tumor Depletion/Apoptosis	Additional comments
Checkpoint Inhibitors	CTLA-4	Yes	Yes	-	Yes	-	-	
	PD-1	Yes	Yes	-	-	-	-	FcR engagement diminishes efficacy
	PD-L1	Yes	-	-	-	Yes	Yes**	
Costimulatory Agonists	CD40	-	-	Yes	-	-	Yes**	FcγRIIB-dependent***
	CD134 (OX40)	-	-	Yes	Yes	-	-	Treg depletion due to ADCC and/or phenotypic conversion
	D137 (4-1BB)	-	-	Yes	Yes	-	-	FcγRIII diminishes efficacy; FcγRIIB-dependent***
	GITR	-	-	Yes	Yes	-	-	Treg depletion due to ADCC and/or phenotypic conversion
PARA	DR5	-	-	-	-	-	Yes	FcγRIIB-dependent****
	CD95 (Fas)	-	-	-	-	-	Yes	FcγRIIB-dependent****

Mechanisms that occur only during particular circumstances are italicized.

^{*} Possibly FcR-independent; not yet characterized.

^{**} Some tumors cells can express surface this target.

^{***} Can be activating FcR-dependent and/or FcR-independent in certain contexts.

^{****} Can be activating FcR-dependent in specific circumstances.

Fc, fragment crystallizable; FcR, Fc receptor; mAb, monoclonal antibody; Treg, regulatory T cell; TME, tumor microenvironment; ADCC, antibody-dependent cell-mediated cytotoxicity; PARA, pro-apoptotic receptor agonists.