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Forum

Fc-optimized checkpoint antibodies for cancer immunotherapy

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The development of checkpoint antibodies for cancer therapy has been guided by the principle of blocking T cell inhibitory signals. Recognition of the role of the Fc domain in therapeutic activities, through the depletion of immunosuppressive populations and myeloid cell activation, prompts a shift toward the development of optimized Fc-engineered checkpoint antibodies.

Immune checkpoint antibodies – potential and limitations

Immune checkpoint blockade (ICB) by monoclonal antibodies (mAbs) has revolutionized the oncology field. Instead of targeting diverse cancer antigens with heterogeneous expression across cancer types and individual patients, ICB harnesses the host immune system to combat immunogenic tumors. Inhibitory immune checkpoints, primarily programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), have become attractive targets for cancer immunotherapy. These antagonistic checkpoint mAbs block inhibitory signaling pathways on T cells, thereby dampening regulatory (immunosuppressive) mechanisms in various cancers. This pancancer therapeutic approach has led to improved prognosis in many advanced cancer patients. However, the clinical application of ICB has presented notable limitations. Due to heterogeneity in their tumor immunity state, not all cancer types show an objective response, and among those that do, only a subset of patients is responsive. The development of new immunomodulatory mAbs has recently slowed down due to inadequate efficacy in clinical trials.

Antitumor immune activity of checkpoint antibodies involves Fcγ receptor pathways

The engagement of Fc receptors by antibodies initiates signaling pathways and diverse immune effector functions, including antibody-dependent cellular cytotoxicity and phagocytosis (ADCC/P) [1]. These mechanisms are used in the antitumor activity of therapeutic mAbs that activate Fcγ receptors (FcR) pathways to eliminate their target cells. While FcγR-dependent elimination of cancer cells by cytotoxic mAbs targeting tumor antigens, such as rituximab and trastuzumab, is well documented, Fc effector functions in the activity of immunomodulatory mAbs have only been recently characterized [2]. Here, we focus on immune checkpoint targets that have already been approved by the US Food and Drug Administration (FDA); that is, CTLA-4 and PD-1/L-1, and the FcγR pathways triggered by their respective blocking mAbs (Figure 1).

FcγR activation of macrophages and natural killer (NK) cells by the Fc domains of anti-CTLA-4 and anti-PD-L1 mAbs promotes their depletion of suppressive immune cell populations with high expression of the targeted checkpoint in the tumor microenvironment (TME). This depletion activity weakens the immunosuppressive nature of the TME, further promoting the Fab-mediated activity of the checkpoint mAbs on effector T cells. Treatment with anti-CTLA-4 mAb mediates the depletion of intratumoral regulatory T (Treg) cells [2–4] and the attenuation of suppressive myeloid cells through activation of FcγR pathways and type I interferon signaling in these cells [2,5]. Treatment with anti-PD-L1 mAb targets myeloid-derived suppressor cells (MDSCs) in the TME, involving their Fc-mediated elimination [2,6,7]. In turn, the numbers and activation state of tumor-infiltrating $CD4^+$ or $CD8^+$ T cells, as well as tumor-reactive myeloid antigen-presenting cells (APCs), increase. These results highlight the significance of TME remodeling in affecting both innate and adaptive immune cell populations by Fc- and Fab-facilitated mechanisms. Thus, immune TME remodeling is a key determinant of antitumor immunity mediated by the checkpoint mAbs. ICB resistance in some cancer patient subsets could be explained by elevated immunosuppressive properties of the TME, which have the potential to be reversed by FcγR-mediated activities. In contrast to anti-CTLA-4 and anti-PD-L1 mAbs, FcγR activation by anti-PD-1 mAbs can impair the antitumor response due to tumor-associated macrophage (TAM)-mediated clearance of tumor-reactive $PD\text{-}1^+$ effector T cells [8,9].

The sole inhibitory FcγR, FcγRIIB, has been identified as playing a limiting role in the cytotoxic antitumor activity of checkpoint mAbs, a finding further substantiated by evidence derived from cancer patient samples [7,10]. FcγRIIB competes with the activating FcγRs for IgG binding. Scientists use the term activating to inhibitory (A/I) ratio to define the magnitude of effector functions of a given antibody [2] (Figure 1). This A/I ratio is mechanistically determined by the sum of inhibitory and activating signaling via the immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based activation motif (ITAM) pathways downstream to the FcγR engagements, respectively. FcγRIIB upregulation by myeloid cells in the TME increases the threshold for FcγR-mediated cytotoxic activities of anti-CTLA-4 and anti-PD-L1 mAbs. In addition, FcγRIIB can promote FcγR-dependent antibody crosslinking that can agonize PD-1 signaling or cause antibody uptake from $PD\text{-}1^+$ T cells, resulting in the reduced potency of anti-PD-1 mAbs [2]. Supporting the

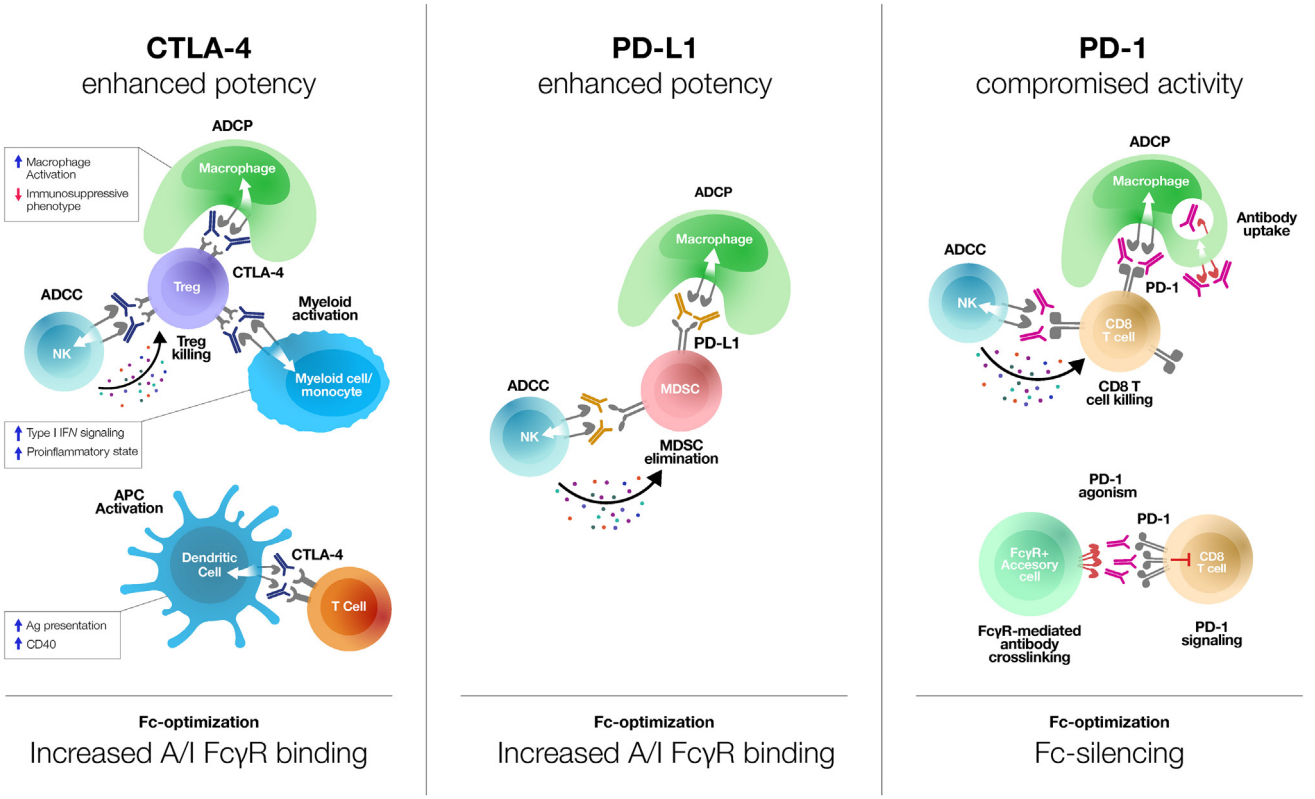

Figure 1. FcγR-dependent mechanisms underlying the antitumor immune response of checkpoint mAbs. FcγR signaling via anti-CTLA-4 mAb engagement enhances antitumor potency by involving (i) macrophage-dependent ADCP and (ii) NK cell-dependent ADCC of CTLA-4$^+$ Treg cells, as well as (iii) IFNγ signaling in activated myeloid cells and monocytes. These mechanisms diminish the immunosuppressive phenotype and increase the pro-inflammatory state of the TME. Another mechanism involves (iv) increased antigen presentation and CD40 expression by dendritic cells interacting with tumor-reactive CTLA-4$^+$ T cells. FcγR signaling via anti-PD-L1 mAb engagement enhances antitumor potency by involving (i) macrophage-dependent ADCP and (ii) NK cell-dependent ADCC of PD-L1$^+$ MDSCs. In contrast, FcγR signaling via anti-PD-1 mAb engagement compromises antitumor activity by involving (i) macrophage-dependent ADCP and (ii) NK cell-dependent ADCC of tumor-reactive PD-1$^+$ CD8$^+$ T cells, as well as (iii) antibody uptake by macrophages. Another mechanism involves (iv) undesired PD-1 agonism in tumor-reactive CD8$^+$ T cells, which is mediated by FcγR-dependent antibody crosslinking, leading to further suppression of antitumor activity. Altogether, these FcγR-dependent mechanisms suggest that scientists should take into consideration an increased A/I when designing Fc-optimized anti-CTLA-4 and anti-PD-L1 mAbs, and Fc silencing when designing Fc-optimized anti-PD-1 mAbs. Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; FcγR, Fcγ receptor; IFN, interferon; mAb, monoclonal antibody; MDSC, myeloid-derived suppressor cell; NK cell, natural killer cell; PD-1, programmed death 1; PD-L1, PD ligand 1; TME, tumor microenvironment; Treg cell, T regulatory cell.

role of FcγRIIB as an inhibitory checkpoint for antibody effector function, the coadministration of anti-FcγRIIB blocking mAbs in preclinical models increases the antitumor activity of anti-CTLA-4, anti-PD-L1, and anti-PD-1 antibodies [7,10]. This concept is currently undergoing evaluation in patients with solid tumors who have progressed following anti-PD-1/L1 treatments in which treatment involves the combination of an anti-FcγRIIB blocking mAb and the anti-PD-1 pembrolizumab (NCT04219254[i]).

Fc-engineering of checkpoint antibodies and future clinical directions

The development of FDA-approved checkpoint mAbs for cancer therapy has been mainly governed by engineering and optimizing antigen-binding domains. The recognition of the role of downstream FcγR activation in potential therapeutic efficacy has prompted a shift toward antibody engineering of the constant region as well. Fc optimization is achieved through amino acid substitutions of the IgG Fc CH2 domain or glycoengineering of the Fc N297-linked glycoform. These modifications can be utilized to remove FcγR binding or modify the binding affinity to specific FcγRs [2].

Preclinical studies have demonstrated that Fc modifications, which manipulate FcγR engagement, impact the antitumor efficacy of checkpoint mAbs. The use of an Fc-null anti-PD-1 IgG prevents the negative effects of FcγR by eliminating TAM-mediated clearance of tumor-reactive PD-1$^+$ T cells

or antibody transfer from the target T cells, resulting in optimal tumor growth inhibition in mice [8,9]. Anti-PD-1 mAbs, such as nivolumab, pembrolizumab, cemiplimab, and dostarlimab, are produced as IgG4 S228P, a stable scaffold of IgG4 with somewhat reduced binding to FcγRs. Since the lack of FcγR binding by anti-PD-1 mAb could enhance its potency,

Table 1. Fc-optimized antagonist checkpoint antibodies in clinical research

Antibody (target)	Fc-optimized IgG	Modified effector functions	Human cancers[a] (clinical trial phase)	Respective clinical trials[b]
Tislelizumab (PD-1)	Fc-null IgG4	Prevention of FcγR$^+$ macrophage-mediated phagocytosis of tumor-reactive PD-1$^+$ T cells [9].	Esophageal squamous cell carcinoma (approved)	NCT03783442 NCT03430843
Budigalimab (PD-1)	Fc-null IgG1	Reduction of excessive complement and antibody-dependent cytotoxicity [6].	Hepatocellular carcinoma, non-small lung cancer, pancreatic ductal adenocarcinoma, gastric, esophageal and gastroesophageal junction adenocarcinoma (phases 2/3)	NCT03000257 NCT05822752 NCT06109272 NCT06236438 NCT06119217 NCT06628310
Prolgolimab (PD-1)			Colorectal cancer, melanoma, non-small cell lung, cervical cancer, Hodgkin lymphoma (phases 2/3)	NCT06428487 NCT03288870 NCT03912389 NCT05783882 NCT03912415 NCT03269565 NCT05757466
Penpulimab (PD-1)			Nasopharyngeal carcinoma, hepatocellular carcinoma, non-small cell lung, Hodgkin lymphoma, gastric and gastroesophageal junction adenocarcinoma (phase 3)	NCT04974398 NCT04344158 NCT03866993 NCT05244642 NCT04385550
Botensilimab (CTLA-4)	Mutated Fc-variants of IgG1 with increased affinity to FcγRs	Enhanced co-engagement of CTLA-4$^+$ T cells and FcγR$^+$ APCs; Treg depletion via antibody-dependent cellular cytotoxicity and phagocytosis [12,14].	Colorectal cancer, melanoma, non-small cell lung, pancreatic ductal adenocarcinoma, renal cell carcinoma, soft tissue sarcoma, gastric, esophageal and gastroesophageal junction adenocarcinoma (phase 2)	NCT05627635 NCT05608044 NCT05672316 NCT06300463 NCT06575725 NCT06322108 NCT06268015 NCT05529316 NCT05630183 NCT04028063 NCT05928806 NCT06279130 NCT05571293
Zalifrelimab (CTLA-4)			Cervical cancer, soft tissue sarcoma, pancreatic ductal adenocarcinoma, non-small cell lung angiosarcoma, urothelial carcinoma (phase 2)	NCT03495882 NCT03894215 NCT05033132 NCT04028063 NCT04827953 NCT03411473 NCT04607200 NCT04430036
Vilastobart or XTX101 (CTLA-4)	Mutated Fc variant of IgG1 with increased affinity to FcγRs (with a covalently linked masking peptide)	Localized activity due to the activity of proteases in the TME.	Advanced solid tumors (phase 1/2).	NCT04896697
BMS-986288 (CTLA-4)	Afucosylated Fc variants of IgG1 with increased affinity to FcγRIIIA (with or without a covalently linked masking peptide)	Localized activity due to the activity of proteases in the TME; enhanced T-cell priming and Treg depletion.	Advanced solid tumors (phase 1/2).	NCT03994601

[a]For the treatment of advanced/metastatic/relapsed/unresectable tumors that are often unresponsive to chemoradiotherapy.
[b]National clinical trial (NCT) number registered at https://clinicaltrials.gov/.

several anti-PD-1 mAbs were developed as Fc variants that completely lack FcγR binding. Ongoing clinical trials may better define whether the Fc-nullification strategy provides an advantage over the IgG4 scaffold of anti-PD-1 mAbs. On the other hand, Fc-null anti-CTLA-4 and anti-PD-L1 IgGs lose their optimal antitumor activity compared to Fc-active IgG isotypes of the same antibody clones [6,11]. This indicates that FcγR enhancement, and not silencing, is crucial for engaging the suppressive immune cell depletion mechanisms for the optimal activity of these mAbs.

Results obtained from tumor-bearing mice with an endogenous immune system treated with murine checkpoint mAbs are informative for studying tumor immunity. However, they are nontranslatable for human cancer immunotherapy. The cross-species differences in the repertoire of FcγR family members, IgG-binding affinities, and their expression profiles in various immune cells underscore the necessity of using preclinical humanized models with better translational potential. One such approach utilizes immunocompromised mice transplanted with a human hematopoietic system and cancer cells [9]. Alternatively, transgenic mice that express human FcγRs and human immune checkpoints instead of the respective mouse homologs have been valuable in characterizing the FcγR pathways and *in vivo* activity of human checkpoint mAbs and optimizing their IgG scaffolds [7,10]. These humanized mouse models have validated the human Fc-optimized variants of ipilimumab (anti-CTLA-4) and avelumab (anti-PD-L1). These variants, which are optimized by either Fc afucosylation to increase FcγRIIIA/B binding or Fc mutations to increase FcγRIIA and FcγRIIIA/B binding, have been identified as superior to their parental FDA-approved IgG1 variants in their antitumor immunity and efficacy.

Given the complexity of the FcγR network, it is advantageous to design next-generation Fc-optimized CTLA-4 and PD-L1 checkpoint mAbs with increased activating/inhibitory FcγR-binding affinity ratio to selectively enhance downstream immune effector pathways. The Fc-optimized anti-CTLA-4 botensilimab, currently undergoing clinical trials involving relapsed/refractory cancer patients (Table 1), is characterized by enhanced binding to activating FcγRs on effector myeloid cells and APCs to promote T cell priming and Treg cell depletion [12]. Application in mice increases the intratumoral effector T cells to Treg ratio and improves antitumor efficacy [12]. Botensilimab can bind both the low-affinity and high-affinity alleles of human *FcγRIIIA*, drawing attention to FcγR polymorphism and its possible role in ICB responsiveness in the population. Aligned with this notion, the response to the anti-CTLA-4 ipilimumab in patients with advanced melanoma and high tumor mutational burden is associated with the high-affinity allele of *FcγRIIIA* [4]. Further, patients with advanced urothelial cancer treated with the anti-PD-L1 avelumab show prolonged overall survival if they carry multiple high-affinity *FcγRIIA/IIIA* alleles compared to those with low-affinity alleles [13]. This pathway is unique to avelumab as a nonmutated IgG1 mAb, in contrast to the anti-PD-L1 durvalumab and atezolizumab, which are mutated IgG1s with reduced binding to FcγRs. Such patient data describing FcγR polymorphism correlations with therapeutic efficacy hold the potential to serve as prognostic biomarkers. Notably, it can also provide the framework for the development of next-generation Fc-engineered checkpoint mAbs tailored to specific FcγR pathways in patients with genetic variation of FcγRs. This strategy could help overcome the challenge of primary or secondary resistance to current ICB modalities. Table 1 lists next-generation Fc-optimized checkpoint mAbs presently undergoing evaluation in clinical trials, illustrating that the field is still in its early clinical development stage.

Concluding remarks

Altogether, new mechanistic insights into FcγR pathways and the technology of Fc-optimization hold great potential in advancing the field of cancer immunotherapy. The fine-tuned Fc-optimization of next-generation checkpoint and other immunomodulatory antibodies may provide a solution for targeting treatment-resistant tumors.

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Declaration of interests

RD is an inventor of PCT patent applications related to Fc optimization of checkpoint antibodies, including CD40, PD-L1, and GITR mAbs. AJK holds equity in Vir Biotechnology and Bristol-Myers Squibb.

Resources

[i]https://clinicaltrials.gov/study/NCT04219254

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