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Unlocking the potential of agonist antibodies for treating cancer using antibody engineering

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Abstract

Agonist antibodies that target immune checkpoints, such as those in the Tumor Necrosis Factor (TNF) receptor superfamily, are an important class of emerging therapeutics due to their ability to regulate immune cell activity, especially for treating cancer. Despite their great potential, to date they have shown limited clinical utility and further antibody optimization is urgently needed to improve their therapeutic potential. Here we discuss key antibody engineering approaches for improving the activity of antibody agonists by optimizing their valency, specificity for different receptors (e.g., bispecific antibodies) and epitopes (e.g., biepitopic or biparatopic antibodies), and Fc affinity for Fcγ**R** receptors. These powerful approaches are being used to develop the next generation of cancer immunotherapeutics with improved efficacy and safety.

Keywords

CD137; 4-1BB; OX40; CD40; mAb; immunotherapy

Optimizing agonist antibody properties to maximize therapeutic potential

In the last decade, therapeutic antibodies have seen exponential growth in treating many diseases including cancer, autoimmune diseases, and inflammatory disorders [1]. These molecules possess attractive biophysical properties such as high specificity, affinity, and solubility that are essential for their success [2]. The Food and Drug Administration (FDA) has approved over 100 antibodies, representing one-fifth of new drug approvals each year [3]. For example, antagonist antibodies that target immune checkpoint inhibitors such as

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programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have shown great promise in cancer treatment and several have been approved by the FDA [4]. On the other hand, agonist antibodies that activate immune receptors have been a subject of many ongoing clinical trials and have faced major roadblocks such as low efficacy and off-target effects despite their importance in mediating anti-tumor immunity [5]. Therefore, further optimization methods are needed to address these challenges in developing potent cancer immunotherapeutics.

The TNF receptor superfamily is a major player in a range of non-immune and immune functions such as cell differentiation and proliferation [6]. This diverse set of receptors consists of at least 29 members, of which several of them, including OX40, CD40, CD137, GITR and DR5, have been shown to play a significant role in cancer treatment. The expression profile of these TNF receptors is highly diverse on different types of immune and cancer cells. Antibodies specific for OX40, CD137 and GITR receptors mainly target T cells subtypes and mediate their activation, while antibodies specific for DR5 mainly target tumor cells and induce cellular apoptosis [7]. Antibodies specific for CD40 target both immune and cancer cells, which mediates activation of antigen-presenting and T cells as well as direct cytotoxic effects against cancer cells.

Several TNF-specific agonist antibodies have shown potent T-cell mediated immunity against cancer in preclinical and clinical trials [8]. However, the development of such antibodies is hindered by numerous and complex factors associated with activating the immunomodulatory receptors. Notably, conventional bivalent antibodies have limited ability to mediate higher-order receptor clustering, which is key for strong receptor activation [5]. While clustering can be achieved via FcγR-mediated crosslinking, there is scant evidence that clinically relevant antibodies are able to achieve sufficient crosslinking *in vivo* for therapeutic use. This issue is further compounded by the varying levels of FcγR expression on different immune cells, leading to a wide range of receptor agonism [9,10]. Therefore, the dependency on FcγR-mediated crosslinking remains a major pharmacologic barrier for clinical success. Finally, key agonist antibody properties include epitope, valency, specificity, Fc-mediated interactions, and isotype, all of which must be critically considered for developing potent agonist therapeutics (Figure 1). In this review, we highlight recent advancements in agonist antibody development for TNF receptors such as OX40, CD40, CD137, GITR, and Death Receptor 5 (DR5) to guide their development and optimization for clinical use (see Clinician's Corner).

Antibody Valency

Receptor clustering is a critical aspect of immune cell activation where monomeric or multimeric subunits are brought together to form higher-order receptor complexes to transduce intracellular signaling [11]. Mounting evidence has demonstrated that multivalent antibodies and Fc-fusion proteins elicit improved agonist function by forming higher-order receptor superclusters compared to their conventional bivalent counterparts (Figure 2). In one study, investigators engineered a novel hexameric Fc-fusion protein (MEDI1873) that targets glucocorticoid-induced TNFR-related protein (GITR, TNFR18) to improve anti-tumor immunity [12]. This novel Fc-fusion protein was constructed by attaching an IgG

Fc region to a trimeric motif and the human GITR ligand ectodomain. *In vitro* studies demonstrated a >2.5-fold increase in T cell proliferation. Next, MEDI1873 was evaluated in a primate model where it induced increased T cell proliferation and elevated levels of immunoglobulin (IgG) circulating antibodies, indicating both strong cellular and humoral immune responses. Given GITR is highly expressed on both human effector and regulatory T cells (Tregs), it represents an important target for developing effective therapeutics [12].

The role of valency is further bolstered by a similar study where multivalent nanobodies were designed to improve DR5 receptor signaling [13]. To do this, researchers engineered nanobodies that were fused together with a flexible glycine-serine linker to create trivalent and tetravalent constructs. These formats significantly reduced tumor cell viability by 40%, demonstrating a greater apoptotic response compared to the conventional bivalent counterpart. Interestingly, the agonist activity of the trivalent nanobody was comparable to the natural ligand as measured by caspase activity. Other studies on TNF receptors, including OX40, further support the claim that higher-order valency mediates strong receptor activation [10,14].

The success of multivalent antibodies in preclinical settings has garnered great interest for their use in early-stage clinical trials. This is exemplified by IGM-8444, an anti-DR5 multivalent antibody, which is currently undergoing a phase 1 study (Table 1, NCT04553692[I]) for relapsed and refractory cancer [15]. Another multivalent protein against OX40 known as MEDI6383 has recently completed phase 1 clinical testing. In this trial, the OX40 agonist is being assessed as a monotherapy and combination therapy with PD-1 antagonist MEDI4736, but study results have not yet been published (NCT02221960[II]) [16]. With an ever-increasing interest in the potency of multivalent antibodies, we expect to see considerable progress in designing these antibodies for therapeutic use.

Multispecific Antibody Targeting

Bispecific antibodies that target both tumor-associated antigens and T cell receptors provide a useful approach for improving the function of agonist antibodies [17]. The key advantage of using antibodies targeting tumor-associated antigens is the ability to minimize off-target effects by directing the therapeutics to the tumor microenvironment. In a recent study, investigators engineered bispecific antibodies that target both DR5 and fibroblast-activation protein (FAP), the latter of which is highly expressed in the tumor microenvironment (Figure 3) [18]. The researchers reasoned that the FAP binding domain would lead to avidity-driven receptor clustering to induce strong antitumor activity. This avidity-driven clustering would also remove the need for Fc-mediated crosslinking, which is a critical limitation of agonist antibodies. In an *in vitro* assay, the bispecific antibody elicited a >2-fold improved apoptotic response compared to its parent DR5-targeting antibody. Additionally, human xenograft mouse models showed that an antibody-induced >4-fold decrease in tumor volume relative to a clinical antibody in an Fc-independent manner. Additional studies on

[I] https://clinicaltrials.gov/ct2/show/NCT04553692
[II] https://clinicaltrials.gov/ct2/show/NCT02221960

bispecific antibodies targeting CD40 and extracellular matrix proteins have shown similar improvements in drug localization and anti-tumor efficacy [19].

Bispecific antibodies have also been designed to target two different TNF receptors, namely CD137 and OX40, to improve antitumor immunity [20]. To achieve this, the bispecific antibody FS120 was engineered to bind CD137 via the Fab region and the OX40 receptor via the Fc region. *In vitro* studies showed that FS120 mediates strong IL-2 production with >20-fold improvement compared to monospecific antibodies. Furthermore, murine studies demonstrated that this bispecific antibody elicited >1.5-fold reduction in tumor volume and resulted in >3-fold higher CD4+ T cell proliferation. These findings are also supported by other studies [21,22] including one that reports a bispecific antibody targeting EGFR and CD137 with potent agonist function [23].

Similarly, biepitopic antibodies that target two different epitopes on the same receptor provide a useful avenue to induce Fc-independent receptor clustering. For example, researchers engineered biepitopic dual-variable domain (DVD) antibodies targeting the OX40 receptor to understand their effect on T-cell proliferation [10]. This unique tetravalent antibody format was constructed by placing an additional set of variable heavy and light domains, which together bind to a distinct receptor epitope, onto the N-termini of the heavy and light chains of the parental antibody. Interestingly, these antibodies demonstrated a >3-fold improvement in CD4+ T cell proliferation compared to their bivalent counterparts. These findings were further corroborated by murine studies that showed comparable results of >2-fold proliferation of effector CD4+ T cells and enhanced IFN-γ production. To understand the mechanism, the investigators conducted structure-based studies on the biepitopic antibodies, which revealed their ability to cluster nearby receptors through daisy-chaining and promote improved agonist function.

One bispecific antibody being evaluated in phase 1 clinical trials is RG7827 (also known as RO7122290), which binds to the CD137 receptor and FAP [24]. It is also being evaluated in a combination trial with atezolizumab (PD-L1 inhibitor) for advanced solid tumors (EUDRACT Number: 2017-003961-83; Protocol Number: BP40087[III]). The tumor biopsies revealed an increased level of proliferative CD8+ T-cells as measured by the Ki67+ proliferative marker, suggesting improved T cell response. Another bispecific antibody (ATOR-1015) that targets CTLA-4 and OX40 has been shown to improve anti-tumor immunity in a phase 1 clinical trial (NCT03782467[IV]) [25]. The preliminary results indicate that the treatment is well tolerated at doses of <200 mg and further dose escalation is currently being explored. Finally, bispecific antibody RO7300490, which targets CD40 and FAP domains (NCT04857138[V]), and FS120, which targets OX40 and CD137 (NCT04648202[VI]), are currently being evaluated in phase 1 clinical settings. Collectively, these clinical trials represent a burgeoning interest in developing bispecific antibodies for diverse cancer applications.

III https://doi.org/10.1016/j.annonc.2020.08.1145
IV https://clinicaltrials.gov/ct2/show/NCT03782467
V https://clinicaltrials.gov/ct2/show/NCT04857138
VI https://clinicaltrials.gov/ct2/show/NCT04648202

Antibody Isotype

Antibody isotypes, comprising four major subclasses, have been implicated in mediating receptor activation [26]. One major theme in isotype selection is the inverse relationship between hinge region flexibility and improved agonist function [27] (Figure 4). A recent study constructed a panel of anti-mouse CD40 antibodies with different human constant domains (i.e., IgG1-4) to evaluate the impact of the C_H1-hinge region on agonist function mediated by antigen-presenting cells. Using a murine model, each human antibody isotype exhibited differential levels of CD8+ T cell activation, including a >6-fold improved response for IgG2 compared to the other isotypes. Next, the researchers sought to investigate the biophysical properties of each isotype in mediating divergent agonist activities. Structural analysis revealed that the IgG2 isotype had the most rigid hinge region whereas the least active isotype (IgG3) was the most flexible. These and other findings demonstrate that the rigidity of the human IgG2 hinge region is responsible for improved clustering of CD40 receptors compared to other isotypes [28].

A unique phenomenon of isotype switching has been observed in a study where the human CD40 IgG4 antagonist antibody (bleselumab) was generated in human IgG1 and IgG2 formats [28]. Interestingly, the IgG1 isotype retained its antagonism as expected whereas the IgG2 isotype was converted to a superagonist. *In vitro* studies illustrated that the IgG2 isotype induced >3-fold improvement in B cell proliferation compared to the most effective clinical CD40 antibody (CP-870,893). These findings were further corroborated in an *in vivo* model, where switching to IgG2 isotype induced > 4-fold CD8+ T cell expansion This phenomenon was also generalized to other CD40 antagonists, which showed improved agonistic activity with isotype switching. Although the exact mechanism of the superagonist properties of IgG2 remains unknown, it is possible that the rigidity of the IgG2 hinge overrides the antagonist nature of the molecule through enhanced receptor clustering.

In a recent clinical trial, a CD137 IgG2 antibody (utomilumab) has been shown to be well tolerated in patients with advanced solid tumors (NCT01307267[VII]) [29]. Thus far, monotherapy treatment has only shown a mild improvement in mediating antitumor immunity. In terms of a combination trial with rituximab, for instance, 20% of patients with advanced solid tumors including colorectal and pancreatic cancer demonstrated complete or partial tumor reduction while >42% maintained stable disease [30]. The tumor biopsy studies showed amplified T cell activation and tumor cytotoxicity, which is consistent with an improved antitumor response. Finally, similar antibodies targeting CD40 in a combination trial have yielded comparable results in early phase clinical trials [31,32].

Antibody Fc Receptor Interactions

The interactions between IgG Fc regions and activating/inhibitory Fcγ receptors critically impact agonist activity [33]. One major antibody engineering approach in this research area is the introduction of favorable mutations in the Fc regions that selectively increase affinity to the inhibitory receptor FcyRIIB (Figure 5). This approach is effective because

[VII] https://clinicaltrials.gOv/ct2/show/NCT01307267

FcyRIIB is solely used as a scaffold for clustering monomeric receptors to meditate strong agonist activity [34]. To demonstrate this point, researchers constructed human anti-CD40 antibodies with Fc mutations such as S267E (SE) and S267E/L328F (SELF), which increased binding to both inhibitory FcγRIIB and activating FcγRIIA receptors [35]. Compared to wild type, these mutants demonstrated >2-fold improvement in CD8 T cell activation, suggesting improved T cell immunity. Given SE and SELF mutations increase affinity to both receptors, the investigators reasoned that selective engagement to FcyRIIB could further improve agonist function. To test this hypothesis, additional Fc mutants were developed to selectively optimize Fc engagement of FcγRIIB over FcγRIIA. The results demonstrated that one variant (V11, contained five mutations) resulted in a >97-fold improvement in affinity to FcyRIIB and 3-fold reduction in affinity to FcγRIIA. This mutant displayed significant improvement in CD8+ T cell activation compared to the wild-type antibody and >5-fold improvement over the SELF variant. *In vivo* tumor studies demonstrated that while the SELF variant reduced tumor volume by 65%, the V11 variant completely abrogated tumor growth. These findings indicated that engineering antibody Fc regions with high FcγRIIB/FcγRIIA binding affinity ratios represents a powerful approach to improve the efficacy of agonist antibodies. Other studies on CD137 and OX40 antibodies highlight comparable results where selective point mutations in the Fc region can dramatically improve agonist activity [36,37].

Although improving affinity to Fcγ receptors is a viable option, this approach inherently depends on the availability of Fc receptors on antigen-presenting cells (APCs). The use of Fc mutations that promote Fc-Fc self-interactions is a unique method for improving agonist activity in an FcγR-independent manner. This is highlighted in an OX40 study where a set of Fc mutations (E345R, E430G, S440Y) promote hexamerization of IgGs [38]. Compared to wild type, the double mutant (E345R/E430G) showed the highest multimerization of receptor clusters in solution followed by the sets of triple and single mutations. An in vitro NF-kB assay was used to assess their biological response, which revealed that a single Fc mutation (E345R) induced the highest dose-dependent response compared to the sets of double and triple mutations. The authors hypothesized that the discrepancy between receptor multimerization in solution and agonist activity was partly due to the E345R mutation forming a favorable hexameric configuration upon antigen binding on the cell surface. Similar studies for other TNF receptors including DR5 demonstrate these favorable Fc mutations led to enhanced receptor agonism [39,40].

While the function of the inhibitory Fcγ receptor is well understood, activating Fcγ receptors are equally as important in mediating anti-tumor immunity. This is because activating FcγRs can induce antibody-dependent cellular cytotoxicity (ADCC) to deplete Tregs, which is a critical component of the anti-tumor response. To demonstrate this point, investigators highlighted the role of activating FcγRs on OX40-mediated Treg depletion [41]. The engagement of an OX40 mAb with activating FcγRs significantly depleted tumor infiltrating Treg cells while maintaining the CD8+ T cell subpopulation in a murine model, indicating the importance of activating receptors for mediating effective anti-tumor response. This observation is further supported by CD137 studies where binding to activating receptors resulted in amplified anti-tumor responses [42].

In terms of Fc-enhanced antibodies, a CD40 agonist mAb (known as CP-870,893 or selicrelumab) engineered with the V11 Fc mutations is currently being evaluated in a phase I combination trial with carboplatin and paclitaxel (NCT00607048[VIII]) [32]. This treatment has shown promise against melanoma cancer, where two patients displayed an increase in T-cell response. In terms of advanced solid tumors, 20% of patients exhibited a moderate decrease in target lesions, suggesting mild improvement in anti-tumor efficacy. Additionally, a CD137 antibody (LVGN6051) containing Fc mutations is also undergoing phase I clinical trials for advanced/metastatic cancer as a single agent and in combination with Keytruda, a humanized PD-1 inhibitor (NCT04130542[IX]) [43]. Thus far, preliminary data indicates a lack of adverse effects in monotherapy and only mild effects in combination therapy. In particular, a patient with metastatic head and neck squamous cell carcinoma has experienced 50% tumor reduction lasting for more than six months in combination therapy. Finally, a DR5 antibody (GEN1029) containing Fc hexamerization mutations is currently being evaluated in a phase I clinical trial (NCT03576131[X]) [44]. Thus, Fc-engineered antibodies have shown great promise in preclinical and clinical settings, strengthening their potential as cancer immunotherapeutics.

Receptor Epitope and Occupancy

The structure of TNF receptors (i.e., OX40, CD40, and CD137) consists of cysteine rich domain (CRD) subunits that can be selectively targeted by antibodies to impart superior agonist activity. Mounting evidence suggests that antibodies targeting the CRD regions outside the ligand-binding domain mediate improved agonist function (Figure 6) [45,46]. In the case of CD40 and CD137 receptors, antibodies that target the membrane distal CRD1 domain have significantly higher agonist activity compared to ligand-blocking antibodies that bind to CRD3 and CRD4. This is because antibodies that target distal domains allow for access to Fcγ receptors with reduced steric hindrance compared to antibodies targeting proximal domains. In a recent CD137 study, the epitope selection of clinical antibodies, namely urelumab and utomilumab, proved influential in mediating potent agonist activity. Structural analysis revealed that urelumab binds to CRD1 while utomilumab binds near the ligand-binding site in CRD3 and CRD4. Functional studies in CD8+ T cells showed that the non-ligand blocking antibody urelumab elicited >2 fold greater IL-2 and IFN-γ cytokine secretion than ligand-blocking utomilumab. The synergistic impact of the native ligand and clinical antibodies were evaluated for their ability to mediate clustering, which resulted in >4 times more receptor clusters for urelumab compared to utomilumab, suggesting a greater enhancement in agonist function [46]. Although urelumab shows superior agonist potential, recent studies have noted increased toxicity profiles in some patients at doses >1 mg/kg, which is much less of a problem for utomilumab for doses up to at least 10 mg/kg [46–48]. Finally, studies of other TNF receptors such as OX40 showed that non-blocking antibodies exhibit potent anti-tumor response [49,50].

VIII https://clinicaltrials.gOv/ct2/show/NCT00607048
IX https://clinicaltrials.gOv/ct2/show/NCT04130542
X https://clinicaltrials.gov/ct2/show/NCT03576131

The importance of receptor epitope selection when designing effective antibodies for cancer therapeutics is highlighted in the case of urelumab (BMS-663513), which binds CD137 via CRD1, and has been the focus of multiple phase 1 clinical trials. In a combination trial (NCT01471210[XI] and NCT01775631[XII]) with rituximab (CD20 mAb), participants with refractory B-cell lymphoma and follicular lymphoma were treated with urelumab monotherapy and combination therapy. The combination trials demonstrated promising results where 35% of follicular lymphoma patients experienced a >30% tumor decrease, and 71% had stable disease, suggesting improved disease outcome. As a result, urelumab is proceeding to further clinical testing in combination with other drugs. The most notable example is an ongoing combination trial (NCT03792724[XIII]) with PD-1 inhibitor nivolumab for patients with B-cell lymphoma.

Receptor occupancy is also an important criterion for establishing optimal agonist function. Unlike antagonist antibodies that show sigmoidal dose-response, agonist antibodies exhibit a bell-shaped dose-response curve, which means that maximum activity occurs at intermediate concentrations [51,52]. To highlight this phenomenon, investigators demonstrated that OX40 antibodies mediated maximal agonist activity at intermediate dosing concentrations using a murine model [53]. To further test this hypothesis, the researchers examined antibody-induced proliferation of human T cells and found that ~40% receptor occupancy led to maximum CD4+ T cell proliferation. Notably, loss in receptor expression was observed at >40% receptor occupancy. Similarly, this general phenomenon has been observed for other T cell receptors, including for CD28, where occupying half of the receptors on the cell surface leads to maximal receptor activation [54,55].

In terms of receptor occupancy, an OX40 antibody (BMS-986178) displays peak agonist function at ~40% receptor occupancy in preclinical settings and is the subject of multiple clinical trials [56]. In a phase I trial (NCT02737475[XIV]), BMS-986178 has been tested as a monotherapy and combination therapy with nivolumab and ipilimumab (CTLA-4 antagonist) in 165 patients with advanced solid tumors. Amongst all treatment cohorts, only a small percentage (<15%) of participants experienced moderate to high-grade adverse effects, and no toxicity was observed even at the highest dose of 320 mg BMS-986178, indicating that the maximum tolerable dosage was not reached in this study. In terms of treatment efficacy, 12% of patients experienced a >30% tumor shrinkage, and one bladder cancer patient achieved complete eradication of cancerous tumors in nivolumab combination therapy. In combination cohorts with nivolumab and ipilimumab, the investigators also observed an increase in proliferating CD8+ T cells along with a decreasing percentage of Fox3+ regulatory T cells associated with an anti-tumor response. More clinical trials are warranted to examine receptor occupancy due to its critical role in enhancing agonist function.

XI https://clinicaltrials.gov/ct2/show/NCT01471210
XII https://clinicaltrials.gov/ct2/show/NCT01775631
XIII https://clinicaltrials.gov/ct2/show/NCT03792724
XIV https://clinicaltrials.gov/ct2/show/NCT02737475

Concluding Remarks

Agonist antibodies have shown great promise in mediating anti-tumor efficacy as cancer therapeutics. Despite their potential, these antibodies have faced many roadblocks that have hindered their progress in clinical settings. Recently, designing antibodies with unique binding epitopes, valency, specificity, Fc-mediated interactions, and isotypes has enabled the optimization of their biological function. These approaches have served a critical role in improving receptor activation while mitigating the current limitations of conventional mAb therapy including poor receptor activation using IgG bivalent antibodies. Their success in a preclinical setting has led to the evaluation of these antibodies in numerous early phase clinical trials. Further research and development are needed to fully uncover their potential as cancer therapeutics.

To develop the next generation of cancer therapeutics, novel engineering approaches highlighted in this review are required for their success in clinical trials (see Outstanding Questions). For example, the receptor structure and biology must be at the forefront of agonist antibody development. A greater understanding of the mechanism of receptor activation may open new avenues for generating optimized agonist antibodies. The trimeric formation of TNF receptors is critical for their intracellular receptor signaling and, therefore, molecular formats such as multivalency are favorable for receptor clustering. Additionally, isotype selection should be an important criterion because the rigid conformation of the IgG2 isotype allows for improved agonist function. Another key challenge for clinical antibodies is the dependency on FcγR-crosslinking, which is inherently variable for in vivo applications. This issue is compounded by varying levels of FcγR expression on different immune cells, which leads to diverse levels of receptor agonism. To overcome these challenges, bispecific antibodies have been developed to mediate potent agonist function in an Fc-independent manner. Beyond engineering the Fab region, optimization to the Fc region to improve its interaction with FcγR is a viable approach to generate improved agonist antibodies. Collectively, these methods can significantly improve T cell immunity to mediate potent anti-tumor response.

Finally, bispecific antibodies that can target tumor-associated antigens can improve tumor site localization and reduce off-target effects. Dosing regimens should also be considered given that partial receptor occupancy has yielded maximum agonist function. We anticipate that the combination of one or more of these optimization approaches can improve their agonist function and therapeutic efficacy.

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Glossary

Receptor clustering

bringing together receptor subunits (monomers or multimers) to form higher-order receptor complexes on the cell surface.

Multivalent antibodies
antibody possessing more than two antigen-binding sites.

Nanobody
a type of antibody that consists of only variable heavy domains, primarily found in sharks and camelids.

Bispecific antibodies
a class of antibodies that engage two distinct receptor epitopes or antigens.

Tumor-associating antigens
antigens that are typically elevated on tumor cells relative to healthy cells.

Isotype
also known as antibody class, which categorizes antibodies based on the heavy chain.

Fcγ receptors
a group of receptors present on immune cells that bind to the Fc region of IgGs to mediate receptor signaling.

Antibody-dependent cellular cytotoxicity (ADCC)
an immune mechanism in which an immune cell lyses a target cell (e.g., tumor cell).

Cysteine-rich domain (CRD) subunits
structural regions within TNF receptors defined by the presence of repeated cysteine-rich sequence patterns.

Receptor occupancy
the fraction of antibody-bound receptors on the cell surface

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Highlights

- Agonist antibodies that activate the tumor necrosis factor (TNF) receptor superfamily on T cells are being broadly pursued for cancer therapy. However, clinical translation is stymied by poor safety and efficacy.
- Clustering of TNF receptors is critical for mediating potent receptor activation and bivalent antibodies have shown limited capacity to mediate receptor clustering on the cell surface. Thus, antibody engineering approaches for improving certain properties (i.e., multivalency and/or biepitopic targeting) are needed to enhance receptor clustering and agonist function.
- Beyond antigen-binding fragment (Fab) engineering, antibody isotype selection and improving FcγR interactions are influential in improving anti-tumor immunity in pre-clinical studies and clinical trials.
- Receptor binding epitopes and occupancy levels must be considered to mediate optimal receptor signaling and anti-tumor immunity.

Outstanding Questions

- Most antibody engineering approaches are geared toward cancer therapy; to what extent are these approaches useful for other diseases, including autoimmune or neurological disorders?
- Given the complexity of receptor signaling to what degree can antibody engineering approaches such as multivalency and bispecificity be generalized across the TNF receptor superfamily? Or across T cell receptors where receptor signaling is dependent on receptor clustering?
- The clinical translation of most agonist antibodies has been modest for cancer therapy. Is this due to poor receptor expression in the tumor microenvironment? Or the lack of tumor-infiltrating T cells? Should adjuvants be considered to boost receptor expression and therefore improve clinical efficacy?
- Factors including receptor epitope and occupancy have been shown to be influential in animal models; how well do they translate to clinical trials? Should dosing be critically considered given that partial receptor occupancy promotes optimal receptor signaling?
- To what degree do different antibody engineering approaches work in concert? For example, would bispecific antibodies that target multiple receptors with enhanced Fc regions work well together to increase efficacy in pre-clinical studies and clinical trials?
- What is the future of cancer immunotherapy with respect to agonist antibody therapeutics? Should we have a more personalized approach using bioinformatics to better understand differences in the tumor microenvironment? Are combination therapies the best way to move forward?

Clinician's Corner

- Agonist antibodies that activate TNF receptors have been shown to mediate potent anti-tumor responses in pre-clinical studies and clinical trials.
- Mounting evidence suggests that antibody engineering may be needed to improve existing antibody therapeutics to enhance their agonist potential.
- Recent clinical trial data suggest that agonists may be most beneficial in combination therapy with chemotherapy agents and inhibitory immune checkpoints (i.e., PD-1, CTLA-4).

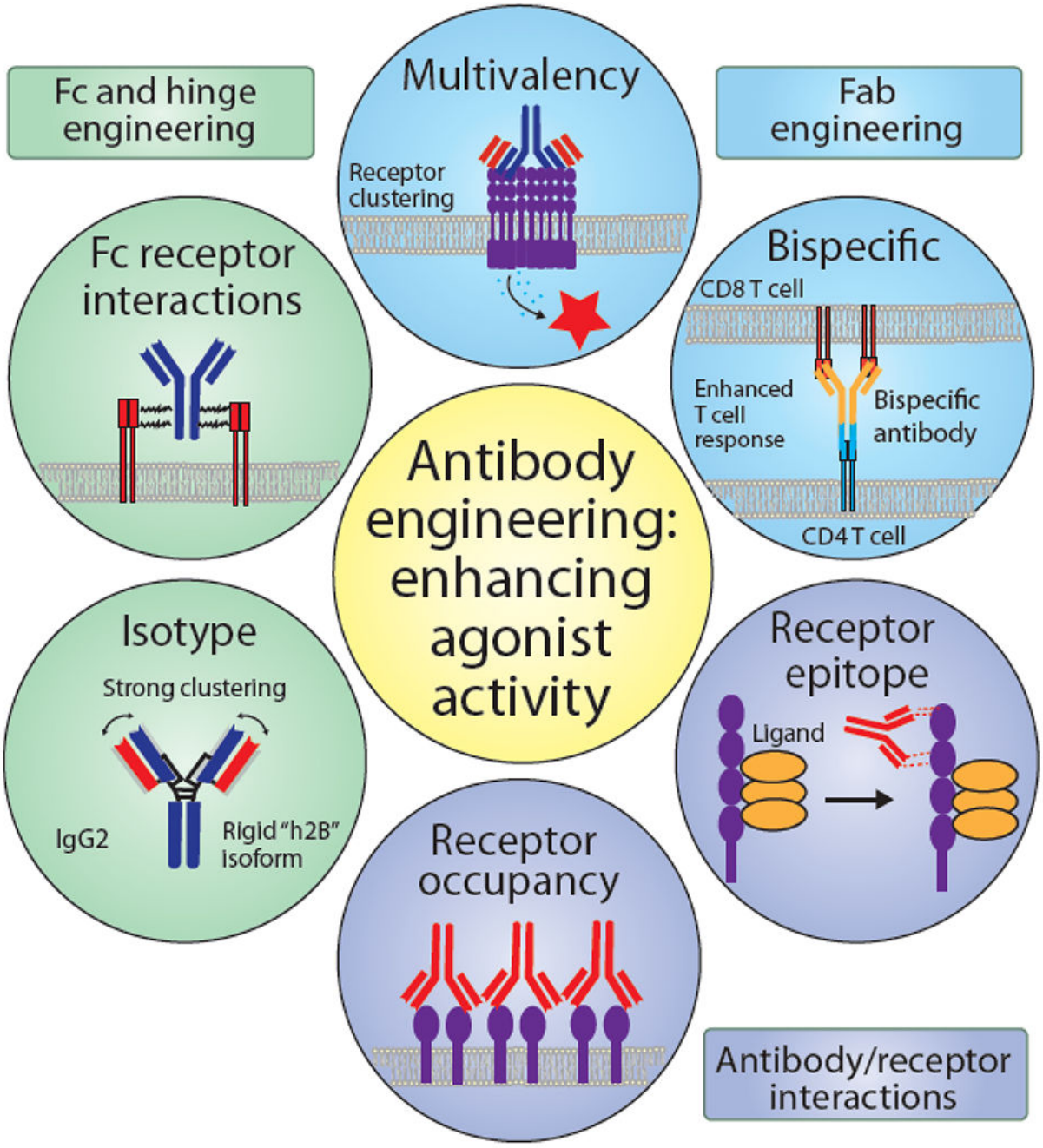

Figure 1.

Summary of antibody engineering approaches for optimizing agonist activity for therapeutic applications. Agonist activity can be optimized via engineering the Fab, hinge and Fc regions to enhance receptor clustering and activation.

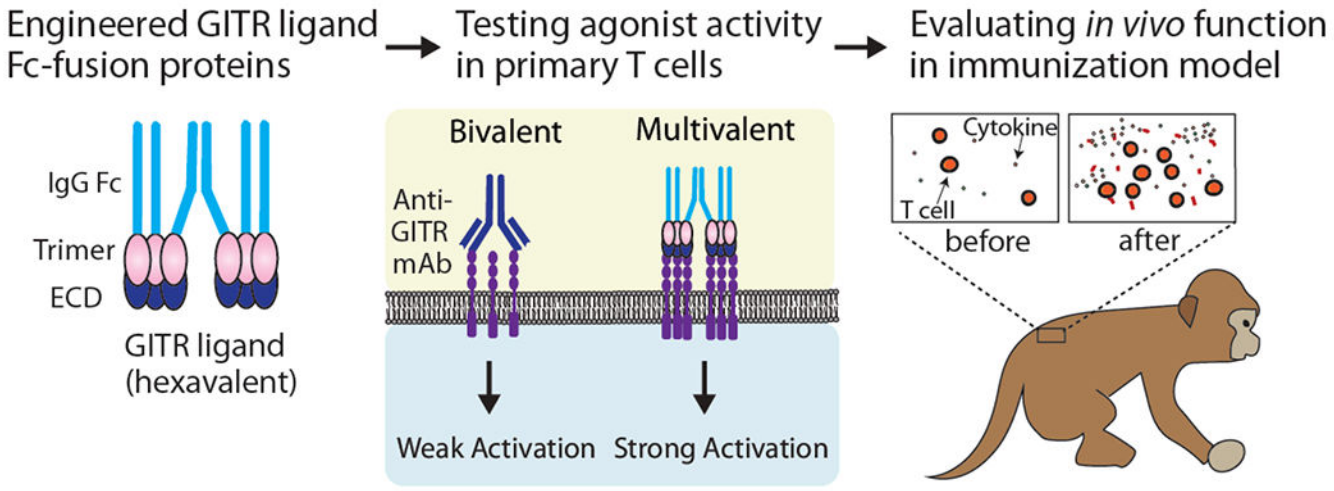

Figure 2.

Multivalent Fc-fusion protein potently activates GITR receptor. Schematic illustration of a hexavalent Fc-fusion protein, which is composed of a trimeric form of the human GITR ligand ectodomain fused on the Fc region of each heavy chain, shows potent receptor clustering and enhanced T cell activation. Adapted from [12].

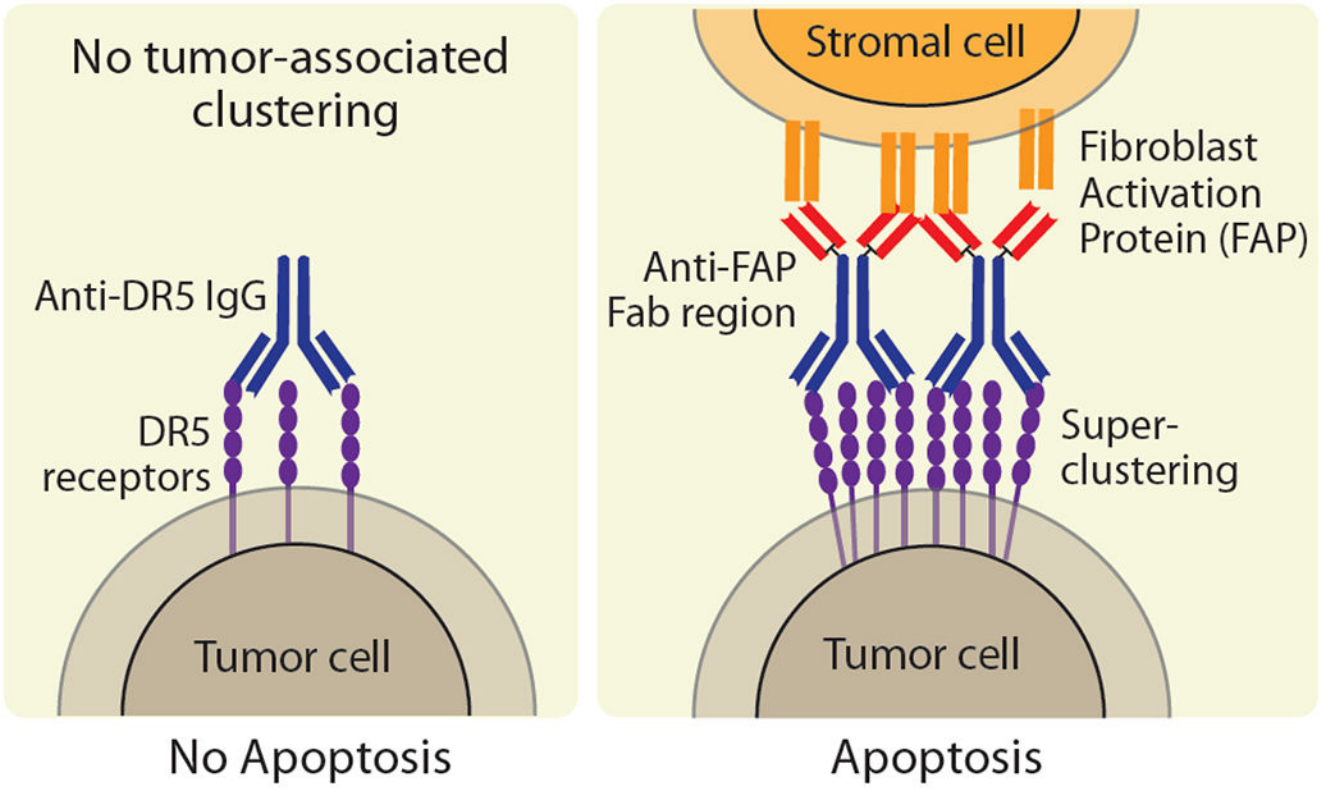

Figure 3.

DR5-FAP bispecific antibodies promote Fc-independent DR5 receptor clustering and activation. Schematic illustration of tumor-specific antibodies targeting (left) only the DR5 receptor and (right) also the fibroblast activation protein (FAP) expressed on surrounding stromal cells to enhance DR5 receptor clustering and activation. Adapted from [18].

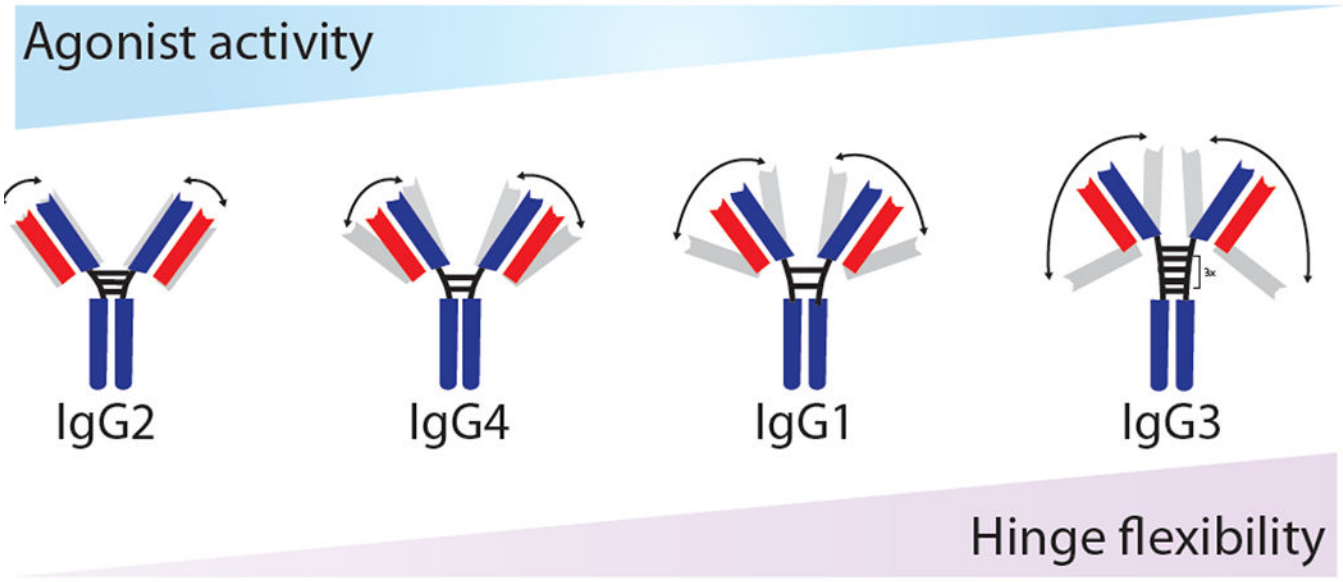

Figure 4.

Inverse relationship between hinge region flexibility and receptor activation for anti-mouse CD40 agonist antibodies with human constant domains. Schematic illustration of the negative correlation between CD40 agonist activity and hinge flexibility of human IgG isotypes. Adapted from [27].

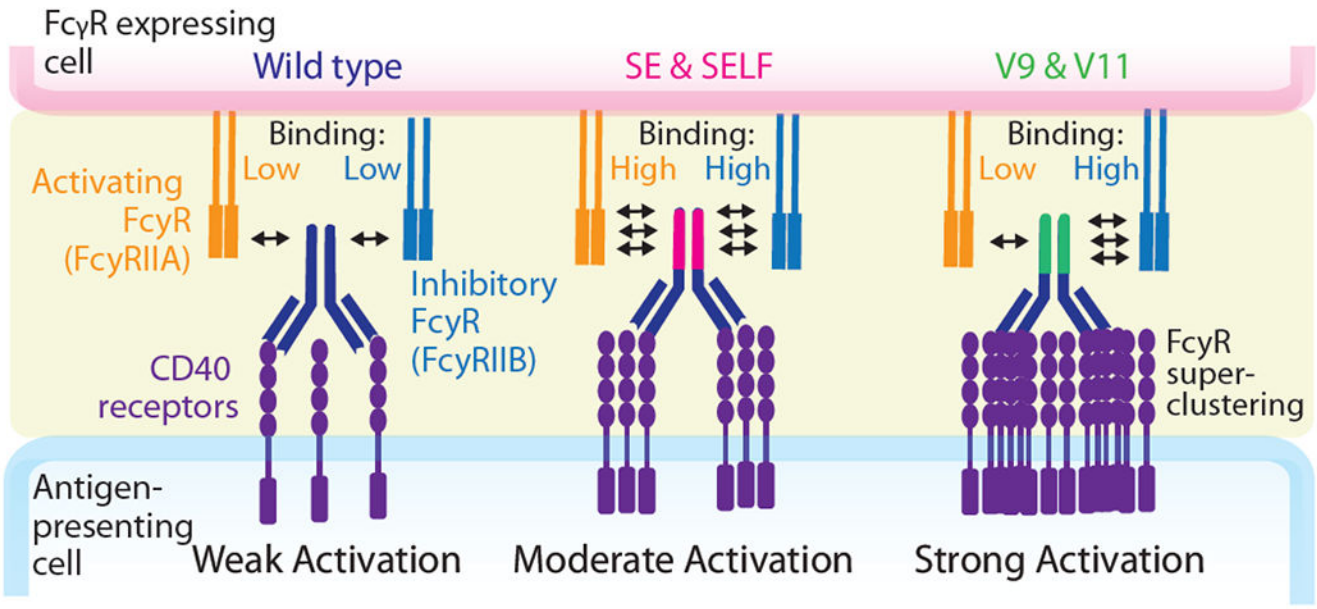

Figure 5.

Selective FcγR engagement is required for optimal human CD40 antibody agonism and anti-tumor activity. Schematic illustration of the impact of activating and inhibitory FcγRs on CD40 receptor agonism. Selective antibody binding to the inhibitory receptor FcγRIIB is positively correlated with receptor activation. Adapted from [35].

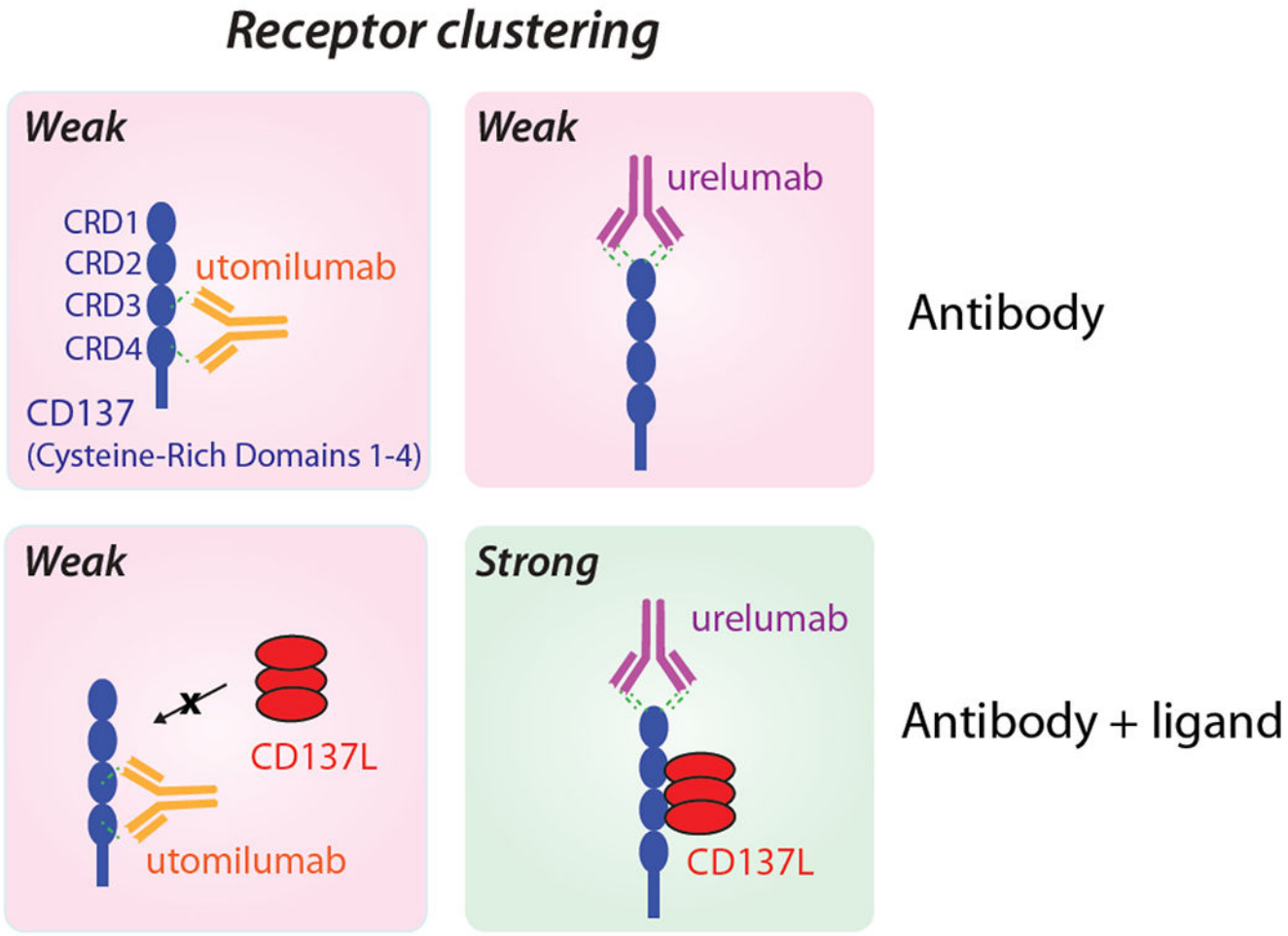

Figure 6.

CD137 epitope strongly influences the activity of agonist antibodies. Schematic illustration of the impact of CD137 receptor epitopes on the activity of two agonist antibodies, namely urelumab and utomilumab. The non-ligand blocking antibody (urelumab) targets membrane-distal cysteine-rich domain 1 (CRD1) and displays enhanced agonist activity. In contrast, the ligand-blocking antibody (utomilumab) binds to CRDs 3 and 4 and displays reduced agonist function. Adapted from [46].

Table 1.

Clinical trials.

Title	Trial ID #	Treatment	Phase	Participants	Age eligibility (years)	Condition or disease
Phase I Study of IGM-8444 as a Single Agent and in Combination in Subjects with Relapsed and/or Refractory Solid Cancers	NCT04553692 I	*Agonist:* IGM-8444 *Other drugs:* FOLFIRI, Bevacizumab (and approved biosimilars), Birinapant, Venetoclax	1	320 *	≥ 18	Solid Tumor, Colorectal Cancer, Gastric Cancer, Non-Hodgkin Lymphoma, Non-Small Cell Lung Cancer, Sarcoma, Chondrosarcoma, Small Lymphocytic Lymphoma, Chronic Lymphocytic Leukemia
A Phase 1 Study to Evaluate MEDI6383 Alone and in Combination with MEDI4736 in Adult Subjects With Select Advanced Solid Tumors	NCT02221960 II	*Agonist:* MEDI6383 *Other drugs:* MEDI4736	1	39	18-99	Recurrent or Metastatic Solid Tumors
First-in-human (FIH) phase I study of RO7122290 (RO), a novel FAP-targeted 4-1BB agonist, administered as single agent and in combination with atezolizumab (ATZ) to patients with advanced solid tumors	EUDRACT Number: 2017-003961-83; Protocol Number: BP40087III	*Agonist:* RG7827 (also known as RO7122290) *Other drugs:* atezolizumab	1	62	Not provided	Advanced Solid Tumors
Phase 1 Study in Patients with Advanced and/or Refractory Solid Malignancies to Evaluate the Safety of ATOR-1015	NCT03782467 IV	*Agonist:* ATOR-1015	1	33	≥ 18	Solid Tumor, Neoplasms
A Study to Evaluate Safety, Pharmacokinetics and Anti-Tumor Activity of RO7300490, as Single Agent or in Combination with Atezolizumab in Participants with Advanced Solid Tumors	NCT04857138 V	*Agonist:* RO7300490 *Other drugs:* Atezolizumab	1	280 *	≥ 18	Solid Tumors
FS120 First in Human Study in Patients with Advanced Malignancies	NCT04648202 VI	*Agonist:* FS120	1	277 *	≥ 18	Advanced Cancer, Metastatic Cancer
A Study Of PF-05082566 As A Single Agent and In Combination with Rituximab	NCT01307267 VII	*Agonist:* PF-05082566 *Other drugs:* rituximab	1	190	≥ 18	Lymphoma (Non-Hodgkin, Follicular, Large B-Cell (Diffuse)) Carcinoma (Non-Small-Cell Lung, Renal Cell, Squamous Cell of Head and Neck), Malignant Melanoma
Dose Finding Study Of CP-870,893, An Immune System Stimulating Antibody, In Combination with Paclitaxel And Carboplatin For Patients	NCT00607048 VIII	*Agonist:* CP-870,893 *Other drugs:* Paclitaxel, Carboplatin	1	34	18-85	Neoplasms

Title	Trial ID #	Treatment	Phase	Participants	Age eligibility (years)	Condition or disease
With Metastatic Solid Tumors						
Phase 1 Trial of LVGN6051 as Single Agent and in Combination with Keytruda (MK-3475-A31/KEYNOTE-A31) in Advanced or Metastatic Malignancy	NCT04130542 IX	*Agonist:* LVGN6051	1	276*	≥ 18	Cancer
GEN1029 (HexaBody®-DR5/DR5) Safety Trial in Patients with Malignant Solid Tumors	NCT03576131 X	*Agonist:* GEN1029	1/2	48	≥ 18	Colorectal Cancer, Non-small Cell Lung Cancer, Triple Negative Breast Cancer, Renal Cell Carcinoma, Gastric Cancer, Pancreatic Cancer, Urothelial Cancer
Safety, Tolerability, Pharmacokinetics, and Immunoregulatory Study of Urelumab (BMS-663513) in Subjects with Advanced and/or Metastatic Solid Tumors and Relapsed/ Refractory B-cell Non-Hodgkin's Lymphoma	NCT01471210 XI	*Agonist:* Urelumab (BMS-663513)	1	124	≥ 18	Cancer - Solid Tumors and B-Cell Non-Hodgkin's Lymphoma
Combination Study of Urelumab and Rituximab in Patients With B-cell Non-Hodgkins Lymphoma	NCT01775631 XII	*Agonist:* Urelumab *Other drugs:* Rituximab	1	47	≥ 18	B-Cell Malignancies
Phase I-II Study of Intratumoral Urelumab Combined with Nivolumab in Patients With Solid Tumors (INTRUST)	NCT03792724 XIII	*Agonist:* Urelumab *Other drugs:* Nivolumab	1/2	32*	≥ 18	Neoplasms
An Investigational Immuno-Therapy Study of Experimental Medication BMS-986178 by Itself or in Combination with Nivolumab and/or Ipilimumab in Participants with Solid Cancers That Are Advanced or Have Spread	NCT02737475 XIV	*Agonist:* BMS-986178 *Other drugs:* Nivolumab, Ipilimumab, Tetanus vaccine Biological: DPV-001, vaccine Drug: Cyclophosphamide	1/2	166	≥ 18	Advanced Cancer

Abbreviations:

* =estimated