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REVIEW

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Rationale and clinical development of CD40 agonistic antibodies for cancer immunotherapy

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ABSTRACT
Introduction: CD40 signaling activates dendritic cells leading to improved T cell priming against tumor antigens. CD40 agonism expands the tumor-specific T cell repertoire and has the potential to increase the fraction of patients that respond to established immunotherapies.
Areas covered: This article reviews current as well as emerging CD40 agonist therapies with a focus on antibody-based therapies, including next generation bispecific CD40 agonists. The scientific rationale for different design criteria, binding epitopes, and formats are discussed.
Expert opinion: The ability of CD40 agonists to activate dendritic cells and enhance antigen cross-presentation to CD8^{+} T cells provides an opportunity to elevate response rates of cancer immunotherapies. While there are many challenges left to address, including optimal dose regimen, CD40 agonist profile, combination partners and indications, we are confident that CD40 agonists will play an important role in the challenging task of reprogramming the immune system to fight cancer.

1. Introduction

One of the key immuno-oncology challenges is to increase the quantity and quality of tumor-infiltrating T cells in non-inflamed tumors [1,2]. In many patients, deficient T cell priming may be responsible for the lack of T cells in the tumor microenvironment (TME) [3]. CD40 provides an opportunity to kick-start the cancer-immunity cycle and promote the priming of tumor-specific T cells [4,5].

CD40, a 48 kDa transmembrane cell surface glycoprotein, is a co-stimulatory receptor belonging to the tumor necrosis factor receptor (TNFR) superfamily [6,7]. CD40 is expressed in diverse cell types and can be detected on antigen-presenting cells (APC), including dendritic cells (DC), B cells, and macrophages. In addition, CD40 is expressed on granulocytes, endothelial cells, smooth muscle cells, fibroblasts, and epithelial cells [6–9]. Consistent with its widespread expression on normal cells, CD40 is also present on the membranes of a wide range of malignant cells, including non-Hodgkin and Hodgkin lymphomas, myelomas, and certain types of carcinomas, including those of the nasopharynx, bladder, cervix, kidney, and ovary [7,10].

CD40 interacts with a single ligand, CD40L (CD154), a transmembrane protein that is expressed by activated T cells, but also on B cells, platelets, mast cells, macrophages, basophils, natural killer (NK) cells, and non-hematopoietic cells (smooth muscle cells, endothelial cells, and epithelial cells) [7,9]. The binding of CD40 to CD40L, as part of a cell–cell interaction, activates an intracellular signal transduction pathway that involves a series of adapter molecules known as TNFR activation factors (TRAF). To initiate this intracellular signal transduction, multiple CD40 receptor trimers must

form a higher order cluster on the cell membrane [8,11]. The CD40 clustering forms a signaling complex that allows multiple TRAF to assemble, which in turn leads to the activation of downstream transcription factors, including NFκB [7,12].

The molecular consequences of CD40 signaling depend on the cell type expressing CD40 and their microenvironment [13]. The 'licensing' of APC, in particular DC, results in up-regulation of membrane co-stimulatory molecules and MHC, as well as the production of pro-inflammatory cytokines [14,15]. Thus, CD40 is involved in the functional maturation of APC and consequently the activation of antigen-specific T cells [16,17]. CD40 also plays a role in humoral immunity by activating resting B cells and by increasing their antigen-presenting function [13,18]. Moreover, CD40 is involved in the induction of innate immunity through stimulation of cells such as macrophages, granulocytes and NK cells [19].

Monoclonal CD40 agonist antibodies are believed to trigger anti-tumor effects via two distinct mechanisms: (i) tumor-specific immune activation; and (ii) direct tumoricidal effects via e.g. apoptosis, antibody-dependent cellular cytotoxicity (ADCC), and/or complement-dependent cytotoxicity (CDC) [20]. Treatment with CD40 agonists induces activation of several different immune cells that contribute to the anti-tumor immune response. T cells, and in particular cytotoxic T lymphocytes (CTL), are essential for the anti-tumor effects induced by CD40 agonists, as demonstrated in a range of preclinical models [21–24]. Activation of DC and subsequent priming of T cells likely plays a central role, as the presence of antigen cross-presenting DC is required for the anti-tumor effects of CD40 agonist treatment in T cell-dependent models [25–28]. NK cells are also capable of cytotoxic killing of tumor

Article highlights

- CD40 addresses a key need in immuno-oncology by enabling activation and expansion of tumor-specific T cells
- The biological effects of CD40 agonists are determined by their binding epitope as well as their Fc format
- By acting on dendritic cells and the myeloid cell compartment, CD40 agonists provide attractive combination opportunities with, for example, chemotherapies, vaccines and checkpoint inhibitors
- Key clinical challenges are the identification of target indications, dosing regimens and combination partners
- Novel approaches to target CD40 include bispecific antibodies allowing tumor-directed target activation or neoantigen-selective T cell priming

This box summarizes key points contained in the article.

cells, and have been shown to contribute to the reduction in tumor growth in response to a CD40 agonist [29]. B cells activated through CD40 can further add to the anti-tumor immune response by presenting antigen to T cells and producing tumor-targeting antibodies [30,31]. Additionally, CD40 agonists have been found to convert tumor-associated macrophages (TAM) to activated macrophages with anti-tumor properties that can promote tumor shrinkage, independent of T cells [25–28].

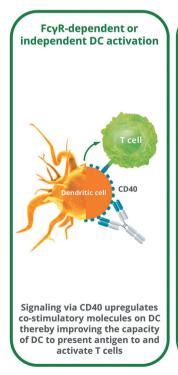## 2. Anti-tumor effects induced by CD40 signaling in different CD40-expressing cell populations
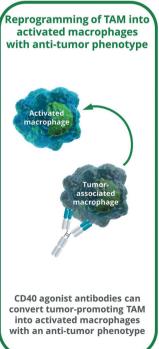
2.1. Dendritic cells

DC are the most important APC for the generation of antigen-specific T cell responses [32]. Their central role in inducing anti-tumor immune responses has been shown in preclinical models, where mice deficient in Batf3 and thereby lacking cross-presenting DC (cDC1), show impaired rejection of immunogenic tumors and fail to respond to immunotherapy due to impaired priming of tumor-targeting CTL [33,34]. In accordance with these data, the presence of cross-presenting DC in human tumors correlates with CD8$^+$ T cell infiltration and is associated with better prognosis as well as better response to immunotherapy [35,36]. Signaling through CD40 on DC induces activation of the antigen presentation machinery and upregulation of co-stimulatory molecules such as CD80 and CD86, thereby improving the capacity of the DC to present antigen to and activate T cells [27,37] (Figure 1), and to produce cytokines, notably IL-12, that helps shape the T cell response.
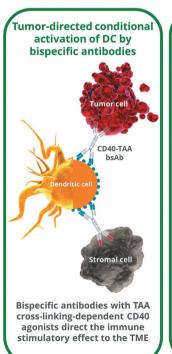
CD40 expression can be detected on all blood DC, with the highest expression found on a subpopulation referred to as cDC1 [38,39]. Recent studies have focused on the role of cDC1 in driving T cell responses to tumors, demonstrating a potential for CD40 agonists alone or in combination with other therapies in enhancing cDC1 priming of tumor-targeting T cells [40–42]. Single-cell RNA sequencing studies confirm the presence of cDC1 with the potential to respond to CD40 agonists in primary tumor tissue [42–44]. Targeting CD40 on DC therefore has the capacity to expand the tumor-specific T cell pool, and potentially represents a way to treat immunologically 'cold' tumors.

2.2. Monocytes and macrophages

Monocytes and macrophages also express CD40 and may promote immune responses against tumors. Indeed, the

FcγR-dependent or independent DC activation

Signaling via CD40 upregulates co-stimulatory molecules on DC thereby improving the capacity of DC to present antigen to and activate T cells

Reprogramming of TAM into activated macrophages with anti-tumor phenotype

CD40 agonist antibodies can convert tumor-promoting TAM into activated macrophages with an anti-tumor phenotype

Tumor-directed conditional activation of DC by bispecific antibodies

Bispecific antibodies with TAA cross-linking-dependent CD40 agonists direct the immune stimulatory effect to the TME

Uptake of neoantigen-loaded tumor debris or EV by bispecific antibodies

Bispecific antibodies targeting CD40 and high density TAA improve uptake of neoantigen-loaded EV and presentation of neoantigen peptides to T cells
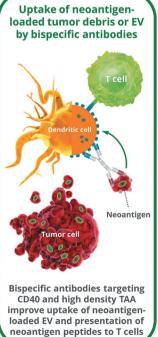
Figure 1. Different approaches to target CD40 in immuno-oncology.

murine anti-CD40 surrogate antibody FGK45 was shown to be capable of mediating anti-tumor activity involving macrophages, independent of T cell and NK cell function [25]. However, the effects of CD40 agonists on macrophages and other myeloid cell populations also result in increased production of IFN-γ and CCL5, which promote improved influx of T cells to the tumor [45].

Several studies have indicated that CD40 agonist antibodies can convert TAM into activated macrophages with an anti-tumor phenotype (Figure 1). FGK45 interacts with TAM following treatment *in vivo*, and results in their increased expression of MHCII and CD86 [26]. Similar effects have been observed on CD11b⁺ F4/80⁺ macrophages in the spleen [46], and the liver, where the treatment may result in hepatotoxicity due to the strong effect on macrophages [47,48]. Interestingly, aged and obese mice were shown to be more susceptible to systemic toxicity after immunotherapy such as anti-CD40, and it was further demonstrated that macrophages were the cells primarily responsible for these effects [49,50]. Macrophage-mediated hepatotoxicity following anti-CD40 treatment was later shown to be alleviated by combination treatment with anti-CSF-1R antibody, which blocked CSF-1R signaling supporting differentiation, proliferation and function of monocytes and macrophages [48]. Combination therapy with anti-CD40 and anti-CSF-1R is currently being explored in clinical studies [51].

2.3. B cells

CD40-activated B cells in tumor-bearing mice are capable of presenting antigens to T cells, as well as activating T cells *in vitro*, and improving anti-tumor T cell responses *in vivo*. This is an MHC II-dependent process, indicating a role for T cell priming by B cells [52]. Still, data on the role of tumor-infiltrating B cells in cancer progression is conflicting and indicate both positive and negative effects in response to CD40 activation [30,53,54].

CD40 agonists induce a rapid reduction of circulating B cells in mice [55,56], non-human primates, and humans [57–64]. This effect appears to be due to margination of activated B cells to secondary lymphoid organs [56], rather than ADCC or antibody-dependent cellular phagocytosis (ADCP), as the effect is not restricted to antibodies that engage activating Fc gamma receptors (FcγR). In humans, CD19⁺ B cell levels are transiently reduced in the blood following intravenous administration of CD40 agonists of both IgG1 and IgG2 isotype, while a dose-dependent upregulation of co-stimulatory molecules can be observed on remaining B cells [65].

2.4. Tumor cells

CD40 was originally identified through an antibody raised against urinary bladder carcinoma [66,67]. CD40 is expressed on several other carcinomas and lymphomas and CD40 antibodies may have a direct apoptotic effect on CD40-expressing tumors [68]. However, on certain types of CD40-positive lymphomas, for example, mantle cell lymphomas and follicular lymphomas, CD40 stimulation demonstrates a proliferative

effect [69,70]. CD40 agonist treatment of lymphomas may thus require a biomarker-based selection strategy [71].

2.5. Endothelial cells and epithelial cells

CD40 is also expressed on endothelial cells [72]. The functional effects of CD40 agonists on endothelial cells are complex and not fully elucidated and may enhance leukocyte adhesion and increase therapeutic efficacy [73,74], but also result in VEGF upregulation [75]. It has been demonstrated that tumor endothelial cells contribute to an IFNγ-driven feedback loop upon CD40 stimulation; however, this mechanism may be shared by other modalities that induce IFNγ [76].

Further, while CD40 is expressed at very low levels on normal epithelial cells, it is also upregulated on epithelial cells in inflamed tissues, for example, in inflammatory bowel disease [77].

3. Clinical development of CD40 agonistic antibodies

3.1. Assessment of clinical efficacy

Several CD40 agonistic antibodies, as well as soluble CD40L, have been evaluated in the clinic over the years. Promising single-agent effects have been reported in clinical studies with dacetuzumab (also known as SGN-40) in patients with non-Hodgkin lymphoma [78,79]. In addition, selicrelumab (also known as CP-870,893) induced immune-activating effects, as well as signs of clinical activity, in a dose escalation study of single intravenous doses in 29 patients with advanced solid tumors [65]. Further, Beatty *et al*. demonstrated an increase in progression-free survival when administering selicrelumab once every three weeks to 22 patients with pancreatic adeno-carcinoma [26,80]. Mitazalimab (also known as ADC-1013 or JNJ-64457107) demonstrated early signs of clinical activity in a phase 1 dose escalation study in solid tumors with one partial response [61]. Sotigalimab (also known as APX005M) and Chi Lob 7/4 reported stable disease as the best response in phase 1a studies [81–83]. In addition, a phase 1 study using recombinant human CD40L in cancer patients provided early signs of clinical activity [84].

3.2. Assessment of clinical safety and tolerability

The safety profile of CD40 agonist antibodies has been evaluated in several clinical studies in cancer patients. Overall, CD40 agonist antibodies are well tolerated, but display different maximum tolerated doses (MTD), or recommended phase 2 doses, depending on their binding epitope, affinity and Fc domain [26,62,65] (Table 1). For example, the FcγR-independent antibody selicrelumab induced cytokine release at relatively low dose levels and had an MTD of 0.2 mg/kg. This antibody was further associated with transient decreases in peripheral lymphocytes, monocytes and platelets, and elevations in serum liver transaminases and total bilirubin [78,79,85,86]. At the other end of the spectrum, the cross-linking-dependent partial agonist dacetuzumab was found to be well tolerated up to 840 mg (12 mg/kg), while Chi Lob 7/4 was tolerated up to 160 mg

Table 1. CD40 agonist antibodies currently in clinical development.

	Mitazalimab [23,59,61]	Sotigalimab [83,87,109]	ABBV-927 [110]	CDX-1140 [57,88,89]	SEA-CD40 [63,111]	Selicrelumab [62,65,80,90,91]
IgG subclass	IgG1	IgG1	IgG1	IgG2	IgG1	IgG2
Fc modification	None	S267E mutation; increased FcγRIIb binding, reduced FcγRIIIa binding	V273Y mutation; increased FcγRIIb binding, reduced FcγRIIIa binding	None	Afucosylated; increased FcγRIIIa binding	None
CD40 epitope	D1b	D3/4	NR	D1	D1b	D1a
CD40L block	Partly	Yes	NR	No	No	No
FcγR-dependent	Yes	Yes	Yes	No	NR	No
RP2D range	0.9–1.2 mg/kg	0.1–0.3 mg/kg	NR	1.5 mg/kg	0.06 mg/kg	0.2 mg/kg
In vitro efficacy	High	High	ND	Weak	High	High
In vivo activity	Yes	Surrogate data only	ND	Yes	Yes	Yes, toxic
Clinical PoM	Yes	Yes	NR	Yes	Yes	Yes
Response as single agent in Phase Ia	Low	None	NR	Low	None	Low
Clinical stage	I/II	II	I	I/II	I	I/II

RP2D, recommended phase 2 dose; NR, not reported; ND, no data; PoM, proof of mechanism

(2.3 mg/kg) [78,79,85,86]. It is likely that FcγR-independent CD40 agonists, such as selicrelumab, will mediate systemic immune activation resulting in a non-optimal therapeutic window (discussed in detail in section 4).

3.3. Combination therapy, the current focus of CD40 agonists in clinical development

To improve the clinical activity of CD40 agonists, and to provide treatment opportunities for indications with poor survival outcome, combination studies with other agents are currently being evaluated in the clinic [92] (discussed in detail in section 5). Combination with chemotherapy in pancreatic cancer is ongoing with several CD40 agonists (ABBV-927, sotigalimab, CDX-1140, SEA-CD40 and selicrelumab). Promising preliminary data was recently presented from a phase 1b clinical study with sotigalimab in patients with pancreatic cancer, in combination with gemcitabine and nab-paclitaxel [82]. Further, mitazalimab will be evaluated in metastatic pancreatic cancer in combination with mFOLFIRINOX, supported by promising preclinical data [93]. Other combinations under assessment include anti-CTLA-4 and anti-PD-1, VEGF inhibitors, anti-CSF-1R, Flt3 ligand, cancer vaccines, and radiotherapy [92].

4. The activity of CD40 agonistic antibodies is determined by their Fc domain and binding epitope

4.1. The role of Fc domain and FcγR engagement for CD40 agonists

For most CD40 agonist antibodies, formation of superclusters and the associated agonistic activity depends on engagement with FcγR [94–97]. There are exceptions to this, such as the IgG2 antibody selicrelumab, which activates CD40 independently of FcγR engagement [98]. In fact, many CD40 antibodies would be capable of activating CD40 in the absence of FcγR engagement if produced in an IgG2 format [98,99], an effect related to the rigidity of the hinge region. Especially the IgG2b variant activates strongly without the need for FcγR binding [98,99]. The antibody CDX-1140 has been designed

based on this principle and activates CD40 in the absence of FcγR cross-linking, albeit with lower efficacy than selicrelumab [57]. However, most CD40 agonistic antibodies, such as ABBV-927, sotigalimab and mitazalimab are dependent on FcγR engagement for their activity.

The Fc domain of CD40 agonists also determines the environment where they will mediate their maximal effect. Commonly used human IgG variants, such as IgG1, IgG2 and IgG4, differ in binding affinity to the different FcγR [100]. This provides an opportunity to direct the activity of anti-CD40 antibodies to different compartments and to induce maximal anti-tumor activity with minimal systemic adverse effects. While data from mouse models suggest that FcγRIIb is important for the agonistic activity of anti-CD40 antibodies [52,53,58], this could be explained by the high density of FcγRIIb in mice [101]. As a comparison, full activity can be achieved *in vitro* by cross-linking CD40 antibodies to any structure that allows efficient superclustering [94]. The subject is further complicated by the fact that engagement of FcγR may induce effector functions such as ADCC, ADCP and CDC [102]. While this may add to efficacy due to direct cytotoxic or phagocytic activity on CD40-expressing tumor cells, there is also a risk for reduced efficacy due to such effects on APC. However, potential ADCC of CD40-expressing DC does not appear to negatively affect the outcome *in vivo* [95], and there is no apparent depletion of DC following treatment with ADCC-competent CD40 agonists [94]. In addition, engagement with FcγRIIb mediates immune-inhibitory signals that may counteract the immune-stimulatory effects [103].

Preclinical efforts aimed at guiding the selection of the optimal Fc domain for CD40 agonists are hampered by the differences in IgG types and FcγR in mice and humans, in terms of expression levels [104], tissue distribution, and affinity. Mice with humanized FcγR have been generated to address these differences [101], but these models do not address the fundamental issue with differences in biodistribution and receptor density in different compartments between mice and humans, or the impact of endogenous IgG levels competing with binding to FcγR [105]. Indeed, a complicating factor is the concentration of free IgG in different compartments, as the levels of IgG determine the

amount of FcγR available for engagement by CD40 antibodies. IgG is one of the most abundant proteins in the body, with a normal IgG concentration in human plasma of approximately 10 g/L [106]. The pools of FcγR in the circulation and extravascular tissues are therefore already saturated (FcγRI), or nearly saturated (FcγRII), by endogenous IgG [107]. Thus, the availability of FcγR engagement will depend not only on their affinity for different Fc domains, but also the concentration of free IgG in each compartment. However, mouse models are, to some degree, translational when it comes to safety parameters, such as ASAT/ALAT, cytokine release and liver function [108].

4.2. Format, function and affinity for FcγR of CD40 agonist antibodies in clinical development

The FcγR-dependent human CD40 agonist antibodies in clinical development are all IgG1 isotype antibodies, but can be subcategorized based on their Fc modifications (Table 1). ABBV-927 and sotigalimab both have mutations in their Fc region (V273Y for ABBV-927 and S267E for sotigalimab), which increase their affinity for FcγRIIb and FcγRIIa and decrease their affinity for FcγRIIIa [109,110]. Mitazalimab is a wild type IgG1, with natural affinity to the different FcγR. Finally, SEA-CD40 is an afucosylated IgG1 with increased affinity to FcγR in general and FcγRIIIa in particular [63]. The activities of the antibodies in these subcategories differ based on the accessibility of FcγR in different compartments. As FcγRIIb is mostly expressed on B cells, ABBV-927 and sotigalimab are both expected to be more active in areas with a high frequency of B cells, for example, the blood or the B cell zones in secondary lymphoid organs. The increased affinity to FcγRIIb will improve their ability to compete with endogenous IgG in the circulation and thus increase activation of CD40 receptors in the blood. The higher activity of sotigalimab in the blood may explain its low phase 2 dose (0.1–0.3 mg/kg) compared to the wild-type IgG1 antibody mitazalimab (1.2 mg/kg). In contrast, mitazalimab will be active in areas with high levels of FcγRI and FcγRIII and thus have a lower activity in the blood, but potentially a higher activity in the TME. Although mitazalimab is tolerable at higher dose levels compared to sotigalimab, the pharmacodynamic biomarker response in patients is very similar [61,82]. *In vitro* studies performed by Filbert *et al.* [109], show large differences in potency between sotigalimab and mitazalimab. This is, however, not in accord with previous studies demonstrating strong immune activation with mitazalimab *in vitro* [23]. The differences are likely due to variations in assay set up and availability of FcγR. The similar pharmacodynamic responses of these antibodies in the clinical setting [61,82], also indicate that the *in vitro* comparison by Filbert *et al.* [109], are of limited translational relevance. The very low MTD of SEA-CD40, 0.06 mg/kg, may be due to its high affinity for FcγR [111], which would allow SEA-CD40 to efficiently outcompete free IgG1 from FcγR in the blood.

In summary, the net effect of CD40 activation and FcγR cross-linking will likely depend on: i) the distribution of cells expressing CD40 and cells expressing the relevant FcγR; ii) the intrinsic properties of the antibody mediating CD40 receptor clustering; and iii) the FcγR density and the sensitivity of different populations of immune cells to ADCC/ADCP/CDC [81].

4.3. Epitope specificity

The role of the binding epitope of various anti-CD40 antibodies, and whether their ability to block the interaction between CD40 and CD40L affects their agonist activity, has been thoroughly studied [112,113]. However, understanding of the optimal binding epitope, and whether blocking the CD40L binding site affects anti-tumor efficacy, is still limited. sotigalimab and mitazalimab both bind to epitopes that completely or partially overlap with the CD40L binding site (Table 1). Sotigalimab binds closer to the cell membrane [109], while mitazalimab binds to the distal domain (D1b) on CD40 [23,114] (Table 1). Selicrelumab binds an epitope located on the outer A-module of the membrane proximal domain (D1a), that is distinct from the epitope of mitazalimab [112]. Generally, it is believed that binding epitopes closer to the membrane are associated with lower efficacy, while more distal binding is associated with higher activity. Epitope-driven Fc-independent activation of CD40 is associated with binding to the most distal parts of CD40 (D1a) [112]. Further, antibodies that bind to CD40 without interfering with the binding of CD40L may enhance CD40L-mediated stimulation [115].

In conclusion, epitope binding and the choice of Fc domain generally determine the agonistic activity of anti-CD40 antibodies [106,112]. By changing the Fc domain, a CD40 antagonist (that blocks the CD40L binding site) can be converted into an agonist [113]. However, this is likely context-dependent, and affected by the choice of Fc domain.

5. Prospects of combining CD40 agonist antibodies with other cancer therapies

CD40 agonist antibodies have demonstrated moderate clinical activity as single-agent therapies and combination therapies will likely be required to unleash the full potential of CD40 targeting in oncology. The key role of CD40 in enhancing T cell priming provides opportunities for a multitude of combination therapies and several combinations have been evaluated in preclinical models [116]. Below, some of the clinically relevant combinations are discussed.

Firstly, therapies that increase the amount of relevant tumor antigens are likely to be synergistic with CD40 antibodies. This includes cancer vaccines as well as therapies resulting in direct tumor killing, such as chemotherapy, radiotherapy, but also antibody-drug conjugates (ADC) and tyrosine kinase inhibitors (TKI). Secondly, immunotherapies that activate T cells and protect from tumor-induced T cell suppression, such as immune checkpoint inhibitors (ICI) and T cell agonists, could enhance the ability of newly primed tumor-specific T cells to reach and kill tumor cells. Finally, CD40 agonist antibody therapies could also benefit from combinations with anti-angiogenic therapies that enhance T cell trafficking, promote access of immune cells to the TME and enhance DC maturation and antigen presentation in the tumor.

5.1. Combinations that increase antigen load

5.1.1. Vaccines
Strategies for improving immune responses to cancer vaccines by targeting CD40 have been explored in preclinical studies. Early reports demonstrated a key role for the interaction between CD40 and CD40L in the induction of immune responses to cancer vaccines [117], such that CD40 activation improved CTL responses to peptide vaccines and resulted in improved eradication of tumors [118]. Delivering vaccines to DC via CD40 antibodies with peptide vaccines chemically linked to the antibody, resulted in superior CD8$^+$ T cell priming when compared to targeting of other receptors such as various c-type lectins, mannose receptors, and integrins [119,120]. Further, the importance of CD40 activation in a cancer vaccination setting has been confirmed in various other preclinical models [121–124].

Mitazalimab was recently demonstrated to result in expansion of antigen-specific CD8$^+$ T cells *in vivo*. In human CD40 transgenic mice immunized with ovalbumin peptide, mitazalimab reduced growth of ovalbumin-expressing tumors both as prophylactic and therapeutic treatment [55]. Thus, CD40 agonists such as mitazalimab have the potential to act as adjuvants and could greatly improve the anti-tumor efficacy of a cancer vaccine. CDX-1140 in combination with a melanoma vaccine is currently the only clinical study assessing this potential synergy (NCT04364230). In addition, CD40 agonists have also been shown to improve T cell responses to non-tumor antigens such as HIV antigens and vaccines to infectious pathogens [32,125,126].

5.1.2. Chemotherapy
In immunologically 'cold' tumors, characterized by low immune cell infiltrates and a low expression of tumor neoantigens, chemotherapy combined with CD40 agonist antibody can improve anti-tumor responses by enhancing immunogenicity and dampening immunosuppression. This has been demonstrated in models of pancreatic cancer, where administration of CD40 agonist antibody following chemotherapy resulted in cancer cell death, tumor shrinkage, and extended survival [127]. Pancreatic cancer is classified as a 'cold' tumor due to its low infiltration of effector CD8$^+$ T cells which are blocked by the desmoplastic tumor stroma surrounding the tumor [128]. Further, the desmoplastic stroma, in addition to functioning as a physical barrier to chemotherapy and immune cell infiltration, also hosts tumor fibroblasts and suppressive myeloid cells that dampen the immune response in the TME [128]. By activating and redirecting the immunosuppressive macrophages into tumoricidal macrophages in the TME [26,28,128], CD40 agonists have the potential to augment the response to chemotherapy and initiate an effective immune reaction by i) stimulating DC and priming T cells reactive to the tumor neoantigens released by the chemotherapy; and ii) inducing degradation of fibrotic stroma surrounding the tumor, thereby enhancing the tumor penetration and effect of chemotherapies [28].

5.2. Combinations with T cell activators
Despite the improvements achieved with the use of ICI, the majority of cancer patients do not respond to these therapies. However, there is growing evidence that combining ICI with other forms of immunotherapy has great potential to improve the efficacy. CD40 agonists combined with ICI, such as anti-PD-1 or anti-CTLA-4 antibodies, have shown synergistic effect on the induction of T cell immunity and anti-tumor responses in several preclinical tumor models, including otherwise immune-resistant tumors [129–131].

5.3. Combination with anti-angiogenic therapies
Another approach to improve T cell immunity is to combine CD40 agonists with agents targeting the growth factors within the angiogenesis pathway, with the aim to block angiogenesis as well as to reduce immunosuppression in the TME [132]. The combination of anti-CD40 with either antibodies or TKI inhibiting the VEGF-A pathway, affects angiogenesis, improves DC maturation and reduces accumulation of myeloid-derived suppressor cells in preclinical tumor models. Collectively, these effects contribute to increased infiltration of CTL in tumors and reduced tumor growth [133,134]. Adding an angiopoietin 2-blocking antibody to this combination also repolarized immunosuppressive TAM into M1-like macrophages, which resulted in an even further improved anti-tumor response [133].

5.4. Other combination opportunities
Additional opportunities include combinations with agents that directly or indirectly provide additive effects on DC, such as CSF-1R inhibitors [51,135], TLR agonists [136–138], Flt3 ligand [40,139,140], and cytokines such as IL-2 [141], and IL-15 [142,143].

6. Dosing and administration route of CD40 agonistic antibodies
No consensus has yet been reached on the optimal target compartment and dosing frequency of CD40 agonist antibodies. Some data indicate that circulating myeloid cells may be an ideal target population [26]. However, activation of DC residing in the tumor or the tumor-draining lymph nodes could potentially be more relevant target cells as these are more exposed to tumor neoantigen. An understanding of which compartment should be targeted is relevant as this influences the interpretation of biomarkers and their utility for dose selection. Studies have demonstrated a bell-shaped curve for the pharmacodynamic biomarker response to CD40 agonist antibodies in the circulation, which has been used as a basis for selecting recommended phase 2 doses [144]. However, preclinical data do not suggest a bell-shaped dose response when measuring anti-tumor efficacy [145], which may reflect activation of DC in the tumor where the antibody concentration in the interstitial fluid can be much lower compared to the circulation [145]. It could thus be argued that

higher dose levels, within a range where the target is fully saturated in the circulation, would likely be required to efficiently activate CD40 in the TME [13].

Another aspect that affects the exposure in the target compartment is the route of administration. In most clinical studies on CD40 agonist antibodies, the antibody has been intravenously administered [116,146]. However, it has been suggested that the risk/benefit ratio of CD40 agonist antibodies can be increased by administering CD40 agonists either subcutaneously or intratumorally [5,23,24,147,148]. Subcutaneous administration reduces and delays the maximum serum concentration (C_{max} and T_{max}), and therefore acute immune-related adverse effects may be reduced [149]. Intratumoral administration results in activation of DC in the TME and has been shown to induce abscopal effects and systemic anti-tumor activity in preclinical models [24,108,148]. This is expected to reduce immune-related adverse effects and possibly increase efficacy. A phase 1 dose escalation study where intratumoral administration of the CD40 agonist antibody mitazalimab was evaluated, demonstrated that injections into superficial lesions was well tolerated at clinically relevant dose levels (0.4 mg/kg) and induced activation of circulating APC, without any detectable antibody levels in the blood [59].

The optimal dosing frequency of CD40 agonistic antibodies has not been defined. A study with selicrelumab showed reductions in total T cell levels upon weekly dosing, indicating that weekly dosing is too frequent and may lead to immune exhaustion [62]. Subsequent clinical studies have used dose regimes administering CD40 agonists every two or three weeks, without negative effects on total T cells [150]. CD40 agonist antibodies display target-mediated drug disposition, generally with half-lives in the range of 24 hours [61,65,81,86]. This means that even at doses well above receptor saturation, drug concentrations will be approaching zero within one week. It is likely that APC require a resting period between CD40 stimulations [55], and this may provide further support to dosing intervals longer than one week.

7. Next generation CD40-targeting compounds

The fact that many CD40 agonists are FcγR-dependent allows for generation of bispecific antibodies with conditional activation. By fusing a tumor-targeting antibody to an FcγR-dependent CD40 agonist, the immune-stimulatory activity can be directed to the TME (Figure 1). The aim of developing such bispecific antibodies is to increase both safety and efficacy.

The first attempt to evaluate this in the clinic was made with ABBV-428, a CD40 x mesothelin bispecific antibody. With this molecule, the relatively tumor-selective expression of the tumor-associated antigen (TAA) mesothelin is used to direct the CD40 agonistic effect to the tumor area, focusing on reducing systemic toxicity. Preclinical data indicated that ABBV-428 could induce similar anti-tumor activity to a monospecific CD40 antibody, while inducing less systemic immune activation and toxicity [151]. ABBV-428 was well tolerated in a phase 1 study, but the clinical efficacy was limited with no confirmed objective responses [152]. While disappointing, the reason for the limited clinical activity may be

attributed to the low expression of the tumor target, as relatively low densities (up to 9×10^4 receptors/cell) of mesothelin have been reported on tumor cells [153,154]. Indeed, preclinical studies using tumor cell lines transfected to express different levels of mesothelin demonstrated the need for much higher mesothelin expression levels to achieve anti-tumor effects with ABBV-428 [151].

In contrast, by designing a CD40 x TAA bispecific antibody which targets a highly expressed TAA, it may be possible to achieve improved safety and higher efficacy. This was recently demonstrated with NEO-X-PRIME™, a bispecific antibody platform targeting DC and a TAA with a high receptor density on the tumor cell. Using the NEO-X-PRIME™ platform, a CD40 x EpCAM bispecific antibody was developed, which demonstrated much higher preclinical anti-tumor efficacy compared to the corresponding monospecific CD40 antibody, or even to the combination of the two monospecific antibodies [155]. Interestingly, by targeting high-density tumor antigens such as EpCAM, CD40 x TAA bispecific antibodies may turn tumor-derived extracellular vesicles (EV) and exosomes into neoantigen-carrying vaccine particles [155]. The TAA-binding domain of such a CD40 x TAA bispecific antibody thus binds to the EV, while the CD40-binding domain delivers the EV to DC. The DC subsequently take up the EV and their neoantigen content and effectively cross-present neoantigen peptide to T cells, thereby expanding the tumor-specific T cell repertoire (Figure 1) [155]. As mentioned above, CD40 is a uniquely well-suited target to drive internalization and cross-presentation of tumor neoantigens, as it induces an efficient CD8$^+$ T cell response superior to that generated by targeting antigens to other receptors expressed by APC [32,119,122].

Additional means of localizing the effect of CD40 activation by use of bispecific antibodies are under investigation. CD40 x PD-L1 is one example where tumor localization could potentially be combined with blockade of the PD-(L)1 pathway. However, CD40-targeted therapies have a short, target-dependent, serum half-life, which may be beneficial for an agonist and could prevent immune exhaustion. However, this may not be ideal for the PD-L1 component as the effect of anti-PD-L1 therapies appears to be dependent on the continuous blockade of the receptor. Another approach is by directing the CD40 agonistic effects to the tumor stroma (Figure 1), which has been attempted with the CD40 x FAP bispecific compounds MP0317 and RO7300490. These bispecific CD40 agonists activate APC in the FAP-expressing stroma rather than directly in the vicinity of the tumor cells, and preclinical data has demonstrated induction of similar anti-tumor responses, but lower systemic toxicity compared to a monospecific CD40 antibody in a transfected tumor model [156,157]. Dual targeting of CD40 and CD47 is yet another approach that combines blockade of the CD47 'don't eat me' signal and localizes the CD40-mediated activity to environments expressing CD47, including the tumor area [158].

Additionally, attempts are ongoing to improve efficacy by cross-linking-dependent dual targeting of two agonistic receptors, CD40 and 4–1BB, by GEN1042, a bispecific antibody currently in clinical development for assessment of safety in a first-in-human study [159].

8. Expert opinion

The success of ICI has clearly demonstrated that the immune system can be 'reprogrammed' to provide meaningful clinical responses in some cancer patients. The coming challenges for the field of immuno-oncology will be to increase the number of patients that respond to cancer immunotherapy. This will require combination of different treatment modalities based on better understanding of the tumor immune microenvironment, where different sets of combinations will be required to overcome immune deficiencies in different tumors and indications. For cancer patients with insufficient T cell priming to tumor neoantigens due to deficiencies in DC activation and function, CD40 stands out as one of the most promising targets. The ability of CD40 agonists to activate DC and enhance antigen cross-presentation to CD8^{+} T cells provides an opportunity to meet a critical biological and medical need in immuno-oncology. However, CD40 agonists have demonstrated limited single-agent activity, and there are several issues that need to be resolved before CD40 agonists can fulfill their potential and benefit cancer patients.

Too frequent administration, that is, weekly administration, may lead to immune exhaustion but, apart from that, the optimal dosing regimen for CD40 agonists remains to be determined. Further, the optimal dosing schedule may differ when combining CD40 agonists with other therapies, in particular how to dose the combination therapies in relation to the CD40 agonist, for example, chemotherapies, where the chemotherapies result in a massive release of tumor antigens, but also affect the myeloid compartment and the quantity and quality of CD40-expressing cells.

From a biological point of view, the central role of CD40 provides a plethora of opportunities for potential combination partners. While multiple combination partners have shown additive or synergistic effects in preclinical models, the lack of translationally relevant clinical models leaves us to test this in clinical studies. Rationally designed clinical studies and comprehensive biomarker analyses will provide a better understanding of this challenge and we believe that the amazing progress achieved over the last decade in the understanding of the TME will allow us to finally make advancements in identifying the ideal combination partners.

Another challenge is to identify the indications where CD40 agonists will provide clinical benefit. While there is a clear case for T cell priming in 'cold' tumors, such as pancreatic and colorectal cancer, the relevant target cells, that is, DC, are much more frequent in 'hot' tumors, such as melanoma, renal and non-small cell lung cancer. In our opinion, more clinical studies of CD40 agonists are warranted in both 'cold' and 'hot' tumors.

There are today several CD40 agonists with different profiles in clinical development. The optimal profile of CD40 agonists will depend on where the main target cell population is located. The TME or the tumor-draining lymph nodes are likely locations, which may support selection of antibodies with a higher tumor-to-blood ratio in terms of activation. Further, activation of tumor-residing DC may require dose levels that are higher than the saturating levels in the circulation. Thus, antibodies that induce strong systemic activation may not be possible to dose at levels that activate DC in the TME or tumor-draining lymph nodes.

Finally, emerging bispecific CD40 antibodies are providing novel opportunities to use the powerful effects of CD40 ligation in a more tumor-directed fashion. By directing the effects of CD40 ligation to the TME, systemic counter-productive feedback mechanisms can be reduced, leading to improved efficacy as well as tolerability. More importantly, improved tumor neoantigen cross-presentation can be achieved, expanding the quantity and quality of the tumor-specific T cell repertoire.

To summarize, while many challenges remain to be resolved, CD40 agonists have the potential to address key biological and medical needs within immuno-oncology and we are confident that CD40 agonists will play an important role in the challenging task to reprogram the immune system to fight cancer.

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Declaration of interest

Reviewer disclosures

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