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CD40 Agonist Antibodies in Cancer Immunotherapy

Robert H. Vonderheide

Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; email: rhv@upenn.edu

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Abstract

CD40 is a cell-surface member of the TNF (tumor necrosis factor) receptor superfamily. Upon activation, CD40 can license dendritic cells to promote antitumor T cell activation and re-educate macrophages to destroy tumor stroma. Numerous agonist CD40 antibodies of varying formulations have been evaluated in the clinic and found to be tolerable and feasible. Administration is associated with mild to moderate (but transient) cytokine release syndrome, readily managed in the outpatient setting. Antitumor activity with or without anti-CTLA4 monoclonal antibody (mAb) therapy has been observed in patients with melanoma, and major tumor regressions have been observed in patients with pancreatic cancer, mesothelioma, and other tumors in combination with chemotherapy. In a recent study of chemotherapy plus CD40 mAb, with or without PD-1 mAb, the objective response rate in patients with untreated, metastatic pancreatic cancer was >50%. Mechanistically, the combination of chemotherapy followed by CD40 mAb functions as an in situ vaccine; in addition, destruction of stroma by CD40-activated macrophages may enhance chemotherapy delivery. Evidence to date suggests that CD40 activation is a critical and nonredundant mechanism to convert so-called cold tumors to hot ones (with prominent tumor infiltration of T cells), sensitizing them to checkpoint inhibition.

INTRODUCTION

CD40 is expressed broadly in hematopoietic and nonhematopoietic tissues and regulates immunity (1) thus providing a tractable target pathway for cancer immunotherapy. Attention has largely focused on the prospect of using CD40 agonists to therapeutically activate dendritic cells (DCs) and other myeloid cells that highly express CD40 (2–5). More than 20 years ago, it was shown that activation of CD40 can license DCs and substitute for T cell help needed to drive CD8 T cell responses in animal models of immunity (6–8). Additional preclinical investigations showed the role of CD40 activation in driving antitumor immunity, whereby CD40-activated DCs are poised to prime or activate tumor-specific T cells (9–11). It has been more recently appreciated that CD40 activation accomplishes immune activation independently of innate immune receptors such as stimulator of interferon genes (STING) or Toll-like receptors (TLRs) (12, 13). These observations have sparked efforts to develop CD40 agonists as novel immune therapy for patients with cancer, most notably agonistic anti-CD40 monoclonal antibodies (mAbs), but also trimeric CD40 ligand (CD40L) or ectopic expression of CD40L using gene therapy of transferred tumor or other cells (2, 4). In each case, the goal has been to activate DCs or myeloid cells via CD40, rather than blocking CD40's interaction with its ligand. This agonist approach poses major challenges around dose and schedule that complicate drug development. By contrast, checkpoint antibodies—such as those against cytotoxic T lymphocyte–associated antigen-4 (CTLA-4), programmed death–1 (PD-1), or PD-1's ligand (PD-L1)—block receptor/ligand interactions. By functioning as inhibitors of inhibitors, checkpoint antibodies permit a more straightforward drug development strategy, at least from a pharmacological standpoint. In general, because of the dosing issues, there are far fewer therapeutic agonists than therapeutic inhibitors in medicine, especially oncology. Nevertheless, in the last few years, CD40 mAbs have begun to show efficacy, particularly in combination with other therapies, and are now seen as a potentially important and as yet untapped mechanism to extend the effective range of current cancer immunotherapy to include tumors in which baseline T cell activation is insufficient. This review describes the preclinical rationale and current status of drug development for CD40 mAbs.

PRECLINICAL MECHANISMS

Activation of cells via CD40 occurs upon receptor crosslinking by CD40L (CD154), which is primarily expressed by activated $CD4^+$ T cells, but also by platelets. The biochemical consequence of CD40 crosslinking—which has been described in detail elsewhere (2, 14–16)—is complex and dependent on second signals (such as simultaneous cytokine signaling via separate receptors). CD40 is not a kinase or phosphatase, and instead signals via a series of adaptor molecules that carry activation mediators from the cell surface to the cytoplasm to the nucleus.

On DCs, CD40 activation results in two main cellular phenotypes. First, CD40 activation leads to upregulation of major histocompatibility complex (MHC) molecules, increased expression of immunoglobulin (Ig) superfamily costimulatory molecules such as CD86, and upregulation of other tumor necrosis factor (TNF) superfamily ligands such as CD137 ligand, GITR ligand, and OX40 ligand. CD40 is therefore described as proximal in the cascade of adaptive immune activation, receiving a signal from CD40L on $CD4^+$ cells, then upregulating on DCs a portfolio of secondary stimulatory molecules that accomplish enhanced antigen presentation and activation of $CD8^+$ T cells. A similar change in cell surface phenotype, especially with regard to MHC, CD80, and CD86, occurs in B cells. It is notable that CD40/CD40L is unique in its physical orientation on the DC:T cell synapse, with the TNF receptor (i.e., CD40) being expressed on the DC whereas, for most other TNF receptor/ligand pairs, the ligand is expressed on the DC, reflecting the proximal role of CD40 in the process.

Second, CD40-activated DCs elaborate an increased level of critical T cell stimulatory cytokines, including interleukin (IL)-12 p70, which is important for $CD8^+$ T cell activation and skewing of the adaptive immune response toward a Th1 polarization. Ectopic delivery of recombinant IL-12 has been challenging in the clinic, with mixed clinical results, so the prospect of CD40 activation to increase IL-12 in the DC:T cell microenvironment is value added. For CD40 agonists being tested in the clinic, the demonstration of IL-12 production has been considered an important biomarker of on-target efficacy.

In mice, there is overwhelming evidence that CD40 activation enables a vaccination effect and the concomitant expansion of antigen-specific $CD8^+$ T cells, dependent on cross-presenting (CD40-expressing) DCs (4). A critical point relates to the timing of DCs being loaded with antigen relative to CD40 activation. In general, inactivated (or immature) DCs are better able to be antigen loaded, whereas CD40-activated DCs poorly take up new antigen; thus, most studies suggest that vaccination before CD40 activation, or at least simultaneously, is optimal and that preactivation with CD40 mAbs prior to antigen delivery can even obliterate T cell activation. Moreover, antigen delivery in the setting of CD40 activation does not need to be restricted to conventional peptide or protein vaccines (17). In tumor models, antigen release can be accomplished by chemotherapy (18), radiation therapy (RT) (13), TRAIL (TNF-related apoptosis-inducing ligand) (19), and likely many other approaches.

In some tumor models, delivery of an agonist CD40 mAb alone can achieve T cell activation and T cell–dependent tumor regression (20–22). (There is also a T cell–independent, macrophage-dependent antitumor effect to consider; see below.) These CD40-sensitive tumor models often include a prominent tumor antigen. In murine models lacking such strong antigens, single-agent CD40 mAb generally fails to trigger T cell–dependent tumor regressions (23, 24). This is an important reminder when considering that among cancer patients, only those with advanced melanoma have been shown to have an objective response rate (ORR) of more than 20% to single-agent CD40 mAb therapy (25) (see below).

Our group has used the KPC model of murine pancreatic adenocarcinoma to understand the CD40 pathway and the prospect of CD40 immunotherapy. In this model, baseline T cell activation and infiltration into the tumor are especially low, and there is no model antigen or prominent display of neoepitopes (26, 27). The impression from these and other studies is that to date, the addition of CD40 enhances every T cell strategy yet tested in the KPC model, with CD40 activation standing out as the critical, independent maneuver to achieve tumor regression and cures. In particular, the addition of CD40 to anti-PD-1 or anti-PD-L1 in many tumor models can achieve tumor regression and cures not obtainable with PD-1 or PD-L1 blockade alone (24, 28). We have observed similar effects from adding anti-CTLA-4 to CD40 mAbs. On one hand, CD40 can be seen as sensitizing tumors to checkpoint therapy by generating or amplifying baseline T cell responses; on the other hand, PD-1/PD-L1 and CTLA-4 mAbs are likely critical adjuncts for CD40 therapy, insofar as new T cells generated with antigen plus CD40 would otherwise be susceptible to checkpoint receptor-mediated exhaustion. These nonredundant immunological roles of CD40, PD-1/PD-L1, and CTLA-4 suggest a potent synergy upon combination therapy.

Preclinical Combinations with Chemotherapy and Radiation

For murine tumors without expression of a strong tumor-rejection antigen, the addition of chemotherapy prior to administering agonist CD40 mAb has been shown to be a potent vaccine-like approach (12, 18, 29). As noted above, the sequence and timing of drug delivery have a major impact; for example, giving chemotherapy too soon after the CD40 mAb results in loss of T cell activation (18) and, for some chemotherapies, lethal hepatotoxicity (30). The type of

chemotherapy is also critical; for example, a CD40 mAb in combination with nab-paclitaxel (with or without gemcitabine) generates potent antitumor T cells in the KPC model but not robustly when combined with gemcitabine in the absence of nab-paclitaxel (12, 23, 31). Although we originally hypothesized this effect of nab-paclitaxel may relate to paclitaxel as a TLR ligand, it was eventually concluded that nab-paclitaxel in the KPC model simply kills more tumor cells (and therefore releases more antigen) than other chemotherapies (12).

Mechanistically, tumor regressions with combination chemotherapy and CD40 are not observed in mice lacking or depleted of T cells, nor in BATF3 knockout (lacking cross-presenting DCs) or CD40 knockout mice. B cells and macrophages are not required (12). Moreover—as expected, given the mechanism of T cell activation—the addition of PD-1 mAb, CTLA-4 mAb, or both to combination chemotherapy and CD40 further enhances the tumor regressions and improves survival (32). Indeed, the most effective CD40-based combination in the KPC model involves gemcitabine, nab-paclitaxel, CD40 mAb, PD-1/PD-L1 mAb, and CTLA-4 mAb (32). Although daunting in clinical complexity, such combinations have entered clinical trials for patients with metastatic pancreatic cancer and have shown early promise (see below) (33).

Likewise, CD40 activation can harmonize with RT, but seems to do so optimally when RT is given on a hypofractionated schedule with single doses of at least 5–6 Gy (13). Some operating features of CD40/RT are similar to CD40/chemo: (*a*) the effect is entirely dependent on T cells and BATF3 DCs, (*b*) the effect is independent of innate immune receptors such as STING or TLR, and (*c*) the addition of PD-1/PD-L1 and/or CTLA-4 mAb further enhances the effects of CD40/RT, but (*d*) the phenotype is lost if CD40 activation precedes the delivery of RT (13). It should be noted that the most commonly used clinical RT schema—including those typically used for the treatment of local tumors with curative intent—do not employ hypofractionation or large single doses. Preclinical studies suggest that the addition of checkpoint and/or CD40 therapies to these conventional RT approaches will be immunologically suboptimal.

Macrophage Activation

CD40-dependent antitumor effects that are independent of T cells have been well described (34). CD40 activation in mice has also been shown to activate host macrophages (which express high levels of CD40) (23, 35), leading in the KPC model to a concomitant involution of desmoplastic tumor stroma and transient tumor regressions. This effect is entirely independent of T cells; it is instead linked to interferon γ (IFNγ) and CCL2 released systemically in response to CD40 agonist, redirecting a $CCR2^{+}$ monocyte and macrophage population to infiltrate tumors, upregulate matrix metalloproteinases, and degrade fibrosis until the CD40 signal abates (36). Although this effect is transient and alone can result in tumor regressions, there is a further therapeutic opportunity to enhance delivery of chemotherapy during this time of stroma degradation and achieve even greater tumor regressions. One proposed schedule, as validated in KPC mice, is to administer a CD40 mAb followed five days later by gemcitabine (36). Waiting five days or more between CD40 and gemcitabine avoids hepatotoxicity.

Seen another way, these detailed studies of CD40-dependent macrophage activation in the KPC model point to the fact that re-educating tumor-associated macrophages, rather than depleting them, is possible and therapeutically tractable. Across multiple tumor models, but especially in mutant *Kras*-driven tumors such as the KPC model, macrophages and other myeloid cells are chief mediators of T cell suppression (27, 37). Tumor-intrinsic factors, especially tumor-derived chemokines, drive the establishment of a myeloid-dominant tumor microenvironment in KPC mice and actively exclude T cells (38–41). Dense macrophage infiltration is observed as early as (noninvasive) neoplastic lesions in these mice (42), and macrophages accompany lone tumor cells

attempting metastasis to the liver and other sites (43). Interrupting these tumor factors quickly leads to loss of macrophages in the tumor microenvironment and sensitivity to CD40 and checkpoint therapy, with or without chemotherapy (41). The simultaneous ability of CD40 mAbs to license DCs and re-educate macrophages (e.g., from M2 state to M1 state) represents the prospect of a dual CD40 mAb mechanism of action that may be especially critical for immunotherapy of *Kras* oncogene-driven tumors.

CLINICAL APPROACHES AND SINGLE-AGENT ACTIVITY

Multiple approaches have been formulated to activate CD40 in patients with cancer. The first CD40 therapeutic agonists, more than 20 years ago, were based on multimeric versions of CD40L itself, given subcutaneously (44). In the first-in-human study of 32 patients treated with recombinant human trimeric CD40L, two objective clinical responses were observed, including one durable complete response in a patient with squamous cell carcinoma of the head and neck. The maximum tolerated dose (MTD) of this agent was defined by transient grade 3–4 liver function test abnormalities (44), which would turn out to be a class effect of CD40 agonists.

Subsequent approaches have largely been based on agonist CD40 mAbs, delivered either intravenously or, more recently, subcutaneously and intratumorally (4). **Table 1** outlines the distinguishing characteristics of six such antibodies. The largest clinical trial experiences reported have been with selicrelumab, formerly known as CP-870,893 and RO7009789, which is a fully human IgG2 mAb (25, 45–50). CDX-1140 is also a fully human IgG2 (51), but four other CD40 mAbs brought forward to clinical trials are chimeric, humanized, or fully human IgG1 antibodies. One of these, APX005M, was uniquely derived from rabbits (52). In two cases (APX005M and SEA-CD40), Fc engineering was undertaken to enhance the potential for Fc receptor crosslinking. CD40 mAbs vary with regard to activation potency, ranging from very high (APX005M) to weak (SEA-CD40). Some CD40 mAbs block the CD40L binding site (APX005M), and others (selicrelumab and CDX-1140) do not (51).

Importantly, CD40 mAbs vary in their requirements and capacities for FcR crosslinking; for example, selicrelumab and CDX-1140 do not require crosslinking and ADC-1013 does (51). Fab'2

Table 1 Six agonist CD40 monoclonal antibodies in clinical testing

	Selicrelumab	APX005M	ChiLob7/4	ADC-1013	SEA-CD40	CDX-1140
Developer	Roche	Apexigen	University of Southampton	Janssen/Alligator	Seattle Genetics	Celldex
Antibody class	fully human IgG2	humanized rabbit IgG1	chimeric IgG1	fully human IgG1	humanized IgG1	fully human IgG2
Potency	high	very high	NR	NR	weak	NR
Engineered Fc	no	yes	no	no	yes	no
Requires crosslinking	no	yes	NR	yes	NR	no
Binds CD40L binding site	no	yes	NR	NR	NR	NR
FIH reported	yes	yes	yes	yes	yes	yes
Combinations in trials	PD-L1, CTLA-4, CSF1R, chemotherapy	PD-1, chemotherapy	NR	NR	NR	Flt3L

Abbreviations: FIH, first-in-human study; NR, not reported.

fragments of selicrelumab are as active in vitro as the IgG2 (53). Boosting signaling by intentionally crosslinking in vitro or in vivo is possible (54–56). Given some investigators' concerns that systemic delivery of CD40 mAbs may never reach an optimal therapeutic level because of toxicity, Fc engineering has been tested to improve the therapeutic index (56, 57). However, it was discovered that FcR-independent activity of human IgG2 CD40 mAbs can be provided by a conformationally distinct arrangement of disulfide bonds in the hinge region (58). Nevertheless, the vast majority of preclinical studies have utilized anti–mouse CD40 mAbs that require FcR crosslinking in vivo and compete with the CD40L binding site, which contrasts with features of selicrelumab and CDX-1140. APX005M is an Fc-mutated, humanized IgG1 that requires crosslinking for activity and competes with the CD40L binding site (52) such that APX005M closely mirrors the molecular and pharmacodynamics features of classic anti–mouse agonist CD40 mAbs such as FGK4.5, upon which most preclinical studies are based. There is no consensus on which formulation of CD40 mAb is best.

Antitumor Clinical Responses

Overall, single-agent CD40 mAbs have yielded minimal rates of objective tumor response. One exception is selicrelumab, which, in the first-in-human, single-dose study, produced objective partial responses in 4 of 15 (27%) patients with advanced melanoma, although none of 14 patients with nonmelanoma solid tumors responded (25). One of these patients with refractory metastatic melanoma subsequently received repeated doses of selicrelumab every 1 or 2 months for 1 year and remains in complete remission 15 years later, without receiving additional therapy (59). The adaptive immune response in this patient has been extensively documented (59). Interestingly, when intravenous selicrelumab was given weekly in a second trial using essentially the same eligibility criteria for patients, the ORR was zero, including among 11 patients with advanced melanoma (45). Biomarker analysis of the weekly study provided evidence for chronic B cell activation and, in some patients, T cell depletion (45), suggesting that longer dosing intervals may be most desirable for optimal immune pharmacodynamics. More is not necessarily better for immune agonists.

In the first-in-human study of APX005M, infusional side effects were dose dependent and manageable, and immune pharmacodynamic studies revealed strong activation of antigen-presenting cells, increased systemic levels of IL-12, and increased T cell activation after treatment (52). There were no clinical responses. Single-agent, first-in-human experiences have also been reported for ChiLob7/4 (60), SEA-CD40 (61), and CDX-1140 (62), each resulting in minimal antitumor clinical activity, despite alternative routes of administration in some cases. However, stable disease, as best response, is observed consistently at a rate up to 24–50% (e.g., for selicrelumab, APX005M, SEA-CD40 and ChiLob7/4) in highly refractory solid tumor patient populations.

There has been a more limited experience in treating patients with B cell malignancies with CD40 mAbs (62, 63). Although CD40 is reliably upregulated on such tumor cells, activation of CD40 in this case may be growth promoting (64). However, CD40 activation of B cell malignancies has been shown to enhance antigen presentation by the tumor cells (63, 64) and confer potential sensitivity to direct cytotoxicity or antibody-dependent cellular cytotoxicity with CD40 mAbs of the appropriate Fc effector capability (4, 51). Nevertheless, objective responses in patients with hematological malignancies treated with CD40 mAbs have been only rarely reported.

Toxicities with Agonist CD40 Antibodies

These multiple phase I studies of CD40 agonists revealed a common set of adverse events and laboratory abnormalities that are dose dependent and transient. Chief among these are mild to

moderate cytokine release syndrome (CRS), manifesting typically in the minutes to hours after infusion with fever, rigors, chills, and other symptoms such as headache or back pain. The likely mediator is IL-6 (25). These symptoms resolve with supportive care within the first hours or days and rarely if ever require hospital admission (25, 52). Hemodynamic instability in the setting of this CRS is highly uncommon. Nonsteroidal anti-inflammatory drugs, antihistamines, and acetaminophen are used to minimize the development and severity of CD40 mAb-mediated CRS. Corticosteroid premedication can prevent CRS [e.g., as tested with ChiLob7/4 (60)], but there is concern that corticosteroids may dampen immune activation. Many trials have excluded the use of corticosteroids outside of a medical emergency. CD40 mAb-related CRS is logarithmically less clinically severe than that often observed with chimeric antigen receptor modified T cell (CART-19) therapy, even though mouse models of CART-mediated CRS have revealed a critical role of CD40/CD40L activation and IL-6 in that setting (65).

Agonistic CD40 mAb infusion is associated with mild to moderate, transient liver function test abnormalities, typically grade 1–3. To my knowledge, and based on our institution's extensive experience with selicrelumab and APX005M, these elevations quickly resolve and have never been clinically meaningful or required medical intervention (4, 52). Although there is a learning curve associated with the clinical use of CD40 mAbs, infusion unit teams able to manage rituximab-related infusional reactions can easily manage CD40 mAbs. The molecular basis of liver function test abnormalities has not been elucidated, although Kupffer cells in the liver express CD40. In mice, high intravenous doses of agonist CD40 mAbs can produce hepatic necrosis (66), which is lessened when similar doses are given intratumorally (57, 67). In the clinic, hypothetical concerns that the therapeutic index of CD40 mAbs may be too narrow to permit drug development have fortunately not been realized.

CD40 mAb infusion is also associated with a transient decrease in the platelet count (25). The decrease in platelet count is nearly universally observed at sufficient doses, regardless of the baseline platelet count, but is registered as thrombocytopenia only if the starting count is relatively low. We have found it useful to chart changes in platelets and other blood parameters relative to baseline, rather than only relying on the absolute grade thresholds in the National Cancer Institute's common toxicology criteria. Changes relative to baseline are considered to reflect CD40 mAb pharmacodynamics rather than adverse effects.

Autoimmune events similar to those commonly observed with CTLA-4, PD-1, or PD-L1 mAbs (such as colitis, hypophysitis, pneumonitis, or uveitis) have either not occurred or occurred rarely with CD40 mAbs (4). One patient treated with CDX-1140 developed treatment-related grade 3 pneumonitis (62). Several patients treated with selicrelumab (47, 49) have developed arterial or venous thromboses, but it was difficult to differentiate these events from embolic events commonly seen in similar patients with advanced solid tumors. Still, many trials of CD40 agents have excluded patients with a history of thromboembolic events.

Chemotherapy and Other Clinical Combinations with CD40 Antibodies

To test the hypothesis that pretreatment with chemotherapy enhances the immune activation achievable with CD40 mAbs, selicrelumab at the single-agent MTD has been extensively tested in combination with chemotherapy. These published studies include selicrelumab with carboplatin/paclitaxel in patients with advanced solid tumors (ORR 20%) (47), with cisplatin/pemetrexed in patients with malignant pleural mesothelioma (ORR 40%) (49), and with gemcitabine in patients with newly diagnosed metastatic pancreatic cancer (ORR 24%) (48). In each case, combination therapy was feasible and tolerable with no new or unexpected side effects beyond those already observed with chemotherapy or selicrelumab alone.

Results from a phase Ib trial of patients with newly diagnosed metastatic pancreatic cancer, treated with chemotherapy and APX005M—with or without nivolumab—are promising (33). Based on preclinical studies in KPC mice, this trial was designed so each patient received gemcitabine and nab-paclitaxel weekly (days 1, 8, and 15 of each cycle per standard of care) and intravenous APX005M (at either 0.1 mg/kg or 0.3 mg/kg on day 3); half the patients additionally received the PD-1 mAb nivolumab on days 1 and 15 of each cycle. In the dose-limiting toxicity-evaluable population ($n = 24$ patients), the ORR was 54%. As a comparison, in a similar first-line metastatic patient population, the ORR of gemcitabine and nab-paclitaxel, with or without nivolumab, ranges from 18% to 23%. A randomized phase II study of gemcitabine/nab-paclitaxel with or without APX005M (0.3 mg/kg), and with or without nivolumab, in first-line metastatic pancreatic cancer is under way, sponsored by the Parker Institute for Cancer Immunotherapy (Overall PI, Vonderheide).

CD40 mAbs have also been combined with other immunotherapies. For 22 patients with checkpoint therapy–naïve metastatic melanoma, treatment with selicrelumab and the CTLA-4 mAb tremelimumab produced an ORR of 27% (50). Two patients (9%) had complete responses and nine patients are long-term survivors (>3 years). Immunologically, selicrelumab/tremelimumab was associated with T cell activation and increased tumor T cell infiltration (50), which was achieved without PD-1 or PD-L1 mAbs.

Other combinations being tested in the clinic include CD40 mAbs in combination with mAbs against PD-1, PD-L1, Flt3 ligand, colony-stimulating factor 1 receptor (CSF1R), or vascular endothelial growth factor (VEGF).

Treatment-Related Biomarkers and Immune Activation

Flow cytometric monitoring of peripheral blood cells before and after CD40 mAb infusion has shown, with several CD40 mAb agents, transient depletion of circulating B cells, in some cases more than 80% (25, 60, 68). Comparing the residual B cells to those at baseline, investigators note increased expression of CD80, CD86, CD54, and MHC classes I and II, consistent with enhanced antigen presentation capability. Similar findings on activation of circulating DCs before and after CD40 mAbs have also been reported (69).

Evidence supporting treatment-induced T cell activation has also been reported. The most extensive evidence in this regard was for the patient with refractory metastatic melanoma who remains cancer free more than 15 years after receiving about a year of selicrelumab (50). Post-treatment increases in Th1 cytokines such as macrophage inflammatory protein (MIP)-1β and TNFα have also been observed (60, 68), and cyclical upregulation of the Ki-67 proliferation marker as well as inducible T cell costimulator on circulating CD8 cells has been observed in response to each CD40 mAb infusion (69). Importantly, in the case of APX005M and ChiLob7/4, a statistically significant burst of IL-12 p70 was observed in the circulation on the first day after infusion, most consistent with DC activation (52, 60).

Across many different CD40 mAbs, significant immune modulation has been observed at doses well short of the MTD. Thus, it seems unlikely that the MTD of agonistic CD40 mAb is the maximum biological dose.

SUMMARY AND FUTURE DIRECTIONS

Agonist CD40 mAbs have a clear scientific rationale, and multiple compounds have demonstrated clinical safety and feasibility. It remains unlikely that CD40 mAbs will exhibit substantial single-agent antitumor activity in patients, outside of perhaps melanoma or other highly mutated cancers.

Given that CD40 mAbs clearly hit their target in patients, low single-agent activity should not, in my view, hinder further development of CD40 mAb therapy in combination with chemotherapy, RT, or immune therapy. Such combinations when used in murine tumor models have shown great promise if the therapeutic agents are dosed and sequenced properly. Moreover, toxicities such as mild to moderate CRS may have unfortunately dissuaded some parties from advancing clinical development of CD40 mAbs, even though biological effects and clinical responses have been observed below the MTD, and CD40 mAb-mediated CRS is readily managed in the clinic.

DC dysfunction in cancer patients remains a rate-limiting biological lesion for many patients with cancer—a deficit in the cancer immunity cycle that is not addressed by checkpoint therapy. It is becoming increasingly difficult to expect that stacking more and more negative immune checkpoint inhibitors together will alone accomplish tumor regression in so-called cold tumors. Agonist and T cell priming maneuvers are likely needed. This is the niche where CD40 agonists and second-generation Fc-engineered CD40 mAbs may show the greatest promise.

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LITERATURE CITED

1. Grewal IS, Flavell RA. 1998. CD40 and CD154 in cell-mediated immunity. *Annu. Rev. Immunol.* 16:111–35
2. Vonderheide RH. 2007. Prospect of targeting the CD40 pathway for cancer therapy. *Clin. Cancer Res.* 13:1083–8
3. Khong A, Nelson DJ, Nowak AK, et al. 2012. The use of agonistic anti-CD40 therapy in treatments for cancer. *Int. Rev. Immunol.* 31:246–66
4. Vonderheide RH, Glennie MJ. 2013. Agonistic CD40 antibodies and cancer therapy. *Clin. Cancer Res.* 19:1035–43
5. Remer M, White A, Glennie M, et al. 2017. The use of anti-CD40 mAb in cancer. *Curr. Top. Microbiol. Immunol.* 405:165–207
6. Bennett SR, Carbone FR, Karamalis F, et al. 1998. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 393:478–80
7. Ridge JP, Di Rosa F, Matzinger P. 1998. A conditioned dendritic cell can be a temporal bridge between a CD4+ T- helper and a T-killer cell. *Nature* 393:474–78
8. Schoenberger SP, Toes RE, van der Voort EI, et al. 1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 393:480–83
9. French RR, Chan HT, Tutt AL, Glennie MJ. 1999. CD40 antibody evokes a cytotoxic T-cell response that eradicates lymphoma and bypasses T-cell help. *Nat. Med.* 5:548–53
10. Diehl L, den Boer AT, Schoenberger SP, et al. 1999. CD40 activation in vivo overcomes peptide-induced peripheral cytotoxic T-lymphocyte tolerance and augments anti-tumor vaccine efficacy. *Nat. Med.* 5:774–79
11. Sotomayor EM, Borrello I, Tubb E, et al. 1999. Conversion of tumor-specific CD4+ T-cell tolerance to T-cell priming through in vivo ligation of CD40. *Nat. Med.* 5:780–87

12. Byrne KT, Vonderheide RH. 2016. CD40 stimulation obviates innate sensors and drives T cell immunity in cancer. *Cell Rep*. 15:2719–32
13. Rech AJ, Dada H, Kotzin JJ, et al. 2018. Radiotherapy and CD40 activation separately augment immunity to checkpoint blockade in cancer. *Cancer Res*. 78:4282–91
14. van Kooten C, Banchereau J. 2000. CD40-CD40 ligand. *J. Leukoc. Biol*. 67:2–17
15. Matsuzawa A, Tseng PH, Vallabhapurapu S, et al. 2008. Essential cytoplasmic translocation of a cytokine receptor-assembled signaling complex. *Science* 321:663–68
16. Elgueta R, Benson MJ, de Vries VC, et al. 2009. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol. Rev*. 229:152–72
17. Ma HS, Poudel B, Torres ER, et al. 2019. A CD40 agonist and PD-1 antagonist antibody reprogram the microenvironment of nonimmunogenic tumors to allow T-cell-mediated anticancer activity. *Cancer Immunol. Res*. 7:428–42
18. Nowak AK, Robinson BW, Lake RA. 2003. Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. *Cancer Res*. 63:4490–96
19. Uno T, Takeda K, Kojima Y, et al. 2006. Eradication of established tumors in mice by a combination antibody-based therapy. *Nat. Med*. 12:693–98
20. van Mierlo GJ, den Boer AT, Medema JP, et al. 2002. CD40 stimulation leads to effective therapy of $CD40^-$ tumors through induction of strong systemic cytotoxic T lymphocyte immunity. *PNAS* 99:5561–66
21. Mangsbo SM, Broos S, Fletcher E, et al. 2015. The human agonistic CD40 antibody ADC-1013 eradicates bladder tumors and generates T-cell-dependent tumor immunity. *Clin. Cancer Res*. 21:1115–26
22. Sandin LC, Orlova A, Gustafsson E, et al. 2014. Locally delivered CD40 agonist antibody accumulates in secondary lymphoid organs and eradicates experimental disseminated bladder cancer. *Cancer Immunol. Res*. 2:80–90
23. Beatty GL, Chiorean EG, Fishman MP, et al. 2011. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 331:1612–16
24. Ngiow SF, Young A, Blake SJ, et al. 2016. Agonistic CD40 mAb-driven IL12 reverses resistance to anti-PD1 in a T-cell-rich tumor. *Cancer Res*. 76:6266–77
25. Vonderheide RH, Flaherty KT, Khalil M, et al. 2007. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. *J. Clin. Oncol*. 25:876–83
26. Vonderheide RH. 2018. The immune revolution: a case for priming, not checkpoint. *Cancer Cell* 33:563–69
27. Morrison AH, Byrne KT, Vonderheide RH. 2018. Immunotherapy and prevention of pancreatic cancer. *Trends Cancer* 4:418–28
28. Zippelius A, Schreiner J, Herzig P, Muller P. 2015. Induced PD-L1 expression mediates acquired resistance to agonistic anti-CD40 treatment. *Cancer Immunol. Res*. 3:236–44
29. Nowak A, Mahendran S, Van der Most R, Lake R. 2008. Cisplatin and pemetrexed synergizes with immunotherapy to result in cures in established murine malignant mesothelioma. *Cancer Res*. 68(Suppl.):2073 (Abstr.)
30. Byrne KT, Leisenring NH, Bajor DL, Vonderheide RH. 2016. CSF-1R-dependent lethal hepatotoxicity when agonistic CD40 antibody is given before but not after chemotherapy. *J. Immunol*. 197:179–87
31. Beatty GL, Winograd R, Evans RA, et al. 2015. Exclusion of T cells from pancreatic carcinomas in mice is regulated by $Ly6C^{low}$ $F4/80^+$ extratumoral macrophages. *Gastroenterology* 149:201–10
32. Winograd R, Byrne KT, Evans RA, et al. 2015. Induction of T-cell immunity overcomes complete resistance to PD-1 and CTLA-4 blockade and improves survival in pancreatic carcinoma. *Cancer Immunol. Res*. 3:399–411
33. O'Hara MH, O'Reilly EM, Mick R, et al. 2019. *A phase 1b study of CD40 agonistic monoclonal antibody APX005M together with gemcitabine and nab-paclitaxel with or without nivolumab in untreated metastatic pancreatic ductal adenocarcinoma (PDAC) patients*. Paper presented at the American Association for Cancer Research Annual Meeting, Atlanta, GA, Mar. 29–Apr. 3, Abstr. CT004

34. Rakhmilevich AL, Buhtoiarov IN, Malkovsky M, Sondel PM. 2008. CD40 ligation in vivo can induce T cell independent antitumor effects even against immunogenic tumors. *Cancer Immunol. Immunother.* 57:1151–60
35. Buhtoiarov IN, Lum H, Berke G, et al. 2005. CD40 ligation activates murine macrophages via an IFN-gamma-dependent mechanism resulting in tumor cell destruction in vitro. *J. Immunol.* 174:6013–22
36. Long KB, Gladney WL, Tooker GM, et al. 2016. IFNγ and CCL2 cooperate to redirect tumor-infiltrating monocytes to degrade fibrosis and enhance chemotherapy efficacy in pancreatic carcinoma. *Cancer Discov.* 6:400–13
37. Balachandran VP, Beatty GL, Dougan SK. 2019. Broadening the impact of immunotherapy to pancreatic cancer: challenges and opportunities. *Gastroenterology* 156:2056–72
38. Bayne LJ, Beatty GL, Jhala N, et al. 2012. Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. *Cancer Cell* 21:822–35
39. Pylayeva-Gupta Y, Lee KE, Hajdu CH, et al. 2012. Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. *Cancer Cell* 21:836–47
40. Chao T, Furth EE, Vonderheide RH. 2016. CXCR2-dependent accumulation of tumor-associated neutrophils regulates T-cell immunity in pancreatic ductal adenocarcinoma. *Cancer Immunol. Res.* 4:968–82
41. Li J, Byrne KT, Yan F, et al. 2018. Tumor cell-intrinsic factors underlie heterogeneity of immune cell infiltration and response to immunotherapy. *Immunity* 49:178–93
42. Clark CE, Hingorani SR, Mick R, et al. 2007. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. *Cancer Res.* 67:9518–27
43. Aiello NM, Bajor DL, Norgard RJ, et al. 2016. Metastatic progression is associated with dynamic changes in the local microenvironment. *Nat. Commun.* 7:12819
44. Vonderheide RH, Dutcher JP, Anderson JE, et al. 2001. Phase I study of recombinant human CD40 ligand in cancer patients. *J. Clin. Oncol.* 19:3280–87
45. Ruter J, Antonia SJ, Burris HA 3rd, et al. 2010. Immune modulation with weekly dosing of an agonist CD40 antibody in a phase I study of patients with advanced solid tumors. *Cancer Biol. Ther.* 10:983–93
46. Gladue RP, Paradis T, Cole SH, et al. 2011. The CD40 agonist antibody CP-870,893 enhances dendritic cell and B-cell activity and promotes anti-tumor efficacy in SCID-hu mice. *Cancer Immunol. Immunother.* 60:1009–17
47. Vonderheide RH, Burg JM, Mick R, et al. 2013. Phase I study of the CD40 agonist antibody CP-870,893 combined with carboplatin and paclitaxel in patients with advanced solid tumors. *Oncoimmunology* 2:e23033
48. Beatty GL, Torigian DA, Chiorean EG, et al. 2013. A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin. Cancer Res.* 19:6286–95
49. Nowak AK, Cook AM, McDonnell AM, et al. 2015. A phase 1b clinical trial of the CD40-activating antibody CP-870,893 in combination with cisplatin and pemetrexed in malignant pleural mesothelioma. *Ann. Oncol.* 26:2483–90
50. Bajor DL, Mick R, Riese MJ, et al. 2018. Long-term outcomes of a phase I study of agonist CD40 antibody and CTLA-4 blockade in patients with metastatic melanoma. *Oncoimmunology* 7:e1468956
51. Vitale LA, Thomas LJ, He LZ, et al. 2019. Development of CDX-1140, an agonist CD40 antibody for cancer immunotherapy. *Cancer Immunol. Immunother.* 68:233–45
52. Johnson M, Fakih M, Bendell J, et al. 2017. First in human study with the CD40 agonistic monoclonal antibody APX005M in subjects with solid tumors. *J. ImmunoTher. Cancer* 5(Suppl. 3):89 (Abstr.)
53. Richman LP, Vonderheide RH. 2014. Role of crosslinking for agonistic CD40 monoclonal antibodies as immune therapy of cancer. *Cancer Immunol. Res.* 2:19–26
54. Li F, Ravetch JV. 2011. Inhibitory Fcγ receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies. *Science* 333:1030–34
55. White AL, Chan HT, Roghanian A, et al. 2011. Interaction with FcγRIIB is critical for the agonistic activity of anti-CD40 monoclonal antibody. *J. Immunol.* 187:1754–63
56. Dahan R, Barnhart BC, Li F, et al. 2016. Therapeutic activity of agonistic, human anti-CD40 monoclonal antibodies requires selective FcγR engagement. *Cancer Cell* 29:820–31

57. Knorr DA, Dahan R, Ravetch JV. 2018. Toxicity of an Fc-engineered anti-CD40 antibody is abrogated by intratumoral injection and results in durable antitumor immunity. *PNAS* 115:11048–53
58. White AL, Chan HT, French RR, et al. 2015. Conformation of the human immunoglobulin G2 hinge imparts superagonistic properties to immunostimulatory anticancer antibodies. *Cancer Cell* 27:138–48
59. Bajor DL, Xu X, Torigian DA, et al. 2014. Immune activation and a 9-year ongoing complete remission following CD40 antibody therapy and metastasectomy in a patient with metastatic melanoma. *Cancer Immunol. Res.* 2:1051–58
60. Johnson P, Challis R, Chowdhury F, et al. 2015. Clinical and biological effects of an agonist anti-CD40 antibody: a Cancer Research UK phase I study. *Clin. Cancer Res.* 21:1321–28
61. Grilley-Olson J, Curti BD, Smith DC, et al. 2018. SEA-CD40, a non-fucosylated CD40 agonist: interim results from a phase 1 study in advanced solid tumors. *J. Clin. Oncol.* 36(Suppl.):3093
62. Sanborn RE, Gabrail NY, Bhardwaj N, et al. 2019. *First-in-human phase 1 study of the CD40 agonist monoclonal antibody (mAb) CDX-1140 alone and in combination with CDX-301 (rhFLT3L) in patients with advanced cancers: interim analysis*. Paper presented at the American Association for Cancer Research Annual Meeting, Atlanta, GA, Mar. 29–Apr. 3, Abstr. LB-194
63. Vonderheide RH, Butler MO, Liu JF, et al. 2001. CD40 activation of carcinoma cells increases expression of adhesion and major histocompatibility molecules but fails to induce either CD80/CD86 expression or T cell alloreactivity. *Int. J. Oncol.* 19:791–98
64. Schultze JL, Michalak S, Seamon MJ, et al. 1997. CD40 activated human B cells: an alternative source of highly efficient antigen presenting cells to generate autologous antigen-specific T cells for adoptive immunotherapy. *J. Clin. Investig.* 100:2757–65
65. Giavridis T, van der Stegen SJC, Eyquem J, et al. 2018. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat. Med.* 24:731–38
66. Medina-Echeverz J, Ma C, Duffy AG, et al. 2015. Systemic agonistic anti-CD40 treatment of tumor-bearing mice modulates hepatic myeloid-suppressive cells and causes immune-mediated liver damage. *Cancer Immunol. Res.* 3:557–66
67. Fransen MF, Sluijter M, Morreau H, et al. 2011. Local activation of CD8 T cells and systemic tumor eradication without toxicity via slow release and local delivery of agonistic CD40 antibody. *Clin. Cancer Res.* 17:2270–80
68. Irenaeus SMM, Nielsen D, Ellmark P, et al. 2019. First-in-human study with intratumoral administration of a CD40 agonistic antibody, ADC-1013, in advanced solid malignancies. *Int. J. Cancer.* 145:1189–99
69. McDonnell AM, Cook A, Robinson BWS, et al. 2017. Serial immunomonitoring of cancer patients receiving combined antagonistic anti-CD40 and chemotherapy reveals consistent and cyclical modulation of T cell and dendritic cell parameters. *BMC Cancer* 17:417