Prospect of Targeting the CD40 Pathway for Cancer Therapy

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Abstract The cell surface molecule CD40 is a member of the tumor necrosis factor receptor superfamily and is broadly expressed by immune, hematopoietic, vascular, epithelial, and other cells, including a wide range of tumor cells. CD40 itself lacks intrinsic kinase or other signal transduction activity but rather mediates its diverse effects via an intricate series of downstream adapter molecules that differentially alter gene expression depending on cell type and microenvironment. As a potential target for novel cancer therapy, CD40 may mediate tumor regression through both an indirect effect of immune activation and a direct cytotoxic effect on the tumor, resulting in a "two-for-one" mechanism of action of CD40 agonists. Several drug formulations that target the CD40 pathway have undergone phase 1 clinical evaluation in advanced-stage cancer patients, and initial findings show objective clinical responses and immune modulation in the absence of major toxicity.

Background

CD40 is best appreciated as a critical regulator of cellular and humoral immunity via its expression on B lymphocytes, dendritic cells, and monocytes (1, 2). CD40 is also expressed on the cell surface of many other normal cells, including endothelial cells, fibroblasts, hematopoietic progenitors, platelets, and basal epithelial cells; the global physiologic effect of the CD40 signaling pathway is profound (1–4). CD40 ligand (CD40L), also known as CD154, is the chief ligand described for CD40 and is expressed primarily by activated T lymphocytes and platelets (2, 5). Atherosclerosis, graft rejection, coagulation, infection control, and autoimmunity are all regulated by CD40-CD40L interactions (1, 2). Curiously, many tumor cells also express CD40, including nearly 100% of B-cell malignancies and up to 70% of solid tumors. Successfully developing novel cancer therapies that target CD40 with an acceptable therapeutic index depends on an understanding of the complex biology of CD40.

CD40 signaling. The physiologic consequences of CD40 signaling are multifaceted, and even biologically opposed, depending on the type of cell expressing CD40 and the microenvironment in which the CD40 signal is provided. For example, CD40-CD40L engagement induces activation and proliferation of B lymphocytes but triggers apoptosis of carcinoma cells. Like some other members of the tumor necrosis factor (TNF) receptor family, CD40 signaling is mediated in large part by an intricate series of downstream adapter molecules rather than by inherent kinase or other signal transduction activity of the CD40 cytoplasmic tail (Fig. 1). As a consequence of CD40 signaling, a number of well-characterized signal transduction pathways are activated, including the nuclear factor-κB, p38 mitogen-activated protein kinase, c-Jun-NH_2-kinase, Janus kinases/signal transducers and activators of transcription, and phosphoinositide 3-kinase pathways (6). These pathways, in turn, regulate alterations in gene expression that are themselves extensive, dynamic, and variable. CD40 is well known to cooperate with, and even require in some cases, other extracellular signals that either induce overlapping downstream pathways or integrate others (Fig. 1). Some attempts have been made to characterize the global integration and regulation of CD40 signaling and gene expression (6); however, overall, a full appreciation of the CD40 circuitry at the level of systems biology remains incomplete. A great need exists for studies in primary cells other than B lymphocytes, primary malignant cells rather than transformed cell lines, and animal models.

CD40-induced immune activation. Signaling via CD40 activates antigen-presenting cells both *in vitro* and *in vivo*. Physiologically, this signal represents a major component of the process known as T-cell "help." Ligation of CD40 on dendritic cells, for example, induces cellular maturation and activation as manifested by increased surface expression of costimulatory and MHC molecules, production of proinflammatory cytokines such as interleukin 12, and enhanced T-cell activation (2, 4). CD40 ligation of resting B cells also increases antigen-presenting function and, in addition, induces proliferation and immunoglobulin class switching (2, 4). Patients with germ line mutations in either CD40 or CD40L are markedly immunosuppressed, susceptible to opportunistic infections, and have deficient T-cell–dependent immune reactions, including IgG production, germinal center formation, and memory B-cell induction (7–9). Similar immunophenotypes are observed in mice deficient in CD40 or CD40L (10–13).

In three articles published simultaneously in *Nature* in 1998, agonist CD40 antibodies were shown to mimic the signal of CD40L and substitute for the function of $CD4^+$ lymphocytes in murine models of T-cell–mediated immunity (14–16). A key

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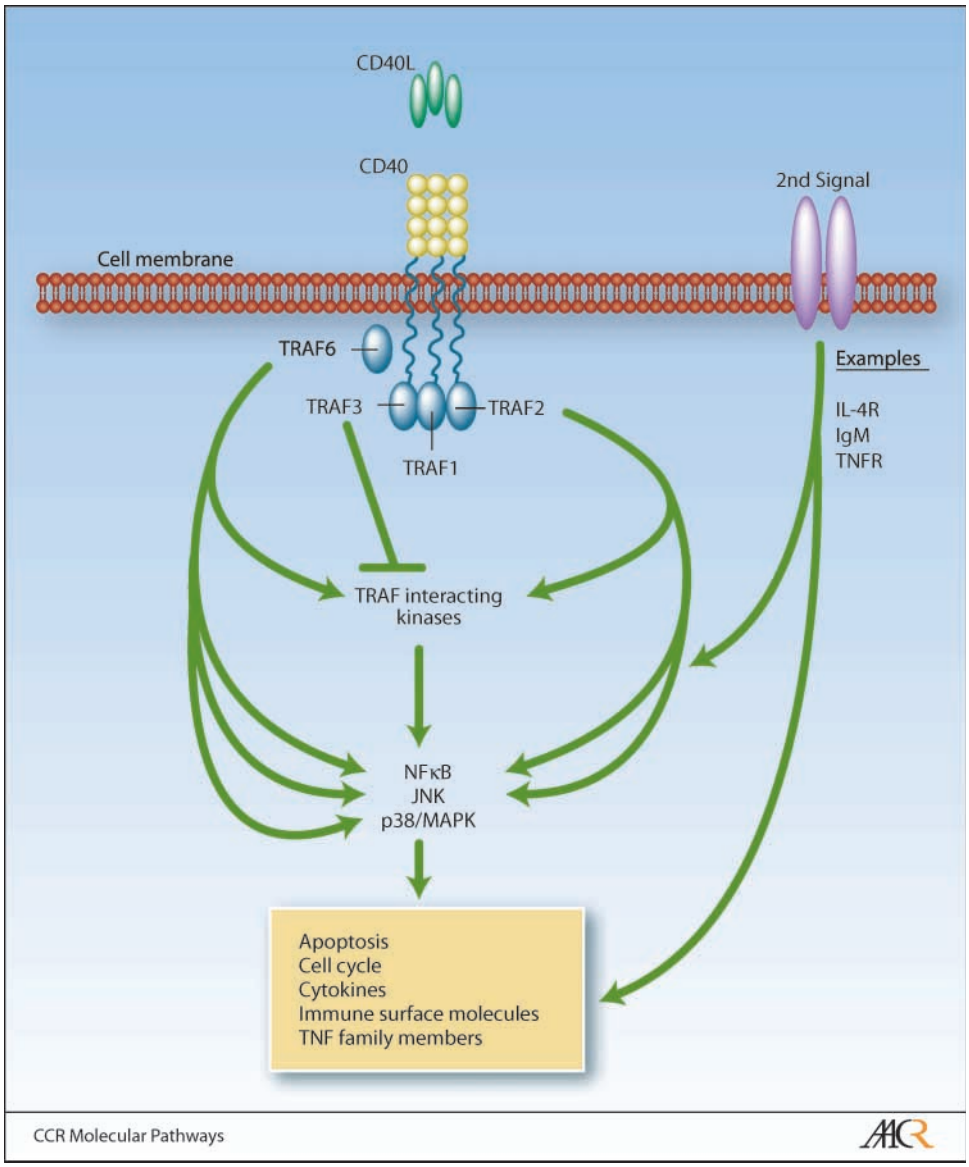

Fig. 1. The CD40 signaling pathway. Engagement of CD40 by multimeric CD40L causes redistribution of CD40 to membrane lipid rafts and a conformational change that recruits adapter molecules known as TNF receptor (*TNFR*)–associated factors (*TRAF*) to at least two distinct binding sites on the CD40 cytoplasmic tail (67, 68). TRAFs then recruit TRAF-interacting kinases and together influence a number of well-characterized signal transduction pathways, including the nuclear factor-κB, p38/mitogen-activated protein kinase (*MAPK*), and c-Jun-NH_2-kinase (*JNK*) pathways (6). TRAF2, for example, interacts with germinal center kinase in B cells and contributes to CD40-induced c-Jun-NH_2-kinase activation and cell proliferation (not shown; ref. 69). In another example, TRAF6 acts in concert with ubiquitin-activating/conjugating enzymes to activate TAK1 kinase complex and ultimately to induce nuclear factor-κB and p38/mitogen-activated protein kinase pathways (refs. 70, 71; not shown). In other circumstances, CD40-TRAF interactions have been shown to be inhibitory (72). Target genes of CD40 signaling regulate apoptosis, cell cycle progression, cytokine production, expression of cell surface immune modulators, and TNF family members and other pathways. Second extracellular signals cooperate with the CD40 signaling pathway, inducing overlapping responses or triggering others. Independent of TRAF-dependent signaling shown here, CD40 signaling can activate the Janus kinases/signal transducers and activators of transcription pathway, for example, via the binding of JAK3 to the CD40 cytoplasmic tail (73), as well as the phosphoinositide 3-kinase pathway (74–76).

mechanism of this effect was felt to be CD40/CD40L–mediated activation of host dendritic cells. These findings raised the hypothesis that CD40 agonists, together with signals involving toll-like receptors, might rescue the function of antigen-presenting cells in tumor-bearing hosts and trigger or restore effective immune responses against tumor-associated antigens. In 1999, three landmark articles in *Nature Medicine* provided the evidence for this hypothesis: agonist CD40 antibodies overcome T-cell tolerance in tumor-bearing mice, evoke effective cytotoxic T-cell responses, and enhance the efficacy of antitumor vaccines (17–19).

Consequences on survival and proliferation. Both proapoptotic and antiapoptotic genes affecting either the intrinsic or extrinsic pathways can be influenced by CD40 (20). In normal and certain malignant B cells, CD40 ligation rapidly rescues cells from apoptosis, an effect involving increased expression of bcl-xL, A20, and Bfl-1, each downstream from CD40-mediated nuclear factor-κB activation (21–24). The antiapoptotic protein survivin is also up-regulated by CD40 in some cells (25). On the other hand, CD40 may induce apoptosis in breast carcinoma cells by increased expression of Bax and in other cells by cooperation with members of the TNF family (26–30).

CD40 target genes also regulate cell cycle progression in certain cells, and, at least in B cells, seem to do so distinctly from the regulation of survival (31). For example, CD40 signaling in B cells increases expression and activation of the cyclin D–dependent kinases 4 and 6 and decreases expression of the cyclin-dependent kinase inhibitor p27kip-1 (32, 33). Pim-1, c-myc, Fas, and telomerase are other important gene products regulated by CD40 signaling (34–37), often in cooperation with second signals such as antigen-receptor ligation in B cells.

CD40-mediated tumor cell death. CD40 ligation on the surface of many tumors mediates a direct cytotoxic effect in the absence of immune accessory cells. CD40 expression is found in nearly all B-cell malignancies and many solid tumors, including melanoma and carcinomas of the lung, breast, colon, prostate, pancreas, kidney, ovary, and head and neck. Engagement of CD40 *in vitro* inhibits the growth of solid tumor cells and high-grade B cell lines, which in most experimental systems has been attributed to the induction of tumor cell apoptosis (29, 30, 38–41). CD40-mediated tumor inhibition has also been observed *in vivo*, including inhibition of breast carcinoma or B-cell lymphoma xenografts in immunocompromised mice in which there is no potential for confounding activation of lymphocytes (26, 38, 42, 43). It has always been puzzling why tumors, particularly epithelial tumors, express CD40, unless it is a remnant from ontogeny, organogenesis, or some other normal process of growth, differentiation, or response to inflammation (3). In primary cutaneous melanoma, CD40 expression has been reported to be a negative prognostic factor (44), yet the expression of CD40 in metastatic melanoma *in situ* is far weaker than in primary melanoma (41).

CD40-mediated tumor cell death seems at least additive and possibly synergic with chemotherapy both *in vitro* and *in vivo* (40, 43, 45). The combination of anti-CD40 agonist antibody and gemcitabine cures most mice with established implanted tumors, and cured mice are resistant to tumor rechallenge (45). This effect is absolutely dependent on CD8 T cells and independent of CD4 T cells and is only seen *in vivo* in the setting of tumor cell death. These findings highlight the hypothesis that immune activation and direct tumor cytotoxicity after systemic CD40 activation can be synergistic for antitumor effects.

Clinical Translational Advances

Several drug formulations that target the CD40 pathway have undergone phase 1 clinical evaluation in advanced-stage cancer patients, and initial results have been promising (Table 1). Most of these investigational drugs are designed as CD40 agonists, with a 2-fold rationale: First, CD40 agonists can trigger immune stimulation by activating host antigen-presenting cells, which then drive T-cell responses directed against tumors to cause tumor cell death. Second, CD40 ligation can impart direct tumor cytotoxicity on tumors that express CD40. Synergy develops if tumor antigens that are shed after a direct cytotoxic hit can be taken up by antigen-presenting cells during the activation process and confer tumor specificity to the resulting T-cell response.

Recombinant human CD40L, engineered with an isoleucine zipper motif to facilitate trimerization, was the first such investigational agent to be tested (46). In collaboration with two other clinic sites, we treated 32 patients with advanced solid tumors or non–Hodgkin's lymphoma with recombinant human CD40L s.c. daily for 5 days of each cycle (46). Transient elevations in serum transaminases were dose limiting, and serum half-life was ~24 h. Two patients had an objective partial response, one of whom was subsequently found to have a complete response several months after discontinuing recombinant human CD40L therapy although the patient had not initiated additional anticancer therapy.

Clinical efforts to target CD40 have accelerated in the past year with the development of anti-CD40 monoclonal

Table 1. Phase 1 studies of CD40-targeted therapy in cancer patients

Drug	Formulation	CD40 signaling	Patient population	Clinical trial findings	Reference
Recombinant CD40L	Recombinant human trimer	Agonist	Solid tumors or NHL (n = 32)	• Increased AST/ALT • Injection site reactions • 2 PR	Vonderheide et al. (46)
CP-870,893	Fully human IgG2 mAb	Agonist	Solid tumors (n = 29)	• CRS • 4 PR	Vonderheide et al. (49)
SGN-40	Humanized IgG1 mAb	Weak agonist	NHL (n = 29; ongoing)	• CRS • 4 PR, 1 CR	Forero-Torres et al. (53)
			Multiple myeloma (n = 23; ongoing)	• CRS • 4 patients with decreases in M-protein	Hussein et al. (54)
HCD 122	Fully human IgG1 mAb	Antagonist	CLL and multiple myeloma		Byrd et al. (77)* Bensinger et al. (78)*
CD40L-expressing CLL cells	Adenovirus gene therapy	Agonist	CLL (n = 11)	• Flu-like symptoms • Reductions in tumor burden	Wierda et al. (57)
Leukemia cells with CD40L and IL-2–expressing fibroblasts	Adenovirus gene therapy	Agonist	Acute or lymphoblastic leukemia in remission (n = 10)	• Well tolerated • 9 patients disease-free at median follow-up of 41 mo	Rousseau et al. (58)

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; NHL, non–Hodgkin's lymphoma; PR, partial response; CR, complete response; CRS, cytokine release syndrome; CLL, chronic lymphocytic leukemia.
*See note added in proof.

antibodies (mAb). CP-870,893 (Pfizer, New London, CT) is a fully human CD40 agonist mAb that has both immune-mediated and nonimmune-mediated effects on tumor cell death (47, 48). CP-870,893 is an IgG2 immunoglobulin (in contrast to most approved mAbs, which are IgG1 immunoglobulins) and as such is unlikely to activate complement or bind Fc receptors efficiently. Any potential biological effect is felt to be primarily related to CD40 signaling. In collaboration with Scott Antonia and colleagues at Moffitt Cancer Center, we completed a first-in-human, dose-escalation trial of 29 patients with advanced solid tumors given single doses of CP-870,893 i.v. (49). The most common adverse event was cytokine release syndrome, which manifest as transient chills, rigors, and fevers on the day of infusion and associated with elevations of serum TNF-α and interleukin 6. CP-870,893–associated cytokine release syndrome most likely reflects CD40 activation of immune and vascular cells, rather than acute target lysis or hypersensitivity, given its fully human IgG2 formulation. Four partial responses were observed upon restaging at 7 weeks; all partial responses were in patients with melanoma. With repeated dosing every 6 to 8 weeks, one patient has a continued response ongoing at 14 months, associated with complete resolution of abnormal tracer activity on positron emission tomography scan.

Pharmacodynamic studies showed that CP-870,893 infusion results in a marked, rapid, and dose-dependent decrease in the percentage of B cells. Among B cells remaining in the blood, there was a rapid and dose-related up-regulation of CD86, a costimulatory molecule fundamental to T-cell activation (49). At the highest dose levels, the percentage of $CD86^+$ B cells increased >8-fold. From these and other findings, we hypothesize that CP-870,893 infusion activates (rather than destroys) peripheral blood B cells, leading to the extravasation of most B cells from the blood. A similar effect may occur for peripheral blood monocytes and dendritic cells after CP-870,893 infusion. Whether CP-870,893 infusion is associated with the induction of cellular tumor-specific immunity remains to be explored. A study of repeated doses of CP-870,893 is under way.

A second CD40 mAb, SGN-40 (Seattle Genetics, Bothell, WA), has been evaluated in two phase 1, dose-escalation studies in patients with relapsed or refractory non–Hodgkin's lymphoma and multiple myeloma, two diseases in which CD40 is nearly uniformly expressed. SGN-40 is a humanized IgG1 immunoglobulin and a weak agonist of CD40 signaling in blood mononuclear cells, including B cells (50). Against a panel of high-grade B-cell lymphoma cell lines, however, SGN-40 mediates potent growth inhibition and apoptosis and facilitates antibody-dependent cellular cytotoxicity (50–52). In one ongoing study, 29 patients with non–Hodgkin's lymphoma received weekly doses of SGN-40 over 4 to 5 weeks, with some patients treated with an intrapatient dose-loading schedule (53). Like CP-870,893, SGN-40 is associated with cytokine release syndrome, most pronounced with the first infusion and extinguished with subsequent dosing in the patients described thus far. Five non–Hodgkin's lymphoma patients have achieved objective tumor responses (four patients with partial response and one with a complete response after one cycle of SGN-40 ongoing at 20 weeks). The experience of 16 multiple myeloma patients treated thus far with SGN-40 has also been reported, with similar adverse events and encouraging antitumor activity (Table 1; ref. 54).

The clearance of both SGN-40 and CP-870,893 seems unusually rapid for IgG1 or IgG2 immunoglobulin molecules administered i.v. CP-870,893, for example, is detectable in serum for <24 h in the single-infusion study, possibly reflecting antibody binding to a broadly expressed target on normal cells (49). SGN-40 investigators suggest that there may be a rapid elimination pathway of SGN-40 or a redistribution volume that is not saturated at certain doses (54). Interestingly, the maximum tolerated dose of a single infusion of CP-870,893 is estimated at 0.2 mg/kg, but doses of SGN-40 at least up to 4 mg/kg have been tolerated in patients (49, 53). These findings may reflect differences in dosing schedules or differences in the agonistic or structural properties of the two mAbs that affect pharmacology or pharmacodynamics.

A third CD40 mAb, HCD 122 (formerly known as CHIR-12.12; Novartis/XOMA, Berkeley, CA), is a fully human IgG1 mAb that mediates antibody-dependent cellular cytotoxicity and blocks CD40L-induced cell survival and proliferation of normal and malignant B cells (55). Distinct from CP-870,893 or SGN-40, HCD 122 does not show any agonist activity in cell proliferation assays. HCD 122 is being tested in phase 1 clinical trial for patients with advanced B-cell malignancies.[1]

Other clinical approaches targeting CD40 in cancer include gene therapy to achieve expression of CD40L in autologous tumor cells before reinfusion. Engagement of CD40L enhances the antigen-presenting function of malignant B cells and enables these cells to generate antitumor immune responses (56). In one study, patients with chronic lymphocytic leukemia were administered autologous leukemia cells transduced with adenovirus encoding recombinant CD40L without major toxicity (57). Reductions in leukemic burden in some patients were associated with the induction of leukemia-specific T cells and increased serum interleukin 12. In another study, leukemic blasts administered with skin fibroblasts transduced with adenoviral vectors encoding human interleukin 2, and CD40L induced leukemic-specific T cells and antibodies after repeated injections (58). Gene therapy and other means can also be used *ex vivo* to activate antigen-presenting cells with CD40L (59, 60). Loaded with a tumor antigen payload, CD40-activated dendritic cells or B cells hold promise as novel cancer vaccines (61).

Most encouraging from these initial clinical trials has been the absence of major toxicity, in light of understandable concerns regarding the potential for CD40-mediated systemic inflammation and autoimmunity (62). In our studies with an agonist CD40 mAb, we have not observed enterocolitis, dermatitis, or hypophysitis that has been observed with other immunomodulatory agents, in particular, blocking anti-CTLA4 mAb (63). Although cytokine release syndrome has been observed with agonist CD40 mAb, the effects have been moderate and transient, and clinically and mechanistically distinct from multiorgan failure observed recently in subjects receiving a single dose of anti-CD28 agonist mAb (64).

Future Directions

Although initial phase 1 studies of CD40 agonists have already achieved objective tumor responses, possibly the

[1] Two additional studies have recently been published. Please see Note Added in Proof for further details.

greatest potential for these drugs will be in combination with other agents. These include chemotherapy, tumor vaccines, toll-like receptor agonists, cytokines, and other TNF receptor family agonists such as DR5 and CD137 mAb. Data from multiple preclinical models suggest the prospect of synergistically enhancing immune activation with such combinations (18, 19, 43, 45, 65, 66). CD40 agonists could also be combined with agents that block negative immune checkpoints (e.g., anti-CTLA4 mAb). Clinical trial designs testing these hypotheses will require careful consideration of both the basic immunology involved and the pharmacology and pharmacodynamics of the agents being investigated.

Note Added in Proof

Additional clinical trial data for SGN-40 and HCD 122 were reported at the annual meeting of the American Society of Hematology in December 2006. Of particular note, HCD 122 investigators reported two partial responses among 24 evaluable patients with chronic lymphocytic leukemia or multiple myeloma (77, 78). Treatment with HCD 122 was associated with transient infusion reactions.

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