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# New emerging targets in cancer immunotherapy: the role of Cluster of Differentiation 40 (CD40/TNFR5)

Manuel Piechutta,[1,2] Anna Sophie Berghoff[3,4]

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[1]Clinical Cooperation Unit Neurooncology, German Cancer Research Centre, Heidelberg, Germany
[2]University of Bonn Medical Center, Bonn, Germany
[3]Department of Medicine I, Clinical Division of Oncology, Medical University of Vienna, Vienna, Austria
[4]Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria

**Correspondence to**
Dr Anna Sophie Berghoff, Department of Medicine I, Clinical Division of Oncology, Medical University of Vienna, Vienna, Austria; Anna.berghoff@meduniwien.ac.at

## ABSTRACT

Cluster of differentiation 40 (CD40) is a member of the tumour necrosis factor family and a new immune-modulating target in cancer treatment. B cells, myeloid cells and dendritic cells can express CD40 and mediate via the ligand cluster of differentiation 40 ligand (CD40L) cytotoxic T cell priming under physiological conditions. Therapeutically, recombinant CD40L molecules, intratumour application of adenoviral vectors leading to CD40L expression and agonistic monoclonal CD40 antibodies are currently tested in various cancer entities for their immune-modulating potential. Early clinical trials suggest safety for agonistic CD40 antibodies with encouraging antitumour effects. Adverse events encompass cytokine release storm, hepatoxicity, thromboembolic events and were so far reported to be clinically manageable and transient. Ongoing studies investigate CD40 activation in combination with chemotherapy, radiation, targeted therapies and immunomodulatory agents. Further studies are awaited to specifically identify patients with the greatest clinical benefit based on predictive biomarkers.

## CD40 IN THE PHYSIOLOGICAL IMMUNOLOGICAL CONTEXT

Cluster of differentiation 40 (CD40) is a cell surface molecule of the tumour necrosis factor receptor family. Under physiological conditions, CD40 is expressed on antigen-presenting cells (APCs), for example, dendritic cells and myeloid cells, and is critical for their activation and proliferation. In addition, CD40 expression can be found on other cell types including platelets, fibroblasts, epithelial cells and endothelial cells.[1–4] The natural ligand for CD40, CD40 ligand (CD40L, CD154), is expressed on activated CD4 T cells, B cells and natural killer cells as well as memory CD8 T cells.[5–10] Relevant CD40 levels were also found on granulocytes, macrophages, endothelial cells and activated platelets.[3 11 12] The CD40 axis is essential to initiate a specific immune response, as CD40 is a pivotal side signal to mediate the antigen-specific activation of naïve B and T cells. Here, CD40 expression on the surface of APCs greatly increases their antigen presentation and co-stimulatory capacity, resulting in a more effective activation of cytotoxic T cells even in the absence of a CD4 T cell helper signal.[5–7] Further, the CD40/CD40L axis bridges signalling between the innate and the adaptive immune system as it was shown to activate natural killer cells.[10] In consequence, CD40 activation results in upregulation of co-stimulatory molecules (like CD80 and CD86) and the major histocompatibility complex receptor as well as the release of immunostimulatory cytokines.[5–7] The hyper-immunoglobulin (Ig) M syndrome is caused by an X chromosomal gene defect causing a CD40L mutation and manifests as an early, primary immune deficiency.[13]

## CD40 EXPRESSION AND SIGNALLING IN CANCER

Substantial CD40 expression was detected in a variety of frequent solid cancer types including bladder cancer (102/131, 78%), melanoma (41/71, 57,7%), breast cancer (53%), lung cancer (67/129, 51,9%), colon cancer (87/110, 79%) as well as B-lineage malignancies.[14–19] CD40 expression was frequently shown to be associated with prolonged survival; however, contrary results were evident in lung and oesophageal cancer as CD40 expression correlated with poor prognosis.[17 20] Interestingly, compared with cancer tissue, normal tissues exhibited very low to no CD40 levels, underscoring the potential of CD40 as a cancer-specific immunological target.

## PRECLINICAL MODELS ON CD40-DIRECTED IMMUNE-MODULATORY ANTICANCER TREATMENTS

A wide range of preclinical studies using immune competent cancer mouse models so far underscore CD40-directed therapies as a next-generation immune-modulating therapy. In the immunogenic B16 melanoma mouse model, intratumourally applied adenoviral CD40L gene therapy plus immune checkpoint inhibition led to systemic tumour eradication including brain metastases.[21] Agonistic CD40 antibodies were shown to generate a strong, durable cytotoxic T cell response in a

syngeneic lymphoma mouse model. Higher antigen presence was postulated to enhance the applied vaccination strategy substantially.[22] Moreover, this effect was abrogated when depleting CD8 cells, but not when depleting CD4 cells underscoring that the immunological function of the CD40/CD40L axis mainly involves CD8 cytotoxic T cells, whereas the mediated immune response is rather independent of CD4 helper T cells.[7] In line, adoptively transferred CD8 T cells from CD40 monoclonal antibody-treated tumour-bearing mice effectively eradicated tumour cells in non-treated mice.[22] In order to generate an effective activation signal, agonistic CD40 antibodies are required to crosslink. In vivo crosslinking is facilitated by the inhibitory Fc gamma receptor (FcγR) IIB and enhanced by increased binding affinity of IgG1 isotype CD40 agonist compared with IgG2 agonists. Furthermore, activating FcγRs, which are induced by a toll like receptor 3 agonist, crosslink IgG1 CD40 agonists and Fc-independent chemical crosslinking, were shown to generate a CD40-mediated immune response in a murine lymphoma model.[23]

Interestingly, activation of CD40 can also enhance immunosurveillance by the innate immune system. In a syngeneic, immunocompetent mouse model, a subset of C-C chemokine receptor type 2 (CCR2)and lymphocyte antigen 6 complex locus c (Ly6C)-positive tumour-infiltrating monocytes were postulated to mediate an immunological antitumour effect as monocytes are recruited to the tumour side by CD40 agonist treatment-induced release of C-C motif chemokine ligand 2 (CCL2). Monocytes were shown to be activated in the lymph node and infiltrated the tumour within hours, while tumour-associated macrophages remained unaffected, which indicates that only systemic CD40 antibody application is sufficient to generate this effect.[24] Further, the downstream-activated matrix metalloproteases, in response to CD40 antibody-mediated elevated systemic interferon gamma levels, can degrade tumour-associated fibrotic tissue potentially leading to better drug delivery in the used pancreatic adenocarcinoma animal model.[24 25] Importantly, the innate immune system effect was not observed using lymphocyte-deficient mouse neuroblastoma and melanoma models.[26 27]

Preclinical investigation also addressed potential combination boosting the antitumour efficacy of CD40 axis-targeting therapies. Combination with local radiotherapy was postulated to enhance CD40 activation and tumour response[28] and is currently further investigated in a clinical study (NCT03165994). Moreover, preclinical data report efficacy of the combination of agonistic CD40 antibody with anti- programmed cell death protein 1 (PD1) antibodies resulting in reprogramming an initial suppressive tumour microenvironment and facilitating promising tumour eradication.[29] Recently, a new mouse model with humanised Fc receptors and CD40 was developed,[30] which can mimic the platelet and hepatocyte toxicity seen in human trials.[31]

## CD40 AS A THERAPEUTIC TARGET IN CANCER

To activate the CD40 pathway in the clinical therapeutic setting three major approaches are used:

(i) recombinant human CD40L molecules[32 33]; (ii) intratumour application of adenoviral vectors leading to CD40L expression[34 35]; (iii) recombinant agonistic CD40 antibodies (table 1).

Recombinant CD40L was so far investigated in a phase I study in patients with advanced solid tumours and non-Hodgkin's lymphoma. Partial response could be observed in 2 out of 32 patients and stable disease for at least 4 months in 4 out of 32 patients.[32] An alternative approach represents the specific linkage of multiple CD40L molecules which can activate CD40 and thereby promote potent antitumour T

**Table 1** List of clinically used agonistic CD40 antibodies

| Name of the compound | Mechanism of action | Phase of clinical trial development | Company | Reference |
|---|---|---|---|---|
| Selicrelumab (formerly known as RO7009789 and CP-870,893) | Agonistic IgG2 CD40 antibody | I | Hoffmann-La Roche | 38 |
| APX005M | Agonistic IgG1 CD40 antibody | II | Apexigen | 50 |
| JNJ-64457107 (formerly ADC-1013) | Agonistic IgG1 CD40 antibody | I | Janssen R&D | 51 |
| SEA-CD40 | Non-fucosylated humanised agonistic IgG1 CD40 antibody | I | Seattle Genetics | 48 |
| ChiLob 7/4 | Chimeric agonistic IgG1 CD40 antibody | I | None | 52 |
| CDX-1140H | Human agonistic IgG2 CD40 antibody, may act synergistically with naturally expressed CD40L | I | Celldex Therapeutics | 47 |
| Dacetuzumab (SGN-40) | Humanised partially agonistic IgG1 CD40 antibody | II | Seattle Genetics | 53 |
| ABBV-428 | CD40/anti-mesothelin bispecific monoclonal antibody | I | Abbvie | NCT02955251 |

CD40, cluster of differentiation 40; Ig, immunoglobulin.

cell-mediated immune responses. HERA-CD40L comprises six CD40L molecules linked by a Fc-silenced human IgG1 and proved crosslinking-independent antitumour properties in the preclinical stage.[36] MEDI5083, a homodimeric fusion protein consisting of 3 scCD40L domains and an IgG4P Fc domain, is currently tested alone and in combination with durvalumab in a phase I trial (NCT03089645).[33]

Local intratumour administration of adenoviral vectors enhancing the expression of CD40L was investigated in advanced urothelial carcinoma and malignant melanoma patients and resulted in increased T cell infiltration into the tumour despite lacking clear antitumour effects.[34 35] A trial investigating the combination of intratumoural viral particle injection of adenovirus encoding chimeric CD154 (Ad-ISF35) with pembrolizumab in melanoma was recently withdrawn (NCT02719015). In chronic lymphocytic leukaemia, local injection of Ad-ISF35 was used to restore antigen presentation in malignant cells and overcome immune evasion.[37]

Most ongoing studies investigate agonistic CD40 antibodies, which enhance the activity of the CD40/CD40L axis. The first clinical study using the agonistic CD40 antibody CP-870,893 (selicrelumab, Hoffman-La Roche) determined a maximum tolerated dose (MTD) of 0.2 mg/kg body weight as a single infusion and showed a partial response in 4 out of 29 (13.8%) patients.[38] The follow-up study applied CP-870,893 (selicrelumab) on a weekly basis and proved safety for the previously determined MTD. However, it failed to reproduce the promising clinical data of the first study: the best response was stable disease in 7 of the 27 (25,9%) enrolled patients.[39] Higher antigen levels were associated with higher efficacy of CD40 antibody treatment in preclinical models, resulting in the theory that combination with chemotherapy could enhance the clinical efficacy via the increased antigen availability. Three studies reported data for CP-870,893 (selicrelumab) in combination with chemotherapeutic agents.[40–42] Clinical response was described in all studies but did not exceed the results of CP-870,893 (selicrelumab) or chemotherapy treatment alone. In one of the studies, the antitumour effect was linked to monocyte activation and not to an enhanced T cell response as described previously.[41] An ongoing phase I study currently investigates the combination of CP-870,893 (selicrelumab) with gemcitabine/nab-paclitaxel in a neoadjuvant and adjuvant setting in pancreatic adenocarcinoma (NCT02588443). CP-870,893 (selicrelumab) was further investigated in combination with other immune-modulating therapies. A study investigating CP-870,893 (selicrelumab) combined with the anti- cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibody tremelimumab represented one of the first clinical trials combining immune checkpoint inhibitors with agonistic immunomodulatory antibodies in tumour treatment.[43] In 22 advanced melanoma patients, an overall response rate (ORR) of 27,2% with two complete responses was achieved. Although comparison between trails should be avoided, the here observed ORR is highly promising given the so far observed ORR of the two phase III studies with anti-CTLA4 alone (tremelimumab 10,7%,[44] ipilimumab 19%[45]). Notably, T cell infiltration in patients with disease progression under CD40/anti-CTLA4 therapy often exhibited programmed death-ligand 1 (PD-L1) upregulation in the tumour tissue, which might reflect a potentially targetable resistance mechanism. Five patients who progressed on CD40/anti-CTLA4 were treated with anti-PD1 out of which four achieved long-term survival. CD40/anti-CTLA4 treatment showed to be effective, well tolerable and potentially increased subsequent PD-1 inhibition.[43]

Three clinical phase I trials further investigating the therapeutic potential of CP-870,893 (selicrelumab) efficacy in patients with advanced solid tumours, each combining CP-870,893 (selicrelumab) with another immune-modulating or targeted antibody, are currently recruiting. The investigated combinations include (i) emactuzumab, an anti- colony-stimulating factor 1 receptor (CSF1R) antibody (NCT02588443), (ii) vanucizumab, a bispecific antibody targeting angiopoietin 2 and vascular endothelial growth factor (VEGF), or bevacizumab, an anti-VEGF monoclonal antibody (mAb) (NCT02665416) and (iii) atezolizumab, an anti-PD-L1 mAb (NCT02304393). Interestingly, the latter two studies include trial arms to administer CP-870,893 (selicrelumab) subcutaneously in order to limit unwanted adverse events.

Second-generation agonistic CD40 antibodies were postulated to achieve a better antitumour/adverse event ratio.[46] By improved pharmacokinetics and pharmacodynamics, CDX1140 was reported to bind outside the CD40L motif enabling additional CD40L binding, thereby enhancing APC activation, while side effects including the cytokine release syndrome were reduced.[47] Another new agonistic CD40 antibody, SEA-CD40, has a non-fucosylated Fc region compared with the ancestor first-generation antibody. Thereby, binding affinity to FcλRIIIa and immune activation was improved.[48]

Further, second-generation agonistic CD40 antibodies take advantage of utilising an IgG1 instead of an IgG2 Fc domain to facilitate FcλR interactions. Increased binding to FcλRIIB enhances CD40 signalling through crosslinking, thus improving tumour immunity.[46] The second-generation agonistic CD40 antibody APX005M, developed by Apexigen, takes advantage of this particular advancement and has been so far investigated in nine early clinical trials. The dose-finding phase I study already finished recruiting at the end of 2018 (NCT02482168). Three phase II studies are currently recruiting and aim to test the safety and clinical efficacy of APX005M in combination with standard of care in several solid cancer types (metastasized pancreatic adenocarcinoma: NCT03214250; advanced soft tissue sarcoma: NCT03719430; resectable (gastro-)oesophageal carcinoma: NCT03165994). In patients with melanoma and non-small cell lung cancer (NSCLC), several studies currently investigate the safety and antitumour efficacy of APX005M, applied either systemically (NCT03123783) or intratumourally (NCT02706353), in combination with PD-1 blockade. A phase I study currently aims to investigate the efficacy of APX005M in combination with cabiralizumab,

an anti-CSF1R antagonist, with and without nivolumab (NCT03502330) in patients who previously failed anti-PD-1 or anti-PD-L1 treatment in order to overcome anti-PD-1/anti-PD-L1 therapy resistance in melanoma, NSCLC and renal cell carcinoma. Another innovative study utilises APX005 with the goal to boost the effectivity of a personalised vaccine (NEO-PV-01) approach with or without checkpoint blockade in patients with advanced melanoma (NCT03597282). Notably, a phase I study of APX005M for recurrent or refractory paediatric brain tumours and newly diagnosed diffuse intrinsic pontine glioma for patients under 21 of age was launched representing the first and only study so far to target the CD40/CD40L axis in childhood cancer (NCT03389802). Two further second-generation CD40 agonistic antibodies are currently investigated in phase I trials: JNJ-64457107, formerly ADC-1013 (NCT02379741, NCT02829099) and SEA-CD40 (NCT02376699). In addition, a bispecific antibody ABBV-428, targeting CD40 and the well-known tumour antigen mesothelin, is currently also investigated in a phase I trial (NCT02955251). A detailed overview of completed and ongoing clinical trials as of March 2019 is given in the online supplementary table 1.

## CLINICAL TOLERABILITY OF CD40-TARGETING IMMUNE-MODULATING THERAPIES

Most knowledge about the safety of CD40 agonist arises from the trials of CP-870,893 (selicrelumab). Here, the most common adverse event described was grade 1 or 2 cytokine release syndrome (CRS), characterised by fever, rigours and chills.[38] Development of CRS did not correlate with an objective response. Treatment with doxylamine fully resolvedCRS in most patients within 24 hours. Hepatoxicity, measured by elevated transaminase levels, elevated D-dimer levels and lower lymphocyte, monocyte and platelet counts were observed after 24–48 hours but recovered within weeks.[39] Long-lasting lymphocytopenia was observed after the weekly application of CP-870,893 (selicrelumab).[39] Thromboembolic events were reported throughout the so far existing clinical studies. All adverse events most likely arise rather through antibody binding of CD40 on immune or vascular cells than through hypersensitivity to the substance itself.[38] Intravenously application of ADC-1013 led in three out of five patients to treatment-emerged adverse events.[49] A detailed safety profile of systemic ADC-1013 application was then decided to investigate in an upcoming trial (NCT02829099). However, when injecting ADC-1013 into well-vascularised liver metastases, they reported similar adverse events to those of CP-870,893 (selicrelumab) including CRS, lower B cell count and elevated liver enzymes.[49] Applying ADC-1013 in superficial metastasis (lymph node or subcutaneous) agonistic adverse events were shown to be diminished and patients tolerated a higher drug dose while the therapeutic effect was supposed to be preserved.[49] Notably, only 1 out of 27 patients who received weekly CP-870,893 (selicrelumab) and no one from the 18 patients who received intratumoural ADC-1013 injections developed immune-related toxicity (grade 3 autoimmune diabetes).[38 49] No deaths were reported linked to agonistic CD40 antibody therapy. Adverse treatment reactions of CP-870,893 (selicrelumab) and ADC-1013 are reported to be transient and clinically manageable encouraging the further development of CD40 axis-targeting immune-modulating therapies. Further safety reports of second-generation agonistic CD40 antibodies are urgently awaited, as the described modification in the molecular formulation were postulated to reduce the side effect rate. Notably, when applying agonistic CD40 antibodies intratumourally instead of intravenously, adverse events were shown to be diminished, while the therapeutic effect was supposed to be preserved.[31 49]

## SUMMARY

In summary, CD40 agonists are new immune-modulating agents with high clinical potential across tumour entities. Future studies need to address (i) predictive biomarkers for CD40-directed therapies and (ii) combination and sequencing strategies with other therapy approaches including immune checkpoint inhibitors.

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