

Characteristics and clinical trial results of agonistic anti-CD40 antibodies in the treatment of malignancies (Review)

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Abstract. Cluster of differentiation 40 (CD40) mediates many immune activities. Preclinical studies have shown that activation of CD40 can evoke massive antineoplastic effects in several tumour models *in vivo*, providing a rationale for using CD40 agonists in cancer immunotherapy. To date, several potential agonistic antibodies that target CD40 have been investigated in clinical trials. Early clinical trials have shown that the adverse events associated with agonists of CD40 thus far have been largely transient and clinically controllable, including storms of cytokine release, hepatotoxicity and thromboembolic events. An antitumour effect of targeting CD40 for monotherapy or combination therapy has been observed in some tumours. However, these antitumour effects have been moderate. The present review aimed to provide updated details of the clinical results of these agonists, and offer information to further investigate the strategies of combining CD40 activation with chemotherapy, radiotherapy, targeted therapy and immunomodulators. Furthermore, biomarkers should be identified for monitoring and predicting responses and informing resistance mechanisms.

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1. Introduction

Cluster of differentiation 40 (CD40), which belongs to the tumour necrosis factor (TNF)/TNF receptor (TNFR) family, is a 277-amino acid, 45-50 kDa transmembrane glycoprotein (1,2). Under physiological conditions, CD40 is primarily expressed on the membranes of various antigen-presenting cells (APCs), including dendritic cells (DCs), B-cells and monocyte-macrophages (3). It can also be expressed on nonimmune cells, including endothelial cells, epithelial cells, haematopoietic progenitors, and platelets (4,5) as well as tumour cells (6,7).

The natural ligand of CD40 (CD40L, otherwise known as CD154, TRAP or T-BAM), which also belongs to the TNF/TNFR family, is a 34-39 kDa type II integral membrane protein that is primarily expressed by active CD4⁺ T helper cells under inflammatory conditions. Previous studies have shown that the soluble form of CD40L, which lacks the transmembrane region, possesses biological activities similar to the transmembrane form (8,9).

The interaction between CD40 and CD40L is critical for the generation of a series of systemic immunizing inflammatory reactions. This includes the class switching and affinity maturation of immunoglobulins; secretion of cytokines; the survival, proliferation, differentiation and adhesion of B cells; and the development of memory B cell generation and germinal centres (10). In addition, CD40 intracellular signalling induces the apoptosis of many transformed cells both *in vitro* and *in vivo*, such as breast cancer and haematological malignancy cells (6,7).

With the inspiring successes of monoclonal antibodies (mAbs) targeting programmed cell death protein (PD-1)/programmed cell death ligand (PD-L1) in cancer therapy, immunomodulation by antibodies has been regarded as an attractive way of boosting anticancer responses (11), CD40 is an emerging immunotherapy target that plays a critical

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role in human immunity. Extensive preclinical research has proven that CD40 ligation stimulates antitumour immunity via several potential mechanisms across various lymphoid (12,13) and solid tumours (14-16).

To date, several anti-CD40 mAbs have been investigated by clinical studies. Here, we present an overview of the physiological and immunological context of CD40/CD40L, emphasizing the clinical outcomes of anti-CD40 mAbs for treating malignant disease.

2. The structure of CD40/CD40L

The human *CD40* gene is located on chromosome 20q11-13 and encodes a polypeptide chain of 277 amino acids, including an extracellular region of 193 amino acid residues, a transmembrane region of 22 amino acid residues and an intracytoplasmic region of 62 amino acid residues (17). As CD40 lacks intrinsic kinase activity in the cytoplasmic tail, the signal is mainly transferred by recruitment of TNF R-associated factors (TRAFs), which are cytoplasmic adapter molecules (18). TRAF trimers specifically recruit tyrosine and serine/threonine kinases to initiate rapid protein phosphorylation, which activates downstream signal transduction pathways, including the phosphoinositide 3-kinase (PI3K), p38/mitogen-activated protein kinase (38 MAPK), nuclear factor- κ B (NF- κ B) and c-Jun-NH2-kinase (JNK)/stress-activated protein kinase pathways (19-22).

The human *CD40L* gene is located on the X chromosome, Xq26.3-Xq27.1, and has five exons that span 12-13 kb, its DNA consists of a 783 bp reading frame encoding 261 amino acids, including an intracytoplasmic region of 22 amino acid residues, an extracellular region of 215 amino acid residues and a transmembrane region of 24 amino acid residues, which has a signalling and anchoring function (23,24). The CD40 ligand amino acid sequence has homology to the amino acid sequences of the extracellular regions of other TNF gene family members, such as TNF- α and tissue growth factor- β . CD40L has a carboxyl terminus located extracellularly and is a type II membrane protein that lacks an amino-terminal signal peptide. There are two soluble forms of CD40L (31 and 18 kDa), which retain the ability to bind CD40 and elicit signals in the form of homotrimers (25).

3. The physiological role of CD40/CD40L

Unlike other TNFR costimulatory targets, CD40 is mainly expressed on APCs including B-cells, macrophages and monocytes (3). In addition, the CD40 is also present on non-immune cells, such as endothelial cells, epithelial cells, hematopoietic progenitor cells and platelets as well as various tumour cells, including malignant lymphoma cells, leukaemia cells and solid tumour cells (4-7).

The key physiological function of the CD40/CD40L pathway is mediated by CD40 ligation on APCs, especially dendritic cells (DCs). CD40L binding with CD40 leads to the activation of DCs, including improving their antigen presentation ability by upregulating the expression of other costimulatory molecules [major histocompatibility complex (MHC) class II, CD58, CD80/86 and CD70] and down-regulating the expression of immunosuppressive molecules

such as PD-L1 (19-21). It also develops a pro-survival signal and increases the release of various cytokines, including interleukin (IL)-1 β , IL-6, IL-8, IL-12, TNF- α and interferon (IFN)- γ by DCs (26-28), and hence further enhances the cytotoxic response and prevents immune tolerance induction.

The binding of CD40L to CD40 also activates macrophages to directly kill tumour cells (29) and leads to the secretion of IL-1 β , IL-6, IL-8, IL-12, TNF- α , IFN- γ and nitric oxide (27). These cytokines mediate the pro-inflammatory response and are crucial to macrophage cross-priming and cytotoxic function (30,31).

The ligation of CD40 activates resting B lymphocytes and causes these cells to differentiate into secretory plasmocytes or memory B lymphocytes but inhibits the growth and immunoglobulin production of active B lymphocytes (32). The differentiation path of activated B lymphocytes is partly dependent on the extent of CD40 activation. Short-term CD40L exposure can differentiate B cells into plasma cell lymphocytes, while long-term CD40L exposure can produce CD40⁺ memory B lymphocytes (33). A previous study found that CD40-stimulated B lymphocytes prolong the lifespan of memory B lymphocytes *in vivo* (34). In addition, patients with X-linked hyper-IgM immunodeficiency syndrome, which is caused by CD154 mutation/dysfunction, are unable to perform immunoglobulin class switching (35).

4. The role of the CD40/CD40L pathway in malignancy

CD40 is expressed on ~100% of malignant B cell tumours (such as Hodgkin's lymphoma, non-Hodgkin's lymphoma, Burkitt lymphoma and multiple myeloma) (36). CD40 can also be identified on the cell surface of 70% of malignant epithelial tumours (including breast cancer, nasopharyngeal cancer and rectal cancer) (36).

There are diverse roles of CD40 pathway activation in cancer (12,37). Activation of the CD40 pathway has been proven to enhance the host antitumour immune response and/or directly induce tumour cell apoptosis in several models, especially in primary high-grade B cell lymphoma models of Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL) or Epstein-Barr virus-driven lymphoma (12,38). This pro-apoptotic effect of CD40 ligation is associated with the activation of cytotoxic ligands of the TNF superfamily (39). However, in some low-grade B cell malignancies, such as follicular lymphoma, chronic lymphocytic leukaemia and hairy cell leukaemia, stimulation of CD40 promotes malignant transformation, tumour proliferation, lymphomagenesis and resistance to chemotherapy (39-43) by inducing overexpression of survival proteins, such as Bcl-x and Bfl-1/A1, and downregulation of FLICE-inhibitory protein (44,45). Collectively, these studies have highlighted that activation of the CD40 pathway can exert either apoptotic or pro-survival effects depending on the type and differentiation state of the cancer cells involved (10). Therefore, care should be taken in clinical trials to exclude tumours for which preclinical trials have shown that activation of the CD40 pathway can lead to tumour progression.

Both preclinical experimental and clinical observations have demonstrated that tumour cells can interfere with an immune response and that deficient activation of antitumour

immunity, such as decreased expression of MHC class I and/or adhesion or accessory/costimulatory molecules and a lack of tumour antigen presentation may facilitate tumour progression (42). Activation of the CD40 pathway can increase antigen presentation, and enhance cytotoxic activity and cytokine secretion, thereby enhancing the host's antitumour immune effect (46).

5. The profiles of agonistic antibodies targeting CD40 for cancer therapy

As a target for cancer treatment, CD40 can be activated by three approaches under clinical research (47): Recombinant human CD40L, adenovirus vector-expressed CD40L and agonistic anti-CD40 mAbs.

Due to its action on both the immune system and tumour cells, agonistic anti-CD40 antibodies have been studied as novel cancer immunotherapy targets, demonstrating potent antitumour immune responses in animal models and cancer patients (13,48-50). Each of the agonistic anti-CD40 mAbs has unique characteristics, including unique binding affinities to CD40, antibody isotypes, Fc modifications and agonistic effects (Table I). However, there is no consensus on which agonist is best for cancer therapy at present (51).

It is worth mentioning that the generation mechanism by which each of these antibodies generates agonism is not exactly the same. IgG1 Fc domain engineering was employed for APX005M based on the finding in a murine model that the potency of a CD40 agonist can be enhanced by increased binding affinity to Fc γ RIIB (52,53). In contrast, agonism of an IgG2 mAb, such as CP-870,893, is believed to be provided by its unique hinge conformation (54,55). A recent study demonstrated that hinge rigidity and selective Fc γ R binding affinity are both critical in regulating antibody agonistic function (56). In addition, other research has shown that the binding site is important in determining CD40 mAb agonistic activities, as membrane CRD1-binding displays stronger agonistic activities when the binding site is distal to the membrane than when it is proximal (57), and this relationship between binding epitope specificity and agonistic activity varies in TNFRs and needs to be resolved on a case-by-case basis (58).

6. Clinical trials of anti-CD40 mAbs for treating the malignancies

To date, a variety of agonistic anti-CD40 mAbs are currently under investigation in clinical trials, as monotherapies or in combination with other agents (51,59-65). All these agonistic anti-CD40 mAbs are in the early developmental stage (Table II).

CP-870,893. CP-870,893 (now licensed to Roche Diagnostics under the names RO7009789 or Selicrelumab) is a fully humanized monoclonal IgG2 antibody that binds CD40 with a very high affinity (K_d of 0.4 nmol/l) (66,67). CP-870,893 has been shown in preclinical studies to be a strong agonist of CD40 without eliciting antibody-dependent cell-mediated cytotoxicity (ADCC), a mechanism through which an antibody induces target lysis by activating host leukocytic effector cells, or complement dependent cytotoxicity, a cascade of complement-related reactions leading to target lysis (67,68).

Table I. Agonistic antibodies targeting CD40.

Drug name (pseudonym)	Isotype	Fc modification	Increased FcγR affinity	Agonistic effect	ADCC	(Refs.)
CP-870,893 (RO7009789)	Fully human IgG2	None	None	Strong	No	(60,61)
Dacetuzumab (SEA-CD40, SGN-40)	Humanized IgG1	De-fucosylation	FcγRIIIa	Weak	Yes	(77,78)
APX005M	Humanized rabbit IgG1	S267E Fc mutation	FcγRIIb	Very strong	No	(87)
ADC-1013	Fully human IgG1	None	None	Moderate	Yes	(84)
CDX-1140	Fully human IgG2	None	None	Strong	No	(85)

ADCC, antibody-dependent cell-mediated cytotoxicity; CD40, cluster of differentiation 40.

Table II. Summary of clinical trials of agonistic anti-CD40 mAbs.

Database identifier	Patient characteristics	Study design	Key tolerability/ safety determinations	Response	Pharmacodynamics
NCT02225002	A total of 29 patients with advanced solid tumours: Melanoma, 52% NSCLC, 17%, Sarcoma, 10%, Cholangiocarcinoma, 7%, Breast cancer, 3% Thyroid cancer, 3% Unknown primary, 3% Mesothelioma, 3%	Phase I Dose-escalation + expansion Single-agent CP-870,893 redosing for responders Six dose levels (mg/kg): 0.01, 0.03, 0.06, 0.1, 0.2, 0.3	MTD: 0.2 mg/kg/ G1-2 CRS 55%	14% PR, including 27% in melanoma 24% SD	Transient decrease in CD19 ⁺ cells Upregulation of the costimulatory molecule CD86
NCT02157831	A total of 27 patients with advanced solid tumours: Melanoma, 41% Breast cancer, 11% Mesothelioma, 7% RCC, 7% Others	Phase I Single-agent CP-870,893 Weekly administration Four dose levels (mg/kg): 0.05, 0.1, 0.2, 0.25	MTD 0.2 mg/kg/CRS of any grade 56%	0% ORR 26% SD	Transient decrease in CD19 ⁺ cells Increase in CD86 ⁺ and CD54 ⁺ B cells Heterogeneous absolute numbers of CD4 T cells, CD8 T cells
NCT00607048	A total of 32 patients with advanced solid tumours: Melanoma, 78%	Phase I Combination of CP-870,893 (0.1 or 0.2 mg/kg) + carbo platin + paclitaxel	MTD of CP870,893 0.2 mg/kg	20% PR	Decrease in CD19 ⁺ cells Upregulation of the costimulatory molecule CD86 on B cells No significant change in the numbers of T cell subsets (CD3 ⁺ , CD3 ⁺ CD4 ⁺ , CD3 ⁺ CD8 ⁺ , CD3 ⁺ CD4 ⁺ FOXP3 ⁺) CD27 ⁺ B cells increased with the proportion of CD86 ⁺ B cells
ACTRN126090 00294257	A total of 15 patients with advanced pleural mesothelioma First-line therapy	Phase I Combination of CP-870,893 (0.1, 0.2, or 0.15 mg/kg as a CRS of any grade de-escalation dose) + cisplatin + pemetrexed	MTD of CP-870,893 0.15 mg/kg/2 DLTs at 0.2 mg/kg 5.1-28.1) months 80%	40% PR 53% SD Median OS 16.5 (95% CI Median PFS 6.3 (95% CI 2.3-10.3) months	Transient decrease in absolute mono cyte count and absolute lymphocyte count Transient decrease in CD19 ⁺ cells Increase in CD86 and HLA-DR
NCT00711191	A total of 22 patients with chemotherapy-naïve advanced pancreatic adenocarcinoma	Phase I CP-870,893 (every 2 weeks for the first six infusions) + gemcitabine	MTD of CP-870,893 0.2 mg/kg/ CRS of any grade 86%	PR 40% SD 53% Median OS 16.5 (95% CI 5.1-28.1) months PFS 6.3 (95% CI 2.3 -10.3) months	

Table II. Continued.

Database identifier	Patient characteristics	Study design	Key tolerability/ safety determinations	Response	Pharmacodynamics
NCT01103635	Metastatic melanoma Checkpoint blockade-naïve	Phase I CP-870,893 + tremelimumab	MTD of CP-870,893 was 0.2 mg/kg in combination with 10 mg/kg tremelimumab	ORR 27.3% CR 9.1% PR 18.2% Median PFS 3.2 months (95% CI, 1.3-5.1 months) Median OS 23.6 (95% CI, 11.7-35.5) months	No changes in the overall frequency of CD3, CD4 or CD8 T cell subsets during the course of therapy. Ki67 ⁺ CD8 T cells were enriched for a phenotype: Tbet-Eomes ⁺ , CD45 RA-CD27 ⁺ , PD-1 ⁺ and granzyme B ⁺
NCT01008527	Melanoma Stage III or IV	Phase I CP-870,893 + peptide vaccine + poly IC:LC			
NCT02760797	38 patients with advanced solid tumours who have no other effective therapy option available	Phase I RO7009789 + emactuzumab (anti-CSF1R)			
NCT02304393	A total of 142 patients with locally advanced/metastatic solid tumours with no other effective therapy option available	Phase I Selicrelumab i.v. and s.c. + atezolizumab (anti-PD-L1)			
NCT02665416	A total of 94 patients with metastatic solid tumours, with no other effective therapy option available	Phase I Selicrelumab + vanucizumab (anti-Ang2)			
NCT03892525	Recurrent/Refractory B-cell Non-Hodgkin lymphoma	Selicrelumab + Atezolizumab (anti-PD-L1)			
NCT01561911	A total of 24 patients with CD40-expressing solid tumours and advanced lymphoma, who have no other effective therapy option available	Phase I ChiLob 7/4 (every 2 weeks) Five doses (μ g/kg): 22.5 i.t., 75 i.t., 200 i.t., 400 i.t., 75 i.v.			

Table II. Continued.

Database identifier	Patient characteristics	Study design	Key tolerability/ safety determinations	Response	Pharmacodynamics
NCT02482168	A total of 43 patients with advanced solid tumours, including urothelial carcinoma, melanoma, squamous cell carcinoma of the head and neck, NSCLC, or any solid tumour with high microsatellite instability status (MSI-high)	Phase I Dose escalation/de-escalation + expansion i) APX005M (every 3 weeks) ii) APX005M (every 2 weeks) iii) APX005M (every week) Approximately 8 dose level cohorts			
NCT02706353	A total of 41 patients with metastatic melanoma	Phase I/II Dose escalation + expansion APX005M (i.t.; every 3 weeks for four doses) + pembrolizumab (anti-PD1, every 3 weeks for five doses)			
NCT03389802	Primary malignant paediatric CNS tumours: Including glioblastoma multiforme, high-grade astrocytoma NOS, CNS primary tumours, ependymoma (NOS), DIPGs, medulloblastoma	Phase I Dose escalation/de-escalation + expansion APX005M (every 3 weeks)			
NCT03214250	A total of 129 patients with untreated metastatic pancreatic adenocarcinoma	Phase Ib/II i) Gemcitabine + nab-paclitaxel + nivolumab ii) APX005M + gemcitabine + nab-paclitaxel iii) APX005M + gemcitabine + nab-paclitaxel + nivolumab			
NCT03719430	Soft tissue sarcoma	Phase II APX005M (0.3 mg/kg i.v. every 3 weeks) + doxorubicin			
NCT03165994	Patients with oesophageal carcinoma or gastroesophageal carcinoma	Phase II Neoadjuvant APX005M + radiation therapy + carboplatin + paclitaxel			

Table II. Continued.

Database identifier	Patient characteristics	Study design	Key tolerability/ safety determinations	Response	Pharmacodynamics
NCT03123783	A total of 174 patients with advanced solid tumours, including NSCLC and melanoma that progressed under chemotherapy/targeted PD-1/PDL-1 treatment.	Phase I/II Dose-escalation portion + tumour-specific portion APX005M+ nivolumab			
NCT03597282	A total of 40 patients with advanced or metastatic melanoma or previously untreated disease	Phase I Trial arms include: i) APX005M + NEO-PV-01 + poly IC:LC + nivolumab ii) APX005M + NEO-PV-01 + poly IC:LC iii) APX005M + nivolumab iv) NEO-PV-01 + poly IC:LC + nivolumab v) NEO-PV-01 + poly IC:LC + nivolumab (alternate schedule) vi) Nivolumab + poly IC:LC vii) NEO-PV-01 + poly IC:LC + nivolumab + ipilimumab (anti-CTLA-4) viii) Nivolumab + ipilimumab Phase I/Ib dose escalation + expansion i) APX005M + cabiralizumab (anti-CSF1R) ii) APX005M + cabiralizumab + nivolumab i) ADC-1013 i.v. ii) ADC-1013 i.t. i) SEA-CD40 i.v. ii) SEA-CD40i.v. + pembrolizumab (only for solid tumours) iii) SEA-CD40 s.c.			
NCT03502330	A total of 120 patients with unresectable NSCLC, melanoma, or renal cell carcinoma that progressed under PD-1/PD-L1 treatment				
NCT02379741	Advanced solid tumours				
NCT02829099	Solid tumours and advanced disease				

Table II. Continued.

Database identifier	Patient characteristics	Study design	Key tolerability/ safety determinations	Response	Pharmacodynamics
NCT03329950	Solid tumours and advanced disease that had failed all standard of care treatments	i) CDX-1140 ii) CDX-1140 + CDX340 [recombinant human FMS-like tyrosine kinase-3 ligand (Flt3L)]			
NCT02955251	Solid tumours, NSCLC and advanced disease	i) ABBV-428 ii) ABBV-428 + nivolumab			
CD, cluster of differentiation; CI, confidence interval; CNS, central nervous system; CRS, cytokine release syndrome; DIPG, diffuse intrinsic pontine gliomas; DLT, dose limiting toxicities; HLA-DR, human leukocyte antigen-DR isotype; i.t., intrathecal; i.v., intravenous; mAbs, monoclonal antibodies; MSI, microsatellite instability status; MTD, Maximum tolerated dose; NOS, not otherwise specified; NSCLC, non-small-cell lung cancer; ORR, objective response rate; OS, overall survival; PD-L1, programmed cell death protein; PD-L1, programmed cell death ligand; PFS, progression free survival; PR, partial response; RCC, renal cell carcinoma; s.c., subcutaneous; SD, stable disease.					

CP-870,893 was administered to 29 patients with advanced solid malignancies as a monotherapy in the first-in-human study (66). A total of six dose levels were investigated ranging from 0.01 to 0.3 mg/kg. Dose-limiting toxicities (DLTs) in this trial were venous thromboembolism (one patient in the 0.3 mg/kg group), grade 3 headache (one patient in 0.3 mg/kg group), and grade 3 transient elevations in serum transaminases (one patient in the 0.2 mg/kg group), and 0.2 mg/kg, was declared as the maximum-tolerated dose (MTD) (66). CP-870,893 was well tolerated and exhibited promising antitumour activity, especially in patients with melanoma. A partial response (PR) was achieved in four patients with advanced malignant melanoma as the best response, and one patient continued to respond for >14 months (66). Pharmacodynamically, CP-870,893 could induce a rapid and temporary decrease in peripheral CD19⁺ B cells and an upregulation of CD86 expression on APCs (66).

According to the clinical pharmacodynamic characteristics of CP-879,893, a weekly dosing schedule was designed for another phase I trial, which was conducted in 27 patients with multiple advanced solid tumours. The MTD of 0.2 mg/kg weekly was administered, and patients showed a good tolerability of CP-879,893. In contrast to the results of the single-dose study, 50% of patients had a significant decrease in CD4⁺ and CD8⁺ T cells, suggesting that the frequent dosing of CP-870,893 led to immune hyperstimulation, which resulted in counterproductive peripheral T cell depletion. No patient in this trial achieved partial responses or complete responses, and 26% of patients achieved stable disease (SD). This outcome indicates that the dose interval of 1 week is too short for cancer therapy (69).

Data from multiple preclinical models indicates that immune activation can be synergistically enhanced by combination with other treatments, including chemotherapy, therapeutic tumour vaccines, agitation of Toll-like receptors, cytokine therapy and blockades of immune checkpoint inhibitors (70-74). Thus, several studies were conducted to investigate the combined treatment of CP-870,893 with other agents. To date, there are three studies investigating the combination of CP-870,893 with chemotherapy in malignancy.

One dose-escalation study investigated CP-870,893 combined with carboplatin and paclitaxel in the treatment of advanced solid tumours (60). A total of 32 patients were enrolled in this trial, of whom 25 patients had metastatic melanoma. The MTD of CP-870,893 was established at 0.2 mg/kg every 3 weeks, as two DLTs were observed at this dose level (grade 3 cytokine release syndrome and transient ischaemic attack). This combination therapy was considered safe in patients with advanced solid tumours. However, PRs were observed in six out of 30 evaluable subjects (20%) as the best response, and thus the study failed to demonstrate an obvious superior efficacy of the combination treatment over chemotherapy (60).

Another phase Ib study showed that CP-870,890 combined with cisplatin/pemetrexed at a dose of 0.15 mg/kg every 3 weeks was safe and tolerable in 15 patients with malignant mesothelioma (61). The efficacy results of this trial included six instances of PR (40%) and nine instances of SD (53%), which were similar to the results achieved with chemotherapy alone.

The third study was conducted in 22 patients who had chemotherapy-naïve advanced pancreatic ductal adenocarcinoma (PDA). The combination of a 0.2 mg/kg dose of CP-870,893 every 3 weeks and standard-of-care gemcitabine was well tolerated in the subjects. The objective response rate (ORR) was 19%, the progression-free survival (PFS) was 5.2 months, and median overall survival was 8.4 months (60). With FDG-PET/CT imaging guidance, the authors found that some lesions responded and others failed to respond during therapy, suggesting that treatment responses to this therapy were heterogeneous (59).

Further studies investigated CP-870,893 combined with other immunomodulators. One study investigated CP-870,893 in combination with tremelimumab, an anti-cytotoxic T lymphocyte-associated protein 4 (CTLA4) mAb, for treating patients with metastatic melanoma. There was no increase in toxicity with the combination treatment compared with the single agents. Moreover, a promising efficacy was observed with an ORR of 27.3% and two complete responses (CRs) in this trial (75). By comparison, tremelimumab monotherapy in the treatment of advanced melanoma led to an ORR of 10.7% in a phase III study (76).

There are several ongoing early-phase trials to further investigate the potential of combination therapies of CP-870,893 and other biological agents. One study was designed to assess emactuzumab, a monoclonal antibody targeting colony-stimulating factor 1 receptor (CSF1R), administered in combination with CP-870,893 to participants with locally advanced or metastatic solid tumours (clinical trial identifier: NCT02760797). Another two trials investigated CP-870,893 combined with vanucizumab, a bispecific antibody targeting angiopoietin 2 and vascular endothelial growth factor (VEGF), or bevacizumab, an anti-VEGF mAb, and in combination with atezolizumab (an anti-PD-L1 mAb) in metastatic solid tumours (clinical trial identifiers: NCT02665416 and NCT02304393, respectively). A clinical study investigating neoadjuvant application of CP-870,893 combined with gemcitabine in resectable PDA is now ongoing (clinical trial identifier: NCT01456585).

Dacetuzumab. Dacetuzumab, also named SEA-40 or SGN-40, is a humanized CD40 targeted IgG1 mAb developed by Seattle Genetics, Inc. As a weak agonist ($K_d \approx 1$ nmol/l), dacetuzumab does not block the CD40/CD40L interaction *in vitro* (77). Dacetuzumab was engineered in an afucosylated IgG1 format to improve the ADCC potential (78). Preclinical results have demonstrated that dacetuzumab induces apoptosis of non-Hodgkin's lymphoma cells *in vivo* by ADCC, antibody-dependent cellular phagocytosis (ADCP), and direct apoptotic signalling (77,79).

The first in-human study of dacetuzumab was conducted in 44 patients with recurrent or refractory advanced multiple myeloma. The patients tolerated dacetuzumab well. The MTD was established at 12 mg/kg/week. No patient achieved CR or PR, and the best clinical response observed in this trial was SD in nine patients (62).

Another phase I trial of dacetuzumab was conducted in 50 patients with the relapsed/refractory Non-Hodgkin Lymphoma subtype. This trial used an intrapatient dose-escalation schedule with a maximum weekly dose of 8 mg/kg in five cohorts. In terms of safety, dacetuzumab was generally

well tolerated with extended therapy in this trial. The ORR was 12%, with one patient with relapsed DLBCL achieving a durable CR for >1 year (80). Next, a phase II study was conducted to assess the efficacy and safety of dacetuzumab in 46 patients with relapsed DLBCL. Subjects in this trial received up to 12 cycles of dacetuzumab; the ORR was 9%, and the disease control rate was 37%, which suggests modest activity of dacetuzumab as monotherapy in unselected patients with relapsed DLBCL (64).

A synergistic effect was observed when dacetuzumab in combination with other agents such as rituximab, was administered *in vivo*, which provided a rationale for combination therapy with dacetuzumab. In a pilot phase Ib study, a regimen of dacetuzumab combined with rituximab and gemcitabine was investigated in patients with relapsed or refractory DLBCLs. The complete response rate in this study was 20%, and the partial response rate was 27% (81). Due to this efficacy outcome, a randomized, double-blind, placebo-controlled, phase IIb clinical trial was conducted to investigate dacetuzumab or placebo in combination with rituximab plus ifosfamide, carboplatin, and etoposide chemotherapy in 151 patients with relapsed or refractory DLBCL. The futility analysis failed to demonstrate a superior CR rate of the dacetuzumab group (36% for the dacetuzumab treatment group compared with 42% for the placebo treatment group), which ended study enrolment (63).

ChiLob 7/4. ChiLob 7/4 (University of Southampton, UK) is a chimeric agonistic anti-CD40 IgG1 antibody. Preclinical studies showed that ChiLob 7/4 has the ability to inhibit the growth of various CD40-expressing human malignant lymphoma and epithelial cell lines (82). A phase I study was conducted in 28 CD40-positive patients with solid tumours or lymphomas. The study showed that ChiLob 7/4 was well tolerated. The MTD was established at 200 mg weekly for 4 doses, and patients with stable disease were observed (83). CD40 staining intensity was not associated with disease stabilization.

Several other agonistic CD40 mAbs are currently being investigated in clinical trials. ADC-1013, sponsored by Alligator Bioscience, is a fully human agonistic anti-CD40 IgG1 mAb with high affinity for CD40 ($K_d=0.01$ nM). A preclinical study demonstrated significant antitumour responses in bladder cancer models (84). A dose-escalation phase I trial is recruiting subjects with advanced solid tumours (clinical trial identifier: NCT02829099). A total of 23 patients received ADC-1013 treatment intravenously (dosing at 75 µg/kg) or intratumourally (dosing from 22.5 µg/kg up to 400 µg/kg). This study demonstrated good tolerability of intratumoural administration of ADC-1013 at a clinically relevant dose. Pharmacodynamic responses, such as a decrease in B lymphocyte levels in peripheral blood and overexpression of CD86, a cell surface activation marker, on remaining B lymphocytes, were observed (65).

7. Other agonistic CD40 antibodies

CDX-1140, developed by Celldex Therapeutics, Inc., is a human IgG2 antibody that stimulates CD40 signalling without the requirement for cross-linking or Fc receptor interactions (85).

This drug is currently being investigated in a phase I clinical trial.

ABBV-927 (AbbVie, Inc.) is an anti-CD40/anti-mesothelin bispecific antibody that is being tested in phase I trials for the treatment of advanced solid tumours, including non-small cell lung cancer, squamous cell carcinoma of the head and neck, cutaneous malignant melanoma, and pancreatic adenocarcinoma, as monotherapy or in combination with other immunotherapies (anti-PD-1 and anti-OX40 antibodies) (86). The estimated primary completion date is 2023.

APX005M, developed by Apexigen, is a humanized mAb IgG1/k against CD40 (87). A preclinical study has demonstrated that APX005M binds to CD40 at the CD40L binding domain with a high affinity in mice ($K_d=0.12$ nM) and monkeys ($K_d=0.37$ nM). The first-in-human phase I study for dose determination was conducted in patients with solid tumours (clinical trial identifier: NCT02482168) in 2015. The trial was completed at the end of 2018 without the results being reported. In 2017, three phase Ib/II studies and one phase II study were launched to investigate the safety and potential efficacy of APX005M combined with immune checkpoint inhibitors or chemotherapy in a variety of solid tumours, including APX005M combined with pembrolizumab for treating metastatic melanoma (clinical trial identifier: NCT02706353), a combination of APX005M and nivolumab in the treatment of solid tumours (clinical trial identifier: NCT03123783), APX005M in combination with chemotherapy with or without nivolumab in the treatment of metastasized pancreatic adenocarcinoma (clinical trial identifier: NCT03214250), and APX005M in combination with concurrent chemoradiation for resectable oesophageal/gastro-oesophageal carcinoma (clinical trial identifier: NCT03165994). In 2018, three phase I studies were launched. One study (clinical trial identifier: NCT03389802) has investigated the potential to overcome resistance to PD-1/PD-L1 blockade immunotherapy by the combination of APX005M with cabiralizumab, an anti-CSF1R antagonist, with and without nivolumab in several solid tumours. Another study (clinical trial identifier: NCT03502330) investigated the therapeutic potential of APX005M for treating paediatric CNS tumours. In addition, an innovative study (clinical trial identifier: NCT03597282) investigated the potential synergistic effect of APX005M with a vaccine (NEO-PV-01) in patients with advanced melanoma. In 2019, a phase II randomized multicentre trial was initiated for neoadjuvant therapy with or without APX005M in patients with locally advanced rectal adenocarcinoma. These trials are still ongoing at time of writing.

8. Safety and clinical tolerability of anti-CD40 agonistic antibodies

At present, most agonistic anti-CD40 antibodies have been demonstrated to be well tolerated both as single agents and in combination in the treatment of solid tumours and haematological malignancies (47,50). Nevertheless, due to most agonistic anti-CD40 antibodies being in the early development stage, the safety information of these agents has not been fully investigated. The most common adverse events associated with CP-870,893, a fully humanized IgG2 antibody and a strong agonist, was transient grade 1 to grade 2 cytokine release syndrome (CRS). This

syndrome, which occurs within minutes to hours after infusion, is characterized by a variety of combinations of chills, rigors, rash, nausea, fever, vomiting, muscle aches and back pain. In a human phase I study of CP-870,893, the CRS occurring in 55% of patients was considered to not be an anaphylactic or allergic reaction by the authors, as normal serum tryptase levels were observed in the subjects (66). There was an association of CP-870,890 with acute elevations in TNF- α and IL-6 in serum. In most cases, this syndrome can be resolved by treatment with doxylamine within 24 h. Similar to CP-870,890, dacetuzumab, a weakly-agonistic humanized IgG1 antibody, showed a high overall incidence of CRS within one day of infusion (41%) in the first-in-human trial, but the agents were generally tolerated well.

Another major safety issue of CP-870,893 is dose-related haematological toxicities, such as a decrease in peripheral lymphocytes, monocytes and platelets. Grade 3-4 lymphopenia and thrombocytopenia were observed in a clinical trial of CP-870,893. Grade 3 anaemia, neutropenia, and thrombocytopenia were observed in a human study of dacetuzumab. Weekly dosing of CP-870,893 may cause long-lasting lymphocytopenia.

Transient elevations in D-dimer levels and two pulmonary embolism cases (grade 4 thrombosis: Of which, one was from the single-dose schedule and one was from the weekly dose schedule) were observed in two phase I clinical trials of CP-870,893.

Aspartate aminotransferase, alanine aminotransferase and total bilirubin were transiently increased after both CP-870,893 and dacetuzumab infusion, which suggests hepatotoxicity of both agents. A study of the weekly dose schedule of CP-870,893 showed that liver enzyme abnormalities returned to baseline in most patients at the time of the next infusion. A human study of dacetuzumab showed that elevations in hepatic transaminases were asymptomatic and not associated with marked changes in bilirubin.

9. Conclusion and outlook

APC dysfunction remains a rate-limiting biological issue in many cancer environments. Targeting CD40 with agonists to enhance APC function and indirectly regulate host immune cells by recruiting innate immune effectors via ADCC/ADCP is considered a promising method to treat cancers. To date, early clinical trials have shown that anti-CD40 antibodies have limited clinical activity and no severe immune-related autoimmune-like toxicities, either as monotherapies or in combination with other treatments. Thus, combination strategies targeting of CD40 for cancer therapy require further investigation in clinical trials with careful designs.

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Authors' contributions

DKL and WW searched and collected relevant literature sources. DKL drafted the initial manuscript. WW reviewed and edited the manuscript. Both authors read and approved the manuscript and agree to be responsible for the accuracy of the information and the relevant sources.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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