



New emerging targets in cancer immunotherapy: CD137/4-1BB costimulatory axis

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ABSTRACT

CD137 (4-1BB) is a surface glycoprotein that belongs to the tumour necrosis factor receptor family (TNFRSF9). Its expression is induced on activation on a number of leucocyte types. Interestingly, for cancer immunotherapy, CD137 becomes expressed on primed T and natural killer (NK) cells, which on ligation provides powerful costimulatory signals. Perturbation of CD137 by CD137L or agonist monoclonal antibodies on activated CD8 T cells protects such antigen-specific cytotoxic T lymphocytes from apoptosis, enhances effector functionalities and favours persistence and memory differentiation. As a consequence, agonist antibodies exert potent antitumour effects in mouse models and the CD137 signalling domain is critical in chimeric antigen receptors (CAR) of CAR T cells approved to be used in the clinic. New formats of CD137 agonist moieties are being clinically developed, seeking potent costimulation targeted to the tumour microenvironment to avoid liver inflammation side effects, that have thus far limited and delayed clinical development.

INTRODUCTION

Cancer immunotherapy is already an important pillar in oncology treatments. T cells are the main mediators of efficacy. These immune cell subsets can specifically recognise tumour cells and kill them when properly activated. The understanding of the underlying biological processes that determine T-cell fate on activation in the tumour microenvironment (TME) represents one of the main cornerstones for the future of cancer immunotherapy. Enabling immune responses by targeting immune inhibitory signals (immune checkpoints) using PD-1/PD-L1 and/or CTLA-4 blockers has shown remarkable clinical efficacy against a large number of malignancies, but responses are still limited to a small fraction of patients.¹ In parallel with T-cell inhibition blockade, immune cell stimulation by agonistic agents acting on costimulatory receptors such as CD137, OX40, TNFR2, CD27, CD40 or GITR, which are mainly expressed on T cells, constitute an area of very active preclinical and clinical research.

CD137 BIOLOGICAL BACKGROUND

CD137/4-1BB (*tnfrsf9*) is a costimulatory receptor and a member of the tumour necrosis factor receptor superfamily (TNFRSF) that was initially discovered on activated T cells.² CD137 is transiently expressed on activated T cells that have encountered cognate antigen, activated NK cells or mature dendritic cells (DCs). To date, a unique functional ligand has been identified for CD137, CD137L/4-1BBL (*tnfsf9*), expressed on the surface of professional antigen-presenting cells such as DCs, macrophages and B cells.³ Structurally, no enzymatic activity has been described for the intracellular domain of CD137 which is believed to function by recruiting TRAF1, TRAF2 and maybe TRAF3 adaptor proteins that mediate K63 polyubiquitination reactions in the CD137 signalosome.⁴ CD137L trimerisation leads to CD137 receptor clustering and TRAFs-mediated activation of NF-κB and MAPK intracellular signalling cascades, ultimately leading to cell activation, proliferation and survival.⁵ Bcl-X_L, Bfl-1 induction and downregulation of BIM have been identified as antiapoptotic mechanisms, that seems to involve a role for interference with activation-induced cell death.⁶

On T cells, T cell receptor (TCR) stimulation and subsequent CD3 signalling induce transient expression of CD137 that on ligation with agonist antibodies or the natural ligand probably favours Th1 responses. Of note, CD28 costimulation results in markedly enhanced CD137 surface expression.⁷ Initial reports using CD137 monoclonal antibodies (mAbs) in mouse and human cells demonstrated that CD137 ligation causes strong costimulatory signals in T cells that in cooperation with TCR signalling gives rise to interleukin (IL)-2 and interferon γ (IFN-γ) production as well as T-cell proliferation.⁸ Further studies have shown that, in addition to inducing effector cytokine production, CD137 costimulation favours T cell memory and effector differentiation,⁵ protects T cells

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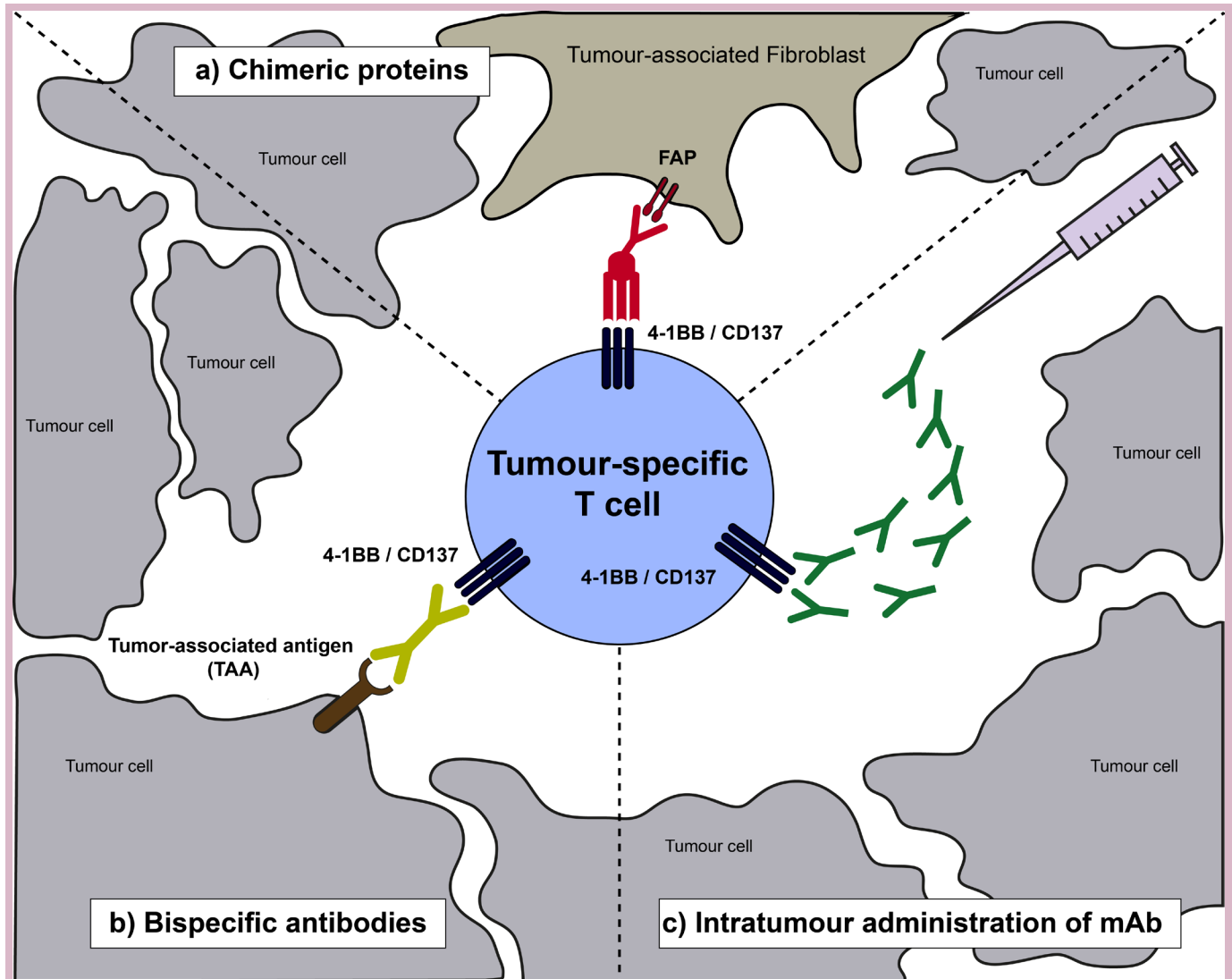


Figure 1 Schematic representation of a tumour-infiltrating T lymphocyte expressing CD137 as a result of priming by tumour-antigen recognition. Then, novel strategies to make the most of CD137 costimulation are represented including bispecifics and direct intratumour injections. FAP, fibroblast activation protein; mAb, monoclonal antibody.

from apoptosis,⁶ changes the mitochondrial metabolism to increase T-cell respiratory capacities^{9 10} and induces overall DNA demethylation and chromatin reprogramming.¹¹ In tumour models, *in vitro* and *in vivo* studies point towards a principal role of CD137 in CD8⁺ T cells rather than in CD4⁺ T cells, even though CD137 is inducible on both CD8⁺ and CD4⁺ T cells, including CD4⁺ FoxP3⁺ regulatory T cells (Tregs).¹² Of note, the functional role of CD137 signalling on Th1 CD4⁺ and Tregs is still poorly understood and it may not be as physiologically relevant as for CD8⁺ T cells. On activated NK cells, CD137 triggering results in increased cell activation.¹³ Reports on enhancement of antibody-dependent cell cytotoxicity and cytotoxic functions have been retracted,^{14 15} but there is evidence that it contributes to NK activation.¹⁶

Because of (1) the strong costimulatory activity on cytotoxic T cells that boosts cell proliferation and survival, (2) the specific expression on antigen-primed tumour-specific T cells in the TME and draining lymph nodes^{17 18} and

(3) the sustained signalling over time causing long-term changes in tumour-specific T cells,¹⁹ CD137 is considered an attractive target to enhance cancer immunotherapy. Furthermore, recent evidence on CD137 expression on CD4⁺ FoxP3⁺ Tregs indicated that CD137 can be used as a target to reduce/deplete Tregs which more brightly express CD137 and thereby offer a window for selective depletion.¹²

CD137-BASED CANCER IMMUNOTHERAPY IN PRECLINICAL MODELS

The first *in vivo* evidence for the therapeutic potential of CD137 targeting in cancer was shown in transplantable tumour models of sarcoma and mastocytoma in which CD8⁺ T cell-dependent complete tumour eradication was attained using agonistic anti-CD137 mAbs.²⁰ Ever since, accumulating preclinical data support the antitumour activity of CD137 agonism in several immunogenic and

Table 1 Summary of combinatorial approaches with agonistic CD137 monoclonal antibody (mAb) under clinical evaluation

Agent	mAb	Main characteristics	Combination under clinical evaluation	Additional biological agent	Condition	Phase	NCT	
Urelumab (BMS-663513)	Fully human IgG4	High agonist activity	Rituximab (anti-CD20)		Chronic lymphocytic leukaemia	II (withdrawn)	NCT02420938	
	Non-ligand blocking	Liver inflammation with doses of ≥ 1 mg/kg			B-cell non-Hodgkin's lymphoma	I (completed)	NCT01775631	
					Nivolumab (anti-PD-1)	Solid tumours (intratumour urelumab)	I and II (not recruiting)	NCT03792724
					Solid tumours and B-cell non-Hodgkin's lymphoma	I and II (completed)	NCT02253992	
					Muscle-invasive urothelial carcinoma of the bladder (neoadjuvant nivolumab)	II (recruiting)	NCT02845323	
					Advanced and/or metastatic malignant tumours	I (active, not recruiting)	NCT02534506	
					Multiple metastases in advanced solid tumours	I (recruiting)	NCT03431948	
					Recurrent glioblastoma	I (active)	NCT02658981	
					GVAX (pancreas vaccine)	Surgically resectable pancreatic cancer	I and II (recruiting)	NCT02451982
					Tumour-infiltrating lymphocytes	Metastatic melanoma	I (active, not recruiting)	NCT02652455
		Cetuximab (anti-EGFR)	Advanced/metastatic colorectal cancer	I (completed)	NCT02110082			
		Elotuzumab (anti-CS1)	Multiple myeloma	I (completed)	NCT02252263			
		Ipilimumab (anti-CTLA-4)	Malignant melanoma	I (withdrawn)	NCT00803374			
Utolimumab (PF-05082566)	Humanised IgG1	Weak agonist activity	Pembrolizumab (anti-PD-1)		Advanced solid tumours	I (completed)	NCT02179918	
	Ligand blocking	No dose-limiting toxicities			Mogamulizumab (anti-CCR4)	Advanced solid tumours	I (terminated)	NCT02444793
					Rituximab (anti-CD20)	Solid tumours or B-cell lymphomas	I (completed)	NCT01307267
					Cetuximab (anti-EGFR)	Advanced colorectal cancer	I (recruiting)	NCT03290937
					Trastuzumab (anti-HER2)	Advanced HER2-positive breast cancer	I (recruiting)	NCT03364348
					Avelumab (anti-PD-L1)	Advanced HER2 +breast cancer	II (recruiting)	NCT03414658
					Avelumab (anti-PD-L1)	Triple negative breast cancer	II (recruiting)	NCT03971409
						Locally advanced or metastatic solid tumours	II (recruiting)	NCT02554812
					PF-8600 (OX-40 agonist)	Advanced solid tumours	I and II (recruiting)	NCT03217747
					Rituximab (anti-CD20)	Relapsed or refractory diffuse large B-cell lymphoma	I (completed)	NCT02951156

Continued

Table 1 Continued

Agent	mAb	Main characteristics	Combination under clinical evaluation	Additional biological agent	Condition	Phase	NCT
				Rituximab (anti-CD20)	Relapsed or refractory diffuse large B-cell lymphoma or mantle cell lymphoma	I (recruiting)	NCT03440567
			PF-8600 (OX-40 agonist)		Advanced or metastatic carcinoma	I (active, not recruiting)	NCT02315066
			ISA101b (HPV16 E6/E7 peptides vaccine)		HPV-16-positive oropharyngeal cancer	II (active, not recruiting)	NCT03258008
			Primed CD8 +tumour antigen-specific T cells	Aldesleukin	Recurrent ovarian cancer	I (recruiting)	NCT03318900

non-immunogenic tumour models. These same tumour models have been crucial for the understanding of the mechanistic principles behind CD137 costimulation in cancer. Among them, it has been shown that CD137 agonists could prevent cytotoxic T lymphocyte (CTL) anergy and thereby break T cell tolerance towards tumour antigens,²¹ restore T cell dysfunctionality in the TME²² and increase persistence of tumour-specific T cells.²³

On their way to the preclinical development of CD137 agonists, initial efforts were made to force expression of CD137L on poorly immunogenic sarcoma cells⁷ or the prostate-specific membrane antigen (PSMA) recognising T cells,²⁴ this strategy resulted in increased immunogenicity and enhanced antitumour activity. However, studies using agonistic mAbs showed stronger antitumour activity probably because of stronger agonistic actions and thus became the focus of translational research. Similar to other TNFRSF agonists such as CD40 mAbs, the road to the development of sufficiently agonistic CD137 has not been straightforward. In order to attain functional CD137 signalling and costimulation, agonist CD137 mAb must trimerise the receptor, a feature that requires effective crosslinking of the Fc to FcγRs. In line with this, Buchan *et al* suggested a sequential mAb treatment scheme using a dual Fc engineering strategy to make the most of the CD137-specific expression pattern, first treating with an activating FcγRs-binding mIgG2a mAb to deplete intratumour Tregs brightly expressing CD137, followed by an inhibitory FcγRs-binding mIgG1 mAb to provide strong costimulation to CD8 CTLs.²⁵

Along with the efficacy of anti-CD137 antibody as a monotherapy, a broad number of synergistic combinations have been reported. Checkpoint inhibitory receptors such as PD-1 or CTLA-4 are commonly found to be upregulated in dysfunctional T cells within the TME across multiple types of malignant diseases.¹ Checkpoint receptors on binding their ligands repress T-cell activation intracellular signals that had frequently been induced by costimulatory receptors. To make the most of these facts, simultaneous blockade of checkpoint inhibitor receptors in conjunction with agonistic mAbs triggering

costimulatory receptors makes sense²⁶. This can be achieved also with bispecific antibodies encompassing both activities in a single moiety. In this regard, true synergistic combinations are a major goal in cancer immunotherapy.²⁷ CD137-resistant ovarian and lung cancer models become amenable to eradication on combined treatment with anti-CD137 mAb with PD-1 blocking mAb, resulting in potent synergistic effects that correlate with increased T-cell survival and effector functions.²⁸ Interestingly, CD137 and PD-1 coexpression is likely to be restricted to neoantigen-specific tumour-infiltrating CD8+ T cells,²⁹ suggesting the rational of combining both pathways for immunotherapeutic purposes. Combination of CTLA-4 blocking mAb with anti-CD137 mAb also resulted in enhanced CD8+ T-cell mediated immune responses in mouse models of melanoma.³⁰ In addition to checkpoint blockade, combination with immunostimulatory mAbs such as CD40,³¹ OX40³² or CD20³³ has been reported to potentiate the antitumour effects.

Other therapeutic strategies including radiotherapy,³⁴ gene therapy³⁵ or adoptive cell therapy synergise with CD137 stimulation. Combination of CD137 mAb with adoptive T cell therapy in mouse models of B16OVA and OVA-specific CD8⁺ T cell (OT1) transfer resulted in long-lasting tumour control elicited by enhanced effector functions of the transferred and endogenous CD8⁺ T cells that when visualised by intravital microscopy behaved more aggressively against malignant cells.²³

CD137 IMMUNOTHERAPY IN CLINICAL DEVELOPMENT

Two approaches encompassing CD137 have been evaluated in the clinic: (1) anti-CD137-targeting mAbs and bispecific Abs and (2) the cytoplasmic CD137 domain forming part of chimeric antigen receptors (CAR). Today, CD19 targeting CAR-T cells are the only CD137-based approach approved by the FDA for the treatment of B-cell paediatric leukaemia and refractory B-cell lymphoma.³⁶ In contrast to CD28 encompassing CARs, CD137 intracellular signalling domain-based CARs are those achieving best adoptive T-cell persistence.³⁷ In addition, stronger

metabolic fitness and beneficial epigenetic reprogramming have been associated with CD137 containing CARs.³⁸

Two agonist anti-CD137 mAb have been tested in the clinical setting; urelumab (BMS-663513), a fully human IgG4-based anti-CD137 mAb that does not block CD137L–CD137 interactions and utolimumab (PF-05082566), a ligand-blocking-humanised IgG1 mAb (table 1).

Initial reports testing urelumab in phase I and II trials showed activity as a single agent in patients with advanced cancer, but trials had to be terminated because of treatment-related severe adverse effects in the form of liver inflammation.³⁹ The hepatotoxicity observed in the patients had not been observed in non-human primates due to lower binding affinities to cynomolgus CD137. An integrated safety analysis of urelumab revealed life-threatening liver inflammation associated with doses of ≥ 1 mg/kg that required a drastic dose reduction to 0.1 mg/kg to be safe.⁴⁰ Such dose reduction maintained little pharmacodynamic activity and resulted in loss of efficacy as monotherapy except for a few B-cell non-Hodgkin's lymphoma cases, where some objective responses were observed. Despite the dose reduction requirement, the obtained safety profile supported evaluation of urelumab in combination with other immune-modulating agents. Low doses of urelumab in combination with the anti-PD1 mAb nivolumab showed acceptable tolerability and promising clinical responses have been reported in patients with metastatic melanoma irrespective of PD-L1 tumour expression.⁴¹ Urelumab is further being combined with cetuximab (NCT02110082), rituximab (NCT01775631) and elotuzumab (NCT02252263) (table 1).

On the contrary, utolimumab has no dose-limiting toxicities and shows preliminary antitumour activity in patients with advanced cancer. Probably because of being a weak CD137-agonist, utolimumab has shown little clinical effect as a monotherapy.⁴² Phase I combination strategies combining utolimumab with rituximab in patients with relapsed or refractory non-Hodgkin's lymphoma⁴³ and utolimumab with pembrolizumab in patients with advanced solid tumours⁴⁴ have demonstrated tolerability, safety and preliminary clinical activity. Another combinatorial strategy being tested is the combination of utolimumab with intravenous infusion of CD8 +tumour antigen-specific T cells (NCT03318900; table 1).

CD137 mAb therapy aims to stimulating the activity of tumour-specific T cells already within the TME. Tumour susceptibility to the mAb treatment is likely to depend on a pre-existing tumour T-cell infiltration and on CD137 expression levels on such tumour-infiltrating T cells. For clinical purposes, well-defined prognostic and predictive markers for CD137 treatment are still missing. Thus, biomarker discovery and validation for CD137-based immunotherapies currently constitute a major research effort.

LESSONS LEARNT: MOVING CD137 AGONISTS TOWARDS SAFER TUMOUR TARGETING

Preclinical studies trying to elucidate the hepatotoxicity related to CD137 agonists showed hepatic polyclonal CD8⁺ T cell activation and IFN- γ secretion as the main mediators of liver damage.⁴⁵ Further studies link CD137 mAb binding to liver resident monocyte and Kupffer cell that secrete tumour necrosis factor α (TNF- α) and IL-27 and activate CD8⁺ T cells, ultimately leading to liver damage.⁴⁶ Prompted by the preclinical mechanistic insight and based on the clinical experiences using CD137 mAb, that when sufficiently agonistic lead to severe liver inflammation, new efforts are in place to specifically bring CD137 agonism to the tumour tissue microenvironment (figure 1).

Different approaches are on the table, among them the most advanced ones under clinical development are the CD137-based bispecific constructs that are designed to bring CD137 costimulation specifically to the TME. A bispecific antibody targeting CD137 on T cells and the oncogenic antigen HER2 expressed on tumour cells (HER2/4-1BB Ab) has been the first CD137-based bispecific Ab tested in the clinic.⁴⁷ Recently, results presented from the phase 1 single-agent dose escalation trial evaluating HER2/4-1BB Ab in patients with HER2+ solid tumours, indicated good tolerability of the construct and showed evidence of clinical activity.⁴⁸ Another tumour targeting bispecific construct being explored in the clinic is the PD-L1 targeting PD-L1/4-1BB Ab (NCT03809624). Preclinically, bispecific Ab targeting the tumour antigen 5T4 and CD137 (5T4/4-1BB Ab) has shown promising activity in cell cultures.⁴⁹ In addition, a chimeric protein consisting on a trimeric form of CD137L integrated into a fibroblast activation protein (FAP), a highly expressed protein on the tumour stroma, recognising bispecific construct (FAP/4-1BBL) has shown impressive anti-tumoural activities against tumour mouse models and tumour-targeting in spontaneous cancer bearing rhesus monkeys, with no signs of toxicity in macaques.⁵⁰ In line with the bispecific designs and to avoid Fc γ R binding mediated liver toxicity, Fc lacking trimeric CD137 constructs with sufficient agonistic activity and without liver toxicity have been successfully evaluated in mouse models.⁵¹

Another approach to specifically provide CD137 costimulation to the TME includes intratumour injections of low doses of the CD137 mAb.⁵² Following preclinical evidence demonstrating the feasibility and antitumour efficacy of the intratumour administration, a clinical trial injecting low doses of CD137 mAb directly into metastatic lesions and in combination with systemic nivolumab is being conducted in patients with solid tumours (NCT03792724).

CONCLUSION

The immunobiology of CD137 provides the basis for our understanding of the role of CD137 costimulation in cancer and arguably presents CD137 as one of the most potent costimulatory receptors for primed CD8⁺ T cells. Preclinical data convincingly show strong antitumour activity of CD137

agonists. Consequently, CD137 emerges as a robust candidate to be targeted for cancer treatment. The beginnings of CD137 agonist mAb in the clinic were overshadowed by hepatic toxicities in a not well-defined fraction of patients. In spite of such caveats, (1) safe combinatorial strategies, involving checkpoint inhibitors and CD137 agonists, designed to simultaneously normalise and stimulate the inhibited antitumour immune responses or (2) strategies to specifically target CD137 agonists to the TME are likely to broaden the therapeutic window. In the next years, we need to harness a promising foal to turn it into a powerful warhorse against cancer.

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