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REVIEW

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The emerging landscape of novel 4-1BB (CD137) agonistic drugs for cancer immunotherapy

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ABSTRACT

The clinical development of 4–1BB agonists for cancer immunotherapy has raised substantial interest during the past decade. The first generation of 4–1BB agonistic antibodies entering the clinic, urelumab (BMS-663513) and utomilumab (PF-05082566), failed due to (liver) toxicity or lack of efficacy, respectively. The two antibodies display differences in the affinity and the 4–1BB receptor epitope recognition, as well as the isotype, which determines the Fc-gamma-receptor (FcγR) crosslinking activity. Based on this experience a very diverse landscape of second-generation 4–1BB agonists addressing the liabilities of first-generation agonists has recently been developed, with many entering clinical Phase 1 and 2 studies. This review provides an overview focusing on differences and their scientific rationale, as well as challenges foreseen during the clinical development of these molecules.

Introduction

The T cell immune response has been shown to be essential in tumor control. To induce a robust and long-term T cell immune response, a T cell receptor (TCR) activation (signal 1) as well as sufficient co-stimulation (signal 2) is needed. Therefore, several costimulatory TCRs including 4–1BB have been evaluated for their possible implementation in cancer immunotherapy.[1] The TNF receptor superfamily member 4–1BB (CD137, TNFRSF9) was first identified in 1989[2] and subsequently described as an important costimulatory receptor on T cells[3,4] as well as on other immune cells.[5] Melero and colleagues showed in 1997 that monoclonal 4–1BB antibodies were able to induce improved anti-tumoral T cell activation that led to the eradication of established tumors in mice.[6] 4–1BB activation on T cells has been shown to improve proliferation via the beta-catenin/TCF1 pathway (CD8 T cells),[7] cytokine secretion,[8] cytotoxicity,[8,9] polarization by EOMES upregulation (ThEO/TcEO),[9,10] long-lived memory formation,[11] survival via up-regulation of Bcl-xL and ERK-dependent Bim down-modulation (CD8 T cells),[12] resistance to exhaustion,[13,14] and mitochondrial biogenesis and function and metabolic fitness.[15,16] Most of the activations seem to preferentially occur in CD8 T cells.

The implementation of intracellular costimulatory 4–1BB domains in the second generation of chimeric antigen receptor (CAR) T cells enabled U.S. Food and Drug Administration (FDA) approval of tisgenlecleucel[17] and ciltacabtagene autoleucel[18] and ongoing development of various new CAR T cell therapies.[19] Other second-generation CAR T cells implement CD28 as a costimulatory domain instead of 4–1BB. Although both costimulatory domains lead to similar tumor control rates in patients mediated by CAR T cells, CD28 seems to induce slightly higher cytokine release, T cell expansion rates,

but also higher neurotoxicity risk. 4–1BB seems to lead to better long-term T cell persistence, but other differences in clinical study and CAR T cell design may contribute to these observed variations.[19] In endogenous T cells, CD28 and 4–1BB differ in both intracellular signaling and expression pattern. CD28 is constitutively expressed on CD4 and CD8 T cells, whereas 4–1BB expression is signal 1 mediated, timewise limited and higher on CD8 T cells.[20] Therefore, data from CD28 and 4–1BB agonists in clinical studies have the potential to give a more diverse picture between CD28 and 4–1BB costimulation than CAR T cells and the rising number of new 4–1BB agonists, as well as new CD28 agonists, entering the clinic[21] will increase our understanding of costimulation in cancer immunotherapy. In this review, we discuss preclinical and clinical results for new 4–1BB agonists that have entered clinical studies.

The first generation of 4-1BB agonists

The clinical development of agonistic 4–1BB antibodies started in 2005 with urelumab (BMS-663513), a humanized anti-human 4–1BB human IgG4 antibody evaluated as a cancer immunotherapy agent (NCT00309023). Although initial results were promising, two fatal adverse events due to hepatotoxicity occurred. Subsequent studies revealed that, when urelumab was administered at a safe dose (0.1 mg/kg), it only mediated very limited efficacy.[22,23] A second monoclonal 4–1BB agonistic antibody, utomilumab (PF-05082566), a fully human anti-human 4–1BB human IgG2, entered the clinic in 2011 (NCT01307267). Unlike urelumab, utomilumab did not induce major toxicities, but it also mediated very limited efficacy, both as a monotherapy and in combination with rituximab[23] so that ultimately clinical development was discontinued.

Urelumab and utomilumab display quite different characteristics that are thought to affect toxicity and efficacy (Figure 1). The affinity for urelumab (22 or 16.6 nM)[24,25] was described to be higher than for utomilumab (69 or 71.2 nM);[24,25] however, the affinity of 4–1BB agonistic antibodies seems not to be critical for agonistic activity and liver toxicity induction, but is rather driven by Fc-mediated crosslinking and epitope binding.[26]

Human IgG2 (used in utomilumab) displays a lower hinge flexibility as human IgG4 (used in urelumab), which was correlated with a higher agonistic activity for CD40 agonistic antibodies.[27–29] Therefore, the hinge flexibility may not explain the higher agonistic activity of urelumab. Fc-mediated cross-linking, especially binding to Fc gamma receptor IIB (FcγRIIB) has been reported to promote the activity of agonistic antibodies.[27,30,31] As an IgG4, urelumab has higher affinity to FcγRIIB, and therefore may mediate stronger FcγRIIB-dependent agonistic activity, but this may not be the only factor related to the agonistic differences between urelumab and utomilumab. Nevertheless, FcγRIIB crosslinking in the liver has been associated with hepatitis induction by anti-Fas or anti-4-1BB agonistic antibodies.[26,32,33]

The different epitopes recognized by urelumab and utomilumab have been investigated extensively to understand their influence on the observed differences. While urelumab binds to an epitope in the membrane-distal cysteine-rich pseudo repeats domain (CRD1) of 4–1BB, without interfering with the ligand, utomilumab binds to the 4–1BB domains CRD2 and CRD3 and competes with the natural ligand.[24,34] Therefore, high levels of endogenous 4–1BBL compete with utomilumab for 4–1BB receptor binding and limit its 4–1BB receptor clustering activity,[34] whereas urelumab activity is potentiated by high levels of endogenous 4–1BBL, leading to a super-agonistic property (Figure 1).[24,35]

The second generation of 4-1BB agonists

During January 2017 to December 2022, at least 41 4–1BB agonistic drugs entered Phase 1 clinical trials according to published reports and data found in the U.S. National Library of Medicine's clinicaltrials.gov registry, the Chinese Clinical Trial Registry and the European Union Trial Register (Table 1, Supplementary Figure S1). The number of molecules entering the clinic experienced a drop in 2020, presumably due to the SARS-CoV2 pandemic, but then rebounded. Additional 4–1BB agonists can be expected to enter the clinic in the future, as more than 40 new 4–1BB agonists are in active preclinical development (Clarivate Cortellis™; www.cortellis.com/intelligence/home.do).

In this review all molecules entering the clinic after urelumab and utomilumab are classified as second-generation 4–1BB agonists. This second generation of 4–1BB agonists can be split into two major groups (Figure 2), IgG-based molecules
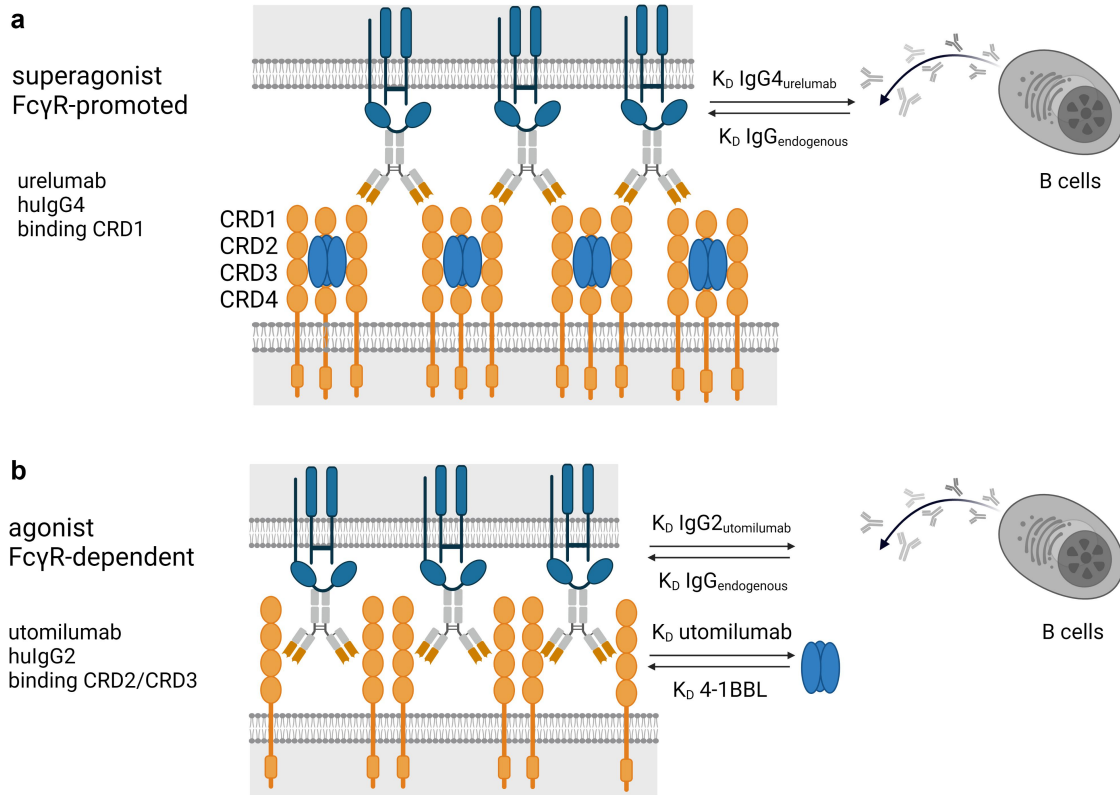

Figure 1. Differences between urelumab and utomilumab. (a) Urelumab binds to CRD1 of the 4–1BB receptor in a non-4-1BBL competing way. If three 4–1BB receptors are trimerized by 4–1BBL, additional binding of urelumab can lead to increased clustering of 4–1BB receptors in a super-agonistic way. This activation will be systemic but can further be potentiated by hyper-crosslinking via FcγRIIB binding. However, urelumab will compete with endogenous IgGs for FcγRIIB binding during this process. (b) Utomilumab binds to CRD2 and CRD3 and therefore competes with 4–1BBL. Only if utomilumab is hyper-crosslinking via FcγRIIb, it will lead to sufficient hyper-crosslinking and activation of 4–1BB receptors. Therefore, utomilumab will compete with soluble and membrane-bound 4–1BBL for binding to 4–1BB receptors as well as with endogenous IgGs for FcγRIIB binding.

Table 1. 4–1BB agonistic drugs in clinical development.

#	molecule	Targets	company	molecule backbone	half-life	clinical trial	Phase	start of trial	application	Ref.
1	PRS-343, Cinrebafusp alfa	4–1BB, Her2	Pieris Pharmaceuticals	IgG4 backbone 2 + 2 anticalin fusions FcγR-silent	antibody-like	NCT03330561 NCT03650348 NCT05190445	1 1 2	**September 2017** August 2018 November 2021	Her2-expressing solid tumors (BC, GC and bladder cancer) Her2-expressing GC or GEJC	36–38
2	RG7827, RO7122290	4–1BB, FAP	Hoffmann-La Roche	IgG1 backbone 1 + 3 4–1BBL fusion FcγR-silent	antibody-like	EudraCT 2017–003961-83 NCT03869190 NCT04826003	1/1b 1b/2 1b/2	**May 2018** July 2019 July 2021	solid tumors, thymoma, mesothelioma, TNBC advanced or metastatic, UC metastatic colorectal cancer	32,39
3	ADG106	4–1BB	Adagene Suzhou	IgG1	antibody-like	**NCT03707093** NCT03802955 NCT04501276 NCT04645069 NCT04775680 NCT05236608 NCT05275777	**1** 1 1b 1 1/2 1/2 1b/2	**September 2018** December 2018 September 2020 March 2021 May 2021 November 2021 April 2022	advanced or metastatic solid tumors and/or NHL metastatic NSCLC Her2 negative BC, advanced solid tumors	40–42
4	INBRX-105/ ES101	4–1BB, PD-L1	Inhibrx/Elpiscience Biopharma	IgG backbone 2 + 2 sdAb fusions FcγR-silent	antibody-like	NCT03809624 NCT04009460 NCT04841538	1 1 1/2	**January 2019** June 2019 July 2021	(metastatic) solid tumors and/or NSCLC, melanoma, HNSCC, GC, RCC, UC, esophageal adenocarcinoma, SCLC, thoracic tumors	43,44
5	CTX-471	4–1BB	Compass Therapeutics	IgG4	antibody-like	NCT03881488	1	**May 2019**	locally advanced solid tumors, metastatic cancer	35
6	Gen1046 / BNT311	4–1BB, PD-L1	Genmab/BioNTech	IgG1 backbone 1 + 1 FcγR-silent	antibody-like	NCT03917381 NCT04937153	1/2 1	**May 2019** June 2021	solid tumors (NSCLC, UC, TNBC, endometrial carcinoma, HNSCC, Cervical Carcinoma)	45
7	MCLA-145	4–1BB, PD-L1	Merus N.V./Incyte Corporation	IgG1 backbone 1 + 1 FcγR-silent	antibody-like	NCT05117242 NCT03922204	2 1	October 2021 **May 2019**	metastatic NSCLC and lung cancer advanced and metastatic solid tumors, B-cell lymphoma	46,47
8	RG6076, RO7227166	4–1BB, CD19	Hoffmann-La Roche	IgG1 backbone 1 + 3 4–1BBL fusion FcγR-silent	antibody-like	NCT04077723	1	**August 2019**	relapsed/refractory B-cell NHL	32
9	MP0310	4–1BB, FAP, HSA	Molecular Partners	DARPins 1 + 2	antibody-like by HSA-binding	NCT04049903	1	**September 2019**	advanced solid tumors	48
10	Gen1042 / BNT312	4–1BB, CD40	Genmab/BioNTech	IgG1 backbone 1 + 1 FcγR-silent	antibody-like	NCT04083599 NCT05491317	1/2 1/2	**September 2019** **Estimated October 2022**	malignant solid tumors, NSCLC, CRC, melanoma, HNSCC, PDAC Metastatic solid tumors	49,50
11	AGEN2373	4–1BB	Agenus	IgG1	antibody-like	NCT04121676	1	**September 2019**	advanced cancer	51,52
12	LVGN6051	4–1BB	Lyvgen Biopharma/Merck Sharp & Dohme	IgG1, improved FcγRIIB binding	antibody-like	NCT04130542 NCT04635995 NCT04694781 NCT05075993	1 1 1 1	**October 2019** December 2020 May 2021 November 2021	advanced and metastatic malignancy metastatic solid tumors: esophageal, GC, HNC, HCC, HPV-related solid tumors, OC, soft tissue sarcoma, uveal melanoma	53,54
13	ATOR-1017	4–1BB	Alligator Bioscience AB	IgG4	antibody-like	NCT05301764 NCT04144842	1/2 1	March 2022 **December 2019**	soft tissue sarcoma solid tumors, neoplasms	55–57

(Continued)

Table 1. (Continued).

#	molecule	Targets	company	molecule backbone	half-life	clinical trial	Phase	start of trial	application	Ref.
14	STA551	4–1BB ATP dependent	Chugai Pharmaceutical	IgG1, improved FcγRIIB binding	antibody-like	JapicCTI-205153, STA101JG, EudraCT: 2019-003329-11	1	**March 2020**	solid tumors	25
15	ND-021/NM21-1480	4–1BB, PD-L1, HSA	Numab Therapeutics / C Stone Pharmaceuticals	VH/VL fusions 1 + 1	antibody-like by HSA-binding	NCT04442126	1/2	**August 2020**	advanced solid tumors, NSCLC	58,59
16	GNC-038 Emfizatamab	4–1BB, PD-L1, CD19, CD3	Sichuan Biokin Pharmaceutical or Sichuan Baili Pharmaceutical or Bailey Pharmaceuticals/SystImmune	IgG 2 + 2 + 2 + 2 scFv fusions FcγR-silent	antibody-like	NCT04606433 NCT05192486 NCT05189782 NCT05485753	1 1/2 1b/2 1/2	**November 2020** December 2021 February 2022 February 2022	relapsed/refractory B-cell NHL, relapsed/ refractory DLBCL	43
17	DSP107	4–1BB, CD47	KAHR Medical	trimeric fusion protein	assumed fast half-life	NCT04440735 NCT04937166	1/2 1	**October 2020** January 2022	PCNSL and relapsed or refractory SCNSL advanced solid tumors, NSCLC, AML, MDS, CMML	60
18	FS120	4–1BB, OX-40 (CD134)	F-star Therapeutics	IgG1 backbone 2 + 2 Fc-region antigen-binding FcγR-silent	antibody-like	NCT04648202	1	**November 2020**	advanced cancer, metastatic cancer, advanced malignancies	61,62
19	FS222	4–1BB, PD-L1	F-star Therapeutics	IgG1 backbone 2 + 2 Fc-region antigen-binding FcγR-silent	antibody-like	NCT04740424	1	**December 2020**	advanced cancer, metastatic cancer, advanced malignancies	63
20	HOT-1030	4–1BB	Shanghai Huabo Biopharmaceutical (Huaota)/ Eutilex	IgG1, abrogated FcγRIIB binding	antibody-like	NCT05060263	1	**March 2021**	advanced solid tumors	
21	ABL503 / TJ-L14B	4–1BB, PD-L1	ABL Bio/I-Mab Biopharma	IgG1 2 + 2 scFv fusions FcγR-silent	antibody-like	NCT04762641	1	**April 2021**	advanced or metastatic solid tumors	64,65
22	IBI319	4–1BB, PD-1	Adimab/Innovent Biologics/Eli Lilly	IgG1 backbone 1 + 1 FcγR-silent	antibody-like	NCT04708210	1	**April 2021**	advanced malignant tumors	66
23	GNC-039	4–1BB, PD-L1, EGFRvIII, CD3	Sichuan Biokin Pharmaceutical or Sichuan Baili Pharmaceutical or Bailey PharmaceuticalsSystImmune	IgG 2 + 2 + 2 + 2 scFv fusions FcγR-silent	antibody-like	NCT04708210	1	**April 2021**	advanced malignant tumors	43
24	EU101	4–1BB	Eutilex/Zhejiang Huahai Pharmaceutical	IgG	antibody-like	NCT04903873	1/2	**May 2021**	solid tumors, colorectal Neoplasms, NSCLC	

(Continued)

Table 1. (Continued).

#	molecule	Targets	company	molecule backbone	half-life	clinical trial	Phase	start of trial	application	Ref.
25	CB307	4–1BB, PSMA, HSA	Crescendo Biologics	sdAb fusions 1 + 1	antibody-like by HSA-binding	NCT04839991	1	June 2021	advanced and/or metastatic PSMA-positive solid tumors	20,67,68
26	ABL111, TJ-CD4B, TJ-CLDN4B, TJ033721	4–1BB, Claudin18.2	ABL Bio /I-Mab Biopharma	IgG1 2 + 2 scFv fusions FcγR-silent	antibody-like	NCT04900818	1	June 2021	solid tumor, advanced cancer, metastatic cancer, GC, GEJC, esophageal adenocarcinoma, PDAC	65
27	GNC-035	4–1BB, PD-L1, ROR1, CD3	Sichuan Biokin Pharmaceutical or Sichuan Baili Pharmaceutical or Bailey Pharmaceuticals/ SystImmune	IgG 2 + 2 + 2 + 2 scFv	antibody-like FcγR-silent	NCT05039931 NCT05160545 NCT05104775	1 1 1	June 2021 November 2021 December 2021	locally advanced or metastatic solid tumors, locally advanced or metastatic breast cancer, relapsed/refractory hematologic malignancy	43
28	PRS-344/ S095012	4–1BB, PD-L1	Pieris Pharmaceuticals	IgG4 backbone 2 + 2 anticalin FcγR-silent	antibody-like	NCT05159388	1/2	September 2021	solid tumors	69,70
29	BI 765179	4–1BB, FAP	Boehringer Ingelheim	IgG backbone 2 + 2 scFv fusions FcγR-silent (all assumptions)	antibody-like	NCT04958239	1	September 2021	advanced solid tumors	
30	QL301/ QLF31907	4–1BB, PDL1	QLSF Biotherapeutics	IgG1 2 + 2 scFv fusions FcγR-silent	antibody-like	NCT05150405	1	September 2021	advanced malignant tumors	
31	ATG-101/YN-051/Ori-Bs-001	4–1BB, PD-L1	Antengene/ Origincell	IgG1 2 + 2 scFv fusions FcγR-silent	antibody-like	NCT04986865 NCT05490043	1 1	October 2021 January 2022	metastatic/advanced solid tumors and B-cell NHL	71
32	BT7480	4–1BB, Nectin-4	Bicycle Therapeutics	Bicycle peptides 1 + 2	reported short half-life	NCT05163041	1/2	November 2021	advanced Nectin-4-expressing solid tumors	72–75
33	PM1003	4–1BB, PD-L1	Biotheus	IgG 2 + 2 VHH fusions FcγR-silent	antibody-like	ChiCTR2100052887	1/2a	November 2021	advanced solid tumors	76
34	YH004	4–1BB	Eucure (Beijing) Biopharma	IgG1	antibody-like	NCT05040932 NCT05564806	1 1	December 2021 Estimated December 2022	advanced or metastatic malignancy advanced solid tumors and NHL	
35	LBL-024	4–1BB, PD-L1	Nanjing Labs	IgG1 2 + 2 scFv fusions FcγR-silent	antibody-like	NCT05170958	1/2	January 2022	advanced solid tumor	77

(Continued)

Table 1. (Continued).

#	molecule	Targets	company	molecule backbone	half-life	clinical trial	Phase	start of trial	application	Ref.
36	PM1032	4–1BB Claudin18.2	Biotheus	IgG 2 + 2 VHH fusions	antibody-like	ChiCTR2200060628	1/2a	**September 2022**	advanced solid tumors	
37	HLX35/BNA035	4–1BB EGFR	Shanghai Henlius Biotech/Binacea Pharma	IgG backbone 2 + 2 scFv fusions FcγR-silent (all assumptions)	antibody-like	NCT05150457 NCT05360381 NCT05442996	1 1 1	**February 2022** June 2022 Estimated January 2023	refractory solid tumors advanced or metastatic Solid Tumors	
38	HBM7008	4–1BB B7H4	Harbor BioMed	IgG1 2 + 2 VH fusion FcγR-silent	antibody-like	NCT05306444	1	**May 2022**	Advanced solid tumor	
41	ABL105/ YH32367	4–1BB Her2	ABL Bio/Yuhan Corporation	IgG1 backbone 2 + 2 scFv fusions FcγR-silent	antibody-like	NCT05523947	1/2	**August 2022**	Her2-expressing advanced or metastatic solid tumors	[78]
39	BGB-B167	4–1BB CEACAM5	BeiGene Ltd	bispecific	antibody-like	NCT05494762	1	**November 2022**	solid tumors (including CRC)	
40	ADG206	4–1BB	Adagene Suzhou	IgG1, enhanced IgG1 binding, 4–1BB binding sites are masked	antibody-like	NCT05614258	1	**Estimated December 2022**	advanced solid tumors	
41	PE0116	4–1BB	Shanghai Hyamab Biotech	IgG4	antibody-like	CTR20201794	1a/1b	**2021 (?)**	solid tumors	[79]

Note: The data were collected from references listed in the table as well as from the NIH US National Library of Medicine (www.ClinicalTrial.gov, indicated by a NCT trial identifier), Chinese Clinical Trial Registry (www.chictr.org.cn, indicated by a ChiCTR identifier) and the European Union Trial Register (www.clinicaltrialsregister.eu, indicated by a EudraCT identifier). Some of the listed information were received from companies webpages like for HOT-1030 (http://www.huabobio.com/en/newsshow/114.html), BI 765179 (https://pro.boehringer-ingelheim.com/inoncology/our-pipeline/cd137-fap-agonist), YH004 (https://biocytogen.com/pipeline/yh004) and HLX35/BNA035 (https://www.henlius.com/NewsDetails-3389-26.html). QL301/QLF31907, ADG206 and PM1032 have been presented at the annual meeting of the American Association for Cancer Research (AACR) in April 2022 in New Orleans (abstract number 2540, 2868 and 5555 respectively). HBM7008 was presented at the annual meeting of The Society for Immunotherapy of Cancer (SITC) 2022 in Boston (abstract number 1057). For PE0116 the clinical trial number as well as the dosing of 16 patients (status January 2022) was communicated at the American Society of Clinical Oncology (ASCO) annual meeting in June 2022 in Chicago (abstract number 2640). 4–1BB agonists are shown in a chronological order by actual study start of the first clinical trial. Combination partners are not listed. Used abbreviations: AML = acute myelogenous leukemia, ATP = adenosine triphosphate, BC = breast cancer, BCL = B cell lymphoma, CEACAM5 = carcinoembryonic antigen cell adhesion molecule 5, CD19 = Cluster of Differentiation 19, CD40 = Cluster of Differentiation 40, CD47 = Cluster of Differentiation 47, CMML = chronic myelomonocytic leukemia, CRC = Colorectal Cancer, DARPins = designed ankyrin repeat proteins, DLBCL = diffuse large B cell lymphoma, EGFRvIII = epidermal growth factor receptor variant III, FAP = Fibroblast Activating Protein alpha, Fc = crystallizable fragment, FcγR = Fc-gamma-receptor, GC = gastric cancer, GEJC = gastroesophageal junction cancer, HCC = hepatocellular carcinoma, Her2 = human epidermal growth factor receptor 2, HNC = head and neck cancer, HNSCC = head and neck squamous cell carcinoma, HPV = human papilloma virus, HSA = Human Serum Albumin, MDS = myelodysplastic syndromes, NHL = non-Hodgkin lymphoma, NSCLC = non-small cell lung cancer, OC = Ovarian Cancer, PCNSL = Primary Central Nervous System Lymphoma, PDAC = pancreatic ductal adenocarcinoma, PD-L1 = Programmed Death-Ligand 1, PSMA = Prostate-specific membrane antigen, RCC = renal cell carcinoma, ROR1 = Receptor Tyrosine Kinase Like Orphan Receptor 1, scFv = single monomeric variable antibody domain, SCLC = small cell lung cancer, sdAb = single-chain variable antibody fragment, SCNSL = Secondary Central Nervous System Lymphoma, TNBC = triple negative breast cancer, UC = urothelial carcinoma, VH = variable domain of heavy antibody chain, VHH = antigen-binding domain of camelid dimeric heavy chain antibodies, VH/VL = variable domain of heavy and light antibody chain.
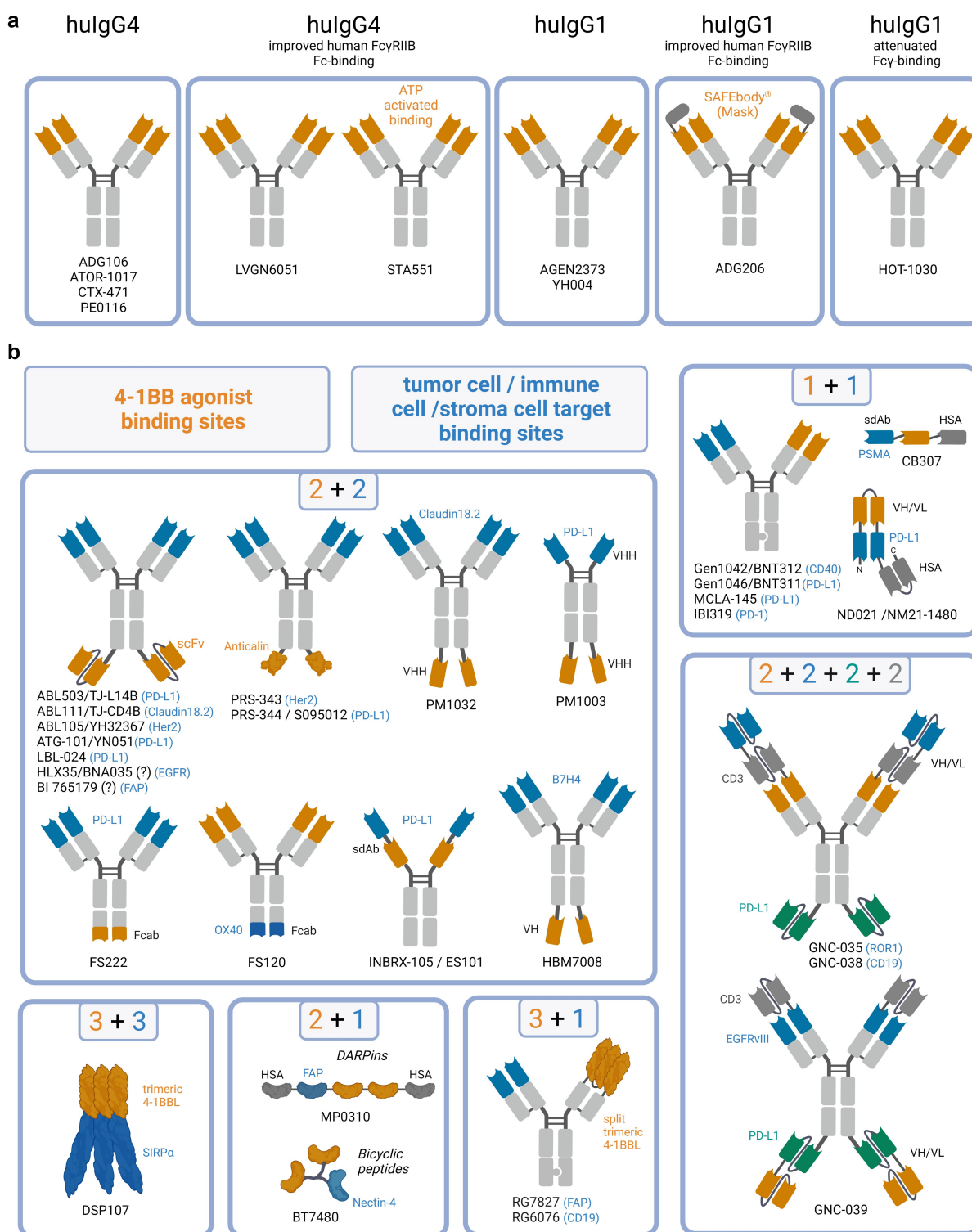
Figure 2. Different molecule designs of second-generation 4–1BB agonistic drugs. (a) Second generation agonistic human 4–1BB IgGs featuring isotypes as indicated. Sometimes an improved or attenuated binding to FcγRIIB is integrated. Some molecules show controlled 4–1BB binding depending on ATP concentration or de-masking of the binding sites. All IgG-like molecules display bivalent agonistic binding to 4–1BB (yellow). EU101 is not included as the isotype is not disclosed. (b) Most of the 4–1BB agonistic drugs display bi-, tri- or tetra-specificity, e.g., in addition to the specificity to 4–1BB (in yellow) they also recognize at least another binding site (blue, green, and dark gray), whereby the main crosslinking target site is highlighted in blue. If molecules implement an antibody-like format, typically mutations are introduced to abolish Fcγ-receptor and complement binding. Other molecules without a Fc-fragment binding to FcRn display binding to human serum albumin (HSA) to improve in vivo half-life. Molecules are sorted by their ratio of the 4–1BB agonistic binding sites (yellow) and the crosslinking target sites (blue). The format of HLX35/BNA035 and BI 765179 are assumptions based on pictures of the companies' webpages. BGB-B16 is not implemented as the structure of the bispecific antibody is not disclosed. Abbreviation: 4–1BBL = trimeric 4–1BB Ligand ectodomain, DARPins = Designed ankyrin repeat proteins, Fcab = Fc-region with antigen binding, FcγRIIB = Fc-gamma-receptor IIB, VHH = antigen-binding domain of camelid dimeric heavy chain antibodies, scFv = single-chain variable antibody fragment, sdAb = single monomeric variable antibody domain, SIRPα = Signal regulatory protein alpha ectodomain, VH = variable domain of heavy antibody chain, VHH = antigen-binding domain of camelid dimeric heavy chain antibodies, VH/VL = variable domain of heavy and light antibody chain.

(Figure 2a) and the bi- tri- or tetraspecific molecules (Figure 2b).

IgG-based 4-1BB agonists

The first group of second-generation 4–1BB agonists is based on a human IgG1 or IgG4 following partially the mode of action (MoA) of first-generation 4–1BB agonistic antibodies, i.e., most of them depend on functional Fcγ-receptor cross-linking, but bind epitopes that are different from urelumab and utomilumab. The antibodies ADG106, ATOR-1070, PE0116 and CTX471 are human IgG4s (Figure 2A) similar to urelumab, but do not recognize urelumab's CRD1 epitope (Figure 3). ADG106, PE0116 and ATOR-1070 are 4–1BBL-blocking and CTX-471 is non-4-1BBL blocking binding CRD3 and CRD4.[35,40,53,55,79] The change of epitope aims at a functionality that is different to urelumab and utomilumab, i.e., being functional but safe ("sweet point" of functionality).[53,79]

The two IgG4 antibodies LVGN6051 and STA551 (Figure 2a) display mutations in the Fc to improve binding and subsequent crosslinking via Fcγ-receptor RIIB (FcγRIIB), therefore promoting the FcγRIIB hyper-clustering and mediating less competition with endogenous IgGs.[27] At the same time they avoid systemic activity by choosing a weaker agonistic 4–1BB binder (LVGN6051)[53] or a modified 4–1BB binder (STA551), which only binds at high adenosine triphosphate (ATP) concentration, as observed in the tumor microenvironment.[25]

The antibodies AGEN2373 and ADG206 are human IgG1, and therefore will display increased flexibility of the hinge. At least for CD40 agonistic antibodies, this has been associated to decreased agonistic activity compared to IgG2 or IgG4.[27] IgG1 displays a similar affinity to the inhibitory human FcγRIIB as IgG4, but higher affinities to activating Type I FcγRs like FcγRI and FcγRIII.[31] The human IgG1 isotype is normally used for antibody-dependent cellular cytotoxicity (ADCC)- and phagocytosis (ADCP)-inducing antibodies like rituximab or trastuzumab.[80] In the case of ADG206, this is compensated by mutations increasing FcγRIIB binding. For AGEN2373, it will be interesting to see if the main clinical MoA is driven by the activation of 4–1BB+ effector T cells via FcγRIIB crosslinking or by the depletion of 4–1BB+ Treg cells via ADCC and ADCP.

HOT-1030 is also a human IgG1, but contains mutations that impede FcγR-binding. Similar to urelumab, it binds to CRD1, and therefore it can cluster 4–1BBL-bound 4–1BB receptors in a super-agonistic way (Figure 1). This allows HOT-1030 to activate 4–1BB systemically in a FcγR-independent manner depending on the presence of endogenous 4–1BBL. This approach is based on the hypothesis that the FcγRIIB-crosslinking and not the epitope is the main driver of hepatitis induction observed for urelumab, although both have been described to determine functionality and liver toxicity.[26]

Bi- Tri- or Tetraspecific 4-1BB agonists

The other group of second generation 4–1BB agonistic drugs are the bi, tri- or tetraspecific 4–1BB agonists (Figure 2B). In addition to an agonistic 4–1BB binding site, they implement at least one other binding site to a second target, thereby making these agents bi-, tri- or tetra-specific. These second targets are either tumor cells surface-expressed targets (Her2, PSMA, EGFRvIII, Claudin18.2, ROR1, Nectin-4, CD47, CD19), tumor stroma and tumor infiltrated lymph nodes surface-expressed targets (fibroblast activating protein alpha (FAP), targets expressed both on tumor cells and antigen presenting cells (PD-L1) or targets expressed on immune cells only (CD40, OX40, CD3). These additional targets lead to specific-targeting and hyper-crosslinking, but can also display agonistic or inhibitory activity leading to further anti-tumoral properties. Here, we refer to these as "crosslinking target sites" as they are intended to mediate 4–1BB hyper-clustering in a targeted manner.

Bi-, tri- or tetra-specific IgG-based drugs typically display modifications of the Fc to abrogate binding and subsequently crosslinking via Fcγ-receptors while maintaining binding to natal Fc receptor (FcRn) to provide antibody-like pharmacokinetics (PK).[32,36,45,46,61,63,64,66,69,70] The abolishment of Fcγ-receptor-binding aims to prevent systemic activity and to inhibit the hepatotoxicity as observed with urelumab.[26,32] Consequently, the rationale for these types of agonists is that crosslinking is strictly provided by binding to the crosslinking target sites, assumingly leading to a better safety profile. Some trispecific molecules lack an Fc (CB307, ND021/NM21-1480, MP0310), instead relying on binding to human serum albumin (HSA) to achieve antibody-like PK.[20,81] Other molecules (DSP107, BT7480) do not display such PK-improving properties, and, at least for BT7480, a reduced half-life has been reported.[82]

Most molecules still display an antibody framework or include antibody-derived binding domains, such as scFv (ABL503/TJ-L14B, ABL111/Tj-CD4B, ABL105/YH32367, ATG-101/YN051, LBL-024),[64,65,71,77,78] VH (CB307),[67] VH/VL (ND021/NM21-1480),[58] sdAb (INBRIX-105/ES101)[43] or VHH (PM1003, PM1032).[49] In contrast, FAP-4-1BB (RG7827) and CD19-4-1BBL (RG6076) are antibody fusion proteins based on human 4–1BBL ectodomains fused to an IgG1 framework[32] and DSP107 is a trimeric fusion protein without the implementation of antibody components.[60] PRS-343 and PRS-344/S095012 have two anticalins (based on human lipocalins) fused at the C-terminus of the heavy chains of an IgG4 backbone.[36,37,70] FS120 and FS222 contain a bivalent binding Fcab (Fc-region with antigen binding) domain, which is C-terminally integrated in the Fc region.[61–63] Novel molecule classes include MP0310, using "Designed ankyrin repeat proteins" (DARPins) as an alternative to antibody framework,[83] and BT7480, which is based on the Bicyclic peptides platform that produces molecules with antibody-like affinity and specificity.[84] The broad variety of approaches might reflect the need to generate new intellectual property rather than a new MoA. Nevertheless, some design features have an impact on functional behavior, such as ratio of binding sites, affinity for targets or molecule weight.

Epitope binding

4–1BB is not constitutively expressed on T cells, but induced after T cell activation via TCR or CD3 (signal 1).[1,20] For optimal co-stimulation (signal 2), 4–1BB has to be clustered on the cell surface, inducing the assembly of an intracellular signalosome.[85] A simple trimerization of 4–1BB receptors is not sufficient because soluble 4–1BBL, being a homotrimer, cannot enable efficient 4–1BB downstream signaling.[32,86] This leads to the conclusion that the assembly of a functional signalosome needs at least 4 or more 4–1BB receptors in close proximity clustered in a synapse (hyper-clustering). This can be provided naturally by membrane-bound trimeric 4–1BBL or artificially by a non-4-1BB competing antibody crosslinking soluble 4–1BBL-trimerized 4–1BB receptors (as described for HOT-1030 or urelumab, Figure 1), or by a 4–1BB agonistic molecule that is crosslinked by another cell expressing FcγRIIB or a crosslinking target. It has been predicted that the size as well as the epitope of the 4–1BB agonist may play a role for optimal synapse formation. For example, for ND021/NM21-1480, targeting the N-terminal (membrane distal) 4–1BB-epitope lead to improved functionality compared to membrane-proximal epitopes.[58] An optimal synapse space of 140 Å has also been predicted, potentially giving smaller molecules an advantage.[72]

Hinner and colleagues have tested different 4–1BB anticalin fusion sites and the C-terminal heavy chain fusion featuring the biggest distance between Her2 binding and 4–1BB binding (see PRS-343 Figure 2B) elicited the best T cell activation measured as IL-2 and interferon (IFN)γ secretion *in vitro*.[36] The optimal epitope and synapse space may thus depend on the molecule design. The diversity of 4–1BB-epitopes (Figure 3) and the variations in molecule sizes (7.2 to ~296 kDa, Figure 5) suggest a flexibility of optimal 4–1BB synapse and signalosome formation.
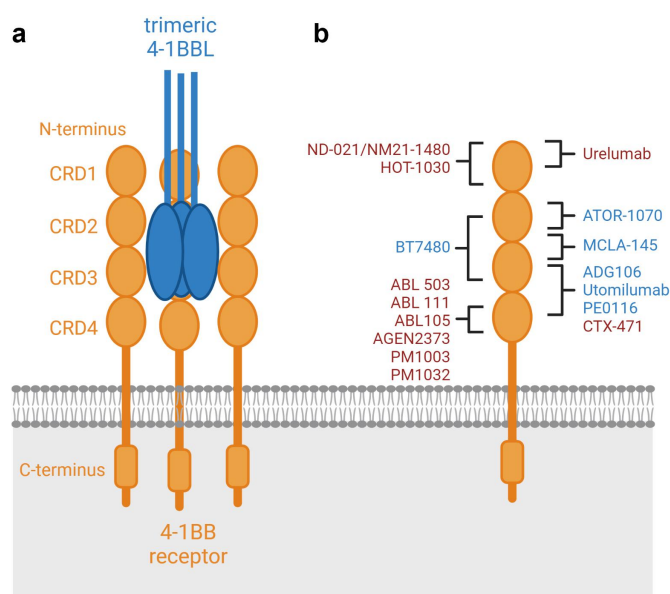
An important feature is the 4–1BBL non-blocking or blocking property of the different agonists (Figures 1 and 3). 4–1BB agonists implementing a 4–1BBL blocking binder (e.g., BT7480,[73] ATOR-1070,[55,56] MCLA-145,[46] IBI319,[66] ADG106[37]) or a 4–1BBL fusion (e.g., DSP107,[60] RG7827, RG6076[32]) will have to compete with soluble or membrane-expressed endogenous 4–1BBL, and high levels of soluble 4–1BBL can hamper their function. On the other side, preclinical data show that inhibition of 4–1BBL reverse signaling leads to a better T cell activation, especially during suboptimal signal 1-mediated T cell activation,[87] as well as improved dendritic cell-mediated T cell priming.[88]

4–1BB agonists implementing at least two non-4-1BBL blocking binders by binding to CRD1 (e.g., urelumab, HOT1030) or to CRD4 (e.g., ABL50, ABL111,[65] AGEN2373,[51] PM1003, PM1032,[49] CTX-471[35]) may lead to systemic 4–1BB activation, especially at high soluble 4–1BBL levels. ND-021/NM21-1480, however, can only bind one 4–1BB receptor and therefore cannot hyper-crosslink 4–1BB receptors in the absence of simultaneous PD-L1 binding. In the presence of PD-L1, soluble 4–1BBL levels can potentiate the functionality of ND-021/NM21-1480.

For most 4–1BB agonist the epitope has not been disclosed, although it has been predicted to be one of the main parameters to determine safety and functionality.[26]

Ratio of binding sites and binding affinities

4–1BB agonism relies strongly on efficient crosslinking of several 4–1BB receptors.[85] Previous publications have shown that a higher ratio of 4–1BB over crosslinking target sites is beneficial for an optimal 4–1BB signaling leading to T cell activation.[32,74,89] Nevertheless, most bi-, tri- or tetraspecific agonists display an even number of 4–1BB and crosslinking target sites, described in Figure 2b as 1 + 1, 2 + 2 or 2 + 2 + 2 + 2 or 3 + 3 ratios. Only four molecules implement an odd number of 4–1BB binding sites in favor of 4–1BB agonistic binding sites, a 2 + 1 (MP0310 and BT7480) or 3 + 1 ratio (RG7827 and RG6076). An uneven ratio can lead to a better 4–1BB hyper-clustering in the case of lower crosslinking target expression (Figure 4) and lower target expression is needed for the same 4–1BB receptor hyper-clustering and 4–1BB signalosome formation.[85]

This could be particularly important in the case of molecules with a higher molecular weight (Figure 5) where activation is desired in tissue with low crosslinking-target expression (like FAP expression on fibroblastic reticular cells in tumor-draining lymph nodes). Although preclinically these advantages of a higher 4–1BB to target binding site ratio have been demonstrated,[32,89] the extent to which this translates into the clinic is an open question. For example, in cases where the target expression is high, the advantage provided by a molecule with a higher 4–1BB-to-target binding site ratio may not be significant. Furthermore, a high target site binding affinity leading to a high-affinity difference between target binding site and 4–1BB binding site (4–1BB K_D/target K_D) has been discussed as a potential advantage to induce better functionality,[59,77] especially in case of low target expression.[77] High crosslinking-target site affinity has been defined to be in the lower pM range, like ND-021/NM21-1480 with a PD-L1

Figure 3. Schematic binding epitopes to the 4–1BB receptor (if disclosed). (a) The trimeric 4–1BBL interacts with CRD2 and CDR3 of three 4–1BB receptors, a type I transmembrane protein. (b) The epitope location of different antibody-based 4–1BB agonists is indicated. Non-4-1BBL-blocking binders are indicated in red, 4–1BBL-blocking binders are indicated in blue.
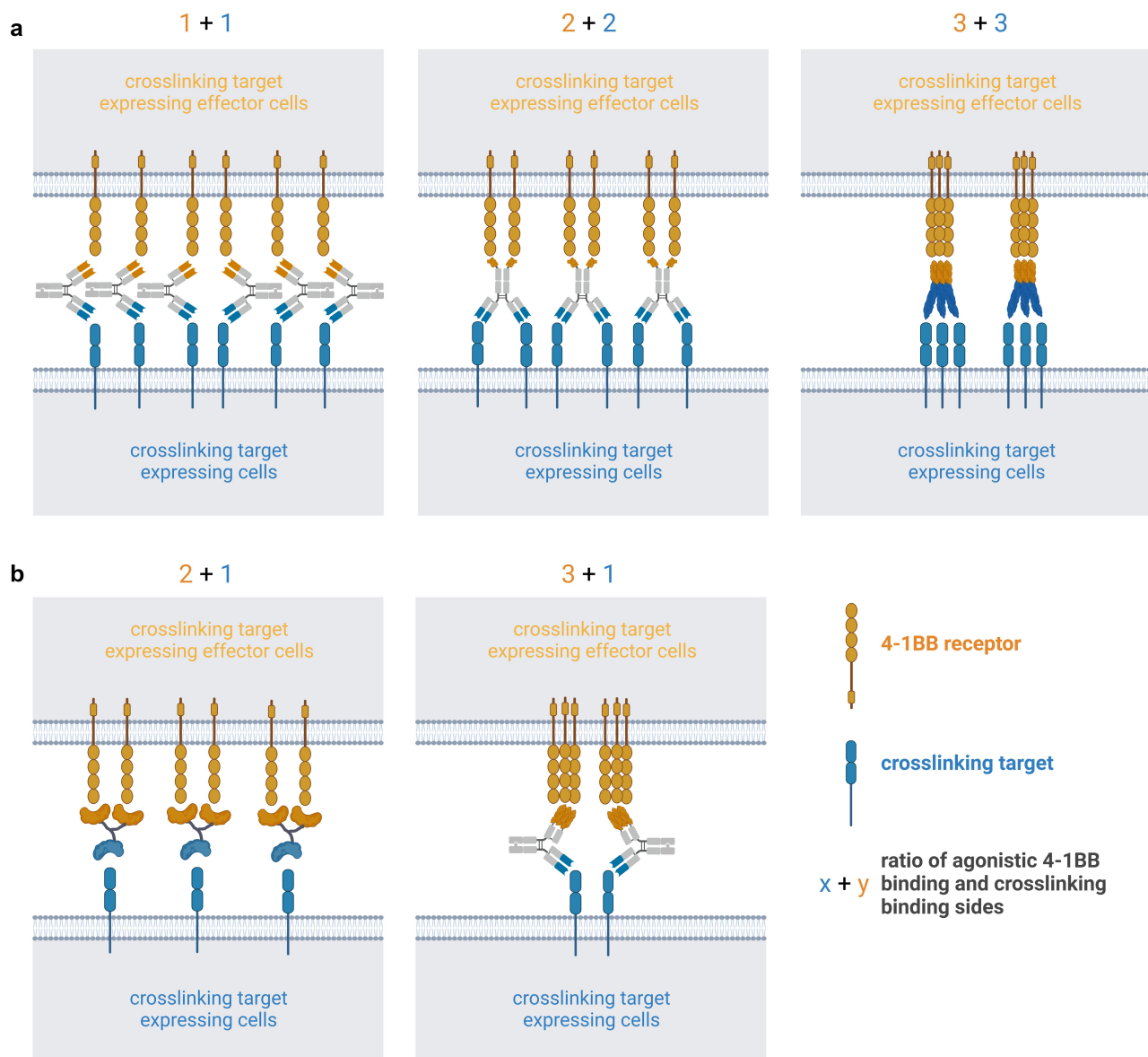
Figure 4. Theoretical impact on hyper-crosslinking based on the different 4–1BB receptor to crosslinking-target binding site ratios. The number of 4–1BB receptors (yellow) is fixed to six receptors, whereas the number of crosslinking targets (blue) and number of drug molecules varies to demonstrate the impact of different binding site ratios. (a) In case of even ratios of crosslinking target to 4–1BB receptor binding sites (1 + 1, 2 + 2 or 3 + 3), a higher number of molecules is needed to gain equal 4–1BB receptor hyper-clustering. (b) An amplification occurs if the ratio of crosslinking target to 4–1BB receptor binding sites is uneven and in favor of 4–1BB binding sites (1 + 2 or 1 + 3). In this case a lower number of crosslinking targets is needed to elicit the same 4–1BB receptor hyper-crosslinking.

target binder displaying an affinity of 7 pM.[59] A favorable 4–1BB K_D/target K_D ratio was reported to be between 100[75] or 500.[77] As shown in Table 2, the reported K_D values display a wide range of affinities and affinity ratios. Nevertheless, Gen1046/BNT311, a PD-L1-targeted 4–1BB agonist with a low 4–1BB K_D/target K_D ratio of 0.94 and a PD-L1 affinity in the higher pM range (150 pM), has already shown functionality in a clinical Phase 1 trial.[45]

Molecular weight and half-life

Most of the molecules show antibody-like PK either by the inclusion of an Fc that binds to FcRn or an HSA binding site.[20,81] Antibody-like PK allows molecules with a high molecular weight to achieve high systemic exposures, a requirement for sufficient tumor accumulation of macromolecules.[90,91] The bicyclic peptide BT7480, however, does not have a half-life stabilizing mechanism. This molecule is 20-times smaller than a normal IgG (Figure 5) and, despite a rapid clearance, it has been shown to penetrate the tumor and tumor-draining lymph nodes in mice rapidly. Indeed, based on PK data collected from mice and cynomolgus monkey, a target coverage of 1–3 days with a once weekly dosing was predicted. In mice this was sufficient to lead to tumor growth reduction and partial regression.[75] Likewise, DSP107 has no half-life improving mechanism. In clinic studies, DSP107 is administered once weekly during a 28-day cycle (NCT04440735, NCT04937166), presumably to compensate for a faster half-life.
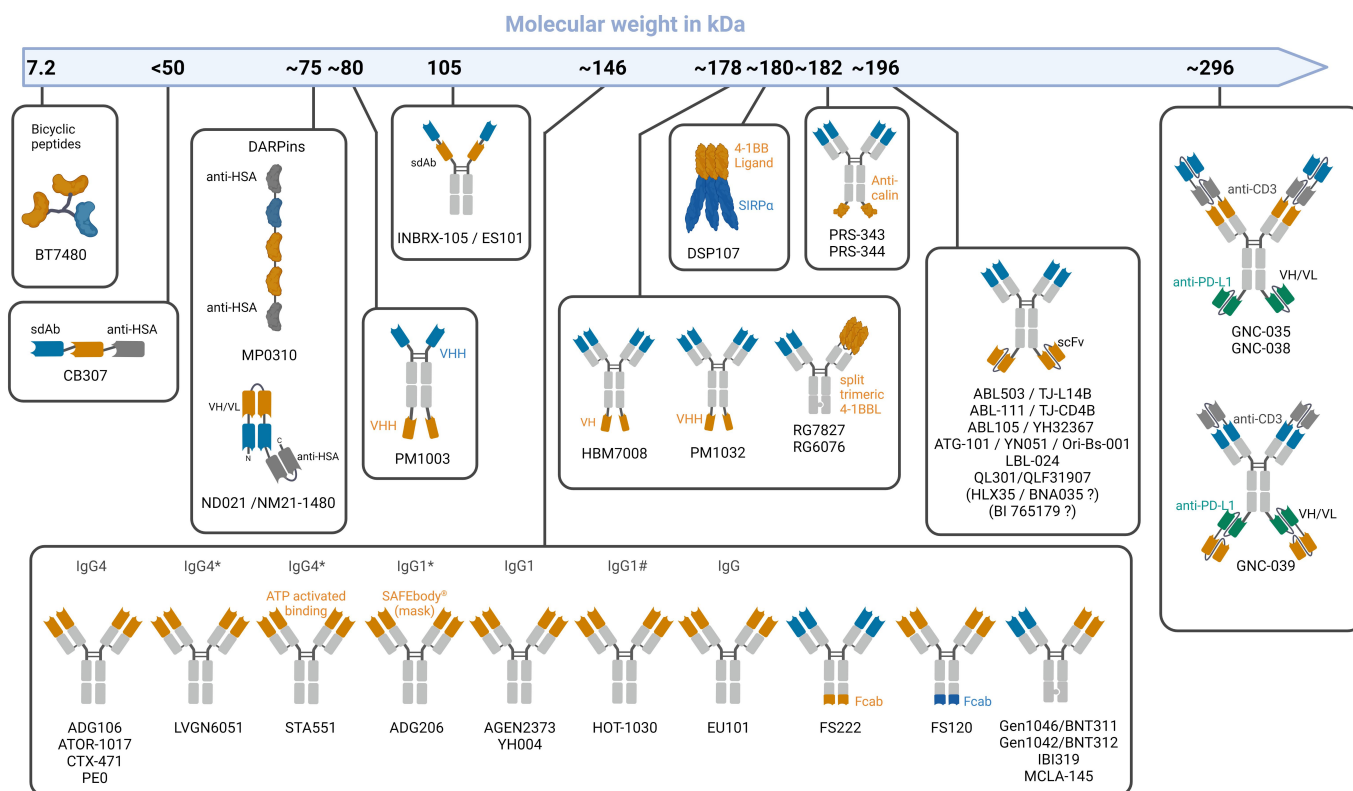
Figure 5. Molecular weight of 4–1BB agonistic drugs. The molecular weight of each molecule was taken from literature or estimated from their formats, and molecules were sorted by their molecular weight in kilo-Dalton (kDa) starting with the smallest molecule (BT7480) on the left and the biggest molecules (~296 kDa) on the right. 4–1BB agonistic binding sites are highlighted in yellow and targeting binding sites are indicated in blue. For the tri-specific molecules binding to HSA is shown in dark gray. For the tetra-specific molecules PD-L1 binding sites are shown in green and CD3 binding sites in dark gray. Abbreviation: 4–1BBL = trimeric 4–1BB Ligand ectodomain, CH/CL = constant domain of heavy and light antibody chain, DARPins = Designed ankyrin repeat proteins, Fcab = Fc-region with antigen binding, scFv = single-chain variable antibody fragment, sdAb = single monomeric variable antibody domain, SIRPα = Signal regulatory protein alpha ectodomain, VH = variable domain of heavy antibody chain, VHH = antigen-binding domain of camelid dimeric heavy chain antibodies, VH/VL = variable domain of heavy and light antibody chain, * = Fc-fragment contains mutations to increase Fcγ-receptor IIB-binding, # = Fc fragment contains mutations to attenuate Fcγ-receptor-binding.

Cis- and trans-setting and localization of 4-1BB agonism

A recent publication implies that 4–1BB agonism works independently of simultaneous signal 1, but the co-localization of 4–1BB receptor and CD3 in one T cell synapse display an additive effect (the so-called cis-setting).[92] Therefore, the crosslinking mechanism plays a role in optimal 4–1BB agonism, as shown in Figure 6. Mechanistically one can distinguish a cis-, a trans- and an autocrine-setting (Figure 6b). A target expressed on tumor cells is supposed to have advantages in the cis-setting; however, 4–1BB co-stimulation will only be delivered in the tumor environment, and not during the T cell priming phase in the lymph nodes. The success of checkpoint inhibitors targeting the PD-1/PD-L1 axis has demonstrated their role in T cell priming,[93] as well as the inability to reverse the status of late exhausted T cells.[94,95] This observation may suggest that 4–1BB agonism is essential not only in the tumor, but also in the tumor draining lymph nodes to elicit an optimal anti-tumoral immune T cell response. Targets like FAP, PD1, PD-L1, OX40 and CD40 will provide 4–1BB co-stimulation during the priming phase, and thus may help to induce a robust long-term memory formation of CD8 T cells.[11] PD-L1 and CD40 are expressed on antigen-presenting cells (cis-setting), whereas FAP is expressed on fibroblastic reticular cells of tumor-draining lymph nodes (trans-setting). Crosslinking via FcγRIIB or CD40 can lead to a cis-setting in the lymph nodes and a trans-setting in the tumor. The autocrine setting was not yet tested directly head-to-head with the cis- or trans-setting, and therefore it remains to be determined how it compares to a trans- or cis-setting. For the PD-1 targeted 4–1BB bispecific antibody IBI319, it has been shown that the molecule is active in a trans- or autocrine setting as part of its MoA.[66]

The tetraspecific molecules GNC-035, GNC-038 and GNC-039 are all tumor-targeted, implementing a ROR1, CD19 or EGFRvIII binding site, respectively (Table 1, Figure 2b). So far, no scientific publications are available describing the full MoA of these molecules. However, the desired MoA has to be a tumor cell-mediated cis-setting, where the molecule crosslink T cell and tumor cell while delivering CD3 (signal 1) and 4–1BB (signal 2) to the T cells and inhibiting PD-L1 expressed by the tumor cell. The risk of such molecules is a polyclonal and unspecific T cell activation outside of the tumor. Therefore, a trans-setting in non-tumoral tissue should be prevented, for example by a high avidity to the tumor target.

Table 2. Reported dissociation constant (K_D) values.

Molecule	4-1BB to crosslinking target ratio Binding sites	Measurement method	K_D in nM for 4-1BB	crosslinking target site	K_D in nM for crosslinking target	ratio 4-1BB K_D / crosslinking target K_D	Reference
Gen1046 / BNT311	1+1	BLI	0.15	PD-L1	0.16	0.94	[42]
Gen1042 / BNT312	1+1	BLI	0.17	CD40	1	0.17	[150]
IBI319	1+1	SPR	394	PD-1	0.1	3940	[45]
MCLA-145	1+1	SPR	1.9	PD-L1	0.51	3.7	[41]
ND-021 / NM21-1480	1+1	SPR	0.48	PD-L1	0.0069	69.6	[57,75]
ABL503	2+2	SPR	13.8	PD-L1	3.07		[44]
FS120	2+2	SPR	0.2	OX40	0.2		[43]
FS222	2+2	SPR	0.665	PD-L1	0.189		[49]
LBL-024	2+2	BLI	146	PD-L1	0.29		[55]
PRS-343	2+2	SPR	5.03	Her2	0.3		[48]
PRS-344 / S095012	2+2	SPR	4.84	PD-L1	0.68		[47]
DSP107	3+3	SPR	0.7	CD47	1.17		[60]
RG6076 / RO7227166	1+3	SPR	310	FAP	0.7		[32]
RG7827 / RO7122290	1+3	SPR	330	CD19	0.4		[32]
BT7480	1+2	SPR	6.3	Nectin-4	12		[67,68]
YH3267 / ABL105	2+2	SPR	3.36	Her2	0.48		[53]
CTX-471	IgG4	BLI	50	n.a.	n.a.		[35]
PE0116	IgG4	SPR	17.6	n.a.	n.a.		[39]
STA551	IgG1	SPR	9.82*	n.a.	n.a.		[25]
urelumab	IgG4	SPR	22 / 16.6	n.a.	n.a.		[24,25]
utomilumab	IgG2	SPR	69 / 71.2	n.a.	n.a.		[24,25]

Notes: K_D values to human 4-1BB or crosslinking-target recombinant protein were collected from literature and values may not be directly comparable due to different assay set ups. Values were determined either by surface plasmon resonance (SPR) or bio-layer interferometry (BLI) as indicated. As it was not always clear if affinity or avidity were measured, only for 1+1 molecules the calculated ratio between K_D (4-1BB) to K_D (target) was calculated and shown. *K_D measured in the presence of 100 μM ATP. n.a.= not applicable.

Limitations of animal models to study 4-1BB agonists

The first agonistic 4–1BB antibody urelumab induced two fatalities related to hepatitis in clinical trial (NCT00612664) at efficacious dose (1 or 5 mg/kg), whereas utomilumab was safe but displayed a lack of efficacy.[22,23] The activation of 4–1BB on liver myeloid cells leading to an IL-27-dependent T cell activation has been described as the liver inflammation-inducing mechanism.[96] Liver macrophages activated by non-specific hepatic memory CD8 + T cells triggered by 4–1BB agonism have also been predicted to be the cause.[37] Furthermore the soluble factor S100A4 secreted by liver macrophages has been shown to be critical, and a neutralizing antibody was able to prevent 4–1BB-induced liver pathology without affecting the antitumor efficacy in mice.[37,97] Independently of the MoA, a strong increase in CD68+ macrophages and proliferating 4–1BB+ CD8 + T cells can be observed in the liver of mice after 4–1BB activation via agonistic 4–1BB mouse IgG1 antibodies.[32]

The isotype and subsequent crosslinking-ability by Fcγ-receptors[32] and the 4–1BB epitope, but not the 4–1BB affinity,[26] were described to be critical for the development of safe 4–1BB agonistic antibodies. Most of these studies have been performed in syngeneic mice[26,35,40,51,53] or human 4–1BB transgenic mice[53,55,64,70,73] with all existing limitations of these models and limited translation into humans. For example the expression and function of human and mouse Fcγ-receptors is different, and therefore the MoA cannot be easily translated into humans.[80,98–100] When 4–1BB agonists, which do not have a mouse IgG but a human or a rat IgG isotype, are used in mice, the cross-species reactivity of mouse Fcγ-receptor binding has to be considered.[101,102] Kamata-

Sakurai and colleagues attempted to overcome some of these limitations by designing mouse surrogates called Sta-MB (for STA551) and Ure-MB (for urelumab) displaying an engineered constant region of mouse IgG1 (MB) to mimic FcγRII-crosslinking in mice, in order to approximate the MoA of FcγRIIB-crosslinking of 4–1BB IgG4 STA551 or urelumab in humans.[25]

Furthermore, it has to be considered that the mouse 4–1BB/4-1BBL complex is dimeric,[103] different to the human trimeric 4–1BB/4-1BBL complex,[24] and human 4–1BB cannot interact with mouse 4–1BBL. Therefore, the human 4–1BB transgenic mouse model cannot be used to predict effects of endogenous 4–1BBL competition or blocking endogenous 4–1BBL reverse signaling. Recently, human 4–1BB/human 4–1BBL double transgenic mouse (C57BL/6-$Tnfrsf9^{tm1(TNFRSF9)}Tnfsf9^{tm1(TNFSF9)}$/Bcgen), have been described that may improve the translatability into humans.

Clinical safety and functionality

The different hypotheses on the impact of affinity, epitope and crosslinking-dependency on safety and functionality, as well as the limitation to study them preclinically in mice, make the clinical outcome more decisive. The available published data on clinical results is limited because most trials are ongoing.[38,39,41,45,47,50,52,54,57,104] As described above, hepatitis induction was the main challenge for first-generation 4–1BB agonist urelumab, a CRD1-binding IgG4 antibody. AGEN2373 is a IgG1 antibody binding to CRD4 in a non-4-1BBL competing manner. So far in a group of 19 patients treated with 0.03–
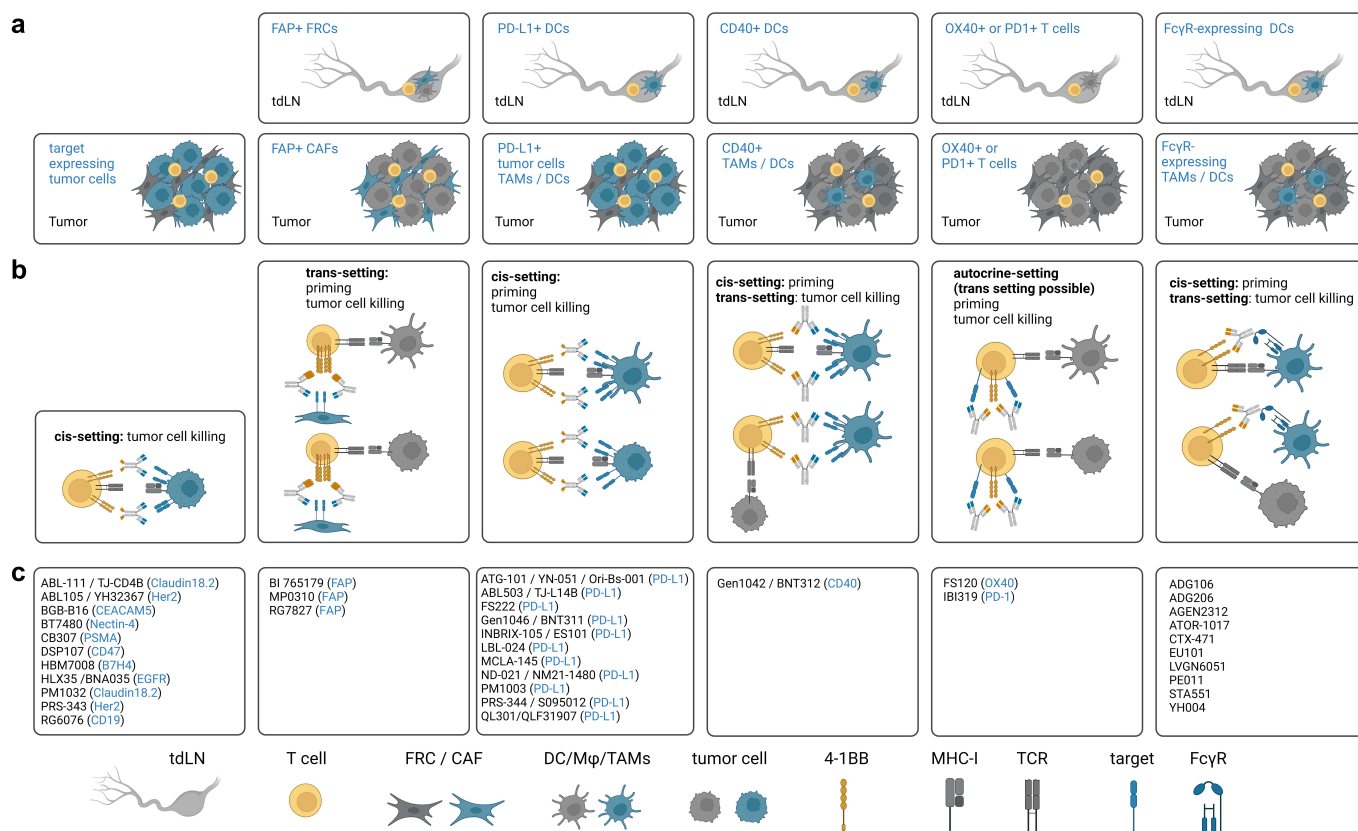
Figure 6. Expected MoA of 4–1BB agonists based on their targeting modality. (a) Summarize the main organs (tumor and lymphoid tissue) where 4–1BB agonists can improve the anti-tumoral immune response as well as the main targeted cells (shown in blue) and 4–1BB+ T cells (shown in yellow). (b) The predicted cellular interaction and MoA are outlined. 4–1BB crosslinking can occur in a cis-, trans- or autocrine-setting. Crosslinking cells can be tumor cells, fibroblasts, macrophages, and dendritic cells (shown in blue) or T cells themselves in an autocrine setting. (c) Names of 4–1BB agonistic drugs in clinical trials, which implement the predicted and outlined MoA. The tetra-specific molecules GNC-035, GNC-038 and GNC-039 are not included. Abbreviations: CAFs = Cancer Associated Fibroblasts, CEACAM5 = Carcinoembryonic Antigen Cell Adhesion Molecule 5, DCs = Dendritic Cells, EGFRvIII = epidermal growth factor receptor variant III, FcγR = Fc-gamma-receptors, FAP = Fibroblast Activating Protein alpha, FRCs = Fibroblastic reticular cells, Her2 = human epidermal growth factor receptor 2, PD-L1 = Programmed Death-Ligand 1, ROR1 = Receptor Tyrosine Kinase Like Orphan Receptor 1, PSMA = Prostate-specific membrane antigen, Mφ = Macrophages, TAMs = Tumor Associated Macrophages, tdLN = tumor draining lymph node.

2 mg/kg AGEN2373 across 5 cohorts, no drug-related elevations in liver transaminases (ALT, AST) or bilirubin beyond 1 grade were observed.[52]

LVGN6051 is a IgG4 antibody with improved FcγRIIB-crosslinking. In a group of 16 patients treated with LVGN6051 (escalating dose cohorts up to 7 mg/kg), no treatment-related adverse events (TRAE) occurred. However, in combination with pembrolizumab one patient with predominant hepatic metastases and history of intermittent grade 2 hepatic impairment experienced grade 3 increased ALT/AST on cycle 1 day 15 and this incidence was reported as dose limiting toxicity (DLT). It resolved back to baseline three days later without corticosteroids.[54]

The combination of 4–1BB agonism with PD-1/PD-L1 inhibition may increase the liver inflammation risk, as liver injury is a well-known side-effect of PD-L1/PD-1 blockage[105] and constitutive PD-L1 expression has been described on non-parenchymal liver cells, including sinusoidal endothelial cells and Kupfer cells.[106]

In patients treated with Gen1046/BNT-311, a PD-L1 and 4–1BB bispecific antibody, treatment-related transaminase elevations occurred in 26.2% of the patients (grade 1–3), whereby grade 3 was observed in 9.8% of patients. Although the transaminase elevations improved rapidly with corticosteroid

administration, 3 of the 6 patients discontinued treatment due to this TRAE.[45] Similarly, in the dose escalation of MCLA-145, another PD-L1 and 4–1BB bispecific antibody, one of 34 patients experienced an ALT/AST grade 3 increase, leading to a DLT at 75 mg flat dose. Grade 1–3 ALT and/or AST elevations were observed in six patients (17.6%), mainly at dose of 50 mg or higher.[47]

During treatment with CD40 and 4–1BB bispecific antibody GEN1042/BNT312, one patient of 50 developed a grade 4 transaminase elevation, which was resolved with corticosteroids. Whether other lower grade transaminase evaluation occurred in the same trial was not disclosed.[50] So far, reported liver-toxicity events remain manageable with second generation 4–1BB agonists and below urelumab-related observations, where 13.5–16.6% of the patients treated with efficacious doses above 1 mg/kg developed a grade 3–4 treatment-related transaminase elevations, including two cases of fatal hepatotoxicity.[22]

All second-generation 4–1BB agonists with published clinical data describe in general a good safety and tolerability profile so far. Common adverse events (AEs) are mostly grade 1 and 2.[38,39,41,45,47,50,52,54,57,104] Some side effects, like pneumonitis, pruritus, rash or injection-related AEs, ALT/AST increase, hypothyroidism, diarrhea and colitis, are common during cancer immunotherapies and could be defined as

common immune-related AEs (irAEs).[107] If these irAEs correlate with a better or a worse outcome is still not fully understood, but a first meta-study indicates that irAEs occurring during cancer immunotherapy correlate with better outcome shown as overall survival (OS) and progression-free survival (PFS).[108] Therefore, in the future, irAEs management will be an important task to improve cancer immunotherapy treatments.[109]

Most immune activations reported are directly linked to increased CD8 + T cells and NK cell activation,[38,39,45,47,50,104] as they are the major cell types expressing 4–1BB.[110] However, other immune cells have also been reported to express 4–1BB,[5] including CD4 + T cells,[110] regulatory T cells (Tregs),[111] dendritic cells,[88,112] monocytes and macrophages,[113,114] mast cells,[115,116] neutrophils[117] and eosinophils from patients with IgG-mediated allergies.[118] There are also reports of 4–1BB expression on nonimmune cells like adipocytes[119] and atherosclerosis- or cancer-induced 4–1BB expression on blood vessel endothelial cells.[120,121] 4–1BB agonism has both pathogenic and protective role in type 1 diabetes mouse models[122,123] and obesity-induced inflammations.[119,124] All this may play a role in the 4–1BB-mediated MoA as well as irAEs, and has to be carefully considered. One obvious irAE is neutropenia, which occurs with a rate of 4.9–17.6%[39,45,47,54,57] and may directly be linked with a 4–1BB MoA, as 4–1BB-activation on neutrophils has been reported to abrogate granulocyte-macrophage colony-stimulating factor-mediated neutrophil survival.[117]

The disease control rate of second-generation 4–1BB agonists in solid tumors seems to be around 56–70%.[38,42,45] Stable disease rates are around 26–70%[41,50,52,54] and overall response rates (ORR) between 3.6–40%.[38,39] Partial (PR) or complete responses (CR) occurred and have been reported with a frequency of 4–21%.[38,41,45,50,54] It has to be kept in mind that these data were collected during dose escalation with different cohorts, mostly in ongoing Phase 1 trials. Some data sets focused on all patients treated, other data sets were cleaned by focusing on patients treated with efficacious doses. Therefore, it is fair to say that the ORR appears until now quite similar between the different trials and a smart clinical strategy focusing on optimal dose prediction and combination strategy may be required.

Receptor occupancy and optimal dose finding

Receptor occupancy has been predicted as a key factor for optimal dose finding of agonistic antibodies. A maximum effect at a receptor occupancy of ~50% has been assumed, as outlined in Figure 7, leading to an optimal 4–1BB hyperclustering supplied by a ternary complex of the target cell, the drug, and the 4–1BB expressing effector cell. Higher concentrations of the drug resulting in a receptor occupancy of 100% will prevent the optimal ternary complex formation and lead to a bell-shaped activation curve.[36,48,59,125,126] Therefore, it is essential to understand the receptor expression in the tumor, though the prediction of 4–1BB expression in tumor
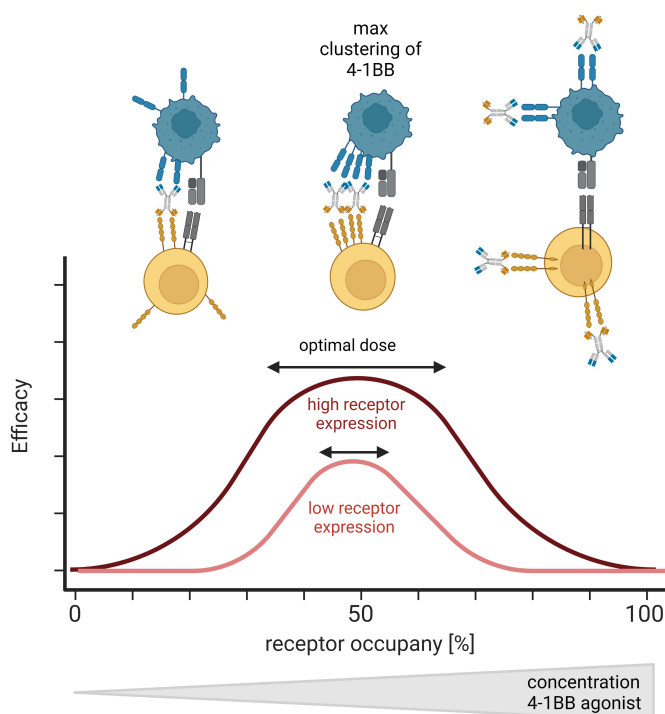

Figure 7. Predicted optimal dose dependent on receptor occupancy leading to a bell-shaped activity curve. Increased concentration of bispecific 4–1BB agonist will lead to saturation of both binding sites while abolishing optimal 4–1BB clustering. Max = maximum.

is challenging, as 4–1BB expression on T cells is strictly controlled. Normally 4–1BB surface expression on CD3-activated peripheral blood mononuclear cell-derived T cells is observed between day 1 to day 3, whereas 4–1BB expression is not detectable after 5 days.[8,32,127] 4–1BB activation prolongs the 4–1BB expression until day 5 and is stronger on CD8 + T cells than on CD4 + T helper cells, leading to a preferential increase of CD8 + T cells in the tumor microenvironment by 4–1BB agonism.[32] At the same time 4–1BB agonism mediated by antibodies may also activate negative feedback loops like 4–1BB internalization,[128] as well as shedding of 4–1BB leading to increased soluble 4–1BB levels.[129,130]

Different published data sets suggest that 4–1BB surface expression on CD8+ tumor infiltrating lymphocytes (TILs) is presumably transient and only a small fraction of TILs display 4–1BB expression. Frequencies of 4–1BB+ CD8+ TILs of total CD8+ TILs have been published for human ovarian cancer (mean ~5-8% with a range of 1–26%), hepatocellular carcinoma (mean ~10% with a range of 0–39%), non-small lung cancer (mean ~2.5% with a range of 0–12%), intrahepatic cholangiocarinoma (mean ~3% with a range of 0–13%), colorectal cancer (mean ~2.5% with a range of 0–10%), glioblastoma multiforme (mean ~1% with a range of 0–3%) and melanoma (mean ~13% with a range of 0–40%).[14, 131, 132] 4–1BB expression on CD8+ TILs has been shown to correlate with PD-1 expression[133] and other exhaustion markers,[134,135] but also with improved anti-tumoral functionality of these CD8+ TILs[131,136–139] assuming that 4–1BB expression
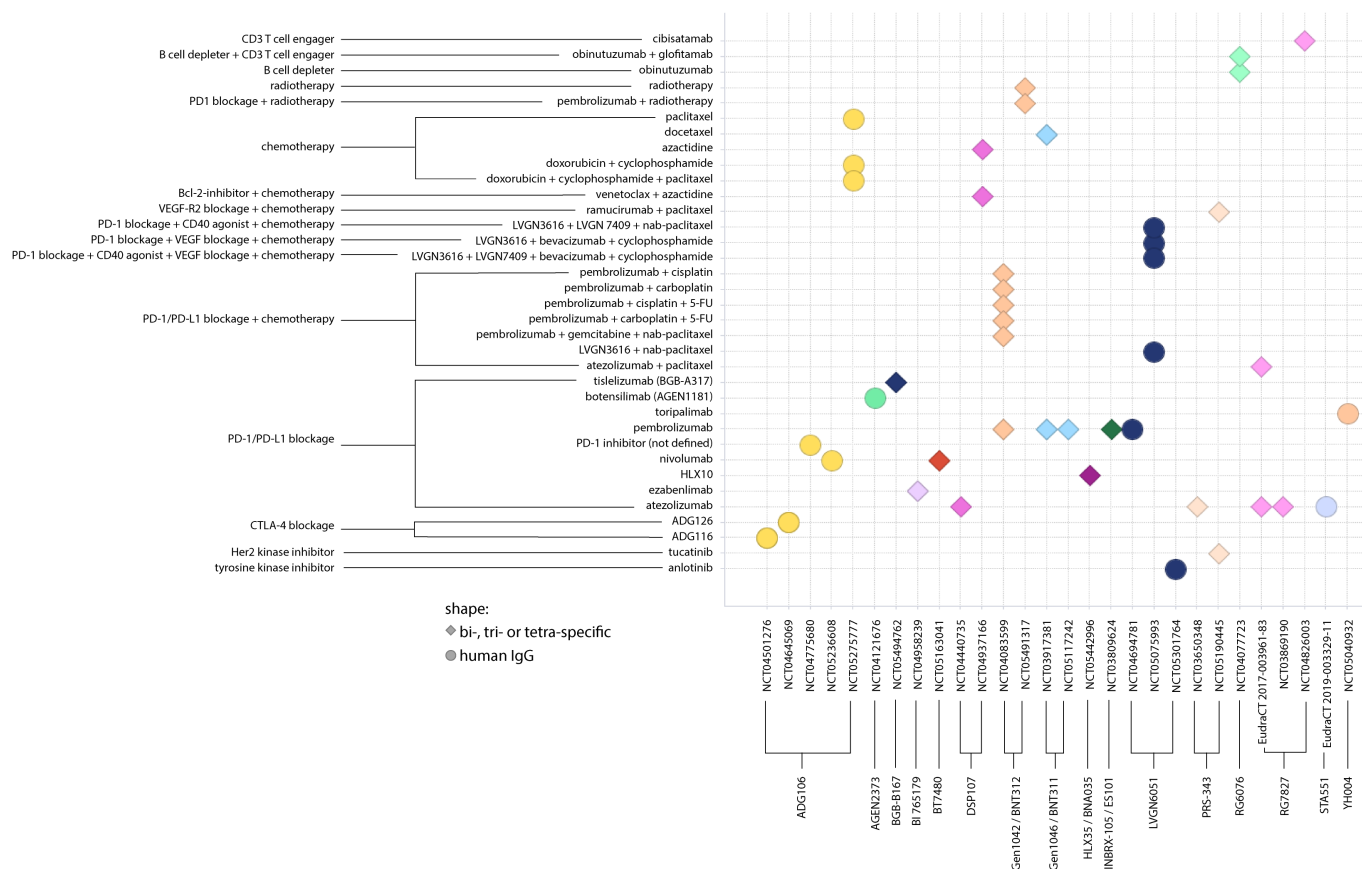
Figure 8. Ongoing or planned combinations in clinical trials. Reported combination partners were set against the clinical trial number and clustered by the used 4–1BB agonist. Most clinical trials combine the 4–1BB agonist with a checkpoint inhibitor (e.g., PD1, PD-L1 or CTLA-4 inhibitor) and/or chemotherapy.

correlates with a contemporary TCR engagement. In the case of bispecific antibodies implementing a high target site binding affinity (favorable 4–1BB K_D/target K_D ratio between 100–500), the receptor occupancy of the target site will become the driving parameter for the optimal dose and less dependent on the 4–1BB receptor occupancy.[59, 77]

The prediction of an optimal dose, however, does not only depend on optimal receptor occupancy and thus receptor expression, but it also depends on possible peripheral sinks mediated by soluble 4–1BB, soluble crosslinking target protein and soluble 4–1BBL. Mechanistic effects will also play a role, like receptor internalization after binding, affinity/avidity relationship between 4–1BB and crosslinking-target binder or factors of tumor tissue penetration like the molecule size and PK.[140] Therefore, the optimal dose finding in the clinic is expected to be challenging, especially in patient population with diverse 4–1BB and crosslinking-target expression, as the receptor expression level will affect the extent of the ternary complex formation.

The reported clinical trials have implemented different doses and scheduling of 4–1BB agonistic injections (Supplementary Figure S2), and in general, either a flat dose or a body weight-based dosing (mg/kg) regime has been chosen. At least for monoclonal PD-1 or PD-L1 checkpoint inhibitory antibodies, the flat dose principle is predicted to provide similar exposure to weight-based dose while reducing the chance of dosing errors and minimizing drug wastage.[141]

Different dosing schedules are being tested, including weekly (Q1W), every two weeks (Q2W), every three weeks (Q3W), every four weeks (Q4W) and/or every six weeks (Q6W) injection. As of today, only limited data on optimal dose finding is available. MP0310, targeting 4–1BB and FAP, has an anticipated therapeutic optimal range between 0.5 and 5 mg/kg.[48] The active dose for PRS-343 (targeting Her2 and 4–1BB) in a Phase 1 dose escalation study was reported to be on a Q2W schedule at or above doses of 8 mg/kg.[38] Gen1046/BNT311, targeting PD-L1 and 4–1BB, was tested in a Phase 1/2a trial Q3W in a flat dose range of 25–1200 mg. Improved pharmacodynamics markers like increased IFNγ and C-X-C motif chemokine ligand 10 (CXCL10) levels, increased Ki67+ and effector memory CD8 + T cells counts, and activated NK cell counts in the peripheral blood were detected at doses at 200 mg or lower. The 100 mg Q3W dose level was chosen as the expansion dose.[45] Gen1042/BNT312, targeting CD40 and 4–1BB, was tested in a Phase 1/2 trial Q3W in a flat dose range of 0.1–400 mg. Two partial responses were reported in melanoma and neuroendocrine lung cancer at doses of 3 and 30 mg, respectively. Based on predictive modeling, the investigators will move forward with 100 mg as the optimal dose in the study's expansion phase. MCLA-145, targeting PD-L1 and 4–1BB, was administered at doses of 0.4–75 mg Q2W and patient enrollment continues at 25 mg Q2W. Pharmacodynamic clinical activity was observed at 25 mg and above. At doses of 10 mg and

above, peripheral T cell activation, including cytotoxic CD8 + T cells were observed.[50] Adagene, testing their anti-4-1BB IgG4 antibody ADG106 (NCT3802955) reported an undisclosed predictive biomarker correlating with 3 of 4 patients displaying tumor shrinkage above 30% after treatment with 3 or 5 mg/kg Q3W ADG106.[42]

Combination therapies

The success of the CAR T cell therapies tisgenlecleucel,[17] ciltacabtagene, and autoleucel[18] underlines the importance of 4-1BB agonism in anti-tumoral CD8 + T cell biology for the treatment of relapsed or refractory hematological cancer. Not surprisingly several 4-1BB agonists target hematological tumors. However, most 4-1BB agonists are tested in solid tumors (Table 1). 4-1BB expression has been positively correlated with functionality of anti-tumoral CD8+ TILs also in solid tumors,[14,131] but 4-1BB expression on T cells seems to be diverse and is presumably dynamic. In line with this, soluble 4-1BB has been reported as a dynamic biomarker to monitor 4-1BB immunotherapies,[85] but a pre-selection of patients with high 4-1BB expression has not been implemented so far.[20] To enable the full potential of 4-1BB agonists and to foster optimal dose finding, a combination partner, which leads to an increased and a more homogeneous 4-1BB expression would be ideal. Possible combination partners are T-cell engagers facilitating a polyclonal CD3-mediated 4-1BB-upregulation on CD8 + T cells[32,142] or ADCC-inducing antibodies leading to 4-1BB upregulation on natural killer (NK) cells.[143] Standard therapies promoting T cell priming and activation like certain chemotherapies[144] and/or radiotherapy[145-147] could also serve as combination partners, though studies investigating 4-1BB expression kinetics after these standard therapies are still missing. Nevertheless, different chemotherapies as well as radiotherapy have already been included in several clinical trial plans (Figure 8).

In preclinical studies the combination of PD1/PD-L1 inhibition with 4-1BB agonism has shown synergism.[137,148] Twelve 4-1BB agonists in clinical trials therefore incorporate inhibition of PD-L1 and PD-1 in the molecule design (e.g., bispecific antibodies targeting 4-1BB together with PD-L1 or PD-1). However, different needs for receptor occupancy toward an optimal MoA are predicted. For example, as shown in Figure 7, ~50% of PD-L1 receptor occupancy will lead to the maximal 4-1BB agonism, whereas an optimal PD-L1 inhibition would be reached at a PD-L1 receptor occupancy close to 100%. A 100% receptor occupancy of PD-L1, however, would reduce the 4-1BB activation based on the bell-shape curve hypothesis. Therefore, the additional combination with a non-competing PD-1 or PD-L1 inhibitor could be beneficial. Not surprisingly Gen1046/BNT311 as well as INBRX-105/ES101, both PD-L1 and 4-1BB bispecific antibodies, are combined with the PD-1 blocking antibody pembrolizumab (Figure 8). Also, PRS-343, a Her2-targeted 4-1BB agonist, was planned to be combined with tucatinib to increase the Her2 inhibition (Figure 8). A skillful biomarker plan to separate target inhibition and 4-1BB agonism effects will allow the prediction of an optimal dose providing 4-1BB agonism while allowing a presumably suboptimal concentration of tumor-target inhibition.

Learning from CAR T cell therapy, the combination of targeted 4-1BB agonists with a tumor-targeted CD3 engager of particular interest and has so far been implemented in two clinical trials (NCT04826003 and NCT04077723). A major challenge of CAR T cells in solid tumors is the absence of truly tumor-restricted targets, leading to on-target off-tumor toxicity.[149] Therefore, targeting the CD3 (signal 1) and 4-1BB (signal 2) agonists to different cross-linking targets can lead to less on-target-off-tumor-mediated healthy tissue damage and a better safety profile. This can further be enhanced by the possibility to dose and schedule both agonists differently.

The combination with a CD3 T-cell engager would also increase the number of 4-1BB+ CD8+ TILs that can benefit from 4-1BB costimulation at baseline, as the number of 4-1BB expressing CD8+ TILs is normally between 0-10% of total CD8+ TILs.[14,32,131,136] At least in humanized mice, a CD3 T-cell engager increased the frequency of 4-1BB+ CD8 TILs from 10% up to 50% of total CD8+ TILs.[32] Furthermore, by not only engaging tumor-specific but also nonspecific bystander T cells,[150] a better T cell to tumor cell ratio can be expected and may lead to a better outcome, especially for the patients with a low count of tumor-specific T cells. Finally, the off-the-shelf-approach will benefit a wider range of patients, which may not have access to CAR T cell therapy.

Conclusions

In the past 5 years, the study of second-generation 4-1BB agonists has substantially expanded and several strategies are being implemented to overcome the liabilities of first-generation 4-1BB agonists, resulting in a high diversity of molecules in development. While these molecules differ in size (molecular weight), half-life, affinities, the crosslinking targets (and therefore mechanisms), as well as the valency of target binding sites, they all aim to achieve safe and potent 4-1BB hyper-clustering. So far, all 4-1BB agonists seem to display good safety and tolerability profiles with manageable irAEs. Which molecule designs are ideal to induce anti-tumor response with a favorable safety profile remains to be proven. However, it can be predicted that, regardless of the design, overcoming challenges in the clinical development like optimal dose finding and optimal combination strategy will be an important aspect to establish 4-1BB agonism in cancer immunotherapy. Therefore, investing into a good preclinical model for optimal dose prediction and a good clinical biomarker plan for optimal dose finding is essential. A good combination partner delivering signal 1 and therefore providing sufficient and more homogenous 4-1BB expression in the tumor will help during dose finding. In general, 4-1BB agonists can be considered as potent immunomodulatory agents, which should be developed right from the start as a combination partner and not as a single agent.

Abbreviations

ADCC	antibody-dependent cellular cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
AEs	Adverse Events
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
4-1BBL	trimeric 4-1BB Ligand
CAR	chimeric antigen receptor
CD19	Cluster of Differentiation 19
CD40	Cluster of Differentiation 40
CD47	Cluster of Differentiation 47
CR	Complete Response
CRD	extracellular Cysteine-Rich pseudo repeat Domains
CXCL10	C-X-C motif chemokine ligand 10
DARPins	Designed Ankyrin Repeat Proteins
DLT	Dose Limiting Toxicity
EGFRvIII	Epidermal Growth Factor Receptor variant III
FAP	Fibroblast Activating Protein alpha
Fc	Fragment crystallizable
FcγR	Fc-gamma-receptor
FcγRIIB	Fc-gamma-receptor IIB
FcRn	neonatal Fc-receptor
FDA	U.S. Food and Drug Administration
Her2	Human Epidermal Growth Factor Receptor 2
HAS	Human Serum Albumin
IFNγ	interferon γ
IgG	Immune globulin
irAEs	immune-related Adverse Events
K_D	Dissociation Constant
MoA	Mode of Action
NK	Natural Killer Cells
ORR	Overall Response Rate
OS	Overall Survival
PD-L1	Programmed Death-Ligand 1
PFS	Progressive Free Survival
PR	Partial Response
PSMA	Prostate-Specific Membrane Antigen
Q1W	once per week
Q2W	every two weeks
Q3W	every three weeks
Q4W	every four weeks
ROR1	Receptor tyrosine kinase like Orphan Receptor 1
SARS-CoV2	Severe Acute Respiratory Syndrome Coronavirus 2
scFv	single monomeric variable antibody domain
sdAb	single-chain variable antibody fragment
TCR	T Cell Receptor
TILs	Tumor Infiltrating Lymphocytes
TNFRSF9	Tumor Necrosis Factor Receptor Superfamily 9
TNFSF9	Tumor Necrosis Factor Superfamily 9
TRAEs	Treatment-Related Adverse Events
Treg	regulatory T cells
VH/VL	Variable domain of Heavy and Light antibody chain

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References

1. Jeong S, Park SH. Co-stimulatory receptors in cancers and their implications for cancer immunotherapy. Immune Netw. 2020;20 (e3). doi:10.4110/in.2020.20.e3.
2. Kwon BS, Weissman SM. cDNA sequences of two inducible T-cell genes. Proc Natl Acad Sci U S A. 1963-1967:86. doi:10.1073/pnas.86.6.1963.
3. Hurtado JC, Kim SH, Pollok KE, Lee ZH, Kwon BS. Potential role of 4-1BB in T cell activation. Comparison with the costimulatory molecule CD28. J Immunol. 1995;155(7):3360–67. doi:10.4049/jimmunol.155.7.3360.
4. Pollok KE, Kim YJ, Zhou Z, Hurtado J, Kim KK, Pickard RT, Kwon BS. Inducible T cell antigen 4-1BB. Analysis of expression and function. J Immunol. 1993;150(3):771–81. doi:10.4049/jimmunol.150.3.771.
5. Vinay DS, Kwon BS. 4-1BB signaling beyond T cells. Cell Mol Immunol. 2011;8(281–284):281–84. doi:10.1038/cmi.2010.82.
6. Melero I, Shuford WW, Newby SA, Aruffo A, Ledbetter JA, Hellström KE, Mittler RS, Chen L. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med. 1997;3(682–685):682–85. doi:10.1038/nm0697-682.
7. Lee DY, Choi BK, Lee DG, Kim YH, Kim CH, Lee SJ, Kwon BS. 4-1BB signaling activates the t cell factor 1 effector/beta-catenin pathway with delayed kinetics via ERK signaling and delayed PI3K/AKT activation to promote the proliferation of CD8+ T Cells. PLoS One. 2013;8(e69677). doi:10.1371/journal.pone.0069677
8. Wen T, Bukczynski J, Watts TH. 4-1BB ligand-mediated costimulation of human T cells induces CD4 and CD8 T cell expansion, cytokine production, and the development of cytolytic effector function. J Immunol. 2002;168(4897–4906):4897–906. doi:10.4049/jimmunol.168.10.4897.
9. Curran MA, Geiger TL, Montalvo W, Kim M, Reiner SL, Al-Shamkhani A, Sun JC, Allison JP. Systemic 4-1BB activation induces a novel T cell phenotype driven by high expression of Eomesodermin. J Exp Med. 2013;210(743–755):743–55. doi:10.1084/jem.20121190.
10. Song C, Sadashivaiah K, Furusawa A, Davila E, Tamada K, Banerjee A. Eomesodermin is required for antitumor immunity mediated by 4-1BB-agonist immunotherapy. Oncoimmunology. 2014;3(e27680):e27680. doi:10.4161/onci.27680.
11. Willoughby JE, Kerr JP, Rogel A, Taraban VY, Buchan SL, Johnson PWM, Al-Shamkhani A. Differential impact of CD27 and 4-1BB costimulation on effector and memory CD8 T cell generation following peptide immunization. J Immunol. 2014;193(244–251):244–51. doi:10.4049/jimmunol.1301217.
12. Sabbagh L, Pulle G, Liu Y, Tsitsikov EN, Watts TH. ERK-dependent Bim modulation downstream of the 4-1BB-TRAF1 signaling axis is a critical mediator of CD8 T cell survival in vivo. J Immunol. 2008;180(8093–8101):8093–101. doi:10.4049/jimmunol.180.12.8093.
13. Woroniecka KI, Rhodin KE, Dechant C, Cui X, Chongsathidkiet P, Wilkinson D, Waibl-Polania J, Sanchez-Perez L, Fecci PE. 4-1BB agonism averts TIL exhaustion and licenses PD-1 blockade in glioblastoma and other intracranial cancers. Clin Cancer Res. 2020;26 (1349–1358):1349–58. doi:10.1158/1078-0432.CCR-19-1068.

14. Kim HD, Park S, Jeong S, Lee YJ, Lee H, Kim CG, Kim KH, Hong S-M, Lee J-Y, Kim S, et al. 4-1BB delineates distinct activation status of exhausted tumor-infiltrating CD8+T cells in hepatocellular carcinoma. Hepatology. 2020;71(955–971):955–71. doi:10.1002/hep.30881.

15. Choi BK, Lee DY, Lee DG, Kim YH, Kim S-H, Oh HS, Han C, Kwon BS. 4-1BB signaling activates glucose and fatty acid metabolism to enhance CD8(+) T cell proliferation. Cell Mol Immunol. 2017;14(748–757):748–57. doi:10.1038/cmi.2016.02.

16. Menk AV, Scharping NE, Rivadeneira DB, Calderon MJ, Watson MJ, Dunstane D, Watkins SC, Delgoffe GM. 4-1BB costimulation induces T cell mitochondrial function and biogenesis enabling cancer immunotherapeutic responses. J Exp Med. 2018;215(1091–1100):1091–100. doi:10.1084/jem.20171068.

17. Ahmad A, Uddin S, Steinhoff M. CAR-T cell therapies: an overview of clinical studies supporting their approved use against acute lymphoblastic leukemia and large B-cell lymphomas. Int J Mol Sci. 2020;21(11):3906. doi:10.3390/ijms21113906.

18. Cilta-cel OK'd for Multiple Myeloma. Cancer Discov. 2022;12:1176. 10.1158/2159-8290.CD-NB2022-0019

19. Cappell KM, Kochenderfer JN. A comparison of chimeric antigen receptors containing CD28 versus 4-1BB costimulatory domains. Nat Rev Clin Oncol. 2021;18(715–727):doi:10.1038/s41571-021-00530-z.

20. Hashimoto K. CD137 as an attractive T cell co-stimulatory target in the TNFRSF for immuno-oncology drug development. Cancers (Basel). 2021;13(10):2288. doi:10.3390/cancers13102288.

21. Skokos D, Waite JC, Haber L, Crawford A, Hermann A, Ullman E, Slim R, Godin S, Ajithdoss D, Ye, X, et al. A class of costimulatory CD28-bispecific antibodies that enhance the antitumor activity of CD3-bispecific antibodies. Sci Transl Med. 2020;12(525). doi:10.1126/scitranslmed.aaw7888

22. Segal NH, Logan TF, Hodi FS, McDermott D, Melero I, Hamid O, Schmidt H, Robert C, Chiarion-Sileni V, Ascierto PA, et al. Results from an integrated safety analysis of urelumab, an agonist anti-CD137 monoclonal antibody. Clin Cancer Res. 2017;23 (1929–1936):1929–36. doi:10.1158/1078-0432.CCR-16-1272.

23. Chester C, Sanmamed MF, Wang J, Melero I. Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. Blood. 2018;131(49–57):49–57. doi:10.1182/blood-2017-06-741041.

24. Chin SM, Kimberlin CR, Roe-Zurz Z, Zhang P, Xu A, Liao-Chan S, Sen D, Nager AR, Oakdale NS, Brown C, et al. Structure of the 4-1BB/4-1BBL complex and distinct binding and functional properties of utomilumab and urelumab. Nat Commun. 2018;9(4679). doi:10.1038/s41467-018-07136-7

25. Kamata-Sakurai M, Narita Y, Hori Y, Nemoto T, Uchikawa R, Honda M, Hironiwa N, Taniguchi K, Shida-Kawazoe M, Metsugi S, et al. Antibody to CD137 activated by extracellular adenosine triphosphate is tumor selective and broadly effective in vivo without systemic immune activation. Cancer Discov. 2021;11(158–175):158–75. doi:10.1158/2159-8290.CD-20-0328.

26. Ho SK, Xu Z, Thakur A, Fox M, Tan SS, DiGiammarino E, Zhou L, Sho M, Cairns B, Zhao V, et al. Epitope and Fc-mediated cross-linking, but not high affinity, are critical for antitumor activity of CD137 agonist antibody with reduced liver toxicity. Mol Cancer Ther. 2020;19(1040–1051):1040–51. doi:10.1158/1535-7163.MCT-19-0608.

27. Jhajj HS, Lwo TS, Yao EL, Tessier PM. Unlocking the potential of agonist antibodies for treating cancer using antibody engineering. Trends Mol Med. 2022. doi:10.1016/j.molmed.2022.09.012.

28. Liu X, Zhao Y, Shi H, Zhang Y, Yin X, Liu M, Zhang H, He Y, Lu B, Jin T, et al. Human immunoglobulin G hinge regulates agonistic anti-CD40 immunostimulatory and antitumour activities through biophysical flexibility. Nat Commun. 2019;10(4206). doi:10.1038/s41467-019-12097-6

29. Orr CM, Fisher H, Yu X, Chan CHT, Gao Y, Duriez PJ, Booth SG, Elliott I, Inzhelevskaya T, Mockridge I, et al. Hinge disulfides in human IgG2 CD40 antibodies modulate receptor signaling by regulation of conformation and flexibility. Sci Immunol. 2022;7 (eabm3723). doi:10.1126/sciimmunol.abm3723

30. Li FB, Ravetch JV. Antitumor activities of agonistic anti-TNFR antibodies require differential Fc gamma RIIB coengagement in vivo. P Natl Acad Sci USA. 2013;110(19501–19506):19501–06. doi:10.1073/pnas.1319502110.

31. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5(520). doi:10.3389/fimmu.2014.00520.

32. Claus C, Ferrara C, Wei X, Sam J, Lang S, Uhlenbrock F, Albrecht R, Herter S, Schlenker R, Hüsser T, et al. Tumor-targeted 4-1BB agonists for combination with T cell bispecific antibodies as off-the-shelf therapy. Sci Transl Med. 2019;11(496). doi:10.1126/scitranslmed.aav5989

33. Xu Y, Szalai AJ, Zhou T, Zinn KR, Chaudhuri TR, Li X, Koopman WJ, Kimberly RP. Fc gamma Rs modulate cytotoxicity of anti-Fas antibodies: implications for agonistic antibody-based therapeutics. J Immunol. 2003;171(562–568):562–68. doi:10.4049/jimmunol.171.2.562.

34. Li Y, Tan S, Zhang C, Chai Y, He M, Zhang CW, Wang Q, Tong Z, Liu K, Lei Y, et al. Limited cross-Linking of 4-1BB by 4-1BB ligand and the agonist monoclonal antibody utomilumab. Cell Rep. 2018;25(4):909–920 e904. doi:10.1016/j.celrep.2018.09.073.

35. Eskiocak U, Guzman W, Wolf B, Cummings C, Milling L, Wu HJ, Ophir M, Lambden C, Bakhru P, Gilmore DC, et al. Differentiated agonistic antibody targeting CD137 eradicates large tumors without hepatotoxicity. JCI Insight. 2020;5. doi:10.1172/jci.insight.133647.

36. Hinner MJ, Aiba RSB, Jaquin TJ, Berger S, Dürr MC, Schlosser C, Allersdorfer A, Wiedenmann A, Matschiner G, Schüler J, et al. Tumor-localized costimulatory T-cell engagement by the 4-1BB/HER2 bispecific antibody-anticalin fusion PRS-343. Clin Cancer Res. 2019;25(5878–5889):5878–89. doi:10.1158/1078-0432.CCR-18-3654.

37. Su TT, Gao X, Wang J, Tumor-Localized A. Approach to bypass anti-4-1BB immuno-toxicity. Clin Cancer Res. 2019;25(5732–5734):5732–34. doi:10.1158/1078-0432.CCR-19-1799.

38. Ku G, Bendell JC, Tolcher AW, Hurvitz SA, Krishnamurthy A, El-Khoueiry AB, Patnaik A, Shroff RT, Noonan A, Hahn NM, et al. A phase I dose escalation study of PRS-343, a HER2/4-1BB bispecific molecule, in patients with HER2-positive malignancies. Ann Oncol. 2020;31:S462–S463. doi:10.1016/j.annonc.2020.08.639.

39. Melero I, Sanmamed MF, Calvo E, Moreno I, Moreno V, Guerrero TCH, Martinez-Garcia M, Rodriguez-Vida A, Tabernero J, Pedrazzoli ABA, et al. First-in-human (FIH) phase I study of RO7122290 (RO), a novel FAP-targeted 4-1BB agonist, administered as single agent and in combination with atezolizumab (ATZ) to patients with advanced solid tumours. Ann Oncol. 2020;31:S707–S707. doi:10.1016/j.annonc.2020.08.1145.

40. Liu GZ, Du F, She XH, Zhu Y, Tolcher AW, Luo P. A safe and potent agonist ADG106 targeting a unique epitope of CD137 with novel mechanism of actions. Cancer Res. 2020;80. doi:10.1158/1538-7445.Am2020-4538.

41. Zhang L, Zhao HY, Ma YX, Zheng X, Jiang J, Zhang Y, Hong SD, Liu GZ, She XH, Ni QJ, et al. A phase I, dose-escalation study of ADG106, a fully human anti-CD137 agonistic antibody, in subjects with advanced solid tumors or relapsed/refractory non-Hodgkin lymphoma. J Clin Oncol. 2020;38(15_supplement):3105.

42. Zhang L. Identification of a predictive biomarker and two pharmacodynamic biomarkers to ADG106 treatment, a novel anti-CD137 agonist antibody, in phase I clinical trials. J Clin Oncol. 2021;39. doi:10.1200/JCO.2021.39.15_suppl.e14505.

43. Elshiaty M, Schindler H, Christopoulos P. Principles and current clinical landscape of multispecific antibodies against cancer. Int J Mol Sci. 2021;22(11):5632. doi:10.3390/ijms22115632.

44. You G, Won J, Lee Y, Moon D, Park Y, Lee SH, Lee S-W. Bispecific antibodies: a smart arsenal for cancer immunotherapies. Vaccines (Basel). 2021;9(7). doi:10.3390/vaccines9070724.

45. Muik A, Garralda E, Altintas I, Gieseke F, Geva R, Ben-Ami E, Maurice-Dror C, Calvo E, LoRusso PM, Alonso G, *et al.* Preclinical characterization and Phase I trial results of a bispecific antibody targeting PD-L1 and 4-1BB (GEN1046) in patients with advanced refractory solid tumors. Cancer Discov. 2022;12(5):1248–65. doi:10.1158/2159-8290.CD-21-1345.

46. Geuijen C, Tacken P, Wang LC, Klooster R, van Loo PF, Zhou J, Mondal A, Liu JB, Kramer A, Condamine T, et al. A human CD137xPD-L1 bispecific antibody promotes anti-tumor immunity via context-dependent T cell costimulation and checkpoint blockade. Nat Commun. 2021;12(4445). doi:10.1038/s41467-021-24767-5

47. Prenen H, Kyi C, Van Lancker G, Patel SP, Mittag D, Weaver A, Bol K, Stalbovskaya V, Pulini J, Zhou G, *et al.* Phase I dose escalation study of MCLA-145, a bispecific antibody targeting CD137 and PD-L1 in solid tumors. Ann Oncol. 2021;32:S1436–S1436. doi:10.1016/j.annonc.2021.10.155.

48. Link A, Juglair L, Poulet H, Lemaillet G, Reichen C, Schildknecht P, Tosevski I, Robinson J, Veitonmaki N, Herbst J, et al. Selection of first-in-human clinical dose range for the tumor-targeted 4-1BB agonist MP0310 (AMG 506) using a pharmacokinetic/pharmacodynamics modeling approach. Cancer Res. 2020;80 (16_Supplement):2273–2273. doi:10.1002/1521-4141(2000012) 30:12<3441::AID-IMMU3441>3.0.CO;2-L.

49. Tsun A, Zhai TH, Miao XN, Huang WF, Wang C, Xu YF, Yuan ZJ, Wang T, Dai S, Peng SG, et al. Discovery of a safer 4-1bb agonist by targeting a membrane-proximal epitope combined with bispecific-mediated cross-bridging. Journal for Immunotherapy of Cancer. 2021;9(Suppl 2):A939–A939. doi:10.1136/jitc-2021-SITC2021.895.

50. Poh A. Dual Targeting Approach for CD40 and 4-1BB. Cancer Discov. 2022;12(1):9–10. doi:10.1158/2159-8290.Cd-Nb2021 -0403.

51. Galand C, Venkatraman V, Marques M, Strauss J, Carvajal R, Lim M, Morin B, Ignatovich O, Findeis M, Underwood D, et al. AGEN2373 IS A CD137 agonist antibody designed to leverage optimal CD137 AND FC gamma R co-targeting to promote anti-tumor immunologic effects. J ImmunotherCancer. 2020;8:A229–A230. doi:10.1136/jitc-2020-SITC2020.0377.

52. Tolcher AW, Carvajal RD, El-Khoueiry AB, Ortuzar Feliu W, Zang H, Ancukiewicz M, Shapiro I, Strauss JF. Initial findings of the first-in-human phase I study of AGEN2373, a conditionally active CD137 agonist antibody, in patients (pts) with advanced solid tumors. J Clin Oncol. 2021;39(15_suppl):2634–2634. doi:10. 1200/JCO.2021.39.15_suppl.2634.

53. Qi X, Li F, Wu Y, Cheng C, Han P, Wang J, Yang X. Optimization of 4-1BB antibody for cancer immunotherapy by balancing agonistic strength with FcgammaR affinity. Nat Commun. 2019;10 (2141). doi:10.1038/s41467-019-10088-1.

54. Fu SQ, Harb WA, Patel SP, Lu C, Halperin DM, Hsu YH, Shi N, Yamamura Y, Tang T, Jiang L, *et al.* Early safety and efficacy from a phase I open-label clinical trial of CD137(4-1BB) agonistic antibody LVGN6051 as monotherapy and in combination with pembrolizumab. J Clin Oncol. 2021;39(15_suppl):2521–2521. doi:10.1200/JCO.2021.39.15_suppl.2521.

55. Werchau D, Veitonmäki N, Hägerbrand K, Dahlman A, Smith KE, Fritzell S, von Schantz L, Thagesson M, Werchau D, Smedenfors K, *et al.* ATOR-1017, a 4-1BB antibody developed for tumor-directed immunotherapy of cancer. Journal for Immunotherapy of Cancer. 2019;7(1). doi:10.1186/s40425-019-0570-8.

56. Dahlen E, Rosén A, Barchan K, Dahlman A, Ellmark P, Furebring T, Smith KE. ATOR-1017: a 4-1BB antibody designed for superior safety/efficacy profile in cancer immunotherapy. Cancer Immunol Res. 2019;7(2_Supplement):A183–A183. doi:10. 1158/2326-6074.Cricimteatiaacr18-A183.

57. Ullenhag GJ, Yachnin J, Carneiro A, Elison E, Carlsson M, Russell CA, Enell Smith K. A first-in-human, multicenter, open-label, phase 1 study of ATOR-1017, a 4-1BB antibody, in patients with advanced solid malignancies. J Clin Oncol. 2021;39 (15_suppl):2646–2646. doi:10.1200/JCO.2021.39.15_suppl.2646.

58. Snell D, Gunde T, Warmuth S, Lichtlen P, Tietz J, Brock M, Simonin A, Hess C, Christopher W, Heiz R, et al. Preclinical development and mechanism of action studies of NM21-1480, a PD-L1/4-1BB/HSA trispecific MATCH3 therapeutic clinical candidate. Cancer Res. 2020;80(16_Supplement):2276–2276. doi:10.1158/1538-7445.Am2020-2276.

59. Warmuth S, Gunde T, Snell D, Brock M, Weinert C, Simonin A, Hess C, Tietz J, Johansson M, Spiga FM, et al. Engineering of a trispecific tumor-targeted immunotherapy incorporating 4-1BB co-stimulation and PD-L1 blockade. Oncoimmunology. 2021;10 (1). Artn 2004661. doi:10.1080/2162402x.2021.2004661.

60. Cendrowicz E, Jacob L, Greenwald S, Tamir A, Pecker I, Tabakman R, Ghantous L, Tamir L, Kahn R, Avichzer J, et al. DSP107 combines inhibition of CD47/SIRPα axis with activation of 4-1BB to trigger anticancer immunity. J Exp Clin Cancer Res. 2022;41(16_Supplement). doi:10.1158/1538-7445.AM2020-3352

61. Gaspar M, Pravin J, Rodrigues L, Uhlenbroich S, Everett KL, Wollerton F, Morrow M, Tuna M, Brewis N. CD137/OX40 bispecific antibody induces potent antitumor activity that is dependent on target coengagement. Cancer Immunol Res. 2020;8(781–793):781–93. doi:10.1158/2326-6066.CIR-19-0798.

62. Papadopoulos K, Yap TA, Piha-Paul SA, Lorusso P, Hu-Lieskovan S, Shepherd CJ, Marshall S, Holz J-B, Poon E, Grabowska UB, *et al.* A first-in-human phase I study of FS120, an OX40/CD137 tetravalent bispecific antibody, in patients with advanced malignancies. Ann Oncol. 2021;32:S864–S865. doi:10.1016/j.annonc.2021.08. 1417.

63. Lakins MA, Koers A, Giambalvo R, Munoz-Olaya J, Hughes R, Goodman E, Marshall S, Wollerton F, Batey S, Gliddon D, *et al.* FS222, a CD137/PD-L1 tetravalent bispecific antibody, exhibits low toxicity and antitumor activity in colorectal cancer models. Clinical Cancer Research. 2020;26(4154–4167):4154–67. doi:10. 1158/1078-0432.Ccr-19-2958.

64. Jeong S, Park E, Kim H-D, Sung E, Kim H, Jeon J, Kim Y, Jung U-J, Son Y-G, Hong Y, *et al.* Novel anti-4-1BBxPD-L1 bispecific antibody augments anti-tumor immunity through tumor-directed T-cell activation and checkpoint blockade. J Immunother Cancer. 2021;9(7):e002428. doi:10.1136/jitc-2021-002428.

65. Lee Y, Lee S, Kim Y, Chung H, Park K, Park E, Park KS, Jung J, Sung B, Won J, et al. A novel anti-CD137 antibody recognizing the membrane-proximal CD137 domain elicits potent anti-tumor T cell activity in a bispecific antibody format. Cancer Res. 2021;81(13_Supplement):1850. doi:10.1158/1538-7445.AM2021-1850 .

66. Qiao Y, Qiu Y, Ding J, Luo N, Wang H, Ling X, Sun J, Wu Z, Wang Y, Liu Y, et al. Cancer immune therapy with PD-1-dependent CD137 co-stimulation provides localized tumour killing without systemic toxicity. Nat Commun. 2021;12(6360). doi:10.1038/ s41467-021-26645-6

67. Legg JW. Tumor dependent co-stimulation of CD137/4-1BB in PSMA positive tumors: preclinical characterization of CB307, a half-life extended PSMAxCD137 bispecific Humabody therapeutic. Cancer Res. 2020;80(16_Supplement):3352–3352. doi:10.1158/1538-7445.Am2020-3352.

68. Hashimoto K, Pierce AJ, Chau A, Bartlett P, Lloyd P, Maginn M, Machacek M, Vollmer J, Rajbally S, Tilson J, *et al.* A phase I open-label, dose escalation and expansion trial to investigate the safety, pharmacokinetics and pharmacodynamics of CB307, a trispecific Humabody T-cell enhancer, in patients with PSMA plus advanced and/or metastatic solid tumors (POTENTIA). Cancer Res. 2021;81(13_Supplement):CT206–CT206. doi:10. 1158/1538-7445.AM2021-CT206.

69. Morales-Kastresana A, Pavlidou M, Peper J, Pattarini L, Barthels C, Hansbauer EM, Bel Aiba R, Bossenmaier B, Scholer-Dahirel A, Jaquin T, et al. Simultaneous costimulatory T-cell engagement and checkpoint inhibition by PRS-344/S095012, a PD-L1/4-1BB bispecific compound for tumor localized activation of the immune system. Cancer Res. 2021;81(13_Supplement):LB135–LB135. doi:10.1158/1538-7445.AM2021-LB135.

70. Peper-Gabriel JK, Pavlidou M, Pattarini L, Morales-Kastresana A, Jaquin TJ, Gallou C, Hansbauer E-M, Richter M, Lelievre H, Scholer-Dahirel A, et al. The PD-L1/4-1BB bispecific Antibody-Anticalin fusion protein PRS-344/S095012 elicits strong T-cell stimulation in a tumor-localized manner. Clin Cancer Res. 2022;28(15):3387–99. doi:10.1158/1078-0432.CCR-21-2762.

71. Hui YW, Li T, Ren Y, Hoenemann D, Mei J, Shan B, Hou B. Atg-101, a Novel Pd-L1/4-1bb bispecific antibody, augments anti-tumor immunity through immune checkpoint inhibition and Pdl1-Directed 4-1bb activation. Journal for Immunotherapy of Cancer. 2021;9(Suppl 2):A936–A937. doi:10.1136/jitc-2021-SITC2021.893.

72. Upadhyaya P, Hurov K, Kublin J, Ma J, Repash E, Kleyman M, Kristensson J, Kanakia D, You F, Chen L, et al. Bt7480, a Fully synthetic tumor-targeted immune cell agonist (Tica (Tm)) induces tumor localized Cd137 agonism and modulation of tumor immune microenvironment. Journal for Immunotherapy of Cancer. 2020;8:A424–A424. doi:10.1136/jitc-2020-SITC2020.0706.

73. Hurov K, Lahdenranta J, Upadhyaya P, Haines E, Cohen H, Repash E, Kanakia D, Ma J, Kristensson J, You F, et al. BT7480, a novel fully synthetic bicycle tumor-targeted immune cell agonist™ (Bicycle TICA™) induces tumor localized CD137 agonism. J Immunother Cancer. 2021;9(11):e002883. doi:10.1136/jitc-2021-002883.

74. Upadhyaya P, Lahdenranta J, Hurov K, Battula S, Dods R, Haines E, Kleyman M, Kristensson J, Kublin J, Lani R, et al. Anticancer immunity induced by a synthetic tumor-targeted CD137 agonist. J Immunother Cancer. 2021;9(1):e001762. doi:10.1136/jitc-2020-001762.

75. Hurov K, Lahdenranta J, Upadhyaya P, Kanakia D, Repash E, You F, Ma J, Haines E, Cohen H, McDonnell K, et al. Abstract 1728: nectin-4-dependent immune cell stimulation and anti-tumor efficacy by BT7480, a Nectin-4/CD137 Bicycle ®tumor-targeted immune cell agonist (TICA™). Cancer Res. 2021;81(13_Supplement):1728–1728. doi:10.1158/1538-7445.AM2021-1728.

76. Zhai T, Wang C, Xu Y, Huang W, Yuan Z, Wang T, Dai S, Peng S, Pang T, Jiang W, et al. Generation of a safe and efficacious llama single-domain antibody fragment (vHH) targeting the membrane-proximal region of 4-1BB for engineering therapeutic bispecific antibodies for cancer. J Immunother Cancer. 2021;9(6):e002131. doi:10.1136/jitc-2020-002131.

77. Huang X, Sun J, Qin Y, Lin H, Guan J, Lai S, Kang X, Ling H. LBL-024, an anti-PD-L1 and 4-1BB bispecific antibody with highly differentiated binding affinity, shows anti-tumor efficacy in a mouse tumor model. Cancer Res. 2021;81(13_Supplement):1838–1838. doi:10.1158/1538-7445.AM2021-1838.

78. Song MY, Lee E-J, Chung H, Lee Y, Park YB, Jee MH, Kim K, Ju M, Lee JK, Choi BH, et al. A novel HER2/4-1BB bispecific antibody, YH32367 (ABL105) shows potent anti-tumor effect through tumor-directed T cell activation. Cancer Res. 2020;80(16_Supplement):6524–6524. doi:10.1158/1538-7445.Am2020-6524.

79. Gao Y, Yang T, Liu H, Song N, Dai C, Ding Y. Development and characterization of a novel human CD137 agonistic antibody with anti-tumor activity and a good safety profile in non-human primates. FEBS Open Bio. 2022;12(12):2166–78. doi:10.1002/2211-5463.13494.

80. DiLillo DJ, Ravetch JV. Fc-receptor interactions regulate both cytotoxic and immunomodulatory therapeutic antibody effector functions. Cancer Immunol Res. 2015;3(704–713):704–13. doi:10.1158/2326-6066.CIR-15-0120.

81. Steiner D, Merz FW, Sonderegger I, Gulotti-Georgieva M, Villemagne D, Phillips DJ, Forrer P, Stumpp MT, Zitt C, Binz HK, et al. Half-life extension using serum albumin-binding DARPin(R) domains. Protein Eng Des Sel. 2017;30(583–591):583–91. doi:10.1093/protein/gzx022.

82. Hurov K, Upadhyaya P, Lahdenranta J, Kublin J, Ma J, Repash E, Kleyman M, Kristensson J, Chen L, Haines E, et al. BT7480, a novel fully synthetic tumor-targeted immune cell agonist (TICA (TM)) induces tumor localized 4-1BB agonism. Cancer Res. 2020;80(16_Supplement):5552–5552. doi:10.1158/1538-7445.Am2020-5552.

83. Stumpp MT, Amstutz P. DARPins: a true alternative to antibodies. Curr Opin Drug Discov Devel. 2007;10(2):153–159.

84. Rhodes CA, Pei D. Bicyclic peptides as next-generation therapeutics. Chemistry. 2017;23(12690–12703). doi:10.1002/chem.201702117.

85. Zapata JM, Perez-Chacon G, Carr-Baena P, Martinez-Forero I, Azpilikueta A, Otano I, Melero I. CD137 (4-1BB) Signalosome: complexity Is a Matter of TRAFs. Front Immunol. 2018;9(2618). doi:10.3389/fimmu.2018.02618.

86. Wyzgol A, Muller N, Fick A, Munkel S, Grigoleit GU, Pfizenmaier K, Wajant H. Trimer stabilization, oligomerization, and antibody-mediated cell surface immobilization improve the activity of soluble trimers of CD27L, CD40L, 41BBL, and glucocorticoid-induced TNF receptor ligand. J Immunol. 2009;183(1851–1861):1851–61. doi:10.4049/jimmunol.0802597.

87. Eun SY, Lee SW, Xu Y, Croft M. 4-1BB ligand signaling to T cells limits T cell activation. J Immunol. 2015;194(134–141):134–41. doi:10.4049/jimmunol.1401383.

88. Macdonald DC, Hotblack A, Akbar S, Britton G, Collins MK, Rosenberg WC. 4-1BB ligand activates bystander dendritic cells to enhance immunization in trans. J Immunol. 2014;193(5056–5064):5056–64. doi:10.4049/jimmunol.1301723.

89. Fellermeier S, Beha N, Meyer J-E, Ring S, Bader S, Kontermann RE, Müller D. Advancing targeted co-stimulation with antibody-fusion proteins by introducing TNF superfamily members in a single-chain format. Oncoimmunology. 2016;5(e1238540):e1238540. doi:10.1080/2162402X.2016.1238540.

90. Muik A, Adams HC, Gieseke F, Altintas I, Schoedel KB, Blum JM, Sänger B, Burm SM, Stanganello E, Verzijl D, et al. DuoBody-CD40x4-1BB induces dendritic-cell maturation and enhances T-cell activation through conditional CD40 and 4-1BB agonist activity. J Immunother Cancer. 2022;10(6):e004322. doi:10.1136/jitc-2021-004322.

91. Thurber GM, Dane Wittrup K. A mechanistic compartmental model for total antibody uptake in tumors. J Theor Biol. 2012;314(57–68):57–68. doi:10.1016/j.jtbi.2012.08.034.

92. Otano I, Azpilikueta A, Glez-Vaz J, Alvarez M, Medina-Echeverz J, Cortés-Domínguez I, Ortiz-de-Solorzano C, Ellmark P, Fritzell S, Hernandez-Hoyos G, et al. CD137 (4-1BB) costimulation of CD8(+) T cells is more potent when provided in cis than in trans with respect to CD3-TCR stimulation. Nat Commun. 2021;12(7296). doi:10.1038/s41467-021-27613-w

93. Oh SA, Wu D-C, Cheung J, Navarro A, Xiong H, Cubas R, Totpal K, Chiu H, Wu Y, Comps-Agrar L, et al. PD-L1 expression by dendritic cells is a key regulator of T-cell immunity in cancer. Nat Cancer. 2020;1(681–691):681–91. doi:10.1038/s43018-020-0075-x.

94. Blackburn SD, Shin H, Freeman GJ, Wherry EJ. Selective expansion of a subset of exhausted CD8 T cells by alphaPD-L1 blockade. Proc Natl Acad Sci U S A. 2008;105(15016–15021):15016–21. doi:10.1073/pnas.0801497105.

95. Paley MA, Kroy DC, Odorizzi PM, Johnnidis JB, Dolfi DV, Barnett BE, Bikoff EK, Robertson EJ, Lauer GM, Reiner SL, et al. Progenitor and terminal subsets of CD8+T cells cooperate to contain chronic viral infection. Science. 2012;338(1220–1225):1220–25. doi:10.1126/science.1229620.

96. Bartkowiak T, Jaiswal AR, Ager CR, Chin R, Chen C-H, Budhani P, Ai M, Reilley MJ, Sebastian MM, Hong DS, et al. Activation of 4-1BB on liver myeloid cells triggers hepatitis via an interleukin-27-dependent pathway. Clin Cancer Res. 2018;24(1138–1151):1138–51. doi:10.1158/1078-0432.CCR-17-1847.

97. Zhang J, Song K, Wang J, Li Y, Liu S, Dai C, Chen L, Wang S, Qin Z. S100A4 blockage alleviates agonistic anti-CD137 antibody-induced liver pathology without disruption of antitumor immunity. Oncoimmunology. 2018;7(e1296996):e1296996. doi:10.1080/2162402X.2017.1296996.

98. Kerntke C, Nimmerjahn F, Biburger M. There is (scientific) strength in numbers: a comprehensive quantitation of Fc gamma

receptor numbers on human and murine peripheral blood leukocytes. Front Immunol. 2020;11(118). doi:10.3389/fimmu.2020.00118.

99. Vonderheide RH, Glennie MJ. Agonistic CD40 antibodies and cancer therapy. Clin Cancer Res. 2013;19(1035–1043):1035–43. doi:10.1158/1078-0432.CCR-12-2064.

100. Buchan SL, Dou L, Remer M, Booth SG, Dunn SN, Lai C, Semmrich M, Teige I, Mårtensson L, Penfold CA, et al. Antibodies to costimulatory receptor 4-1BB enhance anti-tumor immunity via T regulatory cell depletion and promotion of CD8 T cell effector function. Immunity. 2018;49(5):958–970 e957. doi:10.1016/j.immuni.2018.09.014.

101. Derebe MG, Nanjunda RK, Gilliland GL, Lacy ER, Chiu ML. Human IgG subclass cross-species reactivity to mouse and cynomolgus monkey Fcgamma receptors. Immunol Lett. 2018;197(1–8):1–8. doi:10.1016/j.imlet.2018.02.006.

102. Dekkers G, Bentlage AEH, Stegmann TC, Howie HL, Lissenberg-Thunnissen S, Zimring J, Rispens T, Vidarsson G. Affinity of human IgG subclasses to mouse Fc gamma receptors. MAbs. 2017;9(767–773):767–73. doi:10.1080/19420862.2017.1323159.

103. Bitra A, Doukov T, Destito G, Croft M, Zajonc DM. Crystal structure of the m4-1BB/4-1BBL complex reveals an unusual dimeric ligand that undergoes structural changes upon 4-1BB receptor binding. J Biol Chem. 2019;294(1831–1845):1831–45. doi:10.1074/jbc.RA118.006297.

104. Piha-Paul S, Bendell J, Tolcher A, Hurvitz S, Patnaik A, Shroff R, Pohlmann P, Zettl M, Hahn N, Krishnamurthy A, et al. A Phase 1 Dose Escalation Study of Prs-343, a Her2/4-1bb Bispecific Molecule, in patients with her2-positive malignancies. Journal for Immunotherapy of Cancer. 2020;8:A1–A2. doi:10.1136/Lba2019.2.

105. Shojaie L, Ali M, Iorga A, Dara L. Mechanisms of immune checkpoint inhibitor-mediated liver injury. Acta Pharm Sin B. 2021;11(3727–3739):3727–39. doi:10.1016/j.apsb.2021.10.003.

106. Iwai Y, Terawaki S, Ikegawa M, Okazaki T, Honjo T. PD-1 inhibits antiviral immunity at the effector phase in the liver. J Exp Med. 2003;198(39–50):39–50. doi:10.1084/jem.20022235.

107. Albandar HJ, Fuqua J, Albandar JM, Safi S, Merrill SA, Ma PC. Immune-related adverse events (irAE) in cancer immune checkpoint inhibitors (ICI) and Survival outcomes correlation: to rechallenge or Not? Cancers (Basel). 2021;13(5):989. doi:10.3390/cancers13050989.

108. Zhou X, Yao Z, Yang H, Liang N, Zhang X, Zhang F. Are immune-related adverse events associated with the efficacy of immune checkpoint inhibitors in patients with cancer? A systematic review and meta-analysis. BMC Med. 2020;18(87). doi:10.1186/s12916-020-01549-2.

109. Conroy M, Naidoo J. Immune-related adverse events and the balancing act of immunotherapy. Nat Commun. 2022;13(392). doi:10.1038/s41467-022-27960-2.

110. Etxeberria I, Glez-Vaz J, Teijeira A, Melero I. New emerging targets in cancer immunotherapy: CD137/4-1BB costimulatory axis. ESMO Open. 2020;4(e000733). doi:10.1136/esmoopen-2020-000733.

111. Bartkowiak T, Curran MA. 4-1BB agonists: multi-potent potentiators of tumor immunity. Front Oncol. 2015;5(117). doi:10.3389/fonc.2015.00117.

112. Choi BK, Kim YH, Kwon PM, Lee SC, Kang SW, Kim MS, Lee MJ, Kwon BS. 4-1BB functions as a survival factor in dendritic cells. J Immunol. 2009;182(4107–4115):4107–15. doi:10.4049/jimmunol.0800459.

113. Stoll A, Bruns H, Fuchs M, Völkl S, Nimmerjahn F, Kunz M, Peipp M, Mackensen A, Mougiakakos D. CD137 (4-1BB) stimulation leads to metabolic and functional reprogramming of human monocytes/macrophages enhancing their tumoricidal activity. Leukemia. 2021;35(3482–3496):3482–96. doi:10.1038/s41375-021-01287-1.

114. Jiang P, Gao W, Ma T, Wang R, Piao Y, Dong X, Wang P, Zhang X, Liu Y, Su W, et al. CD137 promotes bone metastasis of breast cancer by enhancing the migration and osteoclast differentiation of monocytes/macrophages. Theranostics. 2019;9(2950–2966):2950–66. doi:10.7150/thno.29617.

115. Wensman H, Kamgari N, Johansson A, Grujic M, Calounova G, Lundequist A, Rönnberg E, Pejler G. Tumor-mast cell interactions: induction of pro-tumorigenic genes and anti-tumorigenic 4-1BB in MCs in response to Lewis lung carcinoma. Mol Immunol. 2012;50(210–219):210–19. doi:10.1016/j.molimm.2012.01.009.

116. Nishimoto H, Lee S-W, Hong H, Potter KG, Maeda-Yamamoto M, Kinoshita T, Kawakami Y, Mittler RS, Kwon BS, Ware CF, et al. Costimulation of mast cells by 4-1BB, a member of the tumor necrosis factor receptor superfamily, with the high-affinity IgE receptor. Blood. 2005;106(4241–4248):4241–48. doi:10.1182/blood-2005-04-1358.

117. Heinisch IV, Daigle I, Knopfli B, Simon HU. CD137 activation abrogates granulocyte-macrophage colony-stimulating factor-mediated anti-apoptosis in neutrophils. Eur J Immunol. 2000;30(3441–3446):3441–46. doi:10.1002/1521-4141(2000012)30:12<3441::AID-IMMU3441>3.0.CO;2-L.

118. Heinisch IV, Bizer C, Volgger W, Simon HU. Functional CD137 receptors are expressed by eosinophils from patients with IgE-mediated allergic responses but not by eosinophils from patients with non-IgE-mediated eosinophilic disorders. J Allergy Clin Immunol. 2001;108(21–28):21–28. doi:10.1067/mai.2001.116864.

119. Tu TH, Kim C-S, Goto T, Kawada T, Kim B-S, Yu R. 4-1BB/4-1BBL interaction promotes obesity-induced adipose inflammation by triggering bidirectional inflammatory signaling in adipocytes/macrophages. Mediators Inflamm. 2012;2012(972629):1–10. doi:10.1155/2012/972629.

120. Yuan W, Xu C, Li B, Xia H, Pan Y, Zhong W, Xu L, Chen R, Wang B. Contributions of costimulatory molecule CD137 in endothelial cells. J Am Heart Assoc. 2021;10(e020721). doi:10.1161/JAHA.120.020721.

121. Palazon A, Teijeira A, Martínez-Forero I, Hervás-Stubbs S, Roncal C, Peñuelas I, Dubrot J, Morales-Kastresana A, Pérez-Gracia JL, Ochoa MC, et al. Agonist anti-CD137 mAb act on tumor endothelial cells to enhance recruitment of activated T lymphocytes. Cancer Res. 2011;71(801–811):801–11. doi:10.1158/0008-5472.CAN-10-1733.

122. Foda BM, Ciecko AE, Serreze DV, Ridgway WM, Geurts AM, Chen Y-G. The CD137 ligand is important for Type 1 diabetes development but dispensable for the homeostasis of disease-suppressive CD137(+) FOXP3(+) regulatory CD4 T cells. J Immunol. 2020;204(2887–2899):2887–99. doi:10.4049/jimmunol.1900485.

123. Forsberg MH, Ciecko AE, Bednar KJ, Itoh A, Kachapati K, Ridgway WM, Chen Y-G. CD137 plays both pathogenic and protective roles in type 1 diabetes development in NOD mice. J Immunol. 2017;198(3857–3868):3857–68. doi:10.4049/jimmunol.1601851.

124. Le NH, Kim C-S, Tu TH, Choi H-S, Kim B-S, Kawada T, Goto T, Park T, Yoon Park JH, Yu R, et al. Blockade of 4-1BB and 4-1BBL interaction reduces obesity-induced skeletal muscle inflammation. Mediators Inflamm. 2013;2013(865159):1–10. doi:10.1155/2013/865159.

125. Mayes PA, Hance KW, Hoos A. The promise and challenges of immune agonist antibody development in cancer. Nat Rev Drug Discov. 2018;17(509–527):509–27. doi:10.1038/nrd.2018.75.

126. Betts A, van der Graaf PH. Mechanistic quantitative pharmacology strategies for the early clinical development of bispecific antibodies in oncology. Clin Pharmacol Ther. 2020;108(528–541):528–41. doi:10.1002/cpt.1961.

127. Laderach D, Movassagh M, Johnson A, Mittler RS, Galy A. 4-1BB co-stimulation enhances human CD8(+) T cell priming by augmenting the proliferation and survival of effector CD8(+) T cells. Int Immunol. 2002;14(1155–1167):1155–67. doi:10.1093/intimm/dxf080.

128. Martinez-Forero I, Azpilikueta A, Bolaños-Mateo E, Nistal-Villan E, Palazon A, Teijeira A, Perez-Chacon G, Morales-Kastresana A, Murillo O, Jure-Kunkel M, et al. T cell costimulation with anti-CD137 monoclonal antibodies is mediated by

K63-polyubiquitin-dependent signals from endosomes. J Immunol. 2013;190(6694–6706):6694–706. doi:10.4049/jimmunol.1203010.

129. Glez-Vaz J, Azpilikueta A, Olivera I, Cirella A, Teijeira A, Ochoa MC, Alvarez M, Eguren-Santamaria I, Luri-Rey C, Rodriguez-Ruiz ME, *et al*. Soluble CD137 as a dynamic biomarker to monitor agonist CD137 immunotherapies. J Immunother Cancer. 2022;10(3):e003532. doi:10.1136/jitc-2021-003532.

130. Seidel J, Leitzke S, Ahrens B, Sperrhacke M, Bhakdi S, Reiss K. Role of ADAM10 and ADAM17 in regulating CD137 Function. Int J Mol Sci. 2021;22(5):2730. doi:10.3390/ijms22052730.

131. Ye Q, Song DG, Poussin M, Yamamoto T, Best A, Li C, Coukos G, Powell DJ Jr. CD137 accurately identifies and enriches for naturally occurring tumor-reactive T cells in tumor. Clin Cancer Res. 2014;20(44–55):44–55. doi:10.1158/1078-0432.CCR-13-0945.

132. Gros A, Robbins PF, Yao X, Li YF, Turcotte S, Tran E, Wunderlich JR, Mixon A, Farid S, Dudley ME, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. J Clin Invest. 2014;124(2246–2259):2246–59. doi:10.1172/JCI73639.

133. Kim HD, Park S, Jeong S, Lee YJ, Lee H, Kim CG, Kim KH, Hong SM, Lee JY, Kim S, et al. 4-1BB delineates distinct activation status of exhausted tumor-infiltrating CD8(+) T cells in hepatocellular carcinoma. Hepatology. 2020;71(3): 955–971. doi:10.1002/hep.30881.

134. Zheng L, Qin S, Si W, Wang A, Xing B, Gao R, Ren X, Wang L, Wu X, Zhang J, *et al*. Pan-cancer single-cell landscape of tumor-infiltrating T cells. Science. 2021;374(abe6474). doi:10.1126/science.abe6474

135. Datar I, Sanmamed MF, Wang J, Henick BS, Choi J, Badri T, Dong W, Mani N, Toki M, Meijas LD, et al. Expression analysis and significance of PD-1, LAG-3, and TIM-3 in human non-small cell lung cancer using spatially resolved and multiparametric single-cell analysis. Clin Cancer Res. 2019;25(4663–4673):4663–73. doi:10.1158/1078-0432.CCR-18-4142.

136. Eiva MA, Omran DK, Chacon JA, Powell DJ Jr. Systematic analysis of CD39, CD103, CD137, and PD-1 as biomarkers for naturally occurring tumor antigen-specific TILs. Eur J Immunol. 2022;52 (1):96–108. doi:10.1002/eji.202149329.

137. Leem G, Park J, Jeon M, Kim ES, Kim SW, Lee YJ, Choi SJ, Choi B, Park S, Ju YS, et al. 4-1BB co-stimulation further enhances anti-PD-1-mediated reinvigoration of exhausted CD39+CD8 T cells from primary and metastatic sites of epithelial ovarian cancers. J Immunother Cancer. 2020;8(2):e001650. doi:10.1136/jitc-2020-001650.

138. Duhen T, Duhen R, Montler R, Moses J, Moudgil T, de Miranda NF, Goodall CP, Blair TC, Fox BA, McDermott JE, et al. Co-expression of CD39 and CD103 identifies tumor-reactive CD8 T cells in human solid tumors. Nat Commun. 2018;9(2724). doi:10.1038/s41467-018-05072-0

139. Ugolini A, Nuti M. CD137(+) T-cells: protagonists of the immunotherapy revolution. Cancers (Basel). 2021;13(3):456. doi:10.3390/cancers13030456.

140. Ovacik M, Lin K. Tutorial on monoclonal antibody pharmacokinetics and its considerations in early development. Clin Transl Sci. 2018;11(540–552):540–52. doi:10.1111/cts.12567.

141. Novakovic AM, Wilkins JJ, Dai H, Wade JR, Neuteboom B, Brar S, Bello CL, Girard P, Khandelwal A. Changing body weight-based dosing to a flat dose for avelumab in metastatic Merkel cell and advanced urothelial carcinoma. Clin Pharmacol Ther. 2020;107 (588–596):588–96. doi:10.1002/cpt.1645.

142. Giffin MJ, Cooke K, Lobenhofer EK, Estrada J, Zhan J, Deegen P, Thomas M, Murawsky CM, Werner J, Liu S, *et al*. AMG 757, a half-life extended, DLL3-targeted bispecific T-cell engager, shows high potency and sensitivity in preclinical models of small-cell lung cancer. Clin Cancer Res. 2021;27(1526–1537):1526–37. doi:10.1158/1078-0432.CCR-20-2845.

143. Houot R, Kohrt H, Levy R. Boosting antibody-dependent cellular cytotoxicity against tumor cells with a CD137 stimulatory antibody. Oncoimmunology. 2012;1(957–958):957–58. doi:10.4161/onci.19974.

144. Opzoomer JW, Sosnowska D, Anstee JE, Spicer JF, Arnold JN. Cytotoxic chemotherapy as an immune stimulus: a molecular perspective on turning up the immunological heat on cancer. Front Immunol. 2019;10(1654). doi:10.3389/fimmu.2019.01654.

145. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst. 2013;105 (256–265):256–65. doi:10.1093/jnci/djs629.

146. Barsoumian HB, Ramapriyan R, Younes AI, Caetano MS, Menon H, Comeaux NI, Cushman TR, Schoenhals JE, Cadena AP, Reilly TP, *et al*. Low-dose radiation treatment enhances systemic antitumor immune responses by overcoming the inhibitory stroma. J Immunother Cancer. 2020;8(2):e000537. doi:10.1136/jitc-2020-000537.

147. Benaduce AP, Brenneman R, Schrand B, Pollack A, Gilboa E, Ishkanian A. 4-1BB aptamer-based immunomodulation enhances the therapeutic index of radiation therapy in murine tumor models. Int J Radiat Oncol Biol Phys. 2016;96(458–461):458–61. doi:10.1016/j.ijrobp.2016.05.013.

148. Qu QX, Zhu X-Y, Du -W-W, Wang H-B, Shen Y, Zhu Y-B, Chen C. 4-1BB agonism combined with PD-L1 blockade increases the number of tissue-resident CD8+ T cells and facilitates tumor abrogation. Front Immunol. 2020;11(577). doi:10.3389/fimmu.2020.00577.

149. Marofi F, Achmad H, Bokov D, Abdelbasset WK, Alsadoon Z, Chupradit S, Suksatan W, Shariatzadeh S, Hasanpoor Z, Yazdanifar M, *et al*. Hurdles to breakthrough in CAR T cell therapy of solid tumors. Stem Cell Res Ther. 2022;13(140). doi:10.1186/s13287-022-02819-x

150. Simoni Y, Becht E, Fehlings M, Loh CY, Koo S-L, Teng KWW, Yeong JPS, Nahar R, Zhang T, Kared H, *et al*. Bystander CD8(+) T cells are abundant and phenotypically distinct in human tumour infiltrates. Nature. 2018;557(575–579):575–79. doi:10.1038/s41586-018-0130-2.