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INSIGHTS

4-1BB (CD137) in anticancer chimeras

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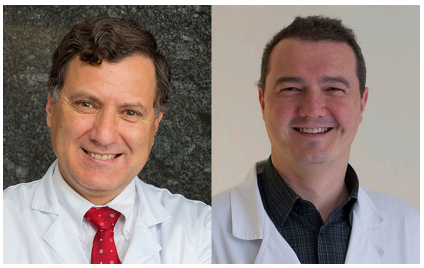
4-1BB (CD137, TNFRSF9) mediates costimulatory signals important for activation and persistence of cytotoxic T lymphocytes. In this issue of *JEM*, Oda et al. (https://doi.org/10.1084/jem.20191166) report on a chimeric construction encompassing extracellular Fas and intracellular 4-1BB to dramatically improve adoptive T cell therapy.

In this issue of *JEM*, Oda et al. report on a remarkably smart approach to enhance adoptive T cell therapy based on a chimeric construct encompassing extracellular Fas and intracellular 4-1BB. The beauty of the approach comes from the following important functional features: Fas undergoes trimerization by Fas ligand, as 4-1BB does when bound by CD137L, to result in caspase-dependent apoptosis in the case of Fas-expressing cells. Hence, the Fas-4-1BB chimera will trimerize upon ligation by FasL, but instead of promoting apoptosis, it confers 4-1BB costimulation (see figure). Moreover, upon expression, the chimera would compete with endogenous Fas activation by ligand in a dominant-negative fashion, thereby further inhibiting apoptosis. Importantly, the system would only function when meeting cells expressing FasL, such as activated sister T cells. This strategy is exploited to retrovirally transduce mouse and human T cells to make the proof of concept. The approach is safe, robust, efficacious, and worthy of clinical development, at least in the context of chimeric antigen receptor (CAR) T cells (CARTs), especially in strategies against solid malignancies that so far are largely refractory to such CARTs.

4-1BB (CD137, TNFRSF9) is a surface glycoprotein discovered by Kwon's group as an activation antigen on the surface of T cells (Kwon and Weissman, 1989). Indeed, its regulated expression is induced upon CD3-TCR engagement and is further stimulated by CD28 ligation (Melero et al., 1998). Its expression is not confined to the T cell lineage since it is inducible on natural killer cells, B lymphocytes, dendritic cells, and even other cell types, including tumor endothelium and adipocytes (Vinay and Kwon, 2011). 4-1BB belongs to the TNF receptor (TFNR) family. This family of surface proteins can be subdivided into those with a death domain able to activate pro-caspases (namely, TNFR1, Fas, and DR4/5) and those without these apoptosis-inducing intracellular sequences (Croft et al., 2013). Signaling involves trimerization by ligand as a general rule in the TNFR family. In the case of the members of the TNFR family that lack the death domain, signaling by trimerization orchestrates a cascade of polyubiquitination reactions that permit docking and activation of downstream signaling moieties. To achieve so, TNFR family members have to engage TNFR-associated factor (TRAF) signaling transducers since they lack any intrinsic enzymatic function. In the case of 4-1BB, TRAF-2 and TRAF-1 have been found to associate with the cytoplasmic tail (Wortzman et al., 2013). This association is promoted upon interaction with agonist antibodies or the natural ligand. The only relevant natural ligand so far discovered is 4-1BBL, which forms trimers on the membrane of mature macrophages, dendritic cells, and activated B cells (Croft et al., 2013). The key role of 4-1BB costimulation in CTL biology is best perceived considering the phenotype of CD137$^{-/-}$ mice upon viral infection (Wortzman et al., 2013) and the alterations in the control of herpes viruses such as Epstein-Barr in patients with homozygous CD137 mutations (Rodriguez et al., 2019). Simple experiments in transplantable tumor models indicated that rejection of established tumors can be elicited by systemic administration of agonist anti–4-1BB mAb (Melero et al., 1997). The mechanism is mainly executed by artificially costimulated tumor-specific CTLs, cross-primed by cDC1 dendritic cells against tumor antigens (Sánchez-Paulete et al., 2016). Although other members of the TNFR family, such as OX40, CD27, or glucocorticoid-induced TNF-related protein, may unleash antitumor immunity (Croft et al., 2013), overall results indicate that 4-1BB is the most potent. Importantly, 4-1BB agonists are synergistic with a variety of cancer immunotherapies, including checkpoint inhibitors (Etxeberria et al., 2020). Agonist anti-CD137 mAbs have been



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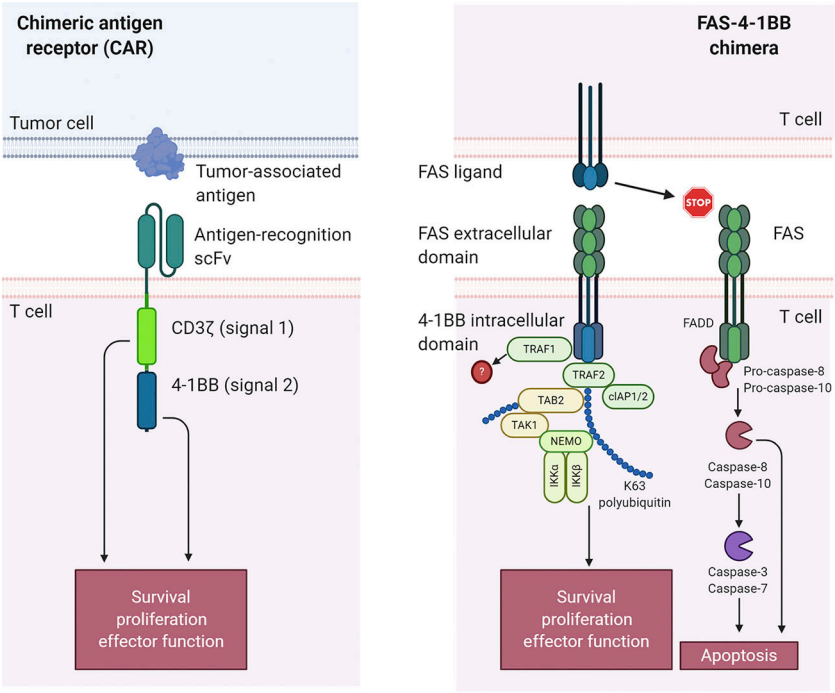

Schematic representation of the molecular physiology underlying chimeric surface proteins encompassing a 4-1BB intracellular domain. In the case of CARs containing 4-1BB, CD3-TCR signaling (signal 1) is provided by the CD3ξ intracytoplasmic sequence, while 4-1BB provides costimulation (signal 2). In the case of the FAS-4-1BB chimera reported by Oda et al. (2020) in this issue of *JEM*, two major mechanisms apply. Fas ligand would elicit costimulatory signals while reducing apoptosis elicited by endogenous FAS in a dominant-negative fashion.

in clinical trials but unfortunately caused Fc receptor (FcR)–dependent immune-mediated liver toxicity (Segal et al., 2017). New formats and constructs of CD137 agonists devised to be safer to the liver are back in the clinic (Etxeberria et al., 2020).

So far, the most clinically successful application of 4-1BB has been the inclusion of its intracellular domain in CARs targeting antigens expressed on malignant cells in hematological neoplasias (see Table 1 and figure). June and colleagues discovered that it confers persistence and powerful activation surpassing that provided by the cytoplasmic tail of CD28 (Carpenito et al., 2009). The field of intracytoplasmic signals via CD137 is incompletely understood. TRAF-2 encompasses an E3 ligase region (RING domain) that presumably K63-polyubiquitinates downstream protein substrates, including TRAF-2 itself, NF-κB essential modulators, the inhibitor of NF-κB kinase kinase complex, TAB1/2, and perhaps others. Unfortunately, the role of TRAF-1 in the 4-1BB signalosome complex remains ignored, albeit TRAF-1 probably mediates an activatory role (Wortzman et al., 2013). Importantly, CD137 signaling induces antiapoptotic moieties such as Bcl-xL, Bfl-1, down-regulation of BIM, and AKT signals. Moreover, CD137 ligation potentiates T cell mitochondrial function (Teijeira et al., 2019), remodels chromatin (Aznar et al., 2018), and enhances cytokine secretion, including IL-2 and IFNγ. CARTs have not been the only adoptive T cell therapy to be experimentally enhanced by 4-1BB costimulation, since stable or transient transfection of 4-1BBL to T cells also enhances antitumor performance of tumor-infiltrating lymphocytes (Etxeberria et al., 2019).

4-1BB offers opportunities to make the most of anticancer immunity. In the recent past, new agonists have been engineered to be given systemically. They are devoid of FcR binding capabilities to avoid liver toxicity but conjugated to antibodies targeting tumor molecules or tumor tissue (Etxeberria et al., 2020), or given as probodies activable by tumor tissue–restricted proteases (unpublished data). Indeed, fibroblast activation protein–targeted 4-1BBL is already in early clinical trials (Claus et al., 2019) as well as anti-PD-L1-CD137 bispecific antibodies (NCT03917381), and more will join this translational adventure soon (Etxeberria et al., 2020). 4-1BB is also exploited to prepare tumor vaccines. Perhaps the most elegant route to exploit this fascinating immunobiology is to potentiate adoptive T cell therapies or CD3-based T cell

Table 1. **FDA-approved and phase III CARTs containing a 4-1BB costimulatory domain**

Name	Company	Target	Indication	Efficacy in phase I/II	FDA status
Tisagenlecleucel	Novartis	Anti-CD19/FMC63	DLBCL, ALL	DLBCL: 49% OS at 12 mo	Approved
				ALL: 76% OS at 12 mo	
Lisocabtagene maraleucel	BMS (formerly Cellgene)	Anti-CD19/FMC63	ALL	83% CR at 6 mo	Phase III (NCT03575351)
JNJ-68284528	Janssen	Two BCMA-targeting single-domain antibodies	MM	90% PF at 9 mo	Phase III (NCT04181827)
Idecabtagene vicleucel	BMS (formerly Cellgene)	BCMA	MM	31.3% CR at 2 yr	Phase III (NCT03651128)

BCMA, B cell maturation antigen; DLBCL, diffuse large B cell lymphoma; ALL, acute lymphocytic leukemia; OS, overall survival; CR, complete response; PF, progression free.

engagers (Claus et al., 2019). Chimeric constructions offer very elegant and creative options worthy of clinical testing. The work published in this issue from Greenberg and colleagues (Oda et al., 2020) is very relevant, since the biology of the Fas-4-1BB chimera probably reaches its optimal functional capabilities because of its components staying within the TNFR family, thereby exploiting a dual role as a signaling module and as a dominant-negative decoy receptor for Fas ligand (see figure). CD137-based cancer immunotherapy is no longer a myth or a futuristic chimera since, as a part of CARTs and in other examples, it is becoming clinical reality.

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