Trends in
Pharmacological Sciences

Spotlight

'Living drugs' target CD70 in advanced renal tumors

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Cellular therapies against solid tumors face three major barriers: low persistence, insufficient specificity, and high costs. In a recent study, Pal *et al.* tackle these challenges in kidney cancer by using novel, 'persistence-tuned' allogeneic chimeric antigen receptor (CAR) T cells directed against a stable antigen.

Autologous (see Glossary) **CAR T cells** brought excitement and hope for millions of cancer patients worldwide. These 'living drugs' are manufactured from the patient's own T cells and are reinfused to treat advanced tumors. Although the excitement persists because durable responses in selected hematologic entities have been achieved, high costs and long manufacturing times prevent their broader use. Therefore, efforts are being made to establish **allogeneic** CAR T cell therapies which could be prepared in advance and stored locally, thus reducing both the costs and the time to application [1]. Allogeneic CAR T cell therapies are being explored preclinically and clinically, mostly in hematologic cancers. Nevertheless, one issue persists in both the autologous and allogeneic settings – what is the ideal target?

In the key article, Pal *et al.* [2] target CD70 that is expressed on renal tumors. CD70, a member of the TNF family, is expressed in several malignancies and also (mostly transiently) on immune cells [3]. Along with several inflammatory mediators, CD70 is induced by **hypoxia-inducible factor (HIF)** signaling under hypoxia or through **von Hippel–Lindau (VHL)** mutations in normoxia. Thus, the very high expression of CD70 by VHL-mutated clear cell renal cell carcinoma (RCC) [4,5] is not surprising. CD27, a ligand for CD70, is expressed by (mostly hematologic) tumors [3]. Importantly, CD27 is also expressed by immune cells and its interaction with CD70 can both activate and inhibit immune cell function, although its inhibitory (**immune checkpoint**) and proapoptotic roles seem to dominate [5]. Thus, given its role in malignancy and immune regulation, the CD70/CD27 duo is a highly interesting target for tumor therapy. Accordingly, strategies to target CD70 with monoclonal antibodies and antibody–drug conjugates have been established in preclinical and clinical studies [3]. The landscape of allogeneic CAR T cells is still small – six studies are currently active or recruiting, and $CD70^+$ RCC is the main entity targeted (Table 1).

Pal *et al.* studied the specificity, safety, and efficacy of CTX130, the first in class, allogeneic, **CRISPR/Cas9 gene-edited**, CAR T cell product targeting CD70 [2]. To prevent **graft-versus-host (GvH) disease and host-versus-graft (HvG) reaction**, and to prevent **fratricide**, CTX130 has a disrupted T cell receptor (TCR), no major histocompatibility complex class 1 (MHCI) expression through β2-microglobulin (B2M) disruption, and CD70 knockout, respectively. Using coculture and *in vivo* models, the authors prove a high specificity of CTX130 for CD70. Furthermore, unedited control T cells induce fatal GvH disease in 21 days, proving successful TCR disruption in CTX130. Lastly, the necessity of CD70 knockout is shown by a superior proliferation, persistence, and activity of CTX130 compared to $CD70^+$ controls.

The improved expansion and activity through CD70 knockout might be for three reasons: (i) averted fratricide through neighboring CAR T cells, (ii) CD70 acts as an T cell inhibitory receptor, or (iii) CD27 coexpressed on the same cell is ligated and its ligation is inhibitory, possibly proapoptotic. This phenomenon deserves further exploration and bears translational potential. A provocative question arises – is the CD70/CD27 interaction a 'physiological' feedback regulation of a T cell? As there is only limited evidence to support this claim, further research is warranted, especially because soluble CD27 can be found in RCC patients [4,5].

Given the encouraging murine model results, CTX130 was employed in COBALT-RCC, a Phase 1 study with 16 heavily pretreated, metastatic RCC patients. The

Glossary

Allogeneic: donated from another individual – genetically different and potentially immunologically incompatible with the host.
Autologous: derived from the same individual.
CAR T cells: engineered T cells equipped with a chimeric antigen receptor (CAR) that recognizes specific antigens expressed on tumor cells, for example CD70 in clear cell renal cell carcinoma (RCC).
CRISPR/Cas9 gene editing: a genetic engineering technique using DNA cutting and deletion/insertion of genes of interest through the use of bacterial clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9).
Fratricide: killing of neighboring cells, for example killing of CAR T cells expressing both CD70 and the anti-CD70 CAR.
Graft-versus-host (GvH) disease and host-versus-graft (HvG) reaction: immunologic reactions of donor cells against the host (GvH), or of host cells against the donor graft (HvG), potentially leading to disease (GvH) or graft deletion (HvG).
Hypoxia-Inducible factors (HIFs): transcription factors induced by hypoxia and other mechanisms that regulate cellular metabolism and growth; HIFs are also associated with malignancies.
Immune checkpoints: receptor/ligand pairs expressed on immune/cancer cells. Receptor binding transmits a (mostly) inhibitory signal to immune cells, for example via the PD1–PDL1 interaction between T cells and cancer cells.
Lymphodepletion: reduction of donor lymphocytes before infusion of CAR T cells to decrease HvG reaction.
Off-the-shelf: cellular products such as CAR T cells that are readily available because they are mass-produced and broadly applicable.
von Hippel–Lindau (VHL) protein: a tumor suppressor that is responsible for ubiquitination of HIFs in normoxia; loss-of-function VHL mutations are found in clear-cell RCC.

Table 1. Currently active or recruiting studies employing allogeneic CAR T cells for the treatment of solid tumors[a]

NCT Number	Location	Status	Tumor entity	Intervention		
				Target	CAR T cell product	Additional treatment[b]
NCT05239143	USA	Recruiting	Advanced or metastatic epithelial derived solid tumors	MUC1C	P-MUC1C-ALLO1 CAR T cells	Rimiducid
NCT04991948	USA, Belgium	Recruiting	Unresectable metastatic colorectal cancer	NKG2D	CYAD-101	FOLFOX, pembrolizumab
NCT03692429	USA, Belgium	Recruiting	Unresectable metastatic colorectal cancer	NKG2D	CYAD-101	FOLFOX, FOLFIRI
NCT04696731	USA	Recruiting	Advanced/metastatic clear cell renal cell carcinoma	CD70	ALLO-316	ALLO-647
NCT04438083	USA	Active, not recruiting	Renal cell carcinoma	CD70	CTX130	
NCT05795595	USA	Recruiting	Relapsed or refractory solid tumors	CD70	CTX131	

[a]Data from to ClinicalTrials.gov.
[b]Abbreviations: FOLFIRI, folinic acid, fluorouracil, and irinotecan; FOLFOX, folinic acid, fluorouracil, and oxaliplatin.

trial has shown convincing safety results with no GvH disease and only a minor cytokine release syndrome in 50% of patients. The absence of side effects is (positively) surprising because CD70 can be found on healthy tissues [3]. The activity of CTX130 was encouraging, and 81% of the patients showed disease control, defined as a complete response (CR) or a partial response and stable disease according to response evaluation criteria in solid tumors (RECIST). Interestingly, one patient (receiving the lowest CTX130 dose) achieved a CR. Of the 16 patients studied, 15 received another therapy in the later course and eight died.

This study, similarly to other allo-CAR T cell studies, encountered the major hurdle of this approach – low persistence of CAR T cells [1,6]: these were not detectable after day 28. Although the authors describe this phenomenon as similar to two autologous CAR T cell studies, low persistence might explain the lower efficacy compared to the COBALT-LYM study where CTX130 was used in lymphoma patients (20% CR). Lastly, the authors nicely show that CD70 continued to be expressed in tumor tissue 42 days after treatment, and CD70 downregulation is therefore an improbable escape mechanism. Nevertheless, CD70 loss at a later timepoint could contribute to immune escape and might be addressed in further studies.

Next, the authors aimed to increase the persistence of CAR T cells by two additional modifications, leading to a new construct, CTX131. Using CTX130 as the backbone, they disrupted the transforming growth factor (TGF-β) receptor TGFβR2, thus preventing the suppressive effects of TGF-β [7]. In addition, they disrupted the endoribonuclease Regnase-1, a T cell regulatory protein which can both activate and, in the case of overactivation, inhibit T cells. Importantly, knockout of Regnase-1 has been shown to prolong T cell persistence [8]. Using an elegant experiment setup, Pal *et al.* tested the isolated role of both modifications in coculture and *in vivo*, and demonstrated superior persistence and activity of CTX131. A Phase 1/2 study (NCT05795595) with CTX131 is currently recruiting patients, and it will be exciting to see whether the preclinical data translate to patient benefit.

Are the knockouts of Regnase-1 and TGFβR2 the key to improving allo-CAR T cell persistence? This cannot be answered yet, but several other approaches are being studied to tackle this barrier [1,6]. These include (i) optimized or more aggressive **lymphodepletion**, for example by using anti-CD52 antibodies, (ii) knockout of molecules such as the 'don't-eat-me' signal through CD47 or the removal of HLA-II, (iii) optimizing the product by using or inducing long-living memory-like cells, and (iv) increasing resistance to oxidative, hypoxic, and metabolic stress, such as through the expression of catalase. One avenue, not yet under exploration, is to combine allo-CAR T cells with pharmacologic modulation. We speculate that targeting the factors that drive RCC, such as HIF signaling, either pharmacologically through belzutifan or through a novel approach using short interfering RNA (siRNA), can reduce RCC tumor burden and thus increase CAR T cell efficiency. Because these approaches might also affect the CAR T cells or decrease the target antigen, sequential application might be preferable. Nevertheless, the use of **off-the-shelf** allogeneic CAR T cells remains an area of high interest and might bring the long-awaited breakthrough in the treatment of solid tumors.

Declaration of interests

The authors declare no competing interests.

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