Antitumor CAR T-cell Screening Platform: Many Are Called, but Few Are Chosen

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Treatment with T cells expressing chimeric antigen receptors (CAR) is a promising anticancer therapy. However, this approach has several limitations and has not yet been effectively applied to treat solid tumors. The study by Panowski and colleagues represents the first comparative analysis of multiple single chain fragment variable (scFv)-based anti-CD70 CAR T-cell clones for the development of a clinical product to treat renal cell carcinoma (RCC). Despite the risk of T-cell fratricide due to CD70 expression on T cells, CD70 CAR T cells were produced successfully thanks to the protective CD70 masking phenomenon. Two distinct classes of CAR T cells were identified with different memory phenotypes, activation statuses, and cytotoxic activity. CD70 CAR T cells presented high cytotoxic activity against RCC both *in vitro* in RCC cell lines and *in vivo* in patient-derived xenograft mouse models. The off-target effects expected on the lymphoid compartment were confirmed by tissue cross-reactivity staining and in a cynomolgus monkey preclinical model with CD3-CD70 bispecific antibody treatment. The efficacy and the toxicity profile of the lead CD70 CAR T-cell candidate instigated the researchers to proceed with upscaled clinical production. This article emphasizes the influence of the scFv of the CARs on their efficacy:toxicity balance. Ultimately, they successfully managed to develop a highly effective CAR T-cell candidate to treat a solid tumor by an allogeneic approach, thereby overcoming two major hurdles to broaden application of CAR T-cell therapy.

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Chimeric antigen receptor (CAR) T-cell therapy represents a true revolution in the field of cancer treatment, especially for hematologic malignancies. Nowadays, there is a gain in momentum for CAR T-cell market authorization, which has grown from two approvals by the FDA in 2017 (Kymriah and Yescarta) to five FDA and EMA approvals in April 2022 (in addition to 2017, Tecartus, Abecma, and Breyanzi). All these market-authorized CAR T cells are produced in an autologous manner, which means the patient is the source of T cells which are then genetically modified. While this approach has resulted in substantial clinical results, the well-established disadvantages have limited the widespread administration of CAR T-cell therapy to patients. Among them, we can cite three major hurdles: the ultra-personalized production process, the selection of the most effective CAR construct, and the failure of the effective treatment of solid tumors (1).

The duration of the manufacturing process of approximately 10 days, along with the manufacturing failure in some patients, delays the availability of treatment by autologous approaches. T-cell variability in terms of quality and quantity inherent to the biological starting material could be considered as the major cause of these disadvantages. Hence, the allogeneic approach is getting increased attention to address these limitations. Thanks to genome-editing nucleases, chronologically zinc finger nucleases and transcription activator-like effector nucleases followed by CRISPR-Cas9, it is now possible to knockout (KO) various genes involved in the allogeneic immune response, namely, the alpha chain of the T-cell receptor (2). Disruption of endogenous T-cell receptors (TCR) also led to improved expression and functionality of transgenic TCRs with a significant increase in the *in vivo* antitumor activity of these modified cells. These were the first steps towards the generation of "off-the-shelf" adoptive T-cell products. There are several applications for these useful DNA-specific molecular scissors, especially in adoptive T-cell transfer settings (3). For instance, multiplex gene editing has allowed the development of TCR, PD-1, CTLA4, and beta-2 microglobulin–deficient allogeneic CAR T cells *in vitro* (4). Recently, Carl June's team demonstrated PD-1 and both alpha and beta endogenous TCR chain KO in a first-in-human study (5).

The study by Panowski and colleagues used a high throughput screening platform to select an optimal single chain fragment variable (scFv) candidate to produce a CAR T cell targeting CD70 (**Fig. 1**; ref. 6). The authors describe the efficacy and safety of fratricide-resistant allogeneic anti-CD70 CAR T cells targeting renal cell carcinoma (RCC). Interestingly, the various scFv could give rise to distinct subsets of CAR T cells with different memory phenotypes, activation statuses, and cytotoxic activity.

The CD70 CAR T cells effectively eliminate renal cancer cells in *in vitro* and mouse *in vivo* models. Unexpectedly, the expression of CD70 on T cells did not induce fratricide of a subset of CAR T cells with specific scFvs. Fratricide resistance is perhaps due to the *cis* masking of CD70 by the CAR. Although the demonstration of masking phenomenon was well-conducted by Panowski and colleagues (6), confocal imaging showing the coexpression of anti-CD70 CAR and CD70 on the surface of CD70 CAR T cells could reinforce this hypothesis (7). Even if it is not the case in this preclinical study, this phenomenon of epitope masking could also be detrimental, as in the case where leukemic cells were transduced by a CAR and could hinder the recognition by antitumor CAR T

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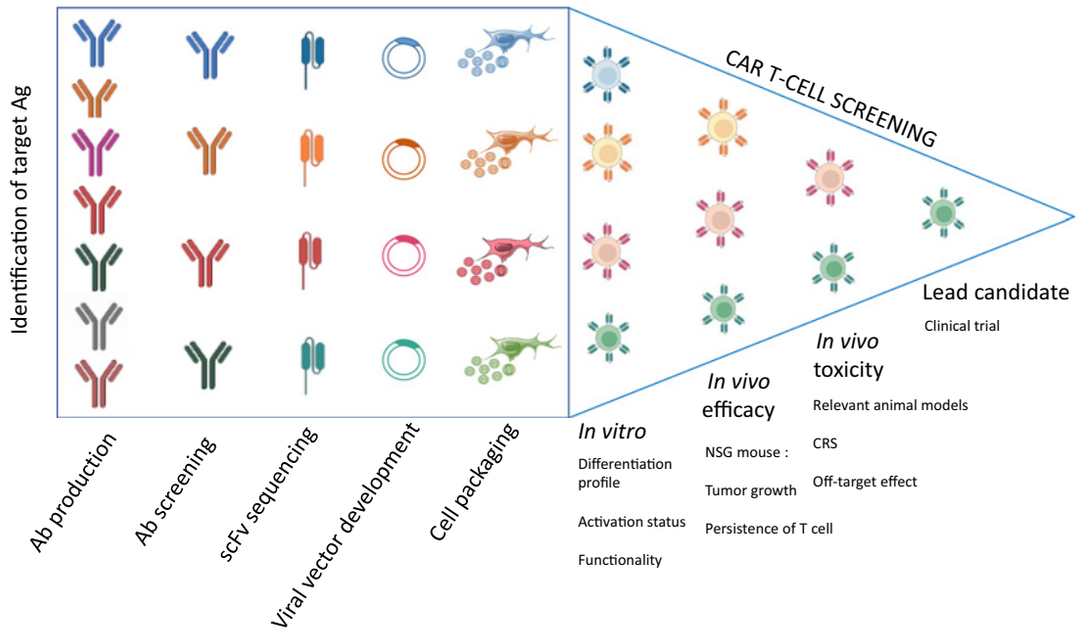

Figure 1.

Antitumor CAR T-cell screening platform. After selecting an antigen (Ag) target of interest, severals steps have to be accomplished before clinical evaluation of CAR-T cells. The first step relies on production of monoclonal antibodies. The second step consists of selection of monoclonal antibodies able to effectively and specifically bind target Ag. Then, scFv from the top monoclonal antibody candidates are sequenced to be integrated into a viral vector, and a packaging cell line is used to produce viral particles to engineer CAR-T cells. The resulting CAR-T cells are then screened. Various readouts can be used to find the best CAR candidate including *in vitro* and *in vivo* assays for evaluating functionality, potency, therapeutic activity, and toxicity.

cells (7). It is noteworthy that this protection is dependent on CAR scFv and highlights the necessity to test several scFv with different CAR constructs to obtain an optimal candidate on all critical points such as toxicity (on-target off-tumor/lymphopenia or treatment resistance) and functionality (fratricide). Although the harmful impacts of fratricide are not fully established, it could decrease the viability of the cells in culture and increase the risk of production failure. As many markers are upregulated on T cells after their activation, fratricide protection of CARs could broaden the possible antigen targets without excluding those expressed by T cells. Another strategy to confer resistance to CD70 CAR fratricide in a controlled manner could be to KO CD70 in T cells. In fact, the authors showed that CD70 KO could improve the functionality of CD70 CAR T cells in an scFv dependent manner. However, given the role of CD27-CD70 signaling in priming, expansion, and memory differentiation of T cells, the effect of CD70 KO or CD70 masking by CAR deserves further consideration during clinical trials.

Targeting CD70, however, does not seem to be completely safe in cynomolgus monkeys treated with a bispecific antibody targeting CD70 and CD3, although the authors mention the CD3 from bispecific antibody might lead to increased cytokine release syndrome compared with CAR T cells.

The choice of CD70 as the target antigen relies on high expression in RCC and minimal expression in normal tissues. However, its expression on activated T cells has to be taken into account as it is necessary to still be cautious about expected lymphopenia toxicity in humans.

So far, the ideal target antigen for CAR T cells in terms of tumor specificity and range of expression by various tumor cells does not exist. Furthermore the severity and outcome of autoimmune side effects linked to off-target effects also depends on the healthy cell type that expresses the target antigen and the availability of supplemental therapy. For instance, B-cell aplasia related to anti-CD19 CAR T-cell therapy is easily manageable with polyclonal antibody treatment (8). As reported recently, the regional delivery of CAR T cells could further improve their efficacy and safety by limiting their systemic side effects (9). Altogether, this highly selective preclinical design of an allogenic CAR T-cell targeting CD70 encourages its future evaluation in clinic.

In conclusion, the CD70 CAR T-cell development presented by Panowski and colleagues combines numerous requirements to improve the CAR T-cell therapy approach and also paves the way for developing other allogenic CAR T-cell therapies in solid tumors.

Authors' Disclosures

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