TanCAR design for PDAC enhances antitumor effects and reduces antigen escape

Miltenyi Biotec

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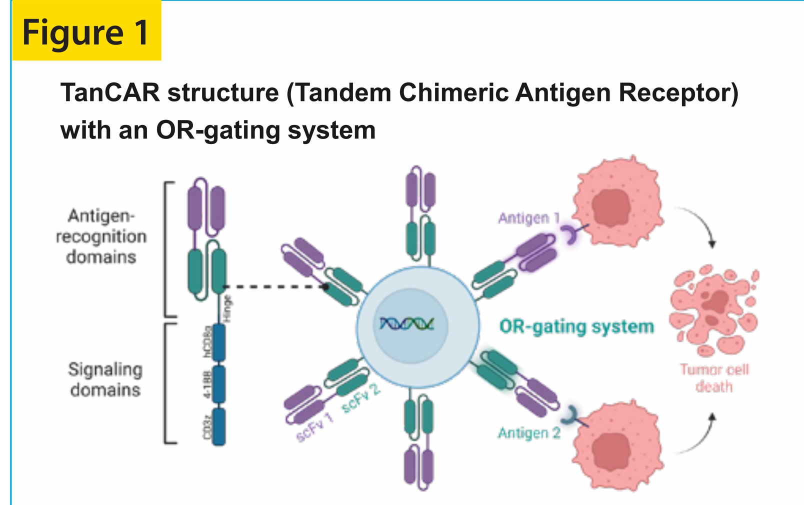
Introduction

Pancreatic cancer is the 12th most common cancer worldwide, with an estimated 500,000 new cases in 2020[1]. With a 5-year survival rate of only ≈11.5%, it is the fourth most common cause of cancer-related deaths[2]. Pancreatic ductal adenocarcinoma (PDAC) accounts for about 90% of all pancreatic cancer cases. Current treatment options are limited and around 80% of cases are unresectable, diagnosed at a late stage and/or metastatic due to lack of specific symptoms, leading it to be resistant to standard drugs or therapies[3,4].

Genetically engineered cell therapies have become a newly established pillar of cancer treatment, with unprecedented success against leukemia. However, they still lack efficacy when it comes to solid tumors.

Additionally, a major obstacle remains the limited spectrum of T cell specificity in the face of tumor heterogeneity and potentially dynamic antigen escape variants, which can lead to tumor recurrence after initial treatment with CAR T cells directed to a single tumor antigen.

Figure 1

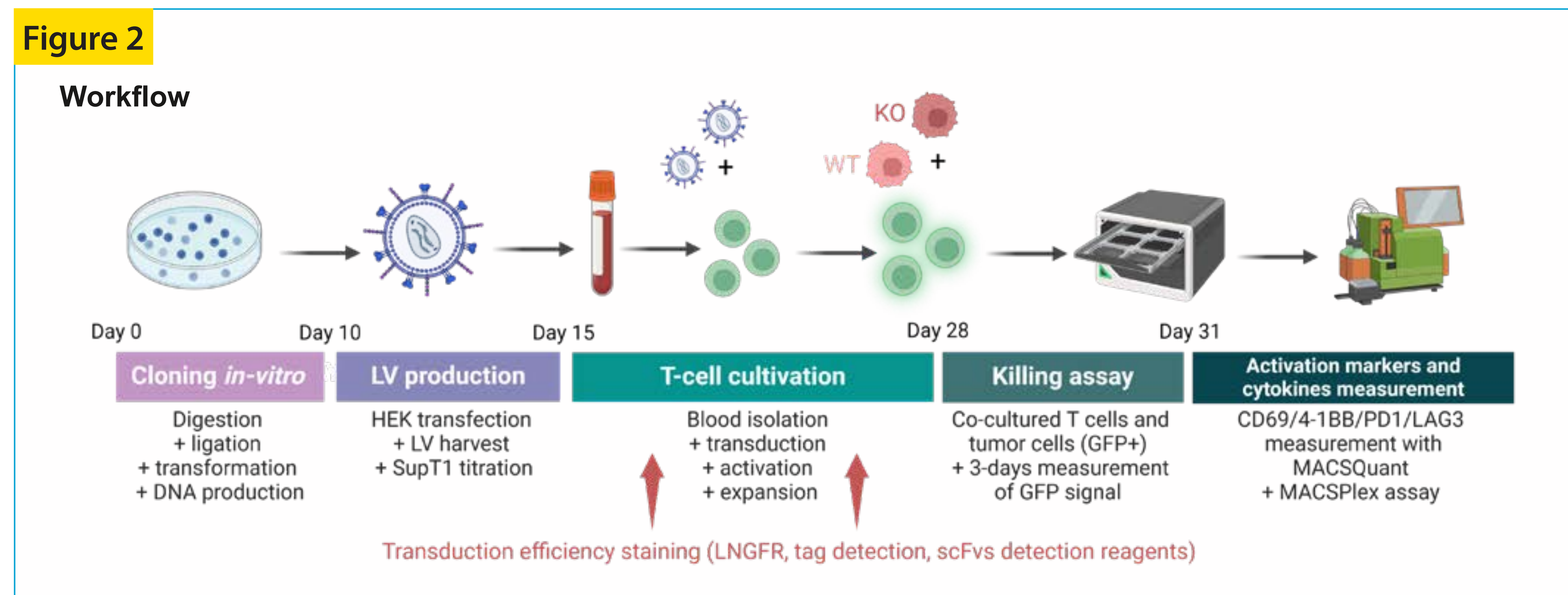


A bispecific CAR molecule with an OR-gated system (tandem CAR, TanCAR) could mitigate antigen escape and improve the anti-tumor activity of CAR T cells. The proof-of-concept has been previously established[5] and we aimed to implement it for PDAC by targeting pre-identified PDAC antigens A, B, and C.

Material and methods

TanCARs were designed with variations of scFv combinations (A+B or A+C), spacer lengths (short hCD8α or very short IgG4 hinge), myc-tag position (C- or N-terminal) and CAR detection methods (tag or detection reagents). TanCARs were cloned *in vitro*. Then, lentivectors (LV) were produced and used for T-cell transduction. After activation and expansion, transduced T cells were co-cultured for 3 days with wild-type (WT) and knock-out (KO) tumor cell lines, reflecting target escape variants. We assessed CAR functionality based on transduction potential, scFv detection, cytotoxic potential, marker upregulation and cytokine release. Finally, data were processed using GraphPad and MACSQuantify to evaluate the functional combinations.

Figure 2

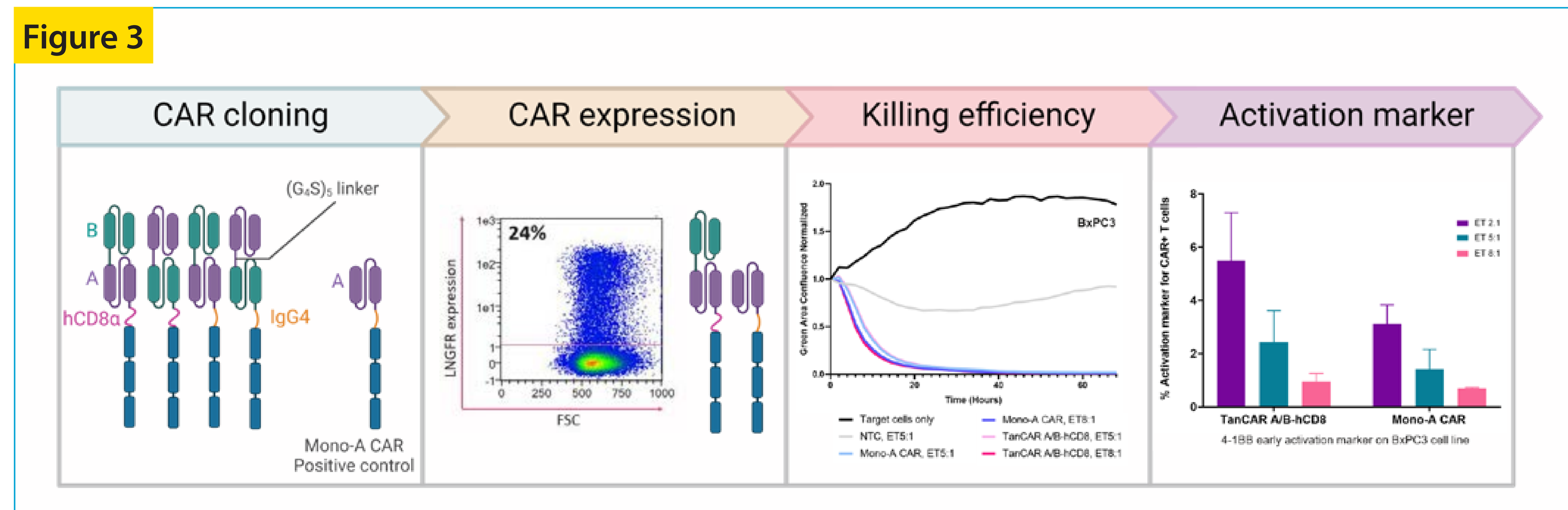


Results

A TanCAR A/B for PDAC proof-of-concept

We first designed TanCARs against targets A and B, without tag. Only the orientation A/B was clonable for both hCD8a and hIgG4 hinges. CAR cassette expression was verified through LNGFR expression and exhibited similar levels as the mono-A CAR positive control. The functionality of A/B-hCD8a TanCAR was assessed by cytotoxicity assays and activation marker staining, for 3 different cell lines, 2 donors and 2 varying E:T (effector-to-target) ratios. Although TanCAR(s) is/are functional, for now it is still unclear due to which scFv.

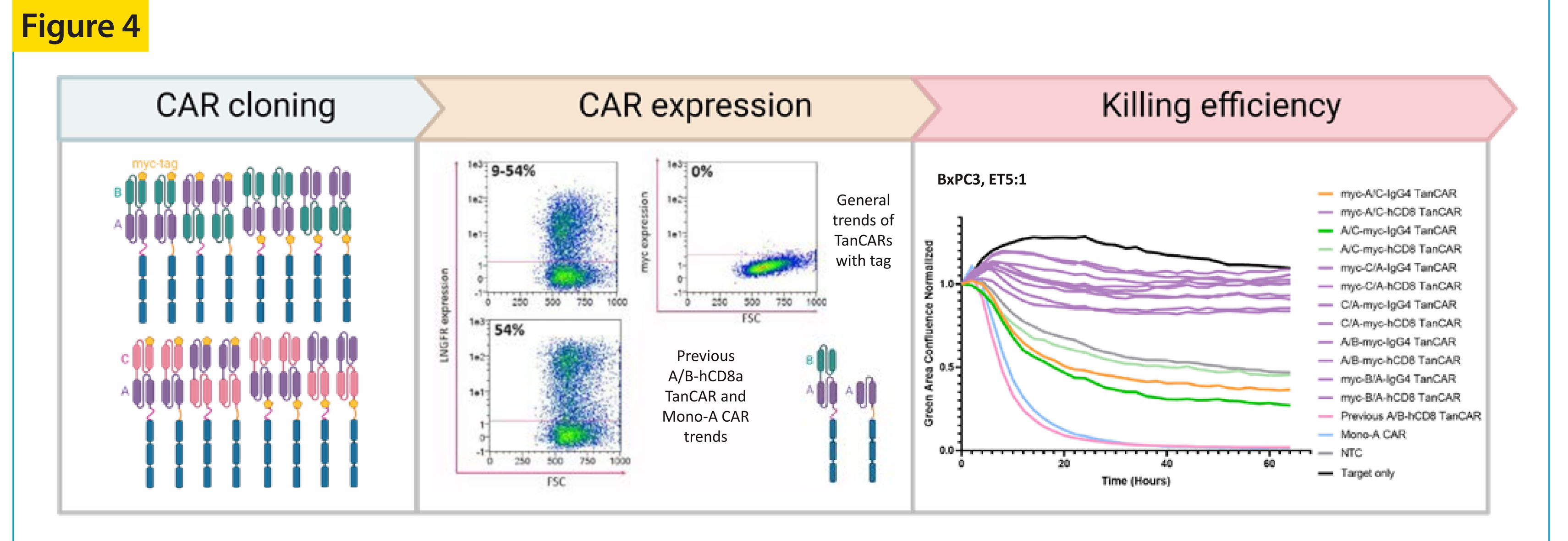
Figure 3



B TanCARs panel with tag

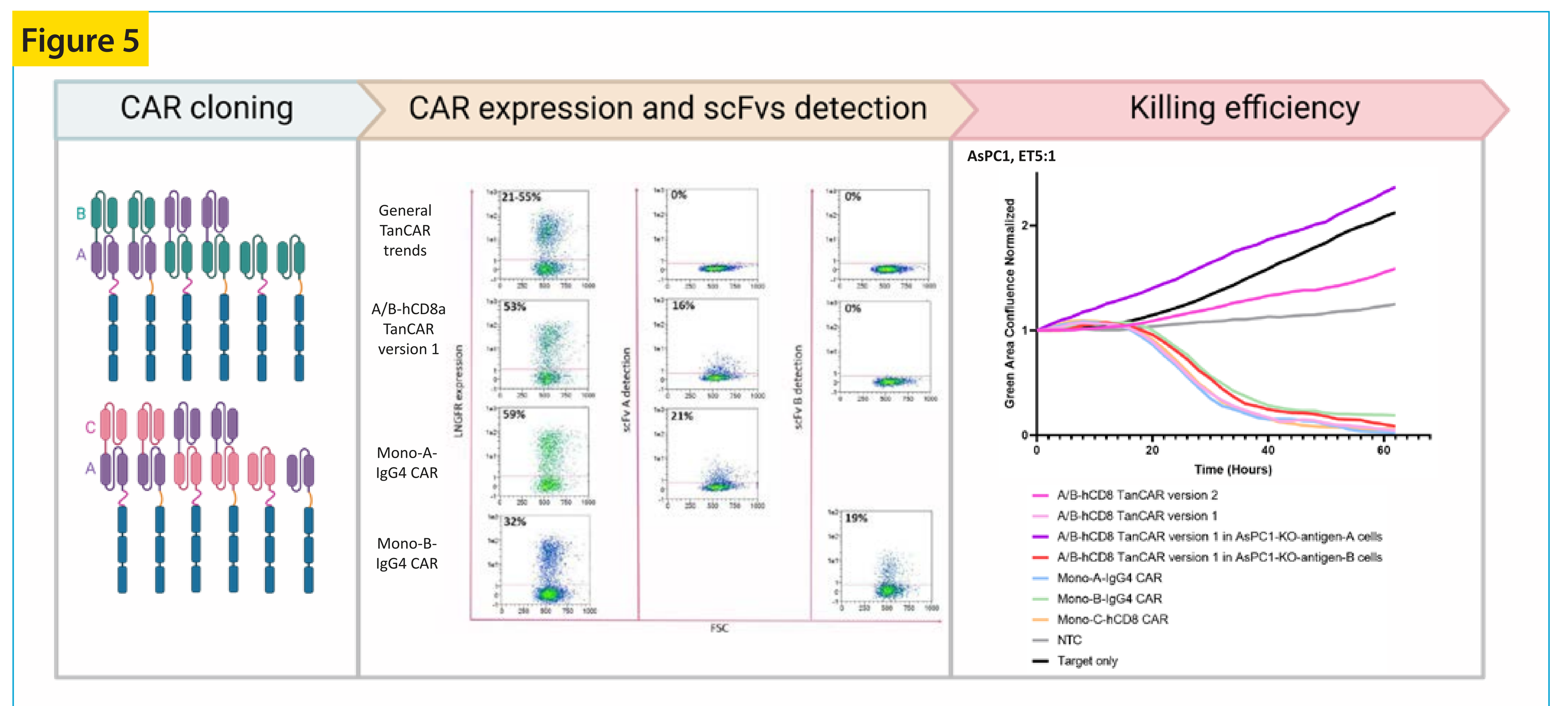
A new panel of TanCARs was designed including a third target (C) and a myc tag (C- or N-terminal) in order to better monitor the TanCAR expression on the cell surface, and not only the CAR cassette expression through LNGFR molecule. We observed as a general pattern, that tagged CARs were less expressed, the tag was not detectable and we got lower or no killing efficiencies as compared to previous non-tagged CARs, leading to the hypothesis that the tag interferes with proper CAR folding. This inspired us to develop detection reagents specific to our scFvs (A, B and C), to enable robust CAR detection, while not impeding CAR folding.

Figure 4



C TanCARs panel using detection reagents to monitor scFv expression

Figure 5



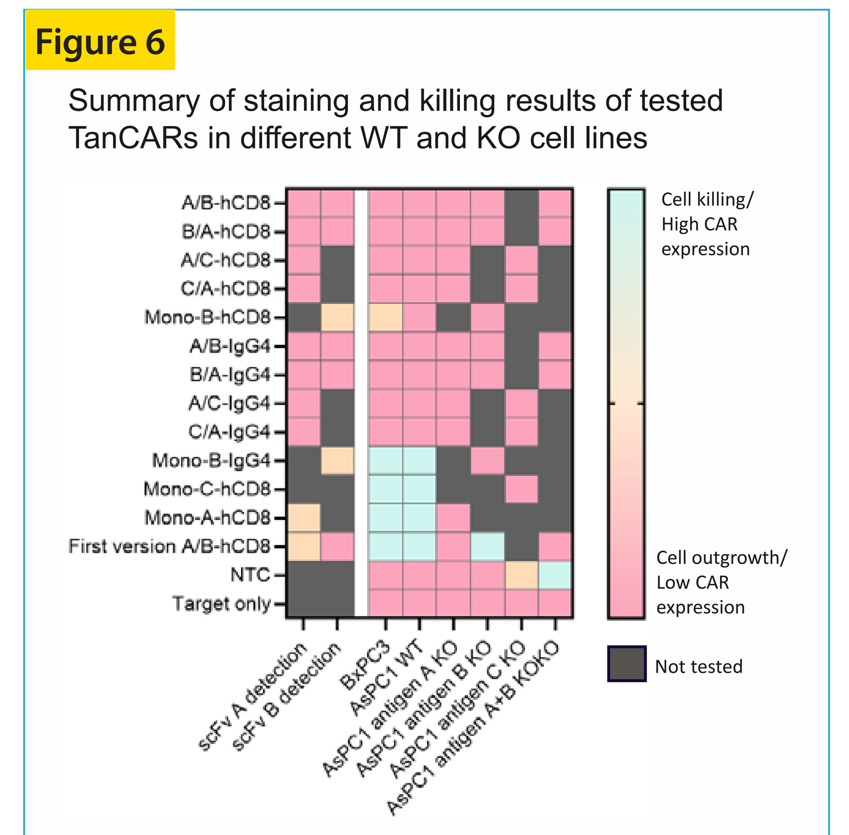
A last panel of TanCARs against targets A, B and C but without tag was designed, including control single CARs (Mono-B and Mono-C), to check functionality of all scFvs. KO cell lines for targets A, B and C, and double KO cell lines for targets A+B and A+C were generated to better assess the influence of each scFv on the functionality respectively.

Expression of scFv A and scFv B was assessed using self-developed detection reagents. No detection reagent against CAR C was available at this time. Screened conditions as well as staining and killing results are recapitulated in the figure 6.

In general, the functionality of all scFv was proven by showing scFv detection abilities and killing efficiencies. However, when these scFvs are combined in TanCARs, the detection and killing is not for all scFvs possible anymore. An exception is the A/B-hCD8a TanCAR without tags. Moreover, this first version of A/B-hCD8a TanCAR was functional only thanks to the N-terminal scFv A.

Figure 6

Summary of staining and killing results of tested TanCARs in different WT and KO cell lines



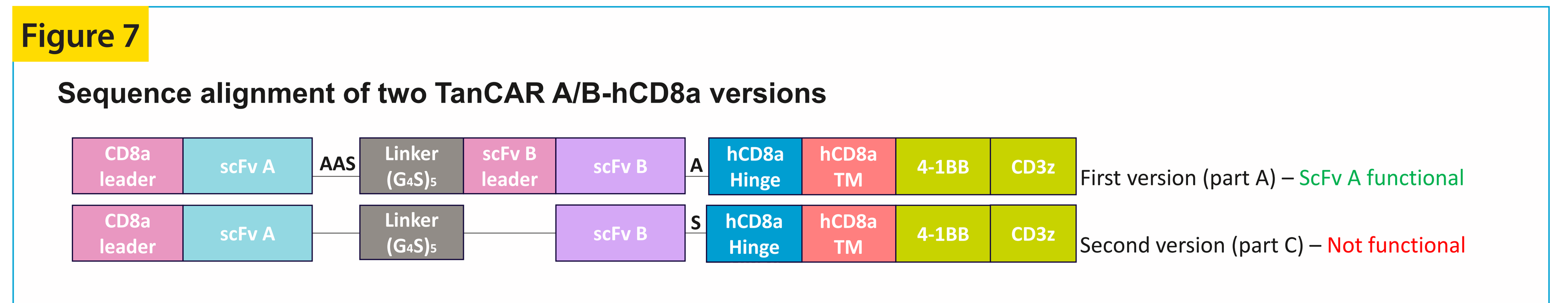
Conclusion and outlook

TanCARs specific for PDAC could enhance immunotherapeutic effects by reducing the chances of tumor antigen escape and increasing T cell functionality. However, we show that some technical aspects need to be considered. We observed that the ability to bind the detection reagent is crucial, and the lack of binding most likely correlates with dysfunction as reflected in the killing assay.

The different behavior of the two A/B-hCD8a CARs remains unclear. One lacks cleavage sites and a second leader. Since the CAR without these modifications was functional, one of these modifications has an impact on the overall functionality of the CAR. Future experiments will investigate G4S linker lengths and VHH domains as binding moieties. In addition, a detection reagent against scFv C will be developed.

Figure 7

Sequence alignment of two TanCAR A/B-hCD8a versions



Once the best functional combinations have been identified, the TanCAR concept can be extended to other target combinations and other solid tumor entities. Future studies should investigate whether our *in vitro* results can be replicated in murine models. In addition, the use of dual antigen-specific CARs, TanCARs, carries an increased risk of on-target/off-tumor toxicities that should be carefully managed.

References
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