Ultrahigh-content imaging helps to identify CAR target candidates against pancreatic adenocarcinoma

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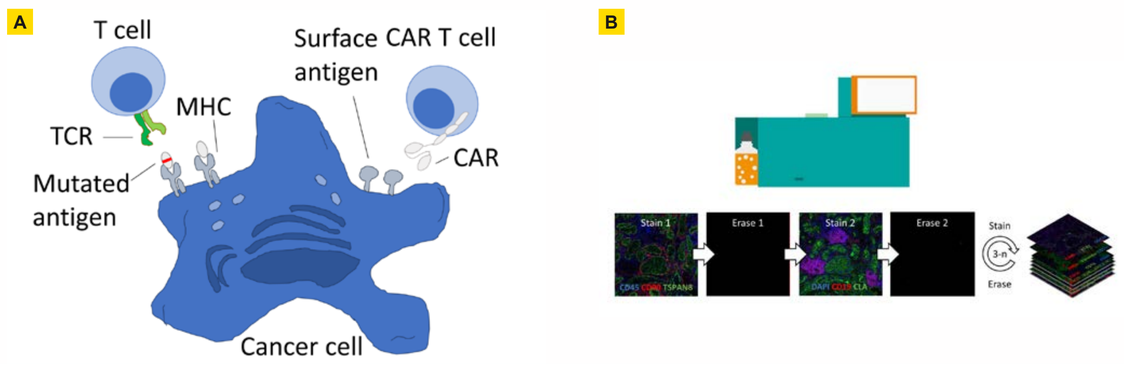
Introduction

Chimeric antigen receptor (CAR) T cells have become a new pillar of cancer therapy. They proved outstanding efficacies in leukemic patients, formerly believed to be beyond treatment. However, their remarkable success in the context of liquid tumors could not yet be translated to the field of solid malignancies. CARs enable T cells to unfold their cytotoxic potency independent of the natural T-cell receptor (Fig. 1a). One major issue in CAR therapy remains the restricted availability of safe tumor-associated antigens (TAA) with restricted healthy tissue expression, that can be targeted by CARs. Here, we show how the newly developed cyclic immunofluorescence microscopy platform MACSima can be integrated in a wholesome and comprehensive workflow to evaluate target expression on tumor cells, as well as healthy tissue expression and to support functional studies. The MACSima platform operates by iterative fluorescence staining, imaging, and signal erasure, enabling the operator to identify and compare the expression of dozens of targets on the very same tissue section (Fig. 1b).

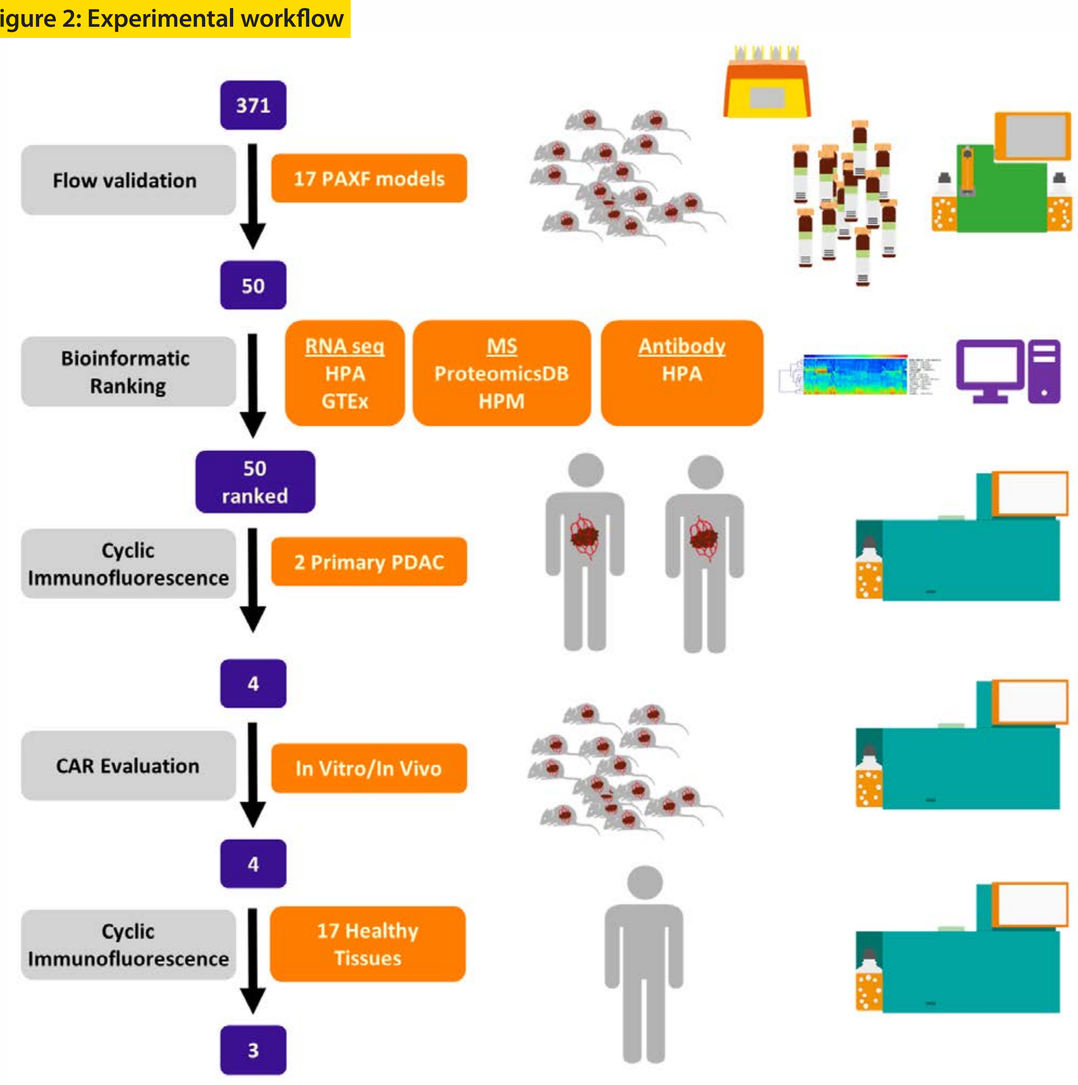
Figure 1: Principle of CAR therapy and MACSima workflow



Methods

We screened around 400 surface antigens on 17 patient derived xenografts (PDX) using flow cytometry (Fig. 2). Expressed target candidates were prioritized based on healthy tissue expression using RNAseq, mass spectrometry and antibody databases. The novel MACSima platform was used to verify the expression of target candidates on human pancreatic tumors and dissecting the different cell linages within. 32 CAR constructs were designed specific for suitable targets and evaluated *in vitro* using cytokine release, marker expression and killing as read-outs. Most promising constructs were challenged *in vivo*. Here, MACSima helped to investigate differences between treatment groups and escape mechanisms. Finally, a healthy tissue multiarray was analyzed, to confirm low expression of the chosen targets.

Figure 2: Experimental workflow

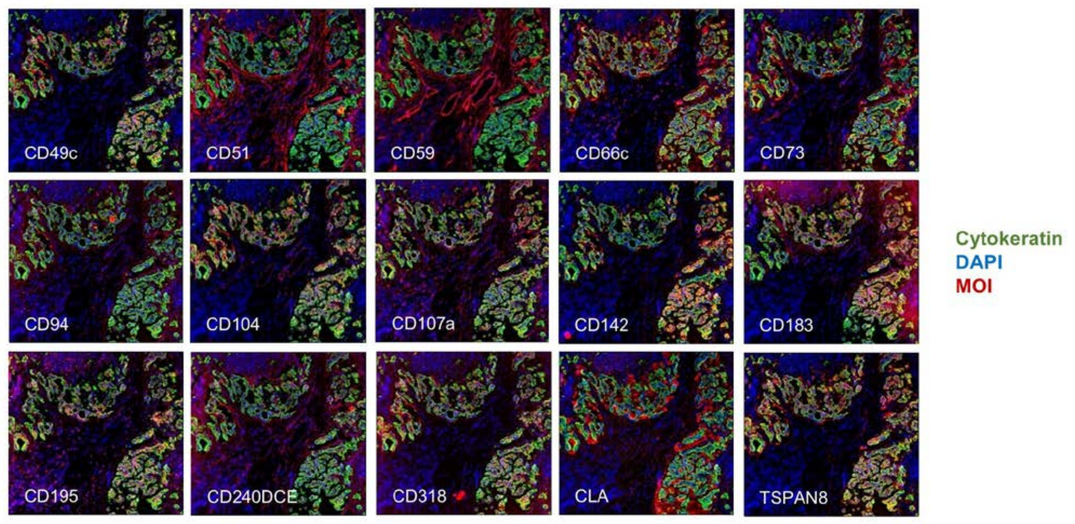


Results

1 Identification of tumor specifific markers

We identified a set of 50 surface antigens on the PDXs in the initial flow screening. RNAseq, mass spectrometry and antibody databases were used to prioritize the target candidates and the MACSima platform helped to reveal, that CLA, CD66c, CD318 and TSPAN8 were the targets with the highest tumor specificity and safety profile (Fig. 3).

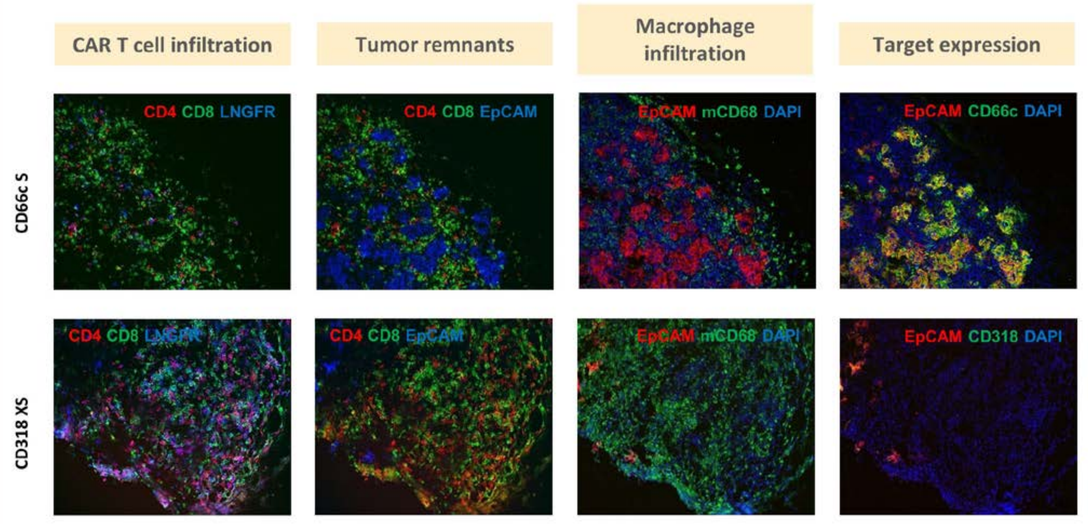
Figure 3: Evaluation of 15 markers of interest on the same tissue section



2 Functional analysis of CAR efficacies

While for CD66c, CD318 and TSPAN8 most efficient CAR constructs were determined *in vitro*, no effective CAR T cells specific for CLA could be generated, due to self-antigenicity. These CARs were also efficient *in vivo* and MACSima was used to show that low efficacies are not caused by target downregulation, but are rather caused by intrinsic CAR function (Fig. 4).

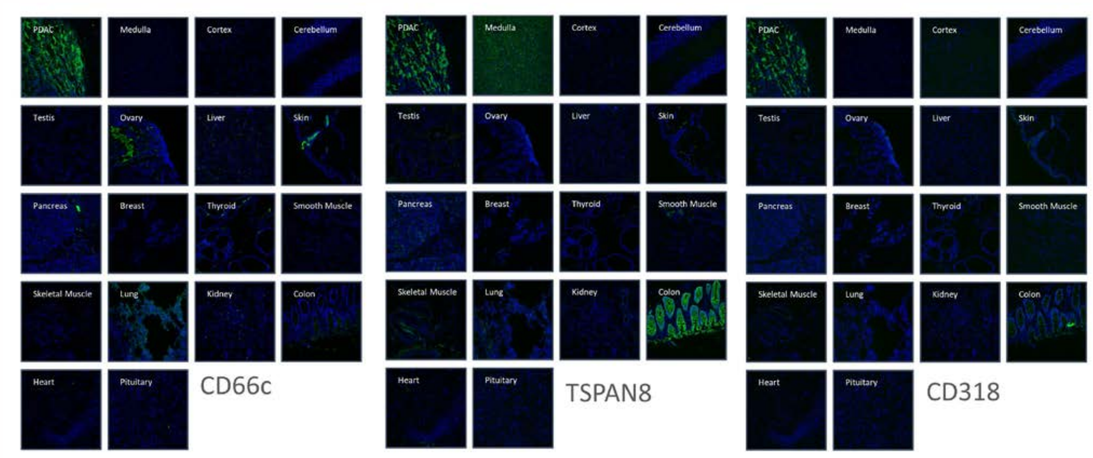
Figure 4: Multiparameter comparison of ex vivo xenofrafts on therapeutic efficacy



3 Evaluation of target expression on healthy tissues

MACSima unravelled also the restricted healthy tissue expression of CD66c, CD318 and TSPAN8, making them interesting targets for CAR treatment (Fig. 5). Overall, the newly developed cyclic immunofluorescence platform proved to be a powerful and versatile tool, when it comes to multiplexing on the very same tissue section.

Figure 5: Healthy tissue expression of target candidates



Conclusion

Here we introduce a novel comprehensive workflow for target identification in an immunotherapy setting. The novel MACSima platform proofed to be a useful tool throughout the whole procedure, resulting in the discovery of novel CAR targets. MACSima facilitates target discovery and evaluation by:

- High-content imaging of tissue samples, enabling analysis of hundreds of markers in a single experiment.
- Allowing for verification of target specificity and helping to understand healthy tissue expression.
- Helping to understand escape mechanisms during *in vivo* trials.

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Data from: Schäfer et al. Identification of CD318, TSPAN8 and CD66c as target candidates for CAR T cell based immunotherapy of pancreatic adenocarcinoma. (2021) *Nature Communications*