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Integrated workflow solutions for the early screening of target candidates for immunotherapy of pancreatic cancer

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Background

The development of successful therapeutics demands an accurate identification of biological targets. This is particularly challenging in cancer, where the effectiveness of novel immunotherapies, such as those based on chimeric antigen receptor (CAR) T cells, is hampered by the lack of suitable tumor-specific antigens. A valid model for systematic screenings of therapeutic targets is represented by primary tumor cells derived from patient-derived xenograft (PDX) and primary solid tumors.

However, a prior consideration of related experimental challenges is necessary. The dissociation of tissues to release intact individual cells with preserved surface antigen epitopes is a crucial step in every investigation of drug targets and biomarkers. Equally important is the purification of target human tumor cells from the mouse cell suspension, regardless of the cancer cell phenotype, for a systematic tumor-specific antigen expression analysis. Samples with contaminating mouse cells, degraded surface antigen epitopes, clumps, debris, and dead cells are indicative of poor quality and can lead to a lack of biological understanding and thus to a difficult translation into clinical utility, while increasing overall experimental costs. It is therefore of utmost importance to utilize tissue and cell preparation approaches which provide high-quality samples while still ensuring a quick, streamlined process. Last but not least, lack of proper analysis tools can slow down the target identification process. Screening of large numbers of targets with manual processing can not only be extremely laborious and time consuming, but also can result in poor-quality data that are often irreproducible.

To address all the challenges described above, our research teams combined automated methodologies for gentle tumor dissociation, immunomagnetic untouched isolation of tumor cells, and high-throughput flow-cytometric analysis from Miltenyi Biotec (fig.1). As a result, we identified novel target candidates for immunotherapy of pancreatic cancer. This application note is based on reference 1.

Methods

Sample collection
All PDAC PDX models were obtained from Charles River Discovery Research Services Germany GmbH. Tumor tissues from PDAC patients undergoing surgical resection of tumor mass were collected at the University Medical Center Göttingen.

Automated dissociation of xenograft and primary tumors
Xenograft and primary tumors were dissociated into single-cell suspensions using the Tumor Dissociation Kit, human, on the semi-automated gentleMACS™ Octo Dissociator with Heaters as per manufacturer's instructions.

Automated immunomagnetic mouse cell depletion
Human tumor cells were automatically purified from the dissociated PDXs by immunomagnetic mouse cell depletion using the Mouse Cell Depletion Kit on the autoMACS® Pro Separator, as per manufacturer's instructions.

Flow-cytometric antigen expression analysis
As quality control of each workflow step, one aliquot of the dissociated PDX and PDAC samples, as well as one of the isolated tumor cells, was stained with REAfinity™ Recombinant Antibodies and analyzed for purity via flow cytometry. For the high throughput screening of antigens, isolated tumor cells were analyzed using the MACS® Marker Screen, human, a monoclonal antibody panel containing 371 pre-titrated antibodies with nine isotype controls, or candidate antibodies selected from this panel for subsequent screening steps.

All samples were measured on the automated MACSQuant® Analyzer 10 and analyzed using the MACSQuantify™ Software v2.13.0, FlowLogic v10.7.1 and Microsoft Excel for Windows 2016.

Results

Generation of pure, untouched, and generic human tumor cells from xenograft tumors
Overall, we analyzed 17 PDX models representing 15 different mutational backgrounds.

Reliable drug target discovery on primary solid tumor specimens requires effective, yet gentle dissociation of the tumor tissue into single-cell suspensions with preserved antigen epitopes. Combined mechanical and enzymatic dissociation with the automated gentleMACS™ Octo Dissociator with Heaters and the Tumor Dissociation Kit resulted in cell samples that were appropriate for subsequent purification of tumor cells and further downstream analysis.
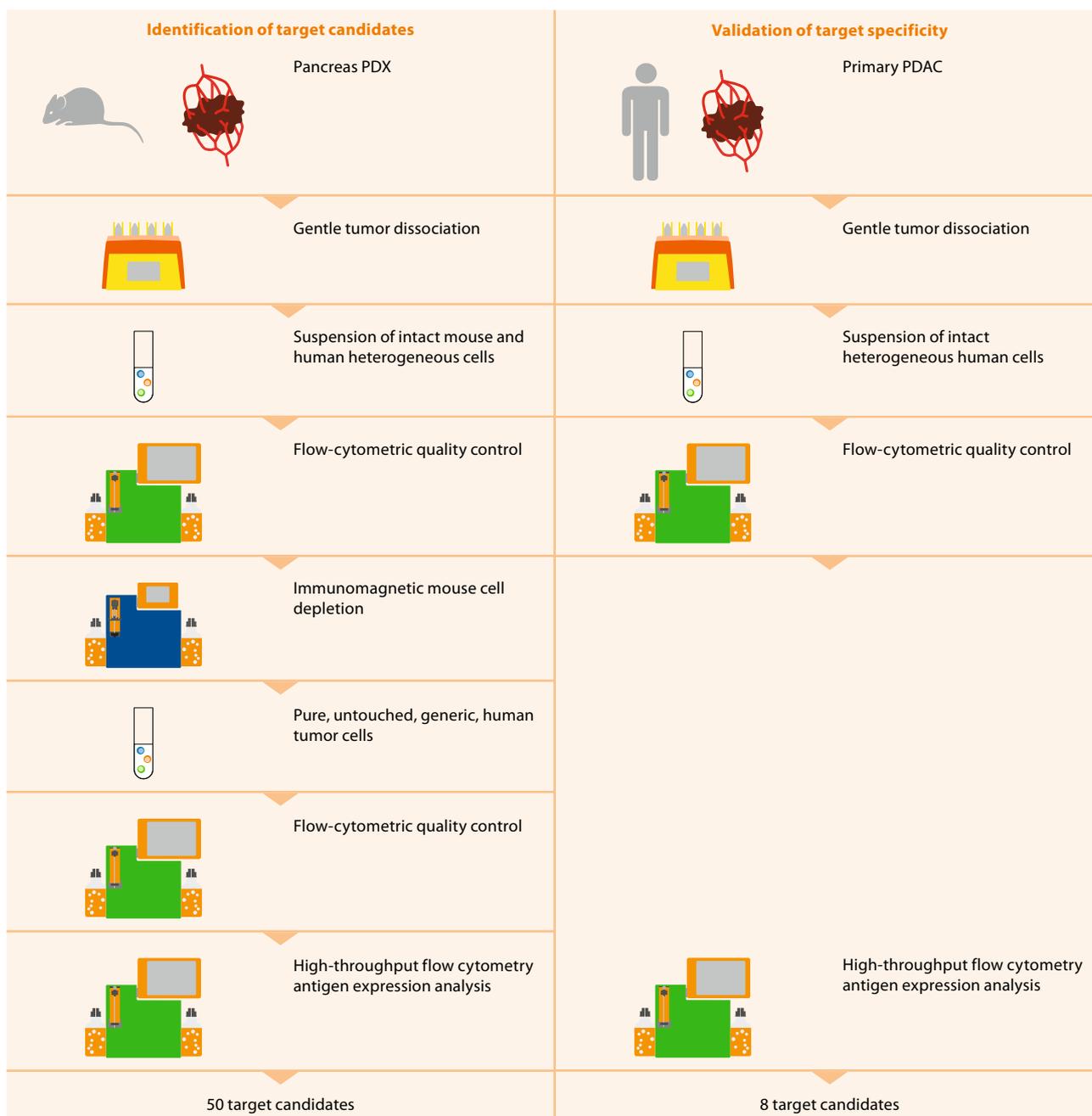

Figure 1: Overview of integrated workflow solutions for drug target screenings in cancer. For the identification of target candidates, the automated workflow starts with the gentle dissociation of PDX tumor specimens into viable and intact heterogeneous cell suspensions. Generic human tumor cells are then separated from the mouse cells with an immunomagnetic, untouched approach. A total number of 371 tumor-specific antigens are then screened via high-throughput flow cytometry, narrowing down the field to 50 target candidates. Initial observations are then validated with regards to expression and specificity of selected targets. Their differential expression is assessed on dissociated primary cells of pancreatic ductal adenocarcinoma (PDAC) patients via flow cytometry, leading to eight target candidates. After each step of the workflow, flow-cytometric quality control is performed.

The Mouse Cell Depletion Kit and the autoMACS® Pro Separator can be used to effectively deplete mouse cells from dissociated mouse xenograft tumors in less than 20 minutes, thereby providing highly pure target human tumor cells. The MACS® negative selection approach ensured the collection of generic, untouched cancer cells which were then truly representative of the tumor heterogeneity and have no blocked key antigen epitopes. Furthermore, the high purity of the target tumor cells (>99%) enabled downstream high-resolution flow cytometric analysis. The use of REAfinity™ Antibodies on the MACSQuant® 10 Analyzer guaranteed a background-free, reliable, and yet fast assessment of cell composition and purity (fig. 2).

Identification of tumor-specific target candidates on pancreatic cancer PDXs

The combination of the MACS Marker Screen on the MACSQuant 10 Analyzer allowed the systematic, standardized, and high-throughput screening of hundreds of markers on multiple samples in parallel directly from the 96-well plate in a completely automated manner. We started by screening 371 antigens (including some in clinical investigation for CAR T cell–based treatment of PDAC) on purified and untouched human tumor cells, and we found 105 antigens

to be expressed on more than 10% of tumor cells on at least one of the PDXs (data not shown). We then used antibodies specific for these 105 antigens and measured their expression on additional PDX models, followed by a manual exclusion of non-suitable target candidates. We measured the remaining 50 surface antigens, which were expressed in at least 20% of all tumor cells of at least three out of four PDX models, on further PDX models (fig. 3).

Next, we investigated the RNA and protein expression profiles of these target candidates, which are available in public online data banks, and we prioritized the 50 target candidates with respect to their off-tumor expression.

Validation of target expression and specificity on primary PDAC tumors

We further investigated the spatial distribution of target candidate expression in PDACs with the proprietary ultra-high content MACSima™ Imaging Platform (data not shown). Our findings led to the decision to further verify the specificity of eight target candidates, namely CD49c, CD66c, CD73, CD104, CD142, CD318, CLA, and TSPAN8, on primary PDAC tumors via flow cytometry.
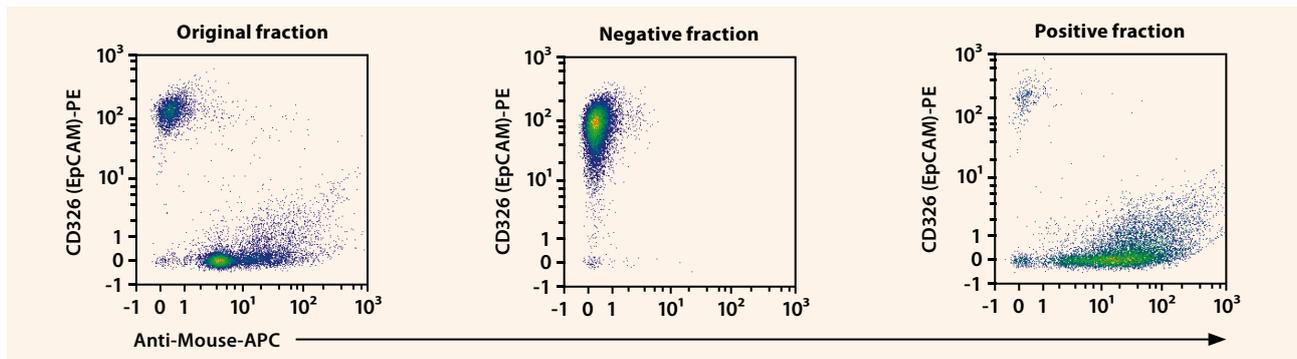

Figure 2: Exemplary depletion of mouse cells from human tumor xenografts. After depletion, both positive and negative cell fractions were stained with REAfinity APC-conjugated murine antibody cocktail anti- CD31, -CD45, -CD81, -CD9, and -Ter119, as well as with the anti-human CD326-PE, and processed via flow cytometry for purity assessments. It was possible to successfully eliminate >99% of the contaminating mouse cells.
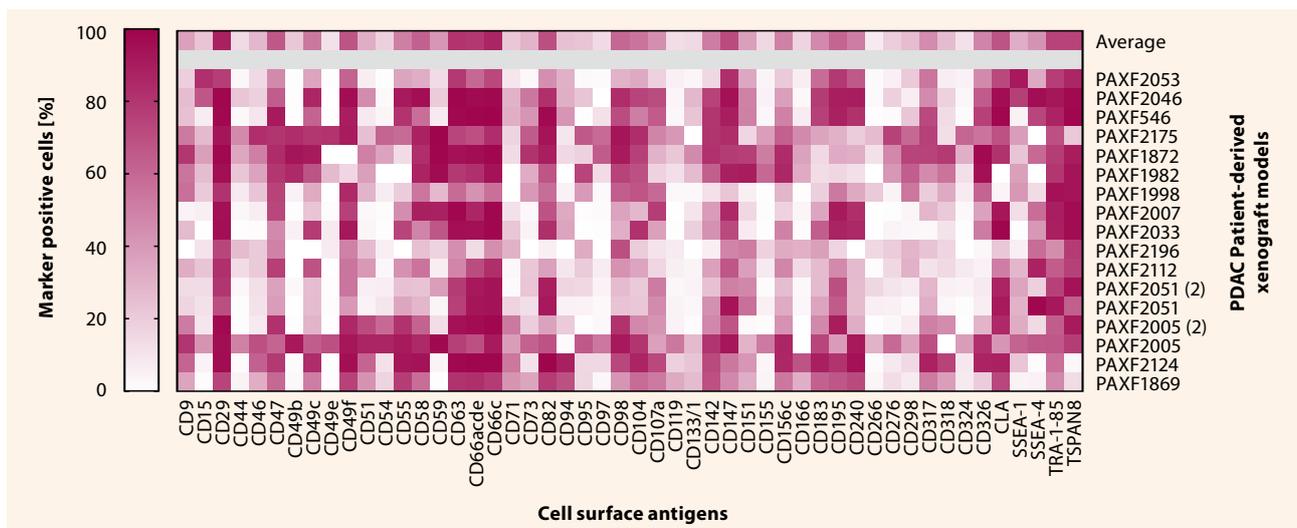

Figure 3: Top 50 antigens expressed on the cell surface of PDAC PDX models as determined by flow cytometry-based screening of a 371 monoclonal antibody panel. Adapted from Schäfer *et al.*, 2021.
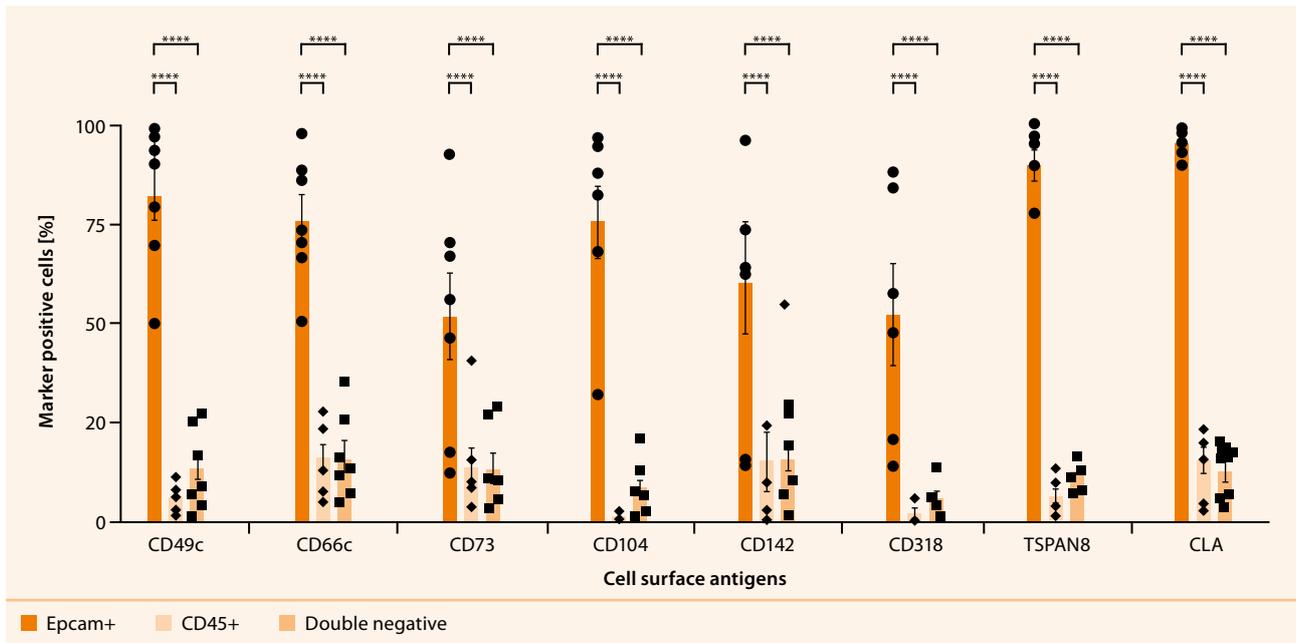
Figure 4: Flow-cytometric analysis of expression for eight target candidates on primary human PDAC samples. Data represent mean ± s.e.m. (n = 7, ****P < 0.0001 determined through two-way ANOVA with Tukey's test). Adapted from Schäfer *et al.*, 2021.

We dissociated seven human PDAC specimens and gated either on tumor cells (EpCAM+), leukocytes (CD45+), or other cells (double negative). It was shown that these eight candidates indeed showed a significant enrichment of expression on tumor cells (fig. 4).

Conclusion

The present data show that our integrated workflow solutions based on automated technologies allow for a fast and yet reliable systematic screening of drug target candidates on solid tumors:

- The gentleMACS™ Portfolio enables the effective dissociation of human and mouse tumors while preserving the cell viability and key antigen epitopes

- MACS® Technology enables the purification of tumor cells regardless of their marker expression. The isolated generic tumor cells are then representative of the tumor heterogeneity and are truly untouched with all antigen epitopes still available

- For high-quality target screening and analysis, our REAfinity™ Recombinant Antibodies offer background-free and reproducible results. Moreover, the MACSQuant® Flow Cytometer allows full automation with high-throughput features for efficient acquisitions

MACS Product	Order no.
Gentle Tumor Dissociation	
gentleMACS Octo Dissociator with Heaters	130-096-427
gentleMACS C Tubes	130-093-237
Tumor Dissociation Kit, human	130-111-570
Untouched Generic Tumor Cell Isolation	
autoMACS™ Pro Separator – Starter Kit	130-092-545
Mouse Cell Depletion Kit	130-104-694
Flow cytometry high-throughput analysis	
MACSQuant® 10 Analyzer	130-096-343
MACS® Marker Screen, human	130-127-043
REAfinity™ Recombinant Antibodies	https://www.miltenyibiotec.com/recombinance

References

1. Schäfer, D., Tomiuk, S., Küster, L.N. *et al*. Identification of CD318, TSPAN8 and CD66c as target candidates for CAR T cell based immunotherapy of pancreatic adenocarcinoma. Nat Commun 12, 1453 (2021). https://doi.org/10.1038/s41467-021-21774-4

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