A TGF-β-directed peptide vaccine induces T cell activation & drives anti-tumor activity by modulating the architecture of the tumor microenvironment

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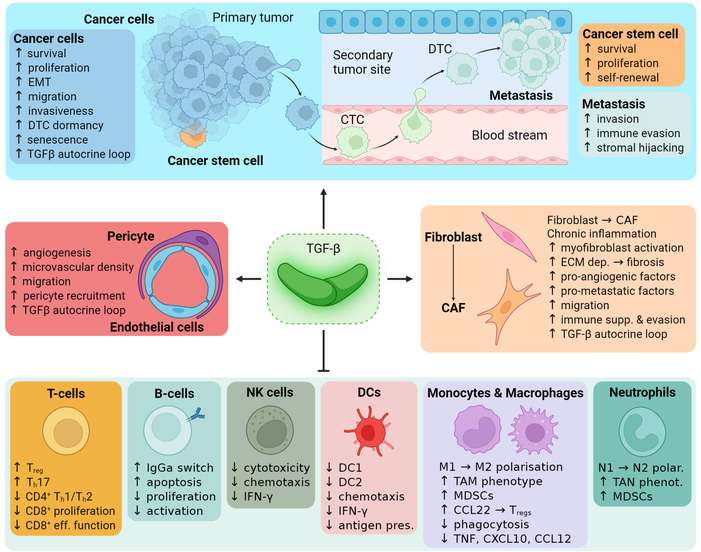
Background: why TGF-β?

Aberrant Transforming Growth Factor (TGF)-β signaling is linked to all hallmarks of cancer, with molecular cues that are cell type-specific & context-dependent.



In clinical studies, global inhibition strategies fell short of the anticipated success mainly due to systemic toxicity, suggesting that TGF-β modulation –rather than its inhibition—could be the key in sustaining meaningful antitumoral activity. In support of this, naturally occurring T cell against TGF-β were reported in man[1,2].

Here, we report the preclinical development of mIO170, a peptide vaccine against TGF-β epitopes based on our proprietary T-win® platform. T-win® is the first immune-modulating vaccine platform directed against both tumor cells and the most important immune-suppressive cells in the tumor microenvironment (TME).

Conclusions

We utilized our T-win® platform to develop a TGF-β vaccine that showed promising *in vivo* results: the vaccination led to a significant tumor growth inhibition in both the PDAC and prostate cancer models, coupled with immune responses and changes in the local tumor microenvironment. The treatment did not affect the overall well-being of the mice, and did not cause overt off-target effects.

Moreover, in the PDAC model, the vaccination ameliorated the fibrotic response measured as α-SMA positivity and increased the infiltration of F4/80+ macrophages. In the prostate cancer model, the preliminary observations from the spatial analysis suggest that the TGF-β peptide vaccine enriched for a tissue ecology where malignant cells and specific components of the immune system intermingle to actively limit tumor progression.

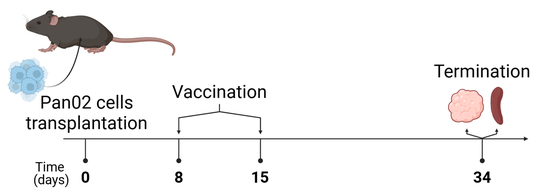
Collectively, our data warrant further interrogation to better understand the efficacy & the safety profile of this novel TGFβ-targeting strategy.

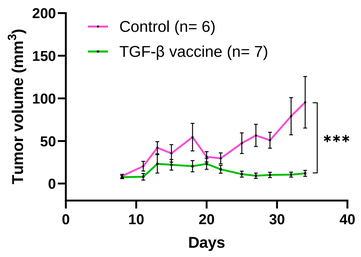
Acknowledgments & References

We would like to thank Associate Professor Martin Kristian Thomsen at Aarhus University (Denmark) for the access to the prostate cancer model.

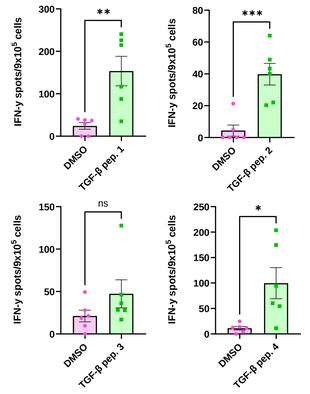
1. Holmström *et al.,2021*
2. Mortensen *et al., 2021*
3. Cai *et al., 2024*
4. Stoltzfus *et al., 2020*

1. mIO170 inhibits the growth of pancreatic ductal adenocarcinomas and induces immune responses against TGF-β epitopes



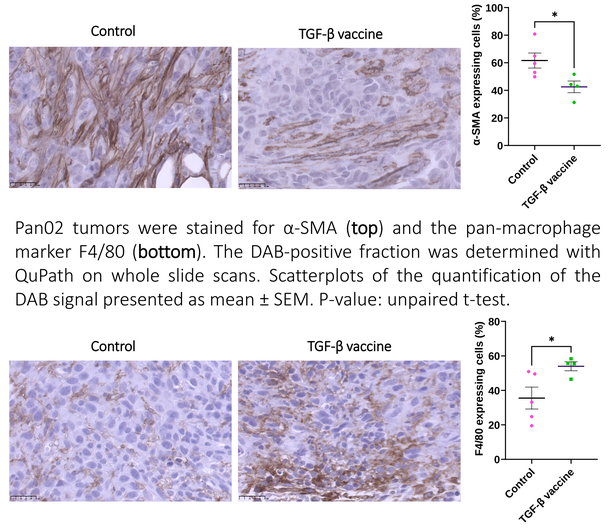


Top: Pancreatic adenocarcinoma (PDAC) Pan02 cells (5 x 10^5) were transplanted *s.c.* into the right flank of 10-week-old C57BL/6 female mice (n = 13). At randomization, mice received a control vaccination (DMSO in Montanide) or a TGF-β vaccine (4 peptides in Montanide) at day 8 and 15.
Left: Average tumor growth of the two groups presented as mean ± SEM. P-value: unpaired t-test.



Top: The presence of TGF-β specific T cells was quantified in total splenocytes *via* IFN-γ ELISpot assay after overnight stimulation. Scatterplots for the quantification of each peptide presented as mean ± SEM. P-value: unpaired t-test.

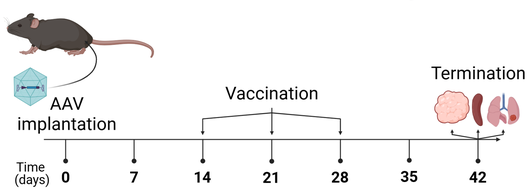
2. Immunization against TGF-β peptides alters the TME

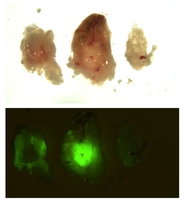


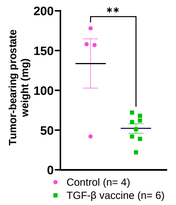
Pan02 tumors were stained for α-SMA (**top**) and the pan-macrophage marker F4/80 (**bottom**). The DAB-positive fraction was determined with QuPath on whole slide scans. Scatterplots of the quantification of the DAB signal presented as mean ± SEM. P-value: unpaired t-test.

3. mIO170 slows progression of prostate cancer *in vivo*

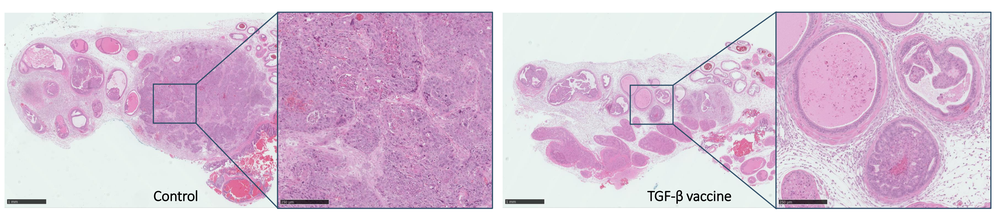
B6J.129(B6N)-*Gt(ROSA)26Sor*$^{tm1(CAG-cas9*,-EGFP)Fezh}$/J x STOCK Tg(Pbsn-cre)4Prb/J



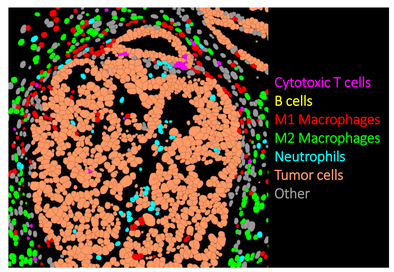




Top: Adeno-associated virus particles (AAV, 10^9 virus genome) were orthotopically injected in the lateral prostatic lobes of 9-to-12-week-old Pb4-Cas9/EGFP male mice (n = 10) as previously established[3]. Following randomization, mice received a control vaccination (DMSO in Montanide) or a TGF-β vaccine (4 peptides in Montanide) at day 14, 21, and 28. The GFP signal was used to guide tumor identification. Average weight of the tumor-bearing prostate lobes presented as mean ± SEM. P-value: unpaired t-test.
Bottom: Hematoxylin and Eosin staining of tumors collected at sacrifice. This histological assessment showed that the TGF-β vaccine delayed the progression to overt carcinomas, as exemplified by several hyperplastic glands that have not yet breached the basement membrane to invade the surrounding tissue (see inlet).

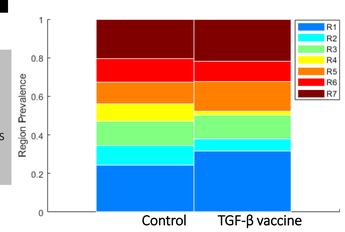


4. The TGF-β vaccine promotes spatial niches with specific immune signatures



Left: As a proof-of-concept, one prostate tumor slide from each cohort was stained with the PhenoCode Signature Discovery Mouse FFPE Immuno-Oncology Panel for PhenoCycler-Fusion (Akoya Biosciences). The 24 markers were analyzed with QuPath for cell segmentation and phenotyping of seven different cell types.

Region 1: other cell types
Region 2: M2 macrophages & B cells
Region 3: Tumor cells, cytotoxic T cells, neutrophils
Region 4: Neutrophils, M1 macrophages, B cells
Region 5: Tumor cells, cytotoxic T cells, B cells, other cells
Region 6: cytotoxic T cells, tumor cells, neutrophils
Region 7: other cell types



Top: Cell phenotypes and tissue coordinated were used to generate neighborhoods with CytoMAP[4]. Neighborhoods inform about cell composition, density, & marker expression. Neighborhoods are clustered into regions, *i.e.* areas with similar cellular composition. The prevalence of the 7 regions we obtained are presented in the barplot. The TGF-β vaccine group showed a lower proportion of region 2 (M2 macrophages), and region 4 (neutrophils/M1 macrophages/B cells), and an increased prevalence of region 5 (cytotoxic T cells, B cells and tumor cells), indicative of active immunosurveillance against tumor cells.