A TGFβ-directed immune-modulatory vaccine induces T cell activation and drives antitumor activity by modulating the tumor microenvironment

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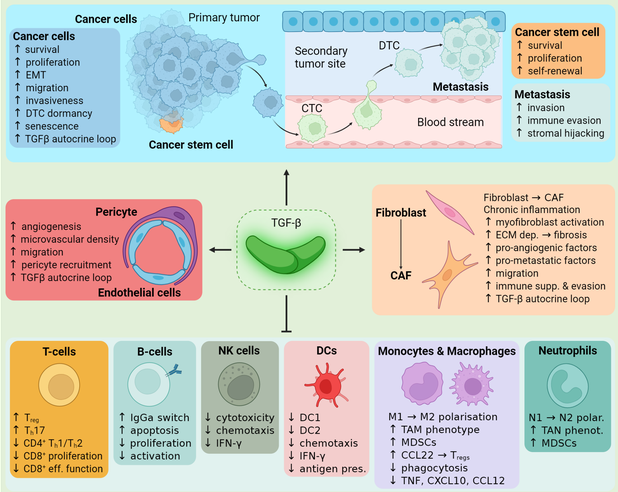
Poster 2257

Background

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 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). The first T-win® clinical program IO102-IO103 against IDO1+/PDL1+ cells, is now being investigated in a Phase 3 clinical trial in advanced melanoma and in a Phase 2 trial in other tumor types.

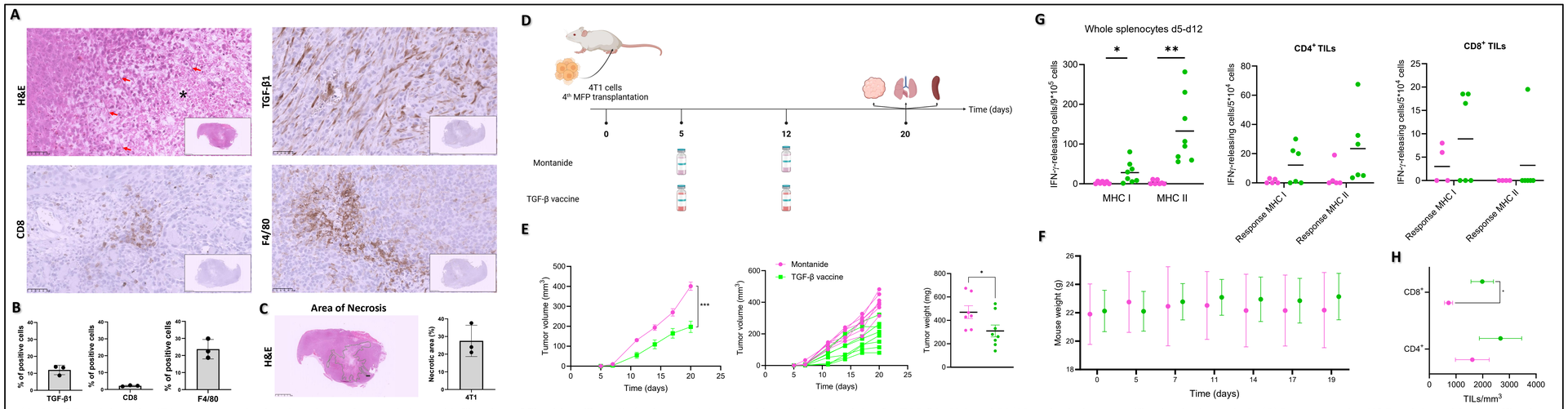
Conclusion

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 and immune responses in both the breast and prostate cancer models. The treatment was well tolerated and did not affect the overall body weight and well-being of the animals.

Moreover, in the breast carcinoma model, the vaccination led to significantly lighter tumors with a higher density of CD8⁺ TILs, and confirmed active intratumoral infiltration of TGF-β-specific T cells. In the prostate cancer model, the preliminary observations from the spatial analysis suggest that the TGF-β peptide vaccine enriched specific components of the immune system to actively limit tumor progression.

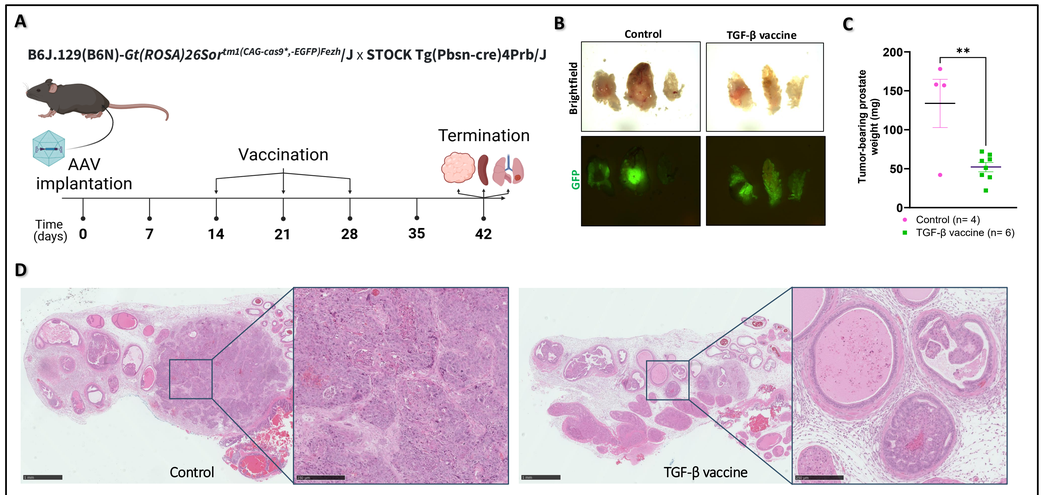
Collectively, our data warrant further investigation to better understand the efficacy & the safety profile of this novel TGFβ-targeting strategy.

1. TGF-β vaccine inhibits the growth of 4T1 mammary carcinomas and induces immune responses against TGF-β epitopes



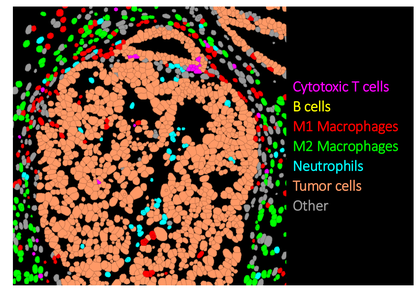
(A, B) H&E staining of a 4T1 tumor sample showing elevated necrosis (*) and infiltration of fibroblast-like cells (red arrows). Immunohistochemical staining for TGF-β1, CD8 and F4/80 and relative quantification is shown in **B.** **(C)** Representative H&E staining with quantification of necrotic area. **(D)** 4T1 cells (1 x 10^5) were transplanted into the inguinal mammary fat pad of 10-week-old BALB/c female mice (n = 16). At day 5, mice were randomized to receive a control vaccination (DMSO in Montanide) or a TGF-β vaccine (4 peptides in Montanide-ISA 51), including a booster shot at day 12. **(E)** Average tumor growth of the two groups presented as mean ± SEM. P-value: unpaired t-test. **(F)** Body weight of animals during the experiment **(G)** The presence of TGF-β specific T cells was quantified in total splenocytes and tumor-infiltrating lymphocytes (TILs) *via* IFN-γ ELISpot assay after overnight stimulation. Whole tumors were dissociated to single cell suspensions, which were used to sequentially isolate CD4⁺ and CD8⁺ cells through positive magnetic labeling (**NB**: for TILs, some mice were pooled (in different combinations in CD4 *vs* CD8), so one dot does not represent one individual mouse). Scatterplots for the quantification of each peptide presented as mean ± SEM. P-value: unpaired t-test. **(H)** Density of CD4⁺ and CD8⁺ TILs within the tumor.

2. TGF-β vaccine slows progression of prostate cancer *in vivo*



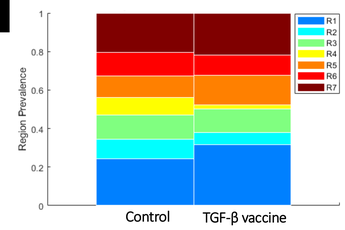
(A) Adeno-associated virus particles (AAV, 10^9 virus genome) were orthotopically injected in the lateral prostatic lobes of 9-to-12-week-old Pb4-Cas9/EGFP male mice (n = 10) as previously established[3]. Following randomization, mice received a control vaccination (DMSO in Montanide) or a TGF-β vaccine (TGF-β peptides in Montanide) at day 14, 21, and 28. **(B)** The GFP signal was used to guide tumor identification. **(C)** Average weight of the tumor-bearing prostate lobes presented as mean ± SEM. P-value: unpaired t-test. **(D)** 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).

3. The TGF-β vaccine promotes spatial niches with specific immune signatures in prostate cancer



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 were used to generate neighborhoods with CytoMAP[4]. Neighborhoods inform about cell composition, density, & marker expression. Neighborhoods were 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.

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Acknowledgements & References

We would like to thank Associate Professor Martin Kristian Thomsen at Aarhus University (Denmark) for the access to the prostate cancer model. We extend our gratitude to Lea Svendsen, Dr. Inés Lecoq Molinos and Dr. Rasmus Agerholm-Nielsen from IO Biotech for their help with the experiments.

1. Holmstrom et al., 2021
2. Mortensen et al., 2021
3. Cai et al., 2021
4. Stoltzfus et al., 2020