A TGFβ-directed immune-modulatory vaccine leads to T cell activation, tumor growth inhibition and reduces metastasis

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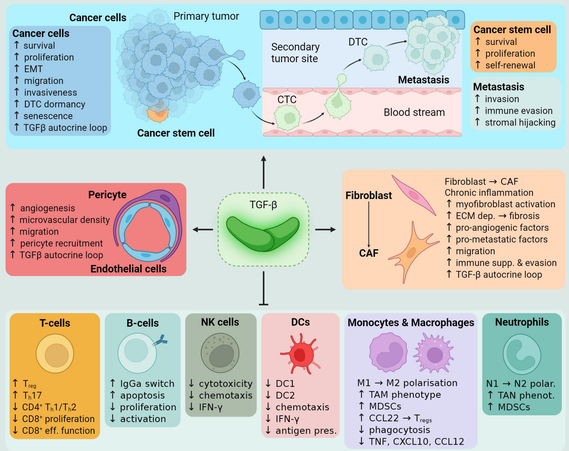
Background

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 cells against TGF-β were reported in man[1,2].

Herein, 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, has shown clinical activity and favorable safety profile across various tumor types and is currently being tested in phase 3 for advanced melanoma.



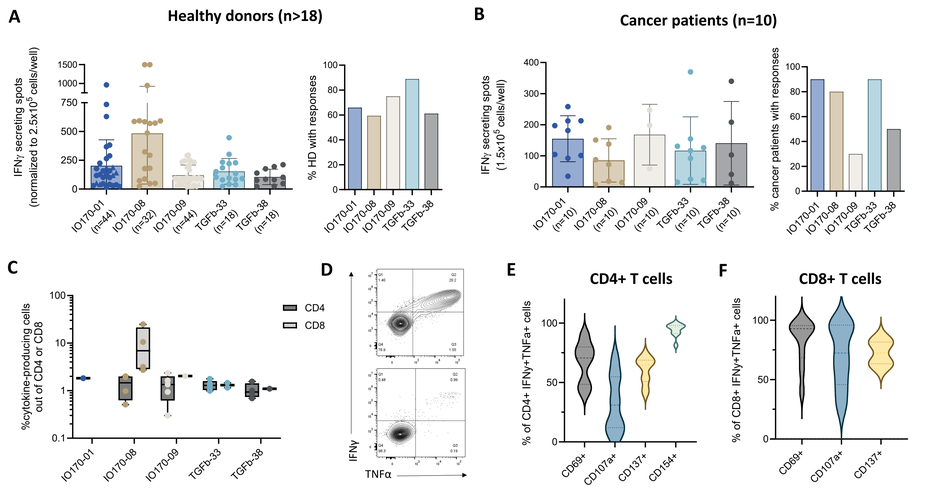
Conclusion

We utilized our T-win® platform to develop a TGF-β vaccine and evaluated its immunogenicity in both human (*in vitro*) and mouse (*in vivo*).

- All 5 peptide candidates selected for the human study elicited TGF-β specific responses in both healthy donors' and cancer patients' peripheral blood samples, supported by an activated and functional phenotype of such specific T cells.
- Vaccination in the CRISPR/Cas9-driven murine model of prostate cancer led to an induction of immune responses towards TGF-β peptides which slowed tumor growth. Moreover, the vaccine reduced intra-tumoral TGF-β1 expression and lung metastasis. The treatment was well tolerated and did not affect the overall body weight and well-being of the animals.

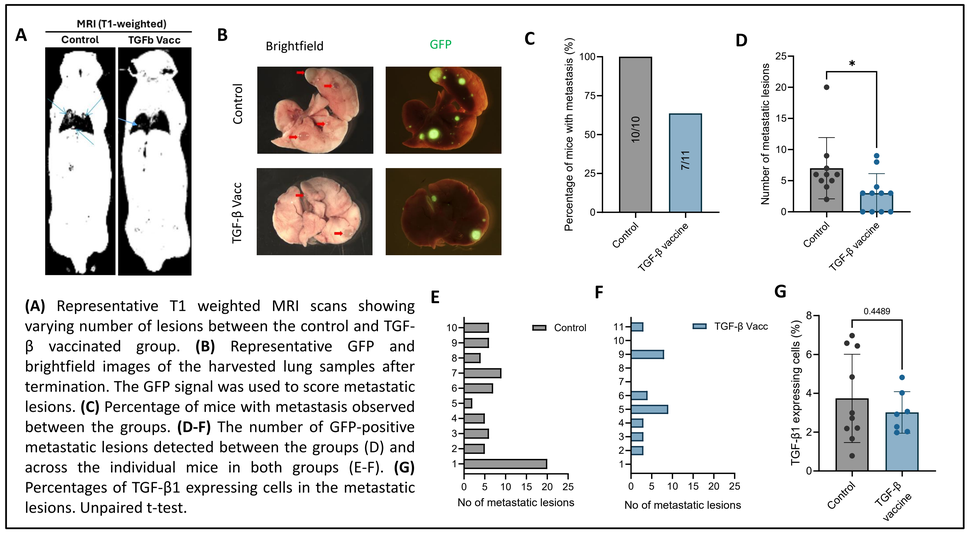
Collectively, our data warrant further investigation to better understand the activity and the safety profile of this novel TGF-β-targeting strategy.

1. TGF-β peptides elicit T cell responses in human blood samples



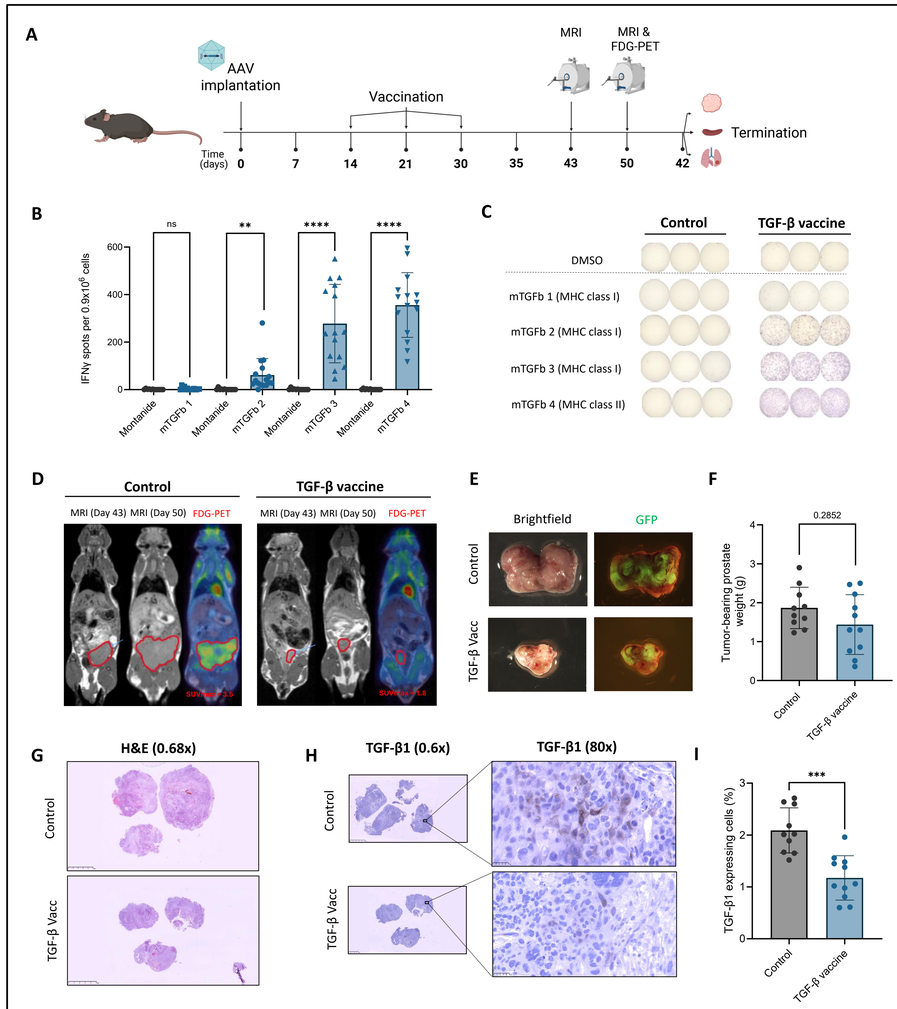
(A, B) Five TGF-β peptide candidates were selected based on previously findings[1] and in-house screenings, and tested in an *in vitro* IFNγ ELISpot in PBMCs from healthy donors (A) and cancer patients (B). Only spot counts significantly increased from background levels using a DFR test are depicted with background subtracted. The percentage of healthy/cancer donors responsive to the peptides are presented in the graphs to the right. **(C)** Frequencies of IFNγ and/or TNFα producing cells in *in vitro* stimulated PBMCs from selected healthy donors following an 8-hour restimulation and ICS. **(D)** Representative flow plots showing a cytokine-producing CD8+ population (top) and CD4+ population (bottom) **(E-F)** Phenotype of the cytokine-producing cells during the ICS.

3. TGF-β vaccine reduces lung metastasis *vivo*



(A) Representative T1 weighted MRI scans showing varying number of lesions between the control and TGF-β vaccinated group. **(B)** Representative GFP and brightfield images of the harvested lung samples after termination. The GFP signal was used to score metastatic lesions. **(C)** Percentage of mice with metastasis observed between the groups. **(D-F)** The number of GFP-positive metastatic lesions detected between the groups (D) and across the individual mice in both groups (E-F). **(G)** Percentages of TGF-β1 expressing cells in the metastatic lesions. Unpaired t-test.

2. The TGF-β vaccine slows tumor progression in a prostate cancer model



(A) Experimental outline of the murine study where 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 = 21) as previously established[3]. Mice received a control vaccination (DMSO in Montanide) or a TGF-β vaccine (TGF-β peptides in Montanide) at day 14, 21, and 30, and scanned on day 43 and 50. **(B)** IFNγ ELISpot responses measured in spleenocytes against the set of 4 mTGF-β peptides. Unpaired t-test. **(C)** Triplicate ELISpot well images for negative (DMSO) and peptide stimulated (4 different peptides) wells for a representative control and vaccinated animal. **(D)** Representative MRI and FDG-PET scans from control and vaccinated mice divided into responding and non-responding. **(E)** Representative GFP and brightfield images of the tumor after termination. **(F)** Average weight of the tumor-bearing prostate lobes presented as mean ± SEM. Unpaired t-test. **(G)** Representative H&E stainings of harvested tumor tissue between the two groups. **(H)** Representative IHC images showing TGF-β1 staining of harvested tumor tissue between the two groups. Only medium to high expressing cells were quantified. **(I)** Percentages of TGF-β1 expressing cells in the tumor. Unpaired t-test.

Acknowledgements & References

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1. Holmstrom et al., 2021
2. Mortensen et al., 2021
3. Cai et al., 2024

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