Using CRISPR to improve T cell cancer therapies

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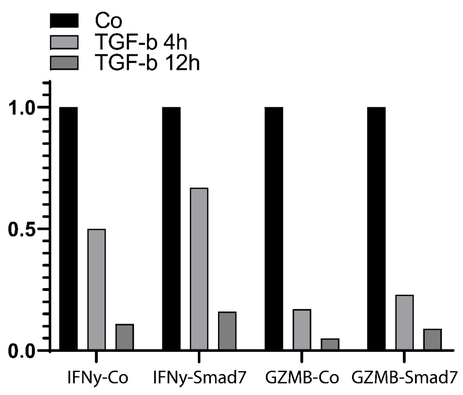
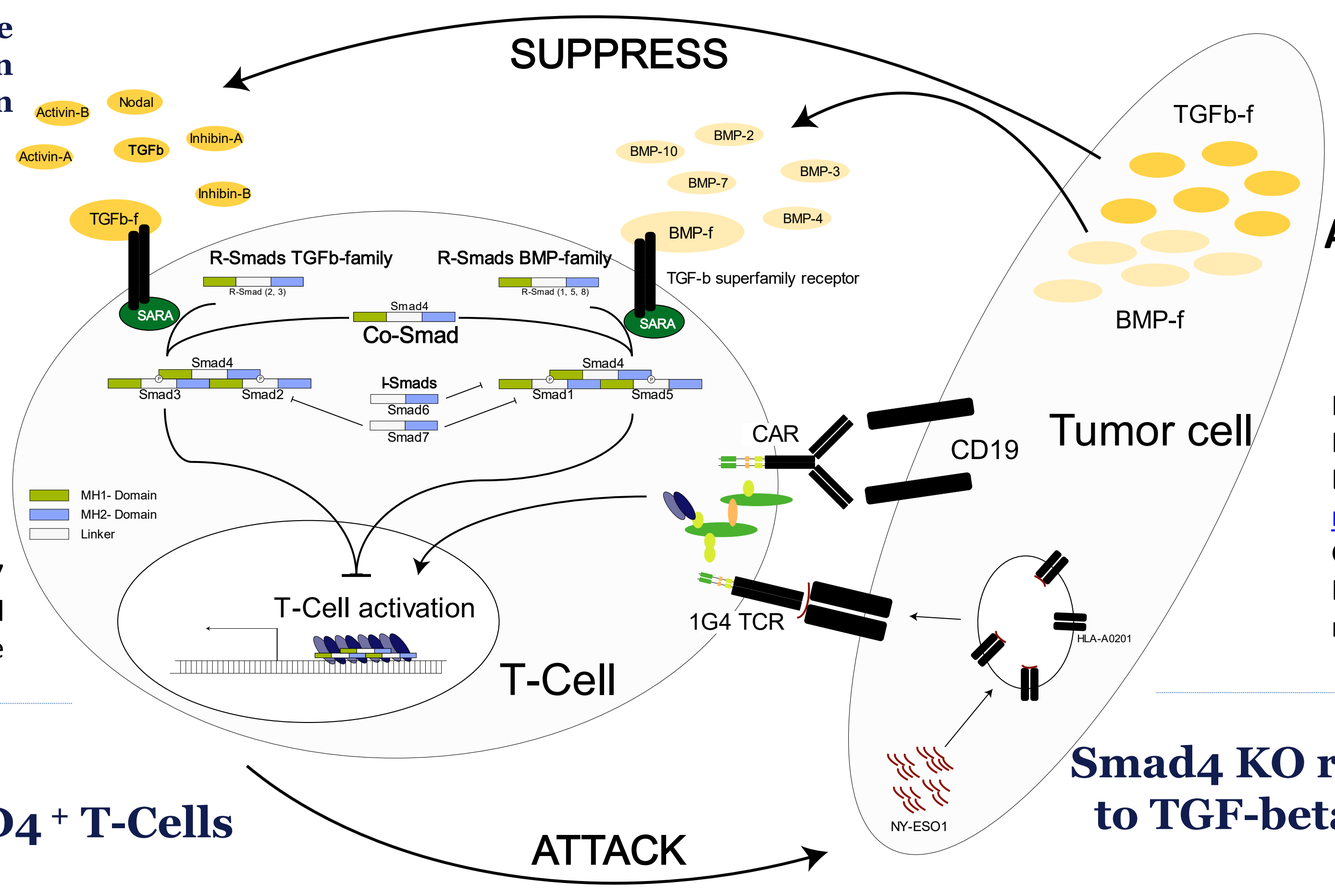
Introduction: Cytokines of the TGF-beta superfamily (TGFb-sf) are responsible for the immunosuppressive microenvironment of many cancer entities. Overcoming this barrier constitutes a major goal in the therapy with adoptively transferred immune cells to target cancer, especially solid tumors. Thus, strategies to counteract signaling induced by factors of the TGF-beta superfamily could help to improve adoptive cell therapy. TGF-beta subfamily (TGFb-f) signaling is mediated by phosphorylation of R-Smads (Smad2, Smad3) and formation of complexes with the Co-Smad (Smad4). BMP-subfamily (BMP-f) signaling is mediated by R-Smads 1, 5 and 8 together with Smad4. Thus, Smad4 acts as common integrator of TGFb-sf cytokines, while inhibitory factors such as Smad6 and Smad7 act as physiological antagonists. In order to target all cytokines of the TGFb-sf by only one genetic modification, we aimed to overexpress inhibitory Smads in primary human $CD4^+$ and $CD8^+$ T-cells and assess the effect of this manipulation on T-cell function and polarization. In parallel, we abrogated TGF-beta superfamily effects by CRISPR/Cas9 mediated knockout of Smad4.

Smad7 does not antagonize TGFb induced downregulation of activation associated genes in $CD8^+$ T-Cells



Expression of IFNy, GZMB, PRF1, NKG7 was measured in Smad7- and Control transduced $CD8^+$ T-Cells in the presence of 5ng/ml TGFb



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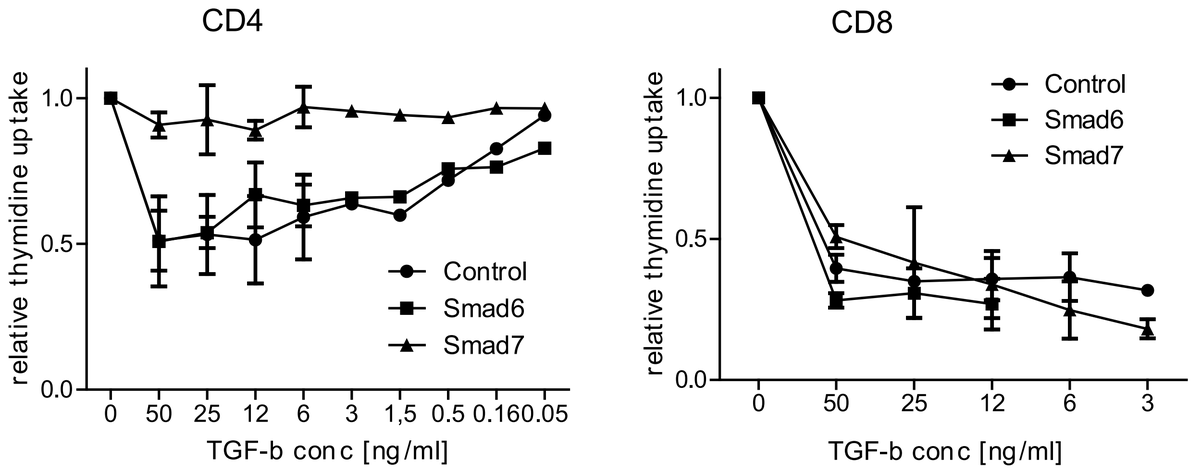
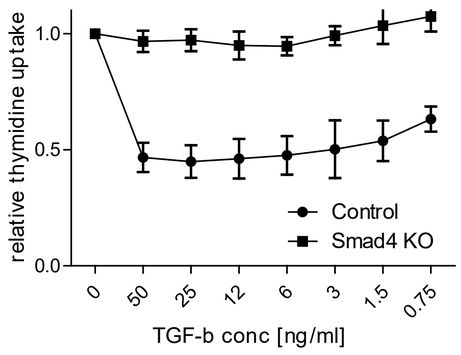
Smad7 overexpression antagonizes TGFb in $CD4^+$ T-Cells but not in $CD8^+$ T-Cells

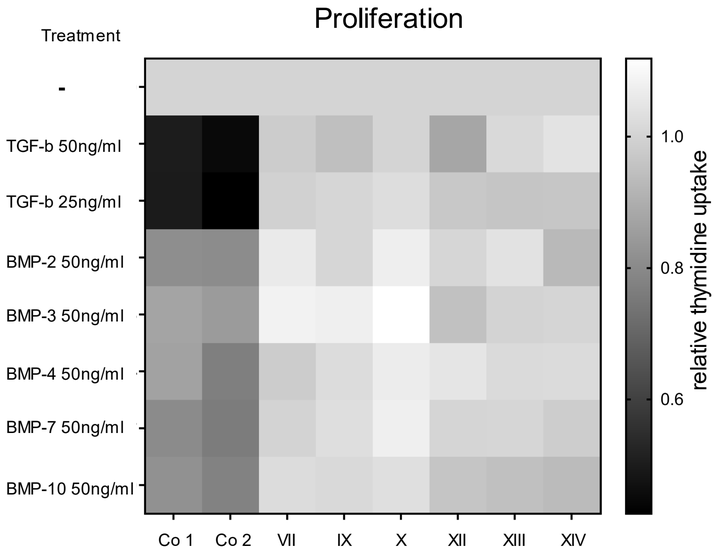


Smad6-, Smad7- and control transduced T-cells were anti-CD3/anti-CD28 activated in the presence of indicated concentrations of rec. human TGFb. 72h after activation, proliferation was measured by thymidine incorporation. While Smad7 overexpression almost completely restored proliferative capacity in $CD4^+$ T-Cells, no effects on $CD8^+$ T-Cells were observed.

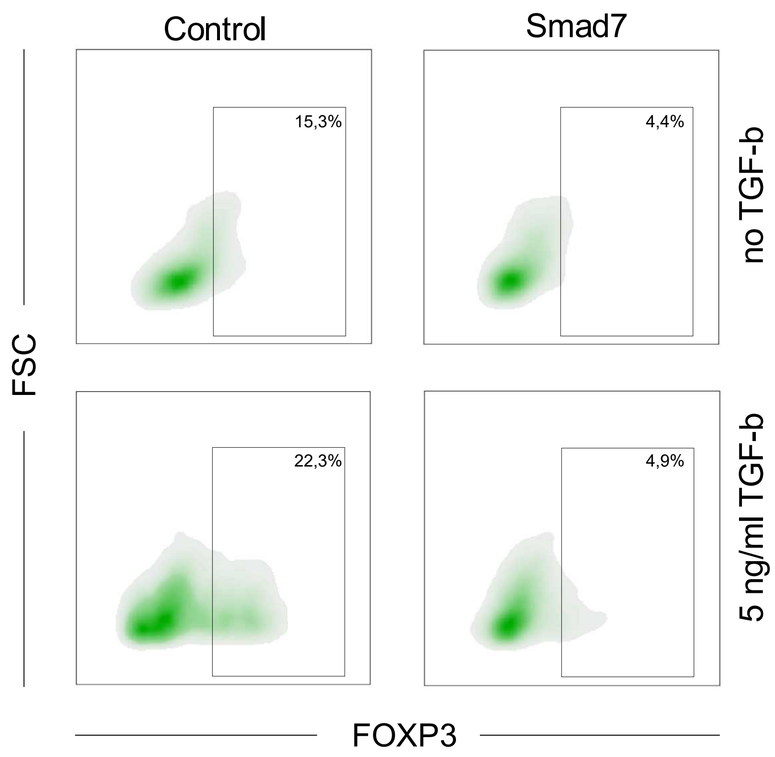
Smad4 KO renders T-cells resistant to TGF-beta superfamily cytokines

Knockout of Smad4 was performed using electroporation of *in vitro* preassembled CRISPR/Cas9 RNP. T-cells were reactivated one week after electroporation and proliferation was measured by thymidine incorporation.



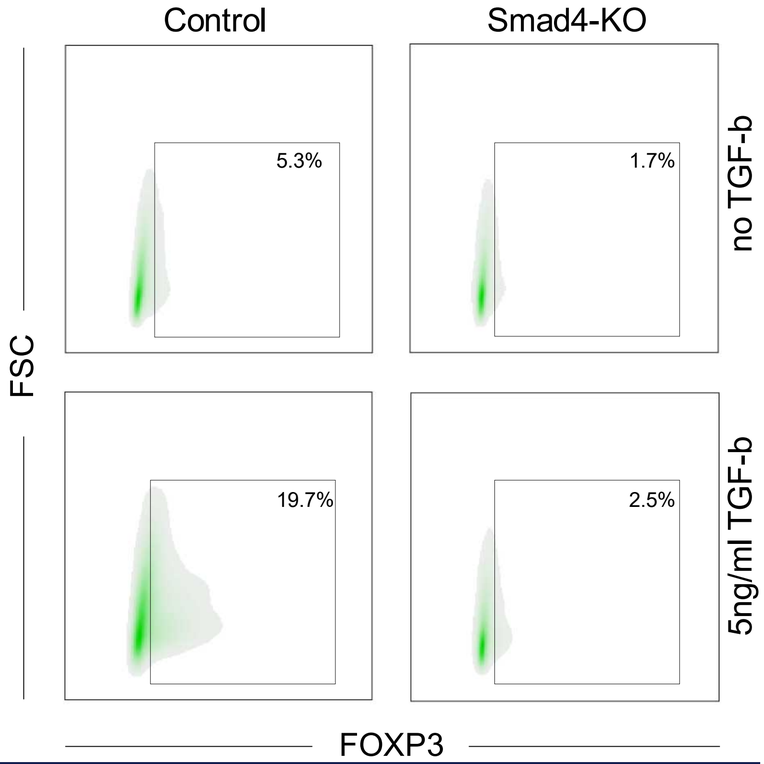


Right: selection of gRNA that recover proliferation under TGFb-sf cytokines. Left: gRNA X was chosen and used for Smad4 knockout over a broad concentration range of TGFb.



$CD4^+$ T-Cells were transduced with a retroviral Smad7-IRES-GFP expression vector or a control vector. One week after transduction, T-cells were re-stimulated in the presence of 50 IU/ml IL-2 with or without 5ng/ml TGF-b to induce regulatory T-cells. FOXP3 expression was assessed by flow cytometry one week after polarization.

$CD4^+$ T-cells were transfected by electroporation of Smad4- or non-targeting (Control) Cas9 RNP. One week after electroporation, T-cells were re-stimulated in the presence of 50 IU/ml IL-2 with or without 5ng/ml TGF-b to induce regulatory T-cells. FOXP3 expression was assessed by flow cytometry one week after polarization.



Induction of regulatory T-Cells is abrogated by Smad7 overexpresion and Smad4 KO

Conclusion: The role of TGFb and cytokines of the TGFb-sf on the immune response against tumors is well documented and intensively investigated. Adoptive cell therapies, such as CAR T-Cells, represent successful therapeutics in lymphoma treatment. Several factors however limit the success of these therapies in solid tumors, among them a TGFb-sf driven tumor microenvironment. Thus, we aim to adoptive immunotherapy by intrinsically rendering T-cells resistant against all cytokines of the TGFb-sf. While targeted Smad7 overexpression and SMAD4 KO reverts TGFb mediated effects on proliferation and T cell differentiation, these approaches did not affect TGFb induced upredulation of PD-1 (data not shown). In the Marson Lab, we plan to use pooled CRISPR screenings to identify common regulators of TGFb-sf cytokine mediated T cell dysfunction and apply the acquired knowledge to improve T cell mediated cancer killing.