REMODELING THE IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT WITH ONCOLYTIC VIRUS-MEDIATED DELIVERY OF A POTENT TGF-B INHIBITOR

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Background Checkpoint blockade often fails due to a lack of pre-existing immune response. Oncolytic viruses (OVs) induce inflammation and generate a tumor specific immune response which is critical for patients with immune desert or excluded tumors. Further, OVs can be engineered to deliver therapeutic cargo in the tumor microenvironment. However, mechanisms of resistance to OVs are not well understood. To study OV resistance, we developed derivatives of the head and neck cancer line MEER: one sensitive to oncolytic vaccinia virus (VV, MEER<sup>vvS</sup>), and one resistant to VV (MEER<sup>vvR</sup>). Using these, we determined mechanisms which suppressed the anti-tumor immune response and engineered a new VV to target them.

Methods A MEER<sup>vvS</sup> tumor that grew out post-treatment was passaged in mice with a single intratumoral dose of VV given at each passage. This was repeated until a VV-resistant line was generated. For flow-cytometric tumor infiltrating lymphocyte analysis, MEER<sup>vvR</sup> and MEER<sup>vvS</sup> tumors were implanted in mice, treated with a single dose of VV<sub>ctrl</sub>, VV<sub>TGFßi</sub>, or PBS, and harvested 7-days post-treatment.

Results After VV treatment, we found increased T cell infiltration in both tumor types; however, MEER<sup>vvS</sup> infiltrate had significantly increased function, suggesting local suppressive mechanisms may define VV resistance. Indeed, in mice bearing both MEER<sup>vvR</sup> and MEER<sup>vvS</sup> we found the regulatory T (Treg) cells in the sensitive tumors had lower Nrp1 expression and higher STAT1 signaling, suggesting they may be primed for fragility. Resistant tumors were characterized by high intratumoral TGFß, a driver of resistance to VV. We found TGFß increased Nrp1 on Treg cell surface and led to maintained Treg suppression in the presence of IFNγ. We then generated a VV expressing a TGFß inhibitor (VV<sub>TGFßi</sub>), which outcompetes TGFß for receptor binding, preventing receptor signaling. When MEER<sup>vvR</sup> were treated with VV<sub>TGFßi</sub> there was a significant increase in tumor clearances and survival compared to VV<sub>ctrl</sub> (figure 1). Response to VV<sub>TGFßi</sub> was linked to a fragile Treg cell phenotype. Further, VV<sub>TGFßi</sub> synergized with PD1-blockade in a melanoma model resistant to either therapy alone.

Conclusions Our data suggest that a TGFß-rich microenvironment is a main driver of VV resistance, due in part to stabilization of Treg cells. Targeting TGFß using an inhibitor-armed virus can overcome this resistance. Importantly, virally-encoded TGFß inhibition is local, carrying no toxicity associated with systemic routes. Taken together, our findings confirm TGFß as a major player in immunotherapeutic resistance and that OVs are an attractive means to deliver TGFß inhibition to synergize with other immunotherapies.

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Tumor growth (left) and survival (right) of MEER<sup>vvR</sup>-bearing C57BL/6 mice treated with an intratumoral injection of PBS, VV-ctrl or VV-TGFßi (black arrow) at 2.5x106 PFU/mouse. CR = complete response. Data represent four independent experiments. Each line represents an individual mouse. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by Mantel-Cox. ns, non-significant.