588 TARGETING GITR ENHANCES HUMAN TUMOUR-INFILTRATING T CELL FUNCTIONALITY IN MISMATCH REPAIR PROFICIENT PRIMARY COLORECTAL CARCINOMA AND LIVER METASTASES

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Background Immune checkpoint blockade (ICB; e.g. anti-PD-1/-CTLA-4) has been proven to be clinically effective in mismatch repair deficient (dMMR) colorectal carcinoma (CRC). Yet, the majority of patients carry mismatch repair proficient (pMMR) CRC, especially those with liver metastasis, and do not respond to ICB. Here, we studied the effect of immune checkpoint stimulation via GITR targeting on human tumour-infiltrating lymphocyte (TIL) functionality in pMMR primary CRC and liver metastases (CRLM).

Methods Human TIL were isolated from freshly resected pMMR tumours of patients with primary CRC (stage 1–3) or liver metastases (table 1). GITR expression on TIL was determined using flow cytometry and compared to leukocytes isolated from blood (PBMC) and tumour-free surrounding tissues (tumour-free colon/liver, resp. TFC and TFL). Ex vivo functional assays were used to assess TIL expansion, activation and cytokine/cytotoxic mediator secretion upon CD3/CD28 bead activation and co-stimulation using an antibody-cross-linked recombinant trimeric GITR ligand (GITRL).

Results GITR was overexpressed on TIL when compared to other stimulatory immune checkpoints (4-1BB, OX40). GITR expression was enhanced on CD4+ and CD8+ TIL compared to PBMC and TFC or TFL compartments in both primary CRC and CRLM. Among CD4+ TIL, GITR was increasingly expressed on CD45RA± FoxP3- helper T (Th), CD45RA- FoxP3int activated helper T (aTh), and CD45RA- FoxP3hi activated regulatory T cells (aTreg), respectively. Within CD8+ TIL, GITR expression was higher on TOX+ PD1Hi and putatively tumour-reactive CD103+ CD39+ TIL. Impaired effector cytokine production upon ex vivo PMA/ionomycin stimulation was observed in CD4+ and CD8+ GITR-expressing TIL, hinting to functional exhaustion of the target population. However, recombinant GITRL reinvigorated ex vivo TIL responses by significantly enhancing CD4+ and CD8+ TIL numbers and proinflammatory cytokine secretion in a dose-dependent manner (figure 1). Treg depletion did not fully abrogate the stimulatory effect of GITR ligation on CD4+ and CD8+ T cell expansion, demonstrating that the stimulatory effect was partly exerted via direct targeting GITR on effector T cells. Importantly, GITR-ligation also enhanced expansion of purified CD8+CD39+ TIL. Dual treatment with GITR ligand and nivolumab (anti-PD-1) further enhanced CD8+ TIL responses compared to GITR ligand monotherapy, whereas nivolumab alone did not show any effect.

Conclusions Agonistic targeting of GITR enhances ex vivo human TIL functionality in pMMR CRC and might therefore be a promising approach for novel mono- or combinatorial immunotherapies in primary CRC and CRLM.
Background Checkpoint blockade immunotherapy has dramatically changed cancer treatment; however, these therapies depend on the presence of a pre-existing immune infiltrate. Unfortunately, some patients have few to no infiltrating immune cells, highlighting the need for therapies that can generate antigenic stimuli. Oncolytic viruses, which infect and lyse tumor cells while leaving healthy tissue unharmed, are an attractive means to provide these signals, although the mechanisms of action of these engineered viral therapies remain incompletely understood. Virally induced immunogenic death causes an influx of tumor- and virus-specific effector CD8+ T cells. Many oncolytic viruses also decrease tumor-infiltrating suppressive immune populations, such as regulatory T cells (Treg), however the mechanism for this is unknown. Here we show that an oncolytic strain of vaccinia virus (VV) infects tumor infiltrating Tregs, in contrast to the prevailing idea that oncolytic viruses only infect tumor cells. Infection leads to viral-mediated Treg depletion that is required for tumor regression.

Methods Using a mouse model of head and neck squamous cell carcinoma (MEER), a VV-resistant line was generated through serial treatment of a VV-sensitive MEER line. At varied time points post-intratumoral treatment with VV, tumor infiltrating lymphocytes (TIL) were isolated from both the VV-resistant and VV-sensitive lines and analyzed by flow cytometry.

Results One day post-treatment of VV-sensitive MEER tumors, tumor isolated Tregs were infected by VV as determined by viral GFP expression. Infection was confirmed in vitro with purified Tregs. Four days post-treatment, tumor infiltrating Treg counts were reduced, and active caspase 3 staining was increased, suggesting that infection lead to Treg death. At 7 days post-treatment, the remaining Tregs in the VV-sensitive tumors acquired a fragile phenotype (IFNγ+ Nrp1-). This was not observed in the VV-resistant MEER line. Fragile Tregs are less suppressive and indeed we observed an increase in proinflammatory cytokine production from CD8+ and Tconv cells. Tumor-infiltrating T cells were mostly reovirus-specific and served as effector cells for the subsequently systemically administered CD3-bsAbs. The combination of reovirus and CD3-bsAbs induced regressions up to 70% in all mice with large, established KPC3, B16.F10, and BT474 tumors and significantly prolonged survival. Importantly, the employment of

Abstract 590 Figure 1 Reovirus sensitizes tumors for CD3-bsAb therapy
Reovirus-induced interferon signaling leads to increased T cell influx and subsequent effective CD3-bispecific antibody therapy in solid tumors.