

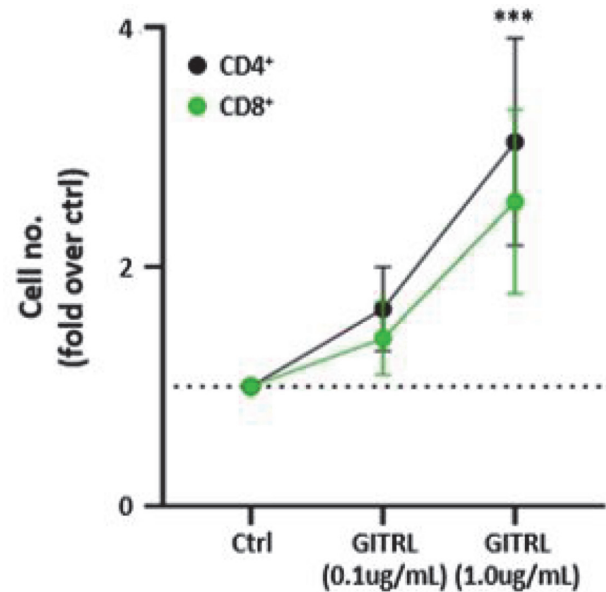
### TARGETING GITR ENHANCES HUMAN TUMOUR-INFILTRATING T CELL FUNCTIONALITY IN MISMATCH REPAIR PROFICIENT PRIMARY COLORECTAL CARCINOMA AND LIVER METASTASES

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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.<sup>1</sup> 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



**Abstract 588 Figure 1** GITR ligation enhances CD4+ and CD8+ TIL expansion

TIL were isolated from CRC or CRLM and cultured upon CD3/CD28 activation with or without GITRL (0.1–1.0 ug/mL) for 8 days. TIL numbers were acquired by flow cytometry and normalized to counting beads. Indicated is fold change relative to ctrl-treated TIL (n=10).

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.

**Acknowledgements** N/A

**Trial Registration** N/A

**Ethics Approval** The study was approved by the medical ethics committee of the Erasmus Medical Center (MEC-2012-331).

**Consent** N/A

#### REFERENCE

- Duhen T, Duhen R, Montler R, *et al.* Co-expression of CD39 and CD103 identifies tumour-reactive CD8 T cells in human solid tumors. *Nat Commun* 2018;**9**(1):2724. doi: 10.1038/s41467-018-05072-0.

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### EFFICACY OF ONCOLYTIC VACCINIA VIRUS REQUIRES INFECTION OF SUPPRESSIVE IMMUNE CELLS IN THE TUMOR MICROENVIRONMENT LEADING TO THEIR REPROGRAMMING AND DELETION

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**Abstract 588 Table 1** Patient characteristics

Patient characteristics of patients included for FACS analysis and/or functional assays. † Pathologic staging was performed according to the AJCC 8th edition criteria

	CRC (N=74)	CRLM (N=34)
Age, years (IQR)	70.0 (59.0-73.0)	68.5 (63.0-73.0)
Male (%)	50 (67.6)	20 (58.8)
Primary tumour site (%)		
Coecum/Colon	58 (78.4)	22 (64.7)
Rectum	16 (21.6)	12 (35.3)
Pathologic disease stage (%)†		
I	23 (31.1)	-
II	26 (35.1)	-
III	22 (29.7)	-
IV	3 (4.1)	34 (100.0)
Pretreatment (%)		
Chemotherapy	13 (17.6)	12 (35.3)
Radiotherapy	7 (53.8)	10 (83.3)
Other	9 (69.2)	-
Other	-	3 (25.0)