CHARACTERIZATION OF TIGIT AND PVR EXPRESSION IN COLORECTAL LIVER METASTASES

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Background Metastatic colorectal cancer (CRC) is common and lethal and generally not responsive to current immunotherapies. We hypothesize that efficacious T cell-based immunotherapy can be developed for this malignancy, provided that immune checkpoints relevant to liver metastasis, the first site of disease progression, are targeted. Here, we characterized CRC liver metastases by RNAseq, FACS and in vitro functional assays to identify candidate immune checkpoints.

Methods We performed deep RNAseq clustering and differential gene expression analysis on bulk RNA extracted from 52 mismatch repair gene proficient CRC liver metastases. By multiparameter FACS, we analyzed the expression of candidate immune checkpoints in cell suspensions derived from 18 liver metastases, matched non-tumoral livers, and pre-operative PBMCs. We evaluated IFN- γ (ELISA) secretion and tumor lysis (Incucyte) of tumor-infiltrating T lymphocytes (TILs) expanded from liver metastases stimulated by autologous cancer cells with or without monoclonal antibodies blocking candidate immune checkpoints.

Results Out of 52 metastases, 21 (40.3%) clustered as immune reactive (IR) defined by concurrent high expression of transcripts related to antigen processing, immune cell lineage, immune checkpoints, interferon-gamma response, cytokines, and chemokines, whereas 25 (48.1%) were classified as non-IR. Of all inhibitory ligands assessed, PVR and PVRL2 had the highest expression, both in IR and non-IR metastases, and higher than PD-L1 and PD-L2 expression. The expression of corresponding receptors TIGIT and CD226 was significantly higher in IR compared to non-IR metastases, at absolute levels higher than PD-1. By FACS analysis, PVR and PVRL2 expression by tumor-infiltrating myeloid and tumor cells was higher than PD-L1 and PD-L2 expression. High PVR expression was also found in hepatocytes, liver macrophages and circulating monocytes in the same patients. In TILs, TIGIT was significantly overexpressed in activated CD4+CD25+ (74.8 $\pm$ 3.0%) and CD8+CD25+ (68.7 $\pm$ 8.4%) compared to resting CD25neg T cells, an expression pattern that was not seen for PD-1 or in T cells infiltrating the liver or circulating in the blood. The majority of cancer cell lines derived from liver metastases expressed PVR, but low levels of PD-L1. TIL clones expanded from liver metastases expressed TIGIT at various levels inducible by TCR stimulation. Upon co-culture with autologous cancer cell lines, TIL clones were more lytic and secreted more IFN- γ in presence of anti-TIGIT blocking antibody.

Conclusions By expression and functional data, the TIGIT/PVR immune suppressive axis appears as a biologically promising target for the development of immunotherapy in patients with CRC metastatic to the liver.

Acknowledgements This work is supported by Bristol Myers Squibb and by the Quebec Cancer Consortium. A.B. holds a postdoctoral scholarship award from the Institut du cancer de Montréal. S.T. holds a Junior 2 clinical-scientist salary award from the Fond de recherche Santé-Québec. The University of Montreal Roger des Groseillers Research Chair in hepatopancreatobiliary surgical oncology supports the biobanking and clinicopathological database associated with this project.

Ethics Approval Institutional review board approvals were obtained to conduct this project (16.262) and all patients provided informed consent to contribute to this project with biospecimens and clinicopathological data (09.237).

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.302>