Background Colorectal cancer (CRC) is the 2nd cause of cancer-related death. Despite standard therapies, more than 50% of patients experience relapse, eventually with metastatic disease. Colorectal tumours are densely infiltrated by immune cells that have a role in surveillance and modulation of tumour progression, correlating with an improved overall survival. However, exhaustion mechanisms acting within the tumour microenvironment impede their functional capacity against tumour cells.

Methods We paired high-dimensional flow cytometry, RNA sequencing, immunohistochemistry and immunofluorescence to describe the T cell functional landscape in tumour and peritumoral tissues from primary colorectal cancers and liver metastases. By CRISPR/Cas9 genome engineering techniques, we redirected the specificity of T cells towards a tumor-specific antigen while disrupting inhibitory molecules, to counteract the immune-suppressive tumor microenvironment.

Results Analysis of the healthy, peritumoral and neoplastic tissues of treatment-naïve primary CRCs and of the peritumoral and tumoral tissues of CRC patients undergoing surgery for liver metastases revealed extensive transcriptional and spatial remodeling across tumors, being metabolic pathways among the major drivers of this variance. Regarding the immune infiltrate, we found that T cells are mainly localized at the front edge and that tumour-infiltrating T cells co-express multiple inhibitory receptors. Unsupervised analysis of flow cytometry data performed by an advanced pipeline of data handling by dimensionality reduction and clustering algorithms allowed the definition of a peculiar inhibitory receptors signature in TILs enriched both in primary CRCs and liver metastases. Among the highly co-expressed inhibitory receptors, CD39 was found to represent the major driver of exhaustion in both primary and metastatic colorectal tumours. CD39 is a diphosphohydrolase converting ATP into AMP that is emerging as exhaustion marker for tumor-specific T cells, thus highlighting its relevance as molecular target for T cells engineering. By CRISPR/Cas9 genome editing tools, we simultaneously redirected T cell specificity by disrupting the alpha and beta genes of the endogenous T cell receptor with >90% efficiency, and disrupted CD39 with 100% efficiency, generating triple-knockout engineered lymphocytes. By lentiviral transduction, we redirected the specificity of our engineered T cell product employing a novel T-cell receptor targeting the HER-2 antigen. Gene-edited, HER2-redireceted T cells were challenged against HER2+ patient-derived organoids (PDOs) in vitro and in vivo: CD39-disrupted, HER2-redireceted T cells displayed a functional advantage in recognizing and killing CRC PDOs and enhancing mice survival, compared to CD39-competent, HER2-redireceted T cells.

Conclusions The CD39 axis is relevant for further exploitation in adoptive T-cell therapy to treat primary and metastatic colorectal cancer.