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TILs from panNET liver metastases: in search of novel adoptive transfer strategies for the treatment of NETs

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BACKGROUND

The anti-tumor activity of tumor-infiltrating lymphocytes (TILs) in NETs is currently unknown.

METHODS

We collected matched blood, FFPE and cryopreserved or fresh samples of liver metastases from 29 patients with well-differentiated panNETs (7 G1;19 G2;3 G3). FFPE samples were subjected to WES and RNAseq to predict number and identity of tumor neoantigens. IHC was used to assess HLA-I and HLA-II expression in tumor samples, and findings were validated by TMA in an independent cohort of 49 panNETs. Digital quantification of CD3+ staining was performed using the Aperio tool. Multi-region analysis of individual tumor samples was carried out and in vitro-mapped TILs outgrowth was contrasted with tumor zonal characteristics. TILs were expanded up to 105 days and weekly enumerated and phenotyped by flow cytometry. TCR sequencing was performed to assess over time TCR skewing. The Seahorse technology was used to evaluate TILs' metabolism. TILs deriving from different tumor regions were co-cultured with autologous tumoroids to assess their antitumor activity. Secretion of IFN-g and Granzyme-B was measured by ELISA.

RESULTS

PanNET liver metastases exhibited a relatively low mutational and neoantigen burden (median of 12 pathogenetic variants per sample; median of 3 HLA-I and 4 HLA-II predicted neoantigens per sample). HLA-I and HLA-II expression was retained in 28/29 and 0/29 samples respectively. By TMA,HLA-I and HLA-II expression was observed in 36/49 and 0/49 samples. PreREP-sufficient numbers of TILs were reached in 18/29 patients (62%). TILs' outgrowth was independent of clinical parameters, being

instead significantly correlated with T cell density by IHC ($p<0.05$) and TLS presence ($p<0.01$).Wide differences were observed in T cell yield according to the different tumor regions analyzed. T cells were the predominant population in the TILs cultures at the time of cryopreservation with CD4+/CD8+ T cells ratio of 5:1. We observed a switch in CD8+ T cell differentiation (from TE to TEM) after 2 weeks of culture. Such a switch was accompanied by a metabolic reprogramming, with reduced efficiency of OXPHOS overtime. When cocultured with autologous tumoroids, TILs deriving from different tumor regions exhibited heterogeneous antitumor activity, spanning from no tumor recognition to massive production of pro-inflammatory cytokines and up-regulation of activation markers such as CD69/CD39. TILs showing anti-tumor reactivity were able to infiltrate co-cultured tumoroids and displayed a significantly higher respiratory capacity and glycolytic capacity. TILs not showing anti-tumor reactivity showed a higher presence of CD8+ Tregs.

CONCLUSIONS

PreREP sufficient TIL numbers were reached in approximately 60% of cases. TILs comprise both anti-tumor reactive clones and bystander lymphocytes. Isolation and expansion of tumor-reactive TILs may enhance the efficacy of TILs adoptive transfer.

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