100P - Patient-derived tissue cultures of esophago-gastric-junction cancer (EGJC) and gastric cancer (GC) – an ex vivo model to study individual response of immunotherapy.

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Background

Immune checkpoint blockade (ICB) achieves limited response rates in EGJC and GC[1]. Human *ex vivo* models containing the tumor microenvironment and modeling response to ICB are urgently needed to study immunotherapy. Patient-derived tissue cultures (PDTC) are suitable to obtain an enhanced understanding of human intercellular processes[2]. PDTC further have the potential to investigate individual response to treatment and have been established for several tumor entities[3,4,5,6,7]. Here, we analyzed the preservation of T cells and potential effects of immunotherapy in EGJC/GC PDTCs.

Method

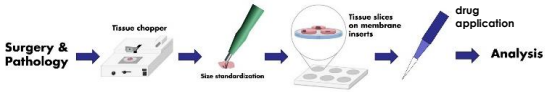


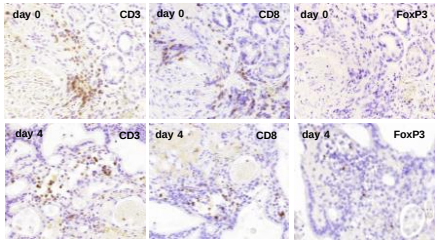
Fig. 1: Standardized procedure for preparing tissue slices and experimental setup Tumor tissue of 15 patients with EGJC or GC was cut in 350 µm thick slices with a tissue chopper. After size standardization of tissue slices, tissue were placed on membrane inserts and cultivated over 4 days[6].

Conclusion

- Resident tissue T cells are preserved in EGJC/GC PDTC *ex vivo* for 96 h
- Individual response to PD-1 Inhibition is represented
- Combinational therapy enhance anti-tumoral activity
- PDTC provide an model to investigate T cells in the tumor microenvironment in EGJC and GC

Results I

Resident T cell subpopulations in tumor microenvironment



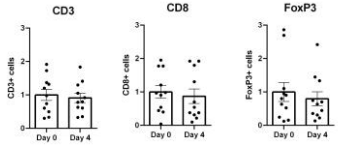


Fig. 2: T cell quantification *ex vivo* Representative images of immunohistochemical stainings and quantification (n=11) of CD3, CD8 and FoxP3 positive T cells at day 0 and after 4 days in culture. Normalized to day 0; error bars: SEM; magnification: 20x.

Migration of patient specific PBMCs in PDTC

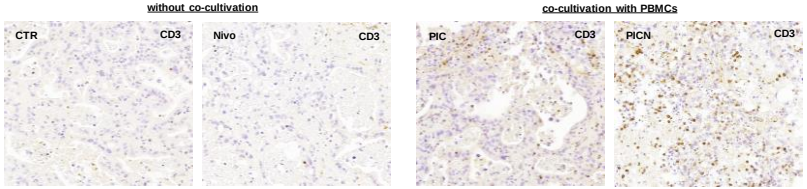


Fig. 3: Co-cultivation of peripheral mononuclear blood cells (PBMC) and PDTC Representative pictures of CD3 expression in PDTC after 4 days *ex vivo*: control (CTR), Nivolumab (Nivo; 3µg/ml), stimulated PBMC (PIC), stimulated PBMC combined with Nivolumab treatment (PICN, 3 µg/ml). Migration of PBMCs is shown by enhanced CD3+ T cell infiltration in co-cultured conditions (PIC, PICN) compared to CTR. Magnification: 20x.

Results II

Individual response to Nivolumab in PDTC

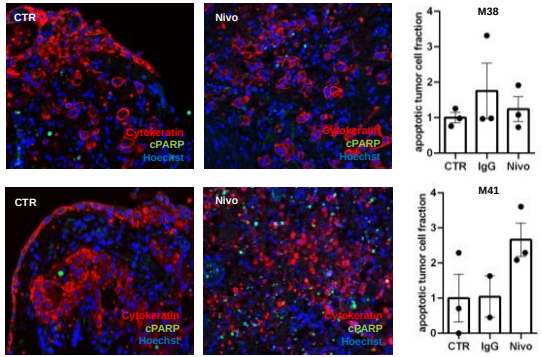


Fig. 4: Individual tissue susceptibility PDTC were treated with Nivolumab (Nivo, 3 µg/ml) and IgG vehicle control (IgG, 3 µg/ml) over 72h. Representative immunofluorescence pictures of apoptotic tumor cell fraction of two selected cases (M38, M41) are presented. Quantification of apoptotic tumor cell fraction after 72 h of treatment normalized to the responding cultured control condition was performed. CTR normalized to 1; error bars: SEM; magnification: 20x.

Enhanced anti-tumoral activity in combinational therapy approaches

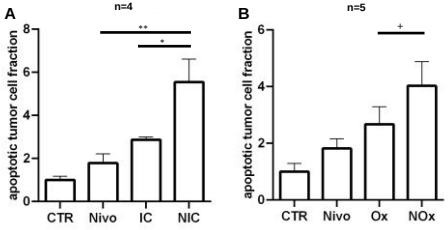


Fig. 5: Combined therapy with CD3/CD28-T-cell stimulation or Oxalipaltin with Nivolumab *ex vivo* Quantification of apoptotic tumor cell fraction after 72 h in various treatment conditions: CTR, Nivolumab (Nivo, 3µg/ml), T cell activator (IC, 25µg/ml), Nivolumab plus IC (NIC), Oxaliplatin (Ox, 20µM) and Nivolumab plus Oxaliplatin (NOx). Enhanced tumoral apoptosis can be observed in the combined therapy either with IC or Ox. (CTR normalized to 1; error bars: SEM; *=one way anova, *: p≤0,05, **: p≤0,01, +=t-test, +: p ≤0,05)

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References: [1]Akyala et al., 2018; [2]Klinghammer et al., 2017; [3]Merz et al., 2013; [4]Gerlach et al., 2014; [5]Koerfer et al., 2016; [6]Soennichsen et al., 2018; [7]Prill et al., 2019

All authors declare no conflict of interest