EIPHT +PHh) in the combined PCT, the patients were divided into following groups: the 1st group - 32 practically healthy individuals; the 2nd group - 36 patients with OC before PCT; the 3rd group - 44 patients with OC after PCT without immunotherapy; the 4th group - 28 patients with OC after PCT combined with EIPHT; the 5th group - 34 patients with OC after PCT combined with EIPHT +PHh.

Results and discussions Cytokines were studied by ELISA test (IL-6, IFN-γ, TNF-α) with the ‘Human’ test systems (Germany) in the dynamics of therapy. The following ratios of cytokines were studied: TNF-α/IL-6 and IFN-γ/IL-6. Normally, TNF-α/IL-6 is 0.69 and IFN-γ/IL-6 is 0.7. In the group of patients with ovarian cancer before treatment: TNF-α/IL-6–0.53, and IFN-γ/IL-6–0.58. In patients after chemotherapy without using immunotherapy: TNF-α/IL-6–0.59, and IFN-γ/IL-6–0.22. In the group of patients with combination of PCT and EIPHT: TNF-α/IL-6–0.66, IFN-γ/IL-6 1.46. In the group of patients with combination of PCT and EIPHT + PHh: TNF-α/IL-6–0.97, and IFN-γ/IL-6–3.22. Consequently, in patients with OC before treatment and after PCT without immunotherapy, there is a pronounced imbalance of cytokines, which is aggravated by PCT.

Conclusion The use of immunotherapy in combination with PCT is, in our opinion, a justified and effective method that leads to normalisation of immunological parameters of the immune system, allows improving immediate results of treatment, to reduce the intoxication caused by disease and makes it possible to carry out a ‘full’ amount of therapy.

Introduction T-cell acute lymphoblastic leukaemia (T-ALL) is a malignancy arising from T-cell progenitors. The molecular cross-talk between thymocytes and stromal cells is key for T-cell development. Although T-ALL cells require stromal cell support to be maintained ex vivo, it is unclear how thymic microenvironmental cues support T-ALL.

We previously showed that stromal lymphotixin-β receptor (LTβR) favours thymic T-ALL development in Eμ-TEL-JAK2 transgenic mice. Mouse leukemic cells expressed lymphotixin (LT) proteins and T-ALL development was impaired in Eμ-TEL-JAK2 mice lacking the Ltbr gene or treated with an LT antagonist. These results suggest that the LT-LTβR signalling axis mediates the crosstalk between malignant and non-malignant cells, thus favouring leukaemogenesis.

Material and methods To test whether LT-expressing leukemic cells can activate LTβR in stromal cells we use an in vitro culture system. Since the main signalling pathway activated by LTβR is that leading to NF-kB transcription factor activation, we have generated luciferase reporter cell lines, by transducing LTβR-expressing stromal cell lines (NIH3T3 fibroblasts and MS5 bone marrow stromal cells) with a lentivirus carrying the luciferase reporter gene linked to an NF-kB promoter. The validation of the in vitro reporter cellular system was performed by LPS and agonist anti-LTβR treatment. For co-culture assays, primary leukemic cells from Eμ-TEL-JAK2 mice were seeded on top of reporter stromal cells. For LT-blocking experiments, a soluble LTβR-Fc fusion protein was used.

Results and discussions Our results demonstrate that LPS and anti-LTβR activate the NF-kB-luciferase reporter in both cell lines. More importantly, mouse leukemic cells activated the NF-kB reporter in the MS5 and NIH3T3 cells. Showing that NF-kB activation was mediated through LTβR stimulation, luciferase activity in co-cultures was blocked by soluble LTβR-Fc protein. In addition, NF-kB reporter induction by co-cultured leukemic cells was impaired in LTβR-deficient mouse embryonic fibroblasts. We are currently generating LTβR knockout (KO) in MS5 and NIH3T3 stromal cell lines by CRISPR/Cas9 which will also carry the NF-kB-luciferase reporter. The LTβR-KO and WT stromal cells co-cultured with leukemic cells will be sorted for RNA-Seq analysis to identify the specific LTβR-dependent transcriptional program.

Conclusion In conclusion, LT-expressing leukemic cells can activate LTβR signalling in neighbouring stromal cells.
regardless of PTEN status, whereas mTOR pathway upregulation is observed mainly in PTEN-competent CRC cells. **Conclusion** The presence of stromal cells (fibroblasts/endothelium) profoundly influences CRC response to PI3K/mTOR-targeting agents. Understanding the mechanisms underlying microenvironmental interactions (tumour, stroma, soluble factors) may be of fundamental importance to overcome therapeutic resistance and develop more effective therapies for patients affected by cancer.

**PO-294 BRAFV600E/PTEN-LOSS STATUS IS ASSOCIATED WITH INTERLEUKIN (IL)–8 EXPRESSION IN PRECLINICAL MODELS OF COLORECTAL CANCER (CRC)**

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**Introduction** Mutational status in CRC is a strong predictor for overall survival; unfortunately, tumour microenvironment (TME) and tumour-stroma interactions (TSI) also increase cancer cells’ drug resistance, leading to an urgent need to better understand the molecular mechanisms of acquired tumor-resistance, which remains crucial to determine overall patient benefit.

**Material and methods** Production of IL-8 and vascular endothelial growth factor (VEGF) was determined by ELISA under standardised culture conditions. Modulation of cytokine production after exposure to selective inhibitors of the MAPK and PI3K pathways was assessed, by both ELISA assay and real time-PCR. BRAF, MEK1, ERK1 and ERK2 expression were modulated using siRNAs specifically targeting these genes.

**Results and discussions** CRC cell lines harbouring both BRAF<sup>V600E</sup> and PTEN-loss expressed the highest levels of IL-8 and a ROC curve-based prediction algorithm based on these two mutations had 68% accuracy in predicting IL-8 production (p=0.002); on the other hand, VEGF levels inversely correlated with KRAS mutational status. IL-8 is tightly and transcriptionally controlled by activation of the MEK/ERK related with KRAS mutational status. IL-8 is tightly and transcriptionally controlled by activation of the MEK/ERK pathway, whereas mTOR pathway upregulation is observed mainly in PTEN-competent CRC cells. **Conclusion** The presence of stromal cells (fibroblasts/endothelium) profoundly influences CRC response to PI3K/mTOR-targeting agents. Understanding the mechanisms underlying microenvironmental interactions (tumour, stroma, soluble factors) may be of fundamental importance to overcome therapeutic resistance and develop more effective therapies for patients affected by cancer.