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P03.16 FUNCTIONAL DEFECTS IN B-CELLS OF PATIENTS WITH VON-HIPPEL-LINDAU SYNDROME

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Von-Hippel-Lindau (VHL)-disease is an inherited cancer syndrome characterized by a variety of benign and malignant tumors, which develop upon mutation of the second allele of the VHL-tumor suppressor gene. The VHL-protein (pVHL) regulates hypoxia-induced transcription factors (Hif) and by this plays a central role for metabolic cellular adaptations to hypoxic conditions. VHL/Hif regulation plays a well-established role in the development and function of immune cells and already VHL-haploinsufficiency can alter gene expression patterns. In contrast, little is known about primary immune cell functions in VHL-patients. In this study, we analyzed the functional capacity of CD40-stimulated B-cells to act as antigen-presenting cells. We confirmed mono-allelic VHL-gene mutations in B-cells from thirteen VHL-patients and found that their response to CD40-stimulation was significantly reduced. On a functional level this translated to an impaired ability to act as antigen presenting cells leading to impaired T-cell responses *in vitro*. Taken together, we demonstrate that VHL-haploinsufficiency deregulates B-cell functions following CD40-activation as a new aspect of VHL-syndrome. (The study was registered in the German Clinical Trial Registry (www.drks.de); ID: DRKS00012413).

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P03.17 UPA-PAI-1 HETEROMERS PROMOTE ADVANCED STAGES OF BREAST CANCER BY ATTRACTING PRO-TUMORIGENIC NEUTROPHILS

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Background High tumor levels of urokinase-type plasminogen activator (uPA)-plasminogen activator inhibitor-1 (PAI-1) heteromers independently predict poor survival in early breast cancer. The pathogenetic role of this protein complex, however, remains largely obscure.

Materials and Methods Neutrophil trafficking was analyzed in orthotopic (multi-channel flow cytometry) and heterotopic (ear; multi-channel *in vivo* microscopy) mouse models of 4T1 breast cancer, in a mouse peritonitis assay (multi-channel flow cytometry), as well as in the mouse cremaster muscle (multi-channel *in vivo* microscopy). Cytokine expression in tumors was determined by multiplex ELISA. Phenotypic and functional properties of primary mouse neutrophils, microvascular endothelial cells (cell line bEnd.3), macrophages (cell line RAW 264.7), and breast cancer cells (cell line 4T1) were characterized in different *in vitro* assays. uPA/PAI-1 expression and neutrophil infiltration in human breast cancer samples were assessed by RNA sequencing, immunohistochemistry, and ELISA.

Results Here, we demonstrate that uPA-PAI-1 heteromerization multiplies the potential of the single proteins to attract pro-tumorigenic neutrophils. To this end, tumor-released uPA-PAI-1 utilizes very low density lipoprotein receptor and ERK mitogen-activated protein kinases to initiate a pro-inflammatory program in peritumoral macrophages. This promotes neutrophil trafficking to cancerous lesions and primes these immune cells towards a pro-tumorigenic phenotype, thus supporting tumor growth and metastasis. Blockade of uPA-PAI-1 heteromerization by a novel inhibitor effectively interfered with these events and prevented tumor progression.

Conclusions Our findings identify an already therapeutically targetable interplay between hemostasis and innate immunity that drives advanced stages of breast cancer. As a personalized immunotherapeutic strategy, blockade of uPA-PAI-1 heteromerization might be particularly beneficial for patients with highly aggressive uPA-PAI-1^{high} tumors.

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P03.18 ABSTRACT WITHDRAWN

P03.19 EVALUATION OF IMMUNOGENICITY DIFFERENCES IN LLC1 AND GL261 TUMOR MODELS FOR EFFECTIVE CHEMO-IMMUNOTHERAPY TREATMENT

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Background Tumor immunogenicity is a critical factor responsible for the limited success of cancer immunotherapy and determine the need for personalized treatment. Correct evaluation of effectiveness of cancer treatments and their combination is inseparable from the proper selection of the experimental tumor model. The lack of knowledge about the immunogenicity of animal tumor models makes it difficult to evaluate the efficacy of cancer immunotherapy and becomes

the reason why the results of experimental studies are not suitable for biomedical research. The goal of our work was to evaluate the immunogenic properties of two murine cancer models - Lewis lung carcinoma LLC1 and glioma GL261 and to select two immunologically different tumor models for further chemo-immunotherapy research.

Materials and Methods Firstly, the immunological properties of GL261 and LLC1 cells were assessed *in vitro*. For this reason, expression of MHC I, PD-L1 and CD44 on LLC1 and GL261 cells surface was evaluated. Then the ability of GL261 and LLC1 lysates to activate immature murine dendritic cells (DCs) was estimated. Murine DCs were generated from bone marrow cells by cultivating them with GM-CSF for 6 days¹ and then maturing them for 24 hours with LLC1 and GL261 lysate supplemented with *E. coli* lipopolysaccharide. Activation status of DCs was assessed by the expression of surface markers CD11c, MHC II, CD80, CD86, CD40 and CCR7. Later C57BL/6 mice were inoculated s.c. into the left side of the back with GL261 or LLC1 cells. Tumor development was monitored every 2–3 days and then tumors reached a size of ~1.5 cm³ mice were sacrificed. Tumors were collected for evaluation of immune cell infiltration and predominant cytokine profile. Also inactivated GL261 and LLC1 cells were inoculated prophylactically before tumor inoculation and their ability to induce antitumor immune memory was investigated.

Results Our study revealed different immunogenic properties of LLC1 and GL261 cells. LLC1 tumors developed significantly faster than GL261 tumors. Infiltration of immune cells, especially CD8⁺ lymphocytes and NK cells, was more prominent in GL261 than in LLC1 tumors. Also MHC I and PD-L1 expression was significantly higher on GL261 cells. They also showed better ability to induce antitumor immune memory and to activate murine dendritic cells. Cytokine profile analysis further confirmed immunological differences between LLC1 and GL261 cells.

Conclusions LLC1 and GL261 tumors possess different immunogenic properties - GL261 tumor reflects immunogenic tumor model while LLC1 tumor - nonimmunogenic model. These results confirm us the idea that the immune subtype of tumour should be taken into account when evaluating the results of various combinations of chemo-immunotherapies.

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P03.20

A MURINE, MYC-DRIVEN LYMPHOMA MODEL EXPRESSING HUMAN CD22 ENABLES TESTING OF TARGETED THERAPIES AND THEIR EFFECTS ON TUMOR IMMUNE MICROENVIRONMENT

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Background The tumor microenvironment (TME) is composed of various cell types which closely interact via cell cell contacts and cytokines leading to tumor promotion, immune cell inhibition and drug resistance. TME is increasingly recognized

for its role in cancer immunotherapies. In B-cell malignancies, myeloid cells play a central role in supporting tumor growth and immune suppression (Roussel *et al.*, 2017, Cancer Immunol Immunother). Despite the importance of a syngeneic TME, preclinical studies with novel drugs have mainly been performed in models lacking a functional immune system. Therefore, we developed an immune competent murine lymphoma model transgenic to human CD22 to study effects of targeted therapies on TME.

Materials and Methods A chimeric CD22 consisting of human extracellular and murine intracellular CD22 (h/mCD22) was introduced in BL6 mice (BL6^{h/mCD22}). Crossbreeding with BL6^{λ-myc} lead to spontaneous development of murine lymphoma that were serially transplanted. Tumor infiltration and TME was characterized by flow cytometry. Mice were treated with Moxetumomab pasudotox, a CD22 targeted immunotoxin and Doxorubicin.

Results Spontaneously developed tumors in lymphoid organs from BL6^{h/mCD22} × λ-myc consist of a monomorphic population of h/mCD22⁺ murine B cells. Three primary lymphoma subclones were isolated from distinct mice and serially transplanted in syngeneic mice. Stable tumor growth was established after subcutaneous (sc) and intravenous (iv) injection. However, TME of sc tumors was infiltrated by less than 1% immune cells, while myc-driven lymphoma in humans usually show substantial immune infiltration. In contrast to sc tumors, systemically growing lymphoma in murine bone marrow (BM) are infiltrated by 30% myeloid cells and 1% T-cells and in murine spleen by 10% and 30%, respectively. Myeloid cells found in these tumors were shown to suppress T cell proliferation *in vitro*. To test functionality of the h/mCD22 transgene, lymphoma-bearing mice were treated with Moxetumomab, which reduced BM lymphoma infiltration by 20 to 100-fold and infiltration in spleen by 5 to 20-fold in the three lymphoma models. Effects of treatment on TME were analyzed after treatment with Doxorubicin which is known to activate myeloid cells *in vivo*. Compared to untreated controls, Doxorubicin increased CD11b⁺ cells in spleen by 1.5-fold. Among these cells, Ly6G⁺ granulocytic cells increased most substantially.

Conclusions We established primary, myc-driven h/mCD22⁺ B-cell lymphoma which stably engraft in syngeneic mice with a TME mimicking myc-driven lymphoma in men. The model responds well to CD22-targeted therapy and Doxorubicin induces expected immunologic changes. Therefore, our unique model provides a platform to test CD22-targeting treatment strategies in an immune competent background.

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P03.21

PROJECTING T CELLS INTO A REFERENCE TRANSCRIPTOMIC ATLAS TO INTERPRET ANTITUMOR IMMUNE RESPONSES

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Background Single-cell transcriptomics is a transformative technology to explore heterogeneous cell populations such as T cells, one of our most potent weapons against cancer and viral