

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

infections. Recent advances in this technology and the computational tools developed in their wake provide unique opportunities to build reference cell atlases that can be used to interpret new single-cell RNA-sequencing (scRNA-seq) data and systematically compare data sets derived from different models or therapeutic conditions.

Materials and Methods We have developed ProjecTILs (<https://github.com/carmonalab/ProjecTILs>), a novel computational method to project new data sets into a reference map of T cells, enabling their direct comparison in a stable, annotated system of coordinates. ProjecTILs enables the classification of query cells into curated, discrete states, but also over a continuous space of intermediate states. We illustrate the projection of several data sets from recent publications over two cross-study murine T cell reference atlases: the first describing tumor-infiltrating T lymphocytes (TILs), the second characterizing acute and chronic viral infection.

Results ProjecTILs accurately predicted the effects of multiple perturbations, including the ablation of genes controlling T cell differentiation, such as *Tox*, *Ptpn2*, *miR-155* and *Regnase-1*, and identified novel gene programs that were altered in these cells (such as a *Lag3-Klrc1* inhibitory module), revealing mechanisms of action behind these immunotherapeutic targets and opening new opportunities for the identification of novel targets. By comparing multiple samples over the same reference map, and across alternative embeddings, our method allows exploring the effect of cellular perturbations (e.g. as the result of therapy or genetic engineering) in terms of transcriptional states and altered genetic programs.

Conclusions The proposed computational method will likely contribute to reveal the mechanisms of action of experimental immunotherapies and guide novel therapeutic interventions in cancer and beyond.

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P03.22 REPOLARIZATION OF TUMOR-ASSOCIATED MACROPHAGES FOR IMMUNOTHERAPY OF TUMORS WITH DIVERSE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I EXPRESSION

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Background Depletion of tumor-associated macrophages (TAMs), which are regarded as M2, pro-tumor cells, is one of the strategies for cancer treatment. However, repolarization of TAMs to the M1 anti-tumor phenotype could constitute an immunotherapeutic alternative for tumors with defective major histocompatibility complex class I (MHC-I), where the anti-tumor effect of cytotoxic CD8⁺ T cells could be limited.

Materials and Methods In this study, we characterized TAMs from mouse tumor models of human papillomavirus 16-associated tumors, characterized by either reversibly (TC-1/A9) or irreversibly (TC-1/dB2m) downregulated MHC-I expression. Tumors were treated with DNA immunization against the papillomaviral E7 oncoprotein combined with intraperitoneal injection of the synthetic oligodeoxynucleotide ODN1826, a Toll-like receptor 9 agonist. TAMs were characterized *ex vivo*

by flow cytometry. *In vitro*, F4/80⁺ TAMs from naïve tumors were stimulated to M1 or M2 phenotype and co-cultures with TC-1/A9 or TC-1/dB2m cells were established. The cytotoxic effect of polarized TAMs was investigated, and the role of nitric oxide (NO) and tumor necrosis factor (TNF)- α was examined. Finally, interleukin (IL)-10, IL-12 and TNF- α concentrations were determined by ELISA in the culture media from polarized TAMs.

Results We demonstrated that TAMs infiltrated both tumor types and this effect was moderately enhanced after combined immunotherapy. Increase in MHC-II molecules, broadly regarded as an M1 marker, was observed solely in TAMs from treated TC-1/A9 tumors. In contrast, TAMs from TC-1/dB2m tumors expressed high MHC-II levels, regardless of the treatment. Therefore, the new CD38⁺/Egr2⁺ classification¹ was applied and showed to be a better descriptive parameter for M1/M2 TAMs, respectively, because the number of Egr2⁺ TAMs decreased in both tumor types after combined immunotherapy. While CD38⁺ TAMs were significantly increased after treatment of TC-1/A9 tumors, they did not increase substantially in TC-1/dB2m tumors. *In vitro*, co-cultures with tumor cells resulted in increase of NO production by M1 TAMs. However, NO and TNF- α contributed to the cytotoxic effect only in TAMs from TC-1/A9 tumor. Finally, *in vitro* polarized M1 TAMs were able to produce TNF- α and IL-10 but not IL-12.

Conclusions Our results showed different effects of immunostimulation on cytotoxicity of TAMs from tumors with distinct MHC-I expression. While TAMs from TC-1/A9 tumors acquired M1 phenotype and became cytotoxic, TAMs from TC-1/dB2m tumors were more resistant to repolarization. This project was supported by grants GA19-00816S provided by the Czech Science Foundation and LQ1604 provided by the Ministry of Education, Youth and Sports of the Czech Republic.

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P03.23 EVOLUTION OF THE IMMUNE LANDSCAPE WITHIN PARTIALLY CONTROLLED MURINE MELANOMA

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Background Regulatory T cell (Treg) depletion with antibodies against CD25 is effective in tumor models but response rates are low in poorly infiltrated B16 melanomas. Combination with a tumor vaccine enhances efficacy, but relapse usually occurs following partial control, similar to what is seen clinically. How resistance develops is unknown.

Materials and Methods C57BL/6 mice were injected subcutaneously with B16 cells. Treatments included a depleting mouse IgG2a α CD25 antibody and/or a genetically modified, granulocyte-macrophage colony-stimulating factor (GM-CSF) secreting whole B16 tumor vaccine (Gvax). Changes in the immune landscape were assessed with high dimensional flow cytometry.