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 

\[ \text{Tox, Ptpn2, mir-155 and Regnase-1,} \]

and identified novel gene programs that were altered in these cells (such as a \( \text{Lag3-Klrc1 inhibitor module,} \)

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.

Disclosure Information M. Andreatta: None. S.J. Carmona: None.

**P03.22**

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

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10.1136/jitc-2020-ITOC7.60

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. TAMs 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 vivo, 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 vivo, 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.

**REFERENCE**


Disclosure Information A. Piataková: None. I. Poláková: None. M. Šmahel: None.

**P03.23**

EVOLUTION OF THE IMMUNE LANDSCAPE WITHIN PARTIALLY CONTROLLED MurINE MELANOMA

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10.1136/jitc-2020-ITOC7.61

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 αCD25 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.
Results Compared to monotherapies, combined Gvax/αCD25 significantly delayed tumour growth and prolonged survival, in association with enhanced infiltration of T cells with an activated phenotype. Approximately 50% of mice achieved partial response with relapse at day 35–45 post tumor injection. To characterize immune evolution prior to relapse, we analysed stable, partially responding tumors and paired draining lymph nodes (DLNs). Over time, activated PD-1 +ICOS+TCF7+- T cells with an effector memory (CD44 +CD62L-) phenotype fell from 30% to 10% whilst resting, TCF7+ early differentiated cells rose in abundance towards levels seen in untreated tumors. Abundance of Ki67+, resting Tregs also recovered. Similar results were obtained in analyses of DLNs.

Conclusions Combined Treg depletion/whole tumor vaccination therapy is effective in a poorly infiltrated B16 melanoma model. Combined treatment promotes T cell infiltration and activation. In mice achieving a partial response, treatment effects on the immune landscape were observed to decay over time suggesting a return to immune equilibrium. Further studies to explore the mechanistic basis of this observation are underway.

Disclosure Information C. Qing: None. E. Ghorani: None. I. Solomon: None. F. Gálvez-Cancino: None. F. Vargas: None. K. Peggs: None. S. Quezada: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; F. HOFFMANN-LA ROCHE LTD.

P03.25 NEUTRALIZING EXTRACELLULAR CHP-1 IMPAIRS TUMOR GROWTH AND METASTASIS FORMATION
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10.1136/jitc-2020-ITOC7.63

Background Found in the extracellular compartment, Heat Shock Proteins (HSPs) are actively secreted proteins that modulate the tumor behavior. Extracellular HSPs play a unique role as extracellular chaperones and receptors-binding molecules, favoring the establishment and maintenance of different cancer hallmarks, including immune modulation and evasion. CHP-1, is a ubiquitously expressed protein with chaperone activity and its high expression correlates with high tumor grade and lymph node positivity in different breast and lung cancer subtypes. In addition, CHP-1 is actively and uncanonically secreted by cancer cells in the tumor microenvironment (TME).

Materials and Methods Sera cancer patients were analyzed for the presence of CHP-1. To assess the role of extracellular CHP-1 (eCHP-1) in the TME, in vitro experiments on different cell populations have been performed. To dissect the molecular mechanisms, through which eCHP-1 induces cancer progression, have been analyzed specific signaling pathways in cancer and immune cells. Immune cell composition in presence of eCHP-1 in tumors has been identified using flow cytometry. The characterization of eCHP-1 inhibition as therapeutic approach has been conducted in breast and colon cancer pre-clinical models.

Results eCHP-1 activates an autocrine signaling through TLR2, TLR4 and LRP1, promoting tumor progression and metastasis formation in different pre-clinical models. Moreover, eCHP-1 can modulate the immune composition of the TME, making interesting the analysis of its inhibition in cancer immunotherapy.

In some settings, cancer cells responding to treatment undergo an immunogenic form of cell death that is associated with the abundant emission of danger signals in the form of damage-associated molecular patterns. Accumulating preclinical and clinical evidence indicates that danger signals play a crucial role in the (re-)activation of antitumor immune responses in vivo, thus having a major impact on patient prognosis. We have previously demonstrated that the presence of calreticulin on the surface of malignant blasts is a positive prognostic biomarker for patients with acute myeloid leukemia (AML). Calreticulin exposure not only correlated with enhanced T-cell-dependent antitumor immunity in this setting but also affected the number of circulating natural killer (NK) cells upon restoration of normal hematopoiesis. Here, we report that calreticulin exposure on malignant blasts is associated with enhanced NK cell cytotoxic and secretory functions, both in AML patients and in vivo in mice. The ability of calreticulin to stimulate NK-cells relies on CD11c+CD14high cells that, upon exposure to CRT, express higher levels of IL-15Rα, maturation markers (CD86 and HLA-DR) and CCR7. CRT exposure on malignant blasts also correlates with the upregulation of genes coding for type I interferon. This suggests that CD11c+CD14high cells have increased capacity to migrate to secondary lymphoid organs, where can efficiently deliver stimulatory signals (IL-15Rα/IL-15) to NK cells. These findings delineate a multipronged, clinically relevant mechanism whereby surface-exposed calreticulin favors NK-cell activation in AML patients.


P03.24 CALRETICULIN EXPOSURE ON MALIGNANT BLASTS CORRELATES WITH IMPROVED NK CELL-MEDIATED CYTOTOXICITY IN AML PATIENTS
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10.1136/jitc-2020-ITOC7.62