P03.30 TUMOR MUTATIONS DRIVE DYSFUNCTIONAL T CELL DIFFERENTIATION IN LUNG CANCER

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Background Effective anti-tumour immunity requires cancer antigen expression, but persistent antigen exposure in chronic viral infections and autoimmunity has a detrimental effect on immune function. This is associated with a decline of early differentiated T cell populations in favour of later differentiated, dysfunctional subsets, resulting in an unfavourable skewing of the immune landscape. It is unknown whether this occurs locally within the antigen rich tumour microenvironment, driving immune failure.

Materials and Methods We combined tumour infiltrating lymphocyte (TIL) high dimensional flow cytometry, bulk exome and RNA sequencing data from multiregional samples obtained from surgically resected tumours of treatment naive patients with non-small cell lung cancer (NSCLC) amongst the first 100 recruited to the prospective, UK-wide lung TRACERx study. Clonal relationship between T cell populations was determined by T cell receptor (TCR) sequencing. We additionally analysed publically available single T cell RNA sequencing data and bulk RNA sequencing data within TCGA.

Results T cell differentiation skewing (T^{DS}) occurred amongst TILs in association with tumour mutational burden (TMB). Surprisingly, this was most evident within the CD4 compartment that had a greater abundance of central memory cells expressing the key transcription factor TCF7. Amongst CD4 cells, loss of a PD1⁺CCR7⁺ T central memory population was accompanied by gain in abundance of PD1⁺ populations with exhausted (CD57⁺ICOS^{hi}CTLA4^{hi}) and terminally differentiated effector (CD57⁺Eomes⁺) features. TCR sequencing revealed early and dysfunctional differentiated populations to be clonally related and CDR3 clustering analysis showed greater similarity of sequences shared vs. non-shared between subsets, consistent with an antigen driven differentiation process. Similar patterns were observed within the CD8 compartment. Identification of these subsets within single T cell RNA sequencing data revealed shared and distinct functional regulators, suggesting the enhanced effector capability of early compared to dysfunctionally differentiated populations. A validated transcriptional signature of T^{DS} generated using TRACERx samples with paired flow cytometry and RNA sequencing data reflected loss of gene expression downstream of TCF7, and predicted worse survival within TRACERx and multiple TCGA cohorts including lung adenocarcinoma (LUAD).

Conclusions Our finding support a model of neoantigen driven T cell differentiation within the tumour microenvironment that drives the depletion of progenitor-like cells and gain in abundance of dysfunctional subsets, resulting in a loss of immune fitness. Our analysis of transcriptomic data

elucidates potential regulatory mechanisms and therapeutic targets within the subsets identified.

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P03.31 SKIN DENDRITIC CELLS IN MELANOMA ARE KEY FOR SUCCESSFUL CHECKPOINT BLOCKADE THERAPY

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Background Immunotherapy of cancer by checkpoint blockade has significantly improved the survival of melanoma patients. However, in patients with tumors that are poorly infiltrated by effector T cells the clinical results are not encouraging. Therefore, combination approaches that enhance pre-existing anti-tumor immunity and reset the patients' immunological status are urgently needed. In this study we used the tg(Grm1)EPv melanoma mouse model that reflects a non-immunogenic tumor microenvironment. In this mouse model, spontaneous melanoma development is driven by the ectopic expression of the metabotropic glutamate receptor-1 in melanocytes, which confers to them a hyperproliferative and anti-apoptotic phenotype. The same alteration has been shown to be present in 40% of melanoma patient samples. The aim of our study was to investigate whether enhancing dendritic cell (DC) numbers and function in the tg(Grm1)EPv mouse model could restore responsiveness to checkpoint blockade.

Material and Methods We used multicolor flow cytometry, gene expression analysis by RNA-seq and microarray to

analyze tumors and tumor-draining lymph nodes (tdLN). With various immunological *in vitro* and *in vivo* assays we determined the functional role of DC in tumor immunity.

Results A loss of skin DC has previously been reported for primary melanoma lesions and we here show that melanoma progression in the tg(Grm1)EPv mouse model coincides with a gradual decrease in the skin cDC2 subset and an upregulation of the inhibitory ligands PD-L1 and galectin-9. Monotherapy with anti-PD-L1 could not delay tumor growth, suggesting that this is a good model to study resistance to checkpoint blockade. We hypothesized that by boosting DC numbers and function we would restore responsiveness to checkpoint blockade. By administering a treatment consisting of systemic Flt3L and intratumoral polyI:C/anti-CD40, we were able to rescue the numbers and function of skin cDC2. Analysis of the treated tumors by flow cytometry showed that the DC boost regimen led to an increased tumor infiltration of activated CD4+ and CD8+T cells. An *in vitro* T cell proliferation assay revealed that dermal cDC2 that had migrated to the tdLN, played a crucial role in this process, since these were able to cross-present endogenous gp100 antigen more efficiently than migratory Langerhans cells and dermal cDC1. CD4+ and CD8+T cells recruited in the tumors of the DC boost treated mice, expressed PD-1 and TIM-3. Therefore, combination therapy with checkpoint blockade of these molecules resulted in increased cytotoxic activity within the tumor and eventually delay of tumor growth.

Conclusions Our results demonstrate that skin DC shape the tumor microenvironment upon immunotherapy and thus, therapies that aim to enhance responsiveness to checkpoint blockade may well benefit from a component that boosts the numbers and the function of skin DC.

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P04 Vaccine Therapy

P04.01 DENDRITIC-CELL BASED IMMUNOTHERAPY TARGETING PANCREATIC AND NSCLC CANCER STEM CELLS

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Background The field of cancer immunotherapy is growing at a fast pace, with new developments in this field leading to a change in cancer therapy. Dendritic cells (DCs) are one of

the central tools in cellular anti-tumour immunotherapy and the production of clinical grade monocyte-derived DCs (Mo-DCs) is the most frequent approach for antitumor vaccines production. However, there is a large space for improvement of protocols and a clear need for the establishment of clinical standard operating procedures (CSOP). Cancer stem cells (CSCs) are a recently identified small cell population present in the tumour, resistant to radio/chemotherapy and known to be responsible for disease recurrence. Here, we aim to contribute to the standardization of CSOPs and to target and eradicate CSCs by developing a DC-based immunotherapy vaccine for pancreatic and non-small cells lung cancer (NSCLC), comparing DC loading with CSCs vs. classical tumour lysates.

Materials and Methods CSCs from PANC-1 (pancreatic cancer) and A549 (NSCLC) cell lines were isolated and characterized by RT-PCR and flow cytometry. CSCs resistance to chemotherapy was also assessed. *In vitro* anti-tumour cytotoxicity assays were performed. We also defined and compared the effect of 4 culture media during human Mo-DCs production. Three Good Manufacturing Practice (GMP) serum-free culture media for clinical use were tested - DendriMACS, AIM-V and X-VIVO 15. RPMI was used as a comparative term given that it is largely used in pre-clinical research. We characterized DC viability, differentiation, maturation, internalization of tumour lysates, cytokines production and autologous T cell stimulatory capacity, as well as metabolomic profiles by Nuclear Magnetic Resonance (NMR) spectroscopy.

Results CSCs from PANC-1 and A549 cell lines were successfully isolated and overexpressed the stem-like markers NANOG, OCT4, SOX2 and CD133, with resistance to gemcitabine. In terms of differentiation, maturation, antigen uptake capacity and metabolic profiles, AIM-V and X-VIVO 15 present similar results. However, the use of X-VIVO 15 shows an enhanced DC production of IL-12. DCs cultured in X-VIVO 15 and AIM-V media are able to induce a superior stimulation of T cells (CTLs and Th1 responses) while DCs cultured in DendriMACS are more prone to induce Treg polarization. Our data show that X-VIVO 15 and AIM-V culture media are preferable to support the differentiation of DCs to be used in immunostimulatory approaches such as in cancer immunotherapy.

Conclusions Overall, our results demonstrate that blood monocytic precursors present considerable plasticity allowing a tailored differentiation of DCs just by changing the nutritive support. This highlights the need of critically defining the culture medium to be used in DC cancer immunotherapy, attaining the desired cell characteristics and consequent robust clinical responses. We are now assessing *in vitro* anti-tumour cytotoxicity to evaluate if DC loading with CSC antigens can be an efficient immunotherapy strategy to target and eliminate this specific and resistant cancer cell population.

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