pembrolizumab therapy and were correlated with response. Effector-type CNs enriched in tumor-infiltrating CD4+ T cells and dendritic cells were significantly increased after treatment in responders. In contrast, a regulatory T cell-enriched CN was significantly increased in non-responders before and after therapy. Furthermore, a spatial signature of cell-cell distances between tumor cells and effector/regulatory immune cells predicted therapy outcome. In addition, CIBERSORTx analysis revealed that tumor cells in responders, but not in non-responders, increased their expression of immune-activating genes. **Conclusions** High-dimensional spatial analysis of CTCL tumors revealed a pre-existing immunosuppressive state in pembrolizumab non-responders. Thorough analysis of the TME therefore enables the discovery of novel spatial biomarkers in a concept that accounts for both cell type information and higher-order tumor architecture. Combining highly multiplexed microscopy with CIBERSORTx allows for the discovery of novel, predictive spatial biomarkers of immunotherapy response and will pave the way for future studies that functionally address these cell types and their interactions.

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On Demand Talk: 'Lost in Translation'

04 MECHANISMS OF LUNG CANCER HYPER-PROGRESSION PROMOTED BY PD-1 IMMUNE CHECKPOINT BLOCKADE

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Background Immune checkpoint blockade (ICB) with antibodies against PD-1 or PD-L1 may provide therapeutic benefits in patients with non-small cell lung cancer (NSCLC). However, most tumours are resistant and cases of disease hyper-progression have also been reported.

Materials and Methods Genetically engineered mouse models of Kras^{G12D}p53^{null} NSCLC were treated with cisplatin along with antibodies against angiopoietin-2/VEGFA, PD-1 and CSF1R. Tumour growth was monitored by micro-computed tomography and the tumour vasculature and immune cell infiltrates were assessed by immunofluorescence staining and flow cytometry.

Results Combined angiopoietin-2/VEGFA blockade by a bispecific antibody (A2V) modulated the vasculature and abated

immunosuppressive macrophages while increasing CD8⁺ effector T cells in the tumours, achieving disease stabilization comparable or superior to cisplatin-based chemotherapy. However, these immunological responses were unexpectedly limited by the addition of a PD-1 antibody, which paradoxically enhanced progression of a fraction of the tumours through a mechanism involving regulatory T cells and macrophages. Elimination of tumour-associated macrophages with a CSF1R-blocking antibody induced NSCLC regression in combination with PD-1 blockade and cisplatin.

Conclusions The immune cell composition of the tumour determines the outcome of PD-1 blockade. In NSCLC, high infiltration of regulatory T cells and immunosuppressive macrophages may account for tumour hyper-progression upon ICB.

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On Demand Talk: Young Researcher Session

05 DECONSTRUCTION OF HAMPERED DENDRITIC CELL DEVELOPMENT BY MICRO-ENVIRONMENTAL CROSSTALK IN AN ORGANOTYPIC HUMAN MELANOMA-IN-SKIN MODEL

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Background Immune suppressive conditions in the melanoma tumor microenvironment (TME) block dendritic cell (DC) development and lead to the accumulation of M2-like macrophages and myeloid-derived suppressor cells (MDSCs). This will effectively hamper T cell priming, recruitment, and effector functions, and so interfere with the efficacy of immunotherapy. Targeting tumor-mediated myeloid suppression represents an interesting therapeutic option to promote the immune attack on tumors. The preclinical human models currently used to study myeloid suppression often fail to reflect the complexity of the TME.

Materials and Methods To study the cross-talk between melanoma and stroma cells and assess its effect on DC differentiation, we therefore established an *in vitro* three-dimensional (3D) reconstructed organotypic human melanoma-in-skin (Mel-RhS) model, allowing the monitoring of tumor growth and progression for up to six weeks.

Results Significantly higher levels of immune suppressive cytokines (IL-10, M-CSF, VEGF, TGF-beta) were detected in the melanoma model, constructed with the BRAF- and PTEN-mutated SK-MEL-28 cell line, as compared to its control (without melanoma cells). Indeed, Mel-RhS culture supernatants interfered with monocyte-to-DC differentiation, leading to the development of M2-like macrophages with a distinct phenotype (CD14⁺CD11a⁺BDCA3⁺CD163⁺CD16⁺PDL1⁺PDL2⁺), as established by polychromatic flowcytometry. Correlation matrix heatmap analysis identified IL-10, TGF-beta and M-

CSF as the main candidate mediators of this skewing of monocytes to an M2-like state. The use of specific neutralizing antibodies against each of these cytokines prevented the observed DC suppression to varying degrees. t-Distributed Stochastic Neighbor Embedding (t-SNE) identified specific shifts between monocytic subpopulations and modulated expression levels of associated surface markers. Neutralization of M-CSF reduced expression of BDCA3, PD-L2, and PD-L1, while increased CD16; whereas blocking TGF-beta led to a concerted reduction in CD14, CD163, PD-L1, and PD-L2 levels, but, unexpectedly, also of CD80. In contrast, IL-10 neutralization resulted in a decrease of all M2-related markers, while CD80 levels were upregulated. Interestingly, while the SK-MEL-28 cell line did not secrete detectable levels of IL-10 in traditional monolayer cultures, RNA *in situ* hybridization revealed *de novo* expression in Mel-RhS in melanoma cells, as well as in keratinocytes and fibroblasts.

Conclusions We conclude that the 3D configuration of the Mel-RhS model results in cross-talk between tumor and stroma, which allows for the delineation of immune suppressive pathways in the melanoma TME. Ultimately, this model could be used as a novel *in vitro* tool for preclinical testing of immune modulatory therapeutic agents.

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On Demand Talks: Combination Therapy

06

EXPRESSION OF ANTI-APOPTOTIC GENE CFLIP TO ENHANCE PERSISTENCE IN CAR T CELLS

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Background CAR T cell therapy has been successful for targeting blood cancers, but treatment of solid cancers has been limited due to the heterogenous nature of tumour-associated antigen expression on solid cancers, and the suppressive tumour microenvironment.¹ Another major obstacle to CAR T cell therapy is activation-induced cell death (AICD) of the CAR T cells.² In this study, we expressed the anti-apoptotic cellular FLICE-like inhibitory protein (c-FLIP short; c-FLIPs) together with the CAR construct to enhance CAR T cell persistence.³

Materials and Methods The anti-Her2 FRP5 CAR T construct with P2A-linked cFLIPs or cFLIPp43 was cloned into the Sleeping Beauty (SB) transposon vector (pSBtet-GP) or lentiviral vector, under the control of either a tet-on or a constitutive promoter. Construct expression was validated by qPCR and immunoblot analysis. CAR T cells were generated by SB transposition or lentiviral transduction of CD3/CD28 stimulated primary human T cells that were subsequently maintained with IL-2. Mitochondrial function and apoptosis were determined by resazurin assay and by flow cytometry using tetramethyl rhodamine (TMRE).

Results Overexpression of cFLIP (cFLIPp43 and cFLIPs) in pSBtet-GP demonstrated protection in both Jurkat T cell line and primary human T cells. pSBtet-GP was modified to

overexpress cFLIPs and cFLIPp43 under tet-on promoter, with the anti-her2 CAR, GFP and rtTA under constitutive promoter. Transfer of the inducible cassette from the SB transposon to a lentiviral system resulted in a significant loss of tightness. Doxycycline treated CAR T cells showed only ~13-fold overexpression of cFLIPs or cFLIPp43 compared to untreated cells, and doxycycline significantly inhibited (approximately 30%) primary CAR T cell expansion. In contrast, constitutive expression of CAR-cFLIPs or cFLIPp43 construct gave a $>3 \times 10^5$ -fold cFLIP overexpression, as compared to CAR-only control. While the transduction efficiency of CAR-only was around 70–80% control in primary T cells, this dropped to 20–25% when using the more genetically complex tet-on system.

Conclusions cFLIP protects T cells from Fas-induced apoptosis. The tet-on system demonstrates several drawbacks in the lentiviral system, including toxicity of the inducer drug (and/or squelching effects resulting in lowered viability), loss of responsiveness and lowered transduction frequencies. Therefore, a constitutive promoter system is preferred in lentiviral systems for the control of genes of interest within CAR T cells, while the SB transposon system may be preferred for tet-on control within CAR T cells.

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07

A BISPECIFIC VHH APPROACH TO LEVERAGE THE POTENT AND WIDELY APPLICABLE TUMOR CYTOLYTIC CAPACITY OF Vγ9Vδ2 T CELLS

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Vγ9Vδ2-T cells include a unique and potent subset of T cells which play an important role in tumor defense. Vγ9Vδ2-T cells recognize and can lyse butyrophilin 3A1-expressing target cells with elevated levels of non-peptide phosphoantigens (pAg), induced by cell stress or malignancy. To date, Vγ9Vδ2-T cell based cancer immunotherapeutic approaches were well tolerated and in some cases capable of inducing relevant clinical responses. In an effort to improve the efficacy and consistency of Vγ9Vδ2-T cell based cancer immunotherapy, we designed a bispecific VHH that binds to both Vγ9Vδ2-T cells and EGFR expressed by tumor cells and results in the target-specific activation of Vγ9Vδ2-T cells and subsequent lysis of colorectal cancer cell lines and primary colorectal cancer samples both *in vitro* and in an *in vivo* mouse xenograft model. Of note, tumor cell lysis was independent of mutations in KRAS and BRAF that are known to impair the efficacy of clinically registered anti-EGFR monoclonal antibodies as well