330 bp covering CDR1, 2 and 3) via the Oncomine TCR Beta-LR Research Assay. To evaluate T cell convergence within each biopsy, we searched for instances where TCRB chains were identical in amino acid space (shared variable gene identity and CDR3 amino acid sequence) but had distinct nucleotide sequences owing to N-addition and exonucleotide chewback within the V-D and D-J junctions of the CDR3. To provide context, we evaluated evidence for T cell convergence in PBL T cell repertoires derived from healthy donors and 63 additional individuals with melanoma.

Results and discussions Sequencing of melanoma biopsy research samples yielded an average of 6029 clones per sample. 11 of 85 samples yielded fewer than 100 clones and were eliminated from downstream analysis. Convergent T cell receptors were identified in 68/74 (92%) of tumour infiltrating T cell repertoires having greater than 100 detected clones. The frequency of convergent T cell rearrangements was greater in melanoma tumour biopsies than healthy or melanoma PBL samples.

Conclusion These data suggest that T cell convergence may be a common feature of the melanoma infiltrating T cell repertoire. Convergence was more frequently observed within the TME than T cell repertoires derived from healthy and melanoma PBL, consistent with elevated antigen-driven T cell selection within the TME. The extent to which convergence is a feature of the TME in other cancers is not yet known. T cell receptor convergence may be driven by T cell responses to tumour neoantigen within the TME. In such case, in silico identification of convergent T cell receptors by long-amplicon TCRB sequencing may serve as a means for rapid identification of antigen-specific T cell receptors for future therapeutic use.

Introduction Studies in the past few years have suggested a key role for neo-antigens in cancer immunotherapy. Since neo-antigens are specifically expressed on the tumour, targeting them is not likely to induce tolerance or normal tissue toxicity, making them candidates for immunotherapy. Despite encouraging results in clinical trials using neo-antigens, peptide or RNA vaccines and adoptive cell transfer (ACT), only a handful of neo-antigens and their corresponding T-cells have been identified in patients.

Material and methods In this study we are using a combination of a novel neo-antigen prediction pipeline and human leukocyte antigen (HLA) peptidomics to unbiasedly identify tumour associated antigens (TAAs) and neo-antigens in tumours derived from melanoma patients, characterise their interactions with their tumour infiltrating lymphocytes (TILs) and identifying their matched T-cell receptor (TCR) sequences.

Results and discussions For the analysis we used few metastases derived from the same patients and observed high similarity in the metastases antigenic and T-cells repertoires. Using interferon gamma release measurements, tetramer staining and killing assays in vivo and in vitro we were able to show that neo-antigen-specific T-cell clones are more abundant, reactive and proliferative than T-cell clones against TAAs. These methods in combination with TCR sequencing of the neo-antigen clones and bulk TILs enabled us to identify the specificity and identity of 87.4% of the TIL population. Furthermore we were able to show that only few reactive neo-antigens are responsible for the complete killing of the autologous melanoma cells and the rejection of the tumour in vivo.

Conclusion Suggesting that our combined pipeline can help to characterise patient antigen and T-cell repertoires and select antigens that would be beneficial for designing personalised immunotherapy.

Symposium: Mechanisms of Resistance to Tumour Therapy

25 STRATEGIES TO OVERCOME PHENOTYPE SWITCH-DRIVEN THERAPY RESISTANCE IN MELANOMA

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Introduction Phenotype switching is a key mechanism contributing to therapy resistance and metastasis in melanoma. It is marked by high expression of several receptor tyrosine kinases, most prominently AXL, and associated with resistance to the clinically oft-used BRAF and MEK inhibitors. Furthermore, it has recently been suggested that phenotype switching also contributes to resistance to immune checkpoint blockade in melanoma.

Results and discussions We have shown previously that upregulation of AXL occurs concurrently with the downregulation of the melanocytic lineage transcription factor MITF and its downstream protein Melan-A1. Furthermore, we recently showed that most melanomas are highly heterogeneous for AXL expression, both untreated tumours and treatment-resistant ones. This observation provided the framework for a concept to differentially target these distinct AXL-high and -low populations. On the one hand, we targeted AXL-expressing melanoma cells by a novel antibody-drug conjugate, AXL-107-MMAE. On the other hand, the MITF-high, AXL-low melanoma cells were targeted with MAPK pathway inhibitors. Therefore, by eliminating distinct populations within heterogeneous melanoma cell pools, AXL-107-MMAE and MAPK pathway inhibitors cooperatively inhibited tumour growth in vitro and in vivo. Lastly, by inducing AXL transcription, BRAF/MEK inhibitors further potentiated the efficacy of AXL-107-MMAE2.

Although the correlation between phenotype switching and resistance to targeted agents such as BRAF/MEK inhibitors has been well described, it is not well established how melanoma
cells, especially in patients or in vivo, can acquire this state. Furthermore, it is as unclear if there is any functional interaction between the immune system and the phenotype switch. Investigating any such relationship may contribute to the development of therapies that aim to prevent the acquisition of an AXL-high, treatment-refractory state. We will present results of recent efforts to better understand the role that immune cells may play in the establishment of a phenotype switch and how we may re-sensitise tumour cells that have become resistant to T cell therapy as a consequence.

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OVERCOMING THE LIMITATIONS OF LUNG CANCER RADIOThERAPY BY TARGETING THE CD73/ADENOSINE IMMUNE CHECKPOINT
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Introduction Radiotherapy (RT) is part of standard treatments for locally advanced lung cancer. Biological factors such as intrinsic or microenvironment-mediated radiation resistance and tumour immune escape limit the success of lung cancer RT. Moreover, adverse late effects in the highly radiosensitive lung such as pulmonary fibrosis limit the use of curative doses resulting in suboptimal local control, metastases and decreased quality of life. We recently showed that genetic or pharmacologic inhibition of the immune regulatory CD73/adenosine (Ado) system attenuates the development of RT-induced fibrosis. Wirdsöfer et al., Cancer Research 2016 Of note, CD73/Ado are considered as a novel immune checkpoint relevant to cancer.

Material and methods Here we used our preclinical immunocompetent murine in vivo models (C57BL/6 wildtype and knockout mice) to study the impact of CD73 and Ado on the accumulation of fibrosis-associated mediators and phenotypic changes in macrophages and lymphocytes recruited to the lung tissue. Moreover we studied the effect of ionising radiation on CD73 expression in cancer cells and the role of signalling in the host on growth and RT response of cancer cells in vitro and in vivo.

Results and discussions CD73/Ado promote a time-dependent accumulation of fibrosis-associated mediators (hyaluronan, fibronectin, osteopontin, and TGF-b). Radiation-induced lung fibrosis in WT mice was associated with the accumulation of CD4+ regulatory T cells (Treg) and the accumulation of macrophage mannose receptor-positive alternatively activated macrophages (AAM) in organised clusters expressing pro-fibrotic mediators during the fibrotic phase. The failure of irradiated CD73/- mice to accumulate Ado abrogated the CD73/Ado-mediated amplification of the profibrotic cross-talk between resident cells, recruited immune cells and pro-fibrotic mediators, and the formation of pre-fibrotic foci. Moreover, exposure of cancer cells to ionising radiation triggered up-regulation of CD73 in cancer cells. The analysis of the in vivo experiments is under current investigation.

Conclusion CD73/Ado influence shapes the irradiated microenvironment to promote fibrosis. Tumour intrinsic or RT-induced upregulation of CD73 in cancer cells may dampen anti-tumour immune responses during therapy. Pharmacologic modulation of the CD73/Ado system may thus provide a clear therapeutic gain in cancer treatment by protecting normal tissues against the adverse effects of RT and reinstating antitumor immunity.

VEHICLE CANCER CELL DYNAMICS OF COLORECTAL CANCER PROGRESSION
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Introduction Most currently available colorectal cancer (CRC) mouse models are not suitable for studying progression towards the metastatic stage of the disease. Recently, establishment of tumour organoid lines, either from murine CRC models or patients, and the possibility of engineering them with genome-editing technologies, have provided a large collection of tumour material faithfully recapitulating phenotypic and genetic heterogeneity of native tumours.

Material and methods To study tumour progression in the natural in vivo environment, we developed an orthotopic approach based on transplantation of CRC organoids into the intestinal epithelium. With this method we dissected the adenoma-carcinoma sequence (i.e. the consecutive genetic and phenotypic steps that lead to CRC progression and ultimately, formation of distant metastasis) of CRC. Using intravital microscopy we captured the migratory cells of CRC and characterise which cancer cells are capable to intravasate and lead to metastasis formation at distant sites.

Results and discussions We developed an in vivo model of CRC which enables growth of transplanted organoids into a single tumour mass within the intestinal tract. Due to long latency, tumour cells are capable of spreading through the blood circulation and forming metastases at distant sites. With this model we demonstrated that sequential accumulation of specific oncogenic mutations facilitates efficient tumour growth, migration, and metastatic colonisation. We showed that reconstitution of specific niche signals can restore metastatic growth potential of tumour cells lacking one of the oncogenic mutations.

Conclusion Our method is designed to generate tumours suitable for studying CRC progression, thereby providing the opportunity to visualise tumour cell dynamics in vivo. Our findings imply that the ability to metastasize—i.e., to colonise distant sites—is the direct consequence of the lack of dependency on specific niche signals.