suggest that upregulation of TIM3 represents a mechanism of immune evasion in response to A2aR blockade. Critically, co-blockade of A2aR and TIM3 produces complete and durable tumour regression.

Conclusion Expression of TIM3 represents a key mechanism of tumour-mediated immune evasion in response to A2aR blockade. The fact that co-blockade of A2aR and TIM3 mediates complete and durable tumour regression, with no obvious adverse effects, strongly supports the use of such dual blockade in the treatment of cancer.

PO-401 TUMOUR STEM CELL CHARACTERISTICS ARE NEGATIVELY ASSOCIATED WITH ANTI-CANCER IMMUNITY IN DIVERSE SOLID CANCERS
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Introduction The majority of clinical responses to immunotherapies appear to be restricted to tumours displaying a pre-existing T cell-infiltrated tumour microenvironment. Consequently, understanding the molecular mechanisms leading to a T cell-poor microenvironment will be crucial for the development of novel treatments to increase the number of patients benefiting from immunotherapy. Increasing evidence has suggested that tumours are organised in a hierarchical structure of phenotypically heterogeneous cell populations. Cancer stem cells (CSCs) are at the top of this tumour cell hierarchy and sustain the long-term maintenance of neoplasms. The ability of CSCs, as well as physiological stem cells, like embryonic stem cells and mesenchymal stem cells, to resist immune-mediated destruction is unviralled by more differentiated cells. Despite this, the potential impact of a cancer stem-like tumour phenotype on the ability to drive immune exclusion and avoid immune rejection has not been systematically explored.

Material and methods We calculated an RNA-based metric of stemness for >8000 TCGA solid tumour samples. We assessed the association of this metric with transcriptomic signatures of immune cell infiltration and other genomic, transcriptomic, and clinical parameters.

Results and discussions Tumour stemness varied strongly across cancers and negatively associated with patient survival both within and across cancers. We found that high stemness tumours show reduced inferred infiltration of multiple immune cell types, particularly of anti-tumour effector cells such as CD8 + T cells, B-cells, and NK cells. Within well-defined cancer molecular subtypes, we observed recurrent negative associations between stemness and immunity. We also detect negative correlations between immunity and defined stem cell regulatory pathways that reflect the activity of specific stemness transcription factors. Screening for potential stemness associated axes of immunosuppression, we found that enrichment of extracellular matrix organisation process could be a possibly mechanism of stemness-immune interference. Using published data from clinical trials of immune checkpoint blockade therapy, we showed that tumour stem cell transcriptional programs negative correlates with patient survival.

Conclusion Our findings reveal the landscape of stemness across human solid cancers, show that tumour stemness can predict anti-cancer immunity in diverse settings, and may help to identify patients that will have improved response to immunotherapy.

PO-403 THE TUMOUR SUPPRESSOR P53 AS A GUARDIAN OF IMMUNE TOLERANCE AND SUPPRESSION

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Introduction Pancreatic cancer is one of the poorest understood cancers with <5% chance of survival after 5 years of diagnosis. Cell autonomous changes in signalling pathways that promote cancer cell proliferation, growth and survival are being successfully targeted by novel cancer therapies that show efficacy, yet generally fail to provide a durable clinical response. The complexity of the tumour/stromal interaction is key to understanding how cancer cells survive and the interplay between cancer cells and the immune system provides another level of complexity. Cancer cells have escaped immunological checkpoints either through promoting immunosuppressing lineages or supressing anti-tumorigenic mechanisms. Genetically engineered mouse models (GEMM) can recapitulate some aspects of human cancers and allow for the study of tumor-stromal interactions in vivo. A model of pancreatic ductal adenocarcinoma (PDAC) was generated using a Cre recombinase system with the following combinations KRasG12D/p53+/− or KRasG12D/p53−/−. Using a technique of tumour rejection, we demonstrate a role for p53 in PDAC cells in modulating the immune response.

Material and methods In vivo studies were done using PDAC cell lines derived from GEMMs with ectopic expression of iRFP. FVB or CD1 immunodeficient mice were injected subcutaneously with PDAC lines and subcutaneous growth was measured using the Licor Pearl Imager. Immunophenotyping was performed using flow cytometry (i.e. intracellular cytokine staining and surface staining) and acquired on the BD Symphony Fortessa. In vitro studies were conducted on the PDAC cell lines, bone marrow derived macrophages (BMDM) and primary T cells.

Results and discussions Genetic ablation of p53 in PDAC cells provided immunological advantage and delayed tumour rejection. Immunophenotyping results demonstrated a suppression of Th1-like responses in vivo as well as changes in myeloid populations. In vitro studies provided evidence of alterations in macrophage priming and consequential changes in T cell polarisation. Recent studies have demonstrated roles for onco-genes, such as c-Myc and KRas in influencing anti-tumorigenic responses, which support our data of p53-dependent changes.

Conclusion Over the last 50 years immunoediting of the tumour has been shown to be a powerful mechanism of a tumour selection. Loss and mutation of p53 occurs quite frequently in human cancers and understanding the underlying immunological mechanisms of escape in the context of p53 is crucial in developing immunotherapies.

PO-404 USING FUNCTIONAL GENETIC SCREENS TO UNDERSTAND CANCER IMMUNE EVASION

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Introduction With the emergence of immunotherapy as an effective cancer therapy, there is also a growing need to identify...
new biomarkers of response as well as novel therapeutic targets. Functional CRISPR screens have proven to be an important tool to identify key genes in complex biological processes. We use an in vitro and in vivo CRISPR screening strategy to better understand how tumours evade the immune system.

**Material and methods** We collected pre and post treatment biopsies from melanoma and lung cancer patients treated with an anti PD-1 inhibitor. Upon performing whole exome sequencing on biopsies from patients who progressed on therapy, we identified a list of candidate genes. We included genes that fell into one of the three categories: were mutated in more than one patient, were mutated only at relapse and had mutations in both alleles or had a heterozygous mutation at baseline and homozygous on progression.

In addition, we also generated an adenovirus transformed mouse embryonic fibroblast cell line, which does not form tumours in the immunocompetent mouse due to T-cell killing. We use this system to perform a in vivo CRISPR screen, investigating which genes are crucial for immune evasion.

**Results and discussions** We have successfully generated a target gene list from sequences of patient biopsies. We have constructed a focused CRISPR library consisting of multiple gRNAs targeting each of those genes. This library will be used in a genetic screen in melanoma cells co-cultured with matched T-cells that recognise and eliminate the melanoma cells. Moreover, we will also use our in vivo adenoviral system to perform genome wide screens.

**Conclusion** After the screening, we can cross validate hits from both platforms to identify robust hits. Furthermore, we can validate them in a bigger patient cohort to determine their potential as predictive biomarkers for immunotherapy response.

**Tumour Antigens and Immune Effectors**

**PO-406 INVESTIGATION OF THE REPERTOIRE OF PEPTIDES BOUND TO MHC CLASS I MOLECULES IN TASMANIAN DEVIL TRANSMISSIBLE CANCERS FOR THE DEVELOPMENT OF A PEPTIDE VACCINE.**

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**Introduction** The marsupial species Tasmanian devil harbours two contagious cancers, Devil Facial Tumour 1 and 2 (DFT1 and DFT2); both are passed between individuals as an allogeneic graft, initiating tumours around the neck and face. DFT1 emerged 20 years ago, causes 100% mortality and has decimated the devil population. In contrast, DFT2 emerged only recently and is still being characterised. As allografts, both tumours should be recognised by T cells interacting with non-self Major Histocompatibility Complex (MHC) molecules and associated peptides. We aim to isolate tumour-specific MHC-bound peptides in order to determine MHC/peptide complexes breaking or inducing tolerance and design a vaccine.

**Material and methods** We have characterised the immunopeptidomes of DFT1-IFNγ, DFT2 and devil fibroblast cell lines. The DFT1 cell line was stimulated with devil interferon-gamma to up-regulate MHC class I expression. MHC class I molecule-peptide complexes were isolated by immunoaffinity and immune-mediated killing.

**Results and discussions** Ongoing work suggests that 3PO treatment significantly increases intratumoral T cell infiltration compared to controls. Tumor-infiltrating T cells appeared to be more activated and proliferative. Furthermore, upon 3PO treatment, cytotoxic short-lived effector (CD44+ KLRG1+ CD127+) and IFNg+ T cells increased compared to controls. The combination of 3PO with anti-PD1 significantly inhibited tumour growth in comparison to 3PO or anti-PD1 monotherapy.

**Conclusion** Our data suggest that TVN induced by PFKFB3 blockade in TECs: (i) increases the immunogenecity of intrinsically resistant tumours, both in terms of T cell infiltration and activation; (ii) reprograms cancer cells towards a more oxidative metabolism. This results in increased cancer cell susceptibility to immune responses and in overall increased efficacy of immune-checkpoint inhibition.

Further research will elucidate how TVN regulates T cell activation and reprograms cancer cell metabolism.