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Clinical priorities in immunotherapy- it's all about the biology

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Drugging the myeloid-cell kinase HCK improves anti-tumour immunity

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Elevated expression of the myeloid cell-specific Src-family kinase HCK occurs in a majority of solid malignancies and correlates with poor patient survival [Poh et al., *Frontiers Oncol* 2018]. We have recently shown that genetic ablation, or therapeutic inhibition, of HCK suppresses the growth of primary tumours arising in the colon, stomach, lung and other mutagenized epithelia of mice. Mechanistically, we have attributed this to the capacity of HCK activity to support and retain the polarization of a wound-healing, Tie2+ (i.e. alternative activated) endotype of tumour-associated macrophages and concomitant tumour vascularization [Poh et al., *Cancer Cell* 2017].

Here we extend these observations to a pancreatic allograft cancer model, where the growth of the primary lesion and its metastatic spread is suppressed in HCK knockout hosts. More importantly, *HCK* gene deletion or therapeutic HCK inhibition in mouse models of gastrointestinal cancers or melanoma further enhances the anti-tumour effects conferred by single agent immunotherapies with *anti*-PD-L1, *anti*-CTLA4 or *agonistic*-CD40 antibodies. The latter observations are underpinned by increased tumour cell apoptosis, which coincides enhanced expression of *ifng*, *gzmb*, *prf1* and other effector proteins in NK and infiltrating CD8+ cells as well as increased expression of *il12* and *ifng* in tumour-associated macrophages, which adopt a more conventional activated endotype. Because HCK-deficiency of the host also associated with increased abundance of tumour infiltrating effectors cells, we propose that therapeutic targeting of HCK not only "makes tumours hotter", but also improves host anti-tumour immunity. We are therefore developing novel HCK-specific small molecule inhibitors.

3

Targeting the tumour fibroblastic microenvironment to improve T cell function

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Checkpoint inhibitor therapies show enormous promise in the treatment of cancer. Response rates are high for blood-borne cancers but relatively poor for solid tumours. Cancer associated fibroblasts (CAFs) are a hallmark of solid tumours and strongly associate with poor prognosis for patients.

We recently described (PLOS Biology, in press) a T cell immunosuppressive role for activated human fibroblasts within lymphoid organs. We hypothesised that CAFs are likely very similar, if not largely identical to activated fibroblasts elsewhere in the body, and may therefore similarly affect tumor infiltrating T cells, reducing the efficacy of checkpoint inhibitor therapy for solid tumours.

A novel meta-analysis of trial data showed that CAF load within solid tumours significantly correlated with lack of objective response to PD-1/PDL1 and CTLA4 checkpoint blockade. We then performed the first direct comparison of freshly isolated CAFs from human pancreatic, breast, and colorectal tumours, using flow cytometry and RNA-Seq. Phenotypes were strongly conserved, with few differences between tissue activated fibroblasts and intratumoral CAFs, supporting a model where CAFs are activated as a normal physiological response which is not tissue or cancer-dependent. Using in vitro co-cultures, all CAFs from breast, colorectal and pancreatic tumours, as well as all activated fibroblasts from adjacent tissues, strongly suppressed T cell activation at physiologically relevant ratios, regardless of tumour or tissue type. Mechanisms included prostaglandin E2 and IDO, and effects were reversible using available inhibitors. A novel assay developed to validate the reversal of T cell suppression using live tumour slices has shown a response in 2/3 tumours.

Together, this work shows that CAF phenotypes are highly conserved between tumours, tissues and patients; that CAFs are not truly cancer-associated but more likely inflammation associated; and that CAFs alter T cell activation using reversible mechanisms. Fibroblasts likely create a conserved therapeutically targetable T cell activation checkpoint.

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Treatment Advances in Metastatic Merkel Cell Carcinoma

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The role of immune checkpoint blockade in finding a cure for HIV

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Soluble inhibitory immune checkpoint proteins are elevated in untreated HIV infection

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Publish consent withheld

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A novel regulator of the type I interferon signalling pathway with broad-spectrum antiviral activity

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Host recognition of intracellular viral RNA and the subsequent induction of antiviral cytokines is tightly regulated at the cellular level, and is a target for manipulation by pathogens and therapeutics alike. As part of a genome-wide screen of cellular proteins required for Hendra virus infection, we identified an uncharacterized protein C6orf106 (C6) required for infection by Hendra virus, in addition to a diverse range of RNA viruses such as highly pathogenic avian influenza virus and West Nile virus. Subsequent work has shown that the previously uncharacterized protein is a negative regulator of the type I interferons, (IFN) α and β and the pro-inflammatory cytokine tumour necrosis factor (TNF) α, in response to the viral RNA mimic poly I:C. We have shown that C6 interacts with IRF3 and reduces levels of transcriptional co-activators p300 and CBP in the nucleus. Type I interferons can play a crucial role in both the innate and adaptive immune responses as well as immunomodulation and inflammation. As such C6 may play a pivotal role in other autoimmune diseases and cancers. In summary we have identified a novel regulator of the type I IFN antiviral host defence immune response, with implications for antiviral immunity against RNA viruses and potential importance in a variety of other diseases.

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Deletion of TACI protects Lyn-/- mice against autoimmune disease

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Systemic lupus erythematosus (SLE) is a debilitating autoimmune disease driven by the production of autoantibodies, which variably affect multiple organs and tissues. SLE is notoriously heterogeneous, arising from numerous possible mechanisms and there is no current efficient treatment. Previously, our lab has shown that removal of the transmembrane activator and cyclophilin ligand interactor (TACI) in a mouse model of SLE mediated by excessive B cell activating factor of the TNF family (BAFF) protects mice from disease. We investigated whether deletion of TACI in mice deficient for the Src-family protein tyrosine kinase Lyn (Lyn-/- mice) would also be beneficial. Lyn-/- mice develop autoimmunity resembling human SLE, where hyper-reactive B cells are over-activated to produce autoreactive antibodies against cellular elements. Flow cytometry was used to characterise B cell and antibody-secreting plasma cell subsets. Autoantibody detection and serum cytokine levels were measured using ELISA. The severe B cell deficit in Lyn-/- mice was not improved in Lyn-/- TACI-/- mice, however deletion of TACI in Lyn-/- mice prevented disease by significantly reducing plasma cell numbers to levels of healthy WT mice, leading to reduced autoantibody production as measured by both ELISA and immunofluorescence staining. IL6 levels were decreased to WT levels, reducing harmful inflammation and disease progression. These data provide increased support for choosing TACI as a key target for therapeutic intervention, which may be applicable in treating multiple subtypes of SLE. This would offer treatment efficacy without the serious adverse events linked with extensive loss of B cells

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C-peptide of proinsulin is an autoantigen in human type 1 diabetes

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Background

Effective antigen-specific therapy for autoimmune diseases, like type 1 diabetes (T1D), require the antigens and epitopes recognized by $CD4^+$ T cells in people with T1D to be identified. However, the antigen specificity of human autoimmune T-cell responses associated with T1D is poorly defined. Here we investigated the immunogenicity of C-peptide, derived from proinsulin in the peripheral blood of people with, and without, T1D.

Methods

The CFSE dye-based proliferation assay was used to detect C-peptide-responsive $CD4^+$ T cells in PBMC (*1*). $CD4^+$ T cells that responded to C-peptide were sorted into individual wells, cloned and characterized in detail.

Results

$CD4^+$ T-cell responses to full-length C-peptide were detected in: >60% (14 of 23) of recent-onset (£100 days of diagnosis) T1D subjects, 13% (2 of 15) of long-standing (>100 days from diagnosis) T1D subjects and 8% (1 of 13) of HLA-matched healthy subjects. A panel of 22 C-peptide-specific $CD4^+$ T-cell clones were generated from 6 individuals with recent-onset T1D. These clones recognized epitopes across the entire 31 amino acids of C-peptide, although most epitopes were towards the C-terminus. Eighty-six percent (19 of 22) C-peptide-specific clones were restricted by high-risk alleles HLA-DQ8, -DQ2, -DQ8*trans* or -DQ2*trans*. TCR sequencing revealed that these clones used a wide variety of TCR genes. Finally, titration experiments showed that full-length C-peptide was a much more potent agonist of some $CD4^+$ T-cell clones than an 18mer peptide encompassing their cognate epitope.

Conclusion

Our findings support the notion that full-length C-peptide is an important target of pathogenic $CD4^+$ T cells in people with DQ8 and/or DQ2 who develop T1D. Consequently, full-length C-peptide may be useful in T-cell assays and antigen-specific therapy protocols.

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Local immunity by Tissue-Resident Memory T cells

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Innate Lymphoid Cells in Melanoma: Promising New Targets for Cancer Treatment.

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The immune cell infiltration into a tumour is heterogenous. It is composed of multiple cell types that can participate in the response and thus critically dictate the outcome for the patient. Recently discovered innate lymphoid cells (ILCs) are rapidly responding immune cells located in all surface barriers. A key function of this family of cells is to protect our body against pathogens and to maintain tissue homeostasis. ILCs are classified in three main groups based on transcription factors and cytokines they express. We can distinguish IFNg and TNFa-expressing NK cells and ILC1, IL-5 and IL-13-producing ILC2 and IL17 and IL-22-expressing ILC3. This suite of cytokines allows them to play multiple roles by regulating critical biological functions, including wound healing, autoimmunity and, importantly, the removal of cancers. Naturally present in the skin, their involvement in melanoma development and progression is currently unknown. Using pre-clinical models, we assessed their role and functions in this pathology. Firstly, we found that several ILC subsets infiltrate melanoma tumours. Secondly, these cells are functional and produce multiple cytokines that allow them to be major players in shaping the tumour microenvironment. Thirdly, tumour infiltrated ILCs are proliferating cells that express a wide range of immune checkpoints including PD-1, TIGIT, ICOS and CTLA-4. These features suggest that ILCs are likely to be modulated by current immunotherapies and thus impact on melanoma prognosis and a patient's outcomes. They may also be promising targets for future development of therapies.

13

Recognition of non-lipid antigens by CD1d-restricted type 2 NKT cells

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Natural Killer T (NKT) cells recognise lipid antigens presented by the MHC Class I-like molecule CD1d. Following activation, they rapidly secrete a broad range of immunoregulatory cytokines that can influence other mediators of immune responses and thus represent a promising therapeutic target for cancer and other diseases. The most extensively studied are type 1 NKT cells, which recognise a derivative of a marine sponge glycolipid α-galactosylceramide (α-GalCer), express a semi-invariant T cell receptor (TCR), and have a well-established role in the immune system. Much less is known about type 2 NKT cells, which do not recognise α-GalCer and express a diverse TCR repertoire. An early study showed that a non-lipid molecule –*phenyl* 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonate (PPBF)– can also be detected by type 2 NKT cells in the context of CD1d. The structure of PPBF shares similarities with several sulfonamide drugs that have been reported to cause hypersensitivity in humans suggesting that type 2 NKT cells may be involved in such hypersensitivity reactions. Through investigation of various PPBF analogues, we identify *3-chlorophenyl* 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonate (ClPPBF) as a stronger activator of type 2 NKT cells than the original PPBF compound. Importantly, we demonstrate that type 2 NKT cell modulation by PPBF is due to TCR recognition of PPBF-CD1d complexes. Using CD1d-ClPPBF tetramers we identified a novel population of type 2 NKT cells that specifically recognises these non-lipid antigens. Single-cell-sequencing of these cells revealed a polyclonal TCR repertoire distinct from type 1 NKT cells. Through the generation of retrovirally TCR-transduced cell lines we were able to validate the antigen-specificity of sorted cells. This study provides valuable insight into the diversity of antigens recognized by CD1d-restricted NKT cells, suggesting that small non-lipid molecules can modulate CD1d-mediated NKT cell activation. We are now addressing whether NKT cells play a role in hypersensitivity driven by PPBF-'like' drugs.

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Targeting Natural Killer cells via BCL2 inhibition improves stem cell transplant outcomes

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Background: The outcome of allogeneic haematopoietic stem cell transplantation (alloHSCT) is a complex interaction between conditioning regimen intensity, recipient immunology and donor immunology. We must find means to lower conditioning toxicity, promote donor engraftment and limit graft-versus host disease (GVHD) in order to improve alloHSCT outcomes.

Methods: MHC-mismatched alloHSCT: Donor BM and T cells from BALB/c ($H2K^d$) mice were injected into irradiated WT C57BL/6 ($H2K^b$), or $Bcl2^{fl/fl}$ NK cell-deficient recipients. WT mice were treated on day -2 and -1 with 100 mg/kg Venetoclax (ABT-199) or vehicle, before alloHSCT. Mice were monitored for GVHD, and donor cell engraftment. MLL-AF9 acute myeloid leukaemia (AML) cells were injected on day 0 into WT recipients, and treated with 100 mg/kg Venetoclax or vehicle on days 8 and 9, before alloHSCT on day 10.

Results: NK cells are significantly more radio-resistant than $CD8^+$ T or myeloid cells, at both myeloablative (1200 rad) and reduced intensity conditioning (RIC) radiation doses (800 rad). Genetic and pharmacological models of recipient NK cell

suppression during alloHSCT with RIC promoted donor cell engraftment, reduced GVHD, and retained graft-versus leukaemia (GVL) effects. *Bcl2*$^{fl/fl}$alloHSCT RIC recipients showed robust donor engraftment, and significantly reduced GVHD compared to WT recipients. Pharmacological inhibition of BCL2 in WT mice with Venetoclax resulted in rapid depletion of NK cells. A significant proportion of alloHSCT WT recipient mice pre-treated with Venetoclax developed full donor engraftment after RIC, with minimal GVHD, and retained potent GVL effects against pre-established AML.

Conclusions: BCL2 inhibition in WT alloHSCT recipients in combination with RIC was: 1) well-tolerated, 2) associated with low rates of GVHD and 3) resulted in long-term donor haematopoietic cell engraftment. Recipient NK cell inhibition may represent a means by which to deliver alloHSCT more safely.

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PRMT1 is Required for B-Cell Activation and Differentiation

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Arginine methylation catalyzed by protein arginine methyltransferases (PRMT) is a common post-translational modification in mammalian cells, regulating many important functions. PRMT1 is known to regulate signalling from the B-cell receptor (BCR), controlling the balance between proliferation and differentiation early in development. We show that PRMT1 is expressed in mouse and human peripheral B cells and increases in amount and activity on activation, both *in vitro* and *in vivo*. Conditional deletion of the *Prmt1* gene in mature B cells established that while B cell subsets in the periphery appeared normal, *in vivo* immune responses were abolished. *In vitro* activation of *Prmt1*$^{-/-}$ B cells through the BCR revealed diminished proliferation and survival, which correlated with altered signal transduction. Thus, atypical arginine methylation arising from unbalanced PRMT1 activity impacts on multiple cellular processes required for normal humoral immunity.

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An immunosuppressive role for IL-11 during tumourigenesis

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Introduction

Accumulating evidence alludes to a role for IL-11 signalling in tumour development although the mechanisms underlying IL-11 biology in tumourigenesis remain largely unknown. We postulate that IL-11 could be a mode of immunosuppression that can be targeted to mount an effective, immune-mediated anti-tumour response. Here we present data indicating IL-11 signalling drives tumour growth. Moreover, we identify an unrecognised immunological role for IL-11 in modulating T cell anti-tumour activity. Collectively, these findings validate IL-11 as a viable therapeutic strategy.

Method

To assess the therapeutic potential for targeting IL-11 signaling during tumourigenesis, colon and breast cancer cell lines were established in WT and *Il11r*$^{-/-}$ C57BL/6 mice. Mice were euthanised and the harvested tumours were immune profiled by FACs. To ascertain a role for IL-11 signalling in T cell activity, CD8^{+} T cells were FACs-sorted from WT and *il11r*$^{-/-}$ mice, and activated with PMA/ionomycin for 4h. T cell activation was assessed by measuring mRNA and protein levels of activation markers/cytokines by qPCR and ELISA, respectively.

Results

Colon and breast cancer growth was significantly attenuated in *il11r*$^{-/-}$ mice compared to WT hosts. Tumours from *il11r*$^{-/-}$ mice harboured higher numbers of activated T cells. *Ex vivo* T cell activation assays indicated that *il11r*$^{-/-}$ T cells also displayed heightened activation and cytolytic potential compared to their WT counterparts.

Conclusion

Here we report that IL-11 signalling supports tumourigenesis using various *in vivo* allograft mouse models. For the first time, we have characterised a functional role for IL-11 in modulating T cell function. Overall, the findings from this study indicate IL-11 signalling as a potential therapeutic target for the treatment of malignancies.

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Personalised healthcare for cancer treatment.

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Evolution of Cancer Immunotherapy

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Modelling resistance to adoptive T-cell immunotherapy in melanoma using CRISPR/Cas9

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Adoptive T-cell therapy (ACT) has emerged as a powerful treatment option in patients with metastatic melanoma. However, tumor cells frequently relapse from therapy by acquired resistance mechanisms such as loss of target antigen expression. Currently, it is not completely understood how the choice of target antigen influences resistance mechanisms to antigen-specific immunotherapies.

Therefore, we established CRISPR-assisted insertion of epitopes (CRISPitope), a technique that fuses a defined T-cell epitope to endogenous gene products. We applied CRISPitope to murine melanoma cells and tagged endogenous melanosomal TYRP1 and oncogenic $CDK4^{R24C}$ with the human $gp100_{25\text{-}33}$ epitope, which rendered them targetable by gp100-specific pmel-1 TCR-transgenic T cells. This enabled us to investigate melanoma escape mechanisms to ACT targeting non-essential melanosomal and essential oncogenic antigens in direct comparison.

Using experimental mouse models, we could identify different escape mechanisms to gp100-specific immunotherapy in TYRP1 versus $CDK4^{R24C}$ melanomas. Resistance to ACT targeting TYRP1 was mainly caused by hardwired loss of antigen accompanied by a non-inflamed microenvironment or reversible downregulation of the antigen associated with an enforced melanoma phenotype switching. In contrast, $CDK4^{R24C}$ melanomas escaping ACT displayed antigen persistence and were associated with an IFN-rich inflamed tumor microenvironment. In $CDK4^{R24C}$ melanomas IFN-driven feedback inhibition by negative immune-checkpoint molecules promotes resistance to ACT despite persistent antigen expression.

Applying CRISPitope to syngeneic mouse models, we could show that target antigen choice can influence ACT resistance mechanisms, phenotype and immune contexture of melanomas in response to antigen-specific immunotherapies. Thus, our work could help to better understand acquired resistance and optimize personalized cancer immunotherapy.

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Impact of tumour inherent interferons on immune reactivity and personalised therapy in TNBC

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Triple negative breast cancer (TNBC) accounts for 15-20% of all breast cancers and has an increased risk of rapid metastasis within the first two years compared to other subtypes. A feature of TNBC is the high accumulation of tumour infiltrating lymphocytes (TILs) in some patients that predict a favourable prognosis and response to chemotherapy. This, along with the lack of targeted therapeutic options for TNBC, has triggered interest in trialling checkpoint-targeted immunotherapy. However, responses to date have been underwhelming are very difficult to predict, leading to an inability to accurately weigh up the benefit-to-risk ratio for their implementation. In agreement with clinical responses, our recent studies in preclinical TNBC models have demonstrated a lack of efficacy of the checkpoint inhibitor, anti-PD-1, in aggressive, immune cold TNBC tumours. Increasing the heat of the tumour via poly (I:C), a potent type I interferon inducer, sensitised mice to anti-PD-1 and induced a tumour specific T-cell response that extended metastasis-free survival. We have now built on these findings to profile the immune landscape of TNBC in a neoadjuvant sequential biopsy cohort in order to develop immune markers that predict poor chemotherapeutic response, a poor prognosis, and patients that may benefit from immunotherapeutic intervention. Utilizing multiplexed immunohistochemistry we have demonstrated that immune cell characterisation and activation status is a superior prognostic for chemotherapeutic response and risk of relapse than standard TIL score. Furthermore, we identified a novel prognostic marker that indicates presence of an intact tumour-intrinsic type I IFN signalling pathway which is superior to TIL characterisation and predicts survival in 3 independent TNBC cohorts. Our work supports preclinical and clinical trials of immunoactivating therapies in patients with IFN and immune cell cold tumours.

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Targeting the adenosine pathway to enhance CAR T cell therapy against solid tumours

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Redirecting potent T cell responses against cancer by means of a Chimeric Antigen Receptor (CAR) has been very successful against Acute Lymphoblastic Leukemia and Non-Hodgkin lymphoma. Solid tumours however pose a greater challenge as they differ dramatically in the form of the immunosuppressive tumour microenvironment (TME). One mechanism of suppression is through the adenosine pathway. Binding of adenosine to A2A adenosine receptors (A2AR), which are highly expressed on T cells results in suppression of effector T cell responses. Our lab has previously shown that targeted blockade of the A2AR can improve both endogenous T cells [1] and CAR T cell efficacy [2]. This project seeks to re-engineer CAR T cells to be resistant to adenosine mediated immunosuppression in solid tumours by overexpressing the A1R and A3R alternative signalling receptors. Overexpression of the A1R and A3R in CAR T cells drive improved cytokine function in vitro. In an immunocompetent syngeneic mouse model tolerant to the human HER2 target antigen, A3R expressing anti-HER2 CAR T cell infusion results in dramatic reduction in tumour sizes and improved survival of mice bearing the E0771-HER2 breast cancer or MC38-HER2 colon adenocarcinoma cancer cell lines. This was accompanied by increased expression of effector cell markers in vivo including PD-1. Subsequently, PD-1 blockade synergized with A3R CAR T cells to further improve therapeutic efficacy. These results lend insight into the role of the A1R and A3R in CAR T cells and could lead to improved CAR T cell efficacy against solid tumours.

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Breast-implant associated anaplastic large cell lymphoma - is this the one of a few non-viral antigenically driven T cell lymphomas?

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Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is a rare form of T-cell lymphoma that occurs after implantation of breast prostheses typically after a relatively long latency . BIA-ALCL is notable for its aggressive histological appearance but a paradoxical predominance of clinical presentation with early stage disease and a relatively favourable prognosis when compared to its systemic counterpart. Whilst the underlying cause of BIA-ALCL is unknown, a possible contribution from chronic antigen stimulation by a unique implant-biofilm associated microbiome has been hypothesised. Genomic and functional characterisation of systemic ALK-negative anaplastic large cell lymphoma (sALCL) has revealed the importance of STAT3 activation, MYC expression, PRDM1/TP53 abnormalities and recurrent structural variants involving the DUSP22 and TP63 loci. By contrast, the genomic landscape of BIA-ALCL and its relevant pathogenic drivers are significantly less well characterised. We aimed to extend the understanding of this rare lymphoma by performing comprehensive genomic characterisation by targeted sequence variant detection, whole genome copy number assessment, T-cell receptor locus structural variant detection and T-cell receptor repertoire sequencing on a cohort of eleven cases of BIA-ALCL.

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Control of Immunopathology in CNS autoimmunity

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Combination immunotherapy; Looking beyond PD-1/PD-L1 blockade - Promises and Failures

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Venetoclax and Chronic Lymphocytic Leukaemia

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Venetoclax is the first specific BCL2 inhibitor shown to have efficacy in the treatment of B cell malignancies in particular chronic lymphocytic leukaemia (CLL). CLL is the most common leukaemia affecting adults in western populations and when relapsed or refractory to front line chemo-immunotherapy carries with it a relatively poor prognosis especially if associated with high risk genetic features such as TP53 dysfunction. CLL ubiquitously overexpresses BCL2 rendering it resistant to apoptotic cell death, over time this results in inappropriate accumulation of the malignant clone in the bone marrow, peripheral blood and lymph node compartments. By inhibiting BCL2 and inducing apoptosis venetoclax has achieved response rates of 80% including complete response rates of 20% among patients with high risk relapsed and refractory CLL. The Achilles heel of venetoclax treatment however, is secondary clinical resistance, emerging results from combination therapy with monoclonal antibodies and other novel targeted agents such as Burton's tyrosine kinase inhibitors are associated with deeper clinical responses and prolonged progression free survival. However inhibition of BCL2 has wide ranging effects on the immunological milieu. Whilst malignant cells are more susceptible to venetoclax induced apoptosis, non-malignant B cells, and conventional T cells are also reduced by treatment. Interestingly, innate T cell populations and immature NK cells exhibit preferential survival. This may have consequences for the combination of venetoclax with immunotherapies.

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TCF-1 Critically Regulates $CD8^+$T cell Fate and IL-17 Production

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Interleukin-17 (IL-17)-producing T cells are associated with rapid progression of colorectal cancer (CRC) and poor patient survival. Recent reports have linked the development of IL-17 producing T cells with our microbiome and separate studies have established a role for the microbiota in response to checkpoint blockade in cancer patients. Therefore, the study of these cell populations in cancer is now of particular relevance. The transcriptional networks controlling the differentiation of IL-17 producing cells, including IL-17-producing $CD8^+$ T (Tc17) cells is unknown. We show that the transcription factor TCF-1 acts to limit Tc17 differentiation in developing $CD8^+$T cells by binding to the promoter regions of *Maf*, *Rorc* and *Sox13* to suppress their expression. TCF-1 dually acted to induce genomic accessibility of key genes associated with the differentiation and the function of effector and memory $CD8^+$T cells, but also limited the genomic accessibility of key genes essential for Tc17 cell differentiation and function. Tc17 cells exhibited a gene-set signature enriched for tissue repair and lipid metabolism, which is in sharp contrast to the transcriptional profile governing effector $CD8^+$T cells. Collectively, these findings identify new regulators of Tc17 cells and highlight a critical role for TCF-1 in determining $CD8^+$T cell fate through regulation of the MAF-RORgt-SOX13 axis.

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Maintenance of CD8 T cell responses during chronic infection and anti-tumor immunity

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Anti-tumor T cell responses are limited by immunosuppressive mechanisms, including inhibitory receptors (checkpoints), metabolic competition and suppression by regulatory T cells. New therapies aiming at reactivation of antigen-specific T cells by overcoming immune suppression resulted in dramatic improvements in the outcome for some patients. However, what determines success or failure of immunotherapy is still poorly understood. Recent studies from our group and others have shown that effector T cell responses during chronic infection and cancer are maintained and can be boosted by memory-like T cells, which express high amounts of the inhibitory receptor PD1 while also displaying memory T cell characteristics. These cells have been termed 'exhausted memory-like' T cells or T_{MEX}.* In this project, we will identify molecular pathways that control the development of T_{MEX} and could be harnessed to improve patient outcome. Our published and preliminary work identified a number of molecules that are linked to the modulation of cytokine responsiveness, T cell receptor (TCR) signaling and cellular metabolism that control the magnitude and dynamics of T cell responses to chronic infection and tumors. This includes the transcriptional regulator Id3, expression of which we found specifically identified T_{MEX} both in chronic infection and tumors. $Id3^+$ T_{MEX} differ dramatically from their $Id3^-$ 'exhausted' T cell (T_{EX}) counterparts in phenotype and function. They also display substantially elevated mitochondrial metabolism, which we found was required for the beneficial outcomes of checkpoint inhibition. In the course of this study, we will examine pathways that control the development and maintenance of T_{MEX}, devise strategies to promote T cell responses and aim to correlate our findings with patient outcomes. This work will provide fundamental insights into the biology of this essential cell type with far reaching implications for our understanding of T cell responses in chronic infection and cancer.

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Targeting phosphatases to promote ant-tumour immunity

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Harnessing the innate immune response to treat small cell lung cancer.

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Lung cancer is the fifth most diagnosed cancer, and the leading cause of cancer-related death in Australia. Small cell lung cancer (SCLC) is the most aggressive subtype of lung cancer, characterised by early metastatic spread. First-line therapy for SCLC patients is a platinum-based agent and topoisomerase inhibitor. While most patients initially respond to treatment, resistance rapidly develops leading to low 5-year survival rates of less than 7%. Critically, treatment regimens for SCLC patients have remained largely unchanged over the last 30 years, highlighting the urgent clinical need for novel treatment modalities.

Immunotherapy has recently gained attention in lung cancer treatment. Although emerging clinical trials utilising anti-PD1/anti-CTLA4 blockade have shown partial responses, these approaches still need to be explored with other therapies to efficiently treat recalcitrant SCLC. Natural killer (NK) cells are an alternative to T cell-based immunotherapies. Unlike CD8 T cells, NK cells do not require sensitisation to antigens, which are often altered on the surface of tumour cells. Interestingly, by taking advantage of a novel NK cell gene signature, bioinformatic analysis revealed a strong correlation between high NK cell score and survival benefit in SCLC patients.

To address the role of immune subsets in SCLC we depleted mice of CD8 T or NK cells during SCLC progression. While no change in metastatic dissemination was observed following depletion of CD8 T cells, metastasis was significantly increased in mice lacking NK cells. Conversely, metastatic dissemination was limited when NK cells were activated through the inhibition of negative checkpoints. Taken together, our results indicate that enhancing NK cell responses might offer a novel immunotherapeutic approach to prevent metastatic spread in SCLC.

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Rational design of cancer vaccines by targeting dendritic cells

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As dendritic cells (DCs) are potent at inducing T cell responses, they have been studied for the development of immunotherapies to combat cancer. Several DC surface molecules have been successfully targeted *in vivo* using monoclonal antibodies to deliver antigen and induce T cell responses that confer tumour protection. One candidate is the C-type lectin-like receptor Clec9A, which shunts antigen efficiently into the cross-presentation pathway, facilitating MHC class I presentation to $CD8^+$ T cells. Recent tumour vaccines utilise tumour-specific mutated peptides, so called tumour neo-antigens. A report identified immunogenic B16 melanoma neo-antigens. By analysis of the immunopeptidome using mass spectrometry, we identified five novel B16 melanoma antigens presented on MHC class I that vary by one amino acid compared to the wild type sequence. Here, we investigated whether these neo-antigens and novel B16 melanoma antigens can be harnessed for vaccinations using Clec9A-targeting antibodies. We demonstrated that the *in vivo* delivery of these neo- or novel tumour antigens to Clec9A does not induce antigen-specific T cell responses. However, when a pool of these neo- or novel antigens were delivered to Clec9A, significant anti-tumour protection was induced in the B16-metastatic melanoma model. These data suggest that mutant epitopes are poorly immunogenic and that vaccines will require multiple mutant epitopes to induce effective anti-tumour protection. Interestingly, we have also shown that B16 melanoma-bearing mice treated with a CpG adjuvant and checkpoint inhibitors develop a protective T cell-dependent anti-tumour response, yet these protective T cells did not recognise the neo- or novel antigens. These findings highlight that only a small subset of neo-antigens are actively involved in tumour rejection and that these are yet to be identified. From a vaccine perspective, it may become critically important to identify *bona fide*rejection antigens and delineate these from poorly immunogenic mutant epitopes.

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Chronic inflammation and bowel cancer; are your MAITs involved?

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Mucosal associated invariant T cells (MAIT cells) are a recently described innate-like T cell subset which comprise up to 5% of the circulating T cells within humans. These MR1 restricted T cells recognise non-peptide, small molecule antigens derived from microbial vitamin B synthesis. They are also enriched within the mucosal tissues in the body such as the gastro-intestinal tract. Upon activation, MAIT cells can rapidly produce cytokines and are believed to play a role in regulating the local immune milieu.

Cancers of the large bowel have the second highest mortally rates within Australia and these cancers respond poorly to conventional immune based therapy. Bowel cancers arise in a background of chronic inflammation, and the regulation of the immune milieu at the site of these mucosal tumours is currently poorly understood.

Whilst investigating the chronically stimulated MAIT cells potentially seen in bowel cancer we have identified a cytokine response rich in interleukin 13 (IL-13). This IL-13 MAIT cell response has not been previously described and has the potential to skew the local immune milieu away from anti-tumour inflammation and promote tumour growth and spread. This IL-13 response not only has the potential to impact cancer research, but also alter the research of a number of human inflammatory disorders.

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Stat3 inhibition in the tumour microenvironment restricts colorectal tumour growth in mice

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The aberrant activation of the transcription factor Stat3 has been described for many cancers including colon cancer. Tumour cell intrinsic Stat3 signalling drives hallmarks of cancer, such as resistance to cell death and sustained proliferation. The effect of aberrant Stat3 activation among tumour infiltrating immune cells however, is yet to be fully elucidated. We postulate that hyper-Stat3 activation in the tumour microenvironment develops an immunosuppressive, pro-tumorigenic environment. We then hypothesise that Stat3 suppression in the non-tumoural cell compartment will inhibit colorectal tumour growth, by dampening this pro-tumour phenotype. Furthermore, we investigated, whether non-tumoural Stat3 suppression synergistically enhances the effects of immunotherapy.

We generated the shStat3 mouse that utilizes short hairpin (sh) RNAi technology allowing for conditional and reversible Stat3 reduction. To study the effects of Stat3 suppression in the non-tumoural compartment, the shStat3 mice were subcutaneously engrafted with MC38 murine colon cancer cells. Anti-tumour effects of Stat3 suppression were assessed either alone or in combination with immune checkpoint blockade, specifically anti-PD1 antibody treatment.

We found, that Stat3 suppression in the non-tumoural compartment alone suppresses MC38 colon tumour growth. Immunophenotyping of excised tumours revealed an increase of monocytic $Ly6C^{+}Ly6G^{-}$ myeloid cells. While checkpoint blockade alone reduced tumour growth similarly to Stat3 suppression, there was no additive or synergistic effect of Stat3 reduction in combination with anti-PD1 treatment.

Our data suggest that combination of STAT3 inhibitors with anti-PD1 blockade do not offer greater therapeutic outcome when treating anti-PD1 sensitive microsatellite instable (MSI) classified MC38 colon cancer cells, which then advises against the use of this combination for colorectal cancer patients with this classification. However, we identified Stat3 signalling within the tumour microenvironment as part of the anti-tumoural activity of Stat3 targeting strategies, with monocytic myeloid cells being a candidate cell type driving these responses.

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More than a BTK inhibitor: Ibrutinib impairs T cell function

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Aim

Ibrutinib is increasingly being incorporated into the clinical management of B cell malignancies. This study aimed to determine if ibrutinib and more selective BTK inhibitors impact the cytotoxic capacity of T cells. Ibrutinib inhibits all Tec family kinases including ITK at clinically meaningful concentrations and may exert a Th1 selective pressure[1]. However this has not been shown in patients[2]. More selective BTK inhibitors have also been developed including zanubrutinib and acalabrutinib. However the impact of ibrutinib or other BTK inhibitors on T cell function remains unclear.

Method

PBMC were isolated from six treatment-naive CLL patients at the Royal Melbourne Hospital. Healthy donor NKT cells were FACS sorted from PBMC and ex vivo expanded. Cells were treated in vitro with 1uM ibrutinib, zanubrutinib or acalabrutinib. CD8 T cell and NKT cell response to CD3/CD28 stimulation and NKT response to α-Galactosylceramide loaded CLL cells was assessed by flow cytometry (CD107a, Granzyme B and IFN

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The interplay among diverse cell types in prostate tumour microenvironment contributes to the activation of key hallmarks

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Treatments for prostate cancer, including surgery, radiotherapy and androgen deprivation therapy, can lead to variable and often temporary results, mainly due to the heterogeneity of cancer cells. The non-cancerous portion of the tumour microenvironment, such as immune and stromal cells, is key for tumour development, and being a genetically stable target holds the potential for both diagnosis and treatment. Improving our knowledge on the molecular interactions between cancer and non-cancer cells could unlock such potential.

An observational study was designed to identify driver molecular interactions among cancer, stromal and infiltrating immune cells. Such cell types were enriched from biopsies of patients across pathological stages, and their transcriptome was profiled. A novel statistical model allowed the identification of unexpected microenvironmental molecular drivers of several hallmarks in prostate cancer. For example, dramatic transcriptional changes in t cells and fibroblasts allow a sustained hormonal and lipid unbalance in the tumour core; while cancer cells suppress key microenvironmental modulators, of which some novel in prostate cancer.

Furthermore, an innovative computational tool will be presented, which enables large-scale studies of tissue composition and immune cell infiltration on public and novel RNA sequencing data sets. Such tool allowed drawing a landscape of the associations between tumour relapse and abundance of infiltrating cells, for the whole TCGA data base.

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Immunotherapy in monogenic disease.

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Rational design of cancer vaccines

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Checkpoint blockade has cemented the relevance of T cells in the treatment of human cancer. Many types of tumours possess mutations (neo-epitopes) that render them susceptible to destruction by T cells. While checkpoint blockade amplifies pre-existing responses, tumour antigens expressed at low levels may be ineffectively presented for priming T cells and remain immunologically invisible. Vaccines provide a means to elicit robust T cells responses that can further be amplified by blockade immunotherapy. Dendritic cells (DCs) are uniquely equipped to activate T cells and can be harnessed to enhance immunity. An effective method of delivering antigen to DC exploits the fact that DC have unique cell surface receptors, and antibodies against these receptors can be used as vehicles to deliver antigenic cargo. A lead candidate, DEC205, is being assessed in 8 clinical trials for the delivery of cancer vaccines. It was previously assumed that many DC receptors could promote antigen presentation. However, our data reveals that presentation of "peptides" as opposed to "proteins", is relatively poorly facilitated by DEC205. We show that Clec9A, another DC-specific molecule, is particularly effective at facilitating the presentation of peptide cargo, leading us to assess its capacity to present peptides encoding neo-tumour epitopes. We provide evidence that Clec9A-targeted neo-antigens can elicit T cell immunity, but not all neo-antigens are equivalent in their capacity to evoke responses. Intriguingly, many of the published neo-antigens are not recognized during tumour rejection, despite this rejection being mediated by T cells. This raises the important question of which neo-antigens drive tumour rejection and how to discriminate effective vaccine candidates from poorly immunogenic neo-antigens. Using several tumour models, published neo-antigens and a suit of newly discovered neo-antigens identified by immunopeptidome analysis, we analyse T cell immunity and its capacity to protect against cancer.

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Tissue-resident Memory CD8$^+$ T Cells Promote Melanoma-immune Equilibrium in Skin

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The immune system can suppress tumour development by eliminating malignant cells and by preventing the outgrowth of cancer cells that resist eradication. Clinical and experimental data suggest the latter mode of control, termed cancer-immune equilibrium, can be maintained for prolonged periods of time, possibly up to several decades. Although cancers most frequently originate in epithelial layers, the nature and spatiotemporal dynamics of immune responses maintaining cancer-immune equilibrium in these tissue compartments remain elusive. Using a novel mouse model of transplantable cutaneous melanoma, we show that tissue-resident memory CD8$^+$ T (T_{RM}) cells promote a durable melanoma-immune equilibrium confined to the epidermal layer of skin. A proportion of mice transferred with melanoma cells remained free of macroscopic skin lesions long after inoculation and generation of tumour-specific epidermal CD69$^+$CD103$^+$ T_{RM} cells correlated with this spontaneous control. By contrast, mice deficient in T_{RM} formation were more susceptible to tumour development. Despite being tumour-free at the macroscopic level, mice frequently harboured dormant melanoma cells in the epidermal layer of skin long after inoculation, and intravital imaging revealed that these cells were dynamically surveyed by T_{RM} cells. Consistent with their role in melanoma surveillance, tumour-specific T_{RM} cells generated prior to melanoma inoculation conferred profound protection from tumour development independently

of recirculating T cells. Finally, depletion of T_{RM}cells from mice with occult melanomas triggered tumour outgrowth, thereby demonstrating that T_{RM} cells can actively suppress cancer progression. Our results reveal a fundamental role of T_{RM} cells in immune surveillance of subclinical melanomas in skin by maintaining cancer-immune equilibrium. As such they provide strong impetus for exploring T_{RM} cells as targets of future cancer immunotherapies.

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Investigating the role of Mucosal-Associated Invariant T (MAIT) cells in cancer

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The success of immunotherapies such as checkpoint blockade in patients has highlighted the importance of the immune system in controlling tumours. The presence of conventional T cells within the tumour microenvironment have been intensively studied and correlates with patient outcomes, however, the role of mucosal-associated invariant T (MAIT) cells in cancer is relatively unknown. MAIT cells are abundant in humans and enriched in mucosal tissues, such as the colon and lung, and some recent studies have reported the presence of these cells within primary and metastatic tumours. However, it is unclear whether MAIT cells contribute to anti-tumour immune responses, although previously we have shown they are capable of cytotoxic activity against multiple myeloma lines[1]. To extend these studies to an *in vivo setting,* we first compared anti-tumour responses in a B16F10 lung metastasis model where tumour cells were loaded with 5-OP-RU, a potent MAIT cell agonist. Strikingly, given the low frequency of MAIT cells in C57/BL6 mice we observed a significant decrease in lung metastasis, compared to unloaded tumour cells. Interestingly, in the primary tumour setting, intratumoural injections of MAIT cell ligands into B16F10 tumours did not affect tumour growth. These results suggest that MAIT cells have the potential to control tumour metastasis, but play a limited role in the primary tumour setting.

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ALK4 signaling suppresses NK cell function to aid tumor immune evasion

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Emerging strategies to target NK cells in cancer immunotherapy

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Therapies targeting the immune system are revolutionising cancer treatment by reactivating tumor-resident cytotoxic lymphocytes. Current clinical practice and drug development is focused on checkpoints known to suppress tumor-resident CD8 T cells, however given the emerging resistance to such therapies and the unresponsiveness of the majority of cancer patients, interest in other effector populations, such as natural killer (NK) cells is growing. NK cells possess an innate ability to detect and lyse transformed cells and NK cell activity is inversely correlated to cancer incidence in humans and experimental metastasis in mice. In the context of NK cell immune surveillance, it is not clear why some solid tumours generate metastasis while others do not. Our recent generation of the specific NK cell deficient mouse has revealed that several primary tumours models regarded as non-metastatic do indeed generate spontaneous metastasis that are specifically detected and eradicated by NK cells. Furthermore, increasing the activity of NK cells*in vivo* by deletion of NK cell checkpoints identified by our team dramatically reduces tumour burden in highly metastatic cancer models. Genome-wide CRISPR screens using NK cell dependent mouse tumour models have revealed novel pathways to increase NK cell immunosurveillance and pathways of tumour immune-evasion. These pathways are being validated in patient-derived NK cells using a gene-editing approach optimized for primary human lymphocytes.

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Exploring the transcriptome of metastatic melanoma to improve natural killer cell targeting

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Metastatic melanoma represents a major health burden in Australia and New Zealand. Relative to other cancers melanoma is highly-immunogenic and it provides a good target for immunotherapies. By exploring the immune infiltration of melanoma tumours using transcriptomic data, we aim to elucidate intercellular signalling and other molecular features which are associated with immune targeting and improved patient survival.

We have curated a natural killer (NK) cell gene signature from public data sources/tools including LM22 (CIBERSORT) and the LM7 (Tosolini *et al.*) gene sets. Using the gene-set scoring method *singscore* we infer the infiltration of NK cells within metastatic melanoma samples and examine how this varies relative to scores associated with other immune cell subsets. We contrast our results against transcriptomic data from the LM-MEL cell line panel to distinguish factors which may be produced by melanoma cells to modulate immune targeting.

Consistent with recent reports, we observe a strong association between NK cell and dendritic cell infiltration which is required for a robust T cell response. These scores show an intriguing association with epithelial and mesenchymal gene sets which capture melanoma phenotype switching, and with a TGF-β EMT signature which specifically captures mesenchymal behaviours induced by the immunosuppressive ligand TGF-β. Metastatic melanomas with evidence of mesenchymal-like behaviour induced by stimuli other than TGF-β, and robust NK cell infiltration, are associated with significantly improved patient survival. Exploring these data further, we identify a number of transcripts associated with reduced/improved immune targeting which may provide novel immunotherapy targets.

These results show that gene set scoring provides an intuitive approach to reduce the dimensionality of transcriptomic data sets in a manner which captures clinically-relevant phenotypic changes. Such approaches will be essential to leverage large public data sets which are becoming increasingly available and may help identify new targets for modulation with immunotherapy.

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Polymorphisims in P2X7 influence post allogeneic stem cell transplantation outcome

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Allogeneic stem cell transplantation (alloSCT) is a highly effective immunotherapy for haematologic malignancies. However infection, acute organ dysfunction and graft versus host disease (GVHD) impact negatively on patient outcomes. Pre-transplant conditioning regimes are associated with high levels of immunogenic cell death and the release of extracellular ATP which binds to the P2X7 receptor. Signalling through the P2X7 receptor may lead to activation of downstream effectors that influence alloSCT outcome. In this study we examined the effect of gain and loss of function P2X7 SNPs in 333 alloSCT recipients and correlated to acute GVHD, chronic GVHD, relapse free survival and overall survival. Inheritance of loss of function SNPs (Arg307Gln or Glu496Ala) was associated with reduced rates of aGVHD while Ile568Asn was associated with increased aGVHD reflecting the the differential effects of these SNPs on P2X7 function. No one single SNP was associated with changes in relapse free or overall survival. However patients with the major gain of function variant haplotype (homozygous Gln460Arg- Ala348Thr) had significantly reduced relapse free and overall survival compared to other patients. Our findings demonstrate that recipient immunology has a significant impact on alloSCT outcome and with validation, analysis of P2X7 SNPs could be added to alloSCT risk stratification models.

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Manipulation of recipient NK cells using Ruxolitinib to target the IL-15 signaling pathway improves allo-HSCT outcomes.

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Background:Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for haematological malignancies due to its graft-versus-leukemia (GVL) effect. However myeloablative conditioning before allo-HSCT is toxic and promotes graft-versus-host disease (GVHD), the major complication post-transplantation. Natural Killer (NK) cells are the major residual recipient cell population after reduced intensity conditioning (RIC) which is less toxic to recipient organs than myeloablative irradiation. The survival and function of NK cells are dependent on the cytokine IL-15 and the downstream JAK1&2

signaling pathways. We hypothesized reducing survival of recipient NK cells via blocking the IL-15 signaling pathway, in combination with RIC, will promote donor cell engraftment and mitigate GVHD.

Methods:MHC-mismatched allo-HSCT: Donor BM and T cells from BALB/c (H2Kd+) mice were injected into irradiated wild-type (WT) C57BL/6 (H2Kb+) recipients and IL-15 knock out (KO) NK-cell deficient recipients. Before allo-HSCT, WT recipients were also pre-treated on days -2 and -1 by oral gavage with 180 mg/kg Ruxolitinib or vehicle. Mice were monitored for GVHD and donor cell engraftment.

Results:NK cell frequency in WT recipients was significantly decreased after treatment with the JAK1/2 inhibitor Ruxolitinib. The Ruxolitinib treated recipients conditioned with 800 rads RIC irradiation achieved complete donor cell engraftment at day 14 post-allo-HSCT, with similar kinetics as untreated mice given a myeloablative irradiation dose (1200 rads). In contrast, the vehicle-treated recipients irradiated with 800 rads rejected the donor graft. The impact of inhibiting recipient NK cell survival on the onset of GVHD is currently being investigated. The RIC-conditioned IL-15KO recipients exhibited enhanced engraftment compared to WT recipients with myeloablative conditioning very early post-transplantation, but they experienced severe body weight loss and hyperacute GVHD.

Conclusion:Pharmacological inhibition of recipient NK cells via the JAK1/2 inhibitor Ruxolitinib allowed donor cell engraftment in RIC treated WT recipients and showed improved outcomes compared with IL-15 genetically depleted recipients.

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A novel preclinical platform for evaluating targeted therapy and immunotherapy combinations in BRAF^{V600E} melanoma

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Both targeted therapy and immunotherapy have been used successfully to treat melanoma, but the development of resistance and poor response rates to the individual therapies has limited their success. Designing rational combinations of targeted therapy and immunotherapy may overcome these obstacles but requires assessment in preclinical models with the capacity to respond to both therapeutic classes. Herein, we describe the development and characterization of a novel, immunogenic variant of the *Braf^{V600E}Cdkn2a$^{-/-}$Pten$^{-/-}$* YUMM1.1 tumor model that expresses the immunogen, ovalbumin (YOVAL1.1). We demonstrate that, unlike parental tumors, YOVAL1.1 tumors are immunogenic *in vivo* and can be controlled by immunotherapy. Importantly, in contrast to the commonly used mouse melanoma model B16, YOVAL1.1 tumors are sensitive to BRAF^{V600E}- and MEK-targeted therapy, responding in a manner consistent with human BRAF^{V600E} melanoma. The YOVAL1.1 melanoma model is transplantable, immunogenic and sensitive to clinical therapies, making it a cost-effective platform to guide strategic development of combined targeted- and immune-therapy approaches in BRAF^{V600E} melanoma.

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Antibody profiling to predict responses to immunotherapy in melanoma

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Advances in melanoma treatment include targeting key immune checkpoints, such as programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). While highly effective in some, the majority of patients do not respond and immune-related adverse events (irAEs) are common and often severe. Therefore, the ability to predict treatment outcomes is of utmost value. Treatment success is often correlated with an immunologically active tumour, but the majority of the commonly-used immune monitoring techniques to investigate tumour-immune engagement rely on access to patient tissue via invasive surgeries or biopsies. As such, there is a clear preference for a blood-based means of assessing immune engagement, with the intent of obtaining a more accurate representation of the majority of all tumours. Tumour antigens, including tumour-specific antigens (TSAs) and tumour-associated antigens (TAAs), can enable malignant cell recognition by the immune system and subsequently lead to the production of specific antibodies. We hypothesized that these antibodies can be informative markers of immune engagement of tumours. Here we used a novel protein array which represents a high-throughput, sensitive tool capable of profiling antibody repertoires of cancer patients using only 1µl of serum or plasma. Importantly, healthy individuals show no detectable cancer-specific antibody titers. Preliminary data (unpublished) using the array on a small subset of patients (n=15) undergoing ICB treatment with pembrolizumab (PD-1 blockade) was generated. Pre-treatment data shows a trend towards

separation of clinical responders from non-responders using number of antigen specificities and mean antibody intensity. Furthermore, immunotherapy treated patient antibody profiles may be useful to predict irAEs ahead of clinical evidence.