and SMART technology, in conjunction with two novel next-generation sequencing (NGS) library preparation kits, using the same primer pairs, to capture full-length variable regions of TCR-α and -β chains.

Material and methods Method 1, using the SMARTer Human scTCRa/b Profiling Kit, permits NGS library preparation of FACS-sorted cells in 96-well plates. Method 2 is an adaptation of the process above that scales to ~1200 single cells using the SMARTer ICELL8 Single-Cell System, which enables single-cell isolation and nanoliter PCR in a nanowell chip.

Results and discussions We present data showing α/β pairing from Jurkat, CCRF, PBMCs, and CD4 +T cells. In addition to the sensitivity of this method, the ability to pool the cDNA from 96wells into 12 sequencing libraries adds to the ease of use. Consistent with immunology reports, unstimulated CCRF-CEM cells examined with this kit expressed a TCR-β but not a TCR-α chain.

scTCR pofiling with SMARTer ICELL8 Single-Cell System as a proof of principle of scTCRα/β was performed on Jurkat cells and CCRF-CEM cells.

Jurkat cells and CCRF-CEM cells were processed using an ICELL8 chip preprinted with barcoded oligos. Paired TCRα/β Jurkat clonotypes were detected in 77% and 87% cells in mixed and single cell populations, respectively.

Conclusion The ability of the core biochemistry and PCR components of these kits to be used with either FACS-sorted cells in 96-well plates or >1,000 cells in novel ICELL8 chips (in development) points to the general utility and scalability of this approach in understanding paired scTCR clonotype diversity.

PO-392 GALECTIN-9 IS REQUIRED FOR DENDRITIC CELL FUNCTION AND DIRECTLY CONTRIBUTES TO DC-MEDIATED ANTI-TUMOUR IMMUNITY

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Introduction Dendritic cells (DCs) express a variety of cell surface receptors aimed at facilitating recognition, uptake and presentation of tumour antigens, which allows for the initiation of a potent anti-tumour immune response. Galectin-mediated interactions on DC-plasma membrane are emerging as potent modulators of cellular organisation although the underlying mechanisms are ill defined. We discovered that galectin-9 (gal-9) is required for DC phagocytosis and migration indicating that gal-9 controls tumour immune surveillance.

Material and methods

Results and discussions We discovered a novel interaction between Galectin-9 and the phagocytic receptor DC-SIGN and demonstrated that galectin-9 is essential for DCs to take up antigens. Atomic force microscopy experiments uncovered that Galectin-9 directly controls plasma membrane stiffness via the reorganisation of the actin cytoskeleton.

Moreover, we observed that galectin-9 is essential for chemokine-driven DC migration and for their capacity to react to melanoma cells, which may have direct implications for tumour immune surveillance. Supporting this hypothesis, we observed that the expression of galectin-9 is down-regulated in DCs upon co-culturing with melanoma tumour cells.

Conclusion In summary, our work identified Galectin-9 as novel plasma membrane organiser in DCs through reorganisation of the actin cytoskeleton that underlies plasma membrane rigidity. Furthermore, our data postulates galectin-9 as a key modulator of DC function, with implications in the ability of DCs to initiate an anti-tumour response. Based on our findings, we hypothesise that loss of galectin-9 impairs DCs to migrate and respond to tumours hampering anti-tumour immunity, thus facilitating tumour immune escape.

PO-393 NOTCH3 AND CXCR4 CROSS-SIGNALLING SUSTAINS ACUTE T-CELL LEUKAEMIA PROGRESSION

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Introduction Acute T-cell lymphoblastic leukaemia (T-ALL) is a childhood cancer, characterised by infiltration of immature T-cells in bone marrow. Notch hyperactivation is a major driver of T-ALL development where CXCL12/CXCR4 axis plays an important role in T-ALL maintenance. In thymus the lympho-stromal communication drives progressive maturation of T-cells. Notch receptors regulate T-cell fate choices, dominating early steps of thymocyte maturation. In T-cell differentiation, Notch3, in association with pre-TCR and chemochine receptor CXCR4, govern the transition from double negative (DN) to double positive (DP) thymocytes. Previously, our laboratory demonstrated the lymphomagenic potential of Notch3 by creating a transgenic mouse model (N3-ICtg), characterised by the constitutive activation of the intracellular domain (IC) of Notch3 receptor (N3-IC) in immature thymocytes. In order to investigate the oncogenic cross-talk between Notch3 and CXCR4 in T-ALL progression, we analysed DP T-cells in different lymphoid compartments of N3-ICtg mice.

Material and methods Freshly isolated cells from thymus, blood and bone marrow of N3-ICtg and WT mice were analysed by flow cytometry in order to verify the presence of DP T-cells and their cell-surface expression of CXCR4 and Notch3 receptors. Experiments in TALL1, a human T-ALL leukemic $CD3^+/CD4^+/CD8^+$ cell line characterised by the activation of Notch3 and high expression of CXCR4, were also performed. TALL1 cells were treated with γ-secretase inhibitor (GSI) or their gene expression of Notch3 was silenced and then analysed by flow cytometry, RT-PCR and western blot. Statistical interpretation of the results was performed.

Results and discussions DP-gated thymocytes obtained by N3-ICtg mice have shown a high co-expression of Notch3 and CXCR4 and a high migratory ability induced by SDF-1. An anomalous percentage representation of these DP T-cells at different ages in circulating blood, spleen and bone morrow may suggest an interaction between CXCR4 and Notch3 in T-ALL cell propagation. Experiments in human TALL1 cell line with Notch3 targeted inhibition suggest a modulated expression of CXCR4 through a β-arrestin1-mediated mechanism. CXCR4-antagonists treatment will further elucidate the molecular crosstalk between the two receptors.

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Conclusion Notch3 abnormal pathway, through boosting the expression of CXCR4 on cell-surface, may play a role in DP T-cells egress from thymus, and define a possible mechanism of 'pre-leukemic-cells' dissemination.

PO-394 PERFORMANCE OF A TARGETED T CELL RECEPTOR BETA IMMUNE REPERTOIRE SEQUENCING PANEL IN SEVERAL FFPE TISSUE TYPES - A TOOL FOR INTERROGATION OF THE TUMOUR MICROENVIRONMENT

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Introduction T cell receptor beta (TCRβ) immune repertoire analysis by next-generation sequencing is a valuable tool for research studies of the tumour microenvironment and potential immune responses to cancer immunotherapy. Here we describe a TCRβ sequencing assay that leverages the low sample input requirements of AmpliSeq library preparation technology to extend the capability of targeted immune repertoire sequencing to include FFPE samples which can often be degraded and in short supply.
Material and methods This assay targets the highly diverse CDR3 region which allows for T cell clone identification and frequency measurement which, when combined, can provide a broad view of the immune landscape within tissue samples. The assay allows for functionality with either RNA or DNA input, with little modification of the library construction workflow, as well as flexibility in sequencing throughput and sample multiplexing capability.
Results and discussions To evaluate assay accuracy, we sequenced libraries including known amounts of 30 well-studied T cell lymphoma rearrangements, as well as evaluation of samples comprised of known numbers of sorted T cells to ensure the absence of false rearrangement reporting. T cell repertoires were evaluated from as low as 5 ng to as large as 1 ug of input from samples of varying repertoire diversity, such as sorted T cells, peripheral blood leukocytes, fresh-frozen tissue, and FFPE tissue from a variety of normal and cancerous tissues such as lung, colon, brain, spleen, lymph node, and thymus. In parallel, we have developed a low input qPCR assay, based on known T cell markers, which allows for a quantitative measure of the T cell content across all sample types applicable to the TCRβ sequencing assay. This sample quantification test acts to guide optimal sample input ranges for library construction and required sequencing depth. Finally, we present the CDR3 TCRβ sequencing assay including a dual barcoding approach to extend sensitivity for detection of rare clones.
Conclusion These data present a T cell immune repertoire sequencing solution for application in a wide range of sample types, in particular, challenging FFPE samples. We find that the assay is capable of profiling repertoire metrics from FFPE samples over a large range of input amounts from several normal and tumour tissue types. In addition, we demonstrate use of a qPCR assay for quantification of sample T cell content to guide sample input for TCRβ immune repertoire sequencing experiments.

Immune Suppression and Escape

PO-395 CORTISOL TRIGGERS IMMUNE ESCAPE OF HUMAN ACUTE MYELOID LEUKAEMIA CELLS BY INDUCING EXPRESSION OF G PROTEIN-COUPLED RECEPTOR LATROPHILIN 1 WHICH FACILITATES EXOCYTOSIS OF IMMUNE SUPPRESSOR GALECTIN-9

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Introduction Acute myeloid leukaemia (AML) is a blood and bone marrow cancer originating from self-renewing malignant immature myeloid cells. AML cells are capable of escaping host immune surveillance. One of the mechanisms underlying this phenomenon is the ability of AML cells to secrete galectin-9, a tandem protein, which induces variety of intracellular and cell-to-cell signalling events leading to inactivation of NK cells as well as killing of cytotoxic T cells. Exocytosis of galectin-9 is mediated by the G protein-coupled receptor latrophilin 1 (LPHN1). However, molecular mechanisms underlying its expression and functional control remain unknown, and thus became the main goal of our work.
Material and methods We used THP-1 and primary human AML cells, primary leukocytes obtained from healthy donors. Western blot analysis, ELISA and quantitative Real-Time PCR served as main instrumental in experimental set-ups.
Results and discussions We discovered that cortisol induces LPHN1 expression on transcriptional level thus also upregulating its translation in AML cells, but not in primary healthy human leukocytes. We found that secreted form of fibronectin and leucine-rich transmembrane protein 3 (FLRT3, an endogenous LPHN1 ligand) is present in blood plasma of healthy donors and AML patients in equally high amounts. Therefore, LPHN1 expressed by human AML cells is not lacking its ligand and thus triggers galectin-9 secretion on permanent basis in order to provide continuous protection against host immune surveillance.
Conclusion Our results demonstrate a fundamentally novel survival mechanism employed by human AML cells. They use a crucial human hormone (cortisol) to express receptor (LPHN1), which uses blood plasma protein (FLRT3) to escape immune surveillance. Thus AML cells employ normal physiological systems of the human body to support their survival and attenuate anti-cancer activity of cytotoxic lymphoid cells.

PO-396 SOLUBLE MEDIATORS RELEASED BY HUMAN MELANOMA-ASSOCIATED FIBROBLASTS INTERFERE WITH CYTOTOXIC T CELL RESPONSE

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Introduction Melanoma associated fibroblasts (MAFs) represent a highly heterogeneous population of fibroblast-like cells residing in the tumour stroma, the exact origin of which is a matter of ongoing debate. In many aspects, MAFs recapitulate

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