developed a novel workflow combining the single molecule and single cell visualization capabilities of the RNAscopes in situ hybridization (ISH) assay with the highly multiplexed spatial profiling capabilities of the GeoMx™ Digital Spatial Profiler (DSP) RNA assays. Using these methods, we sought to spatially profile and compare gene expression signatures of tumor niches with high and low CTNNB1 expression.

Methods After screening 120 tumor cores from multiple tumors for CTNNB1 expression by the RNAscope assay, we identified melanoma as the tumor type with the highest CTNNB1 expression while prostate tumors had the lowest expression. Using the RNAscope Multiplex Fluorescence assay we selected regions of high CTNNB1 expression within 3 melanoma tumors as well as regions with low CTNNB1 expression within 3 prostate tumors. These selected regions of interest (ROIs) were then transcriptionally profiled using the GeoMx DSP RNA assay for a set of 78 genes relevant in immuno-oncology. Target genes that were differentially expressed were further visualized and spatially assessed using the RNAscope Multiplex Fluorescence assay to confirm GeoMx DSP data with single cell resolution.

Results The GeoMx DSP analysis comparing the melanoma and prostate tumors revealed that they had significantly different gene expression profiles and many of these genes showed concordance with CTNNB1 expression. Furthermore, immunoregulatory targets such as ICOSLG, CTLA4, PDCD1 and ARG1, also demonstrated significant correlation with CTNNB1 expression. On validating selected targets using the RNAscope assay, we could distinctly visualize that they were not only highly expressed in melanoma compared to the prostate tumor, but their expression levels changed proportionally to that of CTNNB1 within the same tumors suggesting that these differentially expressed genes may be regulated by the WNT-β-catenin pathway.

Conclusions In summary, by combining the RNAscopes ISH assay and the GeoMx DSP RNA assay into one joint workflow we transcriptionally profiled regions of high and low CTNNB1 expression within melanoma and prostate tumors and identified genes potentially regulated by the WNT-β-catenin pathway. This novel workflow can be fully automated and is well suited for interrogating the tumor and stroma and their interactions. GeoMx Assays are for RESEARCH ONLY, not for diagnostics.

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MULTIPLE MYELOMA FLOW CYTOMETRY PANEL VALIDATED FOR CLINICAL MONITORING OF PATIENTS

Methods PB and BM aspirates (BMA) were obtained from healthy donors and MM donors who consented to research testing. MM cell lines were also used to spike into donor samples to detect specific antigens (collected in Cyto-Chex™ blood collection tubes). Samples were then transferred to True-Count tubes to enumerate immune populations. Fluorescently labeled antibodies directed against CD38, CD138, CD56, CD45, BCMA were evaluated to assess parameters such as time and temperature stability of the reportable immune populations by monitoring the frequencies of the populations. In addition, the limit of quantitation, intra- and inter-assay precision were determined.

Results The MM Counting Panel was optimized to leverage antigen expression and fluorophore combinations. A gating strategy enabled enumeration of MM cells based on antigens that can be further subdivided based on BCMA expression. Further testing showed that the precision in frequencies and absolute counts of key reportable populations was deemed acceptable (%CV of <30%). The precision was within the acceptance criteria of %CV <30% for populations with ≥100 cells. Stability testing revealed that samples were more stable at ambient temperature relative to 4°C, with stability being maintained for 48 h post-collection, where at least 85% of reportable immune readouts were stable (%change <30% relative to baseline), for BMA and PB from various donors (healthy and MM). The panel was ultimately deployed for use with clinical samples from MM clinical trials. Clinical data generated from the MM Counting Panel allowed the identification of malignant plasma cell populations in BMA of patients from trial assessing a BCMAxCD3 bi-specific antibody (NCT03761108).

Conclusions A flow cytometric assay to enumerate and identify normal and malignant plasma cells in MM patients was successfully developed. The approach used can be applied to develop assays for other indications in which patients are treated with therapies.

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PRE-CLINICAL PHARMACODYNAMIC BIOMARKER ASSAYS OF IMMUNE MODULATION CAN TRANSLATE TO INFORM EXPLORATORY ENDPOINTS OF TARGET ENGAGEMENT IN FIRST-IN-HUMAN CLINICAL TRIAL STAGES OF DRUG DISCOVERY

Background Lack of efficacy is a common cause of failure in Phase I and Phase II clinical trials. Pharmacodynamic (PD) biomarker assays can demonstrate target engagement and proof of mechanism; both key components to improve trial success. Biomarkers established at the pre-clinical phase can serve as exploratory endpoints in early phase clinical trials, to confirm the mode of action of the therapeutic. We show examples of human in vitro assays and murine T cell adoptive transfer models, which can be used to establish potential PD biomarkers for inclusion in the clinical phases.

Methods Human peripheral blood mononuclear cell (PBMC) were incubated with SEB in the presence of Pembrolizumab or Ipilimumab. IL-2 and IFNgamma levels were quantified by
Luminex. To identify biomarkers of checkpoint inhibition, mice transferred with a defined population of ovalbumin (OVA)-specific T cells were challenged with OVA antigen or EG7 tumour. Activation and proliferation of antigen-specific T cells was determined and Nanostring gene expression analysis performed. Flow cytometry staining panels for human immune markers including CD4, CD14, CD25 and FOXP3 were established pre-clinically. As part of the assay validation process for a clinical trial, whole blood SEB activation was performed in normal donors, with Luminex analysis of IL-2, IL-17, IFN-gamma and TNFalpha.

Results Immune checkpoint inhibitors resulted in increased IL-2 and IFN-gamma secretion in human PBMC stimulated with SEB. In the murine PD model, anti-PD-L1 caused upregulation of CD25, IFN-gamma and granzyme B by antigen-specific CD8 T cells. Gene expression analysis of murine tumours elucidated changes in response to a vaccine. Flow cytometry panel staining determined the frequencies of human Treg and monocytes, which are common targets of immune-modulating therapies. Fit-for-purpose validation was performed for a human SEB activation assay resulting in robust changes in cytokine production.

Conclusions The experiments here show the flow of experiments that can be performed to identify a PD biomarker for use in first in man trials; the pre-clinical human PBMC SEB screening assay provides a simple assay demonstrating that a therapy can enhance T cell function and would be translatable to the clinic. The murine PD model provides a platform to screen for biomarkers of T cell function and monitor gene expression modulation. Biomarkers identified in the murine setting provide a good starting point for exploratory assessment in early phase clinical trials, where inclusion of exploratory PD biomarker endpoints in can confirm proof of mechanism and improve study success rates. 

Ethics Approval Human tissues used in this study were collected with ethical approval from UK Research Ethics Committee South West, Bristol (UK), approval number 15/SW/0029.

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### PROGNOSTIC FACTORS FOR OVERALL SURVIVAL IN PATIENTS WITH ADVANCED MELANOMA TREATED WITH ANTI-PD-1 THERAPY – THE MELIMMUNE SCORE


Background Immune checkpoint inhibitors (ICI) have changed the paradigm of advanced malignant melanoma (MM). Several prognostic factors, mostly linked to inflammation, have been determined with ethical approval from UK Research Ethics Committee South West, Bristol (UK), approval number 15/SW/0029.

Methods Baseline characteristics and outcomes on 147 patients with advanced MM treated with an anti-PD1 in monotherapy could be incorporated to the daily clinical practice and clinical trials. We further aim to validate this score in an independent larger sample.

Ethics Approval The study was approved by both institutions’ Ethics Committee.

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### IMMUNE CORRELATES ASSOCIATED WITH CLINICAL OUTCOMES IN PATIENTS WITH ADVANCED MALIGNANCIES TREATED WITH AVELUMAB AND OX40 AGONIST

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Background We evaluated immune correlates of avelumab in combination with PF-04518600 (OX40 agonist) in a phase I/II study (NCT03217747) in patients with advanced malignancies.

Conclusions Using easily accessible parameters from our daily practice, we propose the MELIMMune prognostic score for advanced MM patients treated with anti-PD1 in monotherapy that could be incorporated to the daily clinical practice and clinical trials. We further aim to validate this score in an independent larger sample.