Reconfigurable Separation (MARS®) system – combines novel acoustic cell processing and in-flow immuno-magnetic separation technologies with automation of the entire purification workflow for downstream cellular growth, modification, and analysis prior to being administered to patients.

Methods As a fully automated system, the MARS® system can isolate T-cells with high purity from lysed whole blood in as little as 11 minutes with up to 98% recovery and 98% viability without the need for Ficoll or centrifugation. The process is scalable to 10ML of blood, with complete purification requiring 1 hour. The system is designed to fit into a culture hood for sterile cell handling and has been used to isolate T-cells for expansion for downstream T – cell uses.

Results The tunable microfluidic cell processor is a functional module capable of washing and concentrating various sample types including all white blood cell types from whole blood, bone marrow, and apheresis. Additional uses for thawed frozen PBMC, cultured cells and solid tumor dissociation have also been demonstrated. Comparing with conventional centrifugation process, cell preparation by MARS has demonstrated high level of debris removal (>97%), minimal cell loss (> 90% recovery) and high cell viability with full automation. MARS is the first-to-market fully automated system to integrate sample preparation and cell isolation into a single platform and is designed to be a versatile tool for downstream cell analysis workflows.

Conclusions MARS is the first to market automatic sample preparation system and is designed to be a versatile tool for downstream cell analysis platforms. The MARS® system is an ideal instrument to prepare CAR-T cells due to its ability to isolate and purify these cells from whole blood with high viability in a completely automated process.

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0002

3 BUTYROPHILIN-3A IS EXPRESSED IN MULTIPLE SOLID TUMORS: TRANSLATIONAL RESEARCH SUPPORTING THE EVICTION STUDY WITH ICT01, AN ANTI-BTN3A MAB ACTIVATING VG9VD2 T-CELLS

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Background Butyrophillin-3A (BTN3A) three isoforms (3A1/3A2/3A3) are widely expressed on a variety of tumors.[1] BTN3A1 plays a key role in phosphoantigen activation of Vg9Vd2 T-cells, key mediators of innate and adaptive anti-tumor immunity.[2] Vg9Vd2 T-cell infiltration into tumor tissues is associated with a positive prognosis across multiple cancers,[3] which makes BTN3A an interesting target for enhancing anti-tumor immunity. ImCheck Therapeutics is developing ICT01, an anti-BTN3A mAb that specifically activates Vg9Vd2 T-cells. ICT01 is currently in an international, multi-center Phase 1/2a clinical trial (NCT04243499, EVICTION Study). The level of BTN3A expression required for a clinical response to ICT01 is not known. Therefore, we developed novel immunohistochemistry (IHC) methods to enable a precision-medicine based approach to target population selection for dose escalation and potentially guiding patient selection in the expansion cohorts of the ongoing EVICTION study.

Methods A panBTN3A IHC staining that recognizes the three isoforms was developed on Fresh frozen (FF) tissues, while BTN3A2- and BTN3A3-specific IHC methods were developed on formalin-fixed paraffin embedded (FFPE) tissues. BTN3A1-specific staining is still under development. Transfected knock-out/knock-in cell lines and positive tissues were used to assess antibody specificity. BTN3A expression was then analyzed on both normal and associated tumor tissue using tissue microarrays (TMA) and selected frozen blocks from tumor biopsies. FACS analyses were also performed on dissociated lung and pancreatic cancer biopsies to determine BTN3A (3 isoforms) membrane expression on tumor-infiltrating immune cells and cancer/stromal cells.

Results In normal tissues, BTN3A2 and BTN3A3 specific IHC signals were granular cytoplasmic in epithelial cells, with positive mononuclear and endothelial cells. Higher expression in lung, colon, and small intestine tissues was observed. Regarding panBTN3A expression, inter-indication and inter-patient heterogeneity was observed among head and neck, lung, melanoma, bladder, colon, pancreas, breast, and prostate cancer tissues, with both cytoplasmic and membranous localizations. The major finding was higher expression of BTN3A2 on malignant cells in melanoma, lung, colon, and prostate cancers, as compared to normal tissue. Finally, FACS analyses of lung and pancreatic cancer tissues revealed stronger expression of all BTN3A isoforms at the cell surface of infiltrated immune cells compared to its expression on stromal cells.

Conclusions These validated IHC methods supported the selection of cancer indications for the EVICTION trial and will potentially help identify specific tumor subtypes and patients that will most likely benefit from ICT01 treatment.

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0003

4 MOLECULARLY GUIDED MULTIPLEXED DIGITAL SPATIAL ANALYSIS REVEALS DIFFERENTIAL GENE EXPRESSION PROFILES IN THE WNT-β-CATENIN PATHWAY BETWEEN MELANOMA AND PROSTATE TUMORS

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Background The canonical WNT-β-catenin signaling pathway is vital for development and tissue homeostasis but becomes strongly tumorigenic when dysregulated. and alter the transcriptional signature of a cell to promote malignant transformation. However, thorough characterization of these transcriptomic signatures has been challenging because traditional methods lack either spatial information, multiplexing, or sensitivity/specificity. To overcome these challenges, we

developed a novel workflow combining the single molecule and single cell visualization capabilities of the RNAscope 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 RNAscope 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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0004

5 MULTIPLE MYELOMA FLOW CYTOMETRY PANEL VALIDATED FOR CLINICAL MONITORING OF PATIENTS

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Background Multiple myeloma (MM) is an incurable plasma cell malignancy with significant heterogeneity in clinical presentation. Plasma cells are antibody-producing cells of lymphoid origin that are resident in secondary lymphoid organs and in the bone marrow (BM). The detection of circulating malignant plasma cells using flow cytometry has also been described in patients with MM. Enumerating and phenotyping malignant plasma cells in the BM and peripheral blood (PB) may be of value when evaluating the presence of MM antigens targeted by therapies before and during treatment and at relapse. To this end, a flow cytometric panel was developed to enumerate and characterize malignant plasma cells and additional immune subsets.

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 TruCount 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 4oC, 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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0005

6 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

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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