immunotherapy. We have previously described a novel 27-gene immuno-oncology assay and algorithm which demonstrated significant predictive value in both NSCLC and TNBC. This algorithm utilizes gene expression to assess the tumor immune microenvironment (TIME) by combining aspects of the immune response, surrounding stromal cell signaling, and tumor physiology. We hypothesized that features of this algorithm may not only identify responders to immunotherapy (immunomodulatory, IO subtype) but may better enrich for patients who would benefit from other targeted therapeutics that alter the tumor microenvironment such as VEGF or FAK inhibitors (mesenchymal, M subtype).

**Methods** Pathway analysis was used on TNBC specimens representing both the IO and M subtypes as determined by the 27-gene immuno-oncology algorithm. Expression reads were scaled within each sample and the difference of the mean of expression of each gene within IO and M subtypes was determined to quantify relative expression within each pathway. Finally, the mesenchymal score obtained from the 27-gene immuno-oncology algorithm was used to stratify RNAseq expression data from xenograft models that were either sensitive or resistant to a FAK inhibitor (FAKi).

**Results** Pathway analysis identified stratification between the 27-gene immuno-oncology algorithm subtypes finding with the mesenchymal subtype is associated with higher WNT, TGF-B, and RAS pathways whereas the IO subtype was more highly associated with the JAK/STAT pathway. Additionally, the mesenchymal score from the 27-gene immuno-oncology algorithm was higher in the FAK inhibitor sensitive (0.36) xenograft models than the FAKi resistant (0.076) models ($p = 0.025$).

**Conclusions** The 27-gene immuno-oncology algorithm assesses the TIME to account for the immune response, surrounding stromal cell signaling, and tumor physiology to provide both an immuno-oncology subtype and mesenchymal subtype. We have previously demonstrated improved ability of the IO subtype to predict response to ICIs over current gold standard biomarkers. These data suggest that the M subtype is a distinct feature of the IO subtype which may enrich for patients more likely to respond to targeted therapeutics that act upon the canonical tumor promoting signaling pathways.

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## 10 A MULTIPARAMETER FLOW CYTOMETRY ASSAY TO MONITOR NATURAL KILLER CELL PROLIFERATION AND ACTIVATION IN IMMUNO-ONCOLOGY CLINICAL TRIALS

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**Background** Natural Killer (NK) cells have garnered increasing interest as potential cellular therapies or as targets of biotherapeutic agents due to their ability to kill tumor cells in a non-antigen dependent manner. Hence, measurement of NK cell proliferation and/or activation following treatment can serve as a useful biomarker for assessing the efficacy of immunomodulatory therapies.

**Methods** We developed a novel 13-parameter flow cytometry panel incorporating cell differentiation (CD) markers important for identification of NK cell subsets (CD56, CD16), their proliferation (Ki-67), activation (CD25, CD335, NKG2D) and inhibition (CD159a) status. Additionally, CD markers that identify other cellular subsets known to be amenable to cytokine modulation (e.g., CD3 and CD14) were included for concurrent monitoring of T cell proliferation and monocyte activation. Method validation focused on analytical sensitivity, specificity and precision as key criteria of assay performance using peripheral blood mononuclear cells (PBMCs) stimulated with NK cell-activating cytokines and resting PBMCs from healthy donors.

**Results** The assay design allowed for robust quantitation of NK cell, T cell and monocyte functionalities. Lower limit of quantification (LLOQ) of target biomarker population was determined to be 1.0% of the parent population, based upon an analysis of 110 key target populations that displayed a coefficient of variation (CV) of ≤25% and their frequencies ranged from 0.1% to 97.8% of the parent population. Additionally, ≤25% CV was observed in precision assessments, confirming the repeatability and reproducibility of the assay. Clinical trial utility of the assay was verified on cryopreserved PBMCs from patients with a variety of solid tumor malignancies. In these patients, the assay could clearly identify proliferating and activated NK cells, as well as proliferating T cells and activated monocytes, thus demonstrating its suitability for clinical trial applications.

**Conclusions** We developed and validated a novel multiparameter flow cytometry assay that allows for simultaneous measurement of proliferation, activation and inhibitory status of key immune cell subsets. Thus, this assay can help shed light on the mode of efficacy of novel therapeutic agents that modulate the immune system, aimed at treatment of cancer and autoimmune diseases.

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## 11 A MULTI-PHYSICS APPROACH ENABLING RARE CELL ISOLATION WITH HIGH RECOVERY AND HIGH PURITY

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**Background** Advancements in fields of multi-omics analysis and cell-based therapies depend upon efficient cell processing tools to isolate rare cancer and immune cells from complex biologic samples as an initial step in sample preparation. Conventional technologies are limited in automation, recovery and purity. We present an integrated system based on multiple physics principles with built-in novel technologies to achieve cell purification, concentration and target cell isolation, with high recovery at an unprecedented flow rate. This platform, the Multi-physics Automated Reconfigurable Separation (MARS), combines tunable, acoustic cell processing and in-flow immuno-magnetic separation technologies, enabling automation of the entire cell sample preparation workflow for proteomics and genomics analysis.

**Methods** Circulating tumor cells (CTC) are present in extreme low frequency in blood stream (1–100 in billions of blood cells) thus it has been a challenge to isolate CTCs with high recovery. We have developed protocols on MARS to isolate CTCs from whole blood for multi-color flow cytometry analysis. To demonstrate the extent of enrichment of tumor cells in whole blood, PC3 cells were used for spike recovery. RBC lysed blood sample was then loaded on MARS and automatically processed through cell washing, concentration, and magnetic depletion. Enriched tumor cells were collected and analyzed by flow cytometry.