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 ($p = 0.023$).

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.

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 $\leq 25\%$ and their frequencies ranged from 0.1% to 97.8% of the parent population. Additionally, $\leq 25\%$ 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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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.

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Results Results show > 4 log enrichment of tumor cells and average recovery of spiked CTC > 85% in the clinical relative range <100 cells per ml of whole blood (R2=0.929) with a throughput of 60 ml/hr. Isolated cells were confirmed to be cancer cells with imaging analysis and single cell genomic sequencing. The protocol was also validated with other cell line cells such as A549. The purity of the cells prepared by MARS are ideal for single cell genomics platforms.

Conclusions The fluidics of MARS is also replaceable and can be sterilized to minimize sample to sample contamination. The high molecular debris removal achieved by MARS is ideal for single cell genomics platforms, as is the first-to-market automated and integrated sample preparation and cell separation system designed to be a versatile tool for downstream cell analysis.

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12 DEVELOPMENT OF AN IN VITRO ASSAY TO ASSESS BISPECIFIC T CELL ENGAERUSING T CELLS FROM CD3E HUMANIZED MICE


Background Bispecific T cell engagers (BiTE) is a fast-growing class of immunotherapies. They are bispecific antibody that bind to T cell-surface protein (for example, CD3e) and a specific tumor associate antigen (TAA) on tumor cells, by which to redirect T cells against tumor cells in a MHC-independent manner. A successful example in the clinical is Blinatumomab, a BiTE antibody against CD3/CD19 approved in 2014 to treat acute lymphoblastic leukemia. Currently, many CD3-based BiTE are in clinical trials, including BCMAxCD3, Her2xCD3, CEAxCD3, and PSMAxCD3. To evaluate the efficacy of BiTE in vitro, human peripheral blood monocyte cells (hPBMC) are commonly being used as a source of T cells to co-culture with tumor cells. The disadvantage of using hPBMC is donor-to-donor variability and the availability of the original donor if a study needs to be repeated.

Methods To overcome this, we proposed to replace hPBMC with T cells from human CD3e (hCD3) genetically engineered mouse models mice (GEMM) for in vitro coculture assay. T cells were isolated from hCD3 GEMM mice using negative selection mouse T cell isolation kit. Conventional tumor cell lines or luciferase-engineered patient-derived xenograft (PDX)-derived organoids (PDXO) expressing specific antigens are co-cultured with hCD3 T cells in 96-well plates in the presence of BiTE antibody.

Results We measured the killing of tumor cells using either flow cytometry or luciferase activity as readouts. To analyze tumor-reactivity of T cells to cancer cell line or organoids, IFN-gamma in the culture medium was measured and activation markers on T cells was assessed.

Conclusions Our data showed the feasibility of using humanized mice T cells as a replacement for hPBMCs to assess BiTE antibody in vitro. We are further validating the application of murine hCD3 T cells for in vivo models to test bispecific T cell engagers.

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13 USE OF ANTI-VIRAL T CELLS TO MODEL HLA-RESTRICTED ANTI-TUMOR CYTOTOXIC LYMPHOCYTE RESPONSES

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Background With the success of T cell checkpoint antagonists in treating cancer, we must better understand treatment response heterogeneity and develop more physiologically preclinical models for evaluating the next wave of candidate therapeutics. Several hurdles limit the successful recapitulation of the cellular and molecular interactions between human T cells and tumor cells, not the least of which involves the challenge of access to – and ex vivo manipulation of – bona fide tumor antigen-specific T cells.

Methods In order to improve on our understanding of checkpoint therapy using human model antigens, we developed an antigen-specific T cell-mediated cytolysis model using anti-viral human T cells co-cultured with a human tumor cell line expressing viral peptide epitopes.

Results We found that anti-viral T cells could be used to model cytotoxicity HLA-restricted anti-tumor responses and these responses varied by donor according to peptide antigen density, antigen quality, T cell numbers, and time. By identifying sub-optimal conditions in a donor-specific fashion, we demonstrated enhanced cytolytic function of T cells in vitro when combined with multiple disparate anti-tumor modalities, including immune checkpoint blockade, growth factor blockade, and chemotherapy. This in vitro model was then successfully adapted to an in vivo tumor model system that demonstrated control of tumor growth in an antigen-dependent manner that was responsive to checkpoint blockade.

Conclusions These in vitro and in vivo systems represent a simple, yet elegant and robust platform for testing human T cell-directed immuno-oncology (IO) therapeutics and IO combinations.

Ethics Approval All animal experiments were conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care in accordance with institutional animal care and use committee guidelines and after appropriate approvals.

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14 NOVEL CD3 EPSILON HUMANIZED N-TERMINAL EPITOPE MODEL FOR ASSESSMENT OF EFFICACY OF T-CELL ENGAGERS

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Background T-cell engagers have proved to be a promising therapeutic strategy in immunotherapy, for redirecting T cells activity against tumor cells. To facilitate the preclinical assessment of novel T-cell engagers and their translatability, we have developed an immunocompetent CD3 epsilon N-terminal epitope humanized mouse model.

Methods This model was developed to express the human epitope of the CD3 epsilon chain, which is recognized by approximately 70% of the T-cell engagers (clone SP34). The rest of the extracellular domain was kept from mouse origin to preserve the amino acids involved in the interaction with CD3 gamma and delta. Similarly, the transmembrane domains