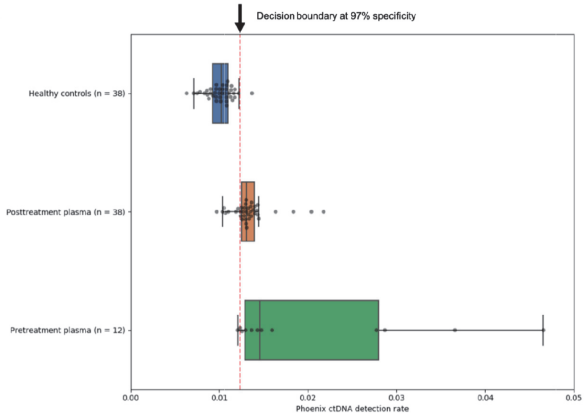
Abstract 33 Table 1 Characteristics of patients at baseline and ctDNA dynamics

Patient ID	Age / sex	Sites of metastasis	Pre-tx detected by iChorCNA	Pre-tx detected by Phoenix	Therapy	Objective response to therapy on imaging	ctDNA response detected at 3 weeks
32	38 M	Lungs, nodes	N	Y	Nivolumab	Y	Y
33	60 M	Nodes	N	Y	Nivolumab	N	N
34	60 M	Nodes	N	Y	Ipi / nivo	Y	N
37	49 M	Liver, soft tissue	Y	Y	Nivolumab	Y	Y
38	73 M	Nodes, lungs, liver, bones	N	Y	Nivolumab	Y	Y
40	70 F	Adrenals, bones, liver	N	Y	Ipi / nivo	Y	Y
41	69 F	Soft tissue	N	Y	Nivolumab	Y	Y
42	58 M	Lungs, nodes, liver	Y	Y	Ipi / nivo	Y	Y
45	70 M	Liver, soft tissue	N	N	Nivolumab	Y	N
53	53 M	Peritoneum, soft tissue	N	Y	Ipi / nivo	Y	Y
55	38 F	Soft tissue	N	Y	Ipi / nivo	Y	N

Baseline characteristics for preliminary validation cohort (n=12)



Abstract 33 Figure 4 Phoenix detects pre- and intratreatment melanoma ctDNA
We evaluated Phoenix post-filter sample-level detection rate. Phoenix detects ctDNA in 92% of pretreatment melanoma plasma samples (green, n=12) at a specificity of 97% relative to held-out noncancerous controls (blue, n=38). Phoenix detected ctDNA in 84% of posttreatment plasma samples (n=38, yellow), indicating full ctDNA clearance in 7/38 samples.

Conclusions Phoenix successfully identified pretreatment melanoma ctDNA without matched tumor tissue and identified response to CB as early as 3 weeks after treatment. Our ongoing studies aim to optimize this technology for early identification of CB response in clinical practice.

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Ethics Approval Use of human data in this study was approved by Memorial Sloan Kettering's IRB, Assurance Number FWA0000499

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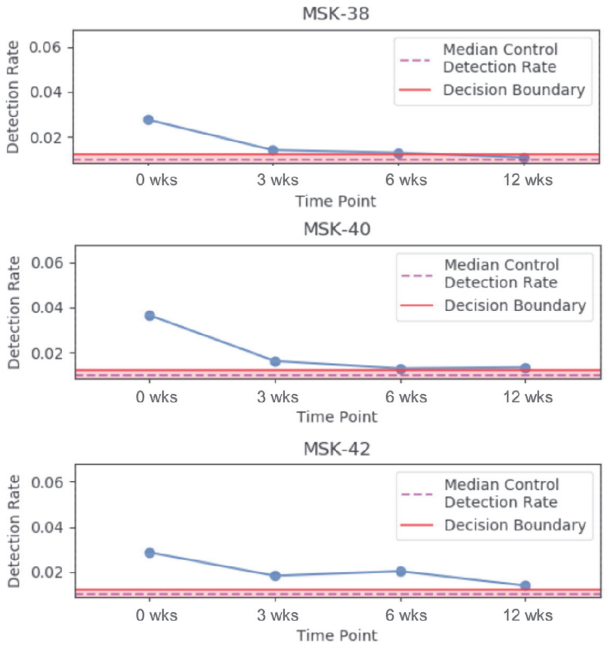

Abstract 33 Figure 5 ctDNA response to checkpoint blockade after 3 weeks
Serial plasma samples were taken from patients on checkpoint blockade (nivolumab alone or with ipilimumab). ctDNA burden was measured as detection rate among post-filter candidate SNVs and compared to a 97% specificity boundary among a panel of healthy controls. Phoenix detects a response to checkpoint blockade, measured as a decrease in ctDNA detection rate, as early as 3 weeks as shown in 3 patients (MSK-38, MSK-40, MSK 42).

34 MULTI-STEP ANTIBODY VALIDATION FOR THE GEOMX® DIGITAL SPATIAL PROFILER

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Background Understanding protein expression patterns within tissue compartments is imperative to investigating a range of biological questions. Historically, low plex immunohistochemical (IHC) approaches have been employed to assess the spatial heterogeneity of protein expression in tissue slices, but these techniques are of limited utility due to the challenge of measuring multiple targets in parallel. Compounding this limitation is the necessity of validating all antibodies which is resource intensive. Antibodies with poor quality have led to wasted time and resources, including false positives and non-reproducible results.[1 2] Here we review the antibody validation process for the GeoMx® Digital Spatial Profiler (DSP) which enables investigation of high-plex, validated, spatially resolved protein targets from a single slide mounted formalin-fixed paraffin-embedded (FFPE) or fresh frozen sample. The robust validation process is in line with recent suggestions for antibody validation from SITC.[3]

Methods Unconjugated and oligo-conjugated antibodies are screened by IHC to assess staining sensitivity, patterns, and more importantly ensure that the oligo-conjugation has not adversely affected antibody performance. Upon approval by a pathologist, the antibodies are incorporated into a core or module and further validated using the GeoMx DSP. Using FFPE cell pellet arrays (CPAs) containing positive and negative control pellets, we assess the specificity as defined as a lack of signal in negative control pellets and a robust signal in positive control pellets. Antibodies with robust signals are then

screened on tissue microarrays (TMAs) composed of healthy and diseased tissues to ensure that they will perform as expected in real samples and yield sufficient signal over background. Finally, after antibodies pass functional validation, we assess the performance of antibodies within panels of antibodies that will be commercialized.

Results In total, approximately 60% of off-the-shelf antibodies tested for use in GeoMx assays pass the entire validation process and are put into commercial assays. Passing requirements include exhibiting a maximum positive signal divided by the limit of detection, plus two standard deviations (SD) that is greater than or equal to 5 in both CPAs and TMAs for individual antibodies; such a threshold gives a false positive rate of less than 10%.

Conclusions Unvalidated or poorly validated antibodies can result in false positives and non-reproducible results. Following the robust validation process outlined here, approximately 40% of off-the-shelf antibodies are removed from panels, underscoring the importance of antibody validation prior to incorporating new antibodies into experiments.

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35 TARGETED NON-VIRAL INTEGRATION OF LARGE CARGO IN PRIMARY HUMAN T CELLS BY CRISPR/CAS9 GUIDED HOMOLOGY MEDIATED END JOINING

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Background Engineered immune cells hold tremendous promise for the treatment of advanced cancers. As the scale and complexity of engineered cell therapies increase, reliance on viral vectors for clinical production limits translation of promising new therapies. Here, we present an optimized platform for CRISPR/Cas9-targeted, non-viral engineering of primary human T cells that overcomes key limitations of previous approaches, namely DNA-induced toxicity and low efficiency integration of large genetic cargos.

Methods A systematic optimization of nucleic acid delivery, editing reagent composition, and culture protocol was performed to overcome DNA toxicity. Targeted knockin (KI) at AAVS1 and TRAC was compared across multiple vector configurations with genetic cargos ranging from 1 to 3 kilobases (kb) in size. Integration efficiency was measured by flow cytometry and sequencing. Off-target editing and integration were evaluated using GUIDE-seq and targeted locus amplification (TLA), respectively. Phenotype and function of non-virally and lentivirus engineered CAR-T cells was compared using flow cytometry, cytokine profiling and cytotoxicity assays.

Results We identified a temporal window following T cell activation where transfection efficiency, cell-cycle-status, and cytosolic DNA sensor expression were optimal for targeted DNA integration and reduced toxicity. Within this window, we targeted a 1kb GFP reporter to the AAVS1 locus with an efficiency of ~45% using homologous recombination (HR). Efficiency was reduced to ~11% with a larger ~3kb TCR cassette targeted to the TRAC locus, consistent with previous reports.[1–3] To improve large cargo integration we employed homology mediated end-joining (HMEJ) and short homology design (48bp vs. ~1kb for traditional HR).[4] Using HMEJ, knockin of the 1kb GFP cassette at AAVS1 reached ~70%. Strikingly, integration of the 3kb TCR at TRAC reached ~50% using HMEJ. Additional optimization of the culture protocol doubled post-engineering survival and proliferation (up to ~35-fold expansion in 7 days). Non-virally engineered TRAC KI CAR-T cells were phenotypically and functionally equivalent to lentivirally engineered T cells in vitro. In vivo assays in xenograft models are underway and results will be presented.

Conclusions Comprehensive, orthogonal optimization of parameters impacting nucleic acid delivery and DNA-toxicity in combination with novel modalities for integration achieved knockin of TCR and CAR cargo at efficiencies equivalent to that of current viral vector platforms without compromising expansion or function. Our protocol is suitable for clinical scale production under GMP conditions and offers an improved methodology over previous methods for non-viral engineering of human T cells.

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36 MOLECULAR EVENTS REGULATING SOLID TUMOR CELL RESPONSES TO NATURAL KILLER CELLS

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Background Natural killer (NK) cells exhibit potent activity in pre-clinical models of diverse hematologic malignancies and solid tumors and infusion of high numbers of NK cells, either autologous or allogeneic, after their ex vivo expansion and activation, has been feasible and safe in clinical studies.

Methods To systematically define molecular features in human tumor cells which determine their degree of sensitivity to human allogeneic NK cells, we quantified the NK cell responsiveness of hundreds of molecularly-annotated 'DNA-barcoded' solid tumor cell lines in multiplexed format (PRISM; Profiling Relative Inhibition Simultaneously in Mixtures approach),[1] correlating cytotoxicity scores for each cell line with the CCLE transcriptional data[2] (RNA-seq), to reveal genes that are