

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.

## REFERENCES

1. Taussig MJ, Fonseca C, and Trimmer JS. Antibody validation: a view from the mountains. *N Biotechnol.* 2018; **45**:1–8.
2. Bordeaux J, Welsh AW, Agarwal S, Killiam E, Baquero MT, Hanna JA, Anagnostou VK, Rimm DL. Antibody validation. *Biotechniques* 2010;**48**(3):197–209.
3. Taube, *et al.* The Society for Immunotherapy of Cancer statement on best practices for multiplex immunohistochemistry (IHC) and immunofluorescence (IF) staining and validation. *J Immunother Cancer* 2020;**8**(1):e000155.

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## 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.<sup>1–3</sup> To improve large cargo integration we employed homology mediated end-joining (HMEJ) and short homology design (48bp vs. ~1kb for traditional HR).<sup>4</sup> 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.

## REFERENCES

1. Roth TL, Puig-Saus C, Yu R, Shifrut E, Carnevale J, Li PJ, Hiatt J, Saco J, Krystofinski P, Li H, Tobin V, Nguyen DN, Lee MR, Putnam AL, Ferris AL, Chen JW, Schickel J-N, Pellerin L, Carmody D, Alkorta-Aranburu G, Del Gaudio D, Matsumoto H, Morell M, Mao Y, Cho M, Quadros M, Gurumurthy CB, Smith, B, Haugwitz M, Hughes SH, Weissman JS, Schumann K., Esensten JH., May AP, Ashworth A., Kupfer G. M., Atma S., Greeley W. & Marson A. Reprogramming human T cell function and specificity with non-viral genome targeting. *Nature* doi:10.1038/s41586-018-0326-5
2. Parker Autoimmune SN, Zuckerberg Biohub C, Francisco S. & Helen U. Polymer-stabilized Cas9 nanoparticles and modified repair templates increase genome editing efficiency. *Nat. Biotechnol.* doi:10.1038/s41587-019-0325-6
3. Schober K, Müller TR, Gökmen F, Grassmann S, Effenberger M, Poltorak M, Stemmerger C, Schumann K, Roth TL, Marson A. & Busch DH. Orthotopic replacement of T-cell receptor  $\alpha$ - and  $\beta$ -chains with preservation of near-physiological T-cell function. *Nature Biomedical Engineering* **3**, 974–984 (2019).
4. Wierson WA, Welker JM, Almeida MP, Mann CM, Webster DA, Torrie ME, Weiss TJ, Kambakam S, Vollbrecht MK, Lan M, McKeighan KC, Levey J, Ming Z, Wehmeier A, Mikelson CS, Haltom JA, Kwan KM, Chien C-B, Balciunas D, Ekker SC, Clark KJ, Webber, BR, Moriarity BS, Solin SL, Carlson DF, Dobbs DL, McGrail M & Essner J. Efficient targeted integration directed by short homology in zebrafish and mammalian cells. *Elife* **9**, (2020).

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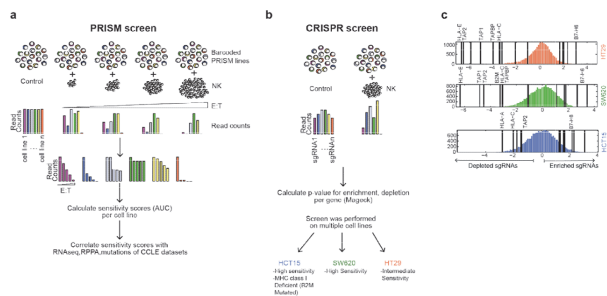
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## MOLECULAR EVENTS REGULATING SOLID TUMOR CELL RESPONSES TO NATURAL KILLER CELLS

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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),<sup>1</sup> correlating cytotoxicity scores for each cell line with the CCLE transcriptional data<sup>2</sup> (RNA-seq), to reveal genes that are



**Abstract 36 Figure 1** Overview of PRISM and CRISPR studies a, Schematic depiction of PRISM study. b, Schematic depiction of CRISPR screens. c, Histogram of gene fold changes (z-scores). Listed are selected genes with most prominent p-values across more than one screen.

associated with resistance or sensitivity to NK cells. In addition, we applied genome-scale CRISPR-based gene editing screens in several solid tumor cell lines to interrogate, at a functional level, which genes regulate tumor cell response to NK cells.<sup>3,4</sup> Figure 1 schematically depicts the two screens.

**Results** Based on these orthogonal studies, NK sensitive tumor cells tend to exhibit high levels of the NK cell-activating ligand B7-H6 (NCR3LG1); low levels of the inhibitory ligand HLA-E; microsatellite instability (MSI) status; high transcriptional signature for chromatin remodeling complexes and low antigen presentation machinery genes. Treatment with HDAC inhibitor reduced the sensitivity of SW620 colon cancer cells, increased antigen presentation machinery, including HLA-E, and reduced B7-H6. Importantly, transcriptional signatures of NK cell-sensitive tumor cells correlate with immune checkpoint inhibitor resistance in clinical samples. Widespread analysis of CCLE transcriptional signatures revealed that cell lines with mesenchymal-like program tend to be more sensitive to NK cells, compared with epithelial-like cell lines. Indeed, mesenchymal tumors tend to have lower expression of antigen presentation machinery in both CCLE and TCGA.

**Conclusions** This study provides a comprehensive map of mechanisms regulating tumor cell responses to NK cells, with implications for future biomarker-driven applications of NK cell immunotherapies. The integration of PRISM and CRISPR identified potential regulators of tumor cell response to NK cell, which upon further validation, may serve as biomarkers in future NK cell-based studies. Moreover, NK cells may complement T-cells, killing tumor cells that do not respond to immune checkpoint inhibitors.

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## REFERENCES

1. Yu C, *et al.*, High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines. *Nat Biotechnol* 2016. **34**(4): p. 419–23.
2. Ghandi M, *et al.* Next-generation characterization of the cancer cell line encyclopedia. *Nature* 2019. **569**(7757): p. 503–508.
3. Doench JG, *et al.* Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat Biotechnol* 2016. **34**(2): p. 184–191.
4. Shalem O, *et al.* Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* 2014;**343**(6166): p. 84–87.

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## MONITORING THE EVOLUTION OF INFLAMMATORY BOWEL DISEASE (IBD) IN PEDIATRIC AND ADULT COHORTS OF PATIENTS TO IDENTIFY BIOMARKERS TO PREDICT CANCER RISK

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**Background** Patients with inflammatory bowel disease (IBD) have increased risk of developing colorectal cancer (CRC), depending on the duration and severity of the disease. The evolutionary process in IBD is driven by chronic inflammation leading to epithelial-to-mesenchymal transition (EMT) events in colonic fibrotic areas. EMT plays a determinant role in tumor formation and progression, through the acquisition of ‘stemness’ properties and the generation of neoplastic cells. The aim of this study is to monitor EMT/cancer initiating tracts in IBD in association with the deep characterization of inflammation in order to assess the mechanisms of IBD severity and progression towards malignancy.

**Methods** 10 pediatric and 20 adult IBD patients, admitted at Sidra Medicine (SM) and Hamad Medical Corporation (HMC) respectively, have been enrolled in this study, from whom gut tissue biopsies (from both left and right side) were collected. Retrospectively collected tissues (N=10) from patients with malignancy and history of IBD were included in the study. DNA and RNA were extracted from fresh small size (2–4 mm in diameter) gut tissues using the BioMasher II (Kimble) and All Prep DNA/RNA kits (Qiagen). MicroRNA (miRNA; N=700) and gene expression (N=800) profiling have been performed (cCounter platform; Nanostring) as well as the methylation profiling microarray (Infinium Methylation Epic Bead Chip kit, Illumina) to interrogate up to 850,000 methylation sites across the genome.

**Results** Differential miRNA profile (N=27 miRNA; p<0.05) was found by the comparison of tissues from pediatric and adult patients. These miRNAs regulate: i. oxidative stress damage (e.g., miR 99b), ii. hypoxia induced autophagy; iii. genes associated with the susceptibility to IBD (ATG16L1, NOD2, IRGM), iv. immune responses, such as TH17 T cell subset (miR 29). N=6 miRNAs (miR135b, 10a196b, 125b, let7c, 375) linked with the regulation of Wnt/b-catenin, EM-transaction, autophagy, oxidative stress and play role also in cell proliferation and mobilization and colorectal cancer development were differentially expressed (p<0.05) in tissues from left and right sides of gut. Gene expression signature, including genes associated with inflammation, stemness and fibrosis, has also been performed for the IBD tissues mentioned above. Methylation sites at single nucleotide resolution have been analyzed.

**Conclusions** Although the results warrant further investigation, differential genomic profiling suggestive of altered pathways involved in oxidative stress, EMT, and of the possible stemness signature was found. The integration of data from multiple platforms will provide insights of the overall molecular determinants in IBD patients along with the evolution of the disease.

**Ethics Approval** This study was approved by Sidra Medicine and Hamad Medical Corporation Ethics Boards; approval number 180402817 and MRC-02-18-096, respectively.

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