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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0034

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

Beau Webber*, Matthew Johnson, Nicholas Slipek, Walker Lahr, Xiaohong Qiu, Blaine Rathmann, Miechaleen Diers, Bryce Wick, R Scott McIvor, Branden Moriarity. *University of Minnesota, Minneapolis, MN, USA*

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.

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, Stemberger C, Schumann K, Roth TL, Marson A. & Busch DH. Orthotopic replacement of T-cell receptor α- and β-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).

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0035

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

Michal Sheffer*, Constantine Mitsiades. *Dana-Farber Cancer Institute, Boston, MA, USA*

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