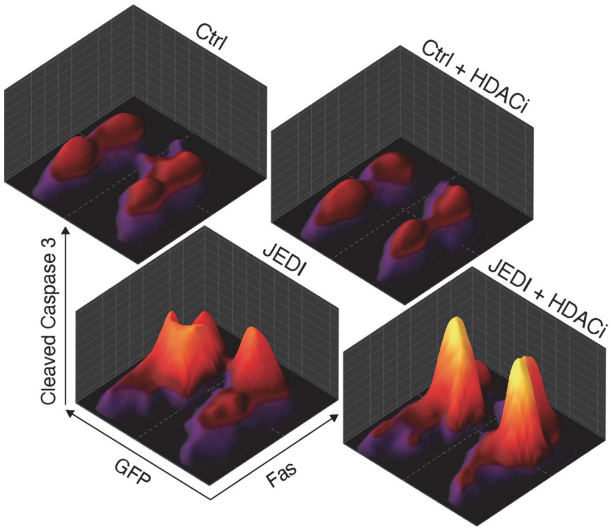

Abstract 90 Figure 3 See text for description

Conclusions T-cell mediated tumor killing can be potentiated with fas pathway modulators. This augmentation improves both fas-dependent Ag^+ and Ag^-tumor cell death. Further studies of modulating the fas pathway alongside T-cell based immunotherapies are needed as potential treatments to prevent antigen escape and improve patient outcomes.

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Ethics Approval The studies were approved by The Mount Sinai Institutional Review Board.

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91 DIRECTLY LINK T CELL PHENOTYPE AND FUNCTION TO GENOTYPE WITH THE OPTO™ CELL THERAPY DEVELOPMENT 1.0 WORKFLOW

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Background The key challenges to developing T cell-based therapies center on the fact that T cell-mediated tumor death relies on complicated cell-cell interactions and several complex mechanisms. These therapies have also been associated with significant side effects related to cytokine release syndrome (CRS) and neurotoxicity, placing importance on understanding T cell anti-tumor functions like cytokine release and killing kinetics. Ideally, T cell therapies would be tailored to mediate the rapid destruction of multiple tumor cells while reducing these side effects.

Methods The Berkeley Lights Opto™ Cell Therapy Development Workflow is a collection of software capabilities, reagents, and protocols that allow scientists to selectively measure cytokine secretion, visualize killing behavior, and sequence TCRs from individual cells in parallel. Here, we demonstrate its use for CAR-T cell phenotypic and functional screening as well as the discovery of TCRs associated with specific T cell behaviors.

Results The cumulative percentage of pens with tumor cell caspase-3 activity increased over time in pens loaded with CD19+ tumors, peaking at 50% tumor cell death after 16 hours of incubation. This is in contrast to only 10% of pens displaying tumor cell death in control pens loaded with CD19- tumor cells; control pens also exhibited slower killing kinetics. The single-cell resolution of the OptoSelect™ microfluidic chip enabled us to analyze each significant T cell-tumor cell interaction. We were able to directly compare differences in killing kinetics of individual T cells and link this tumor killing behavior to IFNγ secretion. We identified fast-killing and slow-killing CAR-T cells in a single-day experiment, which could then be exported for genomic analysis. We highlight an example where TCR alpha and beta sequences are recovered from single T cells after export.

Conclusions The Opto™ Cell Therapy Development Workflow on Berkeley Lights systems enables researchers to correlate cytokine secretion to target cell killing behavior in CAR-mediated antigen recognition, discriminate CAR-T cell subsets based on kinetics of target cell killing, and link cytokine secretion and target cell killing behavior to TCR sequence in TCR-mediated antigen recognition.

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92 ADOPTIVE TRANSFER OF IMMATURE MYELOID CELLS LACKING NF-κB P50 (P50-IMC) SLOWS HIGH-RISK NEUROBLASTOMA TUMOR GROWTH

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Background High-risk neuroblastomas, including those with MYCN gene amplification, harbor abundant myeloid cells that suppress anti-tumor immunity and favor tumor growth. Macrophages lacking the inhibitory NF-κB p50 subunit adopt an M1-polarized, T-cell-activating phenotype. Multiple cancers grow slower in mice lacking p50, and colon cancer grows slower in mice lacking p50 specifically in myeloid cells.

Methods Tumor growth was monitored in mice inoculated subcutaneously with 9464D cells. To generate p50-IMC from $p50^{-/-}$mice, marrow cells were lineage-depleted and then expanded in media with SCF, FL, and TPO, followed by transfer to M-CSF for one day. To generate p50-IMC from wild-type mice, marrow cells were nucleofected with a p50 sgRNA:Cas9 complex, followed by expansion in serum-free media. Mice received 5-fluorouracil on day 27, followed five days later by three doses of 1E7 p50-IMC via tail vein every three to four days. PD-1 antibody was administered twice weekly for four doses, starting on day 32. Azacytidine was administered 5 days per week alternating with ITF-2357 5 days per week, again starting on day 32. CD4 and CD8 antibody was given twice weekly starting on day 21. Tumor T cells were analyzed by flow cytometry.

Results We have now found that murine 9464D neuroblastoma cells, expressing high levels of exogenous human *MYCN*, also grow slower in syngeneic C57BL/6 (B6) p50(f/f);Lys-Cre mice, lacking p50 in macrophages and neutrophils, compared with p50(f/f) littermates. Slowed tumor growth in p50(f/f);Lys-Cre mice was associated with increased total and activated