we describe the development and careful optimization of an in vivo mouse CRISPR-screening platform to identify knock-out targets in primary T cells, with the goal of increasing T cell abundance and persistence in tumors with different TMEs. Using a mouse retroviral system to express single-guide RNA (sgRNA) libraries in T cells from Cas9 transgenic mice, we performed in vivo screens in syngeneic, fully immune-competent mouse tumor models.

Results We identified both known and potential novel regulators of T cell activation and persistence. Importantly, we have discovered knock-out targets that accumulate in multiple, distinct TMEs and other targets that are TME-specific. The use of sub-genomic- focused libraries allowed us to rapidly screen in multiple tumor model systems and reproducibly identify hits across individual mice.

Conclusions We have developed a fully optimized an in vivo genetic screen, which could be a rich source for target discovery, and can enable identification of functional regulators of T cells for rapid incorporation into CRISPR-engineered T cell therapies for different solid TMEs.

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174 AN EX VIVO TUMOROID MODEL OF FRESH PATIENT TUMORS (3D-ACT) TO ASSESS EFFICACY OF CELLULAR THERAPY IN IMMUNO-ONCOLOGY

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Background Adoptive T cell therapy (ACT) strategies have achieved substantial advances in the treatment of malignant tumors. Some of the unique challenges posed to ACT by solid tumors include locating target cells, as well as entering and surviving the complex tumor microenvironment. To develop better ACT applications and identify combination therapies to enhance tumor cell killing efficacy of ACT it is imperative to develop preclinical platforms that recapitulate the complexity of patient tumor microenvironment (TME). The goal of this study was to develop an integrated confocal-based high-throughput, high-content real time imaging platform to assess immunogenic tumor cell killing (TCK) activity of ACT applications such as CAR-T and TCR using fresh patient tumor samples.

Methods All patient tumor samples were obtained with patient consent and relevant IRB approval. For the confocal imaging platform, unpropagated 3D tumoroids with intact TME measuring 150 micron in size were prepared from fresh tumor samples of renal cell carcinoma (RCC), colorectal carcinoma (CRC) and non-small cell lung cancer (NSCLC) using proprietary technology developed at Nilogen Oncosystems. Engineered T-cells were labeled with different fluorescent cell tracker dyes to monitor cell migration and locations within tumoroids by confocal analysis. Comprehensive flow cytometry analysis was performed to corroborate confocal imaging findings from TCK and multiplex cytokine release assays used to assess changes in the TME.

Results Our studies demonstrated that the confocal-based high-content real time imaging platform described here, combined with a custom image analysis algorithm, allowed for monitoring of treatment-mediated tumor cell killing with structural and functional analysis of engineered T-cells in intact 3D tumoroids. The penetration rate of CAR-T and TCR cells into tumoroids as well as associated tumor cell death varied significantly between different tumor types. Flow cytometry analysis allowed for monitoring of the activation status and viability of engineered T-cells, and treatment-mediated changes in tumor resident immune cell populations.

Conclusions Our data indicated that the immunosuppressive tumor microenvironment may have implications for the application of ACT. Use of the ex vivo platform described here (3D-ACT) may aid in the validation of combinatorial therapies that block or deplete suppressive factors present within the TME, allowing these therapies to overcome mechanisms associated with dysfunction in CAR-T and TCR cell applications.

Ethics Approval The study was approved by Chesapeake IRB Pro00014313.

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175 A FAS-4–1BB IMMUNOMODULATORY FUSION PROTEIN CONVERTS A PRO-DEATH TO A PRO-SURVIVAL SIGNAL, ENHANCING T CELL FUNCTION AND EFFICACY OF ADOPTIVE CELL THERAPY IN MURINE MODELS OF AML AND PANCREATIC CANCER

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Background Adoptive cell therapy (ACT) with genetically-modified T cells has shown impressive results against some hematologic cancers, but limited efficacy against tumors with restrictive tumor microenvironments (TMEs). FasL is a particular obstacle for ACT;[1] it is expressed in many tumors and TMEs,[1] including AML,[2] ovarian[3] and pancreatic cancers,[4] and upregulated on activated T cells, where it can mediate activation-induced cell death (AICD).[5]

Methods We engineered T cells to boost function with novel immunomodulatory fusion proteins (IFPs) that combine an inhibitory ectodomain with a costimulatory endodomain. Like current checkpoint-blocking therapies, IFPs can abrogate an inhibitory signal, but also provide an often absent costimulatory signal. Additionally, IFP-driven signals are delivered only to the T cells concurrently engineered to be tumor-specific, thereby avoiding systemic T cell activation. For FasL-expressing TMEs, we developed an IFP that replaces the Fas intracellular tail with costimulatory 4-1BB. We tested the the Fas-4-1BB IFP in primary human T cells and in immunocompetent murine models of leukemia and pancreatic cancer.

Results Fas-4-1BB IFP expression enhanced primary human T cell function and enhanced lysis of Panc1 pancreatic tumor cells in vitro. Fas-4-1BB IFP-engineered murine T cells exhibited increased pro-survival signaling, proliferation, antitumor function and altered metabolism in vitro. Notably, the Fas ectodomain is trimeric[5] and the 4-1BB intracellular domain requires trimerization to signal.[6] In contrast, the CD28 domain is dimeric and did not enhance function when paired with 4-1BB.In vivo, Fas-4-1BB increased T cell persistence and function, and Fas-4-1BB T cell ACT significantly improved survival in a murine AML model. When delivered with a mesothelin-specific TCR, Fas-4-1BB T cells prolonged survival in the autochthonous KPC pancreatic cancer model, increasing median survival to 65 from 37 days (with TCR-only, $^{**}P=0.0042$). Single-cell RNA sequencing revealed differences in the endogenous tumor-infiltrating immune cells, included changes in cell frequency and programming.