tumor $CD4^+$ and $CD8^+$ T cells, and depletion of both $CD4^+$ and $CD8^+$ T cells accelerated tumor growth. PD-1 checkpoint blockade or DNA methyltransferase and histone deacetylase inhibition further slowed tumor growth in p50(f/f);Lys-Cre mice. In addition, adoptive transfer of p50-IMC, generated either from the bone marrow of $p50^{-/-}$ B6 mice or via nucleofection of a p50 sgRNA:Cas9 complex into wild-type B6 hematopoietic progenitors, also slowed neuroblastoma tumor growth, following a dose of myelo-depleting 5-fluorouracil.

Conclusions These findings using a neuroblastoma model further validate the utility of targeting myeloid NF-κB p50 as an immunotherapy strategy for cancer therapy and demonstrate activity of p50-IMC generated by gene editing of syngeneic marrow cells, a cell product relevant to clinical translation. We have also developed means to efficiently gene edit p50 in human marrow CD34 cells, and have demonstrated the feasibility of generating p50-IMC from human induced pluripotent stem cells. We are currently evaluating the efficacy of these gene-edited human cells against human neuroblastoma in immune-deficient mice.

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93 TARGETING SIRT2 RESCUES THE METABOLIC FITNESS AND EFFECTOR FUNCTIONS OF TUMOR-REACTIVE T CELLS WITHIN THE METABOLICALLY RESTRICTED TUMOR MICROENVIRONMENT

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Background The majority of cancer patients remain refractory to existing cancer immunotherapies. Despite the growing evidence that dysregulated metabolism contributes to the exhaustion of tumor-infiltrating T lymphocytes (TILs) and the loss of their effector functions within the metabolically restricted tumor microenvironment (TME), actionable targets to rescue metabolic fitness and anti-tumor activity of TILs remain elusive.Memory T (TM) cells and TILs rely on fatty acid catabolism to preserve their effector functions due to nutrient competition for glucose with tumor cells. Therefore, enhancing fatty acid catabolism of TILs represents an attractive strategy to increase the efficacy of immunotherapies.Sirt2 is an NAD+ dependent histone deacetylase. We previously showed that upregulation of Sirt2 in human TILs negatively correlates with response to TIL therapy in advanced non-small cell lung cancer (NSCLC) and Sirt2 deficiency leads to hyper-reactive T cells with superior antitumor activity.

Methods Sirt2 expression was analyzed by flow cytometry and Western blot. The role of Sirt2 in tumor immunity was studied using in vivo B16F10 tumor challenge models as well as ex vivo analysis including RNA-sequencing, CFSE proliferation assay, DAPI/AnnexinV staining, IFN-γ ELISpot assay, intracellular staining of effector molecules and LDH cytotoxicity assay on WT versus Sirt2KO T cells. Molecular partners of Sirt2 were identified using mass spectrometry (MS) and Co-immunoprecipitation analyses. The role of Sirt2 in T cell metabolism was investigated using seahorse bioanalyzer and LC-MS/MS Metabolomic profiling. AGK2, a Sirt2 selective inhibitor, was used for Sirt2 blockade in human T cells.

Results Sirt2 expression is upregulated during T cell activation, TM stage, and within the TME. Our molecular studies revealed that Sirt2 negatively impacts the acetylation status and the activity of the trifunctional protein, the key enzyme of fatty acid oxidation (FAO). Accordingly, Sirt2 deficiency enhanced FAO and metabolic fitness of activated T cells and mouse TILs isolated from B16F10 tumor nodules. As a consequence of enhanced FAO, Sirt2 deficient mice displayed increased accumulation of TM cells, which was associated with decreased apoptosis and increased survival after tumor challenge leading to superior tumor rejection. Most importantly, pharmacologic inhibition of Sirt2 in human TILs isolated from NSCLC patients enhanced their metabolic fitness and cytotoxic activity against their autologous tumor cells.

Conclusions Our findings indicate Sirt2 as a suppressor of T cell metabolism amenable to therapeutic targeting, and Sirt2 inhibition reprograms T cell metabolic fitness to optimally sustain their effector function within the hypoglycemic TME, thus, leading to an effective anti-tumor immune response.

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94 THE T CELL ANTIGEN COUPLER (TAC) REDIRECTS T CELL ONCOLYSIS WHILE LIMITING TONIC SIGNALING TO CREATE A SAFER ENGINEERED T CELL PRODUCT WITH A HIGHER THRESHOLD FOR ACTIVATION

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Background The T cell Antigen Couper (TAC) is a chimeric receptor that redirects the endogenous T cell receptor (TCR) against a tumor target via an extracellular antigen-binding domain to induce activation and oncolysis. TAC-engineered T cells (TAC-T cells) showed a similar capacity to activate T cells against the tumor-associated antigen HER2 as their classical chimeric antigen receptor (CAR)-engineered counterparts in vitro. However, in a xenograft model, anti-HER2 CAR-T cells gave rise to lethal off-target toxicity while TAC-T cells were efficacious and well-tolerated, despite utilizing the same antigen-binding domain.[1 2] Here, we describe differences in T cell activation by TAC (canonical via endogenous TCR) and CAR (non-canonical independent of TCR) that make CAR-T cells less discriminate towards an off-target stimulus than TAC-T cells.

Methods Paired sets of TAC- and CAR-engineered human T cells, utilizing a variety of antigen-binding domains, were compared in vitro to determine their propensity for tonic signaling and requirements for triggering T cell activation.

Results Transcriptional profiling of CAR- and TAC- T cells in the absence of antigenic stimulus revealed an elevated basal activation status in CAR-T cells. Unstimulated CAR-T cells displayed elevated expression levels of activation and exhaustion markers, as well as basal cytokine production, versus their TAC-T cell counterparts. The degree of basal activation varied with the binding domain incorporated into the CAR, where some binding domains triggered functional exhaustion. Regardless of the binding domain, unstimulated TAC-T cells were indistinguishable from control T cells that expressed no synthetic receptor. Further, TAC-T cells displayed no evidence of functional exhaustion. TCR knock-out studies confirmed that