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

TAC receptors signal via the endogenous TCR, whereas CAR signaling is TCR-independent. Consistent with TCR-dependent signaling, ligation of TAC receptors resulted in the formation of conventional immunological synapses, whereas ligation of CARs produced unconventional synapses. Despite these functional differences, CARs and TAC receptors demonstrated a similar capacity to activate T cells against antigen-positive tumor cell targets. However, CAR-T cells displayed reactivity to antigen-negative cells, due to interaction with a cross-reactive antigen; TAC-T cells displayed no reactivity to antigen-negative cells.

Conclusions Tonic signaling in CAR-T cells reduces their activation threshold and increases their propensity to be activated by cross-reactive antigen. In contrast, TAC receptors do not deliver tonic signals, which increases the stringency of activation and reduces the likelihood of off-target responses. This feature of the TAC platform is advantageous to safeguard against the unexpected cross-reactivity that may occur when a new antigen-binding domain is deployed in vivo.

Ethics Approval Use of human materials was approved by the Hamilton Integrated Research Ethics Board (HiREB).

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95 INHIBITION OF AKT SIGNALING DURING EXPANSION OF TCR-ENGINEERED T-CELLS FROM PATIENT LEUKOCYTE MATERIAL GENERATES SPEAR T-CELLS WITH ENHANCED FUNCTIONAL POTENTIAL IN VITRO

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Background T-cells attributes for adoptive cell therapy of patients with advanced cancer can be optimized during ex vivo expansion culture. Autologous TCR-engineered T-cells targeting the MAGE-A4 antigen with Specific Peptide Enhanced Affinity Receptors (SPEAR T-cells) have shown promise in the clinic.[1] The highly variable leukocyte material obtained from individual patients during apheresis can present a manufacturing challenge for autologous T-cell therapies. The degree of ex vivo expansion and the functional attributes of the expanded T-cell product impact therapeutic efficacy and can be suboptimal for some patient apheresis material. Both TCR and cytokine growth factor signals used for ex vivo T-cell expansion promote robust activation of AKT (Protein Kinase B) signaling, which drives T-cell activation, proliferation, and terminal differentiation. It is hypothesized that inhibition of AKT signaling during T-cell expansion may uncouple proliferation and terminal differentiation, leading to the generation of less differentiated T-cells that may have functional benefit in vivo.[2 3]

Methods We evaluated use of an AKT inhibitor during SPEAR T-cell manufacturing using leukocytes from healthy donors and patients with advanced solid cancers.

Results AKT inhibition resulted in the generation of a more consistent expansion and phenotype of the final T-cell product. This was observed using two SPEAR T-cell constructs, ADP-A2M4 and ADP-A2M4CD8. Ex vivo SPEAR T-cell expansion in the presence of an AKT inhibitor generated CD8+ T-cells that maintained a less differentiated phenotype (based on CCR7+CD45RA+ and CD62L+ expression). AKT inhibition was associated with enhanced antigen-specific responses of SPEAR T-cells in vitro, including effector cytokine production, target-cell killing, ability to proliferate in response to prolonged antigen-stimulation and maintenance of cytotoxic activity following antigen re-stimulation.

Conclusions We plan to introduce AKT inhibition into the GMP manufacturing process, and evaluate the efficacy of the resulting products in ongoing clinical studies.

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Ethics Approval The experimental study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and was approved by local authorities. An independent ethics committee or institutional review board approved the clinical protocol at each participating center. All the patients provided written informed consent before study entry.

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96 DECITABINE GENE MODULATION SENSITIZES HUMAN NON–SMALL CELL LUNG CANCER (NSCLC) TO NY-ESO-1 TCR IMMUNOTHERAPY (LETETRESGENE AUTOLEUCEL; GSK3377794) IN VIVO

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Background NY-ESO-1–specific T cells (letetresgene autoleucel [lete-cel] GSK3377794) are autologous CD4+ and CD8+ T cells transduced to express a high-affinity T-cell receptor (TCR) capable of recognizing NY-ESO-1 and LAGE-1a antigens in complex with human leukocyte antigen (HLA)-A*02. NY-ESO-1 (CTAG1B) and LAGE-1a (CTAG2) are tumor-associated antigens (TAA) that share the SLLMWITQC peptide bound to human leukocyte antigen HLA-A*02 and are expressed in various cancers. Emerging evidence suggests that TCR-engineered T cells targeting NY-ESO-1 hold promise for patients with solid tumors.[1] Approximately 75% of synovial sarcomas can over-express NY-ESO-1 vs 12% of NSCLC,[2] however, NSCLC expression of NY-ESO-1/LAGE1-a may have