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 nucleo-

fication of a p50 sgRNA:Cas9 complex into wild-type B6 hem-
atopoietic progenitors, also slowed neuroblastoma tumor
growth, following a dose of myelo-depleting 5-fluorouracil.

Conclusions These findings using a neuroblastoma model fur-
ther validate the utility of targeting myeloid NF-
kB Sirt2 expression was analyzed by flow cytometry and
cells with superior antitumor activity.

cer (NSCLC) and Sirt2 deficiency leads to hyper-reactive T
response to TIL therapy in advanced non-small cell lung can-
upregulation of Sirt2 in human TILs negatively correlates with
dependent histone deacetylase. We previously showed that
increase the efficacy of immunotherapies. Sirt2 is an NAD+
fatty acid catabolism of TILs represents an attractive strategy
competition for glucose with tumor cells. Therefore, enhancing
lism to preserve their effector functions due to nutrient
metabolism were investigated using seahorse bioanalyzer and

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 impor-
tantly, pharmacologic inhibition of Sirt2 in human TILs iso-
lated 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 sus-
tain their effector function within the hypoglycemic TME, thus,
leading to an effective anti-tumor immune response.

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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 evi-
dence that dysregulated metabolism contributes to the exhaus-
tion 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 elu-
sive. Memory T (TM) cells and TILs rely on fatty acid catabo-
lism 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 can-
cer (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 prolifera-
tion assay, DAPI/AnnexinV staining, IFN-γ ELISpot assay,
intracellular staining of effector molecules and LDH cytotoxic-
ity 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 impor-
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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 sus-
tain their effector function within the hypoglycemic TME, thus,
leading to an effective anti-tumor immune response.

Abstracts

94 THE T CELL ANTIGEN COUPLER (TAC) DIRECTS T CELL
ONCOSIS 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 Coupler (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 oncosis. 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 anti-
gen-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 com-
pared 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
between TAC-T cell counterparts. The degree of basal activation varied
less of the binding domain, unstimulated TAC-T cells were
some binding domains triggered functional exhaustion. Regard-
less of whatever the binding domain, TAC-T cells displayed no evidence of


Abstracts

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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).

REFERENCES

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.

Acknowledgements We are extremely grateful to the patients, who were previously enrolled in our clinical trials, and healthy donors for their consent for R&D studies. This was a collaborative cross-functional project, and we are grateful for the contributions of the following Scientists: Garth Hamilton, Adel Toth, Abigail Kay, Sophie Badie, Josh Griffiths, Kaushik Sarkar, Aanoop Chandran.

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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