SOT302: Dual expression of exogenous glutamine oxaloacetate transaminase (GOT2) and TP53-induced glycolysis and apoptosis regulator (TIGAR) enhance CAR T cell activity in preclinical solid tumor models

Emily Kuiper, Samyabrata Bhaduri, Daniel Garafola, Shalimar Hachem, Kshitij Sharma, Jennifer Coccia, Kathleen Whiteman, and Amy Jensen-Smith

Sotio Biotech Inc., Boston, MA, USA

Abstract 4012

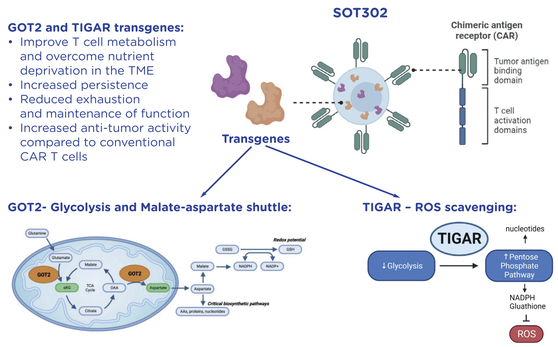
Abstract

Background: Despite success in hematological malignancies, CAR T therapy has had limited efficacy against solid tumors. The suppressive tumor microenvironment limits CAR T activity through various mechanisms including competition for available nutrients and chronic stimulation resulting in reduced effector functions or T cell exhaustion. CAR T cells with enhanced metabolic fitness or more durable memory phenotype could potentially improve the clinical outcome in solid tumors. GOT2 plays an important role in mitochondrial function and maintenance of redox homeostasis. We have previously demonstrated enhancement of CAR T cells overexpressing GOT2. TIGAR is an enzyme known to promote antioxidative activities and reduce reactive oxygen species and has a role in protecting against apoptosis. Here we tested SOT302 CAR T cells expressing both GOT2 and TIGAR in preclinical solid tumor models.

Results: Expression of GOT2 and TIGAR transgenes were confirmed by qRT-PCR and western blot. Activity of GOT2 and TIGAR were confirmed by measuring aminotransferase activity (AST) and glutathione production, respectively. Compared to control CAR T cells, SOT302 is enriched for CD8+ Tscm cells and has a higher percentage functional, non-senescent (CD28+CD57-) cells. SOT302 has similar cytokine expression and cytotoxicity in standard co-culture assays compared to controls. SOT302 cells were chronically stimulated with plate-bound antigen every 3-4 days for 4 rounds. SOT302 had greater expansion of cells and were less exhausted and had better cytolytic capacity compared to controls. SOT302 cells were then evaluated in tumor xenograft mouse models. Consistent with our previous findings, CAR T cells expressing the single GOT2 transgene were more efficacious compared to CAR T cells alone. Expression of the single TIGAR transgene had no apparent benefit over CAR alone, however SOT302 cells expressing both GOT2 and TIGAR had superior anti-tumor activity compared to CAR alone and CAR+GOT2. No overt toxicity or significant body weight loss was observed. Ex vivo analyses to measure peripheral expansion; tumor infiltration and exhaustion were also performed. Preliminary findings indicate that SOT302 has better peripheral expansion compared to CAR alone and similar expansion compared to CAR+GOT2 controls. SOT302 cells had better tumor infiltration compared to both the CAR alone and CAR+GOT2 cells. Tumor infiltrating SOT302 cells also exhibited lower levels of exhaustion compared to CAR alone or CAR+GOT2 controls.

Summary: SOT302 cells express exogenous GOT2 and TIGAR transgenes and have superior anti-tumor activity in preclinical solid tumor models. These data suggest that SOT302 may be a promising candidate for patients with solid tumor cancers.

SOT302: ROR1 targeted, metabolically enhanced BOXR T cell product



GOT2 and TIGAR transgenes:
- Improve T cell metabolism and overcome nutrient deprivation in the TME
- Increased persistence
- Reduced exhaustion and maintenance of function
- Increased anti-tumor activity compared to conventional CAR T cells

Proprietary ROR1 Targeted CAR:
- Fully human scFv
- Specificity for ROR1
- No off-target or off-tumor reactivity
- No/low tonic activity
- Superior/equivalent to benchmark

GOT2 over-expression sustains increased glycolytic flux under nutrient depleted conditions

TIGAR contributes to ROS absorption through generation of scavengers including GSH and NADPH

Figure 1. Proprietary, fully human, ROR1-targeted CAR (1730)

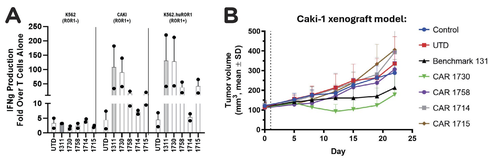
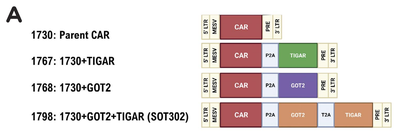


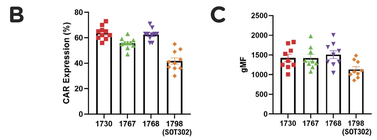
Figure 1. Four proprietary ROR1 targeted CARs (1730, 1758, 1714, 1715) were tested in comparison to the benchmark ROR1 CAR (1311, R12 antibody). **(A)** INFg production was measured in response to antigen positive and antigen negative tumor cell lines. **(B)** In vivo efficacy was measured in the Caki-1 sub-cutaneous xenograft tumor model. A single dose of 5e6 CAR+ T cells were administered on day 1 when tumors were ~100mm^3 and tumor volumes were measured for ~25 days.

Figure 2. T cell expansion, CAR and transgene expression

Constructs tested:



CAR Expression:



Transgene Expression:

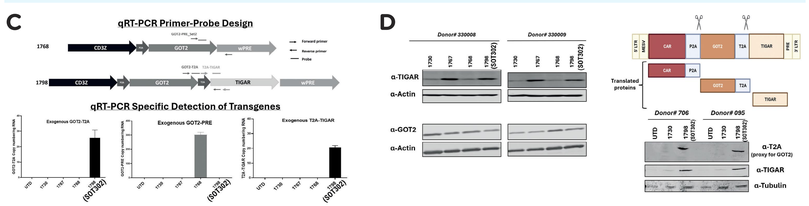


Figure 2. (A) PBMC were either untransduced (UTD) or transduced with parent CAR (1730), CAR+TIGAR (1767), CAR+GOT2 (1768), or SOT302 (CAR+GOT2+TIGAR, 1798). T cells were expanded for 10 days in gas permeable bags. **(B)** ROR1 CAR surface expression and **(C)** MFI were measured on day 10 using ROR1-Fc-biotin + Strepavidin-PE. TIGAR and GOT2 were measured by qRT-PCR **(C)** and western blot **(D)**. *N = 4 unique PBMC donors, 3 independent expansions, data presented as mean +/- SEM*

- CAR expression for CAR+TIGAR and CAR+GOT2 is similar to parent CAR (60%).
- 20% decrease in CAR expression and decreased MFI observed for SOT302 compared to parent CAR.
- Exogenous over-expression of TIGAR and GOT2 confirmed for SOT302 and relevant controls.

Figure 3. SOT302 T cell phenotype

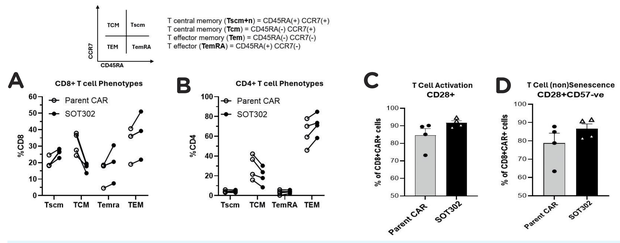


Figure 3. Memory phenotypes of SOT302 T cells were assessed by flow cytometry and compared to parent CAR T cells. **(A)** CD8+ T cells and **(B)** CD4+ T cells. **(C and D)** CD8+CAR+ T cells were stained for CD28 and CD57 surface markers to characterize functionally activated (CD28+) and non-senescent (CD28+CD57-) T cells. *N = 4 unique PBMC donors*

- SOT302 cells have increased CD8+ Tscm and non-senescent population compared to parent CAR control

Figure 4. SOT302 Transgene Function

AST activity (GOT enzymatic function)

Glutathione production (TIGAR activity)

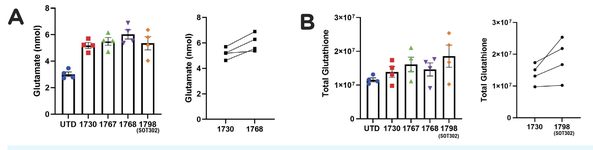


Figure 4. (A) GOT2 transgene activity was measured by aspartate aminotransferase activity (GOT2 catalytic function) and **(B)** TIGAR activity was assessed by glutathione production. Cells were normalized for CAR expression and then stimulated with plate-bound ROR1 antigen for 72hours prior to metabolic assays. *N = 4 unique PBMC donors, mean+/-SEM*

- Trending increase in AST (GOT) activity with GOT2 over-expression compared to CAR alone
- Trending increase in glutathione (GSH) production in SOT302 cells (1798) suggesting an increase in redox potential

Figure 5. SOT302 in vitro functionality following chronic stimulation

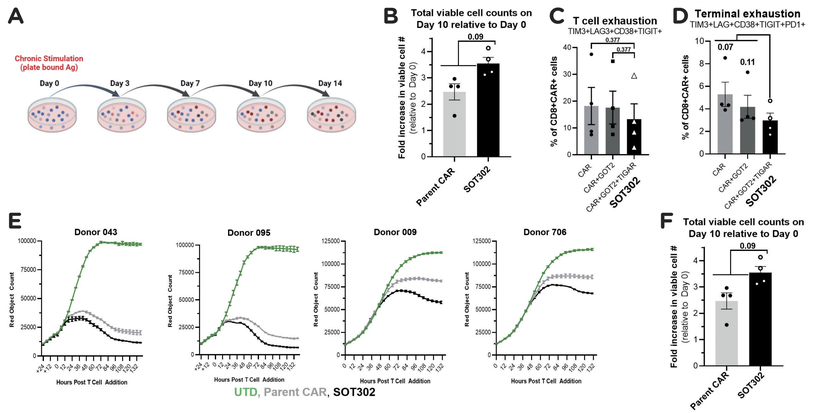
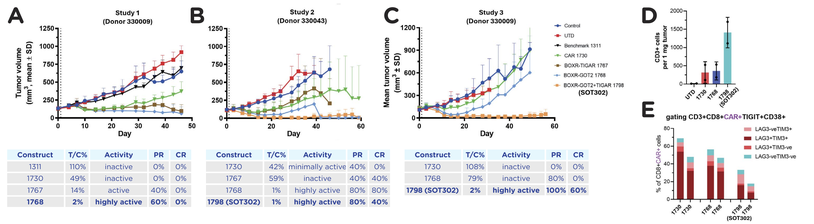


Figure 5. (A) SOT302 or parent CAR control cells were normalized for CAR expression and stimulated with plate-bound ROR1 antigen for several rounds of stimulation lasting 3-4 days each round. **(B)** Total viable cell counts were measured on day 10 and are shown as fold increase relative to day 0. **(C)** Exhausted and **(D)** terminally exhausted T cells were assessed by flow cytometry. **(E and F)** Following 10 days of chronic stimulation, SOT302 cells were assessed for cytotoxicity in coculture with A549 target cells. *N= 4 donors, mean+/-SEM*

- SOT302 yielded more total viable cells following chronic antigen stimulation than parent CAR
- Trend for fewer exhausted and terminally exhausted cells following chronic stimulation observed for SOT302
- SOT302 retained better cytotoxic activity following chronic stimulation compared to parent CAR

Figure 6. SOT302 has superior anti-tumor efficacy, infiltration and less exhaustion



Construct	T/C%	Activity	PR	CR
1311	110%	inactive	0%	0%
1730	49%	inactive	0%	0%
1767	14%	active	40%	0%
1768	2%	highly active	60%	0%

Construct	T/C%	Activity	PR	CR
1730	42%	minimally active	40%	0%
1767	59%	inactive	40%	40%
1768	1%	highly active	80%	80%
1798 (SOT302)	1%	highly active	80%	40%

Construct	T/C%	Activity	PR	CR
1730	108%	inactive	0%	0%
1768	79%	inactive	80%	0%
1798 (SOT302)	2%	highly active	100%	60%

Figure 6. (A-C) Caki-1 cells were implanted subcutaneously in NSG mice and grown to ~100 mm3 prior to treatment. CAR T cells were administered in a single i.v. at a dose of 5e6 CAR+ cells. Tumor growth was measured over the course of ~60 days. Tumor growth inhibition (T/C) percentage was calculated from median tumor volumes of treated (T) relative to untreated control (C) at the group endpoint. PR= partial tumor regression = greater than 50% reduction in tumor; CR = complete tumor regression = tumor volume of 0 mm3. *N= 5 mice per group, 3 independent studies, 2 unique PBMC donors*. **(D and E)** In study 3, two additional mice per treatment group were assessed for tumor infiltration and TIL exhaustion. Tumors were harvested on day 7 post treatment for ex vivo analysis. Single cell suspensions from harvested tumors were assessed by flow cytometry. **(D)** TIL were quantified by huCD3+ cells/mg tumor and **(E)** exhausted TIL were defined as CD3+CD8+CAR+TIGIT+CD38+LAG3+TIM3+

- SOT302 cells are highly active and can induce complete regressions in Caki-1 tumor bearing mice
- SOT302 cells out perform CAR alone, CAR+GOT2 and CAR+TIGAR cells suggesting the combination of two transgenes is superior than either single transgene
- Preliminary data suggest that SOT302 cells infiltrate Caki-1 tumors better than controls and these cells have a less exhausted phenotype

Summary

SOT302 cells express exogenous GOT2 and TIGAR transgenes and have superior anti-tumor activity in preclinical solid tumor models. These data suggest that SOT302 may be a promising candidate for patients with solid tumor cancers.

For more information please contact: jensen-smith@sotio.com