Poster nr. 4147

Combination with IL-15Rβγ superagonist, Nanrilkefusp alfa, enhances CAR T and BOXR T cell anti-tumor activity

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Abstract

Background: Nanrilkefusp alfa (Nanril, SOT101) is an IL-15Rβγ superagonist that is comprised of the IL15 cytokine fused to the IL-15Rα and has demonstrated a favorable safety profile and encouraging efficacy signals as a monotherapy and in combination with KEYTRUDA® (pembrolizumab) in the Phase 1/1b AURELIO-03 trial. SOTIO's BOXR cell therapy platform is designed to improve the functionality of CAR-T cells by incorporating novel transgenes that are co-expressed with tumor-targeting receptors to overcome resistance and improve the function of respective immune cells in the solid tumor micro-environment. Here we tested the combination of nanril with CAR-T or BOXR-T cells in vitro and in in vivo efficacy studies.

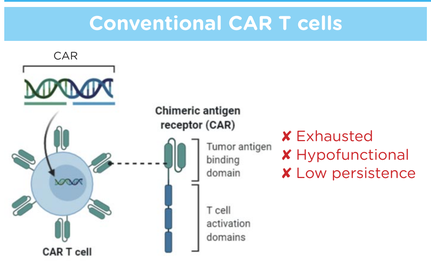
Methods: BOXR-T cells, CAR-T cells or untransduced (UTD) control T cells were treated with 0.1-1 nM Nanril for 3- 7 days and proliferation and memory phenotype were assessed by flow cytometry; RNAseq analysis was also performed. To assess in vitro cytotoxicity, T cells were pre-treated for three days with 0.1 nM nanril and were then co-cultured with target cells and cell killing was monitored using Incucyte analysis. CAKI-1 and NCI-H1975 tumor models were used to assess CAR-T and BOXR-T cell anti-tumor activity in combination with nanril where the nanril dosing regimen was administered 7 days following CAR-T or BOXR-T cells treatment.

Results: Nanril treatment induced proliferation in UTD, CAR-T and BOXR-T cells in a dose-dependent manner. Shifts in T cell memory populations were also observed with increasing nanril concentration, resulting in a higher proportion of effector memory cells and subsequently improved in vitro cytotoxicity. RNAseq analysis findings were consistent with increased proliferation and differentiation with nanril treatment (data not shown). When tested in vivo, BOXR-T cells had superior anti-tumor activity compared to CAR-T cells and combination treatment with nanril further improved both BOXR-T and CAR-T cell efficacy and increased peripheral blood expansion.

Conclusions: These data demonstrate that combination of nanril with BOXR-T and CAR-T cells results in improved T cell function and anti-tumor activity in preclinical models. Combination of nanril with T cell-based therapies may be a promising approach to increase efficacy in difficult-to-treat solid tumors.

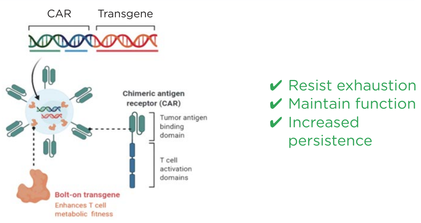
Introduction

Conventional CAR T cells



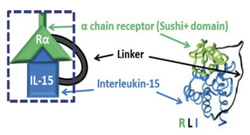
✘ Exhausted
✘ Hypofunctional
✘ Low persistence

BOXR T cells



✔ Resist exhaustion
✔ Maintain function
✔ Increased persistence

Nanril



- Recombinant fusion of IL-15 and the IL-15Ra sushi⁺ domain
- Mimics the high affinity interaction with the IL-15Rβγ subunits bypassing the need for APCs
- Activates IL-15Rβγ expressing cells (NK cells, CD8⁺ T cells, NKT cells, gd T cells) and does not activate Tregs
- Favorable safety profile with positive efficacy signals with monotherapy and in combination Pembrolizumab (ASCO 2022)

IL-15 supplementation can enhance CAR T cell anti-tumor activity and potentially improve clinical outcomes:
- IL-15 increases proliferation and survival of T cells
- CAR T failure can be attributed to limited T cell proliferation and persistence of CAR T cells
- Higher peak serum IL-15 concentrations following CAR T infusion is associated with improved clinical outcomes

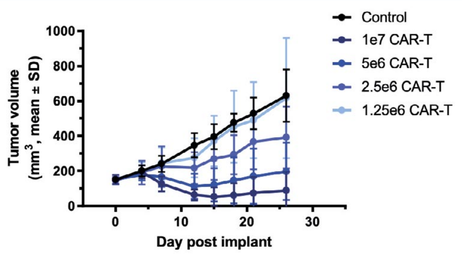
Figure 1: BOXR T cells have enhanced anti-tumor activity vs. CAR-T

A

- ROR1-BBz CAR derived from R12 VH/VL binder (Yang et al. PLoS One, 2011; Hudecek et al. Clin Cancer Res, 2013)
- GOT2: mitochondrial enzyme that enhances CAR-T activity (Hickman et al., PLoS One, 2022)

Conventional ROR1 CAR T cells:

B



BOXR T Cells:

C

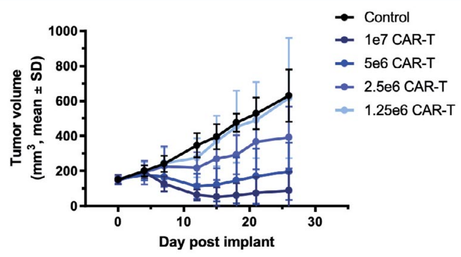
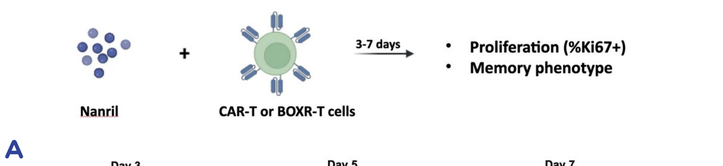


Figure 1: (A) schematic diagram of CAR and BOXR constructs. NSG mice were implanted with ROR1⁺ Caki-1 tumor cells. Once tumor volumes reached ~100 mm³, mice were randomized and treated with CAR-T or BOXR T cells. **(B)** 1.25e6 to 1e7 CAR+ T cells were administered iv, and tumor volumes were monitored. A sub-optimal dose of 3e6 ROR1⁺ CAR T cells was chosen to compare to BOXR T cell activity shown in **(C)**.

- **BOXR T cells have improved tumor clearance compared to conventional ROR1 CAR T cells.**

Figure 2: Nanril treatment increases proliferation and differentiation



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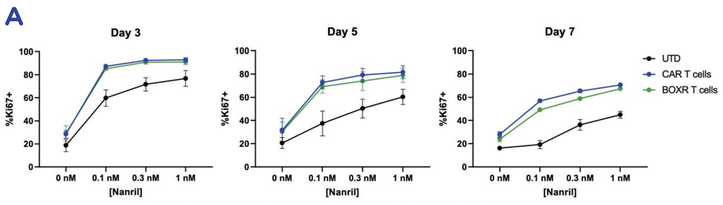

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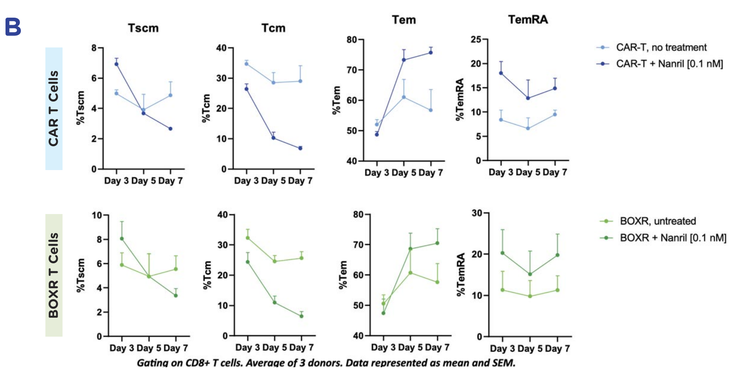
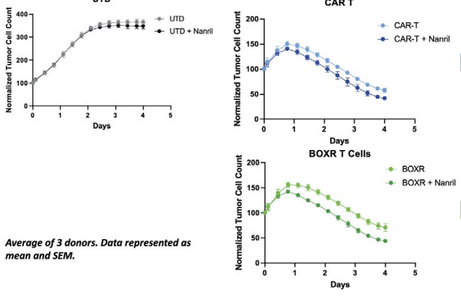


Figure 2: (A) CAR-T or BOXR T cells were treated with 0-1nM Nanril for 3, 5 or 7 days. Proliferation was measured by flow cytometry. Data are presented as %Ki67⁺ CD8⁺ T cells. Peak proliferation was observed at 0.1nM Nanril treatment on day 3. **(B)** CAR-T or BOXR T cells were treated with 0.1 nM for 3, 5, or 7 days and T cell differentiation was assessed by flow cytometry. *CD3⁺CD8⁺ T cells are defined as: Tscm: CCR7⁺CD45RA⁺, Tcm: CCR7⁺CD45RA⁻, Tem: CCR7⁻CD45RA⁻, TemRA: CCR7⁻CD45RA⁺*

- **All T cell conditions proliferate in a dose-dependent manner with nanril treatment; peak proliferation at day 3, 0.1 nM nanril**
- **CD4 T cells also proliferate, but to a lesser extent than CD8 T cells (data not shown)**
- **Comparable proliferation between CAR-T and BOXR T cells**
- **Nanril treatment results in more differentiated T cells**
  - **Decreased Tscm and Tcm**
  - **Increased Tem and TemRA**
- **Similar effect in both CAR-T and BOXR-T cells**
- **Differentiation is nanril dose-dependent (data not shown)**

Figure 3: Nanril treatment may enhance in vitro cytotoxicity

A



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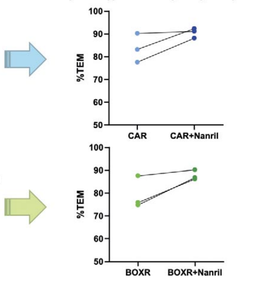
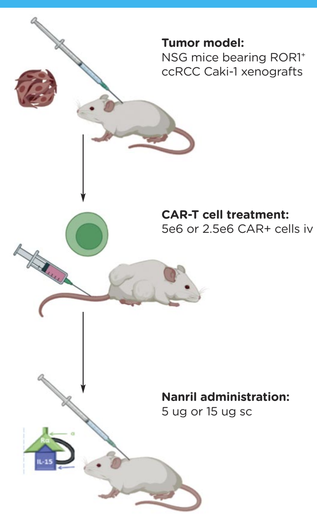
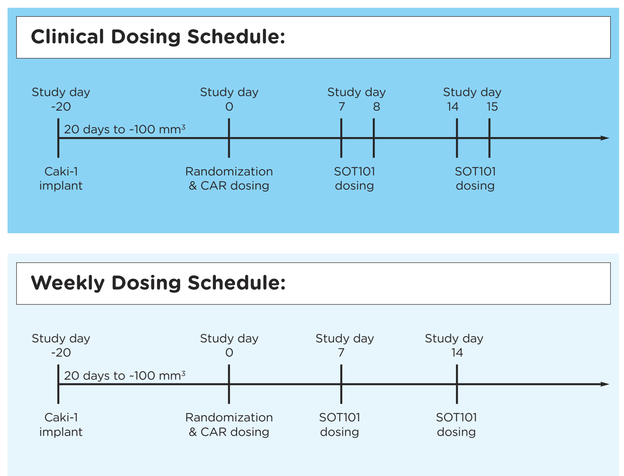


Figure 3: CAR-T or BOXR T cells were treated with 0.1 nM nanril for three days prior to co-culture with Nuclight Red labeled ROR1⁺ tumor cell lines. **(A)** Cytotoxicity was measured using incucyte assay and data are reported as normalized tumor cell count over the course of 4 days. **(B)** CAR-T and BOXR T cell differentiation was assessed following coculture with tumor target cells by flow cytometry.

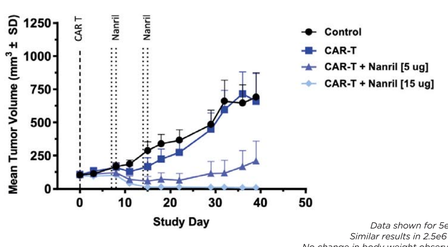
- **A slight enhancement of in vitro tumor cell killing observed with nanril pre-treatment, consistent with a more differentiated phenotype, as observed in figure 1.**
- **Further enrichment in Tem cells was also observed following antigen exposure.**
- **Similar results for both CAR-T and BOXR T cells**

Figure 4: Nanril schedule and dose range finding study

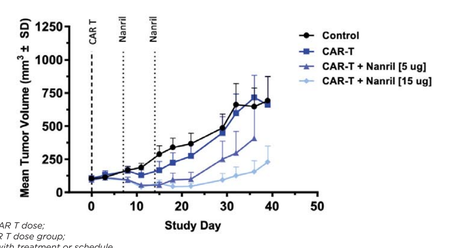




A **Clinical Dosing Schedule:**



B **Weekly Dosing Schedule:**



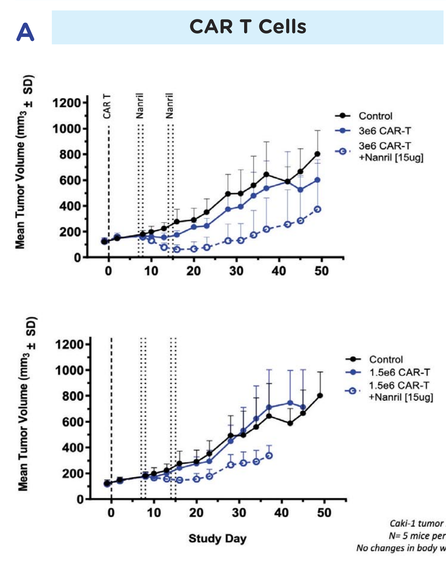
Data shown for 5e6 CAR T dose;
Similar results in 2.5e6 CAR T dose group;
No change in body weight observed with treatment or schedule

Figure 4: Caki-1 tumor bearing NSG mice were treated with either 5e6 or 2.5e6 CAR+ ROR1 CAR-T cells i.v. on day 0. Mice were then treated with 5 ug or 15 ug nanril s.c. following the clinical dosing schedule **(A)** or weekly dosing schedule **(B)**. Tumor volume and body weight were monitored for 40 days.

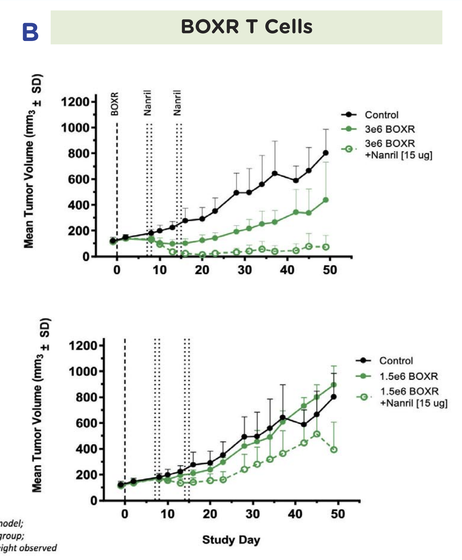
- **Dose-dependent efficacy improvement with nanril treatment using both dosing schedules**
- **Clinical dosing schedule results in better efficacy compared to weekly schedule at both doses of nanril**
- **15 ug dose and clinical dosing schedule selected for further studies**

Figure 5: Nanril treatment enhances both CAR-T and BOXR T cell anti-tumor efficacy

A **CAR T Cells**



B **BOXR T Cells**



Caki-1 tumor model;
N= 5 mice per group;
No changes in body weight observed

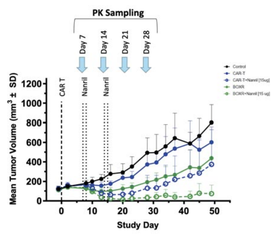
Figure 5: Caki-1 tumor bearing NSG mice were treated with either 3e6 or 1.5e6 CAR+ T cells i.v. on day 0. Mice were then treated with 15 ug nanril s.c. following the clinical dosing schedule as described in figure 4. CAR-T cell **(A)** and BOXR T cell **(B)** treated mice tumor volumes and body weights were measured for 50 days.

- **BOXR-T cells have superior anti-tumor activity compared to CAR-T cells**
- **Nanril treatment enhances both CAR-T and BOXR-T cell anti-tumor efficacy at both T cell dose levels tested**
- **Nanril + BOXR T cells resulted in the best tumor clearance**

Figure 6: Nanril treatment improves peripheral expansion of CAR-T and BOXR T cells

PK Sampling Plan:

A



- Whole blood samples were collected via retro-orbital bleeds on study days 7, 14, 21, and 28
- Blood samples were frozen at the time of sampling
- CD3⁺ cell counts analyzed via flow cytometry in all samples at end of study

PK sample:	Days post nanril treatment (# of doses received):
Day 7	0 (none)
Day 14	7 (2 doses)
Day 21	14 (4 doses)
Day 28	21 (4 doses)

B

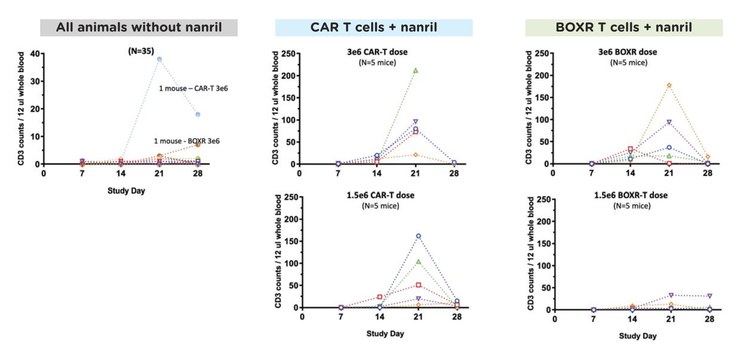
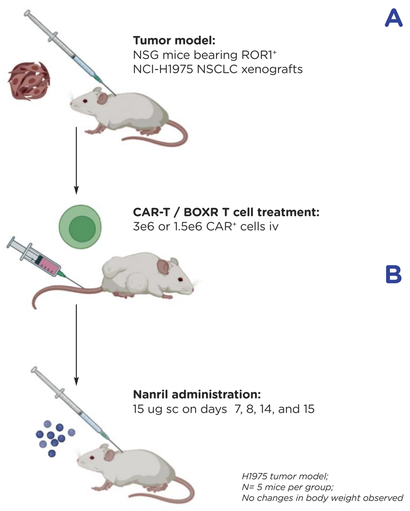


Figure 6: In the same study shown in figure 5, whole blood samples were collected on days 7, 14, 21 and 28 post CAR-T or BOXR T cell treatment as shown in **(A)**. Peripheral blood CD3⁺ cell counts were analyzed by flow cytometry and reported as CD3 counts / 12ul whole blood **(B)**.

- **Superior peripheral expansion with nanril treatment compared to CAR-T or BOXR T cell treatment without nanril.**
- **CAR-T cells expanded more than BOXR T cells at the lowest dose (1.5e6 CAR+ T cells) and at similar levels of expansion were observed between CAR-T and BOXR T cells in the higher dose (3e6 CAR+ T cells).**
- **Minimal expansion was observed in the absence of nanril treatment**

Figure 7: Nanril treatment enhances CAR-T and BOXR T cell activity in H1975 model



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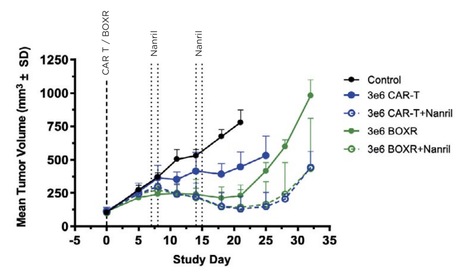

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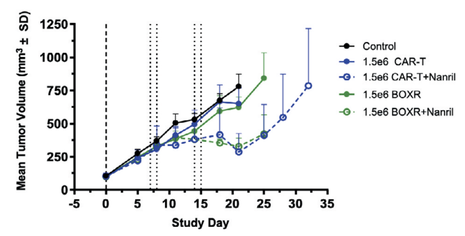

Figure 7: NSG mice were implanted with ROR1+ NCI-H1975 tumor cells. Mice were then treated with either CAR-T or BOXR T cells i.v. on day 0, when tumors had reached ~100 mm³. 15 ug nanril was administered s.c. on days 7, 8, 14 and 15 following the clinical dosing schedule established in figure 4. **(A)** 3e6 CAR⁺ T cell dose. **(B)** 1.5e6 CAR⁺ T cell dose.

- **In a second, hard-to-treat model BOXR-T cells have superior anti-tumor activity compared to conventional CAR-T cells (in the absence of nanril).**
- **Nanril treatment enhanced both CAR-T and BOXR-T cell efficacy at both T cell dose levels tested.**

Conclusions

- Nanril treatment induces T cell proliferation *in vitro*, resulting in further differentiated T cells and slightly enhanced cytotoxicity in both CAR-T and BOXR-T cells.
- BOXR-T cells expressing the additional GOT2 transgene have superior anti-tumor activity compared to CAR-T cells.
- Combination with nanril improves both CAR-T and BOXR T cell anti-tumor efficacy as demonstrated in two different xenograft models.
- Animals receiving nanril treatment showed increased peripheral T cell expansion compared to those without treatment.
- These initial data are promising and suggest that nanril combination with T cell therapies may be an effective approach in the clinic.

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