THOR-707: Using Synthetic Biology to Reprogram the Therapeutic Activity of Interleukin-2 (IL-2)

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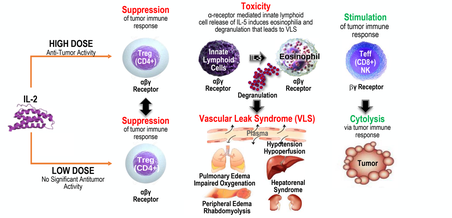
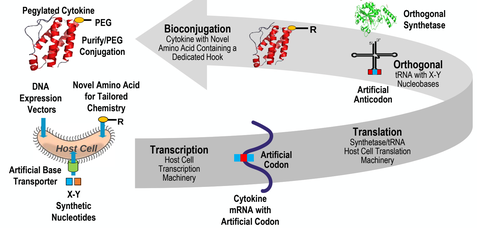
BACKGROUND

Recombinant interleukin-2 (rIL-2 or aldesleukin) is an approved immunotherapy in melanoma and renal cell carcinoma based on complete durable remissions. The anti-neoplastic properties of IL-2 are mediated by interactions with the IL-2 receptor beta-gamma chain complex (IL-2Rβγ), which leads to expansion and differentiation into T effector and T memory cells directed against the tumor. But the widespread use of rIL-2 is limited by interaction with the high affinity IL-2 receptor alpha chain (IL-2Rα) on regulatory CD4+ T cells (Tregs), which leads to immunosuppression, and on type 2 innate lymphoid cells residing in the vascular endothelium, which leads to eosinophilic recruitment and activation, resulting in the often-severe complication of vascular leak syndrome (VLS). A rIL-2 biased toward IL-2βγ affinity with no IL-2Rα interaction could address these needs.

IL-2 Has A Low Therapeutic Index Due to Its Dual Pharmacology at the High Affinity αβγ and Intermediate Affinity βγ Receptor Forms



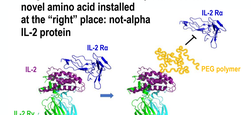
Novel Amino Acids Encoded By Our New DNA Base Pair Enable Precise Bioconjugation of Biologics



THOR-707: IL-2 IO Synthorin

PEG-IL-2 Synthorin Properties

Single, stable PEG covalently attached to the novel amino acid installed at the "right" place: not-alpha IL-2 protein



IL-2 binds to the αβγ receptor form with high affinity because of α chain

Targeted pegylation of THOR-707 at the novel amino acid blocks α chain engagement

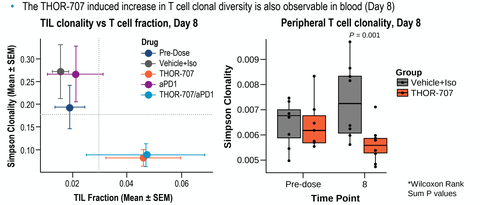
THOR-707's Key Differentiations

- **Improved Selectivity** - Reduced CD4+ Treg bias with retained activity at CD8+ T and NK cells
- **Increased Therapeutic Index** - At least 10 in preclinical non-human primate (NHP) studies
- **Ease of Use** - Expected Q2W dosing or less frequent
- **Reduced Risk of Immunogenicity** - Covalent attachment of stable PEG shields new amino acid; pegylation site devoid of MHC-II anchors

RESULTS

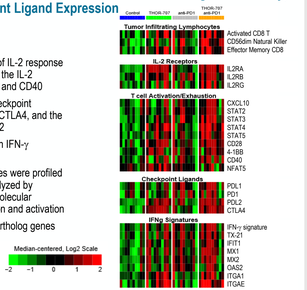
A Single Dose of THOR-707 Increases Intra-tumoral T Cell Fraction and TCR Diversity in Mouse CT-26 tumors

- TCR sequencing was performed on infiltrating T cells via immunoSEQ™ (Adaptive Biotechnologies)
- By Day 8, tumors from mice dosed with 6 mg/kg of THOR-707, alone or in combination with 10 mg/kg BIW of mouse anti-PD-1, showed significantly lower TCR repertoire clonality (higher clonal diversity)
- Post-hoc Dunn's test at Day 8 demonstrates that the THOR-707 and THOR-707 + anti-PD-1 treatment groups show lower clonality compared to either vehicle or anti-PD-1 alone (PKW= 0.0047)
- TCR sequencing also demonstrates that THOR-707 elevates TIL fraction alone or in combo with CPI
- The THOR-707 induced increase in T cell clonal diversity is also observable in blood (Day 8)

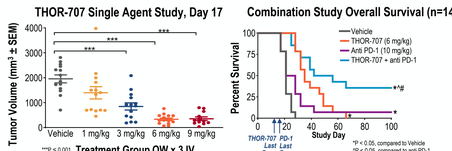


THOR-707 Reprograms the CT-26 Tumor Microenvironment for High Teff Activity, IFN-γ Induction and Checkpoint Ligand Expression

- Key Findings:
  - Induction of multiple markers of IL-2 response and T cell activation, including the IL-2 receptor chains, CD28, 4-1BB and CD40
  - Elevated expression of the checkpoint inhibitory receptors PD-1 and CTLA4, and the PD-1 ligands PD-L1 and PD-L2
  - Induction of genes reporting on IFN-γ signaling pathways
- Methodology: CT-26 tumor samples were profiled via mRNAseq (Omniseq) and analyzed by GeneCentric to identify cell and molecular signatures of lymphocyte infiltration and activation
- Nomenclature shown for human ortholog genes



THOR-707 Is Efficacious as a Single Agent and When Combined With a PD-1 Inhibitor in the CT-26 Mouse Tumor Model

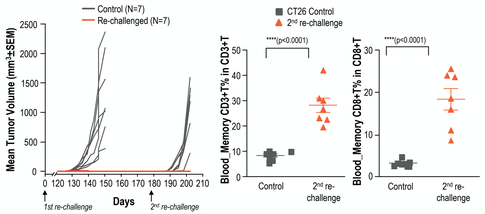


- CT-26 colon tumor-bearing Balb/c mice were treated with THOR-707 (1, 3 or 6 mg/kg QWx3) alone, anti-PD-1 (10 mg/kg, BIW x 3) alone, or the combination of THOR-707 at 6 mg/kg with anti-PD-1
- THOR-707 showed dose-dependent single agent antitumor activity. In combination with anti-PD1, 5/14 animals showed tumor regression and no detectable tumors for 100 days

RESULTS

THOR-707 Promotes the Establishment of Persistent Memory T Cell Responses, Preventing CT-26 Tumor Growth in Surviving Animals Challenged By Re-injection of CT-26 Cells

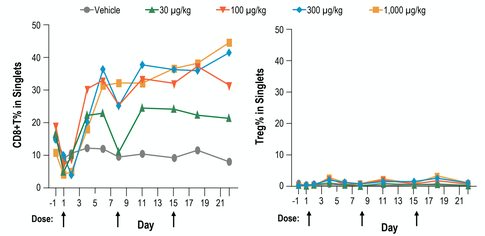
- Surviving Balb/c mice were re-challenged by injection of CT-26 cells at 120 and 180 days after the original challenge
- With no additional THOR-707 treatment over the re-challenge period, CT-26 tumors fail to grow in all surviving animals treated with THOR-707
- THOR-707 promotes the establishment of durable immunological memory against CT-26 tumors, observed as an overall increase in peripheral memory T cells (CD3+), including memory CD8+ T cells



PD Readouts pSTAT5 and Ki67 in CD8+ T and Treg Cells. Ki-67 Correlates With Proliferation of CD8+ T Cells But Not Treg Cells in NHP

- Cynomolgus monkeys were dosed with 30, 100, 300 or 1,000 μg/kg of THOR-707 (IV bolus). Blood samples were collected for measurement of pSTAT5, Ki67 and cell counts via flow cytometry
- All doses induced pSTAT5 at near-maximal levels (>85% cells positive) in CD8+ T and CD4+ Treg cells (Tregs)
- The proliferation marker Ki67 showed dose-dependent elevation at both cell types, reaching near-maximal levels at 100 μg/kg for both cell types
- Peripheral CD8+ T cells reached maximal expansion at 100 μg/kg. Treg cells failed to show significant expansion

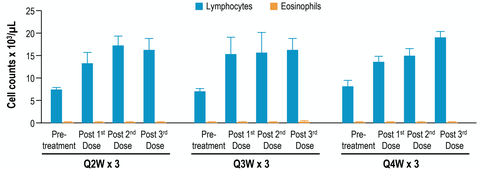
Cell type	Dose level (μg/kg)	%pSTAT5+ at peak day	%Ki67+ at peak day
CD8+ T	30	87.2	44
	100	85.4	70.5
	300	87.9	86.8
	1,000	94.7	70.0
Treg	30	98.3	27.0
	100	97.5	62.3
	300	97.7	52.6
	1,000	99.2	73.9



RESULTS

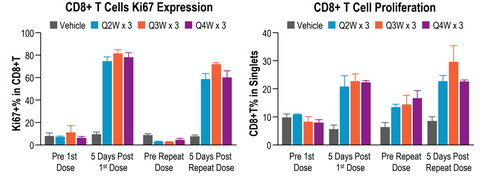
THOR-707 Dosed QW, Q2, Q3W and Q4W Elicited Similar Expansion of Lymphocytes in NHP After Each Dose, With No Expansion of Eosinophils

- Cynomolgus monkeys were given three IV 100 μg/kg doses of THOR-707 QW, Q2, 3 or 4W
- On all three dosing schedules, THOR-707 induced maximal lymphocyte expansion on day 7 following each dose, without expanding eosinophils responsible for VLS



THOR-707 Dosed QW, Q2, 3 & 4W Showed Similar Expansion of Peripheral CD8+ T Cells in NHP. Ki67 Expression Increased ≥60% After Each Dose

- Following each dose, Ki 67 expression reached ≥60% and correlated with expansion of CD8+ T Cells for all dosing schedules
- THOR-707 did not induce the proliferation of peripheral CD4+ regulatory T cells at any schedule



THOR-707 Does Not Induce Vascular Leak Syndrome in NHP

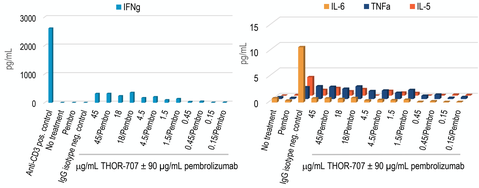
- THOR-707 was well tolerated in cynomolgus monkeys. Diarrhea was observed in some animals at the highest dose level of 1,000 μg/kg
- No indicators of VLS: no elevations in body or lung weights as measures of edema were observed

THOR-707 dose (μg/kg)	Cmax (μg/mL)	AUC_{84} h*mg/mL/ (μg/kg)	EC at CD8+ T cell	Clinical Observations
30	0.674	7.60	97.1	None
100	2.81	39.5	99.3	None
300	7.36	97.6	99.7	None
1,000	29.0	316	>99.9	Diarrhea

RESULTS

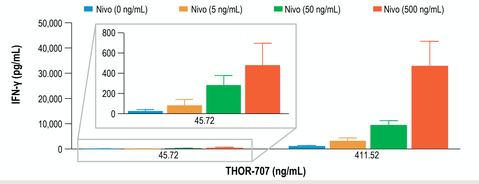
THOR-707 Single Agent or Combined with Pembrolizumab Did Not Elicit Release of Cytokines Associated with Cytokine Release Syndrome from Human Whole Blood

- Human whole blood was incubated with THOR-707 concentrations covering the range of Cmax values observed in the NHP toxicology study (37.9 – 99.5% receptor occupancy at CD8+ T cells, as measured by induction of pSTAT5), and 90 μg/kg of pembrolizumab (expected Cmax of clinical dose)
- After 24 h of incubation, low amounts of IFN-γ were released in a THOR-707 dose-dependent manner relative to positive control anti-CD3 (10 μg/mL). Yet, combination with pembrolizumab did not significantly elicited further increases in IFN-γ release at any THOR-707 level
- The range of THOR-707 concentrations (0.15 to 45 μg/mL) was selected based on monkey exposure data from the GLP NHP toxicity study, the highest dose exposure from which is expected to be higher than the maximum tolerated dose (MTD) in humans. Furthermore, the highest concentration in the assay gives a safety factor of >3X over this high-dose concentration in monkey as can be observed from the Cmax values in Table 2.



THOR-707 Combined With Nivolumab Increased the Strength of T Cell Receptor Signaling in an Ex Vivo Human Mixed Lymphocyte Reaction Model System

- The effect of THOR-707 alone and in combination with nivolumab on TCR signaling was studied using a human mixed lymphocyte reaction model. Two concentrations of THOR-707 reflected 15.7 and 62.6% receptor occupancy at CD8+ T cells, as measured by induction of pSTAT5
- After 5 days of incubation, mismatched lymphocytes released IFN-γ in a THOR-707 concentration-dependent manner. This effect was greatly enhanced by PD-1 blockage with nivolumab. This is expected, because mismatched antigen-presenting cells express PD-L1, which restricts TCR levels of signaling in response to THOR-707
- This study demonstrates the potential for THOR-707 and PD-1 inhibitors to potentiate tumor antigen-driven TCR responses in Teffs



CONCLUSIONS

- THOR-707 is an engineered IL-2 Synthorin with a targeted single site PEG bioconjugate. It has reduced CD4+ Treg bias with retained activity at CD8+ T and NK cells
- The pegylation site in THOR-707 exhibits low potential for MHC-II presentation. The highly stable, irreversible PEG bioconjugate further decreases the risk for immunogenicity
- In the mouse CT-26 syngeneic tumor model, THOR-707 significantly increased the amount of TILs (TIL fraction) as well as the TCR repertoire diversity (lower clonality score) of infiltrating T cells
  - Changes in TIL clonality are observable in the peripheral compartment
- In CT-26 tumors, THOR-707 remodels TILs to Teff, memory Teff and cytolytic NK cells, and promotes T cell and IFN-γ gene expression signature consistent with increased Teff cytolytic function
- THOR-707 showed anti-tumor activity both as single agent and in combination with a PD-1 checkpoint inhibitor
- Anti-tumor responses were durable and protected surviving animals in two consecutive CT-26 re-challenges, demonstrating the establishment of persistent Tmem populations
- In NHP, after repeat dosing at 100 μg/kg, THOR-707 showed similar lymphocyte and CD8+ T cell expansion when dosed QW, Q2, 3 and 4 W
- THOR-707 does not induce VLS in NHP at doses up to 1,000 μg/kg (>99.9% receptor occupancy at cynomolgus CD8+ T cells). In an ex vivo human whole blood system, THOR-707 did not induce the release of cytokines associated with CRS or VLS at concentrations up to 99.5% receptor occupancy at CD8+ T cells
- In that same ex vivo system, the checkpoint inhibitor pembrolizumab did not increase the release of IFN-γ in response to THOR-707, indicating a lowered risk for autoimmune adverse events for this combination
- THOR-707 and another checkpoint inhibitor, nivolumab, demonstrated synergistic effects for antigen-mediated TCR activation in a human mixed lymphocyte reaction culture system