Favorable safety profile of a novel long-acting IL-2, HM16390, with effective control of systemic toxicities via fine-tuned CD25 engagement in animal models

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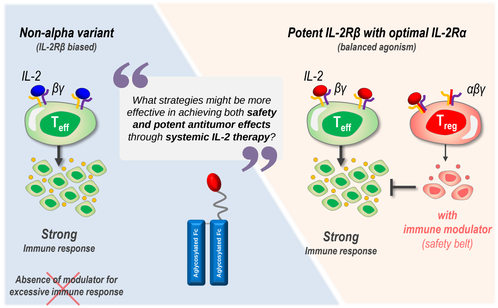
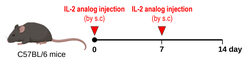
BACKGROUND

Introduction & Objective: **In the development of IL-2 based immunotherapy, it was generally accepted that interaction with the IL-2Rα (CD25) should be eliminated to avoid unwanted toxicity. However, we have been developing a novel long-acting IL-2 applying the opposite strategy of incorporating CD25 engagement. Here, we explored how the CD25 engagement functions to mitigate systemic toxicity in IL-2 based immunotherapies.**



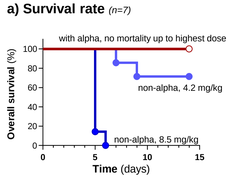
Absence of IL-2Rα binding along with intensified IL-2Rβ binding caused severe toxicity.
Intensified IL-2Rβ binding induces significant anti-tumor effect, but safety belt (IL-2Rα binding) **may be necessary for immune balance.**

Study design

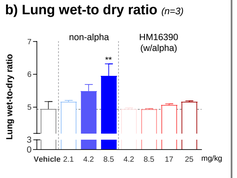


- Vehicle
- Non-alpha variant, 2.1 mg/kg/QW
- Non-alpha variant, 4.2 mg/kg/QW
- Non-alpha variant, 8.5 mg/kg/QW
- HM16390 (w/ CD25), 4.2 mg/kg/QW
- HM16390 (w/ CD25), 8.5 mg/kg/QW
- HM16390 (w/ CD25), 17 mg/kg/QW
- HM16390 (w/ CD25), 25 mg/kg/QW

a) Survival rate *(n=7)*



b) Lung wet-to dry ratio *(n=3)*



**p<0.01 *vs.* vehicle by one-way ANOVA test. Closed bars and circles indicate lethal dose.

METHOD & RESULTS

Effect of CD25 engagement on safety profiles in mouse

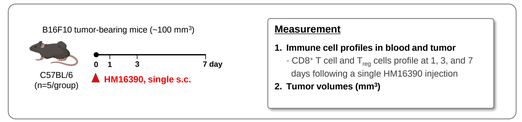
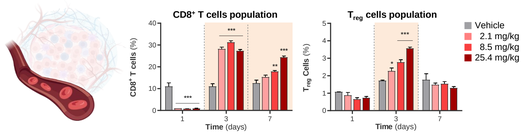
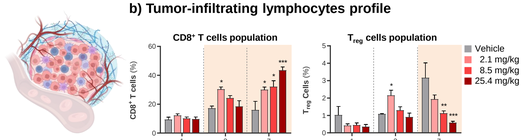


Figure 1. Immune cell profiles in blood and tumors following a single SC administration of HM16390 in B16F10 mice

a) Peripheral immune cells profile

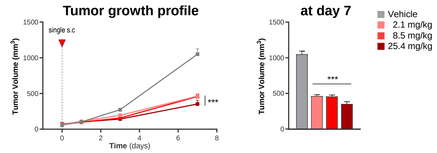


b) Tumor-infiltrating lymphocytes profile



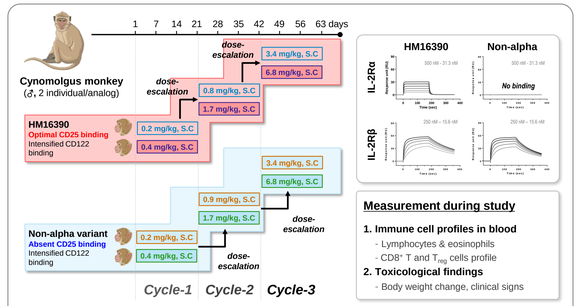
➢ The CD25 binding property of HM16390 induces a dose-dependent expansion of T_{regs}, counteracting for uncontrolled immune responses during circulation, without negative impact on anti-tumor immunity in the tumor microenvironment (TME). ***p<0.001, **p<0.01, *p<0.05 *vs.* vehicle group by one-way ANOVA test.

Figure 2. Tumor growth inhibition following a single SC administration of HM16390 in B16F10 mice



➢ The growth of B16F10 tumors was dose-dependently inhibited by a single subcutaneous administration of HM16390. ***p<0.001 *vs.* vehicle group by one-way ANOVA test.

Effect of CD25 engagement on safety profiles in NHP



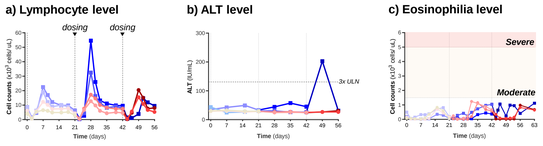
Measurement during study

1. Immune cell profiles in blood
   - Lymphocytes & eosinophils
   - CD8⁺ T and T_{reg} cells profile
2. Toxicological findings
   - Body weight change, clinical signs

➢ HM16390 and its non-alpha variant were administered s.c once every three weeks following a stepwise dose-escalation protocol.

Safety depending on presence of CD25 binding in monkey

Figure 3. Safety profile following IL-2 analogs treatment



Dose	0.2 mg/kg	0.4 mg/kg	0.8 mg/kg	1.7 mg/kg	3.4 mg/kg	6.8 mg/kg
HM16390 (optimal alpha binding)	No notable finding		Body temp. ↑ Hypo-activity (G1-2) N/V	Body temp. ↑ Safe dose (50%)	BWL, -6.0% vs BL Body temp. ↑↑ Hypo-activity (G1-2) Petechiae N/V, Diarrhea	BWL, -6.1% vs BL Body temp. ↑↑ Hypo-activity (G1) Petechiae / Erythema Diarrhea
Non-alpha (No alpha binding)	BWL, -6.1% vs BL Hypo-activity (G1) Chest temp. ↑ Higher than safe dose	BWL, -7.5% vs BL Hypo-activity (G1) Petechiae / Erythema BP ↓	BWL, -4.7% vs BL Body temp. ↑ Hypo-activity (G1) Petechiae / Erythema N/V, Diarrhea, BP ↓	BWL, -6.9% vs BL Body temp. ↑ Hypo-activity (G3) Petechiae / Erythema Diarrhea	BWL, -20.9% vs BL Body temp. ↑↑↑ (≥40°C) Hypo-activity (G4) Petechiae / Erythema N/V, Diarrhea Abdominal wheal	Mortality @D3 Body temp. ↑↑↑ (≥40°C) Hypo-activity (G4) Petechiae / Erythema Diarrhea Shortness of breath

x8.5↑

➢ While the non-alpha variant showed uncontrolled peripheral lymphocyte expansion, HM16390 induced a dose-dependent and stable expansion of lymphocytes up to cycle-3.

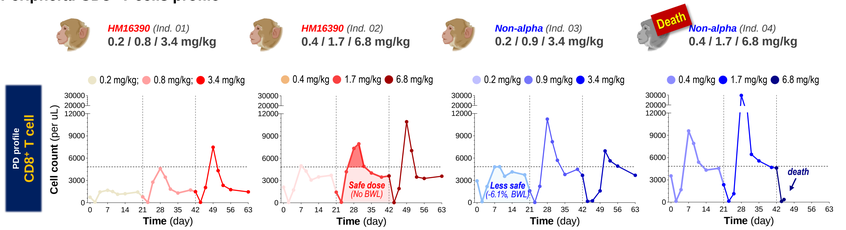
➢ ALT level was stable for HM1690, but significant increases at cycle-3 of non-alpha variant. No notable increase in eosinophil level observed up to highest doses of both compounds.

➢ HM16390 was well-tolerated across all dose ranges, while its non-alpha variant exhibited hypoactivity and body weight loss at the starting dose. Clinical signs progressively worsened, ultimately leading to mortality at the highest dose. BL, base-line; BP: blood pressure; BWL, body-weight loss; N/V, nausea and vomiting; ULN, upper limited of normal

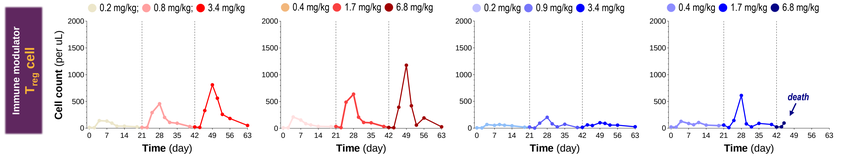
Efficacy profile of HM16390 and its non-alpha variant in Cynomolgus monkey

Figure 4. The proliferation of lymphocytes following HM16390 or non-alpha variant treatment

a) Peripheral CD8⁺ T cells profile



b) Peripheral T_{regs} profile



➢ HM16390 and its non-alpha variant significantly enhanced the proliferative capacity and dose-dependent expansion of CD8⁺ T cells up to cycle-2 and -3, respectively. HM16390, which incorporated optimal CD25 binding property, dose-dependently and gradually increased CD8⁺ T cells, possibly with the help of T_{regs}. The non-alpha variant, however, acutely increased CD8⁺ T cell, leading intolerability, and ultimately lethality was observed at the highest dose due to the absence of T_{reg} modulation.

➢ At the safe dose defined as no weight loss, HM16390 increased CD8⁺ T cell more than the less safe dose of non-alpha variant. This indicates that HM16390 induces a significant effector cell expansion with immune tolerability through its CD25 binding property.

CONCLUSIONS

- **HM16390 is designed to have potent bind affinity to IL-2Rβ, inducing anti-tumor immune responses, while its optimized binding to IL-2Rα helps regulate excessive systemic immune activation.**
- **The CD25 binding characteristics within HM16390 was finely tuned to mitigate unwanted toxicity derived from uncontrolled immune cell expansion. The crucial role of CD25 to transiently modulate peripheral T_{regs} in terms of safety has been demonstrated in rodents and non-human primates.**
- **These findings support HM16390 as a safe and effective immune modulator for anti-tumor activity. Based on the verified safety and efficacy in preclinical studies, the IND has been approved for the first-in-human trial this year.**

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