HM16390, a long-acting IL-2 analog with enhanced IL-2Rβ and optimal IL-2Rα bindings, promotes peripheral T_{reg} expansion to mitigate systemic toxicity while preserving potent anti-tumor immunity

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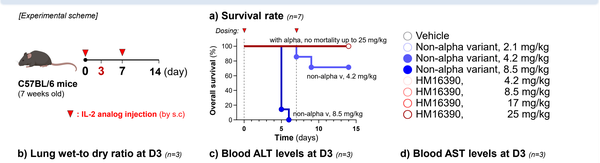
Abstract #859

Background

Introduction & Objective: The development of IL-2 analogs for cancer immunotherapy has primarily focused on reducing systemic toxicity by limiting peripheral regulatory T cell (T_{reg}) expansion. To achieve this, several IL-2 analogs have been engineered to eliminate binding to IL-2Rα (CD25). However, they have failed to demonstrate sufficient safety and efficacy in clinical trials. Given the intrinsic role of T_{regs} in maintaining immune homeostasis and regulating excessive immune activation, T_{regs} may function as a critical safeguard during IL-2 therapy.

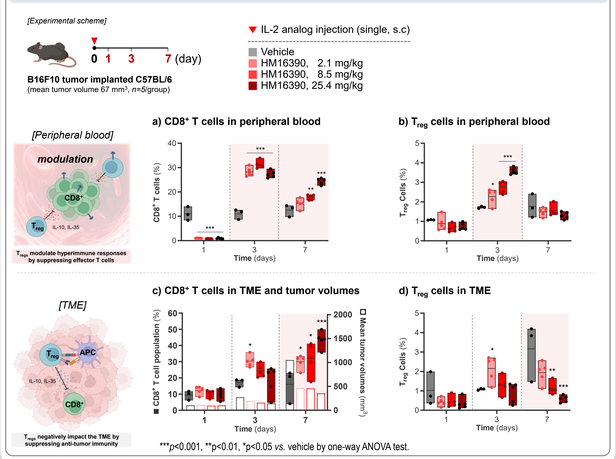
Here, we developed HM16390, a long-acting IL-2 analog with enhanced IL-2Rβ (CD122) binding and optimized CD25 affinity, and investigated how its CD25 engagement contributes to T_{reg} expansion and mitigation of systemic toxicity.

Intensified IL-2Rβ binding elicited a strong anti-tumor response; however, the absence of IL-2Rα engagement, which acts as an immune "safety belt" restraining excessive immune activation, led to immune dysregulation and severe systemic toxicity.



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

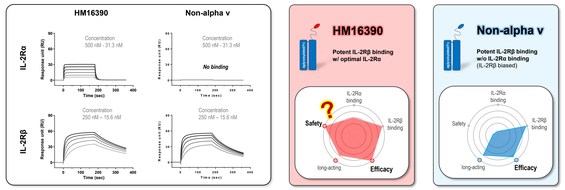
The CD25 binding property of HM16390 induces a dose-dependent expansion of T_{regs}, thereby mitigating uncontrolled systemic immune responses during circulation without compromising anti-tumor immunity within the tumor microenvironment (TME).



***$p<0.001$, **$p<0.01$, *$p<0.05$ vs. vehicle by one-way ANOVA test.

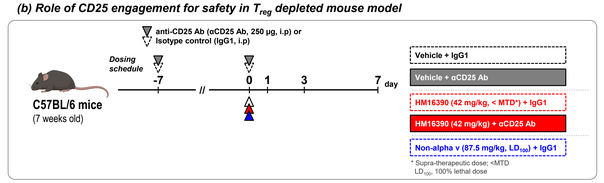
Characteristics of IL-2 analogs and experimental design

(a) Characteristics of long-acting IL-2 analogs

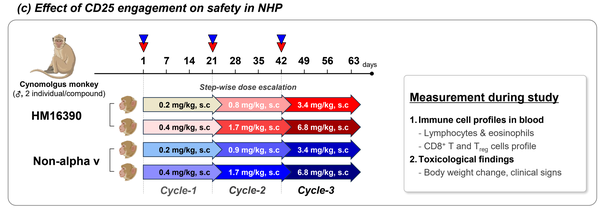


➢ The safety profiles of HM16390—an IL-2 analog engineered with finely tuned receptor-binding affinity to maximize anti-tumor efficacy while minimizing excessive immune activation—and its non-alpha variant (non-alpha v) were comparatively evaluated in animal models.

(b) Role of CD25 engagement for safety in T_{reg} depleted mouse model

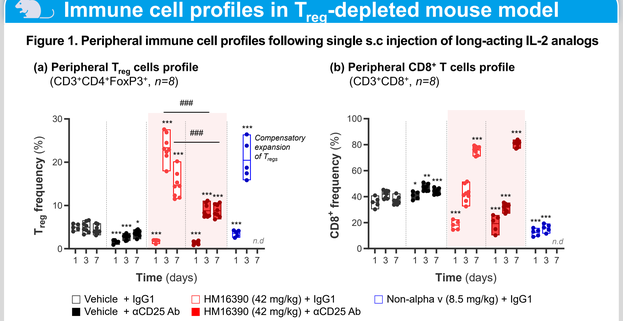


(c) Effect of CD25 engagement on safety in NHP



Immune cell profiles in T_{reg}-depleted mouse model

Figure 1. Peripheral immune cell profiles following single s.c injection of long-acting IL-2 analogs



➢ Administration of an anti-CD25 antibody (αCD25 Ab) significantly depleted peripheral T_{regs} and prevented the expansion of peripheral T_{regs} induced by HM16390 via its CD25 engagement.

➢ Under this condition, the levels of peripheral $CD8^+$ T cells induced by HM16390 remained comparable to those in non-depleted conditions, allowing the interpretation of safety without confounding effects from the $CD8^+$ T cell profile, which is important for anti-tumor efficacy and may, at least in part, contribute to safety. ***p<0.001, **p<0.01, *p<0.05 *vs.* HM16390 + αCD25 Ab treated group by One-way ANOVA test; ###p<0.001 *vs.* HM16390 + IgG1 by unpaired t-test; *n.d*, not determined due to complete mortality in all animals.

CD25-dependent protection from immune-driven toxicity

Figure 2. White blood cells (WBC) composition at Day 3 following administration *(n=8)*

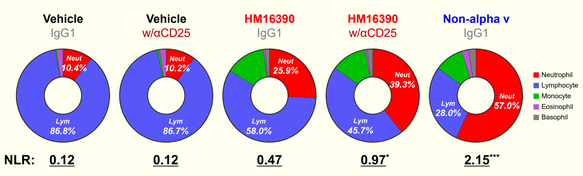


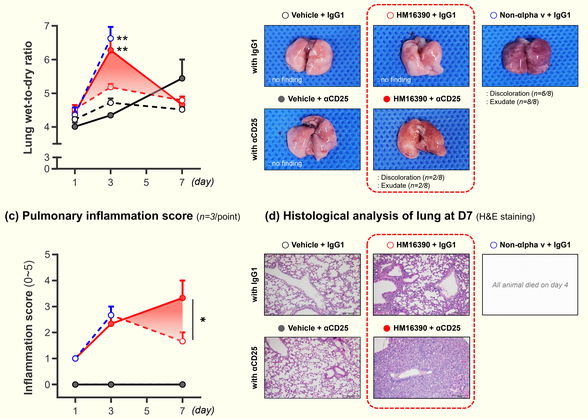
Table 1. Clinical signs during study *(n=8)*

Vehicle -		HM16390 (42 mg/kg)		Non-alpha v (8.5 mg/kg)
IgG1	**αCD25 Ab**	*IgG1*	**αCD25 Ab**	*IgG1*
no finding	*no finding*	*no finding*	**Hypoactivity** *(n=2/8)* @D3	**Found dead** *(n=1/8)* @D3 **Moribund** *(n=2/8)* @D3
			Hypoactivity *(n=2/8)* @D4	**Found dead** *(n=8/8)* @D4

➢ In T_{reg}-depleted mice, high-dose HM16390 (42 mg/kg) treatment resulted in elevated neutrophil-to-lymphocyte ratios (NLR), reflecting uncontrolled systemic inflammation and accompanied by clinical signs such as hypoactivity similar pattern with non-alpha variant. In contrast, HM16390 effectively suppressed immune activation in the isotype control group through peripheral T_{reg} expansion mediated by optimal CD25 interaction. ***p<0.001, *p<0.05 *vs.* HM16390 groups were treated with isotype control by One-way ANOVA test. NLR, neutrophil to lymphocytes ratio (mean value from 8 individuals per group).

CD25-dependent protection from pulmonary toxicity

Figure 3. Changes in lung injury-related profiles following administration



➢ In the absence of T_{reg}-mediated immune modulation, uncontrolled systemic immune activation induced by high-dose long-acting IL-2 therapy ultimately led to multiple organ failure, resulting in pulmonary edema (a, b) and exacerbation of pulmonary inflammation (c, d; 100× magnification) similar with non-alpha variant. Scale bar = 200 μm. Statistical significance was analyzed by one-way ANOVA (**p<0.01, *p<0.05 *vs.* HM16390 with isotype control).
The pulmonary inflammation score was determined on lung tissue slides from each animal based on the extent and density of perivascular inflammatory cell infiltration, using a 0–5 scale: 0 (absent), 1 (minimal, <10% of tissue involved), 2 (mild, 10–25%), 3 (moderate, 25–50%), 4 (marked, 50–95%), and 5 (severe, >95%).

Effects of CD25 engagement on safety in NHP

Table 2. Clinical signs were observed during the study

Dose	0.2 mg/kg	0.4 mg/kg	0.8 (0.9) 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 (MNOAEL)	BWL, -6.0% *vs* BL Body temp. ↑↑ Hypo-activity (G1) Petechiae / Erythema Diarrhea	BWL, -6.1% *vs* BL Body temp. ↑↑ Hypo-activity (G1) Petechiae / Erythema Diarrhea
Non-alpha v (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

Figure 4. The proliferation of lymphocytes following IL-2 analogs treatment in NHP

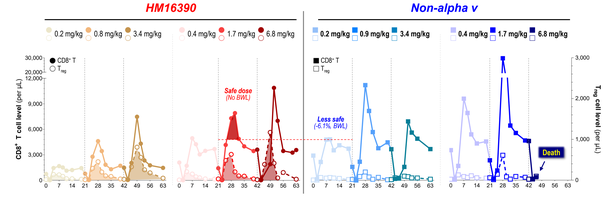
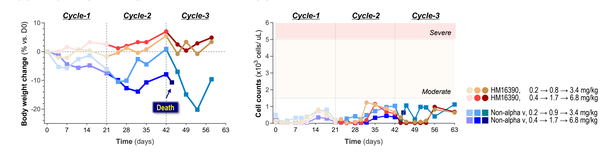


Figure 5. Changes in body weight and blood eosinophil counts over time



➢ In the cynomolgus monkeys, HM16390 induced dose-dependent expansion of peripheral T_{regs} and $CD8^+$ T cells, effectively suppressing excessive systemic immune activation. By contrast, the non-alpha variant (non-alpha v) expanded $CD8^+$ T cells without T_{reg} induction in blood, leading to severe toxicity with body weight loss and mortality.

➢ HM16390 maintained a favorable safety profile at all tested doses, achieving greater $CD8^+$ T-cell expansion than the non-alpha v at non-toxic levels, with no associated body weight loss. Furthermore, the optimal binding interaction with CD25 was not associated with an increase in blood eosinophil counts, which are considered a potential indicator of vascular leak syndrome (VLS).

➢ These results highlight the critical role of CD25-mediated peripheral T_{reg} expansion in maintaining immune balance and preventing systemic toxicity.

Concluding Remarks

- HM16390 is a novel long-acting IL-2 analog that combines potent IL-2Rβ affinity with optimized IL-2Rα binding to effectively control systemic immune activation.
- This balanced IL-2Rα and IL-2Rβ engagement preserves peripheral T_{reg} activation, thereby improving both safety and efficacy compared to non-alpha-binding strategies in mouse and non-human primate models. These results provide a compelling rationale for the clinical development of HM16390, which is currently undergoing a dose-escalation study in patients with solid tumors (NCT06724016).

References

1. McRitchie BR, Akkaya B. Front Immunol. **2022**; 13:940052.
2. Wu J, Bloch N, Chang AY, et al., Cell Rep Med. **2024**;5(1):101747

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