HM16390, a long-acting IL-2 analog with enhanced IL-2Rβ and optimal IL-2Rα binding, selectively expands tumor-specific T cells, resulting in potent anti-tumor immunity in murine tumor models

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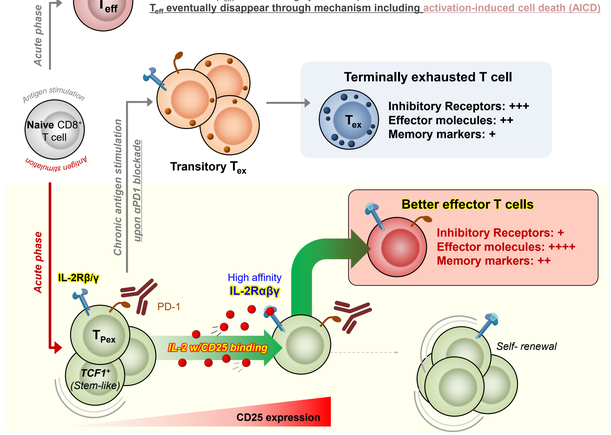
Abstract #850

Background

Introduction & Objective: In the development of IL-2–based cancer immunotherapy, it has long been postulated that eliminating IL-2Rα (CD25) binding is essential to enhance anti-tumor efficacy. However, such approaches have not demonstrated superior clinical benefits compared to wild-type IL-2 therapy, which retains high CD25-binding affinity. Recent studies have proposed an alternative paradigm, suggesting that retention of CD25 binding may be critical for the effective activation and expansion of tumor antigen-specific CD8$^+$ T cells.

To address this, we developed HM16390, a long-acting IL-2 analog engineered with enhanced IL-2Rβ (CD122) binding and optimized CD25 binding affinity, designed to significantly and safely expand cytotoxic effector T cells. In this study, we investigated the immunological impact of IL-2Rα binding property by comparing HM16390 with its non-alpha-binding variant in a murine model, with particular emphasis on the modulation of immune cell subsets and overall immune composition.

Considerations for effective stimulation of tumor antigen-specific CD8$^+$ T cells



HM16390

[Key features of HM16390 as novel long-acting IL-2 analog]

IL-2 analog rationally designed for **intensive anti-tumor activity** with **immune balance**

: Intensified IL-2Rβ binding elicits outstanding cytotoxic effector cells expansion

: Optimal IL-2Rα binding buffers an intensified IL-2Rβ binding derived CRS

Extended half-life allows once per immuno-therapies

Convenient **Subcutaneous treatment** option for patient adherence

Impact of CD25 engagement on anti-tumor efficacy

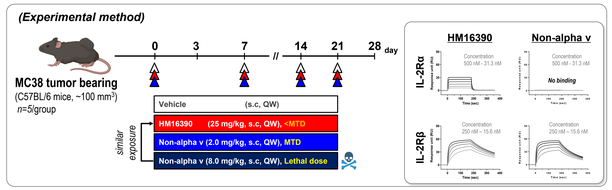
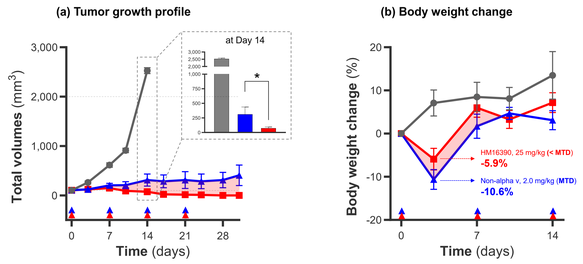


Figure 1. Tumor volumes and body weight in MC38 tumor-bearing mice following s.c treatment



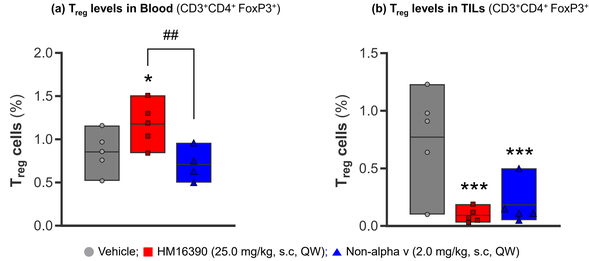
Summarized anti-tumor activity of long-acting IL-2 analogs in MC38 tumor-bearing mice

Group	Vehicle	HM16390	Non-alpha variant	
Dose (mg/kg)	● -	■ **25.0** (<MTD)	▲ **2.0** (MTD)	▲ **8.0** (LD_{100})
TGI (%) @D14	-	**101.2**	**91.4**	*n.d.* (all animal died)
CR	*no*	**5 CR (100%)**	**2 CR (40%)**	

➢ In the MC38 tumor-implanted C57BL/6 mouse model, HM16390 demonstrated significantly improved anti-tumor activity (100% CR at 25.0 mg/kg) with a more favorable body weight change compared to the non-alpha variant at its maximum tolerable dose (2.0 mg/kg). *p<0.05 *vs.* non-alpha variant by unpaired t-test. TGI, tumor growth inhibition rate; CR, complete response; MTD, maximum tolerated dose; LD_{100}, absolute lethal dose; *n.d.*, not determined.

Selective peripheral T_{reg} expansion via CD25 engagement

Figure 2. T_{reg} levels between blood and TILs in MC38 tumor-bearing mice at Day 3 following treatment



➢ HM16390, which exhibits optimal binding affinity to CD25, effectively mitigates systemic toxicity by selectively activating peripheral T_{regs} to suppress excessive immune responses. Meanwhile, within the TME, HM16390 significantly reduced T_{regs}, thereby avoiding potential negative impact on anti-tumor activity. ***p<0.001, *p<0.05 *vs.* vehicle group by One-way ANOVA test, ##p<0.01 *vs.* non-alpha variant (2.0 mg/kg) by unpaired t-test.

Selective Tumor-specific T cell expansion via CD25 engagement

Figure 3. CD8$^+$ T cell levels between blood and TILs in MC38 tumor-bearing mice at Day 7 following treatment

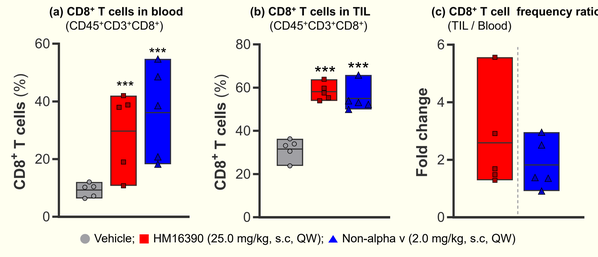
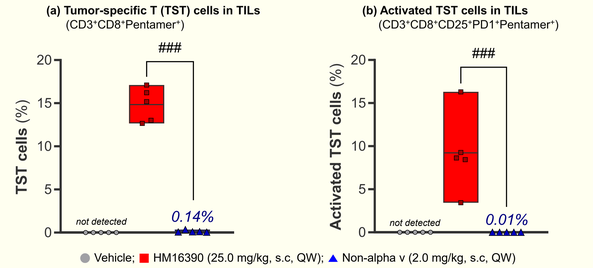


Figure 4. Tumor-specific T cells profile in MC38 tumor at Day 7 following treatment



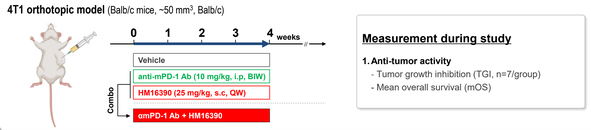
➢ While HM16390 showed relatively lower peripheral CD8$^+$ T cells due to transient T_{reg} expansion upon CD25 engagement, the CD8$^+$ T cells within TILs was comparable to that of the non-alpha variant, supporting TME-selective CD8$^+$ T cell expansion. ***p<0.001 *vs.* vehicle by One-way ANOVA test.

➢ The optimal binding affinity to CD25 further promoted the preferential expansion of tumor-specific T cells. Notably, most of these immune cell subtypes were activated and exhibited PD-1 expression, suggesting a potential synergistic effects when combined with anti-PD-1 antibody therapy. ###p<0.001 *vs.* non-alpha variant (2.0 mg/kg) by unpaired t-test.

Synergistic effect of HM16390 in combination with anti-PD-1

(Experimental method)

(a) Anti-tumor efficacy of HM16390 combined with anti-PD-1 in the orthotopic TNBC model



(b) Anti-tumor efficacy of HM16390 combined with and PD-1 in a lung melanoma metastasis model

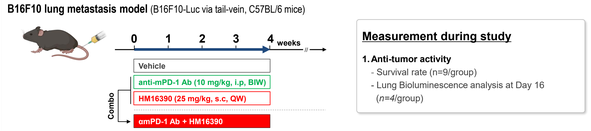
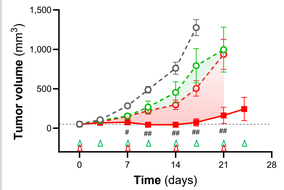


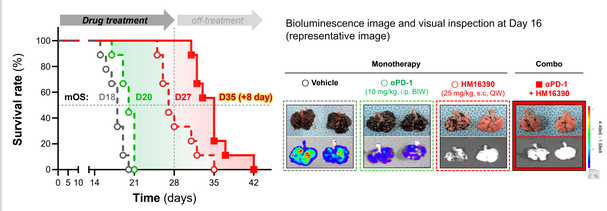
Figure 5. Synergistic effect of HM16390 in combination with anti-PD-1 in various tumor models

(a) Anti-tumor effect of HM16390 and its combo with anti-PD-1 in the 4T1 orthotopic TNBC mouse model



Compound	Vehicle	Mono-therapy: αPD-1	Mono-therapy: HM16390	Combo: αPD-1 + HM16390
Dose	-	10 mg/kg i.p. BIW	25 mg/kg s.c. QW	10 + 25 mg/kg
TGI @D17	-	39.4%	62.9%	102.5%
CR	0%	0%	14.3%	57%
mOS	24 day	28 day	28 day	> 49 day

(b) Anti-tumor effect of HM16390 and its combo with anti-PD-1 in the B16F10 lung metastasis mouse model



➢ Previously, HM16390 (25 mg/kg, subcutaneously QW) effectively overcame the insufficient anti-tumor effect observed with anti-PD-1 (10 mg/kg, intraperitoneally BIW) in highly aggressive tumor-bearing mouse models, including TNBC (a) and melanoma lung metastasis (b).

➢ The combination of HM16390 and anti-PD-1 therapy elicited synergistic anti-tumor efficacy, resulting in complete tumor regression (CR) in 57% of mice in the 4T1 orthotopic TNBC model (a) and prolonged mean overall survival (mOS) in the melanoma lung metastasis model (b). Mice were sacrificed when tumor volume exceed 2,000 mm³. Empty triangles indicate the dosing schedule for each administration. ##p<0.01, #p<0.05 *vs.* HM16390 mono group by unpaired t-test.

➢ These synergistic anti-tumor effect of HM16390 when combined with anti-PD-1 may be attributed to specific immune cell subtypes, such as activated TST, which were selectively expanded by HM16390.

Concluding Remarks

- HM16390 is a novel long-acting IL-2 analog, rationally designed to exhibit potent anti-tumor activity with a favorable safety profile through enhanced CD122 binding and optimized CD25 binding affinities.
- In direct comparison with its non-alpha variant, HM16390 demonstrated a distinct and preferential expansion of activated tumor-specific T cells, which phenotypically have the potential to synergize with anti-PD-1 therapy.
- In aggressive tumor models, the combination of HM16390 with anti-PD-1 led to significantly improved survival and durable anti-tumor responses, supporting that CD25 engagement may be critical aspect of IL-2-based therapies for synergism with PD-1 blockade.

References

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