HM16390, a novel long-acting IL-2 analog with fine-tuned binding affinities to IL-2 receptor subunits for favorable safety profile, exhibits potent tumor killing effect in the various tumor syngeneic models

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Abstract #LB118/6

Introduction

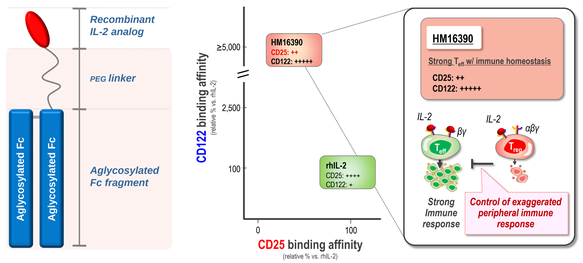
Although recombinant human IL-2 was approved for the treatment of renal cell carcinoma (RCC) and metastatic melanoma, its IL-2Rα (CD25)-biased binding and short half-life require a high dose and frequent dosing interval, leading to systemic toxicity such as vascular leak syndrome (VLS) and cytokine release syndrome (CRS)[1].

To overcome this limitation, various research groups are developing IL-2 muteins, which abolish CD25 binding affinity. However, IL-2 muteins with reduced CD25 binding can decrease CD25-mediated toxicity, but there is a risk of a biased immune response[2].

HM16390, a long-acting IL-2 analog, is developing for subcutaneous (SC) administration once per treatment cycle. It has an increased affinity to IL-2Rβ (CD122) that is aimed to enhance anti-tumor response. Furthermore, optimal binding affinity to IL-2Rα (CD25) is incorporated for marginal action of T_{regs} that prevents the exaggerated and uncontrolled systemic immune responses.

Here, we investigated the comparison of immune cell profiles between peripheral blood and the tumor microenvironment following treatment with HM16390 in the B16F10 syngeneic mouse model. Furthermore, we demonstrated superior and durable anti-tumor efficacy of HM16390 in the orthotopic RCC model.

[Structural features and Development strategy of ^{LAPS}IL-2 analogs]



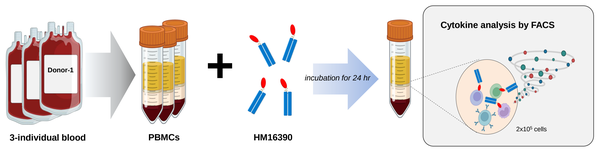
[General profile]

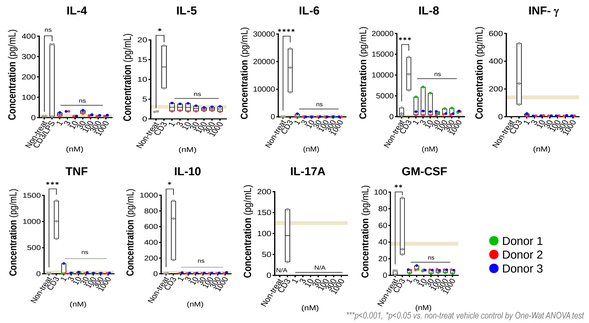
- **Drug moiety rationally designed for intensive anti-tumor effect with immune balance**
  : Intensified IL-2Rβ binding elicits outstanding lymphocyte expansion
  : Optimal IL-2Rα binding minimizes a toxicity such as vascular leak syndrome (VLS) and cytokine release syndrome (CRS)
- **Extended half-life allows once per chemo-cycle**
- **Convenient subcutaneous treatment option for patient adherence**

Method & Result

Preferential stimulation of effector immune cells by HM16390

Figure 1. Cytokine and chemokine release in human PBMCs by treatment of HM16390





***p<0.001, *p<0.05 vs. Non-treat vehicle control by One-Wat ANOVA test

[a]Cytokine and chemokines level in the serum of healthy subjects ≥18 yrs old (Mediators Inflamm. 2013;2013:434010)

- Cytokines and chemokines release profiles of HM16390 have been evaluated *in ex vivo* hPBMCs culture model from healthy volunteers without TCR activation.
- HM16390 generally did not induced multiple cytokines or chemokines associated with cytokine release syndrome.

Immune cell profiles of HM16390 in peripheral blood or TME of melanoma mouse model

Figure 2. Immune cell profile in B16F10 tumor syngeneic mouse model

* Mean tumor volume at 1 day before treatment: 43.7 ± 20.0 mm³

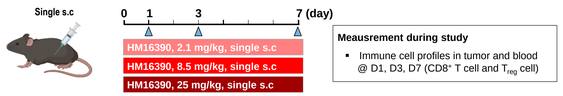
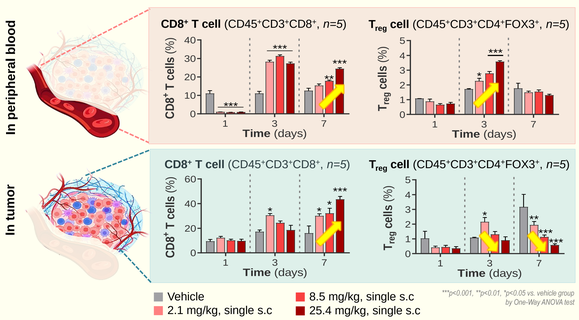


Figure 3. Immune cell profiles in blood and tumor



***p<0.001, **p<0.01, *p<0.05 vs. vehicle group by One-Way ANOVA test

- In the peripheral blood, a temporary increase in T_{reg} balances out the increase in exaggerated immune response caused by excessive immune responses, thus reducing the impact of systemic toxic reactions.
- On the other hand, in tumors, $CD8^+$ T cells peaked on day 7, while T_{reg} levels decreased over time. The decrease in T_{reg} and increase in $CD8^+$ T cells within tumors serve as positive indicators of anti-tumor treatment, implying a shift in the tumor microenvironment towards one conducive to anti-tumor effects.

Anti-tumor effect of HM16390 in orthotopic RCC mouse model

Figure 4. Experimental design for anti-tumor efficacy in orthotopic renal cell carcinoma (RCC) mouse model

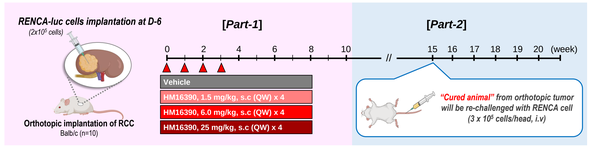
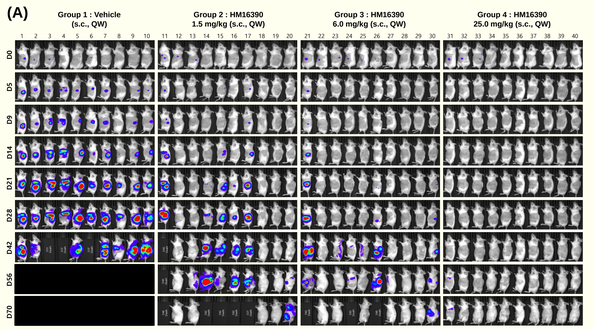
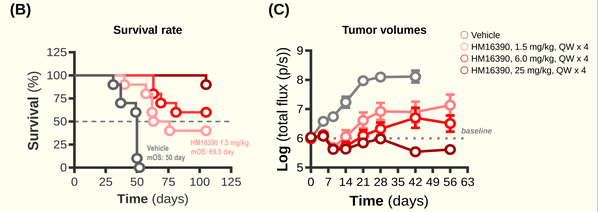


Figure 5. Tumor growth and mOS in orthotopic RCC mouse model



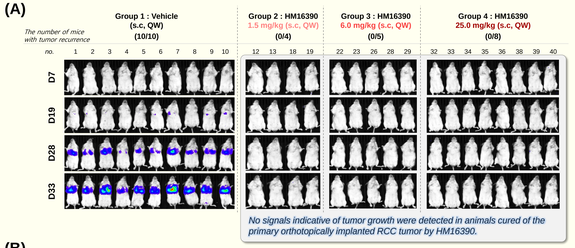


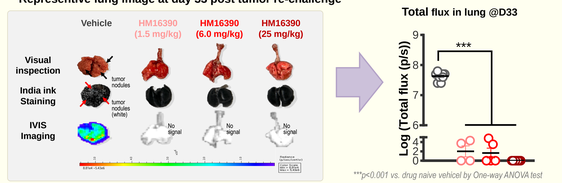
(D)

	Vehicle	HM16390 (QW x 4, s.c)		
Dose (mg/kg)	○	○ 1.5	○ 6.0	○ 25.0
TGI (@D42)	-	74%	63%	100%
Survival rate (@D64)	0%	50%	80%	100%
mOS (day)	50	63.5	> 70	> 70
CR	-	4/10	5/10	9/10

- The HM16390 treatment group exhibited dose-dependent reductions in tumor volume and increased survival rates. Particularly, in the vehicle group, all subjects died by approximately day 56, whereas in the HM16390 group, especially in the higher dosage ranges, the median overall survival (mOS) was not defined. Furthermore, complete regression was also dose-dependent, with CR observed in 90% of cases at high doses.

Figure 6. Experimental scheme for evaluation the memory response in metastatic RCC model





***p<0.001 vs. drug naive vehicel by One-way ANOVA test

- Mice were intravenously injected with RCC cells, and tumor recurrence was observed using IVIS. On the 33rd day of tumor re-challenge, tumors recurred in all individuals in the vehicle group. However, the cured mice by treatment of HM16390 did not experience tumor recurrence. This result suggests that the absence of tumor recurrence in HM16390-treated mice may be attributed to a T-cell memory response.

Concluding Remarks

- HM16390, a novel long-acting and subcutaneous IL-2 analog, is designed to have a strong binding affinity to human IL-2Rβ (CD122) and optimal binding affinity to IL-2Rα (CD25). Furthermore, it did not induce multiple cytokines or chemokines associated with CRS from hPBMCs.
- The immune cell profiles in B16F10 melanoma mouse model supported significant increase in tumor-killing immune effector cells in peripheral blood induced by HM16390, which supplied to the tumors. Simultaneously, it facilitated marginal or transient activation of T_{regs} in peripheral blood, rather than in the TME, thus expected to effectively control excessive immune responses related to systemic toxicity.
- In the orthotopic RCC mouse model, HM16390 not only demonstrated significant inhibition of tumor growth but also completely prevented tumor recurrence through memory T cells.

References

1. Amaria, Rodabe N., et al. *ImmunoTargets and therapy* **2015**, 79-89.
2. Skrombolas, Denise, and John G. Frelinger. *Expert review of clinical immunology* **2014**, 10.2: 207-217.

Acknowledgements

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