

1046

**A LONG-ACTING AND BETA-INTENSIFIED IL-2 ANALOG, HM16390, SIGNIFICANTLY ALTERS TUMORS TO AN IMMUNE FAVORABLE ENVIRONMENT TO POTENTIATE PD-1 BLOCKADE**

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**Background** Anti-PD-1 is most widely used immune checkpoint inhibitors (CPIs) in cancer immunotherapy. However, the response to CPIs depends on the phenotype of the tumor microenvironment (TME). Cold tumor, also known as immune-excluded or -desert tumors, have shown a poor response to PD-1 blockers due to an absence of effector T cells in the TME. Here, we investigated the tumor-infiltrating immune cells composition after HM16390 treatment and the synergistic activity after combination with anti-PD1 in poorly immunogenic tumor syngeneic mice model.

**Methods** B16F10 mice were sacrificed on days 1, 3, and 8 after a single subcutaneous administration of HM16390 or 5 consecutive days via intraperitoneal injections of aldesleukin. The tumor-infiltrating immune cell profile including CD8<sup>+</sup> T cells and regulatory T cells (T<sub>reg</sub>) was assessed by flow cytometry as well as immunofluorescence staining. To investigate synergistic anti-tumor effect in combination with anti-PD1, B16F10 mice were repeatedly given once a week of HM16390 or 5 consecutive days per week of aldesleukin with or without twice a week of anti-PD-1, and tumor growth and survival were monitored up to 49 days.

**Results** The tumor-infiltrating CD8<sup>+</sup>/T<sub>reg</sub> ratio was upregulated by approximately 74 by treatment with HM16390, whereas it was only 6.1 in the aldesleukin-treated group on day 8. The Ki-67 expression was significantly upregulated on tumor-infiltrating CD8<sup>+</sup> T cells following HM16390 treatment. Interestingly, while aldesleukin significantly upregulated Ki-67 expression on T<sub>regs</sub> compared to the vehicle, HM16390 did not induce Ki-67 expression on T<sub>regs</sub> during the observation period. Significantly increased pro-inflammatory molecules such as GrzB and IFN- $\gamma$  were also observed in the HM16390-treated group compared to aldesleukin ( $p < 0.01$  and  $p < 0.001$ , respectively). Next, we investigated synergistic anti-tumor effects in combination with anti-PD-1 in B16F10 mice. After four weeks of treatment, 25% of the B16F10 mice showed a complete response to treatment with HM16390, and this increased significantly to 88% when combined with anti-PD1. However, none of the mice showed a complete response to treatment with the aldesleukin/anti-PD1 combination. The median overall survival with aldesleukin alone was 22.5 days and increased to 34.5 days when combined with anti-PD-1. HM16390 monotherapy showed similar results to the aldesleukin combination group (37.5 days), and all animals survived up to the end of the study in combination with anti-PD1.

**Conclusions** HM16390 efficiently inhibited tumor growth and prolonged survival rate through the significant increase of tumor-infiltrating cytotoxic lymphocytes. This favorable immune alteration in tumors by HM16390 potentiates anti-tumor effect of PD-1 blockade.

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