SITC POSTER #450

Combination of rhIL-7-hyFc and anti-PD-L1xCD3ε bispecific antibody enhances antitumor response in mice

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Background

rhIL-7-hyFc is a hybrid Fc-fused recombinant human interleukin-7 (NT-I7; efineptakin-alfa) with enhanced bioactivity. In a previous study, we found that systemic administration of rhIL-7-hyFc induced antitumor effect by increasing CD8⁺ T cells within the tumor microenvironment. rhIL-7-hyFc monotherapy increased not only PD-1⁺ tumor-reactive but also intratumoral PD-1⁻ bystander CD8+ T cells. Therefore, we hypothesized that the activation of PD-1⁻ bystander T cells in tumors would enhance the antitumor activity of rhIL-7-hyFc. Here we evaluated the antitumor effect of combination therapy with rhIL-7-hyFc and a bispecific antibody (bsAb), anti-PD-L1xCD3ε, targeting both a tumor-associated antigen (PD-L1) and a T-cell stimulatory antigen (CD3ε).

Methods

***In vitro* cell culture.** For analysis of T cell activation and cytotoxicity, splenocytes were isolated from PD-L1 knock-out (KO) mice and co-cultured with either wild type (MC-38^{WT}) or PD-L1-depleted (MC-38$^{\Delta PD\text{-}L1}$) tumor cells in the presence of bsAb for 48 hours

***In vivo* treatment.** Tumor-bearing mice were treated subcutaneously (s.c.) with 1.25 mg/kg of rhIL-7-hyFc. An indicated dose of bsAb was administered daily by intravenous (i.v.) or intratumoral (i.t.) route, starting 3 days after the rhIL-7-hyFc treatment, for a total of 5 times.

Preparation of tumor-infiltrating cells. Tumor tissues were harvested after 7 days of rhIL-7-hyFc treatment. Single-cell suspensions were prepared through mechanical separation followed by collagenase D and DNAse I treatment.

Results

- **Anti-PD-L1xCD3ε bsAb induced PD-L1-specific activation and cytotoxicity of CD8⁺ T cells *in vitro* (Fig. 1).**
- **rhIL-7-hyFc combined with systemic administration of bsAb enhanced antitumor responses, although loss of body-weight was shown with high-dose bsAb combination (Fig. 2)**
- **The combination of rhIL-7-hyFc with a systemic administration of bsAb increased not only the frequency of CD8⁺ T cells in tumors but also the frequency of PD-1⁻ bystander CD8⁺ T cells with enhanced expression of Granzyme B (Fig. 3).**
- **When bsAb was administered systematically, side effects were observed. This is expected because PDL-1 is expressed not only in tumors but also in normal tissues. So, the experiment was designed to target bsAb to tumor by administering by intratumoral route.**
- **Intratumoral administration of high-dose bsAb enhanced antitumor response of rhIL-7-hyFc without body-weight loss (Fig. 4).**

Conclusion

- **The combination treatment of anti-PD-L1xCD3ε bsAb with rhIL-7-hyFc enhances antitumor efficacy.**
- **Both systemic and intratumoral administration of bsAb with rhIL-7-hyFc augments antitumor effects, and intratumoral administration induced less weight loss than systemic administration.**
- **The activation of PD-1⁻ bystander CD8⁺ T cells in tumors by the combination of bsAb and rhIL-7-hyFc suggests that antitumor response may be partially mediated by the targeted activation of bystander CD8⁺ T cells.**
- **Our results serve as a proof-of-concept that the combination of rhIL-7-hyFc, a strong T cell amplifier, with bsAb, a tumor-targeted T-cell stimulator, would be a promising strategy for cancer immunotherapy.**

Acknowledgements

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Anti-PD-L1xCD3ε bsAb activates CD8⁺ T cells by targeting PD-L1 on tumor cells

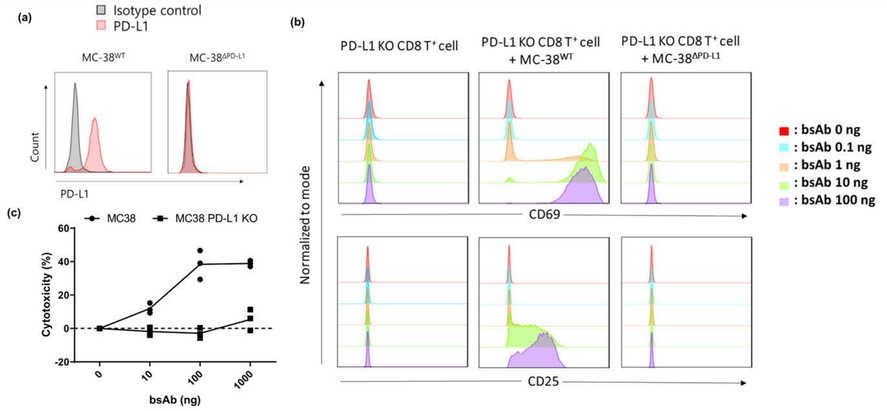

Figure 1. MC-38^{WT} and MC-38$^{\Delta PD\text{-}L1}$ tumor cells were cultured *in vitro*. **(a)** PD-L1 expression levels on each cell line. **(b)** Splenocytes isolated from PD-L1 KO mice were co-cultured with indicated tumor cells (E:T = 20:1) in the presence of bsAb. Expression levels of activation markers, such as CD69 and CD25, on CD8⁺ T cells were analyzed by flow cytometry. **(c)** Cytotoxicity against tumors was analyzed in the presence of bsAb. Cytotoxicity was calculated using the formula: [1 - live target cells(sample)/live target cells(control)] × 100

Combination therapy of rhIL-7-hyFc with systemic anti-PD-L1xCD3ε bsAb enhances antitumor efficacy

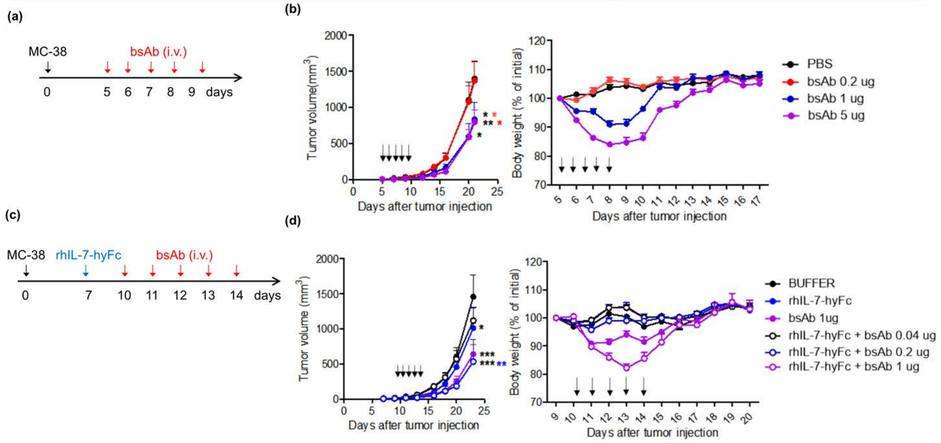

Figure 2. (a-b) Mice bearing MC-38 tumors were treated with different doses of bsAb (i.v.) as indicated in **(a)** (n = 5 per group). **(b)** Shown are mean tumor growth curves (left) and body-weight changes (right). **(c-d)** Mice bearing MC-38 tumors were treated with either 1.25 mg/kg of rhIL-7-hyFc (s.c.), indicated doses of bsAb (i.v.), or combination of each therapy, as indicated **(c)**. In the case of combination therapy with 1 ug bsAb, mice were treated only for the first 3 doses of bsAb because of body-weight loss (n = 5-7 per group). (d) Shown are mean tumor growth curves (left) and body-weight changes (right). Arrows indicate the dosing of bsAb. Data are represented as mean ± SEM. Statistical significance was analyzed by two-way ANOVA with Bonferroni's multiple comparisons for (**b** and **d**) . *P<0.05;**P<0.01;***P<0.001.

Combination therapy of rhIL-7-hyFc with systemic anti-PD-L1xCD3ε bsAb increases the frequency of Granzyme B⁺ bystander CD8⁺ T cells

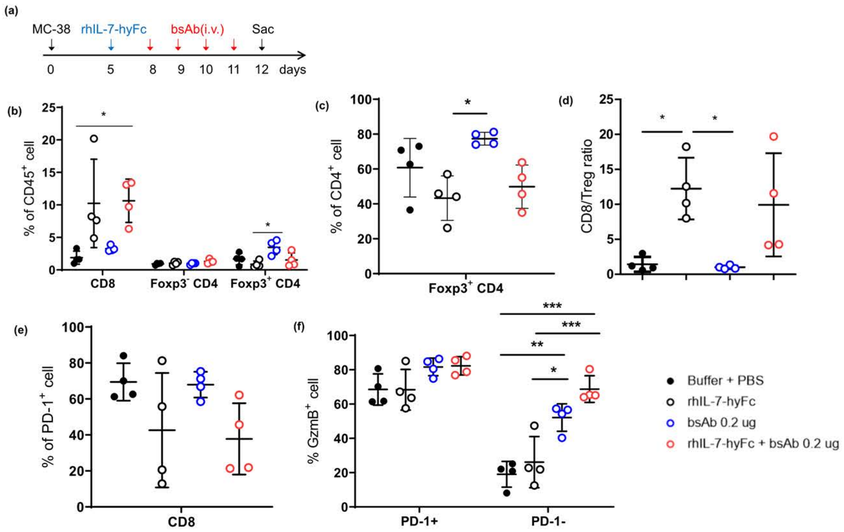

Figure 3. **(a)** Experimental scheme for the analysis of tumor-infiltrating T cells (n = 4 per group). **(b)** Frequencies of CD8⁺, CD4⁺Foxp3⁻ T helper (Th), and CD4⁺Foxp3⁺ T regulatory (Treg) cells among CD45⁺ cells. **(c)** Frequencies of CD4⁺Foxp3⁺ Treg cells among CD4⁺ T cells. **(d)** The ratio of CD8⁺ T cells to Treg cells. **(e)** Frequencies of PD-1⁻ cells among CD8⁺ T cells. **(f)** Frequencies of Granzyme B (GzmB) expressing cells among PD-1⁺ or PD-1⁻ CD8⁺ T cells. Data are represented as mean ± SD. Statistical significance was analyzed by one-way ANOVA with Bonferroni's multiple comparisons. *P<0.05;**P<0.01;***P<0.001.

Combination therapy of rhIL-7-hyFc with intratumoral anti-PD-L1xCD3ε bsAb enhances antitumor efficacy without toxicity

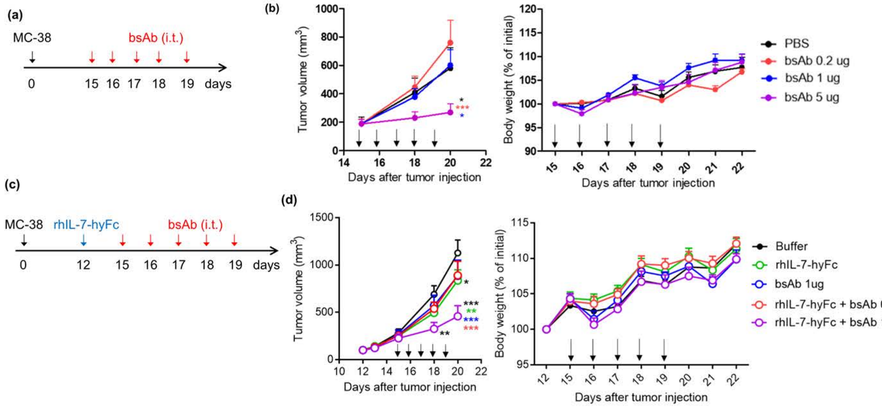

Figure 4. (a-b) Mice bearing MC-38 tumors were treated i.t. with bsAb as indicated in **(a)** (n = 6-7 per group). **(b)** Shown are mean tumor growth curves (left) and body-weight changes (right). **(c-d)** Mice bearing MC-38 tumors were treated either 1.25 mg/kg of rhIL-7-hyFc (s.c.), indicated doses of BsAb (i.t.), or combination of each therapy as indicated in **(c)**. (n = 9-10 per group). **(d)** Shown are mean tumor growth curves (left) and body-weight changes (right). Arrows indicate the dosing of bsAb. Data are represented as mean ± SEM. Statistical significance was analyzed by two-way ANOVA with Bonferroni's multiple comparisons for tumor growth graphs. *P<0.05;**P<0.01;***P<0.001.