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Redirecting IL-7-induced bystander tumor-infiltrating lymphocytes by bispecific T cell engager augments antitumor response

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Background.

rhIL-7-hyFc (efineptakin alfa, NT-I7) is a long-acting form of recombinant human IL-7 and is currently under clinical trials for various cancers in combination with immune checkpoint inhibitors (ICI). We have previously shown that rhIL-7-hyFc monotherapy increases tumor-infiltrating lymphocytes (TILs); however, the majority of CD8⁺ TILs is PD-1⁻ bystander T cells that lack tumor-specific activity.

Therefore, we hypothesized that bispecific T cell engagers (TCE) composed of two single-chain variable fragments simultaneously targeting CD3ε and tumor antigens, including PD-L1, can redirect and activate IL-7-induced bystander TILs to kill tumor cells resulting in enhanced antitumor response.

Methods.

We conducted scRNA-seq paired with TCR-seq of CD8⁺ TILs isolated from tumors after rhIL-7-hyFc treatment to evaluate transcriptomic changes of both tumor-reactive and bystander T cells. We generated various TCEs targeting mouse or human CD3ε and tumor antigens. The efficacy of antitumor responses by combination treatment of rhIL-7-hyFc and TCE was evaluated in immunogenic and non-immunogenic murine tumor models. To address the activation of bystander TILs, we analyzed the expression of effector molecules and cytotoxicity of PD-1⁻ CD8⁺ TILs after co-culturing with TCE and tumor cells. We determined the antitumor response of bystander CD8⁺ T cells with an adoptive transfer experiment in RAG1$^{-/-}$ mice.

Results.

scRNA-seq analysis of CD8⁺ TILs revealed that rhIL-7-hyFc attenuates the dysfunction (or exhaustion) of tumor-reactive cells and recruits bystander cells with the characteristics of cytokine-primed central memory phenotype. TCE can activate CD8⁺ T cells when it simultaneously binds to tumor antigen. The combination of rhIL-7-hyFc and TCE enhanced the antitumor responses by upregulating CD8⁺ TILs. In addition, IL-7-induced bystander CD8⁺ TILs are TCR-activated to gain a cytotoxic activity to tumor cells. Lastly, we observed the antitumor response of IL-7-primed bystander CD8⁺ T cells when redirected in vivo by TCE in RAG1$^{-/-}$ mice

Transcriptomic changes of tumor-reactive and bystander CD8⁺ TILs

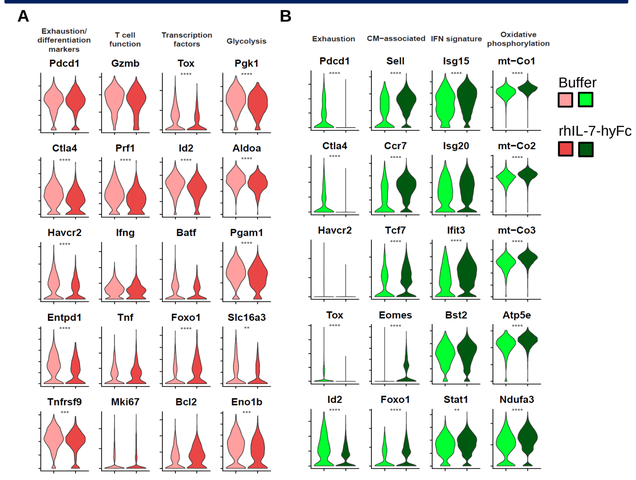

Figure 2. (A) Violin plots representing the expression of genes related to exhaustion, T cell function, transcription factors, and glycolysis in tumor-reactive cells. Tumor-reactive cells are defined as cells with a clone size of 3 or greater. **(B)** Violin plots representing the expression of genes related to exhaustion, central memory T cell, Interferon signaling, and oxidative phosphorylation in bystander cells. Bystander cells are defined as cells with a clone size of 1 or 2 and belonging to clusters 0, 2, 3, and 5.

Enhanced antitumor effects by the combination of rhIL-7-hyFc and TCE

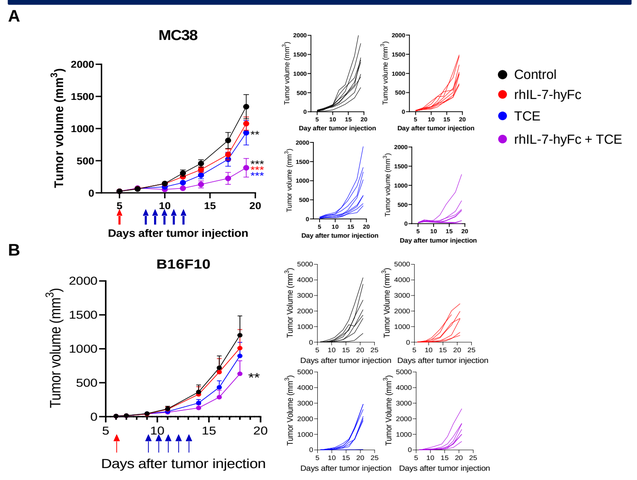

Figure 4. (A) C57BL/6 mice were injected with 1x10^5 MC38 immunogenic colorectal tumor cells s.c. in the right flank. rhIL-7-hyFc (1.25m.p.k) was administered s.c. when the tumors grow palpable. Anti-PD-L1xCD3ε TCE (0.4ug) was administered i.v. 5 times daily from the third day of rhIL-7-hyFc treatment. Average tumor growth curve **(left)** and tumor growth curves for individuals **(right). (B)** C57BL/6 mice were injected with 1x10^5 B16F10 non-immunogenic melanoma tumor cells s.c. in the right flank. rhIL-7-hyFc (10 m.p.k) was administered s.c. when the tumors grow palpable. Anti-PD-L1xCD3ε TCE (0.4ug) was administered i.v. 5 times daily from the third day of rhIL-7-hyFc treatment. Average tumor growth curve **(left)** and tumor growth curves for individuals **(right).**

In-vitro antitumor activity of redirected bystander CD8⁺ T cells

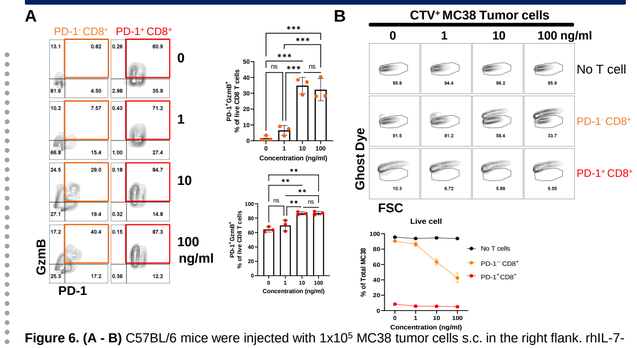

Figure 6. (A - B) C57BL/6 mice were injected with 1x10^5 MC38 tumor cells s.c. in the right flank. rhIL-7-hyFc (10 m.p.k) was administered s.c. when the tumors grow palpable. Tumor-infiltrating PD-1⁻ CD8⁺ and PD-1⁺CD8⁺ T cells were isolated 7 days after treatment, respectively. WT MC38 cells were stained with CellTrace Violet (CTV) and incubated with each T cell in the presence of anti-PD-L1xCD3ε TCE at indicated concentrations for 48 hours. **(A)** Expression of PD-1 and GzmB in CD8⁺ T cells was measured by flow cytometry. **(B)** Expression of ghost dye in tumor cells was measured by flow cytometry.

In-vivo antitumor activity of redirected bystander CD8⁺ T cells

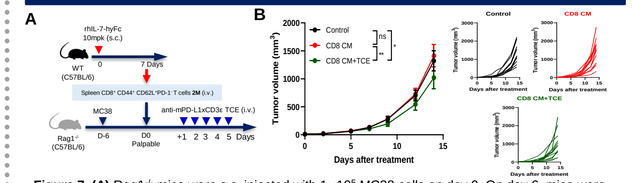

Figure 7. (A) Rag1$^{-/-}$ mice were s.c. injected with 1×10^5 MC38 cells on day 0. On day 6, mice were i.v. injected with 2 × 10^6 Splenic CD8⁺CD44⁺CD62L⁺PD-1⁻ T cells from rhIL-7-hyFc treated C57BL/6 mice. Anti-PD-L1xCD3ε TCE (2μg) or PBS were administered i.v. 5 times daily from the next day after the transfer of T cells. **(B)** Average tumor growth curve **(left)** and tumor growth curves for individuals (**right**).

Characterization of CD8⁺ TILs in MC38 colorectal tumor

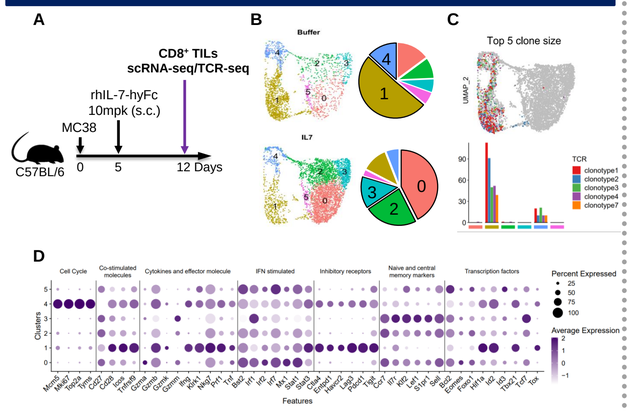

Figure 1. (A) Experimental scheme of single-cell RNA-sequencing paired with single-cell TCR-sequencing of CD8⁺ Tumor-infiltrating T cells from MC38 colorectal tumor-bearing mice. C57BL/6 mice were injected with 1x10^5 MC38 tumor cells s.c. in the right flank. rhIL-7-hyFc (10m.p.k) was administered s.c. when the tumors grow palpable. 7 days after treatment, mice were sacrificed for analyses of CD8⁺ TILs. **(B)** UMAP plots representing each CD8⁺ TIL cluster split by treatment conditions **(left)** and a pie chart representing the proportion of clusters in each treatment condition **(right)**. **(C)** The top five most abundant clones are shown in UMAP **(top)**. Bar plot of the top five most abundant clones by each cluster with clonal size **(bottom)**. **(D)** Dot plot of the expression of various T cell related genes in the 6 different clusters.

In-vitro functionality assay of anti-PD-L1xCD3ε TCE

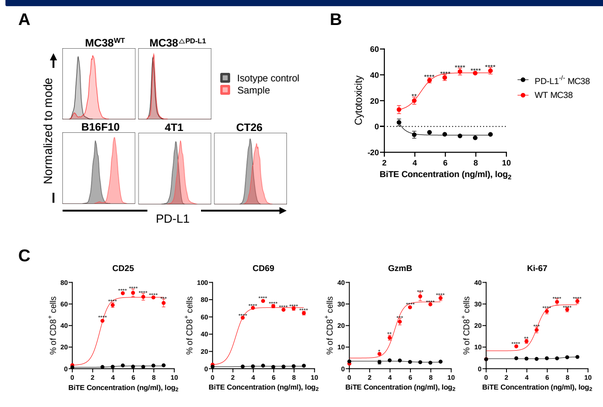

Figure 3. (A) Flow cytometry histograms showing PD-L1 expression of murine tumor cells. Tumor cells were incubated for 48h and stained for PD-L1 expression. **(B –C)** WT MC38 or PD-L1$^{-/-}$ MC38 cells were stained with CellTrace Violet (CTV) and incubated with PD-L1$^{-/-}$ splenocytes in the presence of anti-PD-L1xCD3ε TCE at indicated concentrations for 48 hours. **(B)** Cells were stained with the Ghost dye. % Cytotoxicity = [dead tumor cells (CTV⁺ Ghost Dye⁺)/total tumor cells (CTV⁺)] x 100%. **(C)** T cell activation, cytotoxicity, and proliferation markers are measured by flow cytometric analysis.

Changes in CD8⁺ TILs after combination therapy

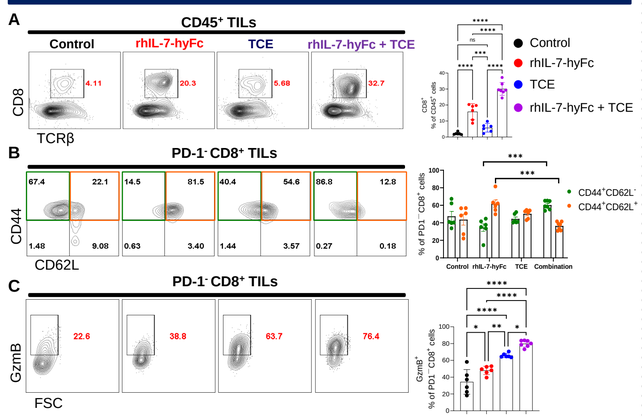

Figure 5. (A - C) C57BL/6 mice were injected with 1x10^5 MC38 tumor cells s.c. in the right flank. rhIL-7-hyFc (1.25m.p.k) was administered s.c. when the tumors grow palpable. Anti-PD-L1xCD3ε TCE (0.4ug) was administered i.v. 2 times daily from the third day of rhIL-7-hyFc treatment. 24 hours after the last treatment, mice were sacrificed for flow cytometry analyses of TILs. **(A)** Frequency of CD8⁺ T cells among CD45⁺ TILs. Expression of CD44, CD62L **(B),** and GzmB **(C)** was analyzed in PD-1⁻ CD8⁺ TILs by flow cytometry.

Acknowledgement

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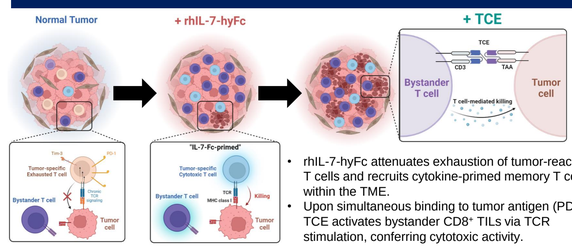
Conclusion



- rhIL-7-hyFc attenuates exhaustion of tumor-reactive T cells and recruits cytokine-primed memory T cells within the TME.
- Upon simultaneous binding to tumor antigen (PD-L1), TCE activates bystander CD8⁺ TILs via TCR stimulation, conferring cytotoxic activity.
- Activated bystander CD8⁺ TILs by TCE participate in antitumor response along with tumor-reactive cells.

Our data suggest that bispecific T cell engagers are promising candidates to augment the antitumor activity of rhIL-7-hyFc by redirecting bystander CD8⁺ TILs.

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