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 dysfunctional (or exhaustion) process 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 MC38, B16F10, and CT-26 models. 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.

Conclusions 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.

REFERENCE

Ethics Approval All animal experiments were performed in accordance with National Institutes of Health guidelines for the care and approved by the Institutional Animal Care and Use Committee of the POSTECH (POSTECH-2022-0052).