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 CD3 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 CD3 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).