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450 COMBINATION OF RHIL-7-HYFC AND ANTI-PD-L1XCD3ϵ BISPECIFIC ANTIBODY ENHANCES ANTITUMOR RESPONSE IN MICE

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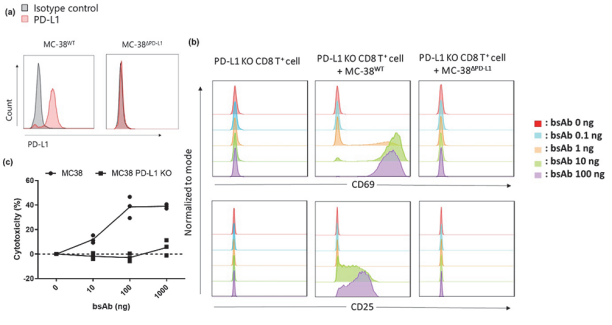
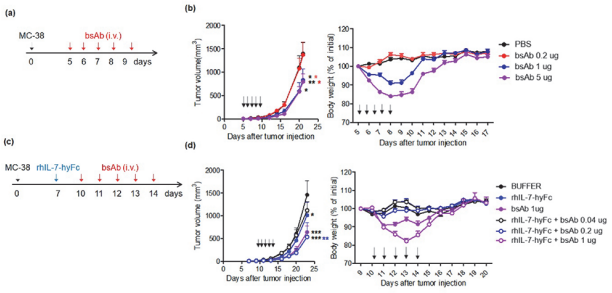
Background rhIL-7-hyFc is a hybrid Fc-fused recombinant human interleukin-7 (NT-I7; efineptakin-alfa) with enhanced bioactivity. In a previous study, we found that a systemic administration of rhIL-7-hyFc induced antitumor effect by increasing $CD8^+$ T cells in the tumor microenvironment. rhIL-7-hyFc monotherapy increased not only $PD\text{-}1^+$ tumor-reactive but also intratumoral $PD\text{-}1^-$ bystander $CD8^+$ T cells. Therefore, we hypothesized that the activation of $PD\text{-}1^-$ 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 knockout (KO) mice and co-cultured with either wild type ($MC\text{-}38^{WT}$) and PD-L1-depleted ($MC\text{-}38^{\Delta PD\text{-}L1}$) 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 daily treated intravenous (i.v.) or intratumoral (i.t.) route starting from 3 days after the rhIL-7-hyFc treatment for a total 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.

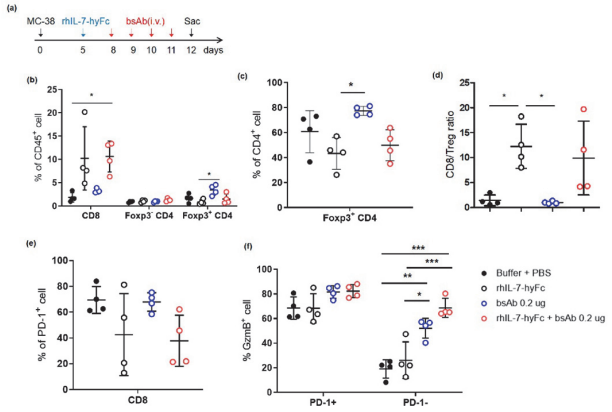
Results Anti-PD-L1xCD3ϵ bsAb induced the PD-L1-specific activation and cytotoxicity of $CD8^+$ T cells in vitro (figure 1). rhIL-7-hyFc combined with a systemic administration of bsAb enhanced antitumor responses, although loss of body-weight was shown with high-dose bsAb combination (figure 2)The combination of rhIL-7-hyFc with a systemic administration of bsAb increased not only the frequency of $CD8^+$ T cells in tumors but also the $PD\text{-}1^-$ bystander $CD8^+$ T cells with enhanced expression of a Granzyme B (figure 3).Intratumoral administration of high-dose bsAb enhanced antitumor response of rhIL-7-hyFc without body-weight loss (figure 4).



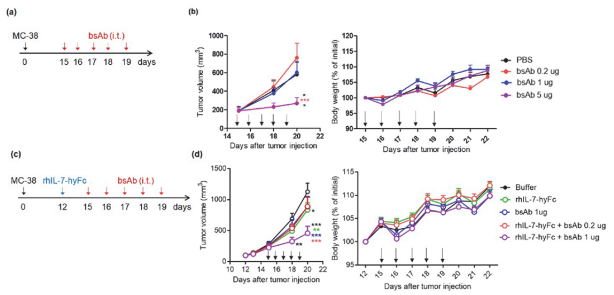
Abstract 450 Figure 1 $MC\text{-}38^{WT}$ and $MC\text{-}38^{\Delta PD\text{-}L1}$tumor cells were cultured in vitro. (a) PD-L1 expression levels on each cell line. (b) Splenocytes isolated from PD-L1 KO mice were co-cultured with indicated tumor cells (E:T = 20:1) in the presence of bsAb. Expression levels of activation markers, such as CD69 and CD25, on the $CD8^+$ 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



Abstract 450 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 either 1.25 mg/kg of rhIL-7-hyFc (s.c.), indicated doses of bsAb (i.v.), or combination of each therapy as indicated in (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



Abstract 450 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 $PD\text{-}1^-$ cells among $CD8^+$ T cells. (f) Frequencies of Granzyme B (GzmB) expressing cells among $PD\text{-}1^+$ or $PD\text{-}1^-$ $CD8^+$ 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



Abstract 450 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

Conclusions 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.

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Ethics Approval This study was approved by POSTECH institutional animal care and use committee; approval number POSTECH-2020-0057.

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451 COMBINING BEMPEGALDESLEUKIN (CD122-PREFERENTIAL IL-2 PATHWAY AGONIST) AND NKTR-262 (TLR7/8 AGONIST) PAIRS LOCAL INNATE ACTIVATION WITH SYSTEMIC CD8+ T CELL EXPANSION TO ENHANCE ANTI-TUMOR IMMUNITY

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Background Previously, we demonstrated that radiation therapy (RT) combined with Bempegaldesleukin (BEMPEG;NKTR-214), a first-in-class CD122-preferential IL-2 pathway agonist, led to enhanced anti-tumor efficacy through a T cell-dependent mechanism. However, we observed only modest systemic responses to BEMPEG/RT across several murine tumor models. Therefore, we explored alternative approaches to improve systemic tumor-specific immunity. We evaluated whether intratumoral NKTR-262, a polymer-modified toll-like receptor (TLR) 7/8 agonist, combined with systemic BEMPEG treatment resulted in improved tumor-specific immunity and survival compared to BEMPEG combined with RT. We hypothesized that BEMPEG/NKTR-262 immunotherapy would promote synergistic activation of local immunostimulatory innate immune responses followed by systemic adaptive immunity to significantly improve tumor regression and overall survival.

Methods Tumor-bearing mice (CT26; EMT6) received BEMPEG (0.8 mg/kg; iv), RT (12 Gy x 1), and/or intratumoral NKTR-262 (0.5 mg/kg). Flow cytometry was used to evaluate CD4+ and CD8+ T cell activation status in the blood and/or tumor (7 days post-treatment) and NK cell activity in the tumor (1, 3 days post-treatment). The contribution of specific immune subsets was determined by depletion of CD4+, CD8+, or NK cells. CD8+ T cell activity was determined in vitro by tracking apoptosis in an Incucyte assay. Data are representative of 1–2 independent experiments (n=5–14/group) and statistical significance was determined by 1-way ANOVA (p-value cut-off of 0.05).

Results BEMPEG/NKTR-262 resulted in significantly improved survival compared to BEMPEG/RT. BEMPEG/NKTR-262 efficacy was NK and CD8+ T cell-dependent, while BEMPEG/RT primarily relied on CD8+ T cells. Response to BEMPEG/NKTR-262 was characterized by a significant expansion of activated CD8+ T cells (GzmA+; Ki-67+; ICOS+; PD-1+) in the blood, which correlated with reduced tumor size (p<0.05). In the tumor, NKTR-262/BEMPEG induced higher frequencies of GzmA+ CD8+ T cells exhibiting reduced expression of suppressive molecules (PD-1+, TIM-3+), compared to BEMPEG/RT. Indeed, CD8+ T cells isolated from BEMPEG/NKTR-262-treated tumors had greater cytolytic capacity than those from BEMPEG/RT-treated mice. CD8+ T cell expansion (blood) and activity (tumor) depended upon the initial NK response, as neither occurred in the absence of NK cells. BEMPEG/NKTR-262 uniquely induced the expansion of early and high effector NK cells.

Conclusions Combining BEMPEG with NKTR-262 lead to an early and robust NK cell expansion not observed in the BEMPEG/RT combination. The improved tumor regression and survival was dependent on the NKTR-262 driven expansion of NK cells. A clinical trial of BEMPEG/NKTR-262 for patients with metastatic solid tumors is in progress (NCT03435640).

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452 COMBINATION TREATMENT USING KISIMA^{TM} PROTEIN-BASED CANCER VACCINE AND SYSTEMIC STING AGONIST RESULTS IN PROFOUND MODULATION OF TUMOR MICROENVIRONMENT AND IMPROVED TUMOR CONTROL

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Background KISIMA^{TM} platform allows the development of protein-based cancer vaccines able to induce a potent, tumor-specific CD8 and CD4 T cells response. While the cell penetrating peptide and the Anaxa portions confer, respectively, the cell delivery and self-adjuvanticity properties, the multiantigenic domain allows the targeting of different cancer antigens, resulting in anti-tumoral efficacy in different murine models.[1] The first clinical candidate developed from KISIMA^{TM} is currently tested, together with anti-PD-1 blockade, in a phase I study in metastatic colorectal cancer patients. Stimulator of