CD25-SPECIFIC APTAMER CONJUGATED WITH CYTOTOXIC PAYLOADS SHOWS THE POTENTIAL TO MODULATE IMMUNE-SUPPRESSIVE ENVIRONMENT IN TUMORS BY DEPLETING TREGS SELECTIVELY

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Background Tregs in tumor microenvironment are associated with increased tumor progression, poor prognosis, and limited responsiveness to immunotherapies. Targeting of Tregs are shown promise in the clinic, though current approaches are not successful due to the on-target and off-tumor induction of autoimmune-related adverse effects. CD25, highly expressed on Tregs, is a potent target for the suppression and depletion of tumor-infiltrated Tregs.

Methods The binding affinity of an aptamer to rhCD25 protein was measured by bio-layer interferometry. The flow cytometry was applied to demonstrate the binding specificity and selective depletion to CD25-positive cells, and used for evaluating the effect of hPBMC activation. For in vivo study, Karpass299 subcutaneous xenograft mouse model was generated.

Results Aptamer bound specifically to rhCD25(IL-2Rα) with 9.63nM of Kd value, but not to other members of IL-2R (IL-2Rβ and IL-2Rγ) and showed no species cross-reactivity. It inhibited the function of Treg-dominant IL-2Rαβγ by blocking IL-2 binding to the receptor, and subsequently reducing pSTAT5 and TGFβ, whereas Teff-dominant IL-2Rβγ was not affected at all. Unlike anti-CD25 daclizumab, CD25-specific aptamer did not affect the activation of both CD4+ and CD8+ cytotoxic T cells in human PBMC. Along with inhibitory effects of CD25-specific aptamer itself, we designed an aptamer-drug conjugate (ApDC) to deplete Treg selectively to anticipate synergy with tumor-infiltrated Treg suppression. We generated CD25-MMAEs by using vc-MMAE as a linker-pay-load, selective depletion of CD25-positive cells was demonstrated and the increase of drug-to-aptamer ratio (DApR) from 1 to 3 showed the improvement of IC50 from 121nM to 25.5nM. Moreover, in the co-culture system with CD25-high Karpass299 and CD25-low HuT8, CD25-MMAEs showed the preferential depletion of CD25-high Karpass299 resulting in increasing the ratio of HuT89/Karpass299 in time-dependent manner. In vivo study with mouse bearing subcutaneous tumor demonstrated that CD25-MMAE (DApR 1) showed the excellent activity of tumor growth inhibition with biweekly dosing of 4 and 12 mg/kg for only 2 weeks. The complete regression of tumors was observed in both cases.

Conclusions CD25-specific aptamer and its drug conjugate CD25-MMAE showed their potential to modulate immune-suppressive tumor microenvironment by selectively inhibiting and depleting CD25-high Treg. Moreover CD25-MMAE could be developed as an option for some types of blood cancers with the high expression of CD25.

Acknowledgements This research was supported by Korea Drug Development Fund funded by Ministry of Sciences and ICT, Ministry of Trade, Industry, and Energy, and Ministry of Health and Welfare (RS-2022-00166270, Republic of Korea)

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1081