Background Interleukin-2 (IL-2) immunotherapies have revolutionized cancer treatment, but their clinical use is limited due to systemic adverse effects, such as vascular leakage syndrome.\(^1\)\(^2\) Notably, the high affinity of IL-2 for the IL-2 receptor (IL-2R)\(^\alpha\) activates regulatory T cells (Treg cells), resulting in unintended outcomes.\(^2\) To address this challenge, multiple strategies are being investigated including many non-binding drugs, and further the anti-PD-1 x IL-2 variant (IL-2v) therapeutics for cis-delivery of its IL-2v specifically to PD-1 enriched T cells.\(^3\)\(^4\) However, their clinical efficacy is still limited due to concerns about peripheral toxicity. Thus, here we present a novel immunokine MB501, which combines anti-PD-1 and IL-2v with an improved safety profile. MB501 might minimize the potential off-target immune activation by attenuating the binding affinity of the common receptor IL-2R\(^\gamma\), which is widely and highly expressed in various immune cells.

Methods The intracellular phosphorylated STAT5 level and Immune cell expansion were measured by flow cytometry using human PBMC. The secretion of granzyme B and inflammatory cytokines were measured using a cytometric bead array. The in vivo efficacy was assessed with MC38-bearing C57BL/6 and the drug tolerability was also measured in the same strain.

Results MB501 showed retained its high affinity for PD-1 while exhibiting reduced binding to IL-2R\(^\gamma\) and no binding affinity to IL-2R\(^\alpha\). MB501 showed a significant increase in pSTAT5 activity in activated CD8\(^+\) T cells comparable to anti-PD-1 x IL-2v (non-\(\alpha\)) while weakly activated pSTAT5 in resting CD8\(^+\) T cells unlike anti-PD-1 x IL-2v (non-\(\alpha\)). In addition, MB501 strongly expanded CD8\(^+\) T cells in the TME mimic condition, but not in resting CD8\(^+\) T cells in contrast to anti-PD-1 x IL-2v (non-\(\alpha\)). Moreover, the MB501 exhibited comparable IFN-\(\gamma\) and granzyme B secretion in co-culture model with PBMC and tumor, while less induction of inflammatory cytokines compared to anti-PD-1 x IFN-\(\gamma\) (WT) or anti-PD-1 x IL-2v (non-\(\alpha\)) treatment in only PBMC condition. Additionally, the murine surrogate of MB501 showed remarkable anti-tumor efficacy without body weight loss compared to the anti-muPD-1 monotherapy or combination therapy with rIL-2 in C57BL/6 mice bearing MC38 tumor. MB501 therapy yielded a complete regression (6/6) in the MC38 tumor and maintained tumor-free in rechallenge trials and well-tolerated up to a 10-fold higher dose compared to the effective dose in the same strain.

Conclusions MB501 represents a promising potential as an immunotherapeutic approach, as it has demonstrated both strong efficacy and good tolerability, along with improved safety margins.

REFERENCES

