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Background 4-1BB and Programed cell death protein-1 (PD-1) are both T cell costimulatory receptors/immune checkpoint regulators. 4-1BB/4-1BBL provides stimulatory signals while PD-1/PD-L1, suppressive ones to T lymphocytes. Various forms of agonists to 4-1BB or blockers to PD-1 have shown a potent anti-cancer activity by modulating mainly CD8\(^+\) T cells. Thus, synergistic or additive anti-cancer effect may be achieved by combining the agonists to 4-1BB and blockers to PD-1.

Methods We have tested various combinations of antibodies and proteins with consideration of overall size of the therapeutic candidates. To obtain molecularly-evolved PD-1 we first used the 3D complex between PD-1 and PD-L1 to select the major contributing PD-1 amino acids, a library targeting selected amino acids and random mutations were constructed and screened using yeast surface display. To demonstrate functions of EU505, Cell binding assay, 4-1BB bioassay and cytotoxicity assay have been conducted. Furthermore, 4-1BB/PD-1 double Knock-in (DKI) mouse and humanized mouse tumor models were used to evaluate the inhibitory activity of EU505.

Results The best anti-cancer activities were obtained by combination of an agonistic anti-4-1BB antibody and soluble PD-1 that binds to PD-L1 with high affinity. we confirmed that EU505 binds both targets independently at the same time. The result of 4-1BB bioassays, a biologically relevant MOA-based assay, showed that potent PD-L1-dependent T cell activation with EU505. In-vitro killing assays showed that EU505 selectively activated T cells which in turn killed high PD-L1-expressing cells, but not low-PD-L1-expressing ones. EU505 demonstrated much stronger tumor-killing effect compared with each component alone when we tested against human PD-L1-expressing tumor cells in a 4-1BB/PD-1 DKI mouse model and humanized mouse model. Furthermore, it was observed that number of effector CD8\(^+\) T cells increased in the peripheral blood upon EU505 injection and consequently tumor size was reduced.

Conclusions EU505, a promising anti-cancer drug, appears to enhance CD8\(^+\) T cell infiltration and activate T cells in situ at the tumor sites by binding two different targets of 4-1BB and PD-L1 simultaneously.