709 PEPTIDYL IL-2/15RβγC-RESTRICTED AGONISTS, HIGHLY-ATTENUATED AND LINKED TO ANTI-PD-1 ANTIBODIES TO ACHIEVE SELECTIVITY AND AMPLIFIED POTENCY IN STIMULATING PD-1HIGH LYMPHOCYTES

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Background Recent reports demonstrate that directing a "non-alpha" IL-2 mutant to a PD-1high CD8+ stem-like population induces proliferation, and differentiation into a highly functional cytotoxic phenotype. We previously reported small synthetic peptides, unrelated to IL-2 or IL-15, that bind IL-2/15Rβγc to induce receptor signaling. These peptides do not bind IL-2Rα and are therefore IL-2/15Rβγc-restricted agonists. We now describe fusion of potency-attenuated peptide agonists to an anti-PD-1 antibody (α-PD-1) to achieve selective targeting to PD-1high lymphocytes, and enhanced potency of IL-2R agonists acting in Cis with α-PD-1 binding.

Methods Peptidyl IL-2/15Rβγc agonists with attenuated potency due to weakened binding to either IL-2/15Rβ or γc were fused to the C-termini of both heavy chains of an α-PD-1 IgG and expressed in CHO cells. The fusion proteins retained PD-1 binding affinity comparable to the α-PD-1; and were evaluated for potency of IL-2Rβγc-dependent STAT5 phosphorylation in TF-1β cells (with undetectable PD-1 expression), and in TF-1β-derived lines expressing varying levels of PD-1. The fusion proteins were also assessed for Rβγc stimulation of CD8+ cells treated with anti-CD3 and anti-CD28 to induce elevated PD-1 expression.

Results An analysis of pembrolizumab (Pem) fused to MDK1169, a potent IL-2Rβγc agonist, showed a 15-fold increase in potency in TF-1β/PD-1+ cells. This served as an initial demonstration of the PD-1-directed, cis-acting mechanism; but the potency of MDK1169 in this construct (~500pM, EC50 pSTAT5 induction) is too high (relative to the affinity of Pem for PD-1) to achieve a more substantial selectivity for PD-1+ cells. To improve selectivity, fusions of α-PD-1 to peptide agonists with potencies as weak as 1uM on TF-1β cells were constructed. Some of these fusion proteins exhibited up to 100-fold increase in potency when tested on TF-1β/PD-1high compared to parental TF-1β cells; and addition of an excess of α-PD-1 blocked this gain in potency in the PD-1high cells. When tested on CD8+ cells activated to express elevated PD-1 levels, potency of the PD-1-directed agonists correlated with PD-1 expression.

Conclusions The malleability of peptidyl agonists makes them useful for optimizing antibody-targeted cis-acting agonists designed to produce minimal activity on non-targeted cells and high potency at targeted cells. IL-2/15Rβγc agonists directed by PD-1 binding to a stem-like highly cytotoxic tumor infiltrating CD8+ population may have useful anti-tumor applications.

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