564 POTENCY-REDUCED AND EXTENDED HALF-LIFE IL12 HETERODIMERIC FC-FUSIONS EXHIBIT STRONG ANTI-TUMOR ACTIVITY WITH POTENTIALLY IMPROVED THERAPEUTIC INDEX COMPARED TO NATIVE IL12 AGENTS

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Background Interleukin-12 (IL12) is a proinflammatory cytokine produced by activated antigen-presenting cells that induces differentiation of Th1 cells and increased proliferation and cytotoxicity of T and NK cells. Stimulation of these cells by IL12 leads to production of high levels of IFNγ. These immune-stimulating aspects of IL12 may help to establish an inflammatory tumor microenvironment critical for anti-tumor responses. Preclinical studies in mice revealed that native IL12 can dramatically shrink syngeneic tumors, however clinical studies in humans resulted in severe toxicity and a small therapeutic window, limiting response rates. Prior work at Xencor demonstrated that reduced-potency IL15/IL15Rα-Fc fusion proteins exhibited superior pharmacokinetics, pharmacodynamics, and safety in non-human primates through reduction of receptor-mediated clearance. Applying similar principles to IL12, we created IL12 heterodimeric Fc-fusions (IL12-Fc) with reduced potency to improve tolerability, slow receptor-mediated clearance, and extend half-life.

Methods IL12 is a heterodimeric protein consisting of two subunits, so we engineered IL12-Fc fusions by fusing the IL12p35 subunit to one side of a heterodimeric (and inactive) Fc domain, and the IL12p40 subunit to the other side. These Fc-fusions were tuned for optimal activity by introducing amino acid substitutions at putative receptor-interface positions and screening for reductions of in vitro potency. In vitro activity was assessed on human PBMCs by measuring signaling in a STAT4 phosphorylation assay and IFNγ production in a mixed-lymphocyte reaction (MLR). In vivo anti-tumor activity was assessed by engrafting MCF-7 cells into PBMC engrafted NSG MHC class I and II double-knockout mice and by measuring tumor volume, lymphocyte activation/proliferation, and IFNγ production over time.

Results IL12-Fc were produced with good yield and purity. An IL12-Fc potency series was created, and variants had up to a 10,000-fold reduction in STAT4 signaling potency and IFNγ production in an MLR assay compared to native IL12-Fc. Anti-tumor activity in the huPBMC-MCF7 model was achieved with potency-reduced IL12-Fc as a single-agent and in combination with anti-PD1, with weaker variants maintaining anti-tumor activity at higher dose levels. Analysis of peripheral lymphocytes indicated increased numbers of T and NK cells as well as activation of CD8+ T cells, as evidenced by upregulation of CD25. Increased expression of immune checkpoints including PD1 was also observed. Analysis of serum indicated up to 200-fold increases in IFNγ levels.

Conclusions Combined, these data indicate that potency-reduced IL12-Fc retain strong anti-tumor activity, while potentially overcoming safety and tolerability issues related to small therapeutic index associated with recombinant native IL12 or IL12-Fc agents.

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565 A NOVEL LONG-ACTING INTERLEUKIN-7 AGONIST, NT-I7, INCREASES CYTOTOXIC CD8+ T CELLS AND ENHANCES SURVIVAL IN MOUSE GLIOMA MODELS

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Background Radiation (RT) and temozolomide (TMZ), which are standard of care for patients with glioblastoma (GBM), can cause prolonged severe lymphopenia. Lymphopenia, in turn, is an independent risk factor for shorter survival. Interleukin-7 (IL-7) is a cytokine that is required for T cell homeostasis and proliferation. IL-7 levels are inappropriately low in GBM patients with lymphopenia. NT-I7 (efineptakin alfa) is a long-acting recombinant human IL-7 that supports the proliferation and survival CD4+ and CD8+ cells in both human and mice. We tested whether NT-I7 rescues treatment-induced lymphopenia and improves survival.

Methods Immunocompetent C57BL/6 mice bearing two intracranial glioma models (GL261 and CT2A) were treated with RT (1.8 Gy/day x 5 days), TMZ (33 mg/kg/day x 5 days) and/or NT-I7 (10 mg/kg on the final day of RT completion). We profiled the CD3, CD8, CD4, FOXP3 cells in peripheral blood over time. We also immunoprofiled cervical lymph nodes, bone marrow, thymus, spleen, and the tumor 6 days after NT-I7 treatment. Survival was monitored daily.

Results Median survival in mice treated with NT-I7 combined with RT was significantly longer than RT alone (GL261: 40d vs 34d, $p<0.0021$; CT2A: 90d vs 40d, $p<0.0499$) or NT-I7 alone (GL261: 40d vs 24d, $p<0.008$; CT2A: 90d vs 32d, $p<0.0154$). NT-I7 with RT was just as effective as NT-I7 combined with RT and TMZ in both GL261(40d vs 47d) and CT2A (90d vs 90d). Cytotoxic CD8+ T cells were increased in both peripheral blood (0.66 x 105 to 3.34 x 105; $P\leq0.0001$) and tumor (0.53 x 103 to 1.83 x 103; $P\leq0.0001$) in mice treated with NT-I7 when compared to control. Similarly, NT-I7 in combination with RT increased the CD8+ T cells in peripheral blood (0.658 x 105 to 1.839 x 105 $P\leq0.0001$) when compared to RT alone. There were decreases in tumor infiltrating FOXP3+ T-reg cells in mice treated with NT-I7 (1.9 x 104 to 0.75 x 104 $P\leq0.0001$) and NT-I7 + RT (1.9 x 104 to 0.59 x 104 $P\leq0.0001$) when compared to the control group without NT-I7. In addition, NT- I7 treatment increased CD8+ T cells in thymus, spleen, and lymph nodes.

Conclusions NT-I7 enhances cytotoxic CD8+ T lymphocytes systemically and in the tumor microenvironment, and improves survival. A phase I/II trial to evaluate NT-I7 in patients with high-grade gliomas is ongoing (NCT03687957).

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566 MDK-202: AN EMPIRICALLY-DESIGNED PEPTIDYL AGONIST OF THE IL-2/15βγC RECEPTOR, DEVOID OF Rα INTERACTION, UNRELATED TO IL-2 OR IL-15, AND FUSED TO AN FC-DOMAIN FOR PK ENHANCEMENT

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