Background Interleukin-12 (IL-12) is a potent cytokine that can promote innate and adaptive anti-cancer immunity, but its clinical development has been limited by toxicity when delivered systemically. Intratumoral (IT) administration can expand the therapeutic window of IL-12 and other cytokines but is in turn limited by rapid clearance from the tumor, thereby reducing efficacy, necessitating frequent administration, and increasing systemic accumulation. We recently described an approach called ‘anchored immunotherapy’ in which an engineered IL-12 variant is complexed with the vaccine adjuvant aluminum hydroxide (alum) to form a locally retained cytokine depot that induces potent therapeutic activity in syngeneic murine tumors after only 1 or 2 IT injections. Here, we provide additional characterization of the retention kinetics and mechanism of action of alum-complexed IL-12.

Methods Human or murine IL-12 was genetically fused to a phosphorylated alum-binding peptide (IL-12-ABP) and mixed with aluminum hydroxide to form the therapeutic complexes ANK-101 (human) or mANK-101 (mouse). Local retention and biodistribution of labeled mANK-101 after IT administration in syngeneic murine tumors was measured by IVIS and SPECT imaging. Intratumoral immune changes were detected by flow cytometry, IHC, Nanostring, and scRNA-seq to characterize mANK-101’s mechanism of action. Safety of ANK-101 was assessed in cynomolgus macaques after subcutaneous injection. Statistical comparisons between groups were performed using one-way ANOVA.

Results ANK-101 induces IFNγ expression from human PBMCs, purified T-cells, and NK-cells with similar potency as native IL-12. Following IT administration in murine tumor models, mANK-101 complexes are retained locally for multiple weeks while unanchored IL-12-ABP protein is cleared within hours as detected by IVIS or SPECT imaging. This extended retention leads to prolonged expression of IFNγ and other pro-inflammatory cytokine and chemokines for >1 week. We hypothesized that this would result in improved CD8+ T cell recruitment, which was tested by intratumoral immunophenotyping by IHC and flow cytometry. We found that mANK-101 treatment led to robust CD8 T-cell infiltration and an increased CD8/Treg ratio that correlated with anti-tumor efficacy. Gene expression and scRNA-seq analyses suggested a profound remodeling of the tumor microenvironment after mANK-101 treatment with T-cell and NK-cell activation, shifts to pro-inflammatory myeloid cell phenotypes, and increased markers of antigen presentation and co-stimulation. Subcutaneous administration of ANK-101 in cynomolgus macaques was well tolerated.

Conclusions Anti-tumor activity of locally retained IL-12/alum is mediated by recruitment and activation of lymphocytes and myeloid immune cells. Anchored immunotherapy may represent a general approach to improve the therapeutic potential of immuno-oncology agents.

REFERENCES