INTRATUMORAL ADMINISTRATION OF ALUM-TETHERED ENGINEERED INFLAMMATORY CYTOKINES SAFELY ELICITS POTENT LOCAL AND SYSTEMIC IMMUNITY

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Background While immune checkpoint blockade therapy has improved progression-free survival in patients suffering from cancer over other treatments,^{1–4} these typically elicit durable responses in only minority of patients, in part because of the highly immunosuppressive tumor microenvironment (TME).^{5–6} Rational combinations with inflammatory cytokines can relieve some immunosuppression,^{7–8} but systemic dosing of these proteins is impeded by severe immune-related adverse events (irAE).^{9–14} One approach to focus the activity of immunostimulatory agents in tumors while lowering systemic toxicity is to administer these drugs intratumorally. However, intratumoral injection alone generally achieves limited persistence in the TME, as drugs quickly clear from the tumor via lymphatics and the tumor vasculature, rapidly leading to harmful accumulation in the circulation.^{15–16} Thus, approaches to promote in vivo retention of intratumorally administered drugs are necessary to maximize local stimulation.

Methods We engineered Interleukin-12 (IL-12) with a peptide tag containing multiple phosphoserine (pSer) residues, through in-cell phosphorylation during recombinant expression in mammalian cells. We then inoculated mice with B16F10, or Ag104A tumors, treated established tumors intratumorally with a single dose of IL-12 mixed with alum, and monitored the tumor size and weights over time. Immunophenotyping of tumors and draining lymph nodes (dLNs) was conducted at several timepoints after treatment. Tumors and serum were also collected to perform bead-based Luminex analysis of many cytokines (including IL-12 and IFN- γ).

Results Cytokines with pSer tags bind tightly to the common vaccine adjuvant aluminum hydroxide (alum) via ligand exchange (72% pSer-IL-12 vs 3.5% IL-12, $P < 0.0001$). Alum particles form a physical depot at injection sites that is persistent over weeks. So, intratumoral injection of pSer-IL-12-loaded alum led to >400-fold greater retention of protein relative to unanchored pSer-IL-12 with 2-fold lower serum ALT (a biomarker for IL-12 systemic toxicity). Further, a single dose of alum-tethered pSer-IL-12 induced 5-fold greater IFN- γ secretion ($P = 0.0031$) at the tumor primarily by CD8⁺ T cells and doubled ($P < 0.0001$) the proportion of tumor antigen-carrying, CD86-expressing CD103⁺ DCs in dLN relative to free IL-12. Further, intratumoral alum/pSer-IL-12 therapy enhanced responses to checkpoint blockade (anti-PD1), leading to a cure rate of 52% in poorly immunogenic B16F10 tumors compared to 0% for free IL-12. Local intratumoral treatment of ipsilateral tumors in mice also led to clearance of large, untreated contralateral tumors in 9/15 animals for alum/pSer-IL-12 vs. 5/17 animals for unanchored IL-12 ($P = 0.04$).

Conclusions Thus, intratumoral treatment with alum-anchored cytokines presents a safe, tumor-agnostic approach to improve local and systemic anti-cancer immunity.

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Ethics Approval All animal studies and procedures were carried out following federal, state and local guidelines under an institutional animal care and use committee-approved animal protocol (Protocol no. 0720-070-23) by the Committee of Animal Care at MIT.

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