Background Immune-stimulating antibody conjugates (ISACs) consist of antibodies conjugated to immune stimulants and are designed to induce antitumor immune response. Despite promising preclinical results, ISAC clinical development has been hampered by systemic toxicities or lack of efficacy. Substituted purines have previously been identified as a privileged scaffold to elicit TLR7 activation. Here, we demonstrate newly designed purine-based TLR7 agonists conjugated to trastuzumab which show significant tumor volume reduction in a HER2-high gastric cancer xenograft model without associated body weight loss (BWL) in healthy mice.

Methods A library of TLR7 agonists was generated by varying substituents at C2- and N9-positions of a common 6-amino-8-hydroxy-purine scaffold, and the structure-activity relationship was studied in vitro using human and mouse TLR7 reporter gene assays (RGAs) as well as measuring cytokine secretion from human peripheral blood mononuclear cells (PBMCs) and mouse splenocytes. Lead TLR7 agonists were conjugated to trastuzumab, and the resulting ISACs were evaluated in vitro for their abilities to induce the production of interleukin-6 (IL-6) from human PBMCs or mouse splenocytes co-cultured with NCI-N87 tumor cells. Lead TLR7 agonists were conjugated to trastuzumab with a drug-to-antibody ratio of ~4 using cleavable or non-cleavable linkers. ISACs capable of inducing IL-6 production from PBMCs or splenocytes co-cultured with tumor cells were further tested in an NCI-N87 xenograft model in comparison to unconjugated trastuzumab and trastuzumab conjugated with the same linker-payload as NJH395, a clinical benchmark ISAC. Selected ISACs were tested for efficacy (single iv injection at 2.5 mg/kg) in mice bearing NCI-N87 tumors (figure 1) and for tolerability (single iv injection at 3, 15, and 45 mg/kg) in healthy mice (figure 2).

Results We prepared ~220 new TLR7 agonists with different substituents at C2- and N9-positions of the purine scaffold. Compounds with IC50 <100 nM in both human and mouse TLR7 RGAs were further screened for their abilities to induce production of cytokines in PBMCs and mouse splenocytes. Certain substituents were found to be highly immunostimulatory in both human and murine settings. Lead TLR7 agonists were conjugated to trastuzumab with a drug-to-antibody ratio of ~4 using cleavable or non-cleavable linkers. ISACs capable of inducing IL-6 production from PBMCs or splenocytes co-cultured with tumor cells were further tested in an NCI-N87 xenograft model in comparison to unconjugated trastuzumab and trastuzumab conjugated with the same linker-payload as NJH395, a clinical benchmark ISAC. Selected ISACs were tested for tolerability in healthy mice with a lead ISAC (Trastuzumab-MTkPABC-P5) identified, capable of inducing tumor regression without causing BWL.

Conclusions We demonstrated the potential of using novel purine-based TLR7 agonists as payloads for ISACs. In contrast to other TLR7-agonist conjugates, our lead ISAC appears to have a sufficiently wide therapeutic window displaying efficacy in an NCI-N87 xenograft model at 2.5 mg/kg without causing BWL in healthy mice at 45 mg/kg.

Ethics Approval All animal studies were performed in accordance with Institutional Animal Care and Use Committee (IACUC)-approved protocols.