ABSTRACT 1559

TLR7/8 immune stimulating antibody conjugates elicit robust myeloid activation and durable anti-tumor immunity

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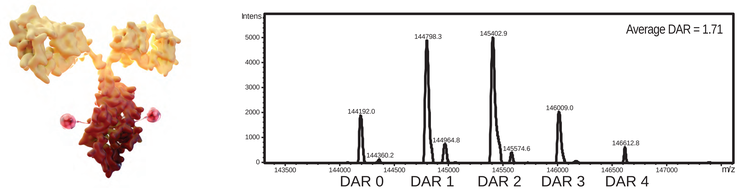
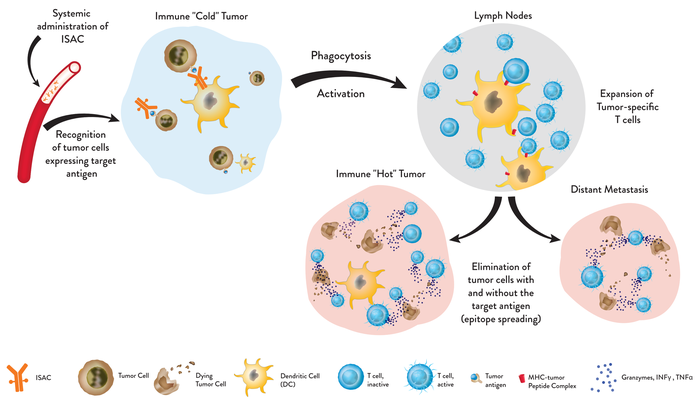
ABSTRACT

Recent studies indicate that local delivery of immunostimulatory adjuvants can activate tumor resident antigen presenting cells (APCs), driving uptake, processing and presentation of tumor neoantigens to T cells that mediate anti-tumor immunity. To overcome challenges associated with intratumoral delivery of such adjuvants, we developed a novel class of systemically delivered TLR immune-stimulating antibody conjugates (ISACs) that comprise a TLR7/8 agonist conjugated to tumor-targeting monoclonal antibodies. *In vitro* co-cultures of human cancer cell lines and leukocytes revealed that ISACs potently activate primary myeloid APCs, leading to increased co-stimulatory molecule expression and secretion of pro-inflammatory cytokines. Surprisingly, ISACs also induced DC differentiation from monocytes, as measured by changes in cellular morphology and DC associated surface markers. Finally, we demonstrated *in vivo* efficacy in xenograft and syngeneic tumor models in which ISAC treatment led to tumor clearance and development of immunologic memory. These results provide a strong rationale for this technology as a platform for cancer immunotherapy.

IMMUNE STIMULATING ANTIBODY CONJUGATES



PROPOSED MECHANISM OF ACTION



HUMAN *IN VITRO* EXPERIMENTAL DESIGN

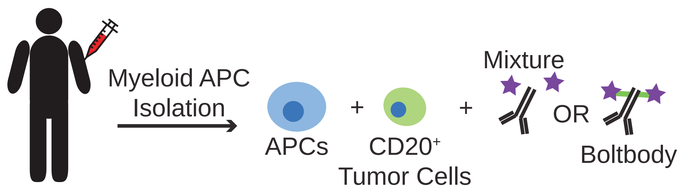


Figure 1: Assessment of ISAC Activity. Myeloid APCs were isolated from healthy donor via negative selection and co-cultured with CFSE-labeled CD20+ tumor cells. After 18 hours, cells were assessed for cellular activation and differentiation via flow cytometry and ELISA (Figures 2-4).

HUMAN *IN VITRO* RESULTS

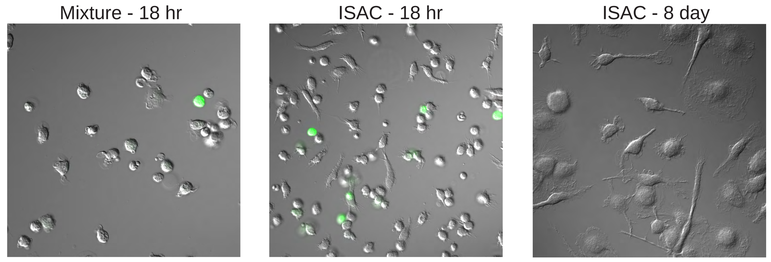


Figure 2: ISACs elicit distinct changes in cellular morphology.

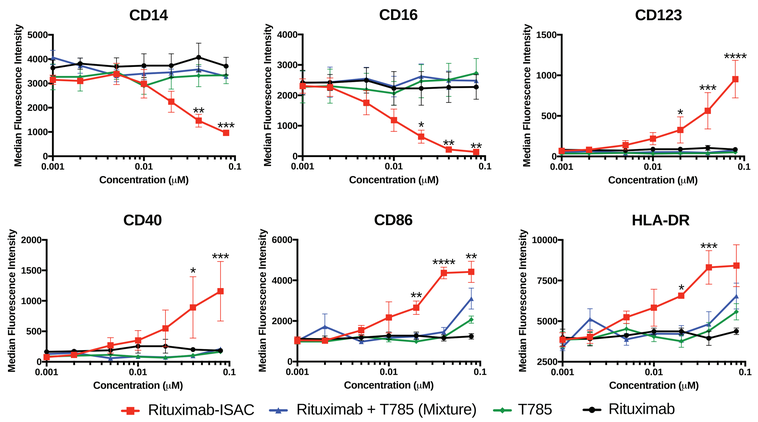


Figure 3: ISACs elicit myeloid activation and DC differentiation.

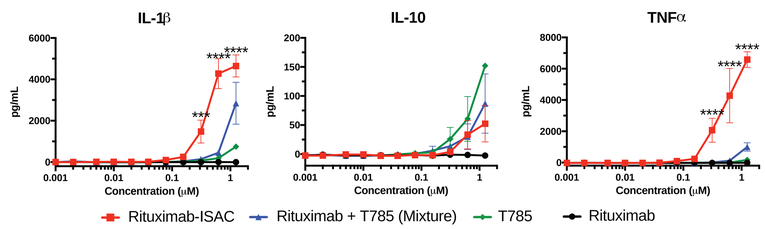


Figure 4: ISACs elicit proinflammatory cytokine secretion.

IN VIVO EXPERIMENTAL DESIGN

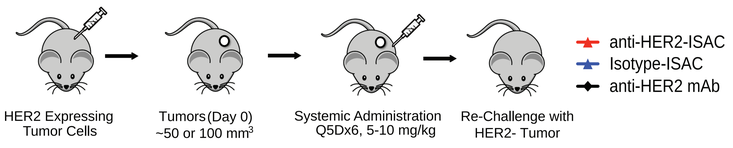


Figure 5: Assessment of ISAC Anti-Tumor Activity. Myeloid-mediated anti-tumor efficacy was assessed in mice that lacked B/T/NK cells with huHER2+ tumor cell lines (Q5Dx6, 5 mg/kg - Figure 6). Immunologic memory was assessed in WT mice with rHER2+ CT26 cells (Q5Dx6, 10 mg/kg) following rechallenge with parental CT26 cells (Figure 7).

IN VIVO RESULTS

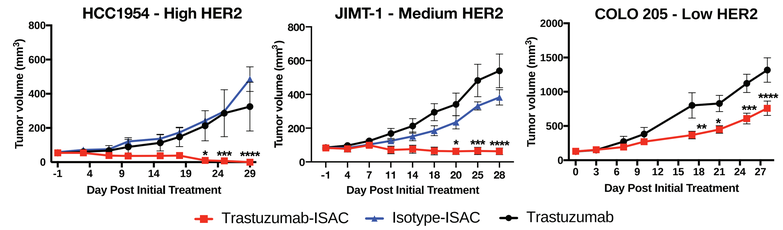


Figure 6: ISACs mediate efficacy in trastuzumab-resistant models.

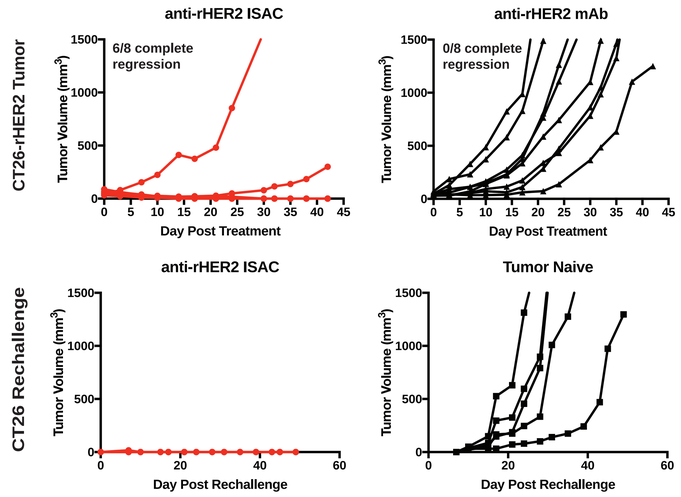
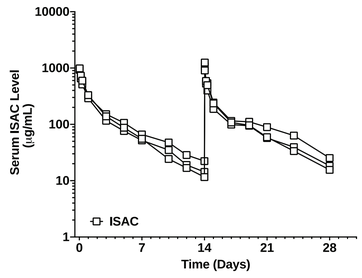


Figure 7: ISACs lead to tumor clearance, epitope spreading and govern immunologic memory in syngeneic models.

NHP PHARMACOKINETICS



3 cynomolgus monkeys dosed every 14 days by intravenous infusion with 30 mg/kg ISAC

Figure 8: ISAC pharmacokinetics in multi-dose NHP study.

KEY FINDINGS

1. ISACs elicit robust myeloid activation and DC differentiation in human cells
2. ISAC treatment leads to anti-tumor efficacy in trastuzumab resistant models
3. ISAC-mediated anti-tumor immunity supports epitope spreading beyond ISAC-target antigen
4. Tumor clearance following ISAC treatment generates immunologic memory
5. ISAC has acceptable pharmacokinetics and is well tolerated in NHP study