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

ABSTRACT
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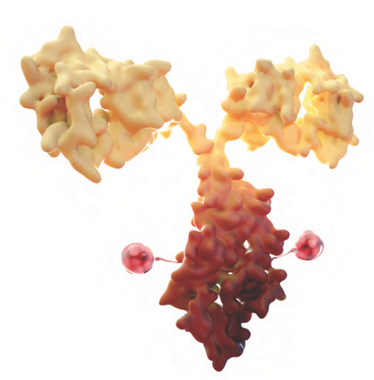
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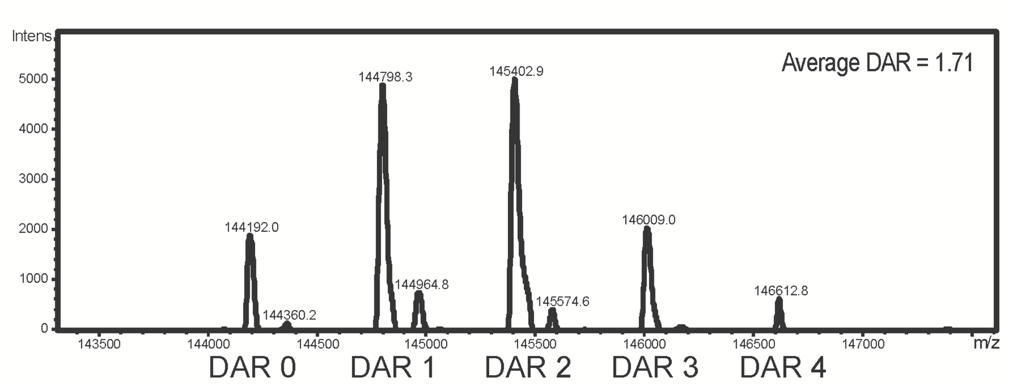
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ABSTRACT

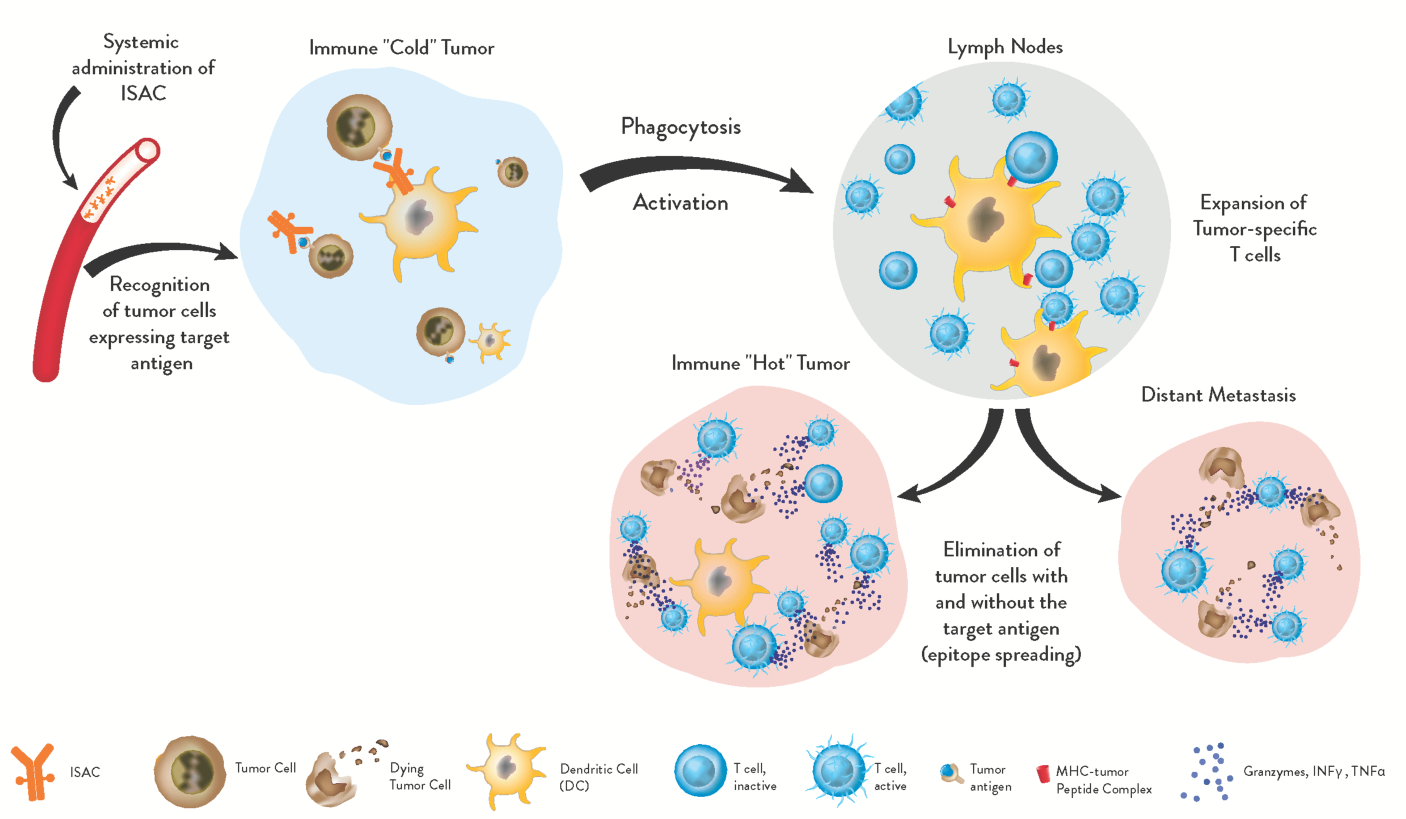
Recent studies indicate that local delivery of immunostimulatory adjuvants can activate tumor resident antigen presenting cells (APCs), driving uptake, processing and presentation of tumor neoantigens to T cells that mediate anti-tumor immunity. To overcome challenges associated with intratumoral delivery 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




Intens.
Average DAR = 1.71
144192.0
144360.2
144798.3
144964.8
145402.9
145574.6
146009.0
146612.8
m/z
DAR 0
DAR 1
DAR 2
DAR 3
DAR 4

PROPOSED MECHANISM OF ACTION


Systemic administration of ISAC
Immune "Cold" Tumor
Recognition of tumor cells expressing target antigen
Phagocytosis
Activation
Lymph Nodes
Expansion of Tumor-specific T cells
Immune "Hot" Tumor
Distant Metastasis
Elimination of tumor cells with and without the target antigen (epitope spreading)
ISAC
Tumor Cell
Dying Tumor Cell
Dendritic Cell (DC)
T cell, inactive
T cell, active
Tumor antigen
MHC-tumor Peptide Complex
Granzymes, INFγ, TNFα

HUMAN *IN VITRO* EXPERIMENTAL DESIGN

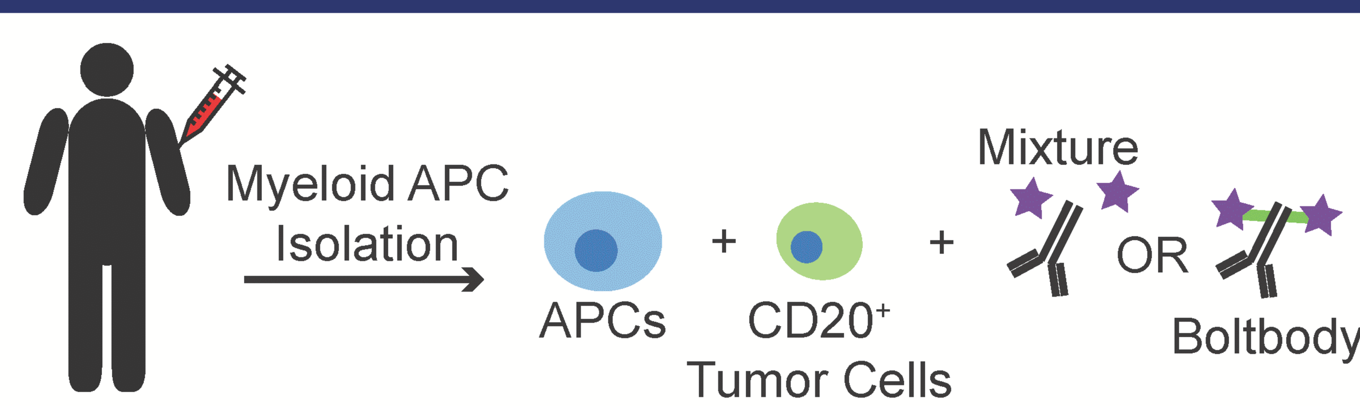

Myeloid APC Isolation
APCs
CD20+ Tumor Cells
Mixture
OR
Boltbody

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

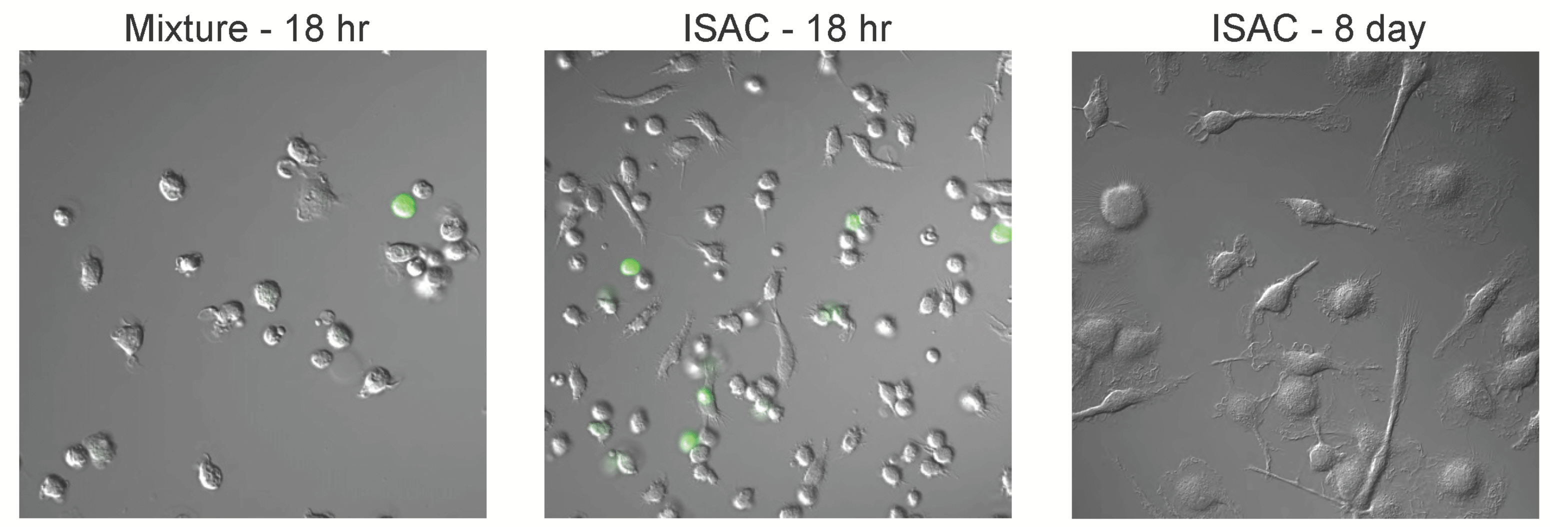

Mixture - 18 hr
ISAC - 18 hr
ISAC - 8 day

Figure 2: ISACs elicit distinct changes in cellular morphology.

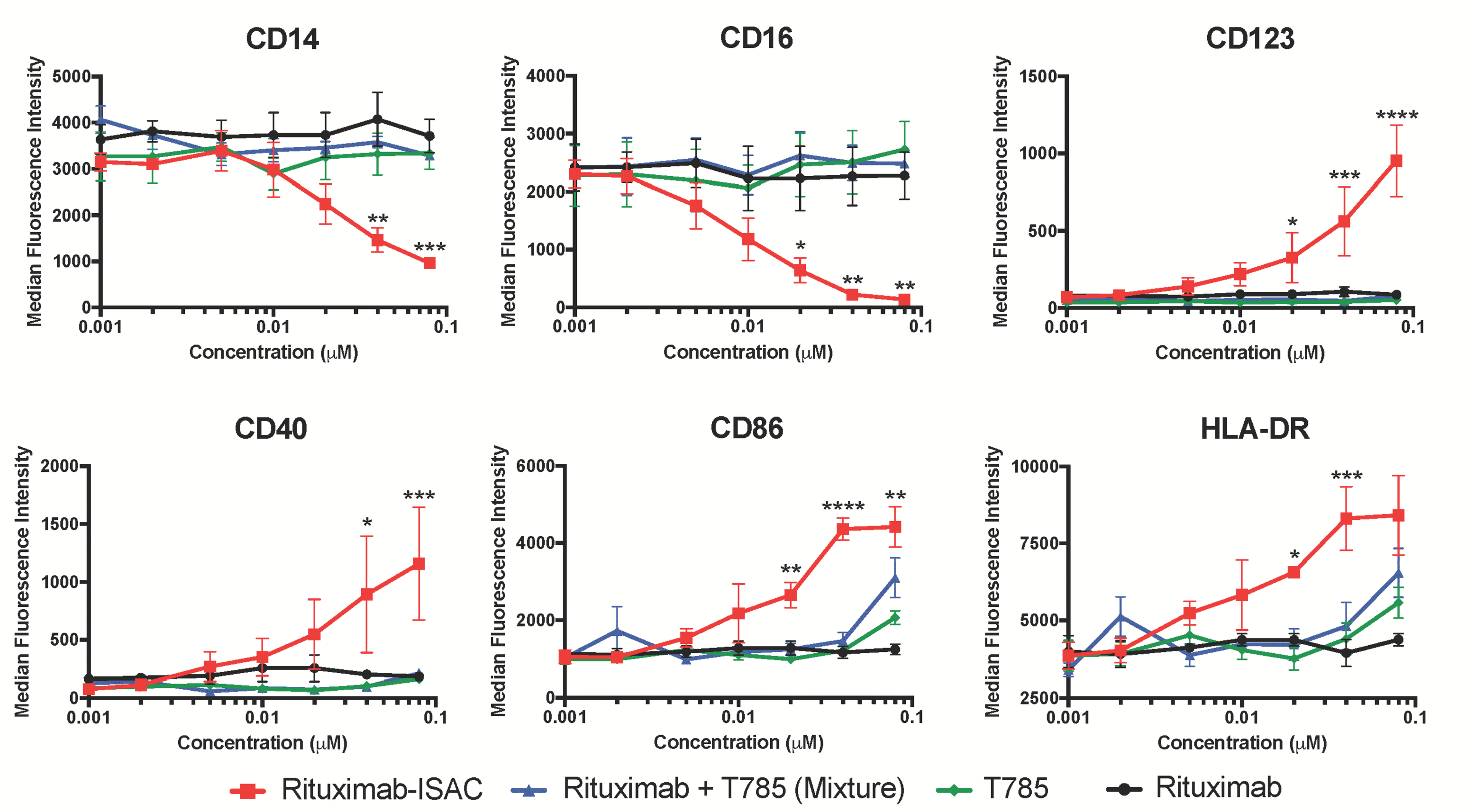

CD14
CD16
CD123
CD40
CD86
HLA-DR
Median Fluorescence Intensity
Concentration (μM)
Rituximab-ISAC
Rituximab + T785 (Mixture)
T785
Rituximab

Figure 3: ISACs elicit myeloid activation and DC differentiation.

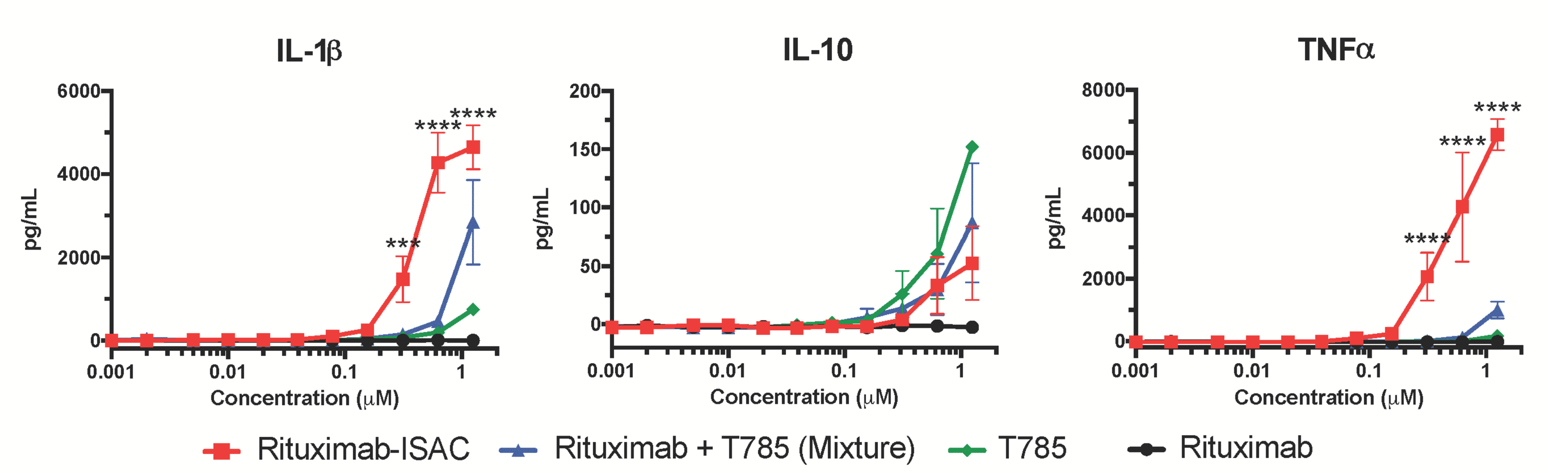

IL-1β
IL-10
TNFα
pg/mL
Concentration (μM)
Rituximab-ISAC
Rituximab + T785 (Mixture)
T785
Rituximab

Figure 4: ISACs elicit proinflammatory cytokine secretion.

IN VIVO EXPERIMENTAL DESIGN

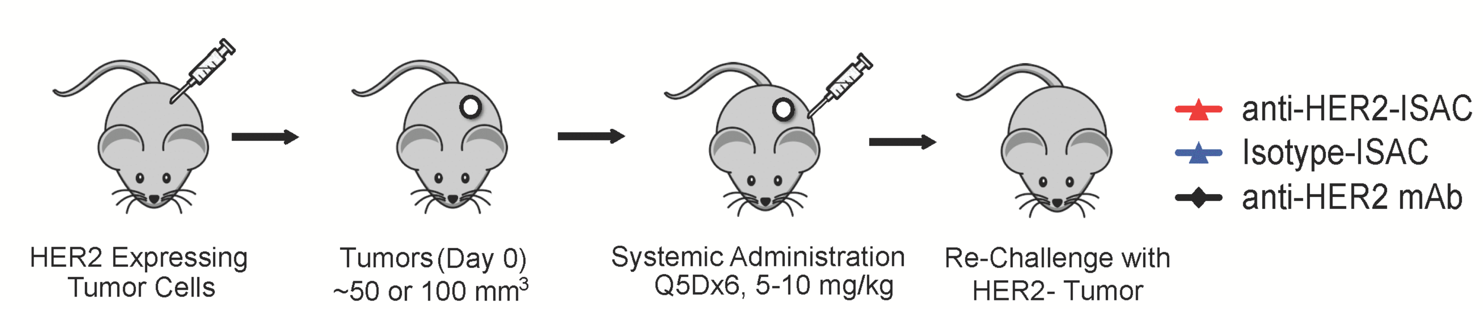

HER2 Expressing Tumor Cells
Tumors (Day 0) ~50 or 100 mm3
Systemic Administration Q5Dx6, 5-10 mg/kg
Re-Challenge with HER2- Tumor
anti-HER2-ISAC
Isotype-ISAC
anti-HER2 mAb

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

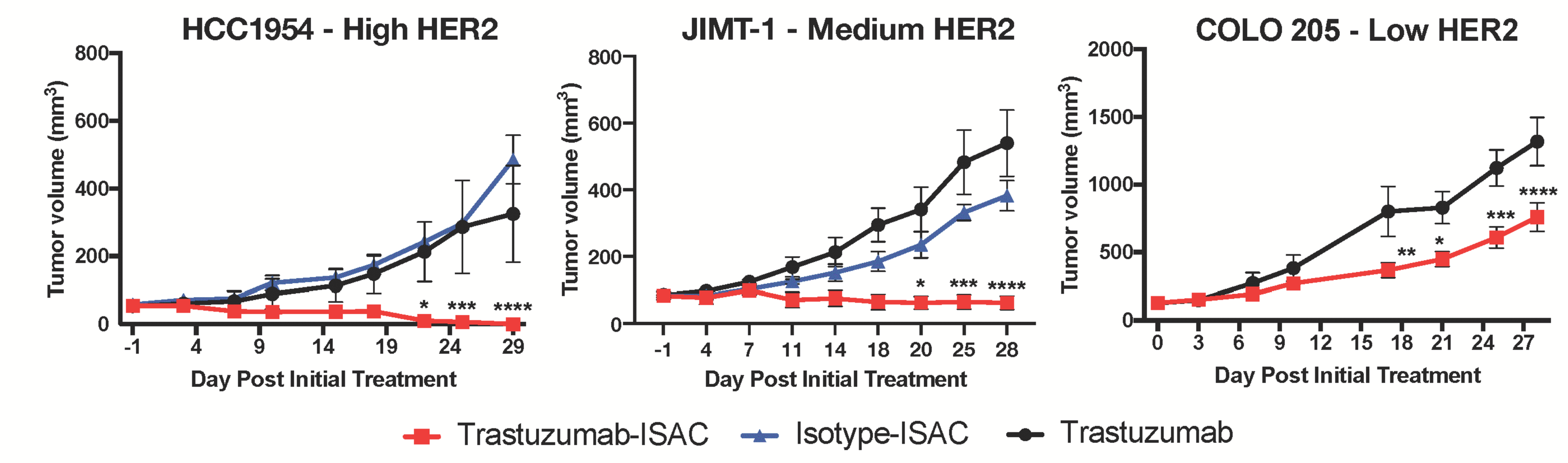

HCC1954 - High HER2
JIMT-1 - Medium HER2
COLO 205 - Low HER2
Tumor volume (mm3)
Day Post Initial Treatment
Trastuzumab-ISAC
Isotype-ISAC
Trastuzumab

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

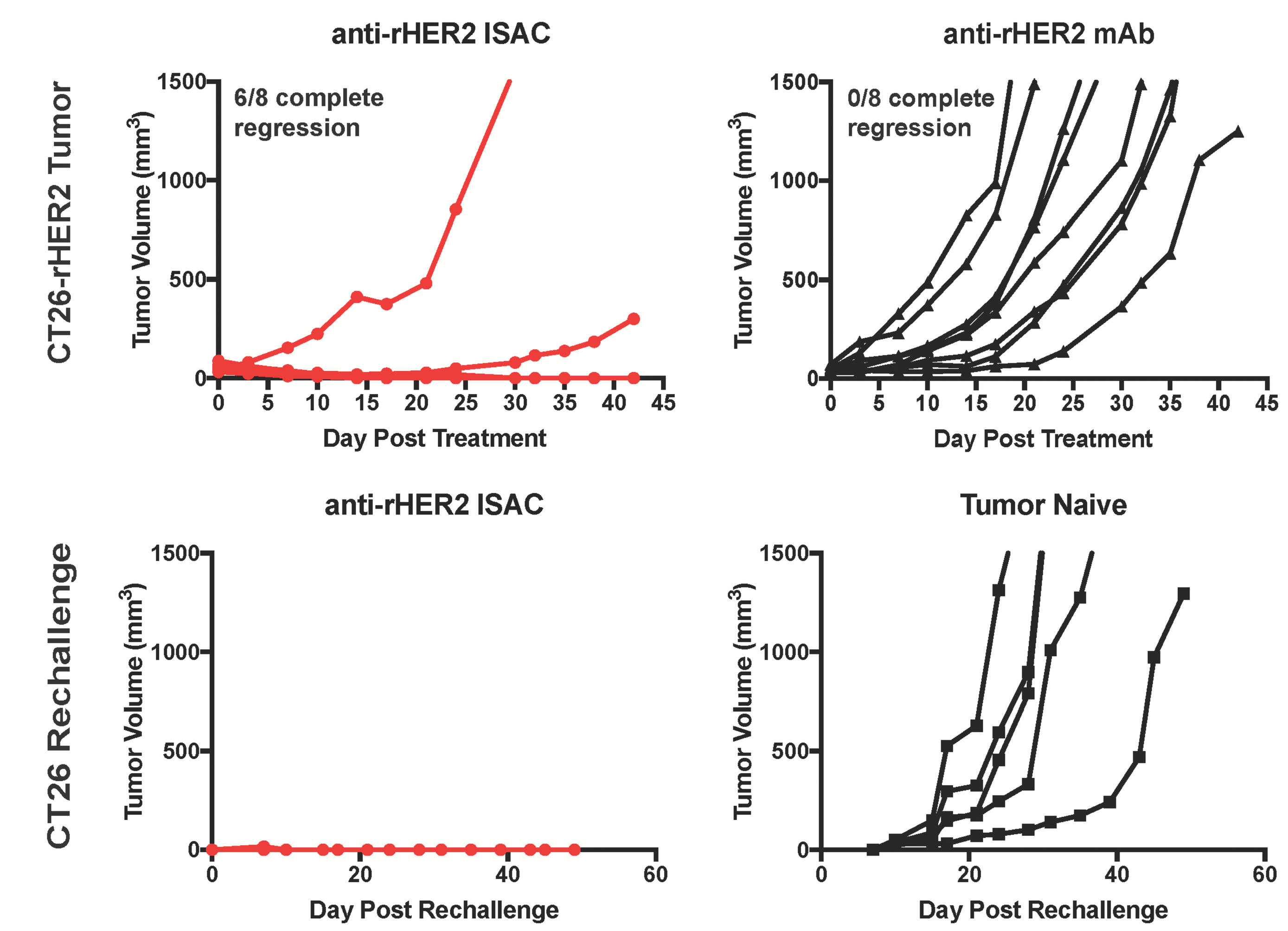
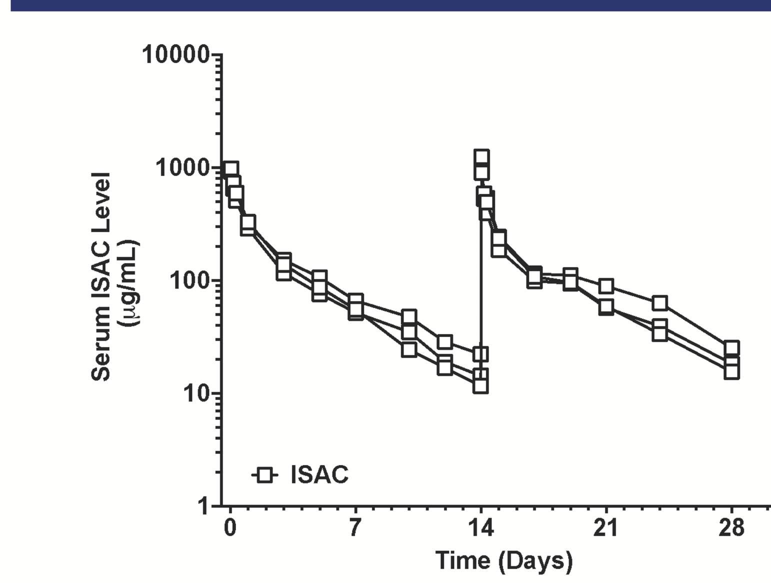

anti-rHER2 ISAC
anti-rHER2 mAb
CT26-rHER2 Tumor
6/8 complete regression
0/8 complete regression
Tumor Volume (mm3)
Day Post Treatment
anti-rHER2 ISAC
Tumor Naive
CT26 Rechallenge
Day Post Rechallenge

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

NHP PHARMACOKINETICS


Serum ISAC Level (μg/mL)
ISAC
Time (Days)

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