Covalent attachment of a TLR7/8 agonist to tumor-targeting antibodies drives potent anti-tumor efficacy by synergistically activating FcγR- and TLR- signaling and enables safe systemic administration

ABSTRACT 603

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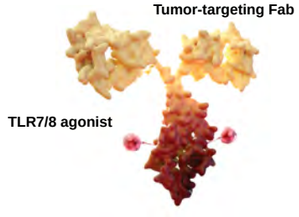
Introduction

Immune stimulating antibody conjugates (ISACs) covalently attach TLR7/8 immune stimulants to tumor-targeting antibodies. ISACs can be delivered systemically and act locally in the tumor microenvironment by requiring the following biological steps to elicit immune activation: 1) tumor antigen recognition, 2) Fc receptor mediated phagocytosis by myeloid antigen presenting cells (APCs), and 3) activation of endosomal TLR7 and TLR8. Here, we demonstrate that covalent attachment of our TLR7/8 agonist to tumor-targeting antibodies not only enables the resulting ISACs to be safely administered systemically in preclinical models, but also unexpectedly promotes synergy between the FcγR and TLR pathways that results in amplified anti-tumor immunity in mice and robust immune activation in human leukocytes as compared to the co-administration of the components.

Boltbody™ Immune-Stimulating Antibody Conjugates

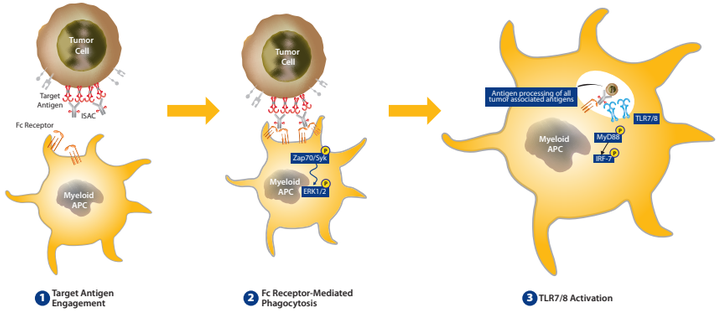


ISAC stimulation requires three-step authentication to mediate effective activation of myeloid APCs:

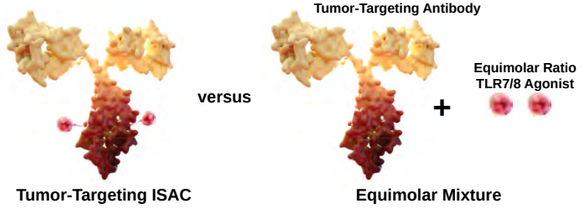
1. Tumor antigen binding via Fab region of monoclonal antibody

2. Fc receptor mediated phagocytosis by myeloid antigen presenting cells

3. Activation of endosomal TLR7 and TLR8



Comparing covalently-linked ISACs to an equimolar mixture



Systemically delivered ISACs outperform mixture in vivo

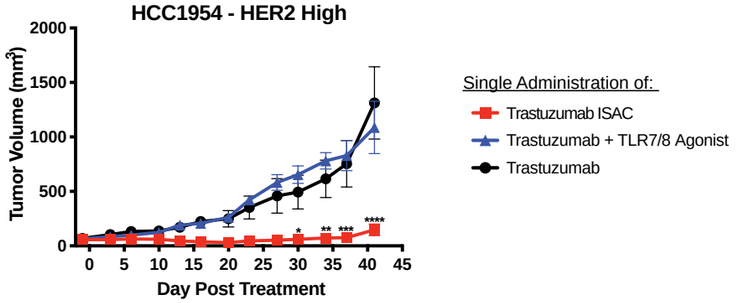


Figure 1: Systemically-delivered ISACs outperform locally-administered mixture. SCID/beige mice were dosed once with 5 mg/kg of ISAC (intraperitoneal), trastuzumab (intraperitoneal), or an equimolar mixture of trastuzumab (intraperitoneal) and TLR7/8 agonist (intratumoral). Data are shown as mean ± SEM with 3-5 mice per group.

ISACs promote amplified signaling in FcγR and TLR pathways

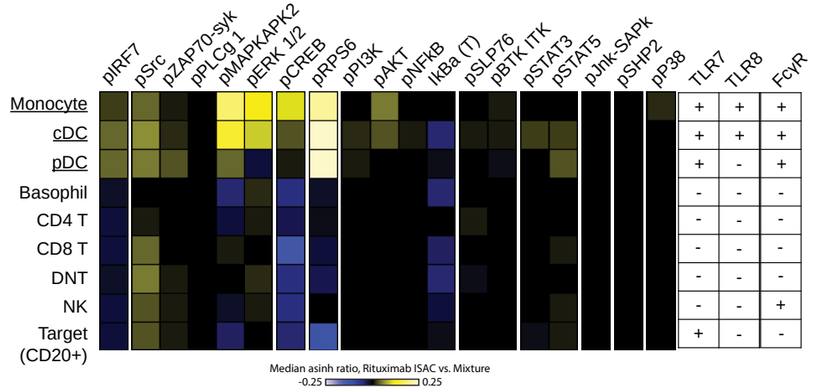


Figure 2: Rituximab ISACs elicit amplified intracellular signaling relative to mixture in leukocytes expressing requisite TLR and FcγRs. Human PBMCs were co-cultured with CD20+ Toledo tumor cells at a 1:1 ratio and stimulated for 15 minutes with rituximab ISAC or an equimolar mixture of rituximab and TLR7/8 agonist.

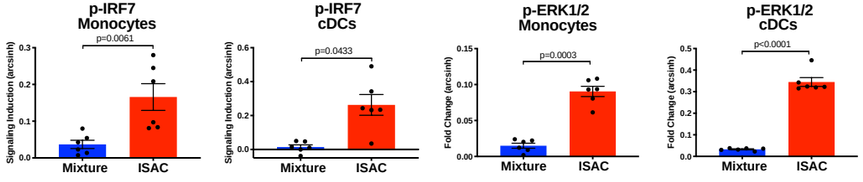


Figure 3: Rituximab ISACs elicit amplified FcγR and TLR-related intracellular signaling in monocytes and cDCs. Human PBMCs were co-cultured with CD20+ Toledo tumor cells at a 1:1 ratio and stimulated for 15 minutes. Signaling induction is reported as the arcsinh fold change relative to the unstimulated co-culture; data shown as mean ± SEM.

ISACs promote synergy between FcγR and TLR signaling pathways

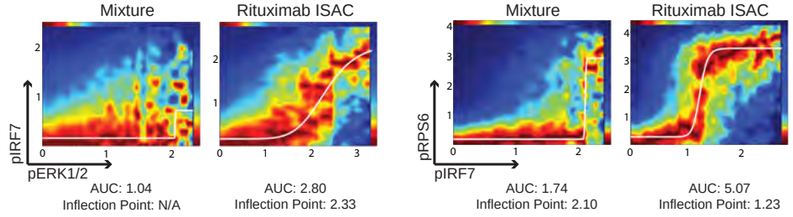


Figure 4: DREMI/DREVI analysis of monocytes reveals ISAC-induced synergy between FcγR and TLR-related pathways. ISAC stimulation led to a reduced threshold for activation (inflection point of the curve fit) and stronger signal intensity (AUC) as compared to the mixture. Signal density is visually scaled as high (red) to low (blue).

ISAC-induced signaling requires FcγR-mediated phagocytosis to access and activate TLR7/8

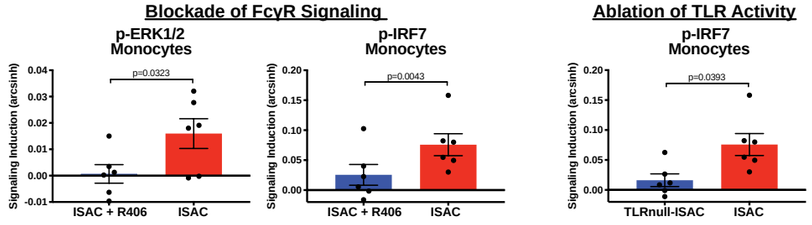


Figure 5: Rituximab ISACs require Fc-mediated entry and TLR ligation to induce amplified signaling. Human PBMCs were co-cultured with CD20+ Toledo tumor cells were stimulated for 15 minutes and analyzed by CyTOF. For FcγR signaling blockade, cells were pre-treated with R406, a small molecule inhibitor of Syk.

ISACs require FcγR-mediated entry and functional TLR7/8 agonism to mediate anti-tumor efficacy

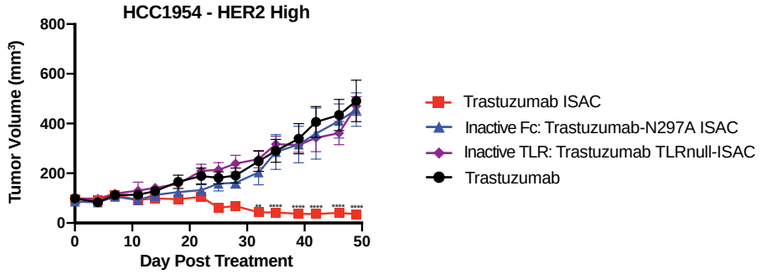


Figure 6: ISACs require functional Fc and TLR agonist in vivo. NSG mice were dosed systemically with 5 mg/kg every 5 days through day 25. Data are shown as mean ± SEM with 5 mice per group.

Conclusions

1. ISACs provide unexpected biological advantages over mixture of antibody and TLR agonist through promotion of synergy between FcγR and TLR pathways (see LeBlanc et al, Abstract #605 for further in vivo demonstration).
2. Systemically-administered ISACs outperform the locally-administered mixture in vivo, and rely on Fc receptor-driven phagocytosis and functional TLR agonism to mediate anti-tumor efficacy.
3. BDC-1001, a HER2-targeted ISAC, is being assessed in an ongoing Phase 1/2 trial (NCT04278144), as described in Sharma et al, Trial in Progress Abstract #401.