Systemically administered HER2-targeted ISACs provoke a rapid, local response that engages the innate and adaptive arms of the immune system to eradicate tumors in preclinical models

ABSTRACT 605

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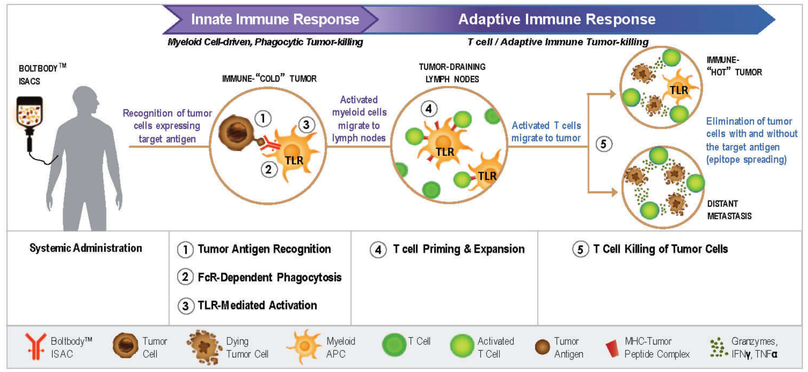
Background

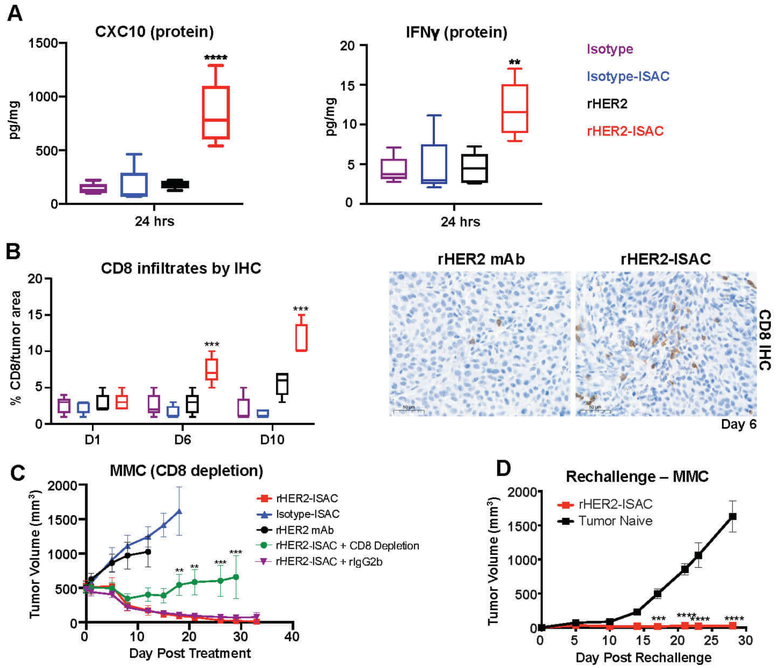
Immune-stimulating antibody conjugates (ISACs) covalently attach immune stimulants to tumor-targeting antibodies such as trastuzumab. We have shown that HER2-targeted TLR7/8 ISACs elicit robust myeloid activation and tumor eradication in a TLR- and Fc-dependent manner in trastuzumab-resistant and HER2-low models. Upon treatment with ISACs, T cell-mediated immunological memory extends to tumor antigens beyond HER2 [1]. Here we describe the ISAC mechanism of action in vivo that leads to eradication of tumors in mice.

Methods: Established HCC1954 (trastuzumab-resistant xenograft) and MMC (rHER2+ syngeneic tumor in a rHER2-expressing host) tumors treated with anti-HER2 ISACs or appropriate controls were assessed for gene expression by NanoString Pan-Cancer Immune Profiling panel comprising 750 genes related to tumor immune biology. Tumor cytokines were measured using MesoScale Discovery (MSD) technology, and immune cell infiltrates were assessed by immunohistochemistry (IHC). Anti-tumor efficacy was assessed after depletion of CD8+ T cells and phagocytes. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

Mechanism of Action



ISACs activate adaptive immunity and T cell memory



rHER2-targeted ISAC stimulates a T cell response in MMC syngeneic tumors.

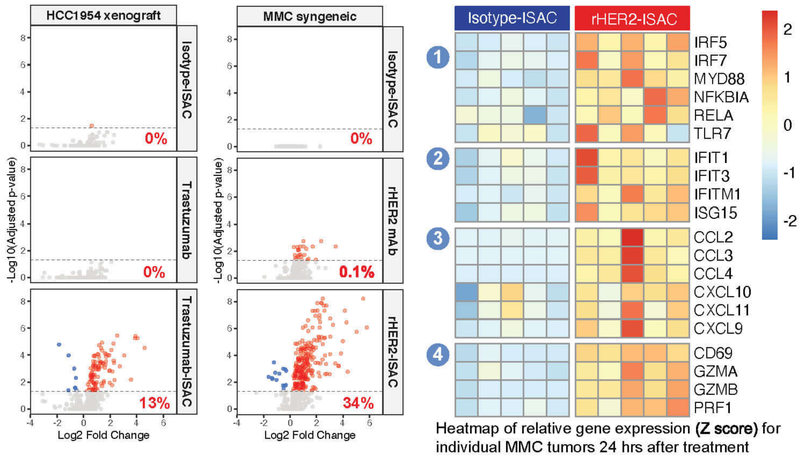
(A) T cell chemokine CXCL10 and **the marker of T cell activation** interferon-γ **increase** in the tumor 24 hours after rHER2 ISAC treatment.

(B) CD8+ T cells are recruited after rHER2-targeted ISAC treatment, shifting the TME from **immune 'cold' to 'hot'.**

(C) CD8+ T cell depletion abrogated anti-tumor efficacy of the rHER2-targeted ISAC.

(D) Mice cured by rHER2-ISAC treatment were protected against tumor rechallenge showing **immune memory**.

HER2-directed ISACs induced robust, target-dependent immune activation



% upregulated genes vs isotype mAb in tumors 24 hrs after treatment

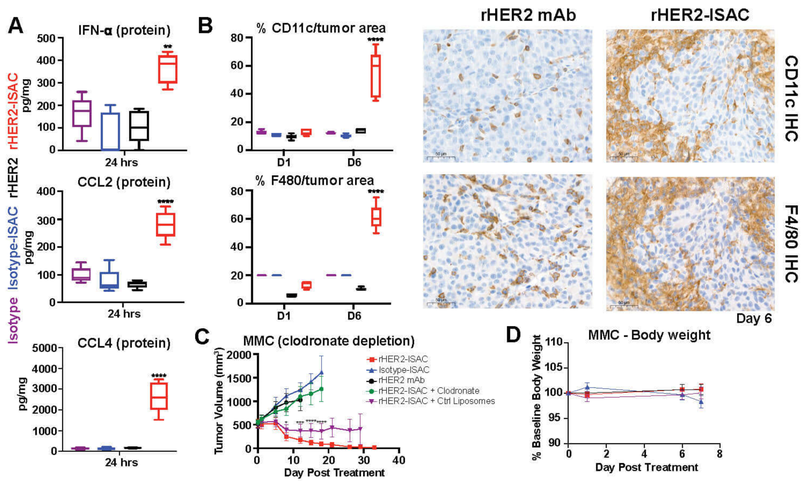
- HER2-targeted ISACs dramatically upregulated immune-related genes in both HCC1954 xenograft and MMC syngeneic tumors.
- In the absence of tumor targeting (Isotype-ISAC) or TLR agonism (HER2 mAbs) changes in gene expression were minimal.

Heatmap of relative gene expression **(Z score)** for individual MMC tumors 24 hrs after treatment

Anti-HER2 ISAC treatment led to activation of pathways indicative of TLR7/8 agonism, FcγR signalling and effector engagement.

1. TLR7/8 transcription pathway
2. Type 1 interferon-inducible genes
3. Markers of myeloid cell activation
4. Markers of effector cells, including T cells

ISACs activate the innate immune system, leading to anti-tumor efficacy



HER2-targeted ISACs provoke a strong myeloid response in the MMC syngeneic tumor model.

(A) rHER2-ISAC treatment provoked expression of interferon-α and the myeloid chemokines CCL2/4, a sign of APC activation.

(B) This was followed by significant recruitment of myeloid cells.

(C) rHER2-ISAC treatment regressed large MMC tumors. Activity was abrogated by depletion of phagocytic cells.

(D) Systemically delivered ISACs were well-tolerated. with no body weight loss.

Similar results were obtained with the HCC1954 xenograft model.

Conclusions

- ISACs trigger a robust, tumor target-dependent activation of the immune system.
- ISACs locally engage both the innate and adaptive arms of the immune system to eradicate tumors.
- This immune activation converts the tumor microenvironment from 'cold' to 'hot'.
- Activation of the adaptive immune system by ISACs results in immunological memory & T cell epitope spreading [1].
- Systemically administered ISACs are well-tolerated **with no body weight loss in mice**.
- The molecular and cellular phenotype associated with ISAC-mediated activation is being evaluated in the on-going BDC-1001 Phase I/II clinical trial [2].

References

[1] Ackerman S et al, Poster# P756, SITC 2019, Ackerman S et al, Poster #603 SITC 2020 [2] Phase 1/2 Study of BDC-1001 as a Single Agent and in Combination With Pembrolizumab in Patients With Advanced HER2-Expressing Solid Tumors; ClinicalTrials.gov (NCT04278144)., Sharma M et al, Poster #401 SITC 2020.