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

Heidi LeBlanc, Cecilia Pearson, Justin Kenkel, Lisa Blum, Po Ho, Angela Luo, Richard Laura, Matthew Zhou, Josh Gregorio, Andrew Luo, Shelley Ackerman, Brian Safina, David Dorman, Michael Alonso, Marcin Kowaniec
Bolt Biotherapeutics, Redwood City, CA

Background

Immune-stimulating antibody conjugates (ISACs) covalently attach immune stimulants to tumor-targeting antibodies such as trastuzumab. We have shown that HER2-targeted TLRT7/8 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 HC1954 (trastuzumab-resistant xenograft) and MMC (HER2+ syngeneic tumor in a HER2-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 CD4+ T cells and phagocytes. *P<0.05, **P<0.01, ***P<0.001

Mechanism of Action

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

Anti-HER2 ISAC treatment led to activation of pathways indicative of TLRT8 activation, Feyr signalling and effector engagement. TLRT8 transcription pathway

Type 1 interferon-inducible genes

Markers of myeloid cell activation

Markers of effectors cells, including T cells

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

- HER2-targeted ISACs dramatically upregulated immune-related genes in both HC1954 xenograft and MMC syngeneic models.
- In the absence of tumor targeting (isotype-specific) or TLRT8 agonist (HER2 mAb) changes in gene expression were minimal.

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

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

(A) iHER2-ISAC treatment provoked expression of interferon-δ and the myeloid chemokines CCL2/4, a sign of APC activation.

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

(C) iHER2-ISAC treatment triggered large MMC tumors. Activity was abrogated by depletion of phagocytic cells.

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

Her2-targeted ISACs stimulate a T cell response in MMC syngeneic tumors.

(A) T cell chemokines CCL5/10 and the marker of T cell activation interferon-ix increase in the 24 hours after HER2 ISAC treatment.

(B) CD4+ T cells are recruited after HER2-targeted ISAC treatment, shifting the TME from immune `cold' to `hot'.

(C) CD4+ T cell depletion abrogated anti-tumor efficacy of the HER2-targeted ISAC.

(D) Mice cured by HER2-ISAC treatment were protected against tumor relapse showing immune memory.

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 epitel 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 III clinical trial [2].

References