The combination of a trastuzumab ISAC and pertuzumab augments anti-tumor efficacy in multiple HER2+ tumor models relative to trastuzumab plus pertuzumab

ABSTRACT # 821

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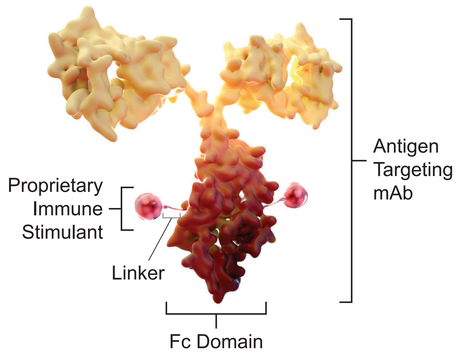
BACKGROUND

Immune-Stimulating Antibody Conjugates

- Immune-stimulating antibody conjugates (ISACs) are comprised of immune stimulants conjugated to tumor-targeting antibodies.
- Trastuzumab-T785 ISAC,[1] referred to as BDC-1001.S, is a murine surrogate of BDC-1001, a HER2-targeting ISAC currently under evaluation in multiple Phase 2 studies.[2,3,4] Trastuzumab-T785 consists of trastuzumab conjugated to a TLR7/8 agonist with a non-cleavable linker.
- Trastuzumab-T785 elicits myeloid activation and tumor eradication in trastuzumab-resistant HER2 IHC3+ models.[1]
- Given that the activity of trastuzumab-T785 is dependent on FcγR-mediated phagocytosis, we hypothesized that trastuzumab-T785 and pertuzumab, which binds a distinct HER2 epitope from trastuzumab, would enhance anti-tumor efficacy by increasing Fc clustering and promoting phagocytosis.



Key Design Criteria of Immune-Stimulating Antibody Conjugates:

- **Safety:** Enable systemic administration through the utilization of non-cleavable and cell-membrane impermeable linker payloads
- **Efficacy:** Mediate target-specific anti-tumor immunity that requires target-engagement and Fc receptor-mediated entry into myeloid effector cells
- **Durable Immunity:** Bridge innate and adaptive immunity by broadly retraining T cells to elicit immunological memory against the tumor

Rationale for Combination of Pertuzumab with BDC-1001

- Combination of trastuzumab, pertuzumab, and chemotherapy is the current standard of care for patients with HER2+ breast cancers
- Multiple mechanisms of action govern the activity of these two antibodies:[5]
  - Direct binding to HER2 inhibits survival signals
  - Pertuzumab inhibits HER2 dimerization with HER3/EGFR
  - FcγR engagement drives antibody-dependent cellular phagocytosis and cytotoxicity
  - Activation of the complement cascade induces complement-dependent cytotoxicity
- The activity of BDC-1001.S is dependent on FcR-mediated phagocytosis[1]
- Addition of pertuzumab to BDC-1001 may enhance anti-tumor efficacy by increasing Fc clustering and promoting phagocytosis

Proposed Mechanism of Action for Combination of BDC-1001 with Pertuzumab

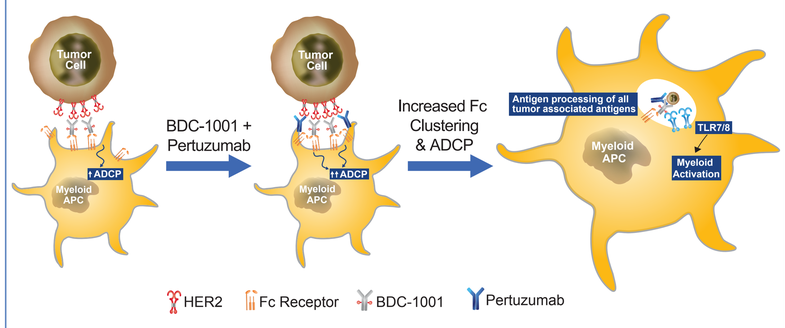


Figure 1. Combination of BDC-1001 with pertuzumab. ISACs mediate activation of myeloid APCs following binding of the targeted antigen and subsequent tumor engulfment via antibody-dependent cellular phagocytosis. Upon entering the myeloid cell, the ISAC mediates TLR7/8 activation. Addition of pertuzumab, which binds a distinct epitope of HER2, increases the number of bound antibodies to the tumor cell surface, increasing Fc clustering, which in turn increases Fc receptor-mediated phagocytosis. Schematic does not represent appropriate scale or binding dynamics.

METHODS

Selected Tumor Models Encompass HER2 High and Low Surface Expression

9 Different Tumor Models Investigated

Tumor Models	HER2 Status
HCC1954, SK-OV-3, Calu-3	$HER2^{High}$
JIMT-1, NCI-H2170, NCI-N87	$HER2^{Medium}$
HCC1187, CFPAC-1, COLO 205	$HER2^{Low}$

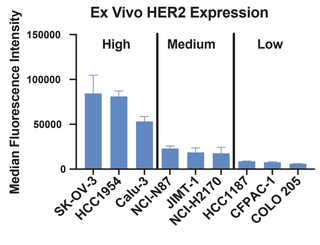


Figure 2. Surface expression of HER2 on the indicated tumors grown in SCID/beige mice was determined by flow cytometry. Upon reaching 100 mm^3, tumors were isolated and dissociated to single-cell suspensions to generate dissociated tumor cells (DTCs) (n=3-5 tumors per cell line). Cells were subsequently stained for tumor and immune cells using anti-HER2 antibody (clone 24D2) and anti-mouse CD45 antibody (clone 30-F11). HER2 expression on CD45- cells is expressed as median fluorescence intensity and is reported as mean with SEM (data are from one experiment and are representative of at least two experiments).

REFERENCES

1. Ackerman SE, et al. *Nat Cancer*. 2021 Jan;2(1):18-33. **2.** Li BT, et al. *J Clin Oncol*. 2023;41(suppl 16):2538.**3.** NCT042878144. **4.** NCT05954143. **5.** Tsao LC, et al. *JCI Insight*. 2022 Mar 22;7(6):e155636.

METHODS

Experimental Design: Efficacy of BDC-1001.S and Pertuzumab Combination

Experimental Treatment Group \ Test Articles (with Dose Levels)	BDC-1001.S 1, 2, or 5 mg/kg	Trastuzumab 5 mg/kg	Pertuzumab 5 mg/kg	Isotype 5 or 10 mg/kg
Isotype				✓
BDC-1001.S Monotherapy	✓			✓
BDC-1001.S + Pertuzumab	✓		✓	
Trastuzumab + Pertuzumab		✓	✓	

Figure 3. Tumor-bearing SCID/beige mice (n=6 per group) were treated systemically with various doses of the indicated test articles q5dx4. Trastuzumab-T785 ISAC (BDC-1001.S) was administered at 1, 2, and/or 5 mg/kg, depending on the tumor model, with the isotype mAb administered at 10 mg/kg in the isotype group and 5 mg/kg in the BDC-1001.S monotherapy group. Percent Tumor Growth Inhibition (% TGI) was calculated relative to the Isotype group with the following formula:

$1-(AverageTV_{Treated}/AverageTV_{Control})*100$, where TV = tumor volume.

RESULTS

Combination of BDC-1001.S and Pertuzumab Enhances In Vivo Anti-Tumor Efficacy

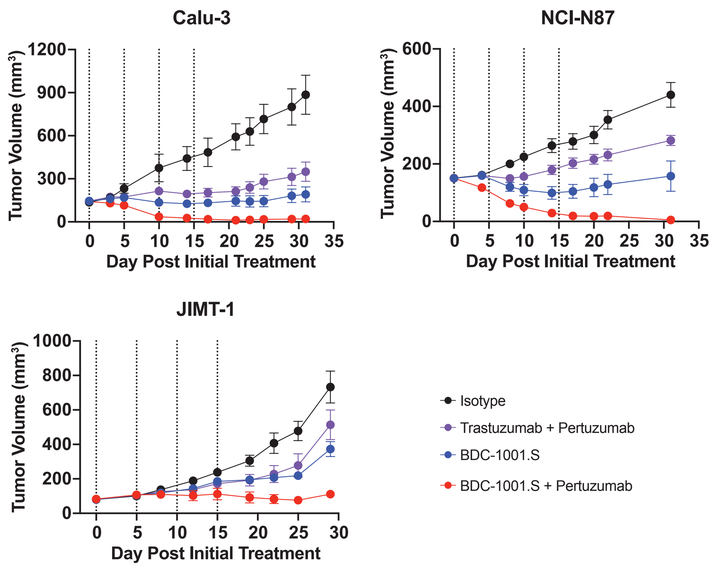


Figure 4. SCID/beige mice bearing the indicated HER2+ xenograft tumors (n=6 per group) were treated systemically with the indicated test articles q5dx4 (dashed lines). All test articles were dosed at 5 mg/kg, except in Calu-3 tumor-bearing mice, where BDC-1001.S and trastuzumab were dosed at 1 mg/kg. BDC-1001.S monotherapy was co-administered with an isotype control antibody. Data are shown as mean with SEM from one experiment and are representative of at least two experiments per tumor model.

BDC-1001.S Combination with Pertuzumab Enhances Efficacy Across Multiple HER2-Expressing Models

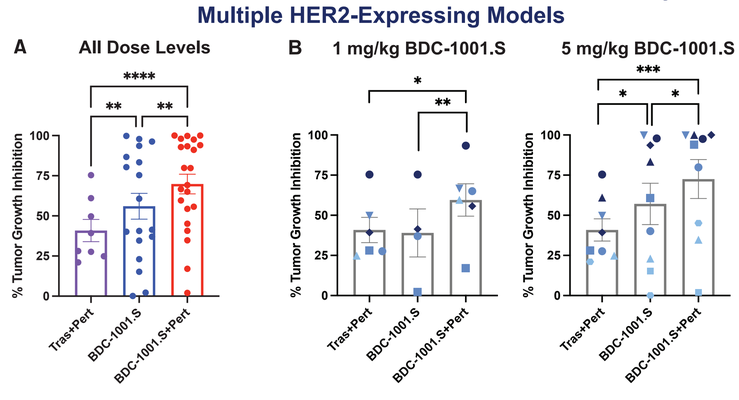


Figure 5. Tumor-bearing SCID/beige mice (up to 9 different tumor models per condition, n=6 mice per group) were treated systemically with the following treatment conditions q5dx4: 5 mg/kg of BDC-1001.S with isotype antibody, 1, 2, or 5 mg/kg of BDC-1001.S with 5 m/kg pertuzumab, or with a combination of trastuzumab and pertuzumab at 5 mg/kg each. % TGI was calculated at Day 20-23 post-treatment relative to the isotype control (data not shown) using the following equation: $1-(AverageTV_{treated}/AverageTV_{control})*100$. **A)** % TGI shown as aggregate data for all dose levels tested.*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 by two-way ANOVA. **B)** % TGI shown for the indicated conditions with BDC-1001.S administered at 1 or 5 mg/kg. *p<0.05; **p<0.01; ***p<0.001 by paired t-test. Each symbol represents a unique tumor model, with dark blue symbols: $HER2^{High}$; blue symbols: $HER2^{Medium}$; light blue symbols: $HER2^{Low}$.

BDC-1001.S Combination with Pertuzumab May Lower BDC-1001.S Dose Threshold for Efficacy

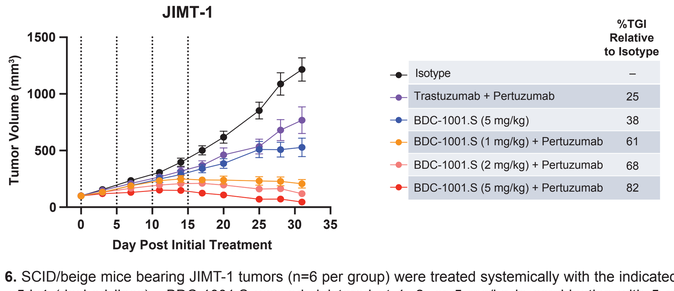


Figure 6. SCID/beige mice bearing JIMT-1 tumors (n=6 per group) were treated systemically with the indicated test articles q5dx4 (dashed lines). BDC-1001.S was administered at 1, 2, or 5 mg/kg in combination with 5 mg/kg pertuzumab. Pertuzumab and trastuzumab were each administered at 5 mg/kg, while the isotype was administered at 10 mg/kg. BDC-1001.S monotherapy was co-administered with an isotype control antibody. % TGI is calculated on Day 20 relative to isotype. Data are shown as mean with SEM from one experiment and are representative of three experiments.

RESULTS

Phagocytes Mediate Enhanced Efficacy in BDC-1001.S + Pertuzumab Combination

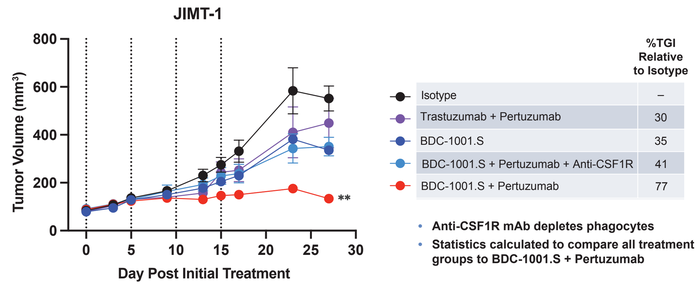


Figure 7. SCID/beige mice bearing JIMT-1 tumors were administered anti-CSF1R or IgG2a isotype antibody at 200 µg per mouse bi-weekly 2 weeks prior to treatment and continuing for the study duration to deplete phagocytes. >90% depletion of CD11c+F4/80+ phagocytes and ~50% depletion of Ly6C+ monocytes observed in the tumor at time of initial treatment. Mice were systemically treated with indicated test articles q5d x 4. BDC-1001.S was administered at 2 mg/kg, while trastuzumab and pertuzumab were administered at 5 mg/kg, and isotype was administered at 10 mg/kg. BDC-1001.S monotherapy was co-administered with an isotype control antibody at 5 mg/kg. % TGI is calculated on Day 23 relative to isotype. Data are shown as mean with SEM and are from one experiment. To compare all treatment groups to BDC-1001.S + Pertuzumab, statistics were determined by an ordinary two-way ANOVA across all time points with Dunnett's multiple comparisons test. **p<0.01.

Pertuzumab Fc-Effector Function is Required for Enhanced Anti-Tumor Activity in Combination with BDC-1001.S

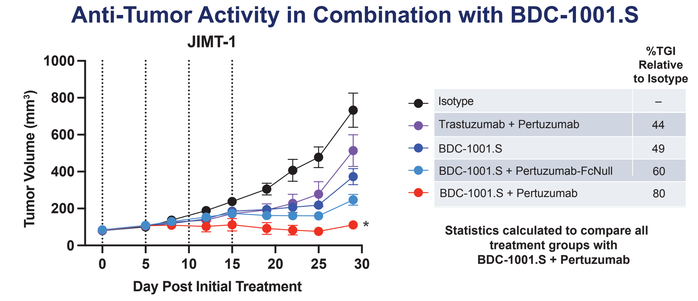


Figure 8. SCID/beige mice bearing JIMT-1 tumors (n=5 per group) were treated systemically with the indicated test articles q5dx4 at 5 mg/kg, except isotype which was administered at 10 mg/kg. Pertuzumab-FcNull is a variant of pertuzumab generated with a non-functional Fc region (mutations D265A and N297A). BDC-1001.S monotherapy was co-administered with an isotype control antibody at 5 mg/kg. % TGI is calculated on Day 22 relative to isotype. To compare all treatment groups with BDC-1001.S + Pertuzumab, statistics were determined by an ordinary two-way ANOVA across all time points with Dunnett's multiple comparisons test. *p<0.05. Data are shown as mean with SEM and are from one experiment.

BDC-1001.S and Pertuzumab Combination Enhances Cytokine Secretion in Tumor

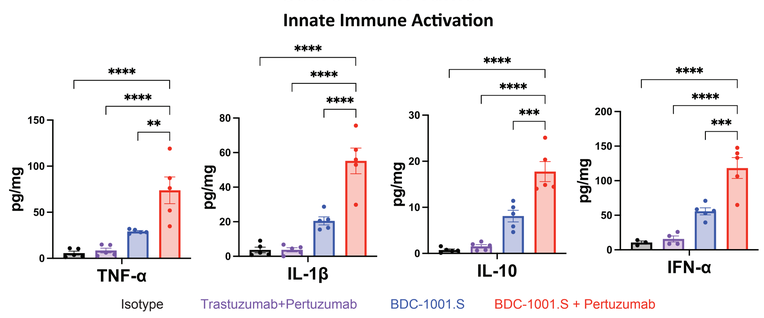


Figure 9. SCID/beige mice bearing JIMT-1 tumors (n=5 per group) were treated systemically with the indicated test articles q5dx2 at 5 mg/kg, except isotype, which was administered at 10 mg/kg. 24 hours after the second dose on Day 6, tumors were isolated and processed into protein lysates. Cytokine levels were measured by multiplex ELISA. Statistics were determined by one-way ANOVA relative to the BDC-1001.S + pertuzumab group; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. Data are shown as mean with SEM and are from one experiment.

BDC-1001.S and Pertuzumab Combination Enhances Chemokine Secretion in Tumor

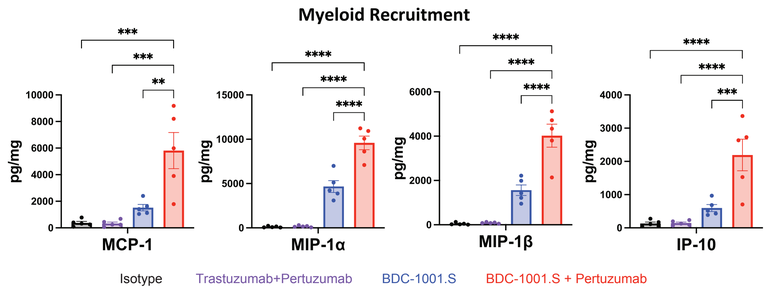


Figure 10. SCID/beige bearing JIMT-1 tumors (n=5 per group) were treated systemically with the indicated test articles q5dx2 at 5 mg/kg, except isotype, which was administered at 10 mg/kg. 24 hours after the second dose on Day 6, tumors were isolated and processed into protein lysates. Chemokine levels were measured by multiplex ELISA. Statistics were determined by one-way ANOVA relative to the BDC-1001.S + pertuzumab group; *p<0.05; **p<0.01; ***p <0.001; ****p<0.0001. Data are shown as mean with SEM and are from one experiment.

CONCLUSIONS

- **Combination of BDC-1001.S and pertuzumab significantly enhances anti-tumor efficacy in multiple HER2-expressing tumor models**
- **Addition of pertuzumab provides an additional source of "eat me" signal that likely enhances antibody-dependent cellular phagocytosis**
- **Anti-tumor efficacy was dependent on antibody-dependent cellular phagocytosis as depletion of phagocytes or the use of a pertuzumab variant lacking Fc effector function reduced efficacy**
- **This combination is being assessed in a multi-national, randomized Phase 2 clinical trial with BDC-1001 and pertuzumab in patients with metastatic HER2+ breast cancer (NCT05954143) who have received prior treatment with Enhertu**