CEA-targeted Boltbody™ ISAC, BDC-2034, drives preclinical efficacy associated with innate immune activation, phagocytosis, and myeloid reprogramming

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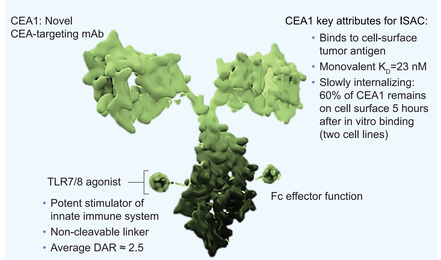
ABSTRACT 2911

BACKGROUND

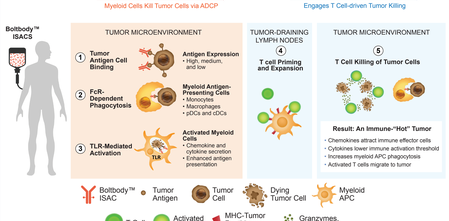
CEA (CEACAM5) is a well-validated cell-surface antigen that is highly expressed in multiple solid tumors. Bolt Bio's pioneering immune-stimulating antibody conjugates (ISACs) direct proprietary TLR7/8 agonists into tumors to activate tumor-infiltrating myeloid cells, initiating a broad innate and adaptive anti-tumor immune response.[1] The favorable properties of CEA, including robust cell surface expression, low internalization rate, and limited normal tissue expression, support the antigen's suitability as an ISAC target. We are evaluating the CEA-targeting ISAC BDC-2034 as a multi-functional approach to treat CEA-expressing cancers.

BDC-2034: Structure and critical properties



Boltbody™ ISAC mechanism targets the innate immune system

Stimulates adaptive immunity for optimal anti-tumor response

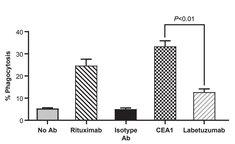


REFERENCE

1. Ackerman S, Pearson C, Gregorio J, et al. Immune-stimulating antibody conjugates elicit robust myeloid activation and durable antitumor immunity. *Nature Cancer*. 2021;2:18-33. https://doi.org/10.1038/s43018-020-00136-x

RESULTS

Phagocytosis: CEA1 efficiently mediates ADCP



CEA1 (used at 6.85 nM) induces phagocytosis of Raji/CEA cells by M-CSF differentiated, monocyte-derived macrophages (E:T = 2:1); mean +/- SEM of three donors shown.

- ADCP activity is superior to reference anti-CEA antibody labetuzumab
- No ADCP detected with Fc-active isotype (does not bind to Raji/CEA)
- Rituximab (anti-CD20) is a positive control for Raji ADCP

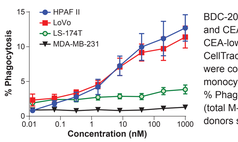
BDC-2034 binds differentially to surface-expressed CEA on a panel of cell lines

Cell line	Lineage	BDC-2034 sites per cell	Binding EC50	IHC H-score
MKN-45	Gastric	>2,000,000	HIGH	30.9 nM	300
HPAF-II	Pancreatic	1,760,000	HIGH	19.5 nM	220
LoVo	Colorectal	166,000	MEDIUM	25.0 nM	110
LS-174T	Colorectal	38,400	LOW	4.7 nM	ND
MDA-MB-231	Breast	0	NEGATIVE	ND	ND

[ND = not determined]

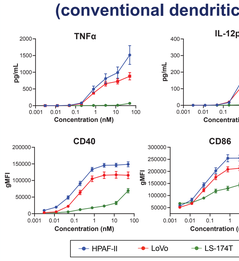
BDC-2034 binding sites per cell for each tumor cell line were quantified by flow cytometry using anti-human IgG Quantum Simply Cellular (QSC) beads standards (Bangs Laboratories). Tumor cell lines and QSC beads standards were stained with Alexa Fluor 488 labelled BDC-2034 and analyzed by flow cytometry to obtain geometric mean fluorescent intensity (gMFI). BDC-2034 binding sites were calculated using QSC beads standard curve.

Phagocytosis: Medium CEA expression is sufficient for BDC-2034 induced ADCP



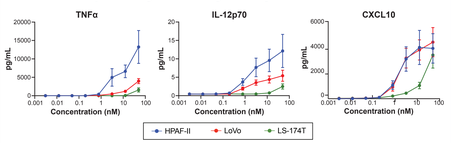
BDC-2034 induces phagocytosis of CEA-high HPAF II and CEA-medium LoVo; minimal ADCP observed for CEA-low LS-174T or CEA-negative MDA-MB-231. CellTracker Green (CTG) labelled tumor cell lines were co-cultured for 4 hours with M-CSF differentiated monocyte-derived macrophages at 2:1 E:T ratio; % Phagocytosis = %(CTG positive macrophages) / (total M-CSF macrophages); mean +/- SEM of three donors shown.

Innate immune activation: Medium tumor CEA expression levels are sufficient for activation of cDCs (conventional dendritic cells) by BDC-2034



cDCs were isolated from donor human blood. BDC-2034 was incubated with enriched cDC-tumor co-cultures (E:T = 10:1) for 18 hours. Secreted cytokine levels were determined using a LegendPlex cytokine bead array kit. Expression of the co-stimulatory surface markers, CD40 and CD86, on cDCs was measured by flow cytometry.

Innate immune activation: Medium tumor CEA expression levels are sufficient for activation of primary monocytes by BDC-2034

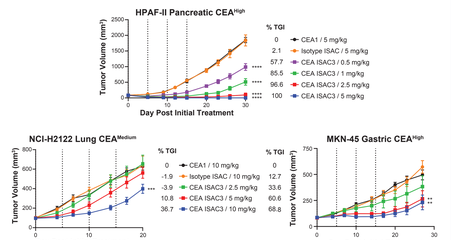


Monocytes were isolated from donor human blood. BDC-2034 was incubated with monocyte-tumor co-cultures (E:T = 10:1) for 18 hours. Secreted cytokine levels were determined using a LegendPlex cytokine bead array kit.

Mouse surrogate CEA ISAC3 inhibits growth of CEA+ xenograft tumors

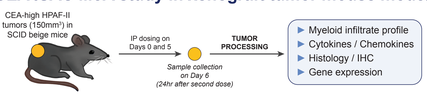
ISAC3: CEA1 ISAC with chemical analog of the BDC-2034 payload

- Improved stability in rodents
- Similar profile in TLR7/8 reporter assays and myeloid activation assays



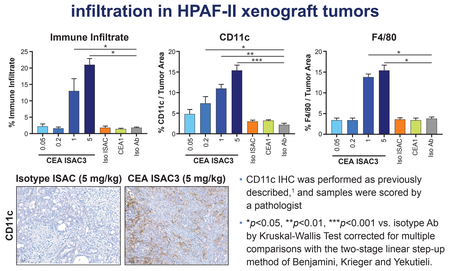
SCID/beige mice were implanted subcutaneously with 5 x 10^6 tumor. Once tumors reached 80 mm^3 (HPAF-II; MKN-45) or 100 mm^3 (NCI-H2122) in size, test articles were administered at the indicated doses q5d x 4 i.p. N = 5 mice per group (HPAF-II) or 8 mice per group (NCI-H2122; MKN-45). Percent tumor growth inhibition (% TGI) was calculated relative to CEA1 at 5 mg/kg (HPAF-II) or 10 mg/kg (MKN-45; NCI-H2122) five days following the last dose using the formula ((Average Control-Average Treated)/Average Control)*100. Data reported are from one experiment per tumor model. Data are presented as mean ± standard error of measurement (SEM). $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ by two-way ANOVA relative to the CEA1 group.

CEA ISAC MoA study in xenograft tumor mouse model



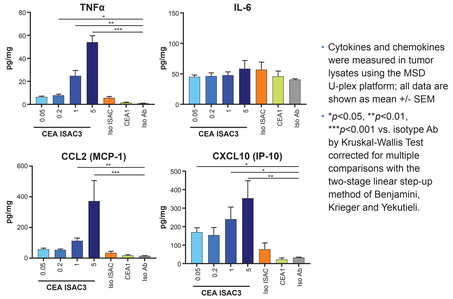
- BDC-2034 surrogate (ISAC3): 0.05, 0.2, 1, 5 mg/kg
- Isotype ISAC, CEA1, isotype Ab: 5 mg/kg
- N=5 per group
- NOTE: Larger tumors than in efficacy studies

CEA ISAC3 promotes CEA-dependent immune infiltration in HPAF-II xenograft tumors



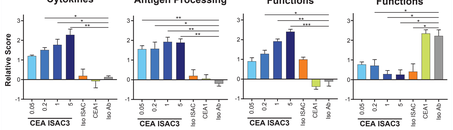
- CD11c IHC was performed as previously described,[1] and samples were scored by a pathologist
- *p<0.05, **p<0.01, ***p<0.001 vs. isotype Ab by Kruskal-Wallis Test corrected for multiple comparisons with the two-stage linear step-up method of Benjamini, Krieger and Yekutieli.

CEA ISAC3 promotes CEA-dependent cytokine secretion in HPAF-II xenograft tumors



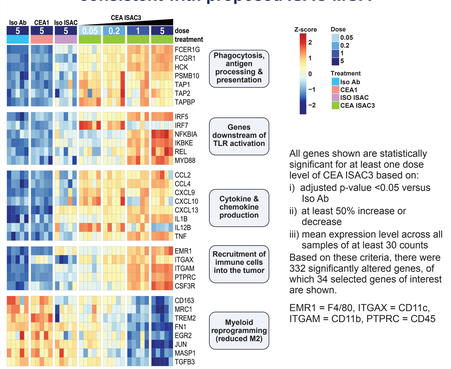
- Cytokines and chemokines were measured in tumor lysates using the MSD U-plex platform; all data are shown as mean +/- SEM
- *p<0.05, **p<0.01, ***p<0.001 vs. isotype Ab by Kruskal-Wallis Test corrected for multiple comparisons with the two-stage linear step-up method of Benjamini, Krieger and Yekutieli.

Gene expression signatures demonstrate myeloid reprogramming in response to CEA ISAC3



Normalized counts of NanoString data were used to generate scaled relative gene signature scores. Scaled relative scores were plotted and analyzed relative to the isotype control Ab by Kruskal-Wallis Test corrected for multiple comparisons with the two-stage linear step-up method of Benjamini, Krieger and Yekutieli. *p<0.05, **p<0.01, ***p<0.001

CEA ISAC3 induces gene expression changes consistent with proposed ISAC MOA



All genes shown are statistically significant for at least one dose level of CEA ISAC3 based on:

i) adjusted p-value <0.05 versus Iso Ab
ii) at least 50% increase or decrease
iii) mean expression level across all samples of at least 30 counts

Based on these criteria, there were 332 significantly altered genes, of which 34 selected genes of interest are shown.

EMR1 = F4/80, ITGAX = CD11c, ITGAM = CD11b, PTPRC = CD45

BDC-2034: DRIVER OF CEA-DEPENDENT IMMUNE ACTIVATION

- ISAC BDC-2034 created by conjugation of CEA1 with dual TLR7/8 adjuvant via a non-cleavable linker
- BDC-2034 and surrogates exhibit promising activity in preclinical models
  - Tumor-dependent induction of immune-stimulating cytokine secretion by primary human innate effector cells
  - Innate immune activation with CEA-medium models (CEA expression levels comparable to human cancers)
  - Anti-tumor efficacy in xenograft models at dose levels as low as 0.5 mg/kg
  - Dose-dependent tumor recruitment of innate effector cells and induction of immune-stimulating cytokines
  - Intra-tumor myeloid reprogramming, including upregulation of antigen presentation and down-modulation of pro-tumor M2 phenotype
- Bolt Bio's preclinical data support further development of BDC-2034 as a therapeutic option for patients with CEA-expressing cancers
- We expect BDC-2034 to enter clinical development in the second half of 2022