BDC-2034: Discovery of a CEA-targeting Immune-Stimulating Antibody Conjugate (ISAC) for Solid Tumors

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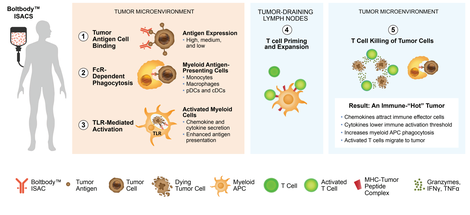
BACKGROUND

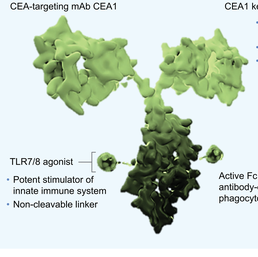
CEA (CEACAM5) is a well-validated cell-surface antigen that is highly expressed in multiple solid tumors. Upon systemic delivery, Bolt Biotherapeutics' pioneering immune-stimulating antibody conjugates (ISACs) use proprietary TLR7/8 dual agonists to activate tumor-infiltrating myeloid cells and initiate 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, suggest that the antigen may be a suitable ISAC target. We are evaluating the CEA-targeting ISAC, BDC-2034, as a novel approach to treat CEA-expressing cancers.

Boltbody™ ISAC mechanism targets the innate immune system

Spreads to Adaptive Immune System for Optimal Anti-tumor Response



BDC-2034: Structure and critical properties

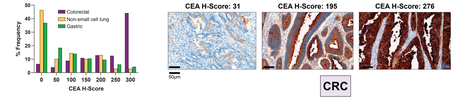


1. Ackerman S, Pearson C, Gregorio J, et al. Immune-stimulating antibody conjugates elicit robust myeloid activation and durable antitumor immunity. *Nature Cancer*. 2021;2:18-33. https://doi.org/10.1038/s43018-020-00136-x

RESULTS

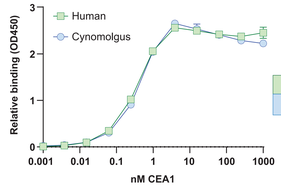
CEA is highly expressed in colorectal cancer and other solid tumors

CRC tumors have low CD8 T cell infiltration but moderate/high myeloid immune cell content



- Human colorectal cancer (CRC) array (n=247), non-small cell lung cancer (NSCLC) array (n=69), and gastric/gastroesophageal (gastric/GEJ) cancer array (n=114) were stained with the CEA31 IHC assay (Ventana/Cell Marque). Representative images are shown from the colorectal cancer array.
- CRC array was also stained with a CD8/PanCK duplex IHC and a custom quad-plex IF assay for identification of myeloid APC sub-populations.
- Consistent with prior literature, IHC staining of TMAs showed >90% CEA+ among CRC tumors, irrespective of MSI status. For NSCLC and gastric/GEJ, >50% of tumors showed CEA+ staining.
- In CRC, myeloid immune cell infiltration was detected irrespective of MSI status, but $CD8^+$ T cell levels were low except in MSI-H tumors.

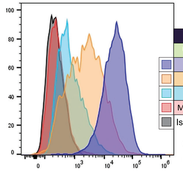
Tumor Antigen Binding: CEA1 exhibits similar affinity to human and cynomolgus monkey CEACAM5 (by ELISA)



	EC50 (nM)
Human	0.31
Cynomolgus	0.34

Tumor Antigen Binding: BDC-2034 binds differentially to surface-expressed CEA on a panel of cell lines

Binding of 200nM Alexa Fluor 488™ labeled BDC-2034 to cell lines



STEP 1 Tumor Antigen Cell Binding — TUMOR CELL

Cell line	BDC-2034 sites per cell		Binding EC50	IHC H-score
(*) MKN-45	>2,000,000	HIGH	30.9 nM	300
HPAF-II	1,760,000	HIGH	19.5 nM	220
LoVo	166,000	MEDIUM	25.0 nM	110
LS-174T	38,400	LOW	4.7 nM	ND
MDA-MB-231	0	NEGATIVE	ND	ND
Isotype (no binding)				

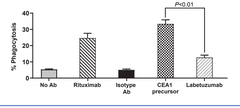
[ND=not determined]

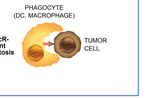
(*) MKN-45 data are from a separate study.
ISAC binding sites per cell correlate with CEA transcript levels (DepMap, Broad (Q3 2021): https://doi.org/10.6084/m9.figshare.15160110.v2)

Phagocytosis: Lead antibody efficiently mediates ADCP

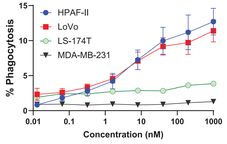
CEA1 precursor antibody (6.85 nM) induces phagocytosis of Raji/CEA cells by M-CSF differentiated, monocyte-derived macrophages (E:T=2:1)

- 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



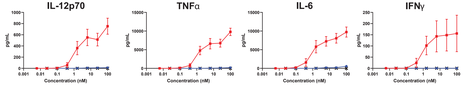


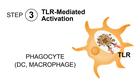
Phagocytosis: Medium CEA expression is sufficient for BDC-2034 induced ADCP



BDC-2034 induces dose-dependent phagocytosis of CEA-high HPAF II (EC50=9.2 ± 2.3 nM) and CEA-medium LoVo (EC50=11.4 ± 3.5 nM); minimal ADCP observed for CEA-low LS-174T or CEA-negative MDA-MB-231. Cells co-cultured with M-CSF differentiated monocyte-derived macrophages; means +/- standard deviations from three donors shown.

Innate Immune Activation: BDC-2034 is superior to reference labetuzumab CEA ISAC and to CEA1 antibody

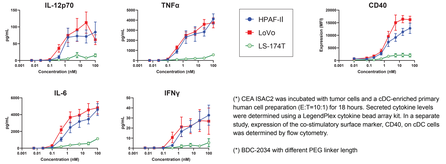




BDC-2034 — Labetuzumab ISAC — CEA1

Test articles were incubated with CEA-high MKN-45 cells and a cDC-enriched primary cell preparation (E:T=10:1) for 18 hours, then supernatants were recovered. Secreted cytokine levels were determined using a LegendPlex cytokine bead array kit.

Innate Immune Activation: Medium tumor CEA expression levels are sufficient for activation of myeloid effector cells by CEA ISAC2 (BDC-2034 linker variant)



(*) CEA ISAC2 was incubated with tumor cells and a cDC-enriched primary human cell preparation (E:T=10:1) for 18 hours. Secreted cytokine levels were determined using a LegendPlex cytokine bead array kit. In a separate study, expression of the co-stimulatory surface marker, CD40, on cDC cells was determined by flow cytometry.

(*) BDC-2034 with different PEG linker length

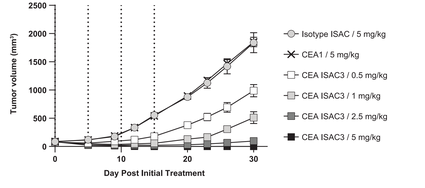
CEA ISAC3 is a mouse-stable surrogate for BDC-2034

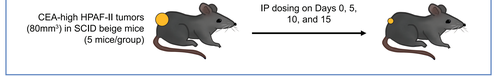
ISAC3 is a CEA1 ISAC using a linker-payload (L-P) that is a close chemical analog of the BDC-2034 linker-payload

- Modified to improve stability in rodents
- Similar activity profile in TLR7/8 reporter assays and myeloid activation assays

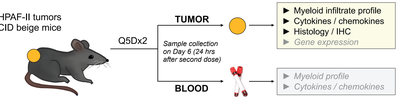
Species	Serum stability: Half-life (hr) BDC-2034 L-P	CEA ISAC3 L-P
Human	>24	>24
Cynomolgus monkey	>24	>24
Mouse	9.2 (± 0.3)	>24

Mouse surrogate CEA ISAC3 inhibits growth of CEA-high HPAF-II tumors



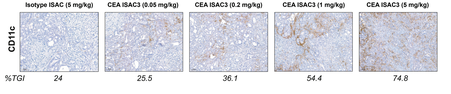


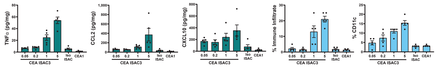
CEA ISAC MoA study in xenograft tumor mouse model



- BDC-2034 Surrogate (ISAC3): 0.05, 0.2, 1, 5 mg/kg
- Isotype ISAC, CEA1, rituximab (isotype Ab): 5 mg/kg
- N=5 per group

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





- CD11c IHC was performed as previously described,[1] and samples were scored by a pathologist
- Cytokines and chemokines were measured in tumor lysates using the MSD U-plex platform; all data are shown as mean +/- SEM
- %TGI (tumor growth inhibition) calculated relative to CEA1 on Day 6 using the formula $((TV^{ISAC3}_{Day\,X}-TV^{ISAC3}_{Day\,6})/TV^{CEA1}_{Day\,X})*100$
  - TV=tumor volume

CONCLUSIONS

- CEA is expressed in solid tumors having robust innate immune infiltrates, including colorectal cancers
- High affinity for CEA, cross-reactivity with cynomolgus antigen, and potent induction of ADCP establishes the CEA1 antibody as a suitable candidate for the ISAC approach
- Conjugation of CEA1 with a TLR7/8 dual agonist payload via a non-cleavable linker generates the ISAC BDC-2034
- BDC-2034 and surrogates exhibit promising activity in pre-clinical 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 a xenograft model at dose levels as low as 0.5 mg/kg
  - Dose-dependent tumor recruitment of innate effector cells and induction of immune-stimulating cytokines
- Bolt Biotherapeutics' pre-clinical data support further development of BDC-2034 as a therapeutic option for patients with CEA-expressing cancers