A highly efficacious next-generation CEACAM5 (CEA)-targeted ISAC for the treatment of colorectal, pancreatic and lung tumors

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Introduction

CEACAM5 (CEA) is a well-characterized cell-surface protein involved in intercellular adhesion. Expressed at low levels on the luminal surface of polarized epithelial cells in normal tissue, CEA expression can be highly upregulated in tumors of epithelial origin including colorectal, pancreatic and lung cancers. Despite favorable properties as a cancer target, no therapies targeting CEA have been approved to date.

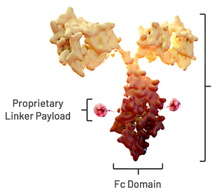
We are developing a CEA-targeted therapy with a distinct mechanism of action based on our next-generation Immune-Stimulating Antibody Conjugate (ISAC) platform (Fig 1). This platform incorporates immune-stimulating payloads with enhanced potency that are active in human, primate, and mouse immune cells. In addition, TLR7/8 specificity and potency of next-generation payloads are tailored to key biology, and conjugation chemistry is optimized with non-cleavable linkers.

Our new CEA targeted therapy combines the enhanced potency of a next-generation payload (Fig. 3) with a novel anti-CEA antibody, CEA Ab 601. This antibody binds with high affinity and selectivity to human and cynomolgus CEA (Fig 4). *In vitro*, CEA Ab 601 drives myeloid-mediated antibody-dependent cellular phagocytosis (ADCP) and macrophage-mediated killing of CEA+ tumor cells (Fig 5) that is superior to a reference anti-CEA antibody, tusamitamab. The next-generation CEA ISAC stimulates cytokine production in human conventional DC (cDC) co-cultures with CEA+ tumor cells and is also superior to tusamitamab carrying the same linker-payload (Fig. 6A). Robust cytokine production in cDC co-cultures is observed with CEA high expressing tumor cells (HPAC and SNU-C1) as well as tumor cells (SW1463) expressing moderate levels of CEA (Fig. 6B). The next-generation CEA ISAC is also active in non-human primate PBMC co-cultures with CEA+ tumor cells (Fig 7) and in mouse cell myeloid cell/tumor cell co-cultures (Fig 8) *in vitro*.

Efficacy was evaluated in CEA transgenic mice using human CEA-expressing MC38 cells. Treatment with the next-generation CEA ISAC results in 100% complete responses (Fig. 9A). Inhibition of tumor growth in cured mice rechallenged with CEA-MC38 tumor cells demonstrates induction of immunological memory (Fig. 9B).

In a non-GLP NHP tox study the next-generation CEA ISAC was well-tolerated with no significant drug-related adverse events observed up to 15 mg/kg, the highest dose tested.

Fig. 1 Properties of Next-Generation CEA ISAC



- **Immune-Stimulating Antibody Conjugates (ISACs):**
  - Antibody against CEA directs ISAC to the tumor
  - Proprietary immune stimulant activates myeloid antigen-presenting cells
  - Myeloid cells kill tumor cells, create a "hot" tumor microenvironment, and initiate an innate & adaptive anti-tumor immune response
- **CEA ISAC**
  - Limited normal tissue expression of CEA reduces toxicity liabilities
  - Antibody cross reacts with NHP CEA to facilitate tox evaluation
  - Potent activity observed *in vitro* and *in vivo* with CEA ISAC
- **Next-Generation Linker-Payload**
  - Increased potency with acceptable therapeutic window

Fig. 2 Immune-Stimulating Antibody Conjugate Mechanism of Action

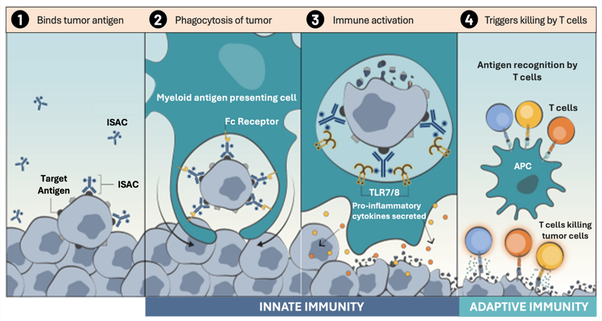
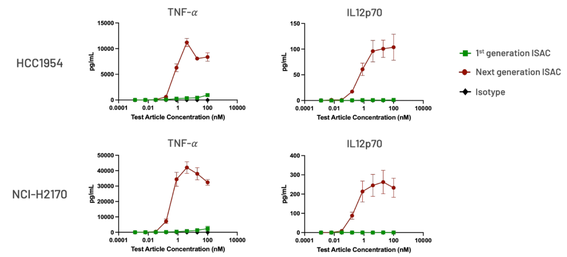
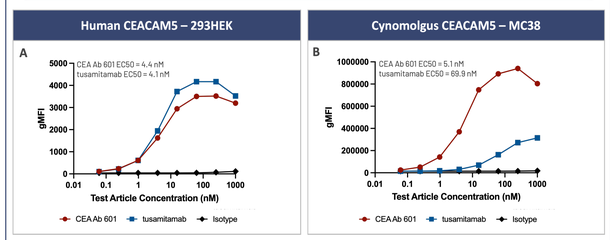


Fig. 3 Next-Generation ISACs Elicit Robust Myeloid Cell Activation



First generation and next-generation linker-payloads were conjugated to trastuzumab and assessed for activity in myeloid/tumor cell co-cultures assays. Conventional dendritic cell (cDC)-enriched primary cells from healthy human donors were co-cultured with Her2+ cell lines HCC1954 or NCI-H2170 at 10:1 (E:T) ratio in the presence of titrated test articles. After 18 hours, supernatants were collected and secreted cytokines quantitated using a LEGENDplex™ multiplex immunoassay. These data were used to generate dose-response curves in GraphPad Prism using a 5-parameter logistic. Data shown is the mean of 3 donors with SEM.

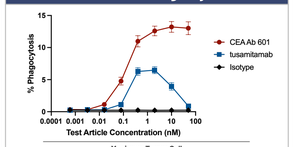
Fig. 4 CEA Ab 601 Ab Binds to Human and Cynomolgus CEACAM5



CEA-negative HEK-293 and MC38 cells were stably transduced with **A)** human or **B)** cynomolgus CEA lentivirus expression vectors. These cell lines were used to evaluate anti-CEA mAb binding. Cells were incubated with titrations of the indicated test articles, followed by a fluorescent secondary detection antibody. Binding was assessed by flow cytometry. "gMFI" = geometric mean fluorescence intensity. Binding curves were generated from gMFI in GraphPad Prism using a 5-parameter logistic. EC50s were calculated from these curves. Human CEA and cynomolgus CEA are expressed at different levels as determined by staining with a pan CEACAM binding antibody. Analyses were performed on different instruments under different acquisition settings; higher gMFI values with MC38/Cyno CEA samples do not necessarily indicate higher antibody binding levels. CEA Ab 601 and tusamitamab were produced recombinantly at Bolt Biotherapeutics.

Fig. 5 CEA Ab 601 Mediates Superior ADCP and MΦ-mediated killing

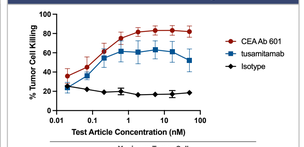
Tumor Cell Phagocytosis



Test Article	Maximum Tumor Cell Phagocytosis (%)	EC50 (nM)
CEA Ab 601	13.1	0.1
tusamitamab	5.6	10.2

ADCP. Raji cells expressing CEA were labeled with Cell Tracker Green (CTG), mixed with M-CSF differentiated macrophages and incubated at 1:2 (T:E) ratio with test articles at 37°C for 4 h. Cells were harvested, stained for CD206 to identify macrophages and analyzed by flow cytometry. Phagocytic potential of effector cells was assessed by gating on CTG and CD206 double positive cells. Percent phagocytosis is the percent of effector cells that are double positive. Data shown are the mean of 3 donors with SEM.

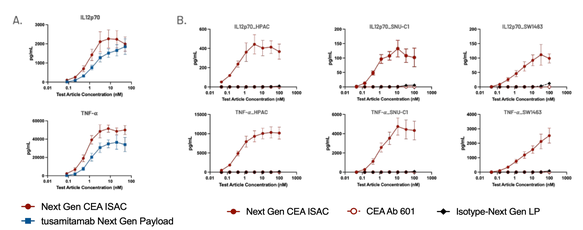
Tumor Cell Killing



Test Article	Maximum Tumor Cell Killing (%)	EC50 (nM)
CEA Ab 601	83	0.2
tusamitamab	63	0.1

Macrophage-mediated tumor cell killing. M-CSF differentiated macrophages from healthy donors were incubated with Luciferase-HEK 293/CEA cells at 5:1 (E:T) ratio at 37°C for 24 h. Cell viability of Luciferase-HEK 293/CEA cells was assessed by BIO Glo. Percent Tumor Cell Killing was calculated relative to no stimulus control group with co-cultured M-CSF macrophages and tumor cells. Data shown are the mean of 3 donors with SEM.

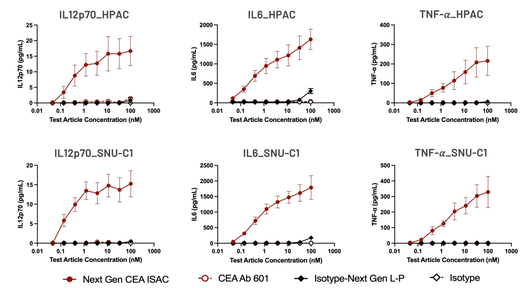
Fig. 6 Next-Generation CEA ISAC Induces Human Myeloid Cell Activation



A. Conventional dendritic cell (cDC)-enriched primary cells from healthy human donors were co-cultured with HPAC tumor cells at 5:1 (E:T) ratio in the presence of indicated test articles. After 18 hours, supernatants were collected and secreted cytokines quantitated using a LEGENDplex™ multiplex immunoassay. GraphPad Prism was used to generate dose-response curves from which EC50s were calculated. Data shown are the mean of 5 donors with SEM. Next-Gen CEA ISAC elicits robust cytokine secretion from cDC + HPAC co-cultures with EC50s of 0.8 and 0.7 nM for IL12p70 and TNF-α, respectively. Tusamitamab conjugated with the same Next-Gen linker-payload is less active with EC50s of 1.9 and 1.1 for IL12p70 and TNF-α, respectively.

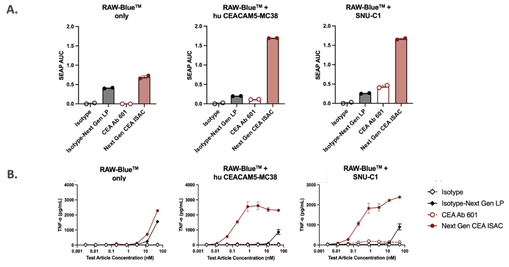
B. cDC from healthy human donors were co-cultured with tumor cell lines HPAC and SNU-C1 expressing high levels of cell surface CEA and SW1463 expressing lower CEA levels at 10:1 (E:T) ratio in the presence of indicated test articles. Data shown are the mean of 3 donors with SEM.

Fig. 7 Next-Gen CEA ISAC Actives Myeloid Cells in Non-Human Primates



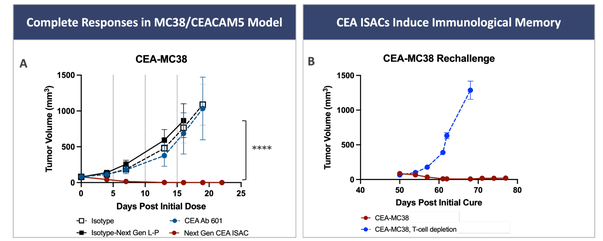
PBMC from cynomolgus macaques were co-cultured with HPAC or SNU-C1 tumor cells at 10:1 (E:T) ratio in the presence of indicated test articles. After 18 hours, supernatants were collected and secreted cytokines quantitated using a Meso Scale Discovery multiplex immunoassay. GraphPad Prism was used to generate dose-response curves. Data shown are the mean of 3 donors with SEM.

Fig. 8 Next-generation ISAC is active in mouse myeloid cells



RAW-Blue™ cells (a mouse macrophage cell line stably expressing an NF-κB/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene) were co-cultured with human CEACAM5 expressing cell lines and test articles titrated 1:4 from 50 nM for 18 h at 37°C. **A)** Supernatants were assessed for secreted SEAP using QUANTI-Blue™ solution according to the manufacturer's protocol and quantitated by absorbance at OD_{630}. Titration curves were generated in GraphPad Prism using a 5-parameter logistic. Results are triplicate mean SEAP Area Under Curve (AUC) with SEM. **B)** Secreted mouse TNF-α in supernatants was quantitated by ELISA and titration curves generated in GraphPad Prism using a 5-parameter logistic. Results shown are triplicate mean TNF-α concentration with SEM.

Fig. 9 CEA ISAC Elicits Complete Responses and Immunological Memory



Next-Generation CEA ISAC elicits complete responses and immunological memory in a CEA transgenic MC38 syngeneic mouse model. **A)** C57Bl/6-CEA transgenic mice bearing CEA-expressing-MC38 tumors (average 100 mm³) were treated systemically with 5 mg/kg of the indicated test articles on days 0, 5, 10, and 15; All mice (n=6) in the Net-Gen CEA ISAC treated group showed complete responses (**** P>0.0001 compared to isotype control with Next-Gen linker-payload on day 16). **B)** Approximately 1 month after tumor clearance, mice were re-challenged with CEA-MC38 tumor cells. Tumor growth was inhibited in mice previously cured by treatment with the Next-Gen CEA ISAC. Tumor growth inhibition was ablated by T cell depletion prior to re-challenge.

Non-GLP Tox in NHP

- Next-Gen CEA ISAC was evaluated in a non-GLP toxicity and toxicokinetic study in NHP
- Three groups (n=3 females per group), 5 and 15 mg/kg Next-Gen CEA ISAC, and 15 mg/kg CEA Ab 601
- All doses were well-tolerated; no significant drug-related adverse events observed
- Findings were minor and generally transient and reversible.
- Evidence of TLR7/8 activation (i.e. CRP)

Summary

Next-generation CEA ISAC

- Combines novel CEA Ab with next-gen TLR7/8 agonist payload via an optimized non-cleavable linker
- Stimulates Ag-dependent induction of immune-stimulating cytokines in primary human and NHP innate effector cells
- Next-generation payload is active in mouse cells
- Complete responses in CEA transgenic syngeneic model demonstrates robust efficacy
- Inhibition of tumor growth in tumor rechallenged mice demonstrates induction of immunological memory

1. Ackerman SE, et al. (2021) Immune-stimulating antibody conjugates elicit robust myeloid activation and durable antitumor immunity. Nat Cancer. 2(1):18-33. doi: 10.1038/s43018-020-00136-x.