Preclinical evaluation of a highly efficacious CEACAM5 (CEA) ISAC comprising a novel CEA-specific monoclonal antibody and TLR7/8 agonist

Rishali Gadkari, Andrew Luo, David T. Omstead, Chi-Ling Fu, Jason Ptacek, Shahid Khan, Katelynn A. McEachin, Karla A. Henning, Han Kim, Robert Veneziale, Lu Xu, Romas Kudirka, William G. Mallet, Shelley E. Ackerman, Michael N. Alonso, Paul D. Ponath

1. Bolt Biotherapeutics, Redwood City, CA, USA

Abstract # 920

Abstract

Background

CEACAM5 (CEA) is a well characterized tumor-associated antigen frequently upregulated in tumors of epithelial origin. CEA-directed therapies have been evaluated clinically including antibodies and antibody-drug conjugates (ADC), but none have been approved to date. We are developing a CEA-targeted therapy with a distinct mechanism of action based on our Immune-Stimulating Antibody Conjugate (ISAC) platform [1]. ISACs offer several advantages over other modalities such as activation of myeloid cells within the tumor microenvironment that can directly kill tumors cells as well as stimulation of a more durable adaptive T-cell response (Fig. 1). Our CEA-targeted ISAC was constructed by conjugation of a next-generation TLR7/8 immune-stimulating payload via a non-cleavable linker to a novel CEA mAb, 601. This CEA ISAC is active in human, non-human primate, and mouse systems and has several advantages over reference CEA antibodies such as tusamitamab and labetuzumab as well as CEA ADC (Fig. 2).

Results

CEA Ab 601 binds to human and cynomolgus CEA+ cells (Fig. 3) and mediates antibody-dependent cellular phagocytosis (ADCP) and macrophage-mediated killing of CEA+ tumor cells that is superior to reference CEA Abs (Fig. 4A). In addition, CEA Ab 601 ISAC (CEA ISAC) stimulates greater cytokine production by human conventional DC (cDC) cocultures with CEA+ tumor cells than tusamitamab with the same payload (Fig. 4B). Our CEA ISAC is active in mouse as shown by cytokine induction in tumor bearing mice (Fig. 5) avoiding the need for a surrogate payload. We used a CEA transgenic (Tg) mouse expressing human CEACAM5 in a tissue specific expression pattern similar to CEACAM5 expression in humans (Fig. 6A) to evaluate anti-tumor activity of our CEA ISAC. Mouse MC38 colon carcinoma cells expressing human CEACAM5 were constructed to serve as models for expression levels observed in human clinical samples. CEA expression on MC38-CEA tumors grown in CEA Tg mice was evaluated by flow cytometry and immunohistochemistry. Two cell lines, MC38-CEA-1 and MC38-CEA-13, were scored by IHC as 2+ and 3+, respectively, and used as models of intermediate and high CEA-expressing tumors (Fig. 6B). The extracellular domain of CEA is shed (sCEA) from cells (Fig. 7A) and is detected in the plasma of CEA Tg mice (Fig. 7B). Elevated levels of sCEA in patient plasma is a well-established biomarker associated with various CEA+ tumors and is correlated with recurrence and poor prognosis. We observed elevated sCEA levels in the plasma of CEA Tg mice implanted with CEA+ tumors (Fig. 7B). sCEA can negatively impact the efficacy of anti-CEA based therapeutics by acting as a target sink reducing the amount of antibody available to bind tumors. However, unlike many anti-CEA antibodies, sCEA has minimal impact on CEA Ab 601 binding to CEA+ cells (Fig. 7C). In vivo we observed dose-dependent inhibition of tumor growth in the IHC 3+ MC38-CEA S13 model with 100% complete remissions at 5mg/kg dosing (Fig. 8A). Growth of MC38-CEA S13 cells implanted in complete responders 45 days after tumor clearance was completely inhibited. However, T cell depletion prior to implant abolished tumor growth inhibition (Fig. 8B) demonstrating induction of T cell-mediated immunological memory. We compared efficacy of our CEA ISAC to that of a CEA ADC (tusamitamab conjugated via cleavable linker to a topoisomerase I inhibitor) (Fig.9). In the IHC 3+ MC38-CEA model CEA ISAC and tusamitamab-ADC showed equivalent efficacy at 10 mg/kg single dose. At 5 mg/kg, 4qd x 3, CEA ISAC had slightly greater TGI than tusamitamab-ADC (97.4% vs 90.7%, respectively) although this difference was not significant. At 2 mg/kg, q4d x 3, CEA ISAC maintained efficacy (TGI = 95.4%, 3 cures) while TGI with tusamitamab-ADC was significantly reduced (TGI = 49.3%, no cures). Further comparison in the IHC 2+ MC38-CEA S1 model (Fig.10) showed CEA ISAC remained efficacious at 2 mg/kg, q4d x 3 (TGI = 68.4%, 3 cures) and at 5 mg/kg, q4d (TGI = 98.3%, 3 cures). However, tusamitamab-ADC was not active at either dose level in this model.

Conclusion

These data show that our CEA-targeted ISAC induces robust innate and adaptive anti-tumor immunity in tumor models with high and intermediate target antigen expression with the potential to treat CEACAM5+ human cancers.

Fig. 1 Immune-Stimulating Antibody Conjugate (ISAC) Mechanism of Action

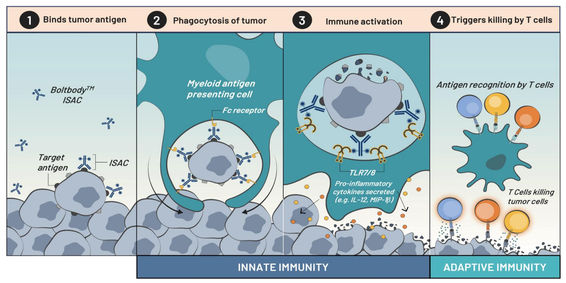
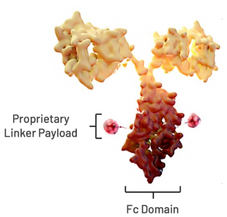
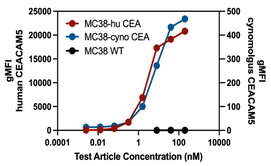


Fig. 2 Key Features of CEACAM5 ISAC



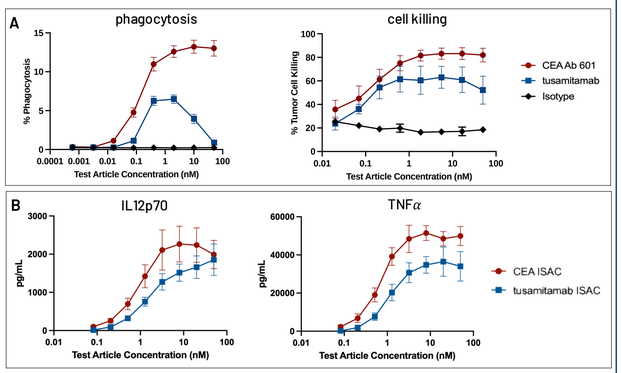
- Immune-Stimulating Antibody Conjugates (ISACs):
  - Antibody against CEA directs ISAC to the tumor
  - Proprietary immune stimulant activates myeloid antigen-presenting cells
  - Myeloid cells kill tumor cells, create a "hot" tumor microenvironment, and initiate an innate & adaptive anti-tumor immune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

Fig. 3 CEA Ab 601 Binds to Human and Cynomolgus CEA



MC38 cells expressing human or cynomolgus CEACAM5 were incubated with test articles for 30 min at 4°C. Cell bound test articles were detected with fluorochrome-labeled anti-human IgG and assessed by flow cytometry. EC50s were calculated from gMFI binding curves using Prism. CEA Ab 601 EC50s = 2.6 nM and 6.4 nM for human and cyno CEACAM5, respectively.

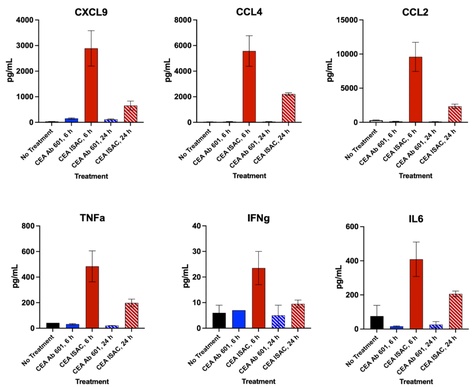
Fig. 4 CEA Ab 601 and CEA ISAC Elicit Superior Tumor Cell Killing and Cytokine Production



A. CEA Ab 601 mediates superior ADCP and tumor cell killing vs. tusamitamab. ADCP (left panel). Cell-tracker green (CTG)-labeled cells expressing CEA were mixed 2:1 (E:T) with M-CSF differentiated macrophages (effector cells) and incubated at 37°C for 4 h. Cells were stained for CD206 to identify macrophages and assessed by flow cytometry. Percent phagocytosis is the percentage of CD206+ cells that are CTG+. Results are mean ± SEM of 3 donors. Macrophage-mediated tumor cell killing (right panel). M-CSF differentiated macrophages were incubated with Luciferase-HEK 293/CEA cells at 5:1 (E:T) ratio at 37°C for 24 h. Cell viability was assessed by BIO-Glo™. Percent Tumor Cell Killing was calculated relative to no stimulus control group of macrophages/tumor cells. Results are mean ± SEM of 3 donors.

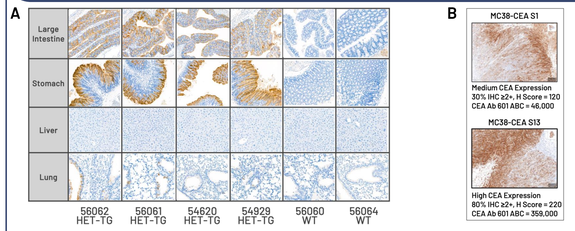
B. CEA ISAC mediates superior myeloid cell cytokine induction vs. tusamitamab in CEA+ tumor cell co-cultures. Conventional dendritic cell (cDC)-enriched primary cells were co-cultured with HPAC cells at 5:1 (E:T) ratio and our CEA ISAC or tusamitamab conjugated with the same payload. After 18 hours, cytokines were quantitated with a LEGEND plex™ multiplex immunoassay. Dose-response curves and EC50s were calculated using Prism. Results are mean of 5 donors with SEM. 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 payload is less active with EC50s of 1.9 and 1.1 for IL12p70 and TNF-α, respectively.

Fig. 5 CEA ISAC is Active in Mouse



CEA Tg mice were implanted with IHC 2+ MC38-CEA cells (see Fig. 6) and treated with a single dose (5 mg/kg) of CEA Ab 601 or CEA ISAC when tumor volume reached 100 mm³. Plasma was collected at 6 h and 24 h post dose and cytokines measured using a LEGEND plex™ assay kit. Plasma from untreated, tumor bearing mice were included for comparison. Treatment with CEA Ab 601 induced no significant change in plasma cytokine levels at any time point. Treatment with CEA ISAC induced a significant increase in plasma cytokine levels at 6 hour which returned to near baseline levels by 24 hours.

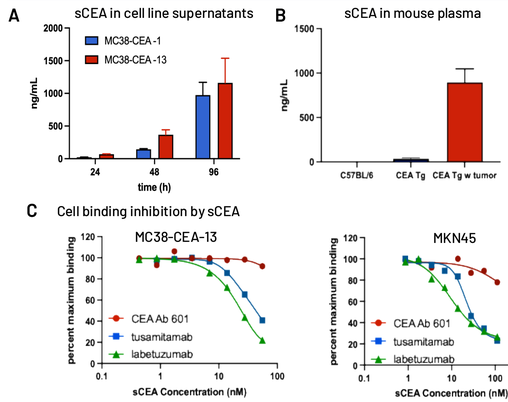
Fig. 6 Expression of Human CEA in Transgenic Mice and MC38-CEA Colorectal Cancer Models



A. CEA expression in transgenic mice was evaluated by IHC and confirmed as previously described [2]. Transgenic CEA expression is observed in large intestine, stomach and lung, but not in liver. In the large intestine, CEA is highly expressed in surface epithelial cells and crypt cells with expression focused in apical or basal regions. CEA expression was observed in stomach epithelial cells of the apical/luminal surface as well as glandular epithelial cells. Weak CEA expression was observed in lung epithelium in bronchi and bronchioles.

B. Tumors (100 mm³) from MC38-CEA S1 and MC38-CEA S13 cells implanted in CEA Tg were excised and processed for CEA expression assessment by IHC and flow cytometry. IHC was performed with biotinylated CEA31 antibody and scored as CEA high expression (≥50% tumor cells at intensity ≥2+) and CEA medium expression (≥25% tumor cells at intensity ≥2+). Flow cytometry was performed with AF647-labeled CEA Ab 601 using Quantum™ Simply Cellular microbeads. Mean Antibody Binding Capacity (ABC) values are reported (MC38-CEA S1, n=2 and MC38-CEA S13, n=2).

Fig. 7 CEA is Shed by Tumor Cells but Does Not Significantly Impact Tumor Cell Binding with CEA Ab 601

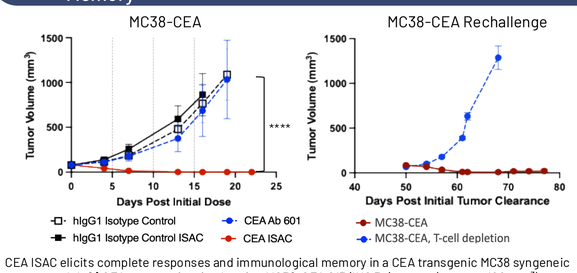


A. MC38-CEA-S1 and MC38-CEA-S13 cells were plated in duplicate and incubated at 37°C, 5%CO_2 for the indicated time. sCEA in supernatants was quantitated by ELISA and normalized to cell number.

B. sCEA in plasma of C57Bl/6, CEA Tg and CEA Tg mice implanted with MC38-CEA-S1 cells for 21 days was quantitated by ELISA. C57Bl/6 ELISA values were used as background and subtracted from CEA Tg and CEA Tg + tumor values.

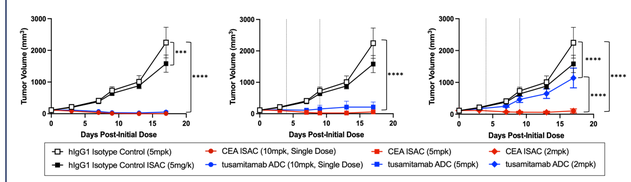
C. The effect of sCEA on antibody binding to MC38-CEA-S13 (left panel) and MKN45 cells (right panel) was evaluated by flow cytometry. Recombinant sCEA (rsCEA) was serially diluted from 55.4 nM for MC38-CEA-13 cells and 110.8 nM for MKN45 cells. Antibody (1 nM final concentration) was added to the diluted rsCEA and incubated 5 min at RT. Cells (1 x 10^5/well) were added to the mixture and incubated at RT for 1 h. Cell bound antibody was detected with PE-labeled anti-human IgG antibody. Data was normalized by setting the maximum MFI for each cell line to 100%.

Fig. 8 CEA ISAC Elicits Complete Responses and Immunological Memory



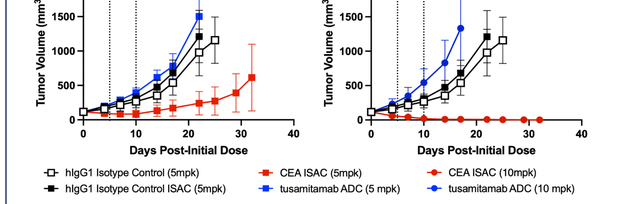
CEA ISAC elicits complete responses and immunological memory in a CEA transgenic MC38 syngeneic mouse model. **A)** CEA transgenic mice bearing MC38-CEA S13 (IHC 3+) tumors (average 100 mm³) were treated systemically with 5 mg/kg of the indicated test articles on days 0, 5, 10, and 15 (n=6 mice/group). All mice in the CEA ISAC treated group showed complete responses [**** P >0.0001 compared to hIgG1 Isotype control-ISAC hIgG1 with the same linker-payload]. **B)** Fifty days after tumor clearance, mice were re-challenged with MC38-CEA tumor cells. Tumor growth was inhibited in mice previously cured by treatment with the CEA ISAC. Tumor growth inhibition was ablated by T-cell depletion prior to re-challenge.

Fig. 9 CEA ISAC Inhibits Tumor Growth More Effectively than CEA ADC at Lower Doses



CEA ISAC efficacy was compared to a CEA ADC, tusamitamab conjugated via cleavable linker to a topoisomerase I inhibitor, DAR8 in the MC38-CEA IHC 3+ model. C57Bl/6-CEA transgenic mice bearing MC38-CEA-13 tumors (n=7, average tumor volume = 100 mm³) were treated systemically with test articles at 10 mg/kg, single dose (left panel), 5 mg/kg, q4d x 3 (middle panel) or 2 mg/kg, q4d x 3 (right panel). Treatment with a single dose of CEA ISAC or tusamitamab ADC at 10 mg/kg resulted in tumor growth inhibition of 99.7% (including 5 cures) and 97.6% (with 4 cures), respectively. Tumor growth inhibition in mice treated with 5 mg/kg, q4d x 3 were 97.4% (5 cures) and 90.7% (4 cures) for CEA ISAC and tusamitamab ADC, respectively. At 2 mg/kg, q4d x 3 the CEA ISAC was more effective at tumor growth inhibition (95.4% TGI, 3 cures) than CEA ADC (50.3% TGI, no cures). **** P < 0.0001, *** P = 0.0005

Fig. 10 CEA ISAC Inhibits Tumor Growth More Effectively than CEA ADC at Lower Doses and Lower Antigen Density



CEA ISAC efficacy was compared to a CEA ADC, tusamitamab conjugated via cleavable linker to a topoisomerase I inhibitor, DAR8, in the IHC 2+ MC38-CEA-S1 model. C57Bl/6 CEA transgenic mice bearing MC38-CEA-1 tumors (n=6, average tumor volume = 100 mm³) were treated systemically with test articles at 5 mg/kg (left panel) or 10 mg/kg (right panel) q5d x 3. At this antigen density the tusamitamab ADC was ineffective at inhibiting tumor growth at the 5 or 10 mg/kg doses. However, the CEA ISAC was efficacious at both doses (75.6% TGI, 4 cures at 5 mg/kg, and 99.3% TGI, 6 cures at 10 mg/kg).

Next-Generation CEA ISAC Summary

- Combines novel CEA Ab with a next-generation TLR7/8 agonist payload via an optimized non-cleavable linker
- Stimulates Ag-dependent induction of immune-stimulating cytokines in human and mouse effector cells
- Shed antigen has minimal impact on tumor cell binding
- Complete responses in CEA transgenic syngeneic models with high (IHC 3+) and intermediate (IHC 2+) CEA expression demonstrate robust efficacy
- Inhibition of tumor growth in tumor-rechallenged mice demonstrates induction of immunological memory
- CEA ISAC is more effective than CEA ADC at lower doses and lower tumor antigen density

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.

2. Eades-Perner AM, et al. (1994) Mice transgenic for the human carcinoembryonic antigen gene maintain its spatiotemporal expression pattern. Cancer Res. 54(15):4169-76. PMID: 8033149