CEACAM5 pre-targeting via click chemistry enables tumor-selective MMAE activation: A first-in-class approach to enhance clinical benefit in targeted therapy

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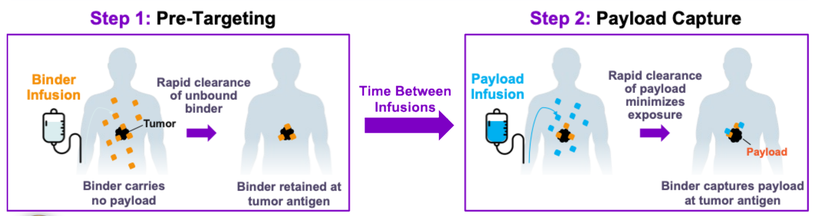
LB-B015

CEACAM5 Pre-Targeting For Tumor Antigen-Selective MMAE Delivery

- Patient benefit from targeted therapies is often limited by off-target toxicities.
- 99% of an ADC dose is catabolized by normal tissues, leading to active payload release and toxicities.[1]
- This reduces patient benefit from targeted therapies, as it limits the dose.
- Shasqi uses a pre-targeting approach to overcome these off-target toxicities.
- Shasqi's Click Activated Protodrugs Against Cancer (CAPAC) separates the binder from the payload and selectively and rapidly reunites them *in vivo* using click chemistry, a Nobel Prize-winning technology.
- Here we present preclinical data on a clickable CEACAM5 antigen-binder and a clickable monomethyl auristatin E (MMAE) payload ("protodrug").
- When united at the tumor, the **clickable CEACAM5 pre-targeting binder** 'clicks' with the **clickable MMAE protodrug**, leading to active therapeutic payload at the tumor.[3-5]

Pre-Targeting Selectively Captures Payloads at Tumors



CEACAM5 Clickable Binder is Not Internalized; Retains its Click Reactivity

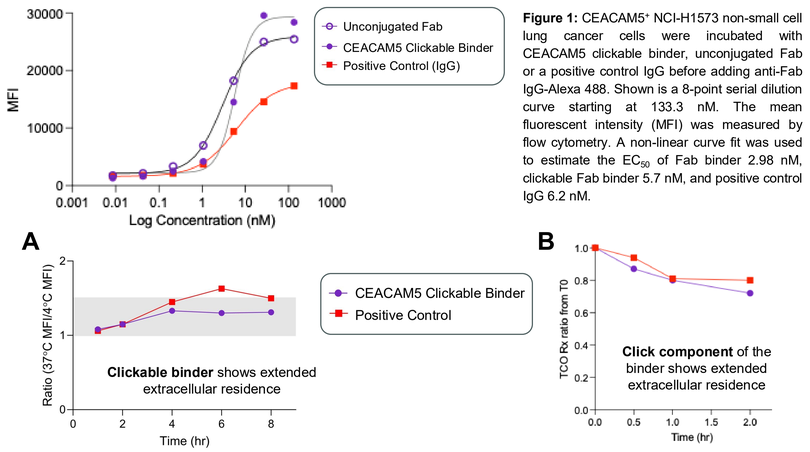


Figure 1: CEACAM5⁺ NCI-H1573 non-small cell lung cancer cells were incubated with CEACAM5 clickable binder, unconjugated Fab or a positive control IgG before adding anti-Fab IgG-Alexa 488. Shown is a 8-point serial dilution curve starting at 133.3 nM. The mean fluorescent intensity (MFI) was measured by flow cytometry. A non-linear curve fit was used to estimate the EC_{50} of Fab binder 2.98 nM, clickable Fab binder 5.7 nM, and positive control IgG 6.2 nM.

Figure 2: (**A**) Binder internalization was measured using a flow cytometry-based *in vitro* assay with CEACAM5⁺ NCI-H1573 cells. pHrodo was conjugated to the CEACAM5 clickable binder or a non-internalizing positive control IgG to monitor endocytosis. A ratio of 1-1.5 (depicted in grey) is considered low internalization. (**B**) Reactivity of the click component on the CEACAM5 clickable binder and a positive control was measured by flow cytometry after incubation for the indicated time using a surrogate, TCO-Biotin, that reacts with the click component and is monitored by streptavidin-Alexa 488.

Clickable MMAE Protodrug Clears Rapidly From Circulation in Rats

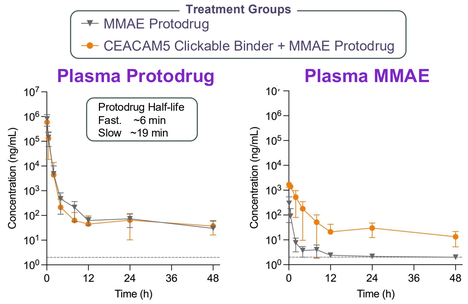


Figure 3: Plasma PK of clickable MMAE protodrug (plasma protodrug) and MMAE (plasma MMAE) in male Sprague Dawley (SD) rats. Rats were treated with vehicle or CEACAM5 clickable binder at 25 mg/kg followed by a single dose of clickable MMAE protodrug at the MTD (100 mg/kg). Plasma protodrug and plasma MMAE were quantified at different time points using LC-MS. Data from two weekly cycles were combined since results showed little inter-cycle variability (n=3 animals/group, 6 data points/ time point). Shown are mean ± standard deviation. Dashed lines indicated lower limit of quantification (LLOQ = 2 ng/mL). Research-grade protodrug has a MMAE contamination of ~0.03%. MTD = maximal tolerated dose.

CEACAM5 Clickable Binder and MMAE Clickable Protodrug Exhibit Antitumor Efficacy Across Multiple Human Tumor Models

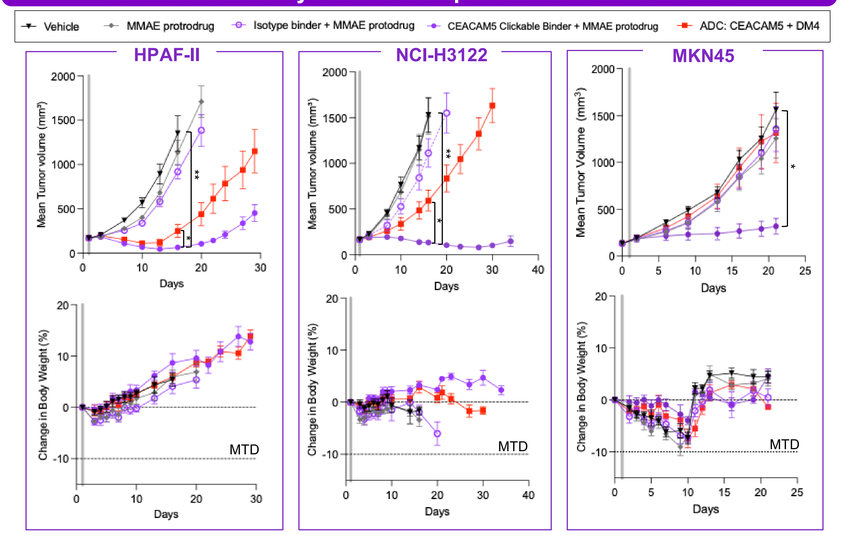


Figure 4: CEACAM5 clickable binder with MMAE protodrug demonstrated antitumor response in multiple CEACAM5⁺ human xenograft tumor models (HPAF-II pancreatic cancer, NCI-H3122 non-small cell lung cancer, MKN45 gastric adenocarcinoma). Tumor-bearing Balb/c nude mice were treated with 50 mg/kg of isotype control or CEACAM5 clickable binder, followed by 30 mg/kg MMAE protodrug 18 hours later; ADC: CEACAM5 + DM4 (monoclonal antibody conjugated to DM4, 3.8 DAR) was dosed at 3 mg/kg. Top: tumor volumes, bottom: mouse body weight changes from initial. Shown are mean ± SEM, n=6/group. Gray bar indicates day of dosing. P-values: two-way ANOVA with Tukey's correction (*p<0.05, **p<0.01). MTD = maximal tolerated dose.

Single Administration of CEACAM5 Clickable Binder and MMAE Clickable Protodrug Induced Regression of Large Tumors

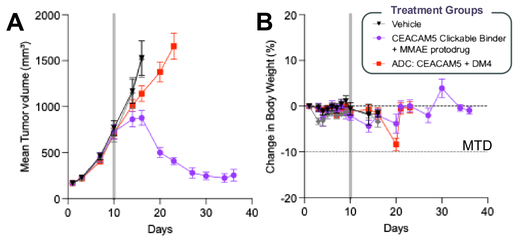
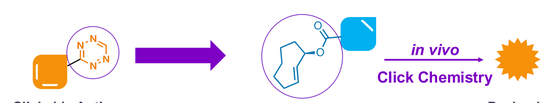
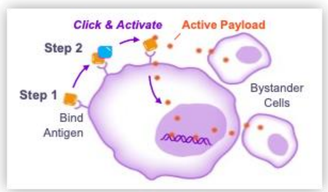


Figure 5:. Regression of larger tumors was observed with a single dose of CEACAM5 clickable binder + MMAE protodrug. NCI-H3122 tumors were dosed when average tumor size reached ~650-700 mm³. CEACAM5 clickable binder was dosed at 50 mg/kg, MMAE protodrug was dosed 18 hours after at 30 mg/kg. ADC: CEACAM5 + DM4 was dosed at 3 mg/kg. (**A**) Mean tumor volumes. (**B**) Mouse body weight changes from initial. Shown are mean ± SEM, n=6/group. Gray bar indicates day of dosing. MTD = maximal tolerated dose.

Click Chemistry

- Rapid
- Covalent
- Selective *in vivo*
- Nonimmunogenic
- **Clinically validated**





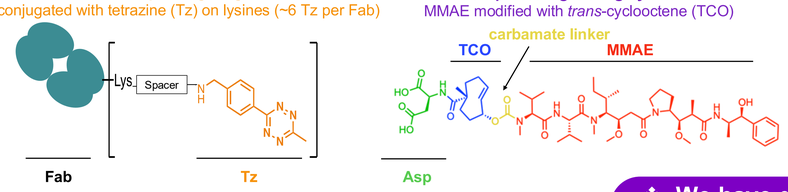
Activation Without Internalization

- Enables new targets (e.g., CEACAM5)
- On-target and bystander killing at the tumor
- Overcomes potential mechanism of resistance

Clickable Pre-Targeting Binder and Protodrug

Clickable CEACAM5 pre-targeting binder is a Fab conjugated with tetrazine (Tz) on lysines (~6 Tz per Fab)

Clickable protodrug is a **highly attenuated**[5] MMAE modified with *trans*-cyclooctene (TCO)



CEACAM5 Clickable Binder in Combination with MMAE Clickable Protodrug is Highly Tolerable in Rats

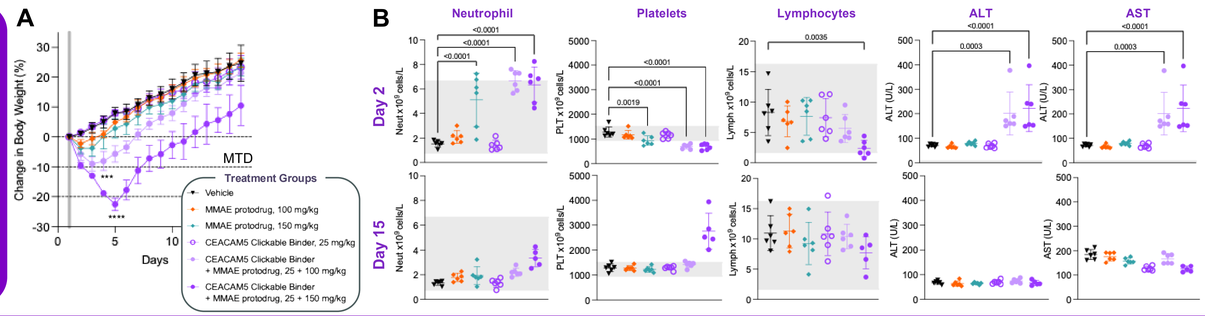


Figure 6: Acute rat toxicology study. Male Sprague Dawley (SD) rats were dosed with either vehicle, MMAE protodrug 100 or 150 mg/kg, CEACAM5 clickable binder alone at 25 mg/kg, or CEACAM5 clickable binder at 25 mg/kg + MMAE protodrug at either 100 or 150 mg/kg. (**A**) Body weight change from initial. (**B**) Select blood markers for neutrophil, platelets, lymphocytes (gray shading indicates normal range), and liver enzymes ALT and AST (shown are mean ± standard deviation, n=6 rats/group). P-value was determined by two-way ANOVA with Dunnett's correction for body weight change vs vehicle and one-way ANOVA with Dunnett's correction for (B). ***p<0.001, ****p<0.0001. MTD = maximal tolerated dose.

REFERENCES

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- ❖ **We have developed a first in class CEACAM5 targeted therapy that utilizes a click chemistry-based pre-targeting approach to selectively activate an MMAE payload at the tumor, avoiding toxicities to normal tissues.**
- ❖ **Data show robust antitumor efficacy, and superiority over an ADC targeting the same antigen with the same class of payload, including in a large tumor model, more representative of tumor burden in patients.**
- ❖ **The drug is well tolerated in rats, with limited hematologic toxicities.**
- ❖ **Pre-targeting with CAPAC can overcome the dose-limiting toxicities associated with ADCs, allowing for improved efficacy.**

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