Tumor-Selective MMAE Activation via CEACAM5 Pre-Targeting Using Click Chemistry: A First-in-Class Strategy to Enhance Clinical Benefit

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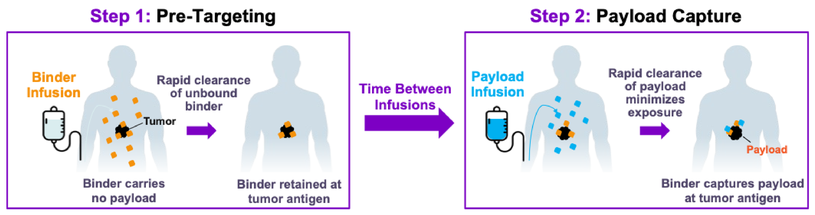
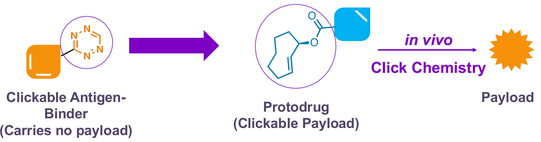
CEACAM5 Pre-Targeting For Tumor Antigen-Selective MMAE Delivery

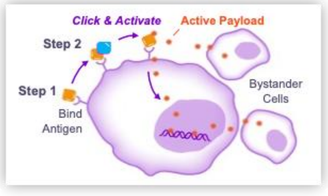
- 99% of an ADC dose is catabolized by normal tissues, leading to active payload release and toxicities.[1]
- This reduces the patient benefit from targeted therapies, as it limits the dose that can be given.
- Shasqi uses a pre-targeting approach to overcome these off-target toxicities.
- Shasqi's Click 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



Click Chemistry:
Rapid
Covalent
Selective *in vivo*
Nonimmunogenic
Clinically validated





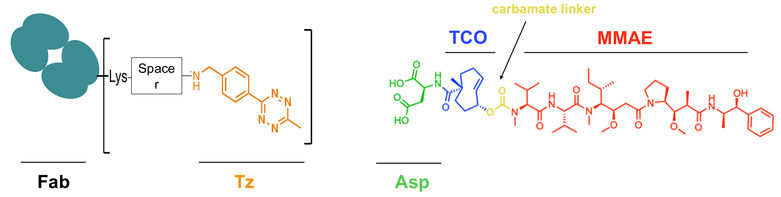
Activation Without Internalization

- 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 Shows Extended Extracellular Residence With Retained Click Reactivity

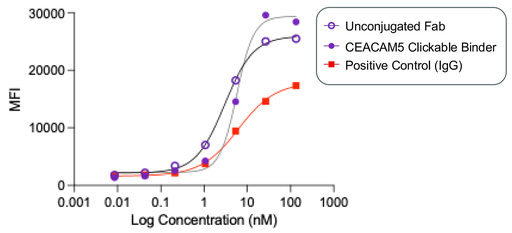


Figure 1: CEACAM5+ NCI-H1573 non-small cell lung cancer cells were incubated with CEACAM5 clickable binder, unconjugated Fab or a positive control IgG before adding anti-Fab IgG-Alexa 488. 8-point serial dilution curve starting at 133.3 nM. The mean fluorescent intensity (MFI) was measured with flow 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.

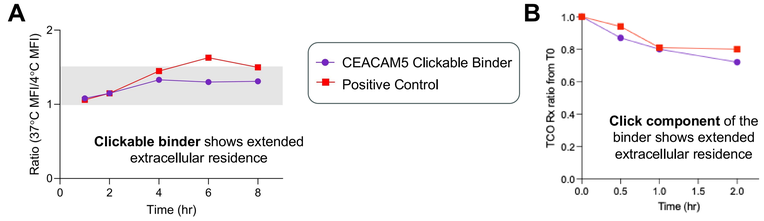


Figure 2: Binder internalization (**A**) was measured using a flow cytometry-based *in vitro* assay with NCI-H1573 cells. pHrodo was conjugated to the CEACAM5 clickable binder or non-internalizing positive control IgG to monitor endocytosis. A ratio of 1-1.5 (depicted in grey) is considered low internalization. (**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 click component and monitored by streptavidin-Alexa 488.

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

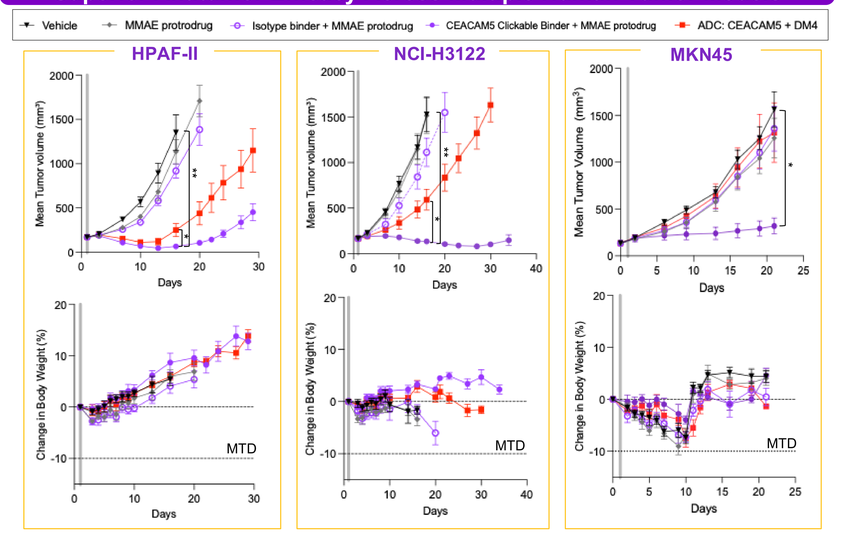


Figure 3: CEACAM5 clickable binder with MMAE protodrug demonstrated antitumor response in multiple human xenograft tumor models (HPAF-II pancreatic cancer, NCI-H3122 non-small cell lung cancer, MKN45 gastric adenocarcinoma). Tumor-bearing Balb/c nude mice were treated with 50 mg/kg of isotype control 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 row shows mean ± SEM tumor volumes n=6/group, bottom row shows mouse body weights changes from initial. P-values: two-way ANOVA with Tukey's correction (*p<0.05, **p<0.01). Gray bars indicate day of dosing. MTD = maximal tolerated dose.

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

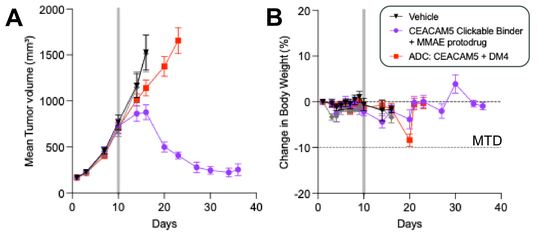
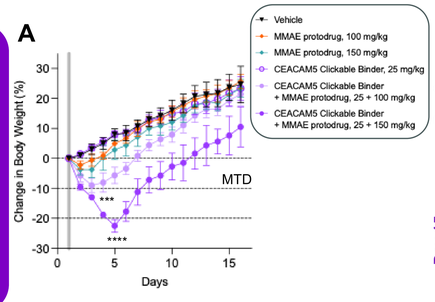


Figure 4. Regression of larger tumors observed with single dose of CEACAM5 clickable Binder and MMAE protodrug. NCI-H3122 tumors were dosed when average tumor size reached ~650-700 mm^3. CEACAM5 clickable binder was dosed at 50 mg/kg and MMAE protodrug was dosed 18 hours after at 30 mg/kg. ADC: CEACAM5 + DM4 was dosed at 3 mg/kg. (**A**) Mean tumor volume (shows mean ± SEM tumor volumes n=6/group). (**B**) mouse body weights changes from initial. Gray bar indicates day of dosing. MTD = maximal tolerated dose.

CEACAM5 Clickable Binder in Combination with MMAE Clickable Protodrug is Well Tolerated in Rats



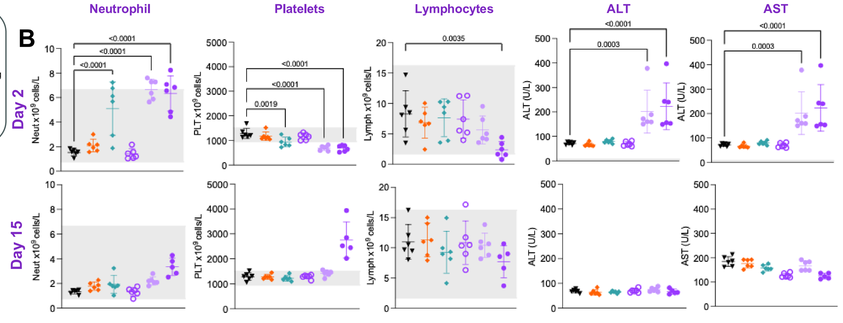


Figure 5. Acute rat toxicology study. Male Sprague Dawley (SD) rats were dosed with either vehicle, MMAE protodrug 100 or 150 mg/kg, CEACAM5 clickable Binder alone 25 mg/kg, or CEACAM5 clickable binder 25 mg/kg + MMAE protodrug at either 100 or 150 mg/kg (**A**) Body weight change from initial (**B**) Select blood markers 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 One-way ANOVA with Dunnett's correction for CBC and two-way ANOVA with Dunnett's correction for body weight change vs vehicle. *p<0.05, **p<0.01, ****p<0.0001. MTD = maximal tolerated dose.

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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 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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