CX-2043, an EpCAM-Targeting Probody Drug Conjugate, Demonstrates Anti-Tumor Activity with a Favorable Safety Profile in Preclinical Models

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ABSTRACT

Background: Ideal targets for antibody drug conjugates (ADCs) are efficiently internalized and are highly and homogeneously expressed on tumor cells in a large proportion of patients across a wide variety of tumor types. EpCAM represents an example of such a target that is highly expressed in the majority of epithelial cancers. However, past efforts to target EpCAM with systemic antibody-based therapeutics have failed in large part due to toxicity in normal tissues with high expression (e.g. gastro-intestinal tract and pancreas).

Probody ® drug conjugates (PDCs) are masked, protease-activatable antibody prodrugs designed to localize drug activity to the tumor microenvironment and minimize interaction with healthy tissues. In this way, PDCs have the potential to deliver potent toxin payloads more precisely to the tumors and mitigate on-target/off-tumor toxicity. Our data demonstrate effective targeting of EpCAM by a PDC with a favorable tolerability profile, which would not be possible with a traditional ADC approach.

Materials and methods: CX-2043 consists of an EpCAM-targeting Probody protein conjugated to a novel maytansinoid payload optimized for bystander activity. Conjugation is via lysine residues on the Probody protein using a peptide linker selected for enhanced stability in circulation (DM21L). CX-2043 was evaluated for cytotoxicity and binding *in vitro* to tumor cells, for efficacy *in vivo* with tumor models, and for tolerability in Cynomolgus monkeys.

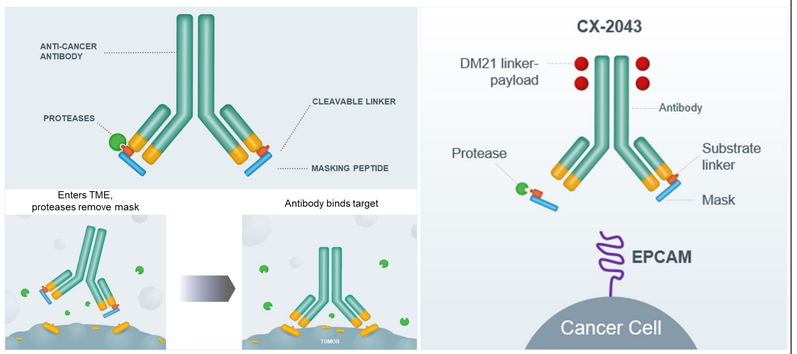
Results: *In vitro* cytotoxicity of activated CX-2043 or unmasked EpCAM-DM21L ADC across cancer lines was mostly in the sub-nanomolar range and correlated with target expression. Consistent with *in vitro* activity, CX-2043 exhibited potent single dose efficacy in cell line-derived xenograft tumor models. Importantly, CX-2043 showed a favorable safety profile in a repeat-dosing toxicology study in monkeys relative to the unmasked EpCAM-DM21L ADC.

Conclusions: Preclinical data suggest that CX-2043 represents a novel and promising therapeutic directed against EpCAM, a compelling but historically "difficult-to-drug" target.

INTRODUCTION

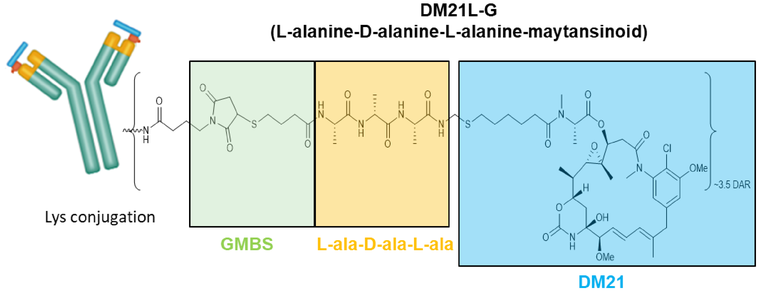
Probody Therapeutics (Pb-Tx) are Designed to be Activated in the Tumor Microenvironment (TME) (Fig. 1)

Pb-Tx are designed to be minimally active when administered systemically and activated in a protease-enriched disease microenvironment. Probody Drug Conjugates (PDCs) have the potential to deliver potent toxin payloads more precisely to tumors and mitigate on-target/off-tumor toxicity.



CX-2043: A Probody Drug Conjugate (PDC) Targeting EpCAM (Fig. 2)

CX-2043 is stochastically conjugated to DM21 linker payload with a DAR 3.5-4 via lysine residues. The tripeptide linker was optimized for stability in mouse models and is cleavable by intracellular lysosomal-associated proteases. The payload is also relatively cell permeable, providing improved bystander activity over previous maytansinoid payloads.

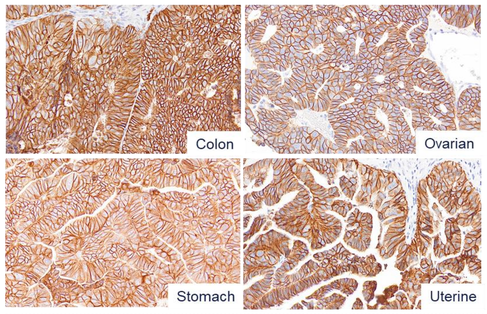


RESULTS

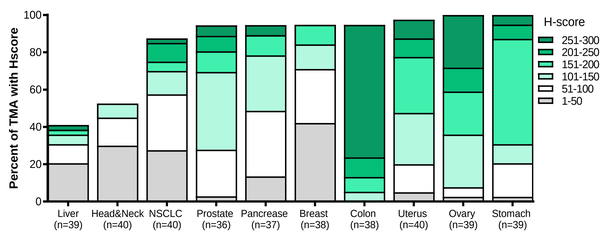
EpCAM is Highly Expressed in Multiple Indications (Fig. 3)

EpCAM is highly and uniformly expressed in numerous epithelial cancers, such as colorectal, ovarian, uterine, stomach, and lung. Its expression is also present in corresponding healthy tissues; thus previous EpCAM-targeted therapeutics have elicited ON-target/OFF-tumor toxicities. The Probody approach is designed to mitigate these toxicities and provide a therapeutic window.

Tumor IHC

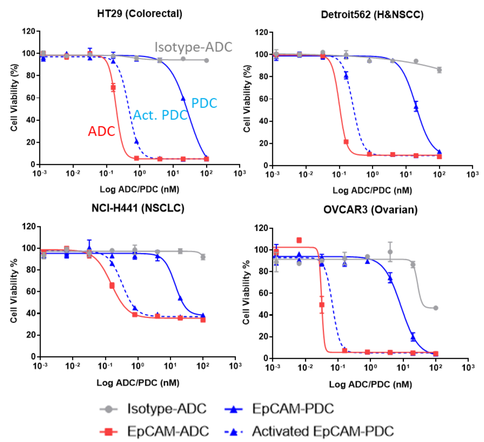


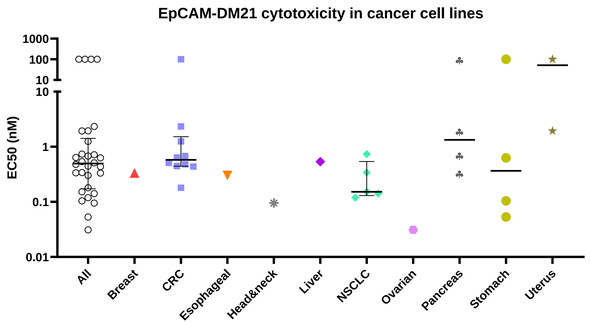
EpCAM IHC



EpCAM expression is also present in corresponding normal tissue, including colon and GI tissues.

EpCAM-ADC/PDC Exhibits Potent Cytotoxicity Across Cancer Cell Lines (Fig. 4)

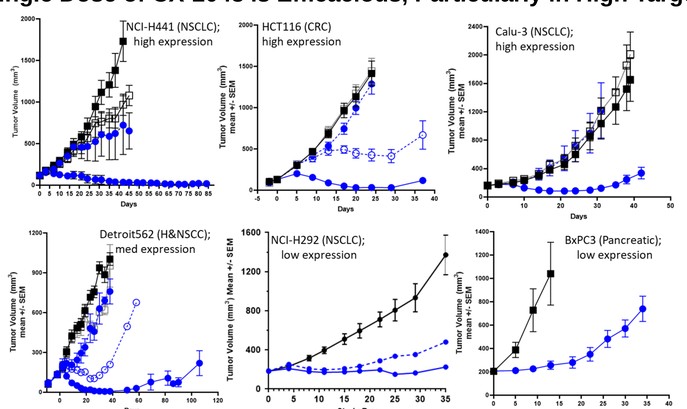




	All	Breast	CRC	Esophageal	Head& neck	Liver	NSCLC	Ovarian	Pancreas	Stomach	Uterus
Median EC_{50} (nM)	0.50	0.33	0.58	0.30	0.10	0.54	0.15	0.03	1.33	0.37	51

The cytotoxicity potency of EpCAM-DM21 is in the sub-nanomolar range. Left panels depict examples of ADC/PDC cytotoxicity in indicated cell lines. The PDC (blue line) activity is attenuated relative to that of ADC (red line), and PDC activity is restored upon activation by protease incubation (blue dotted line). Right panel: ADC potency (EC_{50}) is plotted for cancer cell lines in various cancer indications. Table indicates the median EC_{50} of EpCAM ADC in cell lines of various cancer indications.

Single Dose of CX-2043 is Efficacious, Particularly in High Target Expression Models (Fig. 5)



Tumor-bearing mice were given a single low (0.9-2.5 mg/kg) or high (3-5 mg/kg) dose of CX-2043, or Isotype-DM21 and vehicle controls. Fractionated dosing consisted of 1/3rd of the higher dose given 3 times, once per week (QWX3).

CX-2043 is Well Tolerated in Cyno Up to 9 mg/kg Whereas Only 1 mg/kg of ADC Was Tolerable (Fig. 6)

Dosing (Q2WX2)	ADC	PDC	Isotype
1 mpk	Tolerated		
3 mpk	Not tolerated	Tolerated	Tolerated
6 mpk	Not tolerated		
9 mpk		Tolerated	
12 mpk			Tolerated

Toxicity:

- Isotype-DM21-L-G ADC at 12 mg/kg:
  - Dry, discolored skin; abrasion
  - Liquid feces, mild dehydration
  - ↓ albumin, electrolytes (Na^+, Cl^-), RBC
- EpCAM-DM21-L-G PDC at 9 mg/kg:
  - Dry skin, slight abrasion
  - Mild dehydration
- EpCAM-DM21-L-G ADC at 3 and/or 6 mg/kg:
  - > 10% weight loss, liquid feces
  - Early euthanasia required
  - ↓ albumin, Na^+, Cl^-, RBC; ↑ AST, BUN, CRN

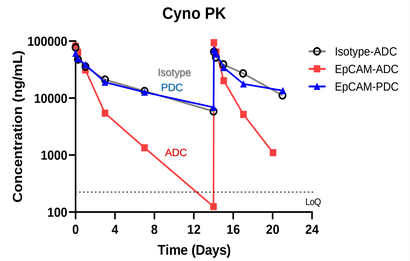
Pharmacokinetics:

- Increased PDC exposure relative to ADC suggests mitigation of target mediated clearance (TMDD)

Summary of tolerated dose is depicted in the table (above). Clinical observations for isotype control, ADC and PDC are shown (left panel).

Cyno PK

The Cyno PK for matched dose of 3 mg/kg is shown for isotype-DM21 (grey line), PDC (blue line), and ADC (red line).



SUMMARY/CONCLUSIONS

1) CX-2043 is a Probody drug conjugate (PDC) targeting EpCAM, a target that is highly expressed on numerous epithelial cancers, as well as on heathy tissues.
2) EpCAM ADC/PDC exhibits potent cytotoxicity across cancer cell lines including colorectal, NSCLC, and ovarian cancers.
3) The Probody technology alleviates ON-target/OFF-tumor toxicities associated with targeting EpCAM, while still retaining efficacy in preclinical models.
4) PDC mitigates target-mediated drug clearance (TMDD) and can increase exposure of the administered drug.

ACKNOWLEDGMENTS

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