Design and development of biparatopic antibody-drug conjugates against protein tyrosine kinase 7 (PTK7)

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Abstract #1565

Introduction

Biparatopic antibodies target two distinct epitopes on the same antigen and have the potential for greater cell surface binding, superior receptor blockade and enhanced receptor-mediated internalization relative to conventional antibodies. When biparatopic targeting is applied to antibody-drug conjugates (ADCs), these benefits can further translate into increased payload delivery and cytotoxic activity.

Protein tyrosine kinase 7 (PTK7) is a member of the receptor tyrosine kinase family and cofetuzumab pelidotin, an ADC against PTK7, has demonstrated antitumor activity in patients with non-small cell lung cancer, ovarian cancer and triple-negative breast cancer.[1,2] Overexpression of PTK7 has also been observed in esophageal, colorectal, head and neck, and cervical cancers.[3] Given the large, multi-domain extracellular region of the protein, antibodies against non-overlapping PTK7 epitopes are available, facilitating the development of biparatopic antibodies and ADCs.

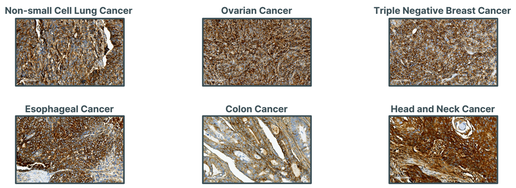
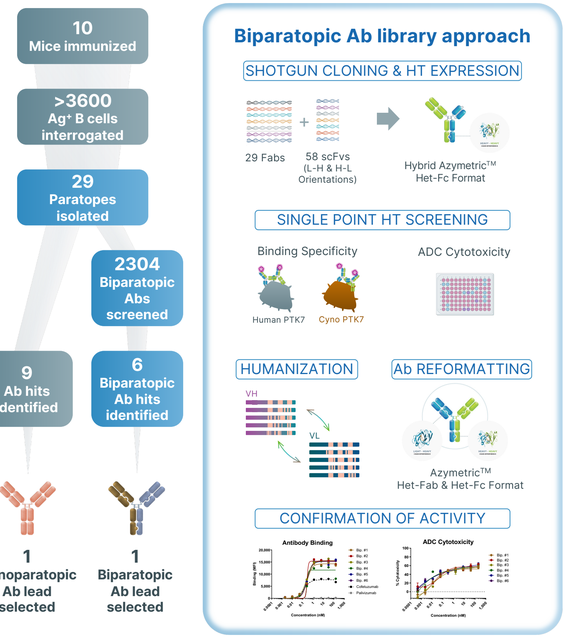


Figure 1. Examples of strong IHC staining of PTK7 in clinical tumor tissue microarrays from select indications.

Integrated antibody library generation and screening workflow



Lead biparatopic engages two non-overlapping PTK7 epitopes

Antibody	Paratopes	Epitopes
Lead Biparatopic (Azymetric™ Het-Fc & Het-Fab)	P1 x P2	Ig4 x Ig3/4
Monoparatopic #1	P1 x P1	Ig4 x Ig4
Monoparatopic #2	P2 x P2	Ig3/4 x Ig3/4
Lead Monoparatopic (Internal Benchmark)	P3 x P3	Ig4 x Ig4
Cofetuzumab (Clinical Benchmark)	Cofe. x Cofe.	Ig4 x Ig4

Lead biparatopic and lead monoparatopic are comprised of different paratopes

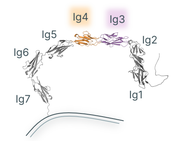


Figure 2. The predicted structure of the extracellular domains of PTK7. [4,5] Hydrogen-deuterium exchange was employed to determine the epitope space bound by each paratope.

Biparatopic targeting can result in beneficial antibody and ADC properties

Greater biparatopic antibody binding leads to higher internalization

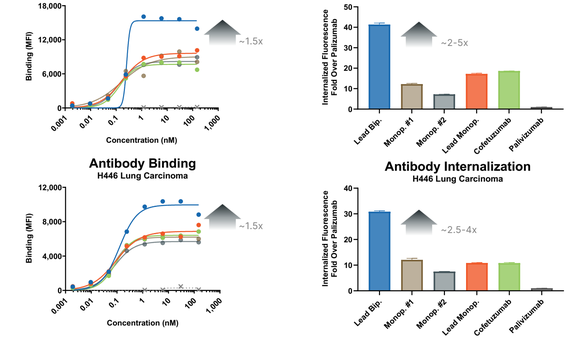


Figure 3. (A) Binding of PTK7 targeting ADCs to cancer cell lines was assessed by flow cytometry. **(B)** Internalization of AF488 labelled antibodies into cancer cell lines after 4 hours at 10 nM.

Increase in payload delivery and corresponding cytotoxicity can be achieved with a biparatopic ADC

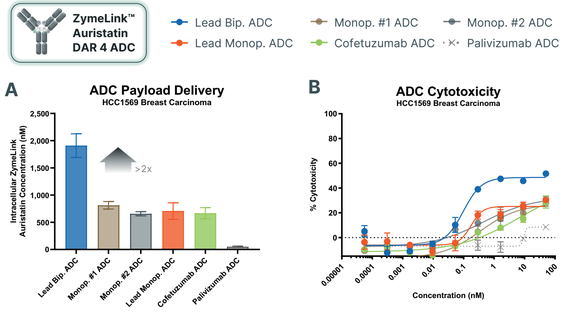


Figure 4. (A) Mass spectrometry quantification of internalized payload following treatment of cells with 20 nM of PTK7 targeting ADCs for 24 hours. **(B)** Cytotoxicity was assessed by treating HCC1569 cells with PTK7 targeting ADCs for 4 days. Cell viability was determined using CellTiterGlo®.

Biparatopic PTK7 TOPO1i ADC demonstrates robust activity

Biparatopic PTK7 topoisomerase 1 inhibitor (TOPO1i) ADC is active across a range of cancer cell lines

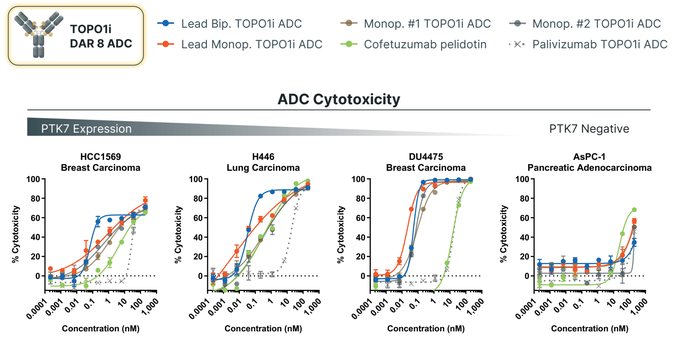


Figure 5. Cytotoxicity was assessed by treating cell lines with PTK7 targeting ADCs for 6 days. Cell viability was determined using CellTiterGlo®. Cofetuzumab pelidotin is a PKT7 ADC comprised of cofetuzumab conjugated to PF-06380101 (Aur0101) with an average drug to antibody ratio (DAR) of 4.

Biparatopic PTK7 TOPO1i ADC displays in vivo anti-tumor activity

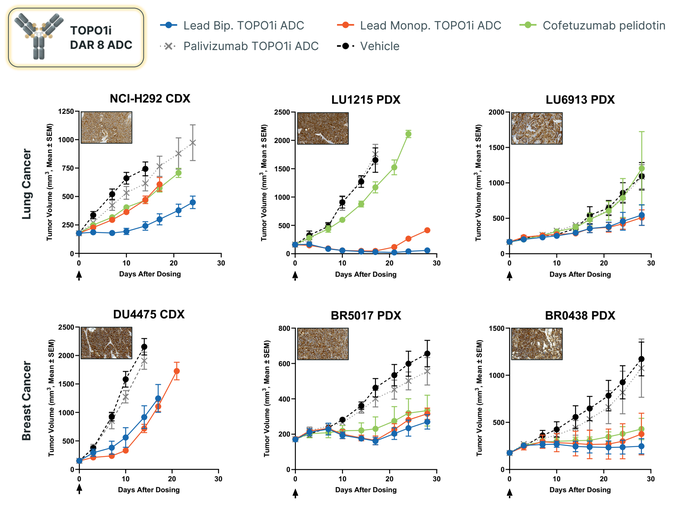


Figure 6. In vivo efficacy of PTK7 targeting ADCs in cell line-derived xenograft (CDX) and patient-derived xenograft (PDX) models of breast cancer and lung cancer. TOPO1i ADCs (DAR 8) were dosed at either 6 mg/kg (NCI-H292 CDX and all PDX models) or 0.6 mg/kg (DU4475 CDX). Cofetuzumab pelidotin (DAR 4) was dosed at 2.5 mg/kg. Mice (n=6 for CDX, n=3 for PDX) were given a single intravenous dose when tumor volumes reached an average of ~150-250 mm³. Growth plots were terminated when >20% of mice (CDX) or 1 mouse (PDX) was lost within a treatment group. PTK7 expression was determined by IHC using an antibody developed at Zymeworks.

Biparatopic PTK7 TOPO1i ADC is well-tolerated in non-human primates

Study design:

Study results:

	Body Weight	Hematology	Serum Chemistry	Organ Weights
Biparatopic Lead TOPO1i DAR 8 ADC 60 mg/kg	Minor ↓ after each dose	↓ lymphocyte & reticulocyte counts	↑ ALT & AST after 1st dose (n=3)	↓ thymus
Monoparatopic Lead TOPO1i DAR 8 ADC 60 mg/kg			↑ ALT & AST after 1st dose (n=1)	

- No mortality or adverse clinical signs observed
- Minor decreases in body weight and food consumption
- Minimal, transient changes in hematology and clinical chemistry parameters for both ADCs
- Thymus weights decreased for both ADCs

Biparatopic PTK7 TOPO1i ADC has a favorable pharmacokinetic (PK) profile

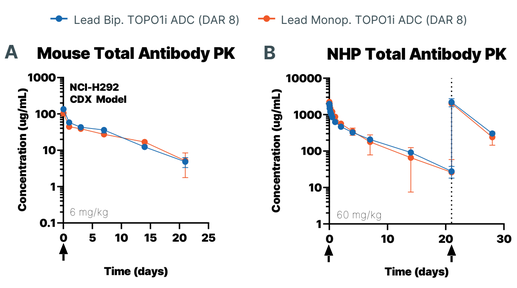


Figure 7. (A) Total antibody PK from a single-dose xenograft study in SCID-beige mice and **(B)** a repeat dose tolerability study in non-human primates (NHPs) show that biparatopic and monoparatopic PTK7 TOPO1i ADCs maintain favorable PK profiles.

Conclusions

- Biparatopic targeting of PTK7 demonstrably increases antibody binding and receptor-mediated internalization relative to monoparatopic counterparts.
- Enhanced antibody internalization of biparatopic ADC allows for increased delivery of cytotoxic payload.
- First in class biparatopic TOPO1i PTK7 ADC is active in breast and lung cancer models and demonstrates potential improvement over cofetuzumab pelidotin.
- Biparatopic TOPO1i PTK7 ADC is well tolerated up to 60 mg/kg in non-human primates.

References

1. Maitland, M.L. et al. First-in-human study of PF-06647020 (cofetuzumab pelidotin), an antibody–drug conjugate targeting protein tyrosine kinase 7, in advanced solid tumors. Clin Cancer Res. 2021 Jun 3;27(16):4511–4520
2. Cho, B.C. et al. A phase 1b study of cofetuzumab pelidotin monotherapy in patients with PTK7-expressing recurrent non-small cell lung cancer. Lung Cancer. 2025 Mar 10;202:108492
3. Kong, C. et al. MTX-13, a novel PTK7-directed antibody-drug conjugate with widened therapeutic index shows sustained tumor regressions for a broader spectrum of PTK7-positive tumors. Mol Cancer Ther. 2023 Oct 2;22(10):1128-1143
4. Varadi, M. et al. AlphaFold Protein Structure Database in 2024: Providing structure coverage for over 214 million protein sequences. Nucleic Acids Res. 2024 Jan 5;52(D1):D368-D375
5. Varadi, M. et al. AlphaFold Protein Structure Database: Massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nucleic Acids Res. 2022 Jan 7;50(D1):D439-D444

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