Preclinical Characterization of PRO1184, a Novel Exatecan-based Folate Receptor α-directed Antibody-drug Conjugate

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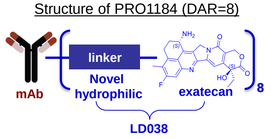
ProfoundBio

Introduction

- Folate receptor α (FRα) is a folate-binding glycoprotein located on cell surface[1] and overexpressed in various solid tumors including ovarian, lung, and breast cancers[2]
- Increasing effort is being made to leverage FRα overexpression to develop a diagnostic marker or novel cancer therapy. Promising clinical activity has been observed with multiple FRα-targeting modalities including antibody-drug conjugates (ADCs)[3]
- PRO1184 is a novel FRα-directed ADC designed with three components:
  - A proprietary human IgG1 monoclonal antibody (mAb) targeting FRα
  - exatecan, a topoisomerase I inhibitor with established antitumor activity and pharmacological attributes[4]
  - A novel hydrophilic and protease-cleavable linker (linker along with the drug, exatecan, is collectively dubbed as LD038)[5,6]

Structure of PRO1184 (DAR=8)



Benchmarking ADC (DAR)	Linker-drug
mAb-soravtansine(4)	SPDB-DM4
mAb-deruxtecan(8)	mc-GGFG-DXd

Cell line	Tumor type	FRα Copy number (X10³ per cell)
OVCAR-3	ovarian	290
KB	oral epithelial	341
JEG-3	choriocarcinoma	113
NCI-H441	NSCLC	47
HCC827	NSCLC	41
OV90	ovarian	0.38
PC-3	prostate	0.041

Binding Affinity and Specificity

A

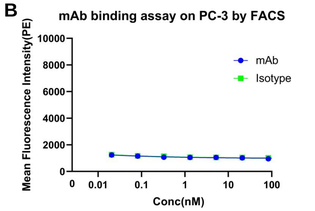
mAb

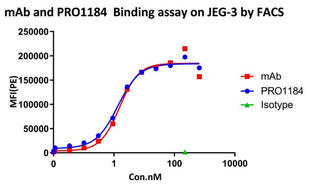
Antigen	KD(M)	ka (1/Ms)	kdis (1/s)
Hu FRα	1.5E-09	2.2E05	3.3E-04
Cyno FRα	8.1E-09	1.9E05	1.5E-03
Rat FRα	NA	NA	NA
Mouse FRα	2.9E-06	1.0E05	3.0E-01

PRO1184

Antigen	KD(M)	ka (1/Ms)	kdis (1/s)
Hu FRα	4.8E-09	2.2E05	1.0E-03
Cyno FRα	8.8E-09	1.8E05	1.5E-03
Rat FRα	NA	NA	NA
Mouse FRα	2.8E-06	9.2E04	2.6E-01

B





	PRO1184	mAb	Isotype
EC50	1.343	1.549	

Fig. 1. A. Affinity of mAb or PRO1184 to recombinant FRα species (human, cyno, rat, mouse) orthologs were measured using Octet RED (Fortbio). B. Binding of mAb to FRα-negative cells, PC-3, or mAb and PRO1184 to FRα-positive cells, JEG-3, were evaluated by flow cytometry. NA, response below range of quantitation.

In vitro Internalization Studies

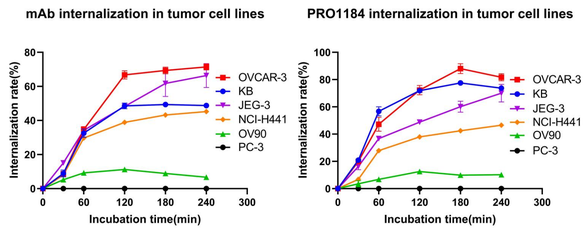
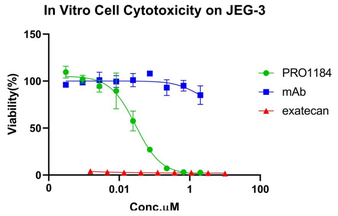
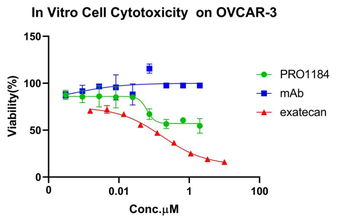


Fig. 2. Internalization of mAb and PRO1184 in tumor cells were determined in a time course manner. Internalization rate was calculated by subtracting the mean fluorescence intensity (MFI) of cell surface-bound antibody at 37°C at each timepoint from the MFI of cell surface-bound antibody at 4°C at time 0, then divided by the MFI of cell surface-bound antibody at 4°C at time 0.

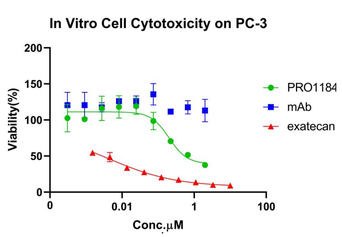
In vitro Cytotoxicity Studies



	IC50(μM)
PRO1184	0.029
mAb	NA
exatecan	~4.9E-15



	IC50(μM)
PRO1184	0.0624
mAb	NA
exatecan	0.1701



	IC50(μM)
PRO1184	0.2007
mAb	NA
exatecan	0.004427

Fig. 3. Cell viability was evaluated 4 days after treatment using the Cell Titer-Glo Assay (Promega Corp.) All readings were normalized as percentage of viable cells in the untreated control wells and the IC50 values were calculated. NA, no appreciable activity.

Anti-tumor Activity in CDX Models

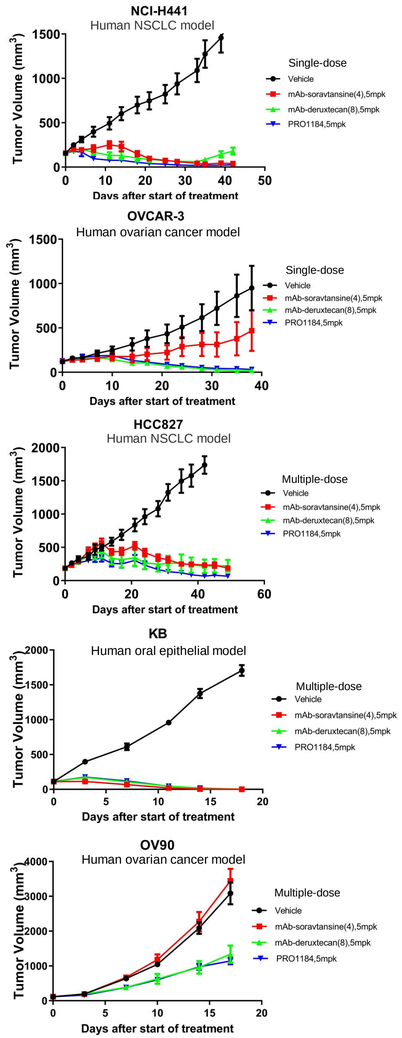
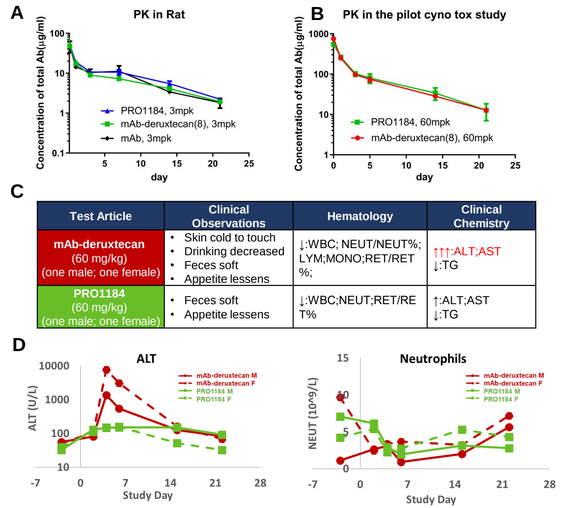


Fig. 4. Anti-tumor activity of the ADCs were examined in various cell-line derived xenograft (CDX) models. Studies of NCI-H441 and OVCAR-3 were single-dose treatment at the specified doses (n=7~8 per treatment group). Studies of HCC827, KB, and OV90 were multiple-dose (on day 1, 4, 8, 11) treatment at the specified doses (n=6~9 per treatment group). None of the ADC-treated animals exhibited appreciable weight loss or apparent distress (not shown).

Plasma PK and Tolerability



C

Test Article	Clinical Observations	Hematology	Clinical Chemistry
mAb-deruxtecan (60 mg/kg) (one male; one female)	• Skin cold to touch • Drinking decreased • Feces soft • Appetite lessens	↓:WBC; NEUT/NEUT%; LYM;MONO;RET/RET %;	↑↑↑:ALT;AST ↓:TG
PRO1184 (60 mg/kg) (one male; one female)	• Feces soft • Appetite lessens	↓:WBC;NEUT;RET/RET T%	↑:ALT;AST ↓:TG

Fig. 5. A. Plasma PK of PRO1184, mAb-deruxtecan(8), and mAb in rat (n=3 per group). B-D. Plasma PK (B), clinical observations (C), ALT and neutrophil levels (D), in the pilot tox study in cynomolgus monkeys (n=2 per group). Concentrations of total antibodies in circulation were determined via an ELISA.

Conclusions

- PRO1184 displayed strong and specific binding to FRα and rapid internalization in target cells
- PRO1184 was highly potent in cytotoxicity studies in vitro and in tumor-growth inhibition in vivo; PRO1184 exerted more anti-tumor effect than F131-soravtansine in vivo
- PRO1184 displayed excellent plasma PK in rat and cyno
- PRO1184 was well-tolerated in cyno with changes in ALT and neutrophils reversed by day 22; transient ALT increase with PRO1184 was at a smaller magnitude than mAb-deruxtecan, suggesting PRO1184 may confer a more favorable therapeutic index in the clinic
- In summary, PRO1184 displays a promising preclinical profile on PK, PD, efficacy, and tolerability. PRO1184 is a promising candidate for further development in the clinic for various cancers

References

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