Proof-of-concept for a Novel ADC Linker-drug Platform, via Preclinical Characterization of PRO1102, a HER2-directed ADC

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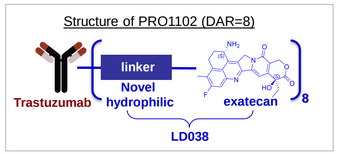
Introduction

- Antibody-drug conjugates (ADCs) are a promising and growing class of therapeutic agents with multiple approved in oncology in recent years[1]
- HER2 is a clinically validated target with several HER2-directed ADCs exhibiting activity in multiple tumor types[2,3], or in HER2-low tumors[4]
- LD038, a novel linker-drug, may further expand therapeutic index of ADCs via its appealing physicochemical properties[5,6]
- PRO1102, a HER2-directed ADC (LD038 conjugated to trastuzumab), was generated and characterized in preclinical studies for initial proof-of-platform
- Two high HER2 expression models (HCC1954, SK-OV-3), one moderate HER2 expression model (JIMT-1), and one low HER2 expression model (Capan-1) were utilized in the studies

Structure of PRO1102 (DAR=8)

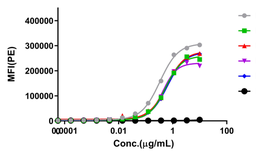


Benchmarking ADC (DAR)	Linker-drug
trastuzumab-deruxtecan(8)	mc-GGFG-DXd
trastuzumab-emtansine(4)	SMCC-DM1
trastuzumab-vedotin(4)	mc-vc-PAB-MMAE

Cell line	Tumor type	HER2 copy number (X10³ per cell)
SK-OV-3	ovarian	467
JIMT-1	breast	70
Capan-1	pancreatic	33
HCC1954	breast	365
Raji	lymphoma	0.18

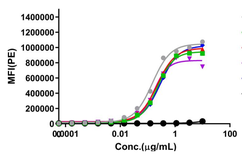
Binding Affinity

Binding to HCC1954 by FACS



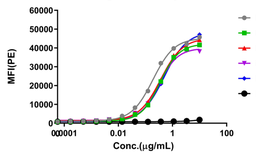
Sample	EC50(μg/mL)
trastuzumab	0.34
trastuzumab-deruxtecan(8)	0.54
trastuzumab-emtansine(4)	0.61
trastuzumab-vedotin(4)	0.43
PRO1102	0.64
Isotype	NA

Binding to SK-OV-3 by FACS



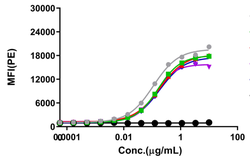
Sample	EC50(μg/mL)
trastuzumab	0.15
trastuzumab-deruxtecan(8)	0.22
trastuzumab-emtansine(4)	0.22
trastuzumab-vedotin(4)	0.17
PRO1102	0.28
Isotype	NA

Binding to JIMT-1 by FACS



Sample	EC50(μg/mL)
trastuzumab	0.19
trastuzumab-deruxtecan(8)	0.35
trastuzumab-emtansine(4)	0.37
trastuzumab-vedotin(4)	0.32
PRO1102	0.49
Isotype	NA

Binding to Capan-1 by FACS



Sample	EC50(μg/mL)
trastuzumab	0.11
trastuzumab-deruxtecan(8)	0.16
trastuzumab-emtansine(4)	0.19
trastuzumab-vedotin(4)	0.13
PRO1102	0.18
Isotype	NA

Fig. 1. Binding of trastuzumab and its conjugates to target cells were evaluated via indirect immunofluorescence on flow cytometry. NA, no appreciable activity.

In vitro Internalization Studies

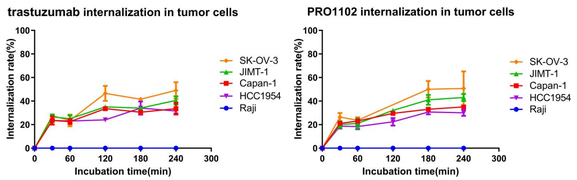
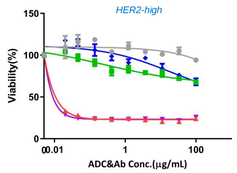


Fig. 2. Internalization of trastuzumab and PRO1102 in tumor cells were determined in a time course manner. Internalization rate was calculated by subtracting the mean fluorescence intensity (MFI) of cell surface-bound antibody at 37°C at each timepoint from the MFI of cell surface-bound antibody at 4°C at time 0, then divided by the MFI of cell surface-bound antibody at 4°C at time 0.

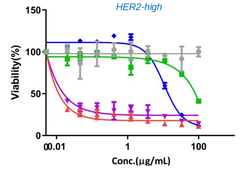
In vitro Cytotoxicity Studies

In Vitro Cell Cytotoxicity On HCC1954



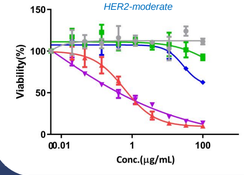
	IC50(μg/ml)
trastuzumab	NA
trastuzumab-deruxtecan(8)	NA
trastuzumab-emtansine(4)	~3.8*10^{-5}
trastuzumab-vedotin(4)	~1.2*10^{-5}
PRO1102	NA

In Vitro Cell Cytotoxicity On SK-OV-3



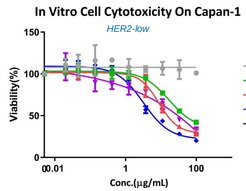
	IC50(μg/ml)
trastuzumab	NA
trastuzumab-deruxtecan(8)	~100
trastuzumab-emtansine(4)	~7.5*10^{-6}
trastuzumab-vedotin(4)	~6.6*10^{-6}
PRO1102	10.7

In Vitro Cell Cytotoxicity On JIMT-1



	IC50(μg/ml)
trastuzumab	NA
trastuzumab-deruxtecan(8)	NA
trastuzumab-emtansine(4)	0.6
trastuzumab-vedotin(4)	0.004
PRO1102	~27.77

In Vitro Cell Cytotoxicity On Capan-1



	IC50(μg/ml)
trastuzumab	NA
trastuzumab-deruxtecan(8)	16.95
trastuzumab-emtansine(4)	8.81
trastuzumab-vedotin(4)	~10
PRO1102	3.22

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

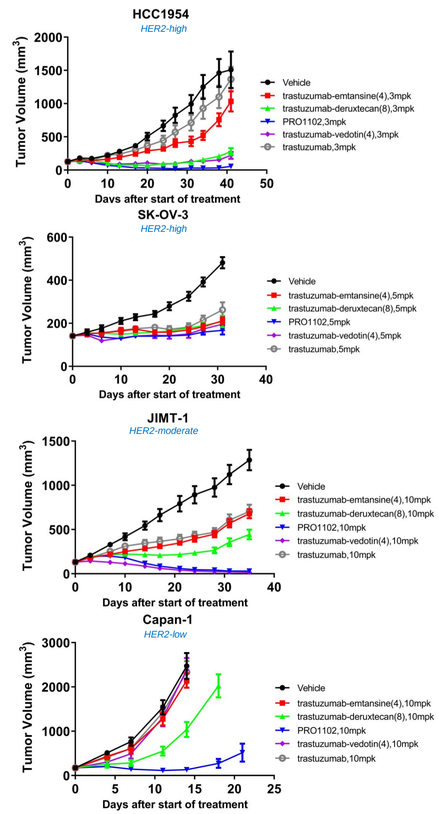


Fig. 4. Anti-tumor activity of the ADCs were examined in cell-line derived xenograft (CDX) models. All studies were single-dose treatment at the specified doses (n=8~10 per treatment group). None of the ADC-treated animals exhibited appreciable weight loss or apparent distress.

Plasma PK and Tolerability

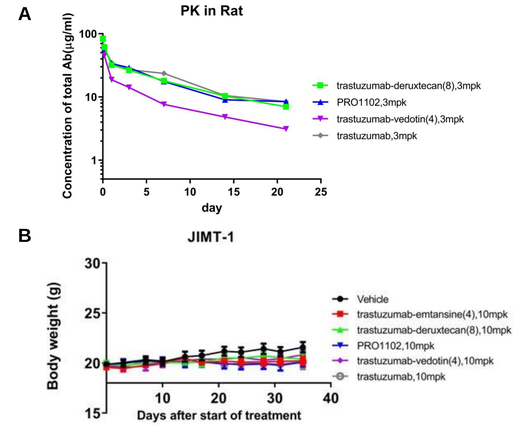


Fig. 5. A. Plasma PK of trastuzumab or its conjugates in rat (n=3 per group). B. Body weight of the mice over the course of the single-dose study in the JIMT-1 CDX model (Fig. 4). PRO1102 was well tolerated in mice at dose levels associated with robust anti-tumor activity.

Conclusions

- PRO1102 displayed strong and specific binding to HER2 and rapid internalization in target cells
- PRO1102 was highly potent in cytotoxicity studies in vitro and in tumor-growth inhibition in vivo; PRO1102 was more potent than trastuzumab-deruxtecan in moderate or low HER2 expression models in vitro or in vivo; PRO1102 exerted more tumor growth-inhibition than the anti-tubulin conjugates in vivo
- PRO1102 exhibited excellent plasma PK characteristics that are similar to the parental mAb, trastuzumab, in rat; PRO1102 was well-tolerated in rodent studies so far
- In summary, characterization of PRO1102 and comparative analyses with the benchmarking ADCs illustrate the promising features of our novel linker-drug platform and support advancement of new ADCs from this platform to the clinic

References

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