Exatecan-based antibody-drug conjugates (DAR8) embedding hydrophilic PSARlink™ drug-linker technology

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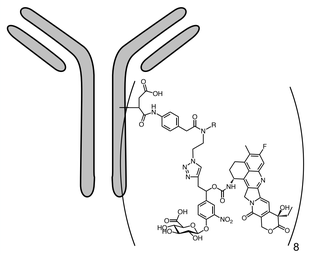
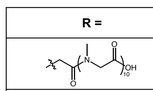
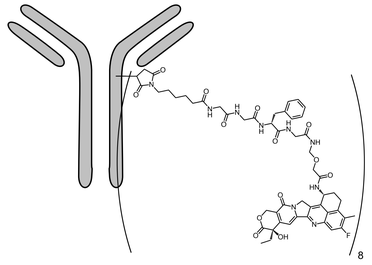
Background

- Two ADCs based on topoisomerase I camptothecin payloads were recently approved: Enhertu® (trastuzumab deruxtecan — DXd payload) and Trodelvy® (sacituzumab govitecan — SN38 payload).
- Camptothecin analogues binds to the topoisomerase I and DNA complex, therefore causing DNA damage and cell apoptosis.
- Exatecan (DX-8951f) is a potent water-soluble camptothecin derivative developed in the 90's.
- In our hands, the straightforward inclusion of Exatecan in cleavable drug-linker designs convey significant ADC aggregation potential and accelerated clearance rates of the conjugates. The steric hindrance around the stereo-defined primary amine attachment point of the molecule certainly plays a role in this observation.
- We herein report a glucuronide-based cleavable Exatecan drug-linker platform embedding our proprietary PSARlink™ hydrophobicity masking technology. DAR8 ADCs having excellent physicochemical properties, pharmacokinetics, efficacy and tolerability were obtained.

Structure of trastuzumab topo I ADCs



R =	ADC name
	TRA-EXATECAN-PSAR10
	TRA-EXATECAN-PSAR0

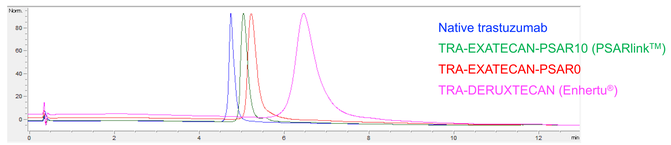


TRA-DERUXTECAN (Enhertu®)

- 3 different DAR8 ADCs based on the mAb trastuzumab (anti-HER2) and were formulated.
- TRA-EXATECAN-PSAR10 includes the PSARlink™ polysarcosine hydrophobicity masking unit (https://doi.org/d8c2). TRA-EXATECAN-PSAR0 is a negative control without polysarcosine. These two ADCs use a glucuronidase-sensitive trigger molecular entity (cleavable linker). These two ADCs includes an hydrolysed plasma-stable aryl-maleimide.
- TRA-DERUXTECAN (Enhertu®) is used as a positive control.

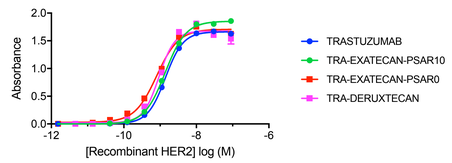
Preclinical in vitro and in vivo proof-of-concept

Low ADC hydrophobicity by Hydrophobic Interaction Chromatography (HIC)

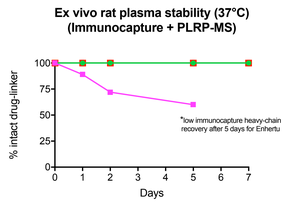


- Exatecan ADCs showed decreased hydrophobicity compared to Enhertu®
- PSARlink™ ADC is more hydrophilic than negative control "PSAR0" ADC
- Aggregation levels <5% is observed for all ADCs by SEC-HPLC

Comparable HER2 binding affinity profiles (ELISA)



High *ex-vivo* plasma stability



- Glucuronide-Exatecan ADCs were stable in rat plasma over the course of seven days.
- Plasma stability compared favourably with deruxtecan drug-linker, which suffered from drug-linker maleimide retro-Michael deconjugation over time on the heavy chain of the antibody.

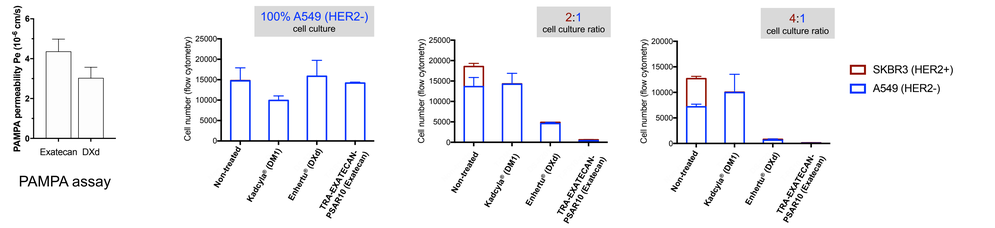
Potent in vitro activity

IC_{50} (nM — ADC conc)	TRA-EXATECAN-PSAR10	TRA-EXATECAN-PSAR0	TRA-DERUXTECAN
NCI-N87	0.3	0.3	0.3
SKBR3	0.2	0.2	0.2
MDA-MB-453	0.3	0.2	0.2
MDA-MB-361	0.8	0.7	0.6
BT-474	0.7	0.9	0.5

Following 6 days incubation, cells were assessed for viability using an MTT assay.

- Identical sub-nM IC_{50} *in vitro* potencies on HER2+ cell lines were observed for these 3 DAR8 topoisomerase-based cleavable ADCs.

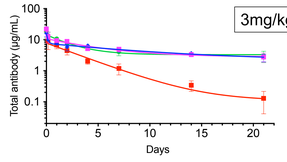
In vitro bystander killing assessment



- Passive membrane diffusion of Exatecan payload is slightly higher than DXd payload used in the deruxtecan drug-linker, as assayed by Corning® Gentest™ PAMPA assay.
- None of the tested ADCs were significantly cytotoxic to the HER2- A549 cell line at the tested concentration, revealing HER2-specific cytotoxicity of the tested ADCs. In co-cultured HER2+/HER2- cells in vitro experiments, Exatecan ADC showed a more pronounced bystander killing activity compared to non-cleavable DM1 ADC (Kadcyla® — SMCC-DM1 drug-linker) or DXd ADC (Enhertu® — deruxtecan linker).

SKBR3 (HER2+) and A549 (HER2-) cells were co-cultured at a 0:1 (A549 cells only), 2:1 or 4:1 seeding ratio, treated with 10nM ADCs for 5 days. After collecting adherent cells, cell number and ratio of HER2+/HER2- cells were determined by flow cytometry. 3 independent experiments were realized.

PK study in Sprague-Dawley rats

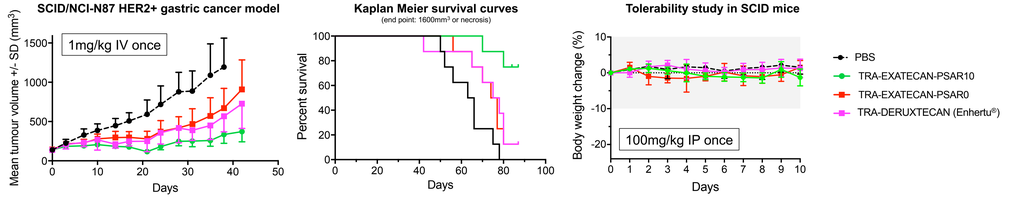


	Trastuzumab	Enhertu®	TRA-EXATECAN-PSAR10 (PSARlink™)	TRA-EXATECAN-PSAR0
Clearance rate (mL/day/kg)	47.3	44.8	48.5	135
AUC_{0-inf} (day x µM)	243	233	216	77.7

Pharmacokinetic parameters

- TRA-EXATECAN-PSAR0 suffers from accelerated plasma clearance, despite its apparent favorable hydrophobicity profile observed by HIC.
- Inclusion of a polysarcosine entity (PSARlink™) in the drug-linker (TRA-EXATECAN-PSAR10) restores the PK profile of the native trastuzumab antibody.

In vivo efficacy study and preliminary tolerability data



- Favorable PK profile conferred by the inclusion of PSARlink™ (ADC-EXA-PSAR10) directly improves efficacy when compared to negative control ADC-EXA-PSAR0 having unfavourable rat PK profile.
- At 1 mg/kg sub-curative dose, ADC-EXA-PSAR10 outperforms Enhertu® in this cancer model.
- All ADCs were tolerated at 100mg/kg (ADC dose) in SCID mice. More tolerability experiments are currently ongoing.

Summary

- TRA-EXATECAN-PSAR10 embedding our PSARlink™ hydrophobicity masking technology (https://doi.org/d8c2) showed excellent physicochemical and pharmacological properties. Inclusion of the hydrophobicity masking chemical moiety seemed mandatory to provide optimized Exatecan-based ADCs.
- TRA-EXATECAN-PSAR10 showed improved plasma stability and in vivo efficacy when compared to Enhertu® (deruxtecan drug-linker based on DXd payload). Bystander killing activity of the Exatecan payload seemed comparable or more pronounced than with DXd payload.
- Mablink is developing two ADC drug candidates that are based on this drug-linker platform.

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