PSARlink - hydrophilic monodisperse polysarcosine drug-linker platform for ADCs

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Abstract

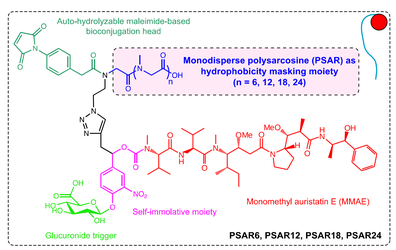
Antibody-drug conjugates (ADCs) convey highly potent anticancer drugs to antigen-expressing tumor cells, thereby sparing healthy tissues throughout the body. Expected improvements in the field rely on the design of new technologies that influence the pharmacological properties of the conjugates. Key parameters such as (i) plasmatic stability, (ii) Drug-Antibody-Ratio (DAR), (iii) conjugation position on the antibody, and (iv) overall hydrophobicity and homogeneity of the conjugates dictates pharmacokinetics (PK) properties, efficacy and tolerability of ADCs.

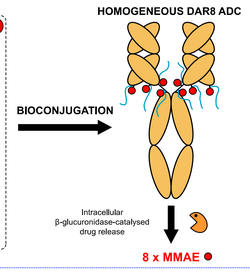
We herein report the use of monodisperse polysarcosine (PSAR) as a hydrophobicity masking entity in hydrophilic drug-linker constructs for high drug-load ADCs (PSARlink ADC platform). This approach improved physicochemical properties, pharmacokinetics, efficacy and tolerability of the resulting conjugates. We showed that the drug-linker platform can be tailored for the use of cleavable or non-cleavable linker constructs and seems especially suited for the use of moderately potent low nM ADC payloads having improved therapeutic indexes and/or differentiated mechanism of action. The platform is directly compatible with any “off-the-shelf” unmodified IgG, yielding homogeneous DAR8 ADCs, but can also be paired with site-specific conjugation technologies if required.

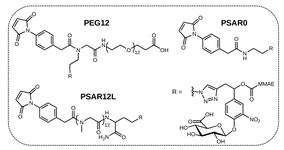
Materials and Methods

- A set of biocompatible polysarcosine (PSAR) hydrophobicity masking entities was synthesized using inexpensive solid-phase methodologies. Molecular length of these oligomers can be varied in order to tune the physicochemical and pharmacological properties of the final ADCs.
- Different cleavable or non-cleavable drug-linkers based on MMAE, Exatecan and PNU-159682 were synthesized.
- Clinically validated trastuzumab (anti-Her2) antibody was used for ADC formulation. ADCs were prepared by total reduction of native disulfide interchain bridges with excess TCEP followed by chemical maleimide-conjugation of drug-linkers in PBS (1.3 molar eq of drug-linker per cysteine). ADCs were purified/buffer exchanged by SEC and/or centrifugal dialysis. When necessary, ADCs were incubated 24h in PBS 8.0 at 37°C to ensure total maleimide hydrolysis and plasma stability (prevents premature deconjugation of the drug-linker by retro-Michael reaction).
- Resulting DAR8 ADCs were analysed by HPLC-SEC and were >95% monomeric. HPLC-HIC and RPLC-QToF analysis confirmed the homogeneous loading of 8 drug-linker per antibody (DAR8).
- For *in vitro* cytotoxicity assays, cells were seeded to a 96-well plate at 1000-5000 cells per well depending on the cell line. After overnight incubation, each diluted substance was added. Cell viability was evaluated after 5 days using MTT or CellTiter-Glo® reagent.
- Pharmacokinetic (PK) experiments were conducted in female SCID mice and female Sprague-Dawley rats at an ADC dose of 3 mg/kg. Quantification of the ADC component was made by anti-human IgG ELISA assay.
- *In vivo* efficacy studies were conducted in a BT-474/SCID breast cancer or NCI-N87/SCID gastric cancer xenografted mouse models. ADCs were dosed **once intravenously** and tumor volume was monitored over time.

MMAE cleavable ADC platform (homogeneous DAR8 ADCs)







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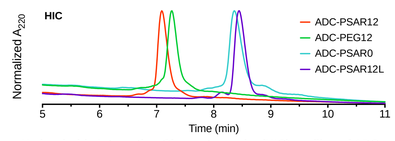
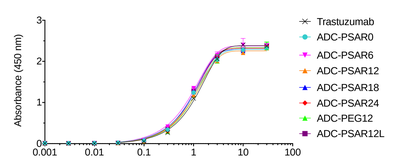


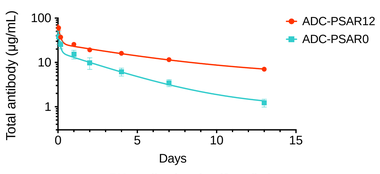
Hydrophobic Interaction Chromatography (HIC) profiles of ADCs



HER2 binding affinity profiles of ADCs assayed by ELISA

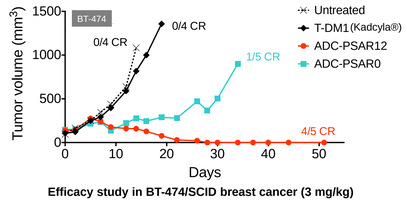
	Architecture	DAR	Hydrophilic moiety
ADC-PSAR6	Orthogonal	Homogeneous DAR8	Polysarcosine (6 residues)
ADC-PSAR12	Orthogonal	Homogeneous DAR8	Polysarcosine (12 residues)
ADC-PSAR18	Orthogonal	Homogeneous DAR8	Polysarcosine (18 residues)
ADC-PSAR24	Orthogonal	Homogeneous DAR8	Polysarcosine (24 residues)
ADC-PSAR0	-	Homogeneous DAR8	None
ADC-PSAR12L	Linear	Homogeneous DAR8	Polysarcosine (12 residues)
ADC-PEG12	Orthogonal	Homogeneous DAR8	Polyethylene glycol (12 residues)

- DAR8 homogeneous ADCs based on trastuzumab were formulated and characterized
- ADC-PSAR0 is a negative control ADC without polysarcosine (no hydrophobicity masking properties). ADC-PSAR12L is a negative control ADC bearing polysarcosine in a serial configuration, compared to an orthogonal orientation in other drug-linkers. ADC-PEG12 is a control ADC bearing polyethylene glycol (PEG) instead of PSAR
- Hydrophobic Interaction Chromatography results show that inclusion of orthogonal PSAR drastically reduce overall hydrophobicity of the conjugates. Inclusion of PSAR in an orthogonal position in relation to the drug payload is mandatory. At equal length (12 monomer residues), PSAR promotes better hydrophobicity masking properties than PEG



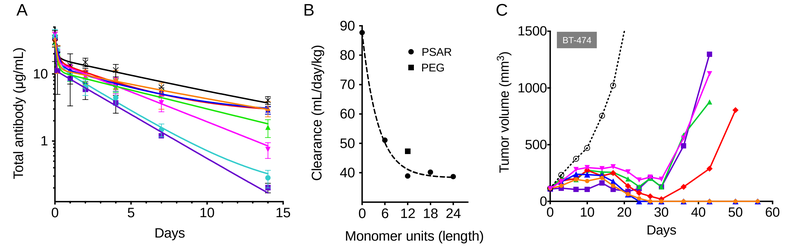
PK studies in mice (3 mg/kg)

- Lack of hydrophobicity masking moiety leads to accelerated plasma clearance (ADC-PSAR0), in accordance with already reported results in the ADC field of research
- Inclusion of a polysarcosine entity in the drug-linker restores a favorable PK profile (ADC-PSAR12)



Efficacy study in BT-474/SCID breast cancer (3 mg/kg)

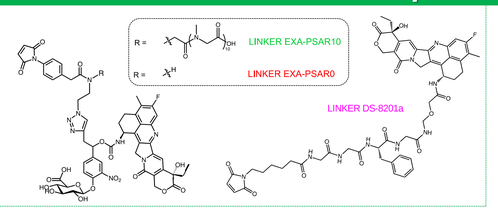
- Favorable PK profile conferred by the inclusion of polysarcosine (ADC-PSAR12) directly improves efficacy when compared to negative control ADC (ADC-PSAR0)
- At the same 3 mg/kg dose, T-DM1 fails to induce tumor regression



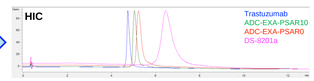
PK studies in Sprague-Dawley rats (3 mg/kg) (A) and efficacy study in BT-474/SCID breast cancer (2.5 mg/kg) (B and C)

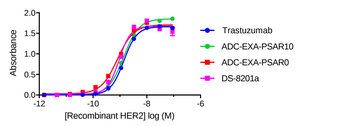
- ADC exposure increases as a function of PSAR length up to 12 monomer residues, eventually reaching a point where further PSAR extension has little influence on the clearance rates of the conjugates (Figures A and B). In accordance with their observed hydrophobicity, ADC-PSAR0 and ADCPSAR12L show unfavorable PK characteristics. At equal length, PSAR more efficiently improves clearance rates when compared to PEG.
- At a 2.5 mg/kg dose, complete tumor regressions were only observed for conjugates having low clearance rates (ADC-PSAR12 and ADC-PSAR18 - Figure C). ADCs having unfavorable (ADC-PSAR12L) or suboptimal (ADC-PSAR6 and ADC-PEG12) clearance profiles were only able to promote delayed tumor growth

Exatecan cleavable ADC platform (homogeneous DAR8 ADCs)



Trastuzumab DAR8 ADCs



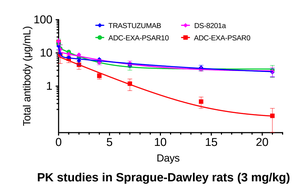


HER2 binding affinity profiles of ADCs assayed by ELISA

IC_{50} (nM - ADC conc)	NCI-N87	SKBR3	MDA-MB-453
ADC-EXA-PSAR10	0.3	0.2	0.4
ADC-EXA-PSAR0	0.3	0.2	0.4
DS-8201a	0.3	0.2	0.3
T-DM1	0.5	0.1	0.3

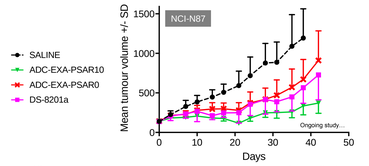
***In vitro* cytotoxicity assays on HER2+ cell lines**

- Identical *in vitro* potency is observed for all the topoisomerase-based ADCs



PK studies in Sprague-Dawley rats (3 mg/kg)

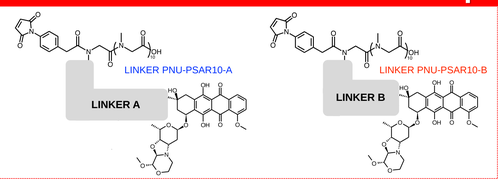
- Lack of hydrophobicity masking moiety (ADC-EXA-PSAR0) leads to accelerated plasma clearance
- Inclusion of a polysarcosine entity in the drug-linker restores a favorable PK profile (ADC-EXA-PSAR10)
- No correlation was observed between HIC retention time (“apparent hydrophobicity”) and PK profiles



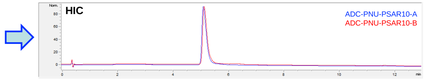
Efficacy study in NCI-N87/SCID gastric cancer (1 mg/kg)

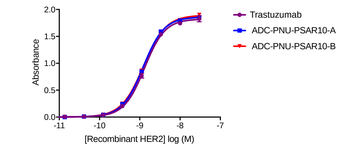
- Favorable PK profile conferred by the inclusion of polysarcosine (ADC-EXA-PSAR10) directly improves efficacy when compared to negative control ADC-EXA-PSAR0 having unfavourable PK profile
- At 1 mg/kg sub-curative dose, ADC-EXA-PSAR10 outperforms DS-8201a in this cancer model

PNU-159682 non-cleavable ADC platform (homogeneous DAR8 ADCs)



Trastuzumab DAR8 ADCs



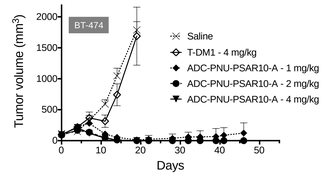


HER2 binding affinity profiles of ADCs assayed by ELISA

IC_{50} (nM - ADC conc)	NCI-N87	SKBR3	MDA-MB-361	BT-474
ADC-PNU-PSAR10-A	0.03	0.01	0.4	0.2
ADC-PNU-PSAR10-B	0.9	0.4	1.3	1
T-DM1	0.2	0.1	0.1	0.3

***In vitro* cytotoxicity assays on HER2+ cell lines**

- *In vitro* potency is retained despite polysarcosine inclusion in a non-cleavable drug-linker architecture
- Linker construct **A** is approximately 5 to 10-fold more potent than linker **B**



Efficacy study in BT-474/SCID breast cancer

- Complete tumor regressions were observed at 1 mg/kg (linker **A**)
- T-DM1 fails to induce tumor regression at 4 mg/kg
- *In vivo* assays are ongoing

Conclusion

- When embedded in an orthogonal configuration in the drug-linker structure, PSAR improves PK profile and efficacy of ADCs.
- The bioconjugation procedure yields homogeneous plasma-stable DAR8 ADCs, from native unmodified monoclonal IgGs.
- The PSARlink platform is compatible with cleavable and non-cleavable drug-linker architectures and with virtually any cytotoxic payloads.
- The platform is especially suited for the use of moderately potent low nM ADC payloads (topoisomerase inhibitors and payloads with differentiated MoA’s…) and very hydrophobic payloads with high aggregation propensity.
- Proof-of-concept studies of the PSARlink ADC platform is still running, with *in vivo* efficacy and tolerability assays currently ongoing.
- Mablink is developing two ADC drug candidates (preclinical stage) that are based on the PSARlink drug-linker platform.