A novel antibody-drug conjugate platform enabling high drug-to-antibody ratios (DARs) and greater payload flexibility.

Ludovic Juen[a], Adam J. Collier[a], Anthony W. Tolcher[b] and Myriam M. Ouberai[a]
[a]Spirea Ltd, Cambridge, UK, [b]NEXT Oncology, San Antonio, USA

In this study, we used a novel and flexible multivalent linker technology to construct two ADCs targeting HER-2 with the monoclonal antibody, trastuzumab and interchain cysteine bioconjugation.

One ADC incorporated monomethyl auristatin E (MMAE) as the drug payload whilst the second incorporated the camptothecin analog, SN38, as the drug payload, both at **average DAR 16**.

The ADCs were characterized and tested for efficacy in a range of HER-2 positive cell lines in vitro, and tumor growth inhibition studies using NCI-N87 cells in a mouse xenograft.

Both ADCs remained > 95% monomeric (no aggregation) and demonstrated greater chemical stability in ex-vivo serum stability studies compared to comparator ADC constructs.

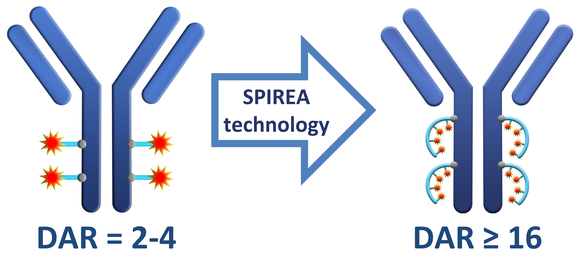
In vitro, the ADCs demonstrated target-specific cell killing with the SN38 average DAR 16 ADC achieving a disproportionate 30-fold increase in efficacy compared to a DAR 8 comparator. Dose dependent tumor growth inhibition was observed in an HER-2 positive NCI-N87 mouse xenograft model with no change in mouse body weight, and good systemic exposure.

These data demonstrate our capability, using a novel multivalent linker technology, to construct ADCs with DARs considerably greater than DAR 8 which are chemically stable, shield payloads from hydrophobic interactions (no aggregation), demonstrate good in vivo exposure profiles and which are highly efficacious *in vitro* and *in vivo*.

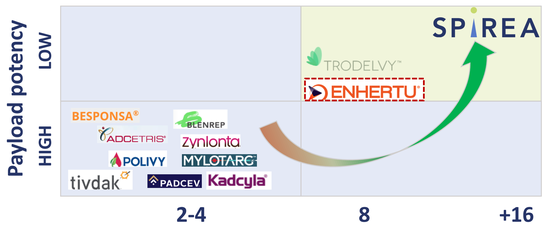
Poster #2012
CH01 Drug Discovery, Design, and Delivery
Section 30

AACR American Association for Cancer Research

TECHNOLOGY



Why is higher DAR better?

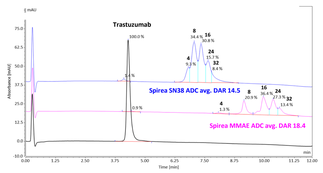


- Higher antibody loading = greater efficacy
- Lower potency payloads = lower toxicity

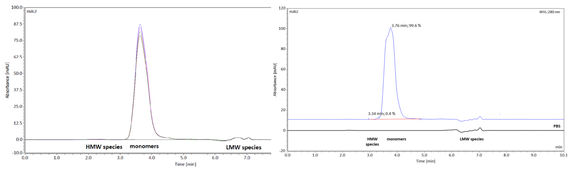
➔ Wider Therapeutic Index

DAR 16 ADCs PROPERTIES

High DAR confirmed by HIC and SDS-PAGE



High monomeric purity (>99%) confirmed by SEC



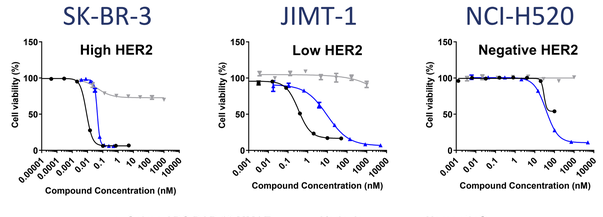
DAR 16 MMAE ADC DAR 16 SN38 ADC

Antibody	Payload	DAR (HIC)	DAR (SDS-PAGE)	SEC purity (%)
Trastuzumab	MMAE	18.4	17.7	>99%
Trastuzumab	SN38	14.5	18.7	>99%

Comparable binding of DAR 16 ADC vs native Ab

Antibody	Payload	DAR (SDS-PAGE)	Kd (nM)
Trastuzumab	SN38	18.4	4.11
Trastuzumab	-	-	4.68

ANTIGEN-DEPENDENT IN VITRO EFFICACY



Compound	SK-BR-3 IC_{50} (nM)	JIMT-1 IC_{50} (nM)	NCI-H250 IC_{50} (nM)
Spirea ADC DAR 16 MMAE	**0.008 ± 0**	**0.228 ± 0.099**	**>100**
Kadcyla®	0.047 ± 0.021	5.921 ± 4.313	37.307 ± 16.075
MMAE	0.284 ± 0.138	0.126 ± 0.053	0.334 ± 0.114
Trastuzumab	0.141 ± 0.138	>1000	>1000

DISPROPORTIONATELY GREATER EFFICACY

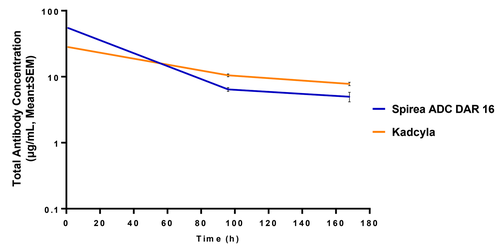
DAR 16 vs DAR 8 SN38 ADCs on HER2+ SK-BR-3 cells

Compound	Antibody	Payload	Release	DAR	IC_{50} (nM) 96h exposure	IC_{50} (nM) 9h exposure
Spirea	Trastuzumab	SN38	pH	16	0.04 ± 0.01	0.18 +/- 0.15
Comparator	Trastuzumab	SN38	pH	8	0.1 ± 0.01	5.56 +/- 2.91
Control	Isotype control	SN38	pH	16	0.11 ± 0.01	12.1 +/- 8.81
SN38 (Free)	-	SN38	-	-	1.17 ± 0.09	5.78 +/- 2.01

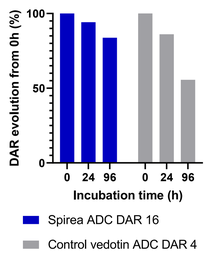
Spirea **DAR 16** SN38 ADC is **30-fold more active** than a comparator SN38 **DAR 8** ADC

PHARMACOKINETICS & EX VIVO SERUM STABILITY

Similar total antibody exposure to Kadcyla



DAR loss 3-fold smaller compared to control ADC

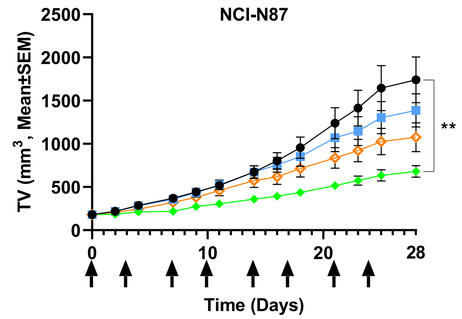


Spirea & control ADCs stability in mouse serum
- Incubation 37 °C for 96 hours
- Affinity capture
- HIC for DAR evaluation

Antibody	Payload	DAR (HIC)	DAR loss (%)
Trastuzumab	MMAE	16	-16
Trastuzumab	MMAE	4	-44

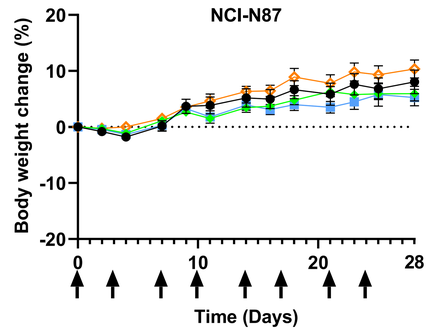
MOUSE IN VIVO EFFICACY

TGI>60% for Spirea SN38 DAR 16 ADC.



dosing adjusted according to MW

All compounds were well tolerated.



CONCLUSIONS

1. Using hydrophobic payloads, Spirea high DAR ADCs display **good physicochemical properties**.
2. Spirea DAR 16 SN38 ADC results in **disproportionate increase of *in vitro* efficacy** compared to lower DAR benchmark.
3. **PK unaffected** by Spirea's multivalent linkers.
4. At equivalent ADC doses, **superior in vivo efficacy** of Spirea DAR 16 SN38 ADCs compared to lower DAR SN38 ADCs.
5. It is anticipated that the use of lower potency payloads will significantly **improve tolerability**.

© POSTER TEMPLATE BY GENSRAPHICS® 1.800.790.4001 WWW.GENSRAPHICS.COM