Innovations in ADC Technology Platform with Legumain-Cleavable KSP-Inhibitor Payloads Adaptable to Various Aspects of Cancer Biology

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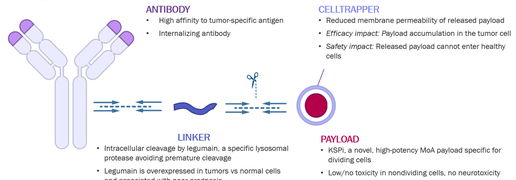
INTRODUCTION

- We have developed a novel technology platform VersAptx™ with tailored solutions for ADCs and SMDCs with intracellular and extracellular cleavage options. The specificity of our platform lies on increasing safety by using tumor-specific enzymes, eg, neutrophil elastase or legumain, for payload release.
- Key features of the ADC technology consist of: (a) kinesin spindle protein inhibitors (KSPi) as a novel payload class, (b) a unique linker selectively cleaved by legumain, and (c) a CellTrapper® modification of the KSPi payload to reduce membrane permeability and provide accumulation in tumor cells to increase efficacy and safety[1,2,3]. Our most advanced ADC, VIP943, is targeting CD123 for the treatment of AML and MDS and is currently in a Phase 1 clinical trial (NCT06034275)[2]. Here we show the extension and applicability of the ADC platform in combination with various antibodies addressing different solid tumor targets.



- Legumain – KSPi has the potential to improve the efficacy of TROP2 and HER2 ADCs.

Brand Name	Substance/ Vehicle	DAR	Linker	Payload	NCI N87 IC_{50} (M)	Fold-Improvement
	Isotype-ADC	5.6	Legumain	KSPi	>1.0E-06	
Trodelvy®	Sacituzumab govitecan	7.6	CL2A	SN38	5.93E-09	1
	Sacituzumab-Legumain-KSPi	5.7	Legumain	KSPi	2.90E-10	20
ENHERTU®	fam-Trastuzumab-Deruxtecan	8.0	Cathepsin B	Dxd	9.62E-10	1
	Trastuzumab-Legumain-KSPi	8.4	Legumain	KSPi	1.24E-10	8

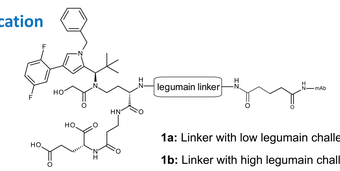
METHODS

- The in vitro cytotoxicity of the ADCs and respective payloads was tested in a panel of cancer cell lines as shown in the tables after a 72h continuous exposure using MTT assays (ATCC). IC50 values were determined as the concentration of compound required for 50% inhibition of cell viability.
- HaCaT cells—immortalized human keratinocytes—were treated with EGFR-ADC. After a 72h continuous exposure, the samples were evaluated in a standard cytotoxicity assay as described above.
- Cysteine coupling of maleimide-modified payload-linker precursors to partially reduced antibodies was performed in PBS buffer at pH 7.2. Subsequent ring-opening of amidomethyl-succinimides was performed by buffer exchange to PBS buffer at pH 8 and subsequent stirring overnight.
- De-conjugation was investigated after incubation of 1 mg/mL ADC in a de-conjugation buffer (150 mM Tris[hydroxymethyl]aminomethane + 10 mM N-acetylcysteine adjusted to pH 8). At defined timepoints, aliquots (25 µL) of the reaction mixture were denatured and reduced. Loaded light chain area and total light chain area were measured using RP-HPLC.
- For the detection of permeable and non-permeable metabolites, HT29-Meso cells were grown in culture medium and incubated with Meso-A2DC **6**. After 3, 24, 48 and 78h supernatant and lysate were collected. Aliquots of supernatants and lysates were taken for analytical testing using an Agilent 1290 HPLC applying MS/MS and analyzed for the presence of metabolites by LCMS.
- To study the antitumor efficacy of ADCs in vivo, immunocompromised female NMRI nu/nu mice were subcutaneously inoculated with human ovarian cancer cells (SKOV-3). Tumors were allowed to establish before assigning the animals to different groups by stratified randomization. Tumor area and body weight were assessed at least twice weekly.

RESULTS

Payload-linker optimization increases selectivity for tumor over healthy cells and potentially overcomes transporter-mediated drug resistance

Linker Modification



1a: Linker with low legumain challenge (fast conversion)

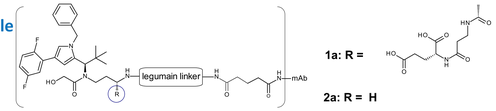
1b: Linker with high legumain challenge (slow conversion)

Effector chemistry	mAb	Legumain challenge	Payload profile	DAR	NCI-H292 tumor IC_{50} [nM]	HaCaT skin IC_{50} [nM]	Selectivity factor
1a	Cetuximab	low	KSPi+CellTrapper	6.4	0.01	0.11	**11**
1b	Cetuximab	high	KSPi+CellTrapper	5.0	0.02	1.27	**52**
Payload	reference		KSPi permeable		0.03	0.02	**0.57**

Table 1. Cytotoxicity of ADCs against NCI-H292 tumor and HACAT skin cells depending on the legumain-cleavable linker

- To further increase selectivity of tumor compared to healthy tissue, we tuned the cleavable linker of a cetuximab-ADC **1a** for a higher challenge of legumain-mediated release (**1b**) with slower conversion to the payload. New ADC **1b** showed significantly improved selectivity for tumor cells versus HaCaT skin cells compared to the standard legumain cleavable ADC **1a** (52-fold selectivity for **1b** compared to 11-fold for **1a** while keeping equipotency.

Payload Profile



1a: R =

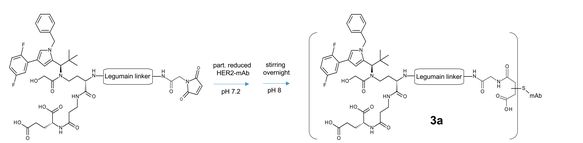
2a: R = H

Effector chemistry	mAb	Linker challenge	Payload profile	DAR	JIMT-1 IC_{50} [nM]
ENHERTU	Trastuzumab	cathepsin low	DxD Permeable	8	>1000
1a	Trastuzumab	legumain low	KSPi + CellTrapper	5.4	4.7
2a	Trastuzumab	legumain low	KSPi permeable	3.4	750

Table 2. Cytotoxicity of ADCs against JIMT-1 cells depending on linker and released metabolite

- A trastuzumab-ADC using a KSPi payload with the CellTrapper **1a** shows low nanomolar potency against JIMT-1 cells resistant to trastuzumab deruxtecan and thus may overcome resistance. An increased potency was not observed with an ADC **2a** releasing a permeable KSPi without CellTrapper.

Antibody Attachment



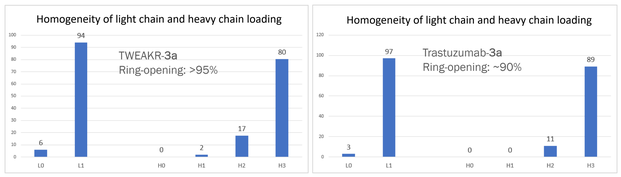


Figure 3. A) Homogeneity of Cys-coupled ADCs with different mAbs and subsequent hydrolysis of succinimide

- Synthesis of homogenous ADCs with subsequent ring-opening of amidomethyl-succinimides can be achieved under mild conditions by stirring overnight at pH 8.
- High degree of homogeneity and efficiency of ring-opening demonstrated by mass spectrometry.

Homogenous and potent DAR 8 ADCs without significant de-conjugation

Effector chemistry	mAb	Linker	Payload	DAR	NCI-N87 [nM]
1a	Trastuzumab	Legumain	KSPi / CellTrapper	8.4	0.12
3a-stochastic	Trastuzumab	Legumain	KSPi / CellTrapper	3.3	0.65
3a-DAR8	Trastuzumab	Legumain	KSPi / CellTrapper	7.8	0.04
ENHERTU	Trastuzumab	Cathepsin	DxD	~8	1.6

Table 3. Cytotoxicity of trastuzumab ADCs against NCI-N87 cells

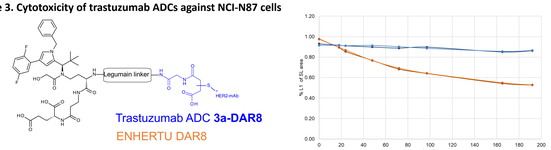
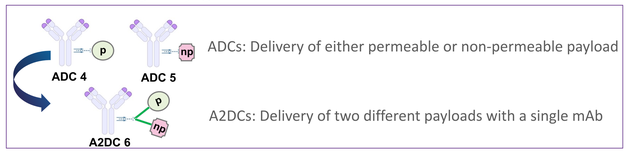


Figure 4: % area of loaded light chain (L1) relative to total light chain area (SL) during deconjugation for trastuzumab-3a-DAR8 ADC and ENHERTU® (trastuzumab deruxtecan)

- Homogenous ADC **3a**-DAR8 with trastuzumab is highly potent and superior to its analog **3a** with stochastic coupling against NCI-N87 cells due to higher DAR.
- No de-conjugation with ring-opened KSPi ADC **3a**-DAR8 in contrast to ENHERTU.

Antibody 2-Drug Conjugates (A2DCs) for Treatment of Solid Tumors



Target	Cell line	ADC **4** permeable payload IC_{50} [nM] / (DAR)	ADC **5** non-permeable payload IC_{50} [nM] / (DAR)	A2DC **6** permeable + non-permeable IC_{50} [nM] / (DAR)	Payload Permeable IC_{50} [nM]
HER-2	KPL4	0.2 (5.2)	0.07 (3.7)	0.04 (2.5x2)	0.3
TWEAK-R	NCI-H292	0.4 (4.5)	0.08 (3.2)	0.1 (2.0x2)	0.1
Mesothelin	HT29-Meso	1.7 (5.5)	500 (3.6)	0.8 (3.5x2)	2.0
CXCR5	REC1	600 (4.3)	0.01 (3.6)	0.003 (1.9x2)	0.3

Table 4. Potency of ADCs and A2DCs against different targets against respective target-expressing cell lines

- HER-2 and TWEAKR-KSP-ADCs (NCI-H292 and KPL4):
  Slightly higher potency of ADC **5** providing a non-permeable metabolite (**5**>**4**)
- Mesothelin-KSP-ADCs (HT29-Meso+):
  Major contribution of permeable metabolite to ADC potency (**4**>>**5**)
- CXCR5-KSP-ADCs (REC-1):
  Major contribution of non-permeable metabolite to ADC potency (**5**>>**4**)

A2DC 6 shows the highest potency independent of the individual target

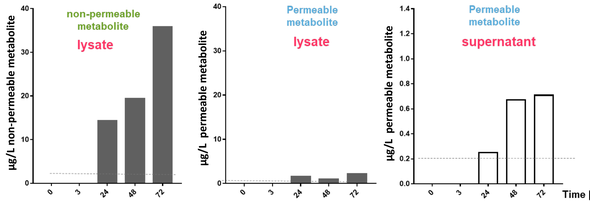


Figure 5. Detection of permeable and non-permeable metabolite of Mesothelin-A2DC 6 in lysate and supernatant of HT29-Meso cells

- **Non-permeable metabolite** significantly increases in the lysate up to 72h
- No metabolite is found in the supernatant above detection limit of 1 µg/L
- **Permeable metabolite** detected in lower concentrations intracellularly
- Increasing concentrations found in the supernatant

In vivo efficacy of stochastic and homogenous KSPi ADCs in comparison to ENHERTU in the ovarian SKOV-3 CDX model

Effector chemistry	mAb	Payload	Linker	Attachment	DAR
1a	Trastuzumab	KSPi / CellTrapper	legumain/low	K random	8.4
1a	Isotype	KSPi / CellTrapper	legumain/low	K random	5.6
1b	Trastuzumab	KSPi / CellTrapper	legumain/high	K random	5.5
3a-DAR8	Trastuzumab	KSPi / CellTrapper	legumain/low	C homogenous	7.5
ENHERTU	Trastuzumab	DxD	Cathepsin	C homogenous	~8

Table 5. ADC composition and DAR

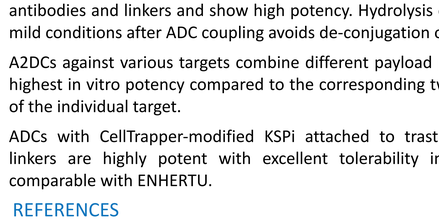


Figure 5. In vivo efficacy of ADCs in HER2 expressing SKOV-3 CDX model

- Homogenous cysteine-linked ADC **3a-DAR8** with trastuzumab as well as the stochastic lysine-linked ADCs **1a** and **1b** are highly potent with once weekly dosing in the HER2-expressing SKOV-3 CDX model comparable to ENHERTU and selective versus isotype control.
- Body weight loss of ≤3% for all treatment groups indicates good tolerability.

CONCLUSIONS

The VersAptx technology platform provides ADCs with high activity in solid tumors and offers multiple options for a tailored design to address the respective target biology. Herein we provided examples that include:

- Legumain-KSPi delivers improved cytotoxicity compared to established effector chemistries on HER2 and TROP2 targeting antibodies. Combined with the improved safety profile of the linker-payload this has the potential to increase the therapeutic window of the ADCs.
- Tuning the linker peptide for a higher challenge of legumain cleavage further increases selectivity of EGFR-targeted ADCs for solid tumor cells over healthy cells while retaining the high potency.
- Tuning the physicochemical profile of the payload with the CellTrapper moiety allows for accumulation in tumor cells and may overcome transporter-mediated drug resistance.
- Homogenous cysteine-linked ADCs with DAR ~8 can be provided with different antibodies and linkers and show high potency. Hydrolysis of succinimides under mild conditions after ADC coupling avoids de-conjugation of DAR8 ADCs.
- A2DCs against various targets combine different payload profiles and show the highest in vitro potency compared to the corresponding two ADCs independent of the individual target.
- ADCs with CellTrapper-modified KSPi attached to trastuzumab via different linkers are highly potent with excellent tolerability in the SKOV-3 model comparable with ENHERTU.

REFERENCES

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2. Kirchhoff et al; Cancers 2020, 12, 3464
3. Sommer at al; OncoImmunology 2022, VOL. 11, e2037216

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