NOVEL HIGHLY POLAR LINKERS FOR ADCs WITH ENHANCED THERAPEUTIC INDEX

Michał Mroczkiewicz,[1] Marcin Zagozda,[1] Aleksandra Błocka,[1] Tadeusz Lemek,[1] Bartosz Setner,[1] Aleksandra Moroz,[1] Anna Maria Maj,[1] Michał Słotwiński,[1] Stanisław Michałek,[1] Krzysztof Dubiel,[1]
Krzysztof Lacek,[2] Małgorzata Choroś,[2] Delfina Popiel,[2] Olga Abramczyk,[2] Joanna Hucz-Kalitowska,[2] Aleksandra Sowińska,[2] Emilia Mróz,[2] Natalia Czerwińska,[2] Anna Jablońska,[2] Filip Mituła,[2] Maciej Wieczorek[2]

[1] Celon Pharma SA, Medicinal Chemistry Dept, Kazuń Nowy, Poland

[2] Celon Pharma SA, Preclinical Research Dept, Kazuń Nowy, Poland

corresponding author: michal.mroczkiewicz@celonpharma.com

16th ANNUAL WORLD adc LONDON 2026

Poster number 56

Shape the future of healthcare

INTRODUCTION

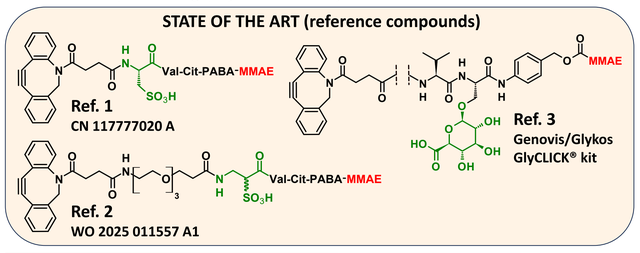
Antibody–drug conjugates (**ADCs**) combine the targeting specificity of monoclonal antibodies with the potency of cytotoxic payloads, but their clinical performance is often limited by conjugation heterogeneity and payload-driven hydrophobicity. **Site-specific** conjugation enables precise control of drug attachment, yielding homogeneous ADCs with defined drug-to-antibody ratio (**DAR**) and improved stability. Linker design plays a critical role in modulating ADC behaviour *in vivo*.

POLAR LINKERS

Polar hydrophilic linkers can offset payload hydrophobicity, reduce aggregation, and limit non-specific interactions with plasma proteins and healthy tissues. The integration of **site-specific** conjugation with highly polar linker architectures represents an effective strategy to improve ADC physicochemical properties, pharmacokinetics, and overall **therapeutic index**.

Highly polar groups such as **sulfonic acids** (e.g., **cysteic acid** in **Ref. 1** or **2-sulfo-b-alanine** in **Ref. 2**) or **glucuronic acid** applied in commercially available Genovis/Glykos linker **Ref. 3** used in GlyCLICK® kit are increasingly used in ADC design to boost hydrophilicity and reduce aggregation. This typically improves **solubility, stability, and pharmacokinetics**, helping ADCs maintain better *in vivo* behaviour without compromising payload delivery.

STATE OF THE ART (reference compounds)



HIGHLY POLAR MULTI-SULFONIC ACID LINKERS FOR SITE SPECIFIC ADCs

HydroBOOST™ – a universal platform for the application of polar linkers for the efficient site-specific conjugation

A new strategy based on the first attempt of the incorporation of two or more sulfonic acid moieties in **α-** or **β-amino acids** derivatives was successfully implemented and evaluated. **L-Cysteic acid** and **2-sulfo-β-alanine** were introduced to the peptide fragment of the linker. A library of more than **70 polar linkers** with variety of high potent payloads (tubulin inhibitors, TOPO1i, DNA-damaging agents) and **„CLICK"-able** metal-free reactive groups (DBCO, BCN) were obtained. Introducing of at least two sulfonic acid derivatives surprisingly increases the productivity of linkers and ADCs in conjugation process. The high polarity of the linkers and their ionic nature facilitate the purification process by precipitation of solid salts in combination of subsequent RP chromatography, if necessary. Combination of at least two sulfonic acids in linker significantly increase their polarity, solubility and stability. **CPL-414-145** is a linker with highly polar di-(L)-cysteic acid fragment which was selected from the wide library for further development. An efficient multigram high yield stereocontrolled synthesis of the linker was successfully developed.

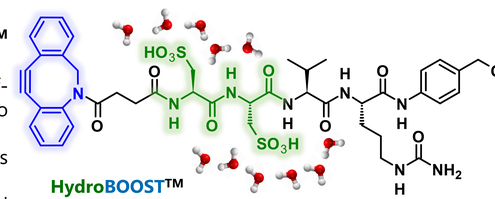
HydroBOOST™

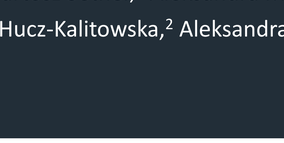
The main benefits of HydroBOOST™ technology application:

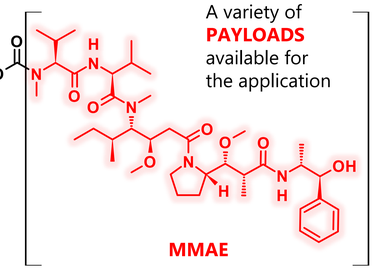
- diversity of payloads and self-immolative fragments possible to attach;
- significant increase of linkers hydrophilicity and water solubility;
- efficient synthesis in stereocontrolled process;
- facilitating the purification of linkers;
- high thermal and chemical stability of the linkers;
- wide application for the metal free „click" conjugation;
- increase of the reactivity in ADC conjugation process.

Reactive group for the site-specific **"CLICK"** conjugation are available: **DBCO, BCN** or **maleimide**

Enzymatically cleavable self-immolative groups are available **-ValCit-PABA-**, -ValAla-PABA-, -GlyGlyPheGly-







A variety of **PAYLOADS** available for the application

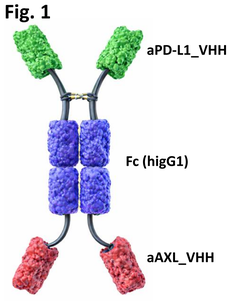
MMAF, doxorubicin, exatecan, SN-38, Dxd

BISPECIFIC ANTIBODY

AXL and **PD-L1** expression levels have been shown to correlate strongly in many cancers. Accordingly, dual targeting of these proteins is expected to improve cancer specificity in the delivery of a toxic payload. We have developed a bispecific antibody **BsAb-1357** that potently promotes receptor internalization (**WO2025080151 A1**).

Potent PD-L1 and AXL binders were isolated from llama VHH libraries and reformatted into multivalent bispecific constructs by fusing them to the *N*- and *C*-termini of a human IgG1 Fc fragment.

Fig. 1



The PD-L1–targeting VHH was fused directly to the hinge region, whereas the anti-AXL nanobody was linked to the Fc *C*-terminus via a flexible glycine–serine (G4S) linker.

Representation of bispecific antibody (**Fig. 1**) Anti-PD-L1 VHH domains (green) were linked to the hinge region (grey) and anti-AXL nanobodies (red) by flexible G4S linker to the *C*-terminal end of the human IgG1 Fc fragment (violet).

NATIONAL RECOVERY PLAN

Republic of Poland

Funded by the European Union NextGenerationEU

MEDICAL RESEARCH AGENCY

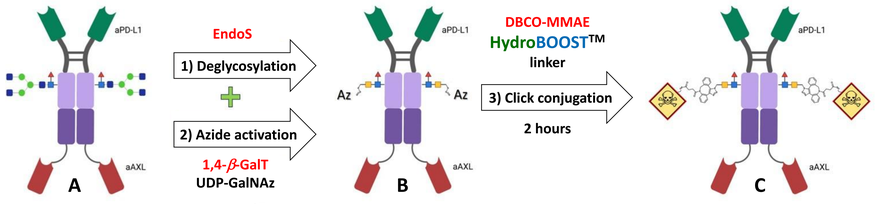
"Design, optimization of production and Preclinical Safety Assessment of a Novel Dual-Action Conjugate with Immunomodulatory and Cytotoxic Properties as a New Anticancer Drug Candidate" no. 2024/ABM/05/KPO/ KPOD.07.07-IW.07-0258/24

The project received support from the European Union under the Recovery and Resilience Facility

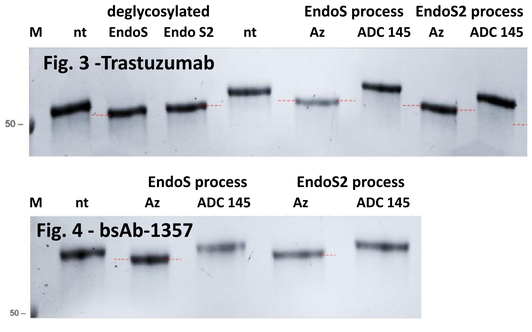
SITE-SPECIFIC CONJUGATION – EndoS vs EndoS2 TRIMMING

Polar linkers were conjugated with **BsAb-1357** aPD-L1/aAXL using optimized glycoconjugation technology (**Fig. 2**). N297 conserved glycoprotein (**A**) was efficiently trimmed with endoglycosidase EndoS and extended with **azide-Gal-NAc** moieties using **UDP-GalNAz** in a process catalysed by Y289L mutant of 1,4-*β*-galactosyl-transferase. Two steps, „one-pot" enzymatic procedure led to the dual azide-activated anchor points (**B**). The activated glycopeptide was conjugated with the aqueous soluble **HydroBOOST™ CPL-414-145** linker in 1,3-dipolar cycloaddition with metal free „click" procedure. Full conversion in 2 hours resulted in ADC (**C**) with DAR 2.

Fig. 2



The deglycosylation with the application of EndoS or EndoS2 was evaluated in a sequential process with **mAb-trastuzumab** (**Fig. 3**). Reducing SDS-PAGE electrophoresis showed high efficiency and complete trimming with both enzymes. The further steps of Ab activation with Gal-NAz and final conjugation with **CPL-414-145** resulted in pure **ADCs**. Both enzymes were also compared in the „one-pot" conjugation process with **bsAb-1357** (**Fig. 4**). Both steps (trimming/activation and conjugation) in case of each endoglycosidase led to full conversion and finally resulted in the ADC products in pure DAR 2 form. High stability and no aggregation of ADCs in the solution were reported.



Literature:

1) Sjögren, J., Cosgrave, E. F. J., *et.al.* EndoS and EndoS2 hydrolyze Fc-glycans on therapeutic antibodies with different glycoform selectivity, *Glycobiology* **2015**, *25*, 1053-1063.
2) Petersen, M. E., Brant, M. G., *et.al.* Structure–Activity Relationships of Bis-Intercalating Peptides and Their Application as ADC Payloads, *J. Med. Chem.* **2023**, *66*, 8288-8309.
3) Duivelshof, B. L., Deslignière, E., *et.al.* Glycan-mediated technology for obtaining homogeneous site-specific conjugated ADCs, *Anal. Chem.* **2020**, *92*, 8170-8177.

IN VIVO STUDIES

Conjugate efficacy was evaluated in a xenograft mouse model. CB17-SCID (CB17/Icr-*Prkdcscid/Rj*) mice were implanted subcutaneously with **MDA-MB-231** human triple-negative breast cancer cells. Once the tumour reached the volume of 100 mm^3 in 3 weeks, then the mice were treated intravenously with the test conjugates to assess tumour growth inhibition. **ADC-145**, **ADC-1**, **ADC-2** and **ADC-3** obtained by the glycoconjugation of linker **CPL-414-145** and reference linkers **Ref. 1**, **Ref. 2**, and **Ref. 3** to **bsAb-1357** were administered twice weekly in total 6 doses (0.8, 2.0 or 6.0 mg of ADC/kg per dose in PBS @ pH 7.4 with 5% glycerol) (**Fig. 5**). After the regrowing period in the cohorts with 0.8 mg/kg dosage of **ADC-2**, and **ADC-145** the second stage of treatment was reassumed. Further significant, continuous and constant tumour regression was observed just only upon the treatment with **ADC-145**. The full remission in the group with 0.8 mg/kg dosage was obtained in 63% of the mice population (20.9 mm^3 mean TV in the rest of mice) vs. 37% remission in **ADC-2** group (182.7 mm^3 mean TV) with the same dosing regimen. In group of **ADC-145** in 6 mg/kg dosage full regression was observed in 75% of mice population after one week after last dose (45th day).

Fig. 5

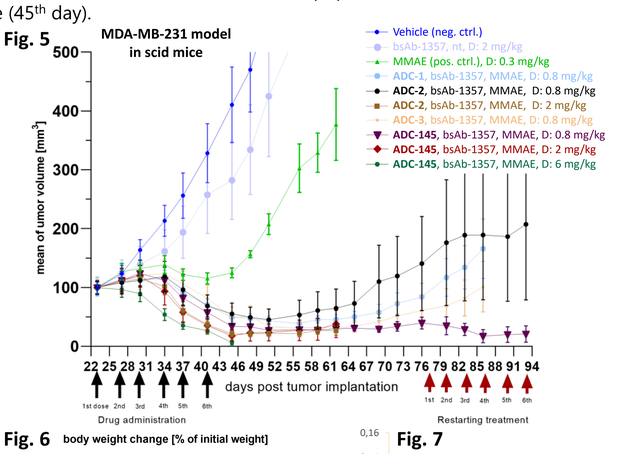


Fig. 6 body weight change [% of initial weight]

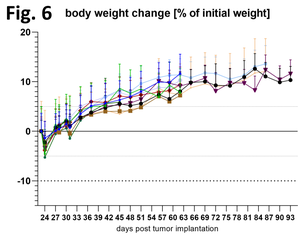
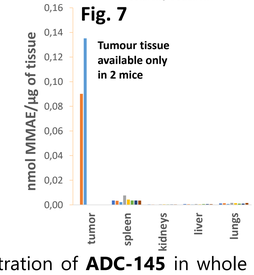


Fig. 7



Safety considerations. Multiple administration of **ADC-145** in whole range of dosage 0.8, 2, and 6 mg/kg and during the whole treatment did not affect any significant adverse effect. No deaths in these groups were recorded. No body weight decrease was also observed (**Fig. 6**). For comparison in every other group with **ADC-1**, **ADC-2**, and **ADC-3** treatment at least one death occurred and cases of necrosis and ingrowth of tumour into the peritoneum were noted. Tissue analysis in **ADC-145** treatment group (6 mg/kg) showed trace amounts of free MMAE compared to tumour (**Fig. 7**).

CONCLUSIONS AND PERSPECTIVES

The application of **HydroBOOST™** polar linkers technology enables the preparation of ADCs that characterise in high treatment efficacy and low toxicity, which translates into an increased therapeutic index. Effective preparation of ADC by the glycoconjugation of **bsAb-1357** (**aPD-L1/aAXL**) with polar linker **CPL-414-145** gave the highly polar stable ADC with extremely high effectiveness in **MDA-MB-231** triple-negative breast cancer model. The variety of available **HydroBOOST™** linkers can be successfully used across the entire range of Ab variants and validated in tumour models.