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Overcoming limitations of current antibody-drug conjugates (ADCs) by a novel linker technology

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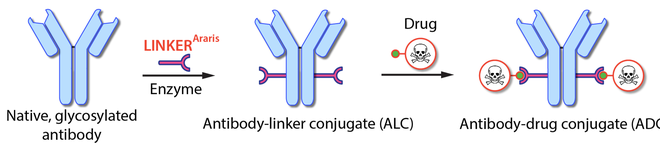
Introduction

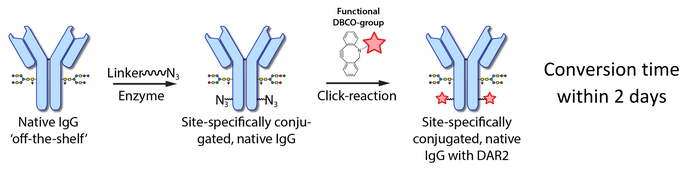
Antibody-drug conjugates (ADC) enable the targeted delivery of cytotoxic drugs. A critical and challenging step of the ADC generation is the drug attachment to the antibody. Currently, various conjugation methods are used that either result in a broad and inhomogeneous drug distribution or in a precisely defined antibody-to-drug ratio (DAR) using site-specific conjugation approaches. However, most common site-specific payload conjugation technologies either 1) require antibody and/or cell line engineering efforts 2) use linkers with limited solubility (suboptimal for hydrophobic payloads) or 3) deploy unstable chemistry for payload-linkage. Together, these limitations lead to a challenging ADC manufacturing process and premature termination of clinical programs due to limited ADC efficacy and/or high toxicity. We here introduce a novel and versatile ADC-linker technology that aims to address the points and enables the following:

The Araris Linker Technology



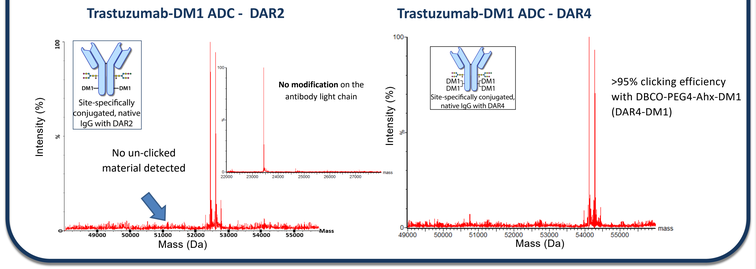
- **ADC generation within 48 hours**
- **Site-specific drug attachment to native Abs "off-the-shelf", no engineering**
- **Abs retain native glycosylation pattern and amino acid sequence**
- **LINKER Araris synthesized within 48 hours by CRO**
- **Enzyme available to produce clinical grade ADC**
- **Highly stable ADCs with favorable physicochemical properties**
- **Drug-to-antibody ratios (DAR) of 2 and 4 possible**
- **Various chemistries tested: Azide, thiol or combinations thereof**
- **Various payloads tested (toxins, dyes, metal chelators…), also combinations are possible**

1 The Araris Linker Technology: Site-specific conjugation to native IgG-antibodies leads to highly homogeneous ADCs



Conversion time within 2 days

Highly efficient and site-specific modification of IgG1 heavy chain – DAR2 and DAR4 in less than 72 hours

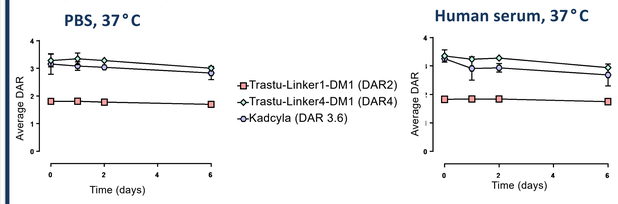


>95% clicking efficiency with DBCO-PEG4-Ahx-DM1 (DAR4-DM1)

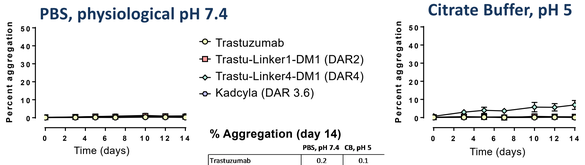
2 DAR2 and DAR4 Trastuzumab-DM1 are stable in PBS and human serum over a week at 37°C

ADCs were incubated in buffer and IgG-depleted human serum. DAR ratio was verified by LC-MS and aggregation by size-exclusion chromatography.

Drug to antibody ratio (DAR) - LC-MS



Aggregation (%) - SEC



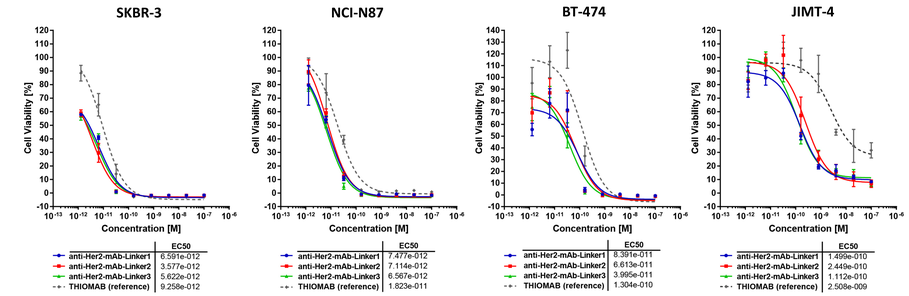
% Aggregation (day 14)

	PBS, pH 7.4	Cit, pH 5
Trastuzumab	0.2	0.1
Trastuzumab-Linker 1-DM1	0.6	0.5
Trastuzumab-Linker 4-DM1	0.7	7
Kadcyla	1.1	0.3

- DM1 remains stably conjugated to Trastuzumab in human serum and in PBS for at least 1 week at 37°C.
- No aggregation of DM1-DAR2/4 in PBS at physiological pH.
- Araris ADCs are highly stable at pH5 (< 10% aggregation).

3 Araris ADCs show superior *in vitro* cytotoxicity

Trastuzumab-Amanitin ADCs (DAR2): Comparison of **Araris Linker Technology** vs **THIOMAB™ conjugation technology**. Cytotoxicity assay on HER2-overexpressing cell lines. Data generated in collaboration with Heidelberg Pharma.

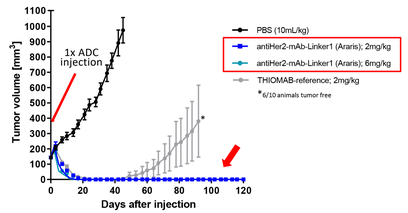


4 Araris ADCs show superior anti-tumor efficacy in JIMT-1 and NCI-N87 mouse models vs THIOMAB™

Therapeutic treatment in HER2+ tumors: comparison Araris-Amanitin ADC vs THIOMAB-Amanitin. Mice were treated with one injection 2 or 6 mg/kg (JIMT-1) and 3 mg/kg (NCI-N87) ADCs on day 0. Data generated in collaboration with Heidelberg Pharma.

JIMT-1 mouse model

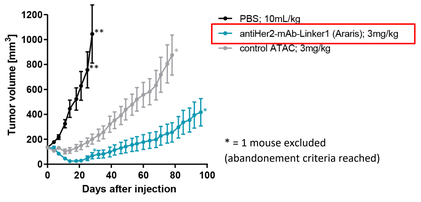
tumor volume ± SEM



- Araris-Trastuzumab-Amanitin ADCs: rapid and long-lasting tumor remission.
- 10/10 animals tumor-free on day 100.

NCI-N87 mouse model

tumor volume ± SEM



* = 1 mouse excluded (abandonement criteria reached)

- Araris ADC anti-tumor efficacy superior to THIOMAB™ control with tumor re-growth starting on day 20.

Conclusion & Contact

We introduced a novel proprietary antibody-conjugation technology that allows for site-specific payload attachment to a single residue within the heavy chain of native IgG-antibodies. With our technology, no antibody engineering is necessary and the light chain is unaffected under the conditions used.
The presented results indicate that our linker technology a) allows for fast (within 48 h) and straightforward manufacturing of ADCs using different payloads without protein engineering efforts, b) results in ADCs with favorable biophysical properties and a **clear defined drug-to-antibody ratio (DAR)**, and c) enables the generation **of highly potent and stable**, thus safer, next-generation ADCs. Further we have data showing that Fc-gamma receptor and FcRn binding is retained upon linker conjugation. Importantly, our ADCs showed superior efficacy in different tumor animal models as compared to control THIOMAB™ ADCs.
For further information, please contact: **pspycher@ararisbiotech.com**

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