SAFC®
Pharma & Biopharma Raw Material Solutions

White Paper

Overcoming the Solubility Challenges of Antibody-Drug Conjugates

Carl Deutsch, PhD

Antibody-Drug conjugates (ADCs) often present solubility challenges due to the hydrophobic properties of the attached payloads; poor solubility can lead to aggregates and impact manufacturability or even pharmacokinetic properties. In an attempt to overcome this hydrophobicity, ADC developers may resort to reducing the drug-antibody ratio (DAR), a strategy which may result in a loss of efficacy, reduce the therapeutic window, and cause unintended side effects. Other opportunities to mitigate solubility challenges are possible and include changes to the formulation, payload, or conjugation site or addition of a co-solvent. Unfortunately, these approaches require additional investments, which can increase development risks as well as have potential intellectual property and freedom-to-operate constraints. If the solubility issue cannot be resolved, the clinical development program may have to be terminated.

Increasing Solubility with ChetoSensar™ Technology

We have developed a chito-oligosaccharide (ChetoSensar™) that dramatically increases the solubility of ADCs when incorporated into the linker-payload construct (Figure 1).

Figure 2 shows the reduction in ADC hydrophobicity when ChetoSensar™ is incorporated into the construct. The top panel illustrates the increase in hydrophobicity of a DAR 4 ADC compared to the unconjugated trastuzumab (Herceptin) monoclonal antibody. When the same DAR 4 ADC has ChetoSensar™ attached, a notable shift towards the unconjugated antibody is observed, indicating that the solubility properties of the ChetoSensar™-ADC are now closer to the unconjugated antibody, facilitating ADC processing in development and manufacturing.

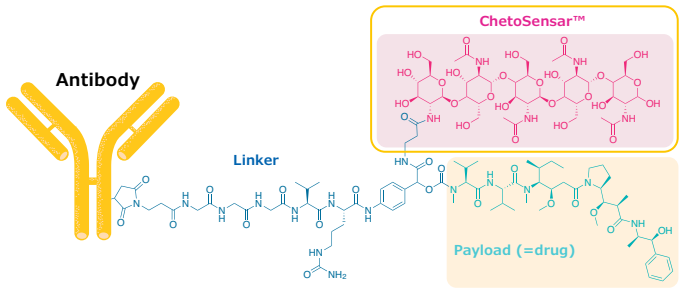


Figure 1. ChetoSensar™ attached to a cathepsin B-sensitive linker and monomethyl auristatin E (MMAE) payload. All ADCs in this report used the monoclonal antibody trastuzumab (Herceptin) as a model.

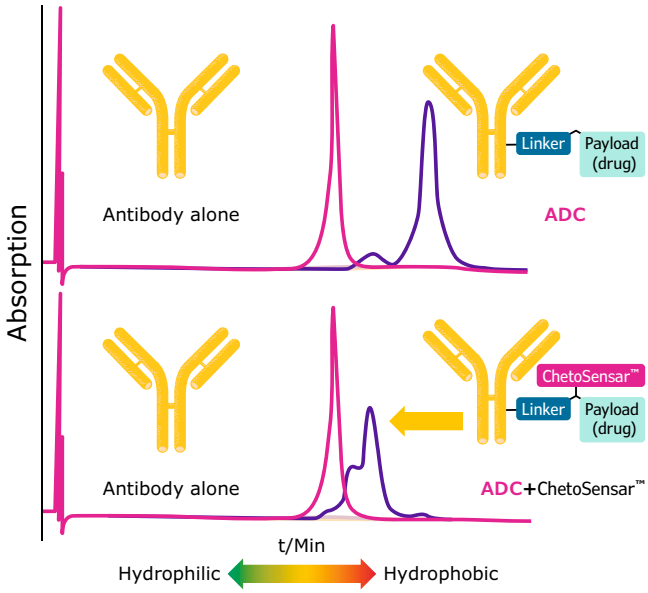


Figure 2. Incorporating ChetoSensar™ into an ADC reduces hydrophobicity. Shown: hydrophobic interaction chromatogram (HIC) with antibody alone (trastuzumab) and DAR 4 ADC with and without ChetoSensar™ (payload is MMAE).

MERCK

ChetoSensar™ offers high flexibility when developing ADCs and enables use of a variety of linkers, payloads, antibodies, and conjugation technologies (Table 1).

Linkers	Payloads	Antibodies	Conjugation Technologies
• Disulfide • Cat B • Maleimide • ß-Gluc	• Dolastatins • Maytansines • Duocarmycin • CBI Dimers • PBD Dimers • Camptothecin	• Various IgG formats • Engineered • Bispecific	• Chemical coupling • Enzymatic coupling • Site specific • Stochastic

Table 1. ChetoSensar™ can be used with a wide variety of linkers, payloads, antibodies, and conjugation technologies.

To demonstrate the benefit of ChetoSensar™ in an ADC construct, two ADCs were compared in an *in vivo* study using an SK-OV-3 xenograft (Figure 3). Tumor volume was measured following a single dose of DAR 4 ADC with and without ChetoSensar™. The results showed that at equal doses, the ChetoSensar™ enabled ADC led to rapid and complete tumor regression through day 80, indicating a dramatic increase in efficacy of the ADC.

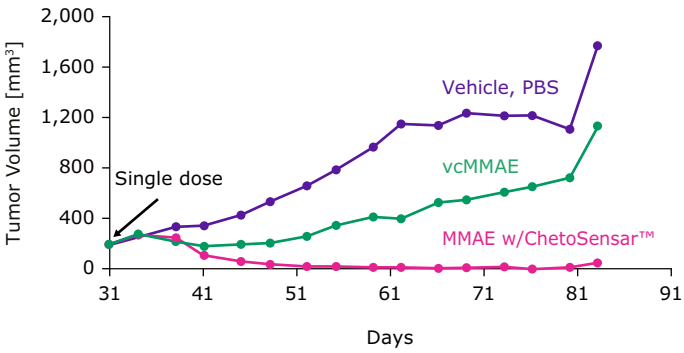
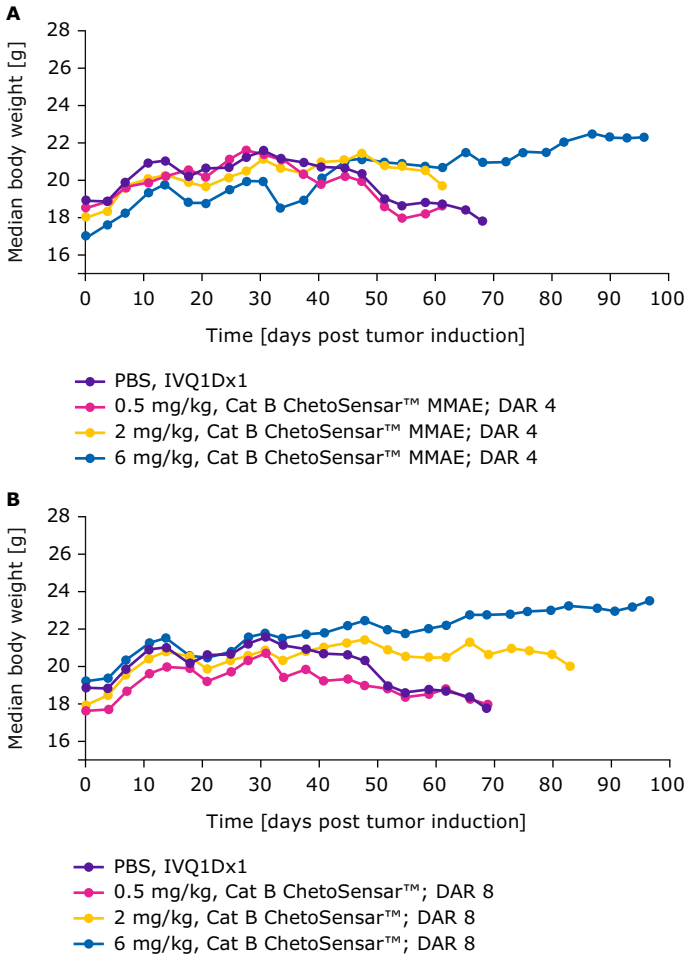


Figure 3. ChetoSensar™-ADC achieves tumor regression close to baseline in SK-OV-3 xenograph. Both ADCs are DAR 4 at 6 mg/kg. Single dose begins at 31 days. All ADCs use traztuzumab as model mAb. Median results report from a group of eight mice.

In addition to efficacy, tolerability of an ADC is critical to its success as a therapeutic. One indicator of tolerability is the body weight of mice throughout the course of an *in vivo* study. With DAR 4 (Figure 4A) and DAR 8 (Figure 4B) ChetoSensar™-enabled ADCs, there was no loss in body weight, indicating the ChetoSensar™-ADCs were well tolerated. In addition, no off-target toxicity of ChetoSensar™ was observed. There were no histological changes in any major organs and no immune response created when added to peripheral blood mononuclear cells (PBMCs) that were isolated from three different human donors (data not shown).

The Basis of Improved Efficacy

We sought to define the reasons for the increased efficacy of ChetoSensar™-enabled ADCs. These ADCs contain the p-aminobenzyloxycarbonyl (PABC) group



- No body weight loss observed
- No side effects observed (e.g. skin rash)

Figure 4. ChetoSensar™-ADCs were well tolerated *in vivo* with repetitive. **A** Body weight of mice in animal study of DAR 4 ADCs with ChetoSensar™. **B** Body weight with DAR 8 ADCs with ChetoSensar™.

that is susceptible to cathepsin B (Cat B) cleavage in the lysosome. PABC linkers have been shown to undergo non-specific clearance and payload loss. However, *in vitro* studies show that ChetoSensar™ may act as a stabilizer against the mouse enzyme carboxyesterase 1C when attached to PABC. The monoclonal antibody Herceptin (trastuzumab) in combination with ChetoSensar™ was shown to be stable in mouse and human cells without any non-specific clearance based on the corresponding carboxylesterase (data not shown). This indicates that in addition to improvements in solubility, the stability of the ADC is driven by improved pharmacokinetics with no negative effect on the binding of the antibody.

We then created a site-specific DAR 2 duocarmycin ADC with ChetoSensar™ and tested it in humanized FcRn mice to predict *in vivo* behavior in humans (Figure 5).

The DAR 2 ChetoSensar™-ADC in buffer solution had a potency in the sub-nanomolar range. *In vivo* plasma samples taken at 4 hours and 168 hours were subsequently tested on antigen-positive cells and showed no loss of efficacy. This indicates that the samples were stable for up to seven days in circulation in humanized FcRn mice. Beyond the DAR 2 studies shown here, ChetoSensar™ empowered the preparation of an aggregate-free DAR 8 duocarmycin ADC, which was previoulsy not possible (data not shown). This reach into higher DAR species with the aid of ChetoSensar™ technology gives optimism to increased potency with other payloads as well.

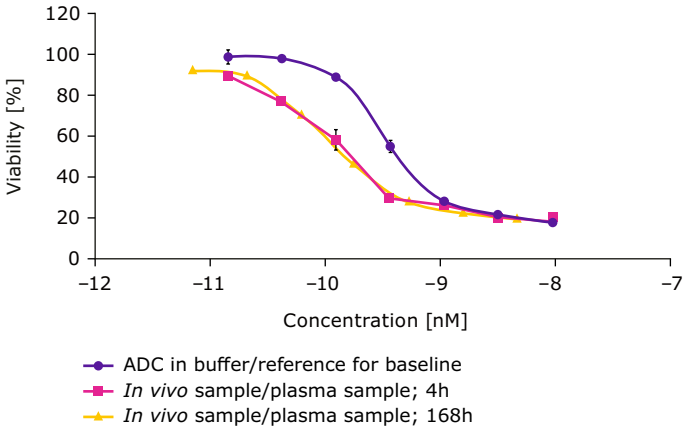


Figure 5. ChetoSensar™-duocarmycin ADC (DAR 2) has favorable pharmacokinetics in humanized FcRn mice. Stability of ChetoSensar™-ADCs is demonstrated with no loss in efficacy after 7 days in antigen-positive cells.

This high degree of efficacy, stability, and robust pharmacokinetics also translated to the *in vivo* setting. As shown in Figure 6, a single dose of DAR 2 ChetoSensar™-duocarmycin ADC led to complete remission of the tumor in a xenograft model with no regrowth through day 100, a notable contrast when compared to the DAR 2 duocarmycin ADC without ChetoSensar™, which had no apparent effect on tumor regression. An ongoing study with a 5 mg/kg dose has also shown complete remission (data not shown).

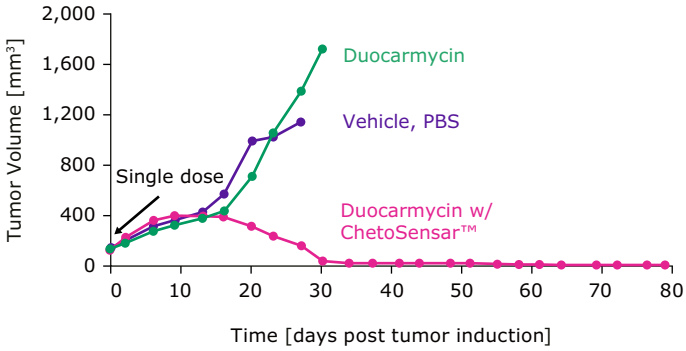


Figure 6. Demonstrated tumor regression with DAR 2 ChetoSensar™-duocarmycin ADC. Purple: Vehicle (PBS); green: ADC without ChetoSensar™; pink: ADC with ChetoSensar™. Both ADCs are dosed at 10 mg/kg; single dose begins at 0 days. ADC construct design referenced in Figure 5.

Importantly, ChetoSensar™ enabled us to make an aggregate-free DAR 8 duocarmycin ADC, which would not have been possible previously.

Solving the ADC Solubility Challenge

The poor solubility of ADCs can put development programs in jeopardy. ChetoSensar™ technology is an innovative option for addressing these solubility challenges. The chito-oligosaccharide linker offers a great deal of flexibility for attaching different kinds of payloads and has been shown to work with a variety of linkers, antibodies, and conjugation approaches. The technology provides access to highly hydrophobic, novel payloads that might not otherwise be considered and presents the possibility of improved efficacy, tolerability, and pharmacokinetics.

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt
Germany

https://www.sigmaaldrich.com/services/contract-manufacturing/adc-bioconjugation
https://www.sigmaaldrich.com/services/contact-safc

Lit. No. MK_WP11961EN Ver. 0.1
02/2023