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White Paper

Use of Monodisperse and Activated PEGs to Accelerate Development of Antibody Drug Conjugates

Introduction

Delivery of a highly toxic drug payload directly to the site of a tumor while minimizing collateral damage to healthy tissue represented a major advancement in oncology when the first antibody-drug conjugate (ADC) was approved in the US in 2,000. Unfortunately, it has proven difficult to translate this innovative concept into wide-ranging clinical success; since the first approval, only five other ADCs have reached the market. With two of these approvals in 2017 and the latest one in 2019 and development challenges being addressed, this unique class of therapeutics is expected to gain momentum, living up to its promise as a breakthrough modality.

A key challenge in the development of ADCs is the impact of hydrophobic payloads on solubility and bioavailability. Issues with toxin solubility can lead to aggregation, drug load limitations and impact pharmacokinetics and pharmacodynamics (PK/PD).

This whitepaper describes the use of monodisperse polyethylene glycols (PEGs) to address the issue of low solubility. We explore how PEGs help to overcome hydrophobicity challenges presented by lipophilic, organic payloads, how they are currently being used in ADCs and the critical quality attributes needed in a monodisperse PEG to address these challenges.

Impact of Toxin Solubility

An essential goal for an ADC is to increase the therapeutic window in contrast to treating with the toxin alone. It has been documented, that higher drug-to-antibody ratios (DAR) and higher lipophilicity can be cleared rapidly and can decrease the overall therapeutic window.

Several problems arise as a result from this toxin solubility including aggregation of the final ADC and corresponding issues with immunogenicity. While the easiest way to remove aggregates is through chromatographic purification, this requires adding processing steps, which can be very complex. As a result of this challenge, R&D is left with focusing on lower DAR analogues, which limits the ability to deliver more drug to the cancer cells.

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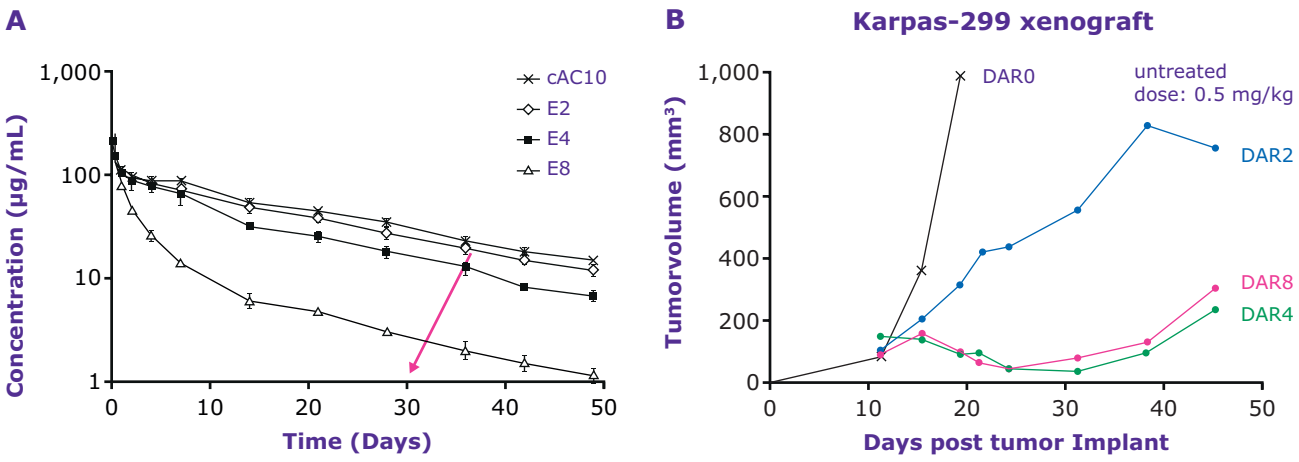
The PK/PD profile is also affected by toxin solubility. With increasing lipophilicity, accelerated plasma clearance of the ADC is observed (Figure 1A), preventing delivery to the target cells and a resulting impact on efficacy (Figure 1B).

Higher DARs – Accelerates ADC clearance

Higher DARs – Non-linear impact on tumor growth



Kevin J. Hamblett et al. Clin Cancer Res 2004;10:7063–7070

Figure 1. Clearance of an unconjugated antibody (cAC10) compared to ADCs with DAR2- DAR4- and DAR8-loaded species shows a significant jump with the highest load DAR8 (A). Increasing the DAR results in reduction of tumor volume, but with diminishing returns (B). With DAR8, there isn't a significant difference when compared to DAR4. Doubling the DAR did not improve efficacy as bioavailability is impacted. From Hamblett, et al., Clin Cancer Res (2004) 10:7063–7070.

Traditionally, the challenges of solubility and hydrophobicity of the toxin have been addressed in several ways including:

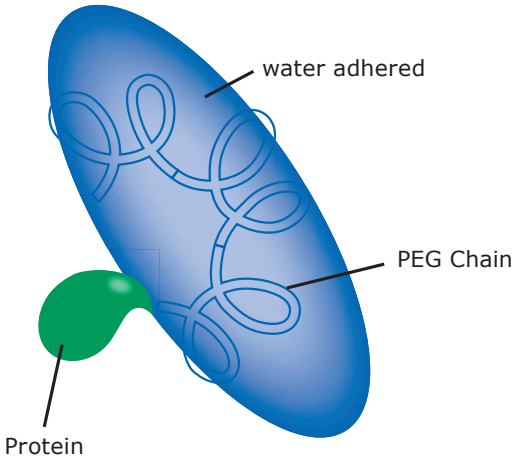
- Alternative scaffolds: attempts can be made to impart some kind of water solubility onto the backbone of the small organic-like molecule. Unfortunately, the structure-activity relationship for small molecules can be very complex and very small changes can result in significant changes in efficacy. Further complicating this approach is that any changes must be made very early as the drug linker needs to be available to go into the conjugation process; this results in a very long lead time when changing the scaffold.
- Optimize linker to offset payload lipophilicity: sulfonates and quaternary ammonium salts can be added to bring water solubility back to the construct.
- Site-specific payload attachment: placement of the payload conjugation can impact manufacturability, stability and aggregation.
- Structure activity relationship (SAR) between antibody, payload, linker and conjugation method: consideration can be given to where the payload will be placed, what kind of linker will be used and the type of payload. While there are many opportunities to improve the construct, an understanding of each component and its impact on the overall product is essential.
- Alternative formulations or additives: while alternative formulations and additives may help address the issue of solubility, they won't resolve the underlying problem with the construct.

Addressing Solubility Issues with PEGs

PEGs are used in many applications and industrial fields as they are generally recognized as safe (GRAS), are biologically inert and very water soluble. Following synthetic activation through addition of functional groups to one or both ends of the polymer, PEGs can be conjugated to proteins, peptides, or small molecules, resulting in "PEGylated" products. Monodisperse PEGs are produced either by a purification of polydisperse PEGs or the specific synthesis of uniform PEG units leading to a defined molecular.

Use of PEGs as a linker between an antibody and a payload molecule can enable higher load ADC species. As shown in Figure 2, PEGs can create a shield, encapsulating the ADC payload from its microenvironment, increasing both solubility and stability. Additional benefits include reduction in aggregation and thus reduced immunogenicity, improved pharmacokinetics, increased circulation time and decreased toxicity.

- **Improved pharmacokinetics**
  - enhanced solubility
  - improved stability
- **Increased circulation time**
  - decreased amount of protein required for therapeutic efficacy
  - decreased dosing frequency due to optimized biodistribution
  - reduced renal clearance increases circulation time
- **Decreased toxicity**
  - improved safety profile
  - reduced immunogenicity
  - reduced antigenicity
  - reduced proteolysis
  - generally recognized as safe (GRAS status by FDA)

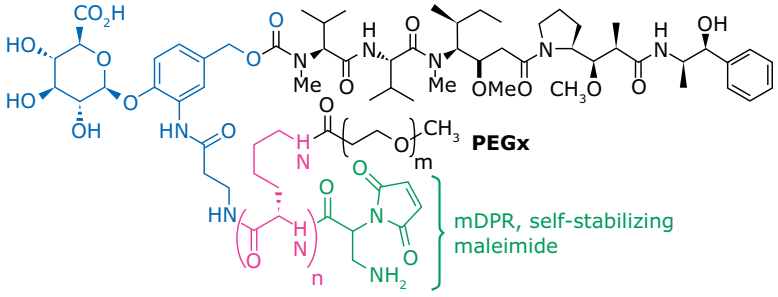


Dumb-Bell like PEG-drug structure

Figure 2. PEGs effectively shield the hydrophobic toxin from the microenvironment, leading to a nubmer of important benefits.

Several publications have demonstrated how PEGs have been used in ADC development to overcome the challenges described above. A study by Burke, et al (Mol Cancer Ther (2017) 16:116) explored use of maleimide-PEG-Glucuronide-MMAE with variable PEG lengths to identify the desired PEG size to stabilize a fully-conjugated ADC with a DAR of 8 (Figure 3). The drug load across the different species is the same; the difference is a branched appendage, close to the maleimide group.

PEGx	m	n
PEG_{24}	24	1
PEG_{12}	12	1
PEG_{8}	8	1
PEG_{4}	4	1
PEG_{2}	2	1
acetyl	0	1
No PEG	–	0



Burke et al., Mol Cancer Ther 16(1) 116

Maleimide-PEG-Glucuronide-MMAE with variable PEG lengths

Figure 3. Maleimide-PEG-Glucuronide-MMAE with variable PEG lengths of 2, 4, 8, 12 and 24.

Impact of the PEG length on pharmacokinetics and plasma clearance for the DAR8 ADC is shown in Figure 4.

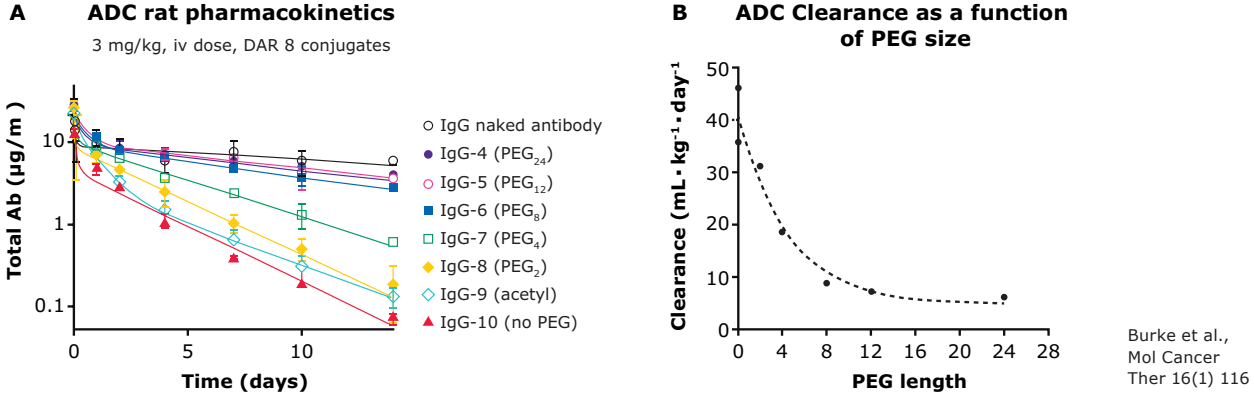
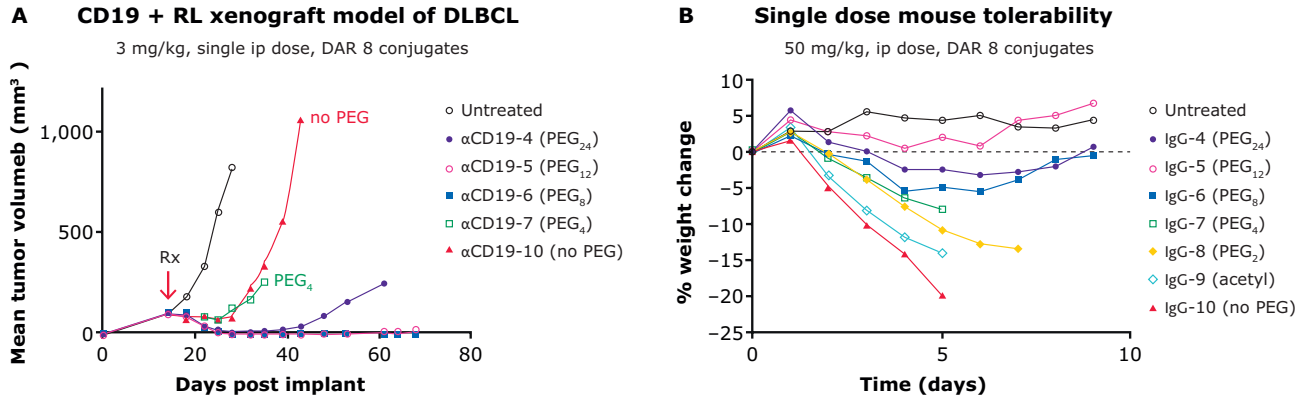


Figure 4. Pharmacokinetics (A) and clearance (B) of the DAR8 ADC are a function of PEG length (2, 4, 6, 8, 12, and 24 PEG units).

Assessing plasma concentration based on radio labelling at 3 mg/kg, the authors showed a strong relationship between drug expsoure and PEG length. The ADCs with the longer PEGS (8, 12 and 24) were able to stay in the plasma at the same concentration as the naked antibody (A). Figure 4B shows ADC clearance as a function of PEG length. With no or limited PEGs, clearance is quite high; in constrast, PEGS at 8, 12 and 24 has stablized clearance – a very clear demonstration of the impact of PEGs.

Increasing PEG length showed a positive effect on pharmacodynamics and increased tolerability and mouse survival rates (Figure 5). The same lengths of PEGs were evaluated for the same DAR8 ADC; dosing of the ADCs was at 14 days post-implant. With no PEG, there was rapid breakthrough of the tumor (A); efficacy was rescued as PEG length increased.

Single dose tolerability as measured by percentage weight change at a 50 mg/kg dose is shown in Figure 5B. At PEG lengths of 8, 12 and 24, weight remained relatively stable while at shorter PEG lengths or no PEG, there was a significant weight loss.



Burke et al., Mol Cancer Ther 16(1) 116

Figure 5. Increasing PEG length shows a positive effect on phamacodynamics (A) and increase tolerabilty and mouse surival rates (B).

Drug Linkers and PEG Incorporation

A wide variety of drug linkers are available, and many have had PEG linkers incorporated to overcome different challenges. PEG linkers have been added to microtubule-targeting agents such as MMAF (Mol Cancer Ther. (2017) 16:116) and amberstatin (Mar. Drugs 2017 15:99) in order to improve and control solubility. DNA-targeting agents such as the highly-lipophilic pyrrolobenzodiazepine (Mol Cancer Ther (2018) 17:2176) can be solubilized using PEG linkers. This approach allows for moderation of the PK/PD profile (Oncotarget (2015) 6:22496).

In addition to the use of a linear PEG chain as a spacer and solubilizing agent in the linker, branched PEGs are also being used in ADC constructs to enable higher DAR species (Figure 6).

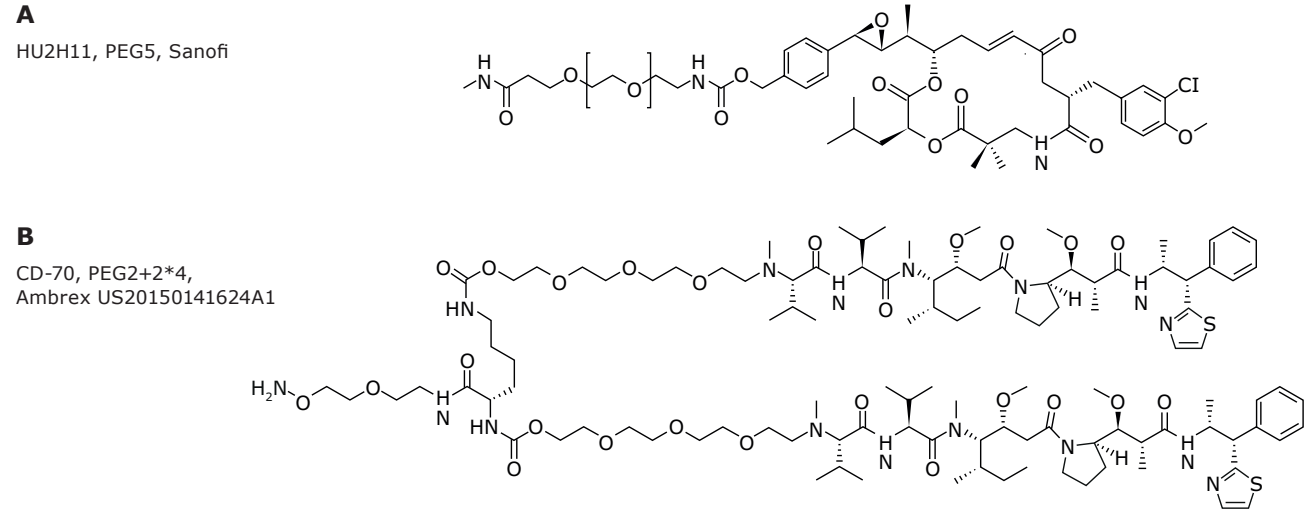


Figure 6. Examples of linear (A; Molecules (2017) 22:1281) and (B) branched PEGs.

Critical PEG Attributes and Analytical Approaches

In a classic synthesis of PEGs, polymerization of ethylene oxide connects randomly and with an equal probability to the built polyethylene chains. The result is a statistical distribution of the molecular rate as shown by MALDI-TOF, where each signal reflects one defined PEG link. The level of distribution depends on the process conditions during the polymerization. This polydisperse PEG, with multiple molecular rate fractions, would be not suitable for use as an ADC linker. This issue is solved with use of highly defined and pure monodisperse PEGs with a uniform molecular mass.

While the typical chain length of PEGs for linkers are 4 to 24 units, larger monodisperse PEGs can be manufactured. Figure 7 shows a PEG52 analyzed by MALDI-TOF with a clear separation of each PEG unit. Maleimide is used as a functional group targeting cysteine on the antibody; at the other end of the PEG, an azide group is used to connect to the payload. In addition to the impact on PK/PD, the uniform molecular rate of the PEG makes it much easier to be analytically characterized. This is beneficial for the registration of the ADC during all clinical phases and supports cGMP requirements which is crucial to commercialization.

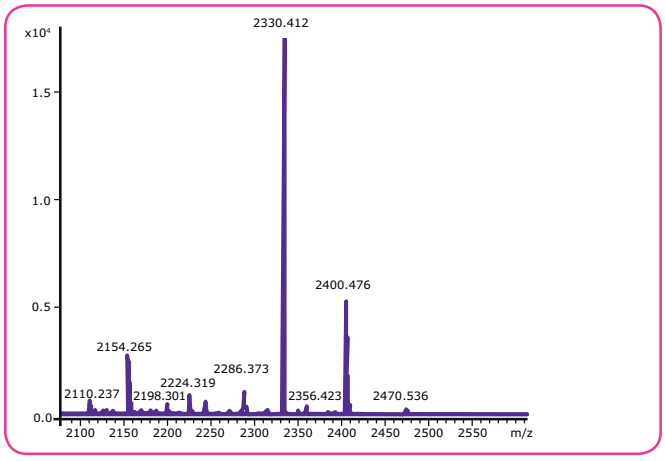


Figure 7. PEG52 analyzed by MALDI-TOF with a clear separation of each PEG unit.

The targeted purity of monodisperse PEGs is usually in the range of 90–95%, and this is the main quality parameter. Monodisperse PEGs don't differ to any extent from polydisperse PEGs in terms of sensitivity of oxidation to hydrolysis. To control this, a chemical derivatization is needed in front of every analytical run. PEGs can easily absorb elemental impurities such as heavy metals, salts, or polar compounds, which is analyzed by state-of-the-art methods via inductively coupled plasma mass spectrometry (ICP-MS), gas chromatography (GC), ion chromatography (IC), Karl Fischer methods or HPLC. Purity is, however, the main quality parameter of monodisperse PEGs.

Some challenges can arise in terms of PEG analytics. Separation of PEG units depends on the molecular rate as shown in Figure 8.

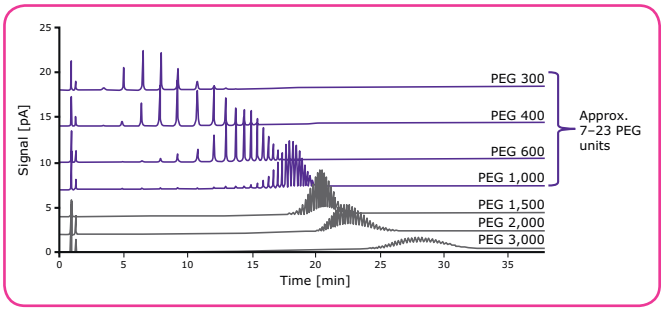


Figure 8. PEG52 analyzed by MALDI-TOF with a clear separation of each PEG unit.

Typical ADCs incorporate polymers of 4–24 PEG units. These various chain link compounds show a significant difference in peak separation, which has an impact on differentiation between purities and impurities of the monodisperse PEG. This must be considered, especially during the derivatization of the optimal PEG length and the respective analytical development.

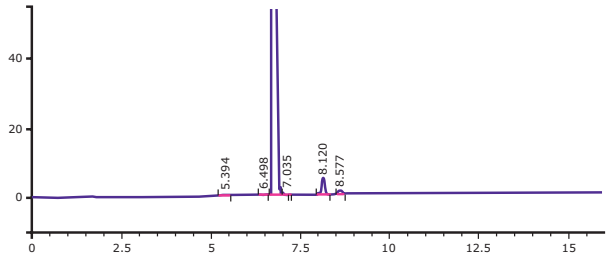
In addition, the use of different detectors of HPLC systems for the determination of purity needs to be taken into consideration. With the absence of double bonds, the most preferred UV light detector cannot be used for PEGs, monodisperse and polydisperse. Applicable detectors are evaporative light scattering detectors (ELSD) and charged aerosol detectors (CAD). Nevertheless, ELSD tends to underestimate low-level signals while CAD overestimates them. With both detectors, small, semi-volatile compounds might be evaporated and therefore not visible or underestimated. Understanding the characteristics of the different detectors is thus crucial for correct interpretation of analytical results.

Figure 9 provides an example of an actual measurement of a monodisperse PEG using HPLC-ELSD. Purity is almost 99 percent and five impurities are detected, the largest with 1 percent area (ELSD).

In comparison, the same sample measured with a CAD shows a purity of only 89 percent (CAD). The increased number of impurities and the area percent is much higher than detected in the ELSD. Gas chromatography is also an option for small PEG units as they are sufficiently volatile up to approximately six ethylene glycol units.

ELSD

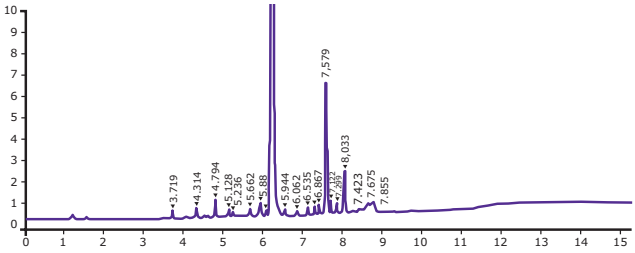
A



- Purity: **98.9% area**
- Largest impurity: 1.0% area
- Number of impurities: 5

CAD

B



- Purity: **89.0% area**
- Largest impurity: 5.8% area
- Number of impurities: 18

Figure 9. The same PEGs sample meaured by (A) ELSD and (B) CAD. The difference in the two methods reinforce the need for expertise when analyzing results.

Conclusion

ADCs represent an important class of therapeutics with significant potential in oncology. With recent approvals and novel solutions to improve their development, we may be at an inflection point where we can now accelerate more of these innovative therapeutics through the clinic and onto the market.

The use of monodisperse PEGs can play a critical role in this new ADC design space in multiple ways. For development of high DAR species; the incorporation of a PEG linker backbone can enable delivery of a significantly higher dose to the target cells resulting in better efficacy and the ability to reach a new cohort of targets. In addition, the use of PEGs can also be utilized to increase the bioavailalbity of any bioconjugate by modification of the solubility of the lipophilic payload. This approach can be both a rescue strategy for exiting constructs as well as a design feature for the development of new compounds. With all the advantages that monodisperse PEGs bring to the development of ADCs, PEGs as linkers look to be a critical factor in the ongoing success of this therapeutic modality.

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