The linker matters in antibody-drug conjugates

It was Paul Ehrlich who first brought up the idea of creating a magic bullet that selectively delivers drugs to malicious tissues while sparing the healthy parts. Roughly a century later, researchers started to attach cytotoxic compounds through linkers to antibodies for a targeted drug delivery approach, given the highly selective binding properties of antibodies.

It turned out though that despite this simple concept, these antibody-drug conjugates (ADCs) are highly complex molecules. To reach optimal results, the components, i.e. the antibody, the drug and the linker need to be carefully controlled.

The first generation of ADCs had up to 50% unmodified antibody, unnecessarily blocking valuable receptor sites on cancer cells. They used linkers which were unstable in blood, leading to limited efficacy, severe side effects and the stopping of clinical trials. Second generation ADCs had less unmodified antibody but resulted in ADCs that were 'over-attached' with drugs, leading to fast blood clearance and potential side effects. Furthermore, the linkers were still unstable. Nevertheless, several ADCs received approval and are successfully being used to treat cancer patients. However, side effects lead to treatment discontinuation in many patients.

To address these issues, third generation ADCs with more stable linkers and conjugation technologies were developed with a well-defined drug-to-antibody ratio (DAR). However, some still showed undesired pre-mature drug loss. All of them required time and costly antibody and/or cell-line engineering for drug attachment. Further, due to limited linker solubility, trial and error was used to identify the best drug attachment site for each antibody and payload combination to get optimal PK, efficacy and manufacturability.

When I started my post-doctoral position at the Paul Scherrer Institute in Switzerland, the enzyme microbial transglutaminase (MTG) was well known to be suitable for site-specific drug conjugation to the conserved glutamine residue Q295 of antibodies[1]. This however, was only efficiently possible when the glycosylation at N297 was genetically or enzymatically removed[1,2] since it was assumed that the neighbouring N297-glycosylation would sterically exclude the MTG from accessing and conjugating the Q295. Despite being a site-specific approach, it still required engineering of the antibody and the linkers still had limited solubility. Thus, there were still two problems to be solved.

We realised that when we used hydrophilic peptides as linkers, antibody engineering was no longer necessary for site-specific conjugation to Q295 and the glycosylation at N297 could be maintained. This was a big surprise as it was commonly believed and assumed that efficient conjugation to Q295 would only be possible upon removal of the antibody glycans given its proximity, or using engineered microbial transglutaminase[3]. Using peptide linkers furthermore offered a broad chemical versatility and flexibility. What is particularly important is that we can tune the hydrophilicity to accommodate all sorts of payloads. Hence, we address the above-mentioned problems of antibody-engineering and low-linker solubility by simply using a peptide linker.

At Araris Biotech AG we are further developing this peptidic-linker technology and have confirmed the many advantages compared to other ADC linker and conjugation technologies. We have achieved site-specific conjugation of five different payload classes to native antibodies in one step within less than a day. We require as little as a five molar excess of the linker-payload compared with the antibody. We believe this is unique in the whole ADC space. Further, we do not see ADC aggregation under various conditions indicating that our hydrophilic linkers well solubilise the hydrophobic payloads.

ADCs carrying our linker not only can be assembled very easily and show excellent biophysical properties but also have an excellent stability in various sera including mouse, cyno and human serum with no payload loss detected over 14 days. Further, our linkers can be designed to be cleaved by Cathepsin B (CatB) as efficiently as the gold-standard di-peptide valine-citrulline of reference ADCs using the same antibody and payload. Even when we incorporate a conventional CatB-cleavage sequence the linker does not get cleaved and the payload remains attached in mouse serum. These observations are remarkable for a cleavable linker which typically gets rapidly cleaved in mouse serum indicating that the payload is located at a sterically occluded site not accessible for certain enzymes and peptidases outside the cell. Overall, we think this may enhance the stability of our ADCs and limit unspecific, premature cleavage of the linker before it reaches the target.

In first head-to-head PK and efficacy studies we could confirm that our linker-payload remains attached to the antibody (using a DAR of 1.9) with a half-life of 10 days. The reference ADC with a DAR of 3.5 using the same antibody and payload but a different linker showed significant payload loss and a half-life of only 5 days. Our ADC essentially was overlapping with the unconjugated native antibody indicating that attachment of the payload did not change the PK of the antibody which is what an ideal ADC should do. In efficacy studies we could see a significantly increased efficacy upon injecting the same amounts of payloads with six out of eight complete tumour remissions, while in the reference, no tumour remission could be observed.

In summary, we believe our novel ADC linker technology based on hydrophilic peptides generates ADCs that show excellent stability with no payload deconjugation, have good biophysical properties, and will greatly facilitate CMC and manufacturing. We therefore hope we can develop novel, fourth generation non-engineered ADCs that are efficient and safe.

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