eBook

Achieving Rapid & Effective Prototyping for Antibody-Drug Conjugate Development

Introduction

Antibody-drug conjugates (ADCs) are a novel type of targeted therapy used for the treatment of cancer. By combining cytotoxins with the targeting capabilities of monoclonal antibodies, ADCs can be used to deliver and release highly-potent cancer killing drugs directly into tumor cells.

The high specificity and targeted delivery afforded by ADCs is making them favorable over conventional chemotherapy approaches within the field of oncology.

Prior to launching an effective antibody-drug conjugate for clinical use, years of rigorous in vitro tests must be conducted to effectively hone in on the optimal antibody-drug pair(s). Given the high costs and time dedicated towards in vivo testing and clinical trials, the preclinical "prototyping" phase of ADCs is crucial to quickly yet effectively identify the top antibody-drug conjugate combination that could yield promising results in further trials.

The ADC development process: high-throughput prototyping during the preclinical phase is crucial yet challenging with traditional antibody labeling technology

Successful ADC development is dependent on many factors including the specificity of the antibody, potency of the drug, drug-antibody ratio (known as the DAR), linker stability, and the drug distribution on the antibody. All these factors will affect the safety, efficiency, and delivery of ADCs to target cells. Therefore, the ability to tightly control the labeling process will greatly influence the success of ADC development.

It is absolutely essential for researchers to use a drug conjugation method that both minimizes uncertainty and maximizes cytotoxicity. With existing antibody labeling technology, two major problems arise during ADC prototyping:

1) The high-throughput capability is limited.

2) Uncertainties arise from non-uniform antibody-drug conjugates.

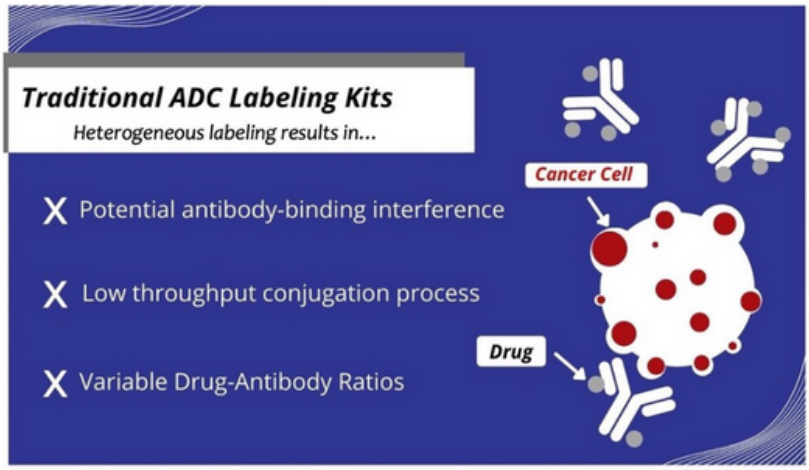
1 Existing antibody-labeling techniques are not suited for high-throughput ADC prototyping

When trying to effectively kill tumor cells for a specific cancer, there are a variety of cellular targets one could choose that might lead to a breakthrough treatment. Thus, it is optimal to test multiple antibodies during the early ADC development stage in efforts to assess the impact on tumor-killing from binding specific targets with their corresponding antibodies. Furthermore, antibodies can vary in their binding affinity or bind to different epitopes on the same target, both of which can have an effect on ADC efficacy. With hundreds or potentially thousands of targets to choose from, it is often necessary to run many assays in parallel with different antibodies in an ADC format to determine which is best.

The drug payload is another crucial component of the ADC: this cytotoxic payload is chaperoned to the tumor cells by the conjugated antibody, ideally leading to high potency killing of the tumor cell. It is important to test different payloads with different antibodies to determine which antibody-drug combination yields the highest tumor cell killing. Given the numerous combinations possible for one ADC candidate, it is essential to have a high-throughput experimental method in which many different conditions can be tested in parallel, allowing researchers to quickly sort out the best antibody-drug combinations for further testing.

The antibody labeling method is a crucial part of this process:

Researchers prefer to have a quick and effective way of attaching their antibody to their drug. Unfortunately with existing labeling technologies, purification of excess drug and linker is often a necessary step prior to testing the ADC in a cell-killing assay.



This extra purification step or steps add additional time, reagents, and costs to the overall experimental plan, which can greatly reduce the high-throughput capability and efficiency of ADC prototyping. Furthermore, the need to purify each ADC individually severely limits how many ADCs can be studied in parallel, thus reducing high-throughput capabilities. Purification can also lead to significant antibody loss, thus requiring more starting material.

Key takeaway

Antibody linker technology should enable high-throughput ADC prototyping, allowing rapid plug and play conjugations with various antibodies and toxic payloads.

2 Antibody labeling techniques often do not yield uniform ADCs, leading to experimental uncertainty

In addition to limited high-throughput capability, the majority of antibody labeling technologies can also lead to highly heterogeneous conjugates and variability in the DAR for different antibodies. This is due to the non-site-specific labeling nature of these common chemical-based antibody conjugation techniques, which typically attach drugs to the lysines on the antibody in a random and uncontrolled manner. Therefore it can lead to interference with antigen binding affinity and reduce the efficacy of the ADC. Furthermore, the number and location of drugs attached to each antibody can vary significantly. Let's look at the impact of DAR on preclinical ADC development in more detail.

The Importance of Defined DAR in Preclinical ADC Development

A high DAR may affect the structure and stability of the antibody, and more drugs per antibody can result in attachment via the antigen binding site, leading to a reduction in antibody activity. Conversely, a low DAR can result in a reduced toxicity profile of the ADC, therefore reducing the overall anti-tumor efficacy.

Whilst a high DAR is often preferential for therapeutic application, the optimum DAR is often dependent on the drug in question. However, at the preclinical testing stages of ADC development, the consistency of the DAR plays a far more important role. Having tight control over the number of drugs per antibody at the in vitro testing stage, allows for more rapid identification of drug candidates that have the potential to yield promising results in the clinical testing stage.

However, control of DAR as a variable within the prototyping phase often proves a challenge for researchers as they struggle to achieve homogeneous ADCs using traditional chemical based antibody-drug conjugation approaches. This often results in two challenges:

1. A heterogeneous mix of antibody conjugates that leads to a variation in the number of drug molecules per antibody, including on occasion completely unconjugated antibodies.
2. The distribution of the drug at different sites on the antibody including the antigen binding site.

In a study by Wang et al. (2005), they analyzed the drug distribution of immunoconjugate huN901–DM1, used in clinical trials for the treatment of small cell lung carcinoma. The drug DM1 is linked to the antibody via random modification at ε-amino groups of the lysine residues, and on average this immunoconjugate contains 3-4 drugs per antibody.

However, the researchers discovered one to six DM1 drug molecules were attached to the antibody molecule and that both light and heavy chains contained linked drugs. In fact, they discovered that the drug molecules distributed over 47% of the 86 lysine residues in the antibody.

This inconsistency in DAR results in uncertainty when testing ADCs in cell killing assays, and can make identification of the optimal antibody-drug pair extremely challenging and time consuming.

Key takeaway

Antibody labeling methods should yield uniform ADCs with a well-defined DAR so that experimental uncertainty can be minimized during prototyping.

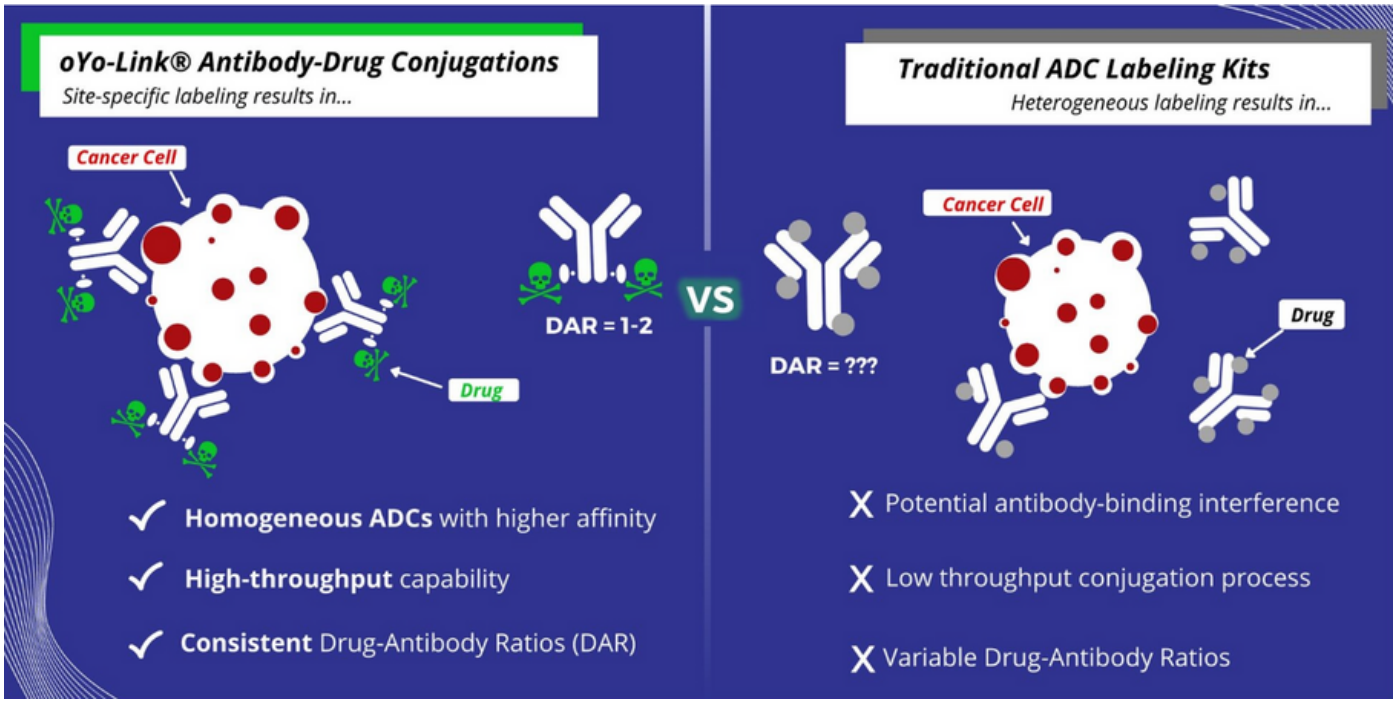
So how do we achieve ADC homogeneity and a defined DAR?

Despite the limitations discussed, there is a solution enabling homogenous ADCs for rapid prototyping. AlphaThera's oYo-Link® Labeling Reagents allow site specific conjugations of drugs to antibodies for use in cancer research applications including targeted cell death and high-throughput screening with cell killing assays.

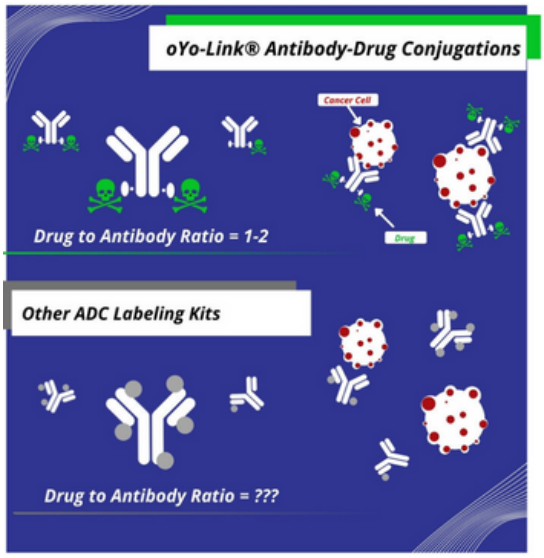
Convenient site-specific conjugations yielding homogeneous ADCs for rapid prototyping

oYo-Link® reagents consist of low molecular weight (~8 kDa), high-affinity antibody-binding domains that possess a photo-crosslinker within their Fc-binding site. Upon illumination with non-damaging Black-light, oYo-Link forms a covalent bond to the antibody.

The resulting conjugates guarantee attachment of 1-2 drugs per antibody specifically in the Fc-constant region.



oYo-Link® provides 3 main advantages for ADC development & prototyping:



A precise DAR is key to understanding results of cell-killing assays when testing your ADCs. oYo-Link assures 1-2 drugs per antibody, removing uncertainty when analyzing your data.

1 Uniform, site-specific conjugates with minimal uncertainty

The site-specific labeling enabled by oYo-Link provides precise control over conjugation sites resulting in a homogeneous ADC. Site-specific labeling attaches the drugs to the Fc-region every time, instilling confidence that the antibody binding region is free. Furthermore, these conjugates have a well-defined DAR of 1-2 drugs per antibody, yielding consistent ADCs with predictable efficacy and toxicity profiles.

2 No Purification of excess drug required

A unique aspect of the oYo-Link drug reagents is that after the antibody-oYo-Link drug conjugation, the free oYo-Link drug will not penetrate into cells, therefore it doesn't cause background cell killing. Thus, no purification is needed following antibody conjugations - you can proceed directly to your cell killing assays. This greatly reduces the time involved with ADC prototyping and affords more flexibility with high-throughput experiments.

3 Use less antibody

oYo-Link can label as little as 1 µg of antibody at a time with oYo-Link ADC reagents, allowing researchers to conduct many more experiments with one batch of antibodies and drugs. This reduces costs and enables more high-throughput screening.

In addition, oYo-Link reagents provide several more advantages when prototyping ADCs. These include:

Robust antibody & buffer compatibility

Works with nearly all off-the-shelf antibody species and subclasses. Reactions are compatible with all common buffers, a wide range of pH values, and the presence of storage proteins (e.g. BSA).

Rapid labeling reaction

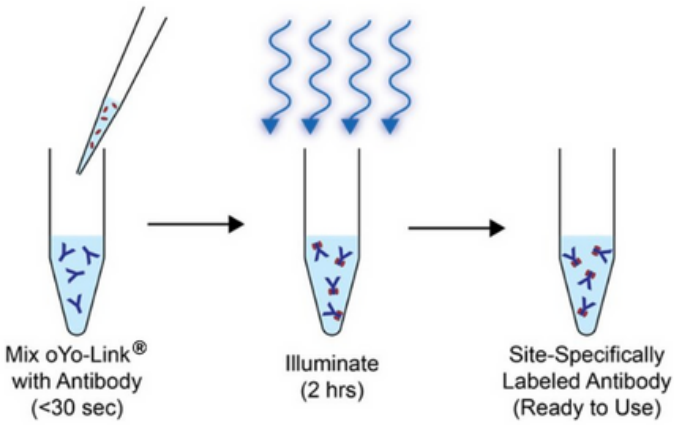
oYo-Link ADC reactions enable extremely quick antibody to drug conjugation, requiring less than 30 seconds hands-on time, with ADCs ready in 2 hours total.

Easily confirm that your Antibody is labeled with SDS-PAGE

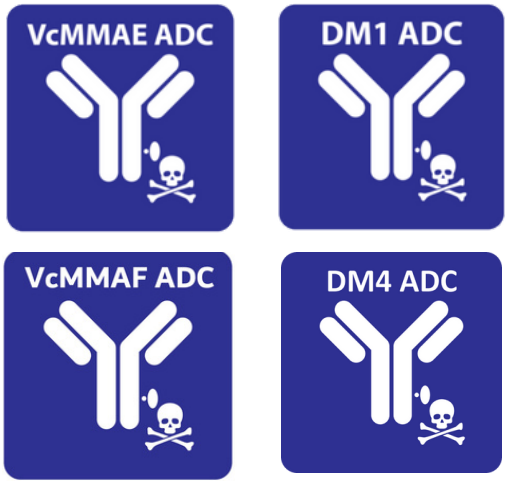
oYo-Link consists of a small (8 kDa) photoreactive antibody-binding domain that site-specifically and covalently labels the heavy chain of antibodies upon Illumination with a black light. Successful photocrosslinking leads to a slight increase in the molecular weight of the antibody heavy chain, which can be easily detected by an upward shift on a gel.

How does oYo-Link work?

Simply mix the oYo-Link drug reagent with your desired antibody followed by photocrosslinking. The entire procedure requires just 30-sec hands on time, 2 hours total.



The oYo-Link range of drug conjugation products includes VcMMAE, VcMMAF, DM1 and DM4, and enables conjugation to the majority of off-the-shelf antibodies.



For more information about oYo-Link ADC reagents get in touch with our technical support team at support@alphathera.com, or visit our website: www.alphathera.com/antibody-drug-conjugation-kits

References

Wang, et al. (2005). Structural characterization of the maytansinoid–monoclonal antibody immunoconjugate, huN901–DM1, by mass spectrometry. *Protein Sci,*14(9), 2436–2446.

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