# What are the ADC drugs?

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The antibody-drug conjugates (ADCs) market continues to rise globally, but ADCs are not a new thing. Why has ADC been surging for so many years? What kind of "magic" power it has, and what makes its growth path so tortuous? Let's take a brief look at the history of ADCs, their features, opportunities, and challenges.

## A brief history of Antibody-Drug Conjugates

ADC (antibody-conjugated drug) is not a new concept. As early as the early 20th century, the Nobel Prize winner in medicine, German scientist Paul Ehrlich, already proposed the concept of ADC and called ADC drugs the "magic bullet". But it continued until the 1950s that the research on ADC drugs improved.

In 1958, Mathe first conjugated an anti-mouse antibody with methotrexate for the treatment of leukemia. Because of the problems of immunogenicity and antibody preparation, progress in ADCs did not go very far until the emergence of monoclonal antibodies in 1975, and later the emergence of humanized antibodies.

In 2000, the first antibody-conjugated drug was approved by the FDA for the treatment of acute myeloid leukemia, but it was limited by issues such as coupling technology, targeting, and effectiveness. Antibody-conjugated drugs are unstable in the blood, and small toxin molecules are released early, resulting in serious toxicity, leading to the withdrawal of the first drug from the market in 2010.

However, the effort paid off. After more than 10 years of precipitation, in 2011, the new antibody-conjugated drug Adcetris was approved by the FDA for the treatment of Hokinson’s lymphoma and systemic anaplastic large cell lymphoma. In 2013, Kadcyla was approved by the FDA for the treatment of HER2-positive breast cancer. The appearance of these two drugs has reignited the enthusiasm for ADC research.

## What are Antibody Drug Conjugates in a nutshell?

ADC generally includes three parts, monoclonal antibody drugs targeting specific antigens, drugs with cytotoxins, linkers linking antibodies, and small toxin molecules. This structure gives ADC drugs more advantages, making them not only have the powerful killing effect of traditional small molecule chemotherapy but also have the tumor-targeting properties of antibody drugs.

Among them, the antibody is responsible for finding tumor cells, and the linker allows the antibody to carry out entrainment, bringing toxic small molecules into the tumor cells, and the small molecules are a sharp sword to kill the tumor cells. In addition, there are also antibodies that have anti-tumor efficacy.

Due to the large differences in the design of different ADC drugs, even for different drugs with the same target, the differences in recognition sites, connection sites, linkers, and small molecules connected will cause large differences in drug toxicity. The factors that need to be considered in the design of ADC drugs are the factors that affect drug toxicity, including:

1. The choice of antibody: a good antibody should have a clear target, high expression in tumor cells, low expression in normal tissues, support drug loading, and load stability, can be well internalized by cells, good PK characteristics, and less non-specific binding.

2. The choice of connection site: whether directional coupling can be carried out, the number of connections, *etc*.

3. Linker: stable in the internal circulation of the body, ensuring that toxins will not be released in the internal environment, can be released well in the cell, can be released by lysosomal digestion, or can be released after the antibody is degraded.

4. Cytotoxic drugs: highly effective pharmacodynamics, non-immunogenic, and can be combined with linkers through modification.

## ADC mechanism of action

**Internal circulation:** due to the poor oral bioavailability of ADC drugs, intravenous injection is used. Because the relative molecular mass of small molecule drugs is very small compared to that of antibodies, the circulation of ADC drugs in the body is similar to that of ordinary antibodies.

**Binding antigen:** The antibody recognizes a specific antigen site for targeted binding.

**Internalization:** After the antibody binds to the antigen receptor, the tumor cell membrane begins to undergo endocytosis, and the ADC drug is swallowed into the cell.

**Drug release:** After the coupling body enters the cell, the linker is cleaved by lysosomes in the cell, and the linker is cleaved to release small-molecule cytotoxic drugs.

**Play the role of drugs:** Small molecule cytotoxics can kill tumor cells and make target cells apoptotic. When the target cell dies, the active cytotoxic load may also kill the surrounding tumor cells, which is also called the bystander effect.

## The development of ADC drugs

The first generation of ADC drugs appeared in the early 1990s, and ADCs based on humanized and chimeric monoclonal antibodies were reported. After that, until 2001, the world's first ADC drug, Pfizer's Mylotarg, was approved by the FDA for the treatment of CD33-targeted acute myeloid leukemia. However, the Phase III study of Mylotarg was launched in 2004 and found that it would cause fatal liver damage. Pfizer voluntarily applied for its delisting in 2010.

Unlike the first-generation ADC drugs using mouse-derived monoclonal antibodies, the second-generation ADC drugs use humanized-derived monoclonal antibodies represented by trastuzumab, which improves the targeting of tumor cells. At the same time, the second-generation ADC uses more toxic small molecules, and the coupling method is still similar to the first-generation, and the stability of the linker still needs to be improved. The representative drugs are Adcetris produced by Seattle Genetics, which went on the market in 2011, and Kadcyla produced by Roche, which went on the market in 2013. Kadcyla is also the first ADC drug approved for the treatment of solid tumors.

The evolution of the third-generation ADC drugs mainly benefited from the development of site-specific coupling technology. More precise coupling technology can control the position and quantity of highly active drug molecules coupled on the antibody, with higher homogeneity and improved drug purity, quality control, etc., ensuring stable and controllable DAR and reducing drug toxicity.

## Several coupling techniques of ADC

1. Lysine residue coupling

This method uses a linker containing active carboxylic acid ester sites to link the payload to the lysine residues of the antibody.

2. Change disulfide bond to reduce cysteine coupling

Taking IgG1 as an example, there are 4 pairs of interchain disulfide bonds that can be reduced, and 8 cysteine sulfhydryl groups are obtained after reduction. Groups such as maleimide on Linker can react with sulfhydryl groups to form stable conjugates. The degree of disulfide bond reduction can optimize the DAR value of the final product.

3. Unnatural amino acid coupling

This method constructs an unnatural amino acid expression system (for example, using tRNAs carrying unnatural amino acids, etc.), introduces unnatural amino acids into antibodies, and connects them with a linker at the unnatural amino acid sites.

4. Enzyme-catalyzed coupling

This method uses enzymes to recognize specific amino acid sequences on antibodies and modify the corresponding sites to generate conjugable sites.

5. Coupling via transpeptidase-mediated transpeptidation

This method relies on the catalytic properties of transpeptidase. Using this feature of Sortase A, various types of molecules can be connected to oligo-G to achieve coupling to specific sites of antibodies.

6. Coupling via MTG enzyme (microbial transglutaminase)-mediated transpeptidation

Using a transpeptidation reaction catalyzed by the MTG enzyme, the linker containing the primary amine is covalently linked to the primary amine of the specific glutamine (Q295) of the deglycosylated antibody. Since each heavy chain of an antibody has a binding site, the DAR value of the ADC molecule obtained by this method is fixed at 2.

7. Coupling by modifying the N-glycan on the aspartic acid residue of the antibody.

## The outlook of ADC Drugs

Due to the advantages of a clear target, mature technology, and good selectivity, the research of antibody-conjugated drugs will still be a hot spot in the future. Existing studies have shown that the proportion of ADC compounds delivering effector molecules to target cells is far less than 1%, but the targeting of drug delivery is still much higher than that of traditional systemic drug delivery, and the incidence of adverse reactions is also significantly lower than that of traditional drugs. It can be seen that ADC has many shortcomings in some places, but it has more advantages than traditional drugs.