Learn More About ADCs From Its Structure

Antibody-drug Conjugates (ADCs) are highly targeted biopharmaceutical drugs that combine monoclonal antibodies specific to surface antigens present on particular tumor cells with highly potent anti-cancer agents linked via a chemical linker. It is a hot topic of drug development in the oncology field. All ADCs contain three core components: an antibody that binds to tumor-associated antigens, a cytotoxic payload, and a linker. Each core component has complex interactions with the tumor and the tumor microenvironment (TME).

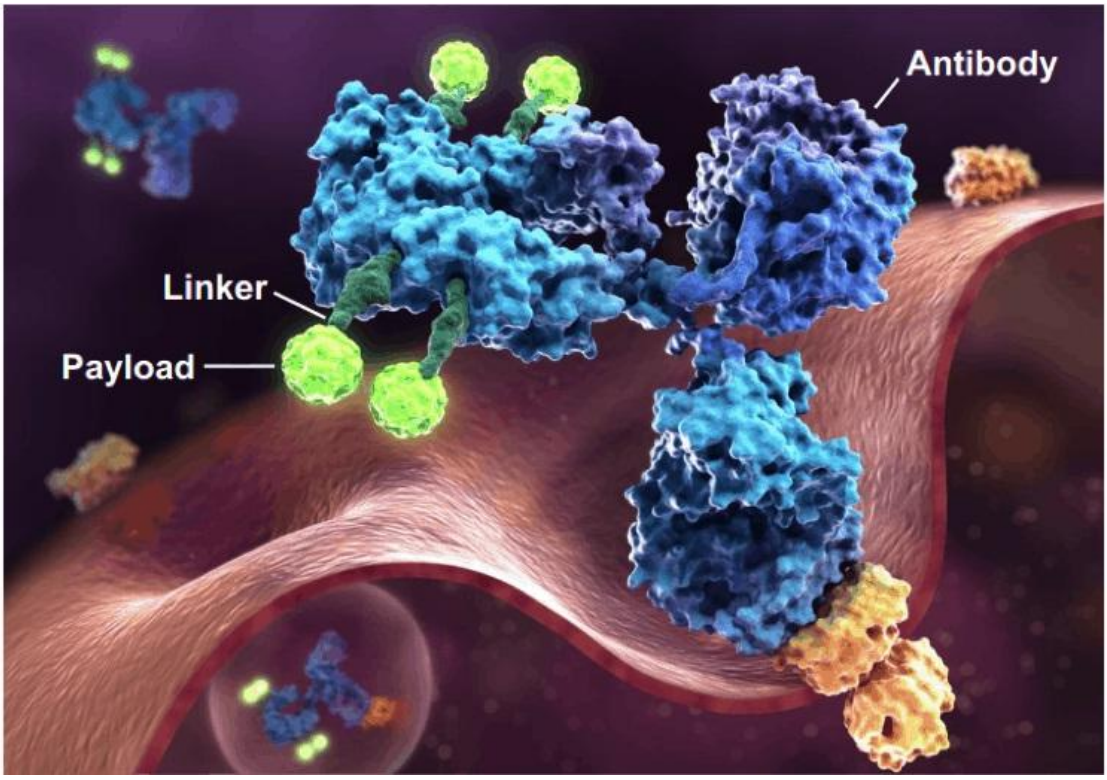


Image from: ADCREVIEW

Different ADCs may have large differences in these three core components, which may affect their pharmacological properties, efficacy and safety. To learn more about ADCs,

start by understanding their structure. Based on a document published by Nat Rev Clin Oncol in June 2021, let's start with 12 ADCs that have already been launched.

A Brief History of ADC Development

The concept of ADC can be traced back to the early 20th century, when scientist Paul Ehrlich conceived of a "magic bullet" that can release cytotoxic drugs. However, the entry of ADCs into oncology clinical trials began in the 1980s. No survival benefit was shown, but significant toxicity was observed, and this continued for 20 years until the CD33-targeting drug gemtuzumab ozogamicin was approved (In 2000, the first indication was relapsed or refractory acute myeloid leukemia), and it was also the first ADC approved by the US FDA. However, it was delisted in 2010 due to adverse reactions.

In 2011, the CD30-targeting drug brentuximab vedotin was approved for the treatment of classical Hodgkin lymphoma and systemic anaplastic large cell lymphoma (ALCL). Soon after, in 2013, the HER2-targeting drug adotrastuzumab emtansine (T-DM1) was launched. Since then, the pace of ADC research and development has gradually accelerated. The table below summarizes the 12 ADCs that have been launched.

The 12 ADCs are: Gemtuzumab ozogamicin, Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Moxetumomab pasudotox, Polatuzumab vedotin, Enfortumab vedotin, Trastuzumab deruxtecan, Sacituzumab govitecan, Belantamab mafodotin, Loncastuximab tesirine and Tisotumab vedotin.

ADC	Target	mAb	Linker	Payload/Payload Class	Payload Action	DAR	Disease Indication (Year of Approval)
Mylotarg® (gemtuzumab ozogamicin)	CD33	IgG4	acid cleavable	ozogamicin/calicheamicin	DNA cleavage	2–3	CD33+ R/R AML (2000) [a]
Adcetris® (brentuximab vedotin)	CD30	IgG1	enzyme cleavable	MMAE/auristatin	microtubule inhibitor	4	R/R sALCL or cHL (2011)R/R pcALCL or CD30+ MF (2017); cHL, sALCL or CD30+ PTCL (2018) [b]
Kadcyla® (ado-trastuzumab emtansine)	HER2	IgG1	non-cleavable	DM1/maytansinoid	microtubule inhibitor	3.5	HER2+ metastatic breast cancer previously treated with trastuzumab & a taxane (2013); HER2+ early breast cancer after neoadjuvant taxane & trastuzumab-based treatment (2019)
Besponsa® (inotuzumab ozogamicin)	CD22	IgG4	acid cleavable	ozogamicin/calicheamicin	DNA cleavage	6	R/R B-ALL (2017)
Lumoxiti®(moxetumomab pasudotox-tdfk)	CD22	unknown	cleavable	Pseudotox (Fragment of Pseudomonas exotoxin-A; PE38)	unknown	N/A	Relapsed or Refractory Hairy-Cell Leukemia (2018)
Polivy® (polatuzumab vedotin-piiq)	CD79b	IgG1	enzyme cleavable	MMAE/auristatin	microtubule inhibitor	3.5	R/R DLBCL (2019) [c,d]
Padcev® (enfortumab vedotin-ejfv)	Nectin4	IgG1	enzyme cleavable	MMAE/auristatin	microtubule inhibitor	3.8	Locally advanced or metastatic urothelial cancer after a PD-1 or PD-L1 inhibitor and a Pt-containing chemotherapy (2019) or are ineligible for cisplatin-containing chemotherapy and previously received 1 or more lines of therapy (2021) [d]
Enhertu® (fam-trastuzumab deruxtecan-nxki)	HER2	IgG1	enzyme cleavable	DXd/camptothecin	TOP1 inhibitor	8	Unresectable or metastatic HER2+ breast cancer after 2 or more anti-HER2 regimens (2019) [d]; locally advanced or metastatic HER2+ gastric or gastroesophageal junction adenocarcinoma after a trastuzumab-based regimen (2021)
Trodelvy® (sacituzumab govitecan-hziy)	TROP2	IgG1	acid cleavable	SN-38/camptothecin	TOP1 inhibitor	7.6	Locally advanced or metastatic TNBC after at least two prior therapies (2020); locally advanced or metastatic urothelial cancer after a Pt-containing chemotherapy and a PD-1 or PD-L1 inhibitor (2021) [d]
Blenrep® (belantamab mafodotin-blmf)	BCMA	IgG1	non-cleavable	MMAF/auristatin	microtubule inhibitor	4	R/R multiple myeloma after at least 4 prior therapies including an anti-CD38 mAb, a proteasome inhibitor, and an immunomodulatory agent (2020) [d]
Zynlonta® (loncastuximab tesirine-lpyl)	CD19	IgG1	enzyme cleavable	SG3199/PBD dimer	DNA cleavage	2.3	R/R large B-cell lymphoma after 2 or more lines of systemic therapy, including DLBCL not otherwise specified, DLBCL arising from low grade lymphoma, and high-grade B-cell lymphoma (2021) [d]
Tivdak® (tisotumab vedotin-tftv)	Tissue Factor	IgG1	enzyme cleavable	MMAE/auristatin	microtubule inhibitor	4	Recurrent or metastatic cervical cancer with disease progression on or after chemotherapy (2021) [d]

12 ADCs currently approved by the US FDA

Antibody And Target Selection

Immunoglobulin G (IgG) is the main antibody backbone in ADCs. Human IgG comprises four subclasses: IgGl, IgG2, IgG3 and IgG4, which differ in their constant domains and hinge regions. These differences affect the solubility and half-life of monoclonal antibodies (mAbs), as well as their affinity for different Fcγ receptors (FcγRs) expressed on immune effector cells.

Currently, most ADCs use IgG1 as the antibody backbone, and a few use IgG2 or IgG4 (eg, gemtuzumab ozogamicin and inotuzumab ozogamicin, both of which use IgG4). Compared with IgG2 and IgG4, IgG1 has similar plasma half-life, but higher complement

fixation and FcγR binding. IgG3 is probably the most immunogenic subclass, but it is generally avoided in ADC design due to its short circulating half-life.

Antibodies	IgG1	IgG2	IgG3	IgG4
Serum half-life	21 days	21 days	7–21 days	21 days
C1q binding	Yes	Yes	Yes	No
Fcγ avidity	High	Low	High	Moderate

Antibodies

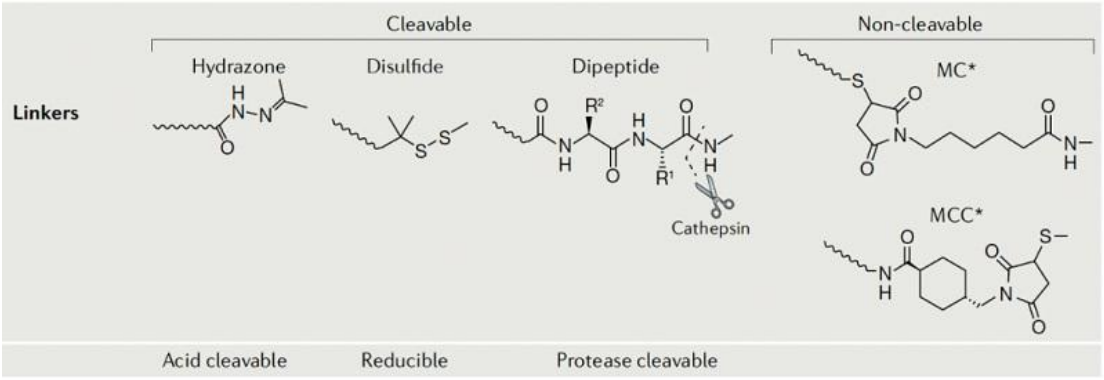
An ideal mAb target should be highly expressed on tumor cells but not expressed on normal cells. ADCs can deliver a payload of cytotoxic substances to any cell that expresses the target antigen. Thus, a target that is preferentially expressed in tumor cells, compared to normal cells, can provide a wider therapeutic window while reducing the chance of systemic toxicity.

Currently, ADC targets approved for the treatment of solid tumors include: HER2, TROP2, and nectin-4. In hematological neoplasms, CD30 is the target of brentuximab vedotin, which is expressed on and characterized by malignant lymphocytes in Hodgkin's lymphoma and ALCL. Likewise, CD22, CD79b, and B cell maturation antigen (BCMA) are all highly specific to the B cell lineage.

In addition to tumor specificity, there are other factors that can influence the efficacy of ADCs. For example, patients with intratumoral or intertumoral HER2 heterogeneity had a poorer T-DM1 response compared with patients with HER2 homogeneous expression. In addition, target turnover rate, internalization rate, lysosomal degradation rate, etc. can all affect the antitumor activity of ADC.

Linker Types And Processes

Linker technology has come a long way over time. The linker has two main functions: first, to ensure that the cytotoxic payload remains firmly attached to the antibody moiety as the drug circulates in the plasma. Linkers are unstable and may release the payload prematurely in plasma, not only increasing the severity of systemic adverse effects, but also reducing the effective delivery of the payload at the tumor site. Second, the payload can be effectively released within the tumor (especially within tumor cells), often through competitive action.



Linkers

Linkers can be roughly divided into two categories: cleavable and non-cleavable. In the presence of tumor-associated factors (eg, acidic environment, abundant proteolytic enzymes, etc.), the cleavable linker can dissociate and release the ADC cytotoxic payload. Cleavable linkers include: pH-sensitive hydrazone linkers (eg gemtuzumab ozogamicin), reducible disulfide linkers, various peptide-based linkers, enzyme cleavable linkers (eg brentuximab vedotin, polatuzumab vedotin, Sacituzumab Govitecan, T-DXd, etc.).

However, in practical applications, the stable forms of cleavable linkers in the plasma circulation are different. For example, the hydrazone linker used by gemtuzumab

ozogamicin, is less stable compared with other cleavable linkers and undergoes a certain degree of hydrolysis at physiological pH, which may partly explain its off-target toxicity.

In contrast, non-cleavable linkers are more stable in plasma, but their antibody-linker structure relies on lysosomal degradation to release the payload, often resulting in the retention of charged amino acids on the payload, which may affect drug efficacy or cell permeability sex. Those using non-cleavable linkers are: T-DM1, belantamab mafodotin.

It is worth noting that there are preclinical data showing that the extracellular release of cytotoxic payloads may be an important component of ADC activity, so optimization of joint stability is a complex issue that depends on target, payload selection, and TME characteristics.

Payload

Early ADC studies used traditional chemotherapy drugs, such as methotrexate, doxorubicin, vincristine, etc. However, studies have shown that these ADCs are no more effective than their traditional models. In addition, the data showed that only a small fraction of the antibody targeted dose reached tumor tissue (0.1%), suggesting that a more cytotoxic payload is required to achieve therapeutic effect.

Therefore, subsequent ADC studies have targeted more effective chemotherapy drugs, such as: auristatins, calicheamicins, maytansinoids, camptothecin analogues, etc. These drugs can show cytotoxicity at concentrations in the subnanomolecular level.

Payloads

Of the 9 ADCs mentioned above, monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF) belong to auristatins, which are tubulin destabilizers. Ozogamicin belongs to the calicheamicins, which can cause double-strand DNA breaks. DM1 belongs to the class of maytansinoids that destabilize tubulin. Finally, DXd and SN-38 are camptothecin derivatives that cause DNA fragmentation by inhibiting topoisomerase I. It should be noted that none of these loads are suitable for systemic delivery as free drug.

The drug-to-antibody ratio (DAR) refers to the average number of payloads per mAb, which is related to the pharmacological activity of the ADC. The current DAR range of approved ADCs is 2-8. In general, high DAR ADCs are more active in vitro, but may be cleared more rapidly from plasma by the liver. Taking brentuximab vedotin as an example, its in vitro activity is related to DAR. In a mouse model, the ADC version with a DAR of 8 had plasma clearance 5 times faster than the version with a DAR of 2, but showed no advantage in anti-tumor activity with increased toxicity.

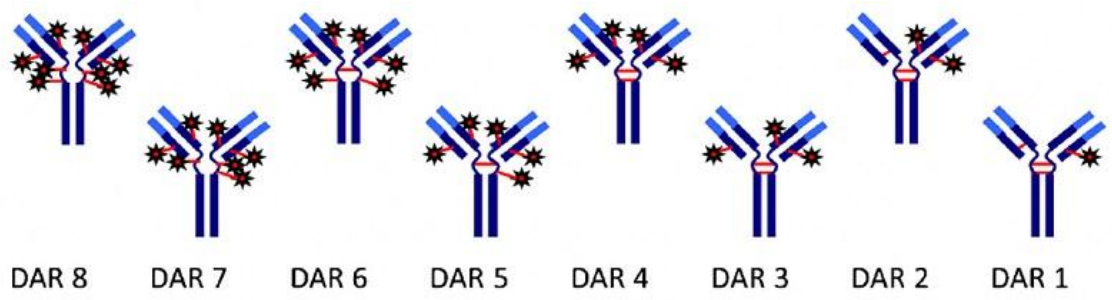


Image from: Pharm Res. 2015; 32(6): 1907-19.

Preclinical studies have shown that the relationship between higher DAR and faster hepatic clearance is associated with increased hydrophobicity of the antibody-linker complex, which can be avoided by the use of hydrophilic structures. For ADCs that drug

structure and DAR do not affect plasma clearance (eg, Sacituzumab Govitecan), higher DAR is directly associated with higher anti-tumor activity in vivo.

The above is the basic structure of ADC. Based on the understanding of ADC structure, the theoretical basis of ADC efficacy, safety, drug resistance and combination therapy can be better understood. Scientists will bring continuous innovation in drug design and clinical application, let us continue to pay attention to the development of ADC.

Biopharma PEG, as a professional PEG derivatives supplier, is dedicated to being your most reliable partner to provide high-quality PEG linkers and chemical synthesis. We are committed to promoting the progress of your ADC discovery and development projects.

Reference:

1、Nat Rev Clin Oncol. 2021; 18(6): 327-344.

Related articles:

[1] The Rise of the TROP2-Directed ADCs for Solid Tumors

[2] Global Antibody-drug Conjugates (ADCs): Approvals & Clinical Trails Review

[3] Progresses Of ADC Technology For Cancer Therapy

[4] ADCs for Clinical Research in the Global Market

[5] FDA Approved Antibody-Drug Conjugates (ADCs) Up To 2022

[6] How To Choose The Best ADC Linker?

[7] ADC Linker: Cleavable vs. Non-Cleavable Linkers