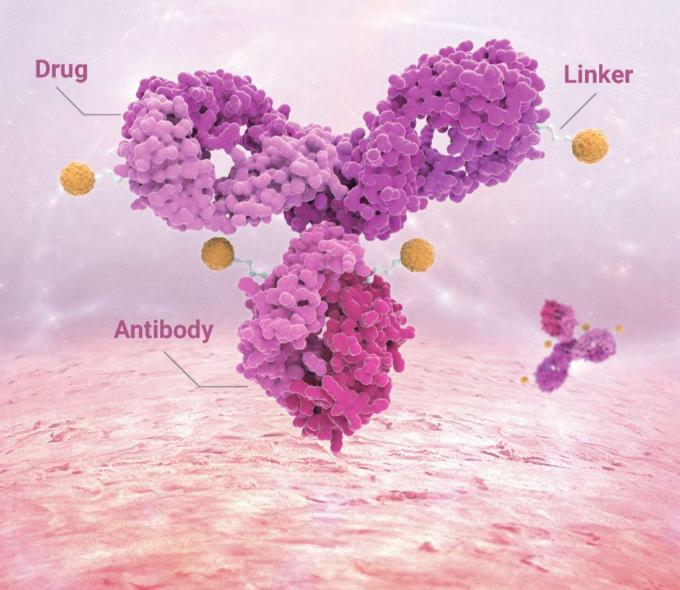


Antibody-Drug Conjugates (ADCs) Handbook

To improve the Specificity of Small Molecules to Tumor Cells



Antibody-Drug Conjugates (ADCs)

Antibody-Drug Conjugate (ADC) is a potent biopharmaceutical cancer-targeted drugs comprised of a humanized or human monoclonal antibody conjugated with cytotoxic drugs (payloads) via a chemical linker.

ADCs exhibit high selectivity and toxicity to the tumor, and become one of the fastest-growing classes of therapeutics. The ADCs are multicomponent molecules. The selection of target antigen, antibody, payload, linker, and conjugation strategies is of vital importance in the design of ADCs.

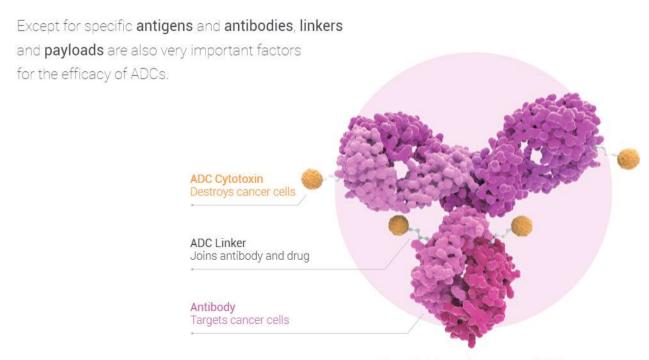


Figure 1. Schematic structure of ADCs,



ADCs Expand the Therapeutic Window

Proteins

ADCs can **increase efficacy** and **reduce the toxicity of payloads** compared with traditional cytotoxic drugs. The selective delivery of cytotoxic drugs to tumor cells increases the percentage of cytotoxic molecules that reach the tumor, thus broadening the therapeutic window. Nevertheless, the therapeutic window remained narrow for second-generation ADCs. This is due to off-target toxicity, competition with unconjugated antibodies and aggregation or fast clearance.

With the coming of new technology, the discovery of the payloads, the optimization of the antibody, the linker and conjugation chemistry plays a key role in improving the therapeutic window of third-generation ADCs^[2].

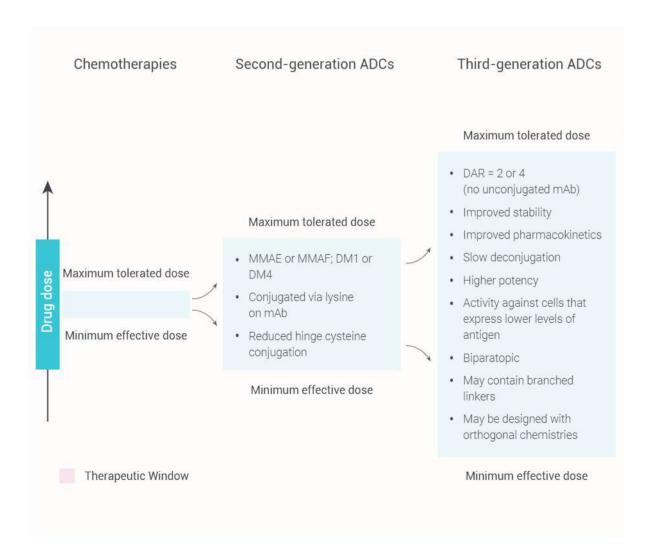
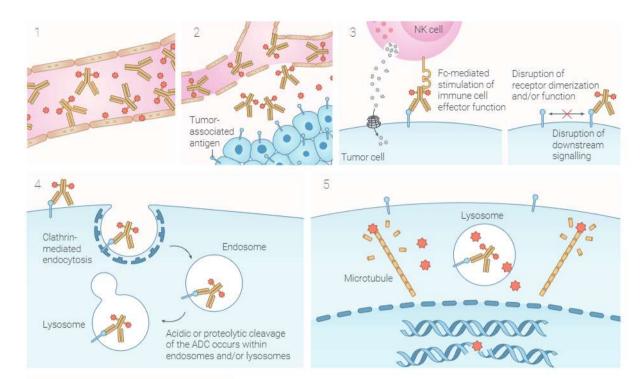


Figure 2. Third-generation ADCs are designed to expand the therapeutic window^[2].

Mechanism of Action



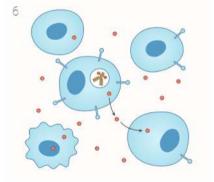


Figure 3. Mechanism of action of ADCs[3].

- 1.The ADC firstly enters the blood system. In this process, the linker must have sufficient stability to reduce the release of the payload and the subsequent off-target toxicity.
- The ADC distributes to tumor tissues. In the tumor microenvironment, some payloads are released to kill the tumor cells.
- 3. The ADC binds to the tumor associated antigen to form an ADC-antigen complex. The antibody can also exert anti-tumor effects.
- The complex is internalized into the cancer cell. A few ADCs can bind to FcRn receptors and can be sent to the
 outside of cells.
- The complex undergoes lysosomal degradation inside the cell. The payload is released and then binds to its target, leading to cell death.
- 6. The cytotoxic payload can also kill the tumor cells through the by-stander effect^[9].

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Antigens for ADCs

Ideally, the target antigen should be highly expressed in tumor tissues as compared with normal tissues. The antigen needs to have limited heterogeneity across the tumor and minimal antigen shedding. The target antigen should be well internalized by receptor-mediated endocytosis. The antigen should not be modulated during endocytosis and down-regulated after treatment with the ADC. In addition, another promising approach to targeting antigens is to target the vasculature and stroma, including stromal cells and extracellular matrix.

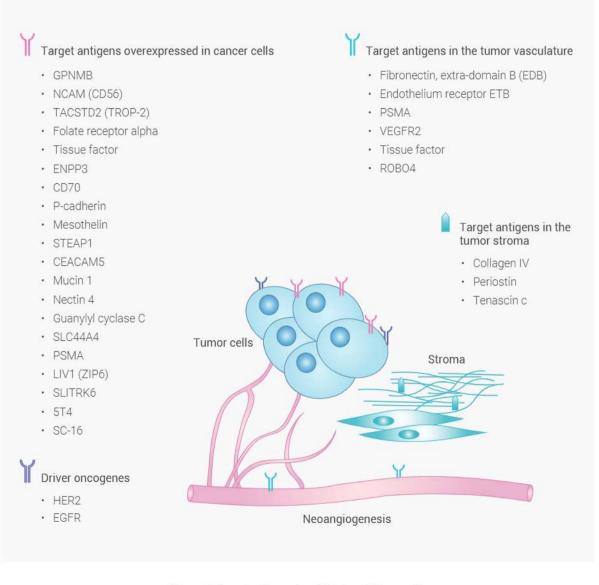


Figure 4. Target antigens for ADCs in solid tumors[4].



Antibodies for ADCs

As the navigation system of ADC drugs, antibodies are mainly responsible for delivering payloads to target cells. Ideal antibodies need to have high specificity and affinity for tumor associated antigens, good stability, low immunogenicity, low cross reaction, long circulating half-life and effective internalization. Currently, all ADCs incorporate antibodies of the immunoglobulin G (IgG) isotype: IgG1, IgG2, IgG3 and IgG4^{Ig}.

	IgG1	lgG2	IgG3	IgG4
Relative natural abundance	60%	32%	4%	4%
# of interchain disulfide bonds	4	б	13	4ª
Serum half-life	~21 days	~21 days	~7 days	~21 days
Immune activation				
via C1q binding	++	+	+++	
via FcγR binding	+++	+	++++	++
Use in clinically- approved ADCs	Kadcyla [®] , Enhertu [®] , Trodelvy [®] , Blenrep [®] , Adcetris [®] , Polivy [®] , Padcev [®]	9	2	Mylotarg® _° , Besponsa®°

^a Hinge region disulfides are labile, enabling spontaneous Fab arm exchange with other IgG4 antibodies in vivo

Figure 5. Overview of IgG subclasses for potential use in ADCs [5].

^b Fab arm exchange is prevented through S228P mutation in the hinge region.

Selection of Payloads and Products

The payloads are the ultimate effector components of therapeutic ADCs. Ideally, the potency of the cytotoxic payload should be extremely high, with IC_{50} values in the subnanomolar range. Their structures should be small in size, and their targets must be located inside tumor cells. They should allow the conjugation of a linker and retain toxic potency after release from ADCs. Also, they should have reasonable solubility, sufficient stability, low immunogenicity, and a long half-life^[6].

The most frequently used cytotoxic agents are **microtubule inhibitors**, **DNA-damaging drugs** and other **toxic** compounds.

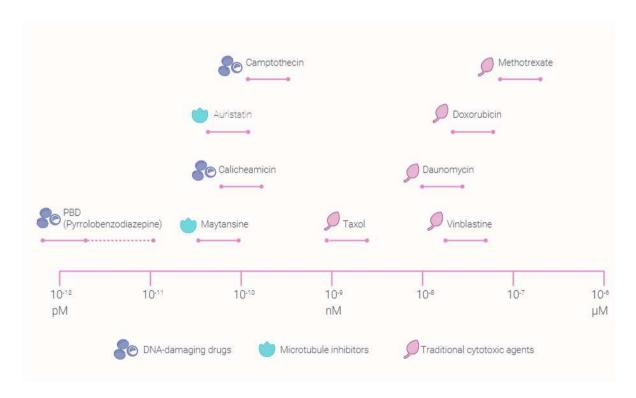


Figure 6. Payload potency of ADCs (IC50, M)[6].

Product Advantages & Features

MCE can provide you with novel and diverse payloads to meet your needs for scientific research. We have the high-quality products and services. The payloads (100+) are expanding.



Payloads/ADC Cytotoxins

Microtubule Inhibitors

DNA-Damaging Drugs

Traditional Cytotoxic Agents

HY-19792

Mertansine

A microtubulin maytansinoid inhibitor. To overcome systemic toxicity and enhance tumor-specific delivery.

HY-19609

Calicheamicin

An antitumor antibiotic.
A DNA synthesis inhibitor.
Causes double-strand DNA breaks.

HY-15142

Doxorubicin

A cytotoxic anthracycline antibiotic and a topoisomerase I/II inhibitor. Induces apoptosis and autophagy.

HY-15581

MMAD

A potent tubulin inhibitor. A toxin payload in ADCs. Coupled through a stable oxime-ligation. HY-13704

SN-38

An active metabolite of the Topoisomerase I inhibitor Irinotecan. Inhibits DNA and RNA synthesis. HY-16261

Aldoxorubicin

An prodrug of Doxorubicin.

Released from albumin under acidic conditions with antitumor activities.

HY-15162

Monomethyl auristatin E

Monomethyl auristatin E (MMAE) is a synthetic derivative of dolastatin 10. Functions as a potent mitotic inhibitor. HY-16560

Camptothecin

A DNA enzyme topoisomerase I inhibitor. Inhibits DNA, RNA synthesis and induces DNA damage.

HY-16700

PNU-159682

A DNA topoisomerase I inhibitor. For EDV-nanocell technology to overcome drug resistance.

HY-15579

MMAF

A tubulin polymerization inhibitor. A cytotoxin of Vorsetuzumab mafodotin and SGN-CD19A. HY-13631D

Dxd

Dxd (Exatecan derivative for ADC) is a DNA topoisomerase I inhibitor. A conjugated drug of DS-8201a. HY-B0015

Paclitaxel

A naturally antineoplastic agent and stabilizes tubulin polymerization. Induces apoptosis and autophagy.

HY-15583

Auristatin F

A MMAF Analog.

A potent microtubule inhibitor and vascular damaging agent (VDA).

HY-107769

Duocarmycin TM

A potent antitumor antibiotic. Induces endonucleolytic DNA fragmentation and apoptosis. HY-14519

Methotrexate

An antimetabolite and antifolate agent. Inhibits the enzyme dihydrofolate reductase and DNA synthesis.

Selection of Linkers and Products

The chemical linker is the short spacer that connects the payload to the monoclonal antibody, which must be stable in the systemic circulation. The properties of the linker are proven to be critical, because they impact greatly on the stability, pharmacokinetics/pharmacodynamics, therapeutic window and efficacy of ADCs. An ideal linker between the cytotoxic payload and the antibody should be stable in circulation. At the same time, it must rapidly and efficiently release the cytotoxic agent inside the tumor cells. In addition, it needs to possess high hydrophilicity. Therefore, the linkers comprise three key parts: a suitable functionality for conjugating to the antibody, a spacer unit containing hydrophilic elements and a trigger for releasing the cytotoxic payload.

The most frequently used linkers are maleimidocaproyl (MC), N-succinimidyl 4-(maleimidomethyl)cyclohexane-1-carboxylate (SMCC), N-succinimidyl-4-(2-pyridyldithio) butanoate (SPDB), N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP), peptides, hydrazones and disulphides. Based on the payload release mechanism, available linkers are categorized into two major classes: cleavable linkers and non-cleavable linkers^[1]

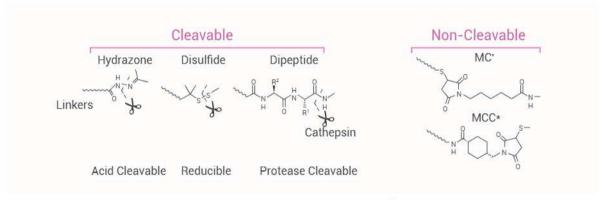


Figure 7. Modular components of ADCs^[8].

		Cleavable Linker		Non-Cleavable Linker
	Hydrazone	Disulfide	Peptide	Stable Linker
Advantages	Intracellular release of payload; Acid sensitive	Intracellular release of payload; GSH sensitive	Intracellular release of payload; Protease sensitive	Stability during circulation
V Disadvantages		remature cleavage durin	g circulation	An amino acid residue attached on the released payload

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MCE can provide you with novel and diverse linkers to meet your needs for scientific research. We have the high-quality products and services. The linkers (700+) are expanding. Related popular products are listed below:

Cleavable Linker -

Protease Sensitive					
HY-78738	HY-12362	HY-20336			
MC-Val-Cit-PAB	Val-Cit-PAB-OH	Mc-Val-Cit-PABC-PNP			
A cathepsin cleavable ADC linker	A cleavable ADC linker	A cathepsin cleavable ADC linker			
HY-78931	HY-41189	HY-130936			
Boc-Dap-NE	Fmoc-Val-Cit-PAB-PNP	DBCO-Val-Cit-PABC-OH			
A dipeptide and cleavable ADC linker	A cleavable ADC linker	A cleavable ADC linker			

GSH Sensitive					
HY-100216	HY-140120	HY-136133			
SPDP A glutathione cleavable ADC linker	Mal-NH-ethyl-SS-propionic acid A cleavable ADC linker	NHS-PEG2-SS-PEG2-NHS A cleavable 4 unit PEG ADC linker			
HY-135966	HY-133413	HY-136031			
Azido-PEG3-SS-NHS A cleavable 3 unit PEG ADC linker	DBCO-CONH-S-S-NHS ester A cleavable ADC linker	Tetrazine-SS-Biotin A biotin-labled, cleavable ADC linker			

			_			
A	100		~	1-1	1 2 1	1/-
- T	1.00	- 60	-		2.4	1 A

HY-136079 HY-136131

Methyltetrazine-PEG4-hydrazone-DBCO NH2-PEG4-hydrazone-DBCO A cleavable 4 unit PEG ADC linker A cleavable 4 unit PEG ADC linker

Non-Cleavable Linker —

Non-Cleavable Linker					
HY-126511	HY-126976	HY-126526			
Propargyl-C1-NHS ester A non-cleavable ADC linker	Propargyl-PEG5-amine A non-cleavable 5-unit PEG linker	N3-PEG2-C2-NHS ester A non-claevable 2-unit PEG linker			
HY-D0975 Sulfo-SMCC sodium A hetero-bifunctional, non-cleavable ADC crosslinker	HY-W008005 Amino-PEG4-alcohol A PEG PROTAC linker and non- cleavable 4 unit PEG ADC linker	HY-130537 Azido-PEG6-alcohol A PEG PROTAC linker and non- cleavable 6 unit PEG ADC linker			

Drug-Linker Conjugates for ADCs

Linker-payload moieties, which consist of a cytotoxic payload and an appropriate linker, can be directly linked to monoclonal antibodies, composing the immunoconjugates for cancer therapy. The payload in linker-payload moieties is a cytotoxic agent (payload/warhead) that is an ultimate effector component of ADCs. The linker in linker-payload moieties is a short spacer that links the payload to the monoclonal antibody⁽¹⁾.

Product Advantages & Features

MCE can provide you with novel and diverse drug-linker conjugates to meet your needs for scientific research. We have the high-quality products and services. The payload-linker conjugates (50+) are expanding. Related popular products are listed below:

Drug-Linker Conjugates

HY-126491

SPP-DM1

A drug-linker conjugate for ADC with potent antitumor activity by using DM1 (a potent microtubule-disrupting agent), linked via the ADC linker SPP.

HY-12460

SPDB-DM4

A drug-linker conjugate for ADC by using DM4 (a tubulin inhibitor) via a SPDB linker, exhibiting potent anti-tumor activity.

HY-15575

VcMMAE

A drug-linker conjugate for ADC by using the anti-mitotic agent, MMAE, (a tubulin inhibitor), linked via the dipeptide, valine-citrulline.

HY-15578

McMMAF

A protective group-conjugated MMAF. MMAF is a potent tubulin polymerization inhibitor.

HY-128946

CL2A-SN-38

A conjugate composed of potent a DNA Topoisomerase I inhibitor SN-38 and a linker CL2A to make antibody drug conjugate (ADC).

HY-101070

SMCC-DM1

A drug-linker conjugate composed of a potent microtubule-disrupting agent DM1 and a linker SMCC to make ADC.

HY-100374

Val-Cit-PAB-MMAE

A drug-linker conjugate for ADC. Contains the ADCs linker (peptide Val-Cit-PAB) and MMAE (inhibiting tubulin polymerization).

HY-15741

McMMAE

A protective group (maleimidocaproyl)-conjugated MMAE, which is a potent tubulin inhibitor. McMMAE is a drug-linker conjugate for ADC.

HY-13631F

Deruxtecan

An ADC drug-linker conjugate composed of a derivative of DX-8951 (DXd) and a maleimide-GGFG peptide linker.

HY-131057

Mc-VC-PAB-SN38

Consists of a cleavable ADC linker (Mc-VC-PAB) and a DNA topoisomerase I inhibitor (SN38).

PROTAC-Linker Conjugates for PACs

Proteolysis-targeting chimeric molecules (PROTACs) are bifunctional molecules composed of a target protein ligand linked to an E3 ligase ligand via an appropriate linker. The mode of action of these compounds depends on their ability to bring the target protein and E3 ligase into proximity, leading to polyubiquitination and subsequent proteasomal degradation of the target protein. Numerous PROTACs are highly efficient degraders. However, they are normally not tissue-specific. Tissue-specific degradation could broaden the therapeutic window and reduce adverse effects for non-selective PROTACs, increasing their potential as therapeutic drugs^[1].

Product Advantages & Features -

MCE can provide you with novel and diverse PROTAC-linker Conjugate for PAC to meet your needs for scientific research. We have the high-quality products and services. Related popular products are listed below:

PROTAC-Linker Conjugate for PAC

HY-129938

PROTAC BRD4 degrader for PAC-1 (SPP-DM1)

Comprises the chimeric BET degrader GNE-987 and disulfide-containing linker.

HY-112100

PAC

PAC, consists the ADCs linker and PROTAC, conjugated to an antibody. PAC extracts from compound LP2.

HY-133736

PROTAC BRD4 Degrader-5-CO-PEG3-N3

A PROTAC-linker Conjugate for PAC, comprises the BRD4 degrader GNE-987 and PEG-based linker.

Antibody-Drug Conjugates (ADCs)

The selection of target antigen, antibody, payload, linker, and conjugation strategies is of vital importance in the design of antibody-drug conjugates. Any inappropriate factor will bring uncertain effectiveness and toxic effects. Therefore, we can also provide FDA-approved ADC drugs for scientific research, for example trastuzumab emtansine and trastuzumab deruxtecan.

Antibody-Drug Conjugates (ADCs)

HY-P9921

Trastuzumab emtansine

An ADC incorporates the HER2-targeted antitumor properties of trastuzumab with the cytotoxic activity of the microtubule-inhibitory agent DM1. Used for the research of advanced breast cancer.

HY-138298

Trastuzumab deruxtecan

An anti-HER2 ADC. Composed of a humanized anti-HER2 antibody, an enzymatically cleavable peptide-linker, and a topoisomerase I inhibitor. Used for the research of HER2-positive breast cancer and gastric cancer.

ADC-Related Customized Synthesis in MCE

Theoretically, the ideal ADC enables targeted delivery of a highly potent cytotoxic payload to the cancer cells, leading to increased activity, reduced systemic exposure and toxicity, and improved pharmacokinetics (PK)/pharmacodynamics (PD) properties compared with traditional chemotherapy drugs. MCE can provide you with novel and diverse antibody-drug conjugates to meet your needs for scientific research. We have the high-quality products and services. Several related popular products are listed below:

ADC Name	Description	Cancer Types
Gemtuzumab ozogamicin	An antibody-drug conjugate (ADC), is a CD33-targeted antibody conjugated to a DNA damaging agent calicheamicin.	Acute Myeloid Leukemia
Inotuzumab ozogamicin	An antibody-drug conjugate (ADC), a CD22-targeted antibody conjugated to a DNA damaging agent calicheamicin, which can be used for precursor cell lymphoblastic leukemia-lymphoma study.	Lymphoblastic Leukaemia- Lymphoma
Enfortumab vedotin-ejfv	An antibody-drug conjugate (ADC), a Nectin-4 -targeted antibody conjugated to a microtubule inhibitor MMAE, which can be used for the treatment of urogenital cancer.	Urogenital Cancer
Depatuxizumab mafodotin	An antibody-drug conjugate (ADC), an EGFR-targeted antibody conjugated to a microtubule inhibitor MMAF, which can be used for the treatment of glioblastoma, gliosarcoma, non-small cell lung cancer, and solid tumors.	Glioblastoma, Gliosarcoma, NSCLC Cancer

ADC-Related Conjugation Services in MCE

Proteins

Currently, the conventional conjugation normally occurs on the antibody backbone (either via lysine side chains exposed on the antibody surface or cysteine residues in the hinge region). These random conjugation strategies can produce a heterogeneous mixture of ADCs with different DARs. resulting in variable pharmacokinetics, efficacy and safety profiles. The DAR can range from zero to eight cytotoxic payloads per antibody. Although high DARs can increase ADC potency, they can also result in aggregation, destabilization, increased off-target effects, and enhanced plasma clearance. Therefore, novel approaches with site-specific conjugation (SSC) are used to overcome the limitations from heterogeneity, broaden the therapeutic window, and ultimately produce more homogenous ADCs. These controlled conjugation strategies include engineered cysteine residues, unnatural amino acids or enzymatic conjugation through glycosyltransferases and transglutaminases.



Figure 9. Conceptualized Relationship between Drug-to-Antibody Ratio (DAR) and Distribution of Payloads^[6].

Conventional Conjugation

Lysine Amide Coupling

Amide coupling is a major ADC conjugation method connecting a payload and solvent accessible lysine residues on the antibody using linkers containing activated carboxylic acid esters. Amide coupling of an amine and an activated carboxylic acid is one of the most reliable, high-yielding chemical conversions in organic synthesis.

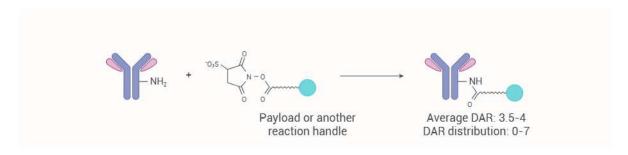


Figure 10. An activated carboxylic acid moiety reacts with a lysine residue, which results in an amide bond linkage between mAb and the payload. Optimized conjugation conditions give an average drug-to-antibody ratio (DAR) value of 3.5-4 with a distribution between 0-7^[1].

Cysteine Coupling

Cysteine-based conjugation methods rely on a specific reaction between cysteine residues of the antibody and a thiol-reactive functional group installed on the payload. In general, antibodies do not possess free thiols, and all cysteine residues form disulfide bonds. Due to the limited number of conjugation sites and the distinct reactivity of the thiol group, cysteine-based conjugation is superior to lysine-based conjugation in terms of controlled DAR and heterogeneity.

Figure 11. (A) Maleimide alkylation. A maleimide moiety reacts with a reduced cysteine residue of a mAb (distribution of DAR: 2, 4, 6, and 8 or predominant at 2 with THIOMAB technology). (B) Rebridging of interchain disulfide bonds. The dibromo (or disulfonate) reagent reacts with the reduced interchain disulfides to provide rebridged mAbs (DAR: predominant at 4). (C) Cysteine arylation using palladium complexes. Aryl-palladium complex reagents undergo aryl-thiol coupling, which affords mAbs containing arylcysteines (average DAR: 4.4)⁽¹⁾.

Site-Specific Conjugation

Non-Natural Amino Acid Incorporation by Genetic Engineering

Installation of non-natural amino acid residues with a reaction handle is a strategy that allows for a site-specific chemical conjugation, leading to strictly controlled DARs. Scientists have developed protein expression systems where p-acetylphenylalanine containing a carbonyl group is genetically encoded by introducing a unique codon-tRNA synthetase.

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Engineered antibodies containing p-acetylphenylalanine residues are produced using either of the expression systems, and the carbonyl groups introduced to react with alkoxyamine-functionalized linkers to provide oxime-conjugated ADCs.

Figure 12. (A) Oxime ligation. (B) Copper-catalyzed or (C) strain-promoted (copper-free) azide-alkyne cyclization[1].

Enzymatic Conjugation

Several enzymes have been used for conjugating the native or genetically engineered antibody with the payload or for installing unique reaction handles on the antibody scaffold for the following chemical conjugation. These enzymes modify the antibody in a site- or amino acid sequence-specific manner. Furthermore, the reaction sites in native mAbs or handles that are genetically introduced are designed to specifically react with counterpart functional groups.

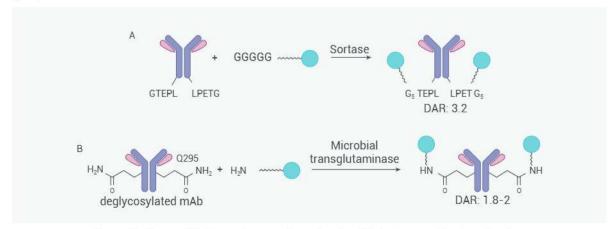


Figure 13. Site-specific (chemo)enzymatic conjugation. (A) Sortase-mediated conjugation. (B) Microbial transglutaminase-mediated conjugation^[1].

ADC-Related Analytical Methods in MCE

The analytical methods employed for monoclonal antibody also applies to ADCs. However, not all approaches can be applied to an ADC without modifications. Especially for ADCs obtained by the conventional conjugation strategies, the resulting drugs are highly heterogeneous. As this may impact the safety and efficacy of drugs, the product commonly contains DAR and degree of homogeneity to strictly control product quality and ensure consistency within various batches.

The DAR can be determined by ultraviolet-visible (UV/VIS) spectroscopy. Other approaches to measure the DAR, the drug load distribution (DLD) and free drug are hydrophobic interaction chromatography (HIC) and Liquid chromatography -electrospray ionization-mass spectrometry (LC-ESI-MS). Peptide mapping can be employed to identify protein modifications that may be caused by conjugation and degradation. The unconjugated drug is traditionally measured

Potency and and Colloidal Stability ADC Biophysical Distribution **Analysis** Analysis of Unconjugated

by using reversed-phase high performance liquid chromatography (RP-HPLO), but capillary electrophoresis and enzyme-linked immunosorbent assay (ELISA) have also been used. Some characterization methods require adaptions for ADCs, for example size exclusion chromatography (SEC). The SEC method is utilized to determine product purity and aggregation profile.

MCE owns a very professional team and state-of-the-art facilities. For ADC-related services, we have rich experience in conjugation techniques and analytical determinations. We continue to make breakthroughs and innovations, develop new analytical methods for ADC products according to customer requirements, and ensure accuracy and repeatability of data and high-quality and efficient services.

Service Advantages



বিটি Quick Delivery

The ready-to-use products will be delivered for using at the earliest time, reducing the intermediate procedures.



(\$) Price Concessions

ADC products have high cost performance and favorable prices.



Product Reports

MCE provide you with real and reliable experimental data related to ADCs.



Instrict Confidence

After confirming ADC products, MCE and you will sign a confidentiality agreement, and your information enjoys absolute security.



Professional Consultation

For pre-sales and after-sales consultation of ADC products, professional ADC technicians will answer and solve your doubts.

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Service Scope -

MCE can provide a one-stop service system for the determination of ADC products. The service content is divided into two parts, including technical services and analytical items. At the same time, MCE can also provide customized services for ADC related products.

ADC Cytotoxin

- Customized synthesis: Help synthesize ADC cytotoxins.
- Cytotoxin modification: Perform modifications of existing cytotoxins.

ADC Linker

- Customized synthesis: Help synthesize ADC linkers
- Linker modification: Perform modifications of existina linkers.

Drug-Linker Conjugates or ADC

- Customized synthesis: Help design and synthesize drug-linker conjugates for ADC.
- Structural modification: Perform modifications of existing drug-linker conjugates.

Antibody-drug Conjugate

- Customized synthesis: Design and synthesize ADCs.
- ADC characterization: Perform the detection of DAR, free drug, endotoxin and so on.

Peptide-Drug Conjugate

- Customized synthesis: Help design and synthesize PDCs.
- PDC characterization: Provide purity, stability, structure and other relevant information about PDCs.

Aptamer-Drug Conjugate

- Customized synthesis: Help design and synthesize ApDCs.
- ApDC characterization: Provide purity, stability, structure and other relevant information about ApDCs.

Conjugation Technologies

- Conventional conjugation: Provide amine-based lysine/thiol-based cysteine conjugation.
- Site-specific conjugation: Provide engineered cysteine residues, unnatural amino acids or enzymatic conjugation.

References:

[1] Protein Cell. 2018 Jan;9(1):33-46. [4] Br J Cancer. 2016 Feb 16;114(4):362-7. [5] Chem Soc Rev. 2021 Jan 21;50(2):1305-1353.

[2] Nat Rev Drug Discov. 2017 May;16(5):315-337.

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