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Quality assessment of antibody drug conjugate

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Outline

- Developmental status of ADC in Japan
- Guideline of ADC
- Quality assessment of ADC

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Advantage of ADCs

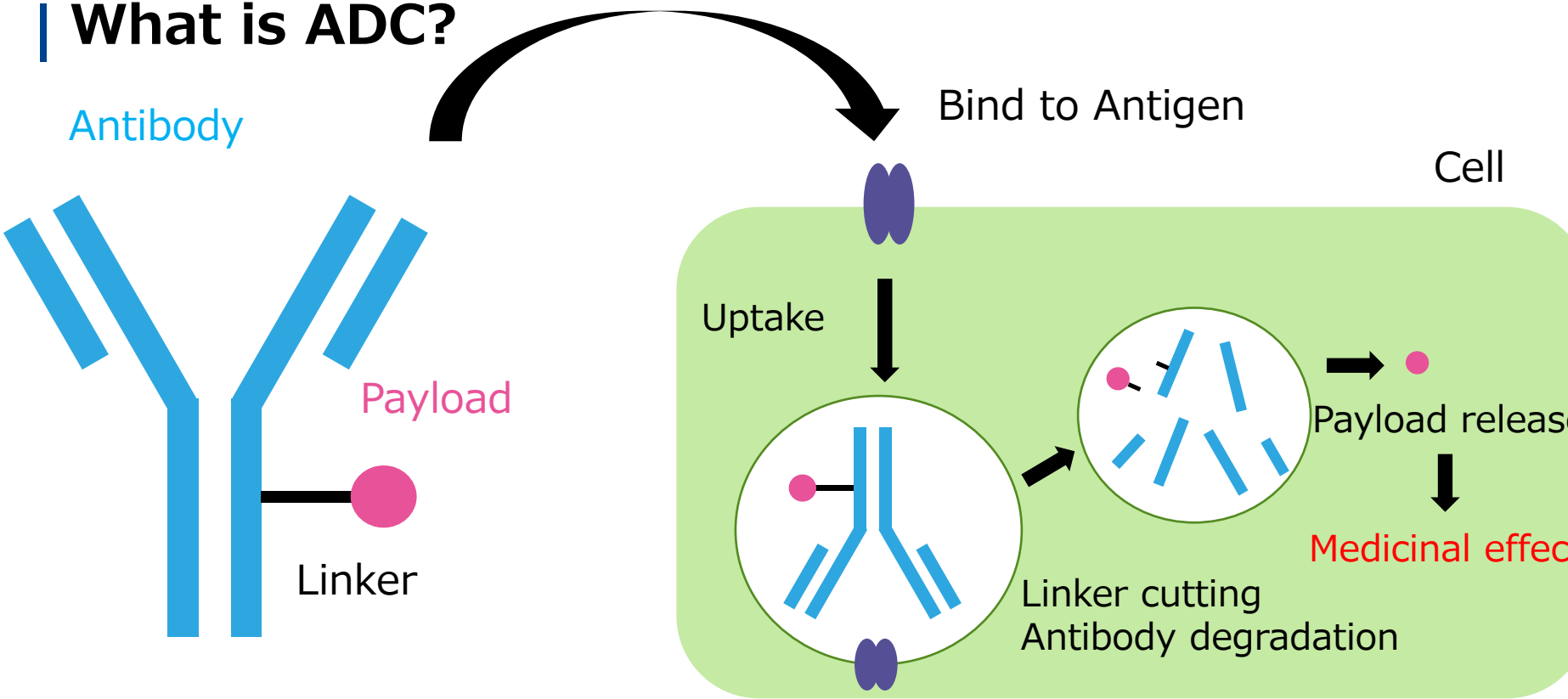
- Conventional chemotherapy

➢Due to safety concerns, the dose cannot increase and the therapeutic area is narrow.

- ADC

➢ADC can directly deliver the cytotoxic small molecule drug to target cells or tissues , and are expected to have high therapeutic effects and a wide therapeutic range.

What is ADC?



ADCs approved in Japan

Name of the medicinal products	Companies	Approval dates
MYLOTARG Injection 5mg	Pfizer	June 2005
ZEVALIN yttrium injection	Mundipharma	November 2008
ZEVALIN indium injection	Mundipharma	November 2008
KADCYLA for Intravenous Infusion	Chugai Pharmaceutical	September 2013
ADCetris for I.V.Infusion	Takeda Pharmaceutical	November 2013
BESPONSA Injection	Pfizer	December 2017
ENHERTU for intravenous drip infusion	DAIICHI SANKYO	March 2020
Akalux IV Infusion	Rakuten Medical	September 2020
POLIVY for Intravenous Infusion	Chugai Pharmaceutical	March 2021
PADCEV for I.V.infusion	Astellas Pharma	September 2021

All are anticancer pharmaceuticals

Antibodies and Payloads of ADCs approved in Japan

Name of the medicinal products	Japanese accepted names for pharmaceuticals	Antibodies	Payloads
MYLOTARG Injection 5mg	Gemtuzumab Ozogamicin	Humanized anti-CD33 antibody	*N*-acetyl calicheamicin
ZEVALIN yttrium injection	Ibritumomab Tiuxetan	Mouse anti-CD20 antibody	MX-DTPA
ZEVALIN indium injection	Ibritumomab Tiuxetan	Mouse anti-CD20 antibody	MX-DTPA
KADCYLA for Intravenous Infusion	Trastuzumab Emtansine	Humanized anti-HER2 antibody	Maytansine
ADCETRIS for I.V.Infusion	Brentuximab Vedotin	Chimeric anti-CD30 antibody	MMAE
BESPONSA Injection	Inotuzumab Ozogamicin	Humanized anti-CD22 antibody	*N*-acetyl calicheamicin
ENHERTU for intravenous drip infusion	Trastuzumab Deruxtecan	Humanized anti-HER2 antibody	Derivative of exatecan
Akalux IV Infusion	Cetuximab Sarotalocan Sodium	Chimeric anti-EGFR antibody	Derivative of phthalocyanin
POLIVY for Intravenous Infusion	Polatuzumab Vedotin	Humanized anti-CD79b antibody	MMAE
PADCEV for I.V.infusion	Enfortumab Vedotin	ヒト抗nectin-4抗体	MMAE

N-acetyl calicheamicin: DNA cutting effect, Maytansine and MMAE: Microtubule inhibition effect
Exatecan: Topoisomerase inhibition effect, Phthalocyanin: Photochemical reaction caused by laser light irradiation

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Current status of guidelines in the development of ADC

- There are no specific guidelines for ADC
- For non-clinical development, refer the following two guidelines:

➢Nonclinical evaluation for anticancer pharmaceuticals（ICH S9、ICH S9 Q&A）

➢Preclinical safety evaluation of biotechnology-derived pharmaceuticals （ICH S6（R1））

Nonclinical evaluation for anticancer pharmaceuticals (ICH S9)

4.1 Conjugated Products

Conjugated products are pharmaceuticals covalently bound to carrier molecules, such as proteins, lipids, or sugars. The safety of the conjugated material is the primary concern. The safety of the unconjugated material, including the linker used, can have a more limited evaluation. Stability of the conjugate in the test species and human plasma should be provided. A toxicokinetic evaluation should assess both the conjugated and the unconjugated compound after administration of the conjugated material.

Current status of guidelines in the development of ADC

- For quality, refer the following guidelines:

- Stability testing（ICH Q1A（R2）～ICH Q1E）
- Impurities（ICH Q3A（R2）～ICH Q3D（R2））
- Test procedures and acceptance criteria（ICH Q6A）
- Quality of biotechnological products（ICH Q5A (R1)～ICH Q5E、ICH Q6B）
- Guidance for quality evaluation of antibodies (PFSB/ELD Notification No. 1214-1 / December 14, 2012)

Guidance for quality evaluation of antibodies (PFSB/ELD Notification No. 1214-1 / December 14, 2012)

3.3.1.4 Artificial modification

For conjugated antibodies bound to modifiers such as chelate compounds for coordinating radioisotopes, compounds with cytotoxicity assay, and polymers such as polyethylene glycol, It is necessary to reveal the number and binding position of the modifiers. In addition, it is necessary to reveal the content of unconjugated antibodies and free modifiers. Comparison of the peptide map of modified antibody and the peptide map of unmodified antibody is useful for analyzing the number and binding position of modifiers.

Guidance for quality evaluation of antibodies (PFSB/ELD Notification No. 1214-1 / December 14, 2012)

3.3.3 Biological properties

The biological activity of monoclonal antibody products differ depending on the product. For example, there are three products;1)suppress or promote antigen action or antigen-mediated in-vivo reactions, 2)has ADCC activity and CDC activity in addition to antigen binding ability, 3) antibodies that are bound to pharmacological compounds are taken up by cells expressing antigens, and the dissociated compounds act within the cells. Based on clinical efficacy and molecular mechanism, it is necessary to establish biological tests to measure biological activity that reflect the expected efficacy and efficacy.

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Selection of antibodies, linkers and payloads

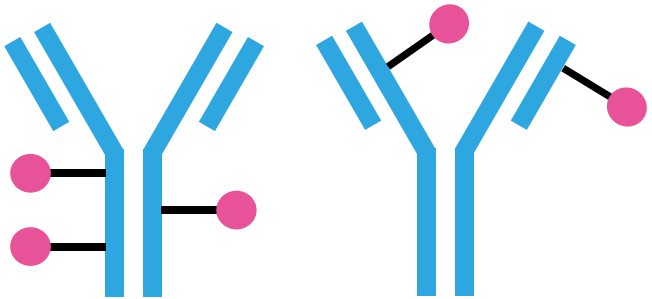
- Antibodies
  - ➢Chimeric antibodies, humanized antibodies, human antibodies・・・
  - ➢Subclass（IgG1～4）
  - ➢Presence or absence of effector functions such as ADCC activity
- Linkers
  - ➢Cutting linkers, non-cutting linkers
- Payloads
  - ➢DNA inhibition effect、microtubule inhibition effect ・・・

Binding of antibodies and payloads

- In the case of using lysine residues of antibodies
- In the case of using free cysteine residues generated by dissociating disulfide bonds between antibody molecules with a reducing agents

Heterogeneity of binding numbers and binding sites

Managing heterogeneity is important



Affects;

- Pharmacological effects
- Conformational structure of antibodies
- Antigen binding activity
- Effector functions such as ADCC activity etc

Binding of antibodies and payloads

- In the case of using lysine residues of antibodies

➢ It is possible that payloads bind to CDR surrounding sequences involved in antigen binding, Fcγ receptors involved in effector functions, and complement C1q binding sites.

➢ A complex mixture is formed when the payload binds to the many lysine residues in antibody.

- In the case of using free cysteine residues generated by dissociating disulfide bonds between antibody molecules under reducing conditions.

➢ It may affects the conformational structure of antibodies.

➢ Compared to the case of using lysine residues of antibodies, the number of free cysteine residues is small, and the binding numbers and binding sites can be easily managed.

Managing heterogeneity

- It is important to develop a manufacturing process that can consistently produce homogeneous ADCs.
- Manufacturing conditions to consider;

➢Antibody to reducing agent ratio、pH of reduction reaction、temperature and time

➢Drug to antibody ratio (DAR), temperature and time of binding reaction

DAR, binding sites,
effect on biological activity, etc.

Identifying critical manufacturing conditions

Managing heterogeneity

- It is important to understand the properties of ADCs.

- Examples of evaluation items for quality attribute analysis, specifications and test procedures.

➢DAR
➢Binding sites of payload
➢Amount of antibody with no payload bound
➢Amount of payload not bound to antibody
➢Antigen binding activity
➢Cytotoxicity assay, etc

ADC evaluation methods

- DAR
  - ➢UV/visible spectrophotometry
  - ➢Hydrophobic interaction chromatography, etc
- Binding sites of payload
  - ➢Peptide map, etc

Evaluation items for Quality attribute analysis

Name of the medicinal products	Evaluation items for Quality attribute analysis
MYLOTARG Injection 5mg	DAR, Cytotoxicity assay, CD33 binding activity
ZEVALIN yttrium injection	Number of chelator bonds, Chelator binding region, CD20 binding activity
ZEVALIN indium injection	Number of chelator bonds, Chelator binding region, CD20 binding activity
KADCYLA for Intravenous Infusion	DAR, HER2 binding activity, Cytotoxicity assay
ADCETRIS for I.V.Infusion	DRA, Number of payload binding, Payload binding site, CD30 binding activity, Cytotoxicity assay
BESPONSA Injection	Cytotoxicity assay
ENHERTU for intravenous drip infusion	DAR, HER2 binding activity, Cell growth inhibition activity
Akalux IV Infusion	DAR, EGFR binding activity, Cell growth inhibition activity
POLIVY for Intravenous Infusion	Conjugatable Impurities, Off-Target Conjugation, Free payload, Unconjugated Impurities, DAR, Payload distribution, Conjugation site distribution, CD79b binding activity, Cell growth inhibition activity
PADCEV for I.V.infusion	Payload distribution, Nectin-4 binding activity, Cytotoxicity assay

https://www.pmda.go.jp/review-services/drug-reviews/review-information/devices/0010.html

Specifications and test procedures of drug substances

- It is necessary to establish test to confirm that homogeneous ADCs are consistently produced.

Name of the medicinal products	Specifications and test procedures
MYLOTARG Injection 5mg	Unconjugated calicheamicin derivative, unbound antibodies, Total calicheamicin derivatives, Cytotoxicity assay, CD33 binding activity
ZEVALIN yttrium injection	Potency
ZEVALIN indium injection	Potency
KADCYLA for Intravenous Infusion	Cytotoxicity assay
ADCETRIS for I.V.Infusion	DRA, CD30 binding activity, Cytotoxicity assay
BESPONSA Injection	Cytotoxicity assay
ENHERTU for intravenous drip infusion	DAR, Cell growth inhibition activity
Akalux IV Infusion	DAR, Potency, EGFR binding activity
POLIVY for Intravenous Infusion	DAR, Cell growth inhibition activity
PADCEV for I.V.infusion	DAR, Nectin-4 binding activity, Cytotoxicity assay

https://www.pmda.go.jp/review-services/drug-reviews/review-information/devices/0010.html

Review team for ADCs

Integrated team

Antibody ADC

Payload

Office of CTBP Bio-CMC team

- Director
- Review Director
- CMC

Office of New Drug

- CMC

Office of New Drug

- Director
- Review Director
- Team Leader
- Deputy Team Leader
- CMC
- ADME
- Pharmacology
- Toxicology
- Biostatics
- Clinical

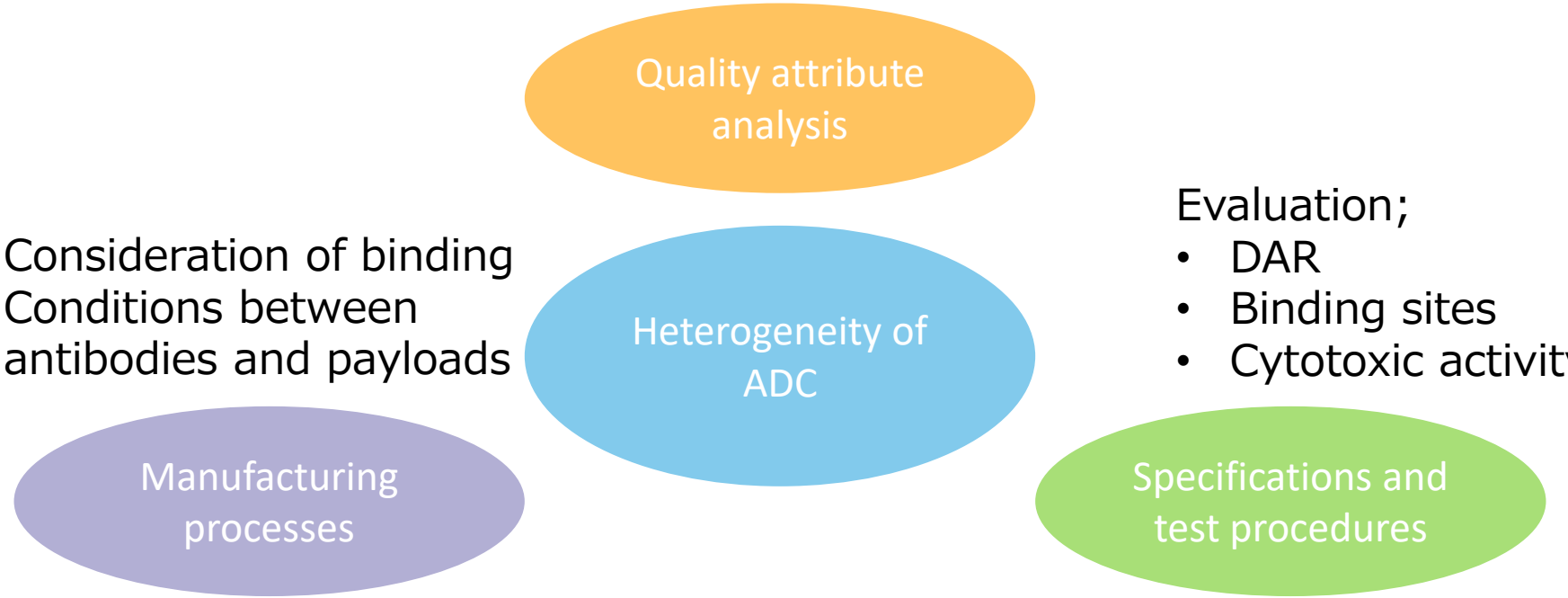
Configuration of CTDs

- Antibodies and payloads fall into the category of important intermediates, and require the comparable explanation as for the drug substance regarding quality control such as manufacturing processes, quality attribute analysis, and stability.
- It is recommended to create CTDs（module2 and module3） for payloads, antibodies, and ADCs separately.

Antibodies
- M2.3.S, M3.2.S – drug substances
- M2.3.P, M3.2.P – drug products

ADC s
- M2.3.S, M3.2.S – payloads
- M2.3.S, M3.2.S – antibodies
- M2.3.S, M3.2.S – ADCs
- M2.3.P, M3.2.S – drug products

Summary



Thank you for your attention!

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