Antibody Drug Conjugates

Requirements for Characterization and Control of ADCs from a (European) Regulator´s Perspective

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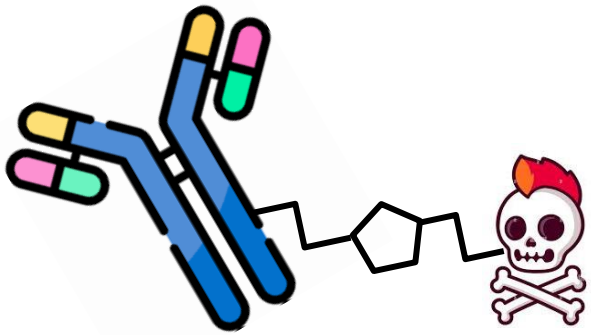

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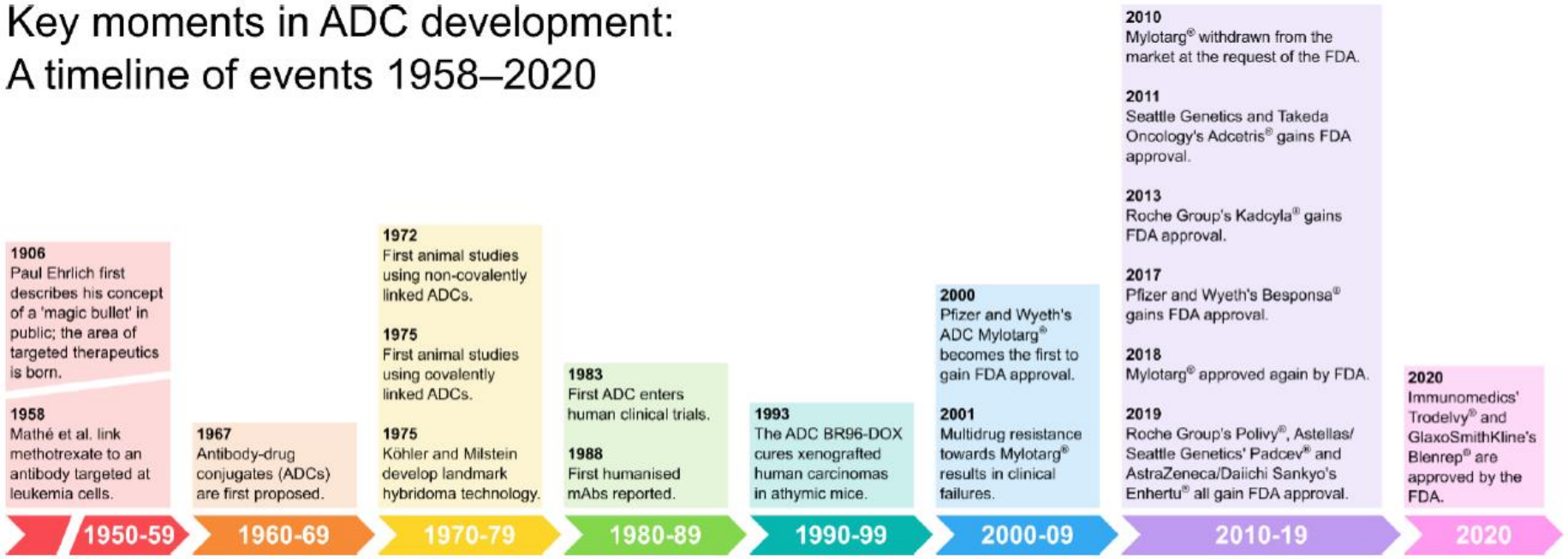
Disclaimer
The view expressed in the following is the one of the presenter and does not necessarily express the view of either the CHMP, BWP, QWP, EDQM or the Paul-Ehrlich-Institut (including other sections)

A brief History of ADC Development



First-generation ADCs

- Conventional chemotherapy drug conjugated to mouse-derived antibody through non-cleavable linker
- Potency (mostly) not superior to free cytotoxic drugs
- Immunogenicity frequent concern

Second generation ADCs (represented by e.g. brentuximab vedotin and ado-trastuzumab emtansine)

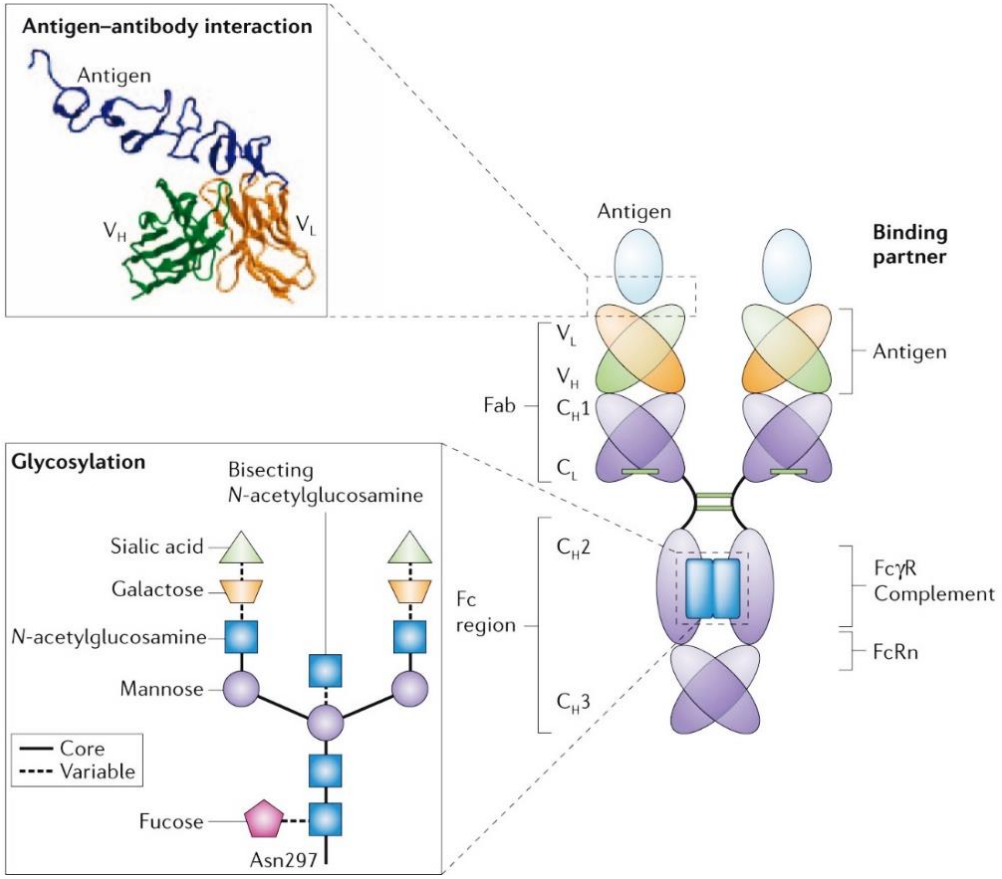
- Humanized mAbs with selected isotypes
- Optimized cytotoxic payloads and new linkers

Third-generation ADCs (represented by e.g. polatuzumab vedotin, fam-trastuzumab deruxtecan)

- Site-specific conjugation technology with well-characterized DARs and desired cytotoxicity
- More hydrophilic linker modulation such as PEGylation

Choice of Antibody Moiety

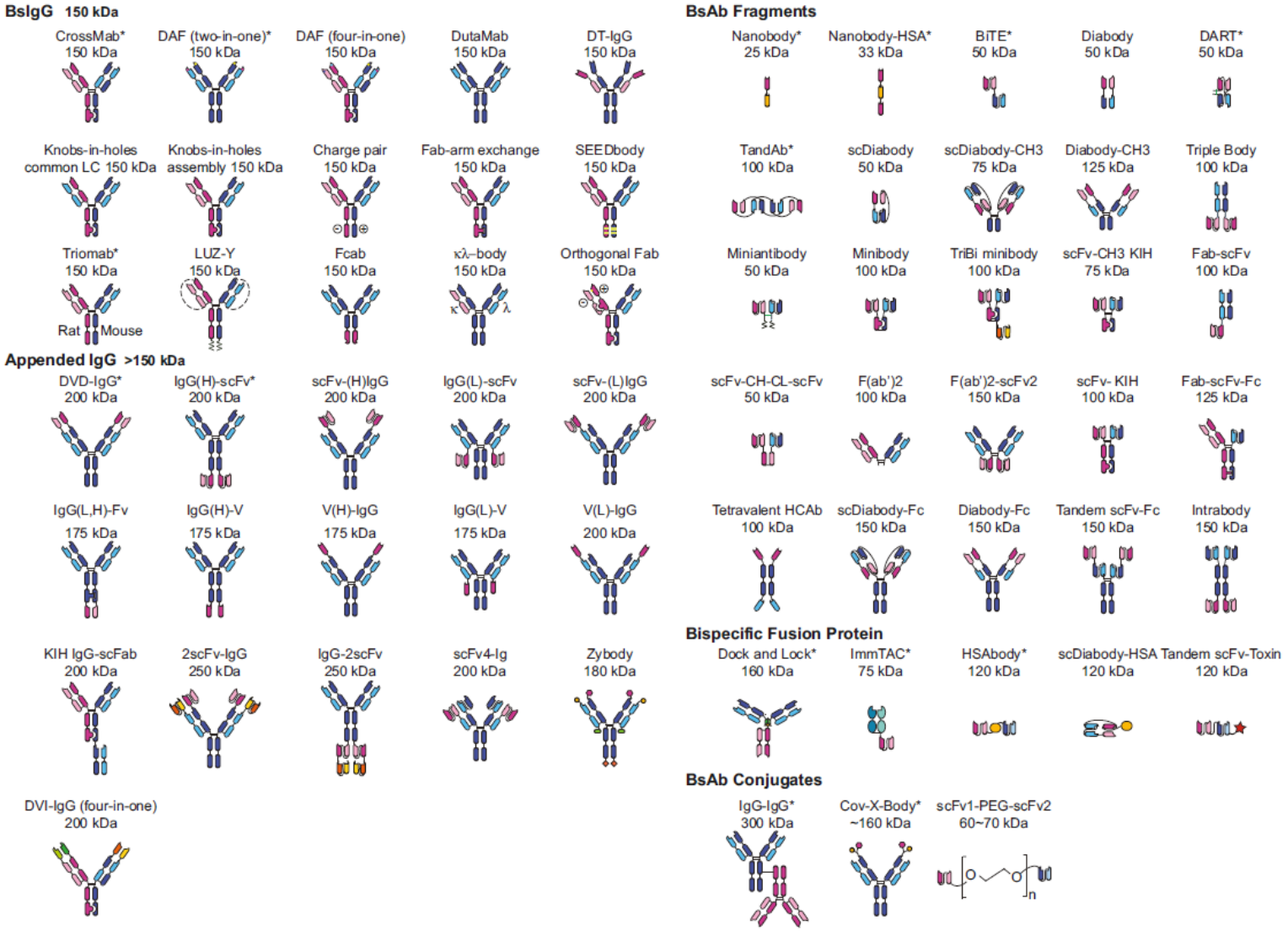
high binding affinity to the target antigen, efficient internalization, low immunogenicity, long plasma half-life, mode-of-action



- IgG1 is the most abundant in serum and could induce strong effector functions such as ADCC, ADCP, and CDC
- IgG2 has reduced effector functions, forms dimers and aggregates *in vivo*, which leads to a decrease of the concentration of ADC drugs
- IgG3, short half-life (7 days) and high propensity to form aggregates during manufacture and in vivo, sensitive to protease cleavage
- IgG4 could induce ADCP, reduced Fc-effector function, Fab-arm exchange (engineering needed)

Figure adapted from Carter PJ: Potent antibody therapeutics by design, Nature Rev Immunology 6, 343 (2006)

Antibody Moiety Formats



From C Siess et al, Alternative molecular formats and therapeutic applications for bispecific antibodies, Molecular Immunology 67 (2015) 95–106

Cleavable vs. non-cleavable Linkers

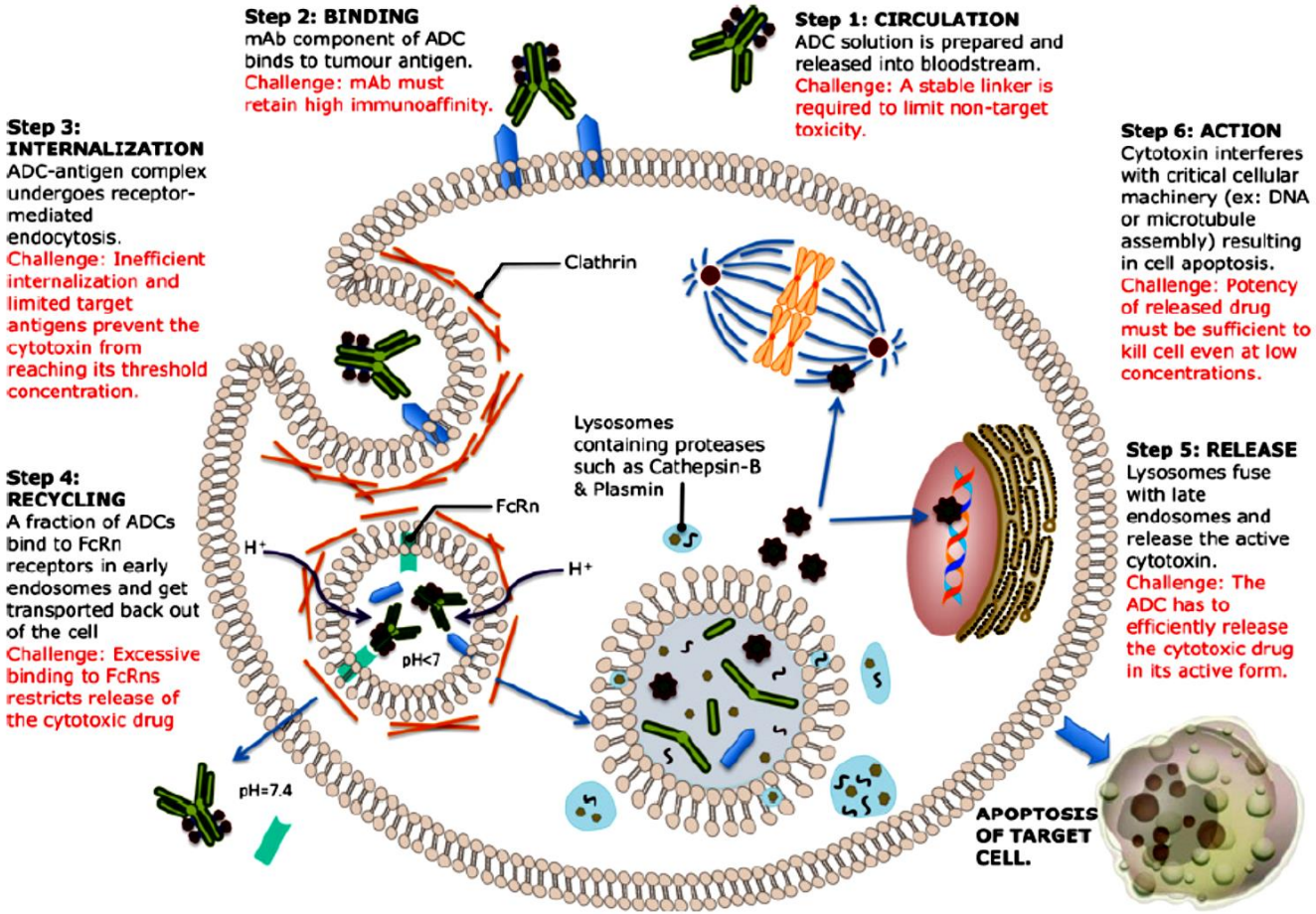


Figure from Peters and Brown, BioSciRep, 2015

Non-cleavable linkers:

- low off-target toxicity due to increase of plasma stability (e.g. T-DM1)

Cleavable linkers:

- chemical cleavage linkers (hydrazone bond and disulfide bond) Hydrazone is a typical acid-sensitive (pH sensitive) linker.
- Enzyme-cleavable linkers (glucuronide bond and peptide bond), sensitive lysosomal protease (e.g. Cathapsin B)

Linker Technology:

- PEG/Polysarcosine for increased solubility/hydrophilic characteristics

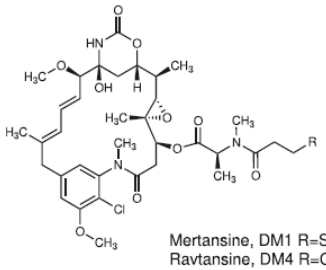
Cytotoxic payloads

potent tubulin inhibitors, DNA damaging agents, RNA synthesis inhibitors and immunomodulators

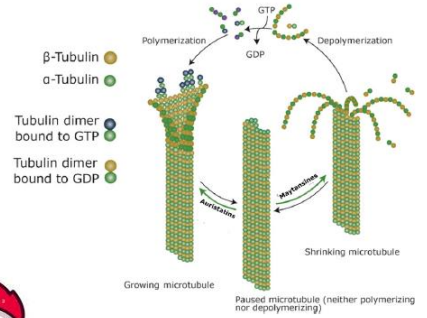
- Tubulin polymerization promoters target at the β-subunits of tubulin dimer to perturb microtubule growth, auristatin derivatives monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF)

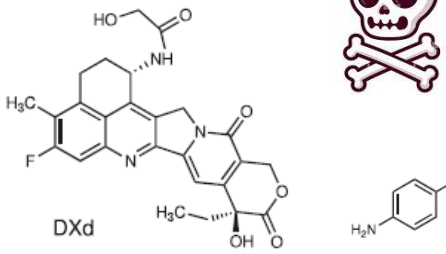
- IC50 values of DNA damaging agents are able to reach picomolar level
  - DNA double strand break, such as calicheamicins;
  - DNA alkylation, such as duocarmycins;
  - DNA intercalation, such as topoisomerase I inhibitors (exatecans)
  - DNA crosslink, such as pyrrolobenzodiazepines (PBD).

- Specific inhibition of RNA polymerase II activity
  - α-Amanitin mechanism of action: Cell-cycle independent mechanism of action, Low intracellular target copies, 1:1 binding

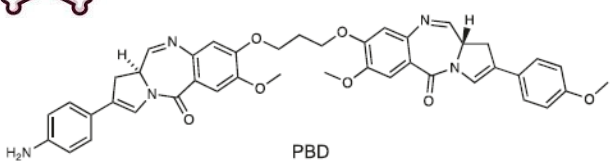


Mertansine, DM1 R=SH
Ravtansine, DM4 R=C(CH$_3$)$_2$SH

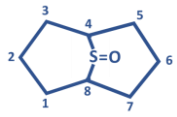




DXd



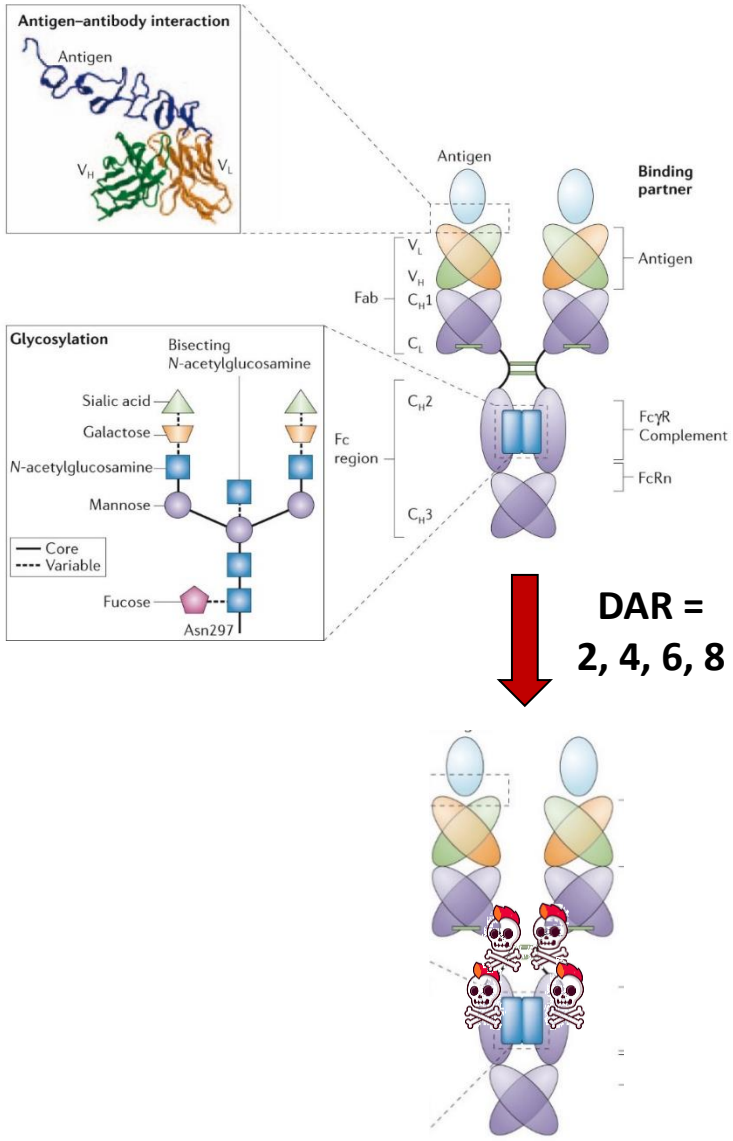
PBD



Amanitin

Conjugation Methods and Drug-load Variants

- stochastic conjugation on pre existing lysine or cysteine residues via appropriate coupling reaction
  - random coupling with lysine residues, varying numbers (0–8) of small-molecule toxins may be attached to an antibody, resulting in a wide drug-antibody ratio (DAR) distribution
  - Cysteine based reaction provides another means of coupling. Due to the limited number of binding sites and the unique reactivity of mercaptan groups, using cysteine as the connecting site helps to reduce the heterogeneity of ADC. Depending on the reduction ratio, products with DAR of 2, 4, 6 and 8 may be generated with better homogeneity compared
- engineered reactive cysteine residues has become a common approach for site-specific conjugation
- Introduction of unnatural amino acids, including N-acetyl-Lphenylalanine, azido methyl-L-phenylalanine and azido lysine, may induce immunogenicity
- from glycan remodeling and glycoconjugation, e.g. N297



Assignment of regulatory starting materials according to ICH Q11

for the synthetically manufactured payload/biological payload and control strategy for starting materials and intermediates

Definition starting material

Part II: Basic Requirements for Active Substances used as Starting Materials

An “API Starting Material” is a raw material, intermediate, or an API that is used in the production of an API and that is incorporated as a significant structural fragment into the structure of the API. A Starting Material can be an article of commerce, a material purchased from one or more suppliers under contract or commercial agreement, or produced in house.

EUROPEAN COMMISSION
HEALTH AND CONSUMERS DIRECTORATE-GENERAL
Health systems and products
Medicinal products – quality, safety and efficacy

Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use Annex 2: Manufacture of Biological Medicinal Substances and Products for Human, Part B.

Definition and Requirements

"starting materials shall mean any substance of biological origin such as micro-organisms, organs and tissues of either plant or animal origin, cells or fluids (including blood or plasma) of human or animal origin, and biotechnological cell constructs (cell substrates, whether they are recombinant or not, including primary cells)."

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

London, 27 June 2013
EMA/CHMP/BWP/429241/2013
Committee for Medicinal Products for Human Use (CHMP)

Guideline on the use of starting materials and intermediates collected from different sources in the manufacturing of non-recombinant biological medicinal products

Draft Agreed by Biologics Working Party	December 2011
Adoption by Committee for medicinal products for human use for release for consultation	16 February 2012
End of consultation (deadline for comments)	31 August 2012
Agreed by Biologics Working Party	May 2013
Adoption by Committee for medicinal products for human use	27 June 2013
Date for coming into effect	1 December 2013

Keywords	*Starting materials, sourcing, intermediates, heparins, urine derived products, plasma derived medicinal products, manufacturing process.*

- The marketing authorisation dossier should include information that adequately describes the manufacturing process and process controls.
- Information on quality and control of all starting materials and process reagents used in the manufacture of a drug substance should be provided.
- GMP measures (e.g. contract between supplier and manufacturer of medicinal product, audit system) should be adequate to ensure an appropriate control while allowing sourcing of starting materials or early intermediate biological products in different locations from third countries.

ICH Q11, Part 5

Selection of starting materials and source materials

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

November 2012
EMA/CHMP/ICH/425213/2011

ICH guideline Q11 on development and manufacture of drug substances (chemical entities and biotechnological/ biological entities)

5.1.3. Selection of source and starting materials

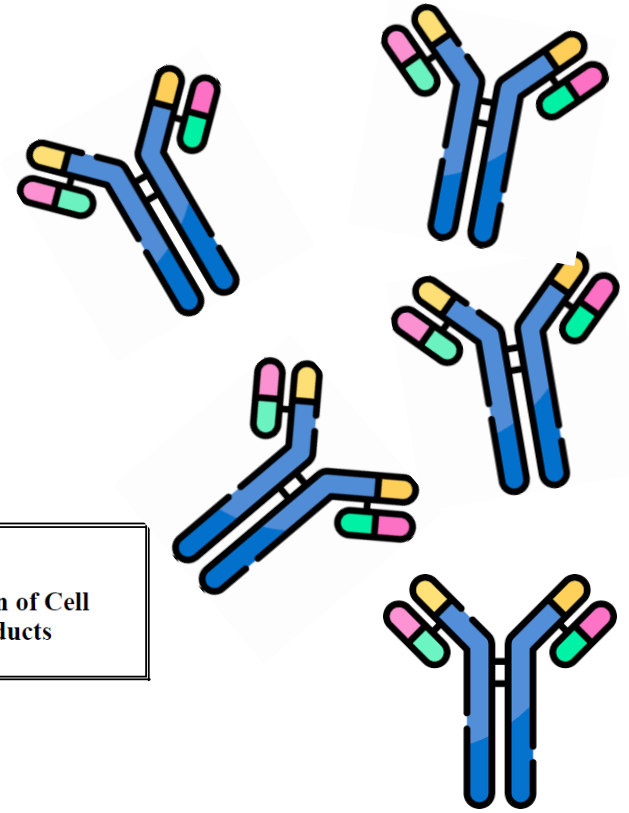
for biotechnological/ biological drug substances

Cell banks are the starting point for manufacture of biotechnological drug substances and some biological drug substances. In some regions, these are referred to as source materials; in others, starting materials. Guidance is contained in ICH Q5A, Q5B, and Q5D.

ICH Topic Q 5 A (R1)
Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

ICH Topic Q 5 B
Quality of Biotechnological Products: Analysis of the Expression Construct in Cell Lines Used for Production of r-DNA Derived Protein Products

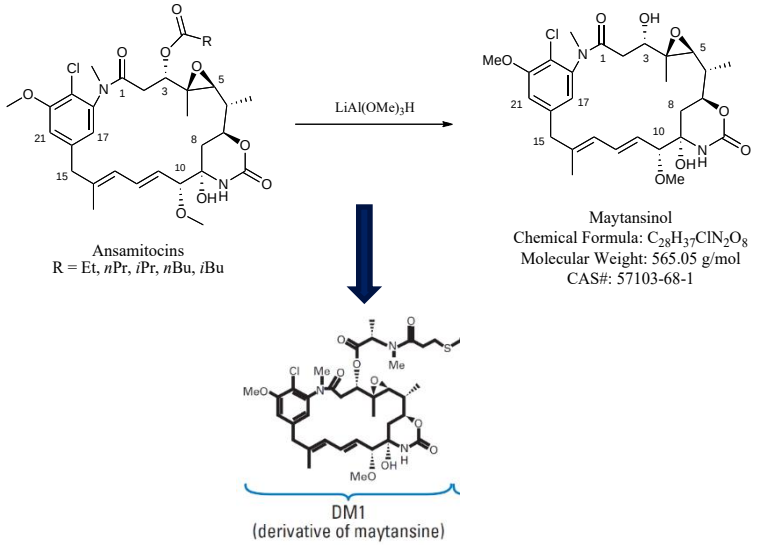
ICH Topic Q 5 D
Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products



DM1/DM4

Tubulin inhibitor payload isolated from fermentation broth

- Ansamitocins are isolated and purified from the fermentation broth of *Actinosynnema pretiosum*
- variable product (ansamitocin mixture)
- MayOH is obtained by cleaving the C-3 ester of ansamitocins
- DM1 is synthesized from Maytansinol (MayOH).
- A consistent purity profile of DM1 is achieved by defined and controlled fermentation process.
- Defining the bacteria working seed lot system/fermentation as starting material is not warranted



Documentation to be provided

- detailed description of the conversion of MayOH to DM1
- Information on the process as well as the in-process controls and/or in-process tests in place
- Information on all reagents and catalyst used in the MayOH conversion to DM1
- Specification, limits and batch data

Test	Acceptance Criterion	Test Method
Appearance/Description		Visual
Appearance	White to yellow solid	
Identity		
Identity by UV	Conforms	UV
Identity by HPLC	Conforms	HPLC
Purity		
Assay % w/w (dry basis)[a]	93.0% - 103.0%	HPLC
Impurities (area%)		HPLC
Individual specified		
Ring Oxidized MayOH	≤ 1.0%	
O-Desmethyl-MayOH	≤ 1.0%	
Deschloro-MayOH	≤ 0.50%	
N-Desmethyl-MayOH	≤ 0.50%	
Descarbamate-MayOH	≤ 1.0%	
Methyl-MayOH	≤ 1.0%	
AP3	≤ 1.0%	
Individual unspecified impurities	≤ 0.5%	
Total unspecified impurities	≤ 1.5%	
Total impurities	≤ 5.0%	
Water Content	≤ 1.0%	KF
Residual Solvents, % w/w		Headspace GC
Ethyl Acetate	≤ 13.0%	
Methanol	≤ 0.3%	
Total Residual Solvents	≤ 15.0%	

[a] anhydrous and solvent-free.

Guideline on Development and Manufacture of Synthetic Peptides

- New guideline on the development and manufacture of synthetic peptides is currently in preparation
- There is consensus on peptide synthesis aspects, e.g. that in general the protected amino acids are considered regulatory starting materials for synthetic peptides.
- It is still under discussion if bigger building blocks, like several amino acids coupled to a solid phase, may possibly serve as starting material

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 September 2022
EMA/CHMP/QWP/735422/2022
Committee for Medicinal Products for Human Use (CHMP)
Committee for Veterinary Medicinal Products (CVMP)

Concept Paper on the Establishment of a Guideline on the Development and Manufacture of Synthetic Peptides

Agreed by Quality Working Party	29 June 2022
Adopted by CHMP for release for consultation	15 September 2022
Adopted by CVMP for release for consultation	8 September 2022
Start of public consultation	20 September 2022
End of consultation (deadline for comments)	20 December 2022

Publishing of the Draft "Guideline on the Development and Manufacture of Synthetic Peptides" was expected end of 2023.

Principles of ICH Q11

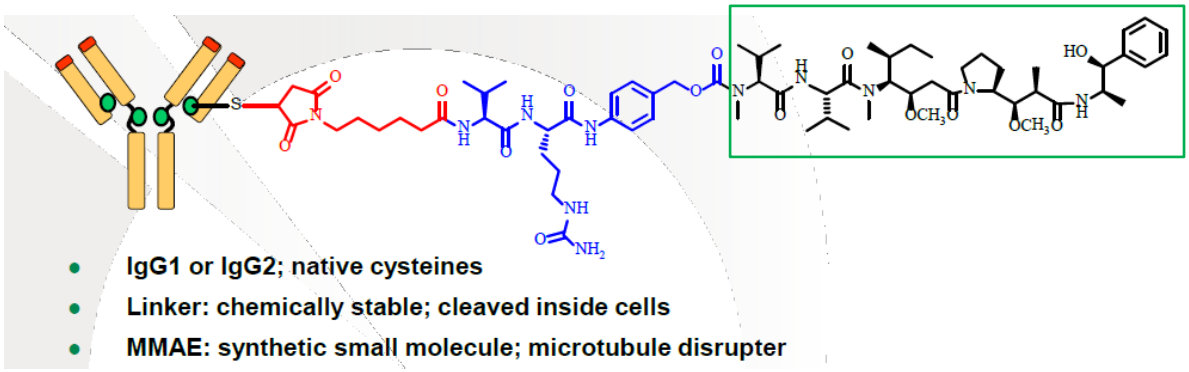
Classifying starting material(s) for synthetic Drug Substances

- The proposed intermediate has defined chemical properties and structure and is incorporated as significant structural fragment into the Payload-Linker
- There is an adequate control strategy in place to control the quality of the proposed starting material
- There is a reduced risk for fate of impurities due to the number of steps after the introduction of the starting material
- The DS manufacturing process is sufficiently described to ensure the understanding of formation, fate and purge of impurities

Starting Material: MMAE

Monomethyl auristatin E is an antimitotic agent which inhibits cell division by blocking the polymerisation of tubulin

convergent, solution phase, fragment-based peptide synthesis.



Example 1: Definition of Starting Material (Payload-linker)

Question 1:

The Payload-Linker intermediate *XYZZZ* is synthesized by the following steps to obtain the final intermediate:

- Four-step synthesis starting from X followed by one purification step by Method A to obtain *Y* (Monocycle)
- Three chemical steps starting from *Y purified* followed by one purification step by Method B to obtain *YZ* (Payload)
- One step to couple *YZ* (Payload) with ZZ (Linker) followed by Method C purification to generate *XYZZZ* (Payload-Linker intermediate)

Considering a future marketing authorization application, does the agency agree to define the Monocycle *purified* as starting material in line with ICH Q11 principles for the synthesis of the payload branch if the applicant demonstrates and ensures well-defined and overall process control and stereo-chemical control as proposed in the applicant's position?

Example 1: Definition of Starting Material (Payload-linker)

Answer to Q1:

The agency stated that a new guideline on the development and manufacture of synthetic peptides is currently in preparation. Although the guideline is not yet officially published, there is consensus on peptide synthesis aspects, e.g. that in general the protected amino acids are considered regulatory starting materials for synthetic peptides. It is still under discussion if bigger building blocks, like several amino acids coupled to a solid phase, may possibly serve as starting material.

However, the structure of *Y* (Monocycle) does not meet the definition of a starting material in accordance with ICH Q11. The proposed starting material contradicts the concept of a starting material since the molecule presents the most significant and efficacy-determining part of the final structure.

- The definition of "significant structural fragment" is not to be used here for justification of the choice of the starting materials, since the intention of ICH Q11 is only to help distinguish starting materials from reagents, catalysts, solvents, or other raw materials.
- The proposed starting material is further in contradiction with the principles of the new guideline as it will be excluded having a starting material which is only further processed by conjugation, hydrolysis, ring closure reactions or other simple modifications.
- Furthermore, the specification of *Y purified* would not enable adequate control of the molecule, but even a more extensive specification of *Y purified* would not allow for definition as starting material.
- Thus, the starting materials should be the activated amino acids and the amino acid derivatives (see also Question 3). Additionally, the first starting material could also be the first amino acid bound to the solid phase since the solid phase does not contribute to the structure of the final molecule.
- The control strategy must start from the starting material onwards: *Y purified* as starting material would bear a significant risk, as the synthesis of this structure, the active moiety of the molecule, would be outside of control of GMP. Thus, the starting materials must be defined earlier in the synthesis.

Example: Documentation of Starting Material Manufacturing and Control

Question 2:
Does the CHMP agree that the nnAA is a starting material for antibody production and Applicant's proposal to manufacture nnAA under (highly documented) HD environment is sufficient for MAA?

Answer to Q2:
The non-natural amino acid is a critical starting material for production of mAb intermediate and DS. In contrast to GMP, the term "HD production process" is not defined for raw/starting materials. The Applicant should therefore provide a detailed control strategy, including specifications for nnAA and thorough justification thereof, and description of the analytical methods used to ensure sufficient quality. In this respect, adherence to GMP principles should be considered. ICH guideline Q11 on development and manufacture of drug substances (EMA/CHMP/ICH/425213/2011) claims that "*The Good Manufacturing Practice (GMP) provisions described in ICH Q7 apply to each branch beginning with the first use of a starting material. Performing manufacturing steps under GMP together with an appropriate control strategy provides assurance of quality of the drug substance*". According to ICH-Q11 it is therefore not mandatory that production is performed under GMP compliant conditions. However, depending on review of the set specification and analytical results data package at the time of MAA, reclassification of nnAA as starting material could apply.
In Conclusion, it can be generally agreed that nnAA not produced under GMP could be acceptable as a starting material for antibody production during the MAA, pending review of the full quality data package at the time of MAA. The Applicant´s position is partially supported.

Example: Characterization and Control of Payload-linker

Question 3:
The **X**-atom in the linker-payload corresponds to a racemic chiral center with a 1:1-ratio of both diasteroisomers. In addition, a double bond is formed upon conjugation, which can have either a Z-or an E-configuration.
Does the PEI agree that it is not necessary to control the stereochemistry on **X** or the E/Z ratio?

Answer to Q3:
PEI agreed that for the planned First-in-human study, a detailed control of neither the stereochemistry nor the E/Z ratio, would be required. However, experience on the E/Z ratio should be gained with increasing numbers of produced ADC batches. Consistency on the 1:1 ratio of both diastereoisomers on X can be shown over increasing numbers of linker-payload batches. PEI stated that if Applicant can demonstrate that the stereochemistry and the E/Z-ratio do not affect payload release by lysosomal proteases, these data would be considered sufficient. However, controls might need to be implemented at the time of an MAA.

Drug linker commercial specification

Tests for description, identification, related substances, residual solvents and assay are in line with ICH Q6A

- Justification for absence of other physicochemical tests, such as polymorphism and particle size may require further
- The stereochemistry is controlled by material specifications, in-process controls and a test for specific optical rotation in the specification of MAAA-1162a. This control strategy is acceptable when thorough discussion on possible stereoisomers and their fate is provided and supported with analytical data.
- absence of racemisation to be shown with batch analysis data and stability studies.
- risk assessment according to ICH guideline Q3D (R1) on elemental impurities
- ICH guideline Q3C (R7) on impurities: guideline for residual solvents

Test
Description (Appearance)
Identification
Specific Optical Rotation (20°C, sodium D line)
Related Substances Specified – NHS-adduct Individual Unspecified Total
Residual Solvents 1-Propanol Acetone Ethyl Acetate Tetrahydrofuran (THF)
Assay

Quality guidance for chemical entities

…applied to the drug-linker intermediate component?

- Drug linker represents an intermediate in the manufacture of the actual DS and might therefore be treated as surrogate API

- It is agreed that for the drug-linker component manufactured using small molecule chemical synthesis, the relevant chemical entity guidance can be applied.

- It is also acceptable to follow Section B.I. Active Substance of the Variation Guidance for "chemical entity drug substance intermediate" for changes concerning the drug-linker intermediate only

Caveat: no DMF (Drug Master File in EU): the concept of the ASMF shall only apply to a well-defined active substance and cannot be used for excipients, finished products and biological active substances – not applicable for intermediate, e.g. drug-linker

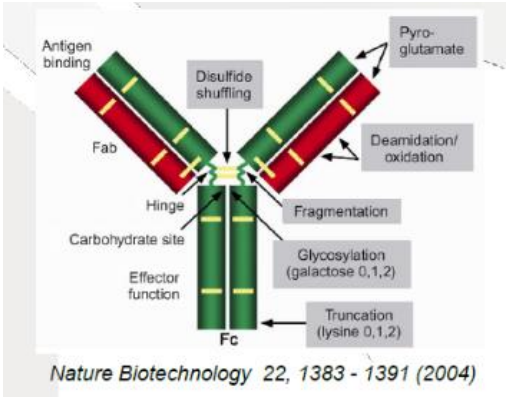
Characterization/Specifications

Antibody Specifications
These specifications have to be established based on principles outlined in ICH Q6B (and ICH Q3D, risk assessment)

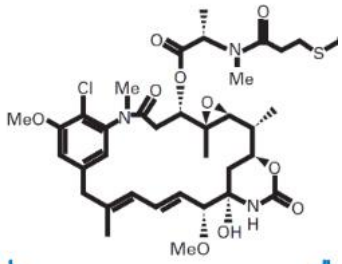
Maytansinol
- MayOH is a well-characterized, stable compound
- MayOH is a well-defined single compound that is produced and tested for conformance against an appropriate specification using qualified reference standards.
- MayOH has a well-defined and consistent impurity profile ICH Q6B

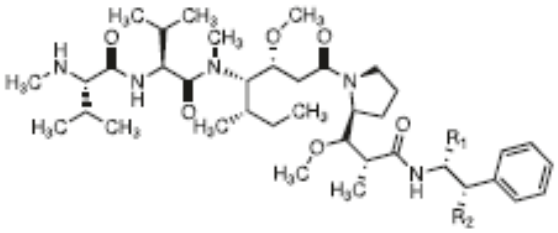
MMAE monomethyl auristatin E
Specifications have been established based on principles outlined in ICH Q6A *Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances Specifications.*

The specifications set for linker and cytotoxic drug should include the recommend acceptable amounts for residual solvents guidance given in the ICH Q3C “Impurities: Guideline for residual solvents” and ICH Q3D “Elemental impurities”should be followed.



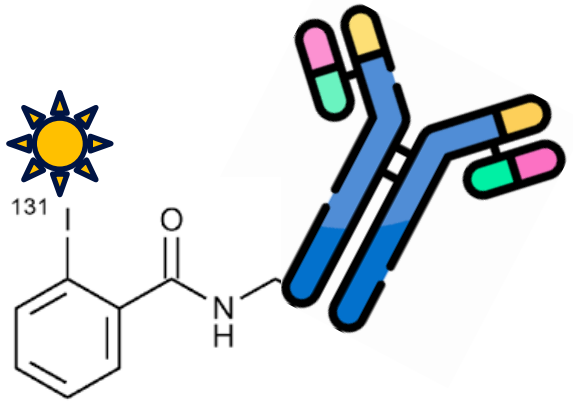
Nature Biotechnology 22, 1383 - 1391 (2004)


DM1
(derivative of maytansine)


MMAE R_1=CH_3, R_2=OH;
MMAF R_1=COOH, R_2=H

Need for Guidance on ADCs ?

- Clear terminology identifying starting materials, intermediates, linkers, active substance and finished product stages
- Structure of CTD quality and non-clinical modules for intermediates, active substance and finished product
- Reference to the dossier of an already authorised medicinal product (e.g., monoclonal antibodies, radionuclide intermediates) and use of an ASMF procedure for radiopharmaceutical precursors
- Specification requirements for radionuclide, e.g., radionuclide characteristics, radionuclide concentration, radionuclide purity, radiochemical purity, specific activity, chemical composition, chemical impurities, chemical stability
- State-of–the-art radiolabelling method (to generate stable conjugate) requirements
- Specification requirements for active substance and finished product, e.g., identity, purity, potency, sterility



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

20 July 2023
EMA/CHMP/BWP/245588/2023
Committee for Medicinal Products for Human Use (CHMP)

Concept paper on the revision of the Guideline on Radiopharmaceuticals Based on Monoclonal Antibodies

Agreed by Biologics Working Party	12 July 2023
Adopted by CHMP for release for consultation	20 July 2023
Start of public consultation	21 July 2023
End of consultation (deadline for comments)	31 October 2023

THANK YOU