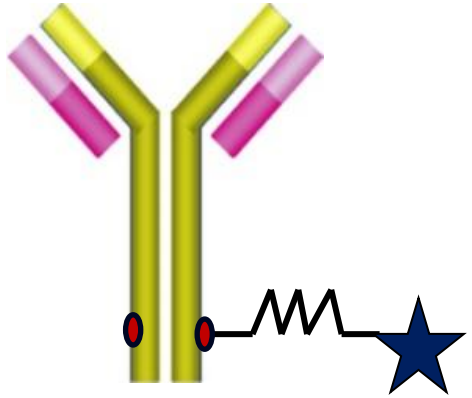
Antibody-X-Conjugates (AXC) as specific and potent medicinal products
- a regulator´s perspective -

www.pei.de



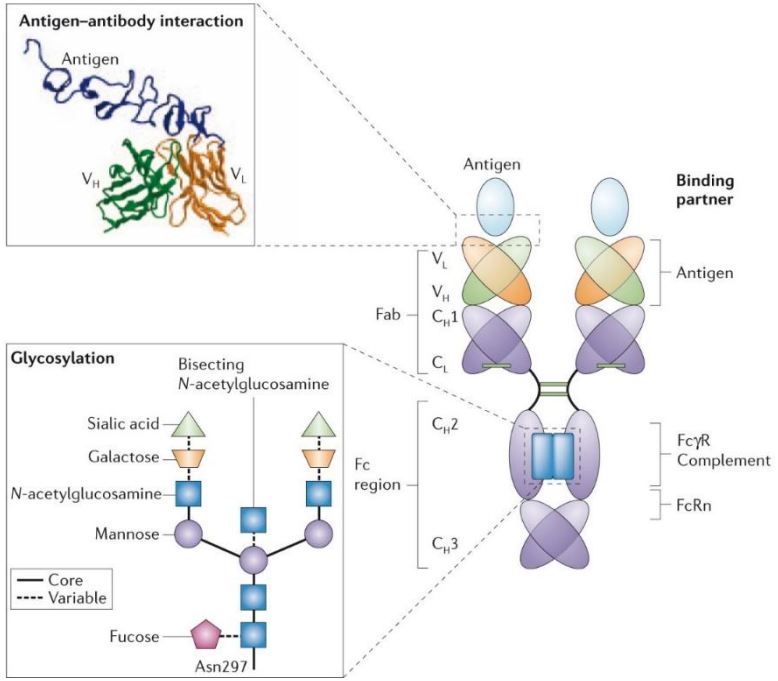
Steffen Gross
Head Section Quality Evaluation of Antibody Therapeutics

PAUL-EHRLICH-INSTITUT
Federal Institute for Vaccines and Biomedicines

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

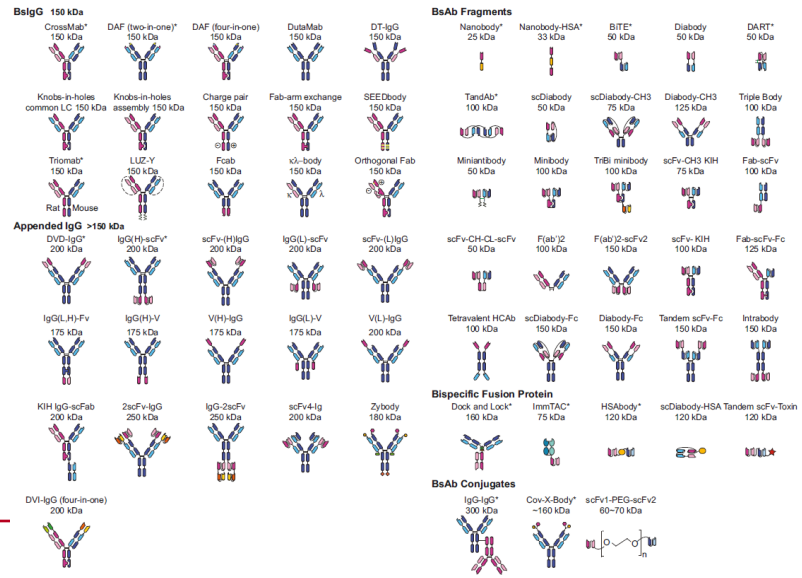
Antibody moiety

high binding affinity to the target antigen, efficient internalization, low Immunogenicity, long plasma half-life



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

- IgG1 is the most abundant in serum and could induce the strong effector functions such as ADCC), ADCP), and CDC
- IgG2 reduced effector function form dimers and aggregations in vivo, which leads to a decrease of the concentration of ADC drugs
- IgG3, short half-life (7 days)
- IgG4 could induce ADCP, reduced effector function



Common Identified Topics for Discussion on AXC

- 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

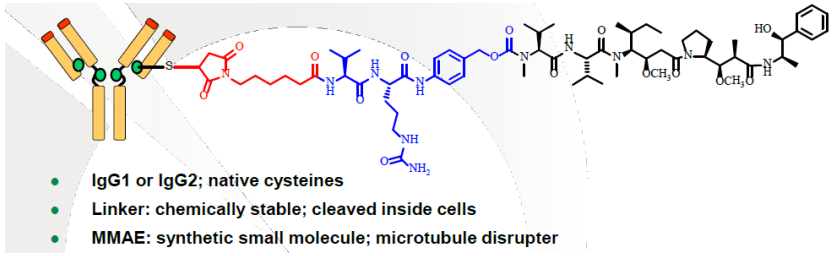
- Reference to the dossier of an already authorised medicinal product (e.g., monoclonal antibodies, radionuclide intermediates) and use of an ASMF procedure for radiopharmaceutical precursors

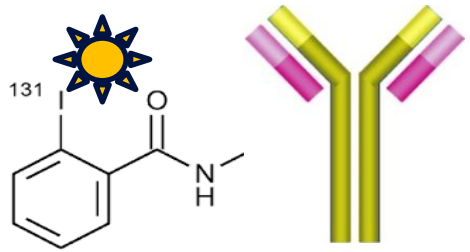
- State-of–the-art conjugation/radiolabelling method (to generate stable conjugate) requirements

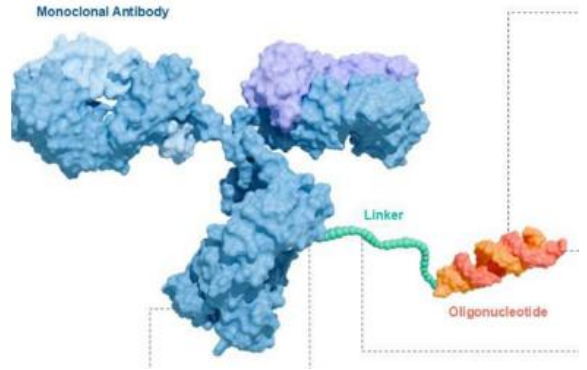
- Specification requirements for intermediates

- Specification requirements for active substance and finished product, e.g., identity, purity, potency, sterility

- Impurities







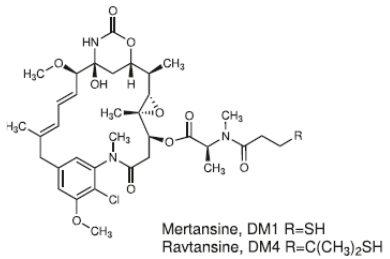
ADC Cytotoxic payloads

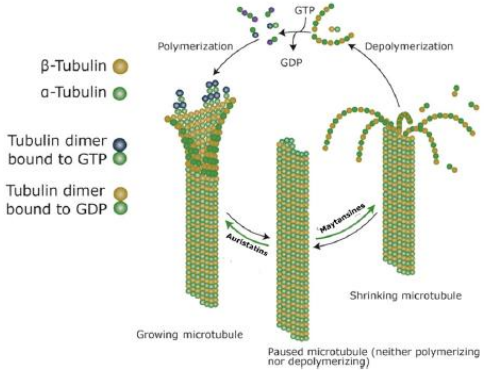
potent tubulin inhibitors, DNA damaging agents, RNA synthese inhibition and immunomodulator

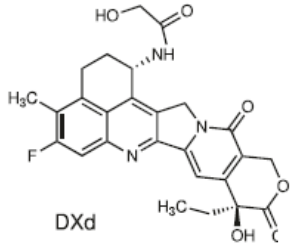
- **Tubulin polymerization promoters target at the β-subunits of tubulin dimer to perturb microtubule growth,**
  - auristatin derivatives monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF)

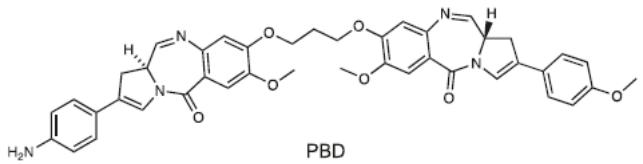
- **IC50 values of DNA damaging agents are able to reach picomolar level**
  - DNA double strand break, such as calicheamicins;
  - DNA alkylation, such as duocarmycins;
  - DNA intercalation, such as topoisomerase I inhibitors (exatecans)
  - DNA crosslink, such as pyrrolobenzodiazepines (PBD).

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

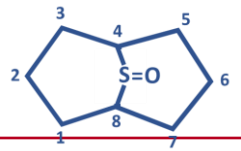








Amanitin



Cleavable vs. non-cleavable linkers

Non-cleavable linkers: low off-target toxicity benefited from an increase of plasma stability (T-DM1)

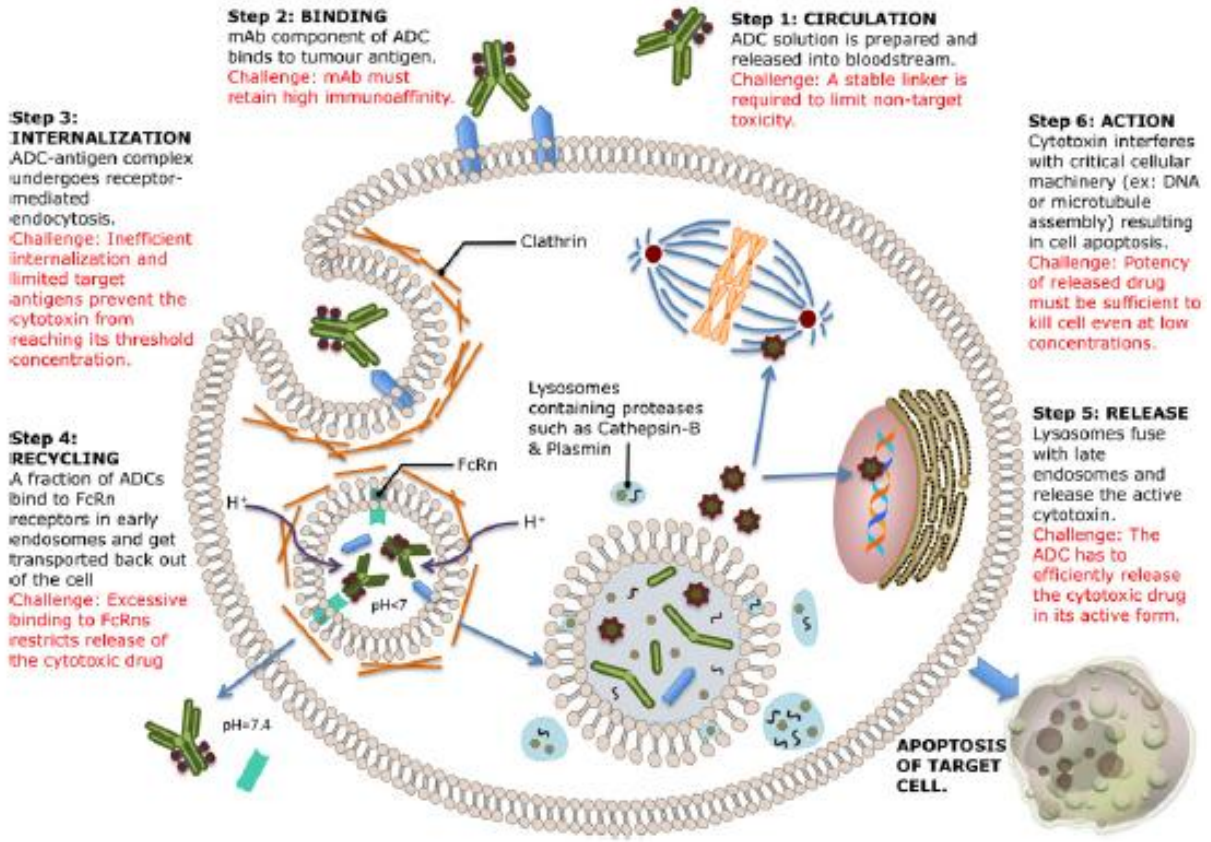
Cleavable linkers:

- **chemical cleavage linkers (hydrazone bond and disulfide bond) Hydrazone is a typical acid-sensitive (pH sensitive) linker.**

- **enzyme cleavage linkers (glucuronide bond and peptide bond), sensitive to the lysosomal protease e.g. Cathapsin B**

Linker Technology:

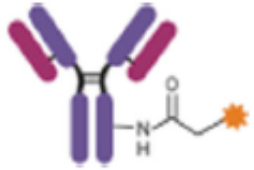
- **PEG/Polysarcosine for solubility/hydrophilic characteristics**



Source (Peters and Brown 2015)

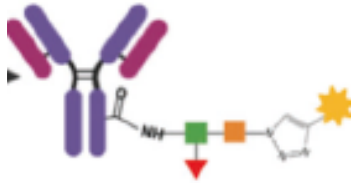
(Site-specific) Conjugation methods

- 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



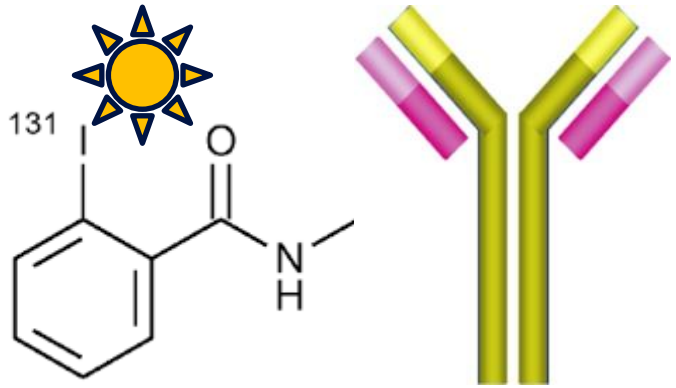
  - Cysteine based reaction provides another means of coupling. Due to the limited number of binding sites and the unique reactivity of mercaptan groups, using cysteine as the connecting site helps to reduce the heterogeneity of ADC. Depending on the reduction ratio, products with DAR of 2, 4, 6 and 8 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



ARC Scope

Provide guidance on strategy and data to be provided in regulatory submissions for radionuclides coupled to monoclonal antibody derivatives. The antibody or antibody fragment is thus only one component of the medicinal product and in the evaluation of quality and safety of this group of products, the radiopharmaceutical and radiation protection aspects must be considered in addition to those of the antibody component.



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

1 21 July 2020
2 EMA/373361/2005
3 <Name of committee (Committee abbreviation)>

4 Guideline on the quality requirements for
5 radiopharmaceuticals based on monoclonal antibody
6 derivatives
7

8 **Draft**

Starting Material Radionucleotide

- The radionuclide precursor used for radiolabelling of the antibody component is manufactured with starting materials typically used in production of radioactive agents in nuclear reactions.

- Usually both, mother and daughter radionuclides sourced by a radionuclide generator are to be considered as active substance intermediates.

Gallium	Ga^{3+} CN = 6, 62 pm	^{66}Ga	9.5	β^+ (56%) EC (44%)	β^+, 4150, 935
		^{67}Ga	78.2	EC (100%)	γ, 93, 184, 300
		^{68}Ga	1.1	β^+ (90%) EC (10%)	β^+, 1880
Indium	In^{3+} CN = 8, 92 pm	^{111}In	67.2	EC (100%)	γ, 245, 172
Lutetium	Lu^{3+} CN = 8, 98 pm[b] CN = 9, 103 pm	^{177}Lu	159.4	β^- (100%)	γ, 112, 208 β^-, 177, 385, 498

Germanium-68/Gallium-68 generator (68Ge/68Ga Radionuclide generator)

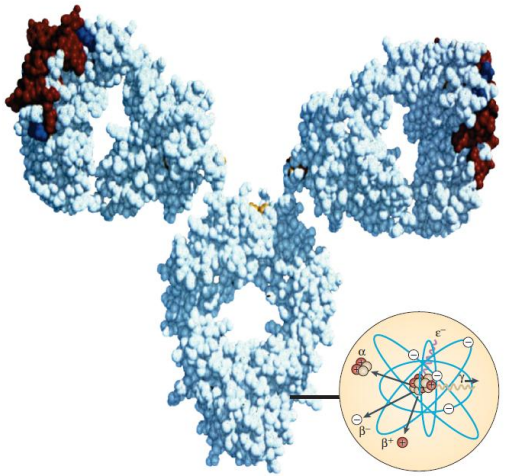
The parent isotope 68Ge has a half-life of 271 days and is utilized for production of 68Ga. Its decay product gallium-68 (half-life of only 68 minutes)

Source Deborah Sneddona and Bart Cornelissen, Current Opinion in Chemical Biology 2021

Conjugation Process

Monoclonal antibodies are linked to various radionuclides through several methods that are primarily based on the chemical characteristics of the specific radionuclide.

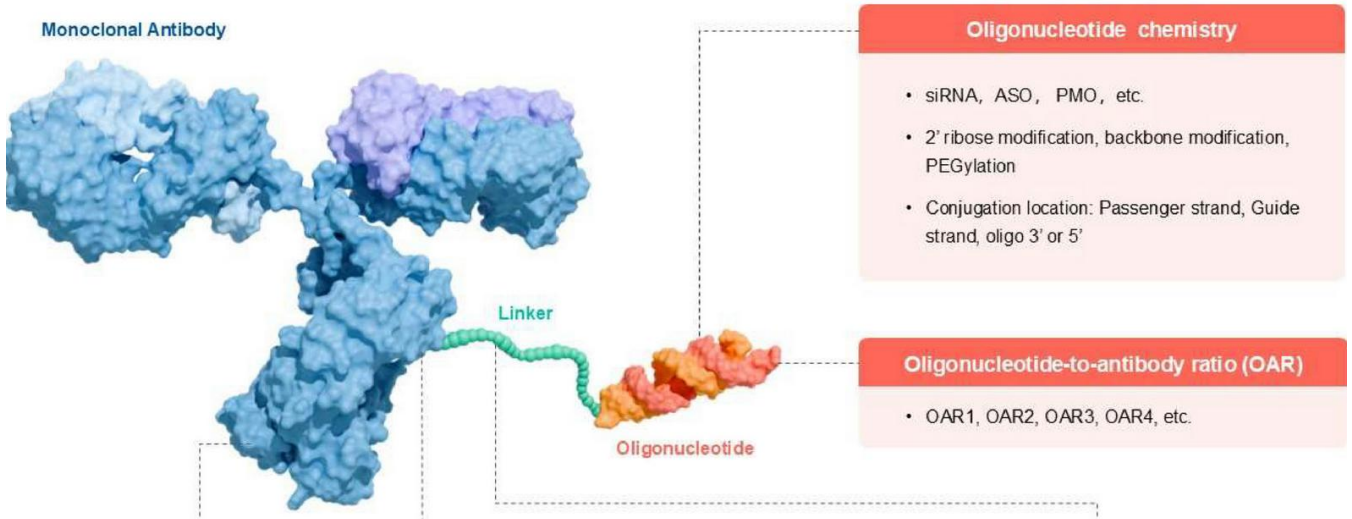
- **Halogens, such as 131I, are routinely introduced by direct halogenation of tyrosine residues of the protein.**



- **Metallic radionuclides, such as 111In or 90Y require chelation of the metal through a suitable ligand. This chelating agent is routinely introduced through a reactive functional group that targets N-terminal and ε-amines of lysine residues**

Source: Diane E.Milenic, Erik D. Brady and Martin W. Brechbiel, Nature Reviews 2004

AOC - Oligonucleotide payloads



Oligonucleotide chemistry

- siRNA, ASO, PMO, etc.
- 2' ribose modification, backbone modification, PEGylation
- Conjugation location: Passenger strand, Guide strand, oligo 3' or 5'

Oligonucleotide-to-antibody ratio (OAR)

- OAR1, OAR2, OAR3, OAR4, etc.

Antibody chemistry

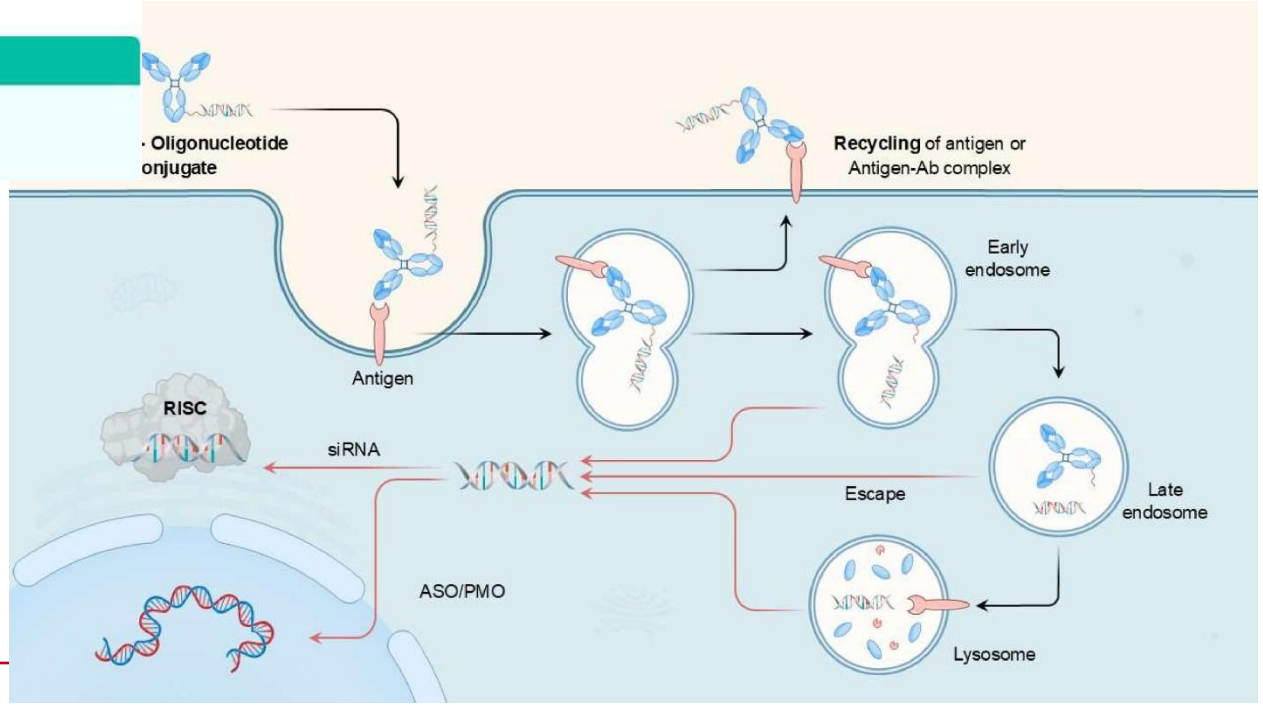
- Antibody isotype

Antibody conjugation location

- Random: lysine
- Site specific: Asn297-glycan, cysteine

Linker selection

- Stable linker: SMCC, MC, bisMal
- Cleavable linker: Val-Cit



From Meng Li, Hongxin An, Jialing Zhang, Weiyu Li, Chuanfei Yu *, Lan Wang **, European Journal of Pharmaceutical Sciences 215 (2025) 107292

Stage classifiaction an definition of CQA

Intermediates

Antibody

- Identification
- Bioactivity
- Protein content
- Charge variants
- Size variants
- Glycosylation
- Residual host DNA
- HCP

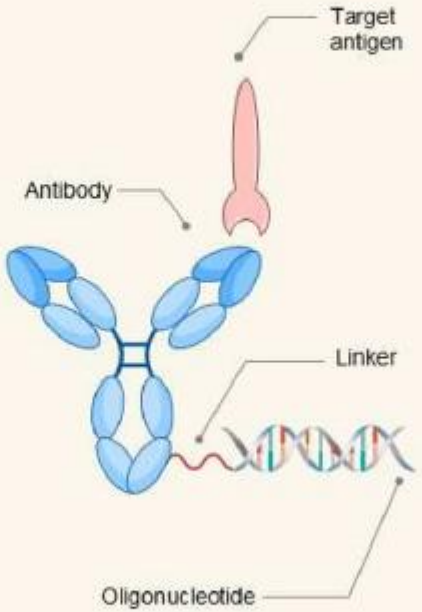
Oligo-linker

- Identification
- Content
- Purity and impurity profile
- Residual solvents

Oligonucleotide

- Identification
  - Mass determination
  - Sequence confirmation
- Assay
- Counter ion testing
- Purity and impurity profile
- Residual solvents
- Heavy metals

AOC



Drug Substance

- Identification
- Bioactivity
- Content
- OAR
- Conjugation Site Distribution
- Free Oligonucleotide
- Unconjugated Antibody
- Charge variants
- Size variants
- Residual solvents
- Endotoxin
- Bioburden
- Other process related impurities

Drug Product

- Identification
- Content
- OAR
- Free Oligonucleotide
- Unconjugated antibody
- Charge variants
- Size variants
- pH
- Osmolality
- Visible particle
- Subvisible Particle
- Endotoxin
- Sterility

ICH Q11, 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)

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.

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.

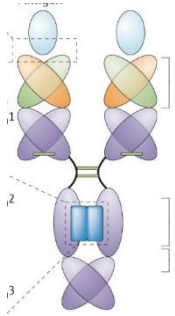
5.1.3. Selection of source and starting materials for biotechnological/ biological drug substances

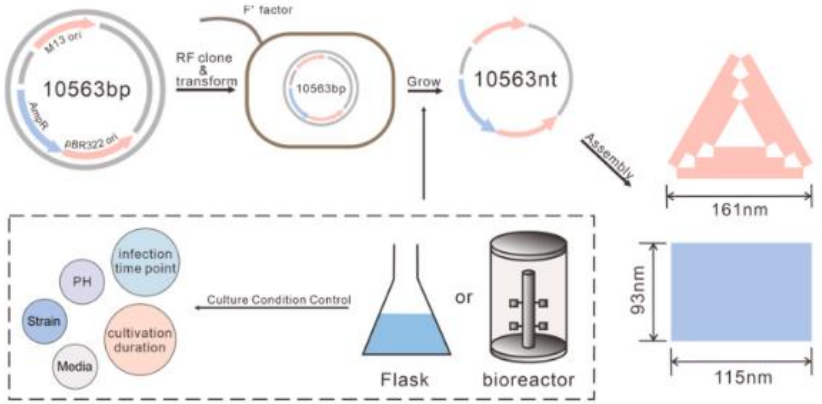
Cell banks are the starting point for manufacture of biotechnological drug substances and some biological drug substances. In some regions, these are referred to as source materials; in others, starting materials. Guidance is contained in ICH Q5A, Q5B, and Q5D. Guidance is contained in ICH Q5A, Q5B and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

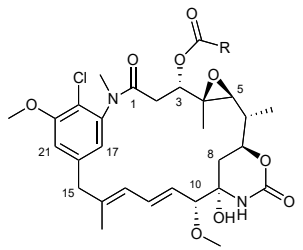
ICH Topic Q 5 D
Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products



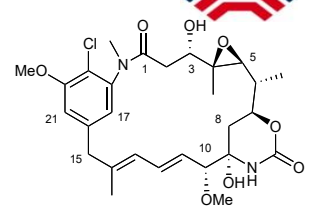


DM1/DM4

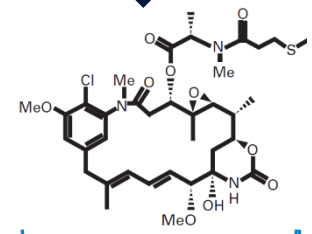
Tubulin inhibitor



Ansamitocins
R = Et, *n*Pr, *i*Pr, *n*Bu, *i*Bu



Maytansinol
Chemical Formula: $C_{28}H_{37}ClN_2O_8$
Molecular Weight: 565.05 g/mol
CAS#: 57103-68-1



DM1
(derivative of maytansine)

- 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

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.

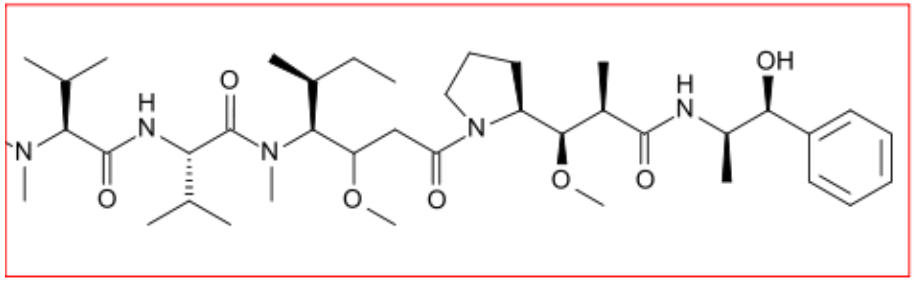
Documentation to be provided

- 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

Starting Material-MMAE

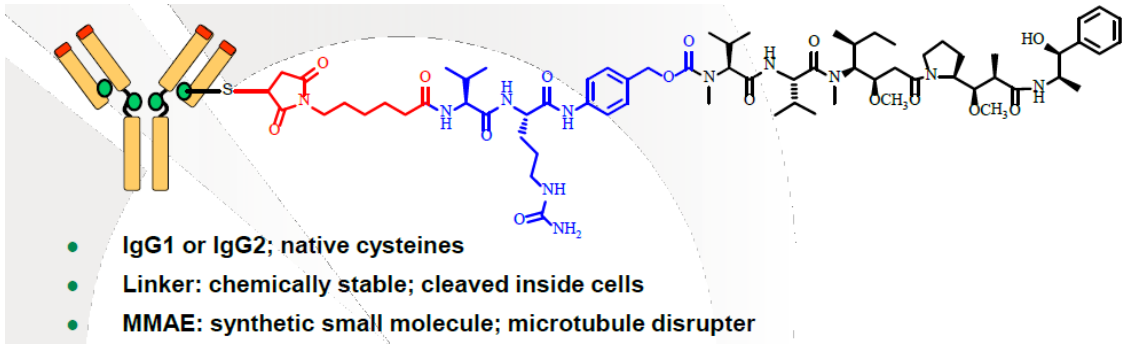
Monomethyl auristatin E is an antimitotic agent which inhibits cell division by blocking the polymerisation of tubulin

MMAE monomethyl auristatin E



MMAE

convergent, solution phase, fragment-based peptide synthesis.



Principles of ICH Q11 for classifying starting material 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.

Guidelines on the Development and Manufacture of Synthetic peptides/oligonucleotides

4 December 2025
EMA/CHMP/CVMP/QWP/367182/2025
Committee for Medicinal Products for Human Use (CHMP)
Committee for Veterinary Medicinal Products (CVMP)

Guideline on the Development and Manufacture of Synthetic Peptides

Draft agreed b	1	17 July 2024
Adopted by CH	2	EMA/CHMP/CVMP/QWP/262313/202424
Adopted by CV	3	Committee for Medicinal Products for Human Use (CHMP)
Start of public	4	Committee for Veterinary Medicinal Products (CVMP)
End of consult	5	Guideline on the Development and Manufacture of Oligonucleotides
Agreed by Qu	6	
Adopted by CH	7	Draft
Adopted by CV	8	
Date of comin		

Draft agreed by Quality Working Party	18 June 2024
Adopted by CHMP/PROM for release for consultation	15 July 2024
Adopted by CVMP for release for consultation	17 July 2024
Start of public consultation	22 July 2024
End of consultation (deadline for comments)	31 January 2025

Guideline on the development and manufacture of synthetic peptides:

- **Protected natural L-amino acid derivatives (with terminal and side-chain protection as relevant) are generally acceptable as starting materials.**
- **In justified cases, short peptide fragments may be acceptable as starting materials.**
- **Longer peptide fragments could be acceptable as starting materials in duly justified cases**

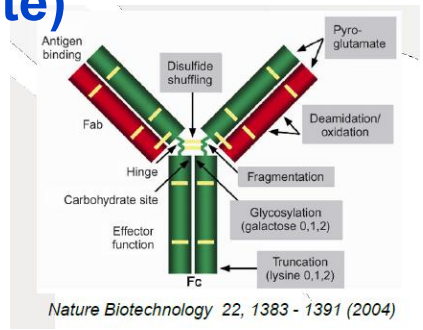
Companies are recommended to request scientific advice to cuss their proposal in advance.

Guideline on the development and manufacture of Oligonucleotides:

- **Protected nucleoside phosphoramidites are generally acceptable as starting materials**
- **For more complex nucleotide derivatives carrying modifications in the phosphate, sugar or base moiety, more detailed information regarding their manufacture and impurity profile is required.**

Characterisation/Specifications

(SM, Intermediates (D-L, Ab, DS, DP, stage appropriate)



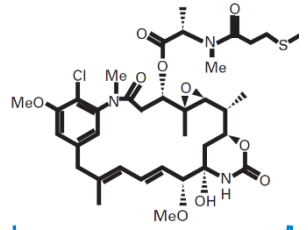
Nature Biotechnology 22, 1383 - 1391 (2004)

Antibody Specifications

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

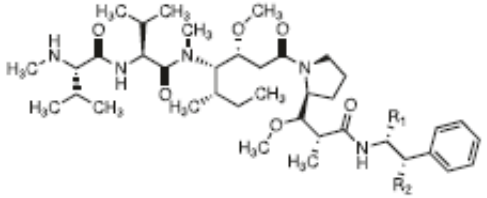
Maytansinol

- 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


DM1
(derivative of maytansine)

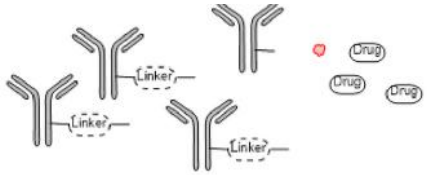
MMAE monomethyl auristatin E

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.*


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

The specifications set for linker and cytotoxic drug should include the recommend acceptable amounts for residual solvents guidance given in the ICH Q3A, ICH Q3C "Impurities: Guideline for residual solvents" and ICH Q3D "Elemental impurities"should be followed.

Impurities: Alternative approach to ICHQ3A qualification limit?

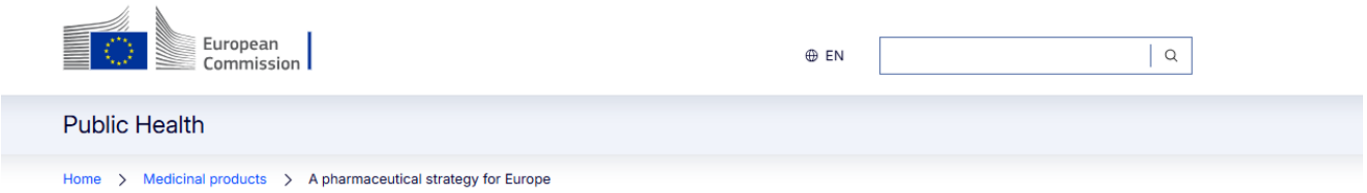


Worst case ADC mole per dose = 0.6/150000 = 0.004 mM
Typical DAR is 4-8
1.0% w/w Imp in D-L results in approx. 0.0003 mM per dose

Acceleration Scenarios….

(Review)-Timelines, „Sandboxes“, classification of post-approval changes…)

Revision of EU variations framework



- Revision of the variation's framework (target revision) included in the 2020 pharmaceutical strategy for Europe.
- Amended variation Regulation (Regulation (EU) 2024/1701) was published on 17 June 2024 and became applicable on 1 January 2025.
- New EC Variations Guidelines were published on 22 September 2025 and will apply from 15 January 2026.
- Second revision of the variations framework is expected once the revision of the basic acts (Regulation/Directive) is complete

- **Expand access and availability of medicines**. The EU's robust, science-based assessment process will continue to underpin all medicine authorisations to ensure the highest safety standards, while streamlined procedures will make it more efficient.
- **Accelerate medicine supply chains,** by cutting red tape for companies, reducing evaluation times for new medicines and reforming the European Medicines Agency (EMA). They will ensure that new medicines get to the market faster and that patients have better access to therapies, especially for unmet needs.
- **Place the EU at the forefront of pharmaceutical innovation**. The reform offers world-leading incentives for innovative products, introduces regulatory sandboxes as a secure testing environment for truly novel medicines and introduces adapted frameworks for certain non-standard treatments, like personalised therapies. In addition, fulfilment of unmet medical needs will receive strong recognition.
- **Enable timely market entry for generic medicines.** The reform brings clarifications regarding the application of the Bolar exemption, which allows certain activities during the patent protection without prejudice to international agreements.
- **Address medicine shortages.** The reform establishes an EU framework to better monitor medicine shortages, with a stronger coordination role for EMA. Companies will be subject to stronger obligations to prevent shortages, while an EU list of critical medicines will be established and vulnerability assessments carried out, amongst other things.

Concept of ASMF?

Active Substance Master File (ASMF)
Assessment Report
Restricted Part
NB: THIS REPORT SHOULD NOT BE DISCLOSED TO THE APPLICANT
Sodium Iodide - ^{131}I (fission), IRE
Sodiumiodide (^{131}I) for radiolabelling

Active Substance Master File (ASMF)
Assessment Report
Applicant's Part

Sodium Iodide - ^{131}I (fission), IRE
Sodiumiodide (^{131}I) for radiolabelling

Concept of AQMF (Additional Quality Master File)

from Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the Union code relating to medicinal products for human use, and repealing Directive 2001/83/EC and Directive 2009/35/EC

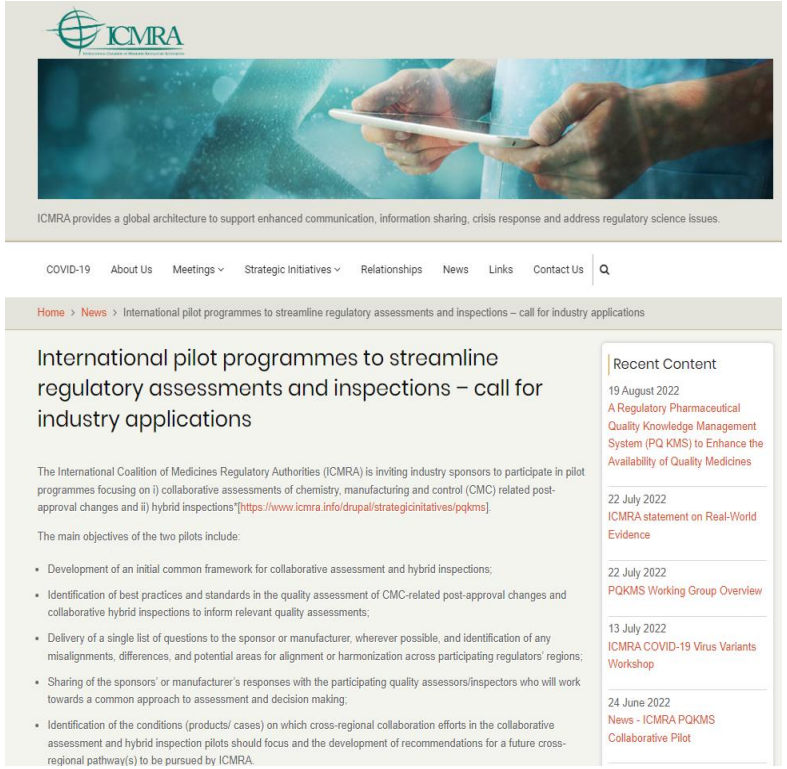
Article 26
Additional quality master files

- 1. Marketing authorisation applicants may, instead of submitting the relevant data on an active substance other than a chemical active substance, or on other substances present or used in the manufacture of a medicinal product, required in accordance with Annex II, rely on an additional quality master file, an additional quality master file certificate granted by the Agency in accordance with this Article ('additional quality master file certificate'), or a certificate confirming that the quality of that substance is suitably controlled by the relevant monograph of the European Pharmacopeia.

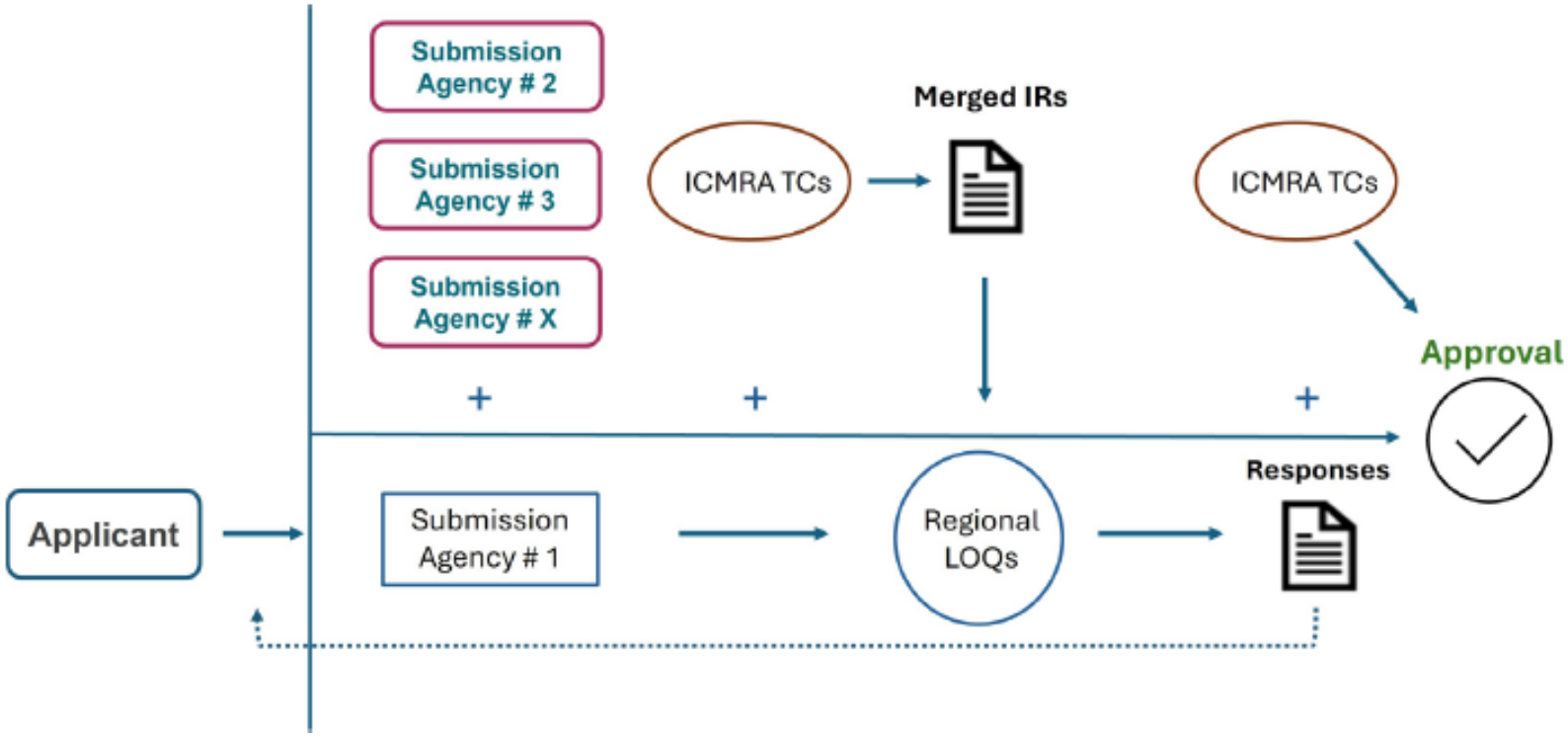
International Coalition of Medicines Regulatory Authorities (ICMRA)

Collaborative assessment and hybrid inspection pilots

- There are (substantial) regulatory divergencies between regions impacting on patient access and supply
- The overall ICMRA aim is to facilitate convergence in assessment practices for key products and to develop collaborative assessment approaches.
- An additional aim is to promote multi-agency GMP inspections



Regional Procedures & ICMRA Pilots: How They Work Together



Streamlined TT
- Common 120 days TT
- Simultanous apporval

Overall duration (days)	Max difference in approval dates between participating authorities
115	0
118	0
105	0
122	2
119	12

Source_ ICMRA presntationhttps://icmra.info/drupal/sites/default/files/2025-10/icmra_industry_pqkms_ws2025_presentations.pdf

There is flexibility within the timeline to schedule Sponsor meetings based on the needs of the Participating Regulatory Authorities

ACTIVITY	TIMELINE
Submission Receipt Date	Day 0
Project Start	Day 3
Initial Filing/Completeness Assessment	Day 10
Sponsor Meeting	TBD
Information requests to Sponsor	Day 20-60
Complete Filing Review	Day 60 or earlier
Information requests to Sponsor	After Day 60
Draft Quality Assessment by Lead Regulatory Authority	Day 106
Complete Quality Review	Day 113
Final/Action Letter Issued	Day 120

Multiple interactions

Outcome

ICMRA Collaborative AssessmentSummary Report January 17, 2025

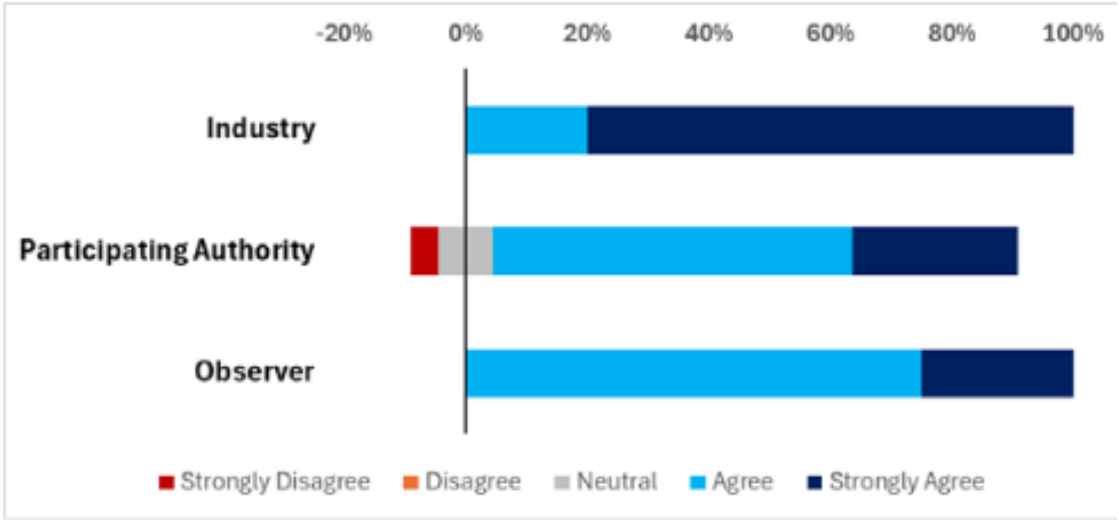
Table 4. Duration of the pilot applications

Pilot	Submission date	Completion date	Overall duration (days)	Max difference in Approval/Positive Opinion dates between participating authorities
Pilot 1	Jan 2023	May 2023	115	Same day
Pilot 2	Jun 2023	Oct 2023	118	Same day
Pilot 3	Nov 2023	Feb 2024	105	Same day
Pilot 4	Sep 2023	Jan 2024	122	2 days
Pilot 5	Jan 2024	May 2024	119	12 days

Table 6. Numbers of harmonised and region-specific information requests

Pilot	#harmonised assessment related IRs	#region-specific assessment related IRs	#region specific administrative IRs
Pilot 1	46	4	3
Pilot 2	7	2	1
Pilot 3	3	0	3
Pilot 4	5	3	2
Pilot 5	12	1	6

The overall experience of the participation to the pilot is considered positive and support its operationalisation into a global regulatory program?



Given the resource requirements for such collaborative assessments, future collaborative assessments should prioritise high-impact changes for medically important treatments and applications which will have a positive impact on medicine supply, and support manufacturing innovations that could also strengthen medicine supply.

Thank You!