(CMC) Challenges of Multispecific Antibodies-Reviewers Perspective

CMC Strategy Forum Europe 2025, Basel
Unlocking the Potential of Multispecific Antibodies
20-22 October 2025

www.pei.de

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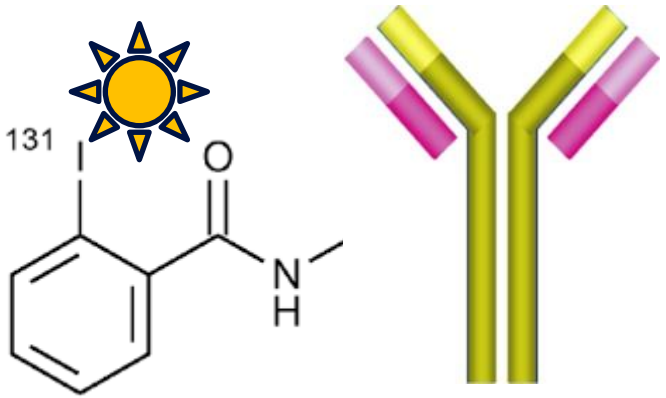
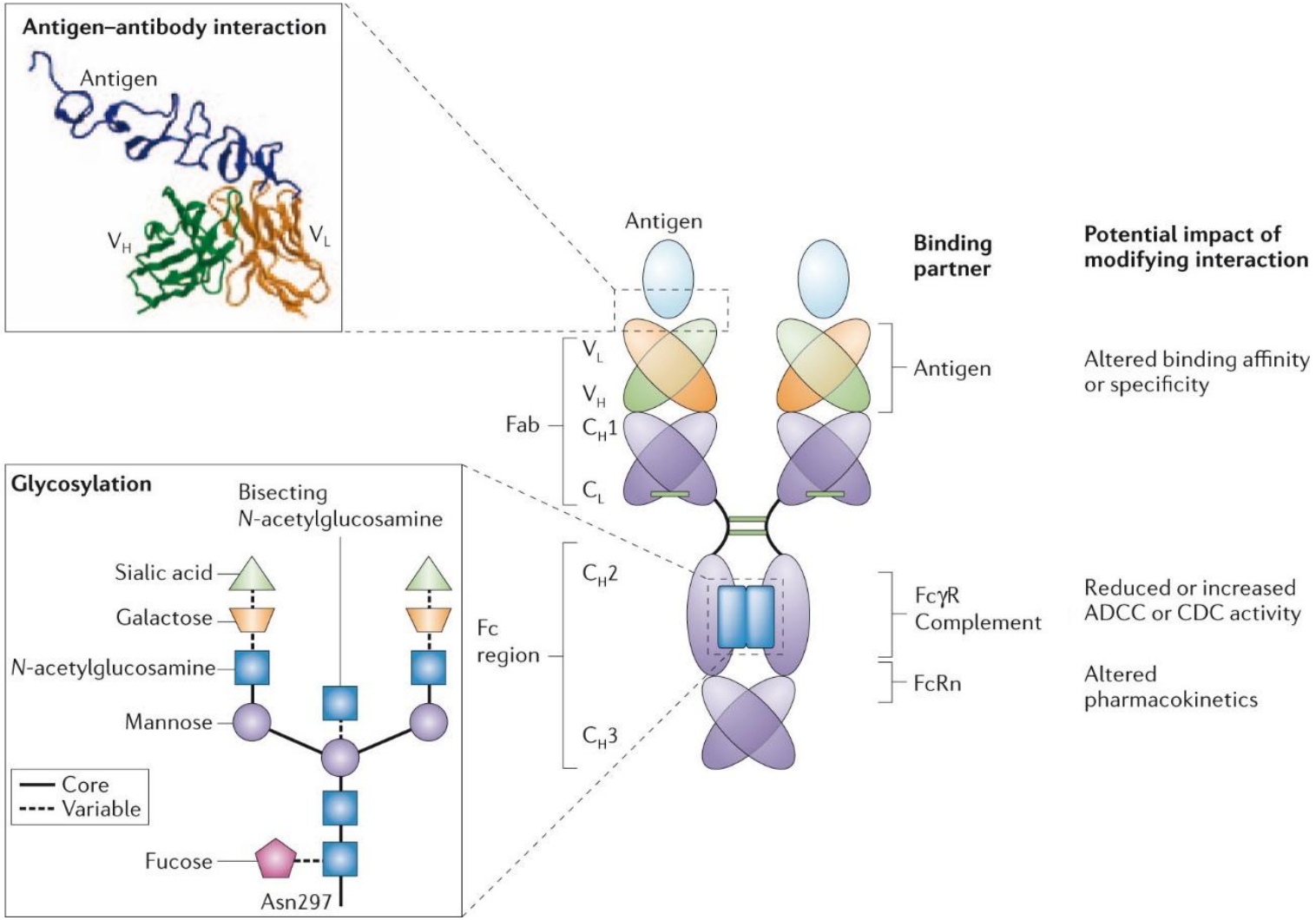
Disclaimer
The view expressed in the following is the ones of the presenter and does not necessary express the view of either the CHMP, BWP, EDQM or the Paul-Ehrlich-Institut (including other sections)

Outlook

- mAb (derivative) evolution and new modalities
- Revision of guideline(s) Radiopharmaceutical(s)
- Need for revision/Emerging topics
  - Definition of starting materials
  - Concept of ASMF
  - Manufacturing
  - Control Strategy



“Classical mAbs“

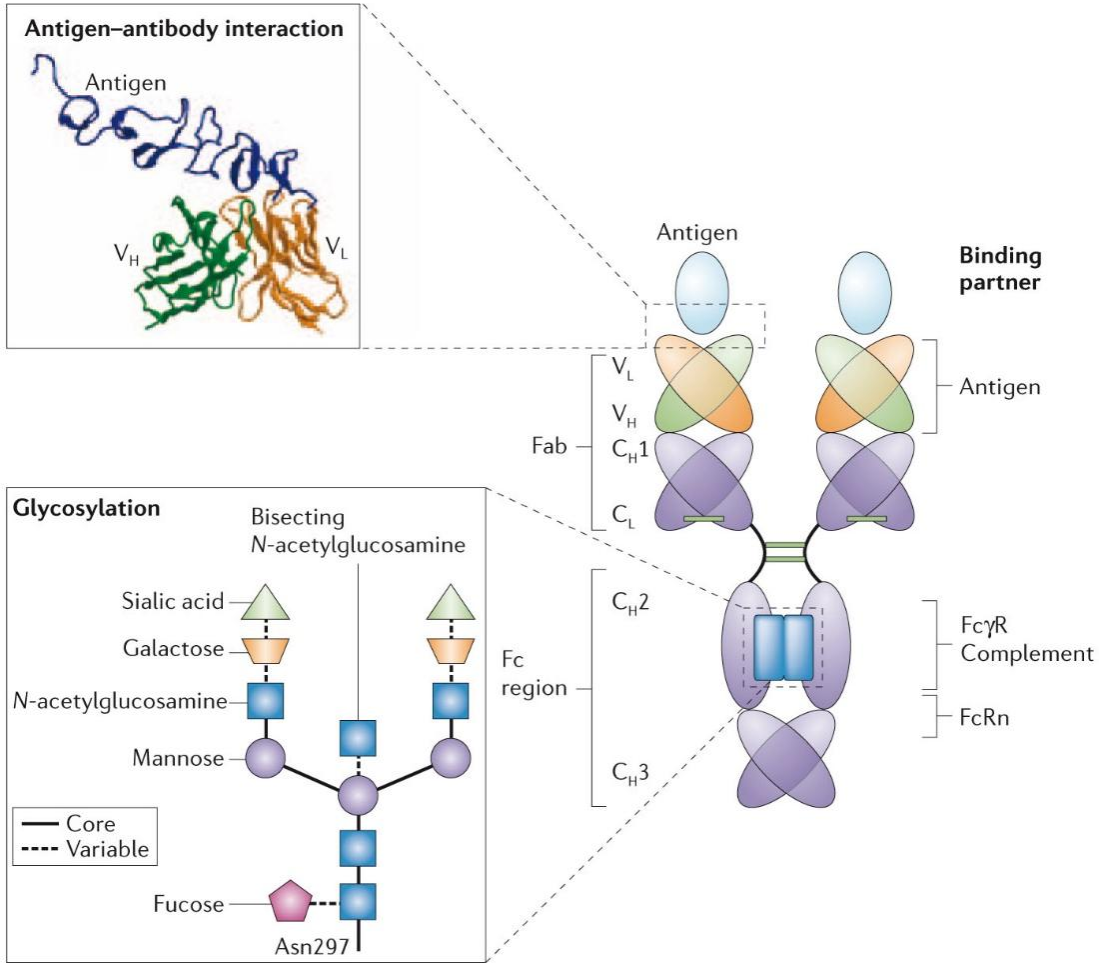


Adapted from Carter PJ: Potent antibody therapeutics by design, Nature Rev Immunology 6, 343 (2006)

- **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**

Monoclonal Antibodies

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



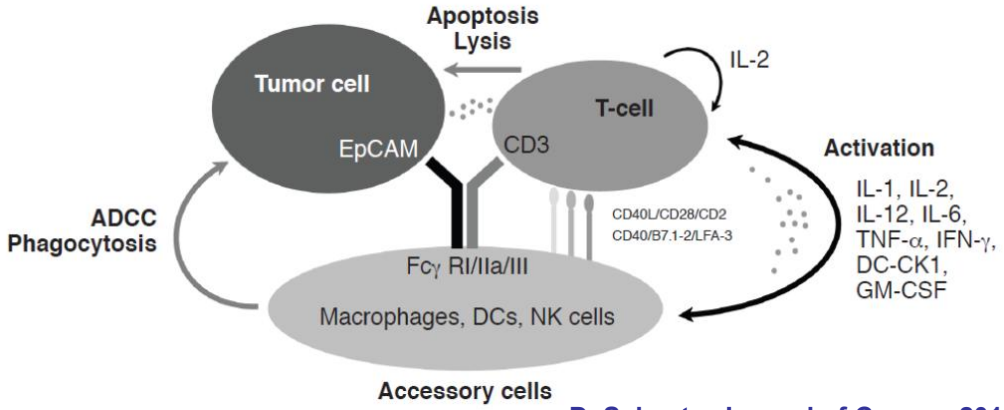
Adapted from Carter PJ: Potent antibody therapeutics by design, Nature Rev Immunology 6, 343 (2006)

Antibody engineering

- enhance/reduce effector function
- overcoming limitations such as limited tissue penetration
- cross barriers (e.g. BBB)
- increase target specificity
  - bs antibodies
  - conjugates
- display pro-longed half lives
  - YTE
  - HSA-fusion

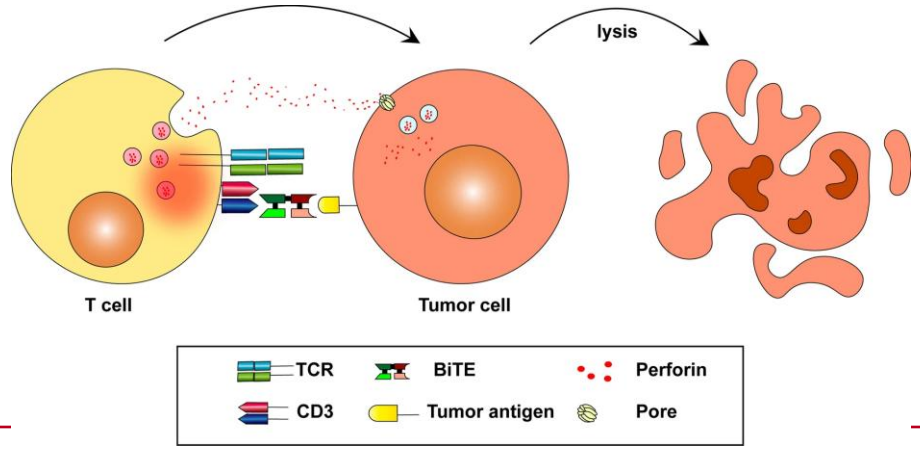
Novel Monoclonal Antibody modalities/multifunctional molecules

Catumaxomab: trifunctional bispecific monoclonal antibody: (1) the mouse Fab fragment binds to human EpCAM; (2) the rat Fab fragment binds to human CD3 on T cells; (3) the hybrid Fc-region selectively binds to and activates Fcγ-receptor I, IIa, and III-positive accessory cells.



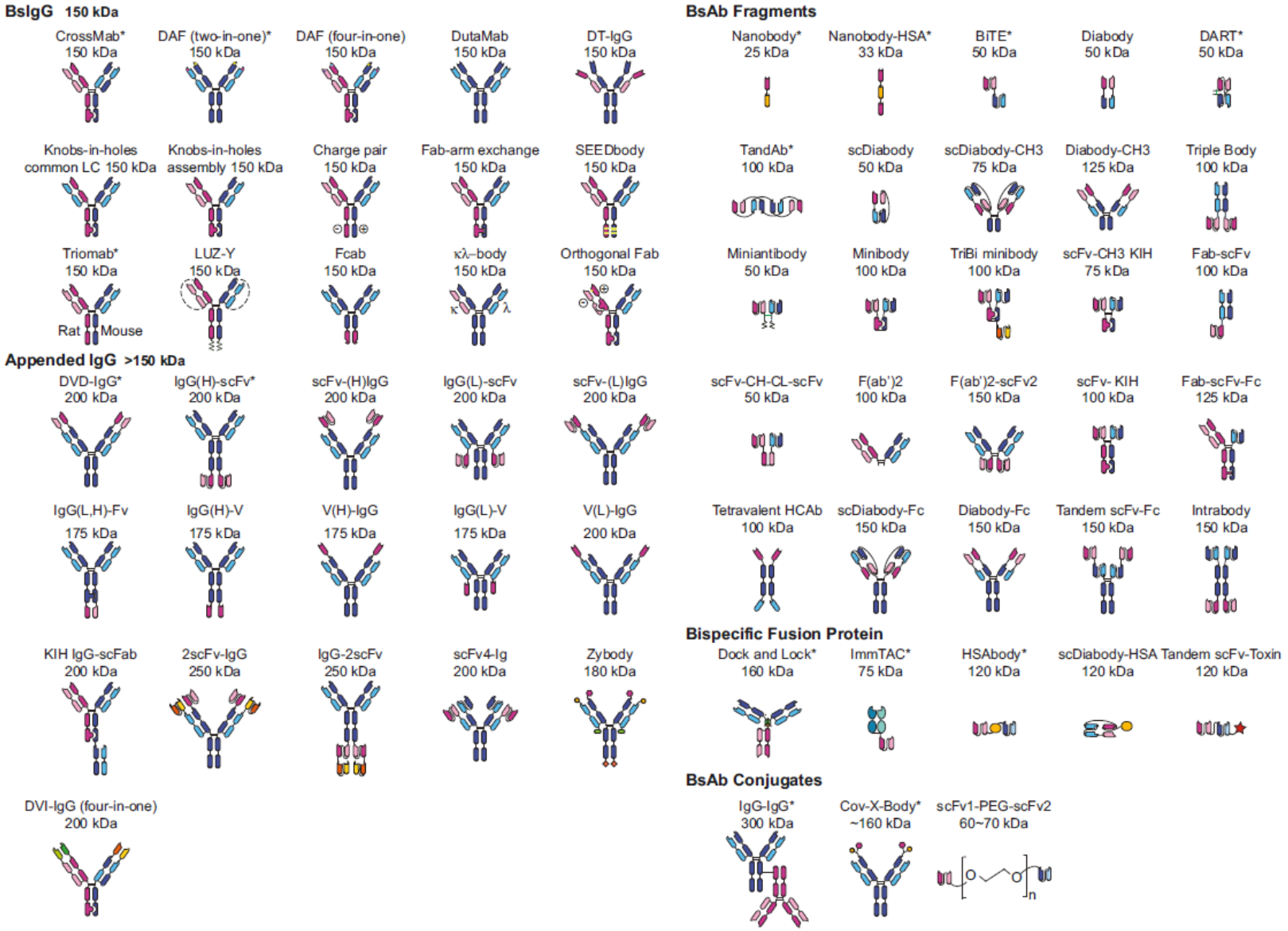
D. Seimetz, Journal of Cancer, 2011; 2:309-316

Blinatumomab, a Bispecific T-cell Engager (BiTE):transiently links CD19-positive B cells to CD3-positive T cells, resulting in induction of T-cell-mediated serial lysis of B cells and concomitant T-cell proliferation



Tian et al. J Hematol Oncol (2021) 14:75

Antibody moiety formats



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

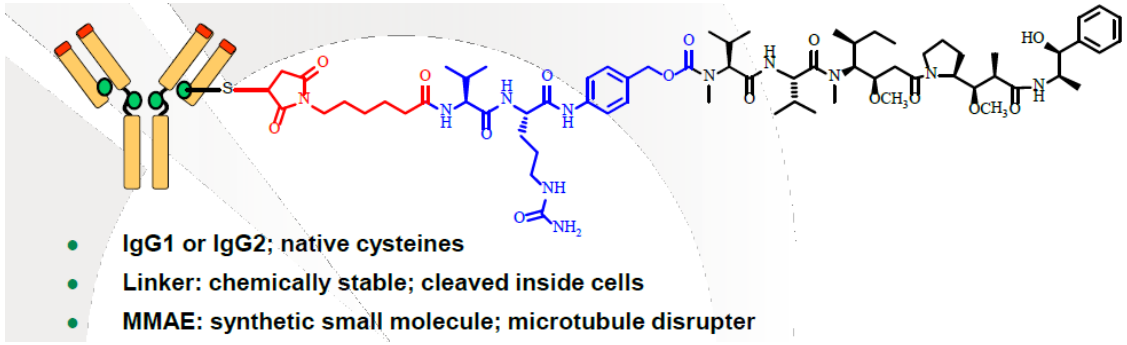
Advances in Manufacturing Technologies

- Expression construct(s)
  - triple mutation (YTE) in the Fc portion to increase binding to FcRn
  - IgG1 variants is L234A/L235A substitutions to reduce binding to the IgG Fc receptors and C1q.
  - Knob in hole to preferential heterodimer formation for bs mAbs
- Expression system
  - Microbial (e.g. E. coli)
  - Leishmania-based expression systems allowing natural folding and perfect post-translation modifications
  - Pichia Pastoris
  - Mammalian cells
- Media composition
  - supplements of d-(+)-glucose (usual feeding component for fed-batch cultures), d-(+)-mannose and d-(+)-galactose impacting growth/PTM
- Conjugation
- Labelling

FAQ-ADC development

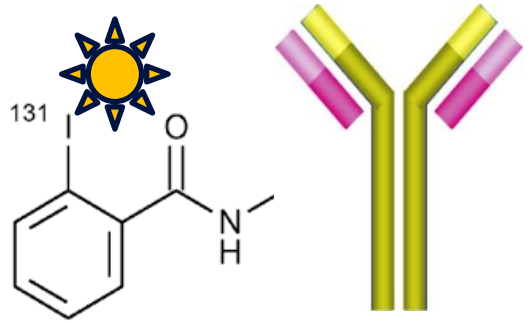
(from CMC Strategy Forum Europe 2023, Stockholm)

- Assignment of regulatory starting materials
- Manufacturing stage definition
  - Intermediate/DS/DP
- Concept of the ASMF (active substance master file)
  - 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
- Control Strategy/Specifications
- Need for Guidance on ADCs?



Recently Identified Topics for Discussion for Radioconjugates

- 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

(Parallel) Revision of GL landscape

European Medicines Agency
Inspections

London, 26 November 2008
Doc. Ref. EMA/441567/2024

COMMITTEE FOR HUMAN MEDICINAL PRODUCTS (CHMP)

GUIDELINE ON QUALITY OF RADIOPHARMACEUTICALS

3AQ21a ■

RADIOPHARMACEUTICALS BASED ON MONOCLONAL ANTIBODIES

Guideline Title	Radiopharmaceuticals based on Monoclonal Antibodies
Legislative basis	Directives 65/65/EEC, 75/318/EEC as amended, Directive 89/343/EEC
Date of first adoption	May 1991
Date of entry into force	January 1992
Status	Last revised May 1991

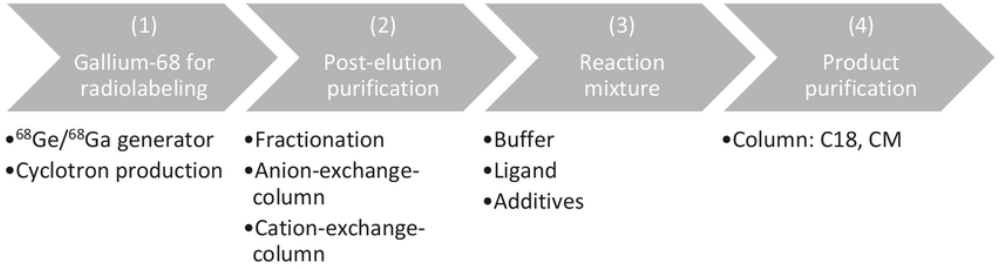
Manufacturing process

„mAb intermediate“ production

Conjugation
(potentially via linker/chelator)

- Optimize parameters such as pH, temperature, precursor peptide content, use of adjuvants for impurity management
- according to the principles of good manufacturing practice

Radiolabelling



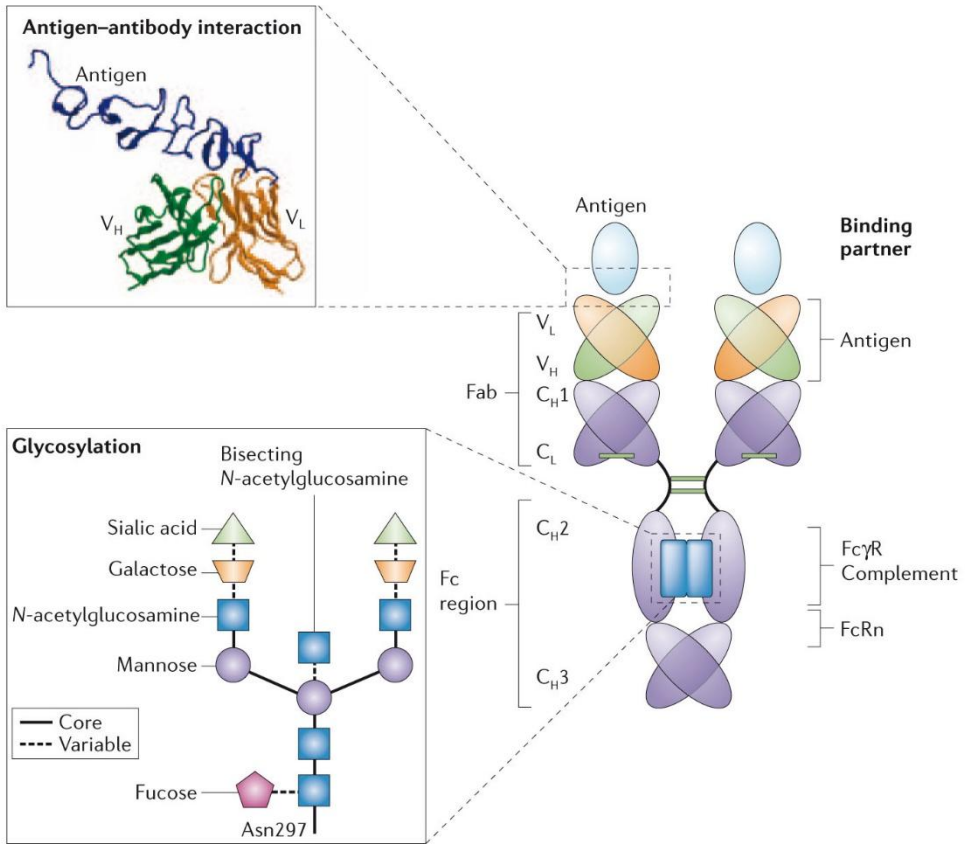
Rusu et al. EJNMMI Radiopharmacy and Chemistry (2023)

carried out by the manufacturer
Shipping validation

carried out by the user
(KIT)

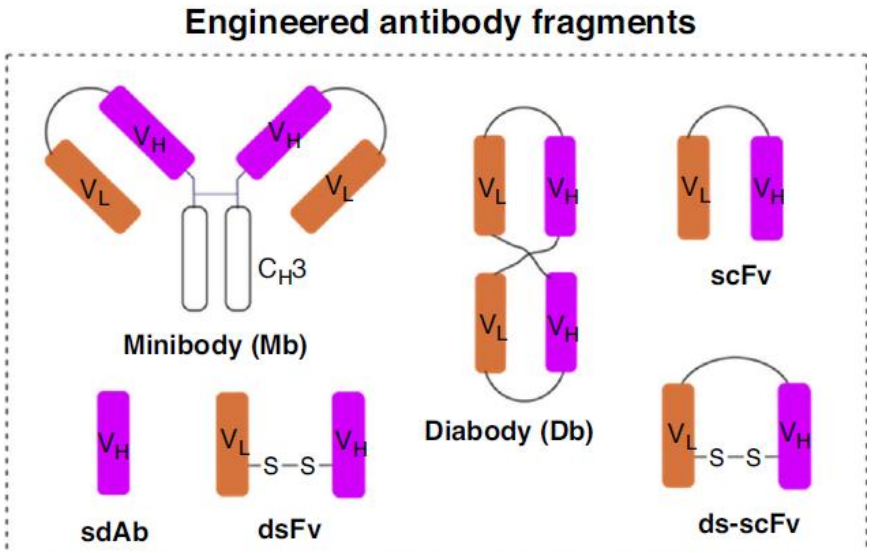
Antibody moiety

high binding affinity to the target antigen

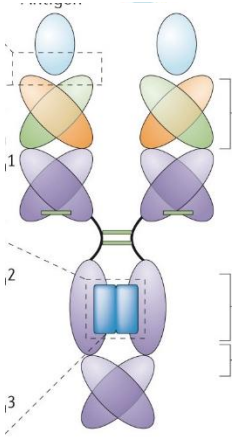


Adapted from Carter PJ: Potent antibody therapeutics by design, Nature Rev Immunology 6, 343 (2006)

- Specificity, tumour penetration and biodistribution may be improved by utilizing mAb derivatives such as bispecific antibodies or smaller antibody fragments such as single domain antibodies for conjugation



ICH Q11, Selection of starting materials and source materials



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

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

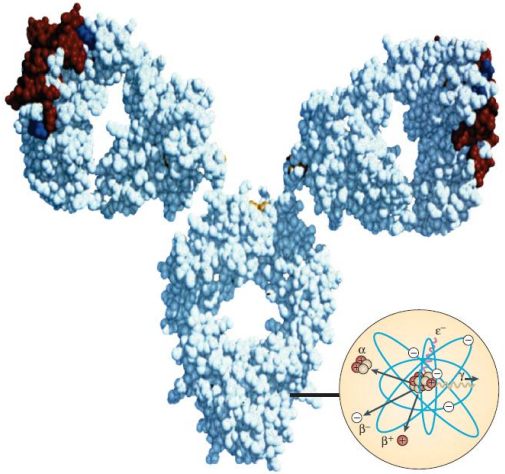
Germanium-68/Gallium-68 generator (68Ge/68Ga Radionuclide generator)The parent isotope 68Ge has a half-life of 271 days and is utilized for production of 68Ga. Its decay product gallium-68 (half-life of only 68 minutes)

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

Conjugation

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.**



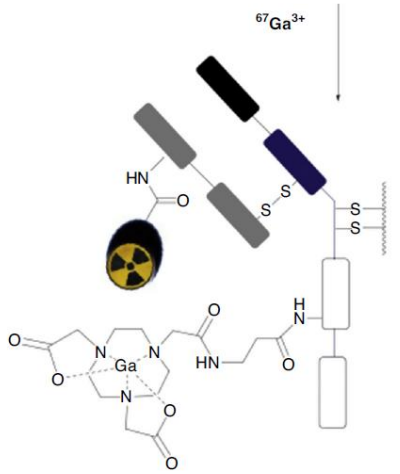
- **Metallic radionuclides, such as 111In or 90Y require chelation of the metal through a suitable ligand. This chelating agent is routinely introduced through a reactive functional group that targets N-terminal and ε-amines of lysine residues**

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

Conjugation Process via chelators

Inorganic radioactive isotopes (radiometals) are conjugated via a chelator that binds to the metal ion

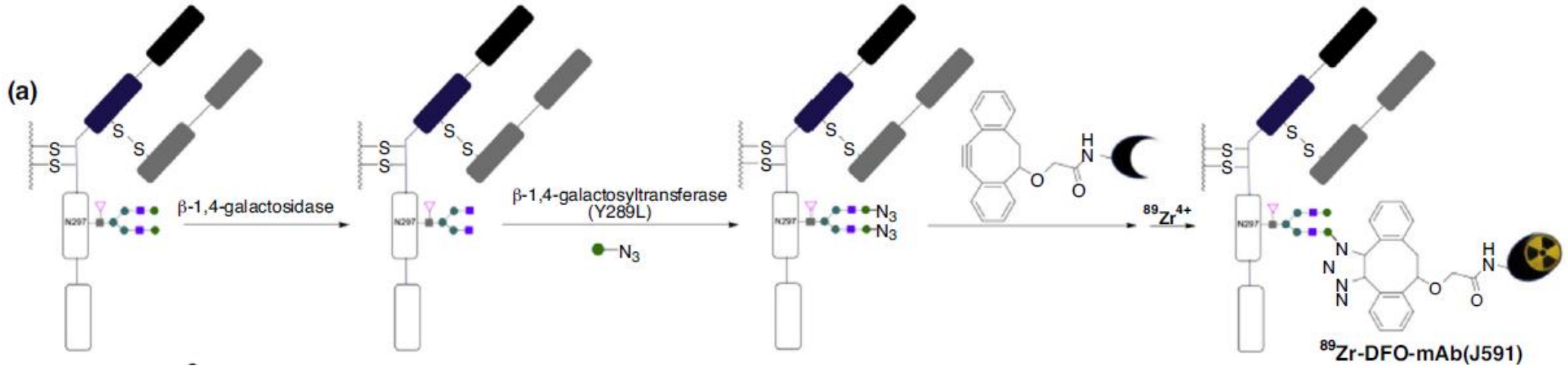
- a bifunctional macrocyclic chelating agent 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA).
- Bioconjugation to the antibody is achieved by the formation of covalent amide linkages using NHS-activated DOTA residues



^{67}Ga-NOTA-mAb

Increase stoichiometric control and site-specificity

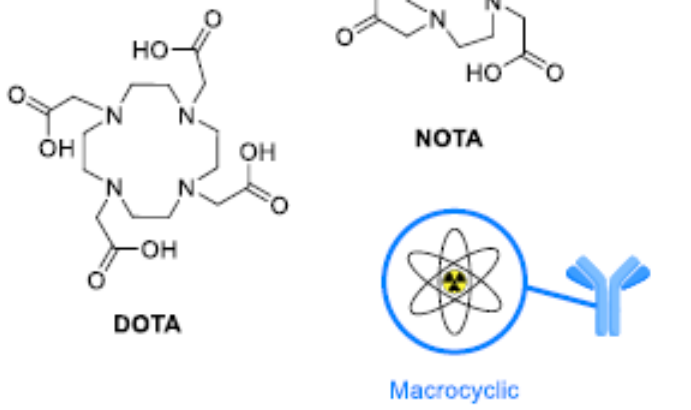
- Cys modification
- Glycan modification including enzyme-mediated conjugation…

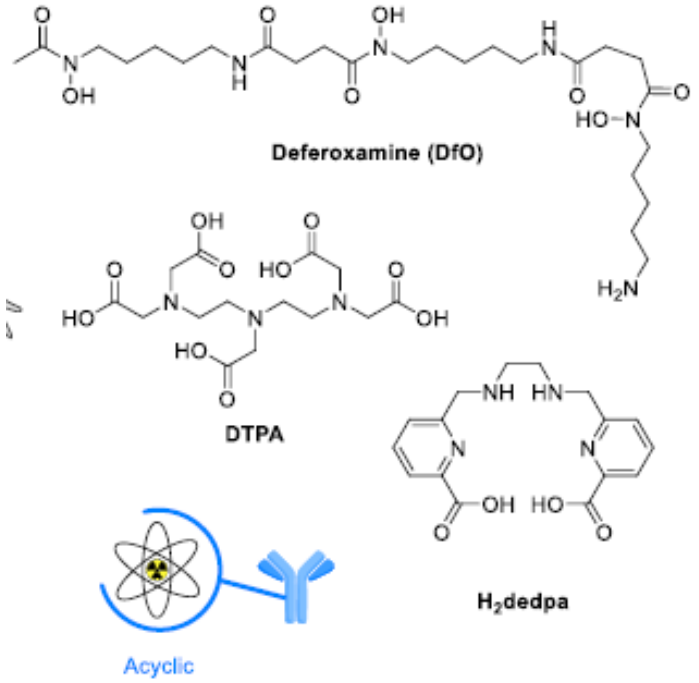


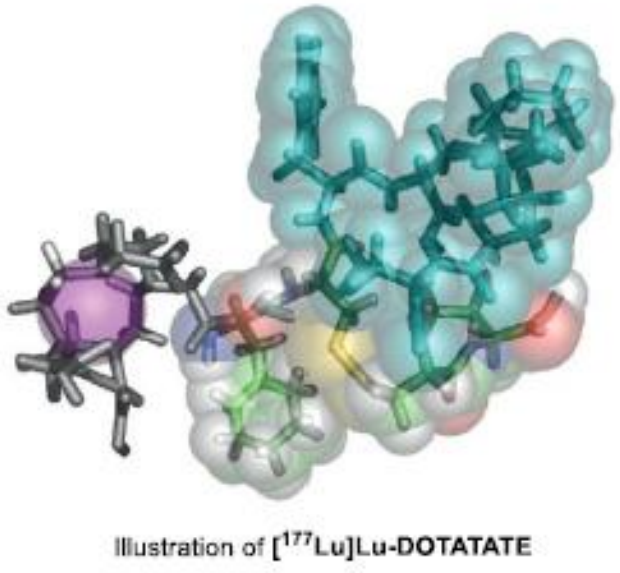
Source: Mauricio Morais*, Michelle T. Ma, Drug Discovery Today: Technologies Vol. 30, 2018

Starting material emerging chelators

- **In case linkers or chelators are utilized, these are in general small synthetic peptides and, in general, the protected amino acids are considered regulatory starting materials for synthetic peptides.**







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

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.

Radiolabelling method

- The relatively short half-life of the used radionucleotide requires that they are to be quickly chelated by suitable ligands, at the same time avoiding harsh conditions in terms of temperature and pH

- For GMP production radiosynthesis carried out as continuous process composed of automatically performed steps is preferred over manual production.

- Radiolabelling might be carried out by the manufacturer or by the end-user

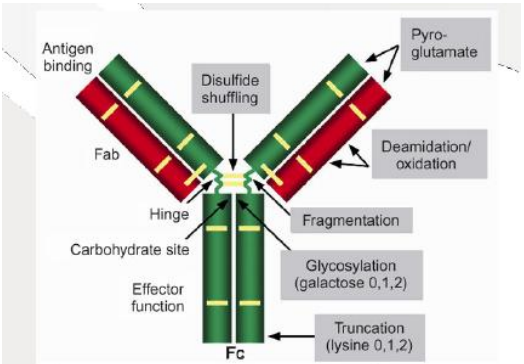
  - carried out by the manufacturer: After labelling, the final product is aseptically dispensed into suitable container closure systems intended for patient administration and immediately stored at the recommended storage condition according to the shelf-life stability

  - carried out by the user: This is likely to be in the form of a radiopharmaceutical kit consisting of a (derivatised) monoclonal antibody, reagents and materials necessary for the radiolabelling procedure, including any necessary purification of the product, plus a package insert giving clear, precise instructions for the use of the kit, quality control and potential hazards

Characterisation/Specifications

Antibody Specifications

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



Nature Biotechnology 22, 1383 - 1391 (2004)

Chelator

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.*

Radionucleotide

Revised guideline on quality for Radiopharmaceuticals

The specifications set for chalator and radionucleotide (and DS/DP?) 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.

Ready for use radiopharmaceutical				
specification parameter	*Radiolabelled active substance*[5]	*chemical precursor*	*radionuclide precursor*	*finished product*
radionuclidic ID	✓		✓	✓ *
radionuclidic impurities	✓		✓	✓ *
radiochemical ID	✓		✓	✓
radiochemical purity	✓		✓	✓
radioactive concentration	✓		✓	✓
specific activity	✓			
chemical purity	✓	✓	✓	✓
chemical ID		✓		
assay		✓		
residual solvents		✓		✓
terility**				✓
bacterial endotoxins**		✓		✓
microbial contamination		✓		
[5] May be tested on the finished product, when the radiolabelled active substance is not isolated during routine manufacture				
* if not tested on radiolabelled active substance				
** If required by the specific dosage form				

Specifications and quality control for the labelled drug product

- Identity:
  - identity of antibody and linker compounds according to characterization data
  - radiochemical identity, e.g. half-life
- Potency and quantity: immunoreactivity (antigen binding), radionuclide concentration at the time of synthesis (EOS) and at the time of calibration (TOC),
- Protein concentration, specific activity determined by dividing the strength (radioactive concentration) by the protein concentration.
- Purity: radiochemical purity, aggregation, chemical impurities (conjugating material, reagents used in derivatization of the antibody, labelling reagents, etc.).
- Understanding of impurities including residual solvents in case they are used in the process are crucial to understand the overall impurity profile and should be reflected in the control strategy at the adequate process stage
- Safety: sterility, endotoxin, dye ingress, filter integrity (IPC).
- Stability: Data need to be provided supporting conformance with the stability specifications limits through the proposed storage time under the proposed storage conditions. Special emphasis should lay on the stability of the conjugate, e.g. amount of free radionucleotide needs to be closely monitored.

Release and Stability Acceptance Criteria for Radiolabeled Drug Product activity (potency?)

Test	Method	Acceptance criteria (release)	Acceptance criteria (Stability e.g. 96+3h)
Protein	UV	x.xx-x.xx mg/ml	x.xx-x.xx mg/ml
Radioactive Concentration at End of Synthesis (EOS)	Dose Calibrator	xxx MBq/ml-xxx MBq/ml (range)	N/A
Radioactive Concentration at Time of Calibration (TOC)	Dose Calibrator	≥xxx MBq/ml	N/A
Antigen Binding	ELISA	80-120%	80-120%

Note: the new pharma legislation: add the *radioactivity* on the label (shielding shall and vial, the amount of radioactivity per dose or per vial)

Thank You!