Comparability strategies for Bioconjugates

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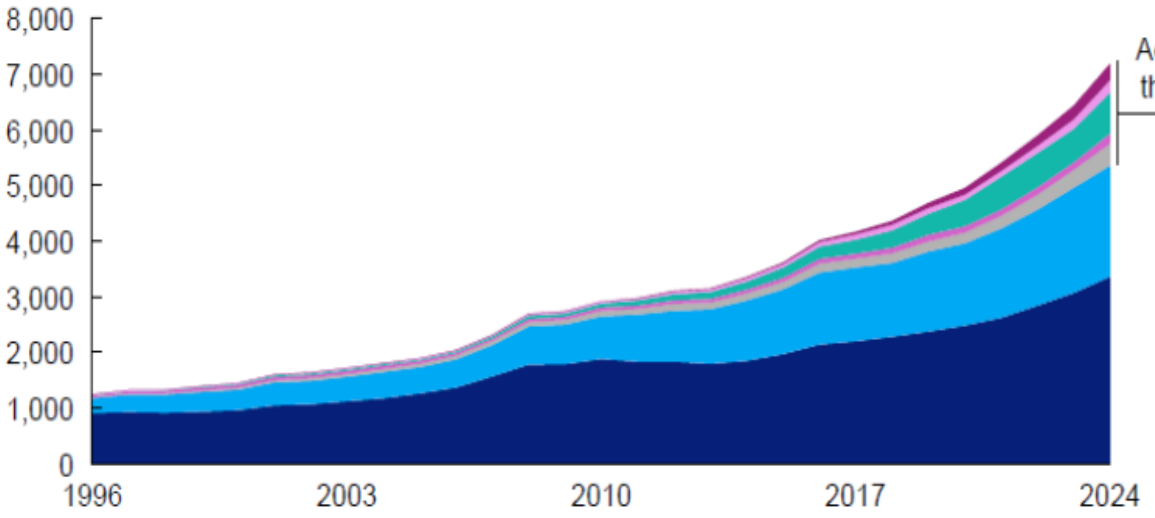
Agenda

1. Introduction to Bioconjugates
2. Comparability considerations
3. Case studies
4. Summary and conclusions

Relevance of Bioconjugates is expected to increase

Size of global pipeline by therapeutic modality
Number of products[1], from Phase I to III



Modality	Portfolio growth CAGR 2020-24	Expected revenue growth CAGR 2020-30
Multispecifics	26%	26%
ADCs and other Bioconjugates	23%	29%
Cell Therapy	12%	39%
Gene Therapy	12%	36%
Others[2]	19%	27%
Conventional Biological therapies[3]	8%	9%
Conventional Chemical therapies[4]	8%	4%

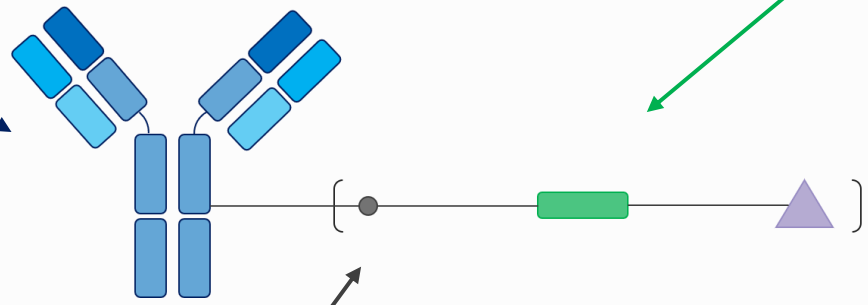
1. Innovative drugs only, excluding reformulations, biosimilars, digital therapeutics, diagnostics & imaging; development status based on most progressed indication
2. Includes Oligos, mRNA, viral terapies (excluding gene therapy), etc.
3. Includes mAbs, Proteins, Biological Peptides, Anti-infective vaccines, etc.
4. Includes small molecules, peptides, etc.

Source: Pharmaprojects; McKinsey analysis

Bioconjugates "*Same Same but Different*"

ANTIBODY
- IgG (native / engineered)
- multi-specific mAbs
- mAb fragments

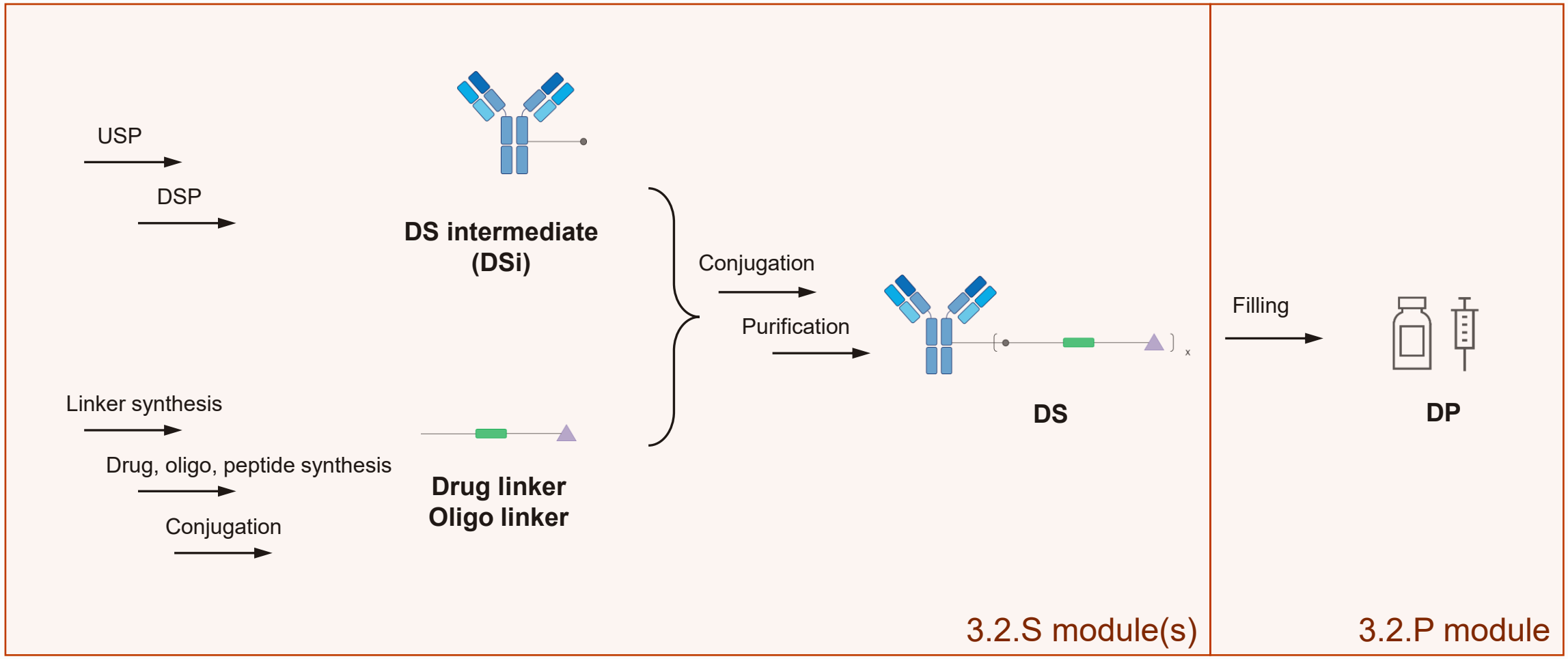
LINKER
- cleavable
- non-cleavable



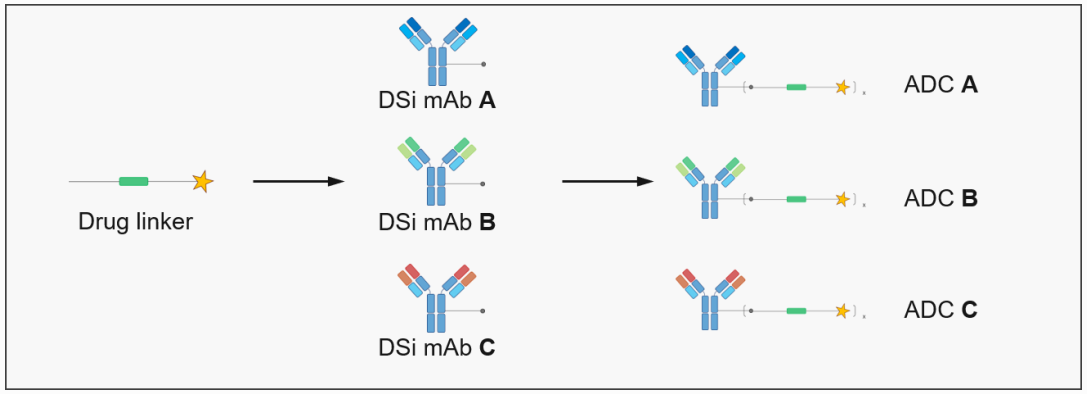
ATTACHMENT
- cysteine (native / engineered)
- lysine (native)
- glycans

ACTIVE MOIETY
- cytotoxic drug
- RNA (siRNA, PMO, ASO)
- peptide
- chelator / radionuclide

Manufacture of Bioconjugates follows a step-wise approach

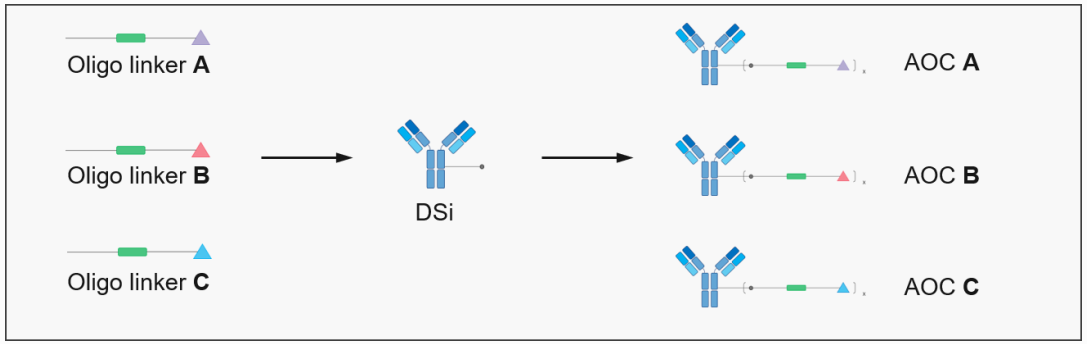


Manufacture of Bioconjugates follows a step-wise approach



ADCs

- mAbs with different target specificity may be leveraged
- deliver the same cytotoxic payload to cancer cell via different surface antigens



AOCs

- different oligos could be conjugated to the same mAb
- address different genetic disorders in the same target cell population

Comparability and supply can become very complex if not managed properly !

Comparability - Basic Principles (ICH Q5E)

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

COMPARABILITY OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

Q5E

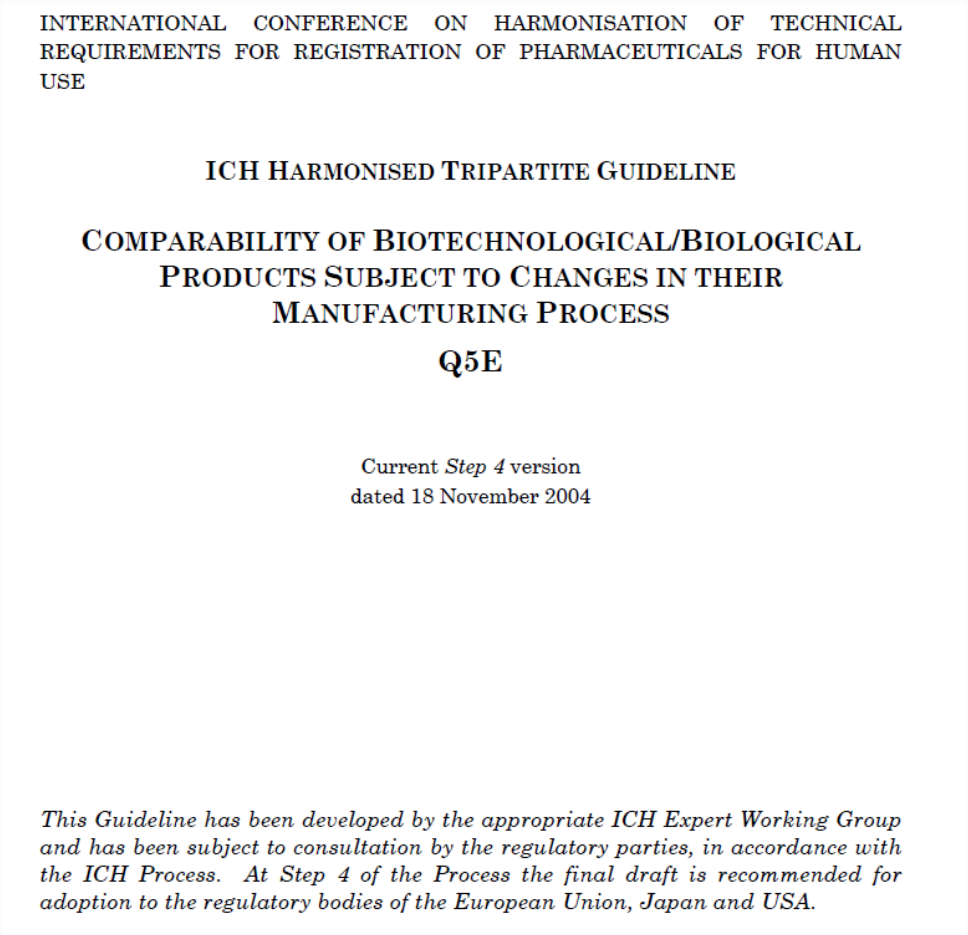
Current *Step 4* version
dated 18 November 2004

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

Scope: "*... Proteins and polypeptides, their derivatives, and products of which they are components,* ***e.g., conjugates*** *...*"

Intention: *"The demonstration of comparability does not necessarily mean that the quality attributes of the pre-change and post-change product are identical, but that they are* ***highly similar*** *and that the* ***existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety or efficacy of the drug product****."*

Comparability - Basic Principles (ICH Q5E)



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*"... the product should be **evaluated at the process step most appropriate to detect a change in the quality attributes**. This may entail evaluating the product at multiple stages of manufacture."*

*"Typically, **re-evaluation/re-validation activities for a simple change might be limited to the affected process step**, if there is no evidence to indicate that there is impact on the performance of subsequent (downstream) process steps, or on the quality of the intermediates resulting from the subsequent steps."*

*"**Critical control points in the manufacturing process** that affect product characteristics, e.g., the impact of the process change on the quality of in-process materials, as well as the **ability of downstream steps** to accommodate material from a changed cell culture process ..."*

Comparability - Risk Based Approach

Iyer & Lequeux, 2020 (GSK & BioPhorum)

CASE STUDY

Risk-Based Approach for Analytical Comparability and Comparability Protocols

KAVITA RAMALINGAM IYER[1,*,#] and ISABELLE LEQUEUX[2]

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ABSTRACT: Chemistry, manufacturing, and control postap
pharmaceutical products. In this paper, the authors exami
quality, safety, and efficacy of biologics. Comparability s
introduced as one of the tools that can support both biom
safety and product safety and efficacy is maintained throu
based review approach based on product and process kno
ensure that the product is "essentially similar", what co
Guidelines principles and definitions are used throughout t
ces. Finally, two case studies are presented: change to the
to the manufacturing process of a drug substance intermed

KEYWORDS: Biologics, Comparability, Postapproval chan
ICH Guidelines.

Blümel *et al.*, 2024 (IQ)

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Review

Patient-centric Comparability Assessment of Biopharmaceuticals

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Bechtold-Peters *et al.*, 2023 (EFPIA)

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Perspective

CMC Regulatory Considerations for Antibody-Drug Conjugates

Karoline Bechtold-Peters[a,*], Andrea Ruggiero[c], Nienke Vriezen[e], Nathan Ihle[f], Armin Klein[g], Charles Morgan[h,k,1], Daniel Schweizer[a], Dengfeng Liu[i,o,1], Fred Jacobson[k,1], Jakob Buecheler[a], Mark Panek[l], Naomi Duggan[g], Padma Malyala[m], Philippe Dupraz[c,1], Priyanka Desai[d,1], Shufang Niu[b], Yiqing Feng[n], Xiangyang Wang[j,o,1]

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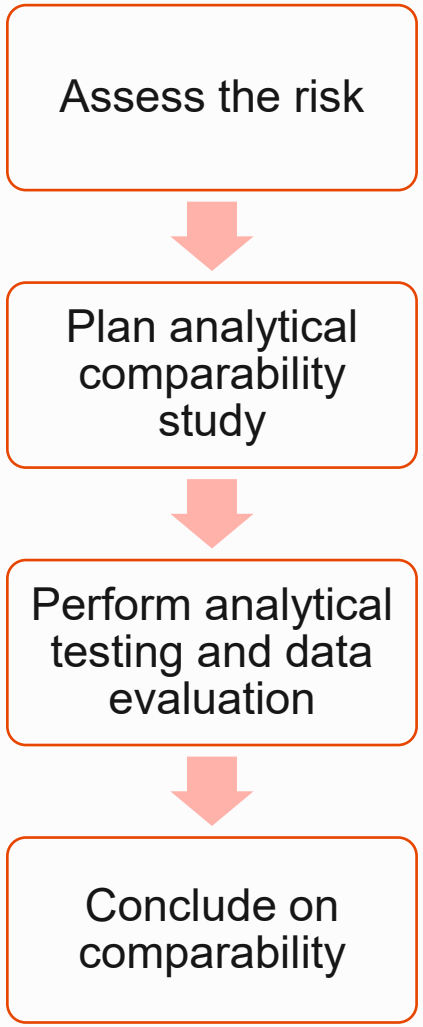
Comparability - Risk Based Approach for Bioconjugates



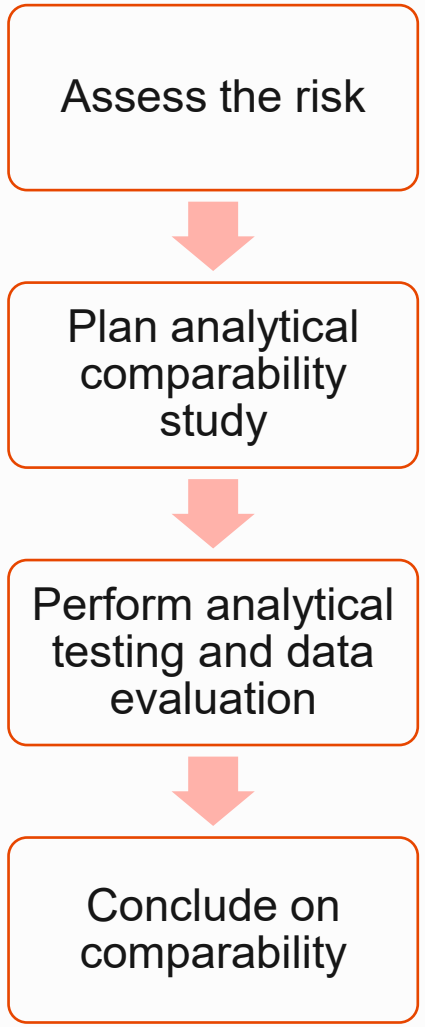
Comparability - Risk Based Approach for Bioconjugates

1. Understand product and process i.e., potential impact of changed production step(s) on critical quality attributes of the product

➢ *Design analytical comparability study to assess quality attributes of pre- and post-change batches at the changed production step*

2. Consider the potential impact of the change on downstream production steps and related quality attributes
   - e.g., downstream purification steps deplete related impurities of the drug-linker intermediate
   - e.g., oxidation status of mAb cysteines may impact DAR

➢ *Identify points of control downstream of the change, which could include in-process tests or formal release / stability testing, to mitigate any remaining risks*



Comparability - Risk Based Approach for Bioconjugates

- Drug-linker intermediates and related impurities are small molecules - they are well characterized
- Checkpoints at the changed production step provide best opportunity for control
  - e.g., small molecule impurities are easier to analyze in the absence of interference through mAb
  - e.g., process related impurities of the mAb production like rDNA or HCP are solely derived from the cell culture process
- Non-conjugatable impurities are effectively cleared by a purification step after conjugation
- Conjugatable impurities cannot be removed after conjugation but due to their small molecular weights they constitute an extremely small quantity of the administered dose

$$\text{Daily Impurity Dose}\left(\frac{\text{mg}}{\text{day}}\right) = \frac{\text{Dose (mg)} \times \frac{\text{Impurity\%}}{100} \times \text{DAR} \times \frac{\text{Impurity MW}}{\text{ADC MW}}}{\text{Dose Frequency (days)}}$$

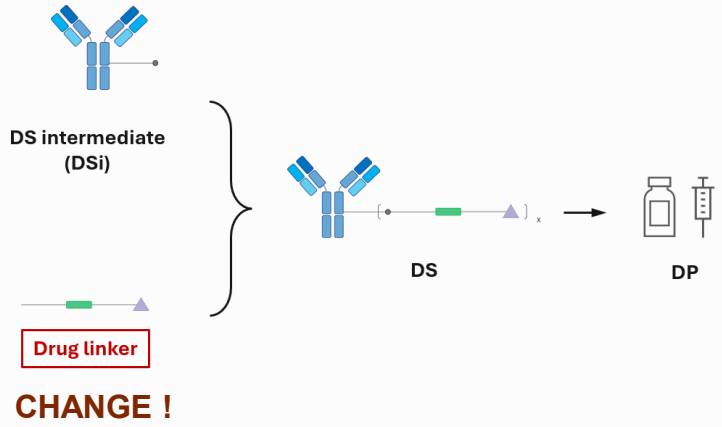
Mass dilution effect see also
Gong *et al*., 2018
Chahrour *et al*., 2026

Case Studies

Case Study 1a: Change in Drug-linker Production (ADC)

Stage of development and manufacturing process change

- Phase 1 resupply, oncology (S9) indication
- weekly dose of 600 mg ADC
- average DAR ~2.0
- Change in drug-linker manufacture
  - same synthesis route
  - optimized drug-linker purification
- No changes in DSi, DS and DP manufacture
- Drug (toxin) may form a structural isomer that is inactive



Case Study 1a: Change in Drug-linker Production (ADC)

Risk assessment

- Generally low risk for presence of new / other drug-linker related impurities
- Overall purity of drug-linker is expected to be higher
- Purification of ADC after conjugation with drug-linker by UF/DF (7 DVs, ~99.9% removal capacity)
- Unspecified impurities at drug-linker release: NMT 3.0%
  - translates into max. dose of impurity of ~57 µg/day (conjugatable) and ~5 µg/day (non-conjugatable), which is well below the 1 mg/day impurity identification & qualification threshold as defined by ICH Q3A
  - ICH M7 does not apply (oncology, S9 indication)
- Free drug-linker, residual solvents, DAR incl. unconjugated antibody and %active drug isomer are controlled at DS release
- A fully formulated DS batch made from post-change drug-linker has been placed on stability

Case Study 1a: Change in Drug-linker Production (ADC)

Conclusion

Based on ...

a) established process understanding and RA of the related change

b) release testing for drug-linker related impurities

c) theoretical considerations on clearance of non-conjugatable and conjugatable impurities

d) additional controls at DS release and stability

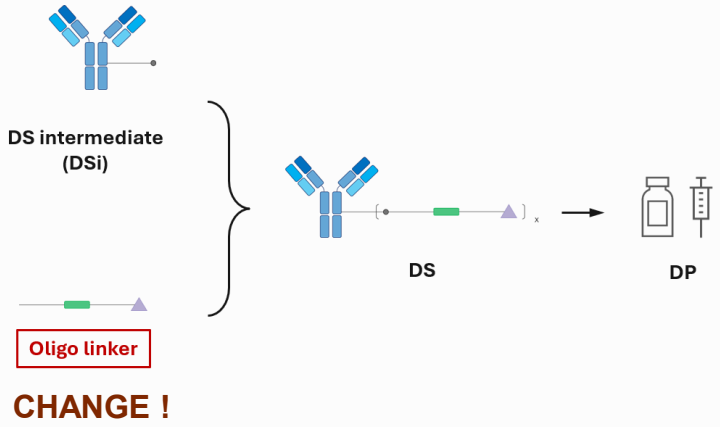
Perform comparability study on drug-linker level + provide comparative data from DS release and stability testing.

No additional comparability study at ADC DS and DP level will be considered.

Case Study 1b: Change in Drug-linker Production (AOC)

Stage of development and manufacturing process change

- Pivotal clinical study stage / commercial supply. non-oncology (non-S9) indication
- dose of 1000 mg AOC every 28 days
- average DAR ~4.0
- Change in oligo-linker manufacture
  - different synthesis route i.e., less synthesis steps
  - RP purification step applied prior to deprotection to enable more efficient impurity separation
  - use of different linker reagent
  - replaced potentially toxic solvents / reagents
- No changes in DSi, DS and DP manufacture



Case Study 1b: Change in Drug-linker Production (AOC)

Risk assessment

- Overall purity of oligo-linker is expected to be higher
- Replacement of potentially toxic solvents and reagents is expected to mitigate safety risks
- Quality of starting materials post-change is equal or better
- Purification of AOC after conjugation with oligo-linker by UF/DF (7 DVs, ~99.9% removal capacity)
- Unspecified impurities at oligo-linker release: NMT 1.0%
  - ensures impurities are below identification & qualification thresholds as defined by ICH Q3A
  - does NOT ensure presence of potential mutagenic impurities below M7 requirements
- Exclude presence of potential mutagenic impurities above M7 requirements by extended characterization of oligo linker batches using LC-MS/MS, qNMR and theoretical considerations
- Free oligo-linker, residual solvents, DAR incl. unconjugated antibody are controlled at DS release
- A fully formulated DS batch made from post-change oligo-linker has been placed on stability

Case Study 1b: Change in Drug-linker Production (AOC)

Conclusion

Based on ...

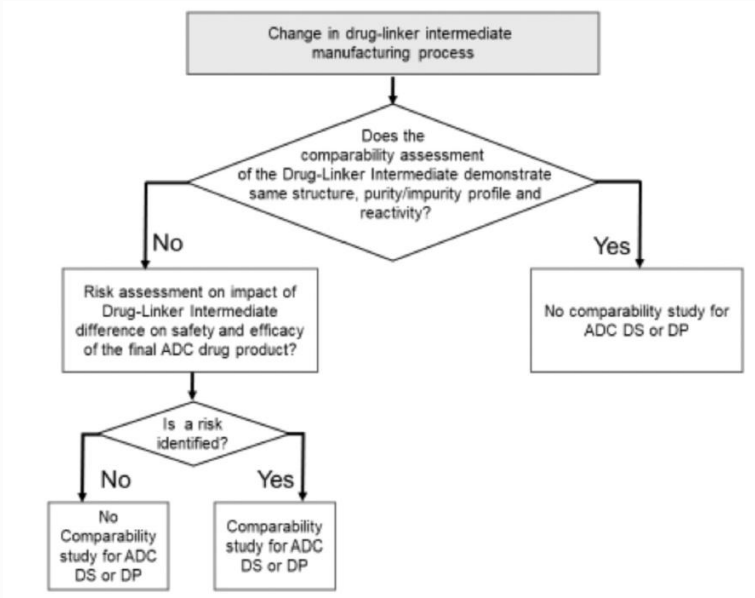
a) the established process understanding and RA of the related change

b) release testing for oligo-linker related impurities

c) extended characterization of oligo-linker batches

d) theoretical considerations on clearance of non-conjugatable and conjugatable impurities

e) additional controls at DS release and stability



see also Bechtold-Peters *et al.*, 2023

Perform comparability study on oligo-linker level + provide comparative data from DS release and stability testing.

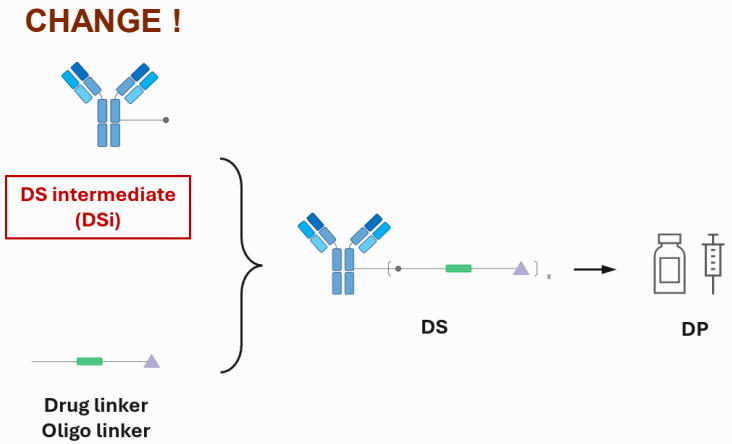
No additional comparability study at AOC DS and DP will be considered.

Extended characterization on DS level to be considered in case potential mutagenic impurities above level of concern were identified by LC-MS/MS characterization of oligo-linker batches.

Case Study 2: Change in mAb Production (AOC)

Stage of development and manufacturing process change

- Pivotal study & commercial supply, non-oncology (non-S9) indication
- Change in DS intermediate (mAb) manufacture
  - DSi manufacturing site transfer (like-for-like)
  - minor change in DSP i.e., increased column load
  - no changes in oligo-linker, DS and DP manufacture
  - conjugation via native cysteines of mAb, target DAR ~4.0



Case Study 2: Change in mAb Production (AOC)

Risk Assessment

- Potential impact on product related substances/ impurities as well as process related impurities
  - product related substances/ impurities are studied at release, stability and by extended characterization of DSi
  - redox status of native cysteines of mAb is studied by reducing and non-reducing peptide mapping mass spectrometry
  - clearance of process related impurities such as HCP, rDNA, and rProA is assessed by IPC testing
- Free oligo-linker, average DAR, oligo-linker distribution, and biological activity (potency by cell-based assay) are controlled at DS release
- 1 fully formulated DS batch made from post-change DSi has been placed on stability

Case Study 2: Change in mAb Production (AOC)

Conclusion

Based on ...

a) the established process understanding and RA of the related change

b) extensive characterization of mAb product related substances and impurities with a focus on residues critical for conjugation

c) comparable clearance of process related impurities

d) additional controls at DS release and stability

Perform comparability study DSi level + provide comparative data from DS release and stability testing.

No additional comparability study at AOC DS and DP will be considered.

Minor differences in product related substances that are understood to not impact downstream production steps were accepted.

Summary & Conclusions

- Comparability of ADCs and next generation bioconjugates require science and risk-based approaches based on ICH Q5E principles to manage complexity
- Regulatory guidelines relevant for chemically synthesized and biotechnology derived molecules need to be interpreted considering all steps of the bioconjugate manufacturing process
- Industry can leverage on deep molecule understanding, prior knowledge and platform approaches to frame science and risk-based approaches
- Potential differences in product quality should be evaluated at the process step most appropriate to detect a change in the quality attributes (point of control)
- Risks to product quality downstream of the manufacturing process change should be addressed by appropriate control strategies to avoid repetitive / partially redundant comparability assessments

Thank You !

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