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CASSS CMC Strategy Forum 2024

Overview of the landscape of bioconjugates: emerging applications and design considerations

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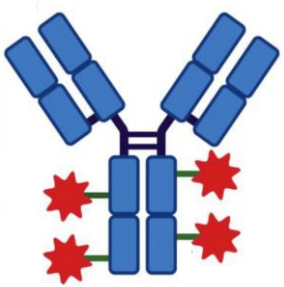
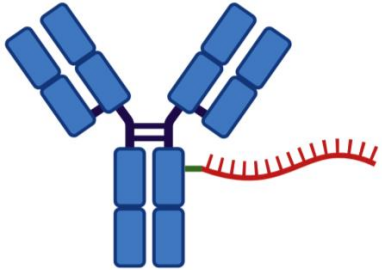
The wide world of bioconjugates

Antibody-drug conjugates

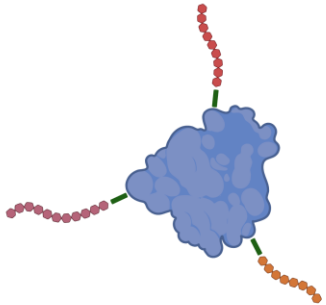


Antibody-oligonucleotide conjugates

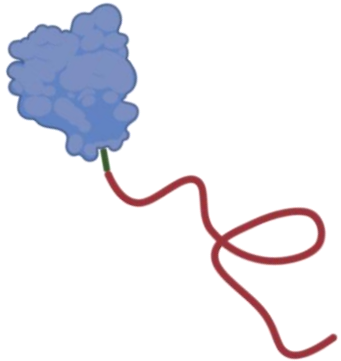


Imaging agents

Protein conjugate vaccines



Polymer-protein conjugates



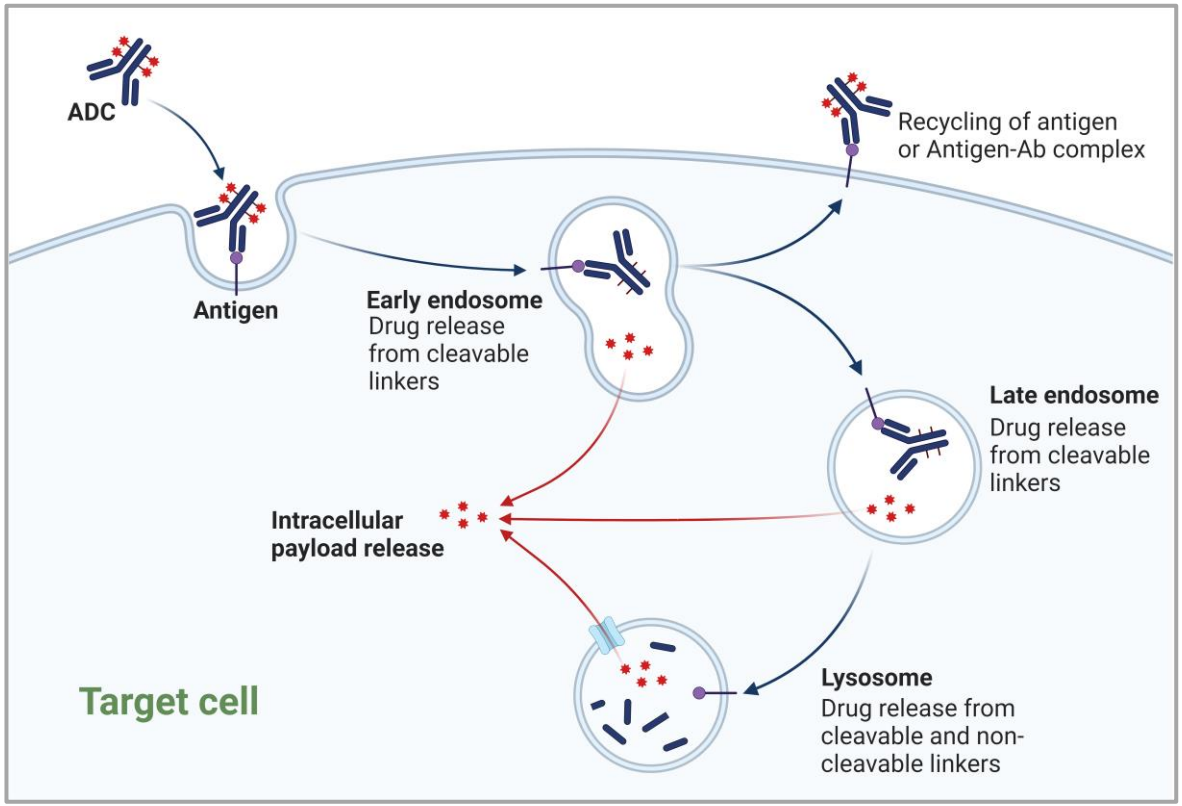
The “Magic Bullet”: Basic MOA of ADCs

Goal of first-generation ADCs:

Improve therapeutic index of potent cytotoxic drugs through targeted delivery to the cell of interest

Key therapeutic challenges:

- Lower than expected therapeutic windows
- Premature/off-target payload release *in vivo*
- On-target, off-tumor payload release

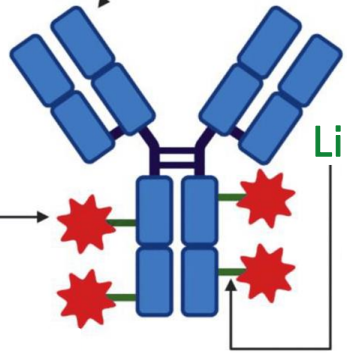


Adapted from “Antibody-Drug Conjugate Drug Release”, by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates

Key components of ADCs

Antibody

- High affinity and specificity for the target cell
- Binds an internalized Ag
- Tumor penetration determined by size, binding affinity, & Ag expression level
- Emerging strategies include bispecific Abs and conditionally activated Abs



Payload

- Microtubule inhibitors, DNA damaging agents, & topoisomerase inhibitors
- Lipophilicity impacts clearance and bystander effect
- Drug-antibody ratio (DAR) needs to be balanced for PK, efficacy, and tolerability
- Next-generation payloads include degraders, immune activators, & antibiotics

Linker

- Linker design is critical for payload stability and release
  - Acid, disulfide, or enzymatically cleavable linkers
  - Non-cleavable linkers may enhance serum stability but prevent bystander effect
- Conjugation chemistry determines DAR and its distribution

CMC challenges

- **Stability:** linker instability leading to payload release; aggregation/insolubility due to hydrophobic payload
- **Manufacturing:** complex with multiple critical intermediates; cytotoxic drugs require special handling
- **Analytics:** characterization of DAR distribution

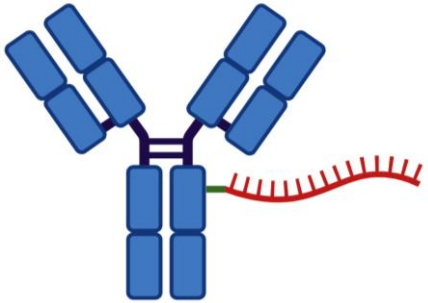
Antibody-oligonucleotide conjugates build on design principles established by ADCs

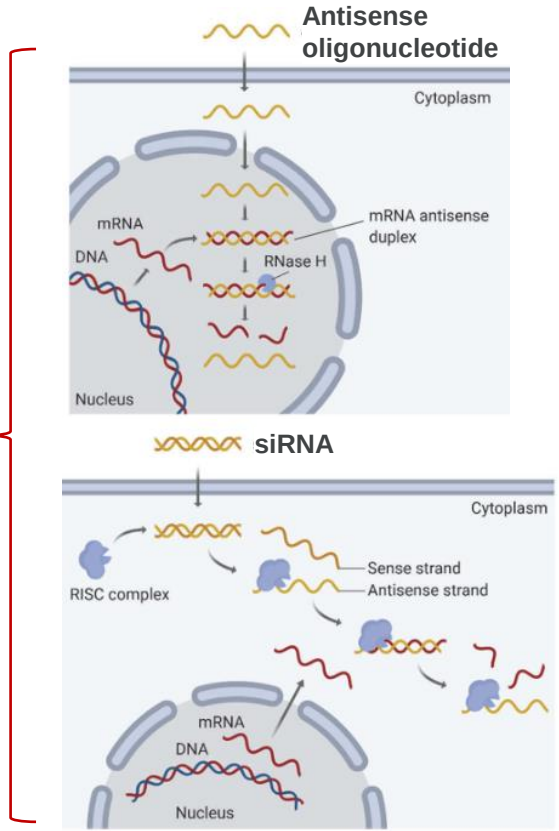
Motivation for AOCs:

- ASOs and siRNAs enable long-term knockdown at the mRNA level, but their ability to cross cell membranes or the blood-brain barrier is limited
- Current clinical applications include delivery to muscle cells and across the BBB

Key therapeutic challenges for AOCs

- Endosomal escape
- Serum stability (e.g. nuclease resistance)
- Limited oligo loading





CMC challenges:

- **Stability:** large, negatively charged oligo may significantly impact physicochemical properties of the antibody
- **Manufacturing:** high cost and low yield
- **Analytics:** oligo-antibody ratio characterization, bioassay development

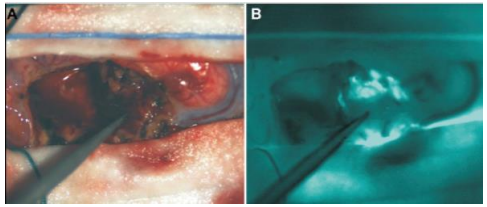
Tromp TR, et al. (2020) Expert Opin Investig Drugs 29:483–493

Design criteria for imaging agents differs significantly from ADCs and AOCs

Applications:

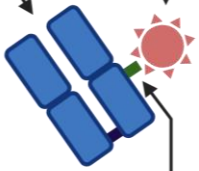
- Diagnostics/theranostics
- Visual surgical aid
- Related: radioimmunotherapy & photodynamic therapy

Glioblastoma imaging with peptide-fluorophore conjugate (Tozuleristide):



Carrier

- mAb, fragment antibody, or peptide
- Smaller size may improve tumor penetration
- Reduced serum half-life decreases background signal



Imaging agent

- Radionuclide
  - PET vs SPECT imaging
  - Half-life selection is critical
  - Supply chain considerations
- Fluorophore
  - NIR dye for better tissue penetration
  - Newer approaches use turn-ON dyes

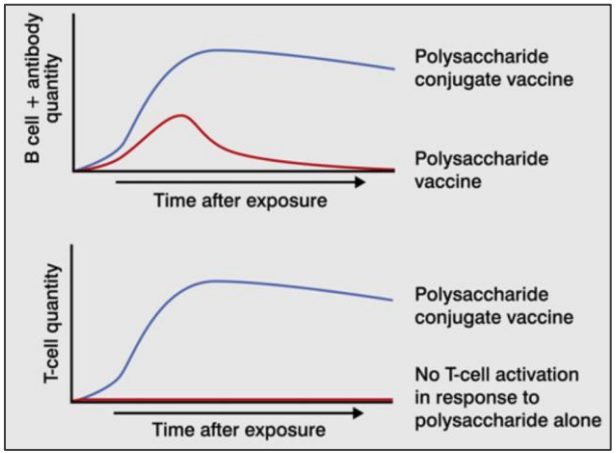
Conjugation strategy

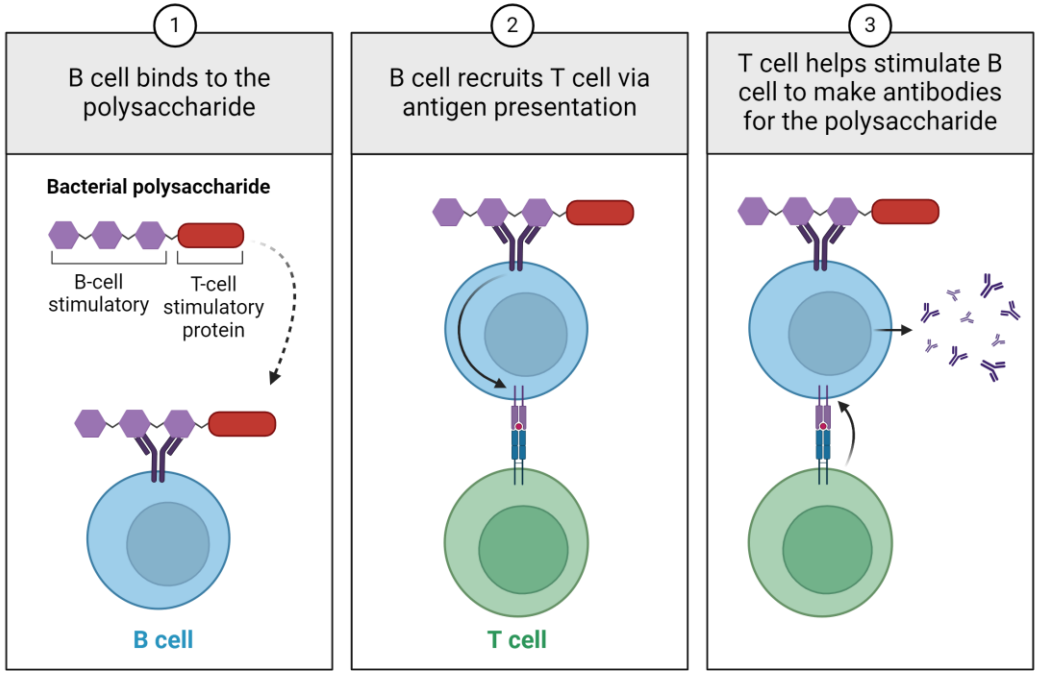
- Stable and non-cleavable: Lys and Cys typically used for conjugation
- Chelators for radionuclides must be stable with fast radiolabeling kinetics
- Conjugation sites & ratio may influence physical stability or biodistribution

Patil, CG et al (2019) Neurosurgery 85: E641-E649

Protein conjugate vaccines enhance the immune response to polysaccharide antigens

MOA: Conjugation of bacterial polysaccharides to immunogenic proteins enhances the immune response by promoting T-cell dependent responses





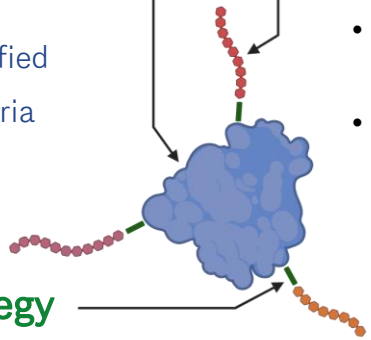
Strugnell R et al (2011). Perspect Vaccinol 1:61–88

Adapted from "Conjugate Vaccine Uses Linked Recognition Between B and T Cells", by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates

Key design criteria for protein conjugate vaccines

Protein

- Protein known to elicit a strong immune response
- Inactivated/purified from pathogen fermentations or genetically detoxified
- Examples: Tetanus Toxoid, Diphtheria Toxoid, or CRM197



Polysaccharides

- Multiple serotypes are typically conjugated
- Most licensed vaccines use polysaccharides purified from microbial cultures
- Recent improvements in chemistry have enabled chemically synthesized antigens

Conjugation/linker strategy

- Protein: conjugation to lysines or site-specific
- Polysaccharide: random vs terminal activation
- Non-specific conjugation strategies can mask T-cell epitopes
- Linker immunogenicity can compromise efficacy

CMC challenges:

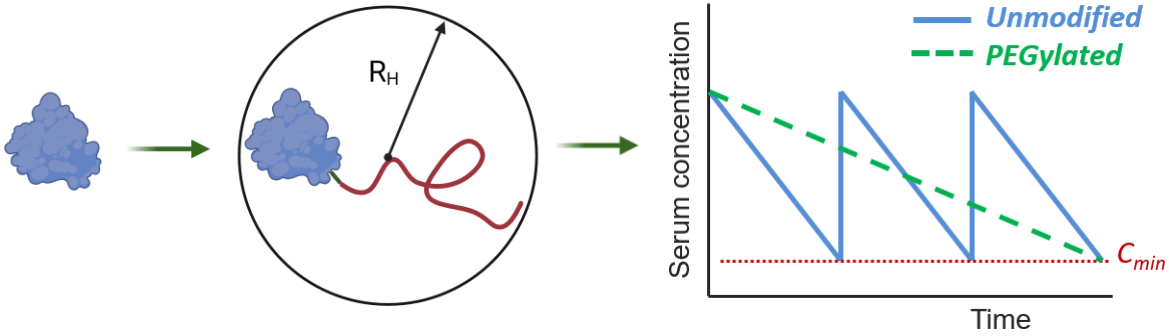
- **Analytics:** significant heterogeneity in the final DP
- **Manufacturing:** high cost and low process yield, reproducibility can be challenging
- **Stability:** polysaccharides may be chemically unstable in aqueous formulations

Polymer conjugates: half-life extension is well-established, but many new applications are emerging

Traditional applications:

1. Systemic half-life extension:
   Increased hydrodynamic size leads to reduced renal filtration
2. Reduced immunogenicity:
   Polymers may mask immunogenicity of non-human proteins

Systemic half-life extension

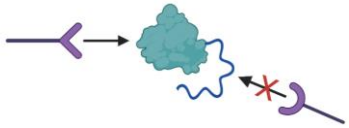


Emerging applications:

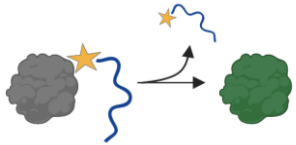
- Ocular delivery
- Altered specificity (e.g. cytokines)
- Polymer prodrugs
- High DAR ADCs
- Multivalent display

Ocular delivery

Altered specificity

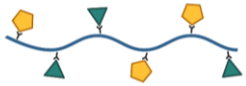


Polymer prodrugs



Next-gen ADCs

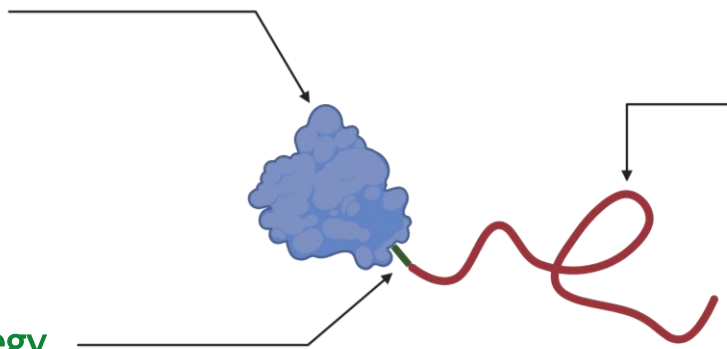
Multivalent display



Key components of polymer conjugates

Therapeutic protein

- Enzymes, growth hormones, cytokines, etc
- Typically lacks FcRn recycling



Polymer

- Majority of applications use PEG
  - Immunogenicity concerns have motivated the development of alternatives (e.g. zwitterionic polymers)
  - Biodegradable polymers extend upper size limit
- Synthetic (typically polydisperse) vs. genetically expressed (monodisperse, e.g. XTEN)
- R_H drives half-life

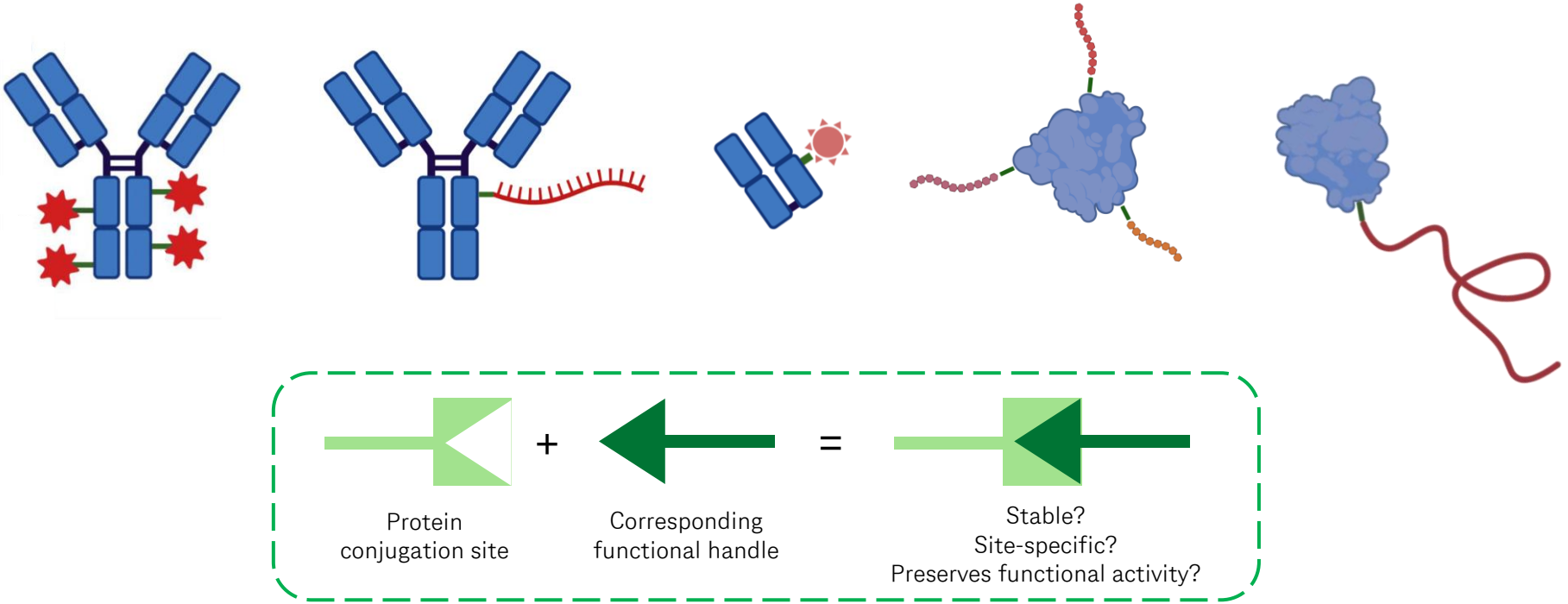
Conjugation strategy

- Conjugation site selection is critical to preserve activity of the protein
- Cleavable (prodrugs) or non-cleavable (half-life extension)

CMC challenges:

- **Analytics:** significant DP heterogeneity (driven by both polymer dispersity + conjugation chemistry)
- **Manufacturing:** low product yield, potential for high viscosity

Evolution of conjugation chemistries



Conjugation strategy is a fundamental challenge shared by all bioconjugates

First-generation bioconjugates used conjugation to primary amines

Chemistries: acylation (e.g. NHS esters), reductive amination

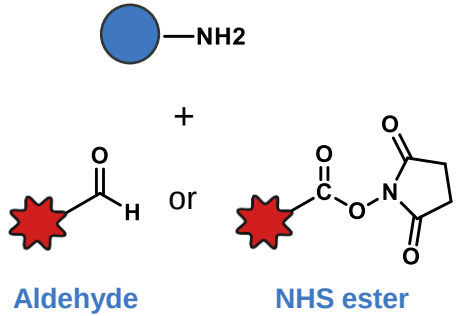
Examples: Kadcyla, Neulasta, Prevnar 13

Benefits:

- Stable covalent bond formation
- N-terminal amine can be targeted (more nucleophilic)

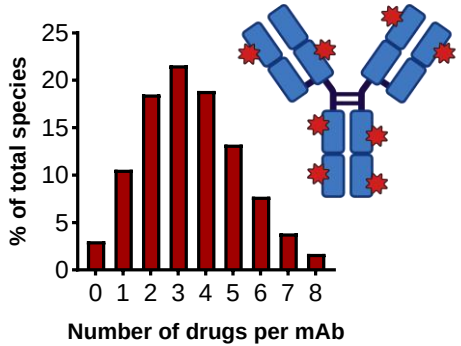
Key CMC challenges:

- Heterogeneous drug product
  - >20 solvent accessible Lys in a mAb
  - Significant optimization required to selectively target N-terminal amine
- Loss of activity upon conjugation (e.g. N-terminal NH_2 often close to binding site)



Aldehyde NHS ester

Conjugation to Lys (ADC example):



Second-generation bioconjugates used interchain disulfides or engineered cysteines

Chemistries: maleimide (covalent), disulfide (reducible)

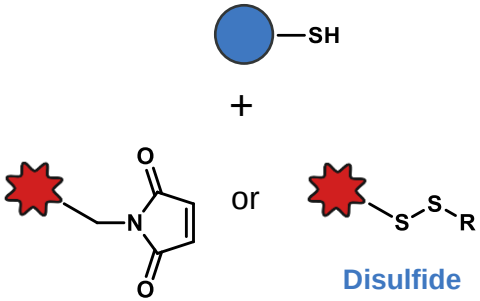
Examples: Polivy, Cimzia

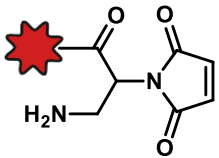
Key CMC challenges:

- Reduction + re-oxidation process adds complexity, may induce disulfide scrambling, and requires exposure to high pH (~8)
- Maleimides & their thiosuccinimide products are susceptible to deconjugation and hydrolysis

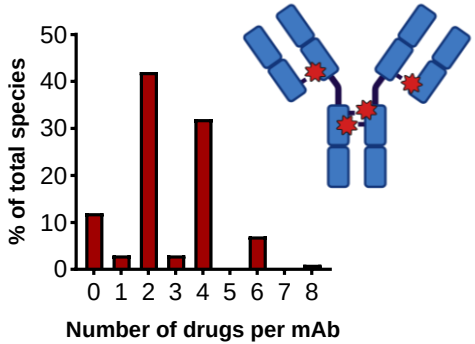
Other design considerations:

- Choice of conjugation site (thiol pK_a) can significantly impact deconjugation rates[1]
- Use of self-hydrolyzing maleimides may limit deconjugation[2]

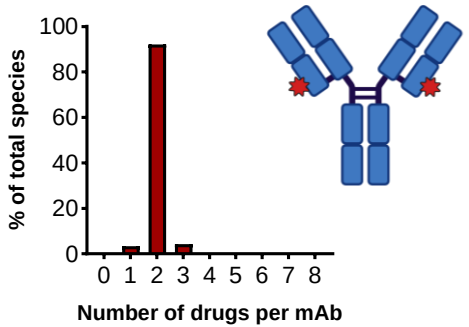




Interchain disulfides:



Engineered cysteines:



1. Vollmar BS et al (2017). Bioconjugate Chem 28:2538–2548
2. Lyon RP et al (2014). Nat Biotechnol 32:1059–1062

Hamblett KJ et al (2004). Clin Cancer Res 10:7063–7070

Next-generation conjugates: emerging strategies

Novel site-specific conjugation strategies may enable more homogeneous, stable conjugates

Strategy	Examples	Advantages	Clinical examples	Challenges
Enzymatic	Transglutaminase Sortase A Glycosyltransferase	Stable covalent bond formation with low molar eq enzyme	SOT-102 (Phase 1/2) ADCT-601 (Phase 1)	1. Requires production & removal of a 2nd protein 2. Optimization required to drive high efficiencies
Unnatural amino acids	Azido-Lys Para-acetyl Phe Para-azido Phe	1. High degree of control over conjugation site 2. Amenable to conjugation under mild conditions, high yield (e.g. click chemistry)	ARX788 (Phase 3) SAR444245 (paused after Phase 2)	1. Requires genetic code engineering 2. Immunogenicity risk 3. Low expression yields
Disulfide rebridging	Bis-sulfones Dibromo-maleimides/ pyridazinediones	1. Restores covalent linkage between chains 2. Does not require protein engineering	OBI-999 (Phase 2)	Need to control stoichiometry to avoid under- or over-conversion

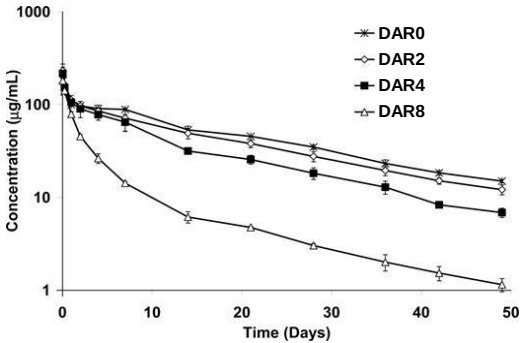
Examples of the diverse impact of site specific conjugation technologies on properties of bioconjugates

ADCs:
Greater control over DAR may avoid higher clearance and/or lower therapeutic windows associated with high-DAR species

Protein conjugate vaccines:
Non-specific conjugation strategies may mask T-cell epitopes on the protein

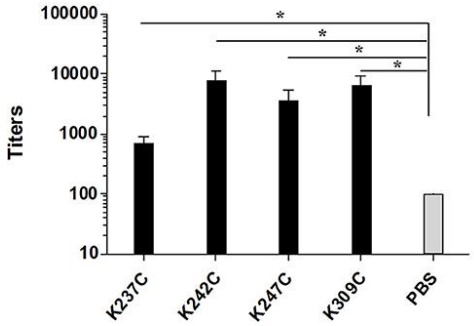
Polymer conjugates:
Site-specific conjugation may enable greater precision when identifying conjugation sites that impact binding or selectivity

Higher DAR species clear more rapidly:



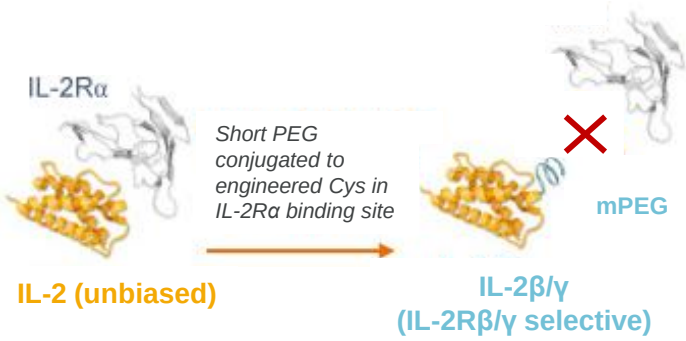
Hamblett KJ et al (2004) Clin Cancer Res 10:7063–7070

Conjugation site influences antibody titers:



Pillot A et al (2019) Front Chem 7:726

Site-selective polymer conjugation can bias cytokine activity:

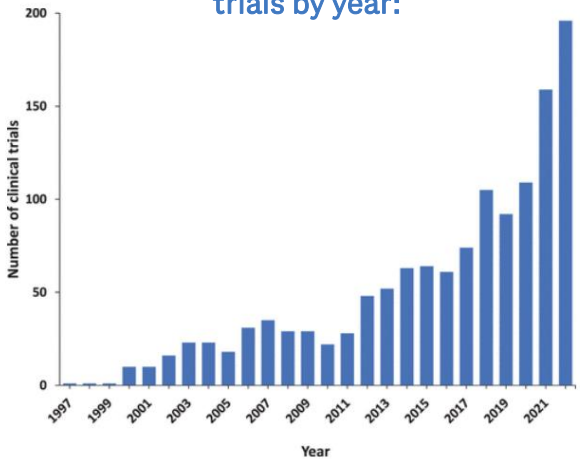


Adapted from Rosen DB et al (2022) J Immunother Cancer 10:e004991

Summary and future prospects

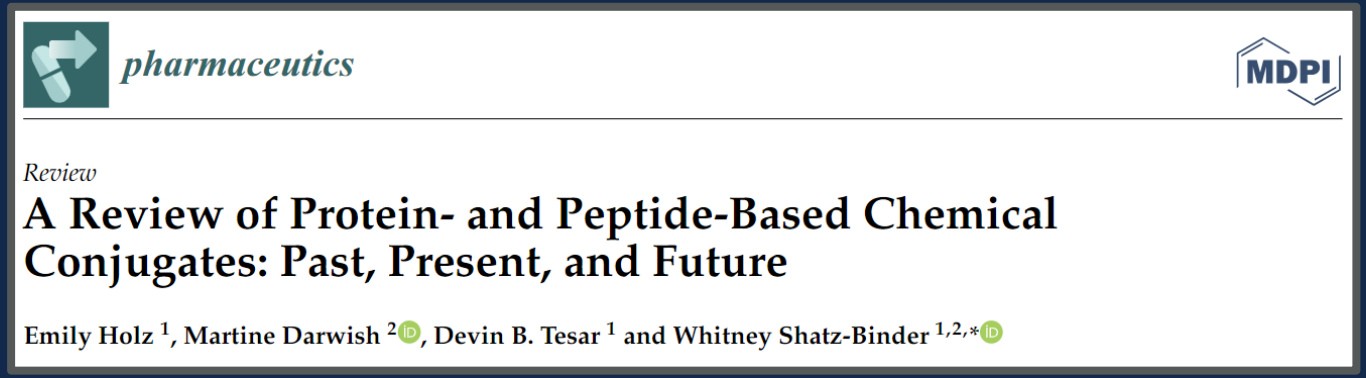
- Bioconjugates comprise a complex and diverse field including antibody-drug conjugates, antibody-oligo conjugates, imaging agents, protein conjugate vaccines, and polymer conjugates
- Recent advances in bioconjugates have been driven by improvements in linker stability, novel payloads, and a deeper understanding of the design criteria governing PK/efficacy
- Site-selective conjugation chemistries may enable lower CMC complexity, better therapeutic activity, or broader applications for bioconjugates
- The therapeutic potential of these conjugates is highlighted by a significant increase in the number of clinical trials today

Number of ADCs in clinical trials by year:



Sasso JM et al (2023) Bioconjugate Chem 34:1951–2000

Thank you!



pharmaceutics

MDPI

Review

A Review of Protein- and Peptide-Based Chemical Conjugates: Past, Present, and Future

Emily Holz [1], Martine Darwish [2], Devin B. Tesar [1] and Whitney Shatz-Binder [1,2,*]

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