Immunome™

Advancing the Next Generation of Targeted Therapeutics

The “5C” Network of Linker-Payloads: Chemistry, Conjugation, Characterization, and Control of the Cytotoxic Intermediate

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ADCs as the Foundation for Emerging Bioconjugate Candidates

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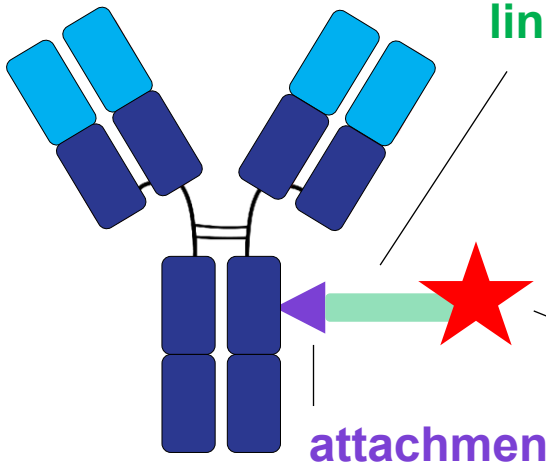
Targeting agent

- ❑ Antibody modality or format
  - **mAb**, HCAb, Fab, F(ab')2, sdAb, sdAb-Fc, ScFv
  - isotype/subclass
  - WT or engineered mutant
  - Fc effector function
  - valency, specificity
- ❑ Other protein binders, peptides, aptamers, etc.



linker

- ❑ non-cleavable
- ❑ **cleavable**
- ❑ spacer length
- ❑ aliphatic, PEGn
- ❑ linear, branched

payload

- ❑ Small molecules
  - **Cytotoxic drugs**
  - Immunomodulators
  - Protein degraders
  - Radiolabels/Chelators
- ❑ Oligonucleotides
  - siRNA
  - ASO
  - CpG
- ❑ Peptides, proteins

attachment chemistry

- ❑ First/second generation
  - Lysine-NHS ester
  - **Cysteine-Maleimide**
- ❑ "Next Gen" Approaches
  - Click chemistries
  - Enzymatic: Sortase, mTGase, Ftase, GlycoConnect®
  - Cysteine re-bridging
  - Proximity/peptide affinity
  - Non-canonical AA

>300 bioconjugates currently in clinical trials

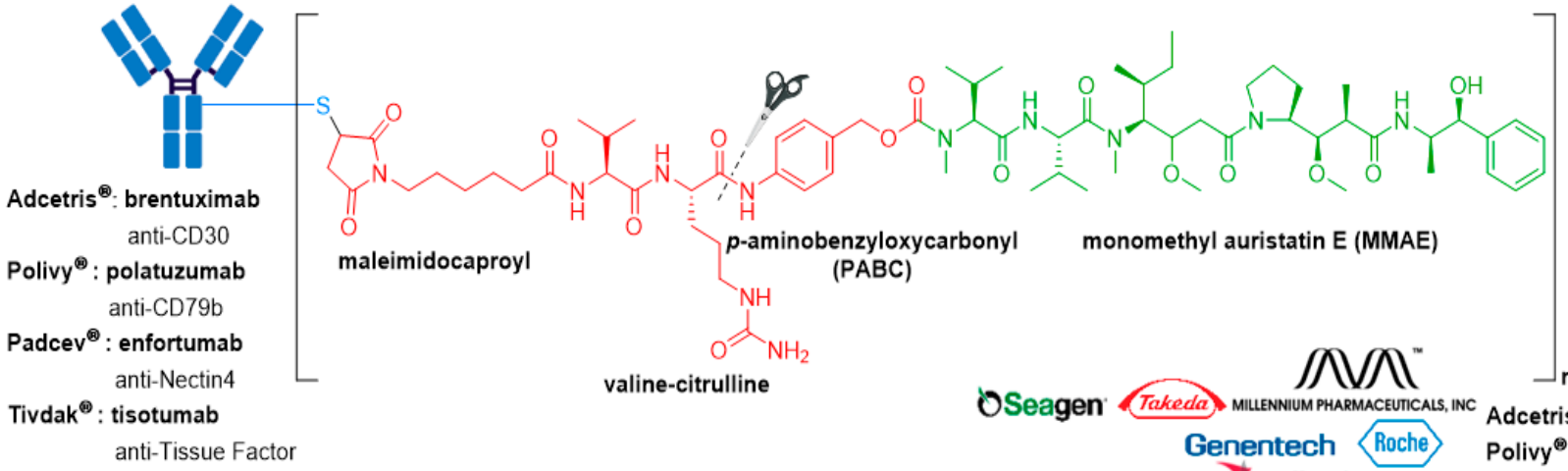
⬇

>200 of these bioconjugates are ADCs

9 of 13 commercial ADCs share many of the same features . . .

Chem. Soc. Rev., **2021**, 50, 1305.
Pharmaceutics **2023**, 15, 600.

Common Features of Commercial ADCs

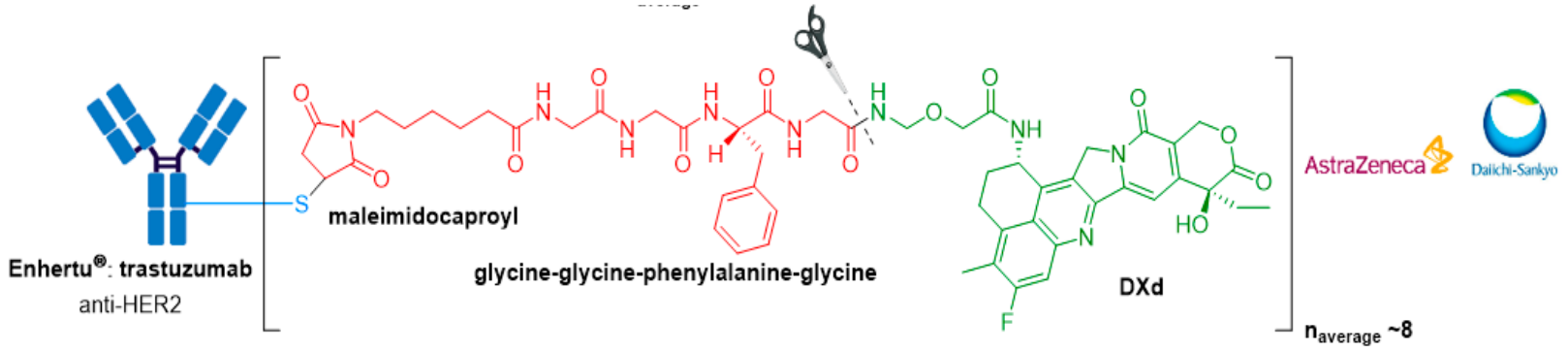


Cathepsin cleavable-linker appended to a small molecule cytotoxic agent imparting either microtubule inhibition or DNA damage

Seagen · Takeda · Millennium Pharmaceuticals, Inc · Genentech · Roche · Astellas · Seagen · Seagen · Genmab

Adcetris® ~4.0
Polivy® ~3.5
Padcev® ~3.8
Tivdak® ~4.0

Maleimide functional handle conjugated to the reduced, native interchain cysteine thiols of the IgG1 mAb at a **DAR of 4 or 8**



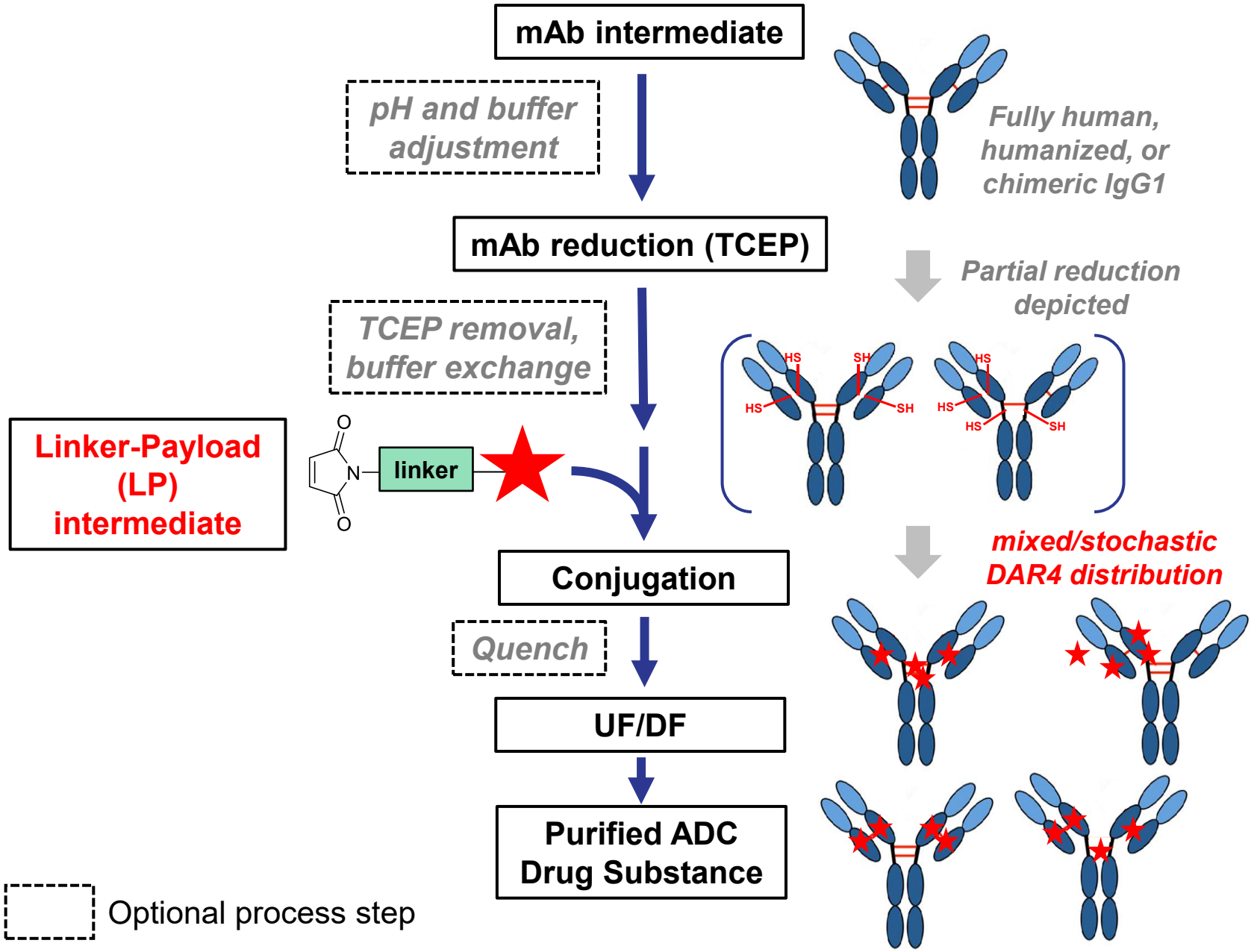
AstraZeneca · Daiichi-Sankyo

All ADC Linker-Payload (LP) molecules are generally . . .

- **Hydrophobic**
- **Highly potent (~nM to pM IC_{50} for free payload)**
- **MW ~1000 Da**

Molecules **2021**, 26, 5847.

General Scheme for ADC DS Manufacturing with Maleimide LP

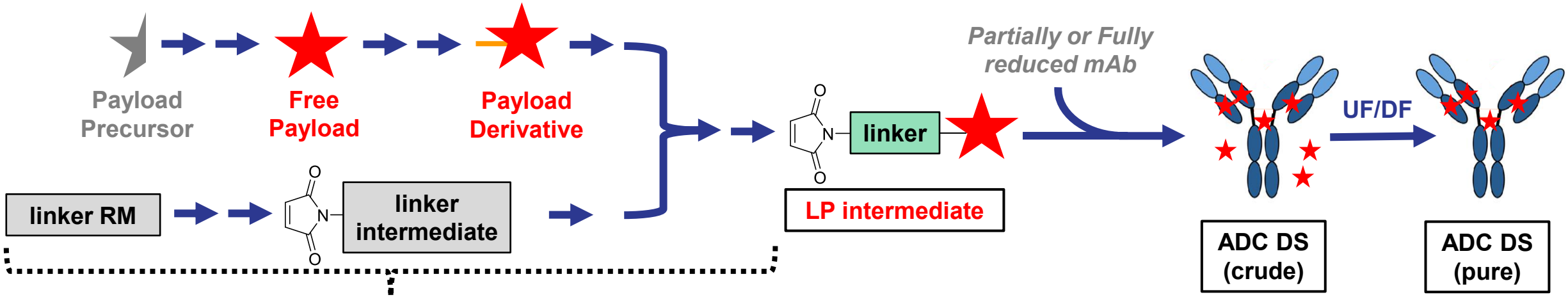


What are the expected Linker-Payload-associated impurities in your ADC DS and DP?

How do you control for them at the clinical and commercial stages?

How does their potency and toxicity influence your control strategy?

Generalized Scheme for Maleimide Linker-Payload Manufacturing



Raw Materials (Non-GMP Steps)

Starting Materials (GMP Steps)

- *Average ~3*
- *Range 0 to >6*
- *≥90% purity typical for both SMs and LP intermediate*

What are the classes of small molecule impurities that emerge during the Linker-Payload manufacturing and subsequent ADC DS/DP production?

Org. Process Res. Dev. **2023**, 27, 1248−1257.

Selected Specifications for LP Intermediate and ADC DS

Quality Attribute/Method	LP intermediate	ADC DS
Appearance (color, clarity)	✓	✓
Protein Concentration (UV-Vis)		✓
Average DAR (HIC/RP-HPLC)		✓
Size (SEC-HPLC/CE-SDS		✓
Charge (icIEF)		✓
Potency (Cytotoxicity)		✓
Residual solvents (GC-MS)	✓	
Chiral purity (if applicable, HPLC)	✓	
Small molecule impurities (HPLC)	✓	

1) Non-conjugatable, Non-Drug
2) Conjugatable, Non-Drug
3) Conjugatable, Drug-related
4) Non-conjugatable, Drug-related

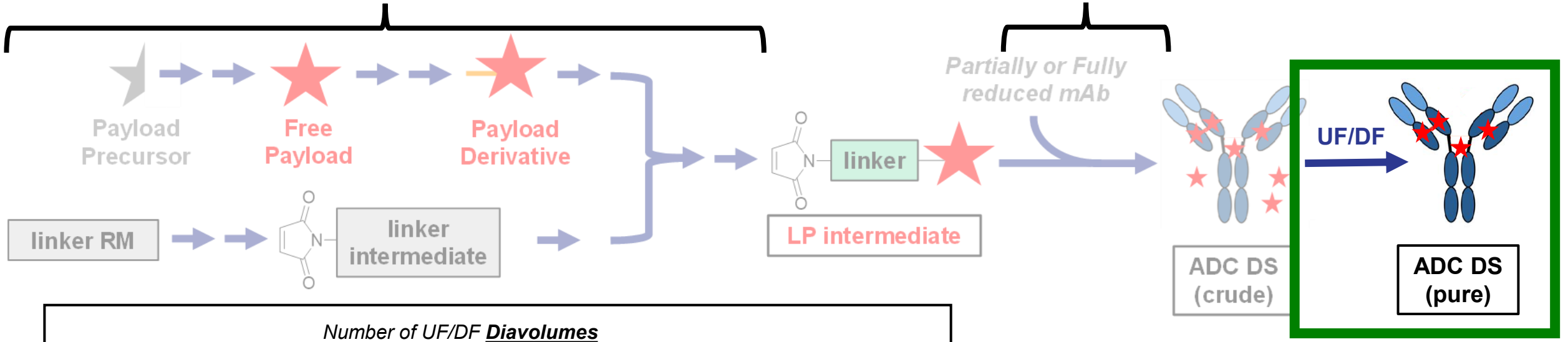
J. Pharm. Sci. **2023**, 112, 2965-2980.

(1) Non-Conjugatable, Non-Drug Impurities

- Residual solvents
- Elemental impurities
- Synthetic reagents
- Synthetic byproducts

Non-Conjugatable Non-Drug Impurities

- Buffer salts
- Conjugation additives (e.g. EDTA, DHAA)
- TCEP, TCEPO
- Organic co-solvents
- Quencher



*Number of UF/DF **Diavolumes***

$$C_{SM} = C_{SM,i}e^{-D(1-R)}$$

***Concentration** of small molecule impurities (final and initial)*

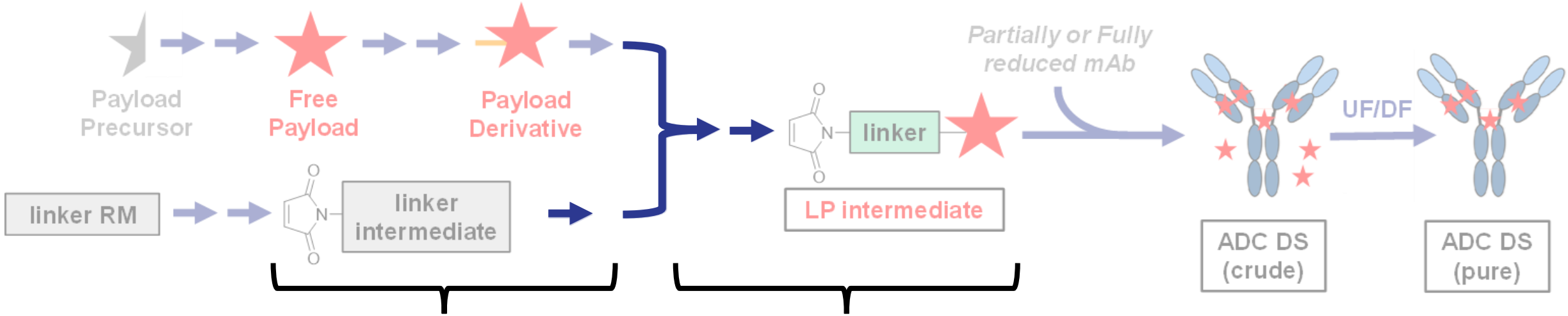
***Retention** of impurity*

Assuming a retention of 0, UF/DF processing with 6.9 DVs will remove 99.9% of impurity

Efficient removal of all Non-Conjugatable, Non-Drug Impurities at UF/DF Stage

AAPS PharmSciTech **2018**, 19, 3, 971.

Conjugatable Impurities



(2) Conjugatable Non-Drug Impurities

(3) Conjugatable Drug-related Impurities

- ***Compete with LP intermediate for conjugation sites***
- ***May not be amenable to UF/DF removal***
- ***Applications of ICH Q3A(R2) and Q3B(R2)control strategies***

ICH Considerations for Conjugatable Impurities in DS

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ICH Q3A(R2) = Impurities in New Drug Substances

Dose	Reporting threshold (%)	Identification threshold	Qualification threshold
≤ 2 g/day	0.05	0.1% or 1 mg/day (whichever is lower)	0.15% or 1.0 mg/day (whichever is lower)
> 2 g/day	0.03	0.05%	0.05

ICH Q3B(R2) = Impurities in New Drug Products

Maximum daily dose	Threshold
Reporting thresholds	
≤ 1 g	0.1%
> 1 g	0.05%
Identification thresholds	
< 1 mg	1.0% or 5 µg TDI, whichever is lower
1–10 mg	0.5% or 20 µg TDI, whichever is lower
> 10–2 g	0.2% or 2 mg TDI, whichever is lower
> 2 g	0.10%
Qualification thresholds	
< 10 mg	1.0% or 50 µg TDI, whichever is lower
10–100 mg	0.5% or 200 µg TDI, whichever is lower
> 100 mg–2 g	0.2% or 3 mg TDI, whichever is lower
> 2 g	0.15%

If you exceed the Qualification threshold for a conjugatable impurity in DS/DP, what is your control strategy?

AAPS PharmSciTech **2018**, 19, 3, 971.

ICH Considerations for Conjugatable Impurities in DS

Impurity % for DS (Q3A)

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

Impurity TDI for DP (Q3B)

Extreme (hypothetical) case:

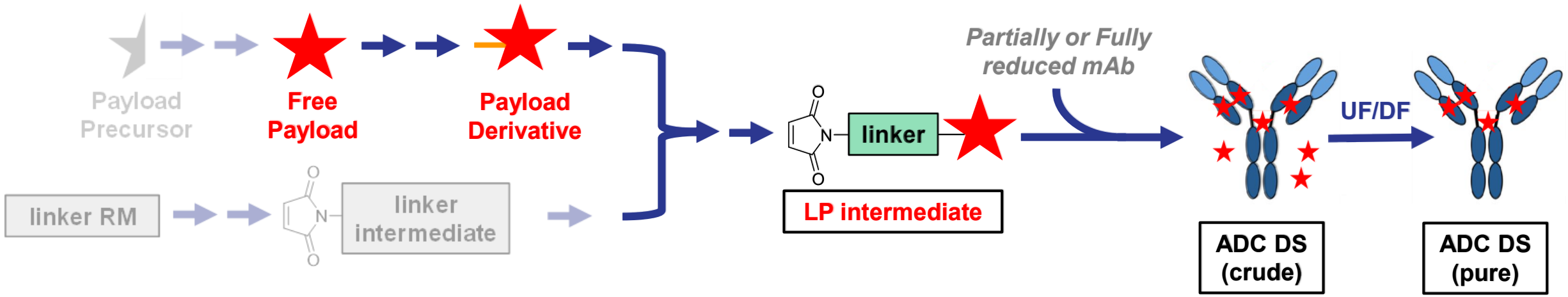
- High, frequent dose (10 mg/kg biweekly), high DAR (8), high MW (2000), ~3% impurity
- Assumes toxic or pharmacological effect at the Identification threshold
- Assumes 100% of impurity attaches to mAb and is then released after DS manufacture

0.3% Impurity
214 µg TDI

- **If impurity is a Conjugatable Non-Drug = may still be low safety risk**
- **If impurity is Conjugatable Drug-related = proceed with appropriate Tox assessment**

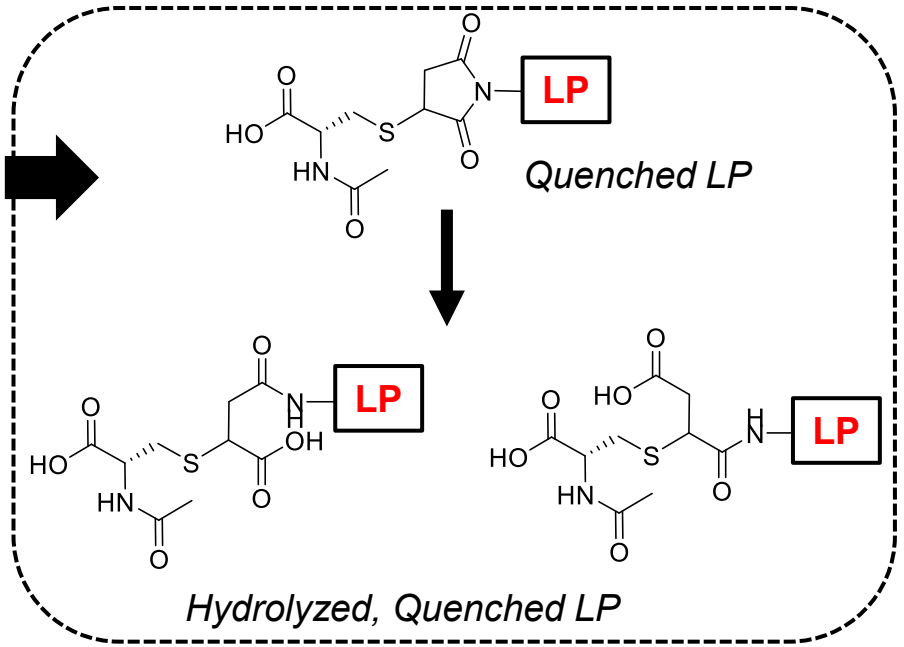
AAPS PharmSciTech **2018**, 19, 3, 971.

(4) Free Drug-Related Impurities (Non-Conjugatable)



General Considerations for Free Drug-Related Impurities:

1) Potency and Toxicity decreases with further elaboration of free payload
   - *Free Payload > Payload Derivative > Linker-Payload + **LP Derivatives***
2) UF/DF generally effective in removal of LP, derivatives, and free payload, but not as efficient as other small molecules (e.g. buffer salts): case-by-case evaluation of UF/DF parameters based on LP properties
3) Appropriate tox assessment should be initiated if Q3A/3B thresholds reached for non-conjugatable impurities



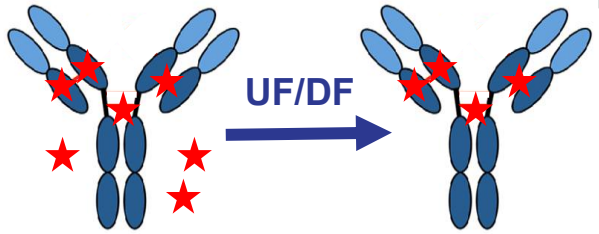
AAPS PharmSciTech **2018**, 19, 3, 971.

UF/DF Considerations for Linker-Payload Removal

Table 3. Comparison of Key UF/DF Processing Parameters between mAbs and ADCs[a]

Parameter	mAbs	ADC
Crossflow rate (L/min/m^2, LMM)	**6** (4–8)	**4** (2–8)
Transmembrane pressure (psi)	**15** (12–18)	**12** (8–25)
Membrane pore size (kDa)	**30**	**30** (10–100)
Membrane composition	**Regenerated cellulose**	**Regenerated cellulose** Polyethersulfone (PES)
Membrane loading (g/m^2)	**600** (500–1000)	**250** (100–600)
Final product concentration (g/L)	**75** (50–150)	**25** (10–150)
Membrane area (m^2)	**4.56** (2–9)	**2.28** (1–9)
Bioreactor/UF/DF recirculating tank volume (L)	**200** (200–1000)	**100** (50–500)
Final pool volume (L)	**75** (50–100)	**30** (10–75)
Number of DVs required	**7** (7–10)	**12** (5–30)
Number of membrane re-uses	**5** (2–10)	**0** (1–11)

[a]Text or numbers in bold represents the most common survey answers; text or numbers in parentheses are the ranges or other options provided by the respondents.



Drug-Related Impurities

- LP intermediate
- Free drug/payload
- Quenched LP
- TCEP-LP adduct

ADC DS (crude)	ADC DS (pure)
~50%	<0.5%

>2-log reduction in Drug-Related Impurity generally required

Free Drug-related DS/DP specification of <0.5 (mol/mol) % for IND is typical.

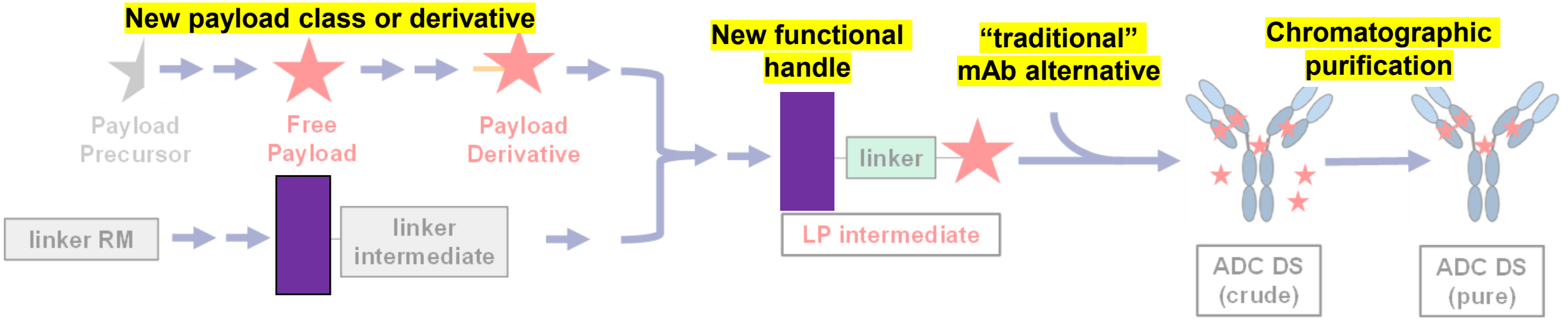
Typical process parameters (membrane loading, required DVs, etc.) driven by highly hydrophobic properties of LP

Reliable predictive models for UF/DF conditions based on simple inputs (e.g. Log D/P values of LP, crude reaction viscosity, conductivity, etc.?

Org. Process Res. Dev. **2023**, 27, 1258−1268.

Conclusions and Future Directions

- Best practices and learnings from commercial maleimide-based ADCs provide a useful benchmark for small molecule impurity control arising in bioconjugate development



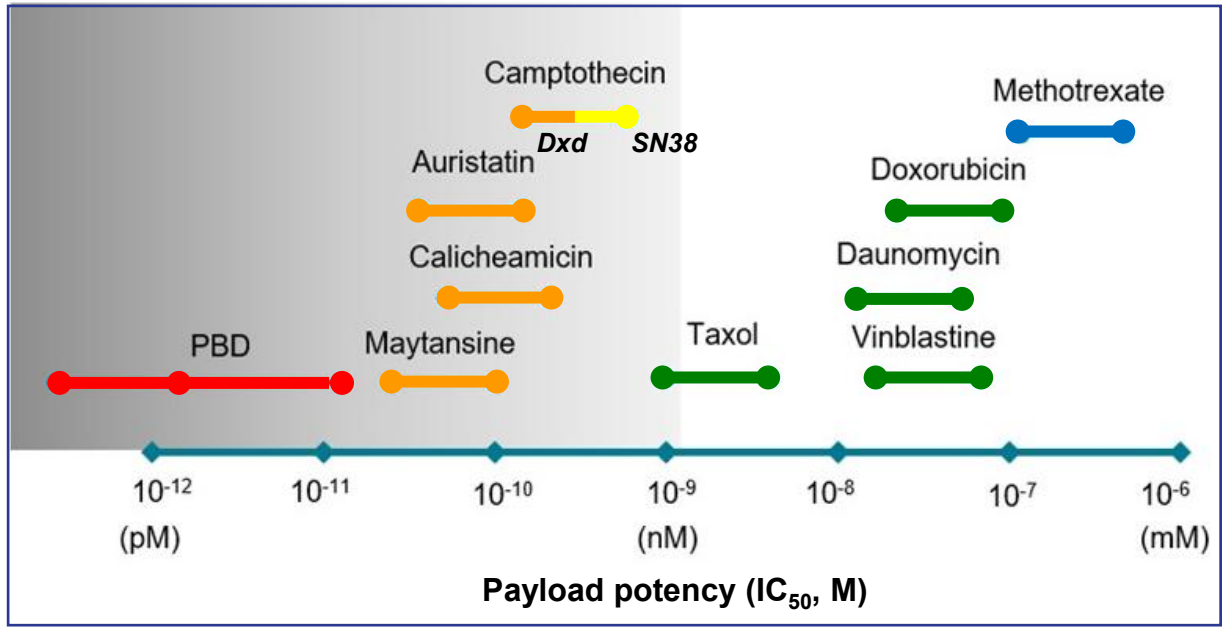
- "worst case scenario" of cytotoxic payloads likely to ease development burden for less toxic antibody-small molecule conjugates (e.g. protein degraders, immunostimulatory agents, etc.)
- New bioconjugate modalities vastly different from ADCs are likely to still borrow some design elements, manufacturing approaches, control strategies, and/or regulatory guidance from commercial ADCs

Thank You!!!

Backup

Relative Potencies of Commercial ADC Payloads

ADC	Target	Linker	Payload	Action	DAR	Indication	Approval year
Mylotarg®	CD33	Acid cleavable	calicheamicin	DNA alkylation	2-3	CD33+ AML	2000ǂ/2017
Adcetris®	CD30	Enzyme cleavable	MMAE	Microtubule inhibition	4	ALCL, cHL, PTCL	2011/2017
Kadcycla®	HER2	Non-cleavable	DM1	Microtubule inhibition	3.5	HER2+ mBC, BC	2013/2019
Besponsa®	CD22	Acid cleavable	calicheamicin	DNA alkylation	6	B-ALL	2017
Polivy®	CD79b	Enzyme cleavable	MMAE	Microtubule inhibition	3.5	DLBCL	2019
Padcev®	Nectin4	Enzyme cleavable	MMAE	Microtubule inhibition	3.8	Urothelial cancer	2019
Enhertu®	HER2	Enzyme cleavable	Dxd	TOP1 inhibition	8	HER2+ mBC/GC	2019/2021
Trodelvy®	TROP2	Acid cleavable	SN38	TOP1 inhibition	7.6	TNBC/urothelial	2020/2021
Blenrep®	BCMA	Non-cleavable	MMAF	Microtubule inhibition	4	MM	2020
Zynlonta®	CD19	Enzyme cleavable	SG3199 PBD	DNA alkylation	2.3	B-cell L, DLBCL	2021
Tivdak®	Tissue factor	Enzyme cleavable	MMAE	Microtubule inhibition	4	Cervical cancer	2021
Elahere®	FOLR1	Enzyme cleavable	DM4	Microtubule inhibition	3.5	Ovarian, FT cancer	2022
Datroway®	TROP2	Enzyme cleavable	Dxd	TOP1 inhibition	4	HR-/HER2- BC	2025



*Excluding Lumoxiti, Akalux and Aidixi

ǂMylotarg was approved in 2000, withdrawn in 2010, and reapproved with a new dosing regimen in 2017

Adapted from Pharmaceutics **2023**, 15, 600.

Free Drug-related DS/DP specification of <0.5 mol% for IND is typical. However, careful re-evaluation is needed for new payload classes and derivatives

Adapted from https://www.bocsci.com/blog/history-of-adc-payloads-and-its-pharmacokinetic-profiles/?srsltid=AfmBOoqbNGiDlBGlyGjkZHum6hLIjvlTw174BYKygqEWkefrI_9K5-iP