Analytical Characterization of Dolaflexin-ADCs

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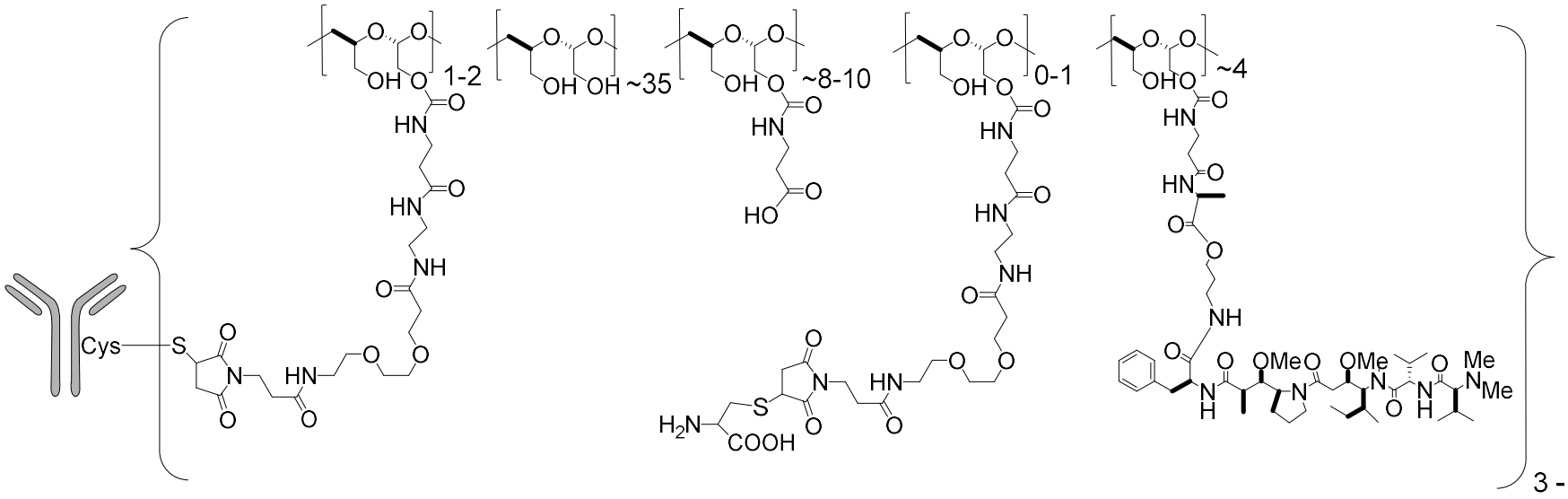
Unleashing the Targeted Power of Antibody Drug Conjugates

Highly Differentiated Platform	Dolaflexin designed to overcome the efficacy and tolerability limitations of existing ADC technologies
Lead Program, XMT-1522 in Ph 1	A HER2 Targeted Dolaflexin ADC addressing large unmet patient needs in breast, NSCLC and gastric
XMT-1536 in Ph1	A NaPi2b Targeted Dolaflexin ADC addressing large unmet patient needs in ovarian and NSCLC
Robust Discovery Effort	1 IND every 12-24 months; new platform innovations
Up to $2 Billion in Partnerships	Technology licensing partnerships with Takeda and Merck KGaA; Major Strategic Expansion of Takeda partnership
Strong Balance Sheet	Successful IPO in June 2017 raised $75mm gross proceeds, Current cash balance adjusted for IPO proceeds is $137mm
World Class Investors	NEA, Wellington, Arrowmark, Cormorant, F Prime, Pfizer Ventures, Rock Springs, Takeda Pharmaceuticals

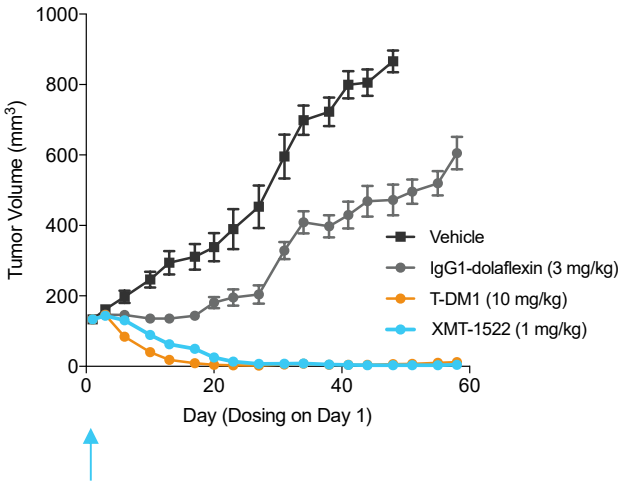
Schematic Structure of a Dolaflexin-Based ADC



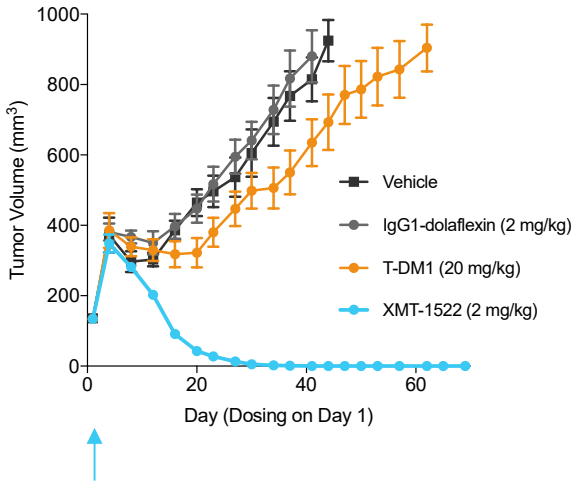
- High DAR, DAR=10-15
- Hydrophobicity of payload offset by polar polyacetal backbone enabling higher DAR
- Novel proprietary auristatin payload

XMT-1522 Achieves Durable Complete Regressions Across Models with Range of HER2 Expression Levels

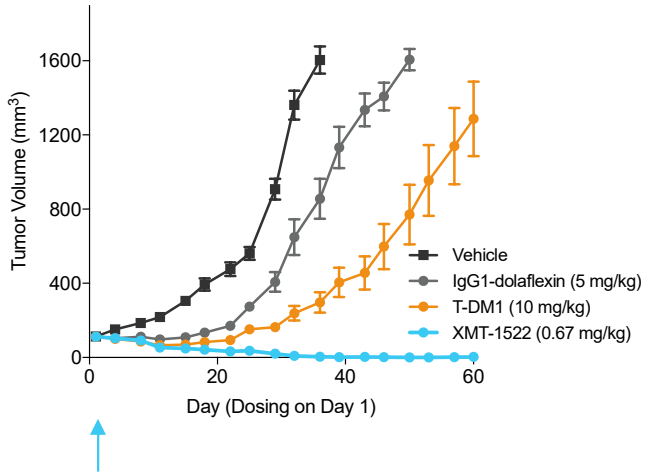
HIGH
N87 Gastric Cancer
800,000 HER2/cell
HER2 3+



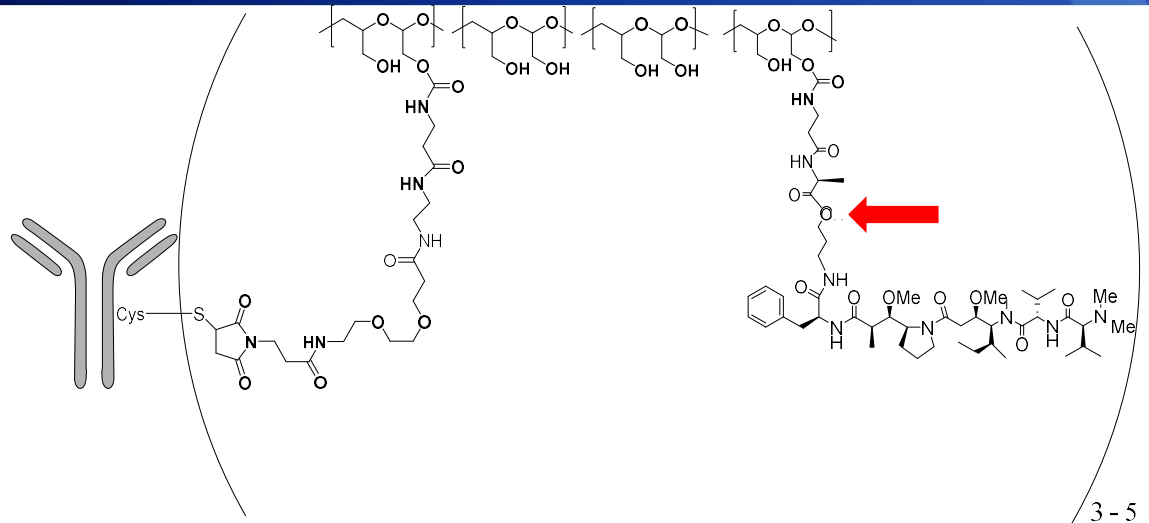
MEDIUM
JIMT-1 Breast Cancer
80,000 HER2/cell
HER2 2+



LOW
SNU5 Gastric Cancer
22,000 HER2/cell
HER2 0/1+



Dolaflexin Intracellular Processing



Bystander Killing

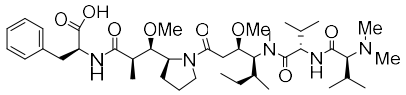
AF-HPA

Primary release product
sub-nanomolar potency; freely cell permeable

Intra-tumor

metabolism

No Bystander Killing



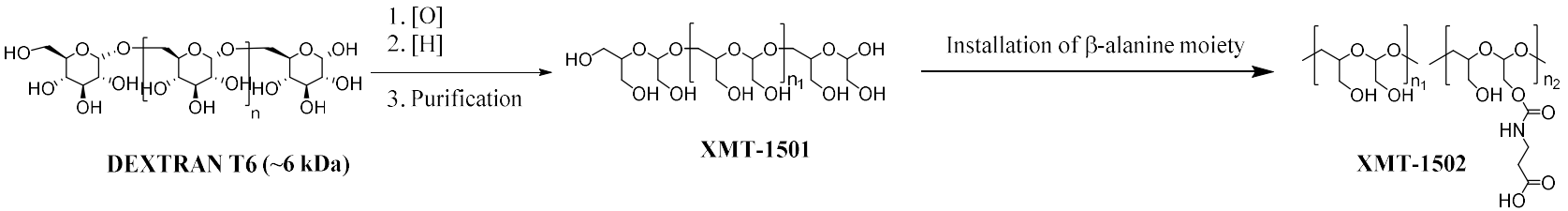
AF

IC50 > 20 nM
Non cell-permeable; not a Pgp substrate

Selected Analytical Considerations for Dolaflexin-ADCs

Selected Attributes	Direct Stochastic Conjugation	Dolaflexin ADC
Linker-drug	• Small simple structure	• Large complex structure • Comparability of Dolaflexin batches important for ADC comparability
Average DAR	• Typically by HIC	• HIC currently not useful for DAR
Positional Isomers / structure	• Limited number of positional isomers, can be identified and quantitated, conjugation sites identified • % DAR=0, 2, 4, 6, 8	• Heterogeneity and reactivity of Dolaflexin complicates structural analysis • ADC structure defined in part by number of Dolaflexins attached, conjugation sites occupied by Dolaflexin

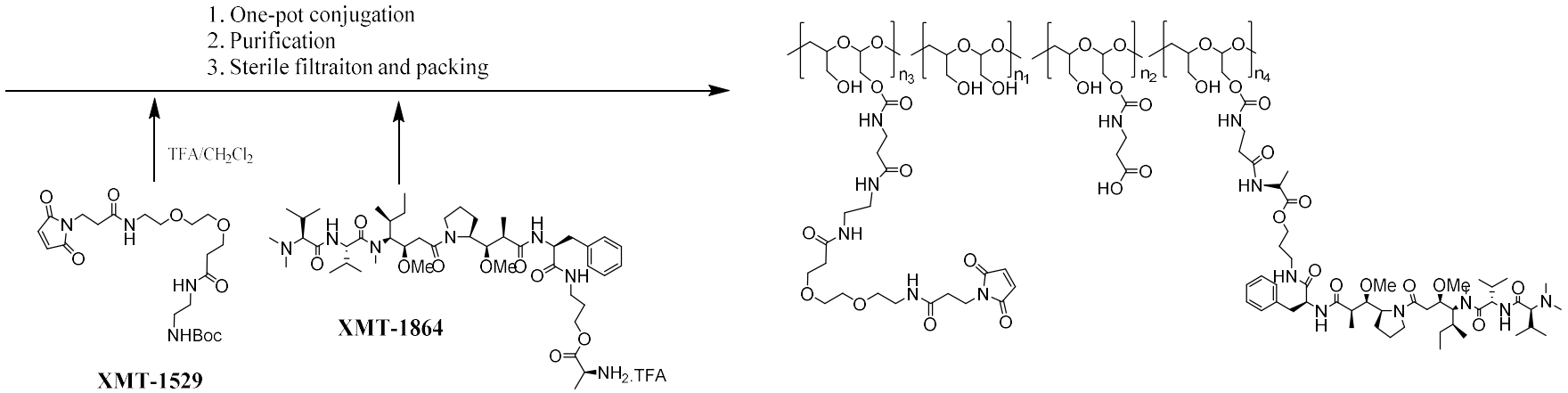
Dolaflexin Process



DEXTRAN T6 (~6 kDa)

XMT-1501

XMT-1502



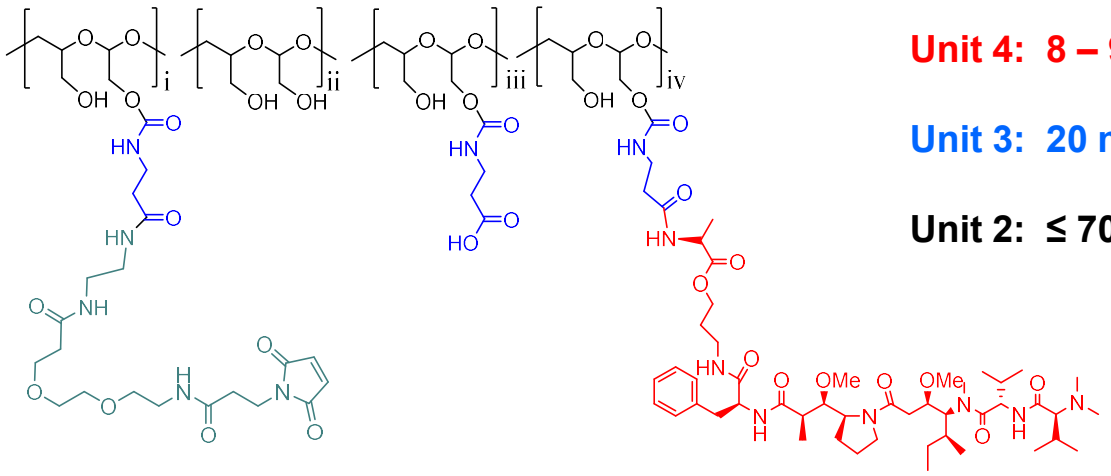
XMT-1529

XMT-1864

XMT-1505

Sources of Heterogeneity in Dolaflexin ADCs

- Heterogeneity due to regiochemistry and loading factors:



Unit 1: 2 – 4 mole% (~ 2 units/polymer)

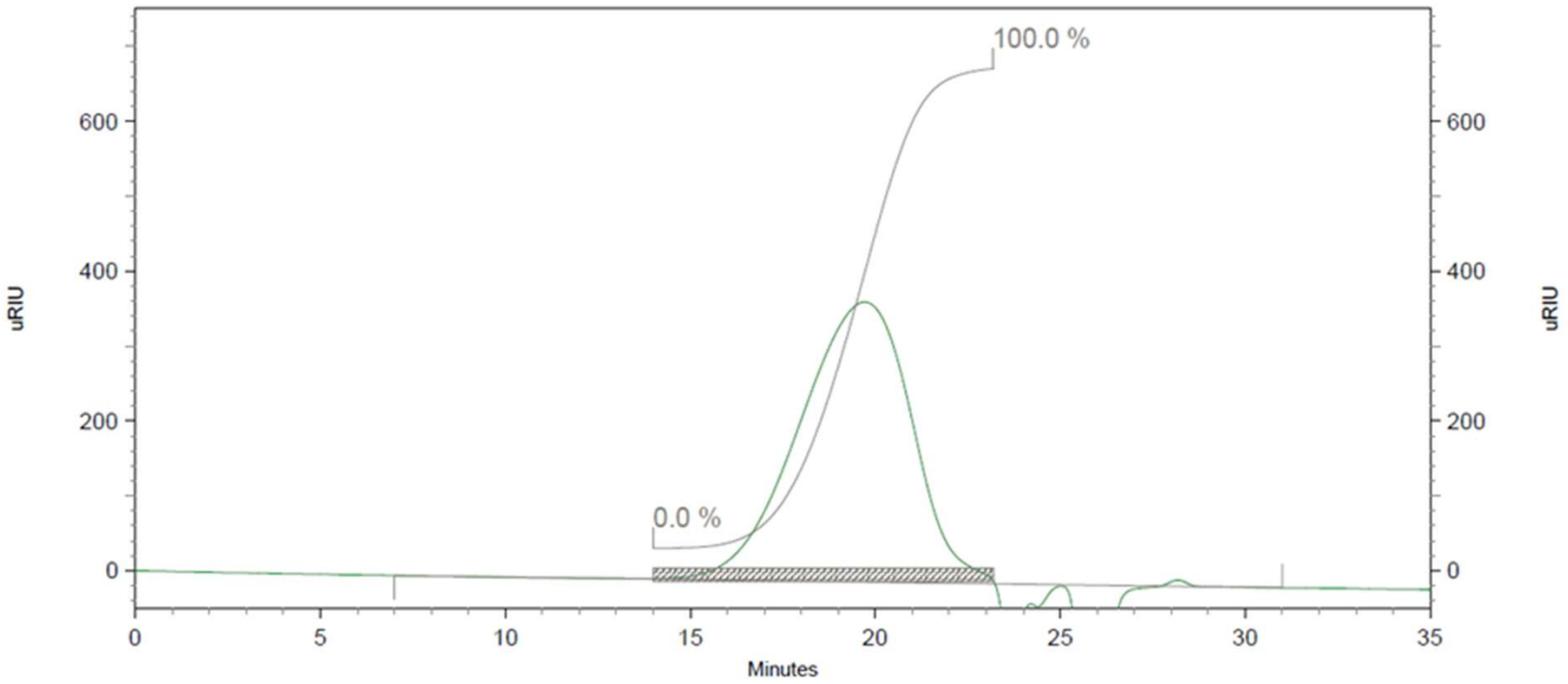
Unit 4: 8 – 9 mole% (3 – 4 warheads/polymer)

Unit 3: 20 mole% (10 – 12 units/polymer)

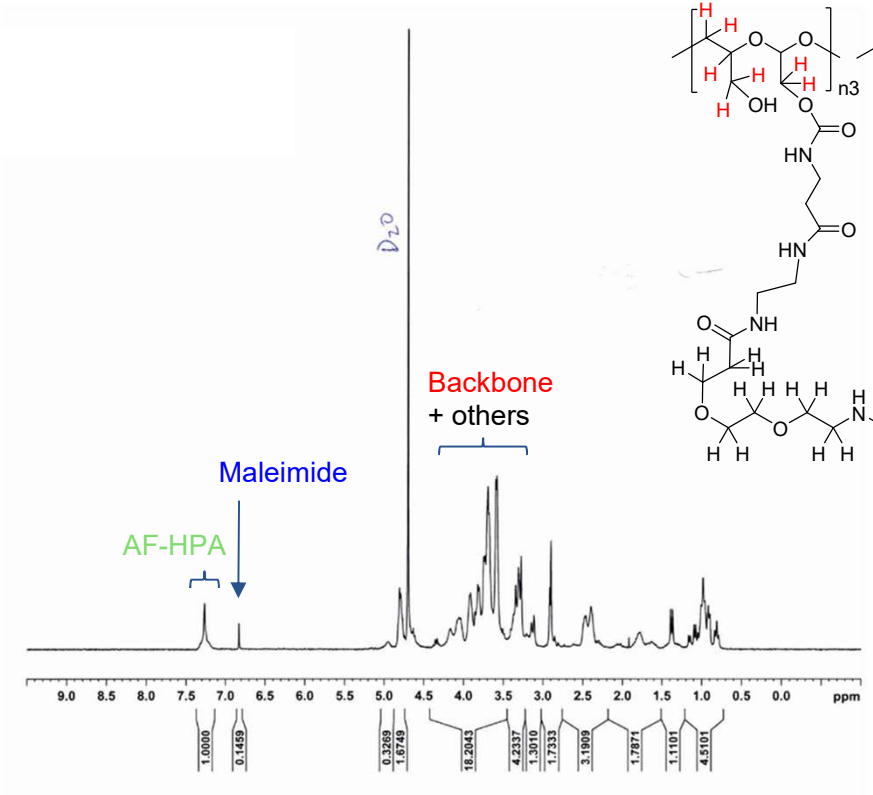
Unit 2: ≤ 70 mole% (~ 35 – 40 units/polymer)

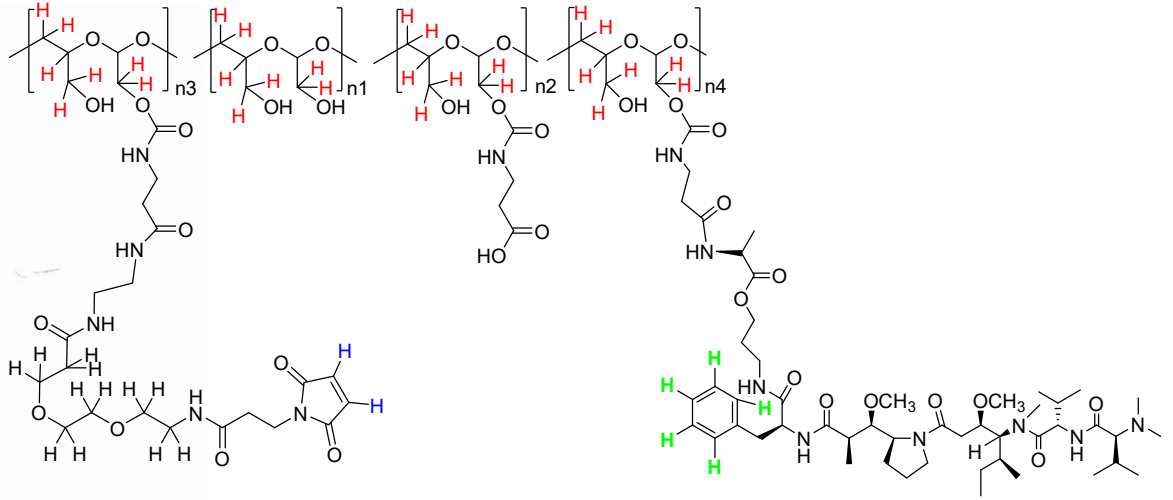
Note: Substitution can occur on either hydroxyl group of monomer, not just as indicated

MW Determined by SEC



NMR Spectrum of Dolaflexin





Selected Batch Data

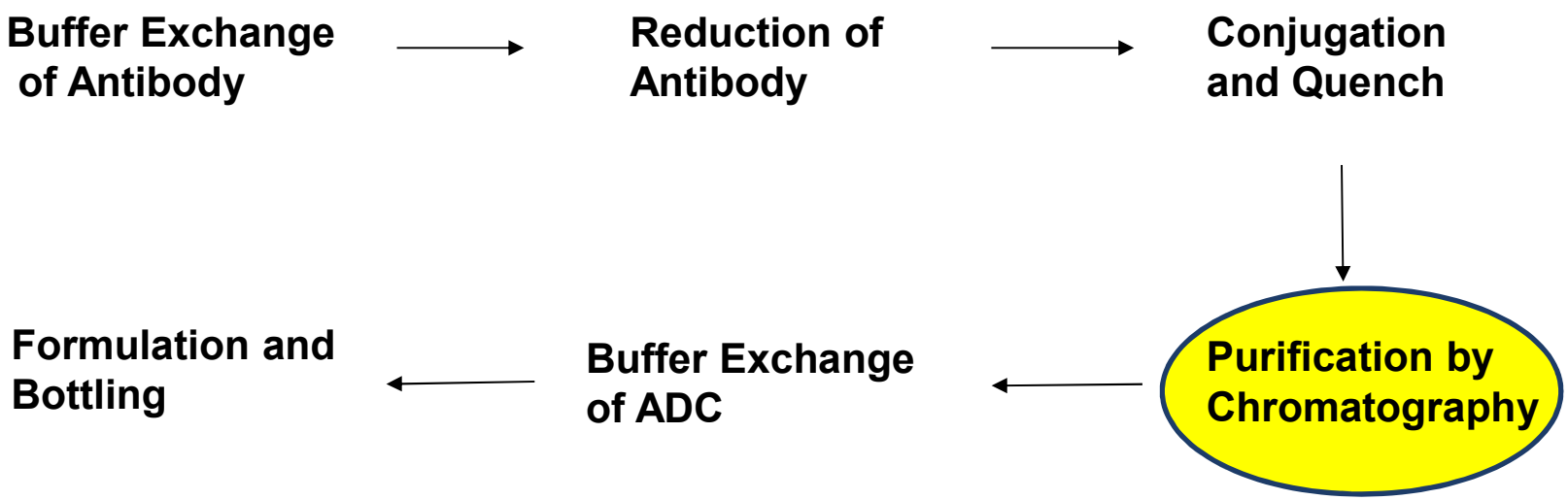
		Batch #						
Attribute	**Assay**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
MW (kDa)	SEC	8.3	12.5	10.8	11.2	11.4	9.3	11.3
PDI	SEC	1.3	1.6	1.5	1.4	1.4	1.3	1.4
Drug Load %	NMR	9.4	9.5	9.0	9.1	9.0	9.1	9.0
Free Drug %	LC-MS	0.4	0.7	0.1	0.2	0.2	0.4	0.1
Linker Load %	NMR	2.4	3.5	3.6	3.5	3.8	3.5	3.5

Controlling Dolaflexin Heterogeneity
Conclusions

- Heterogeneity in Dolaflexin can be minimized and controlled by:
  - Control of raw material properties (e.g. – Dextran MW)
  - Precise control and monitoring of reaction conditions
  - Chromatographic fractionations with established pooling criteria
- Dolaflexin Critical Quality Attributes have shown good batch-to-batch reproducibility

Characterization of Dolaflexin ADCs

Dolaflexin-ADC Process

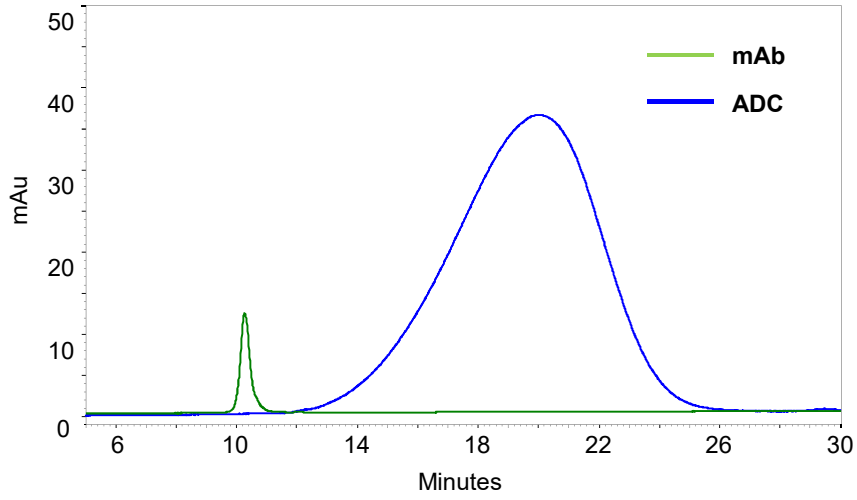


Selected Characterization Test Results

	Attribute	Assay	Result
1	DAR	RP-HPLC	DAR=11-13
2	ADC covalent structure	MALDI-TOF of cross-linked ADC	Verified intact MW of ADC and conjugated polypeptides
3	ADC covalent structure	MSSV (AUC)	Dolaflexin:mAb ~ 3:1
4	ADC covalent structure	Western blot	Verified cross-linking of LC and HC by Dolaflexin
5	ADC covalent structure	Peptide map / MS / MS	Verified correct conjugation sites
6	Secondary structure	Circular dichroism spectroscopy	Verified antibody-like structure, comparable to unconjugated mAb
7	Higher order structure	Disulfide bond mapping	Verified antibody-like SS bond pattern
8	Higher order structure	DSC	Verified ADC has similar thermal stability as mAb
9	Higher order structure	Analytical ultracentrifugation	Confirmed monomeric nature of ADC
10	Biological activity	Biolayer interferometry	Confirmed comparable binding kinetics as mAb
11	Impurities	Free Dolaflexin by HPLC	Dolaflexin not detected

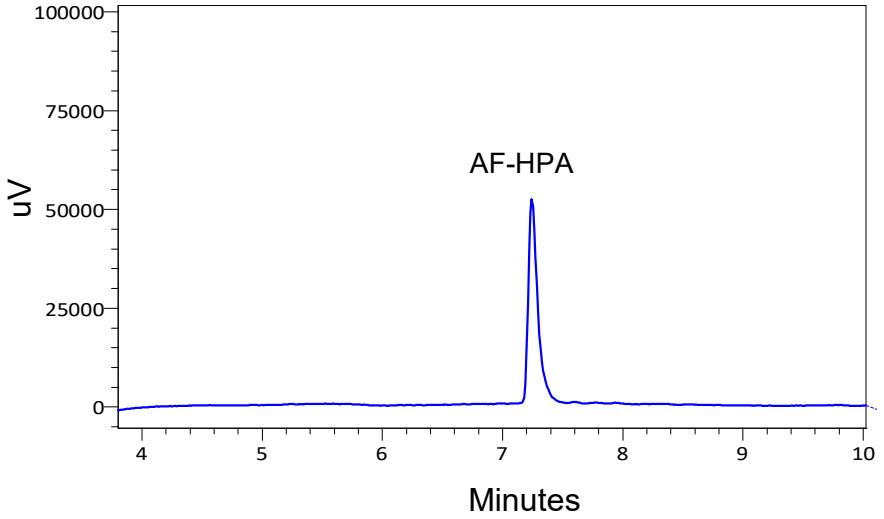
DAR Determination for Dolaflexin ADCs

HIC of a Dolaflexin ADC



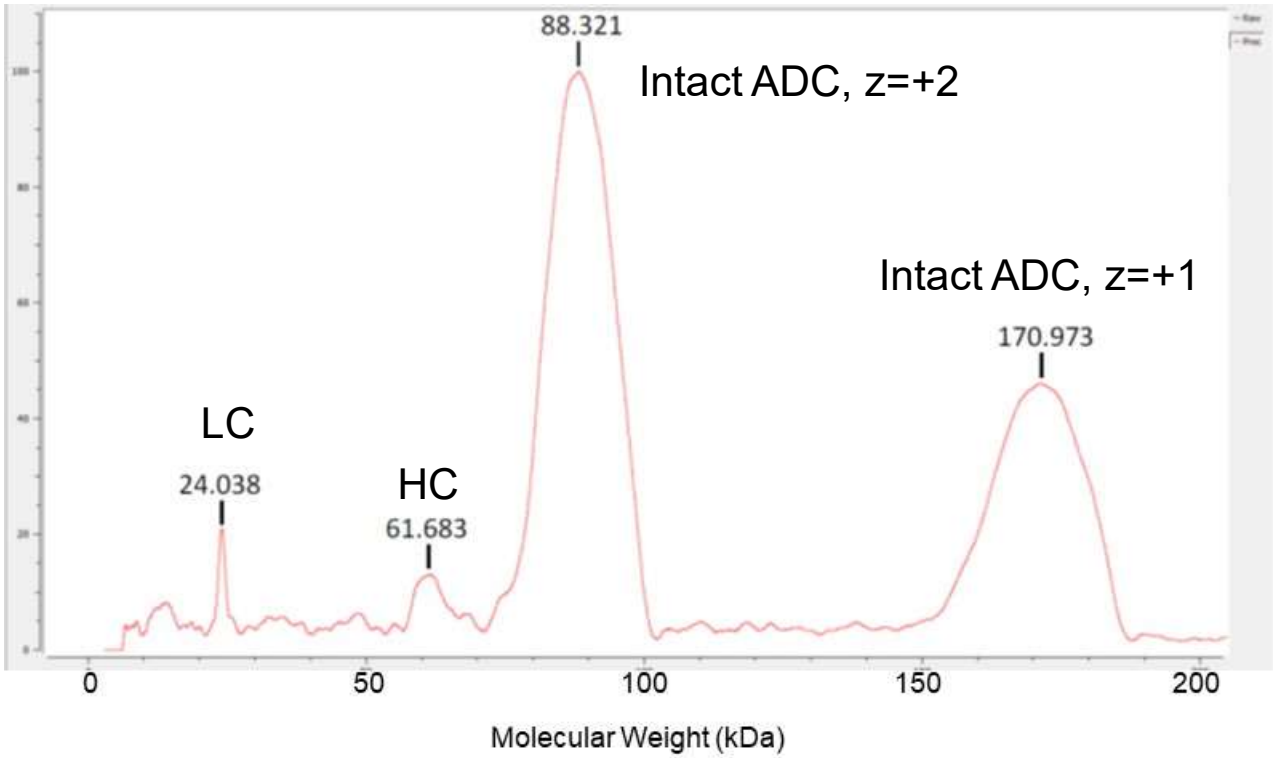
- Current HIC method is uninformative with respect to average DAR

RP-HPLC of a Dolaflexin ADC

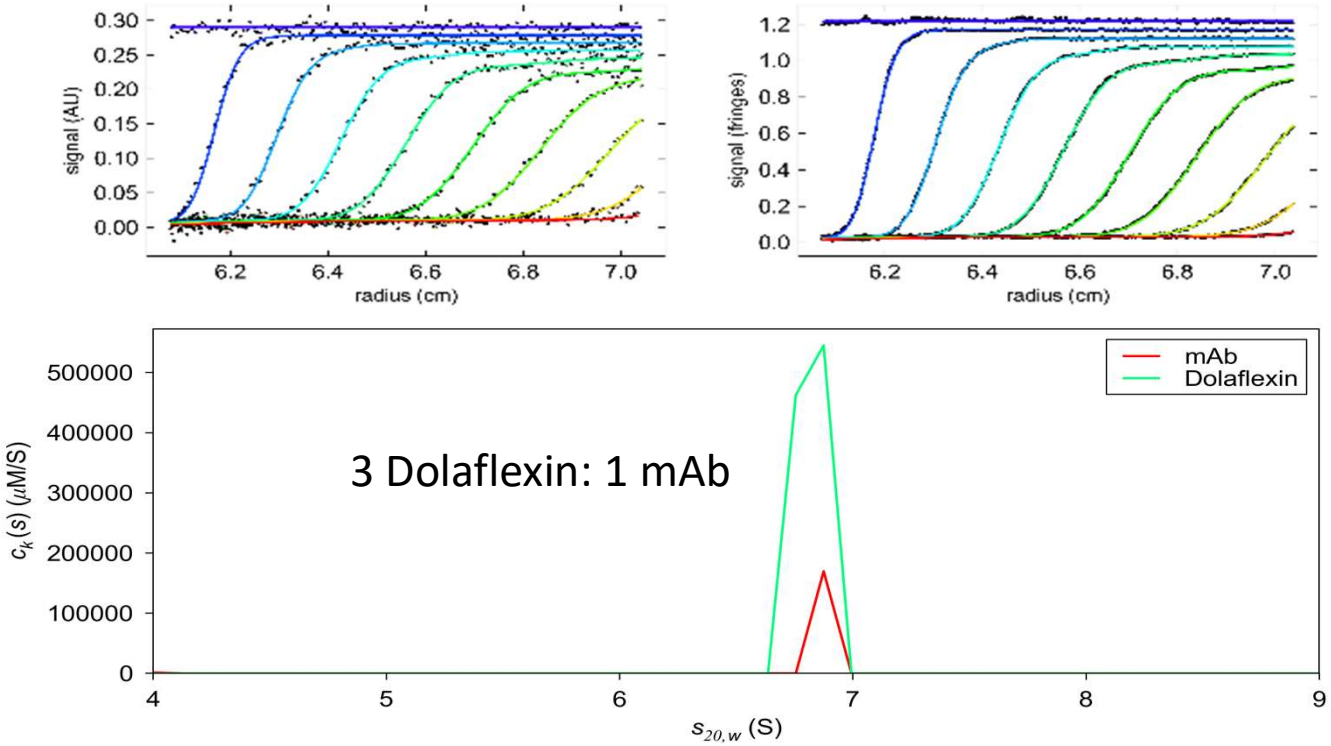


- AF-HPA cleaved from ADC by base hydrolysis
- Protein and polymer is precipitated
- AF-HPA concentration is quantitated by RP-HPLC
- DAR is calculated using known molar protein concentration

MALDI-TOF of Chemically Cross-linked ADC Suggests 1-3 Dolaflexins per Antibody



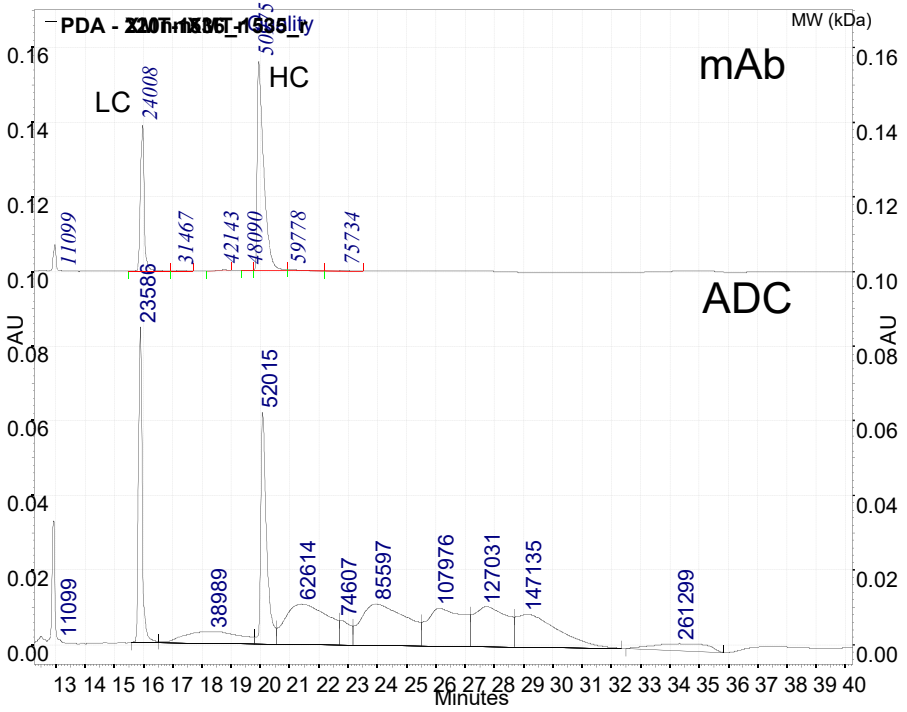
Multisignal Sedimentation Velocity Analysis for Average Dolaflexin:mAb



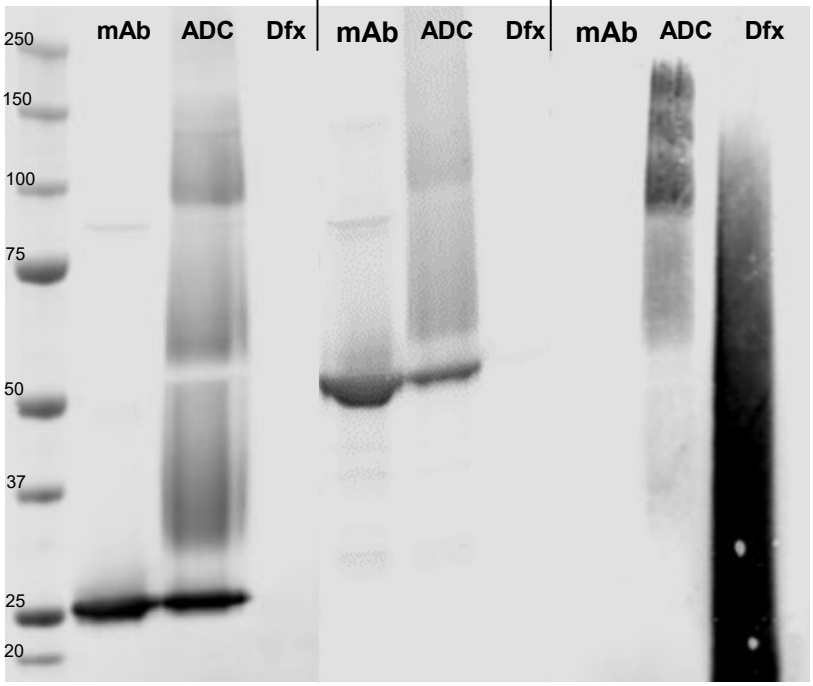
In collaboration with Peter Schuck, NIH

Dolaflexin-ADCs are Likely Intramolecularly Cross-linked by Dolaflexin

R-CE-SDS

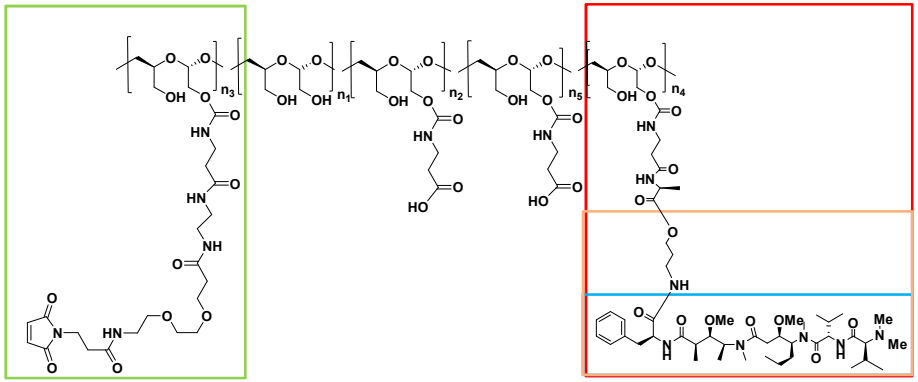


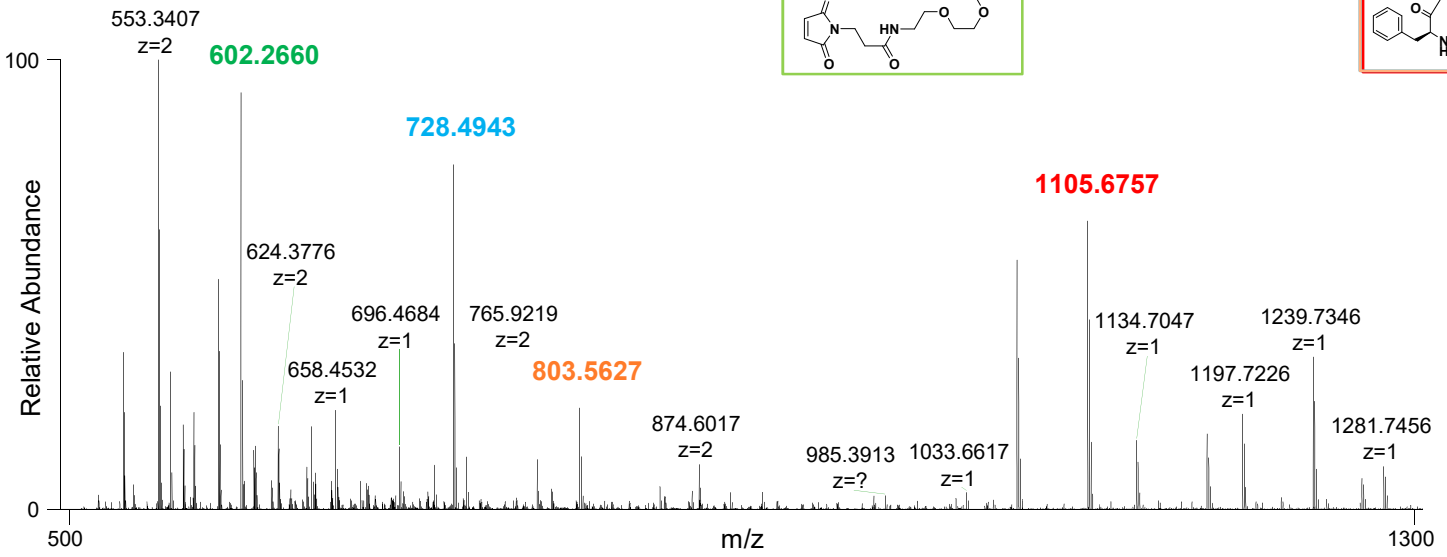
WB



In-Source MS Fragmentation of Dolaflexin

Theoretical Mass (M+H)+	Observed Mass (M+H)+	Mass Accuracy (ppm)
602.2668	602.2660	1.33
803.5641	803.5627	1.74
1105.6755	1105.6757	0.18
728.4957	728.4943	3.28

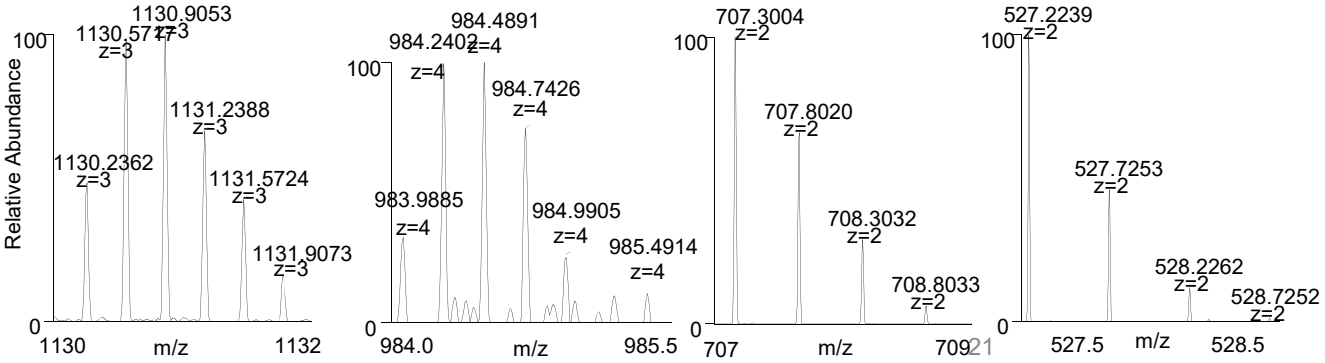


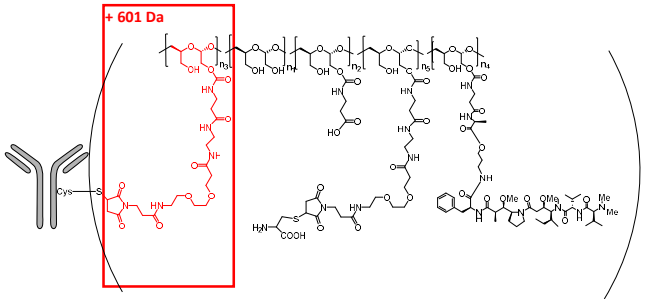


Verification of Interchain Cysteine Conjugation Through In-Source Fragmentation of Df Peptides

Base Formula	Sequence	Z	Observed Mass (m/z)	Theoretical Mass (m/z)	Mass Error
$C_{155}H_{238}N_{36}O_{45}S_2$	THT**C**PP**C**PAPELLGGPSVFLFPPKPK or THT**C**PP**C**PAPELLGGPSVFLFPPKPK	3	1130.2362	1130.2367	0.44
$C_{178}H_{274}N_{40}O_{56}S_2$	THT**C**PP**C**PAPELLGGPSVFLFPPKPK	4	983.9885	983.9889	0.41
$C_{57}H_{88}N_{16}O_{24}S$	SFNRGE**C**	2	707.3004	707.3012	1.13
$C_{41}H_{68}N_{10}O_{20}S$	S**C**DK	2	527.2239	527.2239	0.00

C= Maleimide-conjugated Cys **C**= Unconjugated Cys





- Conjugated peptides containing intrachain cysteines not detected
- ID and conjugation site verified by MS/MS sequencing (data not shown)

Characterization of Dolaflexin ADC
Conclusions

- Dolaflexin ADC has high DAR compared to typical DAR=4 ADCs
- ~ 3 Dolaflexins per ADC
- mAb likely intramolecularly cross-linked by Dolaflexin
- Conjugation occurs only at interchain cysteines

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- Reddy Bollu
- Jacques LeBlanc
- Tom Wagler

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Thank you!