Conjugation Process Improvements - Application of Lock Release Technology

ADCBIO
TO CURE TOGETHER

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ABSTRACT

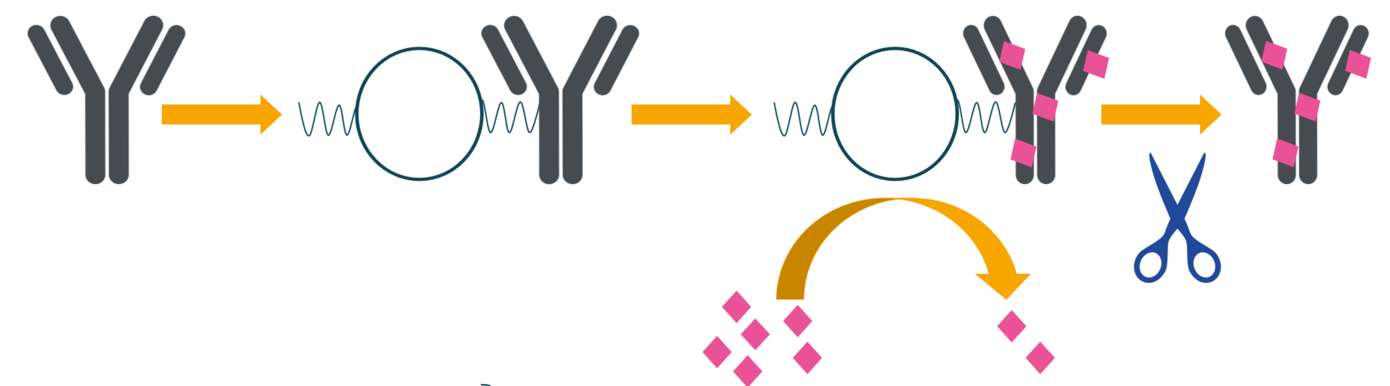
Solid-phase mediated antibody-drug conjugation (Lock-Release) was tested for it's ability to improve process control & product quality (aggregation, level of residuals and overall yield) relative to standard solution phase conjugation.

Conjugations of maytansinoid, auristatin, duocarmycin and PDB payloads to native cysteines, lysines and engineered cysteines of trastuzumab and cetuximab, using 96 well plate, stirred batch and column modes, were used to test the general applicability of this system for antibody-drug conjugate manufacture.

In all combinations of antibody, conjugation method, payload type and Lock-Release mode, the immobilised (locked) antibody was restrained from self-interaction or aggregation during conjugation, consistently resulting in <1.0% soluble dimer over a range of drug-antibody ratios of 0.5 - 4.4. Post conjugation washing of locked ADC removed residual drug and solvent to below the limits of detection. Scalability and batch to batch variation was assessed over 10 lots and a 10 fold scale up, giving an average DAR of 3.6 (SD 0.08), monomer level of 99.0% (SD 0.1%, all batches >98%). Structural and binding studies of lock-release ADC showed an equivalence to solution phase conjugations.

THE LOCK-RELEASE CONCEPT.

Traceless lock-release process where only desired conjugation events modify the antibody.



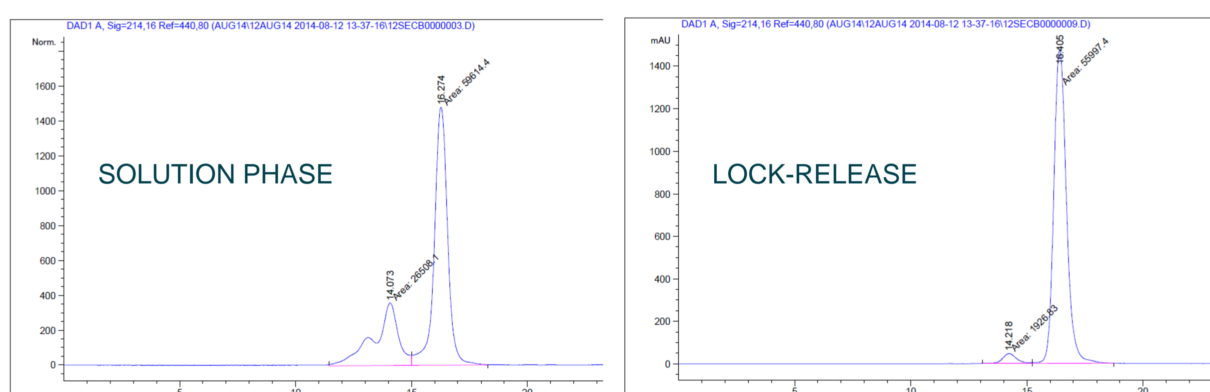
Step 1 Lock Antibody to Resin
Step 2 Conjugate
Step 3 Wash
Step 4 Release

4 Easy Steps
High **quality**, high **purity** ADC

QUALITY

Comparability testing of ADC product quality attributes for the Lock Release process, against solution phase conjugations. Consistency of the site of conjugation was demonstrated by mass spectrometry following Ides fragmentation and reduction (data not shown); further detailed analysis of lock-release conjugated ADC's including RP, SEC and HIC HPLC, antigen binding are reported here; alongside thermal stability and cell killing studies (data not shown).

Lock-Release conjugation improves monomer content (SEC analysis).

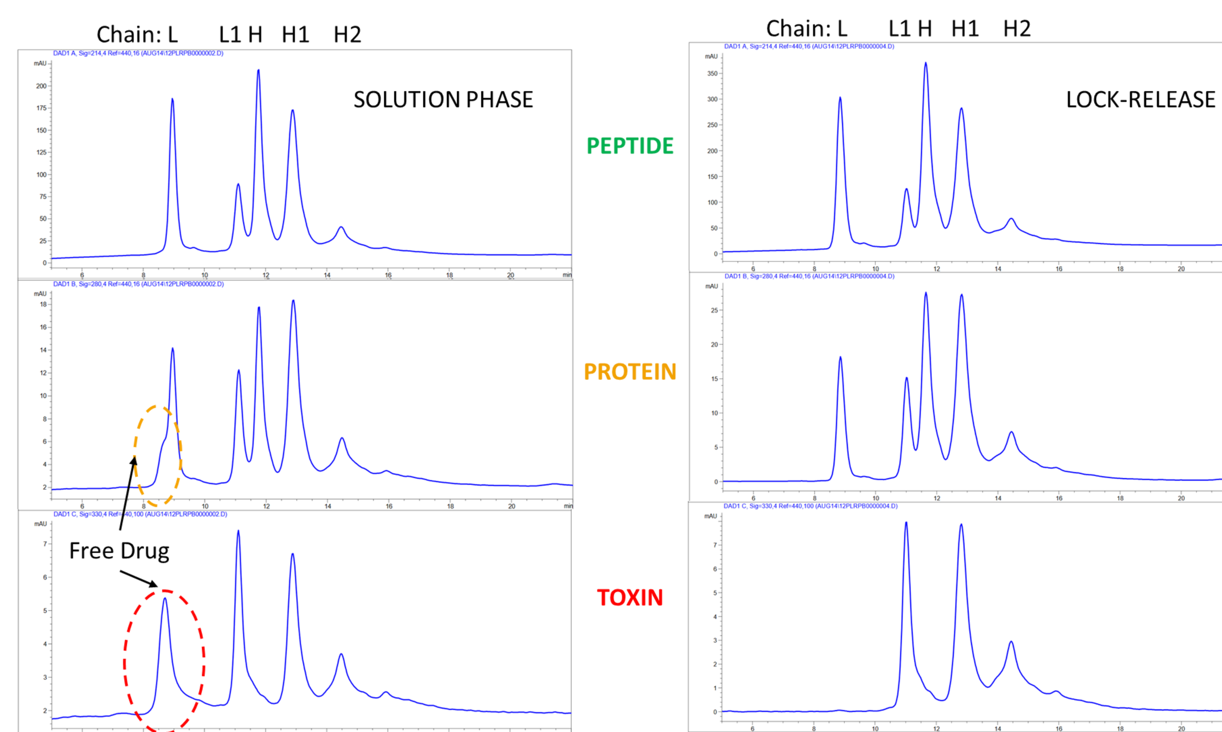


An IgG1 antibody was conjugated with a PBD dimer. Size exclusion chromatography was used to assess quality of the ADC monomer (elution ~16.2 min). High levels of soluble aggregate (11-15 mins) co-purify with the ADC monomer when prepared in solution (Left Hand chromatogram). When prepared on Lock-Release, there is a significant reduction in aggregate (Right Hand chromatogram). The conjugation process is scalable (below).

Maintaining quality throughout scale-up

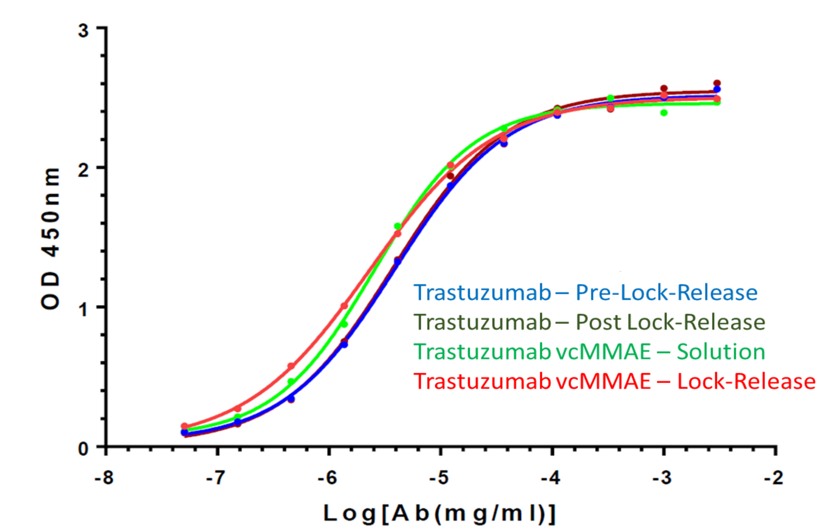
Scale	Average DAR	% Monomer	Free toxin	% Yield
1mg	2.30	97.7	<LOD	76.1
10mgs	2.46	98.2	<LOD	78.0
100mgs	2.36	98.0	<LOD	77.8
750mgs	2.30	95.0	<LOD	79.8

Lock-Release conjugation improves removal of residual drug (PLRP analysis).



Comparison of IgG1 – PBD dimer ADCs made in solution (Left Hand Panel) and on Lock-Release (Right Hand Panel). The solution phase ADC was purified and formulated by G25 chromatography while the Lock-Release ADC was washed 'on-resin' prior to G25 formulation. Analysis of the ADCs by reduced reverse phase chromatography revealed a significant difference in residual PBD dimer. G25 alone left residual PBD as shown by the fingerprint region at 330nm (Toxin) and at 280nm (Protein). Washing of the ADC, with an optimized wash solution, whilst locked onto resin removed residual PBD dimer to below detectable limits.
Chain I.D.: L - light chain, L1 - light chain with 1 PBD; H – heavy chain; H1 – heavy chain with 1 PBD; H2 – heavy chain with 2 PBD.

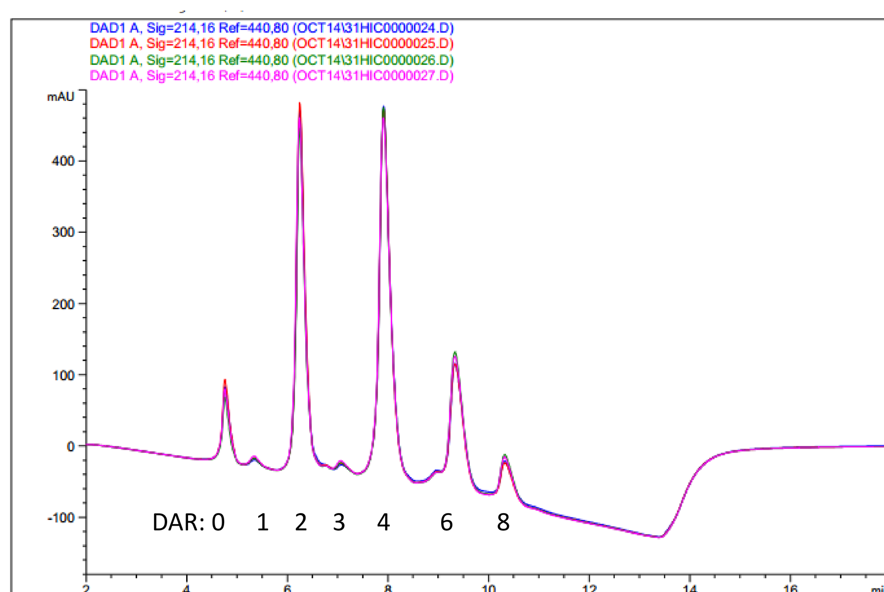
Preservation of antibody function by HER2-binding ELISA.



The binding capabilities of Trastuzumab or Trastuzumab-vcE to HER2 receptor were tested by an indirect ELISA assay. Her2-coated 96-well plates were incubated with control Trastuzumab or test molecules. Post-incubation and washing, binding was detected with anti-human IgG-HRP visualized with TMB. No significant binding difference was observed for ADCs prepared either in solution or Lock-Release and compared to the control antibody. Trastuzumab bound and released from Lock-Release also has very similar binding characteristics to the control.

REPRODUCIBILITY

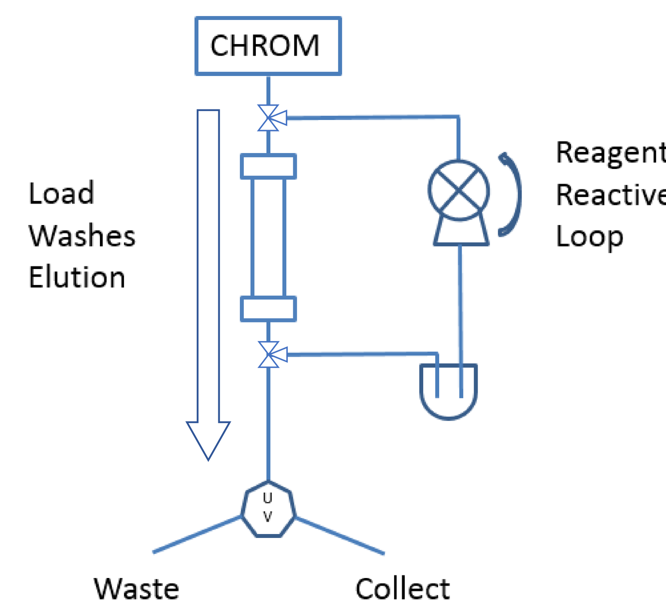
DAR separation analysis over 4 lots of Lock-Release conjugations.



ADC reproducibility by Lock-Release. Batch mode conjugations in a 96-well format of Lock-Release were carried out using Trastuzumab and vcMMAE. The pattern and extent of conjugation is consistent well-to-well as shown by an overlay of Hydrophobic Ion Chromatography (HIC) of the ADC from four individual wells.

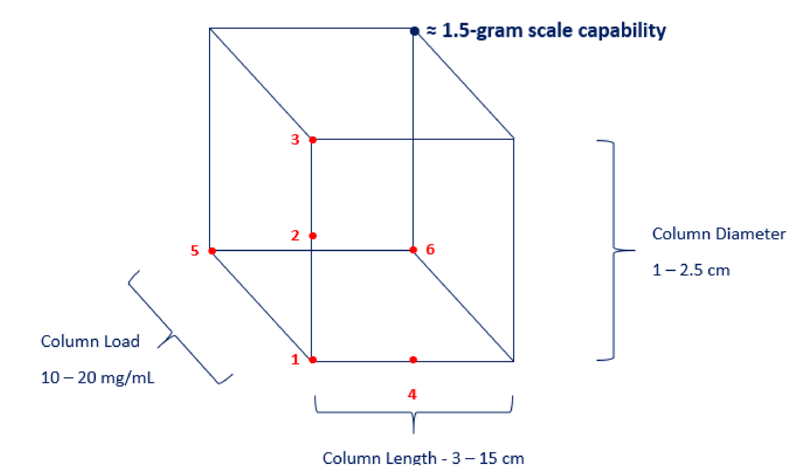
FLOW MODE SCALE-UP

Schematic of flow-mode Lock-Release



Reproducibility of ADC manufacture at different scales. The table shows the data from the 6 experimental set-ups shown on the corners of the cube. The scale ranges from approximately 20 mgs to 235 mgs. The data show we can increase scale with no change to the product quality attributes.
*1 - [DMA] LOD < 0.0001% v/v
*2 – Free MMAE expressed as percentage of total bound and free - [MMAE] LOD < 0.5%
*3 – IC 50 (ng/mL) on SK BR-3 (Equivalent solution phase ADCs = 10.2 ± 0.3 (N=3))

Scaling column dimensions and antibody loading. Column length (3 – 15cm), diameter (1 – 2.5cm) and load (10 – 20 mg/mL) were varied to demonstrate the scalability of Trastuzumab-vcMMAE conjugation via partial reduction in a column / flow mode. Experimental combinations indicated in red in the image below were performed and the consistency of DAR, monomer levels, residuals and cell killing activity was demonstrated – Table below. In bold is the potential input capability that can be achieved using a 2.5 x 15 cm column.



Run No.	Average DAR	% Monomer	[DMA] *1	[MMAE] *2	IC 50 *3
LR1	3.5	99.9	<LOD	<LOD	9.2
LR2	3.7	99.9	<LOD	<LOD	10.4
LR3	3.5	99.9	<LOD	<LOD	10.3
LR4	3.6	99.9	<LOD	<LOD	10.2
LR5	3.7	99.9	<LOD	<LOD	9.6
LR6	3.6	99.9	<LOD	<LOD	9.8

VERSATILITY

Lock-Release technology can be used for used for conjugating a variety of different scaffold antibodies to a variety of toxins with differing linker technologies. The three common models used on the IgG1 scaffold are partial reduction of interchain disulphides followed by thiol-reactive conjugations; site-specific thiol conjugations via introduced Cys mutations and amine-reactive linkers for primary amine cross-linking. Other scaffolds successfully conjugated on Lock-Release are IgG2, bispecifics, scFv (data not shown).

Antibody	Toxin	Toxin MOA	Manf. Process	Chemistry	Cleavable Linker?	Av. DAR	% Monomer	Residual Toxin*
Trastuzumab	DM1	Tubulin	Batch	Lysine	N	3.7	99.7	ND
	MMAE	Tubulin	Column	Cysteine	Y	3.6	99.8	ND
	MMAF	Tubulin	Batch	Cysteine	N	3.6	99.5	ND
	Duocarmycin	DNA Alkylation	Batch	Cysteine	Y	2.1	99.6	ND
	PBD Dimer	DNA Cross-link	Batch	Cysteine	Y	2.2	99.6	ND
Trastuzumab V205C	MMAE	Tubulin	Batch	Cysteine	Y	2.1	99.4	ND

ADCs using Trastuzumab as the scaffold with a variety of drug-linkers. A panel of different drug-linkers were used to conjugate Trastuzumab on Lock-Release either in column flow-mode or batch binding. For cysteine conjugations, partial reduction was used to target specific average DARs typical for the drug class being conjugated. A site-specific, cysteine-engineered scaffold (Trastuzumab V205C Light Chain) was prepared for conjugation by uncapping the introduced Cys by full reduction followed by reoxidation of the native disulphides. *ND: no residual drug-linker was detected after release and formulation.

Antibody	Resin Load	pH	DM1 XS	Average DAR	% Monomer
Trastuzumab	L	L	L	0.6	99
	M	M	M	1.6	99
	H	H	H	3.8	99
Cetuximab	L	L	L	0.5	99
	M	M	M	1.4	99
	H	H	H	3.8	98

Model Lysine Conjugations on Lock-Release. Both Trastuzumab and Cetuximab antibodies are formulated in amine containing buffers. By binding and washing out the buffer, a one-step Lysine conjugation using NHS-activated DM1 can be performed on the locked antibody at low (10g/L), medium (20g/L) and high (30g/L) antibody loadings on resin. Using this platform, different average DARs can be targeted with very high monomer levels obtained post release and formulation.

The advantage of this simplified process is apparent for shear sensitive and aggregation prone antibodies such as Cetuximab which need to go through many process steps for solution conjugation (buffer exchange, modification, buffer exchange, conjugation and buffer exchange for formulation).

CONCLUSIONS

Lock Release technology was conducted using 96-well plates, suspended particles and scaled-up in packed bed format.

SCALABILITY & REPRODUCIBILITY– over 750-fold scaleup with consistent, acceptable DAR, yield, free toxin, monomer attributes, over 10 lots with tight process control.

QUALITY – physicochemical and biological properties showed comparability with standard solution phase techniques.

VERSATILITY – the platform was tested successfully over a range of targeting mAbs, antibody formats and payloads.

PROCESS SIMPLICITY – Lock Release can reduce the number of diafiltration steps in a conjugation process, simplifying the manufacturing process.

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