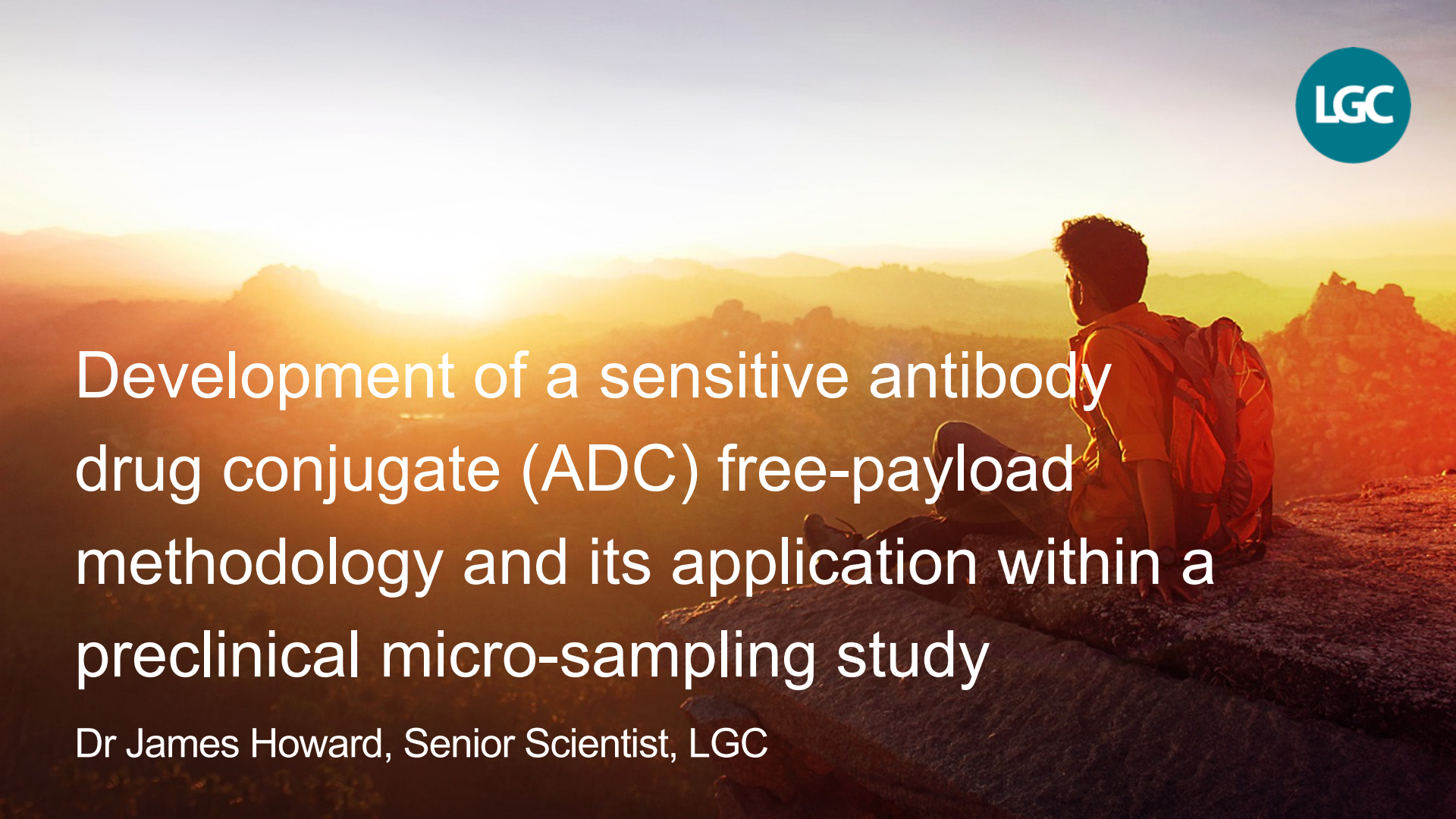


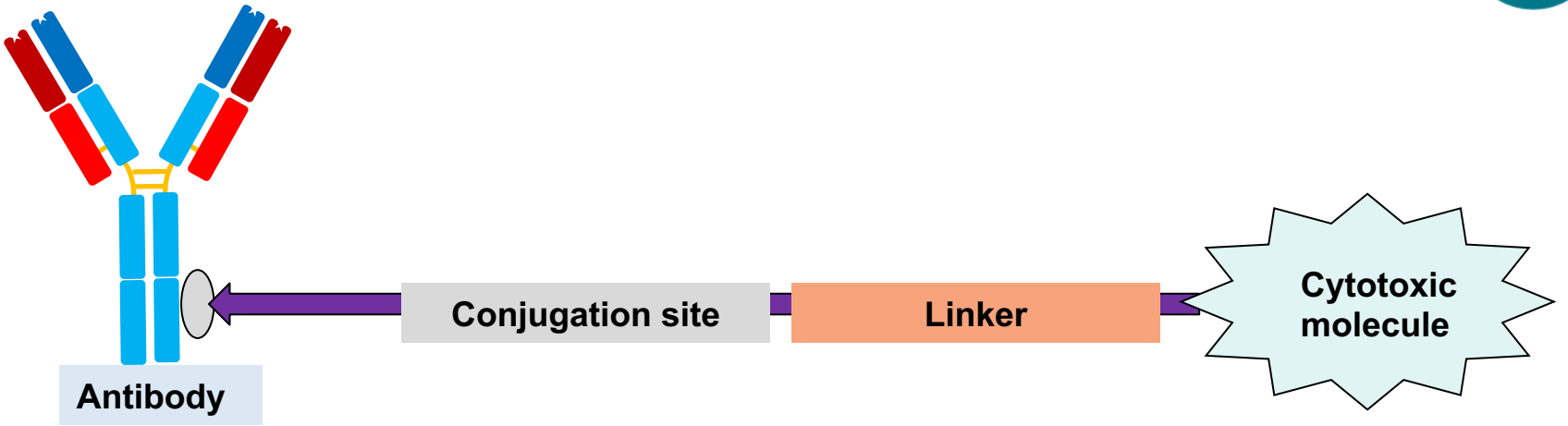
Development of a sensitive antibody drug conjugate (ADC) free-payload methodology and its application within a preclinical micro-sampling study

Dr James Howard, Senior Scientist, LGC

Outline

- Antibody drug conjugates (ADC)
- Safety and ethical considerations for ADC development
- Analytical challenges developing a free-payload methodology
- Assay validation
- Preclinical sample analysis results
- Subsequent ADC work supported at LGC

What are Antibody Drug Conjugates (ADC)?



- Must bind to highly expressed antigen on target cell
- High antigen binding affinity
- High specificity / low cross reactivity

- Most common conjugation site is modification of cysteine or lysine residues on the antibody

- Must be stable while in circulation
- Must be unstable in target cell

- Extremely potent
- Well known mechanism

What types of ADC are there?

Tumour Antigen Recognition Sites
Antigen should be abundantly expressed by tumour cells, but minimally expressed by healthy cells to achieve selective toxicity

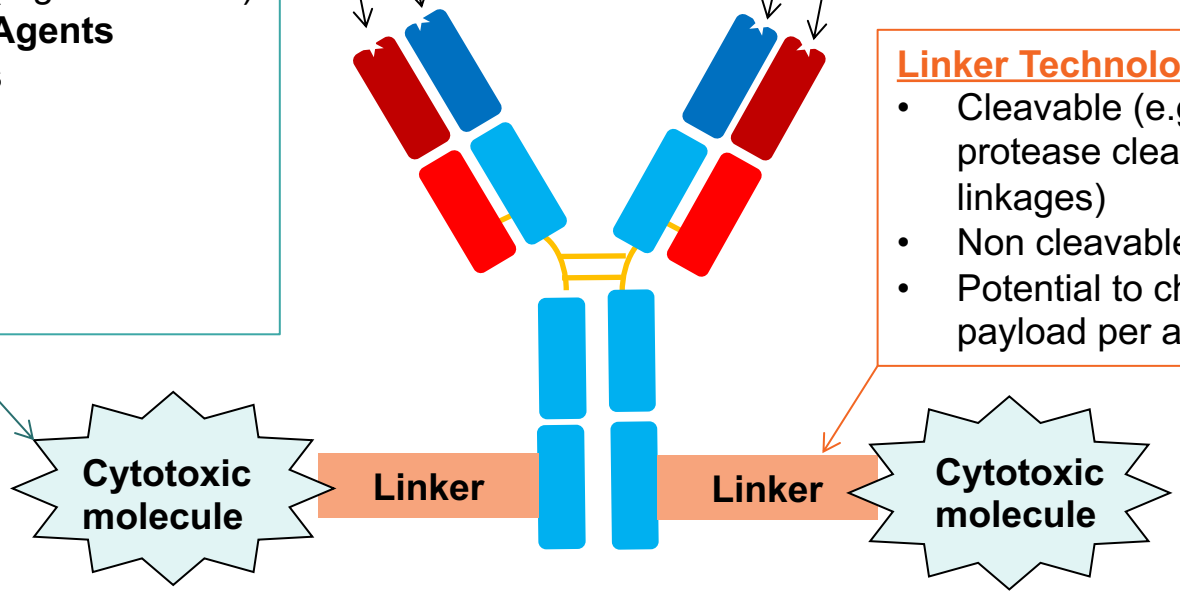
Cytotoxic Payloads

Microtubule (Tubulin) inhibitors
- Auristatins (e.g. MMAE)
- Maytansines (e.g. DM1/DM4)

DNA-Interactive Agents
- Anthracyclines
- Calicheamicin
- Duocarmycins

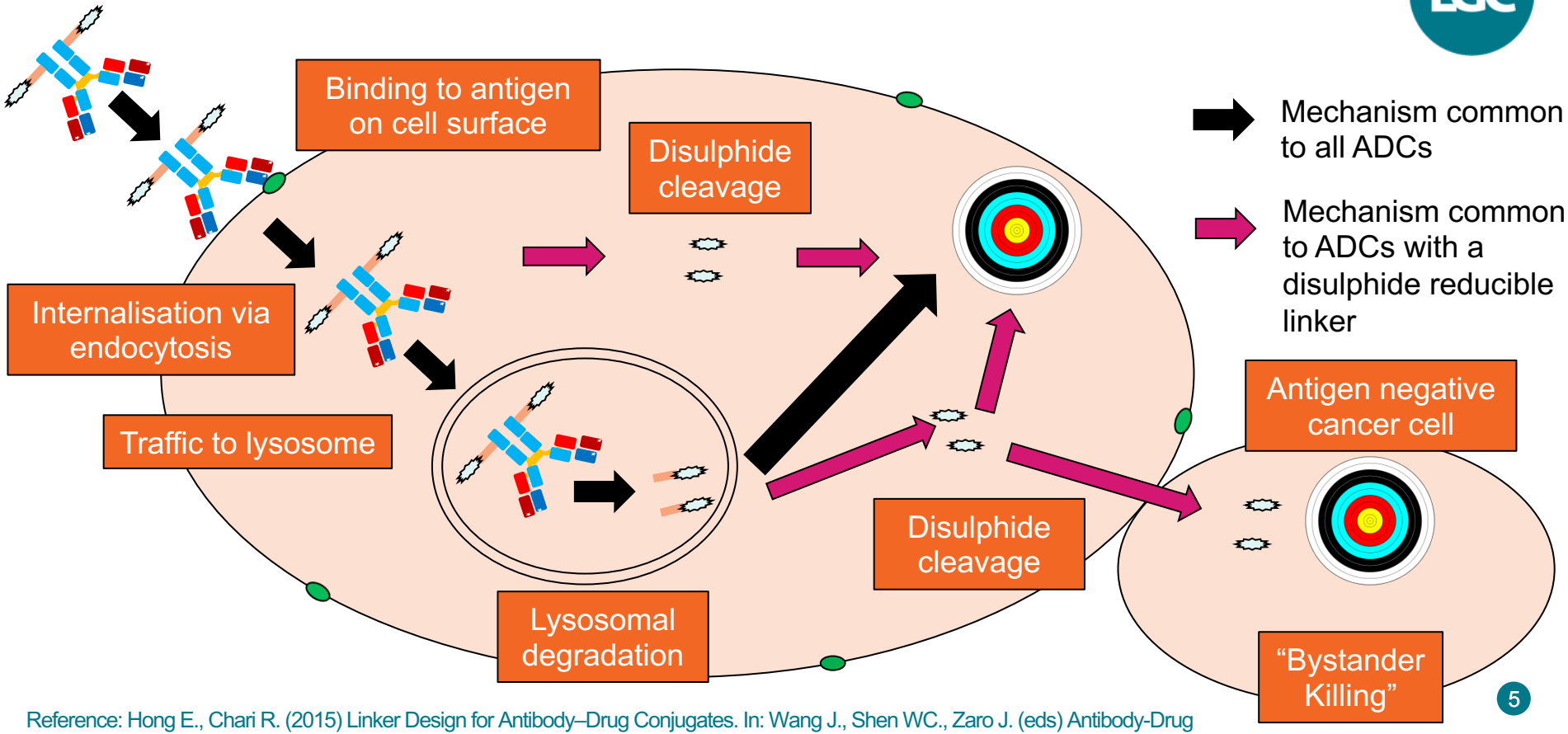
Other
- Toxic protein
- RNA inhibitors

Linker Technologies
- Cleavable (e.g. acid-labile, protease cleavable, disulphide linkages)
- Non cleavable
- Potential to change the number of payload per antibody (i.e. DAR)



Reference: Use of pyrrolobenzodiazepines and related covalent-binding DNA-interactive molecules as ADC payloads: Is mechanism related to systemic toxicity?, Jackson PJM, Kay S, Pysz I, Thurston DE, Drug Discovery Today: Technologies Volume 30, December 2018, Pages 71-83

ADC Mode of Action



Reference: Hong E., Chari R. (2015) Linker Design for Antibody–Drug Conjugates. In: Wang J., Shen WC., Zaro J. (eds) Antibody-Drug Conjugates. AAPS Advances in the Pharmaceutical Sciences Series, vol 17. Springer, Cham

ADCs on the Market and in Development

5 ADCs currently have market authorisation

- 2001 Mylotarg (leukaemia)
- 2011 Adcetris (lymphoma)
- 2013 Kadcyla (breast cancer)
- 2017 Besponsa (leukaemia)
- 2019 Polivy (lymphoma)

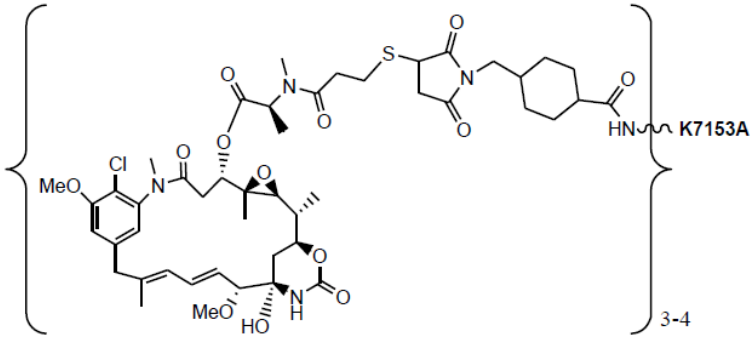
ADCs in development include

- Breast cancer drug
  - > Replacement for Herceptin mab
- Debio 1562

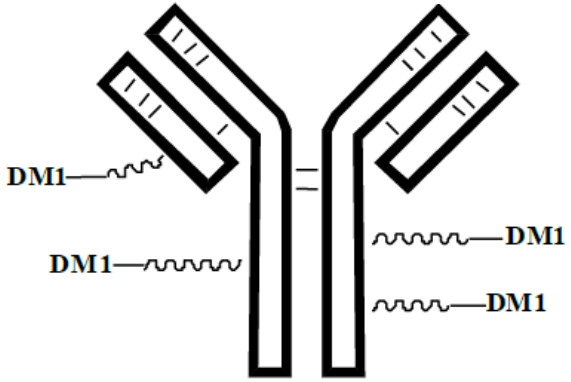
~US$7 billion

Reference: https://en.wikipedia.org/wiki/Antibody-drug_conjugate

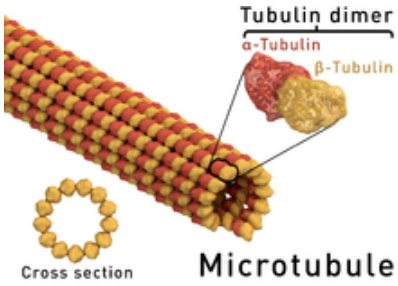
Debio 1562



DM1 is ~1.8% weight by monoclonal antibody (mAb)

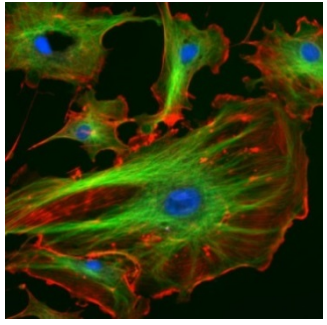


For illustration only – not intended to represent exact conjugation sites



Reference:
https://en.wikipedia.org/wiki/Microtubule

- DM1 toxic payload
- Debio 1562 targets CD37 antigen of non-Hodgkin lymphoma (NHL) cancer cells
- NHL accounts 4% of all cancers, 70,800 new cases in US in 2014 (SEER 2015)
- Patients can show resistance to existing therapeutics (CD20 directed Mab rituximab)



Reference:
https://en.wikipedia.org/wiki/Cytoskeleton

Safety Considerations

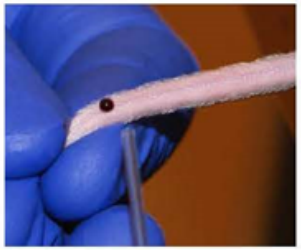
- **DM1 is a highly toxic drug**
  > It should only be significantly released from ADCs when these are inside cancer cells
  > Small amounts released from cells during cell death

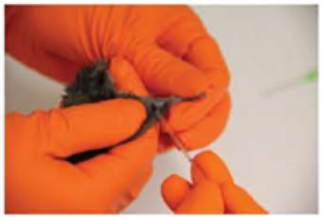
- **Any systemic DM1 must be metabolised and not accumulate with repeat dosing**
  > Measured using a free DM1 LC-MS/MS assay

- **Repeated dose of DM1 in a TK study (rather than Debio 1562) requested by health authorities**
  > Rodents lack the CD37 antigen that Debio 1562 targets, which consequently leads to DM1 release in cells
  > Gene knock-in therapy for CD37 was not successful
  > Toxicity study in the mouse with repeated intravenous (bolus) administration of DM1

Ethical Considerations

- **3Rs: Reduce, Replace, Refine** [1,2]
- **Microsampling**
  - > Blood sample volume 30-60 µL
  - > All time points collected from *all* animals
  - > Composite sampling unnecessary
  - > Fewer animals needed and better PK Data





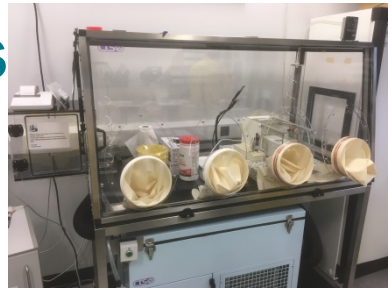
Sampling with Vitrex Micro haematocrit tubes

Wax Plug

Plasma Generation

1) Russell WMS, Burch RL. The Principles Of Humane Experimental Technique. Methuen, London, UK (1959)
2) Diehl K-H, Hull R, Morton D et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J. Appl. Toxicol. 21(1), 15–23 (2001)

Analytical Challenges



- **Safety Challenges**
  > DM1 powder is fatal by inhalation

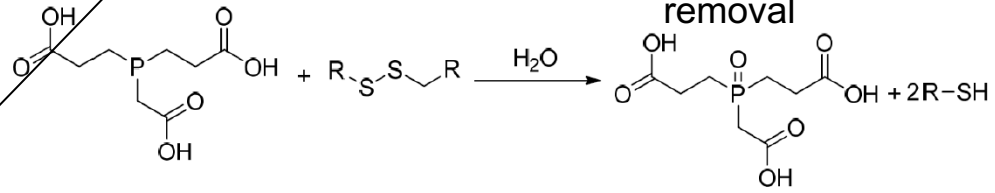
- **Published LC-MS Methods are in human plasma / serum**
  > Increased drug instability in mouse plasma?
  > Increased interferences?
  > Lower sample volume

- **Microsampling**
  > Only 30-60 µL blood obtained, to allow serial sampling in each mouse of at least 4 time points
  > Assay volume needs to be very low
  > Repeat contingency (and ISR?)
    - Backup samples
    - Sample handling challenges
  > Tubes pre-coated with stabilisers are not available

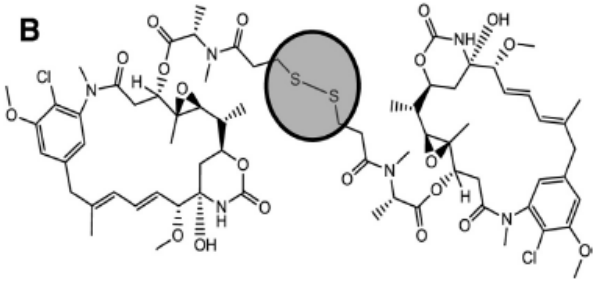
Transfer of Validated Stabilised Human Plasma Methodology

1	Aliquot 100 µL stabilised human plasma (K_2EDTA) sample into ostro plate *(on top of a 1mL lo-bind plate)*
2	Add 12.5 µL 50:50 ACN:H_2O to blanks
3	Add 12.5 µL ISWS to other samples
4	Seal plate with adhesive film and mix – 2 mins
5	Add 12.5 µL 0.1M TCEP in H_2O
6	Seal plate with adhesive film and mix – 10 mins
7	Add 300µL 100:0.1 ACN:FA
8	Seal plate with adhesive film and mix – 10 mins, 900 rpm
9	Push samples through with positive pressure
10	Evaporate at 50°C
11	Reconstitute in 100µL 40:60:0.1 ACN:H_2O:FA
12	Mix – 2 mins, 1200 rpm
13	Centrifuge 1500 rpm +4°C 10mins
14	Inject on LC-MS/MS

Ostro plates allow in-well protein precipitation and phospholipid removal



TCEP (tris(2-carboxyethyl)phosphine)



DM1-dimer

DM1-dimers, glutathione, cysteine and albumin adducts can be formed in plasma

Preparation of Samples for Mouse Assay

- **Plasma samples generated by microsampling**
  > From 30 µL blood, approx **15 µL** plasma (K_2EDTA) generated
  > A 10-fold lower LLOQ could be achieved if needed

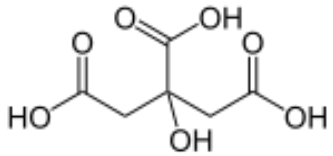
- **10-fold dilution with plasma**
  > 15 µL plasma (K_2EDTA) sample + 135 µL blank plasma (K_2EDTA) = **150 µL** diluted sample
  > Allowed two 75 µL aliquots to be generated
    - Allows multiple analyses per sample (25 µL aliquot volume)
  > Human plasma instead of mouse plasma used for dilution
    - In compliance with 3Rs
    - Method in human plasma already developed
    - Allows stabilisation of DM1 by incorporating any stabiliser directly into human plasma
      – No special capillary microsampling tubes pre-coated with stabiliser required

Stability of DM1 in plasma

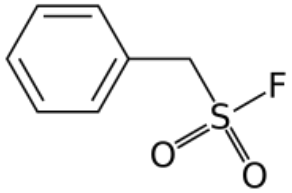
- **Transferred methodology**
  > DM1 was stable in citric acid stabilised human plasma

- ***Stabilised* human plasma (K_2EDTA) used for the 10-fold Dilution**

Stabiliser	Time at 4°C	Spiked DM1 % Change
Citric Acid	6 hr	-55.5



Citric acid denatures degrading enzymes due to lowering plasma pH



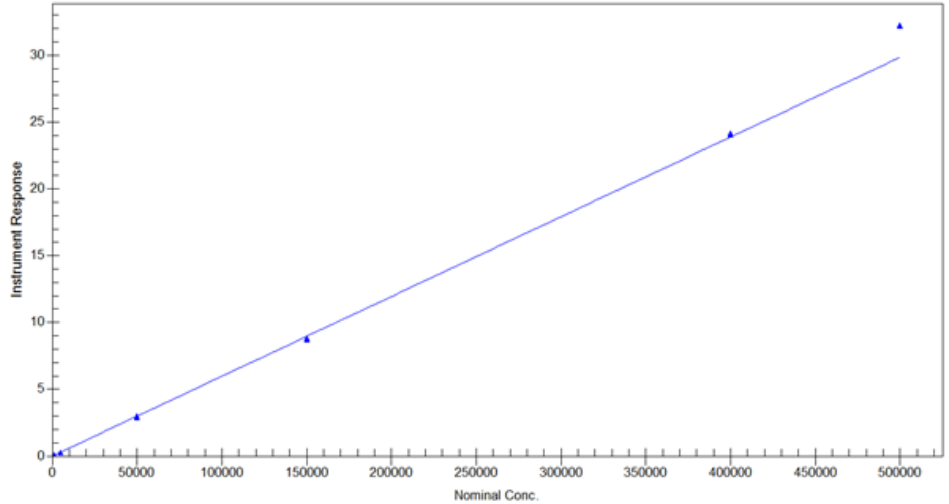
Phenylmethylsulfonyl fluoride (PMSF) is a serine protease inhibitor

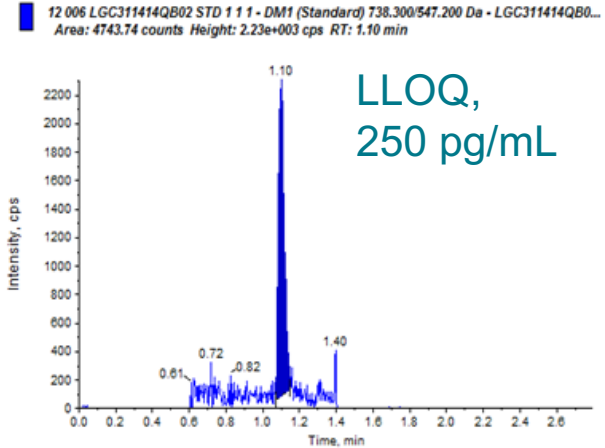
- **LGC provided stabilised human plasma to the test facility, which was added to the microsamples immediately after plasma generation**
  > Clear instructions provided to the test facility
  > Process included in QA audits

Validation

- GLP validation to EMA 2011 / FDA 2001
- 25-50,000 pg/mL range in stabilised (diluted) mouse plasma (K_2EDTA)
- Equivalent to 250-500,000 pg/mL in non-stabilised (non-diluted) mouse plasma (K_2EDTA), due to 10-fold dilution

Batch	Stats	QC LLOQ 250 pg/mL	QC LOW 750 pg/mL	QC MED 15,000 pg/mL	QC HIGH 375,000 pg/mL
1	%CV	4.1	3.0	0.8	0.9
	%RE	-0.4	1.1	0.7	1.3
2	%CV	6.2	5.0	0.8	1.6
	%RE	-4.8	6.4	-2.0	-4.3
3	%CV	4.9	3.3	1.5	1.5
	%RE	-2.4	-8.5	-3.3	-1.9
Mean	%CV	5.2	7.3	2.0	2.7
	%RE	-2.8	-0.4	-2.0	-1.6





LLOQ, 250 pg/mL

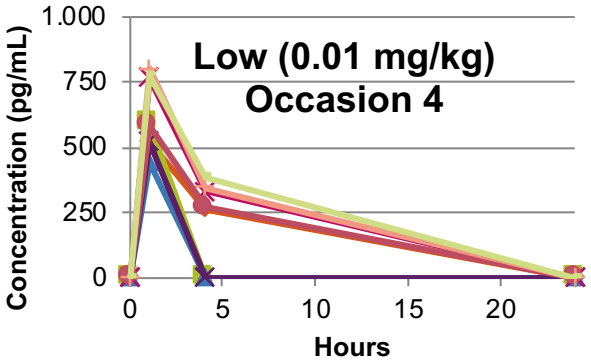
Validation

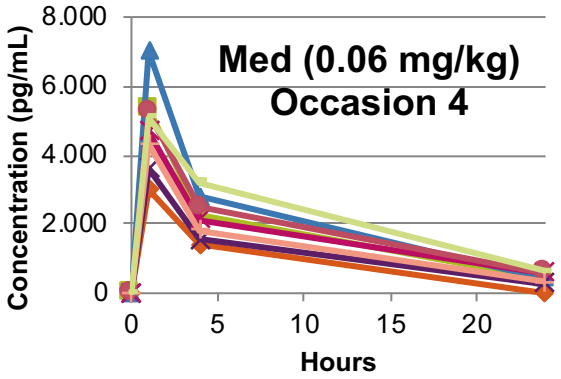
- **Selectivity**
  - > 5/6 stabilised mouse plasma (K_2EDTA) lots completely clear
  - > 1 lot had peak at 1.2 min, but resolved from expected analyte RT (1.1 min)
- **Recovery and matrix Effects**
  - > Passed standard acceptance criteria
- **Stability**

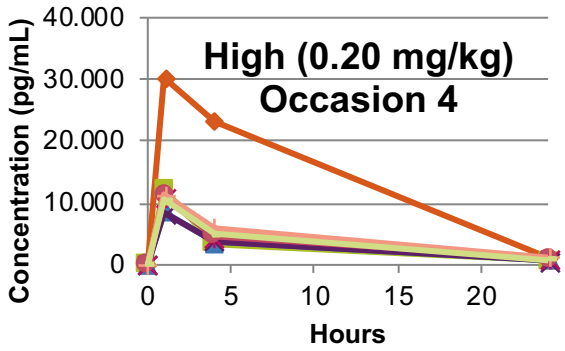
Matrix	Stabilised? (Citric Acid pH3)	Stability condition	Spiked DM1 % Change	
			LOW (750 pg/mL)	HIGH (375,000 pg/mL)
Blood	No	30 min (4°C)	-7.0 % (medium, 15,000 pg/mL)	
Plasma	No	30 min (4°C)	-5.5	-4.4

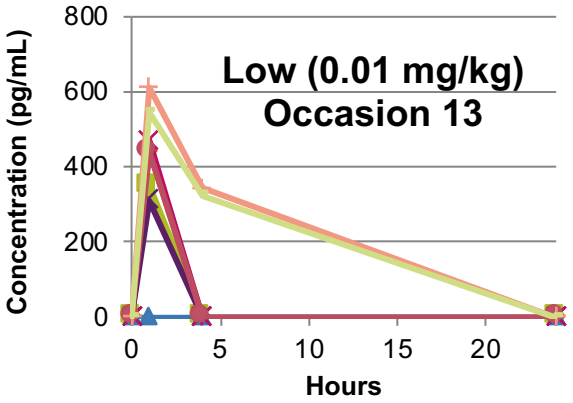
Preclinical Sample Analysis

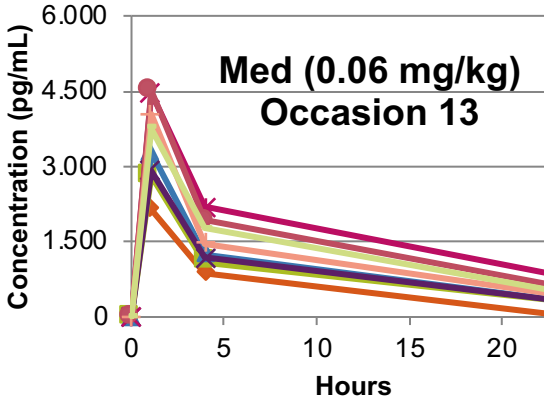
- Repeated IV bolus administration of DM1

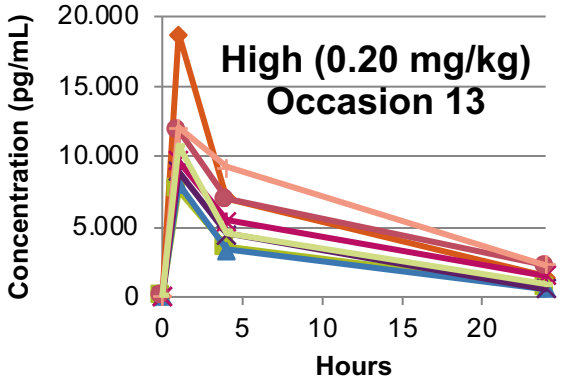






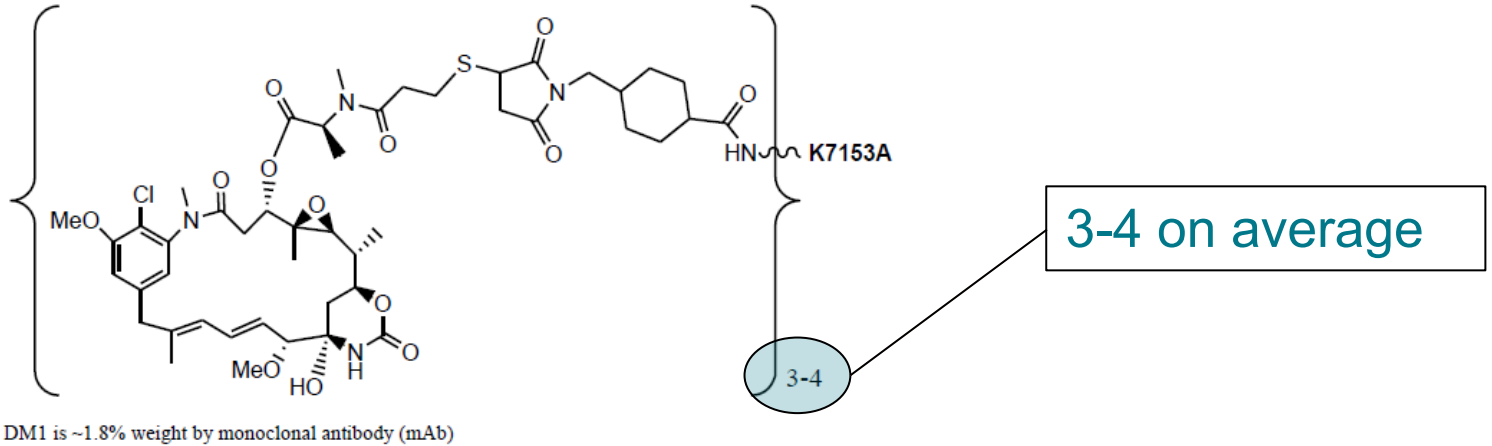






Subsequent Projects at LGC

- **PK analysis from a phase II clinical trial dosing Debio 1562**
  > Free-DM1, with 25 pg/mL LLOQ
- **Drug antibody ratio (DAR) determination of Debio 1562 test materials**
  > Test materials contains mixture of ADCs, with 0,1, 2, 3, 4 or more, DM1 molecules attached
  > Uses high resolution MS



DM1 is ~1.8% weight by monoclonal antibody (mAb)

Conclusion

- ADCs allow highly potent drugs to be used in cancer therapy, with minimal side-effects due to their highly targeted mechanism of action

- A free-payload (DM1) assay has been validated to bioanalytical guidelines

- Sample collection methodology achieved 3R objectives and gave a simple stabilisation methodology
  - > Reduce: Full TK profiles obtained from single animals
  - > Replace: Human plasma used as a stabilisation matrix
  - > Refine: Low blood volumes taken on each occasion
  - > Methodology applied by the sponsor to several GLP and non-GLP preclinical studies, and may also be applied by them for paediatric studies to minimise blood volume collected

- Resulting TK Profiles enabled progression of the ADC development into a Phase II clinical trial

Acknowledgements

LGC

- Geoff Wallace, Senior Scientist, Method Development
- Richard Lucey, Senior Scientist, Validation and Sample Analysis

Debiopharm

- Marie-Claude Roubaudi-Fraschini, Associate Principal Scientist

Any Questions?

Development of a sensitive antibody drug conjugate (ADC) free-payload methodology and its application within a preclinical micro-sampling study