How to develop **A**ntibody **D**rug **C**onjugates: Bioanalysis Contribution

Recommendations and survey results

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on behalf of the EBF TT43

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Barcelona

Topic team 43 - Antibody Drug Conjugates

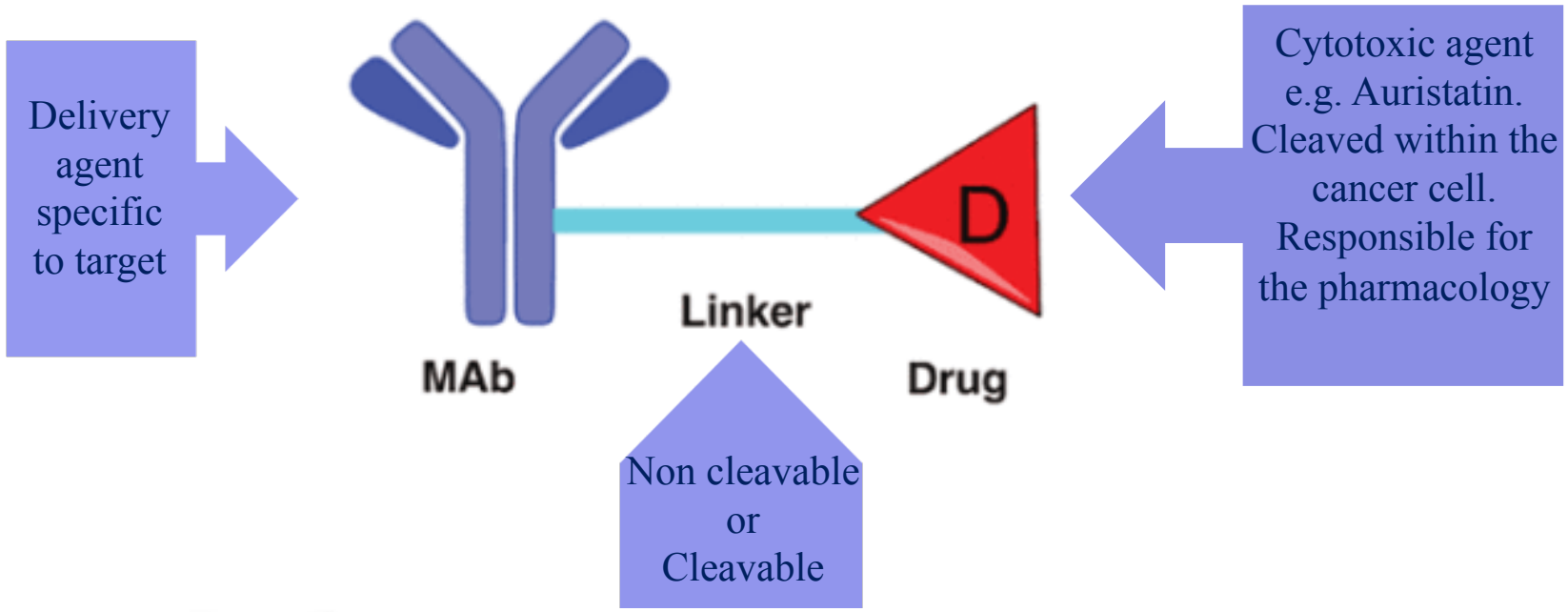
The Team

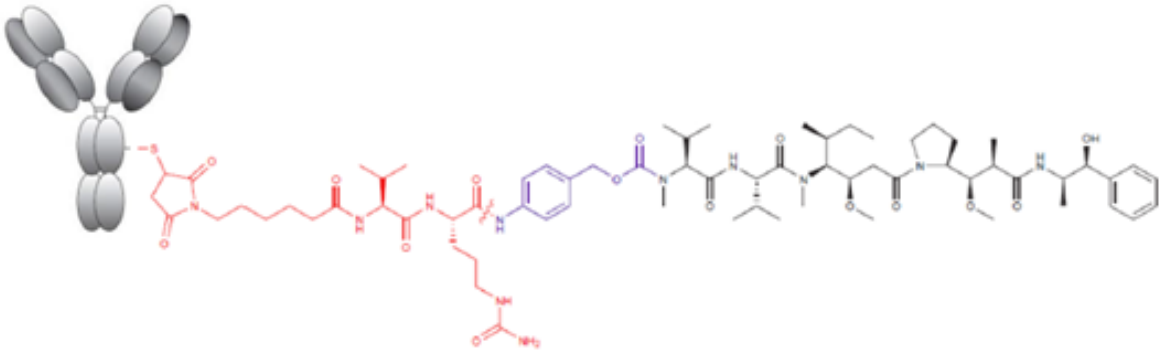
- Matt Barfield, GSK (Lead)
- Bernhard Beckermann, Bayer
- Margarete Brudny-Kloeppel, Bayer (Sponsor)
- Stephanie Fischmann, Abbvie
- Kirsty Jackson-Addie, Astrazeneca
- Martin Nemansky , PRA
- Monique Putman, QPS
- Andrew Roberts, Quotient
- Anne Kleinnijenhuis, TNO

Team goals

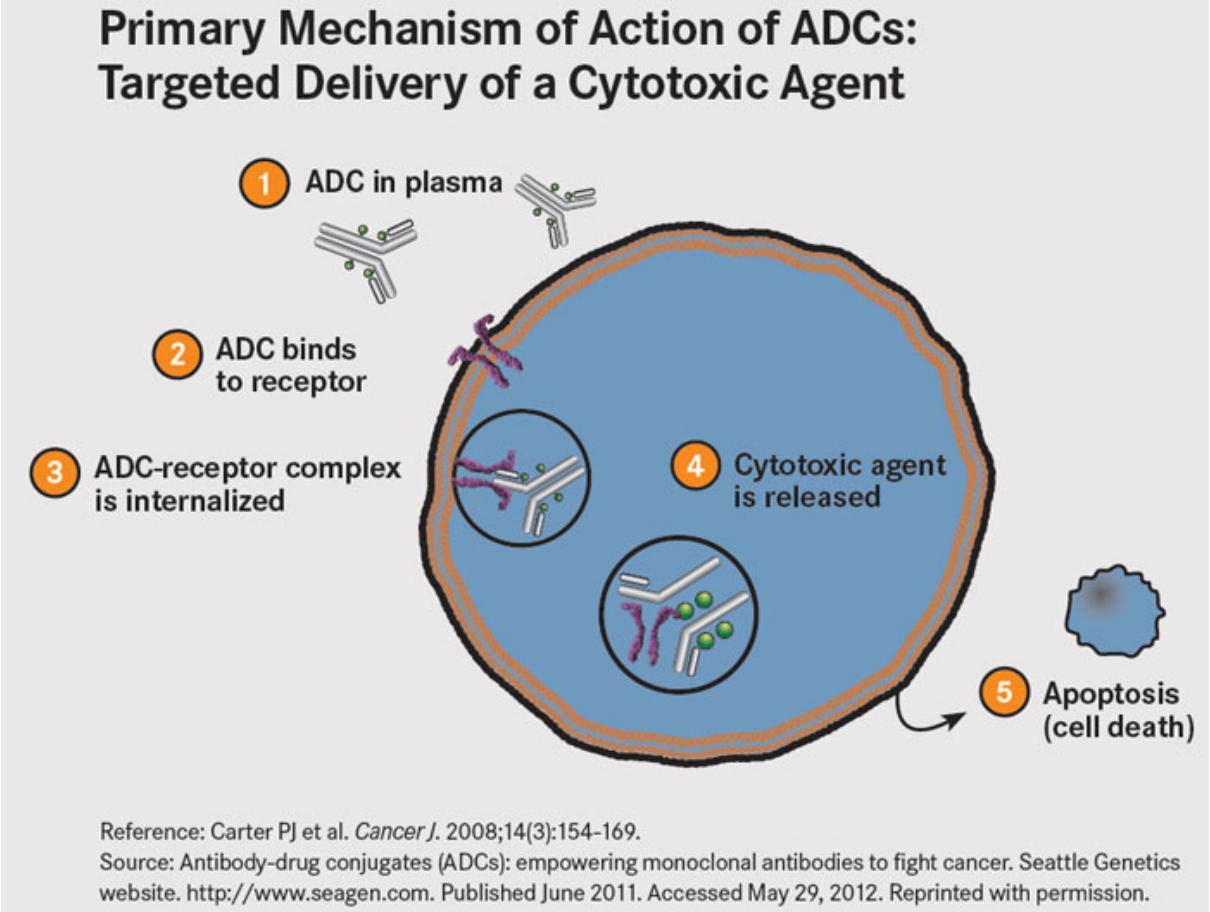
Share best practice, information gathering and disseminate across the community

ADC-Combines the unique targeting capabilities of a mAb with the cancer killing properties of a cytotoxic drug to allow discrimination between healthy and disease tissue



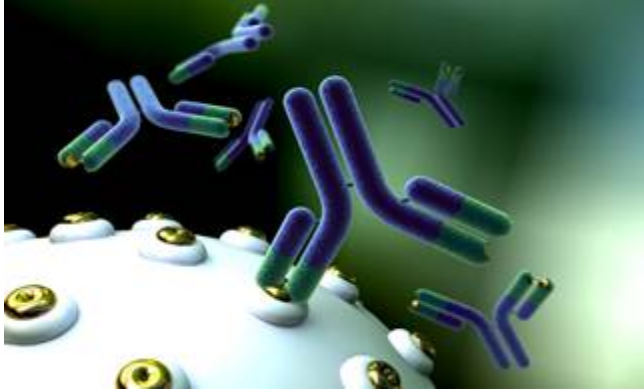


ADC mechanism of action



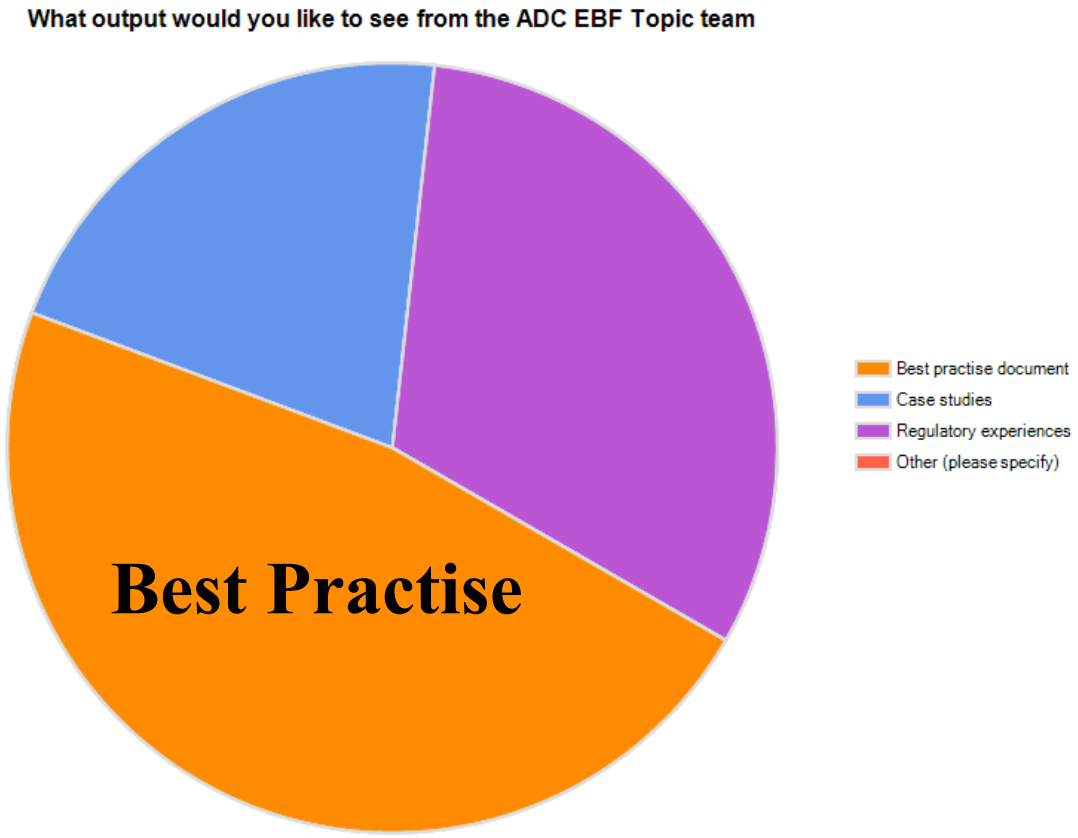
Reference: Carter PJ et al. *Cancer J.* 2008;14(3):154-169.
Source: Antibody-drug conjugates (ADCs): empowering monoclonal antibodies to fight cancer. Seattle Genetics website. http://www.seagen.com. Published June 2011. Accessed May 29, 2012. Reprinted with permission.

Content

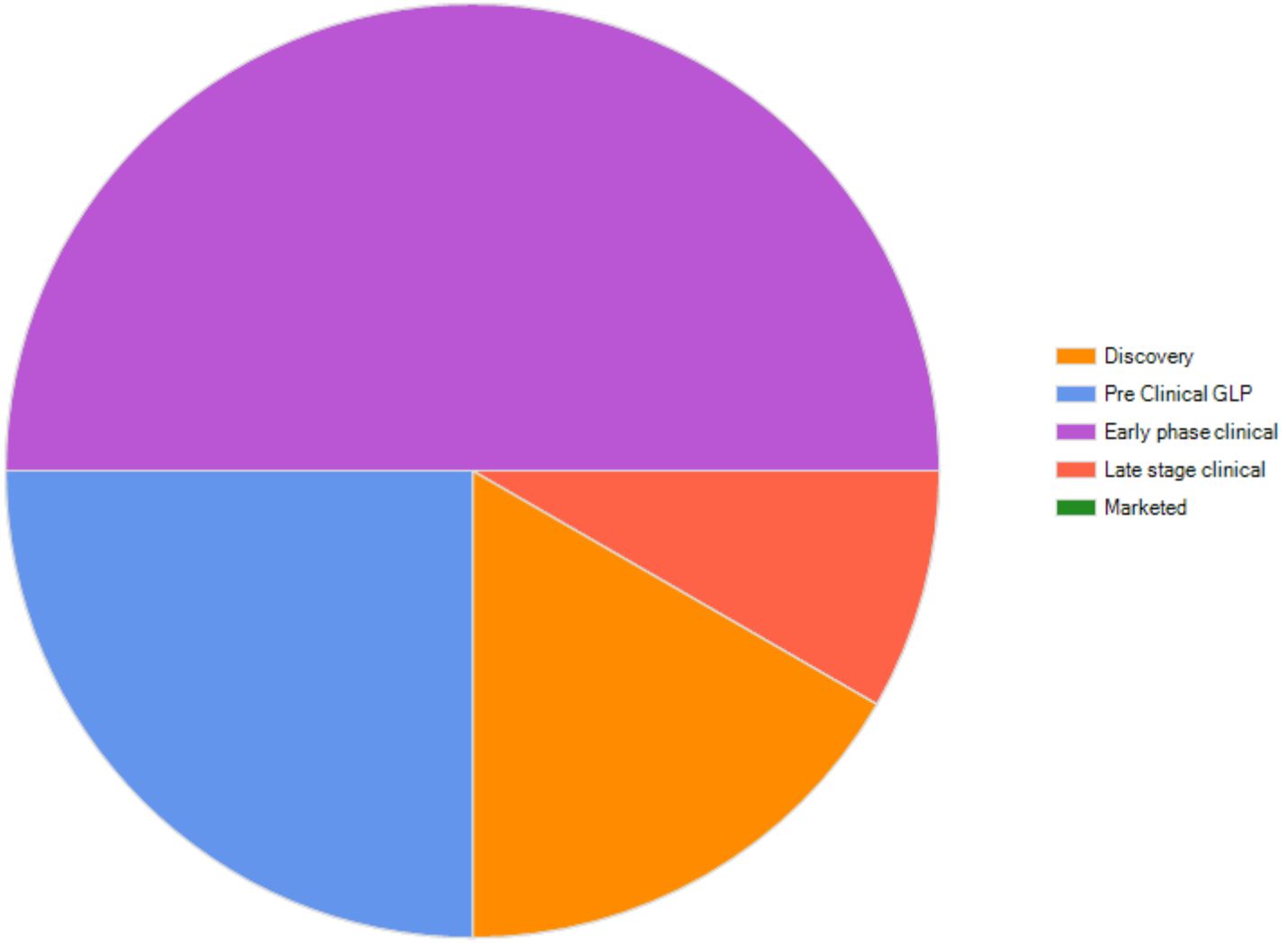


- Survey results
- Basis of a recommendation paper

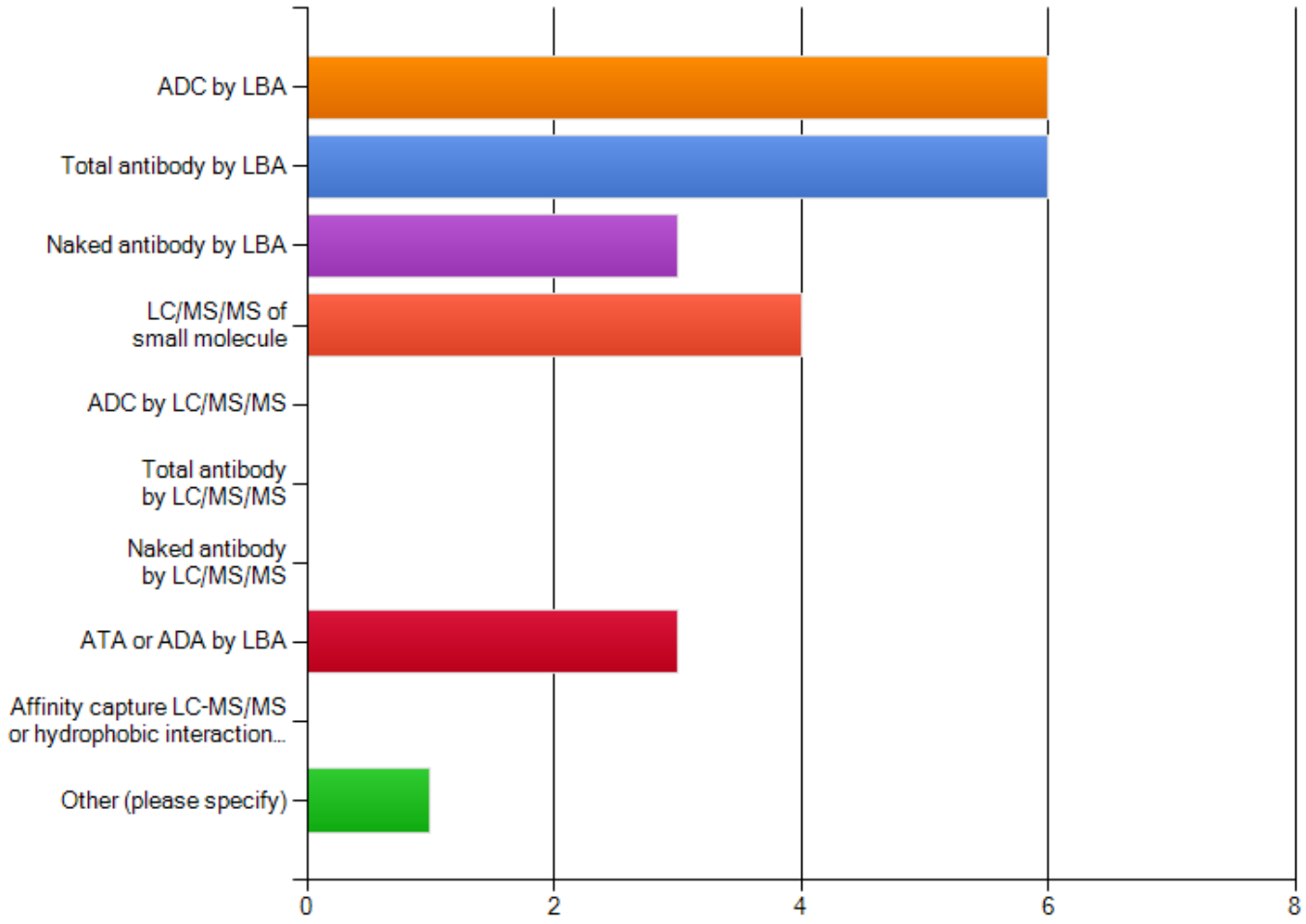
TT-43 Survey learnings
Setting direction



In what phase of development is your ADC method (s)



What bioassays do you conduct



Bioanalytical development for ADC

- Broken down into 4 categories
  - Discovery
  - Preclinical GLP
  - Early clinical (FTIH/repeat dose)
  - Late stage clinical (phase 2B to filing)

Discovery - recommendations

- 3 assays required
  - Total mAb (LBA)
  - ADC assay (LBA)
  - Payload (small toxic molecule, LC/MSMS)
- Species
  - Rodent / Non rodent species used for safety
  - Pharmacology species used for efficacy
- Matrix
  - EDTA Plasma
- Options
  - Drug/Antibody Ratio (DAR, LC-MS) for candidate selection with optimal linker stability
  - LC/MSMS for antibody assays (lead optimization)

Discovery - recommendations

Assay qualification

- Fit for purpose: Tiered approach
- 1 run precision & accuracy
- Assay sets acceptance criteria
- Selectivity (testing of individual's blanks)
- Specificity (testing of reference ADC's)

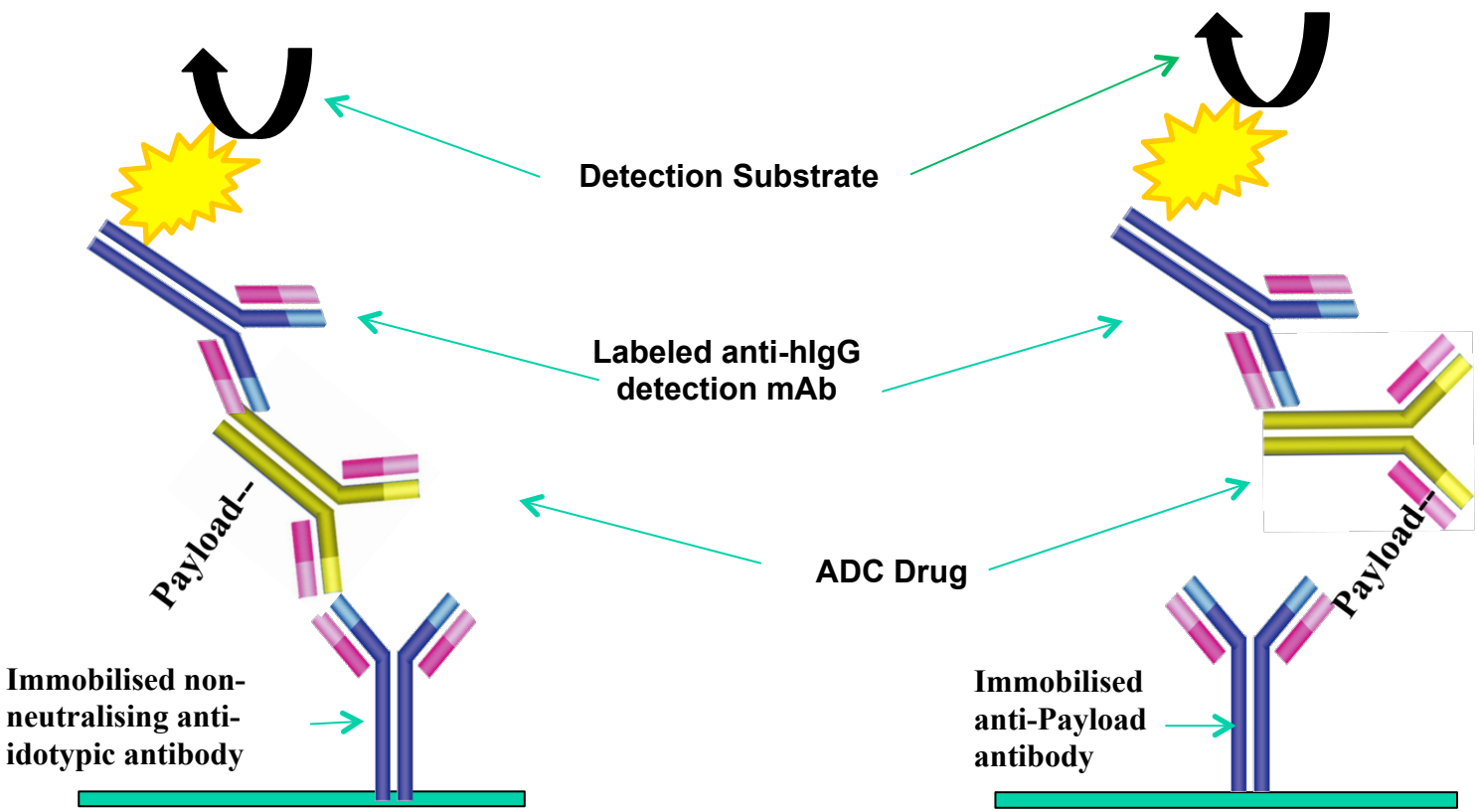
➢ Matrix stability

- Exploratory RT or 37°C plasma stability

Example

Total mAb assay (generic format)

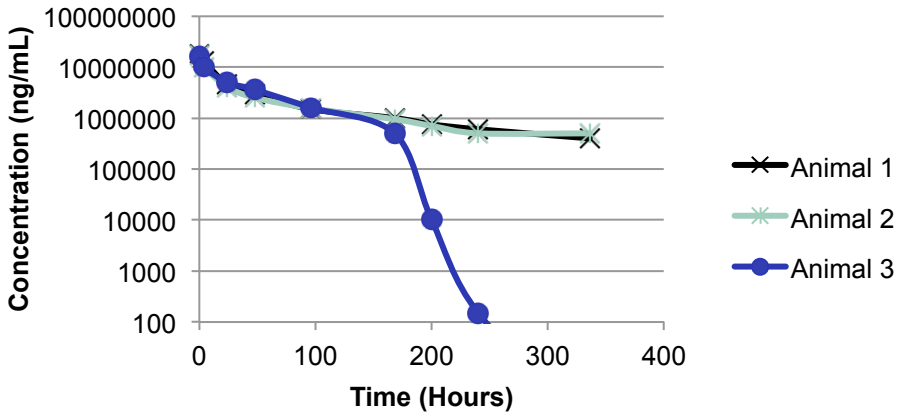
ADC assay



Payload = LC/MS/MS assay

Discovery – recommendations (cont)

- Assays used to support PK, TK, PK/PD studies
- Antidrug Antibody (ADA) assay is developed if PK or toxicology indicates possible ADA



- Possibility of generic assays at this stage to save time (anti human IgG assay)

Preclinical GLP - recommendations

- Same 3 Assays validated to GLP following EMA/FDA guidelines
  - GLP material for validations
  - Stable label internal standard for LC/MS/MS
- ADA assay developed
  - Option of only deploying if PK shows ADA

Preclinical GLP – recommendations (cont)

- ADC Stability – FDA request
  - *In vitro* experiment: 96hour stability of preclinical and human matrix at 37^{o}C
  - Monitor for appearance of free payload
  - Expect <5% appearance

Early Clinical- recommendations

- Validate 3 assays in human matrix
  - Issue: ADC Reference Standards ?
  - Exploratory Metabolite Monitoring ?
- ADA assay developed and deployed

Late phase Clinical- recommendations

- If assays understood remove total mAb assay
- Assays for Mass Balance and Drug metabolism support including DAR ?
- Continue ADA analysis

Unanswered questions

- What is the impact when a different batch gives a different DAR value?
- Late phase experience

Nice to haves – or are they?

- Change in time of DAR value
- Impact of DAR on the assays

Further thoughts around best practice

- Very comprehensive publications now available

1. **Bioanalysis of antibody–drug conjugates: American Association of Pharmaceutical Scientists ADC Working Group position paper - Bioanalysis (2013) 5(9), 997–1006**

- No single package fits all (diverse molecules: mAb, linkers and payloads)
- Need a bespoke approach designed to answer specific questions
- Broad array of assays in the analytical toolbox

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