MedImmune
A member of the AstraZeneca Group

# Challenges and Considerations for the Development of Ligand Binding Assays for Novel Antibody Fusions

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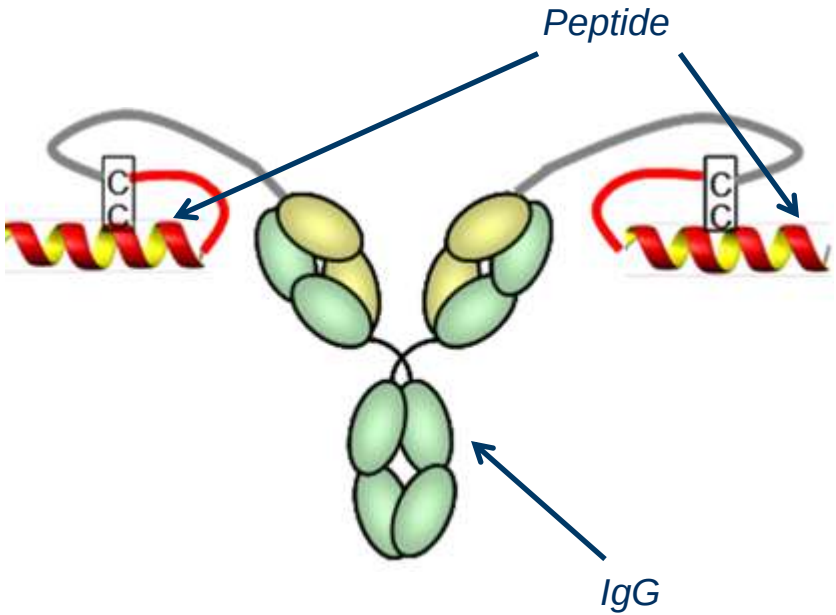
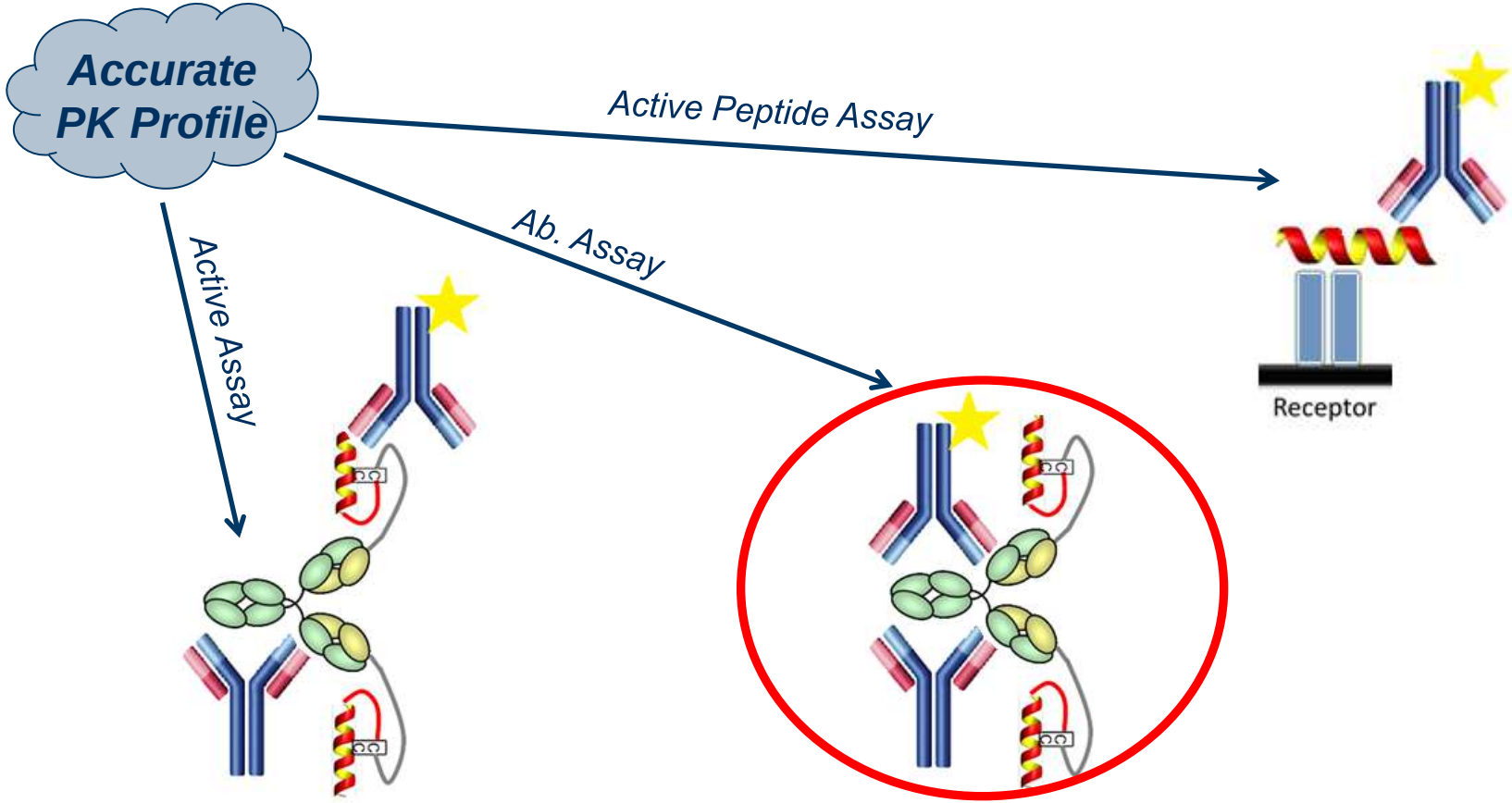
# MedImmune Case Study

- Asset:  An IgG coupled with 2 identical peptide molecules by appropriate linkers (Molecule X)
- Assay Type: Pharmacokinetic (PK)
- Assay Format: Sandwich
  - fluorescence output
- Species/Strain: Cynomolgus Monkey
- Capture: Biotinylated anti-X
  - concentration: 100 µg/mL
- Detection: Alexa-647 labelled anti-X
  - optimised concentration: 20 nM



# First Challenge: Requirement of Multiple Assays



# Second Challenge: Molecule Adherence

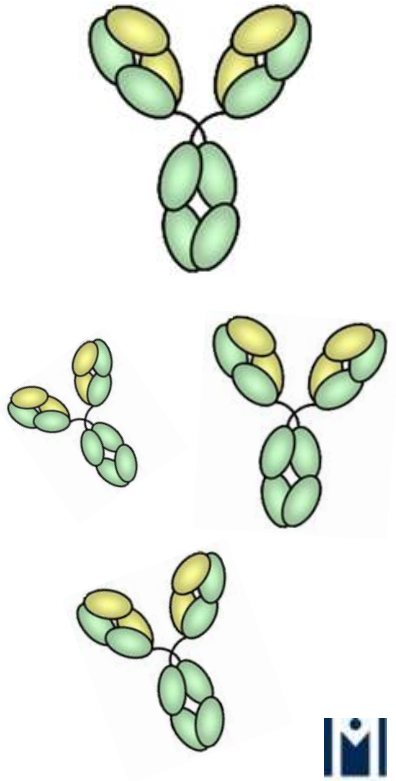
- The peptide portion of the asset was found to be highly amphipathic
- Poor recovery at lower curve, QC and sample concentrations
- Poor precision was also seen in a lot of assay runs

| Ind no. | Ind + Blk (0.00 ng/mL) | | Ind + LQC (50.00 ng/mL) | | Ind + HQC (12500.00 ng/mL) | |
|---|---|---|---|---|---|---|
| | Mean (ng/mL) | % Bias | Mean (ng/mL) | % Bias | Mean (ng/mL) | % Bias |
| 1 | < LLOQ | - | 74.5 | 49.0 | 12900 | 3.2 |
| 2 | < LLOQ | - | 101.0 | 102.0 | 13500 | 8.0 |
| 3 | < LLOQ | - | 80.1 | 60.2 | 13100 | 4.8 |
| 4 | < LLOQ | - | 65.4 | 30.8 | 12500 | 0.0 |

# Molecule Adherence: Changing the Reference Standard

- Reference Standard changed to the MAb scaffold without peptides

| QC ID | Nominal Conc. (ng/mL) | Mean Conc. (ng/mL) | % Bias | % CV |
|---|---|---|---|---|
| HQC | 18750 | > ULOQ | - | - |
| MQC | 750 | 1960 | 161.0 | 0.7 |
| LQC | 75 | 362 | 383.0 | 1.0 |
| LLOQ | 25.72 | 162 | 550.0 | 19.0 |



- QCs made with full molecule do not accurately back-calculate
  - Use of MAb. for curve preparation not appropriate

# Molecule Adherence: Other Options and Solutions

- Change the assay diluent
- Increase the Minimum Required Dilution (MRD)
- Increase the Lower Limit of Quantification (LLOQ)
  - adjust QC concentrations
- Use Lo-Bind plastic-wear or glass tubes
- Special handling conditions
  - e.g. use of cold conditions (perform on ice)

| QC ID (conc. ng/mL) | Average Bias (%) |
|---|---|
| ULOQ (25000) | 11.2 |
| HQC (18750) | 5.6 |
| MQC (1500) | 7.4 |
| LQC (250) | 17.2 |
| LLOQ (97.65) | 12.1 |

# Third Challenge: Freeze/Thaw Stability

- Freshly prepared standards and QCs demonstrated consistently good recovery
- Following only one freeze/thaw cycle, the recovery of the analyte deteriorated

| QC ID | Nominal Conc. (ng/mL) | Mean Conc. (ng/mL) | % Bias | T=0 % Bias | % CV |
|---|---|---|---|---|---|
| ULOQ | 25000 | 28800 | 15.0 | -8.0 | 2.4 |
| HQC | 18750 | 17400 | -7.0 | -5.2 | 4.0 |
| MQC | 1500 | 1320 | -12.0 | -17.3 | 0.8 |
| LQC | 250 | 160 | -35.9 | -23.4 | 11.1 |
| LLOQ | 97.65 | 61.4 | -37.1 | -30.2 | 14.7 |

# Freeze/Thaw Stability: Options and Solutions

- Use of a frozen working stock (WS) to prepare the calibration curve
  - Frozen QCs back-calculated against this curve
- Cold conditions
  - Freeze/Thaws performed on Ice
- Validate the assay with fresh QC preparation limitation
  - All sample analysis to be conducted with freshly prepared QCs as well as standards

# Freeze/Thaw Stability: Results

- The use of a frozen WS in cold conditions greatly improves analyte recovery

| QC ID | Nominal Conc. (ng/mL) | Mean Conc. (ng/mL) | Bias (%) | T=0 Bias (%) | %CV |
|---|---|---|---|---|---|
| ULOQ | 25000 | 24300 | -2.8 | -4.9 | 2.6 |
| HQC | 18750 | 16200 | -13.6 | -1.3 | 1.0 |
| MQC | 1500 | 1420 | -5.0 | -6.7 | 2.4 |
| LQC | 250 | 211 | -15.8 | -16.0 | 3.2 |
| LLOQ | 97.65 | 90.3 | -7.5 | -14.8 | 2.7 |

- **HOWEVER!**
  - Following multiple F/T cycles further deterioration is seen
  - Therefore proven stability for only 1 F/T cycle

# Summary

- Changing landscape in bioanalysis
  - Trend towards more complex molecules
- Longer time needed for method development and validation.
- Think structure and behaviour
- Get creative….

…think outside the box!