Experience of a CRO: Drug Tolerance Case Studies

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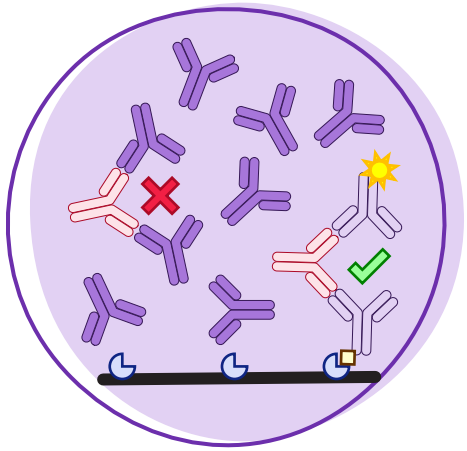
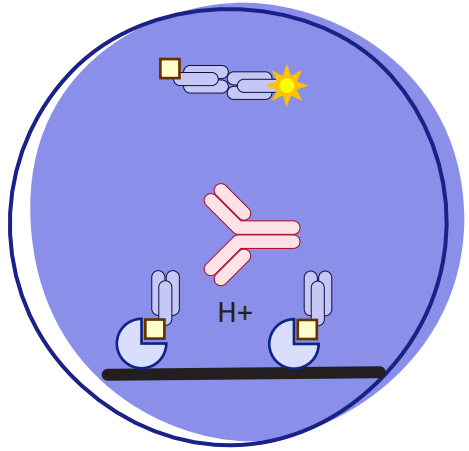
Experience of a CRO: Drug tolerance case studies



Case Study 1

Case Study 2



Case Study 3

Amount of work / time / cost

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Case Study 1

“*Win the race at the slowest speed possible*”

Alain Prost, four-time F1 champion driver

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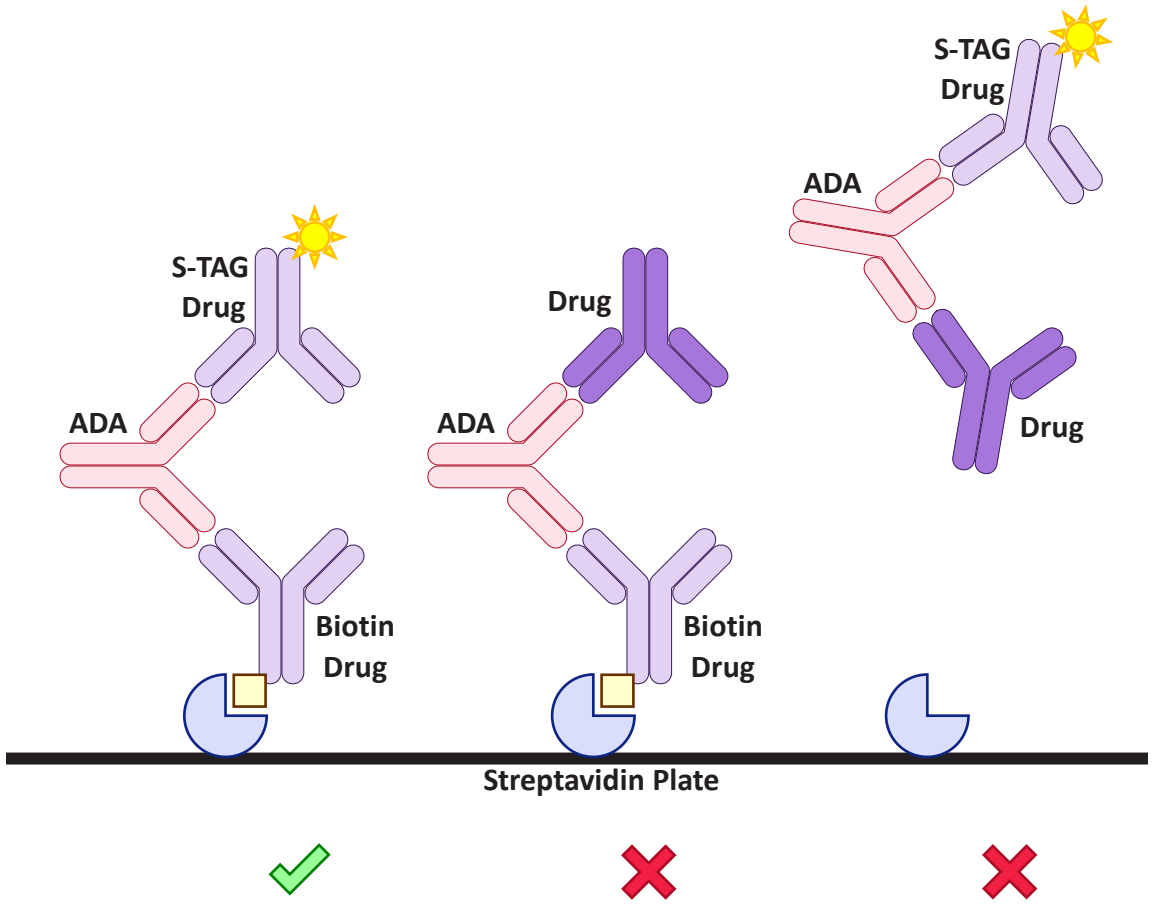
Case Study 1: Increased minimum sample dilution (MRD) in bridging format

Sponsor requirements

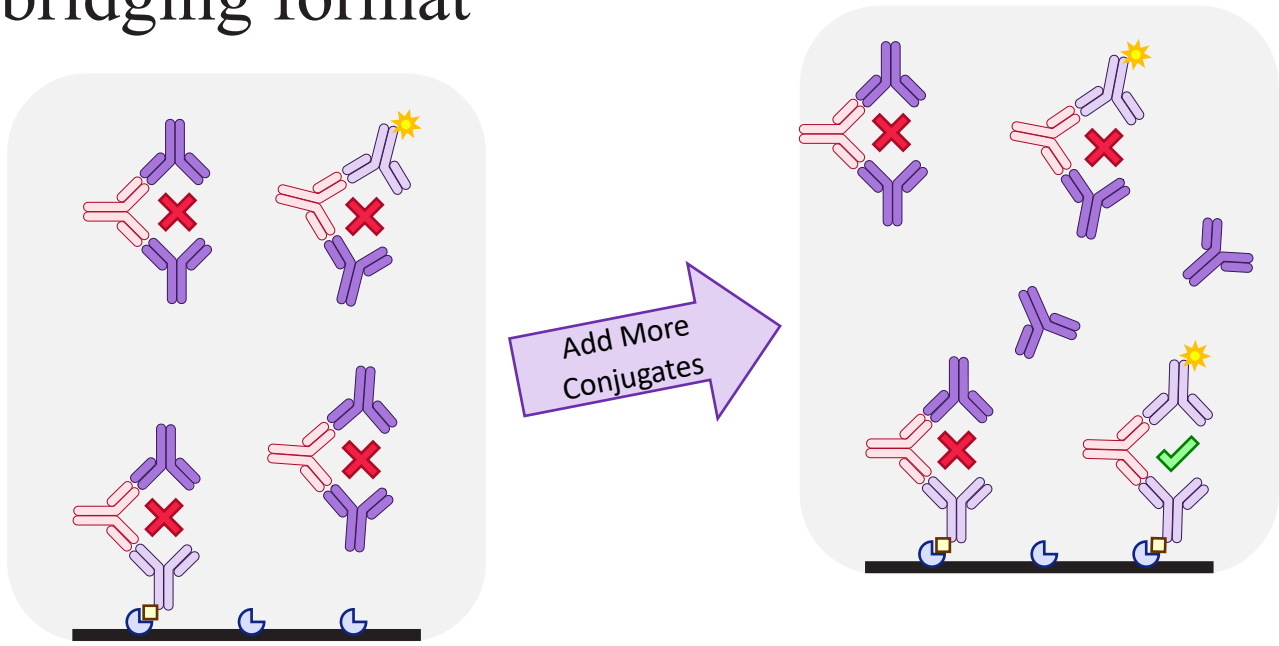
- Monoclonal antibody drug
- GLP preclinical toxicology study in nonhuman primates
  - Very high sensitivity not required, as some ADAs are expected in nonhuman subjects and do not indicate a safety concern
- Estimated drug concentration in samples up to **2.5 mg/mL**
- Existing basic bridging method available to transfer from sponsor, but not achieving drug tolerance requirements for the upcoming study



Case Study 1: Increased MRD in bridging format

Standard optimization experiments

- Introduced acid dissociation to break up pre-existing ADA:drug complexes – various conditions and combinations of acid and neutralization tested
- Increased concentrations of S-TAG and biotin drug conjugates to outcompete the drug



Modest improvements but still not sufficient

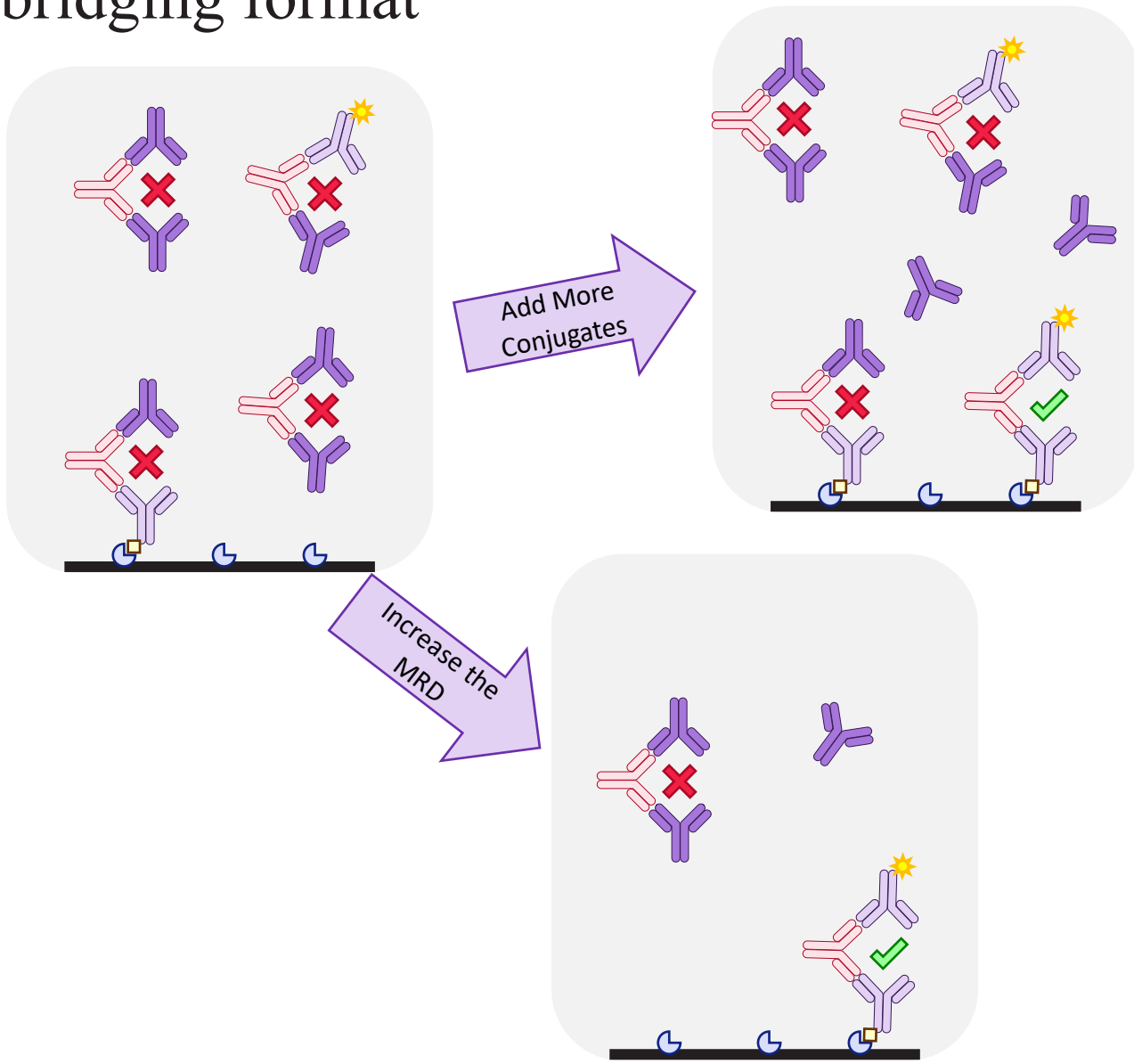
Case Study 1: Increased MRD in bridging format

Dilution is the solution

- Increasing the MRD traded away sensitivity but gave a better ratio of detection reagents/free drug
- Though positive control samples had lower signal, signals from PC+drug samples were higher when the sample was more dilute
- Optimal balance of sensitivity and drug tolerance found to be 1 in 600:
  - 100 ng/mL ADA detected in the presence of 1.5 mg/mL Drug
  - 500 ng/mL ADA detected in the presence of 5.9 mg/mL Drug (target 2.5 mg/mL)

Sufficient drug tolerance achieved

And the acid dissociation step is not even required!



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Case Study 1: Increased MRD in bridging format

Conclusion

- Final assay takes 4 hours with no additional reagents or training for analysts required
- An MRD exceeding 1 in 100 may be useful, when the required assay sensitivity and immunogenicity risk assessment is considered
- Sponsor agreed this was the best approach: clinical significance of immunogenicity in primates was low and the assay had sufficient sensitivity
- Assay successfully validated and used for sample analysis

Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection Guidance for Industry, FDA:
"Although the MRD ultimately selected by the sponsor will depend on the assay design and subject population, FDA recommends that MRD not exceed 1:100. Higher MRD may result in false-negative responses. However, in some instances higher MRD may be required, and the overall effect of such MRD on assay sensitivity and immunogenicity risk assessment should be considered."

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Case Study 1: Increased MRD in bridging format

Conclusion

- Simple assay format was used; more complex or expensive assay avoided
- Time saved developing something more complex
- Sometimes the easiest solution is the best

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Case Study 2

“*Push, without risk*”

Leena Gade, three-time Le Mans winning race engineer

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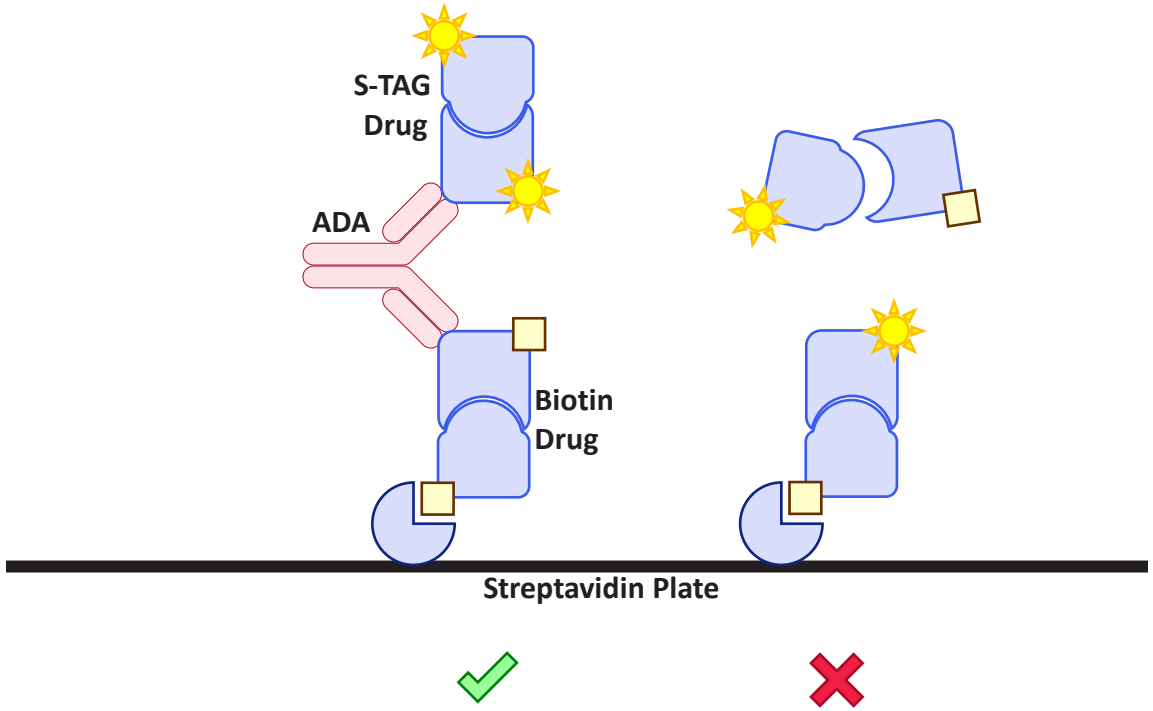
Case Study 2: Affinity-capture and elution

Sponsor's drug consists of two parts which non-covalently bind one another

Sponsor requirements

- Unusual therapeutic molecule consisting of two non-covalently bound proteins associated with one another
- GLP preclinical toxicology study in nonhuman primates
- Estimated drug concentration in samples up to 10 µg/mL
- Sponsor requested a three-tier immunogenicity assay, with screening, confirmation and titer

- "Drug tolerance" challenge comes not from free drug in sample, but from exchange of subunits between tagged drug reagents leading to high assay background in standard bridging assay



Case Study 2: Affinity-capture and elution

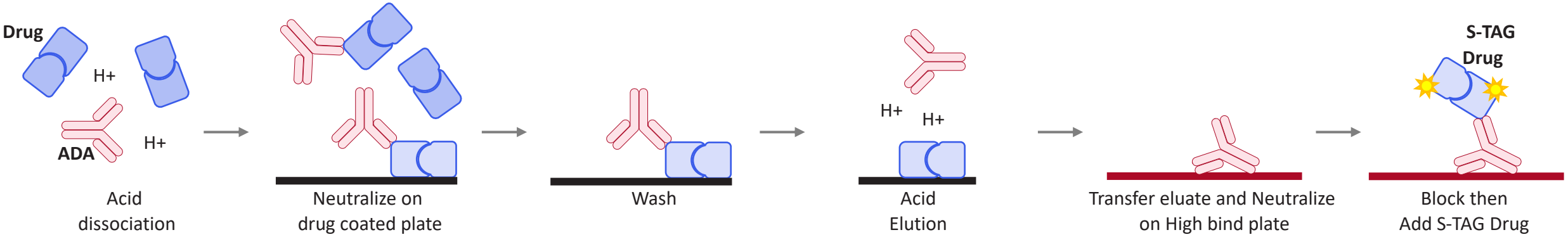
Affinity-capture and elution assay (ACE)

Positives

- No non-standard reagents required and no special equipment
- Drug tolerance better than a bridging assay, and can often help with other forms of interference too
- Not a lot of additional training required for analysts (compared to bead assays or PANDA)

Negatives

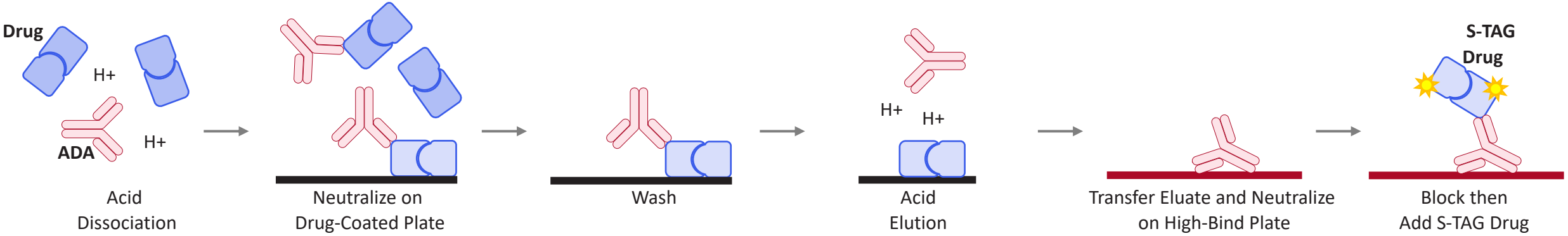
- Repeated use of acid may result in sensitivity loss for some ADAs
- Extra steps can result in increased assay variation
- Long single day assay or two-day assay – harder to schedule and make time for data processing



Case Study 2: Affinity-capture and elution

Rationale for selection for this study

- Washes the free drug out of the assay
- Specific capture reagent and detection reagent never meet
- Theoretically, this removes the ability for drug from sample or capture reagent to bind S-TAG labelled drug and produce high background



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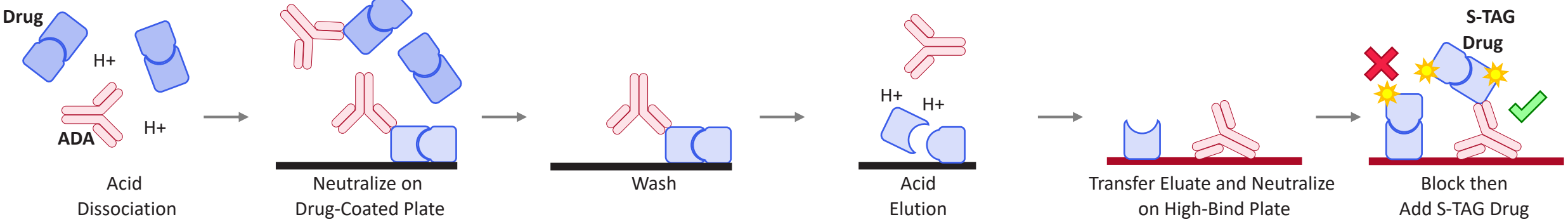
Practicalities

- In practice, a low background remains, which we attribute to carry-over of the drug from the ACE plate
- As background signal is generated by binding of the S-TAG drug to other drug molecules, it can be depleted by addition of excess drug. Therefore, a standard confirmatory tier would not be possible
- **But is a confirmatory tier really required for preclinical?**



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Case Study 2: Affinity-capture and elution

Conclusion

- Confirmatory tier was attempted but proved challenging
- Sponsor originally requested confirmation but was happy with our justification of removal of this tier
- Final assay takes 6.5 hours with no non-standard reagents. One day assay is more cost effective and schedule friendly
- Assay successfully validated and used for sample analysis

ICH guideline S6 (R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals:
"Immunogenicity assessments are conducted to assist in the interpretation of the study results and design of subsequent studies. … animal studies are not relevant in terms of predicting potential immunogenicity … in humans."
ICH guideline S6:
"If the interpretation of the data from the safety study is not compromised … no special significance should be ascribed to the antibody response.

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Case Study 2: Affinity-capture and elution

Conclusion

- Complex format was a consequence of the unusual structure of the drug
- A non-standard therapeutic may require a non-standard solution!
- Successfully balanced the sponsor's desire for a three-tier assay with the need to meet the basic requirements on a reasonable budget and timescale

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Case Study 3

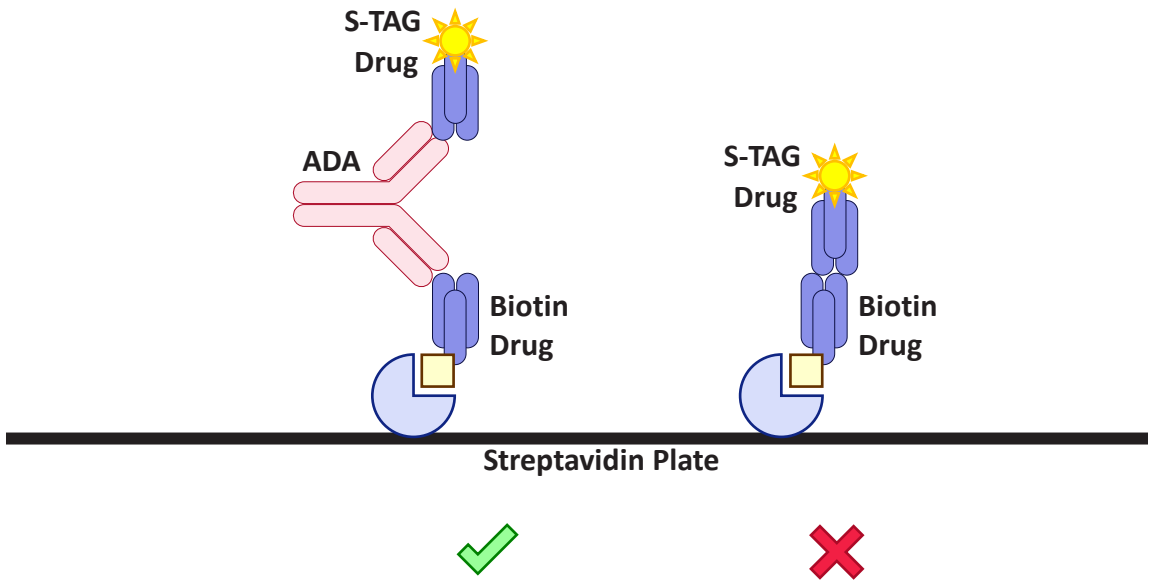
“*It’s Hammer-time*”

Pete Bonnington, seven-time F1 champion race engineer

Case Study 3: Affinity-capture and elution II

Sponsor requirements

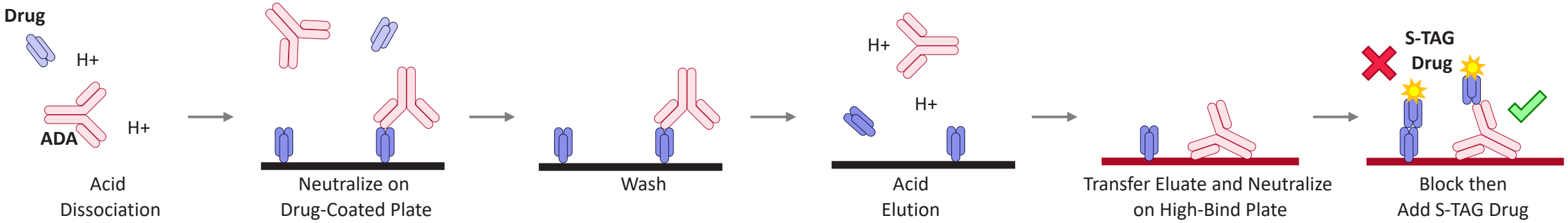
- Medium-length radiolabelled peptide drug
- Clinical study
- Estimated drug concentration in samples up to 10 µg/mL
- Similar challenge as previous case study. High bridging assay backgrounds, attributed to tagged drug reagents associating with each other in solution
- Conjugation of different versions of the drug (derivatized radiolabelling precursor vs. non-derivatized version), and different challenge ratios did not reduce background to an acceptable level
- Step-wise method using Protein A/G detection also yielded high non-specific backgrounds



Case Study 3: Affinity-capture and elution II

ACE

- Similar game plan to previous case study
- Similar result – drug-specific background was still present in the blank samples
- However, this time it is a clinical study, so a confirmatory tier is required



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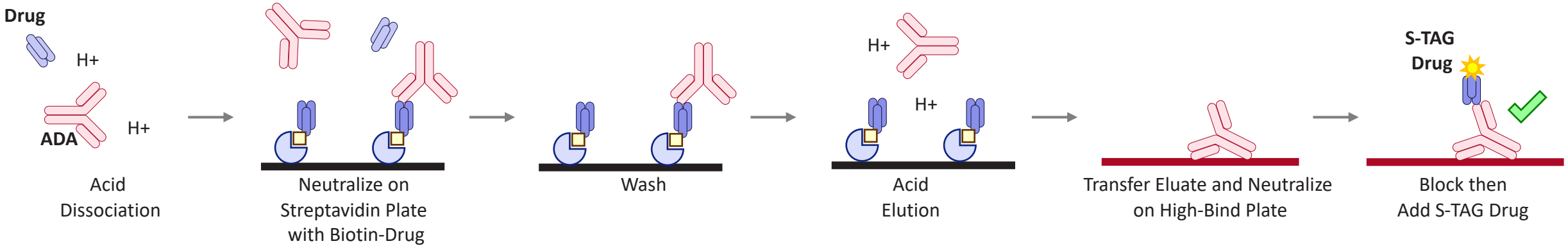
Modifications

- Streptavidin-coated plates with biotinylated drug – less susceptible to acid dissociation of the drug
- Reduction of detection reagent to reduce blank signal
- Confirmatory drug concentration adjusted carefully to achieve optimal distinction between NC and LPC % depletion
- MRD adjusted up to 1 in 40



Case Study 3: Affinity-capture and elution II

Conclusion

- Many options (conjugation of different drug versions, protein A/G detection) were tested before finding a format that worked
- Finding the optimal conditions for the confirmatory assay took many additional days of work
- Could have been streamlined if the drug-specific challenges were understood better from the beginning
- Assay successfully validated and sample analysis expected to start in December 2023

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Case Study 3: Affinity-capture and elution II

Conclusion

- Even though drug levels were not high, the properties of the drug can still cause analytical problems. Understanding those properties up front could save some work
- Finding the optimal conditions for the confirmatory assay took many additional days of work. Was this a must-do?
  - Alternative would be to proceed without a confirmatory tier; regulators would require justification of this

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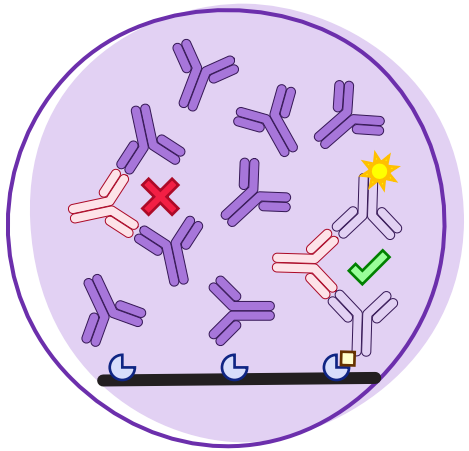
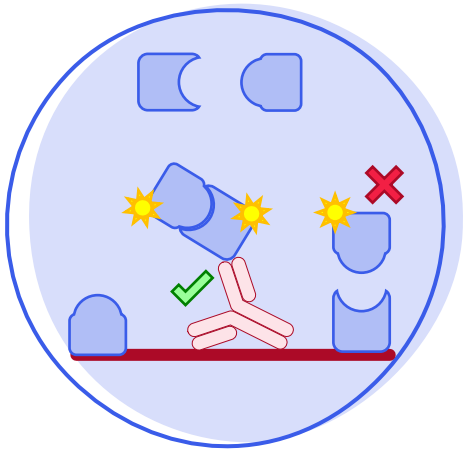


Case Study 1

Preclinical ADA

High drug tolerance requirement

Bridging format with unusually high MRD overcomes drug interference

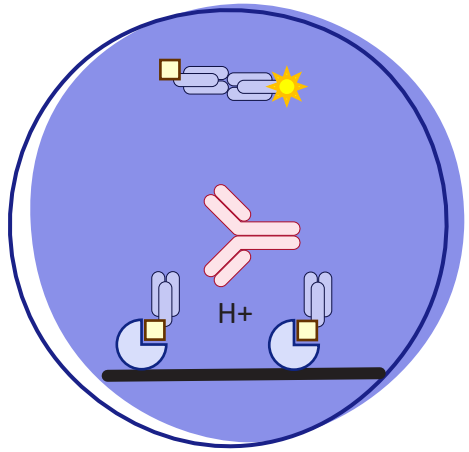


Case Study 2

Preclinical ADA

Drug composed of two parts, leading to high background in bridging assay

Straightforward ACE format, useful for screening/titer only



Case Study 3

Clinical ADA

Drug dimerizes leading to high background in bridging assay

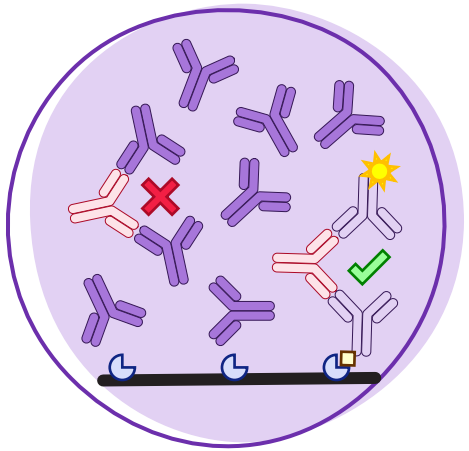
Carefully optimized format performed well in three tiers of ADA analysis

Acknowledgements

- Heather Revell
- Sam Willcox

Thank you

Experience of a CRO: Drug tolerance case studies



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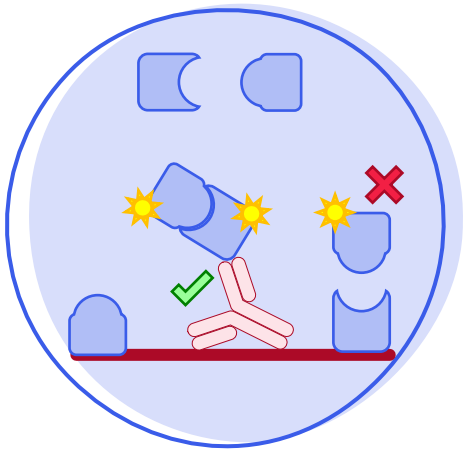

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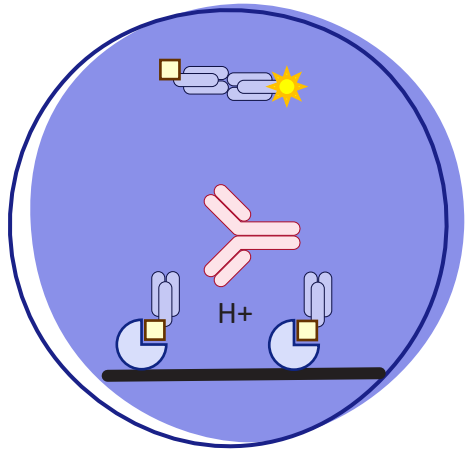

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