AstraZeneca

An Old Dog with New Tricks? Resurrection, Reoptimisation and Refinement to Render Drug and Target Interference Redundant

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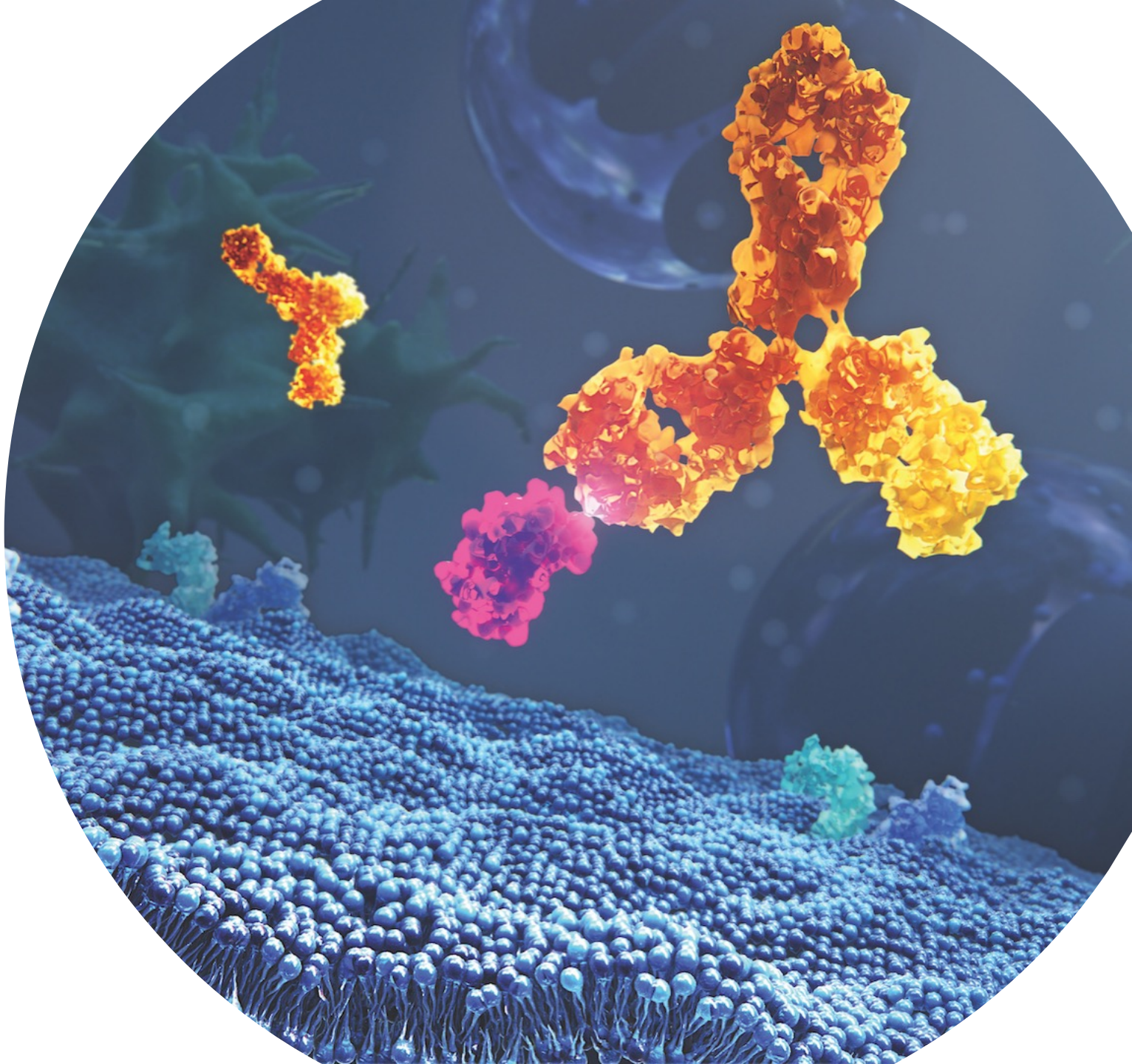
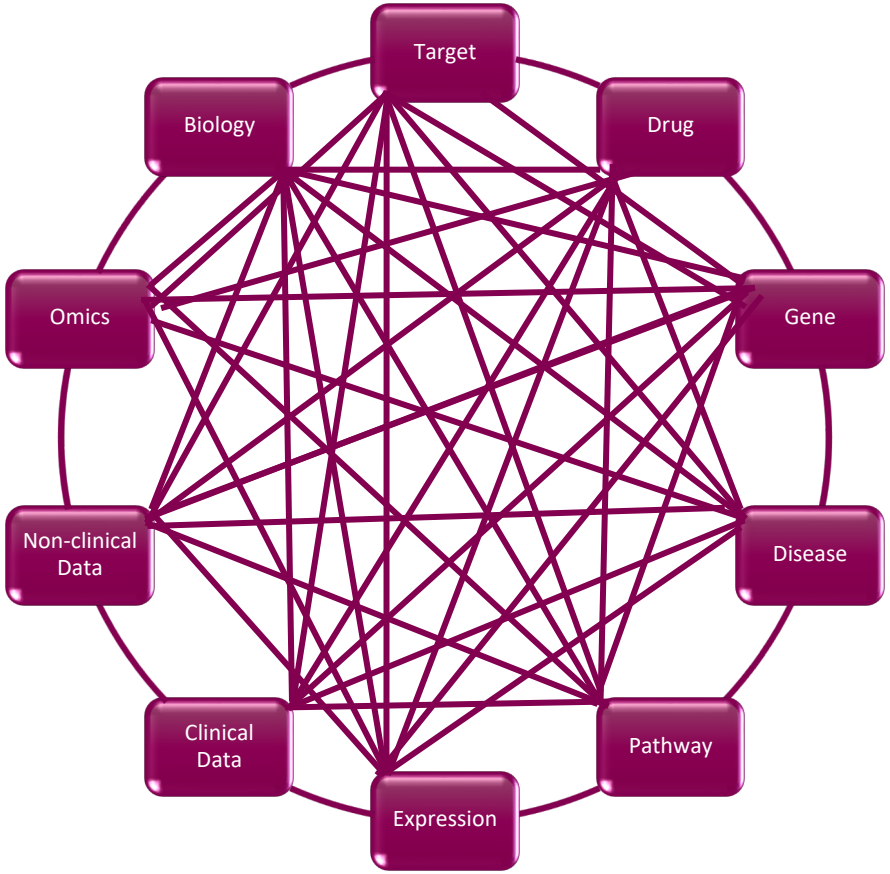
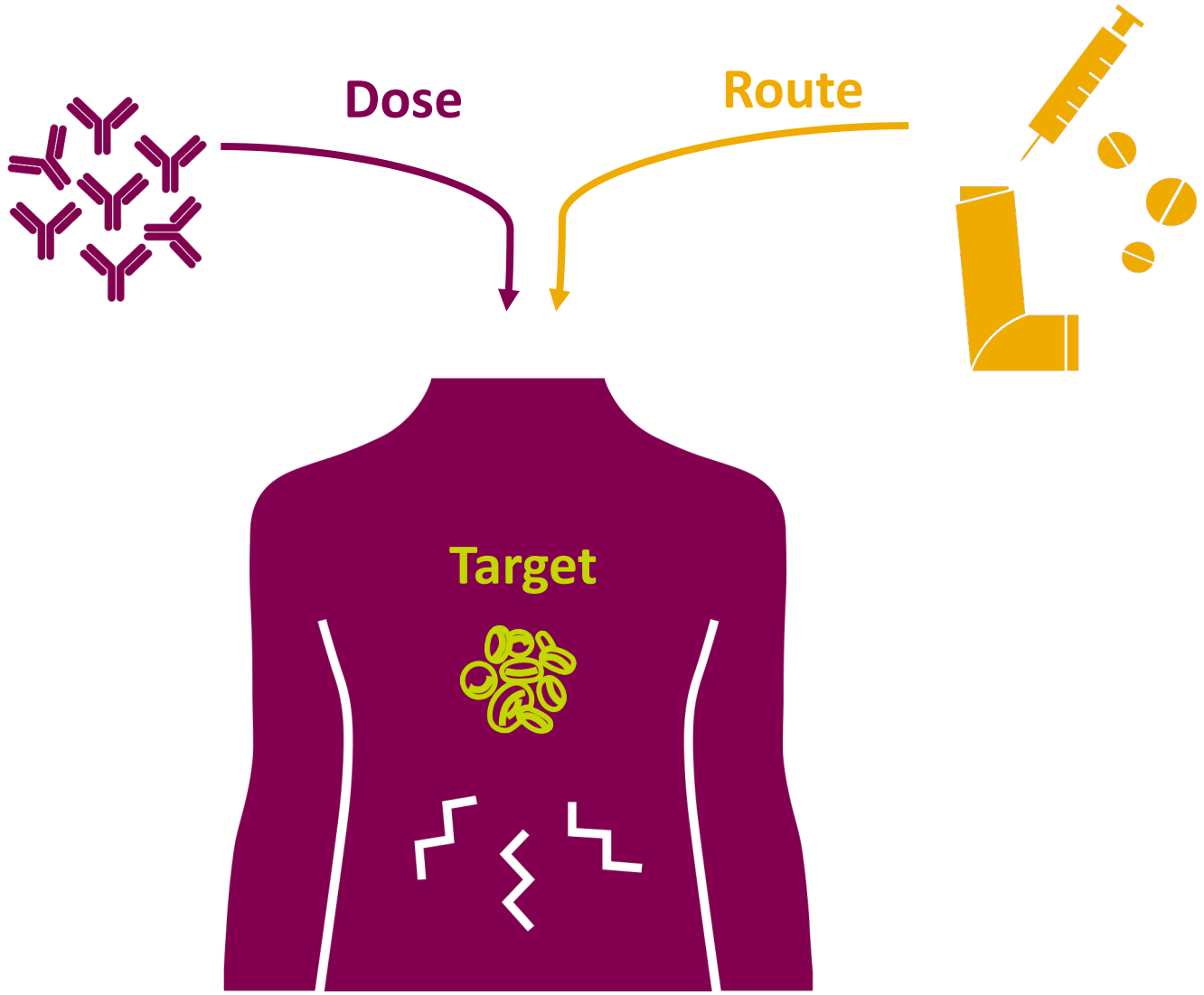


Utilisation of AI & Machine Learning to Aid Target Identification

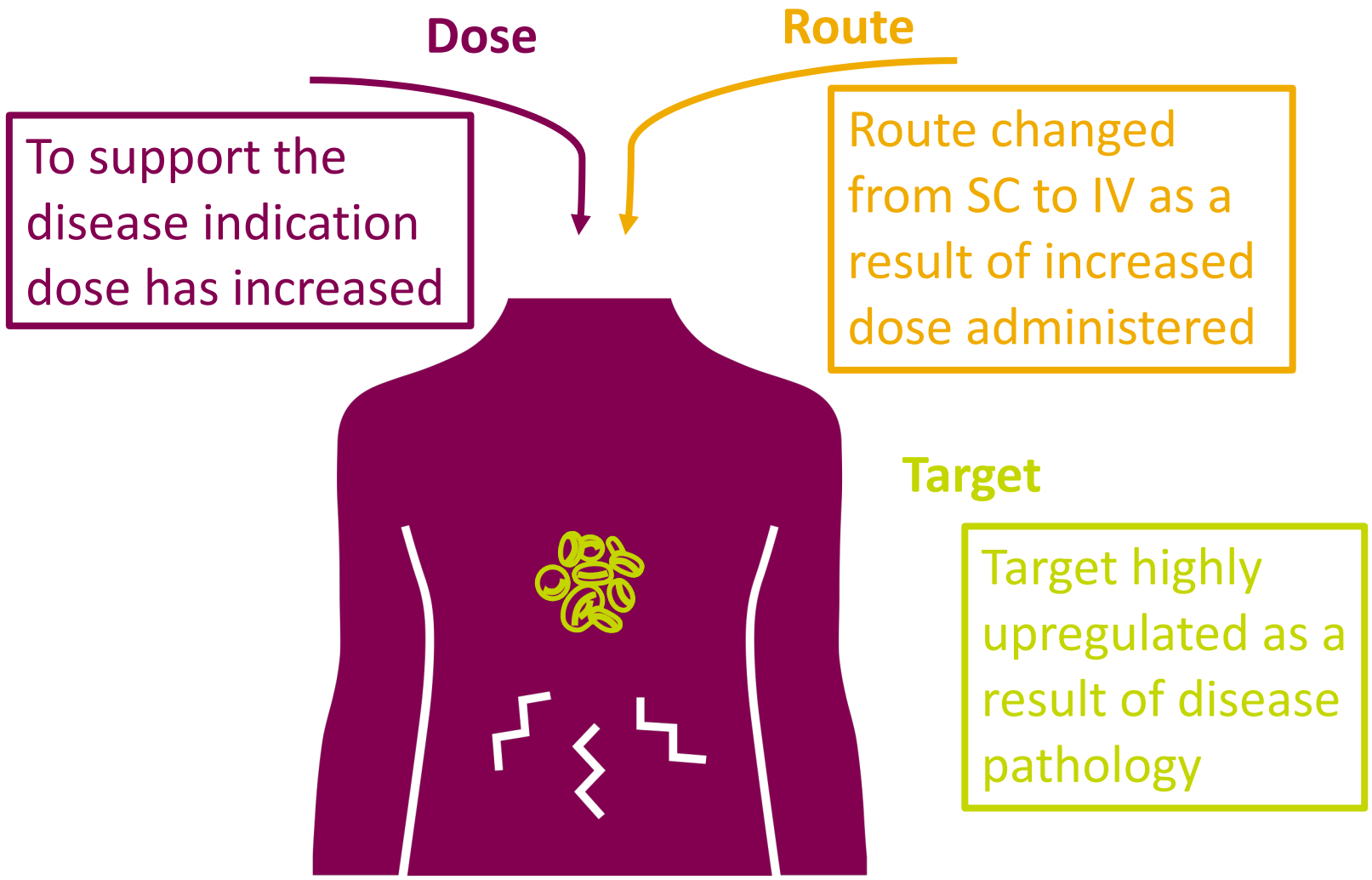


- Over the past decade the use of AI has increased dramatical in the Pharmaceutical Industry
  - Drug ‘repurposing’ is a key end-point
- AI can draw and analyse data from multiple sources rapidly leading to:
  1. Identification of new patient populations
  2. Identification of new indications

Identification of New Indications and New Patient Populations May Impact Existing Validated Methods



Understanding the Clinical Study Design is Key in Evaluating Whether the Original Method is Fit-for-Purpose



Interrogation of the New Patient Population Identified Key Gaps in ADA Bioanalytical Methodology

Original Method

- Homogenous bridging Assay
- Acid Dissociation Step (300 mM Acetic Acid)
- Sensitivity = 25 ng/mL

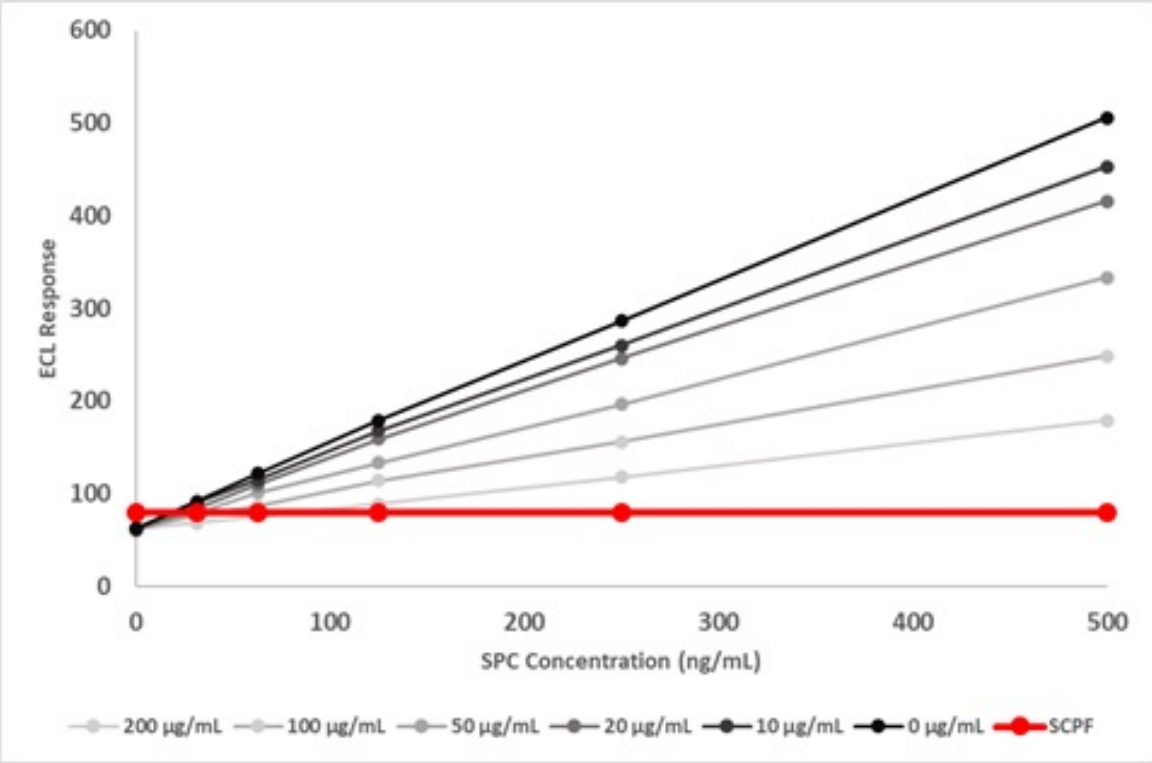
Drug Tolerance

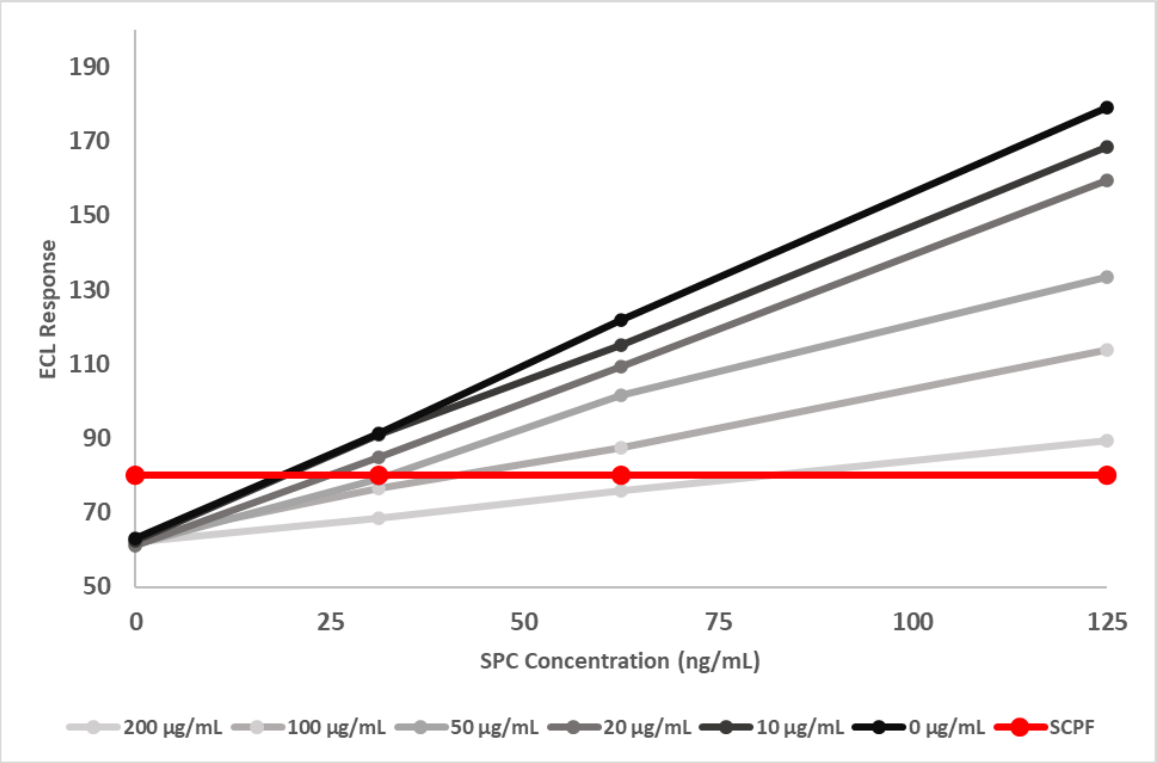
- Can detect 125 ng/mL in the presence of 200 µg/mL drug
- **Increase in dose and route of administration**
- **Multiple dose study design results in elevated Ctrough and potential false negative results**

Target Interference

- Concentrations of Target >31.3 ng/mL lead to false positive results in the screening and confirmatory tiers
- **Significant upregulation of circulating target as a result of disease pathology would result in false positive results**

Drug Tolerance; Acid Dissociation (300 mM Acetic Acid)





Strategies to Improve Drug Tolerance Aim to Break the Reversible Noncovalent-binding Interactions that form ADA-Drug Immune Complexes

Alternative Low pH Solutions[1]

- Use of Glycine HCl has previously been demonstrated to aid in the detection of low, intermediate and high affinity antibodies in the presence of excess therapeutic

Affinity Capture Elution (ACE)[2]

- Affinity capture of ADA on solid-phase drug
- Followed by removal of excess free drug
- Release and transfer of bound ADA and subsequent detection using biotinylated drug

Solid-Phase Extraction Acid Dissociation (SPEAD)[3]

- Sample containing ADA incubated in presence of Biotin-Drug
- Biotin-Drug:ADA complex extracted via streptavidin
- ADA Acid eluted and detected via direct assay

1. Kavita U et al, Immunol. Methods 448, 91–104 (2017) https://doi.org/10.1016/j.jim.2017.06.002
2. Bourdage JS et al, J Immunol Methods. 2007 Oct 31;327(1-2):10-7 https://doi.org/10.1016/j.jim.2007.07.004
3. Smith et al, Regul. Toxicol. Pharmacol. 49(3), 230–237 (2007) https://doi.org/10.1016/j.yrtph.2007.07.005

Affinity Capture Elution (ACE); Purpose to Increase Drug Tolerance to Detect ADA at Trough Concentrations

Diluted Samples + Acid + Neutralisation

Polypropylene Plate

Biotin-Drug Coated Streptavidin Plate

Acid Elution

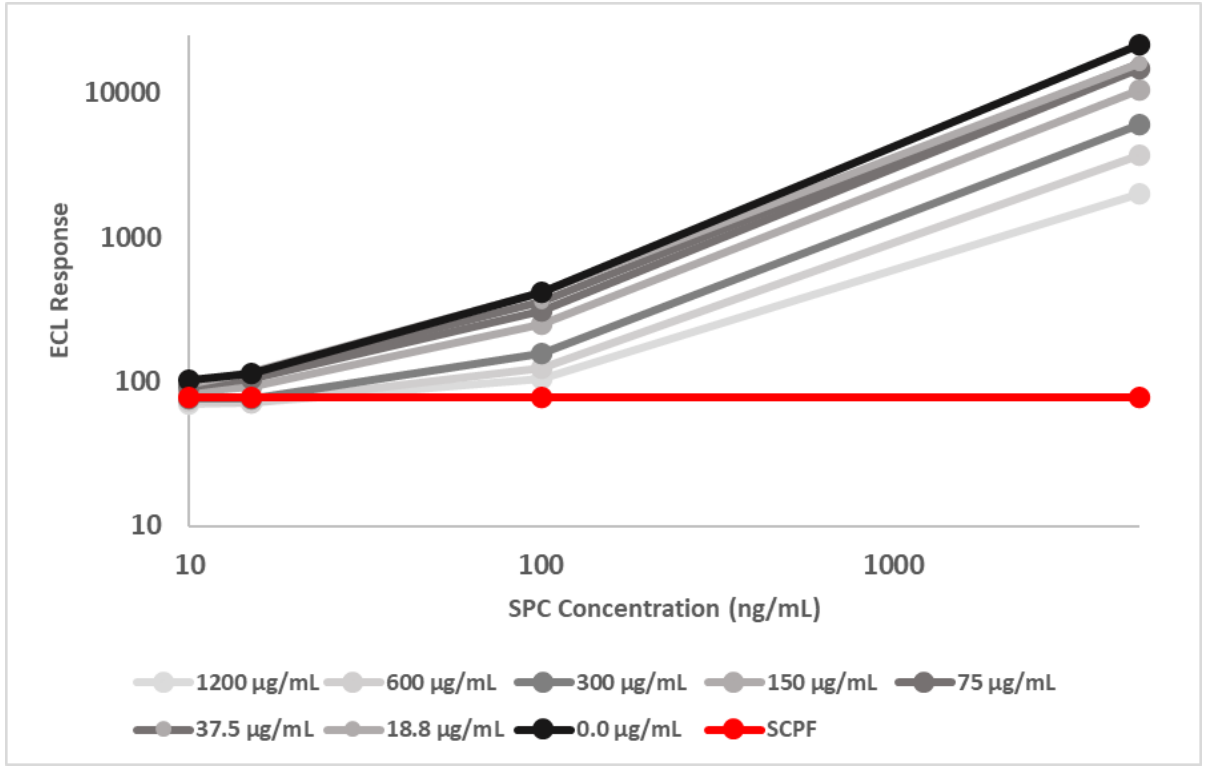
Biotin-Drug Coated Streptavidin Plate

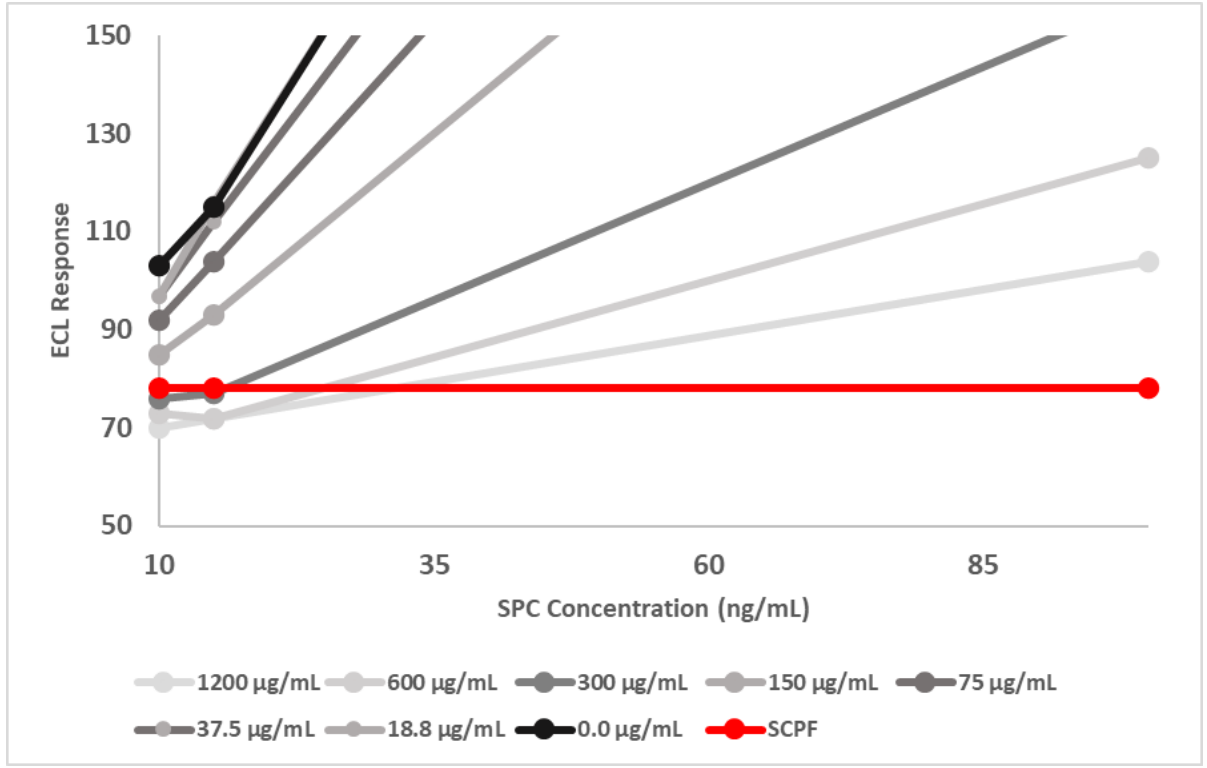
Detection Reagent (Ru-Drug)
Conf Reagent (Ru-Drug + Drug)

Polypropylene Plate

Biotin-Drug Coated MSD-SA Plate

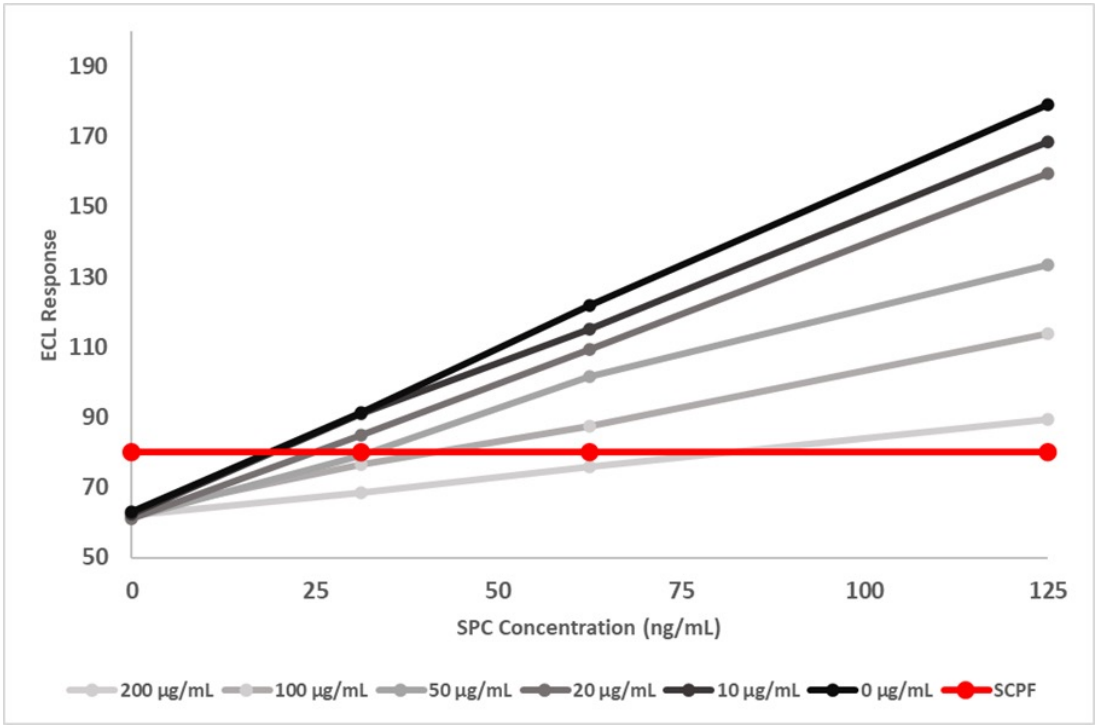
Drug Tolerance; Affinity Capture Elution (ACE) Method



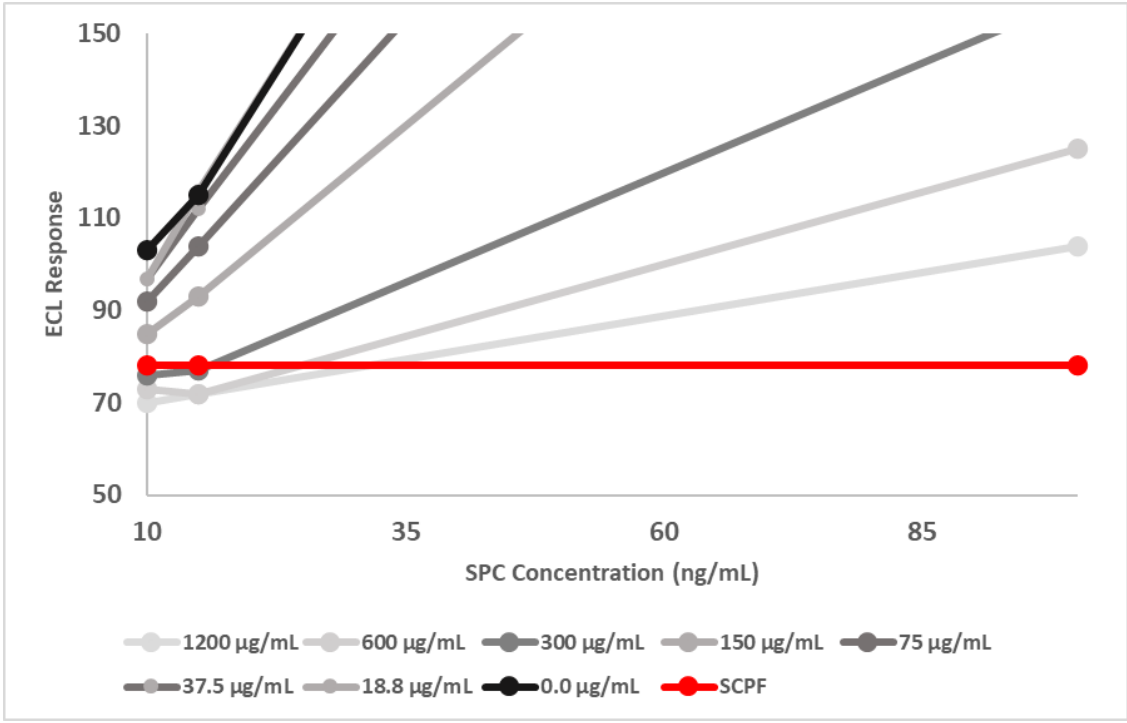


Application of ACE **Significantly** Improves Drug Tolerance (DT)

Method Utilising AD (300 mM Acetic): Detection of 125 ng/mL SPC in presence of 200 µg/mL Drug



ACE method significantly improves DT: Detection of 100 ng/mL SPC in presence of 1.2 mg/mL Drug

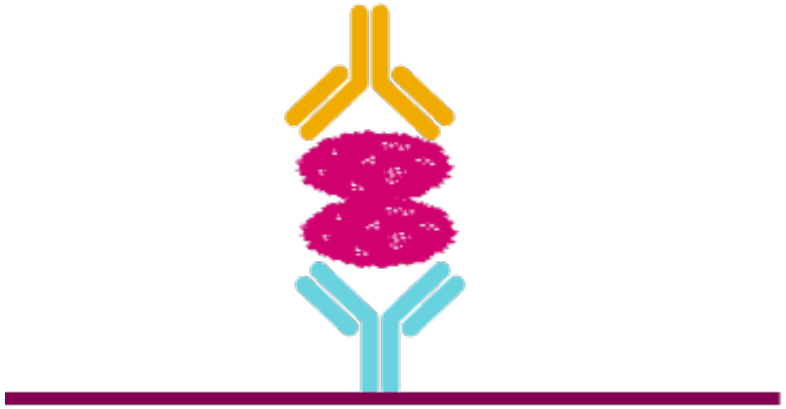


Interrogation of the New Patient Population Identified Key Gaps in ADA Bioanalytical Methodology - Solutions

Drug Tolerance

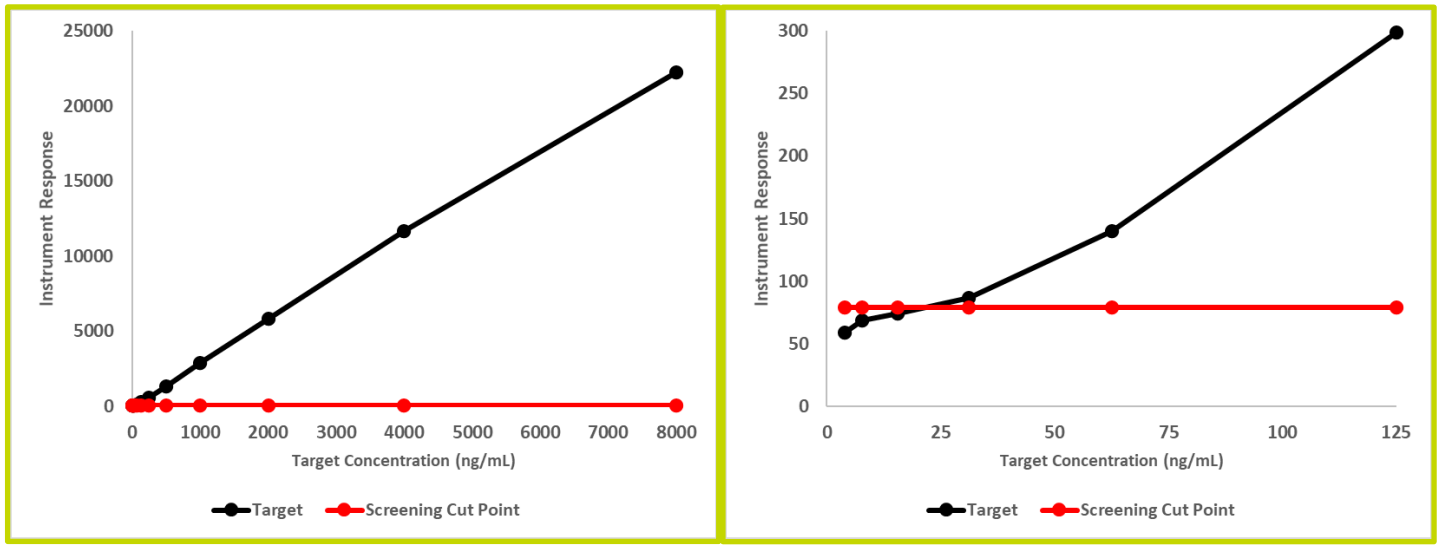
Target Interference

- Significant upregulation of circulating target as a result of disease pathology would result in false **positive** results

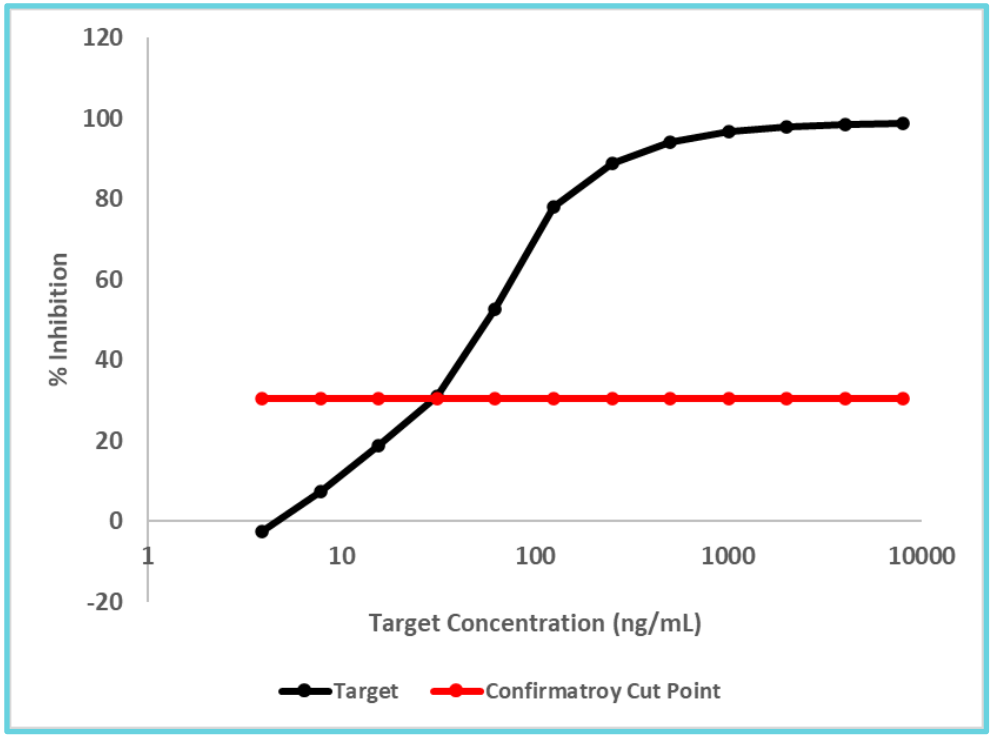


Levels of Target in the Range Expected in New Patient Population Significantly Interferes With Assay Response Leading to False Positive Results

Target at concentrations >31.3 ng/mL leads to false positive results in the screening assay



Target at concentrations >31.3 ng/mL leads to false positive results in the confirmatory assay



Examples of Strategies to Overcome Target Interference in ADA Assays

Remove the Target from Samples

- Pass samples through an affinity capture column prior to analysis
- Use anti-target magnetic beads

Dissociate Drug-Target Interaction

- Break the non-covalent interactions between Drug and Target
- Use high salt concentrations, extremes of pH or detergents

Inhibit Drug-Target Interaction

- Competitive binding of target by addition of receptor or competing/blocking antibody

ACE Method to Detect ADA; Process Map

Diluted Samples + Acid + Neutralisation

Polypropylene Plate

Biotin-Drug Coated
Streptavidin Plate

Acid Elution

Biotin-Drug Coated
Streptavidin Plate

Detection Reagent (Ru-Drug)
Conf Reagent (Ru-Drug + Drug)

Polypropylene Plate

Biotin-Drug Coated
MSD-SA Plate

Addition of Blocking Antibody Out-Competes Target – Drug Interaction Favouring ADA – Drug Binding

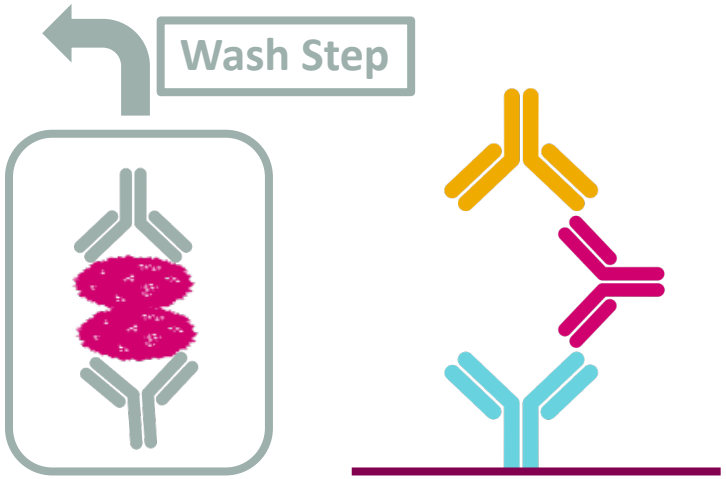
Add Blocking Antibody Detection & Confirmatory Reagent

Detection Reagent:
(Ru-Drug + Blocking Antibody)
Conf Reagent:
(Ru-Drug + Drug + Blocking Antibody)

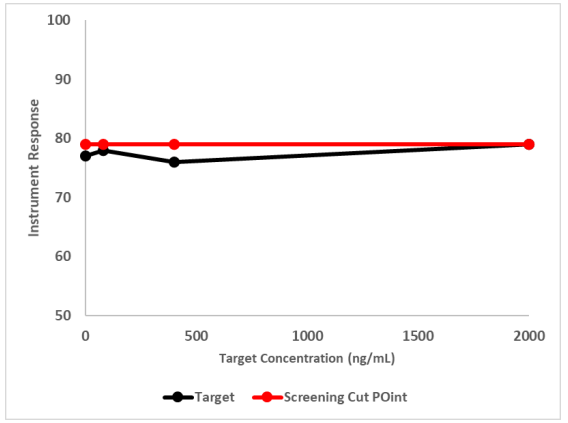
Polypropylene Plate

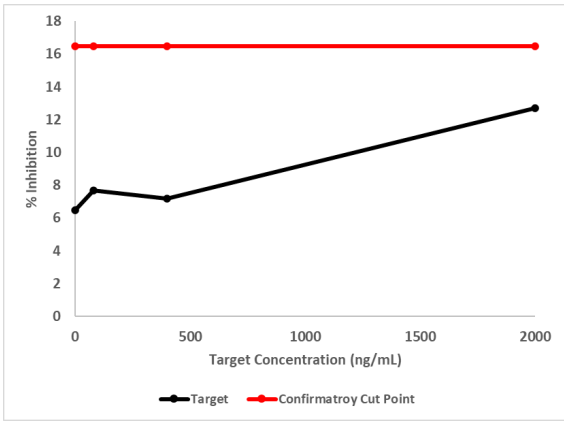
Competitive Inhibition of Target-Drug binding occurs

- Acid dissociation and addition of a blocking antibody in molar excess favours blocking antibody-target binding



Addition of blocking antibody eliminates false positive results





Closing Remarks

AI identified new study designs to address novel target patient population

Resultant novel challenges required refinement of existing bioanalytical methodologies

Inadequate Drug Tolerance	Significant Target Interference
Application of Acid Dissociation Elution (ACE) improved Drug Tolerance allowing adequate characterisation of ADA at PK sampling timepoints	Addition of excess blocking antibody favoured blocking antibody – target binding leading to immunedepletion and favourable target tolerance

Acknowledgments

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