APPROACHES TO IMPROVE DRUG TOLERANCE IN IMMUNOGENICITY ASSAYS

Cara Gunning, Study Director Immunobiology, Charles River Laboratories

Introduction

IMMUNOGENICITY ASSAYS

- Immunogenicity is defined as the ability of a foreign substance to produce an immune response in the body of a human or other animal
- All biotherapeutics have the potential to produce an immune response which can affect the safety and efficacy of a study
- Therefore the assessment of “anti-drug antibodies” (ADA) raised against a biotherapeutic is important to fully understand the entirety of the data collected
- ADA can affect the pharmacokinetics (PK), pharmacodynamics (PD) and/or the biological activity of the biotherapeutic, therefore it is important to ensure interpretation of the data is not compromised by any ADA present in the samples
- In addition to being present as a free antibody, ADA can also from antibody-drug complexes in the bioanalytical sample
- As a result of the potential to form these antibody-drug complexes the presence of high concentrations of drug in samples can prevent the detection of ADA
- For this reason one of the most important factors to consider when developing and validating immunogenicity assays is drug tolerance

WHAT IS DRUG TOLERANCE, AND WHY IS IT IMPORTANT

- Drug tolerance is defined as the measure of the maximum concentration of drug that can be present in a sample and not prevent a positive sample from being detected.
- Drug tolerance is performed to determine the level of biotherapeutic that can be present in a sample and still enable to the sample to be determined as positive for ADA
- Poor drug tolerance can lead to false-negative samples during the screening process of low level positive samples
- This can lead to a misinterpretation of the immunogenicity potential of the biotherapeutic
- Guidance provided by the EMA and FDA highlight the importance of drug tolerant immunogenicity assays for this reason, this is particularly important for non-clinical assays as dosing levels will be much higher

Case Study

Improving Drug Tolerance in a Pre-clinical Assay

ORIGINAL ASSAY SET UP

Case Study – Improving Drug Tolerance

Standard Acid dissociation approach on the MSD platform

Step 1 – Acid Dissociation

Incubate for 1 hour with 300 mM Acetic Acid

Step 2 – Immune Complex Formation and Sample Neutralisation

Samples incubated overnight with biotin and Ruthenium-labelled drug in neutralisation buffer (200 mM Tris-HCl)

Step 3 – Block

Streptavidin plate blocked for 1 hour

Step 4 – Streptavidin:Biotin-Drug Complex Capture

Incubate for 2 hours

Step 5 - Addition of Read Buffer

Excitation of Ruthenium-labelled drug via electrochemical reaction of $RU(bpy)_3$ to generate luminescence

The quantity of luminescence correlates with the level of ADA present in the sample

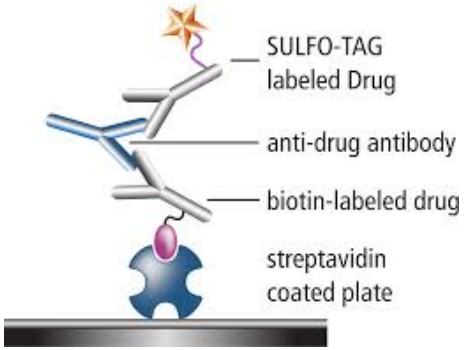

Figure property of Meso Scale Diagnostics LLC and used with permission

DRUG TOLERANCE RESULTS

Case Study – Improving Drug Tolerance

Drug Spike Concentration (µg/mL)	PC Concentration			
	HPC 20000 ng/mL		LPC 100 ng/mL	
	Detector Response		Detector Response	
0	8426	7661	97	101
0.01	8032	7675	98	96
0.1	7097	6563	**97**	**94**
1	2354	2363	76	75
10	**118**	**117**	62	64
100	57	59	61	60
500	60	54	60	54
1000	56	61	57	57
1500	56	56	56	56
2000	55	56	57	57
PSCP	77			

- HPC could tolerate up to 10 µg/mL drug
- LPC could tolerate up to 0.1 µg/mL drug
- Predicted Cmax concentrations > 1000 µg/mL drug
- Drug tolerance levels not suitable in current assay format

NEXT STEP

Case Study – Improving Drug Tolerance

Options investigated

1. Assessing the minimum required dilution (MRD) of the assay – impacts on the sensitivity
2. Acid Investigations – Glycine HCl vs Acetic Acid
3. Acid incubation times

MRD DILUTION

Case Study – Improving Drug Tolerance

Drug Spike Concentration (µg/mL)	PC Concentration	
	HPC 50000 ng/mL	LPC 500 ng/mL
	Detector Response	Detector Response
0	11724	127
0.01	10555	116
0.1	5012	92
1	1589	**69**
10	188	52
25	84	49
50	69	55
75	**64**	54
100	56	50
500	49	49
1000	48	50
PSCP	63	

- 2% assay – MRD of 1 in 50 utilised
- HPC could tolerate up to 75 µg/mL drug
- LPC could tolerate up to 1 µg/mL drug

ACID INVESTIGATION PART 1

Case Study – Improving Drug Tolerance

Acetic Acid (300 mM)

Drug Spike Concentration (µg/mL)	PC Concentration	
	HPC 20000 ng/mL	LPC 100 ng/mL
	Detector Response	Detector Response
0	9493	98
0.01	9674	96
0.1	9822	100
1	6745	**87**
10	1575	73
25	455	72
50	214	66
75	149	69
100	**119**	67
500	68	69
1000	68	64
PSCP	79	

- Improvement on previous occasion
- HPC could tolerate up to 100 µg/mL drug
- LPC could tolerate up to 1 µg/mL drug

Glycine HCl (100 mM)

Drug Spike Concentration (µg/mL)	PC Concentration	
	HPC 20000 ng/mL	LPC 100 ng/mL
	Detector Response	Detector Response
0	4323	68
0.01	4342	63
0.1	4357	67
1	2943	65
10	646	55
25	208	54
50	**109**	49
75	84	51
100	81	60
500	53	53
1000	55	58
PSCP	69	

- HPC could tolerate up to 50 µg/mL drug
- LPC did not give a result above the PSCP

ACID INVESTIGATION PART 2

Case Study – Improving Drug Tolerance

Drug Spike Concentration (µg/mL)	60 min incubation	45 min incubation	15 min incubation	5 min incubation
	PC Concentration		PC Concentration	
	LPC 100 ng/mL	LPC 100 ng/mL	LPC 100 ng/mL	LPC 100 ng/mL
	Detector Response	Detector Response	Detector Response	Detector Response
0	93	92	95	87
0.01	94	93	90	84
0.1	100	91	90	82
1	**90**	**82**	81	**77**
10	71	71	**80**	63
25	63	63	71	57
50	69	70	72	65
75	68	69	69	58
100	65	68	73	58
500	64	66	67	58
1000	71	73	68	58
PSCP	76	75	77	66

- LPC could tolerate between 10 and 1 µg/mL drug
- Various acid incubation times did not show a significant improvement of drug tolerance

Solid-Phase Extraction Acid Dissociation (SPEAD)

SPEAD – SOLID-PHASE EXTRACTION WITH ACID DISSOCIATION

Case Study – Improving Drug Tolerance

- Solid-phase extraction with acid dissociation removes the interfering drug from the sample prior to performing a direct immunoassay to detect ADA
- The interaction between biotin and streptavidin is used to separate the ADA and antibody-drug complexes from the sample
- The acid dissociation step then removes the ADA from the biotin-streptavidin complex
- Acid dissociated samples are then neutralised and a direct detection step is used

SPEAD – IN PRACTICE

Case Study – Improving Drug Tolerance

Step 1 – Biotin-Drug Complex Formation

Overnight Incubation

Step 2 – Streptavidin:Biotin-Drug Complex Capture

Incubate for 1-2 hours

Step 3 – Acid Dissociation

Incubate for 5 min with 300 mM Acetic Acid

Step 4 – Sample Neutralisation

Incubate briefly

Transfer to MSD plate

Step 5 – Coat Neutralised samples on MSD plate

Incubate for 1 hour

Step 6 – Block

Incubate for 1 hour

Step 7 – Ruthenium-labelled drug addition

Incubate for 1 hour

Step 8 - Addition of Read Buffer

Excitation of Ruthenium-labelled drug via electrochemical reaction of RU(bpy)3 to generate luminescence

The quantity of luminescence correlates with the level of ADA present in the sample

SPEAD DRUG TOLERANCE RESULTS

Case Study – Improving Drug Tolerance

Drug Spike Concentration (µg/mL)	PC Concentration	
	HPC 20000 ng/mL	LPC 80.0 ng/mL
	Detector Response	Detector Response
0	15828	135
0.0001	15331	139
0.001	15944	130
0.01	14286	132
0.1	14322	123
1	10732	109
10	7307	95
50	4730	91
100	3341	**79**
500	1226	70
1000	811	61
PSCP	78	

- HPC can tolerate in excess of 1000 µg/mL drug
- LPC can tolerate up to 100 µg/mL drug

charles river

Conclusion

ACID DISSOCIATION VS SPEAD

Case Study – Improving Drug Tolerance

Drug Spike Concentration (µg/mL)	PC Concentration			
	HPC 20000 ng/mL		LPC 100 ng/mL	
	Detector Response		Detector Response	
0	8426	7661	97	101
0.01	8032	7675	98	96
0.1	7097	6563	**97**	**94**
1	2354	2363	76	75
10	**118**	**117**	62	64
100	57	59	61	60
500	60	54	60	54
1000	56	61	57	57
1500	56	56	56	56
2000	55	56	57	57
PSCP	77			

Drug Spike Concentration (µg/mL)	PC Concentration	
	HPC 20000 ng/mL	LPC 80.0 ng/mL
	Detector Response	Detector Response
0	15828	135
0.0001	15331	139
0.001	15944	130
0.01	14286	132
0.1	14322	123
1	10732	109
10	7307	95
50	4730	91
100	3341	**79**
500	1226	70
1000	811	61
PSCP	78	

- By employing SPEAD methodology we improved the drug tolerance of the assay at the LPC level from 0.1µg/mL to 100 µg/mL
- This is a 1000-fold increase in drug tolerance at LPC level

Thank you for listening

CONTACT US

Cara Gunning, BA
Study Director
Department of Immunobiology
Email: cara.gunning@crl.com

charles river