Immunogenicity Case Study

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Disclaimer

This presentation reflects the views of the author. It should not be construed to represent FDA's views or policies.

Learning Objectives

- Identify potential issues with an immunogenicity study based on the background provided.
- Understand why those issue may impact sample analysis.
- Identify what additional data may be available to help evaluate or mitigate the potential issues.

The next few slides will provide some background materials for a bioequivalence (BE) study with pharmacokinetic (PK) endpoints for a biosimilar product. As part of the safety evaluation, an immunogenicity assessment was also performed.

Study Perfect-123

- An open-label, multicenter, parallel study of cureimab (Stars, Inc.) and AB-123 (proposed biosimilar to cureimab; Best Sponsor, Inc.) in patients with chronic video call fatigue (VCF) to assess PK and safety (including immunogenicity).

Study Perfect-123

- Compare AB-123 to cureimab
- Assessed both PK and immunogenicity
- Multiple clinical sites sent all samples to a single analytical CRO (DC Analytical Services)
- DC Analytical conducted method validation and sample analysis

Study Perfect-123

		Samples collected during each visit						
Sample types collected	**Time point**	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Follow -up*
PK	0h (pre-dose)	X	X	X	X	X	X	X
	2.5 h post dose	X		X	X		X	
ADA	0h (pre-dose)	X			X		X	X

*30 days (+/- 2 days) post dose 6, final course only

ADA Assay Validation

Parameters evaluated

- Screening cut-point*
- Confirmatory cut-point*
- Sensitivity (including LPC determination)*
- Inter- and intra-assay precision
- Specificity
- Selectivity
- Drug Tolerance*
- Prozone effect
- Titer precision
- Freeze-thaw stability (5 cycles)
- Bench top stability (6 hours at room temperature)

Case Set 1 – Cut-point Determination

Screening Cut-point

- 50 independent normal plasma lots, analyzed three separate times
- Averaged the three ECL values for each lot
- Up to three iterations of normalization and upper bound outlier exclusion.
- Distribution remained non-normal following exclusion of 3 outliers
- Correction factor: 1.3 (plate-specific/floating cut-point)

Confirmatory Cut-point

- 50 independent normal plasma lots, assessed on three occasions
- Determined % inhibition with AB-123 and cureimab
- Averaged the three % inhibition values for each lot
- No more than one outlier at the upper bound was to be excluded.
- Cut-point calculated based on a 0.1% false positive rate

Case 1:
Discussion Questions

Case Set 2 – Sensitivity and LPC selection

Inter- and Intra-assay Precision

AB-123	Plate	Intra-Assay Precision									Inter-Assay Precision		
		Value 1a	Value 1b	Value 2a	Value 2b	Value 3a	Value 3b	Mean ECL Value	St Dev	CV%	Mean	StDev	CV%
HPC (15,000 ng/mL)	1	10250	9905	7850	7960	7610	8213	8631	1142.07	13	7928	1030	13
	2	9030	9440	9050	8780	8010	8529	8807	494.51	6			
	3	9135	8960	8300	8685	8040	7495	8436	614.16	7			
	5	9130	8670	6467	5680	5980	6100	7005	1496.83	21			
	6	7130	7380	7053	6980	6257	7020	6970	377.24	5			
	7	8370	8400	7450	7365	7005	7115	7618	616.12	8			
LPC 2 (400 ng/mL)	1	229	228	245	222	210	209	224	13.47	6	214	25	11
	2	240	238	249	260	239	239	244	8.75	4			
	3	219	230	236	243	216	220	227	10.76	5			
	5	217	219	170	162	157	174	183	27.64	15			
	6	208	198	196	201	188	197	198	6.54	3			
	7	218	219	203	210	201	192	207	10.50	5			
LPC 1 (250 ng/mL)	1	170	172	170	159	150	161	164	8.55	5	158	19	12
	2	186	187	186	185	172	177	182	6.18	3			
	3	173	173	176	168	158	157	168	8.17	5			
	5	159	164	122	127	120	117	135	20.97	16			
	6	154	151	144	140	146	137	145	6.44	4			
	7	161	168	150	150	145	150	154	8.65	6			

Sensitivity and LPC selection

- Established based on precision data
- Lowest PC with ECL>PSCP
- Sensitivity and LPC set at 250 ng/mL
- False positive rate of 5.7%

Case 2:
Discussion Questions

Case Set 3 – Drug Tolerance

Drug Tolerance

HPC (15, 000 ng/mL)

Drug Concentration (ng/mL)	Screening	Confirmatory	
	Mean ECL	Mean ECL (With Drug)	% Inhibition
800, 000	60	61	-1.67
400, 000	75	76	-1.33
200, 000	112	108	3.57
100, 000	240	212	11.67
50, 000	568	496	12.68
25, 000	1207	912	24.44
12,500	2258	1593	29.45
6,250	3478	2210	36.46
3,130	4142	2293	44.64
1,560	3326	1305	60.76
780	2532	549	78.32
390	1700	345	79.71
200	1357	149	89.02
100	3152	290	90.80
50	11,352	715	93.70
20	23,175	1346	94.19

Screening Drug Tolerance – **100,000 ng/mL**

Confirmatory Drug Tolerance – **25,000 ng/mL**

PSCP: 130

CCP: 20.8%

LPC (250 ng/mL)

Drug Concentration (ng/mL)	Screening	Confirmatory	
	Mean ECL	Mean ECL (With Drug)	% Inhibition
800, 000	46	46	0.00
400, 000	46	45	2.17
200, 000	45	46	-2.22
100, 000	53	54	-1.89
50, 000	54	54	0.00
25, 000	62	60	3.23
12,500	73	70	4.11
6,250	87	83	4.60
3,130	91	86	5.49
1,560	95	89	6.32
780	97	90	7.22
390	107	96	10.28
200	110	97	11.82
100	115	100	13.04
50	122	107	12.30
20	138	115	16.67

Screening Drug Tolerance – **200 ng/mL**

Confirmatory Drug Tolerance – < **20 ng/mL**

PSCP: 108

Case 3:
Discussion Questions

Drug Tolerance

- Optimize your assay
- Know the limitations of the assay
- Monitor study samples
- Accurately report which samples are impacted by drug tolerance issues

Summary

- Cut points should be established with a scientifically justified statistical approach.
- The LPC of an assay should be based on the sensitivity of the assay.
- Ideally, the drug tolerance of an assay covers concentrations observed in subject samples.
  - If not, it is best practice to identify and accurately report samples with drug concentrations above the tolerance of the assay.

Closing Thought

Every assay is different and therefore each requires unique considerations. Use scientific principles to help guide what considerations may be necessary for your assay or study.


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