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Establishment of Generic ADA assay for antibody therapeutics in preclinical studies

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Introduction

Antibody-based therapeutics are protein products, and such drugs tend to induce immune responses when administrated in animals. Evaluation of immunogenicity of an antibody therapeutic is frequently required in a preclinical studies through detection of anti-drug antibody (ADA). To facilitate preclinical ADA assay developed, two generic assay platforms are established in this study.

Methods

Using a custom-made monkey anti-human IgG monoclonal antibody as ADA positive control and a specific mouse anti-monkey IgG monoclonal antibody as the detection reagent, a sandwich assay is developed for monkey ADA analysis (Figure 1). Likewise, using a custom-made mouse anti-human IgG monoclonal antibody as ADA positive control and a specific goat anti-mouse IgG monoclonal antibody as the detection reagent, a sandwich assay is developed for mouse ADA analysis (Figure 2). In either assay, the detection reagent selected does not bind to a humanized or human IgG (i.e., drug itself). Both assays are successfully qualified for their intended use.

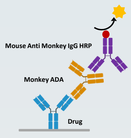


Figure 1. Monkey ADA assay design

Results

Monkey ADA Assay Cut-Points, Sensitivity and Drug Tolerance

A total of 45 data points were generated by analyzing 15 individual monkey serum samples by at least 3 times to determine screening cut point (SCF) values. The 95th percentile of one-sided confidence interval was established as cut point. Sensitivity of the monkey ADA assay was then evaluated with 3 antibody therapeutics of different subclasses (i.e., IgG1, IgG2, and IgG4). The assay sensitivity in monkey serum was observed in a range of 2-9 ng/mL (Table 1).

Table 1. Determination of assay sensitivity of the monkey ADA assay

ADA Conc. (ng/mL)	Anti Her2 Antibody-IgG1 (SCPF=1.12)		Anti RANKL Antibody-IgG2 (SCPF=1.10)		Anti PD-1 Antibody-IgG4 (SCPF=1.14)	
	Signal Value	SNR Value	Signal Value	SNR Value	Signal Value	SNR Value
1000.0	2.993	18.23	2.538	12.30	2.784	20.86
100.0	0.512	3.12	0.504	2.44	0.484	3.62
50.0	0.364	2.22	0.353	1.71	0.318	2.38
25.0	0.263	1.60	0.279	1.35	0.229	1.72
12.5	0.224	1.37	0.248	1.20	0.177	1.32
6.3	0.189	1.15	0.214	1.04	0.159	1.19
3.1	0.193	1.17	0.213	1.03	0.158	1.18
1.6	0.183	1.12	0.205	1.00	0.147	1.10
Mean NC	0.164	1.00	0.206	1.00	0.133	1.00
Sensitivity (ng/mL)	2		9		2	

Drug tolerance profiles of this platform assay was evaluated with the above-mentioned three antibody therapeutics at 500 ng/mL monkey ADA level. Results showed that drug tolerance were all above 1000 µg/mL (Table 2).

Table 2. Drug tolerance assessment of the monkey ADA assay

Drug Conc. (µg/mL)	Anti Her2 Antibody-IgG1 (SCPF=1.12)		Anti RANKL Antibody-IgG2 (SCPF=1.10)		Anti PD-1 Antibody-IgG4 (SCPF=1.14)	
	Signal Value	SNR value	Signal Value	SNR value	Signal Value	SNR value
1000.0	0.199	1.21	0.228	1.10	0.210	1.57
500.0	0.238	1.45	0.286	1.39	0.251	1.88
250.0	0.306	1.87	0.318	1.54	0.330	2.47
125.0	0.392	2.39	0.388	1.88	0.433	3.25
62.5	0.541	3.30	0.490	2.38	0.544	4.08
31.3	0.702	4.27	0.589	2.86	0.719	5.39
0.0	2.026	12.34	1.488	7.21	1.718	12.87
Mean NC	0.164	1.00	0.206	1.00	0.133	1.00
Drug tolerance (µg/mL)	1000		1000		1000	

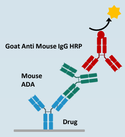


Figure 2. Mouse ADA assay design

Mouse ADA Assay Cut-Points, Sensitivity and Drug Tolerance

A total of 45 data points were generated by analyzing 15 individual mouse serum samples by at least 3 times to determine screening cut point (SCF) values. The 95th percentile of one-sided confidence interval was established as cut point. Sensitivity of the mouse ADA assay was evaluated with 3 antibody therapeutics of different subclasses (i.e., IgG1, IgG2, and IgG4). The assay sensitivity in mouse serum was observed in a range of 3-4 ng/mL (Table 3).

Table 3. Determination of assay sensitivity of the mouse ADA assay

ADA Conc. (ng/mL)	Anti Her2 Antibody-IgG1 (SCPF=1.13)		Anti RANKL Antibody-IgG2 (SCPF=1.05)		Anti PD-1 Antibody-IgG4 (SCPF=1.11)	
	Signal Value	SNR value	Signal Value	SNR value	Signal Value	SNR value
1000.0	2.100	19.26	1.980	26.38	1.789	17.48
100.0	0.367	3.37	0.361	4.80	0.315	3.07
50.0	0.235	2.16	0.216	2.88	0.212	2.07
25.0	0.172	1.58	0.147	1.95	0.156	1.53
12.5	0.147	1.35	0.104	1.38	0.129	1.26
6.3	0.133	1.22	0.090	1.20	0.120	1.17
3.1	0.120	1.10	0.074	0.99	0.115	1.12
1.6	0.115	1.05	0.074	0.99	0.110	1.07
Mean NC	0.109	1.00	0.075	1.00	0.102	1.00
Sensitivity (ng/mL)	4		4		3	

Drug tolerance profiles of this platform assay was evaluated with the above-mentioned three antibody therapeutics at 500 ng/mL mouse ADA level. Results showed that drug tolerance were all above 1000 µg/mL (Table 4).

Table 4. Drug tolerance assessment of the mouse ADA assay

Drug Conc. (µg/mL)	Anti Her2 Antibody-IgG1 (SCPF=1.13)		Anti RANKL Antibody-IgG2 (SCPF=1.05)		Anti PD-1 Antibody-IgG4 (SCPF=1.11)	
	Signal Value	SNR value	Signal Value	SNR value	Signal Value	SNR value
1000.0	0.146	1.34	0.089	1.18	0.121	1.18
500.0	0.169	1.55	0.123	1.63	0.158	1.54
250.0	0.209	1.92	0.162	2.16	0.204	1.99
125.0	0.288	2.65	0.233	3.10	0.276	2.69
62.5	0.394	3.61	0.338	4.50	0.350	3.42
31.3	0.559	5.13	0.467	6.21	0.457	4.47
0.0	1.388	12.74	1.291	17.20	0.906	8.85
Mean NC	0.109	1.00	0.075	1.00	0.102	1.00
Drug tolerance (µg/mL)	1000		1000		1000	

Conclusion

Innovative assay platforms have been established for monkey ADA or mouse ADA analysis in preclinical studies of human antibody therapeutics. As both assay work for all three antibody subclasses, they represent generic immunoassay platforms for detection of anti-human antibodies in monkey or mouse serum. Using the monkey anti-human IgG antibody as the positive control, the assay sensitivity in monkey serum was 2-9 ng/mL and drug tolerance was above 1000 µg/mL when tested at 500 ng/mL ADA level. Using the mouse anti-human IgG antibody as the positive control, the assay sensitivity in mouse serum was 3-4 ng/mL and drug tolerance were above 1000 µg/mL at 500 ng/mL ADA level. Results demonstrated adequate assay sensitivity and drug tolerance in either assay.

Due to the use of specific antibody reagents as generic detection reagents, the platform assays are expected to significantly reduce assay development efforts, while the ADA detected remains drug specific.

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