Development and Validation of Cell-Based Neutralizing Anti-Adalimumab Antibody Detection Methods for Adalimumab Biosimilar Program

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INTRODUCTION

Adalimumab (Humira) is a genetically engineered, fully humanized monoclonal antibody that binds TNF-α, preventing it from activating TNF-α receptor. The drug has been approved for rheumatoid arthritis, psoriatic arthritis and Crohn's disease in United States. Due to these therapeutic values, PF-06410293 is being developed as a potential biosimilar to Adalimumab. We have developed and validated two cell based assays for the detection of either anti-Adalimumab innovator or anti-PF-06410293 antibodies in human serum to support ongoing clinical studies.

MATERIALS AND METHODS

The neutralizing anti-Adalimuab antibody in human serum detection assay was developed in WEHI-13VAR cell line, which has high sensitivity to TNF-α. Adalimumab blocks the TNF-α induced cell cytotoxicity. The neutralizing antibodies to Adalimumab bind to the drug and restore the TNF-α induced cytotoxicity. In this homogenous assay, samples and controls are pre-incubated with Adalimumab and TNF-α, then the mixture is incubated with cells to initiate the TNF-α induced cytotoxicity. The presence of neutralizing antibodies inhibit the function of Adalimumab. The screening cut point was determined from 50 drug naïve individual matrix lots. Drug tolerance, matrix selectivity and TNF-α interference were also evaluated. The assay sensitivity based on human anti-Adalimumab monoclonal antibody, rabbit anti-Adalimumab polyclonal antibody and mouse anti-PF-06410293 monoclonal antibody were determined and compared between 2 assays.

- Intra-Assay and Inter-Assay Precision: Three different positive control (PC) antibodies were evaluated: Human anti-Adalimumab monoclonal antibody (AbD Serotec), rabbit anti-Adalimumab polyclonal antibody (Pfizer, Inc.) and mouse anti-PF-06410293 (Adalimumab BSI) monoclonal antibody (Pfizer, Inc.).
- Screening Cut Point Determination in Normal Population: The parameters needed to calculate the screening cut point value were established by running 50 lots of human serum samples. The individual samples were analyzed over 3 separate days.
- Mass-based Assay Sensitivity: Determined from all three positive control antibodies
- Other Tests Evaluated: Drug Tolerance; Matrix Specificity; TNF-α Interference; PC Stability.

VALIDATION RESULTS

The intra-assay precision was determined from 2 plates performed each day for 3 days by 2 analysts. The inter-assay precision was calculated from all accepted plates (Table 1).

Table 1. Intra-Assay and Inter-Assay Precision

Assay	Anti-Adalimuamab mAb (Primary PC)		Anti-Adalimumab polyAb		Anti-PF-06410293 mAb	
	Intra-Assay	Inter-Assay	Intra-Assay	Inter-Assay	Intra-Assay	Inter-Assay
Innovator EU	0.6-2.8%	10.2%	2.0-25.9%	18.8%	*0.1-2.0%*	*3.4%*
Adalimumab-BSI	0.2-4.1%	6.9%	1.2-7.6%	11.3%	*0.6-2.3%*	*3.8%*

The screening cut point were determined from the individual samples analyzed each at 3 separate days. Individual male and female serum samples were included on each plate. A floating screening cut point factor was adapted to calculate the plate specific cut point (Table 2).

Table 2. Screening Cut Point in Anti-Adalimumab Nab Assays

Drug	Screening Cut Point Factor
Adalimumab Innovator	0.65
PF-06410293	0.71

Drug tolerance was evaluated by pre-incubating the anti-Adalimumab polyclonal antibodies with the innovator drug or PF-06410293 at room temperature before being analyzed. The positive samples (last reading below the plate cut point) were highlighted (Table 3).

Table 3. Drug Tolerance Test in Anti-Adalimumab Nab Assays

PC (ng/mL)	Adalimumab (µg/mL)					PF-06410293 (µg/mL)				
	0	0.1	0.25	0.5	1.0	0	0.1	0.25	0.5	1.0
	RU ($X10^6$)	RU ($X10^6$)	RU ($X10^6$)	RU ($X10^6$)	RU ($X10^6$)	RU ($X10^6$)	RU ($X10^6$)	RU ($X10^6$)	RU ($X10^6$)	RU ($X10^6$)
15000	2.71	2.45	2.61	**2.87**	13.9	1.27	1.30	1.39	**1.94**	21.5
7500	2.88	**3.02**	**5.43**	13.2	22.2	1.54	1.75	**6.71**	18.2	24.6
3750	**3.89**	8.04	12.8	18.4	22.9	2.22	**7.58**	15.4	21.6	24.8
1875	7.64	11.8	16.0	19.4	23.0	**6.28**	12.1	17.9	22.1	24.4
938	9.94	13.1	16.8	19.0	22.7	7.81	13.4	18.5	21.5	24.6
469	11.0	14.2	17.8	20.3	22.4	9.26	14.5	18.9	21.6	24.3
234	10.3	13.1	17.2	18.8	23.2	9.47	14.6	18.9	21.3	24.3
Plate Cut Point	**7.27**				**8.69**	**7.67**				**7.96**

Matrix specificity (recovery) was demonstrated by the analysis of ten individual lots of normal, RA and Crohn's human serum, neat or spiked with anti-Adalimumab antibodies (Table 4).

Table 4. Matrix Specificity (Recovery) in Normal, RA, and Crohn's individual Lots

Recovery in Innovator Assay (%)			Recovery in PF-06410293 Assay (%)		
Normal	RA	Crohn's	Normal	RA	Crohn's
108.8	105.1	100.6	101.0	125.7	99.0
101.3	102.4	78.1	103.4	120.7	79.2
107.0	103.2	102.0	102.8	123.1	99.0
104.4	102.4	96.5	103.5	120.9	93.8
101.7	93.4	102.1	99.7	104.5	96.5
103.6	102.9	101.5	102.9	122.0	94.3
110.1	100.0	106.3	106.8	118.7	99.9
68.4	101.1	98.0	*67.5*	118.8	96.8
103.1	*31.2*	104.2	105.1	*31.7*	91.5
106.8	87.7	103.8	105.8	*25.1*	93.4

To assess TNF-α interference in the assay, TNF-α was spiked into NC pool, anti-Adalimumab monoclonal antibody, and rabbit anti-Adalimumab polyclonal antibody. The spiked NC and PCs were pre-incubated at room temperature before analysis (Table 5). No TNF-α interference was observed up to 5000 pg/mL.

Table 5. TNF-α Interference in Anti-Adalimumab Nab Assays

TNF-α (pg/mL)	Innovator Assay			PF-06410293 Assay		
	NC	polyAb PC	mAb PC	NC	polyAb PC	mAb PC
0	1.44E+07	2.95E+06	2.03E+06	9.91E+06	2.28E+06	5.17E+06
50	9.77E+06	4.06E+06	2.72E+06	8.69E+06	2.50E+06	5.47E+06
250	9.51E+06	3.89E+06	2.50E+06	8.20E+06	2.55E+06	5.71E+06
500	9.44E+06	3.59E+06	2.49E+06	9.02E+06	2.58E+06	5.46E+06
2500	8.55E+06	3.26E+06	2.54E+06	8.05E+06	2.36E+06	5.08E+06
5000	8.43E+06	3.83E+06	2.44E+06	7.60E+06	1.93E+06	5.64E+06
Plate Cut Point	7.61E+06			7.04E+06		

Assay sensitivity was determined by the concentration at which the PC produced a response equal to the cut point determined for the assay. It was reported as the 95% confidence interval value from all acceptable validation runs (Table 6).

Table 6. Mass-based Assay Sensitivity in Anti-Adalimumab Nab Assays

Assay	Human Anti-Adalimumab Ab	Rabbit Anti-Adalimumab Ab	Mouse Anti-PF-06410293 Ab
Innovator	0.25 µg/mL	8.2 µg/mL	0.22 µg/mL
PF-06410293	0.13 µg/mL	1.8 µg/mL	0.20 µg/mL

The stability of the anti-Adalimumab antibody was assessed. It was found that the antibody was stable for at 24 hours at room temperature and over at least 5 freeze/thaw cycles (Data not shown).

CONCLUSIONS

- Two independent cell-based assays to detect the neutralizing anti-Adalimumab innovator and anti-Adalimumab biosimilar (PF-06410293) antibodies were validated in human serum to support ongoing clinical studies.
- Screening cut points were determined for both in normal populations.
- Two anti-Adalimumab innovator antibodies (one monoclonal, one polyclonal) and one anti-PF-06410293 monoclonal antibody were evaluated in both innovator EU and biosimilar Nab assays for cross reactivity and sensitivity.
- The two assays were found to have comparable precision, drug tolerance and assay sensitivity.

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