Assessment of a Novel Biosimilar to Stelara (Ustekinumab), a First Line Anti-Psoriatic Drug

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Introduction

Psoriasis is an auto-immune disease related to an overwhelming production of the pro-inflammatory cytokines Il-12 and Il-23, and it affects 2-4% of the human population. Psoriasis manifests itself as scaling (skin inflammation) or arthritis and may even lead to death in extreme cases. Medications effective against psoriasis are combined IL-12/23 inhibitors like Stelara® and biosimilars thereof.

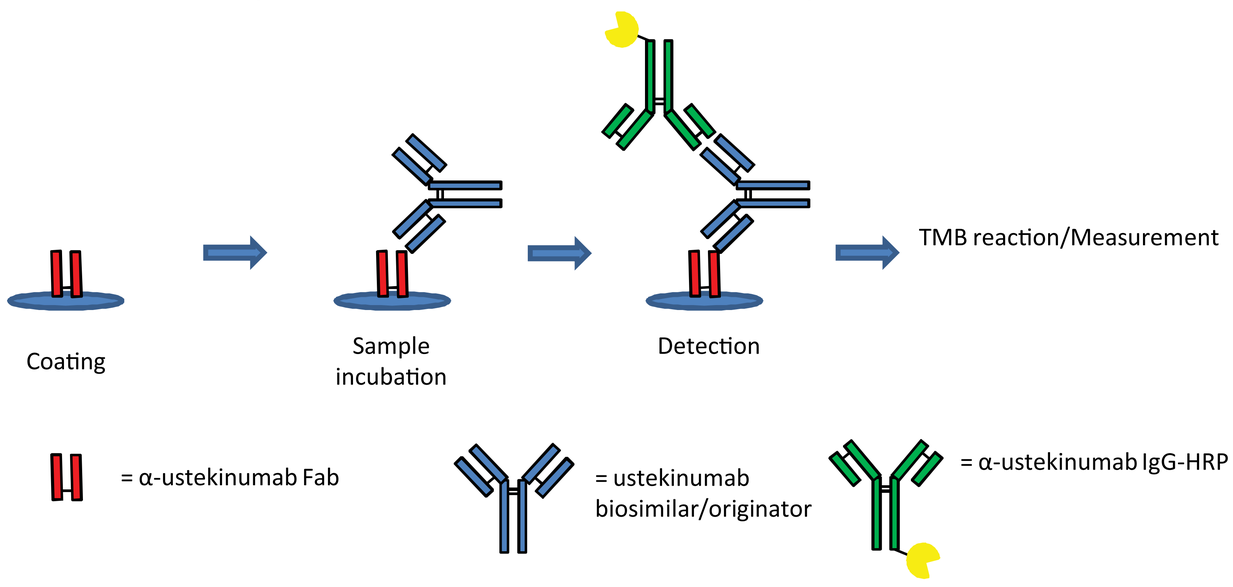
In the present study we evaluated a new biosimilar to the originator Stelara® developing a highly sensitive comparative bioanalytical assay

Analytical Methods

The total ustekinumab biosimilar/originator concentration in minipig serum was measured using quantitative sandwich ELISA (Figure 1). The assay utilizes two anti-ustekinumab antibodies recognizing distinct epitopes on the analyte. One of these antibodies (Fab fragment) is used to capture ustekinumab biosimilar/originator on the ELISA plate whereas the second one, a HRP-conjugate, serves as detection reagent. The assay is developed using TMB and the resulting colorimetric reaction is measured with a spectrophotometer.

Figure 1: Assay Procedure.

Principle of the assay used to measure ustekinumab biosimilar/originator concentration.



Results

Assay development

Assay development involved testing of the optimal concentration of coating (0.5 vs 1 µg/mL) and detection (0.05 vs 0.1 vs 0.15 vs 0.2 µg/mL) reagents as well as evaluation of the minimal required dilution (MRD 10 vs 30). Coating at 1 µg/mL and detection at 0.05 µg/mL enabled to reach high signal to background ratio as well as favorable parameters of calibration curve. MRD 10 was chosen as a good compromise between assay sensitivity and the volume of sample to be utilized. Using the above-mentioned conditions the analytical range was established between 35 and 1200 ng/mL. (Figure 2).

Comparison of ustekinumab biosimilar and originators

To asses the similarity between the investigated ustekinumab biosimilar and originators we followed the recommendations from Marini et al; 2014. Two types of tests were performed: comparison of standard curves as well as precision and accuracy of QC samples. Three independent batches of standard curves and QC were prepared for each of the analytes (biosimilar, originator US, originator EU). The overlap of the curves was assessed by visual comparison whereas the QCs were compared using back calculations.

Figure 2: Biosimilar vs originators - STD curves.

Comparison of three independently prepared batches of standard curves of the biosimilar and two originators. All 3 batches were tested on a given plate (3 duplicates per data-point for each analyte). The assay was repeated on 3 days.

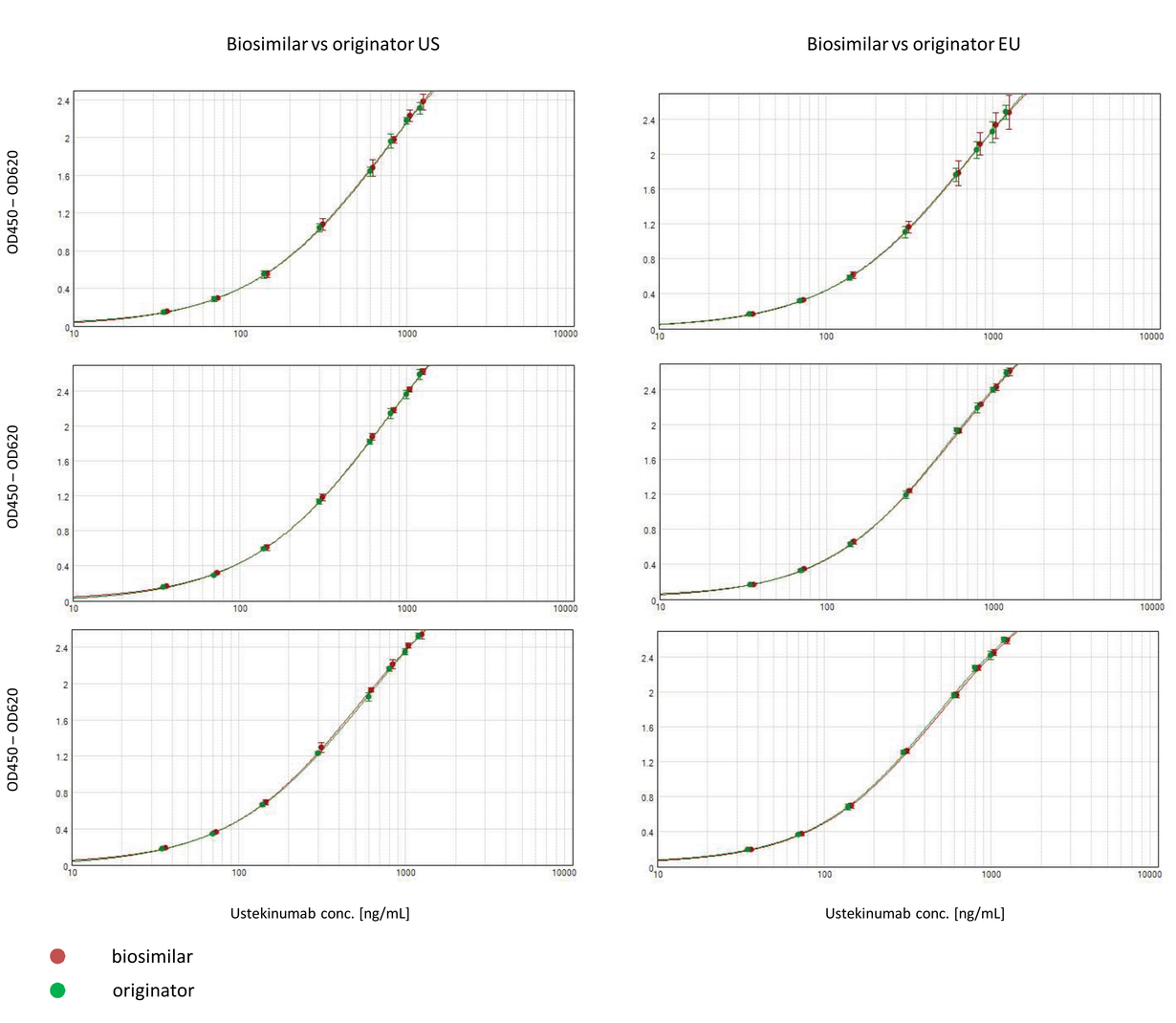


Figure 3: Biosimilar vs originators - QCs.

Comparison of QC samples of the biosimilar and two originators. Three batches of QCs were prepared independently for each analyte. All 3 batches were tested on a given plate. The test was performed in total 3 times on different days with a different arrangement of batches on a plate. Three independently prepared batches of STD curves made of originators were used to fit the QCs. The table presents how the given originator relates to the investigated biosimilar.

	QC	Mean Difference in %RE	90% Confidence Interval on Mean Difference in %RE	
			Lower Limit	Upper Limit
originator US	ULOQ	-3.73	-7.48	0.02
	HQC	-5.39	-8.72	-2.07
	MQC	-5.24	-7.91	-2.56
	LQC	-3.37	-6.66	-0.08
	LLOQ	-5.08	-8.22	-1.95
originator EU	ULOQ	-4.23	-9.29	0.82
	HQC	-4.50	-7.59	-1.42
	MQC	-3.62	-5.48	-1.77
	LQC	-0.95	-3.84	1.93
	LLOQ	-2.49	-4.14	-0.84

Evaluation of STD curve accuracy, assay selectivity and the stability of ustekinumab biosimilar

STD curve accuracy was tested in 4 independent runs by assessing the closeness of individual data-points to the obtained curve fit (Figure 4).

Figure 4: STD curve accuracy.

Reported is the average of a duplicate measurement.

Nominal [ng/mL]	STD01 35	Nom %	STD02 70	Nom %	STD03 140	Nom %	STD04 300	Nom %	STD05 600	Nom %	STD06 800	Nom %	STD07 1000	Nom %	STD08 1200	Nom %
R37	36.51	104.31	68.17	97.38	139.25	99.46	302.55	100.85	605.02	100.84	789.11	**98.64**	989.05	98.90	1223.05	101.92
R38	37.08	105.94	66.44	94.91	140.88	100.63	302.38	100.79	602.44	100.41	796.19	**99.52**	981.50	98.15	1222.73	101.89
R39	35.21	100.61	69.45	99.22	140.39	100.28	300.83	100.28	595.77	99.29	794.56	**99.32**	1033.40	103.34	1177.37	98.11
R40	35.30	100.85	69.22	98.89	141.16	100.83	297.78	99.26	607.34	101.22	796.92	**99.62**	986.96	98.70	1211.99	101.00
mean	36.02		68.32		140.42		300.89		602.64		794.19		997.73		1208.78	
SD	0.92		1.37		0.85		2.21		5.00		3.53		23.99		21.57	
CV [%]	2.55		2.01		0.60		0.73		0.83		0.44		2.40		1.78	
Nom [%]	102.93		97.60		100.30		100.30		100.44		99.27		99.77		100.73	
n	4		4		4		4		4		4		4		4	

Selectivity was assayed using 10 individual minipig serum samples (5 females, 5 males) and a mixed gender minipig serum pool as control. Each sample was tested unspiked or spiked with 35 ng/mL (Low Spike) of ustekinumab biosimilar. Selectivity assessment showed no interference related to matrix content (Figure 5).

Figure 5: Selectivity Selectivity of the assay.

Maximal allowed %Bias is indicated. Reported is the average of a duplicate measurement.

R37	Blank		Low Spike [Ustekinumab (biosimilar)] Nominal [ng/mL] 35.000	Nom %
CM/17-2132	BLQ	CM/17-2132	33.49	95.69
CM/17-2133	BLQ	CM/17-2133	33.83	96.66
CM/17-2134	BLQ	CM/17-2134	32.35	92.42
CM/17-2135	BLQ	CM/17-2135	34.05	97.29
CM/17-2136	BLQ	CM/17-2136	38.18	109.09
CM/17-2139	BLQ	CM/17-2139	32.17	91.91
CM/17-2140	BLQ	CM/17-2140	32.34	92.39
CM/17-2141	BLQ	CM/17-2141	30.37	86.76
CM/17-2142	BLQ	CM/17-2142	30.81	88.02
CM/17-2143	BLQ	CM/17-2143	32.95	94.15
pool: CM/18-2143	BLQ	pool: CM/18-2143	32.09	91.69
		mean [ng/mL]	33.05	
		SD	2.16	
		CV [%]	6.55	
Total BLQ	10	Nominal [%]	94.44	
n	10	n	10	
BLQ: Below Limit of Quantitation				
Evaluation		**Evaluation**		
		± maximal %Bias	25	
	Blank		Low Spike [Ustekinumab (biosimilar)]	
individuals	10 / 10 BLQ	individuals	**10 / 10 acceptable**	
pool	BLQ	pool	acceptable	

To test the stability ustekinumab biosimilar was spiked into mixed gender minipig serum pool at QC Low and QC high levels (3 aliquots for each QC level and test condition). Next, each test sample was submitted to 3 different conditions: BenchTop ON incubation, 3 cycles of freeze/thaw at -20°C or -80°C (Figure 6). The experiment showed that the ustekinumab biosimilar is stable at all tested conditions.

Figure 6: Stability.

Stability evaluation of ustekinumab biosimilar in minipig serum. Reported is the average of a duplicate measurement.

Nominal [ng/mL]	BenchTop, undiluted 16 hours				- 20°C, 3 cycles				- 80°C, 3 cycles			
	QC Low 105.00	Nom %	QC High 900.00	Nom %	QC Low 105.00	Nom %	QC High 900.00	Nom %	**QC Low** 105.00	Nom %	QC High 900.00	Nom %
R40	100.90	96.10	872.64	96.96	103.68	98.75	887.71	98.63	108.80	103.62	910.67	101.19
	103.66	98.72	918.36	102.04	104.85	99.85	936.38	104.04	106.59	101.52	884.38	98.26
	96.85	92.24	884.48	98.28	101.57	96.74	909.59	101.07	104.99	99.99	878.65	97.63
mean [ng/mL]	100.47		891.82		103.37		911.23		106.80		891.23	
SD	3.42		23.73		1.66		24.38		1.91		17.08	
CV [%]	3.41		2.66		1.61		2.68		1.79		1.92	
Nominal [%]	95.69		99.09		98.45		101.25		101.71		99.03	
n	3	3	3	3	3	3	3	3	3	3	3	3

Discussion and Conclusions

In the present study we successfully demonstrated similarity between a novel biosimiliar and its originators applied in Psoriasis therapy. For the characterization of this biosimilar we followed Marini's recommendations. We showed biosimilarity to the originators by overlapping standard curves and the precision and accuracy of QC samples. A 'one assay' setup proved to be sufficient to perform the comparison. Furthermore we have demonstrated that our assay is highly selective and the analyte is stable under various conditions.

The subject of biosimilars has become important in the recent years due to increasing number of those compounds being developed as an alternative to the, usually more expensive, originator substances. Therefore it is crucial to properly evaluate biosimilars in order to have safe and equally effective drugs compared to the originators. Thanks to the assay developed here we can conclude that this novel biosmilar drug for Psoriasis is safe and can be used as an alternative in patient therapy.

Poster presentation at EBF 2018