Adalimumab and Anti-adalimumab Lisa-tracker Immunoassays Performance Criteria for Therapeutic Drug Monitoring of Adalimumab-amgen Biosimilar (Abp501)

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Abstract

Background.

ABP501 is a biosimilar to Reference Adalimumab (HUMIRA®) produced by AMGEN. Adalimumab (ADA) has marketing authorization for Crohn's disease, ulcerative colitis and other inflammatory or autoimmune diseases. The aim of this study was to evaluate the LISA-TRACKER assays developed by Theradiag (France), for the monitoring of ABP501 and anti-ABP501 antibodies in human serum.

Methods.

Accuracy of the LISA-TRACKER was measured using 3 human serum matrices spiked with known levels of biosimilar, 3 levels spanning the dynamic range. Specificity was tested with Biosimilar spiked samples, Biosimilar with Humira® spiked samples, and clinical samples from patients treated with adalimumab biosimilar. All of these samples were spiked with polyclonal antibodies directed against Humira®. Intra-run, inter-run imprecision, inhibition, kit's stability, specificity inhibition and specificity detection steps were also part of the LISA-TRACKER Duo Adalimumab assay validation parameters.

Results.

68 ABP501 clinical samples were measured with the LISA TRACKER Duo Adalimumab assay. LISA TRACKER has been validated as suitable for quantification of ABP501 in human serum samples. Concerning accuracy, percentages of recovery were ranged from 90–120% for biosimilar batch1, and between 93% and 105% for biosimilar batch2. The acceptance criteria (CV < 20%) were met for intra-run (from 3.8–16.5%) and inter-run imprecision (from 4.4–13.9%) including the two batches. All results were comprised within +/-20% from results, obtained with the kit and sample unexposed in order to evaluate stability of the sample, stability of the kit and consistency of the results. In any case but two, all percentages of inhibition were > 50% for specificity. Reagents made with ABP501 gave similar results than reagents made with Humira® meeting acceptance criteria.

Conclusions.

LISA-TRACKER ADA and anti-ADA assays are reliable for the monitoring of patients treated with ABP501.

Background

Reference Adalimumab (R.A.) named HUMIRA®, marketed by AbbVie, is a human monoclonal antibody to tumour necrosis factor alpha (TNFα), part of the same family as Infliximab (Remicade®, Inflectra®, Remsima®) that showed efficacy and safety for treatment of inflammatory bowel diseases (IBD) as Crohn's disease [1] or Ulcerative colitis [1,2], Psoriatic arthritis, Ankylosing spondylitis, Rheumatoid arthritis [3], Juvenile idiopathic arthritis [4], Hidradenitis suppurativa [5] and Uveitis [6]. This therapeutic versatility has made adalimumab the top-selling drug with global sales of $19 billion in 2019 alone [7]. From 2017, with HUMIRA's patent expiration, biosimilars started receiving marketing authorization, positive opinions by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA), and new ones are currently being developed in highly regulated markets. A biosimilar is a molecule highly similar to an already licensed biologic product (referred to as the reference product) with minor differences in clinically inactive components and for which there are no clinically meaningful differences in purity, potency, and safety between the two products [8]. This is the case for, at least, six biosimilars: ABP 501 (Amgevita®, Solymbic®, Amgen, USA), GP2017 (Hefiya®, Halimatoz®, Hyrimoz®, Sandoz, Germany), BI 695501 (Cyltezo®, Boehringer Ingelheim, Germany), SB5 (Imraldi®, Biogen, South Korea), FKB327 (Hulio®, Fujifilm Kyowa Kirin, Japan), and MSB11022 (Idacio® and Kromeya®, Fresenius Kabi, Germany) [9]. ABP501 showed efficacy and safety

comparable with R.A. [10] , and have been approved in Europe in March 2017. The fact that therapeutic drug monitoring (TDM) of adalimumab may be associated with a lower risk of treatment failure compared with standard of care in patients [11] and that biosimilars are increasingly developed, suggests that being able to monitor biologicals as ABP501 in human serum could be interesting. LISA-TRACKER assays, based on ELISA method and developed by Theradiag (France), have already shown suitability for Infliximab biosimilars monitoring [12] and studies exposed suitability of assays for Adalimumab [13] or ABP501[14,15]. The purpose of this study was to evaluate, for the first time, the LISA-TRACKER assays, for the monitoring of adalimumab-amgen biosimilar and anti-adalimumab-amgen-biosimilar antibodies in human serum.

Methods

Reagents

The assay protocol has been conducted in order to evaluate the performance criteria of LISA TRACKER immunoassays for adalimumab and anti-adalimumab biosimilar antibodies. According to the results, the monitoring of the patients treated with ABP501 could be interesting, using the kit developed by Theradiag and based on ELISA method. To this end, 30 clinical samples from patients with R.A. were collected from France territory, 68 serum samples were provided by AMGEN and preparations of ABP501 spiked samples were made with 3 human serum matrices (two from individual healthy donors and one from a pool of healthy donors: M1, M2 and M3 respectively) in addition with ABP501 solutions spiked in to reach 3 levels of drug: 1, 4 and 12 µg/ml (low, medium, high respectively). Accuracy, standard curves comparison, intra-run imprecision, inter-run imprecision, correlation, kit's stability, specificity inhibition and specificity detection step were part of the LISA-TRACKER Duo Adalimumab assay validation parameters. Lower Limit Of Quantification (LLOQ) of ADALIMUMAB was 0.3 µg/ml and LLOQ of Anti-ADALIMUMAB assay was 10 ng/ml. Acceptance criteria, based on FDA guideline "Guidance for Industry - Bioanalytical method validation-ligand binding assay-Sept2013" and EMEA guideline "Guideline on bioanalytical method validation-ligand binding assays-21 July 2011", were similar to the criteria used for the validation of LISA-TRACKER assays. The study was considered exempt by our IRB.

Clinical Samples

All patients signed an informed written consent to the protocol which was approved by the Ethics Committee of Saint-Etienne University and Centre National Informatique et Liberté (CNIL 1849323 v 0).

Results

Measure of HUMIRA®/ABP501 trough levels in spiked samples

Accuracy of the LISA-TRACKER was measured using 3 human serum matrix spiked with known levels of biosimilar, 3 levels spanning the dynamic range. For each level, the percentage of recovery was calculated (Percentage of recovery of Adalimumab assay = [Mean of the six obtained results/theoretical level of Adalimumab)] x 100). An imprecision intra-run has been realized. Imprecision was assessed by using 3 clinical levels (low, medium, high) for 3 human matrices and for each levels, 10 tests per run were performed. An imprecision inter-run has been realized. Imprecision was assessed by using 3 levels (low, medium, high) of clinical samples for 3 human matrices and for each levels, on 6 independent runs, 2 tests per run were performed with LISA-TRACKER Duo Adalimumab assay. Quantification of Adalimumab was performed according to the technical insert of LISA-TRACKER Adalimumab kits (product number LTA 005 or LTA 002). As described in the technical insert, a run performed with LTA kits was validated when the optic density (OD) of the highest standard was above 0.8 and when the result (µg/ml) of the positive control was within its target range. Then, the result of each sample could be interpreted.

Accuracy

Spiked samples made with batch1 gave percentages of recovery comprised between 90% and 120% and spiked samples made with batch2 gave percentages of recovery comprised between 93% and 105%. All results met the acceptance criteria (80–120%). “ABP501 spiked samples” were quantified with LISA-TRACKER Duo Adalimumab assay. **(Table 1)** Quantification of ABP501 was not affected by serum matrix. Similar results were obtained with Humira® during the development of LISA-TRACKER Duo Adalimumab assay.

Intra-run and inter-run imprecision

For the imprecision intra-run, the CV ranged from 4.1–11.1% for samples made with biosimilar batch1 and ranged from 3.8–16.5% for samples made with biosimilar batch2 (Table 2). For the imprecision inter-run, the CV ranged from 4.4–13.9% for samples made with biosimilar batch1 and 4.5–13.0% for samples made with biosimilar batch2 (Table 3). The acceptance criteria (CV < 20%) was met.

Low intra-run and inter-run imprecisions were reached with LISA-TRACKER Duo Adalimumab assay for the quantification of ABP501.

Measure of HUMIRA®/AB501 through levels in clinical samples

Clinical samples with detectable level of Adalimumab (Humira®) were spiked with Humira® or ABP501. Thus, Adalimumab (Humira® or ABP501) were added into the clinical samples in order to increase the level of Adalimumab of 4 µg/ml. All samples from the 3 preparations (3 × 30 “spiked clinical samples”) were quantified with LISA-TRACKER Adalimumab kit (LTI 002, batch: 1847). The results obtained with “ABP501 spiked clinical samples” were compared to the results obtained with “Humira® spiked clinical samples”. Furthermore, the same type of tests was launched with clinical samples with detectable level of adalimumab biosimilar. Thus, ABP501 clinical samples were spiked with Humira® in order to increase the level of 4 µg/ml; spiked samples were quantified and compared to the unspiked samples.

ABP501 levels in patients

There were 30 clinical samples with detectable level of Humira®. All results from clinical samples spiked with ABP501 were within +/-20% of the expected values (levels of Adalimumab from clinical samples spiked with Adalimumab (Humira®). (**Tab. S1a**). The coefficient of determination (R^2) and the slope were calculated for the 3 different preparations. ABP501-batch1 vs Humira® (R^2 = 0.95; slope: 0.94), ABP501-batch2 vs Humira® (R^2 = 0.98; slope: 0.94), ABP501-batch1 vs ABP501-batch2 (R^2 = 0.94; slope: 0.95). **(**Fig. 1a**)**. Thus all the coefficients of determination and Slopes met the acceptance criteria (R^2 > 0.90 and Slope comprised between 0.9 and 1.1). Detection of ABP501 trough levels in the patients treated with ABP501 (34 clinical samples, ID: ABP501-1 to ABP501-34) with the LISA-TRACKER Adalimumab assay was compliant: all ABP501 positive samples were in the expected range after the addition of 4 µg/mL of R.A. (**Tab. S1b**). Therefore LISA-TRACKER Duo Adalimumab assay detect efficiently ABP501 trough levels as the R.A. in serum without any interference.

Specificity Assessment: Adalimumab Assay

“ABP501 spiked samples” inhibition assays were performed with LISA-TRACKER Adalimumab kit (product number: LTA 002, batch: 1847). In order to confirm the positivity of “ABP501 spiked samples” and the positivity of clinical samples, polyclonal antibodies directed against Humira® were added to the kit’s dilution buffer: “poly-anti-ada-buffer”. On one hand, “ABP501 spiked samples” (“ABP501 spiked samples” and “ABP501 + Humira® spiked samples”) and clinical samples were diluted with “poly-anti-ada-buffer”, and on the other hand they were diluted with the kit’s dilution buffer. Quantification of the samples was performed after 60 minutes of incubation at room temperature. For each sample, the percentage of inhibition was calculated. (Percentage of inhibition of Adalimumab assay = [1-(level of Adalimumab from sample diluted with poly-anti-ada-buffer/level of Adalimumab from sample diluted with sample’s dilution buffer)] x 100). Spiked samples (“ABP501 spiked samples” and “ABP501 + Adalimumab (Humira®) spiked samples”) and clinical samples with a percentage of inhibition greater than 50% were considered “positive” (detectable level) for ABP501.

Specificity

For “ABP501 spiked samples”, the levels of Adalimumab were below the limit of quantification when the kit’s dilution buffer spiked with polyclonal was used. All percentages of inhibition were at least above 77%. For “ABP501 + Adalimumab spiked samples” made with Humira® in addition with ABP501, the levels of Adalimumab were below the limit of quantification when the kit’s dilution buffer spiked with the polyclonal antibodies was used. (**Tab. S2a**). For “ABP501 clinical samples” (ID: ABP501-35 to ABP501-68), the levels of Adalimumab were below the limit of quantification when the kit’s dilution buffer spiked with the polyclonal antibodies was used. All percentages of inhibition were at least above 92%. (**Tab. S2b**). Acceptance criteria (percentage of inhibition > 50%) were met. “ABP501 spiked samples” and “ABP501 + Adalimumab spiked samples” and clinical samples gave similar results. Anti-Adalimumab antibodies generated with Humira® have the capacity to block Humira® and ABP501. LISA-TRACKER Duo Adalimumab assay should be able to detect ABP501 as it does for Humira®.

a.

ADALIMUMAB Samples	SAMPLES spiked with ABP501-Batch1				
	No-inhibited*		Inhibited**		% of inhibition***
	µg/ml	mean	µg/ml	mean	
Low	1,3	1,3	< 0,3	< 0,3	> 77%
	1,3		< 0,3		
Medium	4,8	5,1	< 0,3	< 0,3	> 94%
	5,4		< 0,3		
High	18,6	18,5	< 0,3	< 0,3	> 98%
	18,4		< 0,3		
ADALIMUMAB Samples	SAMPLES spiked with ABP501-Batch2				
	No-inhibited*		Inhibited**		% of inhibition***
	µg/ml	mean	µg/ml	mean	
Low	1,3	1,3	< 0,3	< 0,3	> 77%
	1,2		< 0,3		
Medium	5,1	5,0	< 0,3	< 0,3	> 94%
	4,9		< 0,3		
High	15,8	16,1	< 0,3	< 0,3	> 98%
	16,3		< 0,3		
ADALIMUMAB Samples	SAMPLES spiked with ABP501-Batch1 + ADALIMUMAB (Humira)				
	No-inhibited*		Inhibited**		% of inhibition
	µg/ml	mean	µg/ml	mean	
Low	3,8	3,7	< 0,3	< 0,3	> 92%
	3,5		< 0,3		
Medium	12,4	12,7	< 0,3	< 0,3	> 98%
	13,0		< 0,3		
High	> 20	> 20	< 0,3	< 0,3	> 98%
	> 20		< 0,3		
ADALIMUMAB Samples	SAMPLES spiked with ABP501-Batch2 + ADALIMUMAB (Humira)				
	No-inhibited*		Inhibited**		% of inhibition***
	µg/ml	mean	µg/ml	mean	
Low	3,6	3,8	< 0,3	< 0,3	> 92%
	4,0		< 0,3		
Medium	13,6	13,9	< 0,3	< 0,3	> 98%

a.					
	14,1		< 0,3		
High	> 20	> 20	< 0,3	< 0,3	> 98%
	> 20		< 0,3		

ANTI-ADALIMUMAB SAMPLES DILUTED WITH BUFFER SPIKED WITH :							
ANTI-ADALIMUMAB CLINICAL SAMPLES		ADALIMUMAB (Humira)		ABP501-Batch1		ABP501-Batch2	
ID	*Anti-ADALIMUMAB (ng/ml)*	Anti-ADALIMUMAB (ng/ml)	% of inhibition*	Anti-ADALIMUMAB (ng/ml)	% of inhibition*	Anti-ADALIMUMAB (ng/ml)	% of inhibition*
AADA1	*48*	< 10	79%	< 10	79%	< 10	79%
AADA2	*39*	< 10	74%	< 10	74%	< 10	74%
AADA3	*43*	< 10	77%	< 10	77%	< 10	77%
AADA4	*31*	< 10	68%	< 10	68%	< 10	68%
AADA5	*52*	< 10	81%	< 10	81%	< 10	81%
AADA6	*42*	< 10	76%	< 10	76%	< 10	76%
AADA7	*49*	< 10	80%	< 10	80%	< 10	80%
AADA8	*26*	< 10	62%	< 10	62%	< 10	62%
AADA9	*46*	< 10	78%	< 10	78%	< 10	78%
AADA10	*41*	< 10	76%	< 10	76%	< 10	76%
AADA11	*58*	< 10	83%	< 10	83%	< 10	83%
AADA12	*44*	< 10	77%	< 10	77%	< 10	77%
AADA13	*38*	< 10	74%	< 10	74%	< 10	74%
AADA14	*33*	< 10	70%	< 10	70%	< 10	70%
AADA15	*49*	< 10	80%	< 10	80%	< 10	80%
AADA16	*44*	< 10	77%	< 10	77%	< 10	77%
AADA17	*48*	< 10	79%	< 10	79%	< 10	79%
AADA18	*67*	< 10	85%	< 10	85%	< 10	85%
AADA19	*53*	< 10	81%	< 10	81%	< 10	81%
AADA20	*54*	< 10	81%	< 10	81%	< 10	81%
AADA21	*41*	< 10	76%	< 10	76%	< 10	76%
AADA22	*63*	< 10	84%	< 10	84%	< 10	84%
AADA23	*31*	< 10	68%	< 10	68%	< 10	68%
AADA24	*57*	< 10	82%	< 10	82%	< 10	82%
AADA25	*39*	< 10	74%	< 10	74%	< 10	74%
AADA26	*64*	< 10	84%	< 10	84%	< 10	84%
AADA27	*54*	< 10	81%	< 10	81%	< 10	81%
AADA28	*48*	< 10	79%	< 10	79%	< 10	79%
AADA29	*56*	< 10	82%	< 10	82%	< 10	82%
AADA30	*39*	< 10	74%	< 10	74%	< 10	74%

		ANTI-ADALIMUMAB SAMPLES DILUTED WITH BUFFER SPIKED WITH :					
AADA31	*43*	< 10	77%	< 10	77%	< 10	77%

DETECTION REAGENT SPIKED WITH :					
ANTI-ADALIMUMAB CLINICAL SAMPLES		ABP501-Batch1		ABP501-Batch2	
ID	*Anti-ADALIMUMAB (ng/ml)*	Anti-ADALIMUMAB (ng/ml)	% of inhibition*	Anti-ADALIMUMAB (ng/ml)	% of inhibition*
AADA32	*48*	< 10	79%	< 10	79%
AADA33	*39*	14	64%	< 10	74%
AADA34	*32*	< 10	69%	< 10	69%
AADA35	*27*	< 10	64%	< 10	64%
AADA36	*27*	< 10	64%	< 10	64%
AADA37	*24*	< 10	58%	< 10	58%
AADA38	*37*	< 10	73%	< 10	73%
AADA39	*62*	< 10	84%	< 10	84%
AADA40	*31*	< 10	68%	< 10	68%
AADA41	*23*	< 10	56%	< 10	56%
AADA42	*36*	< 10	72%	< 10	72%
AADA43	*94*	< 10	89%	< 10	89%
AADA44	*28*	< 10	65%	< 10	65%
AADA45	*74*	< 10	86%	< 10	86%
AADA46	*35*	< 10	71%	< 10	71%
AADA47	*18*	< 10	44%	< 10	44%
AADA48	*55*	< 10	82%	< 10	82%
AADA49	*30*	< 10	67%	< 10	67%
AADA50	*14*	< 10	30%	< 10	30%
AADA51	*74*	< 10	86%	< 10	86%
AADA52	*53*	< 10	81%	< 10	81%
AADA53	*29*	< 10	65%	< 10	65%
AADA54	*64*	< 10	84%	< 10	84%
AADA55	*52*	< 10	81%	< 10	81%
AADA56	*39*	< 10	74%	< 10	74%
AADA57	*30*	< 10	67%	< 10	67%
AADA58	*64*	< 10	84%	< 10	84%
AADA59	*27*	< 10	63%	< 10	63%
AADA60	*51*	< 10	80%	< 10	80%
AADA61	*70*	< 10	86%	< 10	86%

DETECTION REAGENT SPIKED WITH :					
AADA62	*25*	< 10	60%	< 10	60%
AADA63	*29*	< 10	65%	< 10	65%
AADA64	*21*	< 10	51%	< 10	51%
AADA65	*34*	< 10	71%	< 10	71%
AADA66	*59*	< 10	83%	< 10	83%
AADA67	*29*	< 10	65%	< 10	65%
AADA68	*20*	< 10	51%	< 10	51%
AADA69	*40*	< 10	75%	< 10	75%

	ADALIMUMAB (µg/ml)						
a.	**Kit's stability**						
	Levels	**Unexposed**	Acc. Criteria (+/- 20%)		**7d + 37°c**		
			target Low	target High			
	PC (c+)	**4,1**	1,9	5,6	**5,1**		
ADALIMUMAB ABP501-batch1	Low	**1,8**	1,4	2,2	**1,6**		
	Medium	**5,3**	4,2	6,4	**6,2**		
	High	**19,6**	15,7	23,5	**20,0**		
ADALIMUMAB ABP501-batch2	Low	**1,1**	0,9	1,3	**1,3**		
	Medium	**4,3**	3,4	5,2	**4,8**		
	High	**18,0**	14,4	21,6	**17,5**		
b.	**Specimen's stability**						
	Levels	**Unexposed**	Acc. Criteria (+/- 20%)		**7d + 4°c**	**3d RT**	**5 x f/t cycles**
			target Low	target High			
ADALIMUMAB - ABP501-Batch1	Low	**1,2**	1,0	1,4	**1,2**	**1,0**	**1,1**
	Medium	**4,0**	3,2	4,8	**4,2**	**4,2**	**4,6**
	High	**12,8**	10,2	15,4	**12,1**	**13,0**	**11,2**
ADALIMUMAB - ABP501-Batch2	Low	**1,2**	1,0	1,4	**1,1**	**1,1**	**1,2**
	Medium	**4,3**	3,4	5,2	**4,1**	**4,5**	**4,3**
	High	**12,8**	10,2	15,4	**13,7**	**11,8**	**12,3**

Measure Of Anti-adalimumab In Clinical Samples: Inhibition Assay

Clinical samples with detectable level of Anti-Adalimumab antibodies were diluted with the kit's dilution buffer previously spiked with Adalimumab (Humira® or ABP501 were added to kit's dilution buffer in order to prepare 2 types of "ada-buffer"). Also, the clinical samples were diluted with the kit's dilution buffer. The 3 preparations (2 preparations made with Adalimumab and 1 preparation made without Adalimumab) were incubated 60 minutes at room temperature and quantified with LISA-TRACKER Anti-Adalimumab kit (product number LTA 003, batch: 1844). For each sample, the percentage of inhibition was calculated (percentage of inhibition of Anti-Adalimumab assay = [1-(level of Anti-Adalimumab antibodies from sample diluted with "ada-buffer"/level of Anti-Adalimumab antibodies from sample diluted with kit's dilution buffer)] x 100). The capacity of ABP501 to block the detection of ATA was measured. Clinical samples with a percentage of inhibition greater than 50% were considered to be inhibited by Adalimumab.

Adalimumab and ATA inhibition assay

There were 31 clinical samples (ID: AADA1 to AADA31) with detectable level of Anti-Adalimumab antibodies. Samples with high levels of Anti-Adalimumab antibodies gave high percentages of inhibition because they were well above the LLOQ. In any case, all percentages were above 50% and both batches of ABP501 and Adalimumab gave similar results. Acceptance criteria were met. (**Tab. S3**). ABP501 is able to block Anti-Adalimumab antibodies from patients treated with Humira®.

Measure Of Specificity Detection Step In Spiked Clinical Samples

Clinical samples with detectable level of Anti-Adalimumab antibodies were quantified with LISA-TRACKER Anti-Adalimumab kit (product number: LTA 003, batch: 1849). In order to confirm the capacity of ABP501 to block antibodies directed against Humira®, detection step was performed with or without the addition of ABP501 into the detection reagent (biotinylated Humira®) used for the detection step of Anti-Adalimumab antibodies during the assay). For each sample, the percentage of inhibition was calculated (percentage of inhibition of Anti-Adalimumab assay = [1-(level of Anti-Adalimumab antibodies in the presence of ABP501 into the detection reagent / level of Anti-Adalimumab antibodies)] x 100). Clinical samples with a percentage of inhibition greater than 50% were considered to be inhibited by ABP501.

Specificity detection step

There were 38 clinical samples (ID: AADA32 to AADA69) with detectable levels of Anti-Adalimumab antibodies. Samples with low levels (around 2 x limit of quantification (LLOQ)) of Anti-Adalimumab antibodies gave percentages of inhibition near 50% because they were near the limit of quantification (10 ng/ml). Samples with high levels of Anti-Adalimumab antibodies gave high percentages of inhibition because they were well above the LLOQ. In any case but two, all percentages were above 50%. Both batches of ABP501 gave similar results: acceptance criteria were met. (**Tab. S4**). ABP501 is able to compete with biotinylated Humira®. Anti-Adalimumab antibodies induced by Humira® were able to detect ABP501 during the detection step. LISA-TRACKER Duo Adalimumab assay should be able to detect Anti-Adalimumab antibodies induced by ABP501.

Measure Of Anti-adalimumab/anti-abp501 In Spiked Samples

Clinical samples with detectable level of Anti-Adalimumab antibodies were quantified with the 3 pairs of reagents made with the 3 types of raw materials (pair1: Humira® coated microplate and biotinylated Humira®; pair2: ABP501-batch1 coated microplate and biotinylated ABP501-batch1; pair3: ABP501-batch2 coated microplate and biotinylated ABP501-batch2). Results from the 3 pairs were compared.

Measurement of levels of Anti-adalimumab in patient and spiked samples according to the Anti-adalimumab standard curve

There were 20 clinical samples (ID: AADA70 to AADA89) with detectable level of Anti-Adalimumab antibodies. The coefficient of determination (R^2) and the slope were calculated for the 3 different combinations of reagents. ABP501-batch1 vs

Humira® (R^2 = 0.98; slope: 1.05), ABP501-batch2 vs Humira® (R^2 = 0.93; slope: 1.04), ABP501-batch1 vs ABP501-batch2 (R^2 = 0.97; slope: 0.97) (Fig. 1b). Thus all the coefficients of determination and Slopes met the acceptance criteria ($R^2 > 0.90$ and Slope comprised between 0.9 and 1.1). Reagents made with ABP501 give the same performances as reagents made with Humira® for the detection of Anti-Adalimumab antibodies (from patients treated with Humira®). This demonstrates the similarity of ABP501 and Humira® towards Anti-Adalimumab antibodies. LISA-TRACKER Anti-Adalimumab assay should be able to detect Anti-Adalimumab antibodies induced by ABP501 as it does for Anti-Adalimumab antibodies induced by Humira®.

Measure Of Kit And Sample Stability

In order to evaluate the kit's stability, LISA-TRACKER Duo Adalimumab kit was stored under stress thermic condition (7 days at + 37 °C). Then, "ABP501 spiked samples" were tested with this "stressed" kit. Results were compared to the results obtained with the unexposed kit (stored between + 2 °C and + 8 °C). For specimen's stability, spiked samples from patients treated with adalimumab biosimilar were stored in different conditions until quantification: at -20 °C (unexposed samples), 7 days between + 2 °C and + 8 °C (+ 4 °C storage condition), 3 days between + 18 °C and + 24 °C (room temperature (RT) storage condition), and 5 freeze/thaw cycles undergone.

Kit's stability

The percentages of variation (results from the stressed kit compared to the unexposed kit) were comprised between − 11% and 17% for spiked samples made with ABP501-batch1, and between − 3% and 18% for spiked samples made with ABP501-batch2. All results were within +/-20% from the unexposed kit: acceptance criteria were met. LISA-TRACKER Duo Adalimumab assay is robust. Quantification of ABP501 was not disrupted even if the kit was stored after a long period of elevated temperature (delivery, storage, etc.). (**Tab. S5a**)

Specimen's stability

For all storage conditions (+ 4°c, RT or freeze/thaw cycles), all samples gave levels of Adalimumab comprised within +/- 20% compared to the unexposed samples. ABP501 serum samples can be stored, 7 days at + 4°c, or 3 days at RT, or can undergo up to 5 freeze/thaw cycles, before being quantified with LISA-TRACKER Duo Adalimumab assay. (**Tab. S5b**)

Discussion

In this study, by analysing the results obtained, quantification of ABP501 was not affected by serum matrix. Low intra-run and inter-run imprecisions were reached with LISA-TRACKER Duo Adalimumab assay for the quantification of ABP501 with similar results for the two batches. LISA-TRACKER Duo Adalimumab assay should be able to detect efficiently ABP501 as it does for R.A. in serum without any interference, and also detect Anti-Adalimumab antibodies induced by ABP501 as it does for Anti-Adalimumab antibodies induced by Humira®. The two results out of our acceptance criteria for specificity detection step cannot question the last point because the initial level of ATA was near the LLOQ, underestimating the percentage of inhibition. Regarding the fact that quantification of ABP501 was not disrupted even if the kit was stored after a long period of elevated temperature (delivery, storage, etc.), it expresses the robustness of the collected results. These performance criteria including reproducibility, attest to the suitability of LISA-TRACKER Duo Adalimumab assay for ABP501 and anti-ABP501 measurement. Therapeutic drug monitoring of monoclonal antibodies allows clinicians to more safely, effectively, and efficiently use medications[16]. A recent retrospective cohort study emphasized the importance of measuring adalimumab serum levels early, which may guide dose optimization and prevent immunogenicity with associated treatment failure[17]. Other studies showed that TDM limits unnecessary dose escalation and provides appropriate treatment strategy without compromising clinical outcomes[18]. Researches have been made using LISA-TRACKER immunoassays for different anti-TNFα as Infliximab, Adalimumab, Etanercept[19], Certolizumab and Golimumab[20], but a few presented results about

biosimilars. The increasing development of new biosimilars as ABP501 and the opportunity to switch from the originator adalimumab to a biosimilar compound without affecting treatment efficacy[21–23], leads to the fact that these biologicals actually represent great potential allowing patients greater access to monoclonal antibodies[24] and will have a more important role for TDM in the future. To this end, this study suggests a new option for drug monitoring in order to guide the management of patients with loss of response to ABP501 as it has been described for R.A in Crohn's disease[25].

Conclusion

LISA-TRACKER Duo Adalimumab kit (LTA 005), LISA-TRACKER Adalimumab kits (LTA 002–48, LTA 002–96) and LISA-TRACKER anti-Adalimumab kits (LTA 003–48, LTA 003–96) are suitable for the monitoring of patients treated with ABP501. Our study shows a perfect correlation of dosages between the ATRA and Anti-ABP501, as well as detection of AB501. Thus the monitoring of ABP501 will be useful for the follow-up of patients with inflammatory diseases, for the research and for the therapeutic optimization.

Abbreviations

ATA: antibodies to adalimumab, CV: coefficient of variation, ELISA: enzyme-linked immunosorbent assay, FDA: Food and Drug Administration, ADA: adalimumab, OD: optic density, R.A.: reference adalimumab, ATRA: antibodies to Reference Adalimumab, TNFα: tumour necrosis factor alpha, LLOQ: Lower Limit of quantification, TDM: Therapeutic drug monitoring

Declarations

Ethics approval and consent to participate

All patients signed an informed written consent to the protocol which was approved by the Ethics Committee of Saint-Etienne University and Centre National Informatique et Liberté (CNIL 1849323 v 0).

Consent for publication

All authors consent for publication.

Availability of data and material

All datas and materials are available upon request to the corresponding author.

Competing interests

There is no competing interests.

Funding

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Authors' contributions

FF, LN, XR, AEB and SP contribute equally to the realization of experiments and to the writing/corrections of the paper.

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Figures

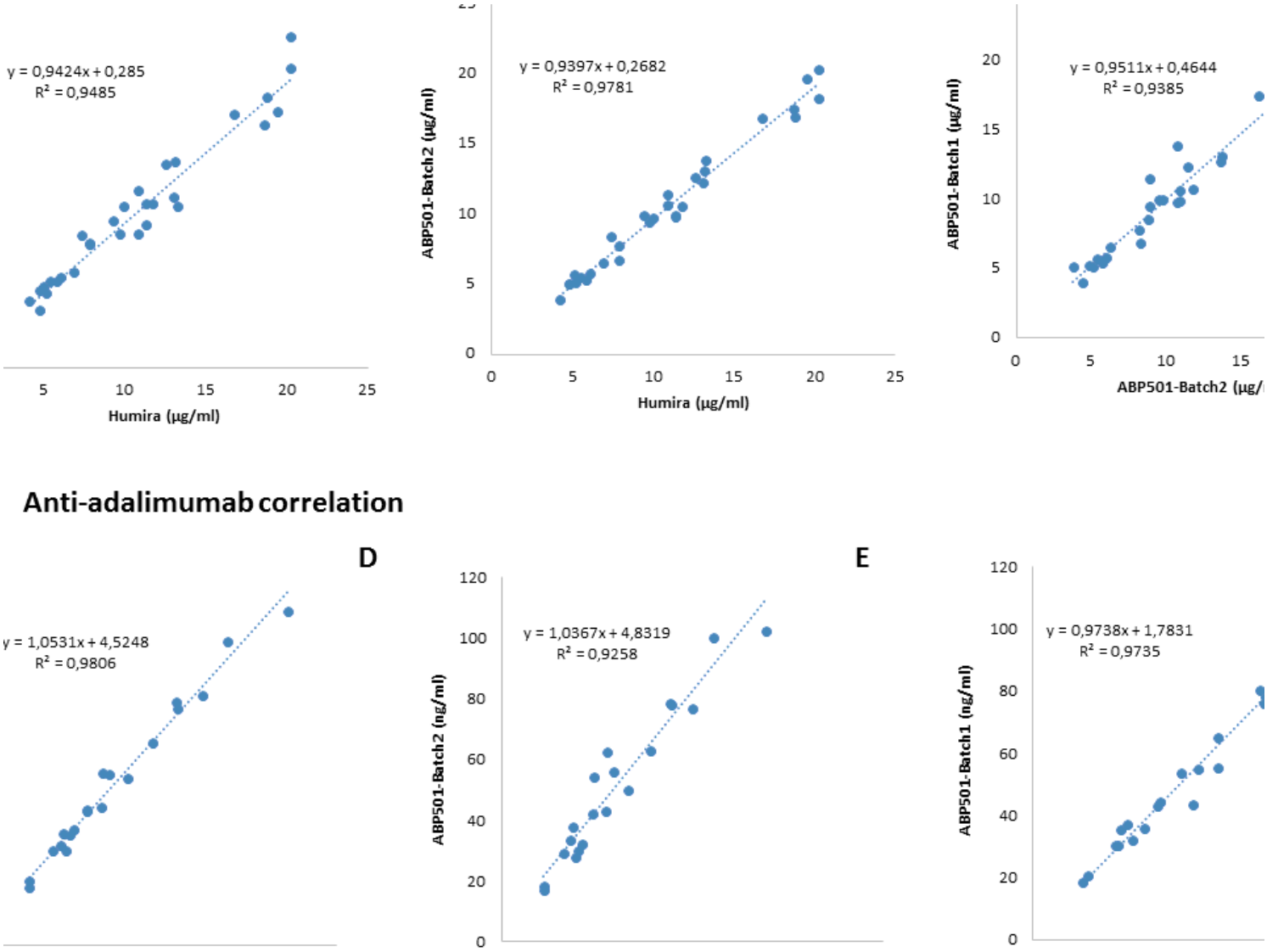

Figure 1

Measurement of levels of adalimumab (ABP501/Humira®) and anti-adalimumab (Anti-ABP501/Anti-Humira®) in patients or spiked samples according to the Adalimumab standard curve. The coefficient of determination (R2) and the slope were calculated for the 3 different combinations. a) Adalimumab correlation: ABP501-batch1 vs Humira® (R2 = 0.95; slope: 0.94) (A), ABP501-batch2 vs Humira® (R2 = 0.98; slope: 0.94) (B), ABP501-batch1 vs ABP501-batch2 (R2 = 0.94; slope: 0.95) (C). b) Anti-adalimumab correlation: ABP501-batch1 vs Humira® (R2 = 0.98; slope: 1.05) (D), ABP501-batch2 vs Humira® (R2 = 0.93; slope: 1.04) (E), ABP501-batch1 vs ABP501-batch2 (R2 = 0.97; slope: 0.97) (F). All the quantifications were measured with the LISA-TRACKER Duo Adalimumab assay.

Supplementary Files

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