



New tools for therapeutic antibodies and anti-drug antibodies (ADA) detection in human serum

Background: The therapeutic use of chimeric or humanized antibodies such as Remicade®, Mabthera® or Roactemra® has greatly improved the treatment of several autoimmune diseases (rheumatoid arthritis, psoriasis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, leukemias, or lymphomas). However, therapeutic failure is observed in a significant proportion of patients treated with therapeutic antibodies. Among several hypothesis, the development of an anti-drug response (Anti-drug antibody (ADA)) may explain the loss of response due either to degradation and/or to neutralization of the drug effect. Therefore, accurate monitoring of drug and anti-drug antibody levels should be implemented in routine clinical practices.

Objective: Development of a panel of murine monoclonal antibodies against Remicade®, Mabthera® or Roactemra® intended to monitor the level of therapeutic antibodies and anti-drug antibodies in patient's sera.

Materials and Methods

Therapeutic antibodies used as immunogen

Therapeutic antibody	Alias	Target	Description	Supplier
Remicade	Infliximab	TNF- α	chimeric IgG1/ κ	Janssen BV
Mabthera	Rituximab	CD20	chimeric IgG1/ κ	Roche
Roactemra	Tocilizumab	IL6-R	humanized IgG1/ κ	Roche



Immunization

The immunization program was performed according to Dendritics routine process.

Briefly, one BALB/c Jico (Charles River's Laboratory) was immunized with Remicade, Mabthera and Roactemra according to the following schedule:

- Day 0 : IP1 3X30µg mAb in complete Freund adjuvant (1ml)
- Day 14 : IP2 3X30µg mAb in incomplete Freund adjuvant (1ml)
- Day 21 : IP3 3X30µg mAb in PBS (1ml)
- Day 29 : IV 50µg pool 3 mAbs inPBS (0.5ml)+ IP 40µg pool 3 mAbs in PBS (0.5ml)
- Day 32 : Splenocytes isolation and fusion with SP2/0 myeloma cells.

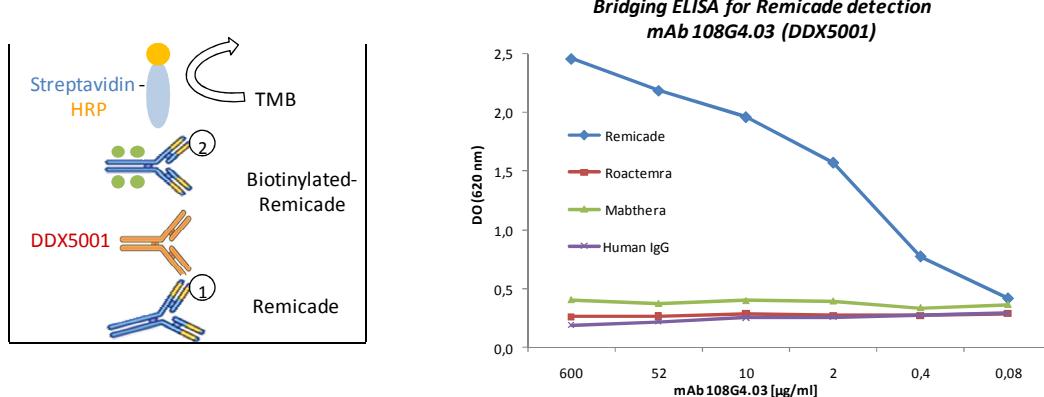
Results

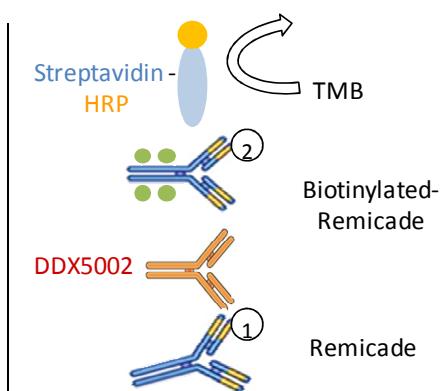
A panel of murine monoclonal antibodies was selected for each therapeutic antibody. These antibodies were set up in ELISA aiming at the dosage of both therapeutic antibodies and anti-drug antibodies in human serum samples.

REMICADE (DDX5001-DDX5002)

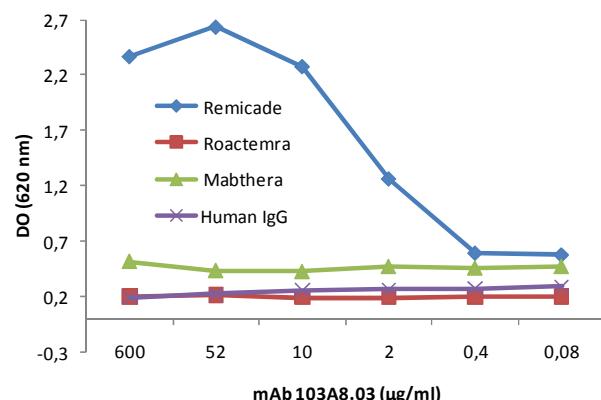
Remicade® is a chimeric monoclonal antibody targeting TNF- α , used for the treatment of autoimmune diseases such as rheumatoid arthritis, ankylosis spondylitis, Crohn's disease, psoriatic arthritis or ulcerative colitis. We have generated a panel of anti-Remicade® monoclonal murine antibodies. These antibodies can be used for the determination of Remicade® levels in biological fluids. In addition, competition ELISA using these antibodies allows the dosage of anti-Remicade® in sera of patient under Remicade therapy.

DDX5001 and DDX5002 are highly specific for Remicade

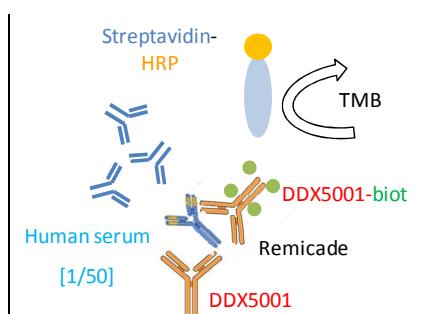




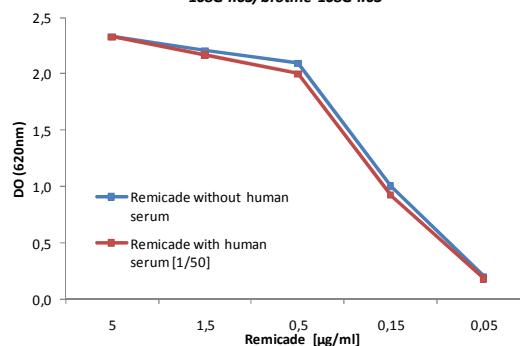
**Bridging ELISA for Remicade detection
mAb 103A8.03 (DDX5002)**



Remicade detection by DDX5001 is not affected by human serum

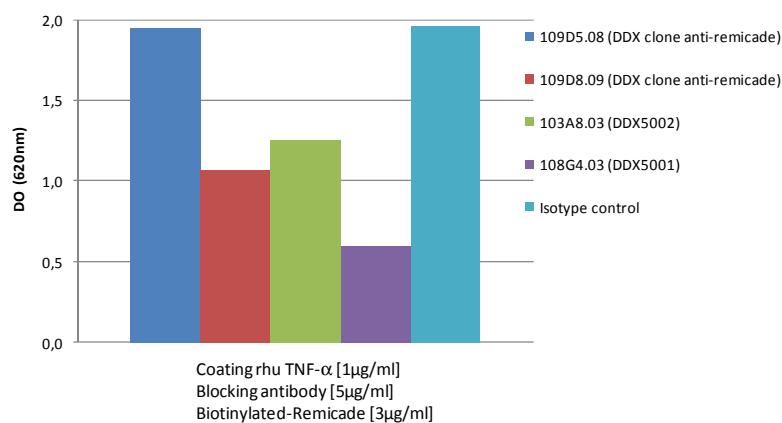


**ELISA detection of Remicade with or without human serum
108G4.03/biotine-108G4.03**



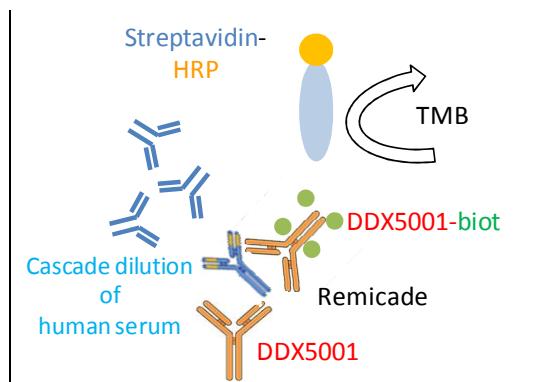
DDX5001 and DDX5002 significantly inhibit Remicade binding to TNF-α

Inhibition of Remicade binding to TNF-α

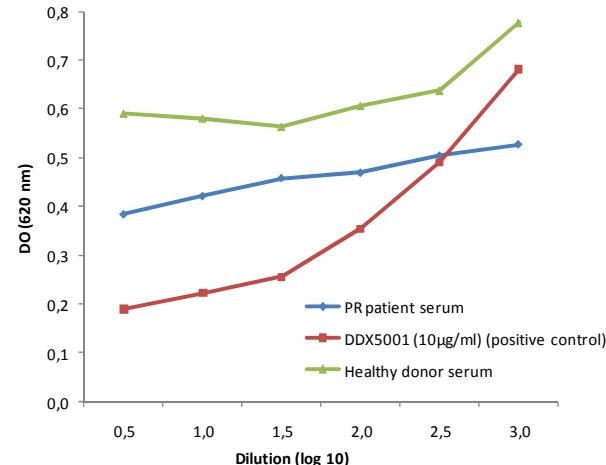




Anti-Remicade (ADA) detection by competition ELISA in serum from Remicade-treated polyarthritis rheumatoid patient (PR)



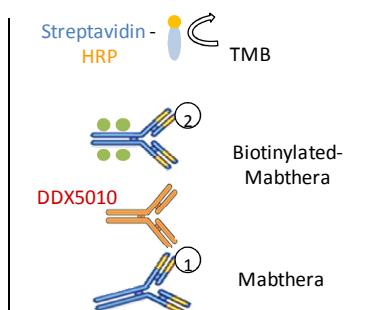
Detection of anti-Remicade (ADA) in human serum



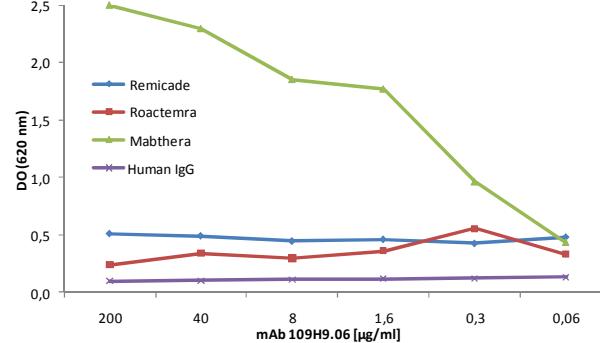
MABTHERA (DDX5010-DDX5011-DDX5012)

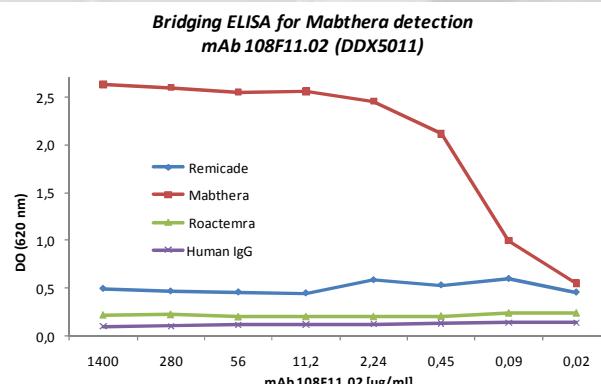
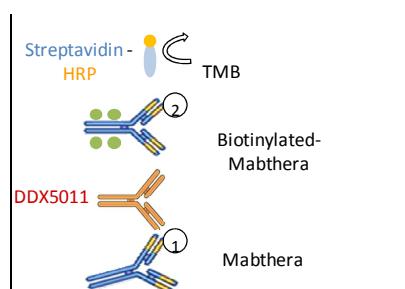
Mabthera® is a chimeric monoclonal antibody targeting the CD20 protein expressed on B cell surface. Mabthera® destroys B cells and is therefore used to treat diseases such as lymphomas, leukemias, transplant rejection, and autoimmune disorders. Mabthera® has also been shown to be an effective rheumatoid arthritis, in cases where anti-TNF- α therapy is not satisfactory. We have generated a panel of anti-Mabthera® monoclonal murine antibodies. These antibodies can be used for the determination of Mabthera® levels in biological fluids. In addition, competition Elisa using these antibodies allows the dosage of anti-Mabthera® in patients sera.

DDX5010 and DDX5011 are highly specific for Mabthera

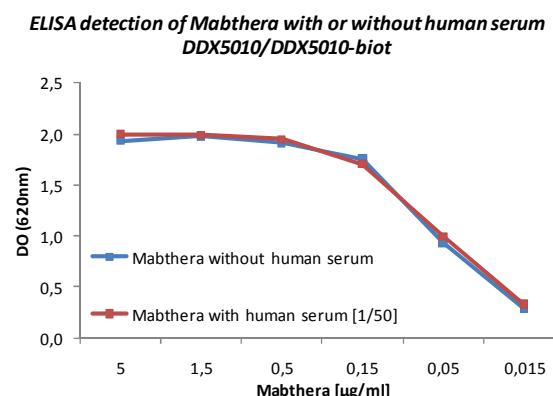
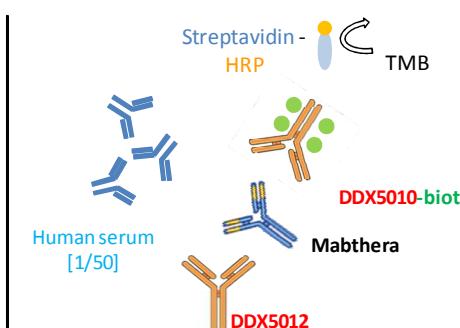


*Bridging ELISA for Mabthera detection
mAb 109H9.06 (DDX5010)*

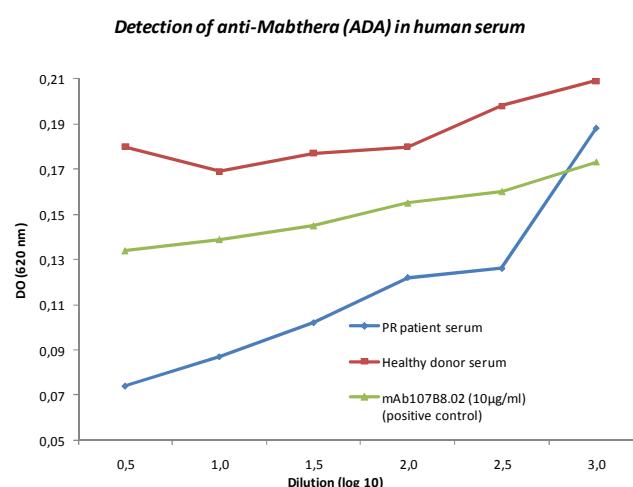
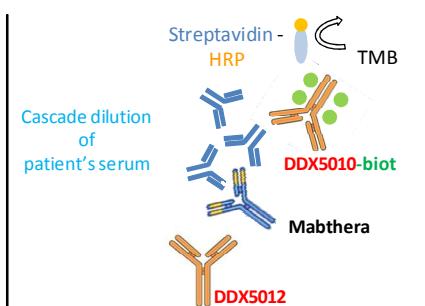




Mabthera detection by DDX5010 is not affected by human serum



Anti-Mabthera (ADA) detection by competition ELISA in serum from Mabthera-treated polyarthritis rheumatoid patient (PR)

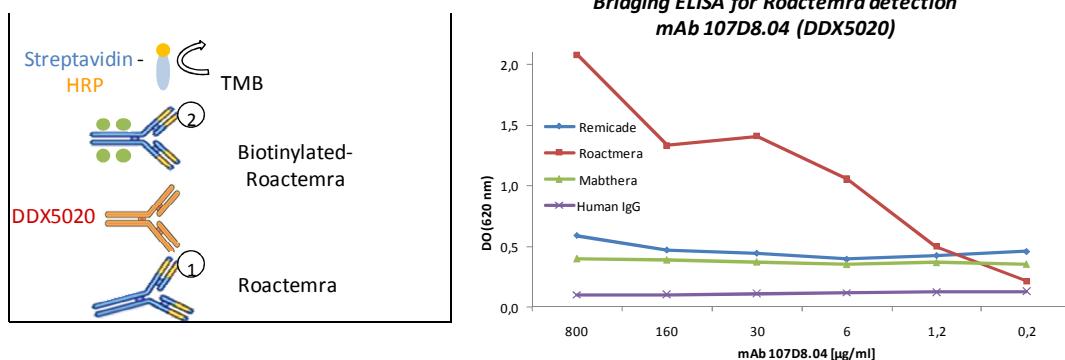


ROACTEMRA (DDX5020-DDX5021)

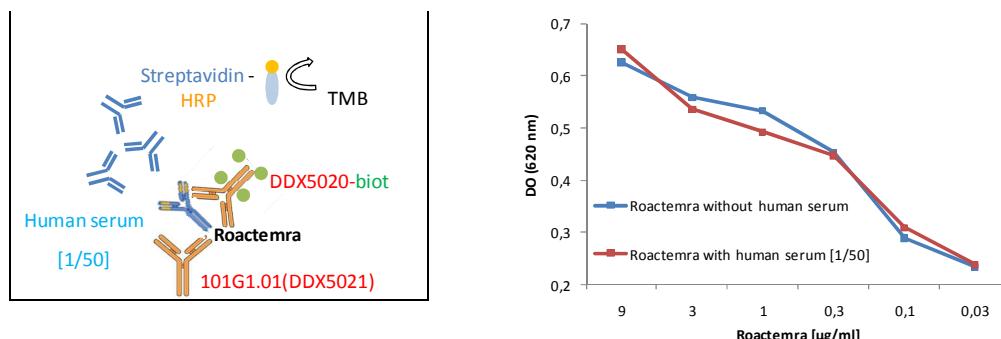
Roactemra® is a humanized antibody targeting the interleukine-6 receptor. This immunosuppressive drug is mainly used for the treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis. We have generated a panel of anti-Roactemra murine

monoclonal antibodies intended for Roactemra and anti-Roactemra (ADA) detection in patient's sera.

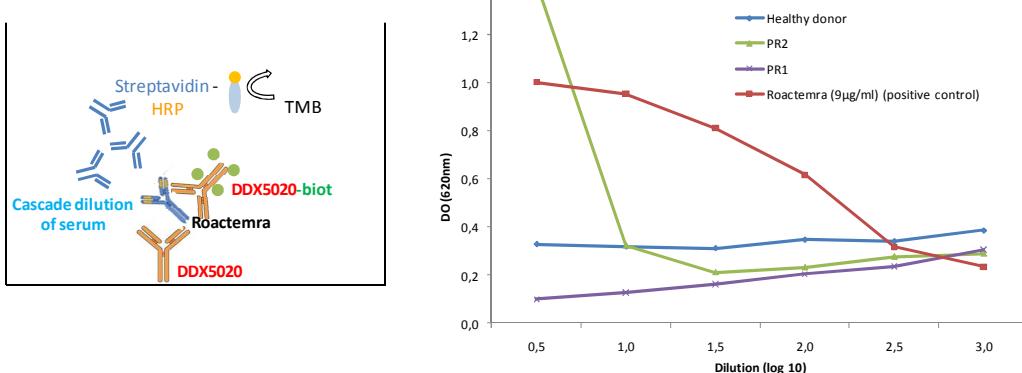
DDX5020 is highly specific for Roactemra



Roactemra detection by DDX5020/101G1.01 is not affected by human serum

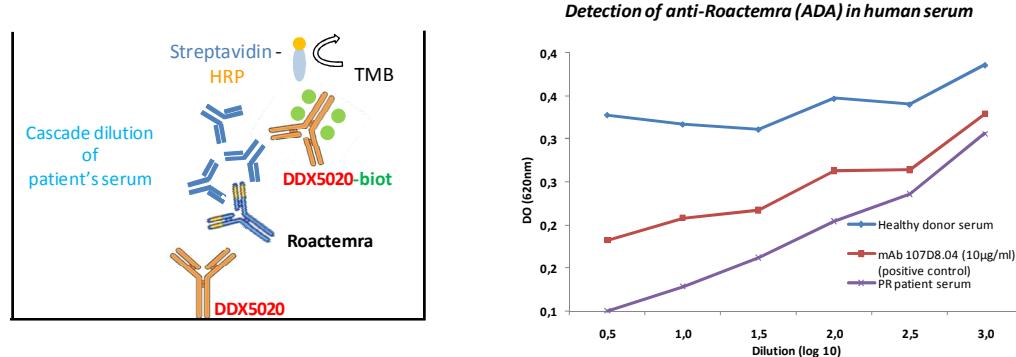


Roactemra detection by capture Elisa in serum from Roactemra-treated polyarthritis rheumatoid patients





Anti-Roactemra (ADA) detection by competition ELISA in serum from Roactemra-treated polyarthritis rheumatoid patient (PR)



SUMMARY TABLE

# catalog	Clone	Specificity			Detection	Capture	Bridging	Inhibition of remicade binding to TNF- α
		ROACTEMRA	REMICADE	MABTHERA				
	102C3.04	-	+	-	+	-	-	-
	107A12.04	-	+	-	+/-	-	-	-
	107C8.11	-	+	-	+/-	-	-	-
	108D12.11	-	+	-	+	-	-	-
	108E3.11	-	+	-	+++	+	-	-
	109D6.07	-	+	-	+/-	-	-	-
	109G3.24	-	+	-	+++	+++	+	+/-
	210D4.10	-	+	-	++	+++	-	-
	210G4.02	-	+	-	+/-	-	-	-
DDX5001	108G4.03	-	+	-	+++	+++	++++	++++
DDX5002	103A8.03	-	+	-	+++	+++	++++	++
	109D8.09	-	+	-	++	+++	++	++
	102B9.03	-	-	+	++	+++	+	
	107E9.03	-	-	+	++++	++++	+/-	
	107G12.06	-	-	+	+/-	-	-	
	107H12.02	-	-	+	++	+++	-	
	108G9.10	-	-	+	+++	+++	+	
	108H2.12	-	-	+	+++	++	-	
	108D1.23	-	-	+	+++	+++	+	
	108H5.02	-	-	+	++	+++	-	
	109A2.02	-	-	+	+	-	-	
	109B9.04	-	-	+	+	-	-	
	110D7.09	-	-	+	-	-	++	
	110B9.02	-	-	+	+++	+/-	+++	
	110F8.09	-	-	+	+/-	-	-	
	110F5.12	-	-	+	++++	++++	+++	
DDX5010	109H9.06	-	-	+	++++	+/-	++++	
DDX5011	108F11.02	-	-	+	+/-	-	+++	
DDX5012	107B8.02	-	-	+	++	++++	-	
	105D9.05	+	-	-	++	-	++	
	107E11.04	+	-	-	-	+/-	-	
	109C12.06	+	-	-	++	++	+	
	109B11.04	+	-	-	-	+/-	-	
	110A3.09	+	-	-	+++	++++	+	
	110E11.15	+	-	-	+++	++++	++	
	110H10.08	+	-	-	+/-	-	-	
	110E9.10	+	-	-	-	+/-	-	
DDX5021	101G1.01	+	-	-	++++	++	++	
DDX5020	107D8.04	+	-	-	++++	++++	+++	