Electronic Supplementary Information (ESI)

A New Lateral Flow Assay to Detect sIL-2R during T-Cell Mediated Rejection after Kidney Transplantation

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Tables

Table S1: *The 15 best scored continuous scaled variables as discriminators for rejection (comprising borderline TCMR/TCMR/AMR) or non-rejection state, scored according to neural net analysis using a perceptron and 250 feature selections.*

In addition to neural network analysis, CXCL9, sIL-2R, TSLP and serum creatinine were statistically relevant as indicated in a hypothesis-driven statistical approach (Wilcoxon test rsp. Mann-Whitney-U-test) in patients with or without rejection.

variable	Mean ± sd rejection versus non rejection	Score	Significance according to Mann-Whitney U--Test
GM-CSF (pg/ml)	5.1 ± 14.7 vs. 10.8 ± 22.7	0.9	Ns
Serum Creatinine at biopsy (µM/l)	**256.8 ± 93.2 vs. 170.8 ± 80.9**	**0.83**	**<0.001**
CXCL9 (pg/ml)	**1586.0 ± 269.3 vs. 818.8 ± 152.2**	**0.79**	**<0.03**
IL-13 (pg/ml)	14.5 ± 12.0 vs.12.9 ± 8.6	0.73	Ns
MIF (pg/ml)	797.4 ± 757.1 vs. 413.9 ± 212.1	0.61	Ns
IFN-2α (pg/ml)	39.8 ± 16.4 vs. 45.0 ± 15.3	0.54	Ns
IL-15 (pg/ml)	9.0 ± 5.7 vs. 9.0 ± 6.0	0.54	Ns
sIL-2R (pg/ml)	**126.4 ± 98.6 vs. 363.6 ± 216.02**	**0.54**	**<0.05**
CCL17 (pg/ml)	11.5 ± 7.8 vs. 16.5 ± 16.9	0.53	Ns
CCL5 (pg/ml)	833.1 ± 447.7 vs. 692.5 ± 300,0	0.52	Ns
CCL26 (pg/ml)	20.6 ± 10.8 vs. 20.9 ± 22.7	0.52	Ns
TSLP (pg/ml)	**1.7 ± 0.7 vs. 2.5 ± 7.2**	**0.51**	**0.01**
HGF (pg/ml)	145.7 ± 110.4 vs. 161.4 ± 100.1	0.50	Ns
TNF-β (pg/ml)	0.5 ± 0.7 vs. 0.7 ± 0.9	0.49	Ns
PDFG-bb (pg/ml)	271.7 ± 471.5 vs. 454.2 ± 667.5	0.48	<0.08

Table S2: Characteristics of the 1st patient cohort categorized according to rejection type. CyA* = standard triple therapy comprising cyclosporine, mycophenolate *(MPA)* and steroids; Tac* = standard triple therapy comprising tacrolimus, *MPA* and steroids; mTOR* = standard triple therapy comprising mTOR-inhibitor, *MPA* and steroids. TCMR: acute T-cell mediated rejection, AMR: Antibody mediated rejection, Borderline TCMR: T-cell driven borderline rejection, non-rejection: non-rejection proven by biopsy

Variable	1st patient cohort			2nd patient cohort			3rd patient cohort		
	n / N	%	Rejection in Follow Up biopsies	n / N	%	Rejection in Follow Up biopsies	n / N	%	Rejection in Follow Up biopsies
Female	48 / 112	42.9		27 / 71	38.0		28 /64	43.9	
Male	64 / 112	57.1		44 / 71	62.0		36/64	56.1	
Protocol biopsy	52 / 112	46.4		20 / 71	28.2		58/64	90.6	
For-cause biopsy	60 / 112	53.6		51 / 71	71.8		6/64	9.4	
Non-rejection	52 / 112	46.4	6, 4 x TCMR, 2 x Borderline TCMR	40 / 71	56.3		26/64	50	n. a.
Borderline TCMR	31 / 112	27.7	11, 4x Borderline TCMR, 2 x AMR, 2 x AMR / TCMR, 3 x TCMR	7 / 71	9.9	3, 3 x TCMR	3/64	2.4	n. a.
TCMR	13 / 112	11.6	6, 3 x TCMR, 1 x AMR / TCMR, 1 borderline TCMR	7 / 71	9.9	3, TCMR	35/ 64	66.6	n. a.
AMR	16 / 112	14.3	8, AMR	17 / 71	23.9	13/17 17 x AMR	n.a.	n. a.	n. a.

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Table S3: Characteristics of the 1st patient cohort categorized according to rejection type. CyA* = standard triple therapy comprising cyclosporine, mycophenolate (MPA) and steroids; Tac* = standard triple therapy comprising tacrolimus, MPA and steroids; mTOR* = standard triple therapy comprising mTOR-inhibitor, MPA and steroids. TCMR: acute T-cell mediated rejection, AMR: Antibody mediated rejection, Borderline TCMR: T-cell driven borderline rejection, non-rejection: non-rejection proven by biopsy.

	Non-rejection	TCMR	Borderline TCMR	AMR
Females	18/52	4/13	16/31	4/16
Age	48.0 ± 14.9	54.5 ± 18.5	52.8 ± 14.3	49.0 ± 12.2
Transplant age (months)	25.4 ± 55.7	11.5 ± 20.7	23.2 ± 55.6	64.3 ± 75.7
Delayed graft function	6/52	4/13	5/31	1/16
Immunosuppressive drug regimen	19 CyA*, 33 Tac*	5 CyA*, 8 Tac*	12 Tac*, 12 Cya*, 2 Cya /MPA, 2 mTOR/MPA, 2 mTOR*, 1 CyA/Aza	3 CyA*, 7 Tac*, 2 Tac / steroids, 2 Cya / steroids, 1 Cya mono, 1 steroids mono
Living donations	22/52	2/13	10/31	3/16
Previous rejections	16/52	3/13	17/31	8/16
Re-graft	9/52	0/13	5/31	4/16

Table S4: Characteristics of the 2nd patient cohort categorized according to rejection type (including 11 children younger than 12 years). CyA* = standard triple therapy comprising cyclosporine, mycophenolate and steroids; Tac* = standard triple therapy comprising tacrolimus, MPA and steroids; mTOR* = standard triple therapy comprising mTOR-inhibitor, MPA and steroids, all other regimens are indicated. TCMR: acute T-cell mediated rejection, AMR: Antibody mediated rejection, Boderline TCMR: T-cell driven borderline rejection, non-rejection: non-rejection proven by biopsy.

	Non-rejection	TCMR	Borderline TCMR	AMR
Females	22/40	3/7	4/7	6/17
Age	59 ± 2.8	43.45 ± 20.3	40.4 ± 23.1	38.9 ± 22.8
Transplant age (months)	110 ± 110.3	94 ± 98.7	71.2 ± 100.6	130.1 ± 103.9
Delayed graft function	3/40	no	no	no
Immunosuppressive drug regimen	25 Tac*, 9 mTOR*, 3 CyA / MPA, 3 CyA-Aza-steroids	4 Tac*, 2 mTOR*, 1 CyA*/ MPA	2 Tac*, 3 mTOR*, 2 mTOR /steroids	10 Tac*, 2 mTOR*, 2 CyA-Aza-steroids, 2 CyA / MPA
Living donations	8	4	2	2
Previous rejections	no	no	no	6
Re-graft	no	no	no	3

Table S5: Characteristics of the 3rd patient cohort; again categorized according to rejection type. CyA* = standard triple therapy comprising cyclosporine, MPA and steroids; Tac* = standard triple therapy comprising tacrolimus, MPA and steroids; mTOR* = standard triple therapy comprising mTOR-inhibitor, MPA and steroids, all other regimens are indicated. TCMR: acute T-cell mediated rejection, AMR: Antibody mediated rejection, Boderline TCMR: T-cell driven borderline rejection, non-rejection proven by biopsy.

	Non-rejection	TCMR	Borderline TCMR
Females	11/26	14/35	2/3
Age	50.4 ± 16.2	51.1 ± 14	16.3 ± 16.4
Transplant age (months)	74.9 ± 279.9	22.6 ± 54.2	14.2 ± 8.7
Delayed graft function	7/26	7/35	0/0
Immunosuppressive drug regimen	4 CyA*, 19 Tac*, 1 mTOR* 2 Tac/mTOR/steroids	16 CyA*, 13 Tac*, 2 mTOR*, 2 MPA / steroids, 1 Tac/mTOR	1 CyA* 2 mTOR*
Living donations	7/26	8/35	2/3
Previous rejections	10/26	13/35	1/3
Re-graft	4/26	4/35	0/3

Table S6: Characteristics of patient samples used for LFAs; categorized according to rejection type. CyA* = standard triple therapy comprising cyclosporine, MPA and steroids; Tac* = standard triple therapy comprising tacrolimus, MPA and steroids; mTOR* = standard triple therapy comprising mTOR-inhibitor, MPA and steroids, all other regimens are indicated. TCMR: acute T-cell mediated rejection, AMR: Antibody mediated rejection, Boderline TCMR: T-cell driven borderline rejection, non-rejection: non-rejection proven by biopsy.

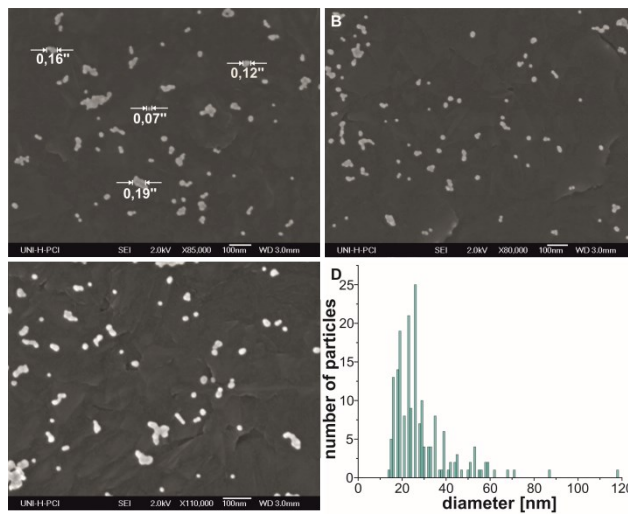|	Non-rejection	TCMR	Borderline TCMR	Mixed AMR/TCMR, mixed AMR/Borderline TCMR
Females	7/13	14/35	2/3	1/2
From 2nd cohort	3/13	4/35	2/3	2/2
From 3rd cohort	10/13	31/35	1/3	0/2
Age	54.9 ± 14.4	51.1 ± 13.9	16.3 ± 16.3	12.5 ± 0.7
Transplant age (months)	33.9 ± 64.8	22.6 ± 54.2	14.2 ± 14.2	34.4 ± 10.3
Delayed graft function	2/13	7/35	0/3	1/2
Immunosuppressive drug regimen	1 CyA*, 10 Tac*, 1 mTOR* 1 Tac/mTOR / steroids	16 CyA*, 13 Tac*, 2 mTOR* 2 MPA / steroids 1 Tac/mTOR/MPA, 1 CyA / steroids	1 CyA*, 2 mTOR*	2 mTOR*
Living donations	4/13	8/35	2/3	0/2
Previous rejections	7/13	13/35	1/3	1/2
Re-graft	2/13	4/35	0/3	0/2
eGFR [mL·min^{-1}]	37 ± 15.9	39.1 ± 14.9	57.9 ± 13.1	88.6 ± 46.2

Figures

Figure S1: Scanning electron microscope of gold nanoparticles (AuNPs). A), B) and C) Three exemplary scanning electron microscope images of AuNPs. In A four exemplary dimensioning markers and a corresponding scale [nm] are shown. D) Distribution of AuNP diameter [nm] determined from these SEM pictures (A, B, C, with 186 gold nanoparticles).
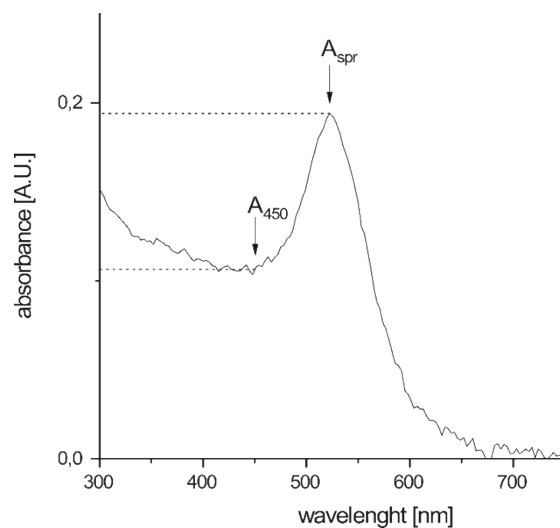

Figure S2: UV-Vis spectra of gold nanoparticles used for conjugation with detection antibody. The size of gold nanoparticles can be measured according to the ratio of the absorbance of AuNP at the surface plasma resonance peak (Aspr) to the absorbance at 450 nm (A450), as indicated by arrows.
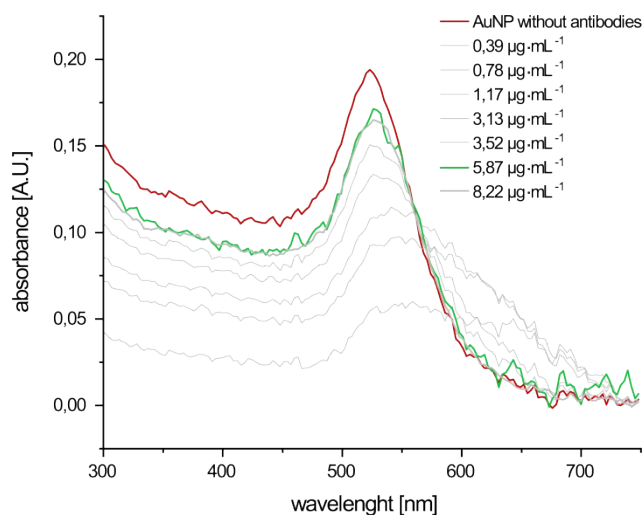
Figure S3: UV-Vis spectra of different detection antibody concentrations conjugated to AuNPs: The red curve shows AuNPs without addition of antibodies, the green curve shows the optimal antibody concentration to cover the AuNPs to create stable conjugates and the other curves show AuNPs conjugated with the antibody concentration indicated.
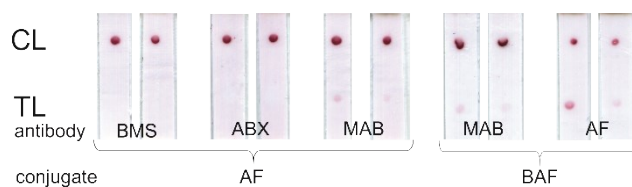

Figure S4: Prescreening to determine a usable antibody pair. In this pre-testing for the spots in the area of the test and control line 0.3 µL antibody (1 mg*mL^{-1}) was applied. Three different monoclonal antibodies (BMS: BMS134, eBioscience, Vienna, Austria; abx015891, abbexa, Cambridge, UK; MAB: MAB623-100, Bio Techne, Wiesbaden, Germany) and one polyclonal antibody (AF: AF-223-NA, Bio Techne, Wiesbaden, Germany) were tested as capture antibodies on the test line. As detection antibody two polyclonal antibodies (AF-223-NA and BAF: BAF223, Bio Techne, Wiesbaden, Germany) were tested. On the control line an antibody against goat was used (31105, ThermoFisher, Rockford, USA). S1). For each pairing, strips were run with 100 µL of 250 pM (left strip) und 50 pM (right strip) sIL-2R.
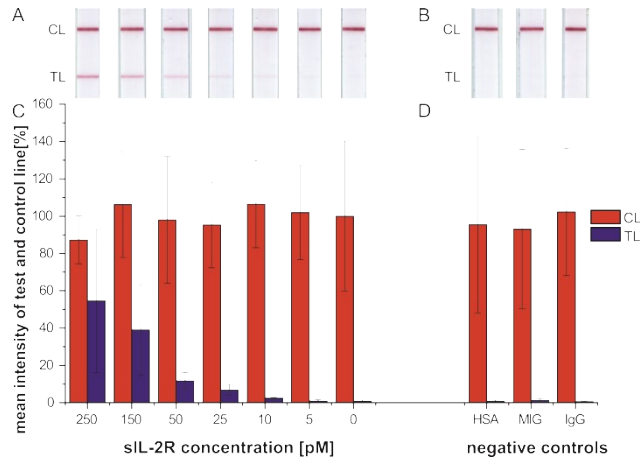
Figure S5: Different LFAs run with various concentrations of sIL-2R and control protein in running buffer without pre-incubation of the analyte or negative control samples with the AuNP-labeled detection antibody. The scans were taken 60 minutes after the sample was applied. A) Lateral Flow Assay using concentrations between 5 and 250 pM of sIL-2R in running buffer. B) Lateral Flow Assay using different negative controls (HSA=human serum albumin, 750 µM; MIG=Monokine induced by Gamma-Interferon, 145 pM; IgG: Immunglobulin G, 67 mM). C) & D) Corresponding diagrams of intensity of test and control line of A and B to compare the different strips with each other, the strips were evaluated with ImageJ and the mean of the control line of 0 pM sIL-2R was set to 100%. Red bars represent the control line (CL) and blue bars represent the test line (TL). N=3; mean ± SD.
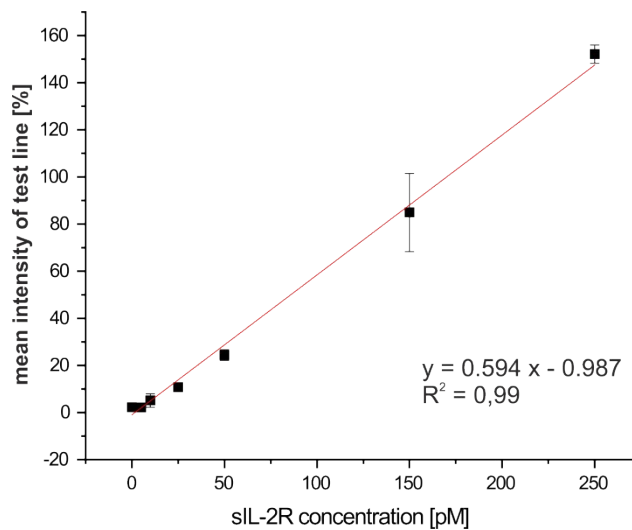

$y = 0.594 \ x - 0.987$
$R^2 = 0{,}99$

Figure S6: Proportional correlation of the spiked concentration and the corresponding intensity of the test line. N=3 ± SD.
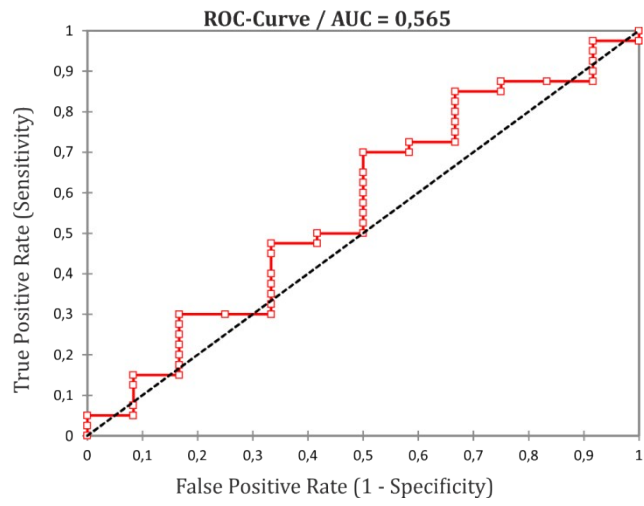
Figure S7: Receiver operating characteristics (ROC) curve of eGFR in kidney transplanted patients with either biopsy proven TCMR or non-rejection (AUC = 0.565).