Renal Function and Regulatory T-cell assessment in Kidney Transplanted Patients Receiving Cyclosporine A versus Sirolimus after 5 years

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Introduction

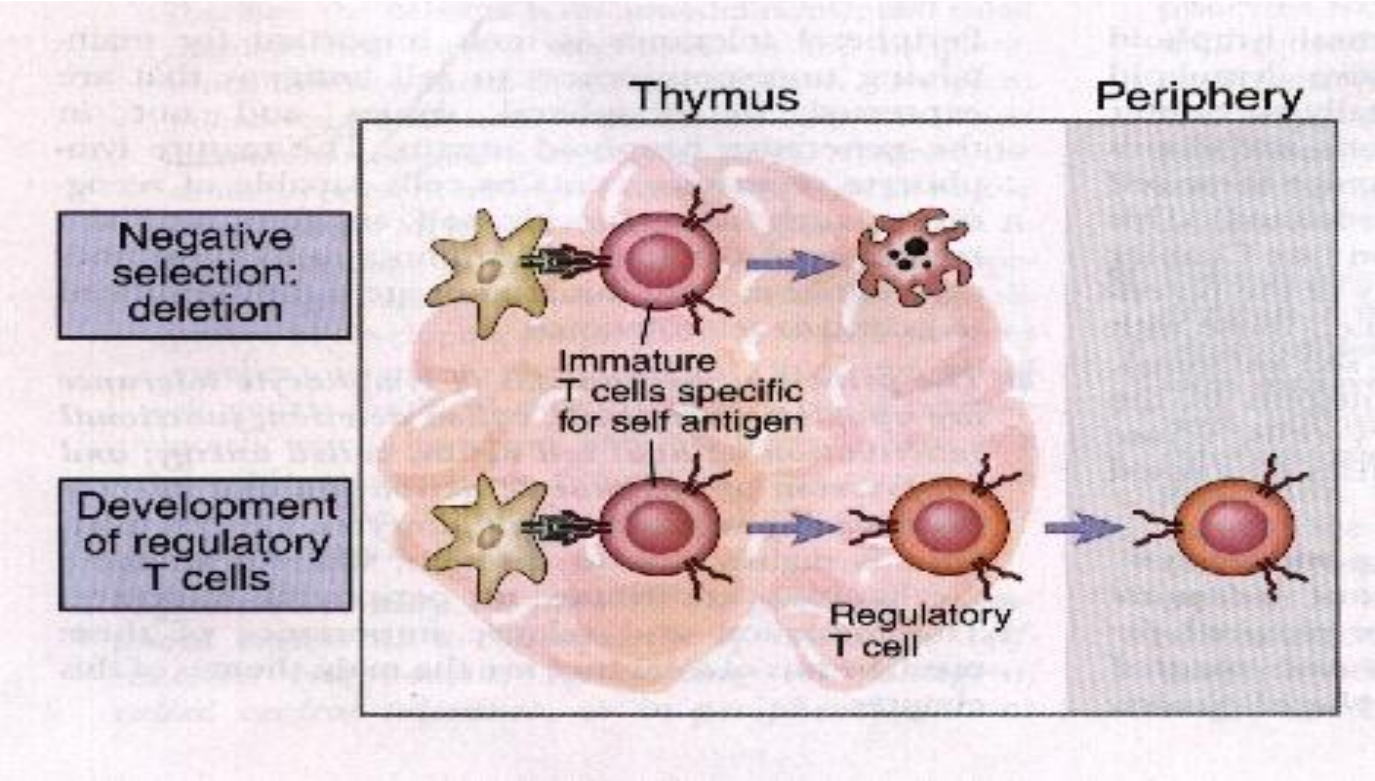
- Current routine maintenance protocol in kidney Tx i.e. CNIs + MMF+ steroids has greatly been able to improve ***one-year survival*** in most centers and to decrease the AR rate.
- But it remains some unsolved problems including:
  - ***chronic rejection***
  - impaired renal function
  - CAN, all caused at least partly by CNIs in such protocol.
- One way to decrease such destructive effects is to replace them with ***mTOR*** inhibitors which are:
  - immunosuppressor
  - not nephrotoxic
  - and in comparison to CNIs, they cause better function and lower tissue chronicity.

Introduction, con.

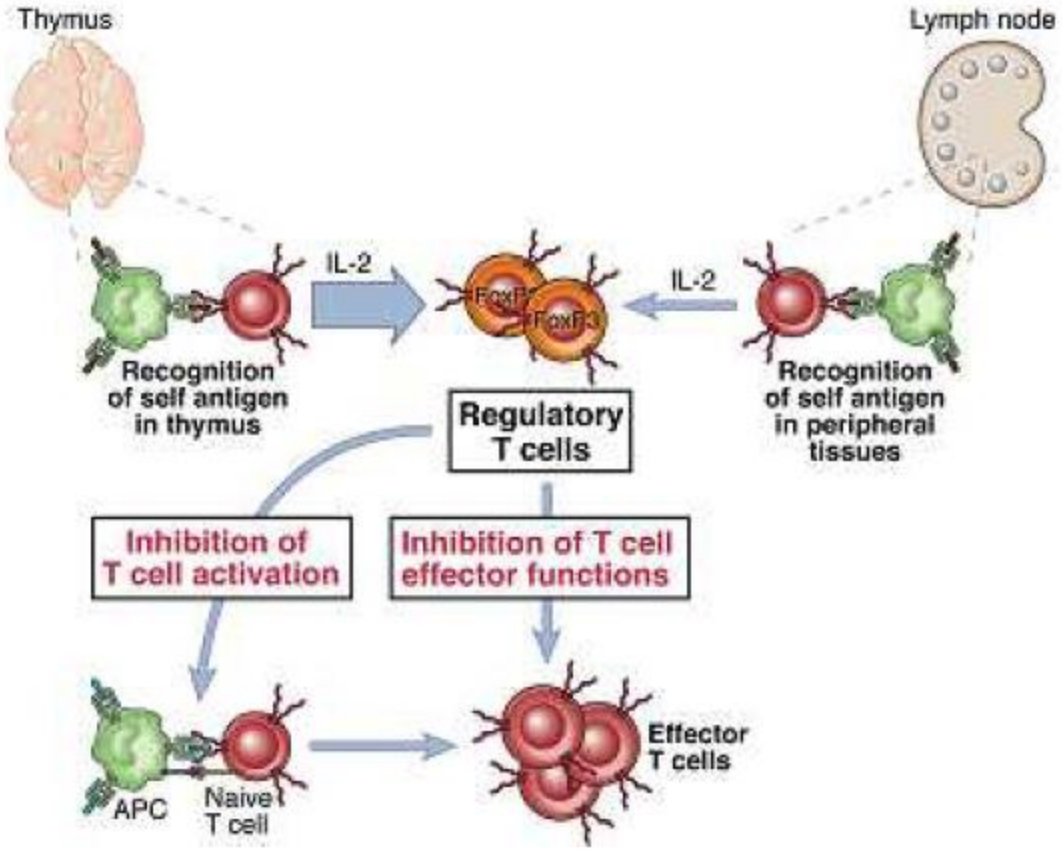
- Such replacement could be beneficial to
  - to preserve the graft function
  - improve CAN grading
  - ***lower incidence of SRL*** side effects such as surgical wound healing delay
  - utilize the protective effects of CNIs on AR
  - ***Increase the numbers and effects of Tregs in prevention of rejection*** processes and tolerance induction.
- This study was conducted to examine such benefits from the point of kidney function and Treg numbers 5 years after kidney Tx.

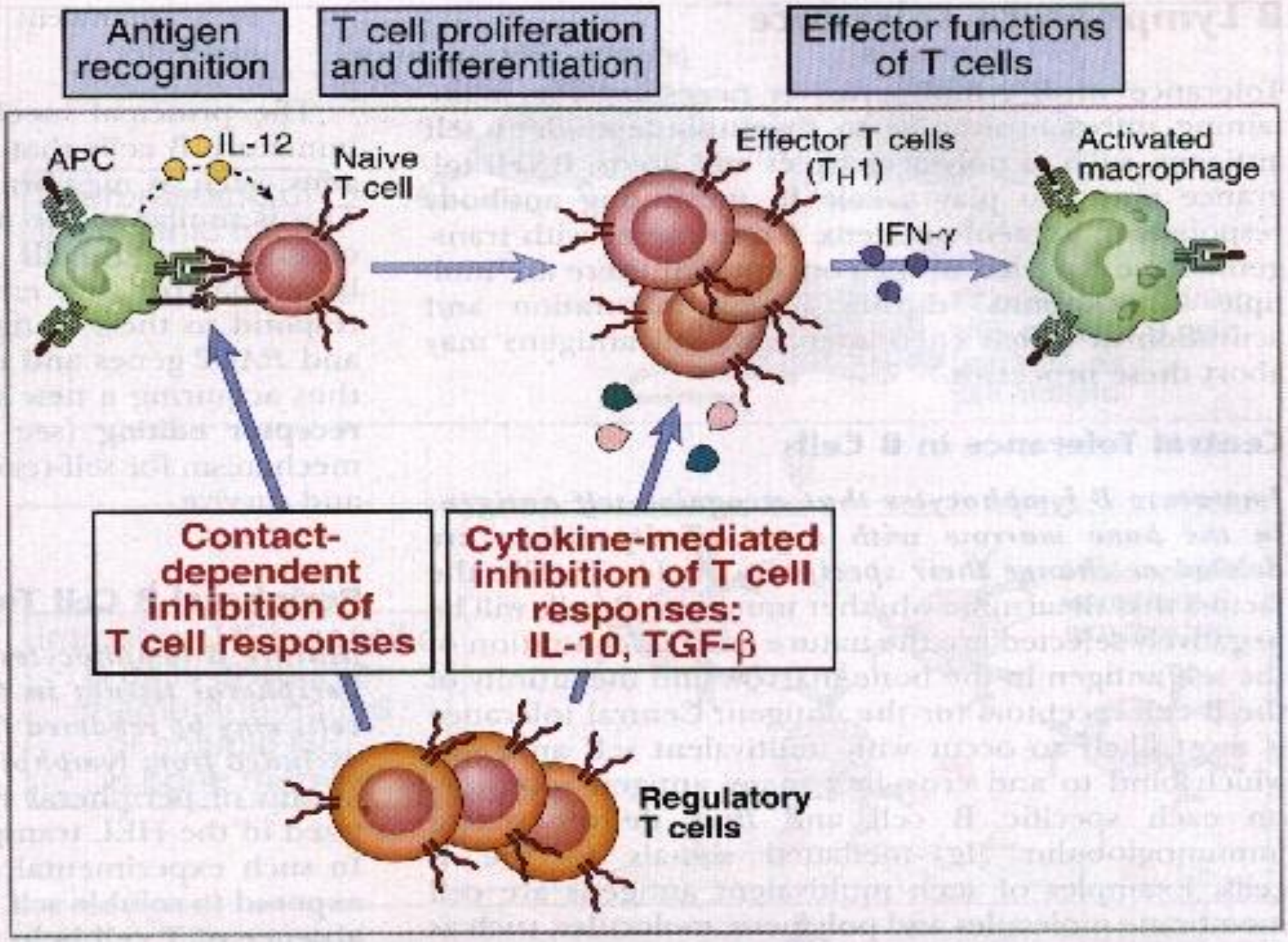
Naturally Occurring Tregs

- **CD4+CD25+FoxP3+ Tregs**
- **CD8+CD25+CD28-FoxP3+ Tregs**
  - Develop in the thymus via a high affinity mode
  - 5-10% of peripheral T cells
  - Constitutive expression of CD4/CD8, FoxP3, CD25hi



Regulatory T cells




Antigen recognition
T cell proliferation and differentiation
Effector functions of T cells
APC
IL-12
Naive T cell
Effector T cells (T_H1)
IFN-γ
Activated macrophage
Contact-dependent inhibition of T cell responses
Cytokine-mediated inhibition of T cell responses: IL-10, TGF-β
Regulatory T cells

Materials and Methods

- **Patients:** 88 adult primary kidney recipients from living donors
- **Exclusion criteria:**
  - prior exposure to the immunosuppressants
  - BPAR/ treatment for AR
  - proteinuria ≥750 mg/day in 24-hours urine collection
  - GFR<50%
  - recent malignancy or treatment for cancer
  - Pregnancy
  - active illness such as MI as well as HBV, HCV, and HIV infection
  - any events leading to hospitalization
  - BMI greater than 35kg/m2
  - WBC<3,000/mm3, HB<8g/dl, platelets <100,000/mm3

Materials and Methods, con.

- **Study design:** recipients receiving clinically adjusted doses of MMF plus steroids were randomized to remain on CsA (*n=59*) or to switch to SRL (*n=29*) ***after early phase of 3-6 months post-transplant.***

Before conversion, none of the subjects demonstrated

- CAN
- Contraindication to conversion, including:
  - a creatinine variation >30%
  - proteinuria ≥750 mg/24 h
  - mean MMF daily dose <1.5 g
  - total cholesterol ≥9 mmol/L and/or triglycerides ≥6 mmol/L despite lipid-lowering treatment
  - tendency to be remained fertile
  - non-repaired wound
  - recent surgical procedures
  - acute illnesses such as recent infections

Materials and Methods, con.

- **Clinical parameters:**
  - The ***SRL dose*** consisted of a loading dose of 6 mg in a day followed by a maintenance dose of 2 mg daily.
  - The average dose of CsA considered 1.7 mg/kg/day provided that we achieve a therapeutic trough and C2 level with no serious side effects of CsA including hyperchloremic acidosis, hyperkalemia, proteinuria, hypertrichosis and vigorous tremor.
  - Tregs and GFR were counted before drug conversion and at year 5 after transplantation in all patients

Before to be continued...

- Our study had some advantages:
  - relatively proper sample
  - there were no essential factors affecting the comparability of the groups including
    - eventful unstable graft
    - differences in time from the point of Tx
    - length of treatment
    - using different kinds of immunosuppression
  - serial samples were performed

Results

Table 1: Baseline and clinical characteristics of the patients before conversion

	Drug group Variable	Cyclosporine A	Sirolimus	P value
Demographic characteristics	Age	41.93±13.78	46.72±14.24	0.33
	Male	32 (54.2%)	24 (82.8%)	0.009
	Female	27 (45.8%)	5 (17.2%)	
	Weight	58.67±11	54.4 ± 11	0.11
	HLA mismatch	4.75±1.12	4.27±1.01	0.15
Cause of renal failure	Glomerulonephritis (29.5%)	17	9	N.S
	Diabetes (27.5%)	15	9	
	Blood pressure (15.9%)	9	5	
	Alport syndrome (10.2%)	6	3	
	Urinary tract infection (9.1%)	6	2	
	Reflux, ADPKD, Lupus (each one: 1 patient, 3.4%)	3	-	
	Unknown	3	1	

Table 2: Clinical characteristics of the patients after conversion

	Drug group Variable	Cyclosporine A	Sirolimus	P value
Clinical complications	PTDM	19 (32.2%)	0	0.001
	Respiratory infection	13 (22%)	6 (20.6%)	0.98
	UTI	39 (66%)	19 (65.5%)	0.792
	Acute rejection	10 (17%)	2 (6.8%)	0.33
Biochemistry	LDL	120.17±40.1	159.9±55.35	0.001
	Tch	242.9±45.9	279.7±51.9	0.005
	ALT	40.01±22.75	43.85±34.37	0.414
	AST	32.11±14.45	29.07±12.73	0.353
	K^+	4.43±0.48	4.2±0.46	0.01
	Cr	2.16±1.46	1.45±1.26	<0.001
Cell counts	WBC	8700±2900	6300±2200	0.001
	Platelets	200000±65000	170000±55000	0.025
	Hb	12.65±1.52	11.8±2.1	0.047
Viral infection	CMV	15 (25.4%)	10 (34.4%)	0.299
	BK	2 (3.3%)	3 (10.3%)	0.182

Table 3: Values of GFR and Tregs in all patients and drug groups at 2 different time points

Group		At conversion time	2 years post-Tx	P-value
All patients	GFR	50.8±17	48.7±19.6	0.37
	$CD4^+CD25^+FoxP3^+$	1.16±0.7	1.86±0.72	<0.001
	$CD8^+CD28^+$	0.58±0.35	0.9±0.5	<0.001
Cyclosporine A	GFR	51±15	44.5±18.4	0.002
	$CD4^+CD25^+FoxP3^+$	1.07±0.59	1.7±0.49	<0.001
	$CD8^+CD28^+$	0.61±0.37	0.78±0.46	0.028
Sirolimus	GFR	50.5±21	58.2±19.2	0.19
	$CD4^+CD25^+FoxP3^+$	1.59±0.66	2.18±0.95	0.018
	$CD8^+CD28^+$	0.56±0.32	1.1±0.52	<0.001

Table 4: linear multivariate regression analysis evaluating the effect of different parameters on $CD4^{-}CD25^{-}FoxP3^{-}$ Tregs between the drug groups

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	P value
		B	Std. Error	Beta		
1	(Constant)	0.646	0.425		1.520	0.134
	Drug	0.068	0.234	0.039	0.292	**0.771**
	GFR change	-0.004	0.005	-0.114	-0.882	0.381
	BK	0.619	0.578	0.149	1.070	0.289
	Respiratory infection	-0.285	0.258	-0.139	-1.104	0.274
	CMV	-0.024	0.271	-0.012	-0.089	0.930
	UTI	-0.113	0.222	-0.065	-0.510	0.612
	Rejection	-0.363	0.338	-0.137	-1.075	0.286

a. Dependent Variable: $CD4^{-}CD25^{-}FoxP3^{-}$changes

Table 5: linear multivariate regression analysis evaluating the effect of different parameters on $CD8^{-}CD28^{-}$ Tregs between the drug groups

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	P value
		B	Std. Error	Beta		
1	(Constant)	1.003	0.269		3.724	0.000
	Drug	-0.418	0.148	-0.360	-2.820	**0.006**
	GFR change	-0.003	0.003	-0.105	-0.837	0.406
	BK	-0.314	0.366	-0.115	-0.858	0.394
	Respiratory infection	-0.063	0.163	-0.047	-0.385	0.702
	CMV	0.036	0.172	0.028	0.209	0.835
	UTI	-0.005	0.140	-0.004	-0.034	0.973
	Rejection	0.038	0.214	0.022	0.179	0.858

a. Dependent Variable: $CD8^{-}CD28^{-}$ changes

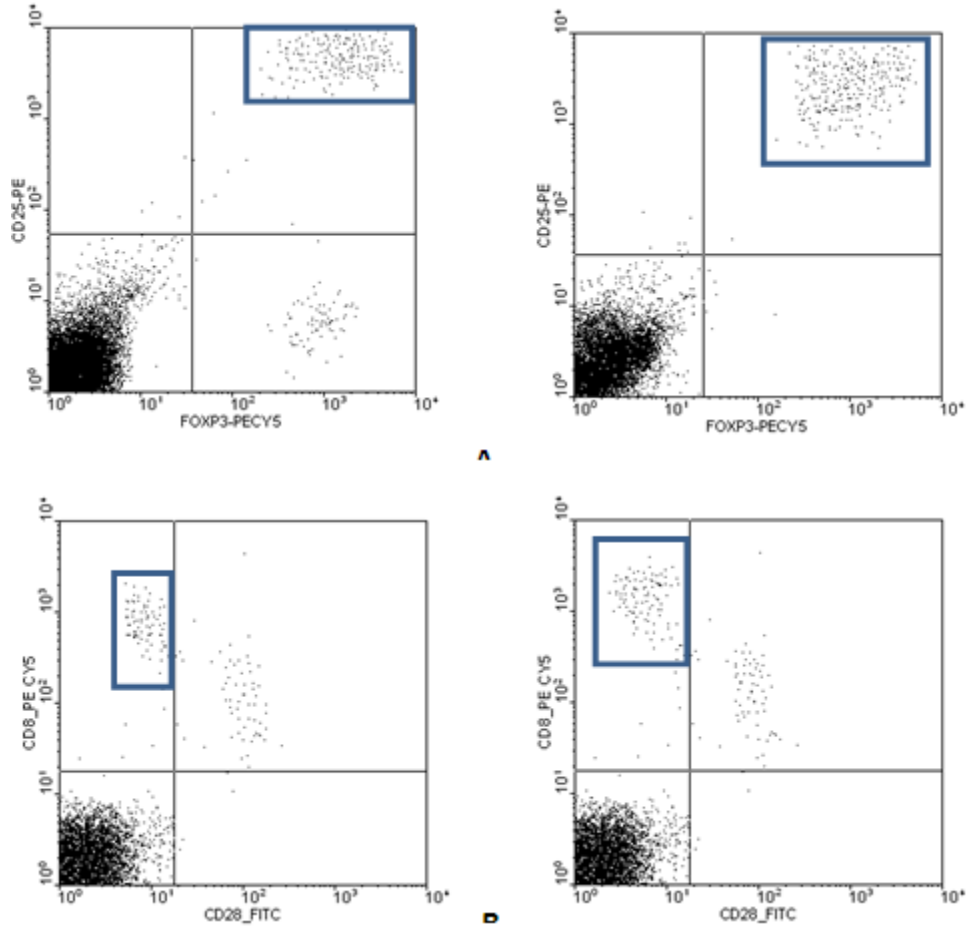
Table 6: linear multivariate regression analysis evaluating the effect of different parameters on GFR between the drug groups

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	P value
		B	Std. Error	Beta		
1	(Constant)	24.391	9.620		2.535	0.013
	Drug	-14.692	5.123	-0.311	-2.868	**0.005**
	BK	-6.139	11.425	-0.065	-0.537	0.593
	Respiratory infection	0.959	5.547	0.019	0.173	0.863
	CMV	-1.567	5.618	-0.032	-0.279	0.781
	UTI	-1.238	5.006	-0.027	-0.247	0.805
	Rejection	-1.686	6.954	-0.027	-0.242	0.809

a. Dependent Variable: GFR changes

Flowcytometry analysis of A) $CD4^+CD25^+Foxp3^+$Tregs after gating on $CD4^+$ cells and B) $CD8^+CD28^-$Tregs after gating on $CD3^+$ cells in a sample patient of Cyclosporine A group. Left: at the time of conversion; Right: 2 years post-transplantation



Conclusion

- In the long run, if it be tolerated by the patient, the CNI may be replaced by an mTOR inhibitor to reduce nephrotoxicity and to help the graft tolerance.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