Clinical validation of a novel ELISpot-based *in vitro* diagnostic assay to monitor CMV-specific cell-mediated immunity in kidney transplant recipients

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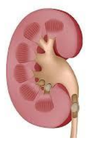
BACKGROUND & OBJECTIVE

Impairment of cytomegalovirus (CMV)-specific cell-mediated immunity (CMV-CMI) by immunosuppressive therapy is a major cause of CMV reactivation and associated complications in solid-organ transplantation. Assessing the functional impairment of CMV-CMI may help to individually adjust immunosuppressive and antiviral therapy. The aim of this study was to evaluate the suitability of T-Track® CMV, a novel immune-monitoring assay, to survey CMV-CMI in kidney transplantation (Tx) patients.

STUDY DESIGN



A prospective longitudinal observational multicenter study was conducted in a cohort of 96 intermediate risk (D-/R+, D+R+) renal transplant recipients over 6 months post-Tx. Patients received standard immunosuppressive (IS) therapy and underwent pre-emptive antiviral treatment following institutional guidelines. CMV-CMI was monitored using T-Track® CMV, a novel IFN-γ ELISpot assay quantifying CMV-reactive immune cells (Th, CTL, NK, NKT-like) in response to T-activated® IE-1 and pp65 CMV proteins. CMV viral load (qPCR or pp65 antigenemia), CMV-related complications, opportunistic infections and graft function were also monitored.

RESULTS

1. Patient characteristics

Full Analysis Set (FAS), N (%)	**96 (100%)**
Gender, N (%)	
Male	64 (66.67%)
Female	32 (33.33%)
Age in years, mean ±SD (range)	53.4 ±13.4 (20-78)
CMV serostatus, N (%)	
D+/R+	41 (42.71%)
D-/R+	50 (52.08%)
D?/R+	5 (5.21%)
Induction therapy (Basiliximab), N (%)	
Yes	57 (59.38%)
No (or not documented)	39 (40.62%)
Immunosuppressive regimen, N (%)	
including CNI, MMF/MPA, mTOR inhibitor, steroid	90 (93.75%)
Not documented	6 (6.25%)
Patients with any visit-related data, N (%)	**86 (89.58%)**
Patients with end-organ CMV disease, N (%)	0 (0.00%)
Patients with CMV syndrome, N (%)	4 (4.17%)
Patients with at least one recorded CMV event[1], N (%)	28 (29.17%)
Time to onset of CMV event[1] in days, median (range)	48 (14-145)
Patients with at least one recorded CMV viral load (VL > 0), N (%)	49 (51.04%)
Time to onset of first CMV VL in days, median (range)	41 (14-145)
Patients with infections other than CMV, N (%)	
Any infection	38 (39.58%)
BKV	9 (9.37%)
Urinary tract infection	19 (19.79%)
Bacteria	10 (10.42%)
Fungi	2 (2.08%)
Other	5 (5.21%)
Delayed graft function	10 (10.42%)
Graft rejection (Banff '09)	13 (13.54%)
T cell-mediated rejection (TCMR)	8* (8.33%)
Borderline changes (suspicious for TCMR)	4* (4.17%)
Antibody-mediated rejection (ABMR)	1 (1.04%)
Unknown	1 (1.04%)
Time to onset of graft rejection in days, median (range)	34.5 (3-140)
Graft loss	1 (1.04%)

[1]defined as CMV viral load requiring treatment (or 'CMV complications'), as per investigator's assessment; *One patient experienced 3 rejection episodes (1 TCMR and 2 Borderline changes), thus contributing to both categories; Abbreviations: CIN, calcineurin inhibitor; D/R, donor/recipient; MMF/MPA, mycophenolate mofetil/mycophenolic acid.

2. T-Track® CMV can measure CMV-CMI before and after kidney transplantation

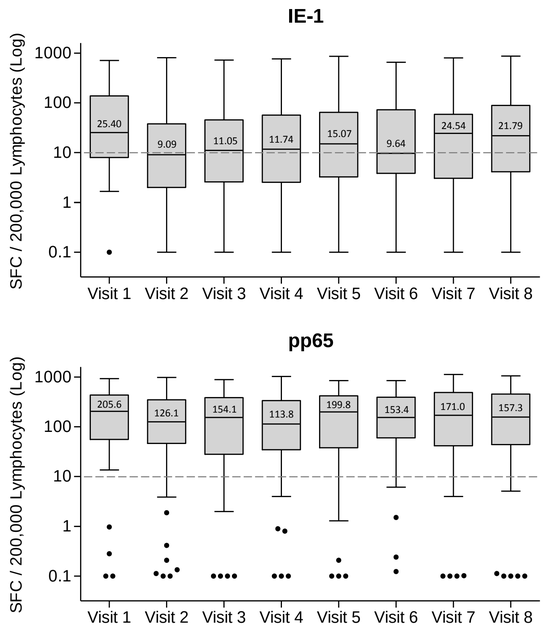


Figure 1. CMV-CMI in renal transplant recipients measured with T-Track® CMV before (Visit 1) and after (Visits 2 to 8) transplantation and start of immunosuppressive regimen.
PBMC were stimulated with T-activated® IE-1 and pp65 CMV antigens and ELISpot were conducted as recommended by manu-facturer.
The dashed line indicates the cut-off of T-Track® CMV positivity (10 spot-forming cells or SFC / 200,000 lymphocytes).

3. T-Track® CMV is a highly sensitive immune-monitoring assay pre- & post-Tx

Visit number	Time to transplantation in days (median)	IE-1-SFC	pp65-SFC	T-Track® CMV	
1	-3 to 0 (-1)	67.6	89.2	**94.6**	Pre-transplantation (prior to IS treatment)
2	13 to 30 (20)	50.0	89.2	**90.7**	
3	21 to 51 (41)	55.9	85.0	**90.2**	
4	42 to 76 (63)	53.0	85.9	**88.1**	
5	63 to 113 (85)	59.4	90.5	**92.2**	Post-transplantation (under IS treatment)
6	89 to 120 (109)	49.1	89.5	**91.4**	
7	102 to 147 (127)	63.6	87.0	**89.1**	
8	126 to 172 (153)	61.5	84.9	**90.6**	

Table 1. T-Track® CMV positive test results per visit (%). According to manufacturer's instructions, the T-Track® CMV test is positive when at least one of T-activated® IE-1 (IE-1-SFC) or/and pp65 (pp65-SFC) test result is positive. IS, immunosuppressive

4. T-Track® CMV can monitor patients' immunosuppressive state

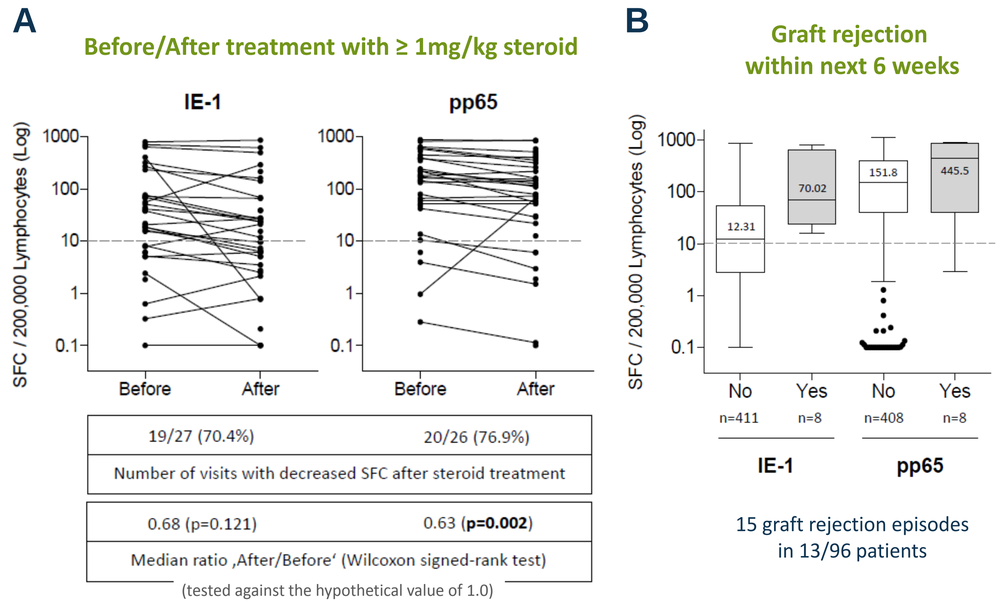


Figure 2. CMV-CMI is reduced following high-dose steroid treatment (A) and increased in association with graft rejection (B). (**A**) IE-1- and pp65-specific ELISpot results at any visits before and after high-dose steroid treatment (N = number of paired measurements. (**B**) IE-1- and pp65-specific CMI at post-transplantation visits (V2-8) not affected by graft rejection with respect to occurrence (Yes vs. No) of graft rejection within the next 6 weeks. Median IE-1- and pp65-specific CMI is 5.7-fold and 2.9-fold higher in case of future graft rejection.

5. T-activated® pp65-specific CMI is a potential immunocompetence marker

Visit preceding first occurence of viral load (VL) among patients with self-limiting VL ('no CMV event') vs. antivirally-treated patients ('CMV event')

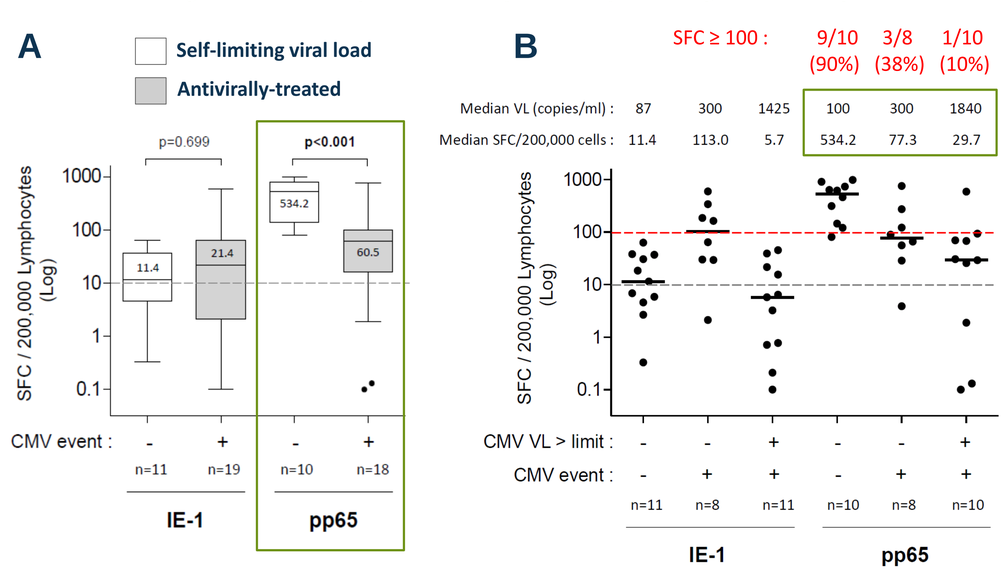


Figure 3. pp65-CMI is increased prior to first detection of viral load (p<0.001) (post hoc analysis; n=79 patients). (**A**) IE-1- and pp65-specific CMI at post-Tx visit preceding first detection of VL in untreated patients ('self-limiting VL') vs. antivirally-treated patients ('CMV event' definition). Groups were compared using a MWU test. Median pp65-specific CMI was 8.8-times higher in patients with self-limiting VL at the visit preceding first occurrence of VL (p<0.001). (**B**) Additionally, median pp65-specific SFC inversely correlated with VL levels (< or > institutional limit).

CONCLUSION

T-Track® CMV is a highly sensitive immune-monitoring tool, enabling the functional assessment of CMV-specific CMI following kidney Tx. In combination with viral load measurement, T-Track® CMV might help identify patients potentially at increased risk for CMV-related complications.

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