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O0381 Clinical validation of a novel ELISpot-based *in vitro* diagnostic assay to monitor cytomegalovirus-specific cell-mediated immunity in immunocompromised transplant recipients

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Background: Impaired cytomegalovirus (CMV)-specific cell-mediated immunity (CMV-CMI) is a major cause of uncontrolled CMV reactivation and associated complications in both solid-organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT). Reliably assessing CMV-CMI is desirable to individually adjust antiviral and immunosuppressive therapy. We demonstrate here the suitability of a novel IFN-γ ELISpot assay (T-Track® CMV), based on the stimulation of peripheral blood mononuclear cells with pp65 and IE-1 CMV proteins, to monitor CMV-CMI in immunocompromised SOT and HSCT patients.

Materials/methods: Two independent prospective, longitudinal, observational, multicenter studies were conducted, the first one in 86 intermediate-risk (D-/R+, D+/R+) renal transplant recipients (ClinicalTrials.gov ID: NCT02083042), the second one in 154 intermediate- or high-risk (D+/R+, D+/R-, D-/R+) HSCT recipients (ClinicalTrials.gov ID: NCT02156479). In both studies, patients underwent pre-emptive antiviral therapy per institutional guidelines. CMV-CMI, CMV viral load and clinical complications were monitored over approximately six months post-transplantation.

Results: In the kidney transplantation setting, CMV-specific response was reduced following immunosuppressive treatment and increased in patients with graft rejection, indicating the ability of the ELISpot assay to monitor the patients' immunosuppressive state. Interestingly, median pp65-specific response was 9-fold higher in patients with self-clearing viral load compared to antivirally-treated patients prior to first detection of viral load ($p<0.001$), suggesting that reactivity to pp65 represents a potential immunocompetence marker. In HSCT recipients, out of 74

patients (24 D+/R+, 3 D+/R-, 47 D-/R+) who experienced a first CMV reactivation, 30 (41%) faced a recurrent CMV reactivation during the observational period. Interestingly, 41/44 patients free of recurrent reactivation had a positive ELISpot test result after resolution of the first CMV reactivation, resulting in a 93% specificity in diagnostic accuracy. Accordingly, a time-to-event analysis indicated a significantly lower incidence of recurrent CMV reactivation in patients with a positive test result following the primary CMV reactivation (Figure 1; Hazard ratio=5.68; Log-Rank Test, $p<0.001$).

Conclusions: Altogether, this novel IFN-γ ELISpot assay (T-Track® CMV) is a highly sensitive immune-monitoring tool, suitable for the follow-up of SOT and HSCT recipients, and with a potential use for the risk assessment of CMV-related clinical complications.

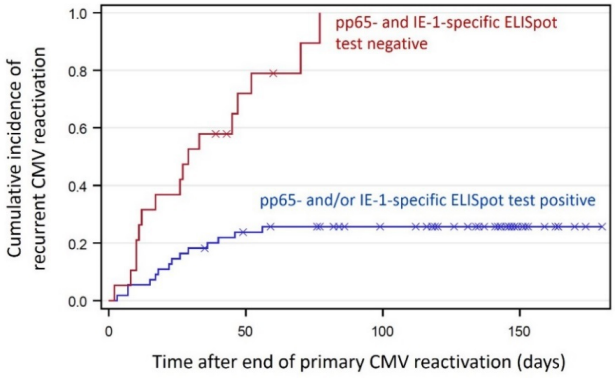

Figure 1. Kaplan-Meier analysis of incidence of recurrent CMV reactivation following resolution of a primary CMV reactivation. IFN-γ ELISpot (T-Track® CMV) assays were conducted between 0 and 17 days after the end of a primary CMV reactivation. Cumulative incidence of at least one further CMV reactivation is plotted in patients with a positive (N=55) and a negative (N=19) T-Track® CMV test result. A minimum 30-day observational period was considered for patients with no recurrent CMV reactivation. x / x indicate censored observations.