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O0414 Technical and clinical validation of a novel, highly sensitive IFN-gamma ELISpot assay to monitor CMV-specific cell-mediated immunity in immunocompromised patients

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Background: Impairment of cytomegalovirus (CMV)-specific cell-mediated immunity (referred to as CMV-CMI) by immunosuppressive therapy is a major cause of CMV reactivation and associated complications in solid-organ and hematopoietic stem cell transplantation. Reliably monitoring CMV-CMI may therefore assist the prognosis of CMV-associated clinical complications and guide individual therapeutic decisions. Aim of this work was to establish an optimised and standardised CMV-specific IFN-γ ELISpot assay and determine its suitability to measure CMV-CMI in immunocompromised patients.

Materials/methods: T-activated® immunodominant IE-1 and pp65 CMV proteins were used as stimulatory antigens for *in vitro* restimulation of peripheral blood mononuclear cells (PBMC). All basic assay parameters and reagents were optimised to establish a user-friendly protocol and maximise the signal-to-noise ratio of the ELISpot assay. The technical performance of the optimised IFN-γ ELISpot assay (T-Track® CMV) was compared to that of QuantiFERON®-CMV and of a cocktail of 6 class I iTAg™ MHC Tetramers in 124 hemodialysis patients, representative of patients prior to kidney transplantation.

Results: The optimised IFN-γ ELISpot assay (T-Track® CMV) demonstrated low intra-assay, inter-assay and inter-operator variability (coefficient of variation CV < 22%). Assay linearity was demonstrated between 6×10^4 and 2×10^5 PBMC per well upon stimulation with T-activated® IE-1 ($R^2 = 0.97$) and pp65 ($R^2 = 0.99$) antigens. The novel T-Track® CMV assay allows the detection of a broad range of CMV-reactive effector cells (Th, CTL, NK, NKT-like cells), resulting in a sensitivity of 97% in a cohort of 45 healthy donors. Sensitivity of T-Track® CMV in CMV-seropositive hemodialysis patients was 90%, compared to 73% with QuantiFERON®-CMV and 77% with CMV-specific iTAg™ MHC tetramers.

Conclusions: T-Track® CMV is a highly sensitive IFN-γ ELISpot assay, suitable for the immune monitoring of immunocompromised patients and with a potential use for the risk assessment of CMV-related clinical complications.