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P0660 Validation of a highly sensitive interferon-gamma ELISpot assay to monitor cytomegalovirus-specific cell-mediated immunity in immunocompromised patients

Sascha Barabas[1], Theresa Spindler[1], Richard Kiener[2], Tamara Lugner[1], Julia Batzilla[1], Mathias Schemmerer[1,2], Josef Koestler[2], Hanna Bendfeldt[1], Anne Rascle*[1], Carsten A. Böger[3], Bernd Krueger[4], Bernhard Karl Krämer[4], Ralf Wagner[2,1], Bernhard Banas[3], Ludwig Deml[1]

[1] Lophius Biosciences, Regensburg, Germany, [2] Institute of Clinical Microbiology and Hygiene, University Medical Center Regensburg, Regensburg, Germany, [3] Department of Nephrology, University Medical Center Regensburg, Regensburg, Germany, [4] Vth Department of Medicine, UMM University Medical Center Mannheim/Heidelberg, Mannheim, Germany

Background: Impairment of cytomegalovirus (CMV)-specific cell-mediated immunity (CMV-CMI) by immunosuppressive therapy, in particular following solid-organ and hematopoietic stem cell transplantation, is a major cause of CMV reactivation and associated complications. Reliably monitoring CMV-CMI may therefore improve the risk stratification of patients and guide individual therapeutic decisions. To do so, a standardised CMV-specific IFN-γ ELISpot assay was established and optimised. Its suitability to reliably measure CMV-CMI was determined in healthy donors, in hemodialysis patients (representative of patients prior to kidney transplantation), and in kidney transplant recipients.

Materials/methods: T-activated® immunodominant IE-1 and pp65 CMV proteins were used as stimulatory antigens for *in vitro* stimulation of peripheral blood mononuclear cells (PBMC). 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. Technical sensitivity was evaluated in a cohort of 45 healthy donors, and in 86 CMV-seropositive kidney transplant recipients.

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), as shown by flow cytometry, resulting in a technical sensitivity (defined as positive agreement with CMV serology) of 97% in healthy donors. Technical 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. In kidney transplant recipients, technical sensitivity was 95% pre-transplantation and 88% to 92% over 6 months post-transplantation.

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.