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Title: Cytokine profiling of specific T cells in patients after BCG-therapy allows distinction between patients with primary infection and prior contact

Mrs. Julia 26247 Elsäßer julia.elsaesser@uks.eu MD 1,2, Dr. Martin 26248 Janssen martin.janssen@uks.eu MD 1,2, Dr. Urban 26249 Sester urban.sester@uks.eu MD 3, Dr. Frank 26250 Becker frank.becker@me.com MD 4, Dr. Henrik 26251 Suttmann henrik.suttmann@gmx.de MD 5, Dr. Carsten 26252 Ohlmann carsten.ohlmann@uks.eu MD 2, Kai 26256 Schmitt kai.schmitt@uks.eu MD 6, Prof. Dr Michael 26257 Stöckle michael.stoeckle@uks.eu MD 2 and Prof. Dr Martina 26270 Sester martina.sester@uks.eu 1. 1 Department of Transplant and Infection Immunology, Saarland University, Homburg, Germany, 66421 ; 2 Department of Urology, Saarland University, Homburg, Germany, 66421 ; 3 Department of Internal Medicine IV, Saarland University, Homburg, Germany, 66421 ; 4 Boxberg Centre, Urological Group and Clinic, Neunkirchen, Germany ; 5 Urologicum Hamburg, Urological Clinic, Hamburg, Germany and 6 Department of Pathology, Saarland University, Homburg, Germany, 66421 .

Body: Specific T cell immunity in active tuberculosis (TB) is associated with a decrease in multifunctionality. However, it is unknown whether cytokine profiles differ in patients with primary infection and those with prior contact. We therefore used BCG instillation therapy in patients with urothelial carcinoma as a model to characterise the induction of systemic immunity towards PPD and to study whether cytokine profiles differ depending on pre-existing immunity. IFNγ and IL2 producing PPD-specific CD4 T cells were analysed longitudinally before each instillation in 18 patients using flow-cytometry. 54 age-matched subjects without TB served as controls. Baseline levels of IFNγ producing PPD-specific T cells were comparable to controls. T cells showed a 5fold increase to 0.23% by week 2/3, and further increased 8fold by week 4/5 (to 0.42%, p<0.0001). Systemic immunity was induced in all patients, although the increase was less pronounced in patients with pre-existing immunity. As in active TB, cytokine profiling during therapy revealed a lower percentage of multifunctional IFN-γ/IL2 double-positive T-cells compared to controls (48.1% vs. 78.2%, p=0.0003). Of note, unlike in patients with pre-existing immunity, cytokine profiles in patients with primary immunity was shifted towards IL2 single producing T cells (35.9% vs. 13.4%, p=0.02). Decreased functionality is a typical feature of specific immunity in both patients with active TB and BCG therapy. Among patients with active infection, the percentage of IL2 single positive cells may allow distinction between patients with primary infection and cases with boosted immunity after prior contact.