

Transplantation and Clinical Virology



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Translational Research in Clinical Virology: From Bedside to Bench and Back to the Patients

"Transplantation and Clinical Virology" is interested in translational research of virus infections to improve clinical diagnosis, prevention, and treatment. This includes

- Respiratory viruses (RV);
- Human herpesviruses; and
- Human polyomaviruses in vulnerable populations (e.g. HIV/AIDS; transplantation; autoimmunity; inherited immunodeficiency).

We aim at characterizing 1) key determinants of virus pathology; 2) potential targets of antiviral intervention; 3) adaptive immune responses; 4) modifiable and non-modifiable risk factors in patients.

The focus on human polyomaviruses (HPyVs) serves as example: In the last decade, 12 new HPyVs have been identified by molecular methods in addition to BKPyV and JCPyV known since 1971. Currently, at least 5 HPyVs have been convincingly linked to diseases, all in immunocompromised patients. Importantly, no specific antivirals are available for the treatment of HPyV disease making immune reconstitution the main stay of any therapeutic approach today.

The polyomavirus non-coding control region (NCCR) harbors the origin of viral DNA genome replication and promoter/enhancers controlling the sequential bi-directional expression of PyV early and late gene expression. We reported that kidney transplant patients with persistent BKPyV viremia showed the emergence of viral variants with rearranged NCCR. We demonstrated that the emerging r-NCCR caused an activated early viral gene expression, higher viral loads, and replication rates (replication capacity) *in vitro*, and more advanced disease in patients. A similar dynamic change of the NCCR was seen in JCPyV of HIV patients with PML linking activated early viral gene expression to replication and pathology. Importantly, non-rearranged JCPyV NCCR is activated by HIV1 explaining the high number of PML among HIV/AIDS patients.

We conducted extensive point mutation analyses of the archetype BKPyV NCCR identifying 3 phenotypic groups whereby Sp1 affinity and orientation governed bidirectional BKPyV early and late gene expression (Fig. 1). The pathologic relevance of the point mutations was supported by their identification in clinical isolates from patients with nephropathy and hemorrhagic cystitis. Thus, similar to HIV/tat in JCPyV, BKPyV (re-)activation does not only result from failure of immune control, but also from activation from the NCCR.

Based on clinical studies, BKPyV viremia and nephropathy has been associated with tacrolimus as main calcineurin inhibitor. We observed that cyclosporine A inhibited/slowed BKPyV replication, whereas tacrolimus activated/accelerated BKPyV replication *in vitro*. Importantly, viral activation by tacrolimus was antagonized by sirolimus competing the intracellular binding protein FKBP-12 (Fig. 2). The data strengthen the theme that activation of virus replication plus failure of adaptive immune control synergize in the emergence of opportunist viral infections in immunocompromised hosts. This knowledge could be taken back to the clinical management of BKPyV not only by reducing immunosuppression, but by switching to low-dose cyclosporine plus mTOR inhibitor combinations.

Characterizing BKPyV T-cell responses through IGRAs to 15mers, we observed that BKPyV Vp1 responses were generally stronger and involved mostly CD4 T-cells, whereas LTag responses were weaker, but contained a larger fraction of specific CD8 T-cells in peripheral blood. Since CD8 T-cells are the main cytotoxic effectors, and since LTag are rate limiting for BKPyV replication *in vitro* and *in vivo* (see NCCR mutant variants), we hypothesized that LTag-specific CD8 T-cells are key effectors of antiviral immunity. We identified 39 of 90 predicted immunodom-

inant 9mers responses that will be developed for T-cell vaccines (Fig. 3), and for clinical assays to guide immunosuppression reduction.

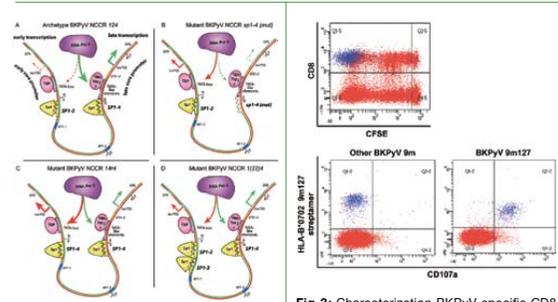


Fig. 1: The host cell factors Sp1, TATA- and downstream core promoter elements form imperfect symmetry underlying bidirectional expression of the early and late viral gene region of BKPyV (see Bethge *et al.* 2016 J. Virol 90: 10083–10101).

Fig. 3: Characterization BKPyV-specific CD8 T-cell responses identifying immune-dominant 9mers epitopes (see Cioni *et al.* 2016 Am J Transplant 16: 1193–1206). CD8+ T cell proliferation (CFSE dye) and cytotoxic degranulation (CD107a) of HLA-9mer-streptamer CD8 T-cells (blue).

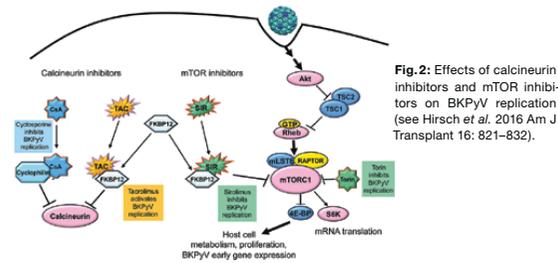


Fig. 2: Effects of calcineurin inhibitors and mTOR inhibitors on BKPyV replication (see Hirsch *et al.* 2016 Am J Transplant 16: 821–832).

Selected Publications

Ljungman P, Boeckh M, Hirsch HH, Josephson F, Lundgren J, Nichols G, Plakis A, Razonable RR, Miller V, Griffiths PD. Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials. *Clin Infect Dis.* 64(1):87–91

Leboeuf C, Wilk S, Achermann R, Binet I, Golshayan D, Hadaya K, Hirzel C, Hoffmann M, Huynh-Do U, Koller MT, Manuel O, Mueller NJ, Mueller TF, Schaub S, van Delden C, Weissbach FH, Hirsch HH. Swiss Transplant Cohort Study. BK Polyomavirus-Specific 9mer CD8 T Cell Responses Correlate With Clearance of BK Viremia in Kidney Transplant Recipients: First Report From the Swiss Transplant Cohort Study. *Am J Transplant.* 2017 Mar 22. doi: 10.1111/ajt.14282.

Hirsch HH, Yakhontova K, LU M, Manzetti J. (2016) BK Polyomavirus Replication in Renal Tubular Epithelial Cells is Inhibited by Sirolimus, but Activated by Tacrolimus through a Pathway Involving FKBP-12. *Am J Transplant.* 16(3):821–32

Bethge T, Hachemi HA, Manzetti J, Gosert R, Schaffner W, Hirsch HH. (2015) Sp1 sites in the noncoding control region of BK polyomavirus are key regulators of bidirectional early and late gene expression. *J Virol.* 89(6):3396–411

Hirsch HH, Martino R, Ward KN, Boeckh M, Einsele H, Ljungman P. (2013) Fourth European Conference on Infections in Leukemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus. *Clin Infect Dis.* 56(2):258–66

Connection to Clinical Practice

Nina Khanna, Manuel Battegay, Joerg Halter, Ulrich Heiningler, Jakob Passweg, Stefan Schaub, Jürg Steiger, Daiana Stolz, Michael Tamm
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Multidisciplinary Clinical Virology Approach to Improve Outcome in Vulnerable Patients

Together with Prof Nina Khanna and Prof M. Battegay; and Profs J Passweg, and J. Halter, a retrospective study on respiratory viruses (RV) in stem cell transplant recipients was conducted with special focus on the therapeutic role of oral ribavirin. Also, we are participating in a multi-center randomized phase 3 study of a fusion inhibitor targeting respiratory syncytial virus in HCT recipients with upper or lower respiratory tract infections (GS-US-218-0108; GS-US-218-1502).

Together with Profs D. Stolz and M. Tamm, a prospective study of RV-multiplex testing in patients exacerbations of asthma and COPD. In a 5-year prospective study with Prof J Gavaldà, Spain, we defined the role of RV for acute and chronic lung disease after lung transplantation.

Together with PD MJ Kim, Prof S. Schaub and the Swiss Transplant Cohort Study (STCS), we are engaged in a prospective randomized trial (Dr O. Manuel, Lausanne; <https://clinicaltrials.gov/ct2/show/NCT02538172>) to study the role of CMV-specific T-cells in guiding the duration of valganciclovir prophylaxis. In collaboration with colleagues from Finland, USA, Italy, Spain, and USA, we aim at improving the traceability and commutability of CMV load tests to define the path forward to uniform criteria for FDA and EMA-approved clinical CMV studies for licensing antivirals and vaccines. Together with the STCS, we organised a multicentre study to identify the BKPyV-specific T-cells in kidney transplantation. By the end of the study, more than 1800 EISpot assays from PBMCs will inform about super-dominant epitopes and their MHC-I context to improve risk assessment and immunosuppression reduction.