Supplementary data

Overview of BKPyV biology, immune response and disease

BKPyV virions are small non-enveloped particles of 40-45 nm in diameter, with an icosahedral symmetry [1-3] that tolerate heating, and remain infectious in the environment including in sewage. The capsid consists of the structural proteins Vp1 on the outside and Vp2 and Vp3 on the inside accommodating the circular 5.1 kb double-stranded DNA genome. Akin to other polyomaviruses, [3,4] the BKPyV genome can be divided into three regions (Figure S1):

i) the non-coding control region (NCCR) which harbours the origin of DNA replication and promoter/enhancers for bidirectional viral gene expression;

ii) the early viral gene region (EVGR) which encodes the non-structural proteins called small tumour antigen (sTag), large tumour antigen (LTag), and spliced variants called truncated Tag;

iii) the late viral gene region (LVGR) encoding the structural proteins Vp1, Vp2, Vp3, and a small accessory protein of unknown function called agnoprotein.

The LVGR transcripts extend into the EVGR, where a pre-miRNA is encoded. The pre-miRNA generates two miRNAs involved in down-regulating the LTag transcripts and the cellular stress-induced ligand ULBP3. [5,6] In immunocompromised patients, the NCCR can undergo partial sequence deletions and duplications, giving rise to new BKPyV variants. Variants found as majority species in blood are associated with increased replicative fitness in vitro and higher viral loads in vivo. [7-9] LTag and sTag regulate entry of the host cell into G2/S-phase in order to provide abundance of host cell enzymes and building blocks for viral replication; and LTag is directly involved in the viral DNA replication.

Notably, BKPyV does not encode classic antiviral targets such as viral nucleoside kinases, DNA polymerase or protease enzymes, which renders current antiviral drugs developed for herpes-or hepatitis viruses suboptimal or ineffective. In vitro studies suggest some potency of cidofovir and brin-

cidofovir (formerly called CMX001), which need to be confirmed in proper clinical studies. [10,11] LTag and sTag play a role in oncogenic transformation in experimental models, and there is limited evidence that BKPyV may contribute to urothelial malignancies in humans. [12,13] Detection of LTag is clinically important for making a BKPyV-specific diagnosis in tissue samples by immunohistochemistry, which often uses cross-reacting antibodies to the simian polyomavirus SV40 LTag. The Vp1 capsid protein represents the main target of neutralizing and non-neutralizing BKPyV-specific antibodies [14-16] and 4 major Vp1 serotypes have been distinguished. [17,18] However, a quasispecies-like variability of VP1 has been described in immunocompromised patients with persisting BKPyV replication. [19,20]

Virus biology, immune response, and disease. BKPyV is commonly acquired during childhood, and the virus then persists in renal tubular epithelial and urothelial cells. [4,21,22] The route of BKPyV transmission is not defined, but most likely occurs via the oral and/or respiratory tract. Symptoms and signs of BKPyV primary infection are not known, either because of its subclinical or unspecific, for example “flu-like”, presentation. Asymptomatic viruria is seen in 5% - 10% of healthy individuals. [23] Using seroconversion as indicator of past exposure, approximately 40% of 5 year-old children have BKPyV Vp1 antibodies increasing to >90% by early adulthood. [15,23,24] BKPyV IgG titres decrease with age resulting in a lower seroprevalence after the forth decade of life. [15,23,24] BKPyV-specific CD4 T-cell responses appear to correlate with declining BKPyV IgG titers, showing decreasing frequencies in the peripheral blood of older age groups. [25] Asymptomatic viruria has been detected in >60% in immunosuppressed patients, in whom the urine viral loads are higher compared to healthy populations. [26-28] In transplant patients, BKPyV replication is viewed as the result of failing BKPyV-specific immune control due to immunosuppressive drugs, and impaired virus-specific T-cell effector functions in the allogeneic setting of transplantation. [22,28-31] Extensive cytopathic damage from virus infection and conditioning regimens, and significant inflammation from innate and adaptive immune responses then lead to organ-invasive disease and

functional compromise. [2,32,33] Although the most frequent clinical manifestation of BKPyV in allogeneic HSCT is HC, [2] other manifestations have been reported including nephropathy, [34,35] ureteric stenosis, [36] encephalitis, [37,38] respiratory tract infections, [39,40] and skin pathologies. [41]

References

Figure S1. BKPyV genome organization.

The genome can be divided in three regions: i) The non-coding control region (NCCR) containing the origin of DNA replication and promoter/enhancers for bidirectional viral gene expression; ii) The early viral gene region (EVGR) encoding regulatory small and large tumour antigens (sTag, LTag), spliced variants called truncated Tag, and miRNA; (iii) The late viral gene region (LVGR) encoding the capsid proteins Vp1, Vp2, Vp3, and a small accessory protein of unknown function called agnoprotein. The BKPyV genome does not encode a viral DNA polymerase or protease.

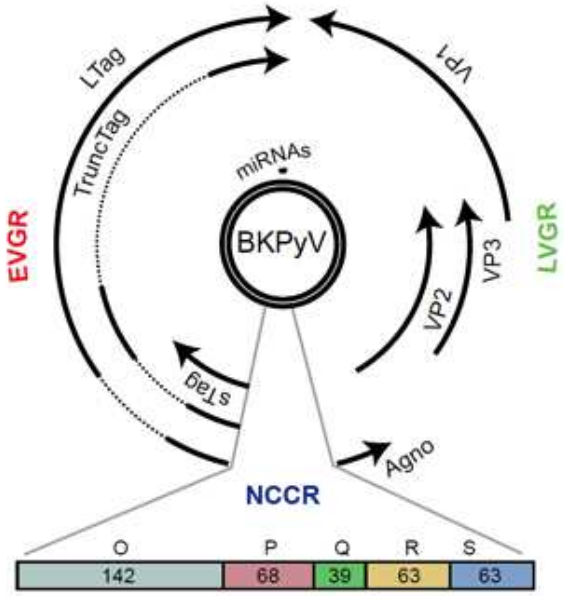

Table S1. Results of non-specific measures: hyperbaric oxygen therapy and fibrin glue application

	Hyperbaric oxygen	**Fibrin glue**
N° of studies	5	1
References	[1-5]	[6]
Type of Study	Retrospective, 5	Retrospective, 1
N° of patients	29	35
Efficacy: - Clinical - Virological	CR (86%) Reduction in BKPyV loads in urine (65%)	CR (83%) BKPyV viruria remained positive in 83% of patients achieving CR
Toxicity (N° episodes)	Ear barotrauma (1), pressure intolerance (1), claustrophobia (1); beware feasibility for earache or claustrophobia	No adverse effects

1 Focosi D, Maggi F, Pistolesi D, et al. Hyperbaric oxygen therapy in BKV-associated hemorrhagic cystitis refractory to intravenous and intravesical cidofovir: case report and review of literature. *Leuk Res* 2009;**33**:556-60.

2 Hosokawa K, Yamazaki H, Nakamura T, et al. Successful hyperbaric oxygen therapy for refractory BK virus-associated hemorrhagic cystitis after cord blood transplantation. *Transpl Infect Dis* 2014;**16**:843-6.

3 Hughes AJ, Schwarer AP, Millar IL. Hyperbaric oxygen in the treatment of refractory haemorrhagic cystitis. *Bone Marrow Transplant* 1998;**22**:585-6.

4 Savva-Bordalo J, Pinho Vaz C, Sousa M, et al. Clinical effectiveness of hyperbaric oxygen therapy for BK-virus-associated hemorrhagic cystitis after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2012;**47**:1095-8.

5 Zama D, Masetti R, Vendemini F, et al. Clinical effectiveness of early treatment with hyperbaric oxygen therapy for severe late-onset hemorrhagic cystitis after hematopoietic stem cell transplantation in pediatric patients. *Pediatr Transplant* 2013;**17**:86-91.

6 Tirindelli MC, Flammia GP, Bove P, et al. Fibrin glue therapy for severe hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2014;**20**:1612-7.