

Clinical Applications of EBV & BK Viral Load Assays in Transplantation

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EBV in Transplantation: Background

- EBV seroprevalence is directly age-related
 - ~95% of adults are seropositive
 - many young children/adolescents are EBV seronegative & at high risk for primary infection
- EBV-associated lymphoproliferative disease (EBV-PTLD) is a rare but serious complication of transplantation
 - Incidence estimates ~1-5%, greatest risk within 1st year post-transplant
 - Histologically variable (early lesions, polymorphic, monomorphic/"non-hodgkins lymphoma")
 - High morbidity/mortality
 - Pathogenesis is closely-linked to EBV (uncontrolled proliferation of latently EBV-infected B lymphocytes)
 - Higher VL, persistent viremia associated with increased PTLD risk
- Risk Factors:
 - EBV serostatus (highest risk in EBV D+R-, ~5% of adult transplant recipients)
 - Specific type of transplant (HCT vs SOT)
 - Immunosuppression type (anti-lymphocyte antibody)
 - Age

Challenges in use of EBV VL Assays in Transplantation

- Evidence for use is significantly lower than for CMV VL assays
- Assay-related issues [Cook] (sample type, assay variability, lack of clinically-defined threshold, ...)
- Biological/non-assay-related issues
 - Relatively uncommon/rare condition overall
 - High rates of EBV reactivation during immunosuppression (specificity)
 - persistent high-grade EBV viremia w/o apparent clinical consequences
 - Non-EBV associated PTLD (small proportion)
 - Lack of safe and proven effective therapies

Consensus/Guideline Recommendations for use of EBV VL Assays in Transplant

- Limited and low level evidence to guide recommendations
- EBV serostatus of donor and recipient should be assessed pre-transplant
- Restrict EBV VL monitoring to highest risk subpopulations
 - HCT: T-cell depleted, >2 HLA mismatches, ATG
 - SOT: EBV D+R-, other “high-risk” (ATG, thoracic transplant)

Indications for EBV VL Testing in Transplantation

1. Monitor EBV VL blood to predict PTLD risk (“Preemptive Therapy”)

- Typically for the first 3-12 months post-transplant
- For patients with “**significant**” EBV viremia
 - Assess clinically for PTLD
 - Interventions: reduction in immunosuppression, rituximab, EBV-specific T-cells
- Used variably at transplant centers
 - Pediatric > Adult

Indications for EBV VL Testing in Transplantation

2. Adjunct to diagnosis of PTLD

- Does NOT replace biopsy (histopathology, genetics)
- PTLD typically associated with high blood VL
- Clinically-significant VL threshold appears to be:
 - Assay-specific
 - Specimen-specific (whole blood vs plasma vs PBMC)

Indications for EBV VL Testing in Transplantation

3. Monitoring response to therapy

- Reduction in VL associated with clinical response to therapy
- Increasing VL associated with treatment failure or progression, or recurrence

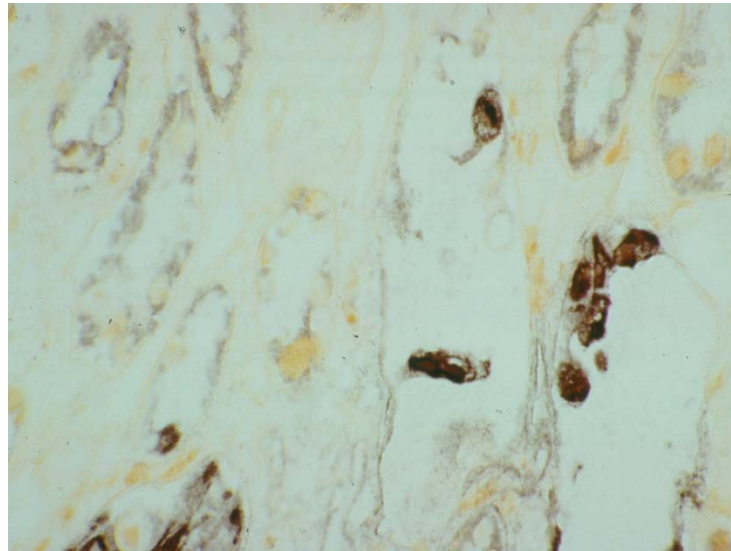
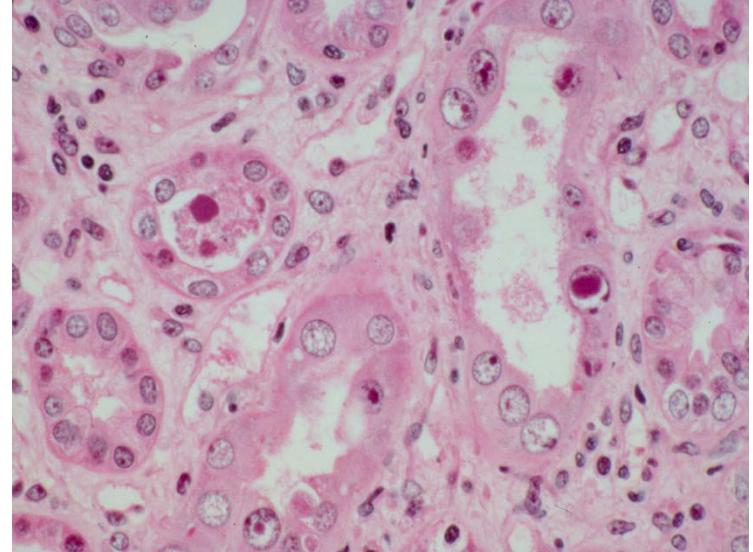
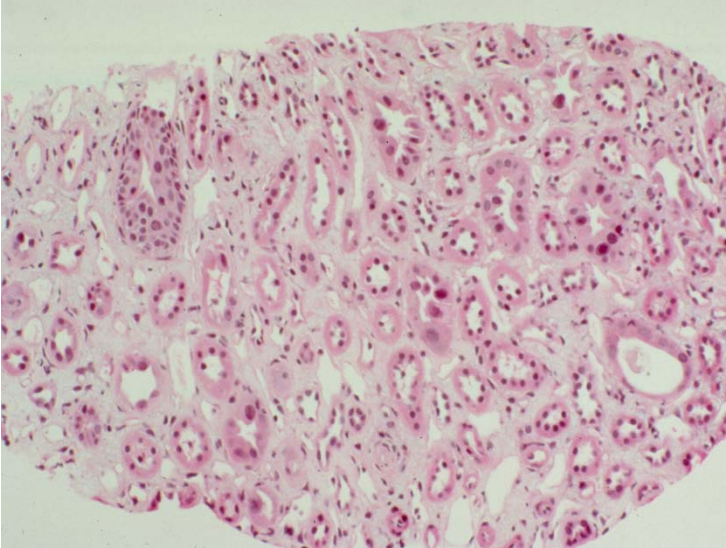
4. Predict risk for relapse

- Recurrent viremia associated with clinical relapse

Current Clinical Use of EBV VL Assays in Transplantation

- Limited and low quality of evidence to guide use
- Widely variable across centers (specimen type, actionable threshold, specific population(s) tested, frequency of testing, indications, etc.)
- Lack of consensus that EBV VL assays should be routinely used
 - Lack of definitive evidence of benefit (reduction in PTLD, improved outcomes of established PTLD, reduction in need for invasive procedures/biopsy, reduced incidence of recurrence)
- Major work required in the field
 - Assay issues (sample type)
 - Clinical evidence to support intended uses

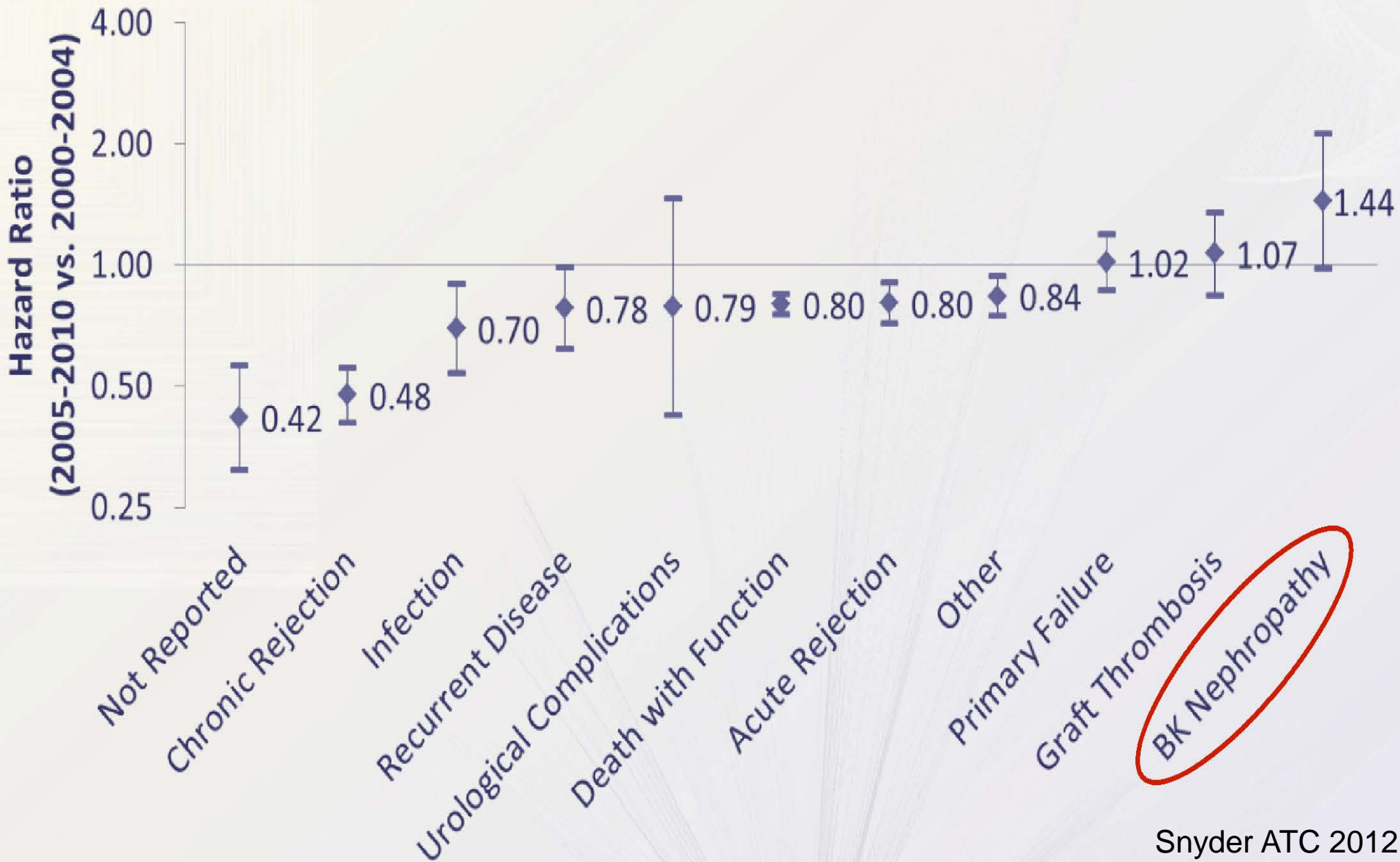
BK Virus in Transplantation



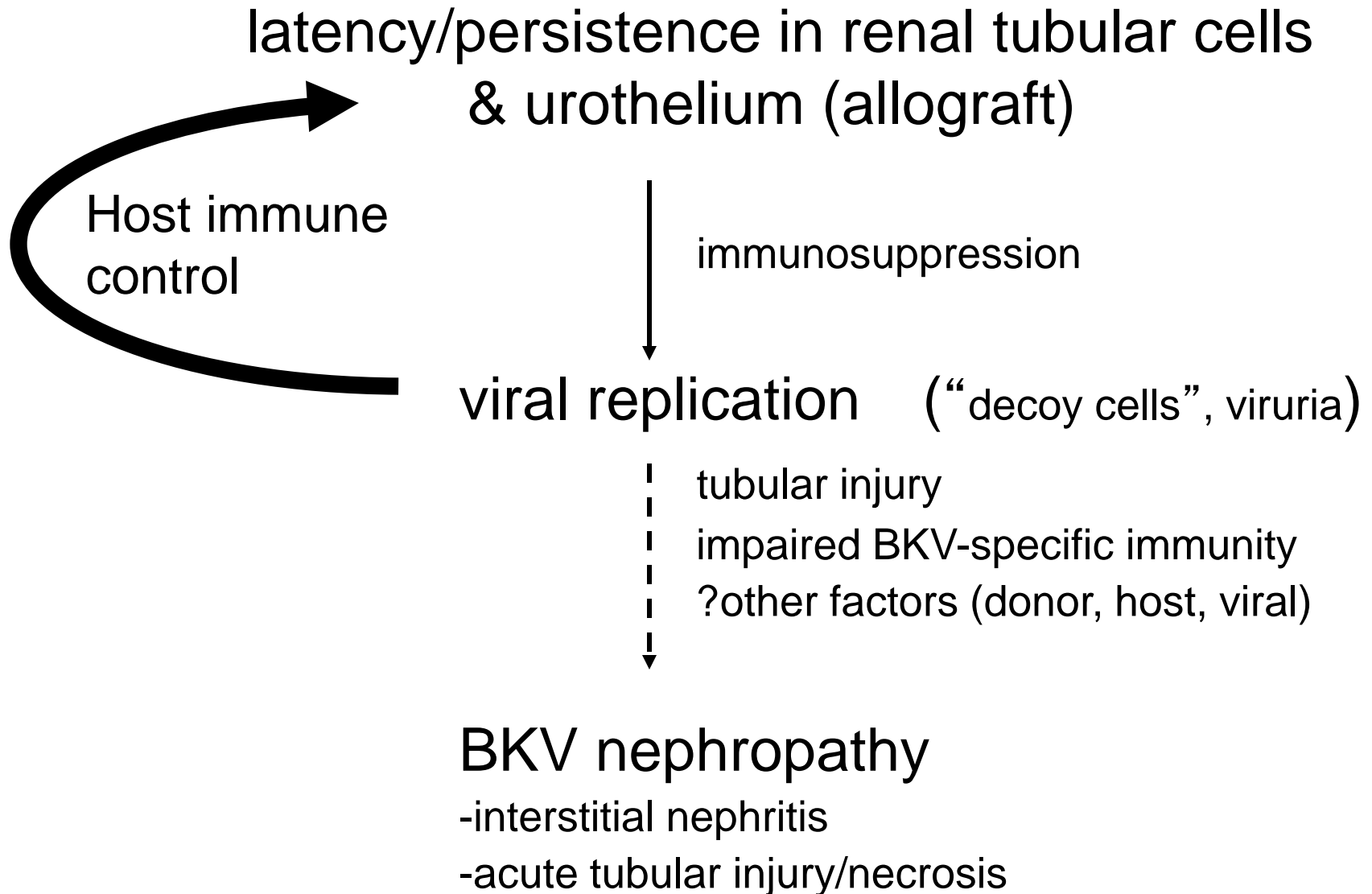
BKV in Transplantation: Background

- BKV has greatest impact in kidney transplant recipients (BKVN)
 - 1-5% incidence, 15-30% with premature allograft loss
 - BKVN occasionally occurs in native kidneys of non-kidney SOTx transplant recipients) [Limaye *Am J Transplant* 2005]
 - No recommendations for routine BKV VL testing in non-kidney SOTx recipients (except as adjunct to biopsy)
- HCT: BKV-associated hemorrhagic cystitis
 - Limited data on association of BKV VL with disease (HC, BKVN)
 - No recommendations for routine BKV VL monitoring in HCT recipients (except as adjunct to diagnosis of HC)

BKVN is the only cause of 1st year graft loss that has increased between 2000-04 and 2005-10



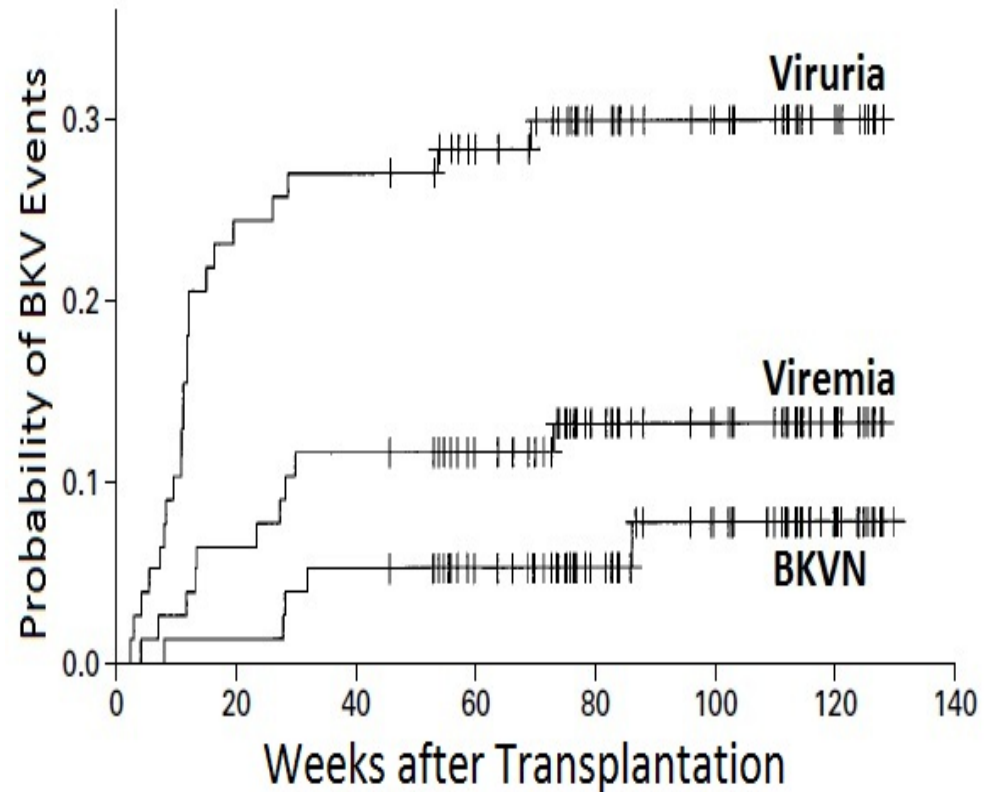
BKV Nephropathy: *Pathogenesis*



Incidence & Timing of BK Virus Replication and Disease in Kidney Transplant Recipients

- Prospective study of 78 Ktx recipients
- ATG induction (40%), FK/Aza/Pred (47%), CSP/MMF/Pred (53%)

Decoy cells	30%	16 wks
BK viremia	13%	23 wks
BKVN	8%	28 wks



Uses of Molecular BKV Assays (blood qPCR)

1. Diagnosis of BKVN

- Adjunct to allograft biopsy (greater sensitivity than biopsy)

2. Monitoring the course of BKVN

- Viral load in blood correlates well with degree of BKV allograft involvement

3. Routine screening & preemptive reduction in immunosuppression

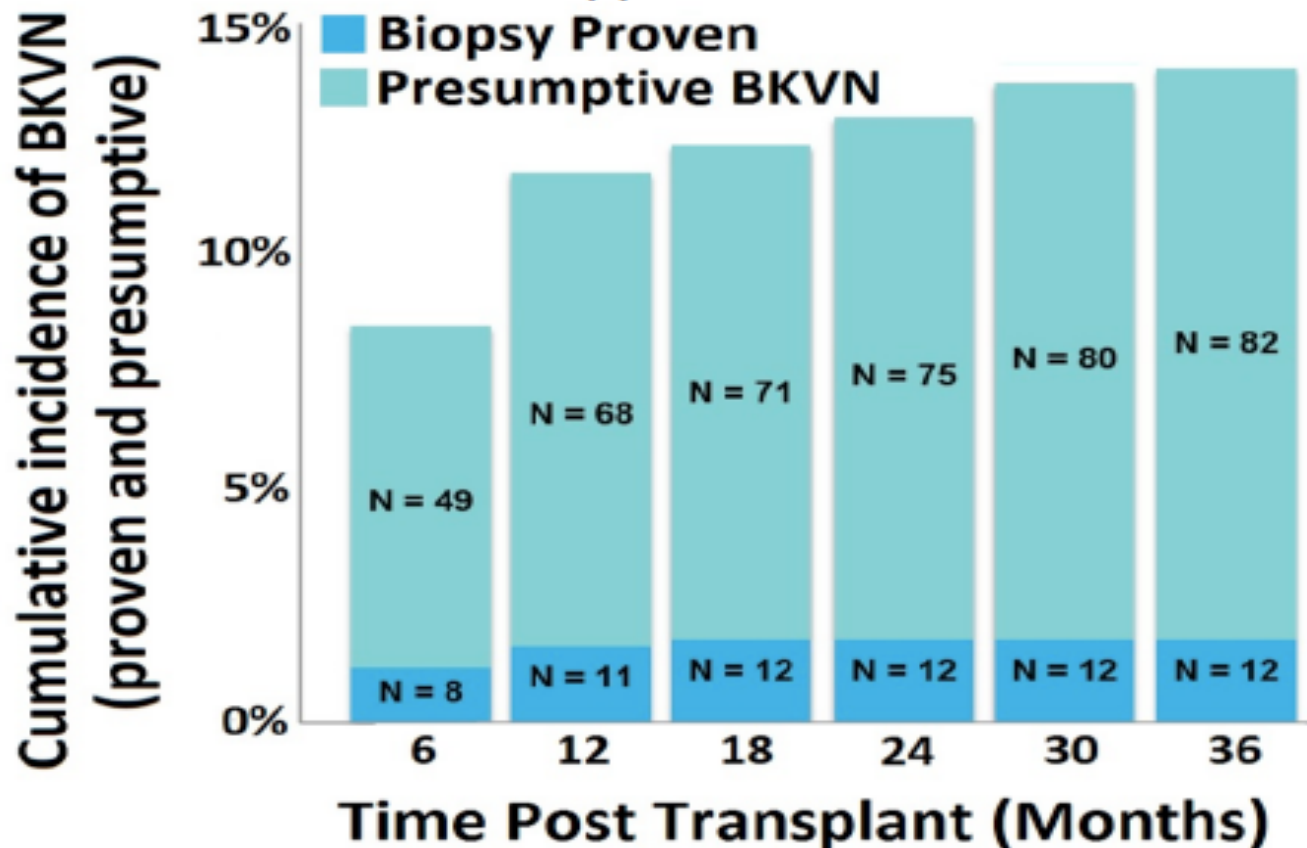
- May prevent progression to BKVN

BKV VL Assays as Adjunct to Diagnosis

- Decreasing incidence of biopsy-confirmed BKVN
 - But, ...increasing rates of “presumptive BKVN” (ie. high VL in patient at risk & compatible clinical scenario)
 - BKV VL measurements are replacing biopsy
- Limitations of current “gold standard” (allograft biopsy)
 - ~35% rate of discordance between biopsy cores for BKVN [Drachenberg *Am J Transplant* 2004]
- Blood BKV VL reflects renal allograft VL [Funk *AJT* 2008, Nicleleit *NEJM* 2000, Limaye *JID* 2001]
 - Specific VL thresholds for biopsy-confirmed BKVN have been defined (for specific assays)
 - BKV DNAemia (even without biopsy-confirmed BKVN) is associated with worse graft function [Hirsch *Am J Transplant* 2013]

Screening for BK virus Nephropathy in Renal Transplant Recipients: Real World Experience

Figure 7. High incidence of biopsy-proven and presumptive BKVN despite BKV screening and reduction in immunosuppression.

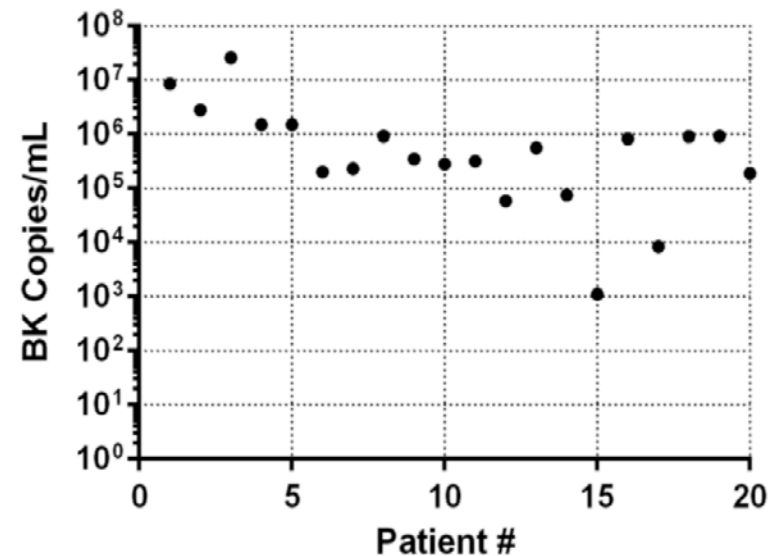


- Retrospective analysis of 671 kidney transplant recipients (2008-2010)
- Blood BKV PCR monitoring per guidelines

Clinically-Significant BKV Blood VL

- Majority of cases of biopsy-confirmed BKVN with BKV DNAemia $\geq 10^4$ copies/mL
- Inter-laboratory comparison Hirsch/Basel and UW assays

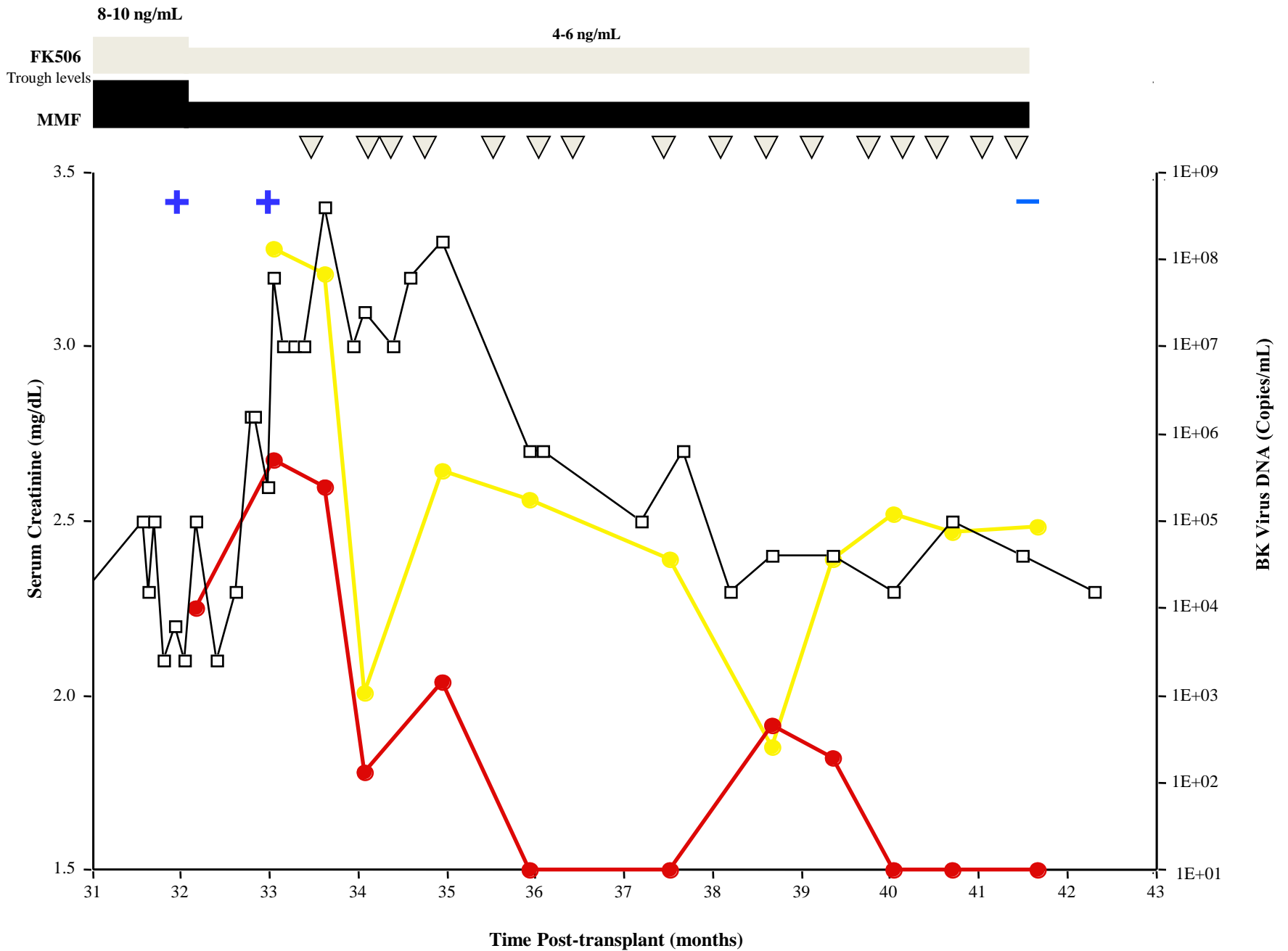
Figure 11. Plasma BKV DNA load in patients with biopsy-proven BKVN is usually $\geq 10^4$ copies/mL.

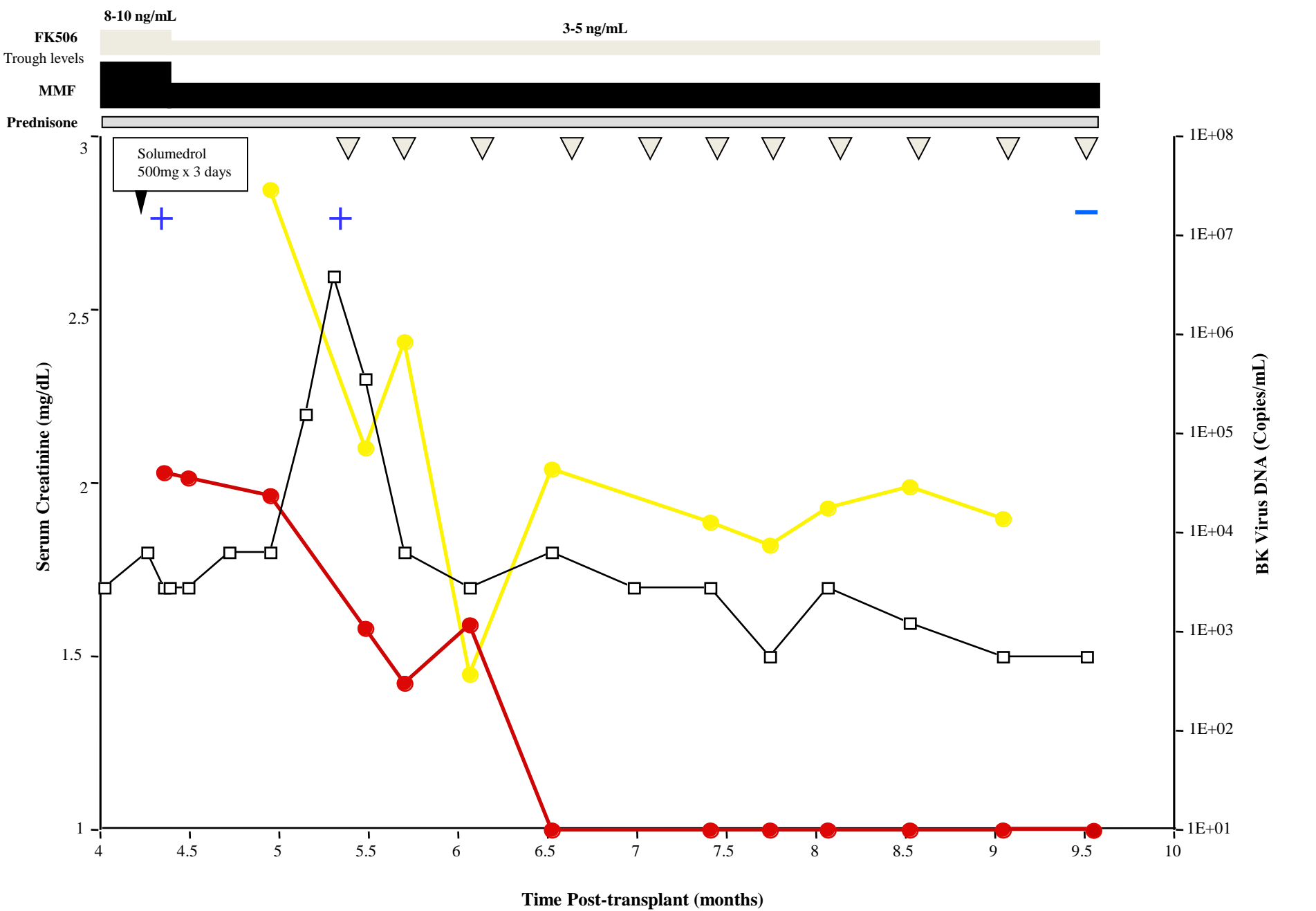


Uses of BKV Blood VL Assays

2. Monitoring the course of BKVN

- Assess the impact of therapy (reduction in immunosuppression, antivirals, etc.)
- Viral load in blood associated with degree of BKV allograft involvement
- Viruria may persist (urothelial reservoirs) even after clearance from allograft





Uses of BKV VL Assays in Kidney Transplant: Rationale for Screening

3. Routine screening post-transplant

- Identify patients at highest risk for progression to clinically-evident disease [BKVN]
 - Allow for preemptive reduction in immunosuppression → prevent BKVN [Brennan *Am J Transplant* 2005]
 - Earlier diagnosis associated with better outcome [Buehrig *Kidney Int* 2003]
- Might be cost-effective in certain populations [Kiberd *Am J Transplant* 2005]

Controversies in screening for BK virus in Renal Transplant Recipients

1. What sample to screen?

- **Blood:** (higher specificity/predictive value, later in course)
- **Urine:** earlier diagnosis, lower specificity/predictive value

2. What test(s) to use?

- Blood: qPCR
- Urine: qPCR, cytology

Screening for BK virus Nephropathy in Renal Transplant Recipients

How often to screen?

1. International Interdisciplinary Guidelines [Hirsch 2005]
 - blood or urine, q3 months x 2years
2. KDIGO Clinical Practice Guidelines [Kasiske 2009]
 - Plasma qPCR monthly x 3-6 months, then q3 months until 1yr
3. Am Soc Transplant ID Guidelines [Hirsch 2013]
 - Blood or urine, at least q3 months x 2yrs
 - Alternatives:
 - Plasma qPCR qMonth x 6mo, then q3mo until 2 yr
 - Urine twice a month x 3mo, then qMonth x 3mo, then q3mo until 2yr

Important Issues in Uses of BKV VL Assays

- Non-assay/biological issues
 - No established clinically significant viremia threshold (association of viremia with worse outcomes) [Hirsch *Am J Transplant* 2013]
 - Specimen type for screening (urine vs blood)
- Assay-related issues [Cook]
 - Calibrator/reference standard
 - Impact of genotype
 - Inter-assay variability