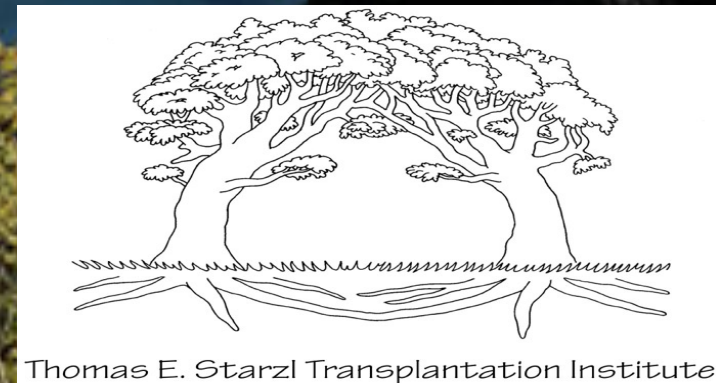


POLYOMAVIRUS IN SOLID ORGAN TRANSPLANTATION

Parmjeet Randhawa
Professor, Division of
Transplantation Pathology
Department of Pathology
University of Pittsburgh

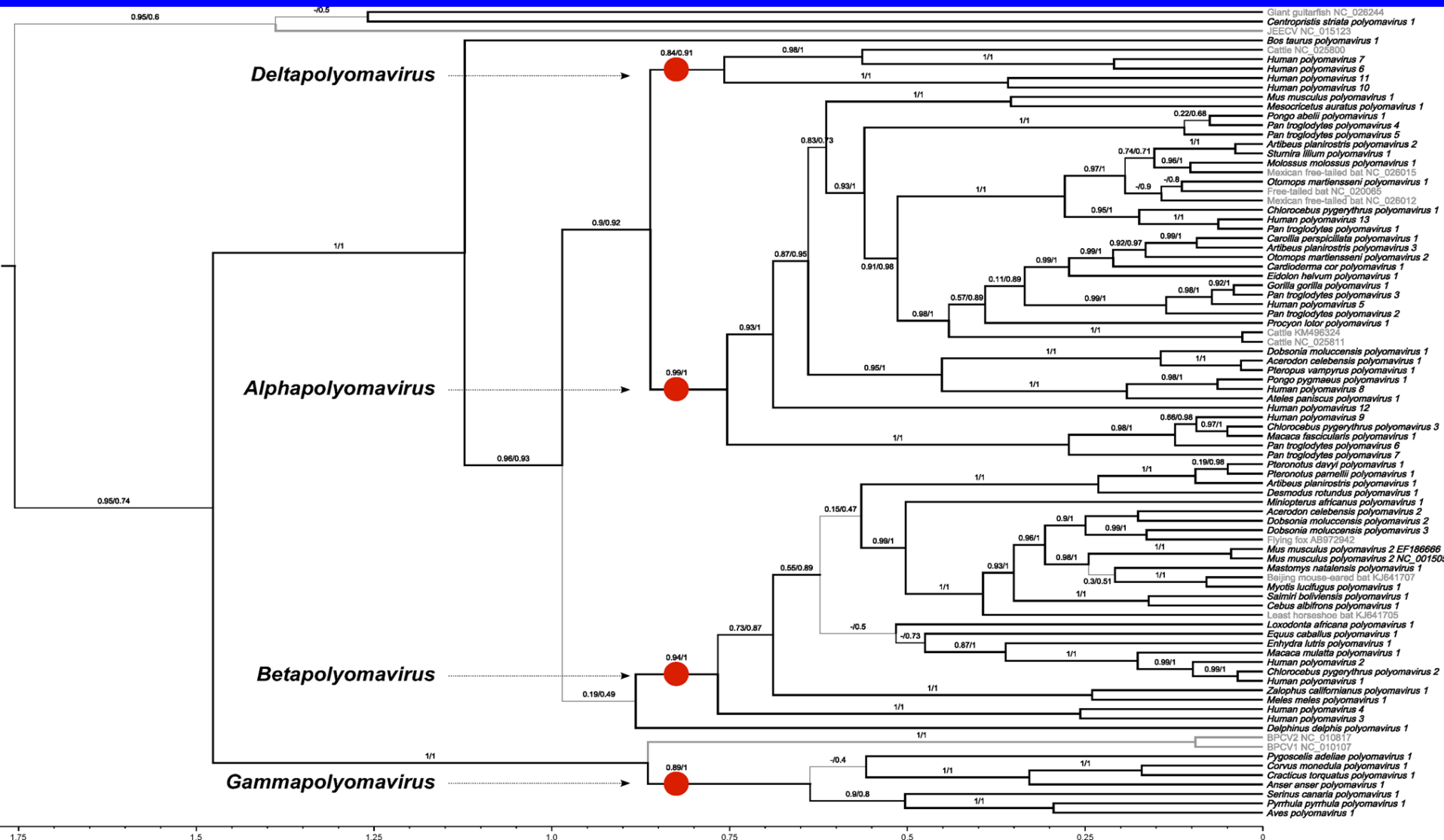


Outline of Talk

- Taxonomy
- Viruses discovered in the new millennium
- Classic polyomaviruses JCV, BKV (1971)

Emphasis: clinical presentation, laboratory diagnosis, pathology, & principles of Rx

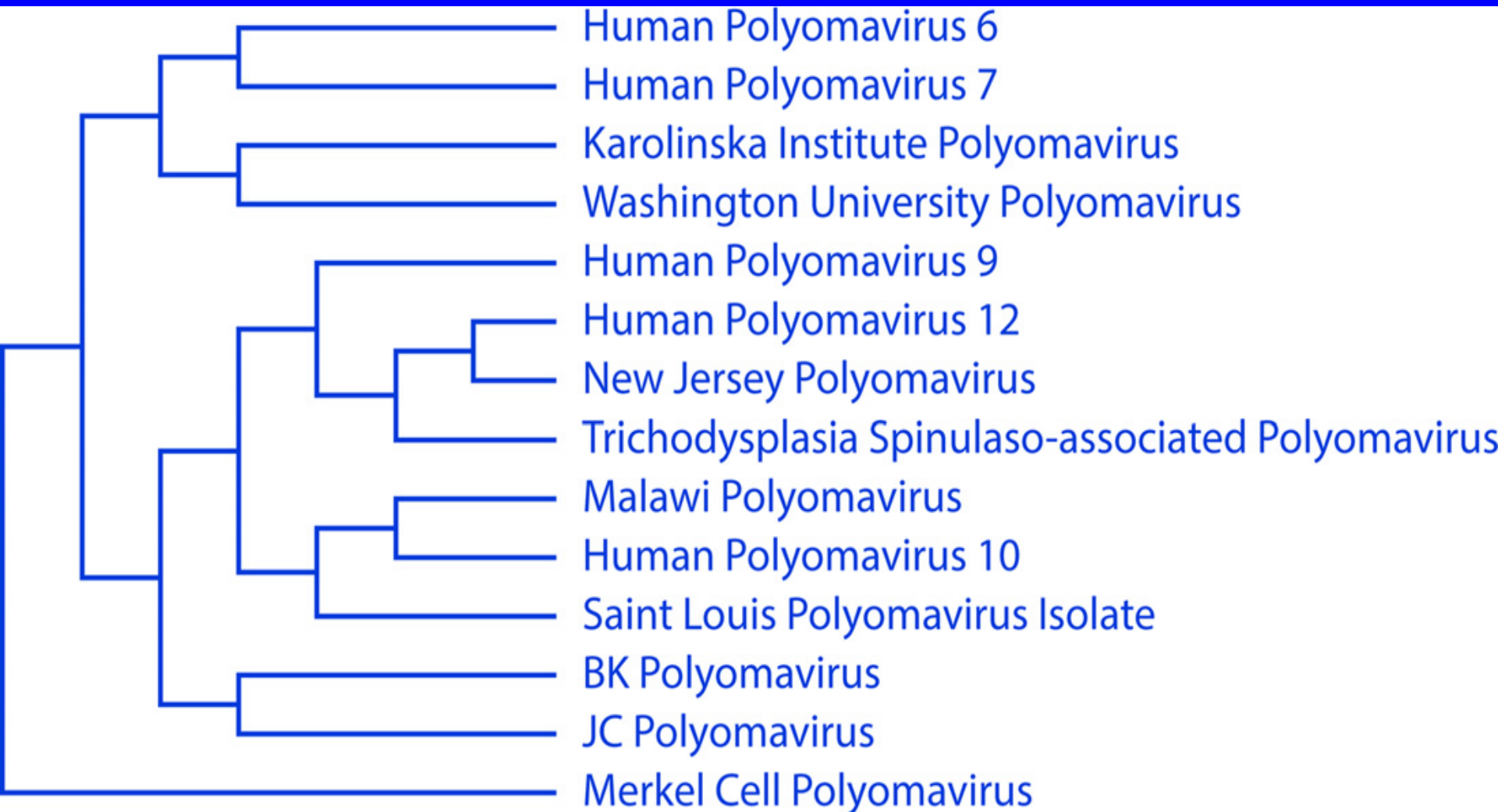
TAXONOMY OF POLYOMAVIRUSES (>117)



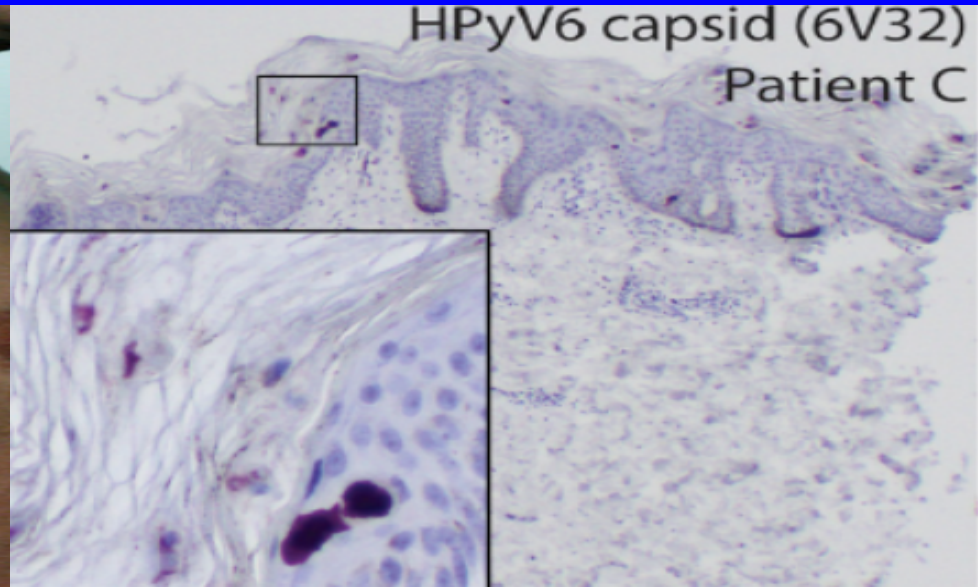
Classification by Genera

- ALPHA: largest group many mammals & human ones viz MePyV, TrDyPyV, HPyV 9,12, NJPyM
- BETA: BK, JC, SV40, KI, WU (bat, badger, baboon, horse, elephant, mouse pneumotropic, lion, SqMonkey, mouse (pneumotropic species))
- DELTA: HPyV6, 7, 10, MwPyV, STL
- GAMMA: birds

HUMAN POLYOMAVIRUSES (N=14)



HUMAN PyV 6 & 7 Inhabit the Skin



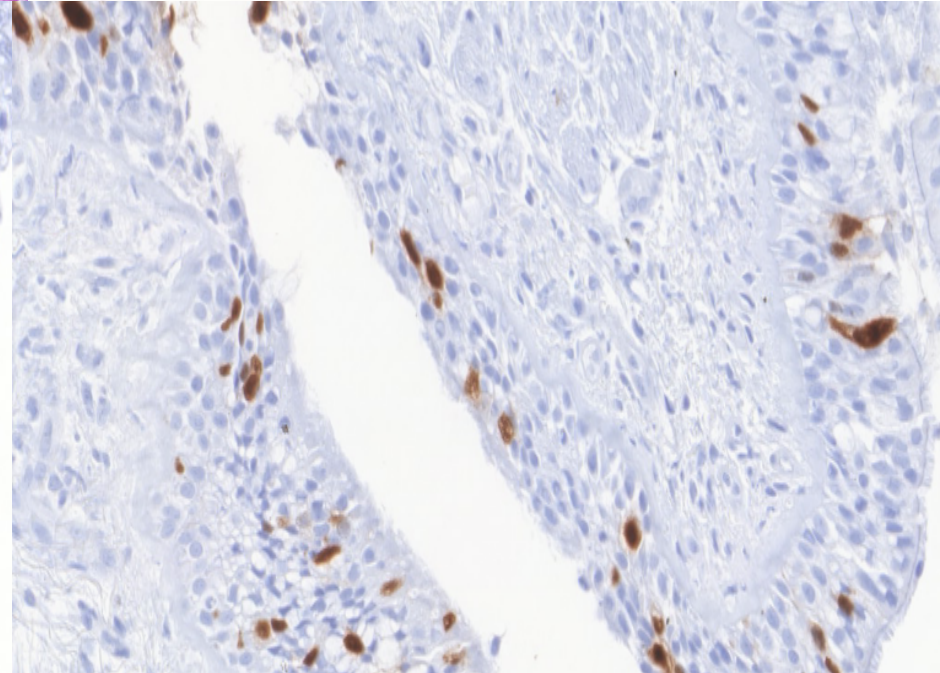
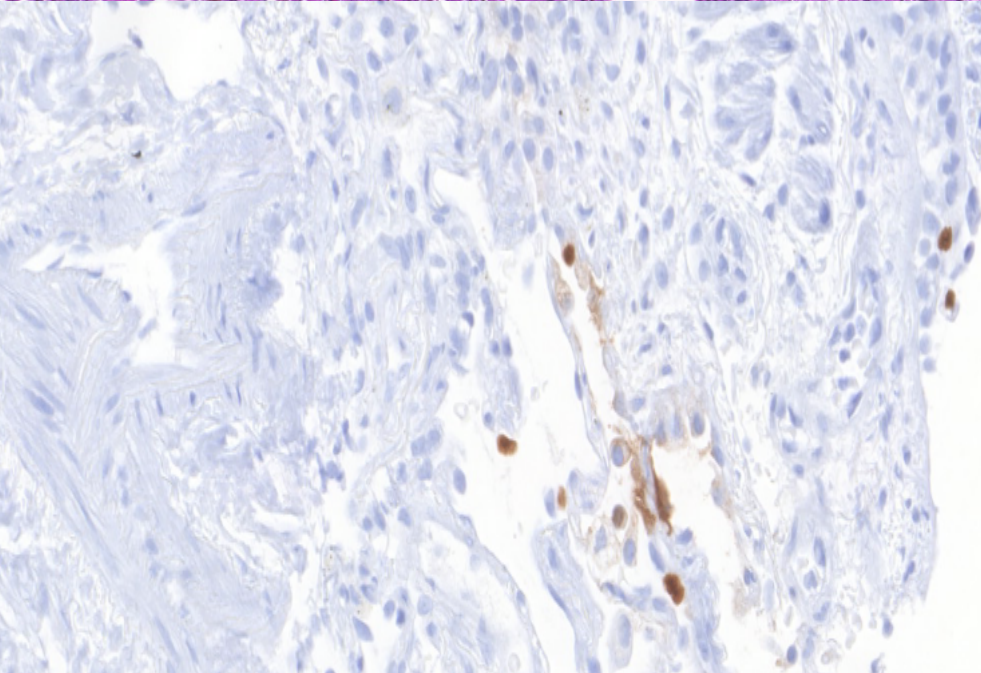
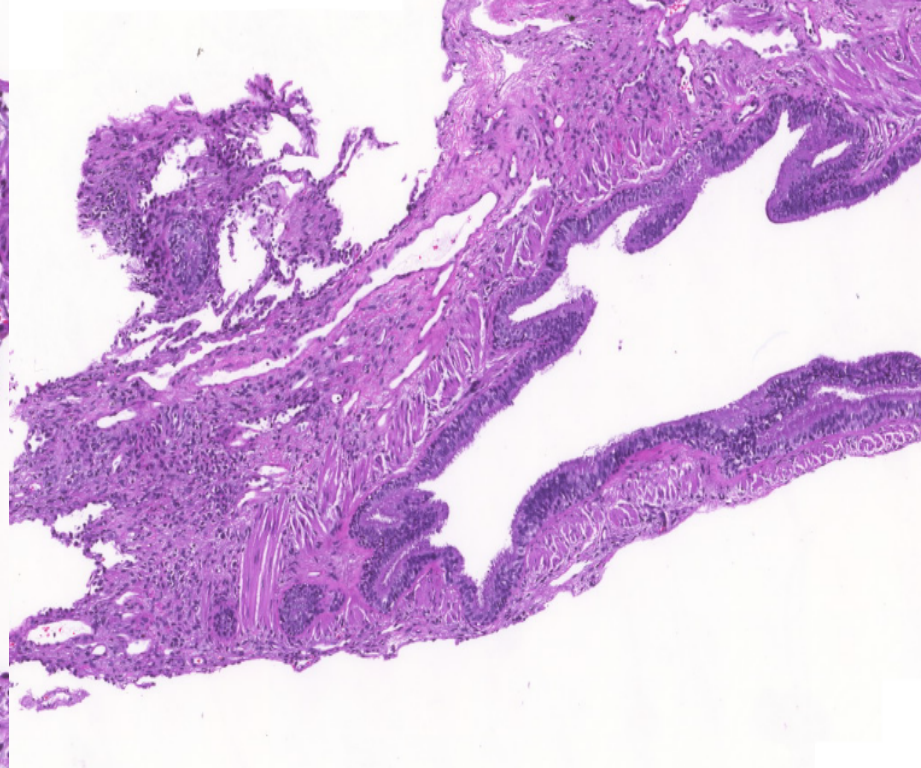
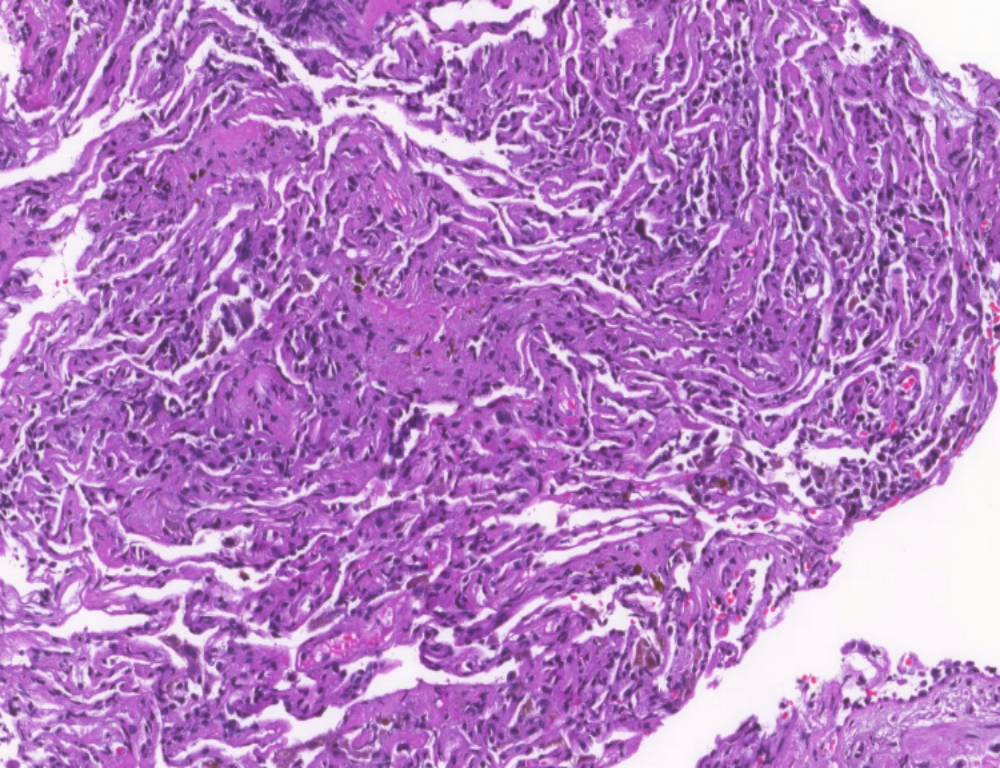
N = 5 (Kidney transplant, HIV+ & NonI.S. patients)

Clinical presentation: generalized pruritic skin rash

Pathology: irregular parakeratosis (feather plume pattern)

Immunohistochemistry: HPyV6 and HPyV7 antigens

Nguyen, Lee et al. J Am Acad Dermatol 2016 ePUB



HPyV6 in Extracutaneous Sites

- Viral DNA has been amplified from:
 - human stool samples
 - bile in patients with stones/cholangiocarcinoma
 - CSF of leukoencephalopathy
 - thymomas

WU and KI PyV

- Seroprevalence 54-70% & 80-89% respectively
- No definitive clinical syndrome but DNA found in 2-8%% of respiratory samples (URIs)

35% of tonsillar/adenoid specimens

14.3% autopsy lymphoid tissue I.S. patients

5% Ca Lung/colon specimens

Human Polyomavirus 9

- Homologous to B-lymphotropic PyV in African green monkeys
- Seroprevalence of 33-46%
- Viral DNA in blood of 2-20% KTx patients
- Skin of Merkel cell carcinoma (n=1)

HPyV10: Isolated from WHIM Syndrome

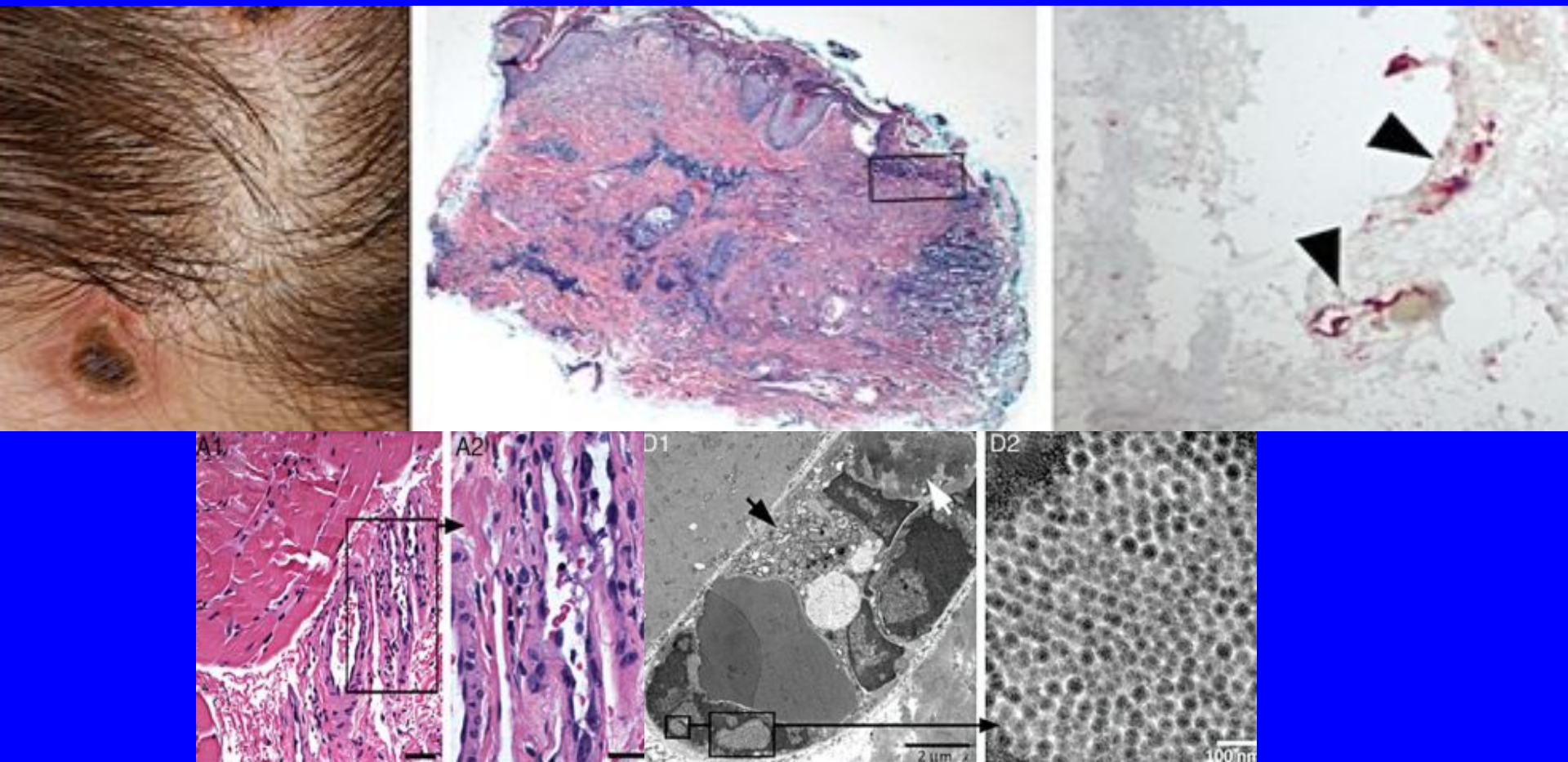
Autosomal dominant: mutations of the *CXCR4* gene.

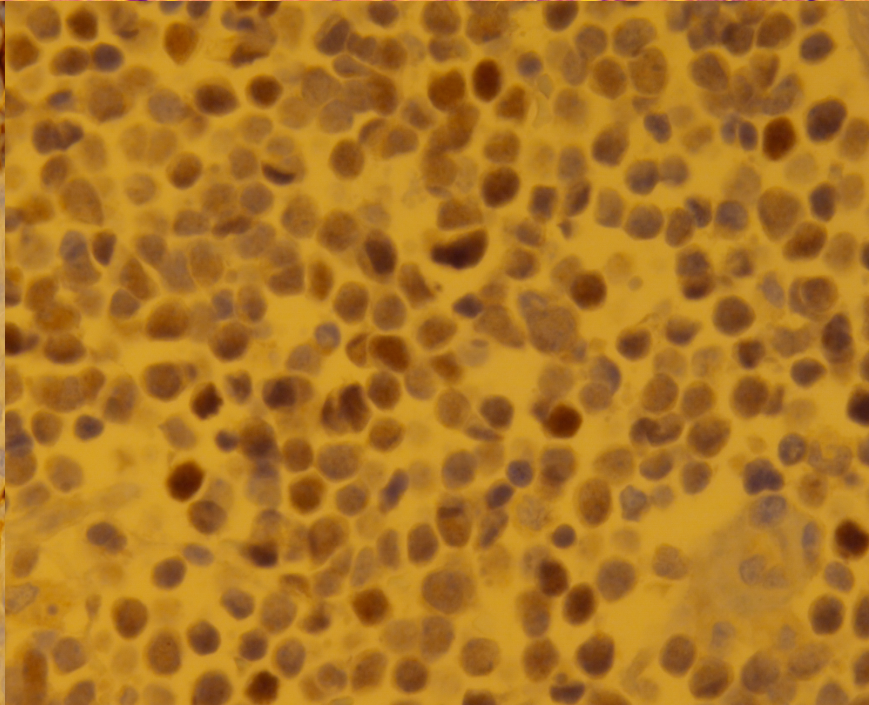
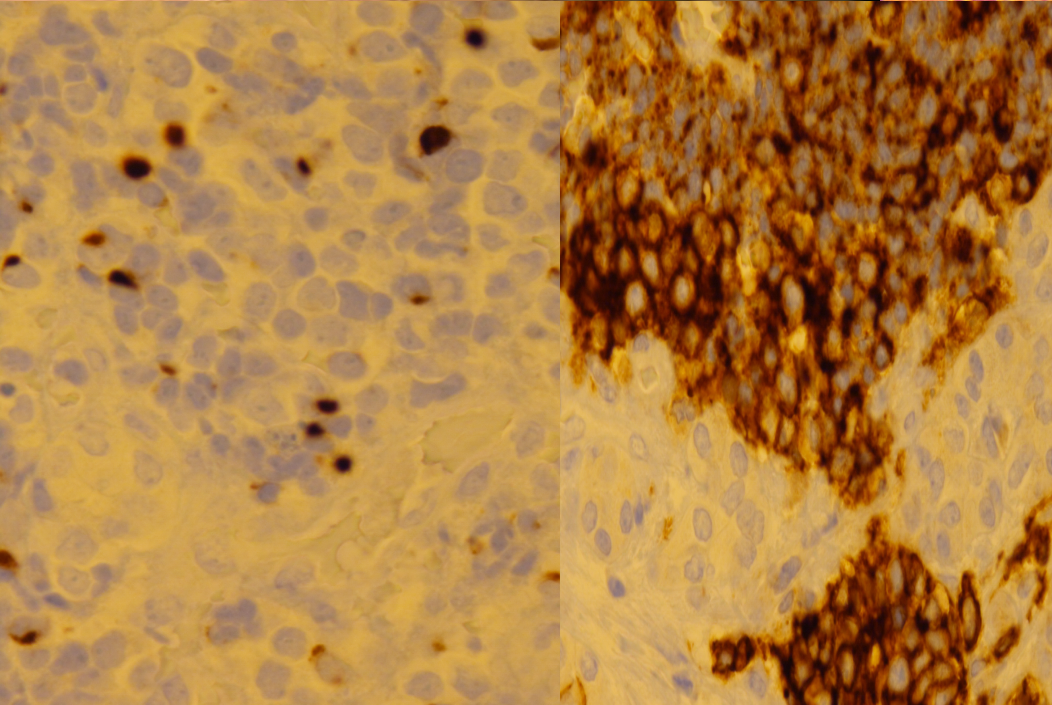
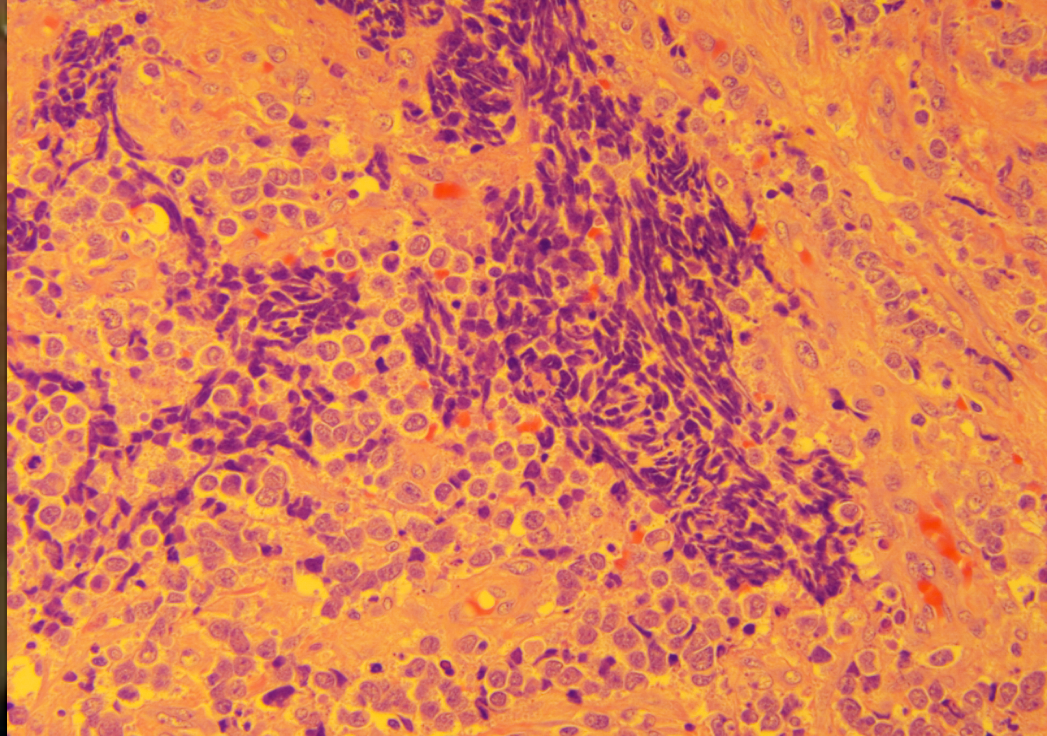
- Warts related to HPV infection.
- Hypogammaglobulinemia
- Infections
- Myelokathexis

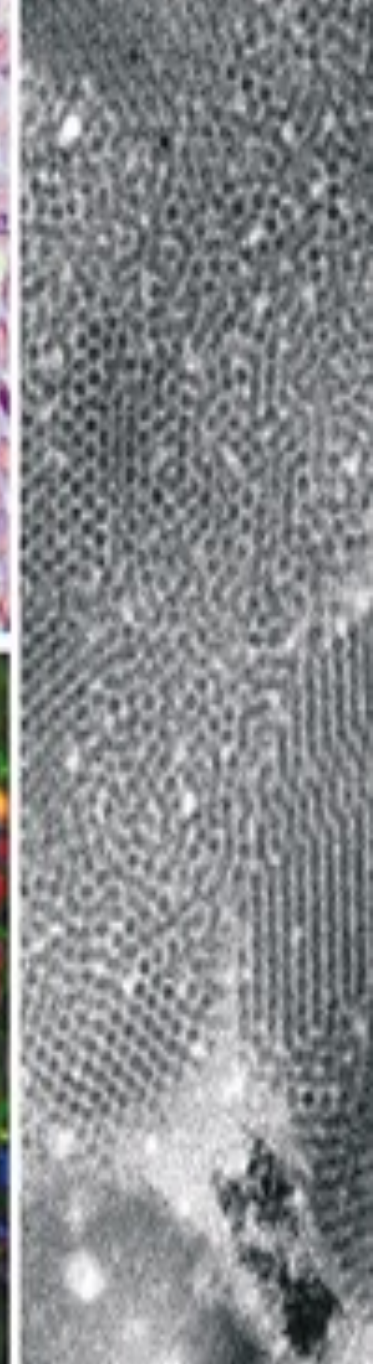
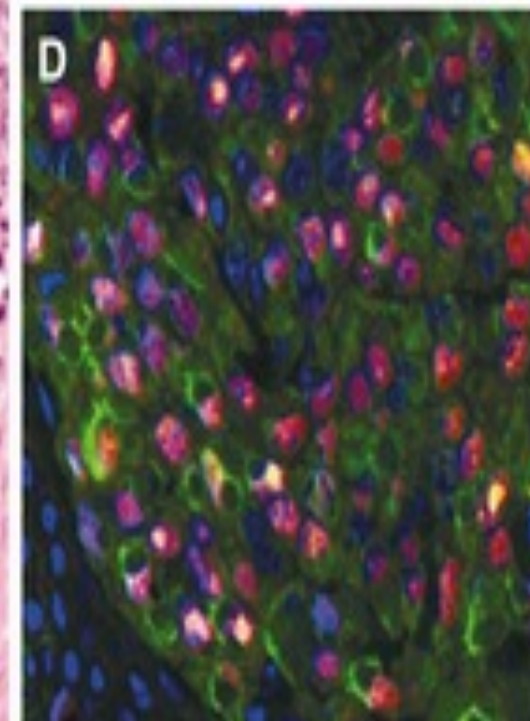
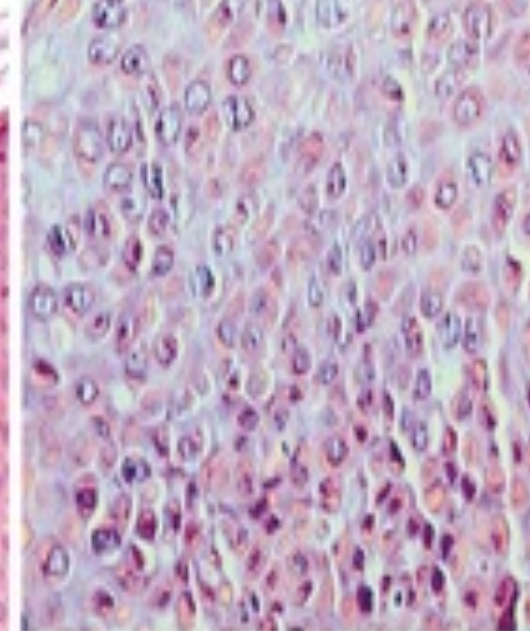
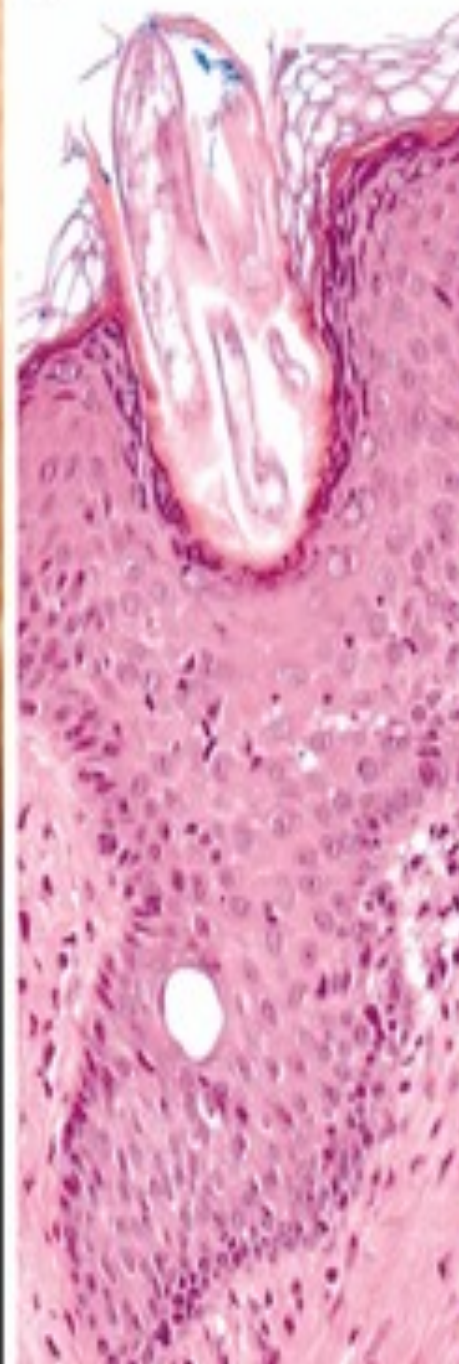
HPyV 11 & 12

- HPyV11= STLPyV: isolated from children with diarrhea (Genome Announce 2013: 24: e00812)
 - Related viruses MWPyV, MXPpyV
- HPyV12: amplified from liver, cecal, and rectal resection specimens

New Jersey PyV









Polyomavirus SV40

- PML-like disease in I.S. monkeys
- Present in stools of healthy kids & hospitalized patients
- SV40 sequences present in FSGS, mesotheliomas, NHL, osteogenic sarcoma
- Not reproduced by all laboratories

B- Lymphotropic Papovavirus (LPV)

- Isolated from lymphoblastic cell lines from African Green Monkey
- HIV+ blood samples, PBLs-HC, PML
- Can infect human B-lymphoblasts & demonstrated in B-cell NHL

Polyomavirus JC

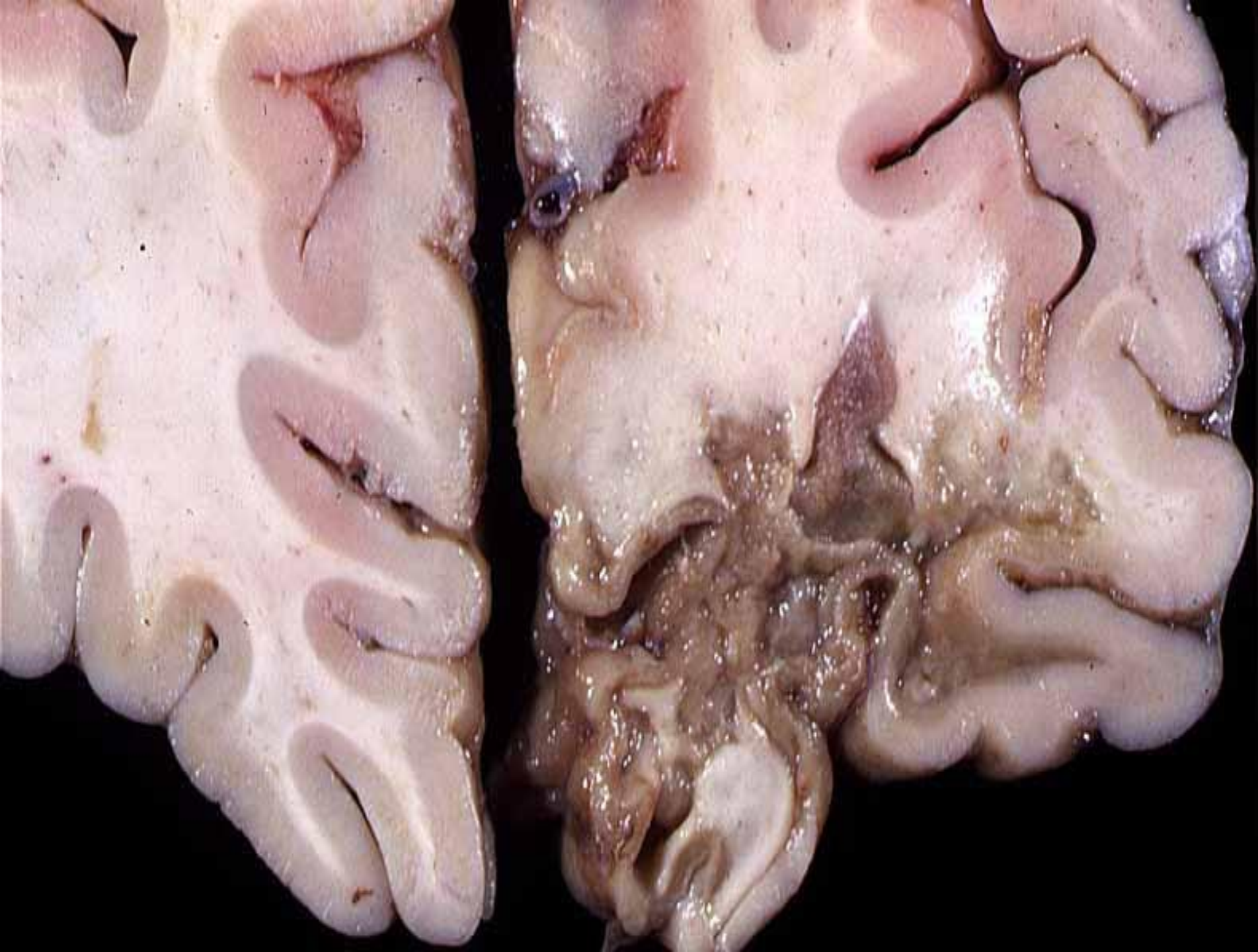
- Diseases associated with infection include:
 - PML, Granule cell neuronopathy (cerebellar)
 - JCV enceph (grey/white matter), JCV cystitis
 - Rare cases of allograft nephropathy (0.9%)
- JCV DNA found in medulloblastoma, glioblastoma multiforme, cancer colon

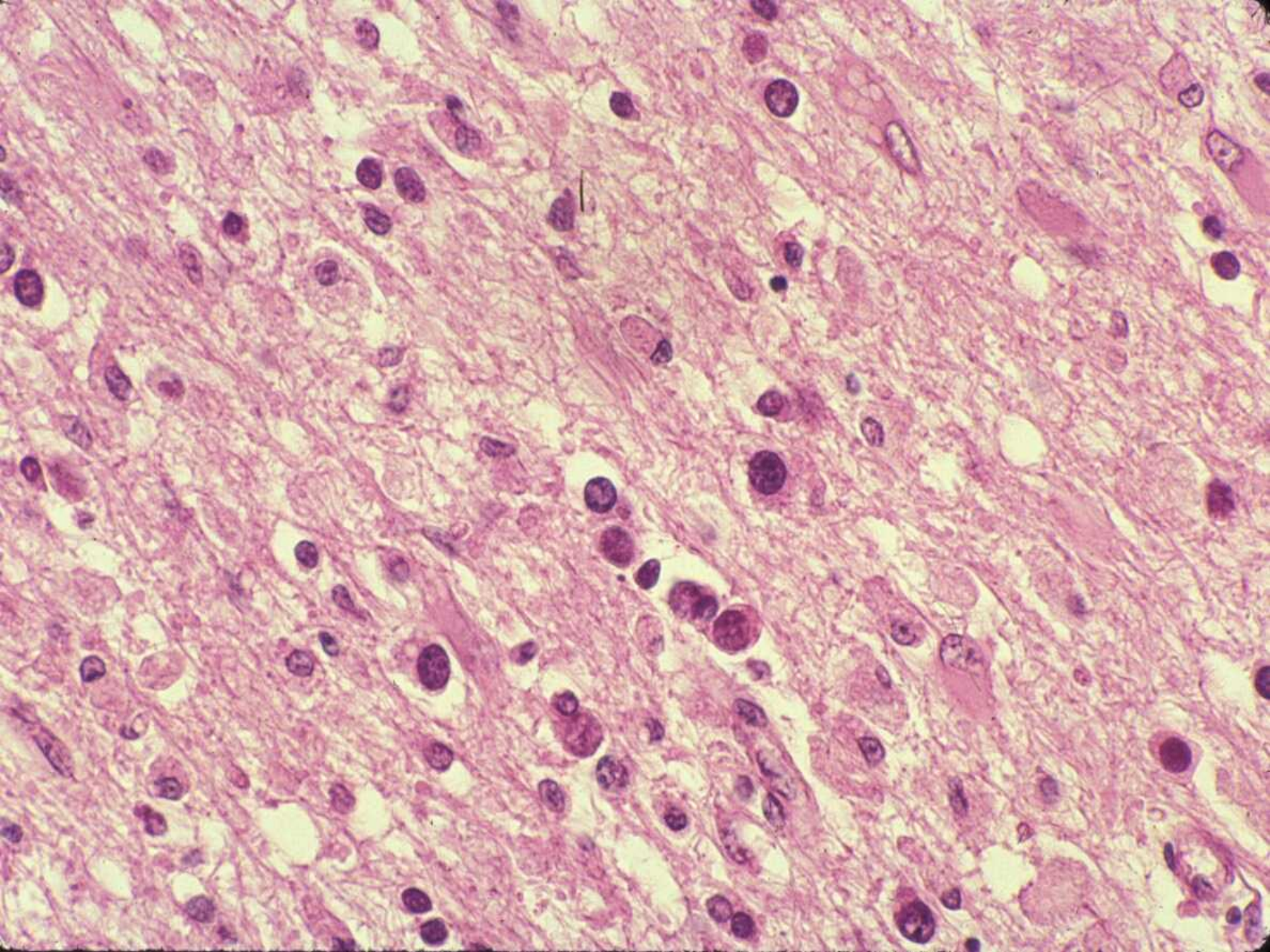
Progressive Multifocal Leucoencephalopathy

- Historically most often in AIDS
- MS patients Rx with Natalizumab (3.85/1000 patients, 19.5 after >24m Rx)
- Psoriasis patients on Efalizumab
- B-cell lymphoma on Rituximab
- Cancer patients on Fludarabine
- Transplant patients on MMF (1.24/1000)

Diagnosis of PML

- Presents as subacute onset neurologic deficits
- Absence of fever, optic nerve involvement & spinal cord disease
- MRI: non-enhancing lesions white matter, no mass effect: suffice for dx **possible PML**
- JCV DNA in CSF allows dx **probable PML**
- Brain biopsy permits diagnosis of **definitive PMLs** but is not always clinically indicated





TREATMENT & PROGNOSIS OF PML

- HAART therapy is started or optimized
- MMF or other I.S. reduced in Tx patients
- Cidofovir, cytarabine, camptohecin, chorpromazine/mirtazapine empirically used
- Historically 80% died < 9 months, now down to ~ 50%; many surviving a few years

Polyomavirus BK

BKV associated syndromes include:

- BK virus nephropathy, stricture ureter (KTx)
- Hemorrhagic cystitis after bone marrow transplant
- HIV associated salivary gland disease
- Not well documented reports: brain, nerves, lung, GIT

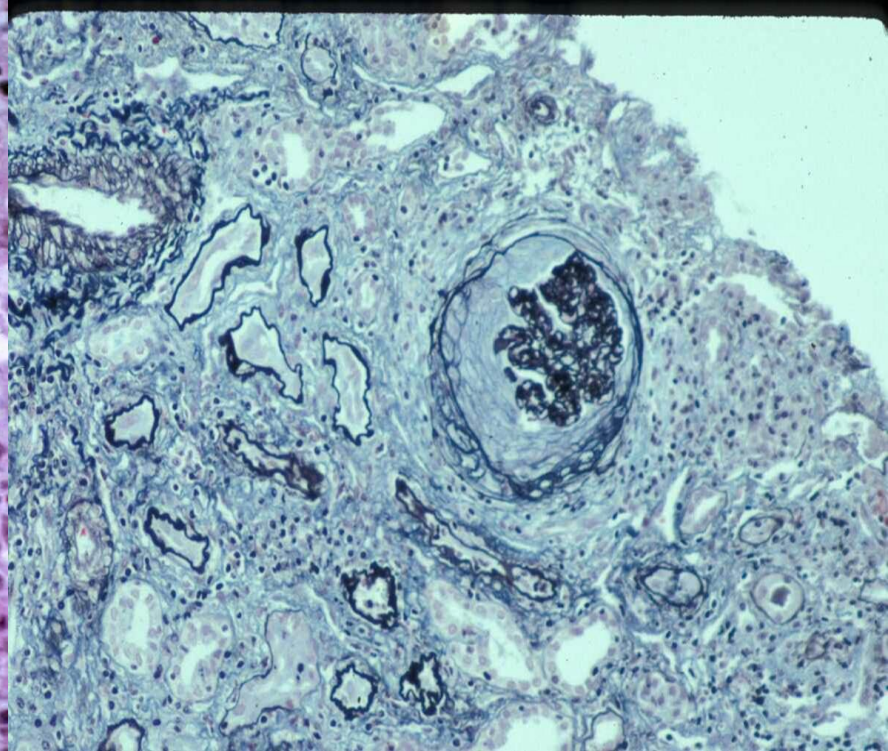
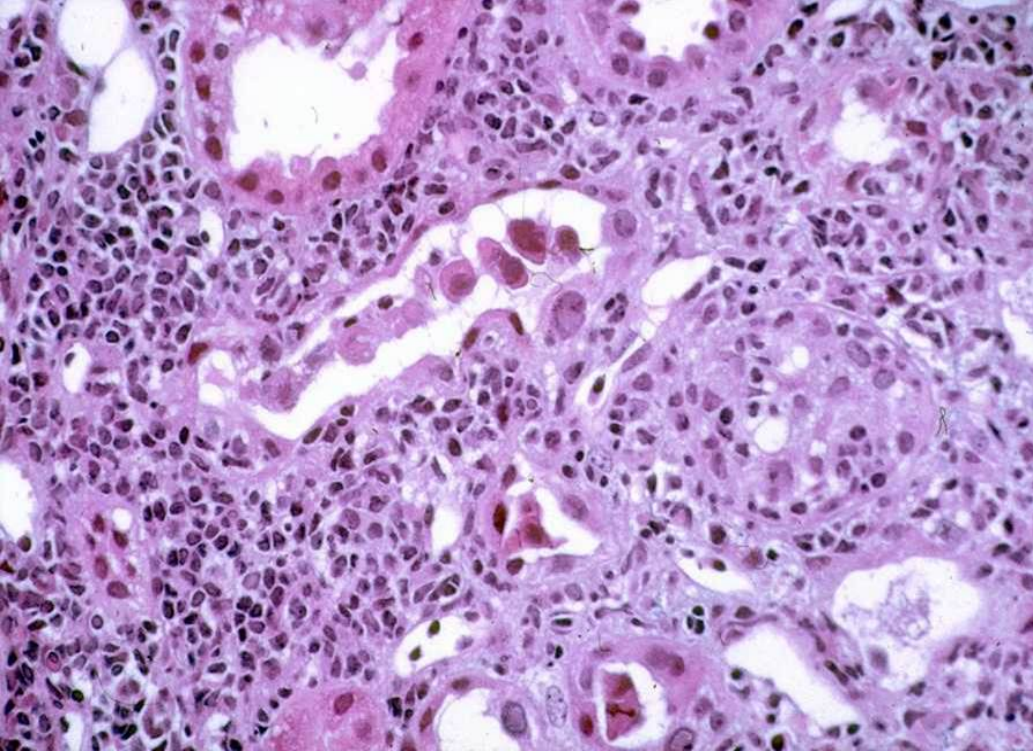
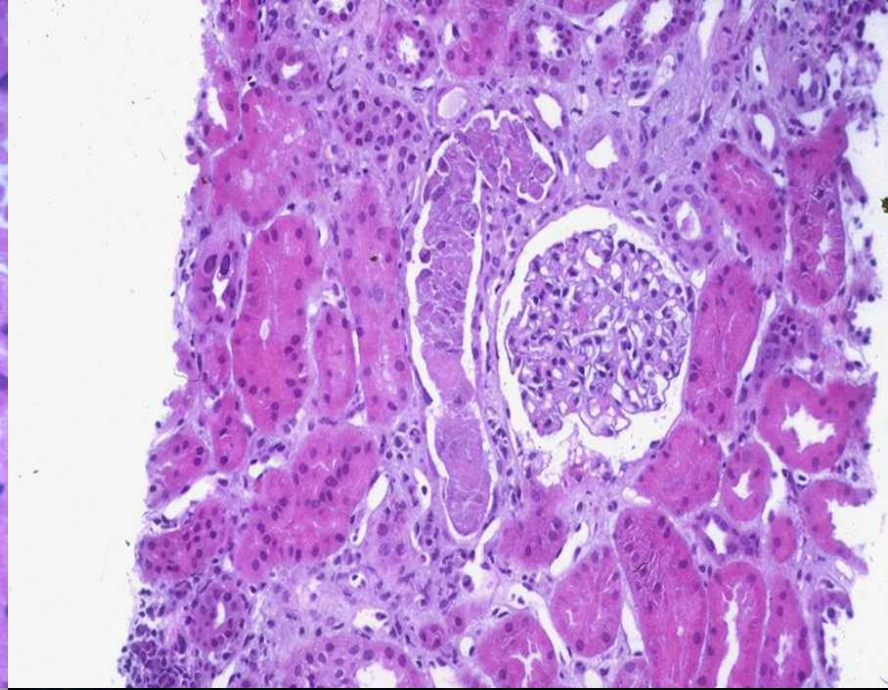
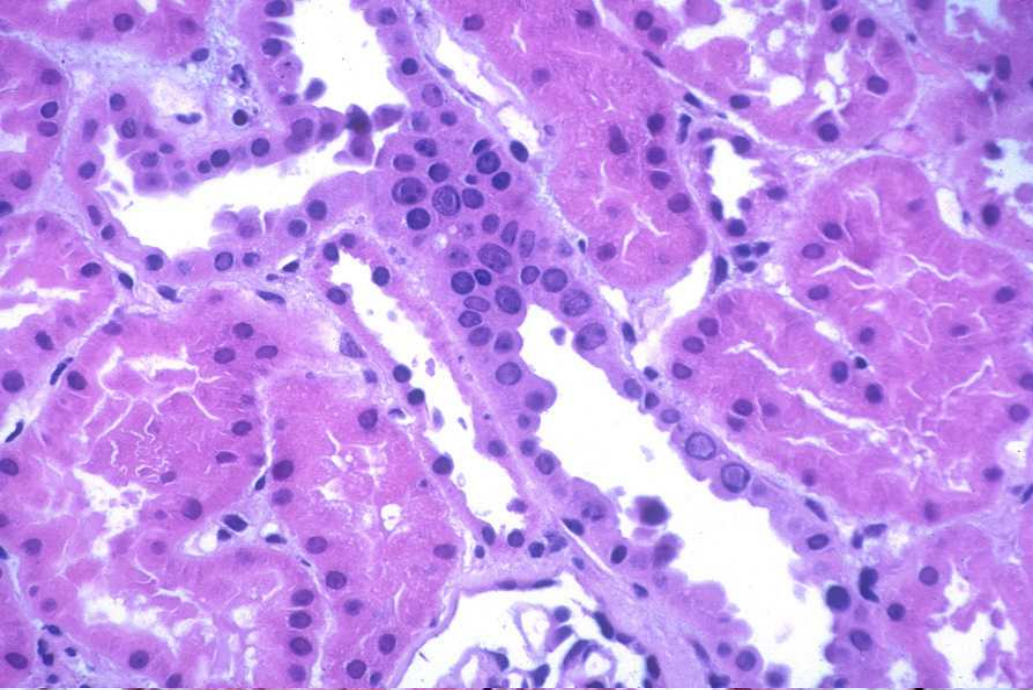
BKV DNA demonstrated in:

- Urothelial, renal cell, prostatic carcinoma
- Lymphoma, oral SCCa

BKV NEPHROPATHY

BKV Infection of the Kidney Has Important Direct & Indirect Effects

- VM up to 29% , BKVN to 10%
- 3 yr GS in SRTR 90% → 79% for TBKV
- Adj OR death censored graft loss 3.1 (~50%VM)
- 2 fold incid AR at 6m; 4.8x at 12m; 85% IFTA
- HR 2.55 for dev class II DSA (50% mixed)



Presumptive BKVN

- Persistent BKV viremia $> 1\text{E}+04$
- High creat required by some but not needed
- Allograft bx without CYTOPATHIC EFFECT
- In-situ hybridization/IHC negative
- Treat in a manner identical to BKVN even if biopsy normal or fulfills criteria for TCMR

Even Definitive BKPyVN can be confused with Rejection

Clinical features favoring nephropathy:

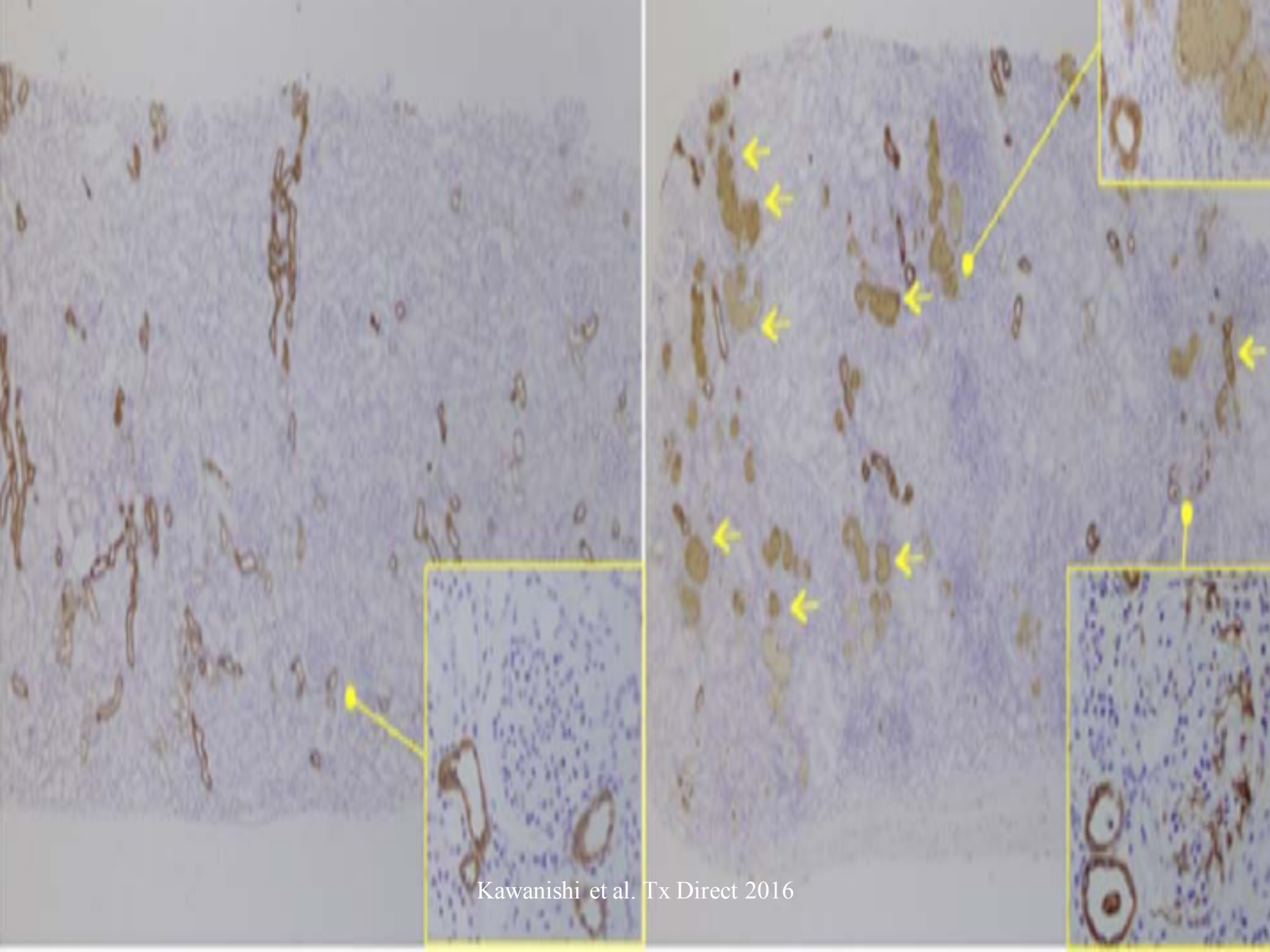
- State of over immunosuppression
- Extremely high viral loads ($E4$ in plasma/ $E7$ U)
- Progressive viremia with one or more log change in viral load over a couple of weeks

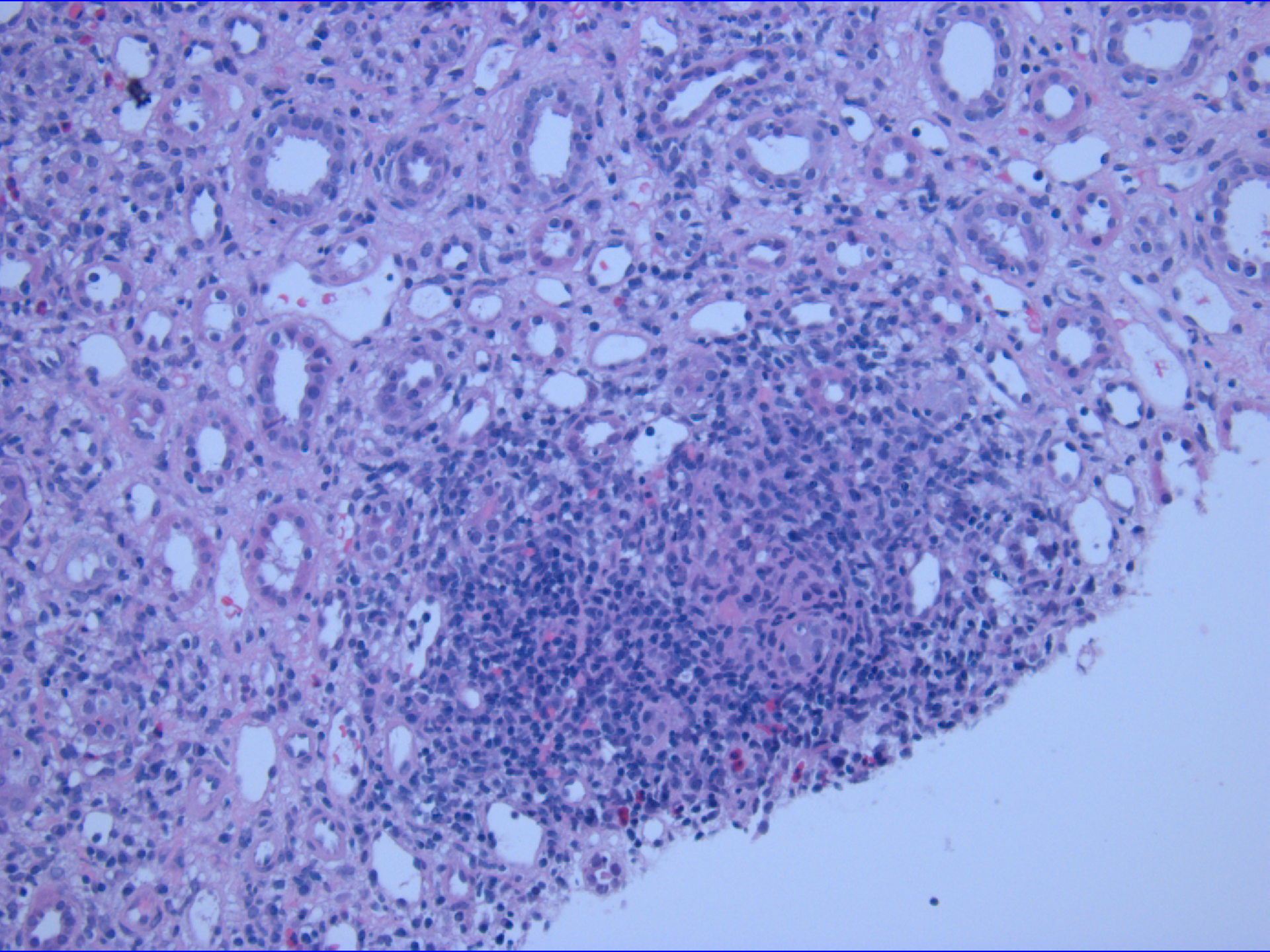
Patterns of Pathologic Injury Favoring BKPyVN

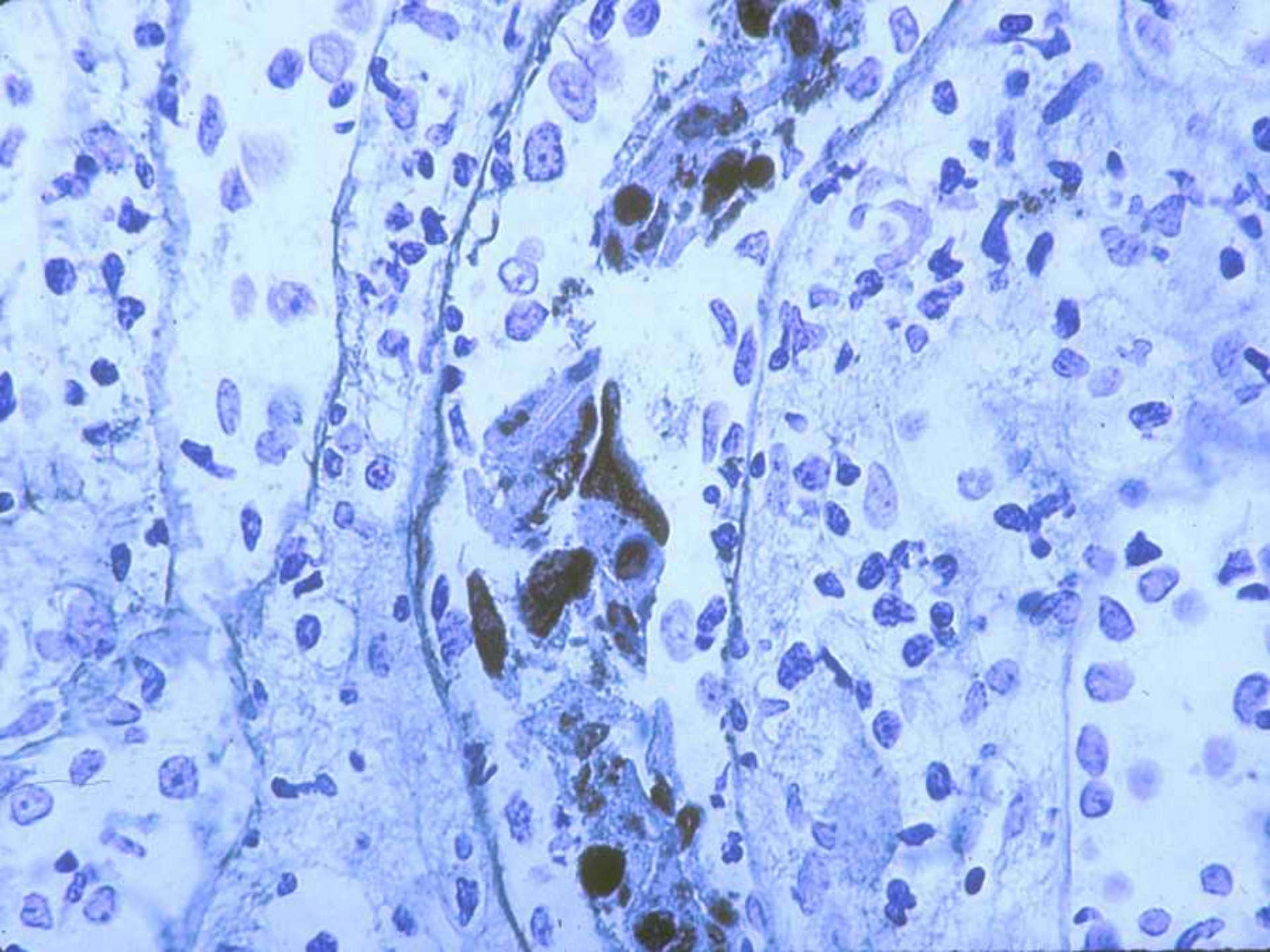
- Relative sparing of cortex
- Medullary ray & CD centered injury
- Sharply defined foci i around tubules with CPE
- Tamm Horsfall Protein cast formation,
extravasation or reflux in glomerular urinary
space

Histopathologic Lesions Favoring BKPyVAN

- Tubular lesions: apoptosis, necrosis, neutrophilic tubulitis
- Interstitial lesions: presence of granulomas
- One glomerular lesion: nuclear atypia in Bowman's capsular epithelium







TREATMENT OF BKVN

- Dose of prednisone: 10 mg/d or less
- CNI 25-50%, A-metabolites 50-100%
- Dose of MMF<1g, Aza<75mg/d)

- Blood target levels <6 ng/ml for Tac, <125ng/ml for CSA, < 6ng/ml Sirolimus
- Switch strategies: Tac-Csa, MMF-Aza-Ev/Sir
- Refractory cases Cido/Lef/FQ/IVIG
(OFF LABEL USES ≠ APPROVED BY FDA)

CIDOFOVIR FOR BKVN

University of Leuven

- N=41 (1 mg/kg weekly, maximum 10w)
- Graft loss 15% Rx vs 73% Non-Rx 73%
- NO effect virus clearance; 3/26 anterior uveitis
- Patient assignment based on ability to pay (838E)
(socioeconomic status, education, nutrition)

LEFLUNOMIDE

French Experience

- MMF replaced by Leflunomide, & blood levels kept 40-80 ug/ml
- Viremia clearance 5/12
- Graft function became worse 4/12 (two lost)
- Anemia, thrombocytopenia 6/12 (D/C 2/12)
- Mild elevation GGT 3/12
- Fungal pneumonia (4) CMV viremia (10)
- Others describe hemolysis, TMP

Faguer et al. Transplant Int 2007; 20:962

QUINOLONES

Cipro/Gati/Levofloxacin

- Inhibit DNA topoisomerase activity.
- Several initial anecdotal case reports showed benefit: could not be confirmed in larger studies.
- Example: Randomized, double blind, placebo controlled, clinical trial using 500 mg/d Levofloxacin for 3 m did not affect incidence of BK viruria

PROLIFERATIVE SIGNAL INHIBITORS

- Sirolimus (Rapamycin), Everolimus
- Rationale to leverage anti-proliferative, and nephrotoxicity sparing action
- Sirolimus also inhibits mTOR pathway and may have **prophylactic** anti-BKV effect, viz a lower incidence BKN in patients on this regimen.

Small studies on established BKVN also show some **therapeutic** effect but I.S. also reduced

Potential Role IVIG in Rx BKV Infection

- Neutralize circulating circ. BKV very effectively
- Lower plasma load even if no penetration tubules
- Should facilitate more effective immune response
- Available studies show no dramatic effect & viral clearance takes weeks to months
- Some patients show progressive disease

Caveats in Existing Studies of IVIG

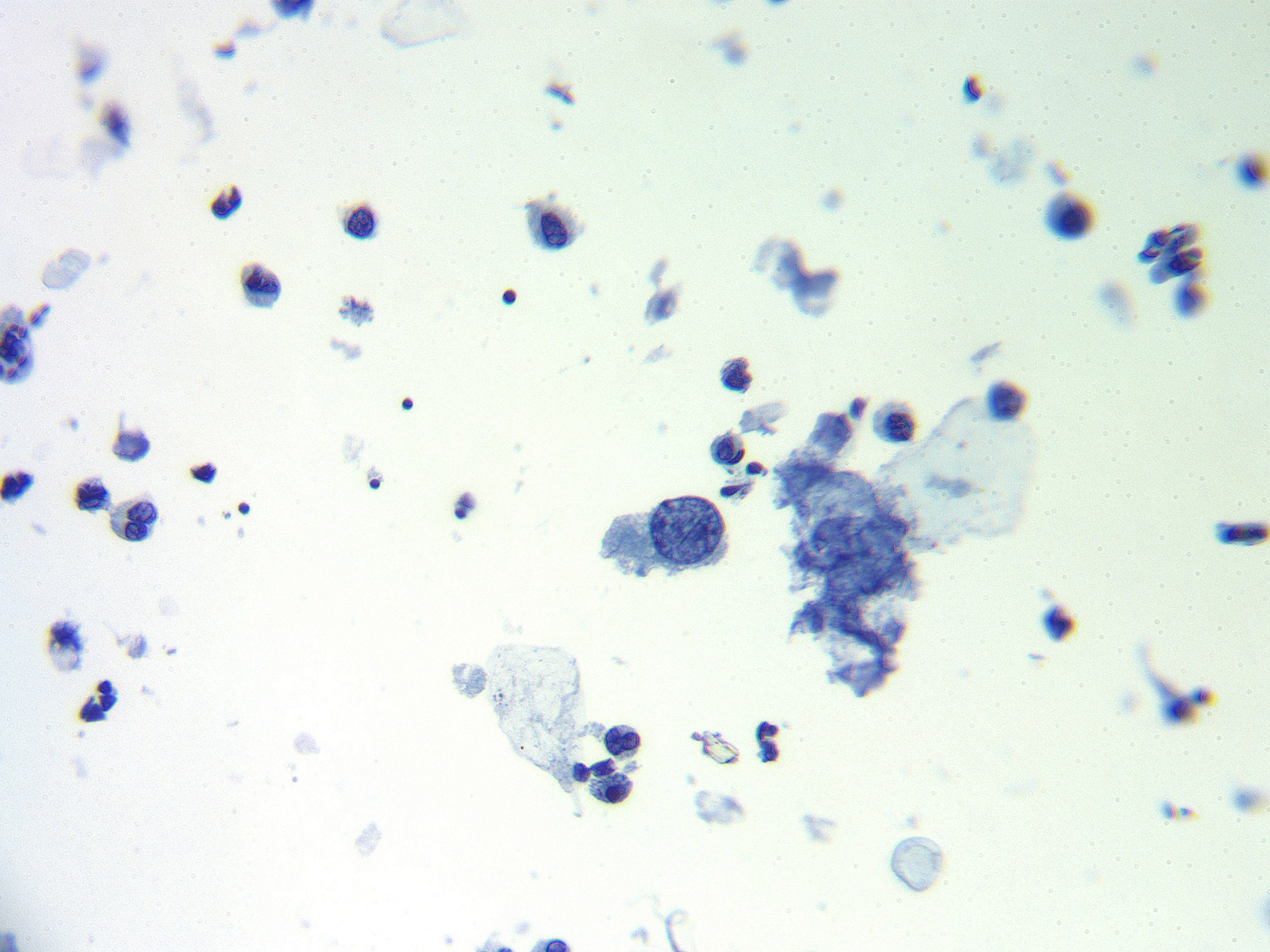
- Treatment was initiated late stage of disease
- Anti-BK titer & Ab $T^{1/2}$ of preparations unknown
- Monitored by PCR based DNA assays
- Dose/duration varied widely (constrained by cost)
- More rationally designed trials are indicated

SCREENING FOR BK VIRUS



MONITORING TECHNIQUES FOR BK VIRUS

- Urine cytology
- Urine BKV DNA by q-PCR
- Plasma PCR

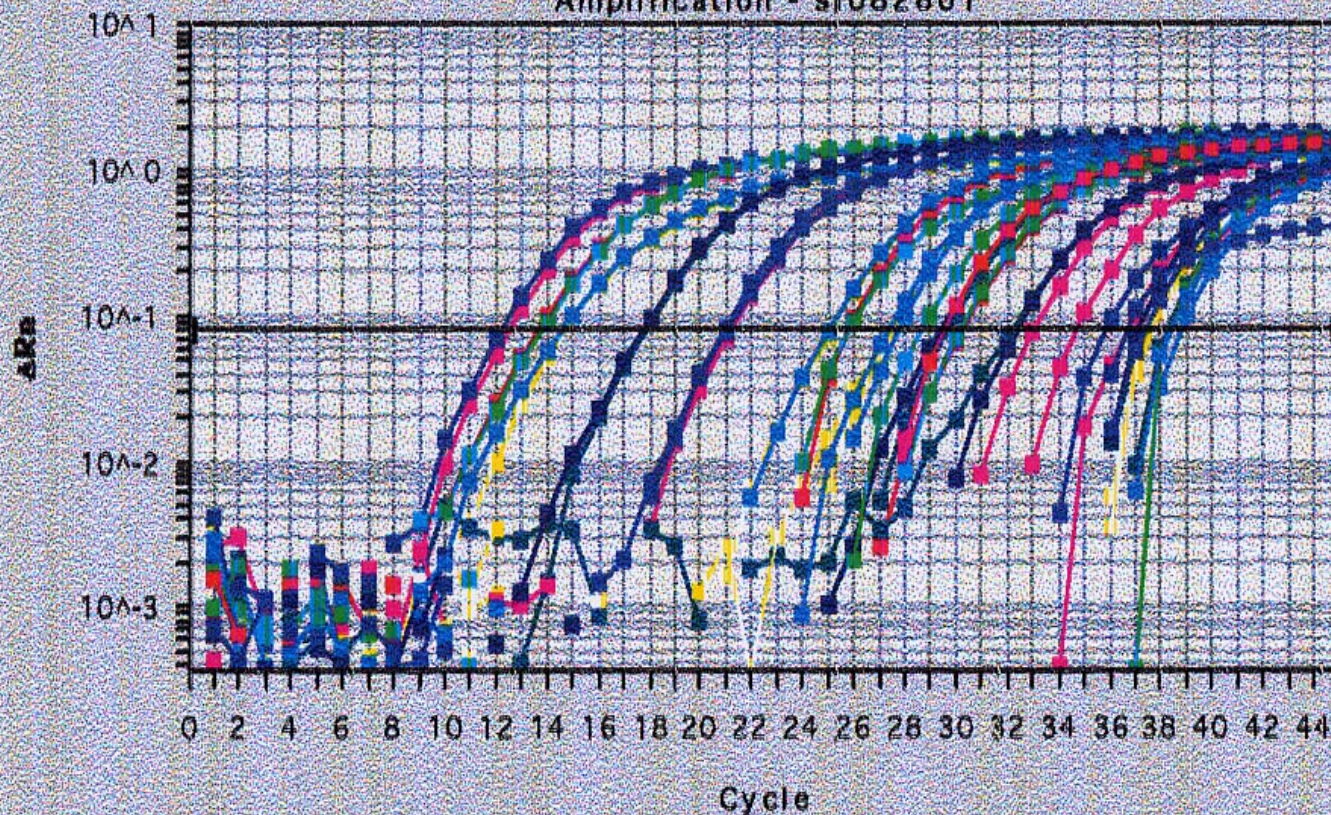


Urine Cytology as a Screening Tool

Technically simple, low cost test, high NPV

1. PPV BKN only ~30% (unless inflammation)
2. Specimen adequacy criteria undefined
3. Requires experienced pathologist
4. Open to subjective interpretation
5. Cannot detect free virus
6. Does not allow distinction BKV & JCV

Amplification - sf082801



Samples

- ☒ FAM - E3
- ☒ FAM - E4
- ☒ FAM - E5
- ☒ FAM - E6
- ☒ FAM - E7
- ☒ FAM - E8
- ☒ FAM - E9
- ☒ FAM - E10
- ☒ FAM - E11

Viewer:

Reporter:

Threshold Cycle Calculation

Threshold

Use Threshold:

Mult. * Stddev: *

Omit Threshold:

Baseline

Start: Stop:

	Ct	Std Dev	
FAM - E3	29.487	0.000	
FAM - E4	28.603	0.001	
FAM - E5	25.130	0.001	
FAM - E6	25.048	0.001	▲
FAM - E7	21.167	0.000	▼

Primer on Interpretation of BKV Viral Loads

- Urine $1e7$ copies/mL
- Plasma 5-10,000 copies/mL BKV
- Each lab needs to develop own cut offs
- Inter-laboratory standardization needed & availability of WHO reference should help

Urine versus Plasma PCR

URINE

- Flags at risk patients at earlier time point
- Picks up occ cases who are not viremic
- Better monitoring of treated patients once plasma negative
- JCVN & HC

BLOOD

- More invasive but
- Detects clinically more significant infection
- Blood testing less prone to false positive results & to inhibitors (vu-vm+)

Frequency of Screening



Basel 2005:

- 3m for 2 yrs

AST 2009, 2013 :

At least 3mx2y;
annually to 5y

KDIGO 2009:

- Plasma 1m x 3-6m,
3m x 1yr

- Unexplained graft
dysf

- After Rx AR

Is Screening Cost-Effective?

- Incidence of BKVN at medical center
- Screening frequency
- Charges incurred by laboratory testing
- Savings from reduced immunosuppression
- Expenses ensuing from any antiviral therapy
- Financial benefits of avoiding graft loss

CONCLUSIONS

- Human PV family grown to 14 members
- Kept in D/D for syndromes affecting CNS, salivary glands, lungs, GIT, UGT, skin
- Commonest PV: BK 20-60% KTx VU, risk BKN
- Early Dx BKVN improves graft outcome
- Facilitated by screening programs (cytology/PCR)
- Cornerstone of Rx for BKVN is reduced IS
- Good anti-viral agents not yet available

