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ON MALIGNANCIES IN AIDS AND
OTHER ACQUIRED IMMUNODEFICIENCIES (ICMAOI)

Disclosures

Christopher Buck, PhD

**No Relevant Financial Relationships with
Commercial Interests**

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NIH Main Campus



Merkel Cell Polyomavirus and Two Novel Polyomaviruses Are Chronically Shed from Human Skin

Chris Buck

**Tumor Virus Molecular Biology Section
Lab of Cellular Oncology
National Cancer Institute, Bethesda, MD
buckc@nih.gov**

Do Polyomaviruses Cause Human Cancer?

• **Answer (pre-2008):** human polyomavirus DNA and T antigen can be found in tumors... sometimes

• **Problem:** practically all adults are seropositive

• **Problem:** the grim specter of “hit and run” etiology

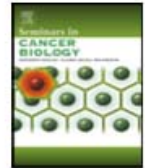
Seminars in Cancer Biology 19 (2009) 261–269



Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer



Review

JC Virus: An oncogenic virus in animals *and* humans?

Melissa S. Maginnis, Walter J. Atwood*

Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, 70 Ship Street Box G-E4 Providence, RI 02903, United States

Seminars in Cancer Biology 19 (2009) 252–260



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Review

BK virus and human cancer: Innocent until proven guilty

Johanna R. Abend¹, Mengxi Jiang¹, Michael J. Imperiale*

Department of Microbiology and Immunology and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI 48109-5620, United States

Do Polyomaviruses Cause Human Cancer?

• Answer (post-2008): Very likely!

• Evidence supports a causal role for a new human polyomavirus.
Key findings confirmed in multiple labs

Clonal Integration of a Polyomavirus in Human Merkel Cell Carcinoma

Huichen Feng, Masahiro Shuda, Yuan Chang,* Patrick S. Moore*

Merkel cell carcinoma (MCC) is a rare but aggressive human skin cancer that typically affects elderly and immunosuppressed individuals, a feature suggestive of an infectious origin. We studied MCC samples by digital transcriptome subtraction and detected a fusion transcript between a previously undescribed virus T antigen and a human receptor tyrosine phosphatase. Further investigation led to identification and sequence analysis of the 5387–base-pair genome of a previously unknown polyomavirus that we call Merkel cell polyomavirus (MCV or MCPyV). MCV sequences were detected in 8 of 10 (80%) MCC tumors but only 5 of 59 (8%) control tissues from various body sites and 4 of 25 (16%) control skin tissues. In six of eight MCV-positive MCCs, viral DNA was integrated within the tumor genome in a clonal pattern, suggesting that MCV infection and integration preceded clonal expansion of the tumor cells. Thus, MCV may be a contributing factor in the pathogenesis of MCC.

Polyomaviruses have been suspected as potential etiologic agents in human cancer since the discovery of murine polyoma virus (MuPyV) by Gross in 1953 (1). However,

although polyomavirus infections can produce tumors in animal models, there is no conclusive evidence that they play a role in human cancers (2). These small double-stranded DNA viruses

[~5200 base pairs (bp)] encode a variably spliced oncoprotein, the tumor (T) antigen (3, 4), and are divided into three genetically distinct groups: (i) avian polyomaviruses, (ii) mammalian viruses related to MuPyV, and (iii) mammalian polyomaviruses related to simian virus 40 (SV40) (5). All four known human polyomaviruses [BK virus (BKV), JCV, KIV, and WUV (6, 7)] belong to the SV40 subgroup. In animals, integration of polyomavirus DNA into the host genome often precedes tumor formation (8).

Merkel cell carcinoma (MCC) is a neuroectodermal tumor arising from mechanoreceptor Merkel cells (Fig. 1A). MCC is rare, but its incidence has tripled over the past 2 decades in the United States to 1500 cases per year (9). It is one of the most aggressive forms of skin cancer; about 50% of advanced MCC patients

Molecular Virology Program, University of Pittsburgh Cancer Institute, University of Pittsburgh, 5117 Centre Avenue, Suite 1.8, Pittsburgh, PA 15213, USA.

*These authors contributed equally to this work. To whom correspondence should be addressed. E-mail: yc70@pitt.edu (Y.C.); psm9@pitt.edu (P.S.M.)

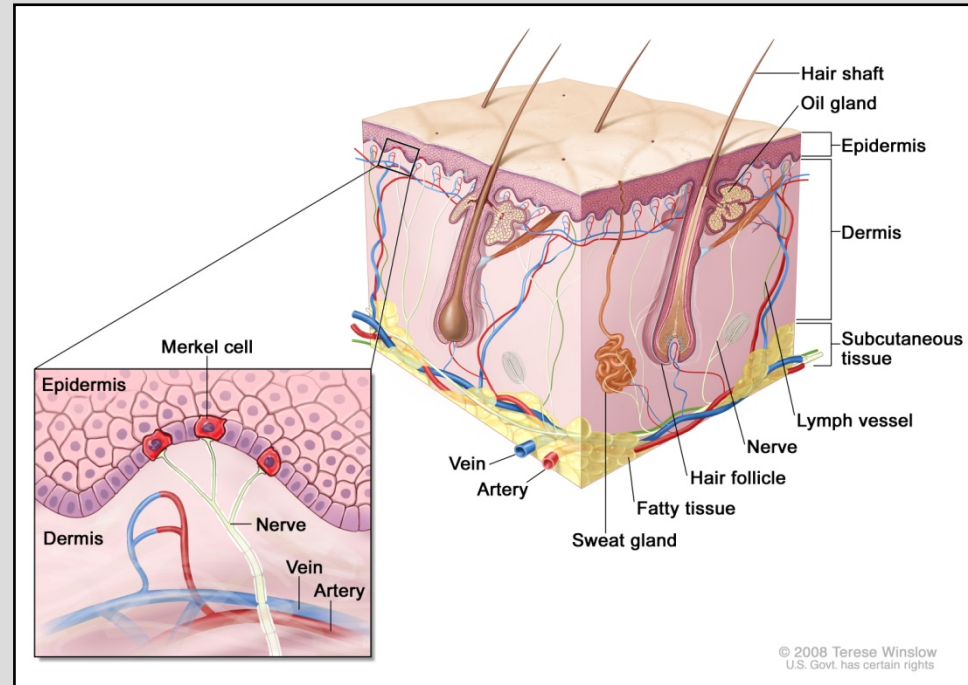
Merkel Cell Carcinoma

- Fast-growing, highly lethal form of skin cancer (>30% mortality)
- 1400 cases per year in the US. Three-fold increase in incidence between 1986 and 2001
- More prevalent among light skinned individuals, usually sun-exposed sites
- More prevalent in aged or immunocompromised subjects. 11-fold increased risk of MCC among AIDS patients (Eric Engels)



Merkel Cells

- Associated with sensory neurons, required for sensation of light touches¹
- Although Merkel cells have some neuroendocrine features recent work shows they are of epidermal origin²
- Merkel cells are somewhat rare, making up only a very small percentage of cells in the epidermal basal layer



¹Maricich et al (2009) *Science* 324:1580

²Van Keymeulen (2009) *JCB* 187:91

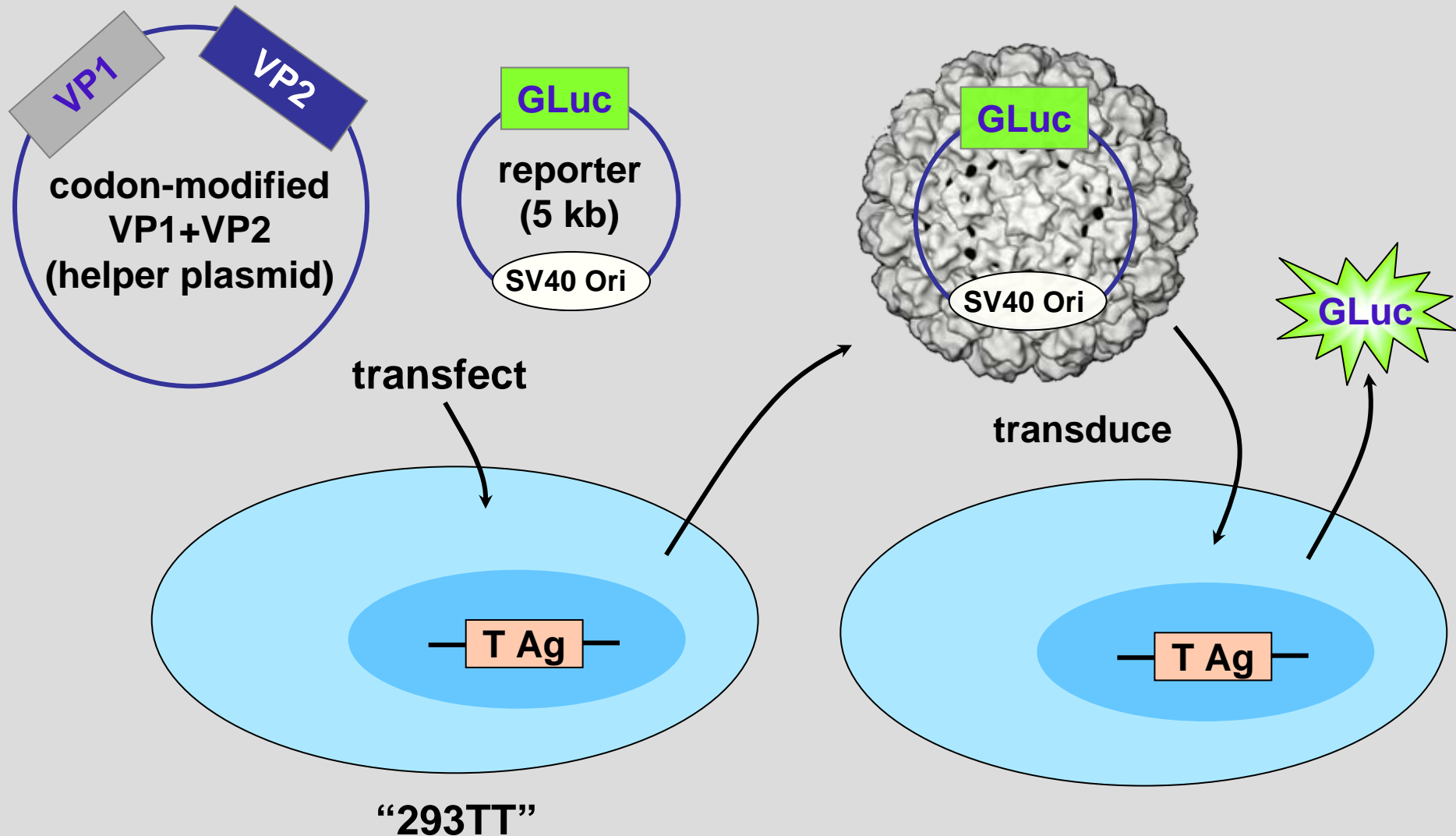
MCV is Probably a Causal Factor in MCC

- **About 80% of MCC tumors harbor MCV DNA**
- **In many instances, the viral DNA is clonally integrated into the DNA of the primary MCC tumor and its metastases**
- **MCV DNA found in MCC tumors has a characteristic pattern of mutations in T antigen that preserve its oncogenic domains but destroy the helicase domain needed to replicate the viral DNA**
- **T antigen protein can be detected immunohistochemically in most MCC tumors**

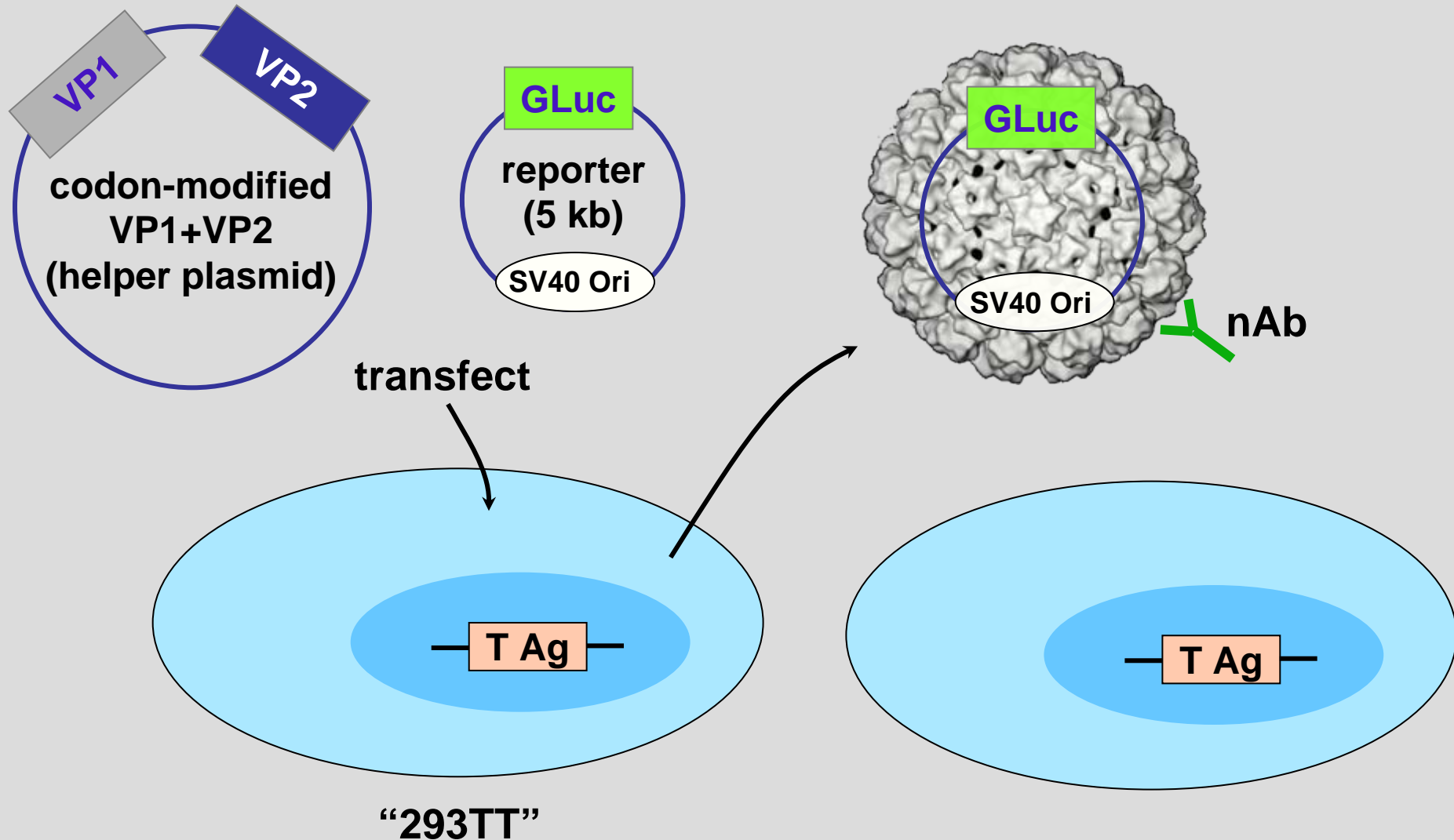
Questions

- How common is MCV infection?
- What tissues does MCV productively infect?
- Is infection acute or chronic?
- Are there distinct MCV genotypes/serotypes?
- Are some genotypes more prevalent in MCC tumors?

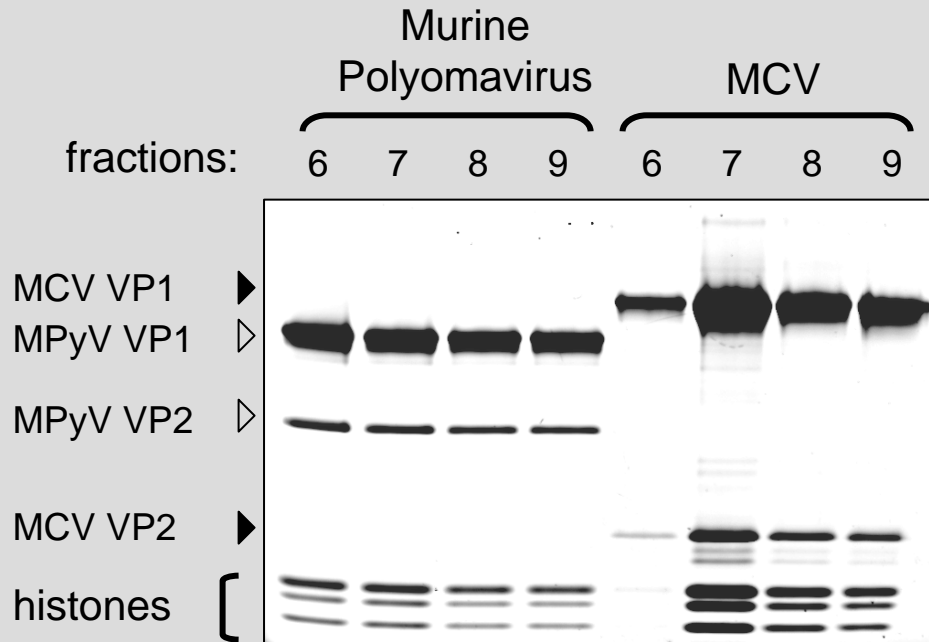
MCV Reporter Vector-Based Neutralization Assay



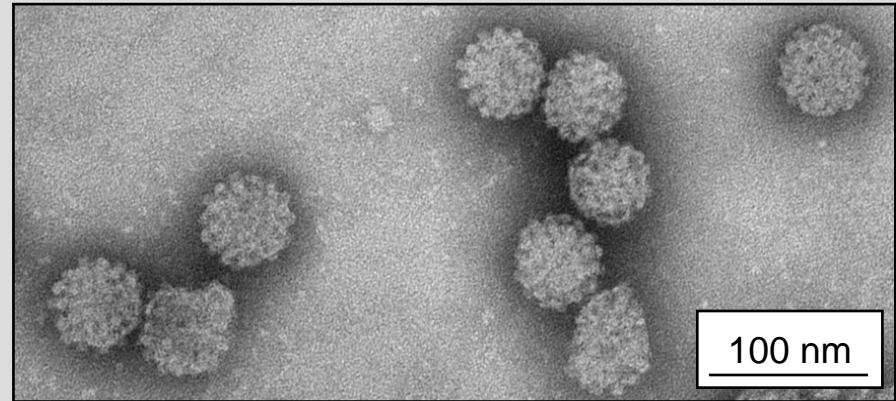
MCV Reporter Vector-Based Neutralization Assay



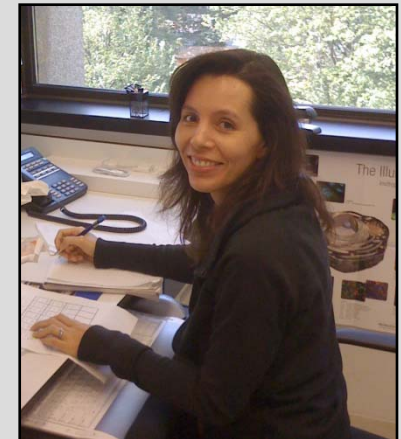
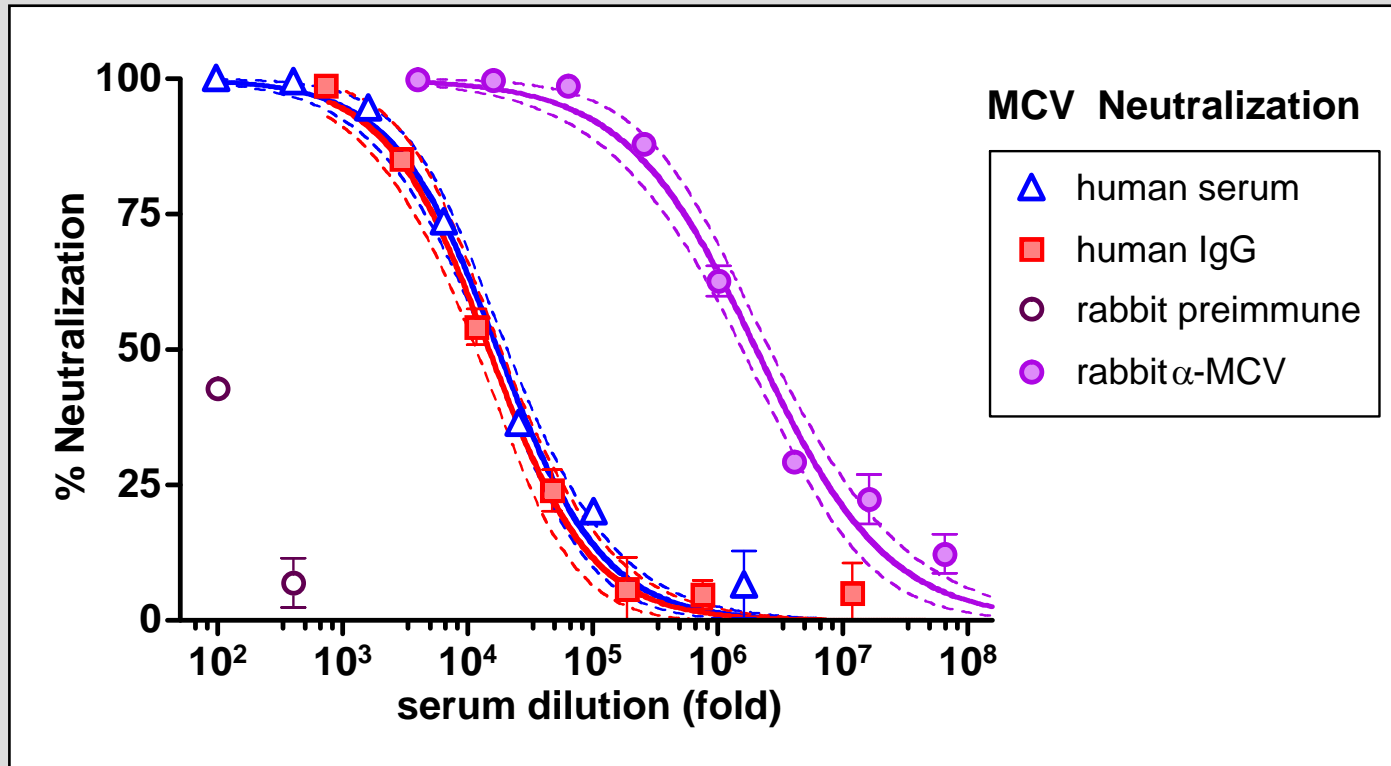
Particle Production



Optiprep velocity/density gradient
SYPRO Ruby stained gel



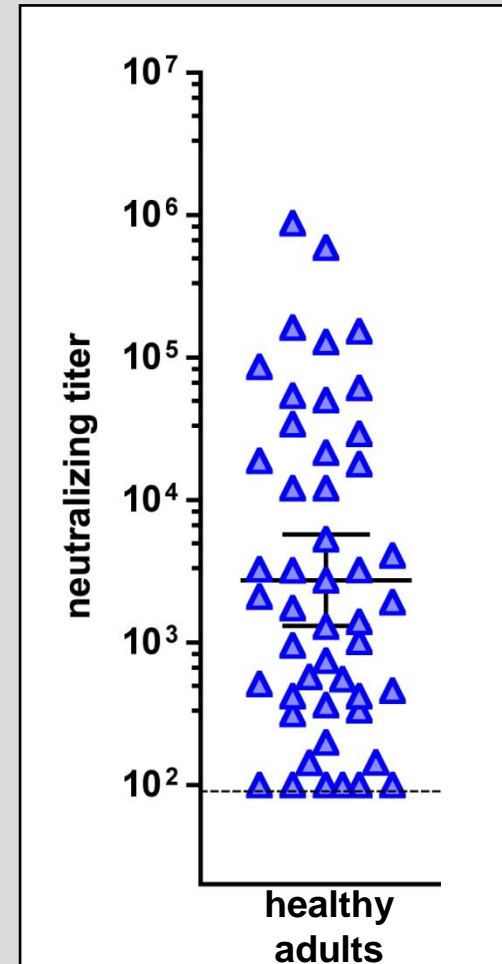
Neutralization Assay



Diana Pastrana

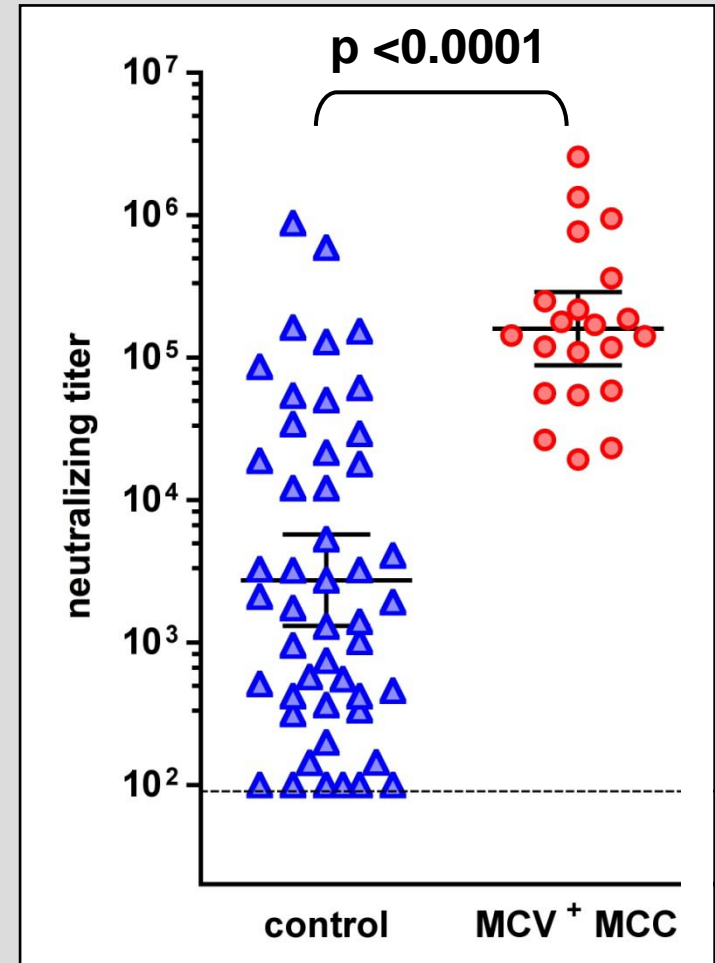
Quantitative Serology

- 85% of healthy adult subjects are seropositive for MCV

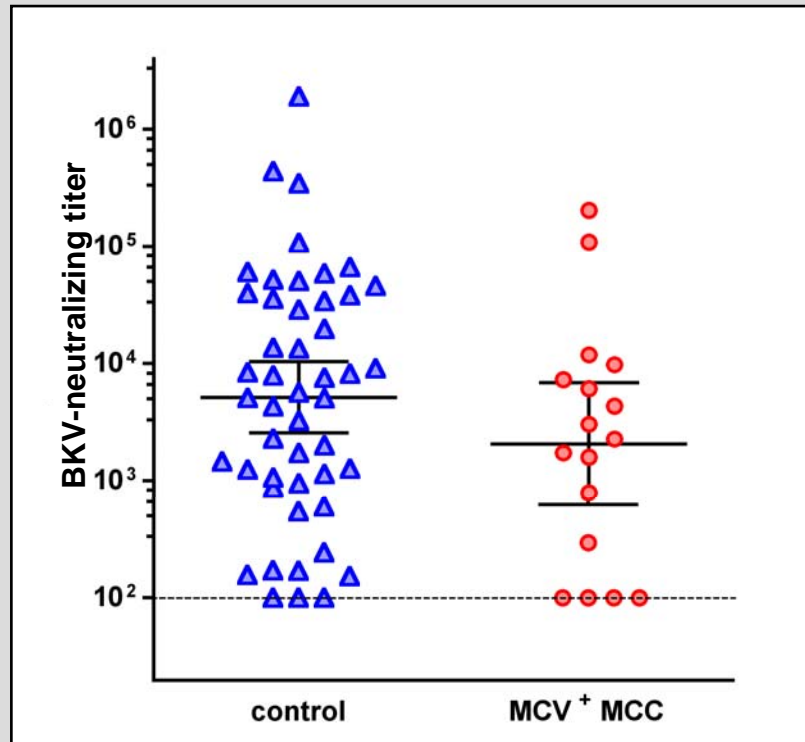


Quantitative Serology

- 85% of healthy adult subjects are seropositive for MCV
- MCC patients have unusually high titers of high-affinity MCV-neutralizing antibodies



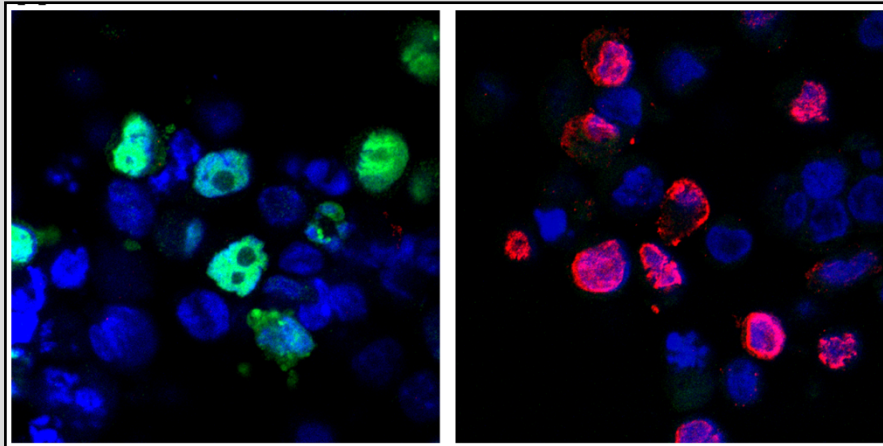
MCC Patients Have Normal Titers Versus BK Polyomavirus



- Generalized loss of immune control over all polyomaviruses is unlikely

MCC Tumors Express Little or No VP1

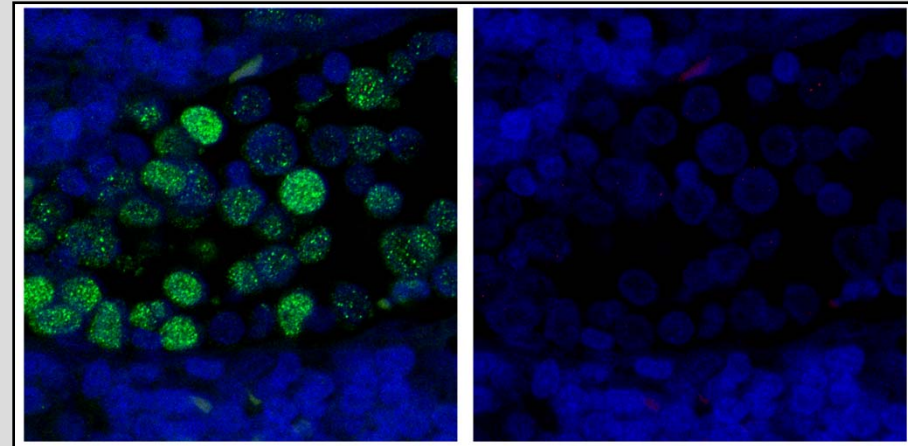
Transfected HeLa



T antigen

VP1

MCC Tumor



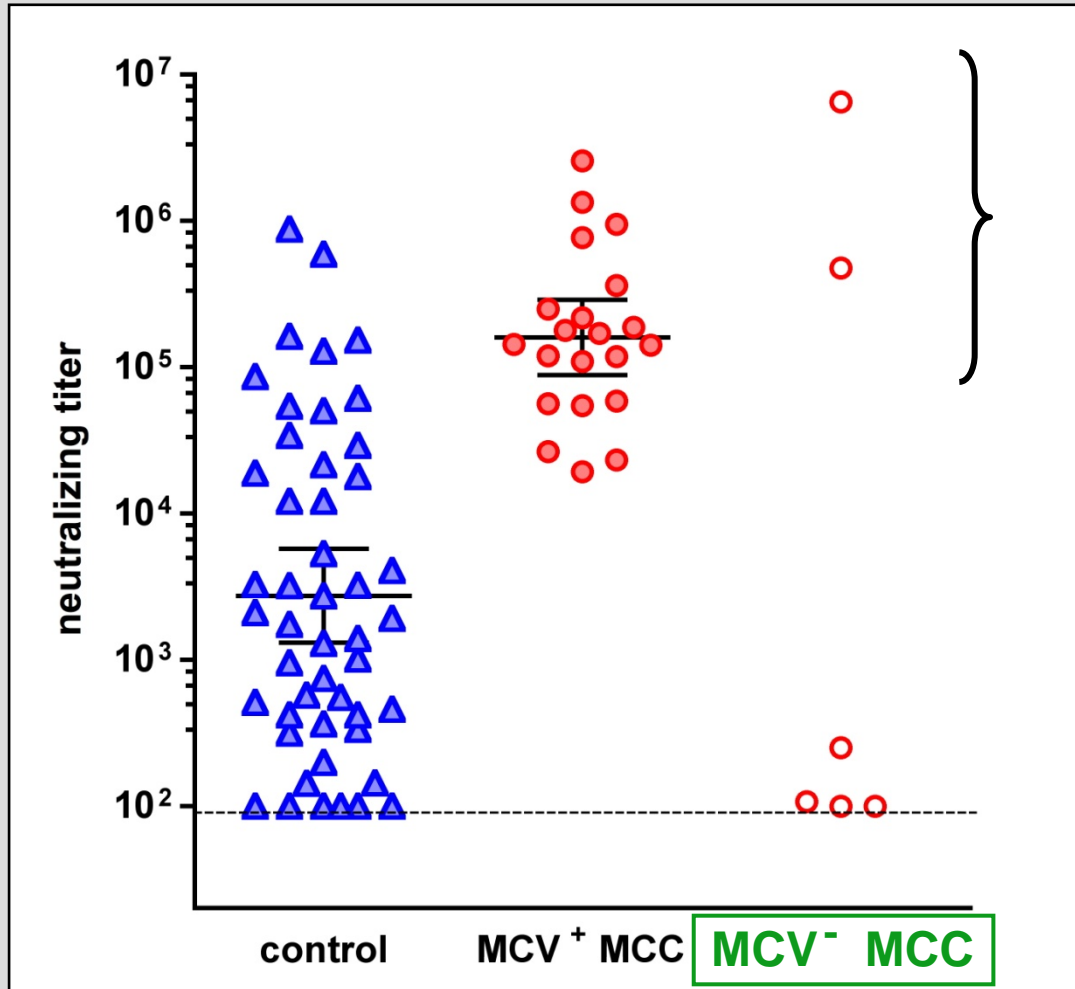
T antigen

VP1

• MCC tumors probably aren't a major source of VP1 immunogen

10/10 MCV T Ag+
MCC tumors scored
VP1 negative

MCV vs MCC: Some Dirty Little Secrets



- Most MCC tumors have less than one MCV genome per tumor cell

- Some patients with MCV-negative MCC tumors show serological evidence of MCV involvement

....hit and run in progress?

- Possible good news: even if the virus disappears from the nascent tumor, the telltale fingerprints of vigorous antibody responses may remain

Working Model

- **Chronically high MCV viral load leads to stronger MCV-neutralizing antibody responses**
- **High viral load correlates with development of MCC**

MCV DNA is Shed from Healthy Skin

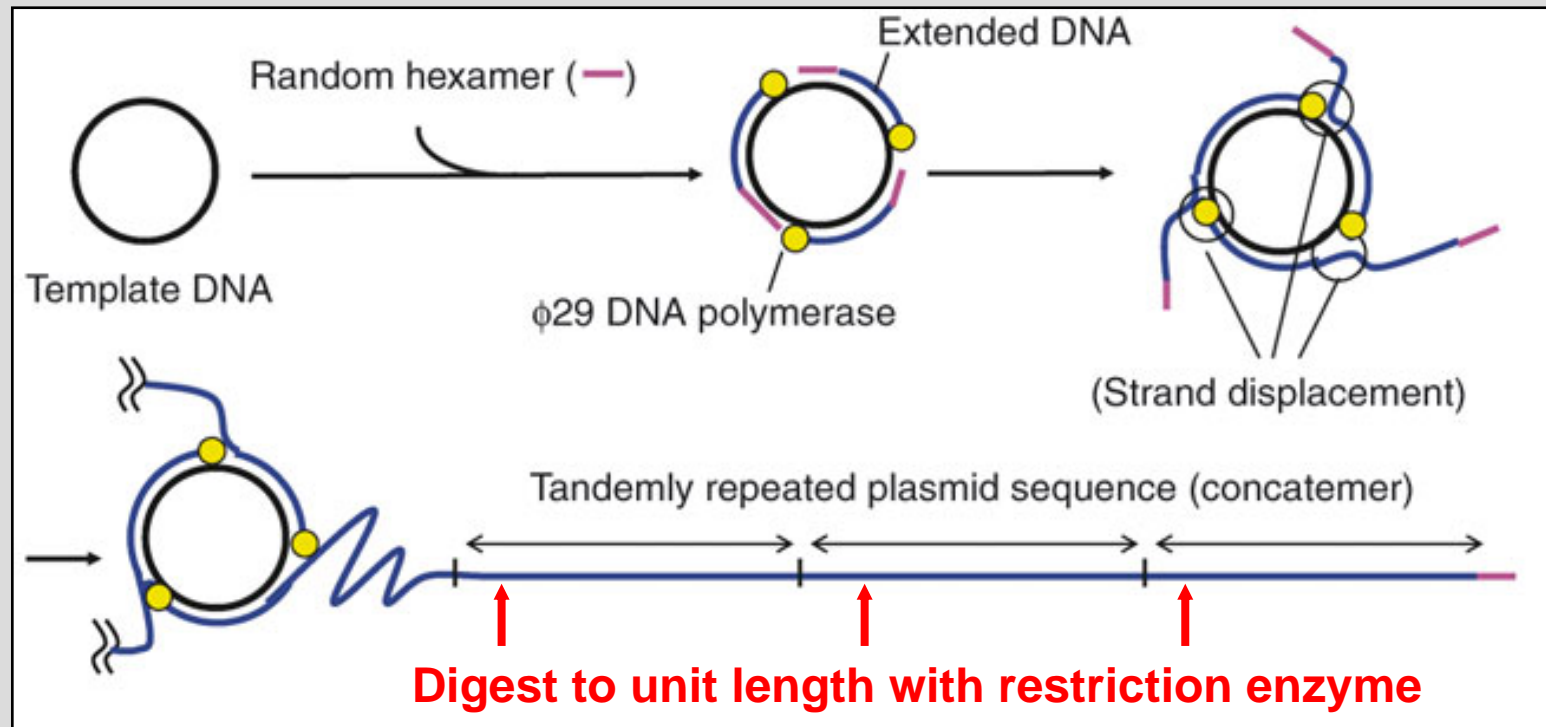
Merkel Cell Polyomavirus DNA in Persons without Merkel Cell Carcinoma

Ulrike Wieland, Cornelia Mauch,
Alexander Kreuter, Thomas Krieg,
and Herbert Pfister

Merkel cell polyomavirus **MCPyV DNA was detected** in 88% of Merkel cell carcinomas in contrast to 16% of other skin tumors. MCPyV was also found in anogenital and oral samples (31%) and eyebrow hairs (50%) of HIV-positive men and **in forehead swabs (62%) of healthy controls.** MCPyV thus appears to be widespread.

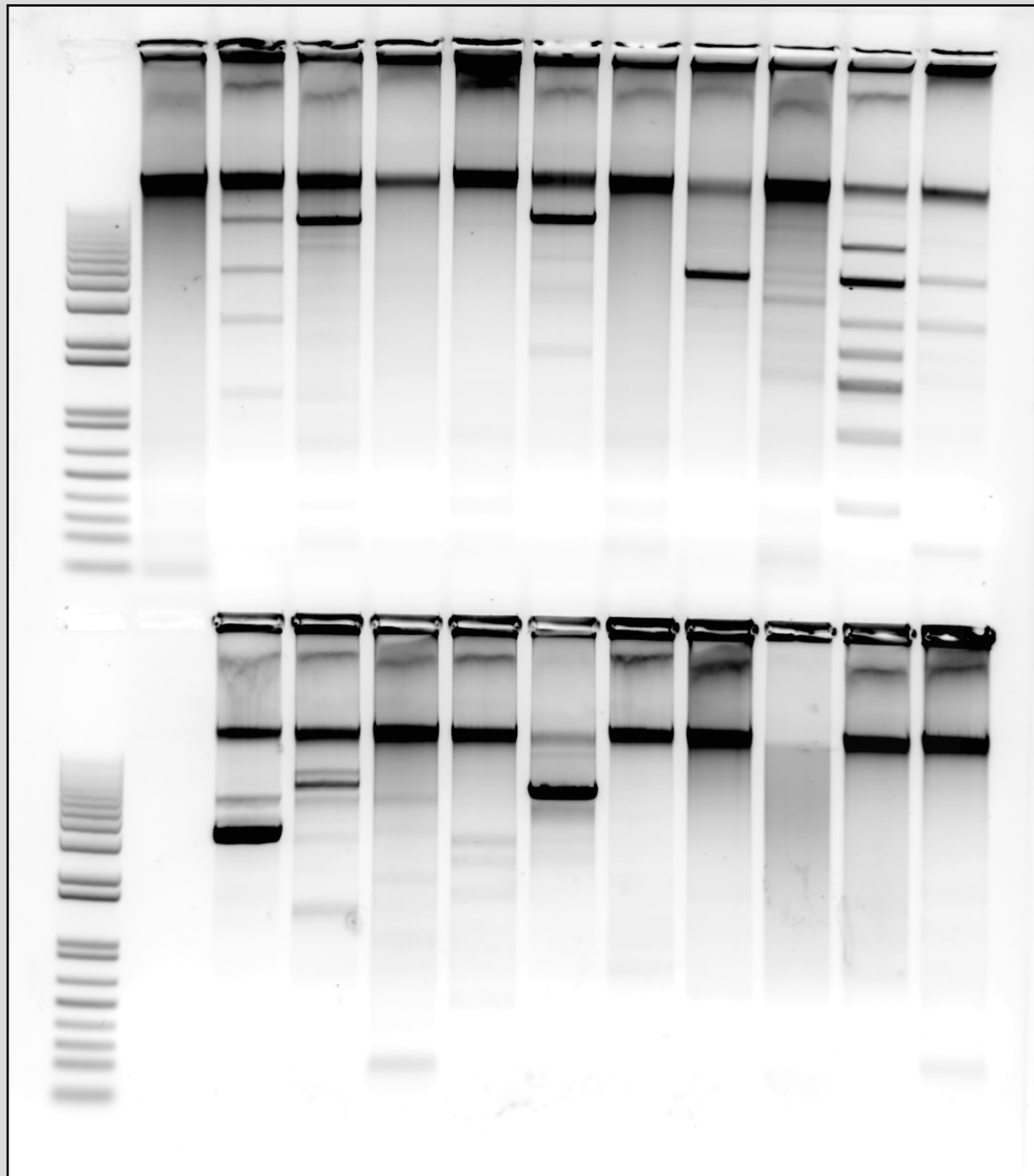
Goal: Isolate w.t. MCVs

- **Problem:** all available full-length MCV clones are derived from tumors
- **Hypothesis:** MCV in tumors may be a different genotype than MCVs in healthy individuals (e.g., HPV16 more common in cervical cancer)
- **Approach:** use rolling circle amplification (RCA) to amplify MCV genomes from forehead swabs of healthy volunteers



RCA Rogues Gallery

•Agarose gel analysis
of BamH1-digested
RCA reactions from
swabs of 22 healthy
subjects



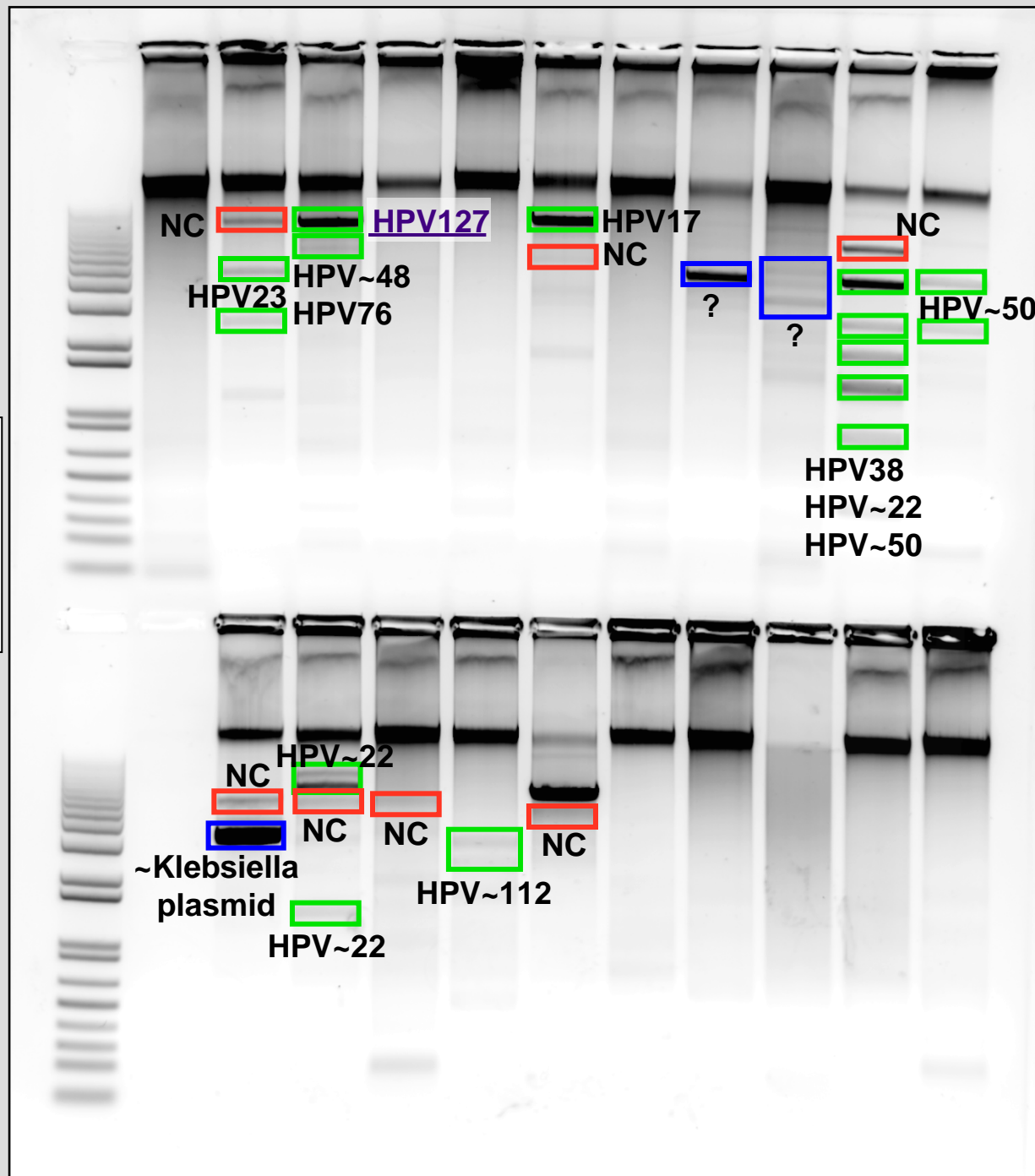
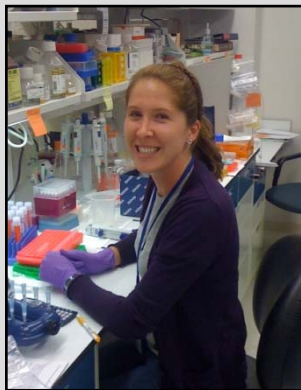
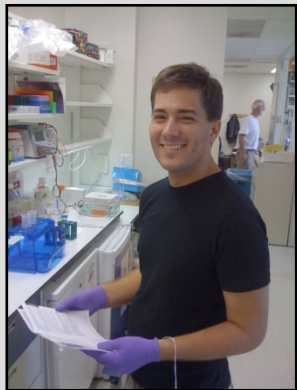
RCA Rogues Gallery

Individual bands cloned and sequenced

HPVs (~possible new types)

? - no homologs in GenBank

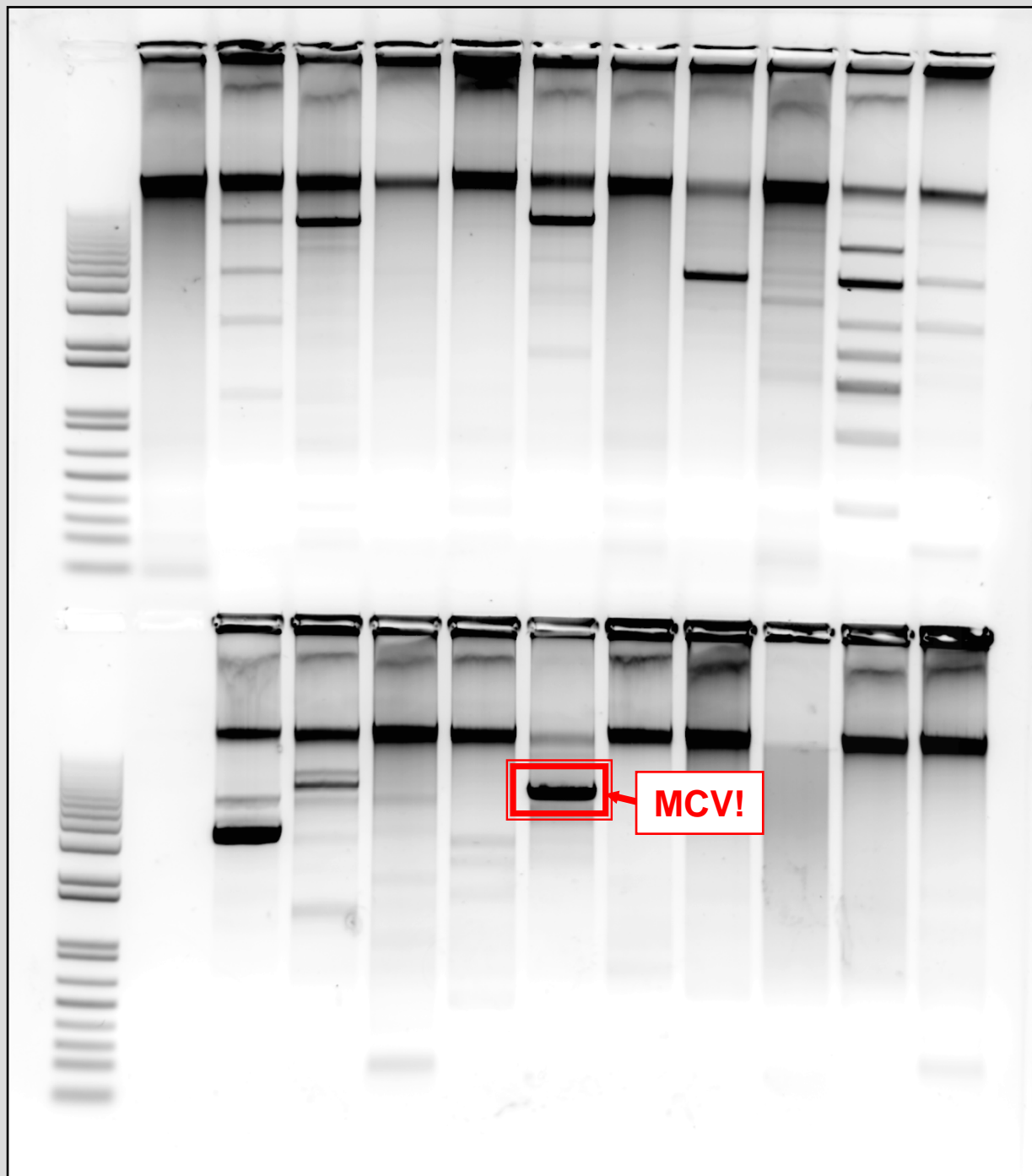
NC - band yielded no clones



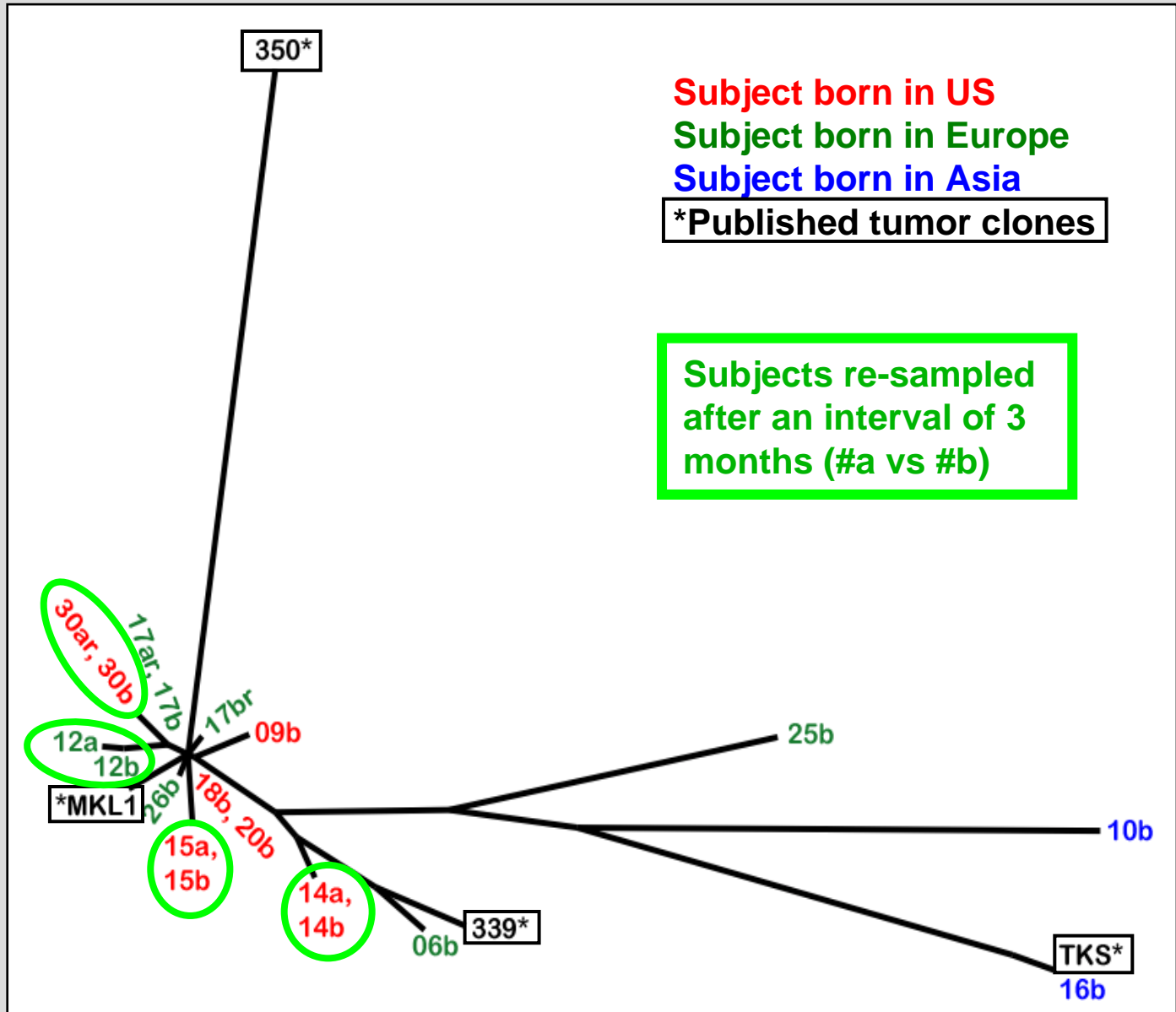
Adam Moyer
Katie Pumphrey

RCA Rogues Gallery

•Used RCA and/or PCR
to clone MCV from
14/35 (40%) subjects



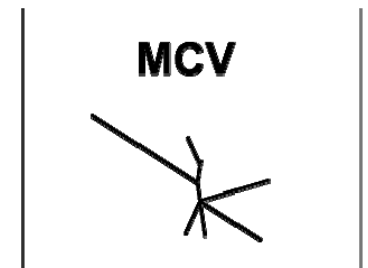
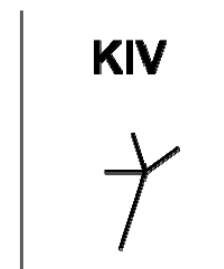
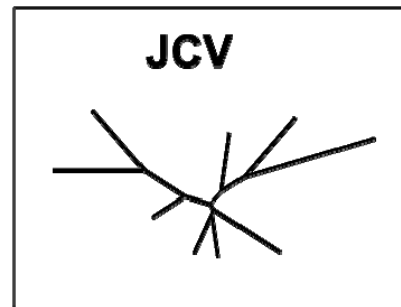
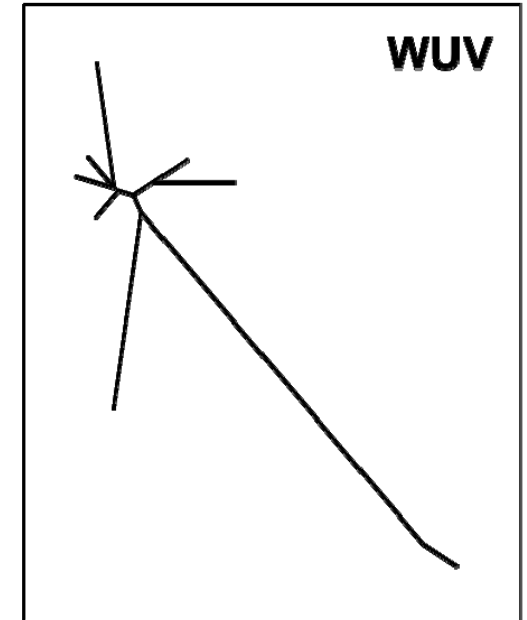
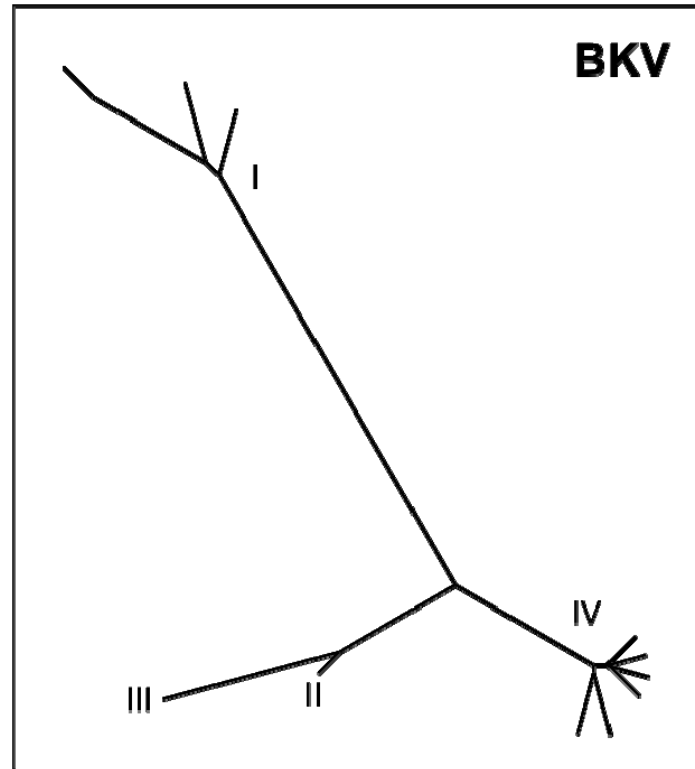
Skin MCVs are Similar to Tumor MCVs



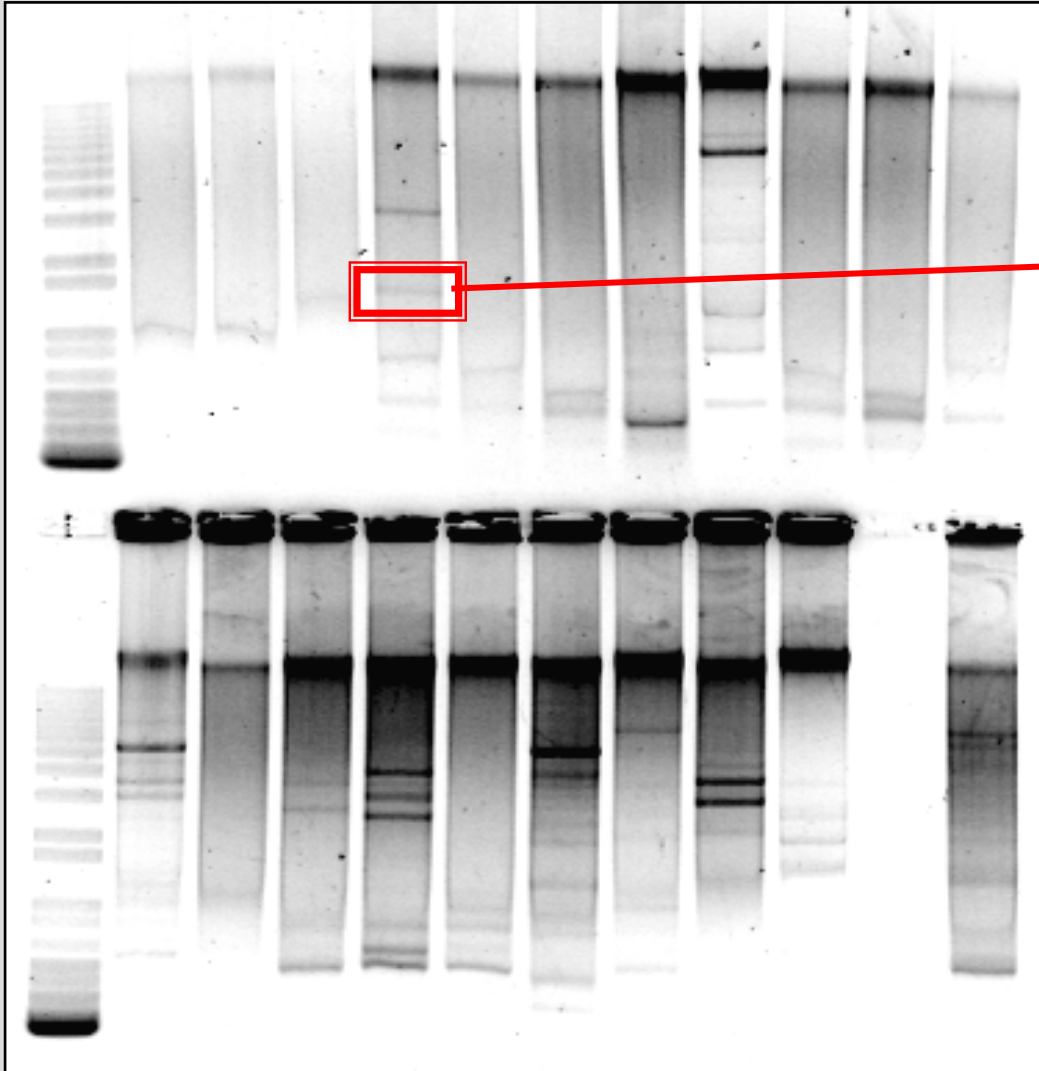
Distinct MCV Serotypes are Unlikely

VP1 (protein) alignments

- All known MCV VP1 proteins are at least 98.6% identical



RCA Rogues Gallery - Jackpot!

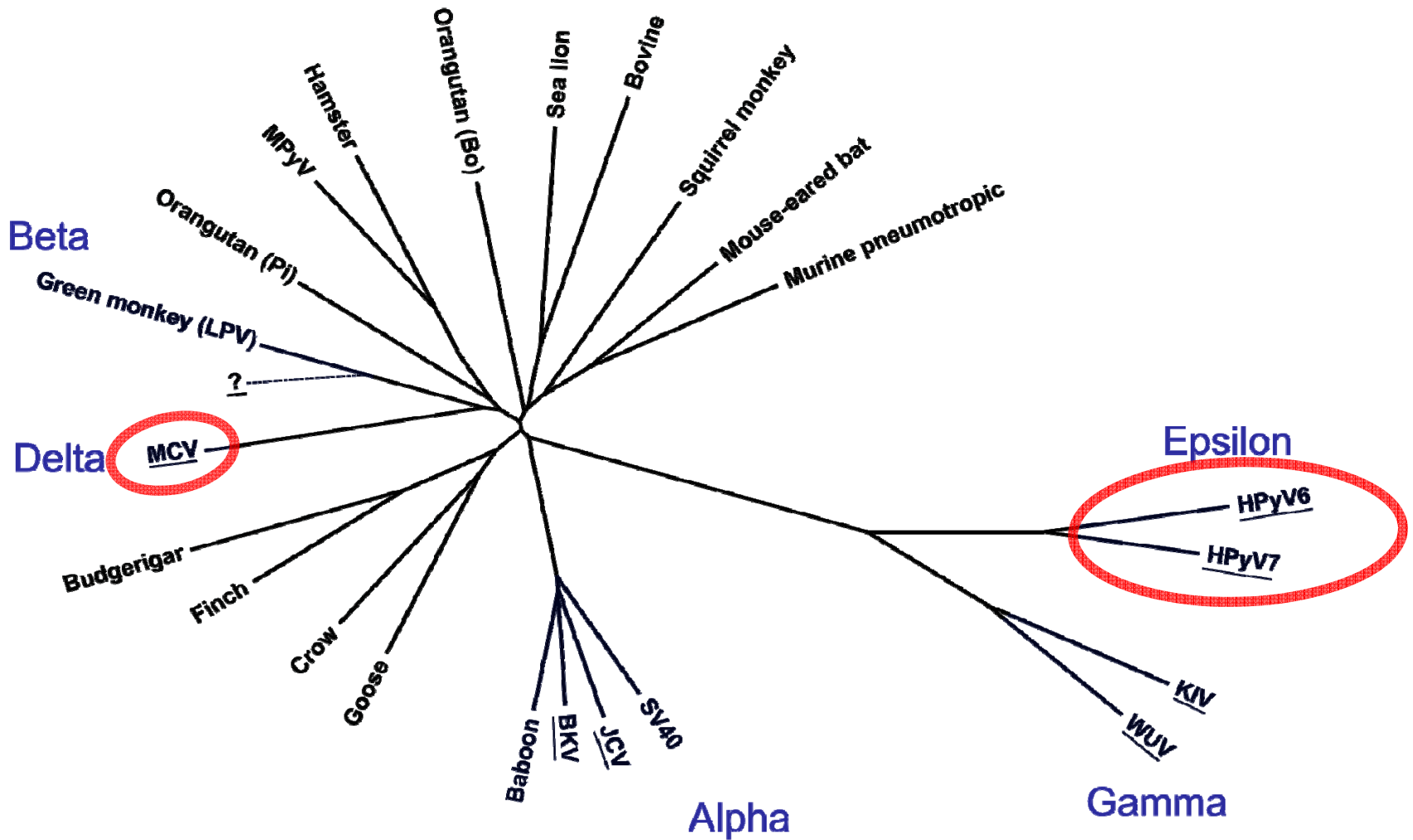


**Fragment of a novel
polyomavirus!**

**PCR reveals new
polyomavirus in 5/30
subjects**

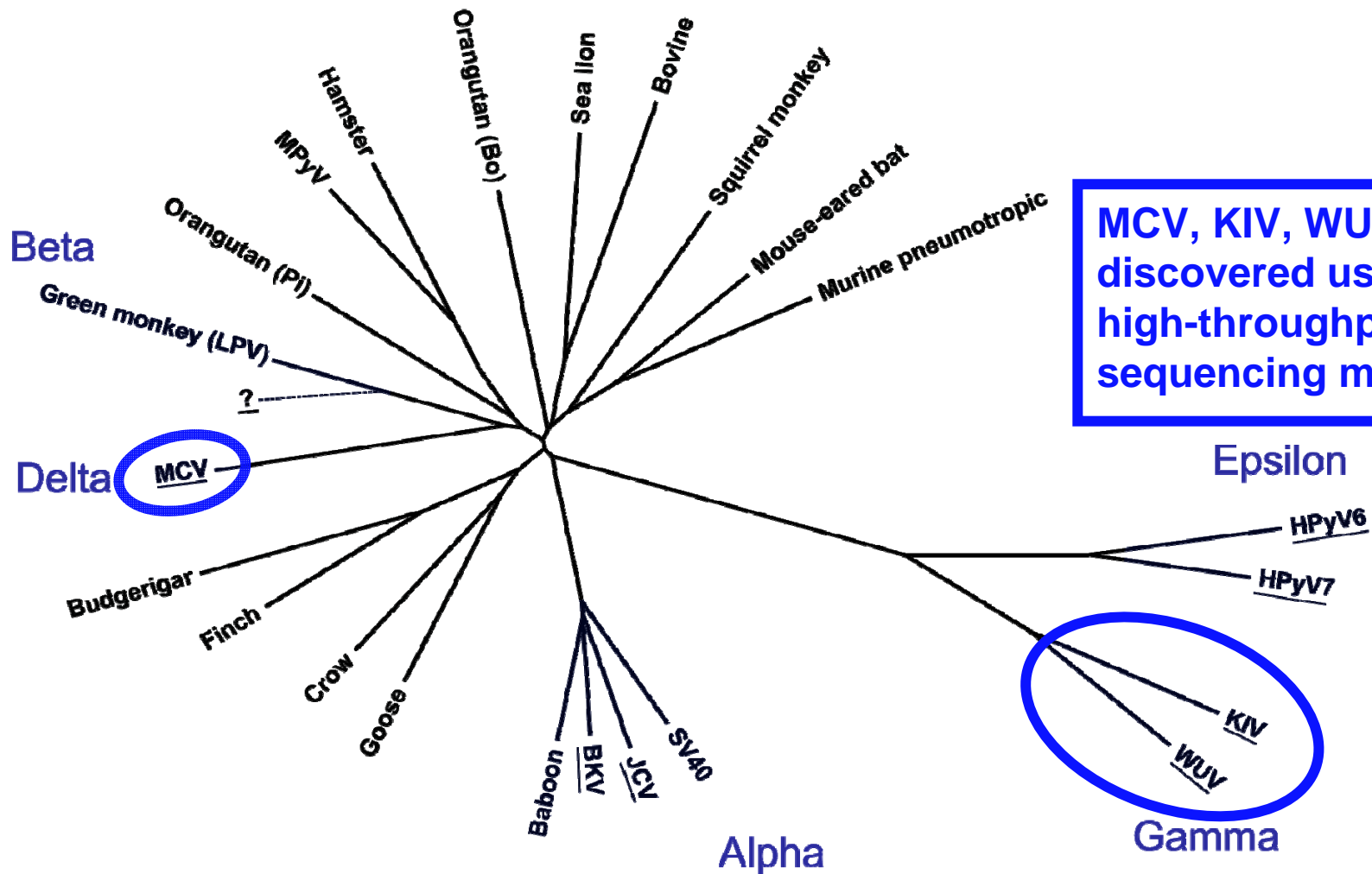
**PCR with degenerate
primers reveals an
additional novel
polyomavirus in 4/30
subjects**

Polyomavirus Phylogeny



0.2

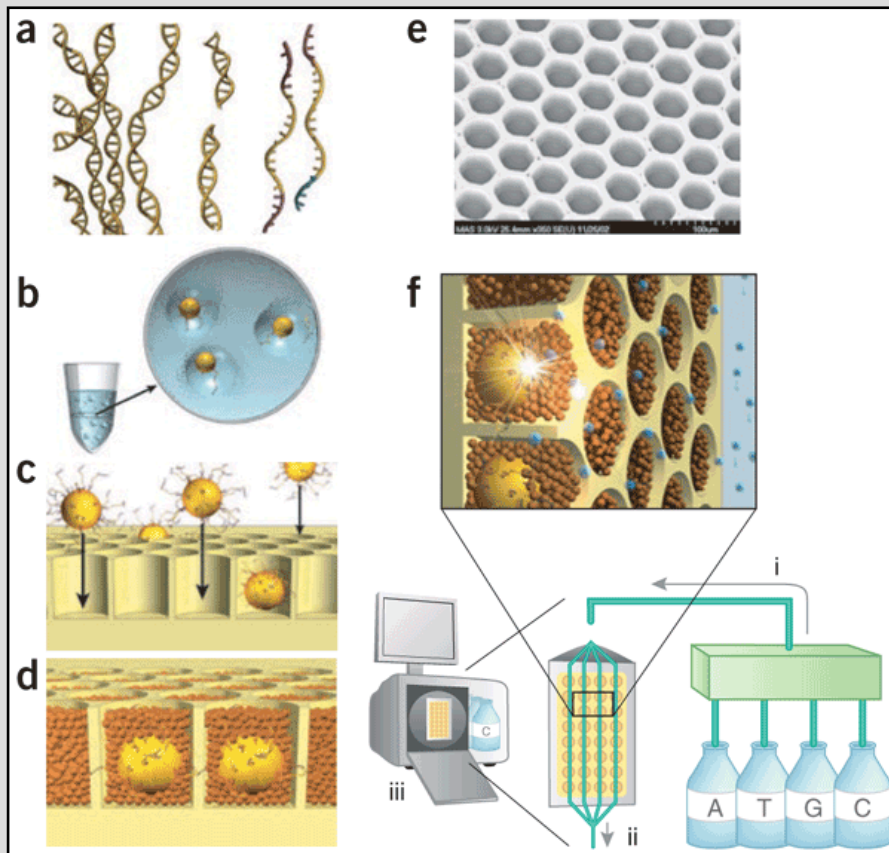
Polyomavirus Phylogeny



MCV, KIV, WUV were discovered using high-throughput sequencing methods

454 Sequencing of RCA Reactions

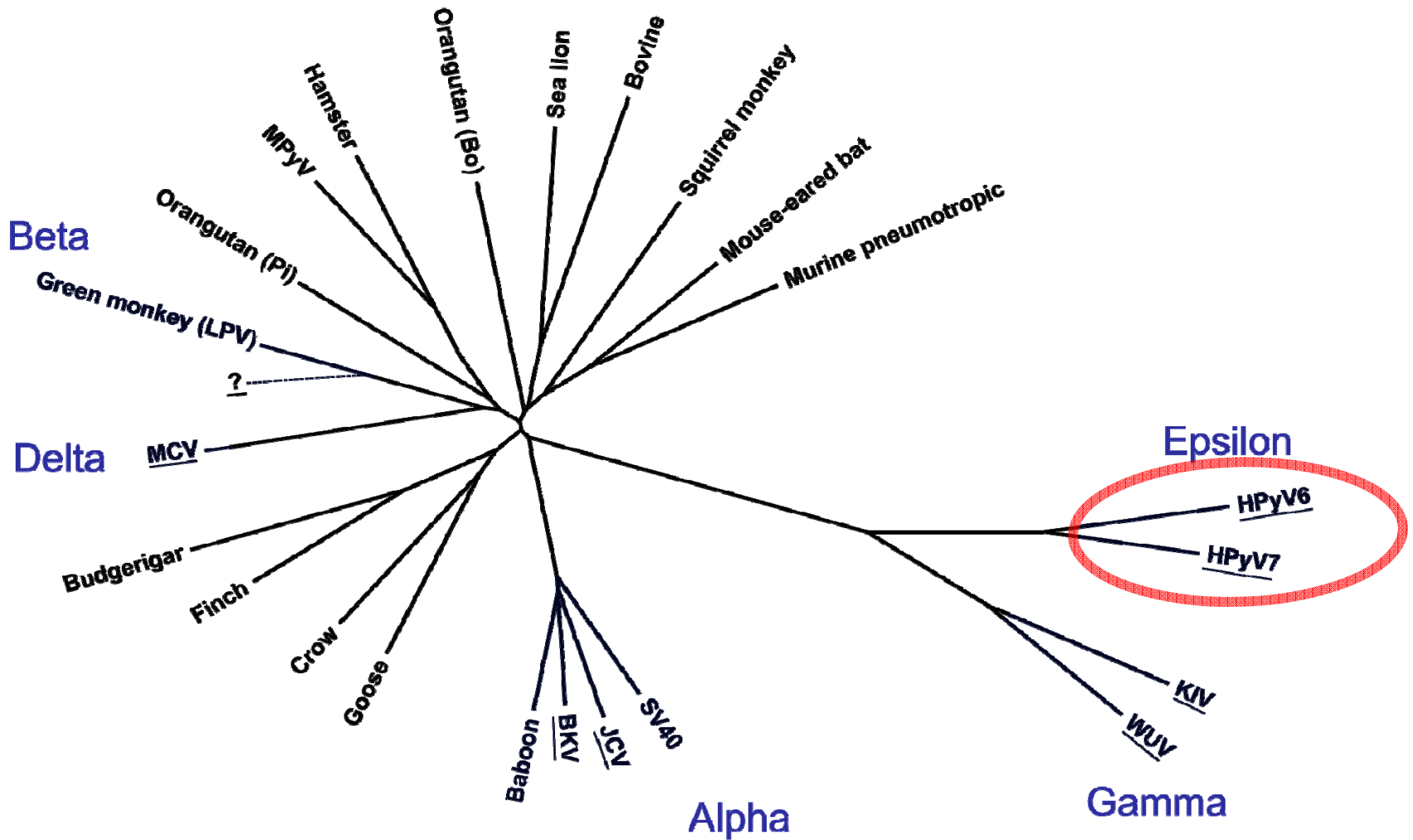
- Performed RCA reactions on forehead and genital swabs from 18 HIV+ volunteers. Subjected to 454 sequencing



| | |
|-------------|-------------|
| read length | 170bp ± 134 |
| human | 333,733 |
| bacterial | 27,165 |
| viral | 7,055 |
| plasmid | 14,851 |
| unknown | 148,433 |

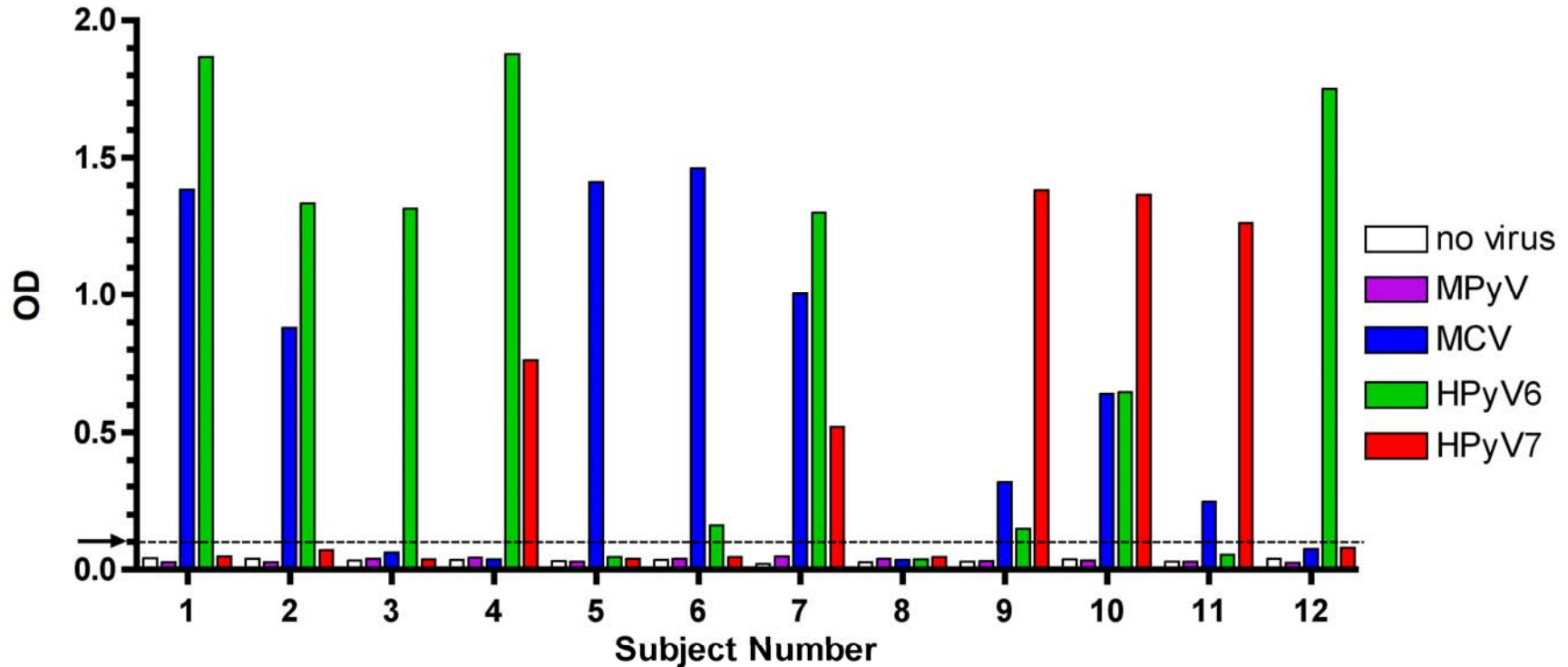
Bob Yarchoan
Kathy Wyvill
Claudia Stewart

Polyomavirus Phylogeny



Most Adults are HPyV6 or 7 Seropositive

- Reactivity of human sera in VLP ELISAs (95 sera)



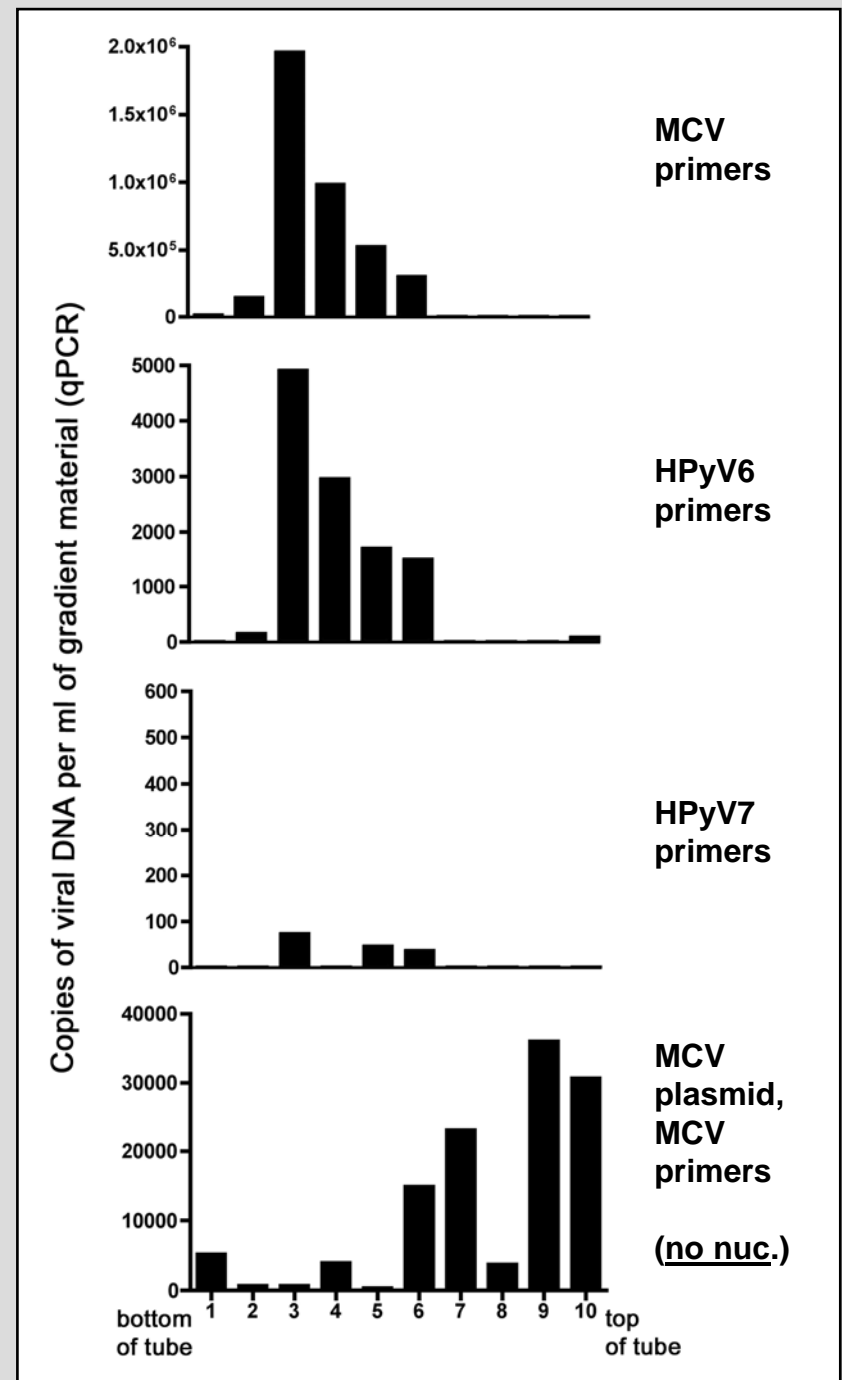
| % positive | MCV | HPyV6 | HPyV7 | MCV+HPyV6+ | MCV+HPyV7+ | HPyV6+HPyV7+ | +++ | --- |
|-----------------|-----|-------|-------|------------|------------|--------------|-----|-----|
| <i>observed</i> | 61 | 69 | 35 | 41 | 21 | 28 | 16 | 8 |
| <i>expected</i> | | | | 42 | 21 | 24 | 15 | 11 |

Detection of Shed Virions

- Swab ten healthy volunteers
- Extract swabs with DNase, non-ionic detergent, high salt
- Run extracted material over Optiprep ultracentrifuge gradients
- Detect viral DNA by qPCR

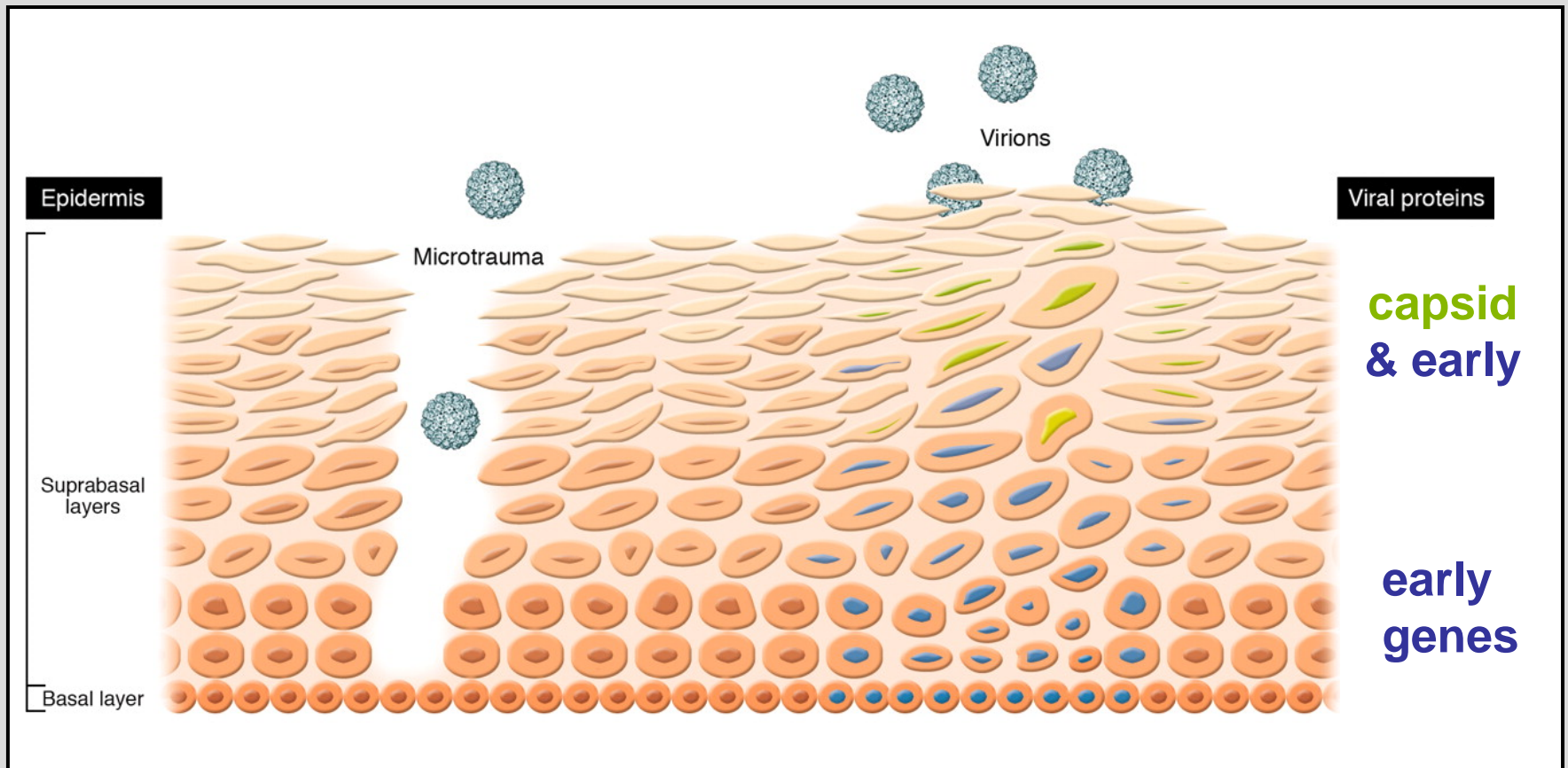


Rachel Schowalter



Working Model

- Some polyomaviruses may lead a “hiding in plain sight” lifestyle reminiscent of papillomaviruses



Conclusions

- **Most adults harbor chronic polyomavirus infections in their skin**
- **MCV genotypes found in tumors closely resemble genotypes commonly found on healthy skin**
- **MCC is a rare side effect of a common infection**
- **Strong antibody responses, likely reflecting an unusually vigorous MCV infection, correlate with the development of MCC**

Conclusions

- Polyomaviruses could theoretically cause cancers (or other diseases) beyond MCC

Hunting strategy:

- Develop a more comprehensive catalog of HPyVs
- Stain tumors for T antigen
- Detect viral DNA in tumors (or their precursors)
- Quantitative serology

Acknowledgements

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Herbert Pfister

The End

