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Disclosures

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

Chris Buck

Tumor Virus Molecular Biology Section

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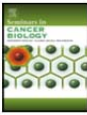
buckc@nih.gov

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

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 **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*

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Seminars in Cancer Biology 19 (2009) 252–260

Contents lists available at ScienceDirect

 **Seminars in Cancer Biology** 

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

Review

BK virus and human cancer: Innocent until proven guilty

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

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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), JC virus (JCV), K1 virus (K1V), and WU virus (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

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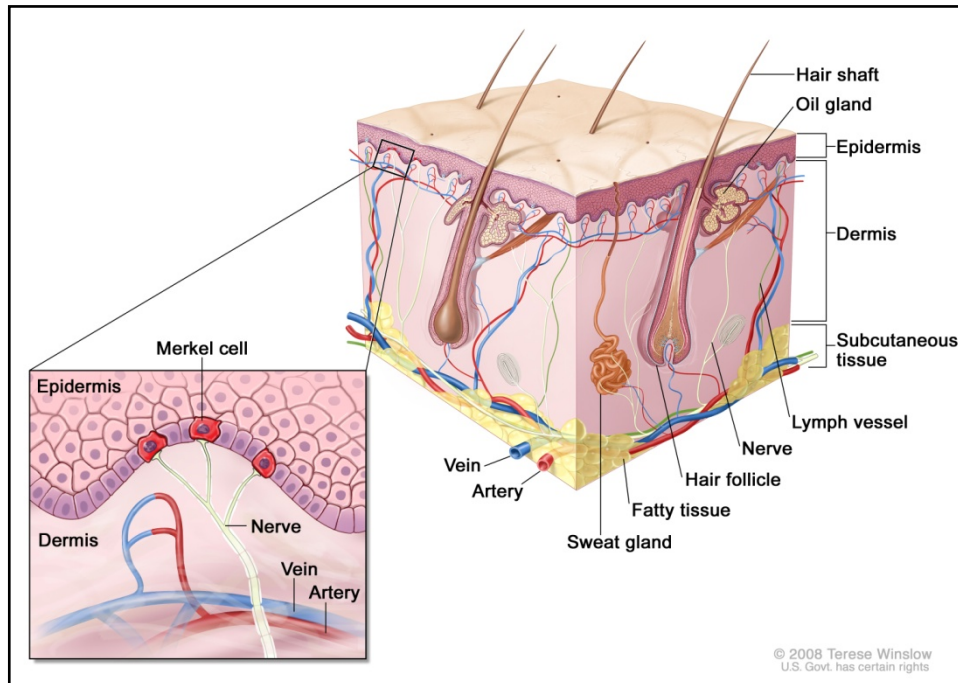
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Merkel Cell Carcinoma

- 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) Science324:1580

2Van Keymeulen (2009) JCB187:91

MCV is Probably a Causal Factor in MCC

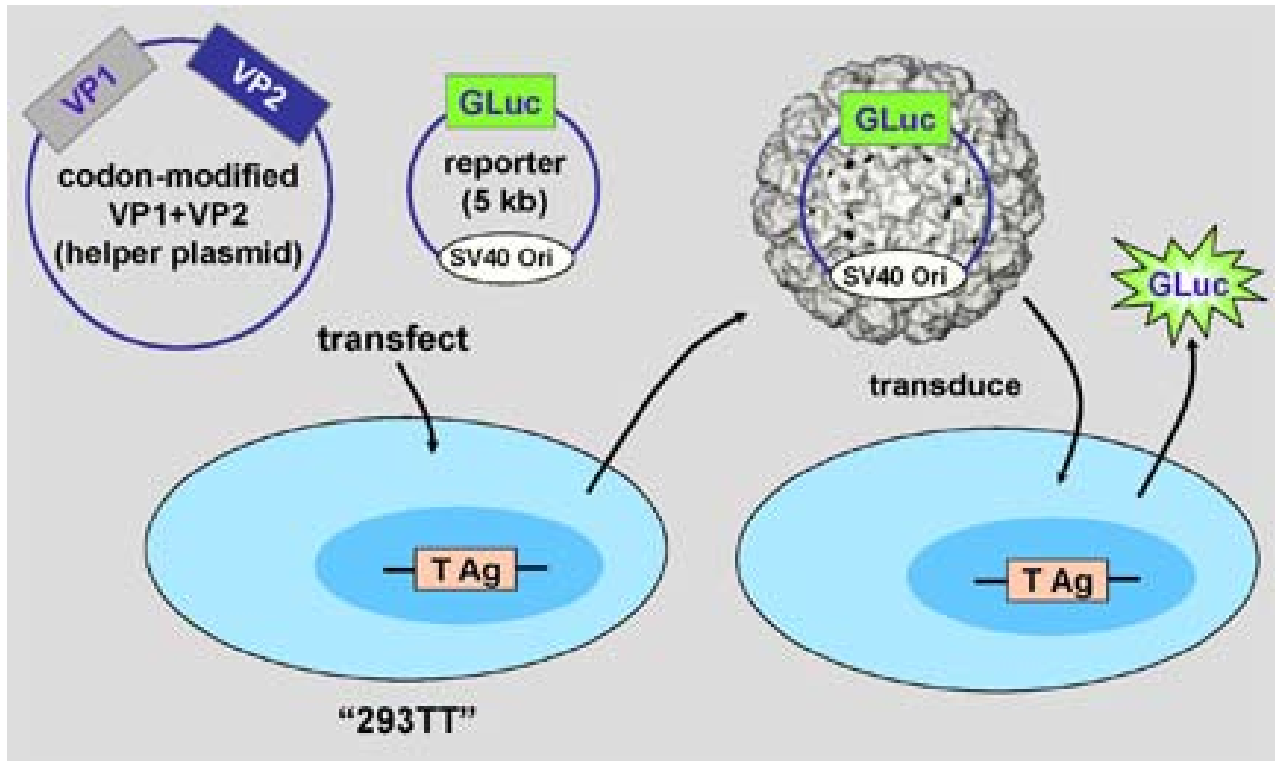
- About 80% of MCC tumors harbor MCV DNA
- In many instances, the viral DNA is clonally integrated into the DNA of the primary MCC tumor and its metastases
- MCV DNA found in MCC tumors has a characteristic pattern of mutations in T antigen that preserve its oncogenic domains but destroy the helicase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

Moore & Chang

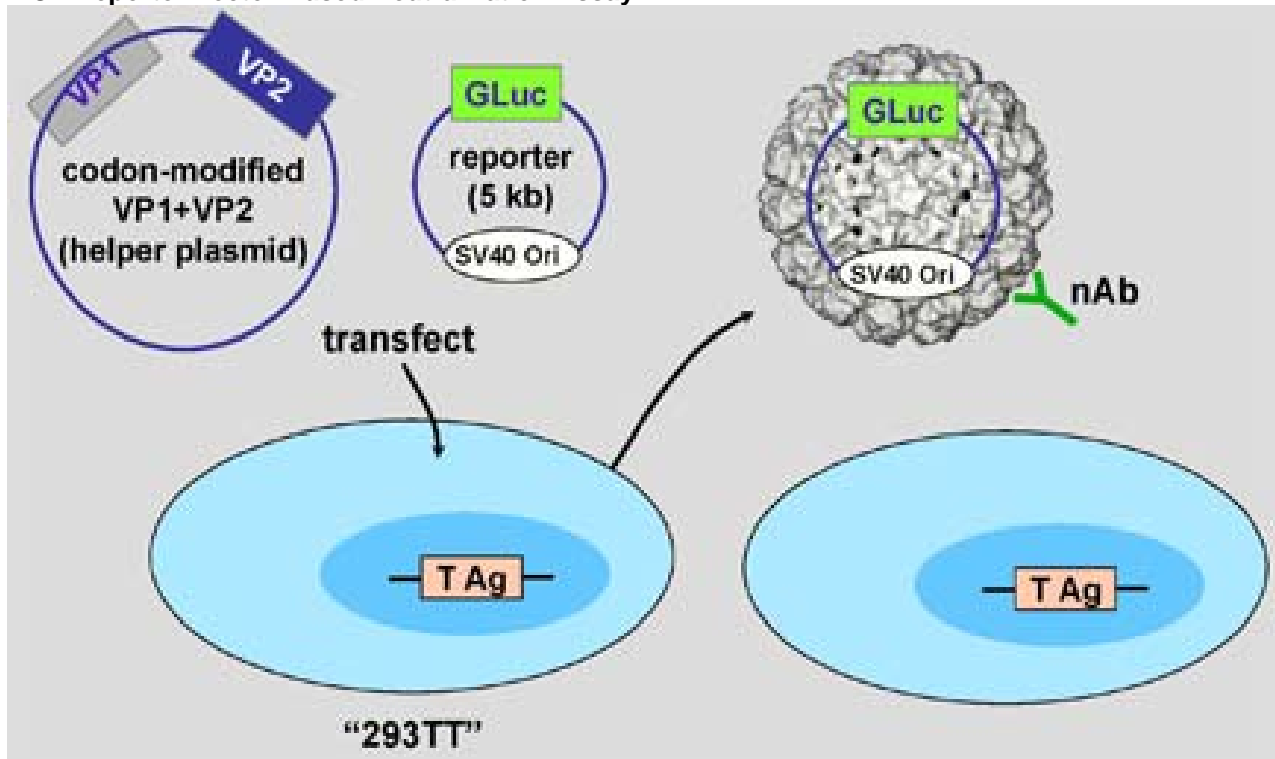
Questions

- 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?

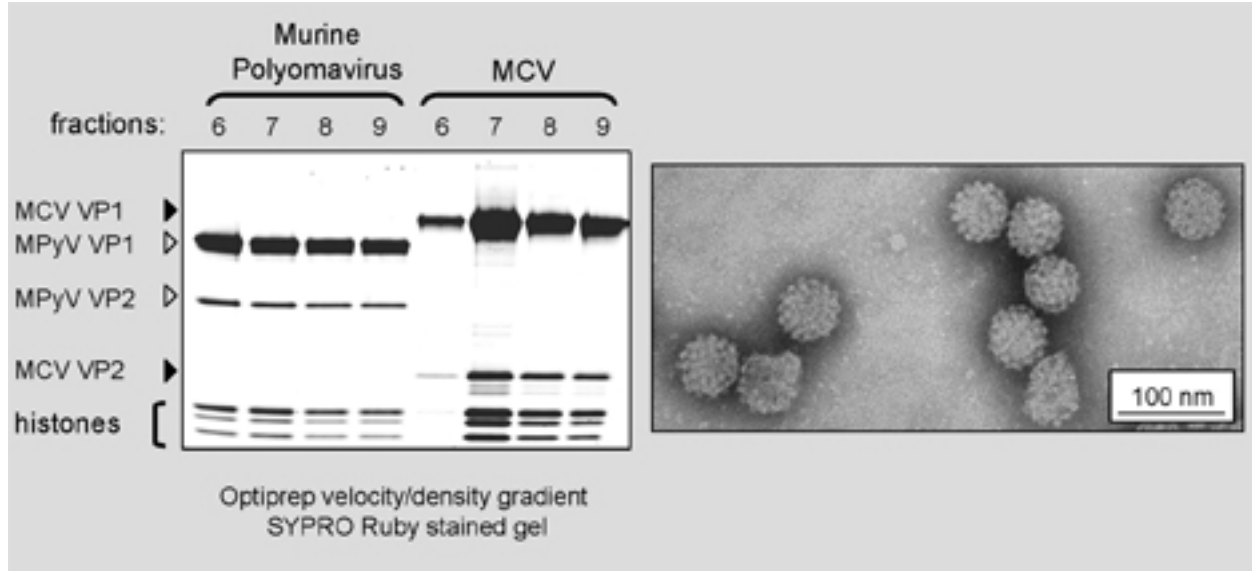
MCV Reporter Vector-Based Neutralization Assay



MCV Reporter Vector-Based Neutralization Assay

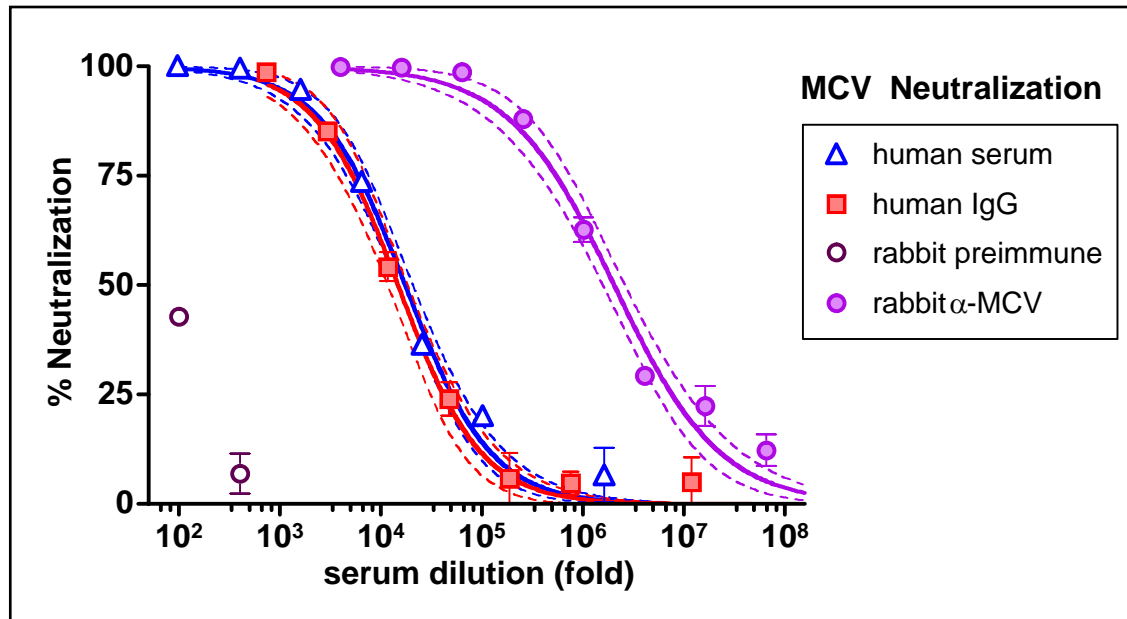


Particle Production



Tolstov et al (2009) Int J Cancer 125:1250

Neutralization Assay

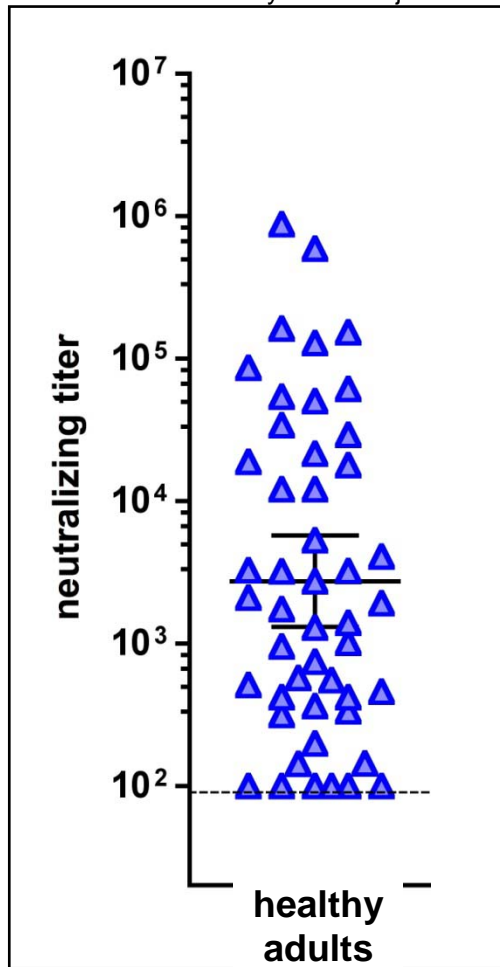


Pastrana (2009) PLoS Pathogens 5e:10000578



Quantitative Serology

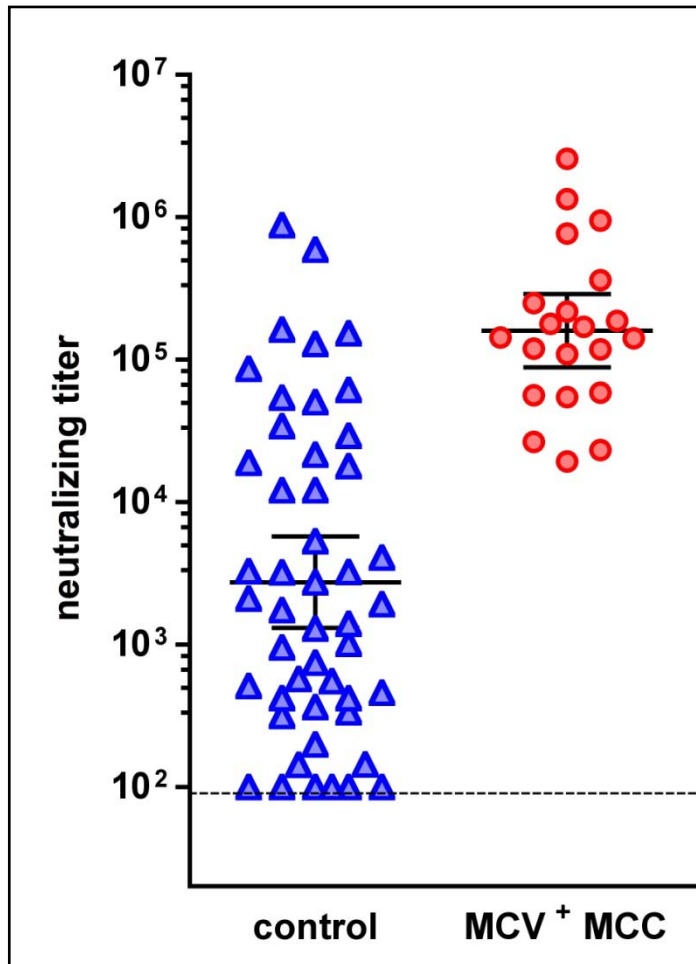
- 85% of healthy adult subjects are seropositive for MCV



Collaboration: Yuan Chang and Patrick Moore

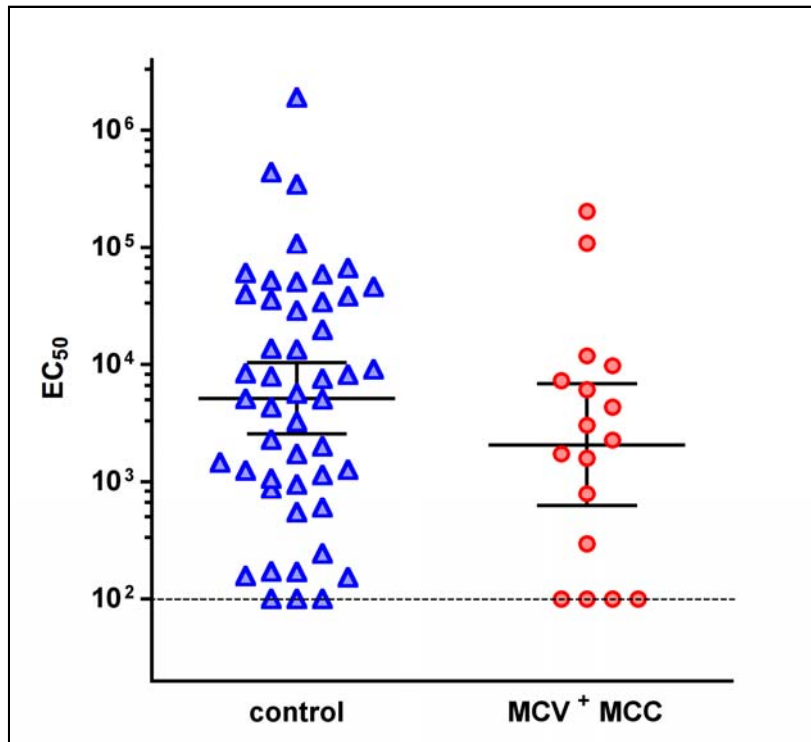
Quantitative Serology

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



Collaboration: Yuan Chang and Patrick Moore
Pastrana (2009) PLoS Pathogens 5e:10000578

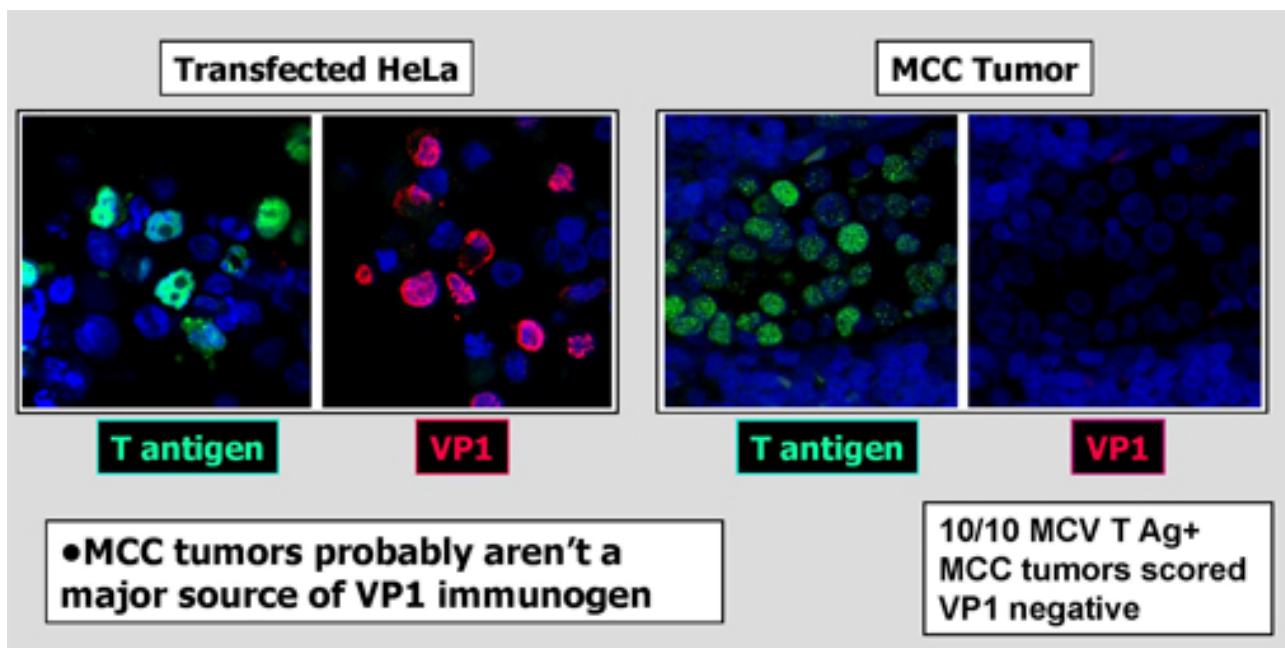
MCC Patients Have Normal Titers Versus BK Polyomavirus



- Generalized loss of immune control over all polyomaviruses is unlikely

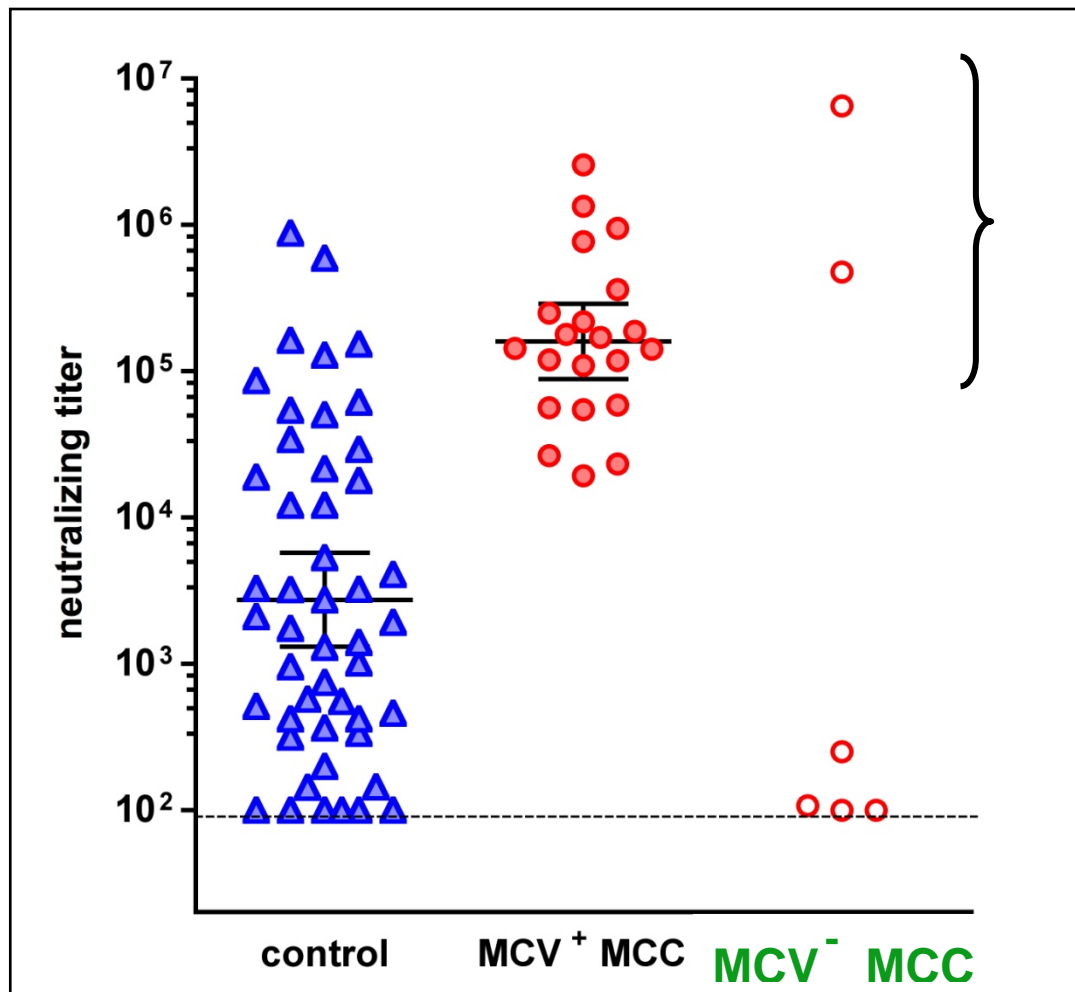
Pastrana (2009) PLoS Pathogens 5e:10000578
 MCC Tumors Express Little or No VP1

MCC Tumors Express Little or No VP1



Pastrana (2009) PLoS Pathogens 5e:10000578 T antigen mAb: Shuda (2009) Int J. Cancer 125:1243

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

Pastrana (2009) PLoS Pathogens 5e:10000578

Working Model

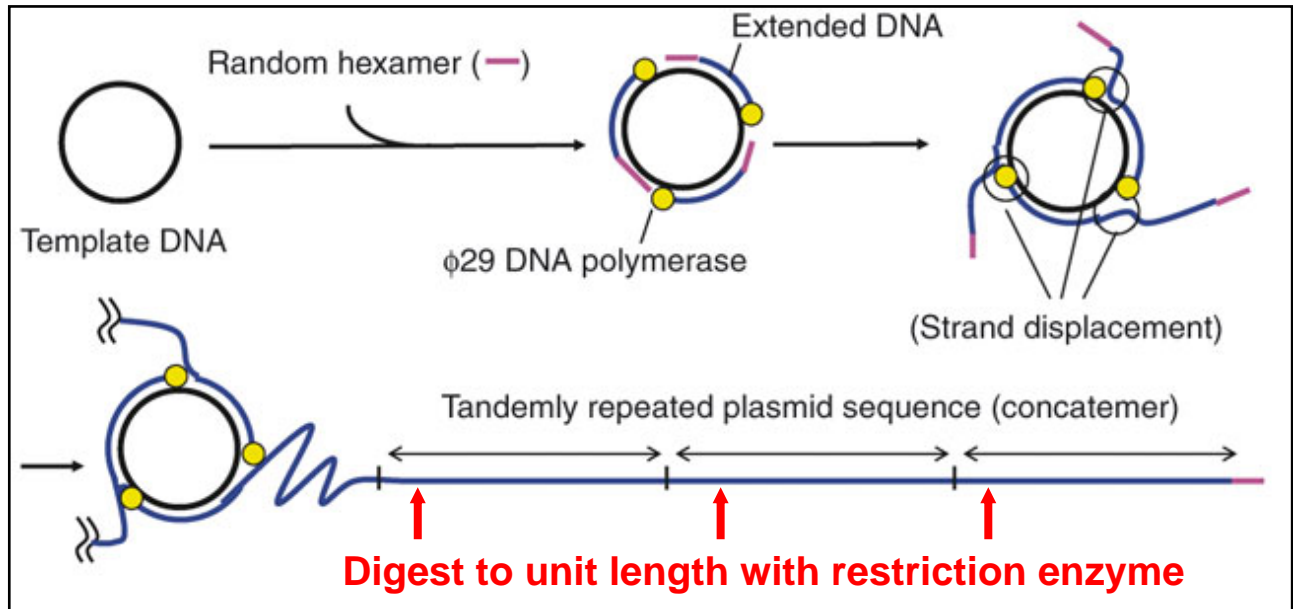
- Chronically high MCV viral load leads to stronger MCV-neutralizing antibody responses
- High viral load correlates with development of MCC

MCV DNA is Shed from Healthy Skin

Merkel Cell Polyomavirus DNA in Persons without Merkel Cell Carcinoma

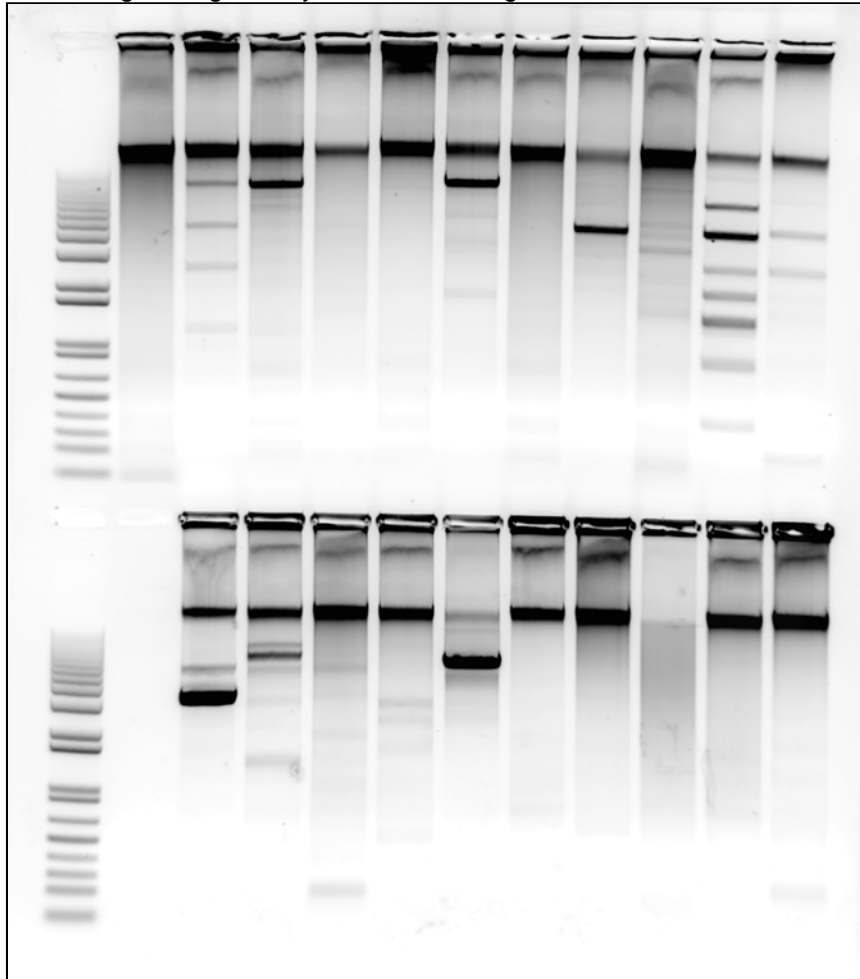
Ulrike Wieland, Cornelia Mauch,
Alexander Kreuter, Thomas Krieg,
and Herbert Pfister

Merkel cell polyomavirus **MCPyV DNA was**
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.



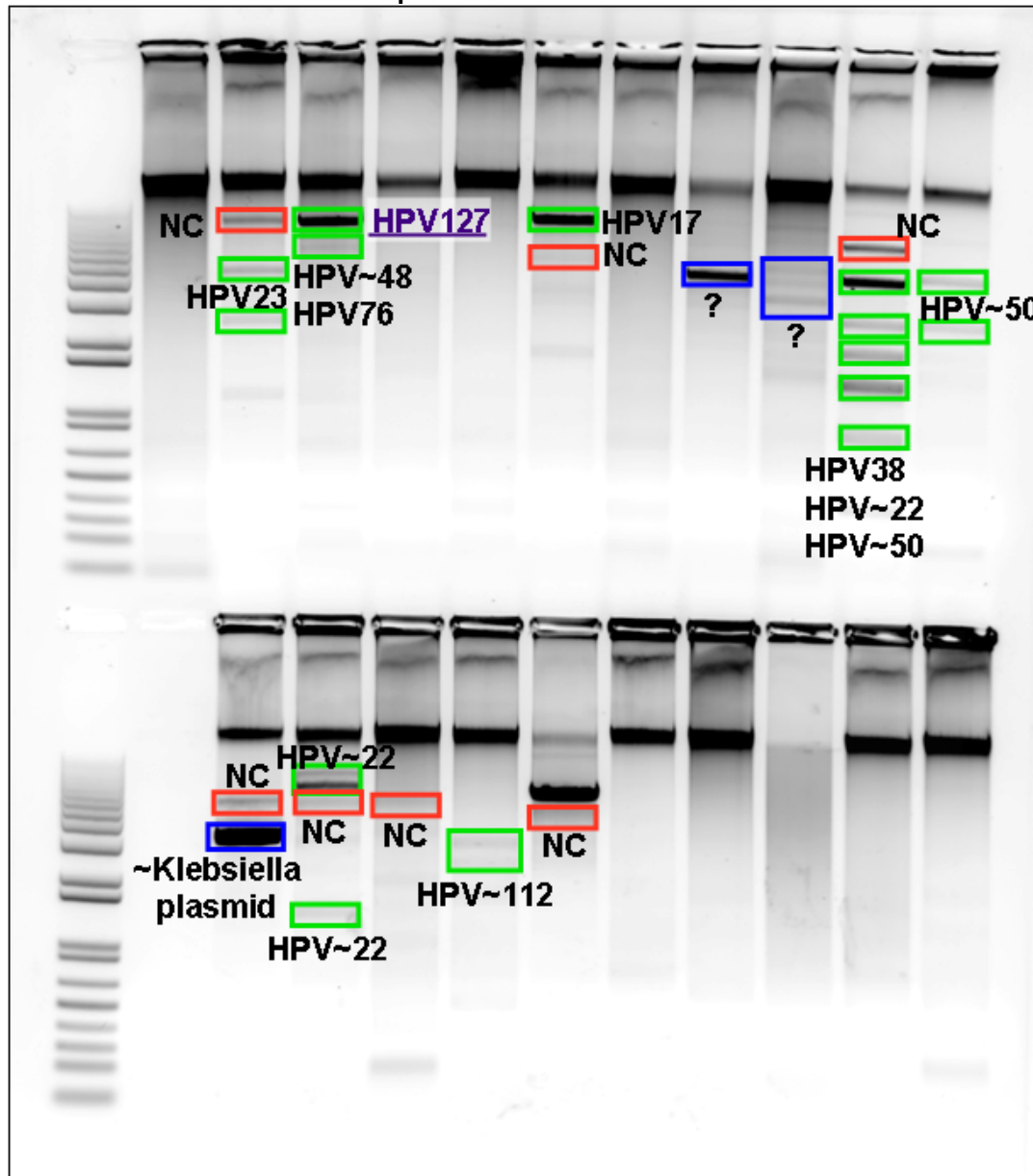
RCA Rogues Gallery

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



RCA Rogues Gallery

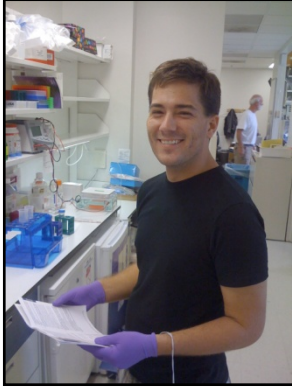
Individual bands cloned and sequenced



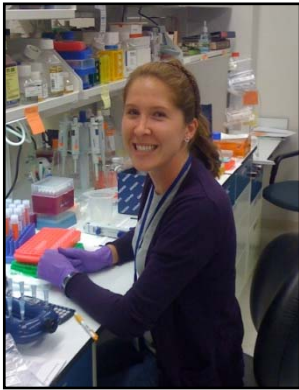
HPVs (~possible new types)

? - no homologs in GenBank

NC - band yielded no clones



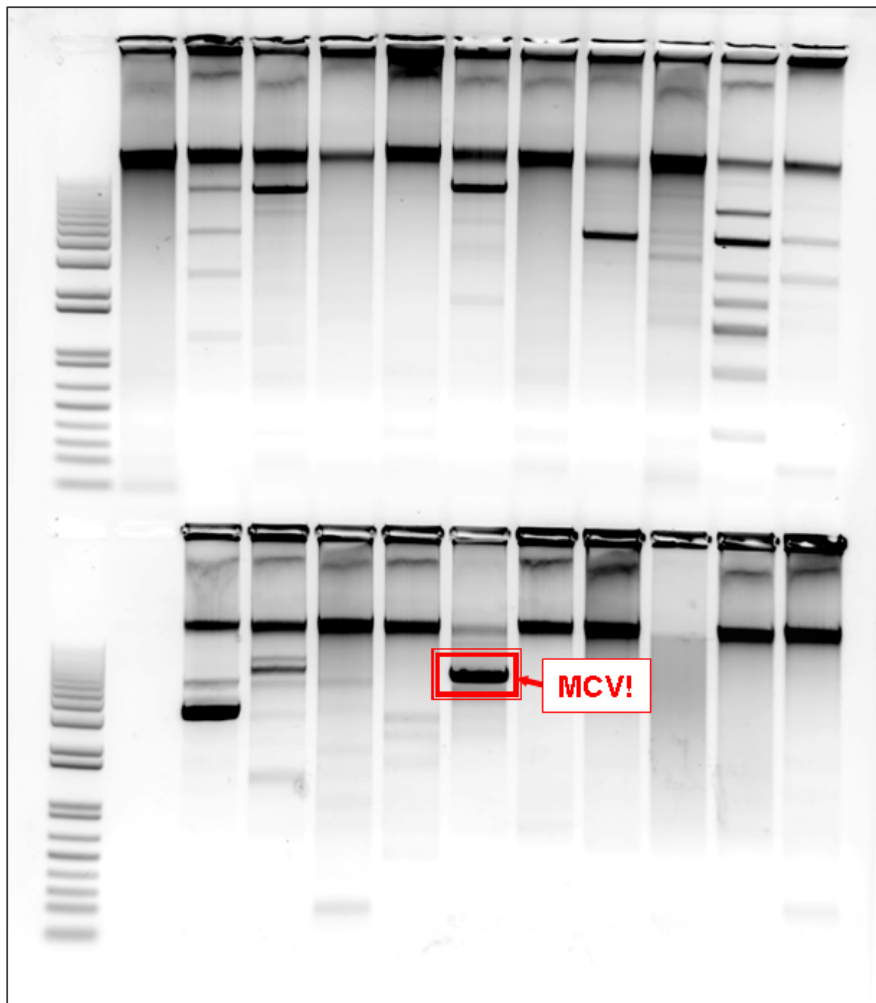
Adam Moyer



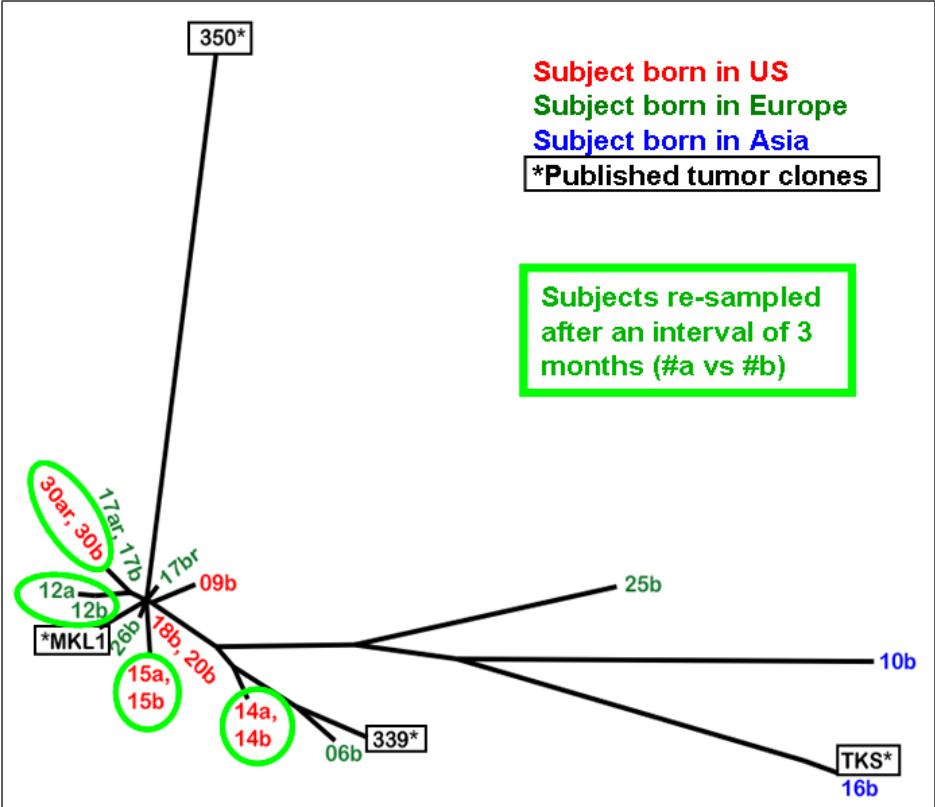
Katie Pumphrey

RCA Rogues Gallery

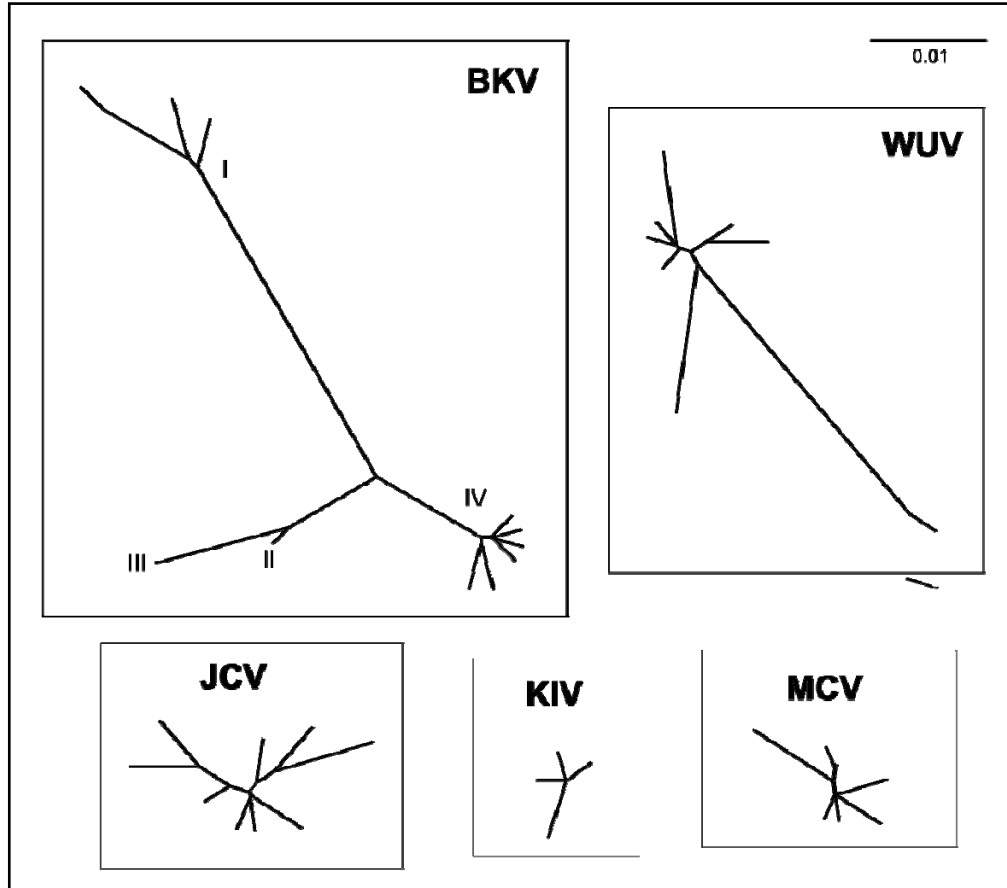
- Used RCA and/or PCR to clone MCV from 14/35 (40%) subjects



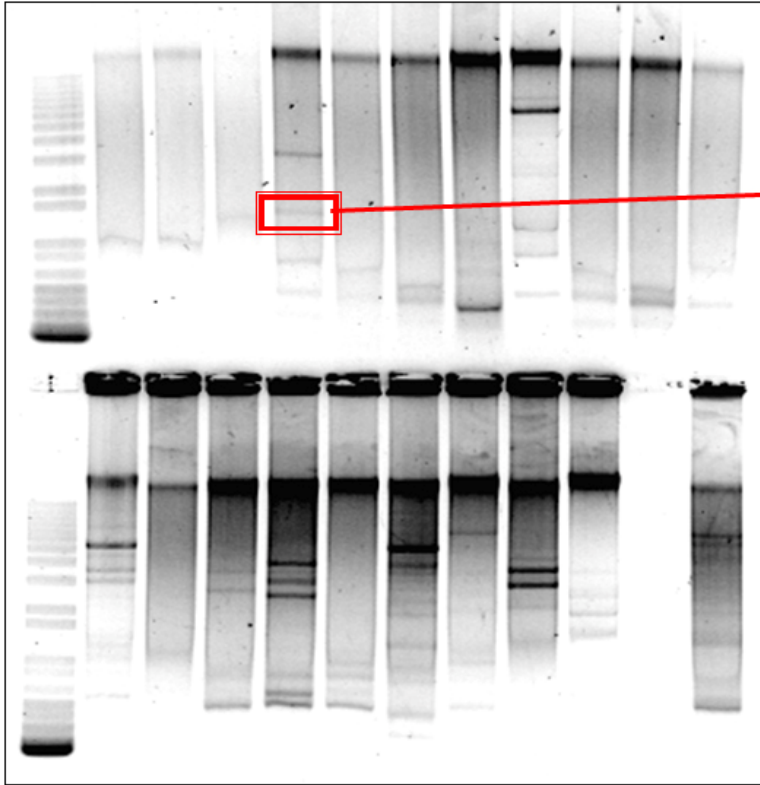
Skin MCVs are Similar to Tumor MCVs



Distinct MCV Serotypes are Unlikely



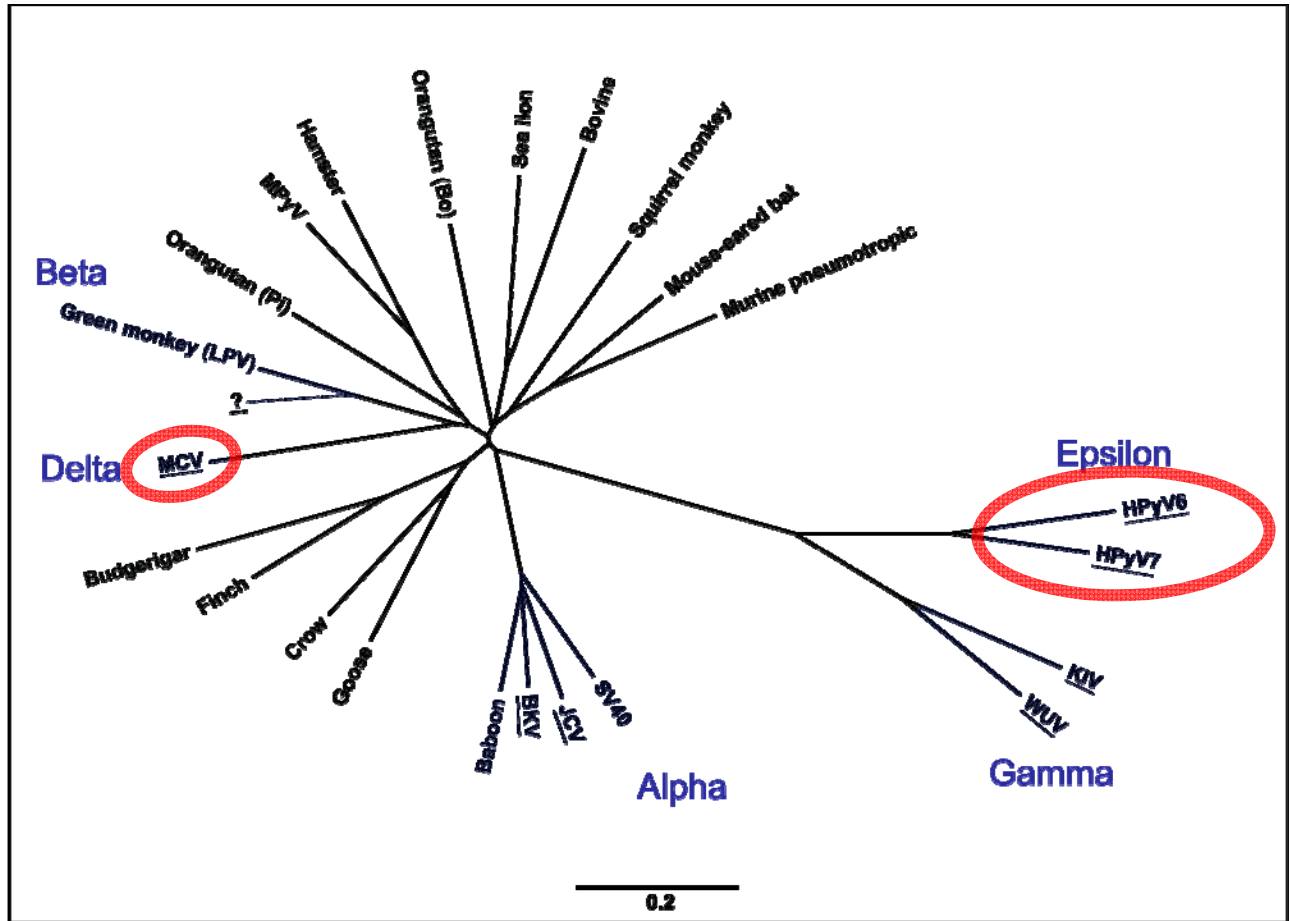
RCA Rogues Gallery -Jackpot!



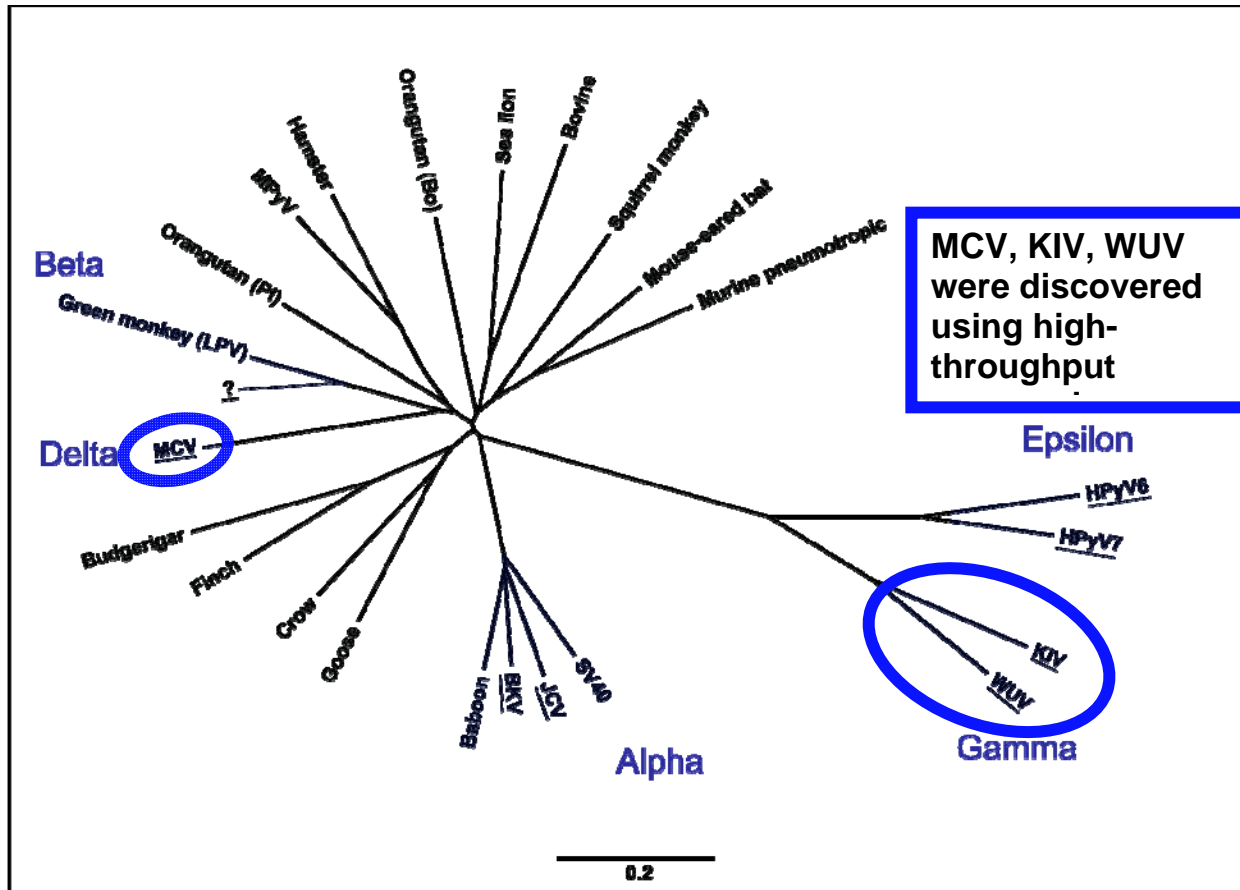
Fragment of a novel polyomavirus!

- PCR reveals new polyomavirus in 5/30 subjects
- PCR with degenerate primers reveals an additional novel polyomavirus in 4/30 subjects

Polyomavirus Phylogeny

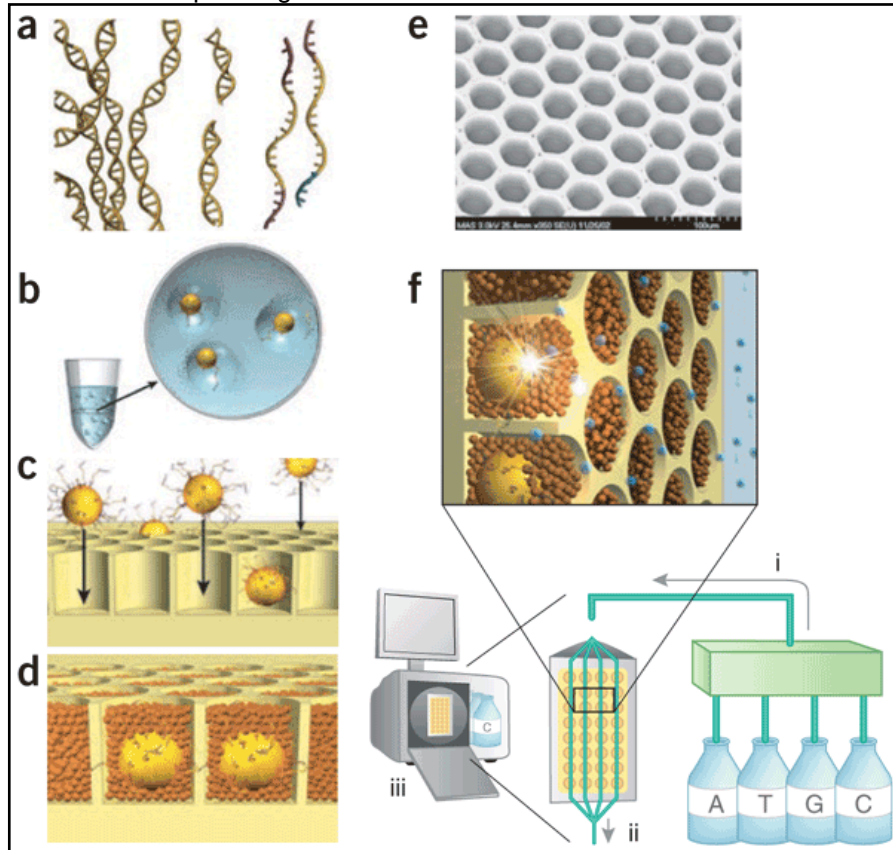


Polyomavirus Phylogeny



454 Sequencing of RCA Reactions

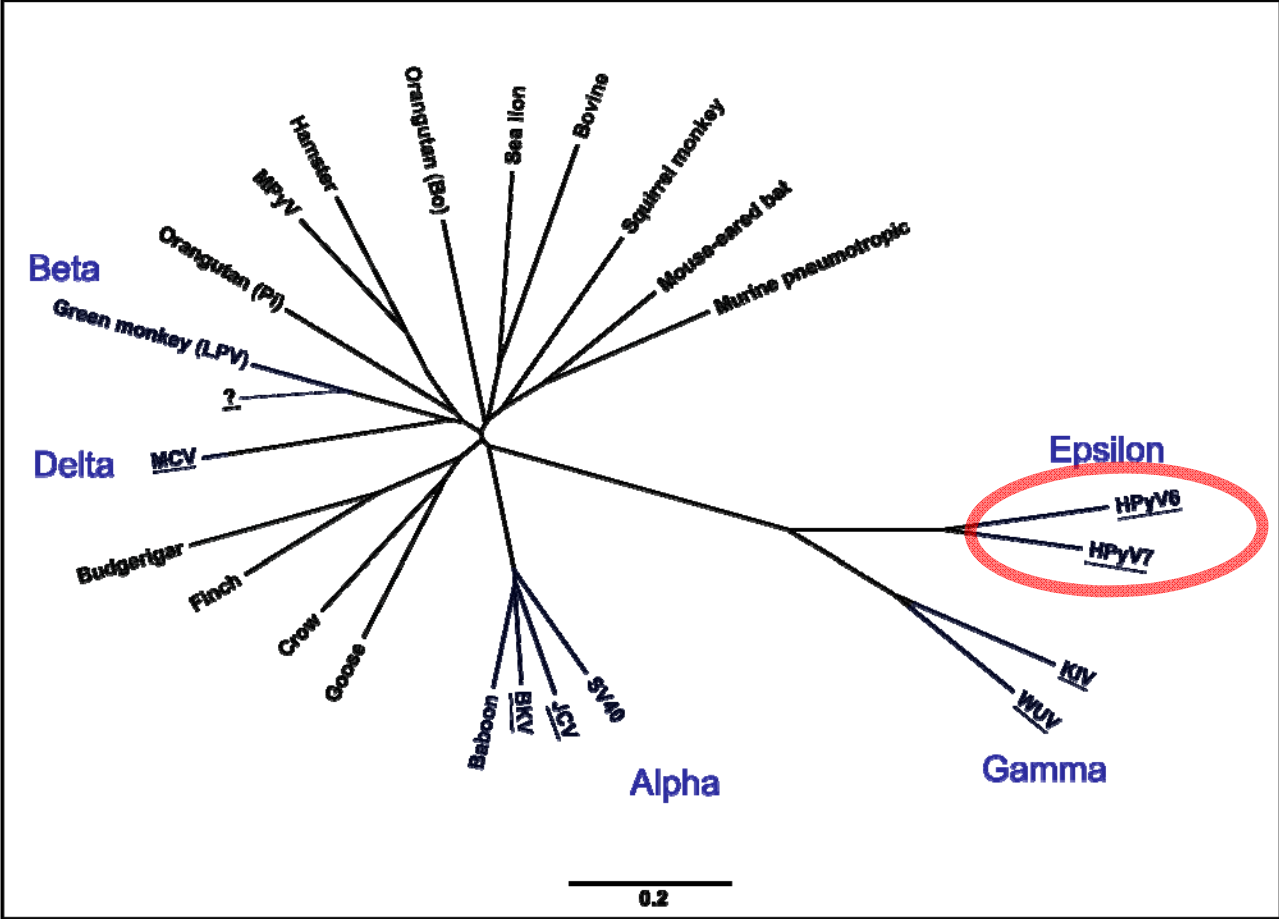
- Performed RCA reactions on forehead and genital swabs from 18 HIV+ volunteers. Subjected to 454 sequencing



read length $170\text{bp} \pm 134$
human 333,733
bacterial 27,165
viral 7,055
plasmid 14,851
unknown 148,433

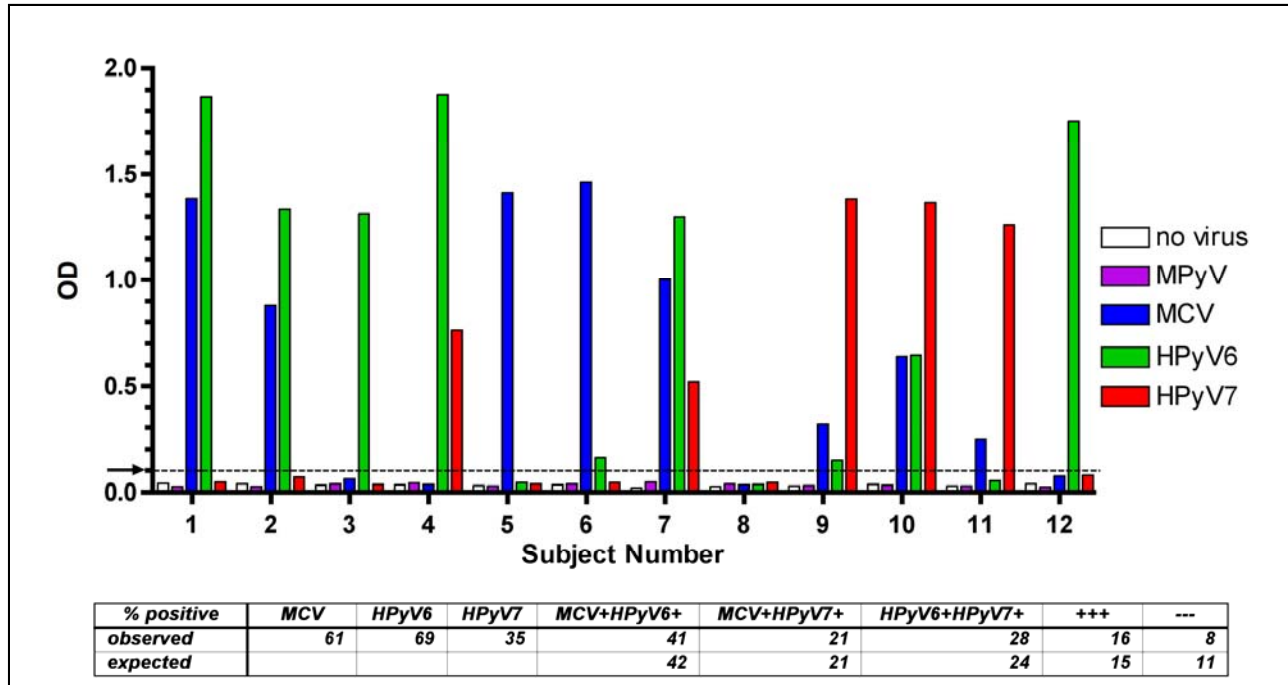
Bob Yarchoan Kathy Wyvill Claudia Stewart

Polyomavirus Phylogeny



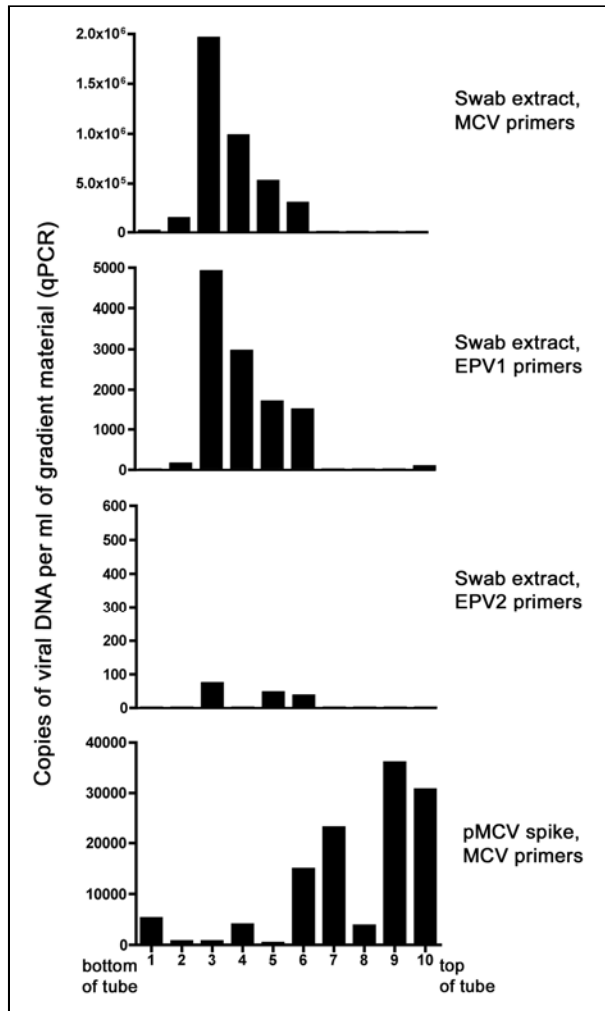
Most Adults are HPyV6 or 7 Seropositive

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



Katie Pumphrey, Diana Pastrana

Detection of Shed Virions



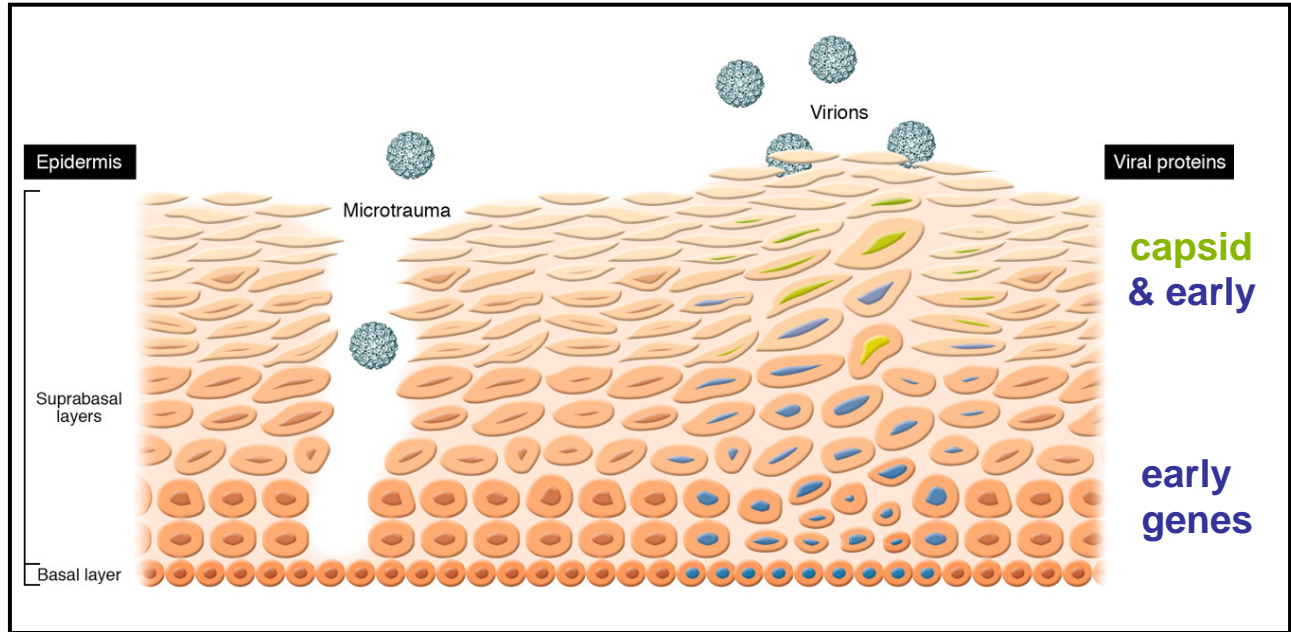
- Swab ten healthy volunteers
- Extract swabs with DNase, non-ionic detergent, high salt
- Run extracted material over Optiprep ultracentrifuge gradients
- Detect viral DNA by qPCR



Rachel Schowalter

Working Model

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



Lowy & Schiller (2006) JCI 116:1167

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

NCI

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Herbert Pfister

The End