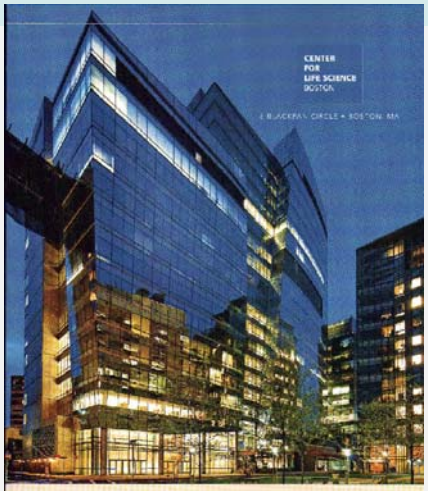
Development of models for possible treatment of PML



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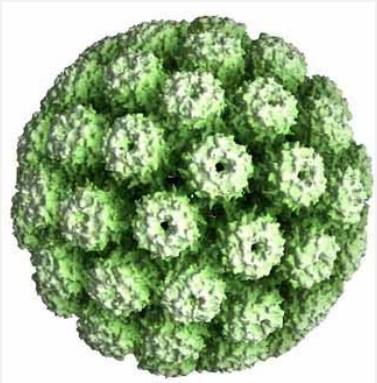

Major obstacles in developing a treatment for PML

- JCV grows very slowly in vitro in primary fetal astroglial cells and limited number of cell lines transformed with SV40 T ag:
  - No plaque assay
  - Low percentage of cells infected/transfected
  - Effect of in vitro compounds difficult to evaluate by IFA or QPCR
- JCV receptors not fully characterized
  - sialic acids and 5HT2a serotonin receptor
- JCV infects only humans
  - no animal model of PML
- PML is a rare disease
  - Need for multicenter studies to gather enough patients for treatment evaluation

In vitro studies are not leading to efficient treatments of PML

- **Cytarabine (Ara-C):**
  - **decreased JCV replication in human astroglial cells in vitro (Hou JNV 98)**
  - **no benefit over cART alone in HIV+ patients (Hall NEJM 98)**
- **Cidofovir:**
  - **decreased replication of murine polyomavirus and SV40 (Andrei AAC 97) but not of JCV (Hou JNV 98) in vitro**
  - **no benefit over cART alone in HIV+ patients (Marra AIDS 02, DeLuca AIDS 08)**
- **Alpha interferon 2b (Geschwind JNV 01), topothecan (Royal JNV 03):**
  - **no benefit over cART alone in HIV+ patients**
- **Mirtazapine:**
  - **5HT2a receptor blocker decreases entry of JCV in astroglial cells in vitro (Elphick Science 04)**
  - **No survival advantage in retrospective analysis (Marzocchetti Neurol 09)**
- **Mefloquine:**
  - **anti-malaria drug decreases JCV replication in vitro (Brickelmaier AAC 09)**
  - **Multicenter PML treatment trial sponsored by Biogen Idec discontinued in 10/2010 for lack of efficacy**

There is no animal model of JCV/PML

- JCV in hamsters:
  - medulloblastoma and other tumors (Ressetar Lab Invest 90)
- Murine polyomavirus and SV40 in immunosuppressed mice
  - No pathology (Koralnik lab)
- JCV T ag transgenic mice
  - Dysmelination and tumors (Gordon Dev Biol Stand 98)
- JCV in owl and squirrel (new world) monkeys
  - cerebral tumors, no demyelination (Houff, London Prog Clin Biol Res 83
- JCV in old world monkeys
  - No pathology

SV40 causes PML in immunosuppressed macaques

- Simian virus 40 (SV40) is the simian counterpart of JCV (69% homology)
- Infects rhesus monkeys in the wild without causing any disease
- Reactivation of SV40 induces PML-like disease in 2.6% SIV-infected monkeys (Simon Am J Path 99)
- Primary infection with SV40 induces a meningocencephalitis (ME) in SIV-infected monkeys
- SV40 inoculated into 10^8 people as contaminant of polio vaccines before 1961 !!!

SV40 primary infection in SHIV-infected rhesus monkeys

Naturally SV40 infected SHIV+ rhesus macaque developed a “PML-like” disease

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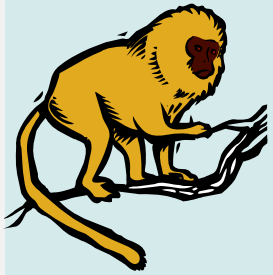

SV40 isolate from Brain

Injected into SHIV+ SV40 neg monkeys

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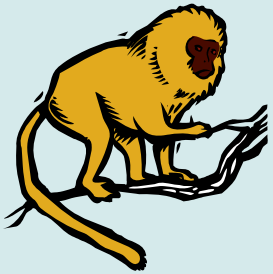

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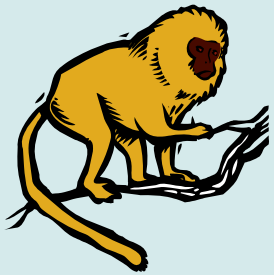
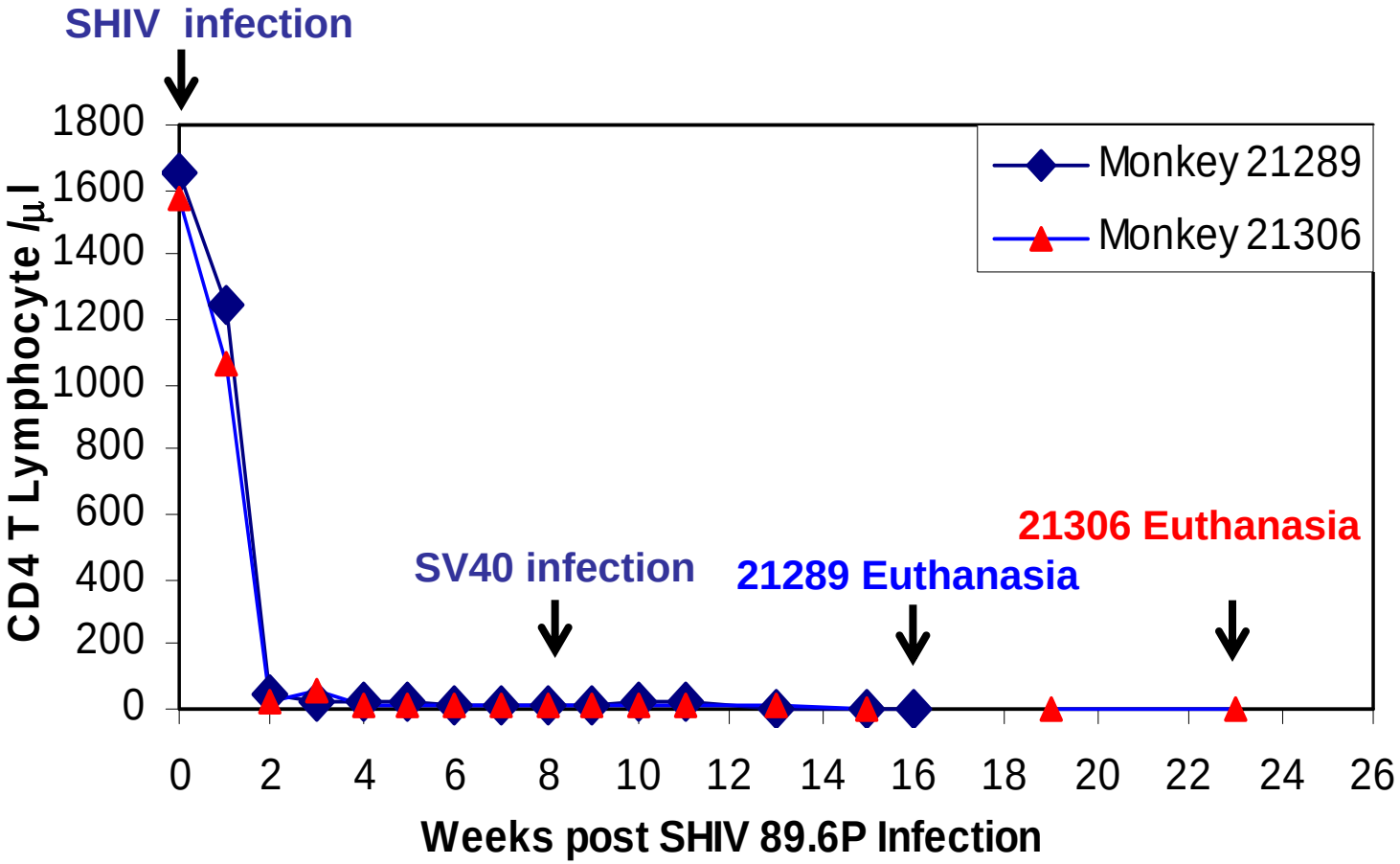


Both animals developed a PML-like disease after 9-11 weeks

SHIV infection causes profound drop of CD4+ T cell counts



Reactivation or primary infection with SV40 cause PML in SHIV+ monkeys

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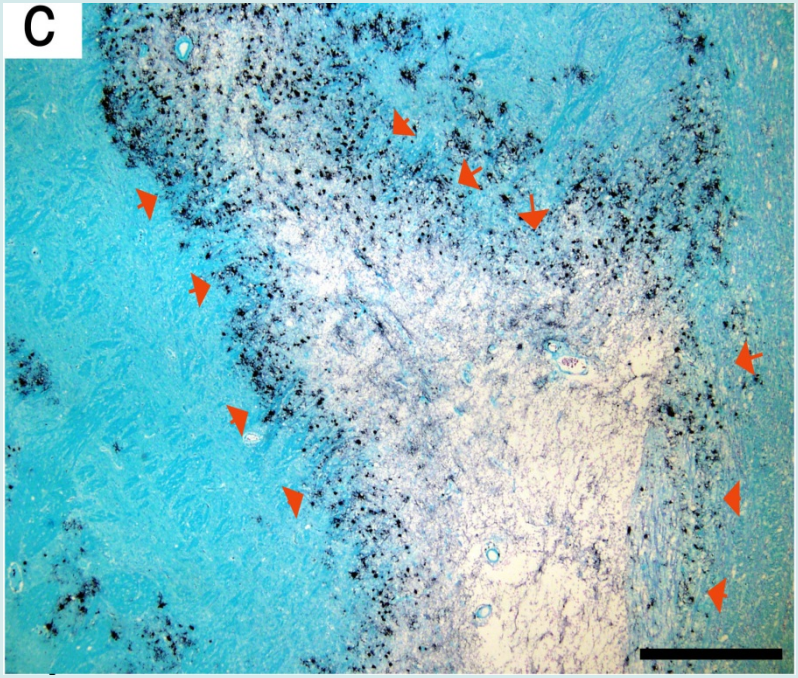

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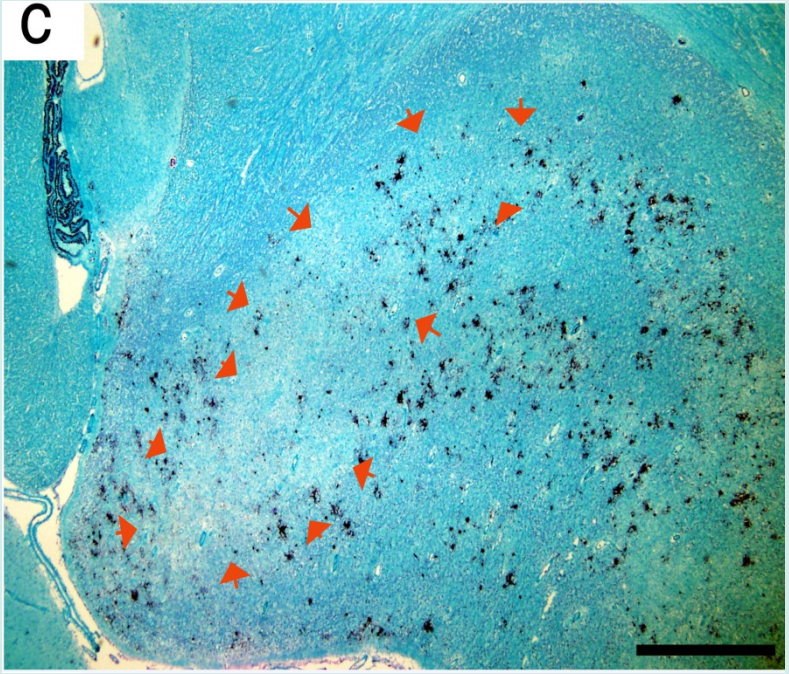


Axthelm JNEN 2004

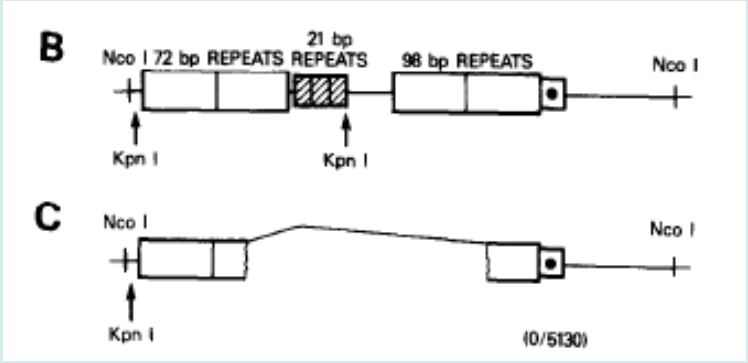
Dang J Virol 2005

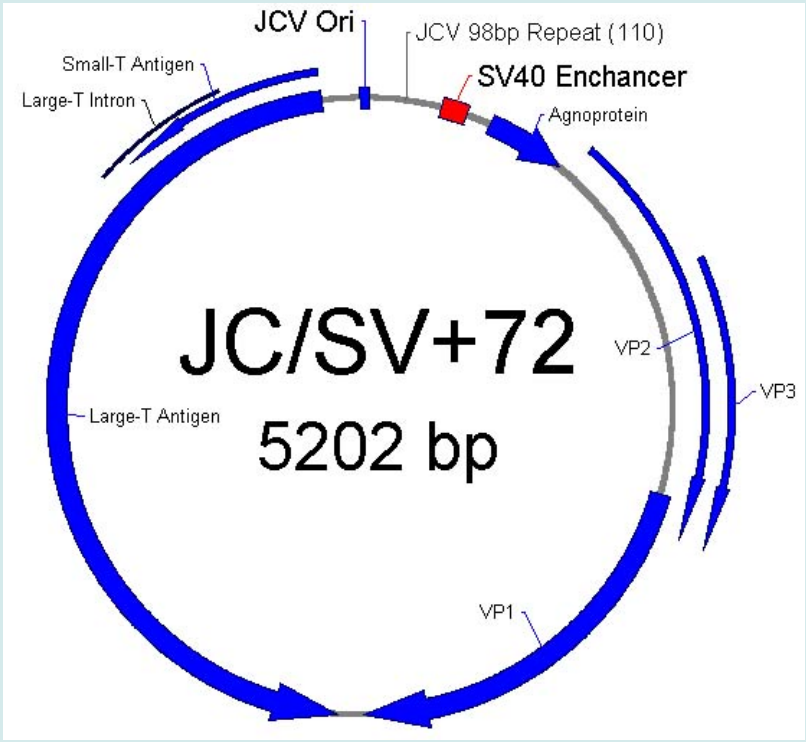
PML-derived molecular clone of SV40 causes disseminated infection

- Full length PCR amplification of SV40 from the brain of a PML monkey
- Isolation of a molecular clone of SV40 that can grow in monkey fibroblasts
- Infection of two SV40 negative SHIV-immunosuppressed monkeys
- Diffuse meningoencephalitis (astrocytes and neurons) and systemic infection **Dang JNEN 2008**

Solution #1: infection of monkeys with JCV/SV40 hybrid viruses

- JCV(M1/SVEDelta) with hybrid SV40/JCV regulatory region “turbo virus “(Vacante 1989)
  - May acquire other mutations (eg: Agnogene)
- JC/SV+72: insertion of one 72 bp element from SV40 in regulatory region of JCV Mad1 (Koralnik lab)





Solution # 2: JCV infection of humanized NOD/SCID mice made from and transplanted human organs and cells

Fetal Thymus

Fetal Liver

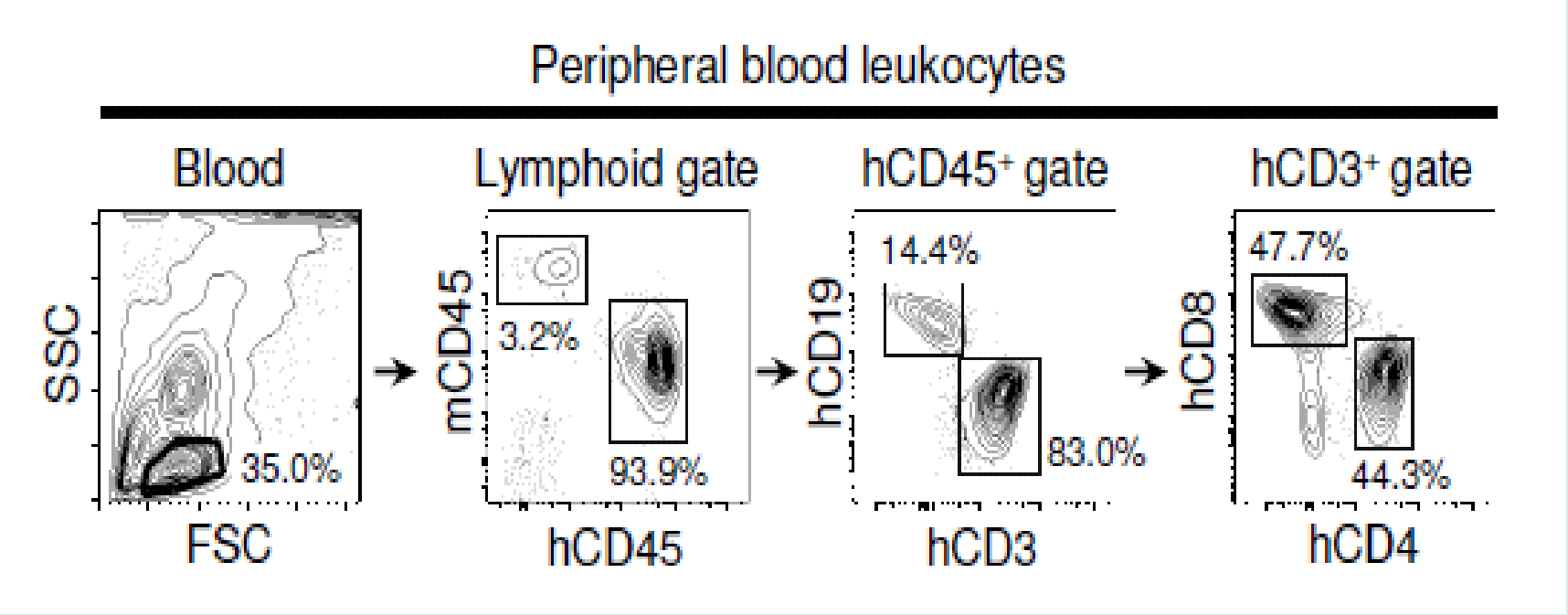
Fetal liver stem cells

NOD/SCID mice

B(one) L(iver) T(hymus): BLT mice

Brainard JV 2009

Reconstituted BLT mice display mostly human cells



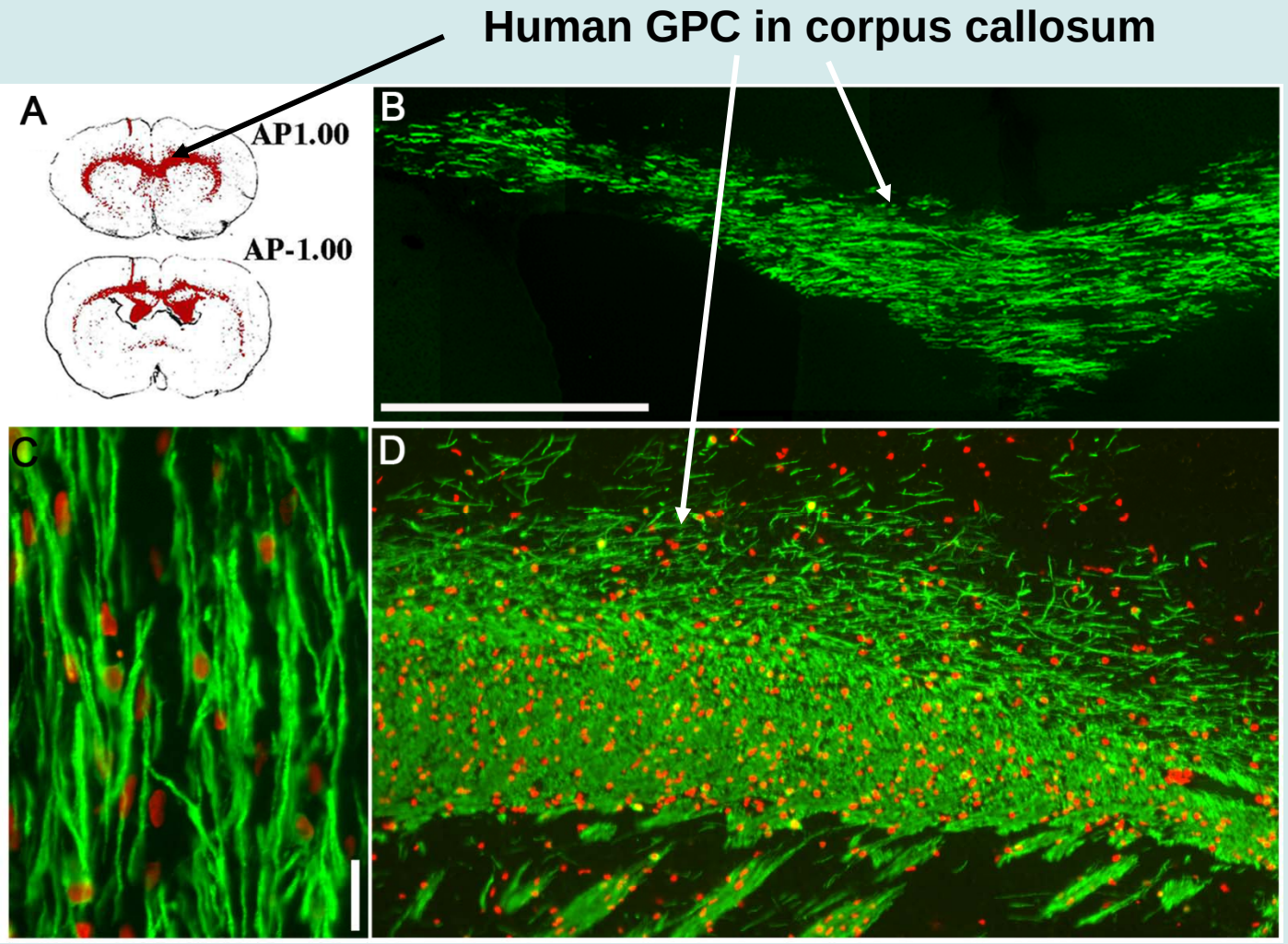
BLT mice PBMC showed 93.9% human lymphocytes

Brainard JV 2009

JCV infection in BLT mice

- Ongoing collaboration with Tager's lab (MGH) and Khalili's lab (Temple Univ)
- Primary infection with various strains of JCV
- Detection of JCV in different compartments
- Measurement of anti-JCV humoral and cellular immune responses
- Model of JCV primary infection, latency and reactivation
- Not a model of PML

Solution #3: JCV infection of demyelinated shiverer rag2 -/- mice remyelinated with human glial progenitor cells



Windrem Cell Stem Cell 08

Chimeric humanized glial-mouse brain model

- human glial progenitor cells engrafted perinatally in forebrain of neonatal hypomyelinated shi/shi mice
- chimeric mice have all oligodendrocytes and myelin from human origin
- majority of resident mouse glia eventually replaced
- Require injection of JCV in brain white matter

Major obstacle to success: Funding

- **NIH funding at all time low**
- **Less than 10% grant applications funded**
- **Stimulus package challenge grants: ~ 2% grants funded**
- **PML is a rare disease**
- **Natalizumab/PML felt to be a “company problem, not an NIH problem”**
- **Collaboration with Industry and other funding agencies (EMEA etc) crucial**
- **Streamlining information and access to funding for collaborative research studies should be a Major Goal of Workshop**

Collaborators

- **Div NeuroVirology**
  - Xin Dang
  - Christian Wuthrich
  - Sabrina Tan
  - Sarah Gheuens
  - Laura Ellis
  - Yiping Chen
  - Evelyn Bord
  - Elizabeth Norton
  - Angela Marzocchetti
  - Thomas Broge

PML patients and their families

- **Div Viral Path**
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- **Temple Univ**
  - Kamel Khalili
  - Jennifer Gordon
  - Mahmut Safak
- **Mount Sinai NY**
  - David Simpson
  - Susan Morgello
- **Washington Univ**
  - David Clifford
- **Univ Kentucky**
  - Joseph Berger
- **Univ Rochester**
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- **Hopkins**
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  - Avi Nath
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  - Rip Kinkel
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- **NEATC**
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