The Rise and Fall of XMRV

Stephen Hughes

HIV Drug Resistance Program

NCI–Frederick

NATIONAL® CANCER INSTITUTE

What Is XMRV?

- Xenotropic Murine leukemia virus-Related Virus
- First isolated from a human prostate tumor line (22Rv1)
- Closely related to, but distinct from, endogenous viruses found in the genomes of both laboratory and wild mice
- MLVs, as their name implies, cause cancers in animals (viral integration causes transcriptional activation of oncogenes)
- Closely related retroviruses (gammaretroviruses) infect a number of mammalian species, including primates (GALV)
- There are no known human gammaretroviruses

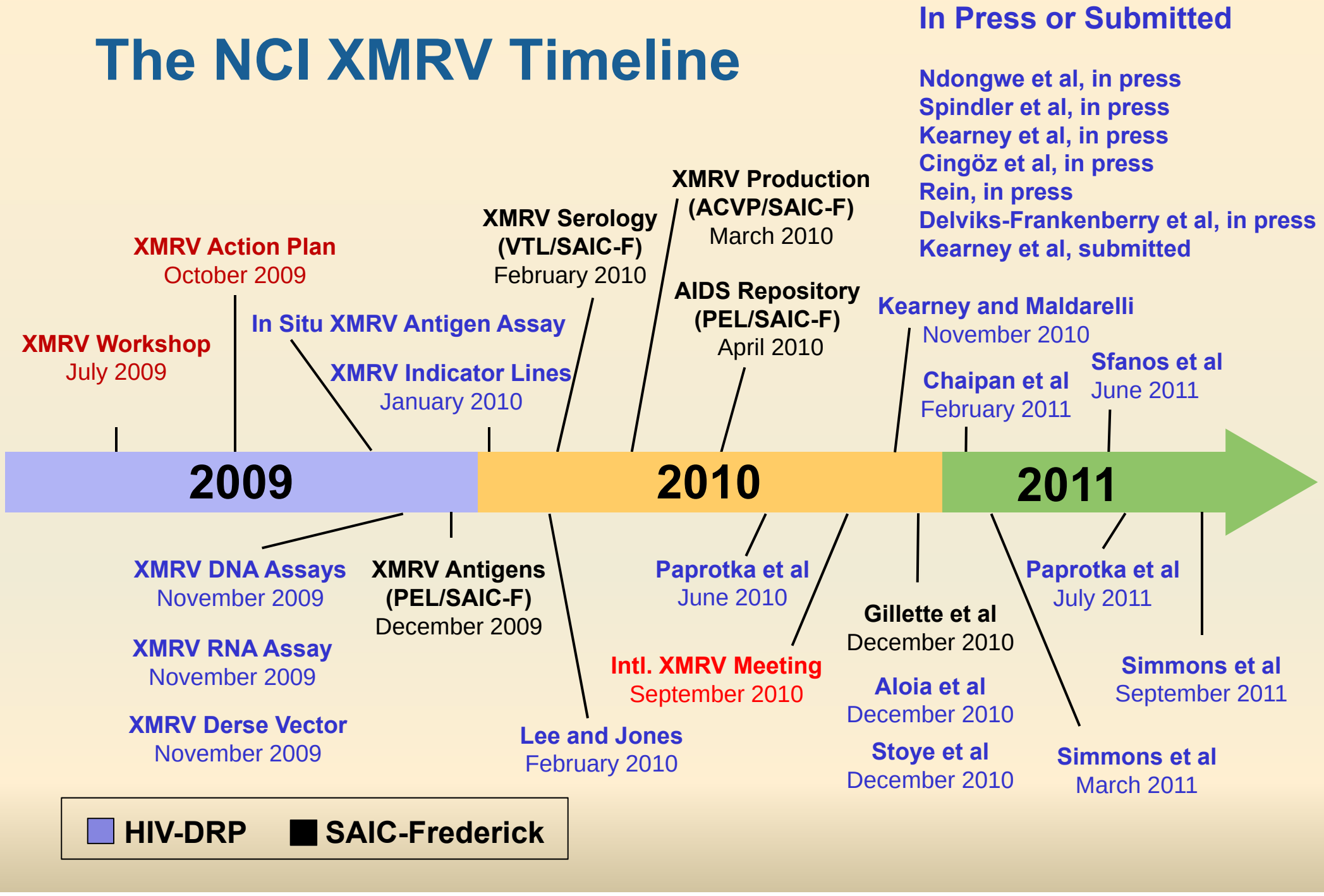
XMRV Questions/NCI Responses

- **Early reports suggested that XMRV is present in a majority of prostate cancer (and CFS) patients and in a small percentage of "normal" controls**
- **To determine if these early reports were correct, adapted assays developed for the detection of retroviruses/HIV (DRP)**
- **If XMRV infects humans, develop reagents and assays that can detect live virus, viral nucleic acids, proteins, and anti-XMRV antibodies (NCI-F=CCR+SAIC)**
- **Lay the foundation for developing vaccines and/or therapies (NCI-F)**
- **NCI/NCI-F has retrovirus expertise, is interested in prostate cancer, and can respond quickly (solving XMRV took about 18 months)**

A Brief History of XMRV

- Urisman et al., Plos Path 2006, report a link between XMRV and prostate cancer; also Schlaberg et al., PNAS 2009
- XMRV reported to be present in CFS patients Lombardi et al., Science 2009
- Several DRP PIs begin work on XMRV in 2009
- The potential importance of XMRV prompted an NCI-sponsored effort to develop reagents and assays (J. Coffin, S. Le Grice)
- XMRV workshop in July 2009 and an XMRV Action Plan (reagents, assays, etc.) in place in October 2009
- By late summer of 2011, there was good evidence that XMRV was not a human pathogen

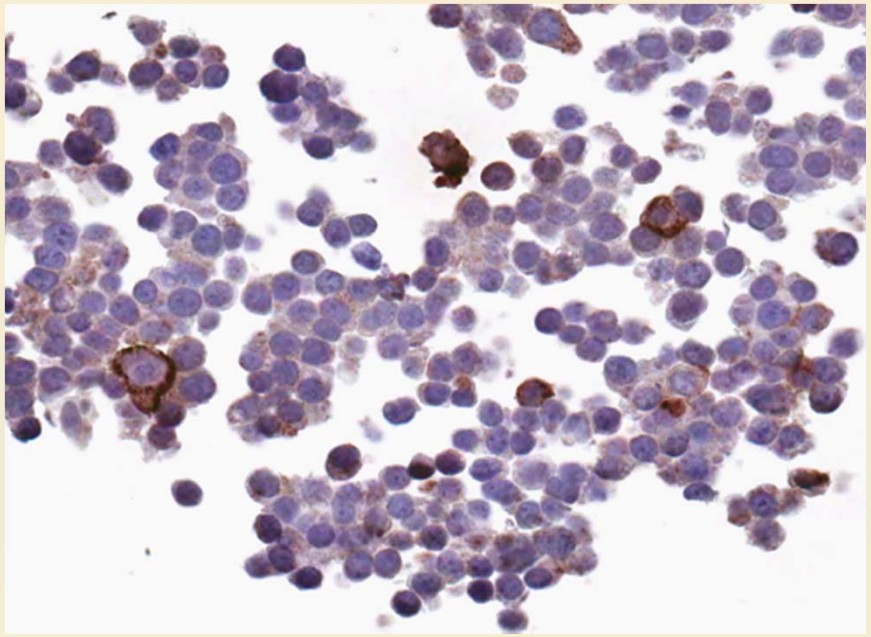
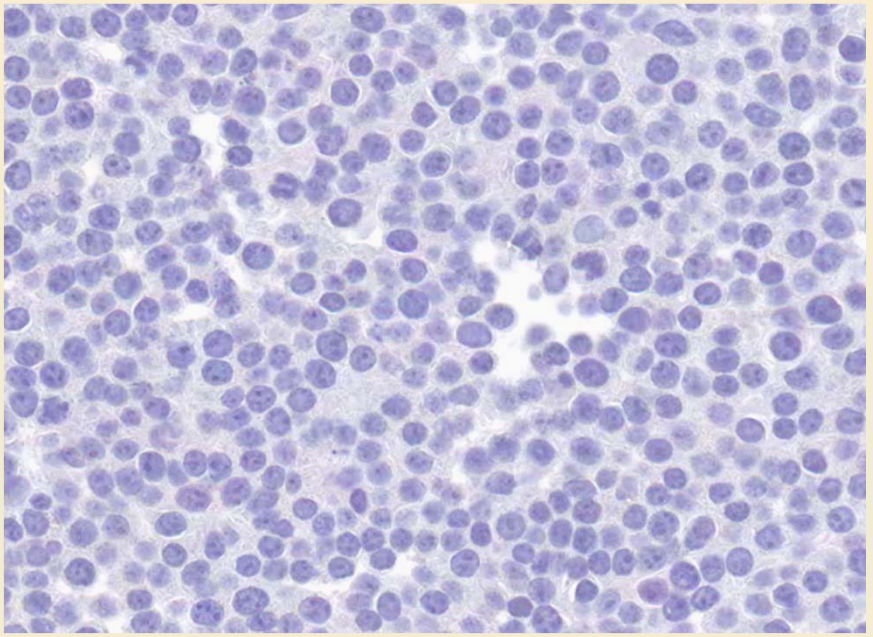
The NCI XMRV Timeline



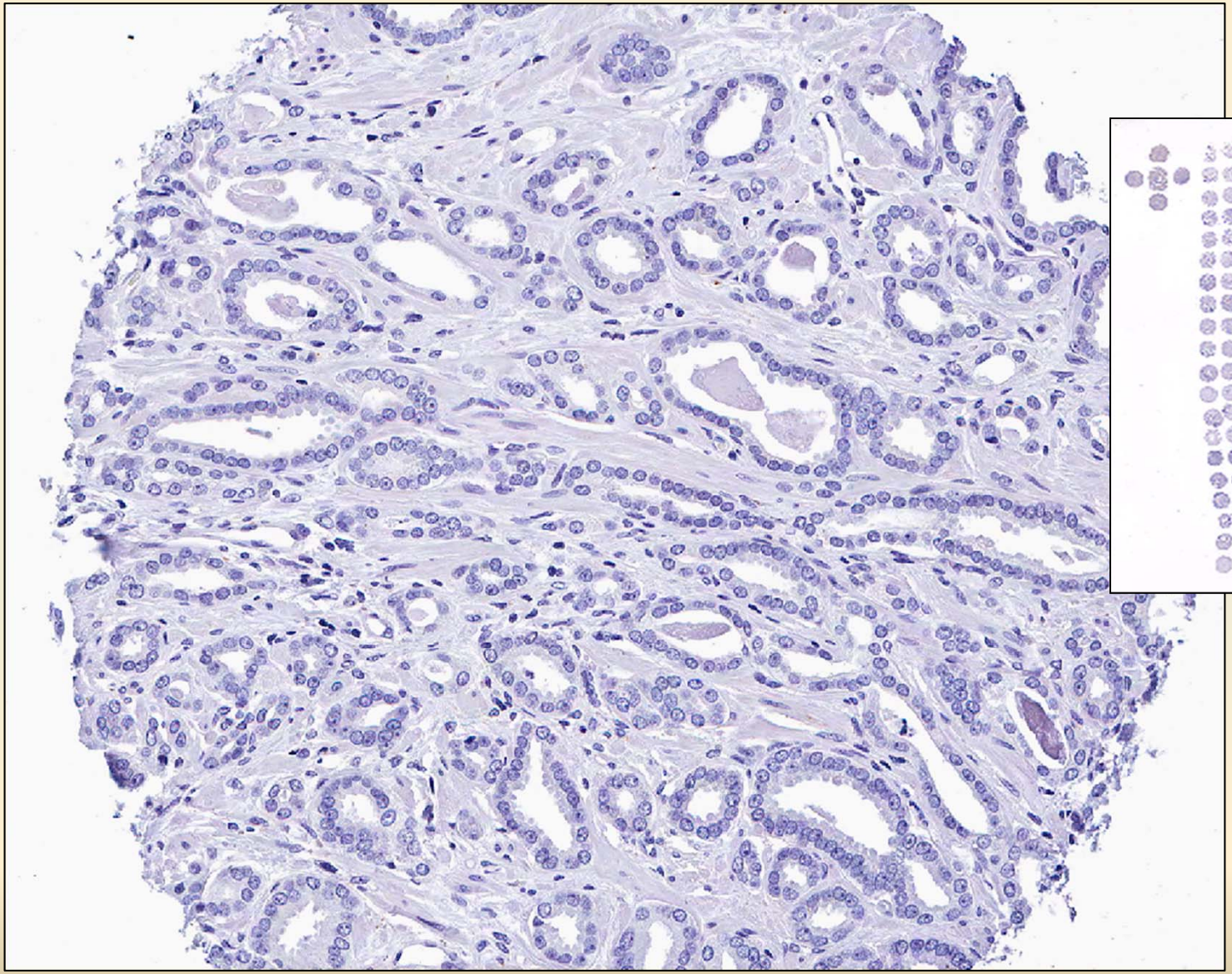
DRP Contributions to the XMRV Problem (Part 1)

- **Alan Rein:** No XMRV DNA in ~160 prostate cancers; no XMRV antigens in sections from ~600 prostate cancers, developed cell lines to detect XMRV
- **Frank Maldarelli:** Clinical protocols, obtained patient samples
- **Stuart Le Grice:** Oversaw preparation of XMRV proteins and mAbs (SAIC), structure of XMRV RT, organized meetings
- **Vinay Pathak:** No XMRV DNA in >100 prostate cancers (with TRU); XMRV is susceptible to human APOBEC and to some anti-HIV drugs; XMRV originated in human cells passed in mice (with J. Coffin), developed cell lines to detect XMRV (with W-S Hu)

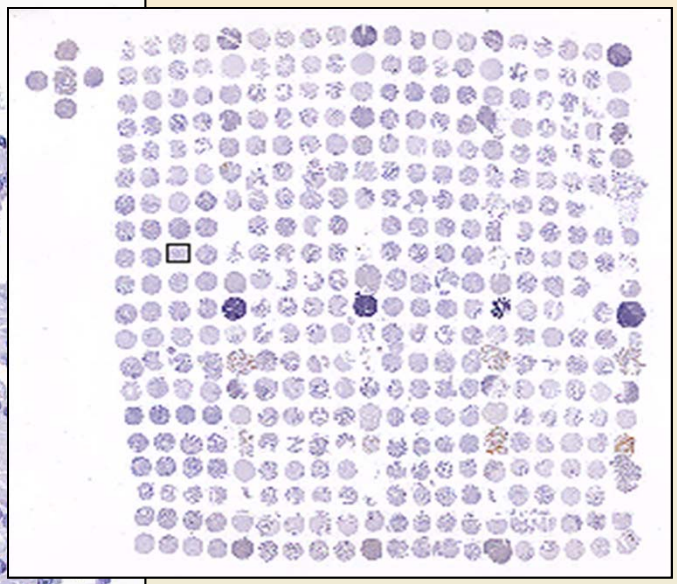
Detecting XMRV Antigens: The Controls

	Positive	Negative
α-p30^{CA}		

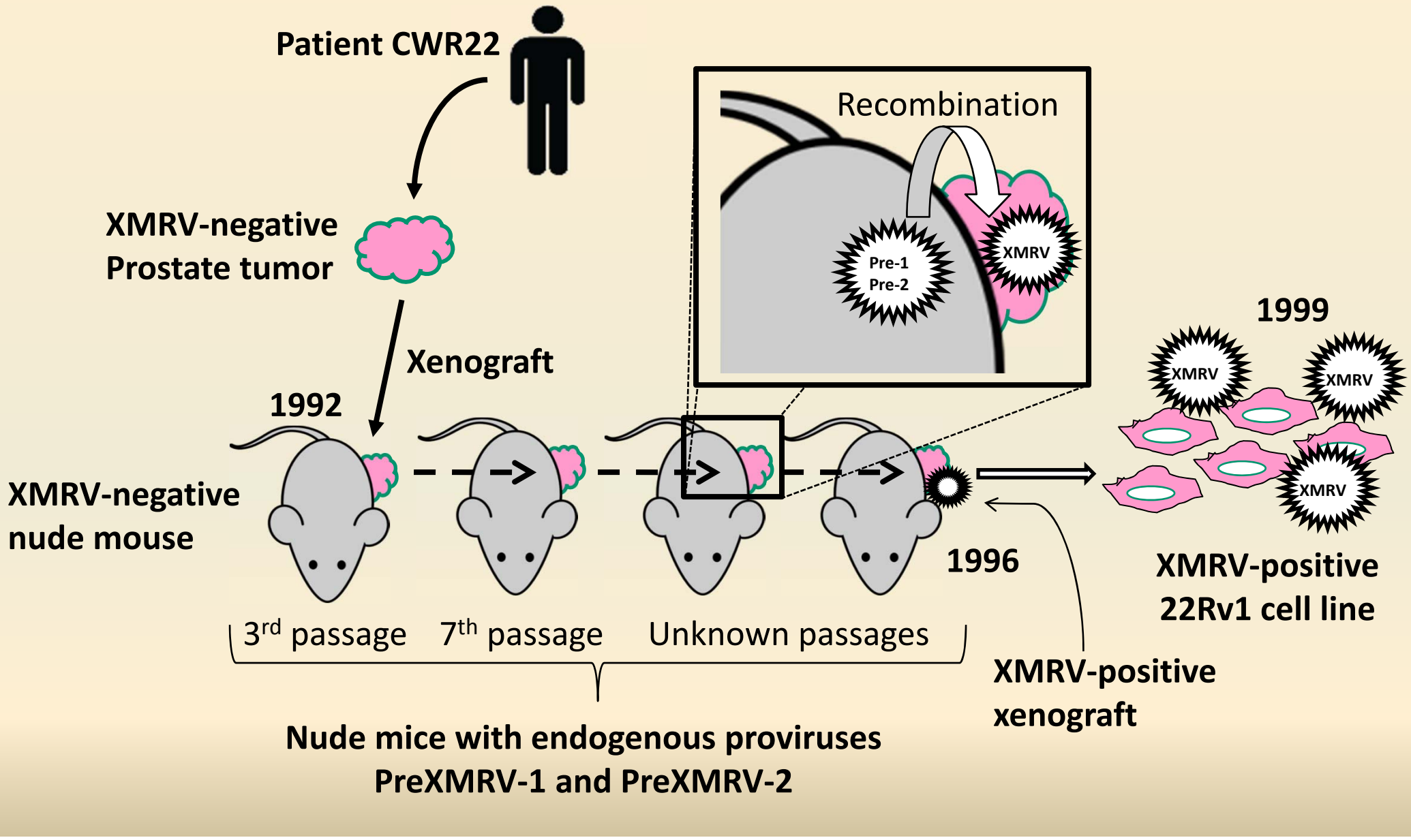
No XMRV Antigens in Human Prostate Cancers



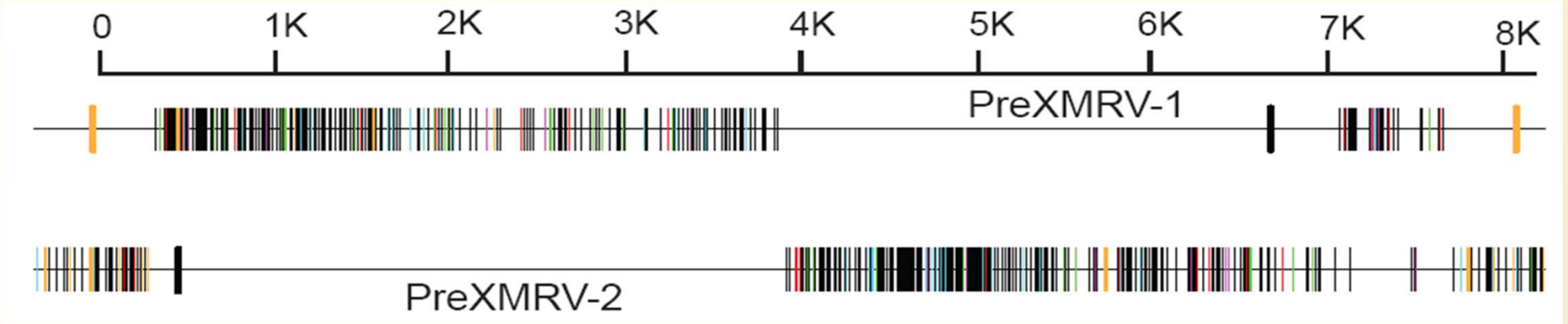
α-p30^{CA}



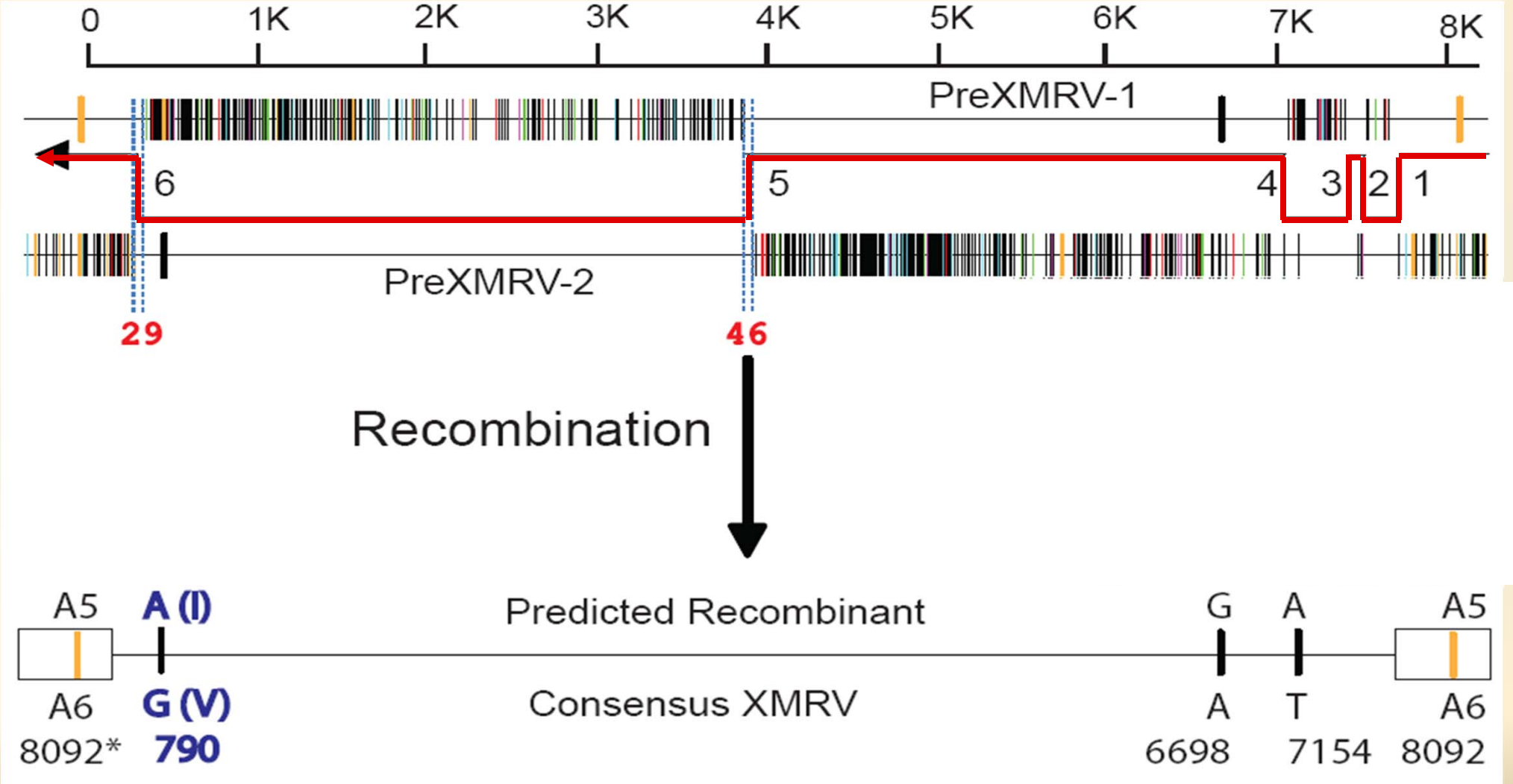
XMRV Originated in a Prostate Cancer Xenograft



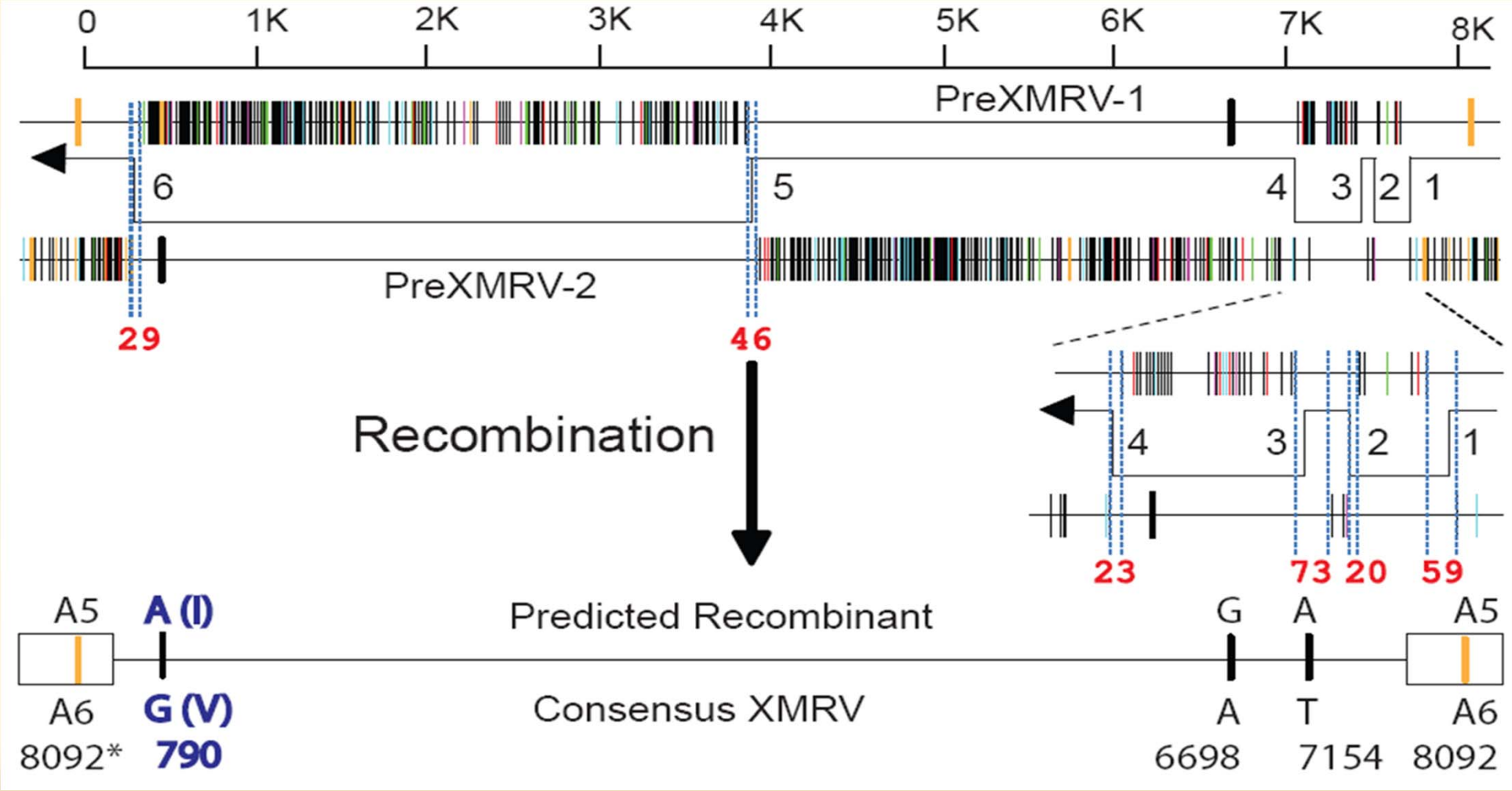
XMRV Arises via Recombination Between PreXMRV-1 and PreXMRV-2



Predicted Recombinant Between PreXMRV-1 and PreXMRV-2



Recombination Junctions Between PreXMRV-1 and PreXMRV-2 Define XMRV

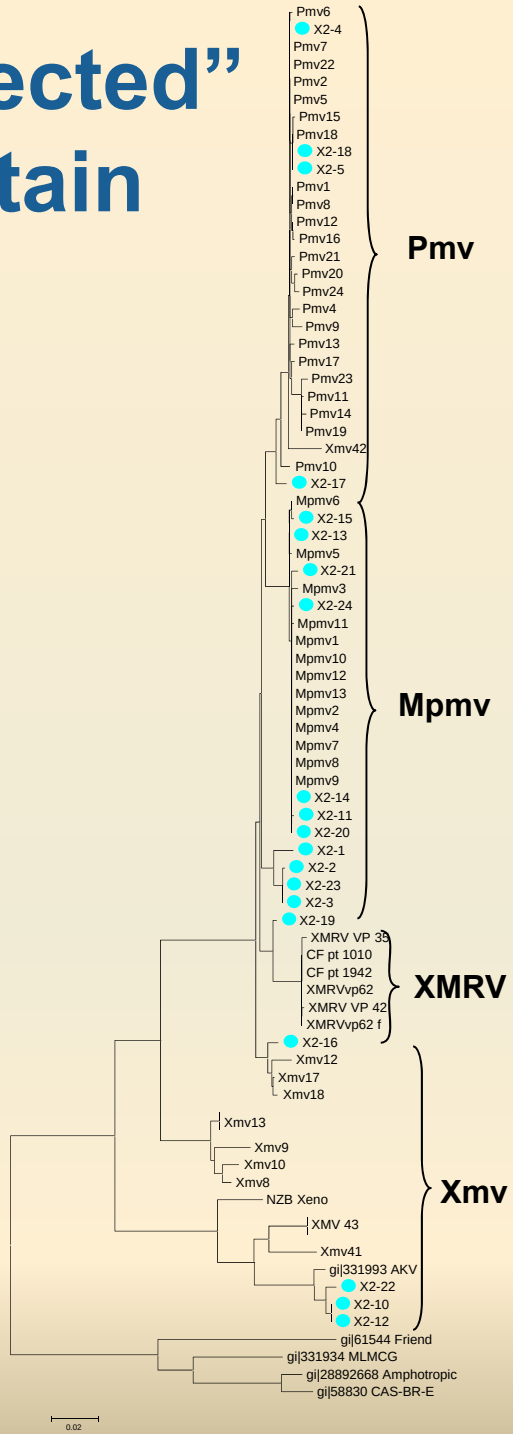


DRP Contributions to the XMRV Problem (Part 2)

- **Gisela Heidecker:** Developed DERSE vectors to detect replicating XMRV
- **Vineet KewalRamani:** Cell lines based on the DERSE vectors, infected macaque (TRU, ACVP)
- **Mary Kearney:** XMRV controlled and mutated in an infected macaque (Vineet, ACVP), mouse DNA is present in CFS patient samples, XMRV in patient samples is due to contamination

New Plasma Samples from "XMRV-Infected" Patients (Lombardi, et al, 2009) Contain Mouse DNA

TRU patient number	CFS status	XMRV status by WPI	X-SGS	Mouse COX2 gene	Mouse IAP
X1	neg	neg	neg	neg	neg
X2	CFS	pos	pos	pos	pos
X3	CFS	pos	pos	pos	pos
X4	neg	neg	neg	neg	neg
X5	CFS	pos	pos	pos	pos
X6	neg	neg	neg	neg	neg
X7	neg	neg	neg	neg	neg
X8	CFS	pos	pos	pos	pos
X9	neg	neg	NT	NT	NT

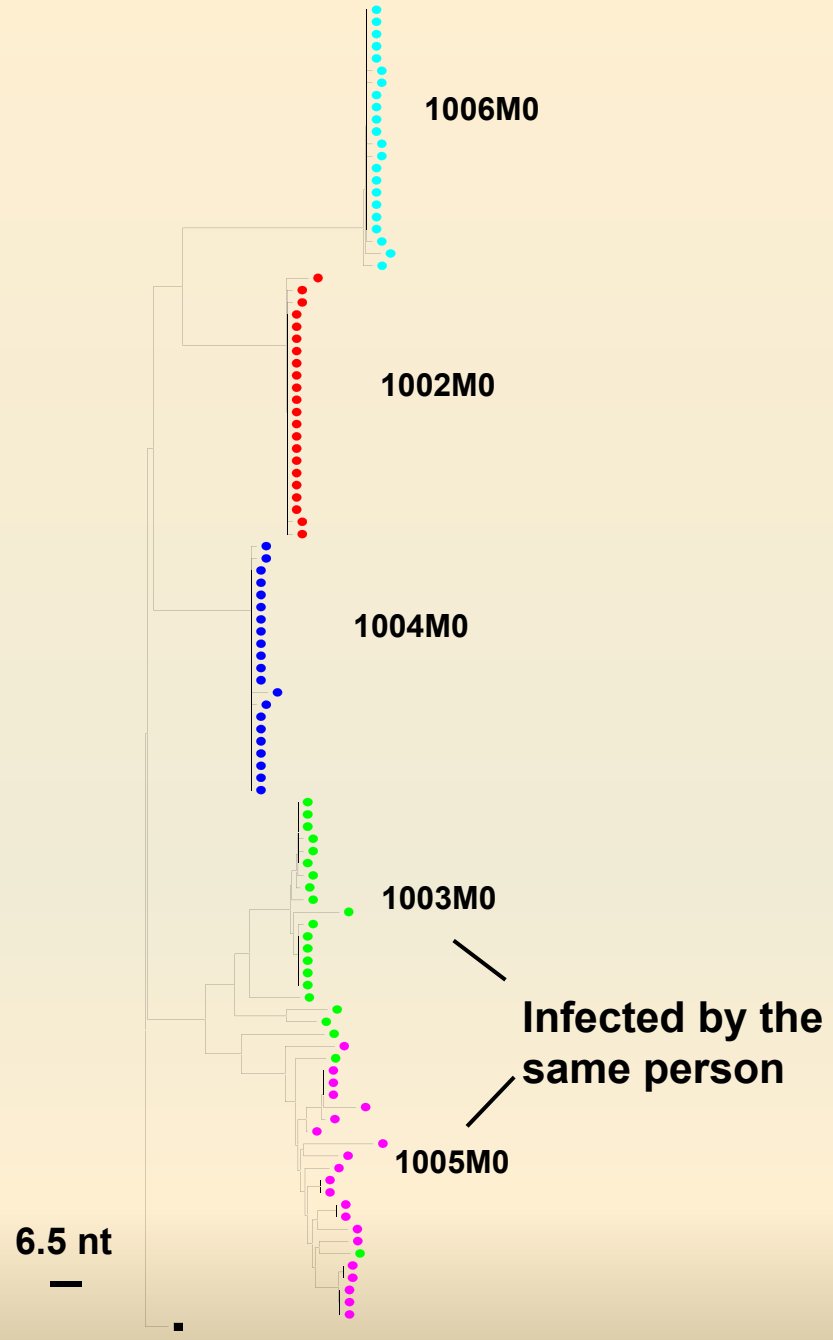


Viral sequences are from various endogenous mouse viruses, but none are XMRV.

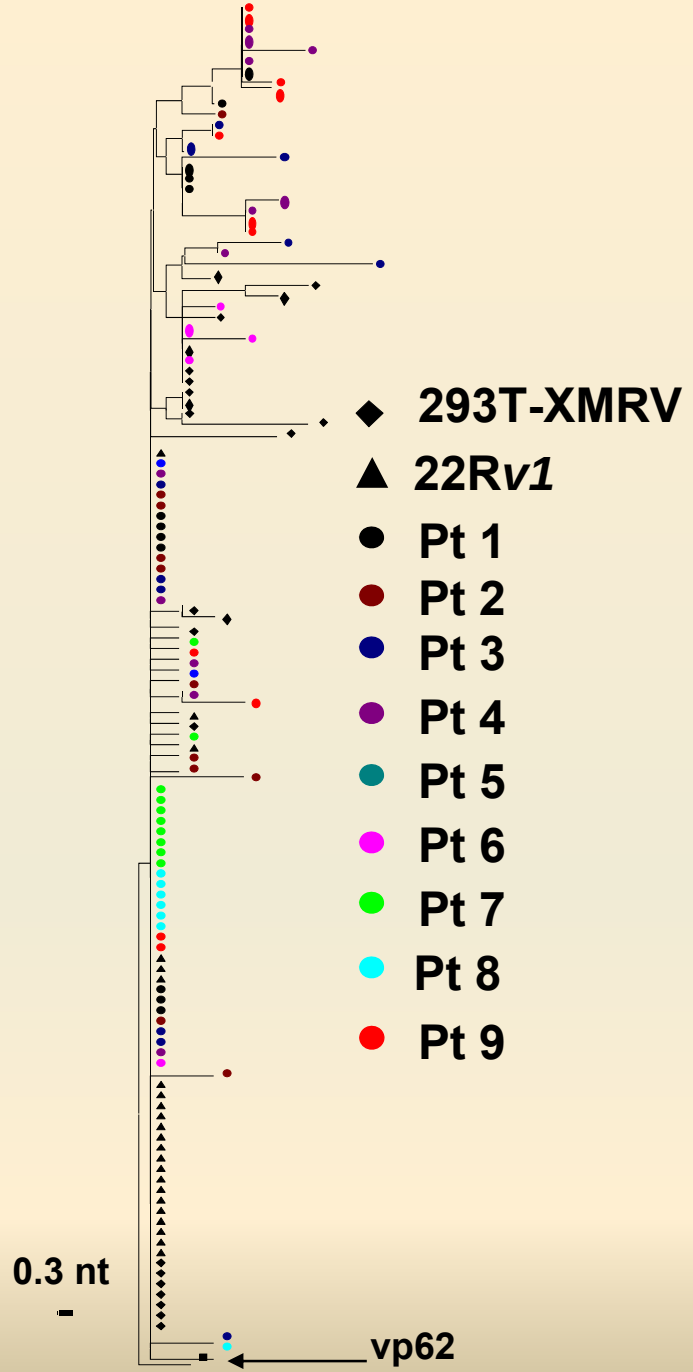
XMRV from "Positive" Cell Cultures Inoculated with Patient Samples Is Due to Contamination

- Sequenced XMRVs isolated from cultures inoculated with samples from 8 patients
- All "patient derived" XMRV isolates are nearly identical to XMRV from 22RV1
- These XMRV isolates have the signature recombination junctions near the 3' end of the (22Rv1) XMRV genome
- The XMRVs do not segregate phylogenetically by patient
- The XMRVs are laboratory contaminants

HIV-1 Sequences Segregate Phylogenetically by Patient



XMRVs in "Positive" Cell Culture Supernatants Do Not Segregate by Patient: The Viruses Are Contaminants



XMRV Is Not a Human Pathogen

- XMRV arose by recombination of two endogenous mouse viruses in a tumor xenograft about 15 years ago
- XMRV is susceptible to human APOBEC in cell culture and is rapidly cleared from the serum of an infected monkey
- Samples from CFS, prostate cancer patients, and controls show no evidence of XMRV infection either by PCR or immunohistochemistry
- All XMRV viruses have very similar sequences, and the same recombination breakpoints, as the virus in the xenograft
- Some of the reports of XMRV in patient samples are due to mouse DNA contamination
- Some of the reports of XMRV in patient samples are due to XMRV DNA contamination

If XMRV Had Been a Human Pathogen

- PCR assays to detect viral RNA and DNA
- Immunohistochemical assays to detect viral proteins
- DERSE vectors and indicator cell lines to detect XMRV replication
- A large panel of recombinant viral proteins for ELISA assays; infected monkey serum as a positive control
- A panel of specific monoclonal antibodies (developed using the recombinant proteins)
- Anti-HIV drugs were screened, and effective anti-XMRV drugs were identified
- The structures of two XMRV enzymes were solved (PR and RT); the corresponding HIV proteins are important drug targets

For the HIV-DRP, it’s back to HIV

Evidence for XMRV/pMLV Infection in Humans

1. **Urisman et al. (2006)**
   - Detection of viral DNA/RNA by Virochip
2. **Schlaberg et al. (2009)**
   - Viral protein detection by immunohistochemistry
   - PCR
3. **Lombardi et al. (2009)**
   - PCR
   - Detection of antiviral antibodies in patients
   - Isolation of infectious virus by culture
4. **Lo et al. (2010)**
   - PCR

XMRV, Prostate Cancer, and CFS: Negative Studies

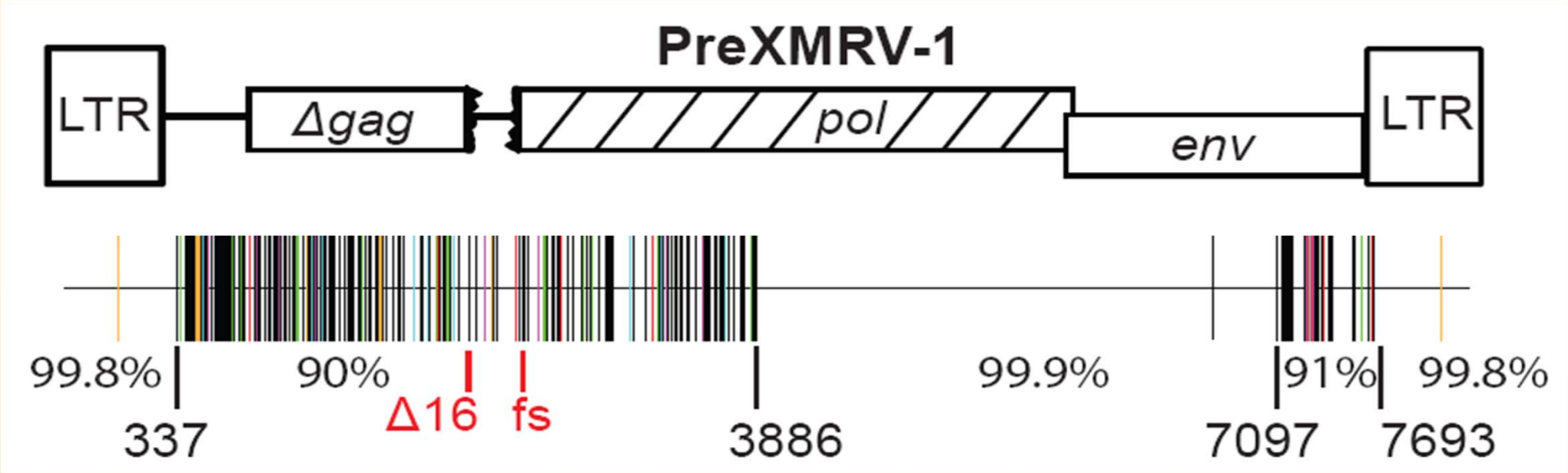
Prostate Cancer

Fischer et al., *J. Clin. Virol.* 2008; (1/105 pts; 1/70 cont.) German patients
Hohn et al., *Retrovirology*, 2009; (0/589 pts) German patients
Verhaeghet al., *Prostate*,2010; (3/74 pts) Dutch patients
Aloia et al., *Cancer Res*. 2010; (0/800 pts) US patients
Switzer et al, *PLoS One* 2011; (3/162) US patients

CFS

Erlwein et al., *PLoS One*, 2010; (0/186 pts) UK patients
van Kuppeveld et al.,*BMJ*, 2010;(0/32 pts) Netherlands patients
Groom et al., *Retrovirology*, 2010; (0/299 pts) UK patients
Switzer et al., *Retrovirology* 2010; (0/251) US patients
Hong et al., *JV* 2010; (0/65); Chinese patients
Satterfield, et al. Retrovirology, 2011; (0/45 pts; 0/42 cont.) US patients
Shin et al., *JV,* 2011; (0/100 pts; 0/200 cont.) US patients
Knox et al., *Science*, 2011; (0/61 pts) US patients

Early Xenografts Contain an XMRV-related Provirus: PreXMRV-1



Distribution of XMRV in Mouse Strains

Laboratory Strains (48)

129P1/ReJ	DBA/2J
129P3/J	HRS/J hr/+
129S1/SvImJ	Hsd nude*
129X1/SvJ	I/LnJ
A/J	LP/J
AKR/J	LPT/LeJ
AKR/J nude	MA/MyJ
B6.129	NCRNU-M*
B6CByF1/J	NFS/N
BALB/c nude	NIH Swiss*
BALB/cByJ	NIH-III
BALB/cJ	NU/J
BTBR	NU/NU*
C3H/HeJ	NUJM nude
C57BL/6	NZB/BinJ
C57BR/cd	NZW/Lac
C57L/J	P/J
C58/J	RIIIS
CBA	SJL/J
CByB6F1/J	SJLSmn.AK
CByJ.Cg	SM/J
CE/J	ST/bJ
CWD/Le	STOCK Ces1c
DBA/1J	SWR/J

94 Strains
170 Mice

0/170

**outbred*

Wild-derived Strains (46)

M. m. domesticus	SF/CamEi
	SK/CamEi
	SK/CamRk
	PERA/Ei
	PERC/Ei
	CALB/Rk
	WSB/Ei
	BIK
	ZALENDE/Ei
	TIRANO/Ei
	Poschiavinus
	BFM
	WMP/Pas
M. m. castaneus	CTA
	CASA/Rk
	CAST/Ei
M. m. molossinus	MOLC
	MOLD/Rk
	MOLE/Rk
	MOLF/Ei
	MOLG/DN
	MSM/Ms
	JF1/Ms
M. m. musculus	CZECH/I
	CZECH/II
	SKIVE/Ei
	MPB
	PWK/Ph
M. spretus	SFM
	SPRET/Ei
M. spicilegus	J131
	PANCEVO/Ei
	ZRU
M. caroli	KAR
	CAROLI/Ei
	J135
M. cookii	COK
	J136
M. cervicolor	CRV
	J53
M. platythrix	PTX
M. bactrianus	BIR
M. famulus	FAM
M. macedonicus	XBS
M. dunni	MDTF
M. pahari	*Mus pahari*/Ei

Distribution of PreXMRV-1 and PreXMRV-2

Laboratory Strains (48)

129P1/ReJ
129P3/J
129S1/SvImJ
129X1/SvJ
A/J
AKR/J
AKR/J nude
B6.129
B6CByF1/J
BALB/c nude
BALB/cByJ
BALB/cJ
BTBR
C3H/HeJ
C57BL/6
C57BR/cd
C57L/J
C58/J
CBA
CByB6F1/J
CByJ.Cg
CE/J
CWD/Le
DBA/1J
DBA/2J
HRS/J hr/+
Hsd nude*
I/LnJ
LP/J
LPT/LeJ
MA/MyJ
NCRNU-M*
NFS/N
NIH Swiss*
NIH-III
NU/J
NU/NU*
NUJM nude
NZB/BinJ
NZW/Lac
P/J
RIIIS
SJL/J
SJLSmn.AK
SM/J
ST/bJ
STOCK Ces1c
SWR/J

PreXMRV-1
PreXMRV-2
Both
Neither
**outbred*

Wild-derived Strains (46)

M. m. domesticus	SF/CamEi
	SK/CamEi
	SK/CamRk
	PERA/Ei
	PERC/Ei
	CALB/Rk
	WSB/Ei
	BIK
	ZALENDE/Ei
	TIRANO/Ei
	Poschiavinus
	BFM
	WMP/Pas
M. m. castaneus	CTA
	CASA/Rk
	CAST/Ei
M. m. molossinus	MOLC
	MOLD/Rk
	MOLE/Rk
	MOLF/Ei
	MOLG/DN
	MSM/Ms
	JF1/Ms
M. m. musculus	CZECH/I
	CZECH/II
	SKIVE/Ei
	MPB
	PWK/Ph
M. spretus	SFM
	SPRET/Ei
M. spicilegus	J131
	PANCEVO/Ei
	ZRU
M. caroli	KAR
	CAROLI/Ei
	J135
M. cookii	COK
	J136
M. cervicolor	CRV
	J53
M. platythrix	PTX
M. bactrianus	BIR
M. famulus	FAM
M. macedonicus	XBS
M. dunni	MDTF
M. pahari	*Mus pahari*/Ei

Distribution of PreXMRV-1 and PreXMRV-2

Laboratory Strains (48)

129P1/ReJ
129P3/J
129S1/SvImJ
129X1/SvJ
A/J
AKR/J
AKR/J nude
B6.129
B6CByF1/J
BALB/c nude
BALB/cByJ
BALB/cJ
BTBR
C3H/HeJ
C57BL/6
C57BR/cd
C57L/J
C58/J
CBA
CByB6F1/J
CByJ.Cg
CE/J
CWD/Le
DBA/1J
DBA/2J
HRS/J hr/+
Hsd nude*
I/LnJ
NU/NU*
NUJM nude
NZB/BinJ
NZW/Lac
P/J
RIIIS
SJL/J
SJLSmn.AK
SM/J
ST/bJ
STOCK Ces1c
SWR/J

Wild-derived Strains (46)

M. m. domesticus
SF/CamEi
SK/CamEi
SK/CamRk
PERA/Ei

European

M. m. castaneus
BFM
WMP/Pas
CTA
CASA/Rk
CAST/Ei

M. m. molossinus
MOLC
MOLD/Rk
MOLE/Rk
MOLF/Ei
MOLG/DN
MSM/Ms
JF1/Ms

Asian

M. m. musculus
CZECH/I
CZECH/II
SKIVE/Ei
MPB
PWK/Ph
SFM
SPRET/Ei
J131
PANCEVO/Ei
ZRU
KAR
CAROLI/Ei
J135
COK
J136
CRV
J53
PTX
BIR
FAM
XBS
MDTF
Mus pahari/Ei

M. cookii
M. cervicolor
M. platythrix
M. bactrianus
M. famulus
M. macedonicus
M. dunni
M. pahari

C57BR/cd
Hsd nude*
NU/NU*

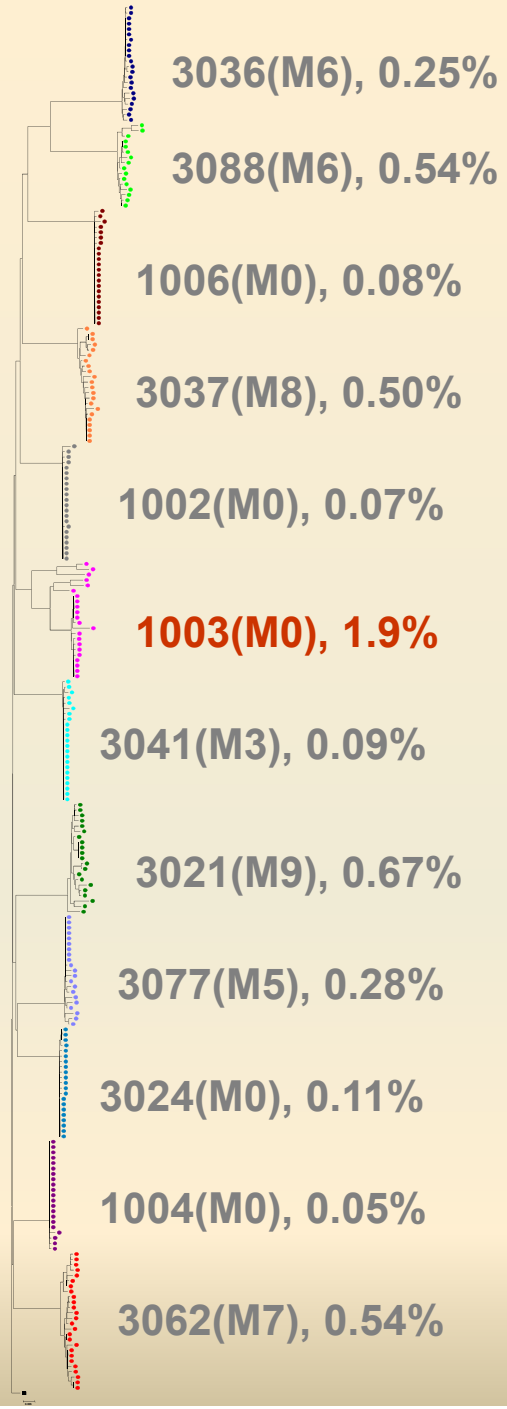
PreXMRV-1
PreXMRV-2

Wild-derived (n=46)

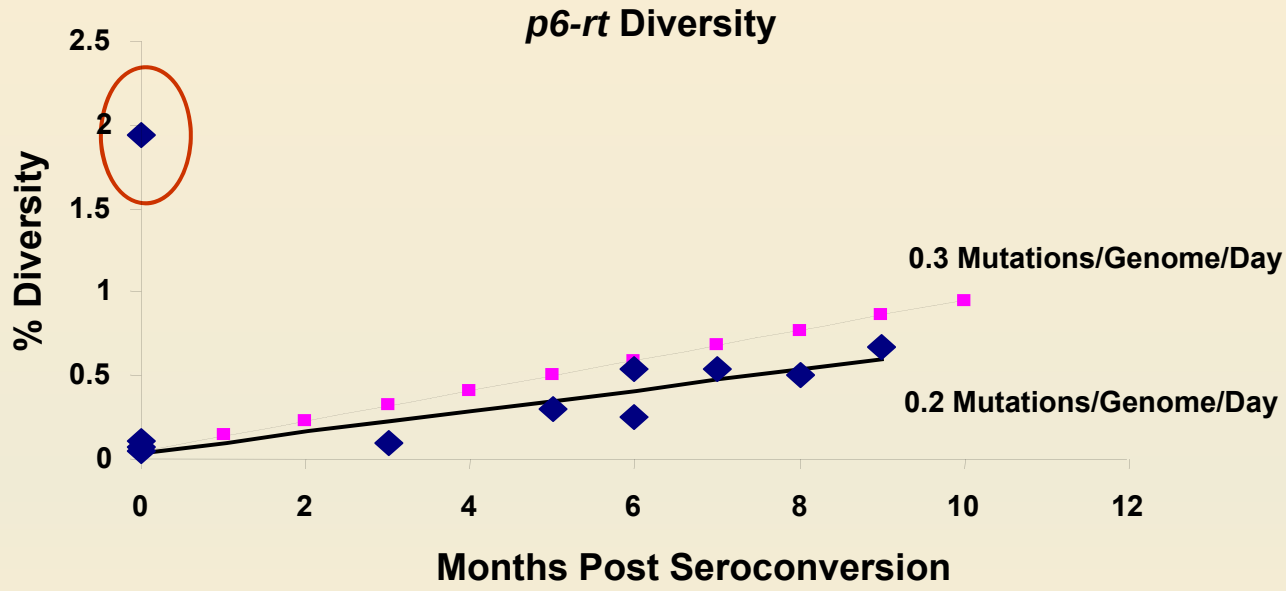
17.4% (8)
4.3% (2)

outbred

Genetic Diversity in Early/Acute HIV Infection



p6-rt



Kearney et al 2009

New Plasma Samples from "XMRV-Infected" Patients (Lombardi, et al, 2009) Contain Mouse DNA

TRU patient number	CFS status	XMRV status by WPI	X-SGS	Mouse COX2 gene	Mouse IAP
X1	neg	neg	neg	neg	neg
X2	CFS	pos	pos	pos	pos
X3	CFS	pos	pos	pos	pos
X4	neg	neg	neg	neg	neg
X5	CFS	pos	pos	pos	pos
X6	neg	neg	neg	neg	neg
X7	neg	neg	neg	neg	neg
X8	CFS	pos	pos	pos	pos
X9	neg	neg	NT	NT	NT

Viral sequences detected in the X-SGS assay are from various endogenous mouse viruses, but none are XMRV.

XMRV Originated, by Recombination, in a Prostate Cancer Xenograft