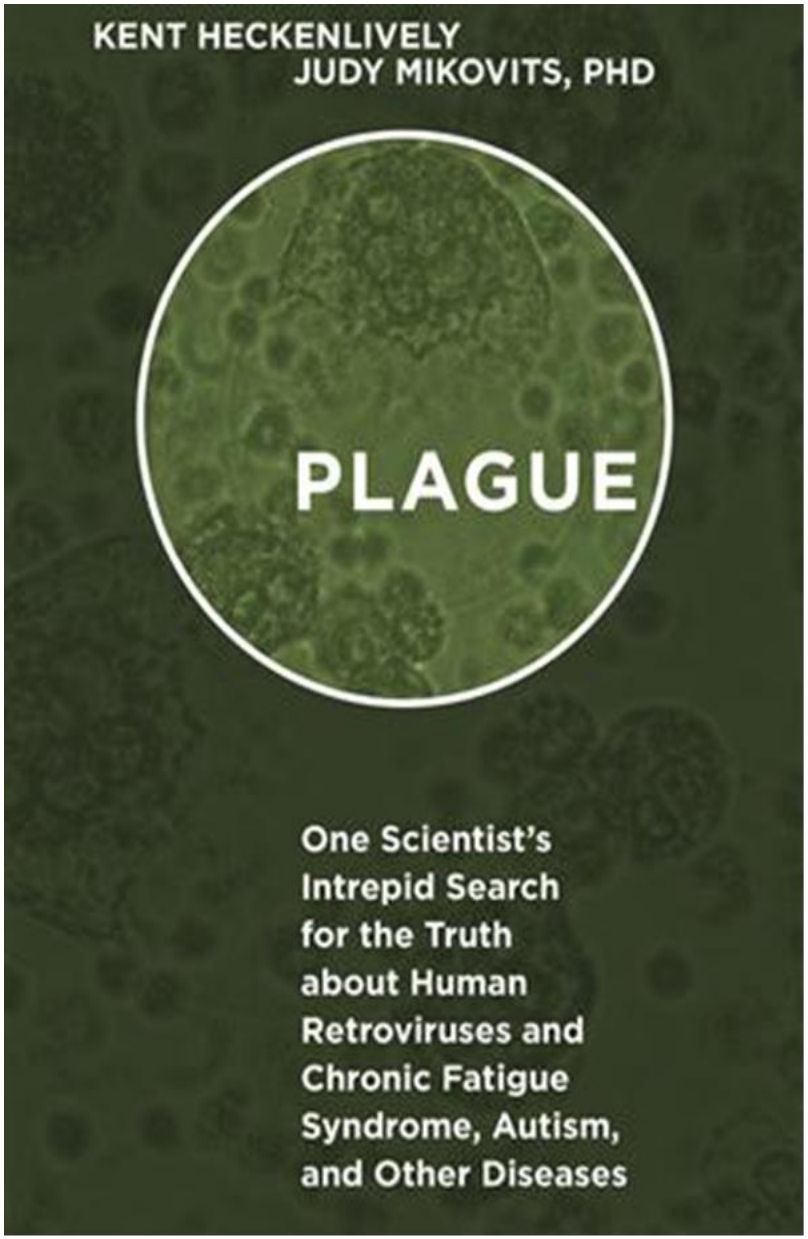
THE PROMISE AND PERIL OF BIG SCIENCE

The best scientist in jail story since Galileo.



How politics corrupts scientific research

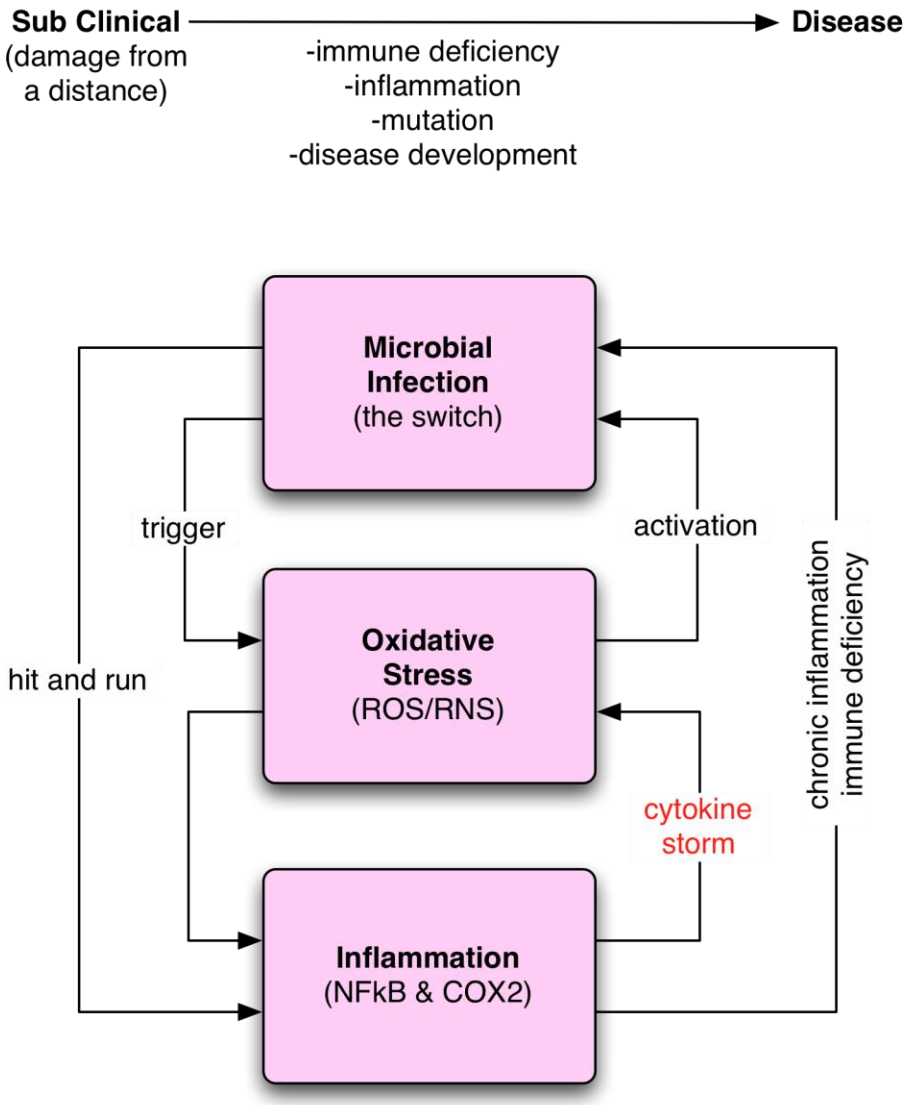
Particularly research involving human subjects

"they find what they want to find"

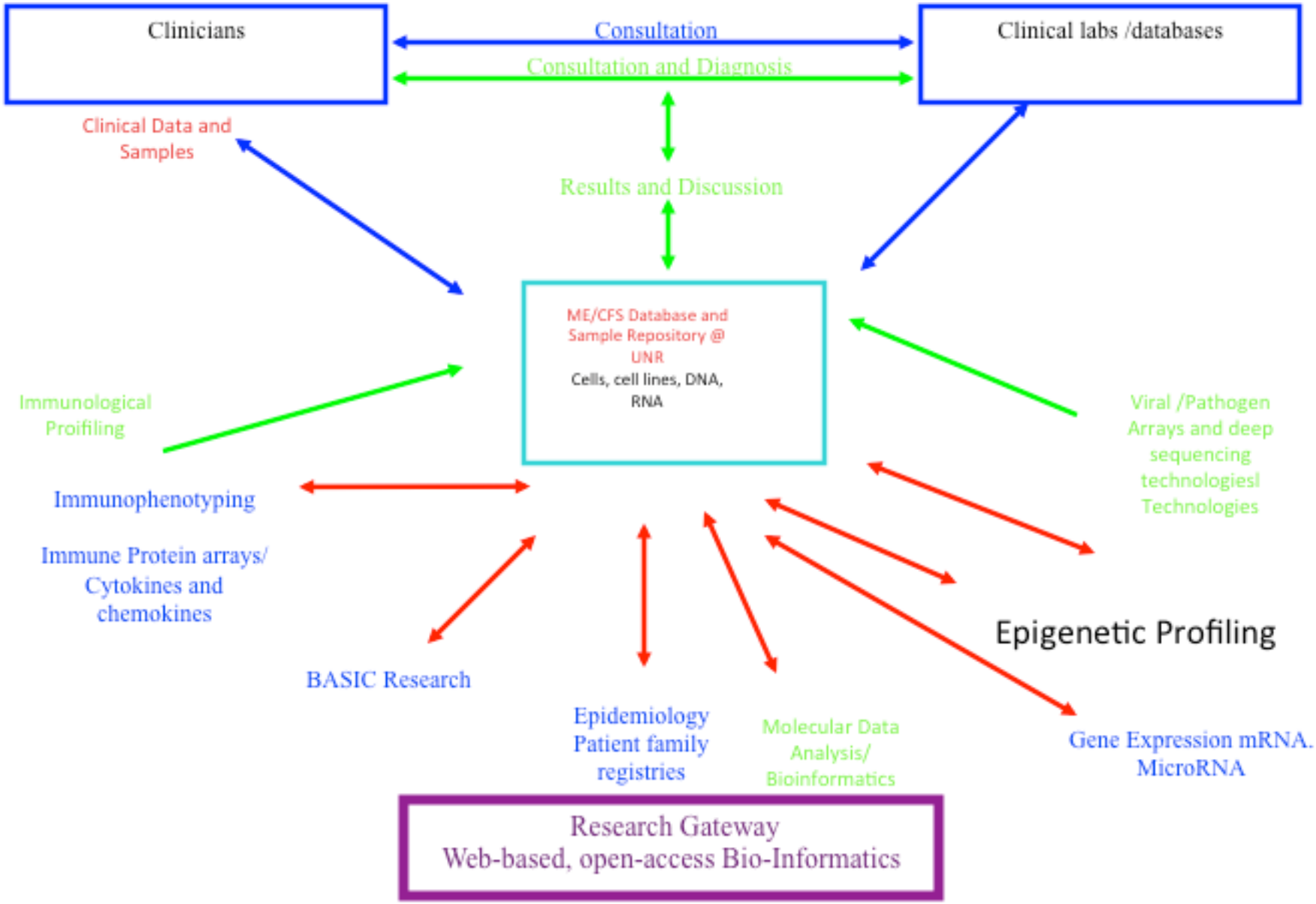
No funding = no associations

Difference between associations found in research associations found in MEDIA

Key Contributors to Chronic Diseases



Systems Biology Approach to Chronic Disease

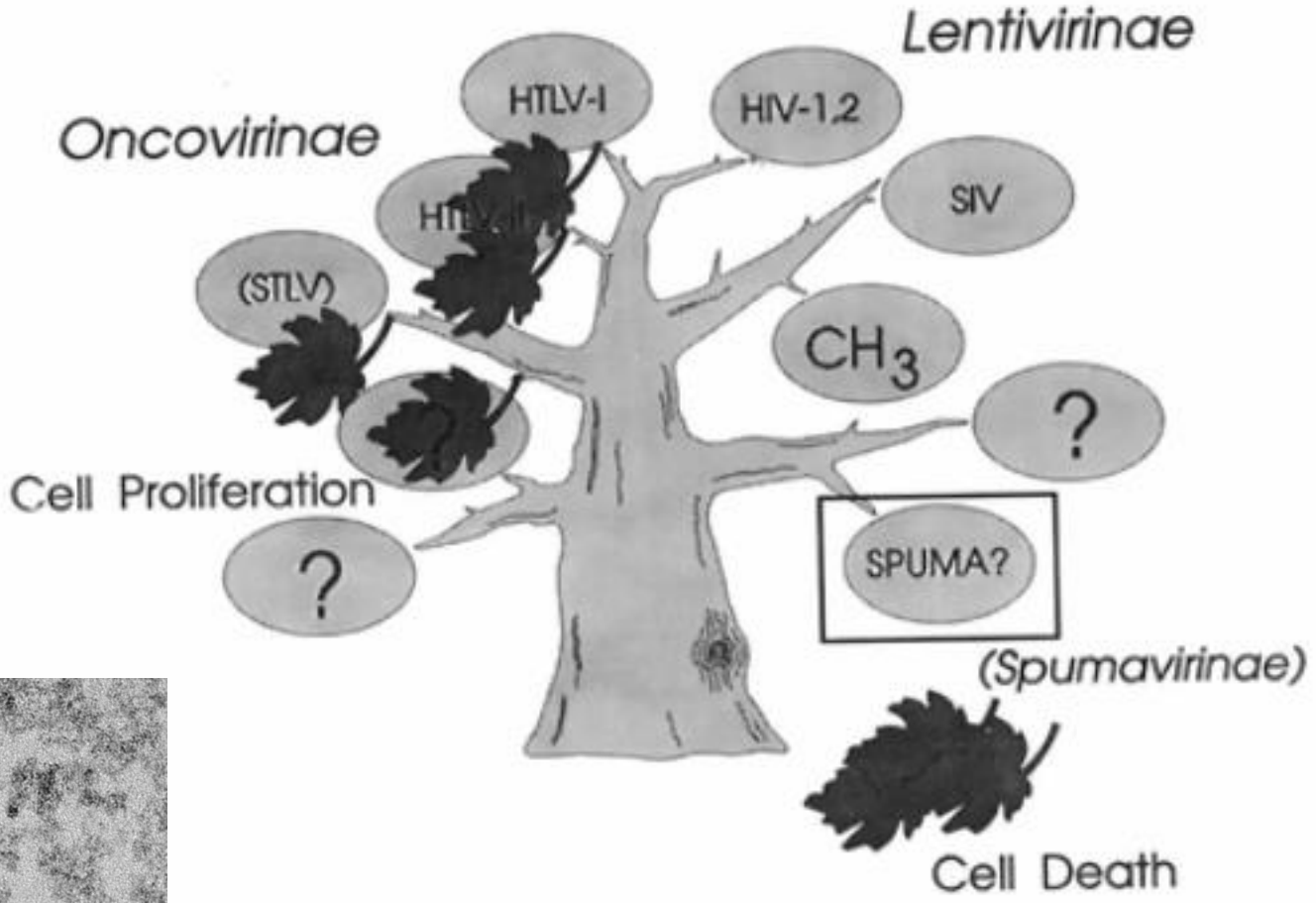


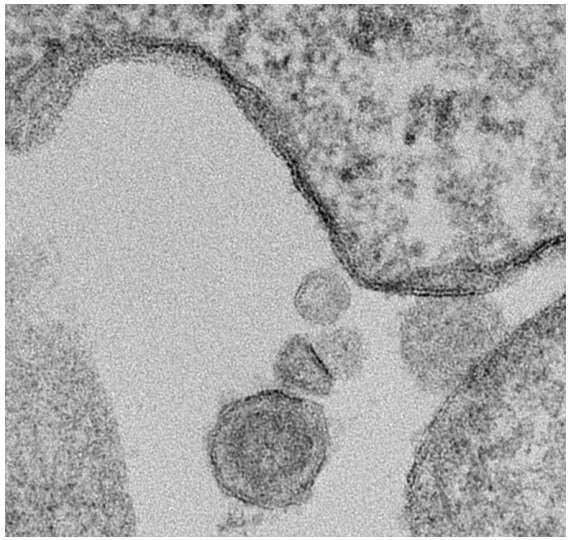
The Environment ME/CFS, ASD All Chronic Disease?

- More than 200 genes associated with Autism
- 2008 Kerr associated 88 genes with ME
- Many subtypes
- Pesticides
- Toxins
- EMF
- **Lessons learned from Other human retroviral Infections**
- **Zoonotic transmission exposures**
- **Heavy metals in water-Example from the Silver state**
- **GMO**
- **Vaccinations-The Anti-hygiene Theory**
- Microbiome.

ALL *ON TOP OF* THE GENETICS

Retrovirus Phylogeny

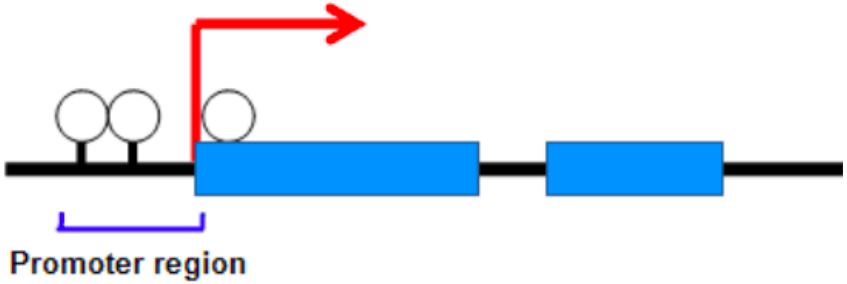




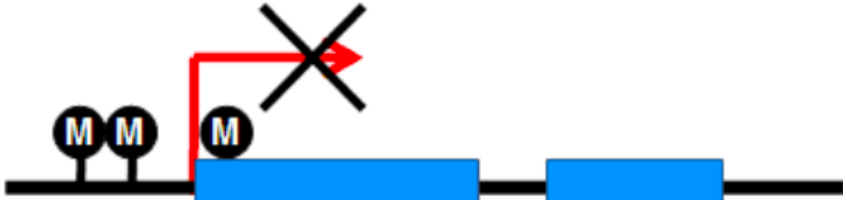
Retroviruses Integrate into genome..forever part of DNA of host

As much as 15% of human genome is made up of endogenous Retroviruses that have been crippled by the immune system That is they are not replication competent

Genes that can be expressed



Genes inactivated by DNA methylation



Molecular and Cellular Biology

Infection with Human Immunodeficiency Virus Type 1 Upregulates DNA Methyltransferase, Resulting in De Novo Methylation of the Gamma Interferon (IFN-γ) Promoter and Subsequent Downregulation of IFN- γ Production

Judy A. Mikovits, Howard A. Young, Paula Vertino, Jean-Pierre J. Issa, Paula M. Pitha, Susan Turcoski-Corrales, Dennis D. Taub, Cari L. Petrow, Stephen B. Baylin and Francis W. Ruscetti
Mol. Cell. Biol. 1998, 18(9):5166.

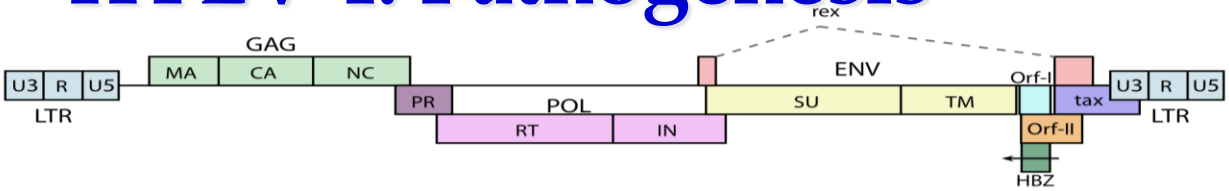
Mechanisms of Pathogenesis:

- Lessons learned from 30 years of Human retrovirus study

- Lessons learned from 40 years of MLV study

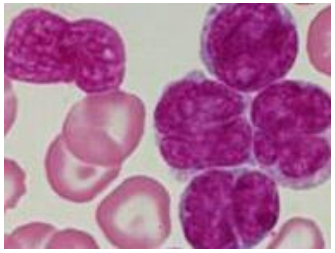
HTLV-I: Pathogenesis

HTLV-I



Genus: Deltaretrovirus (complex)

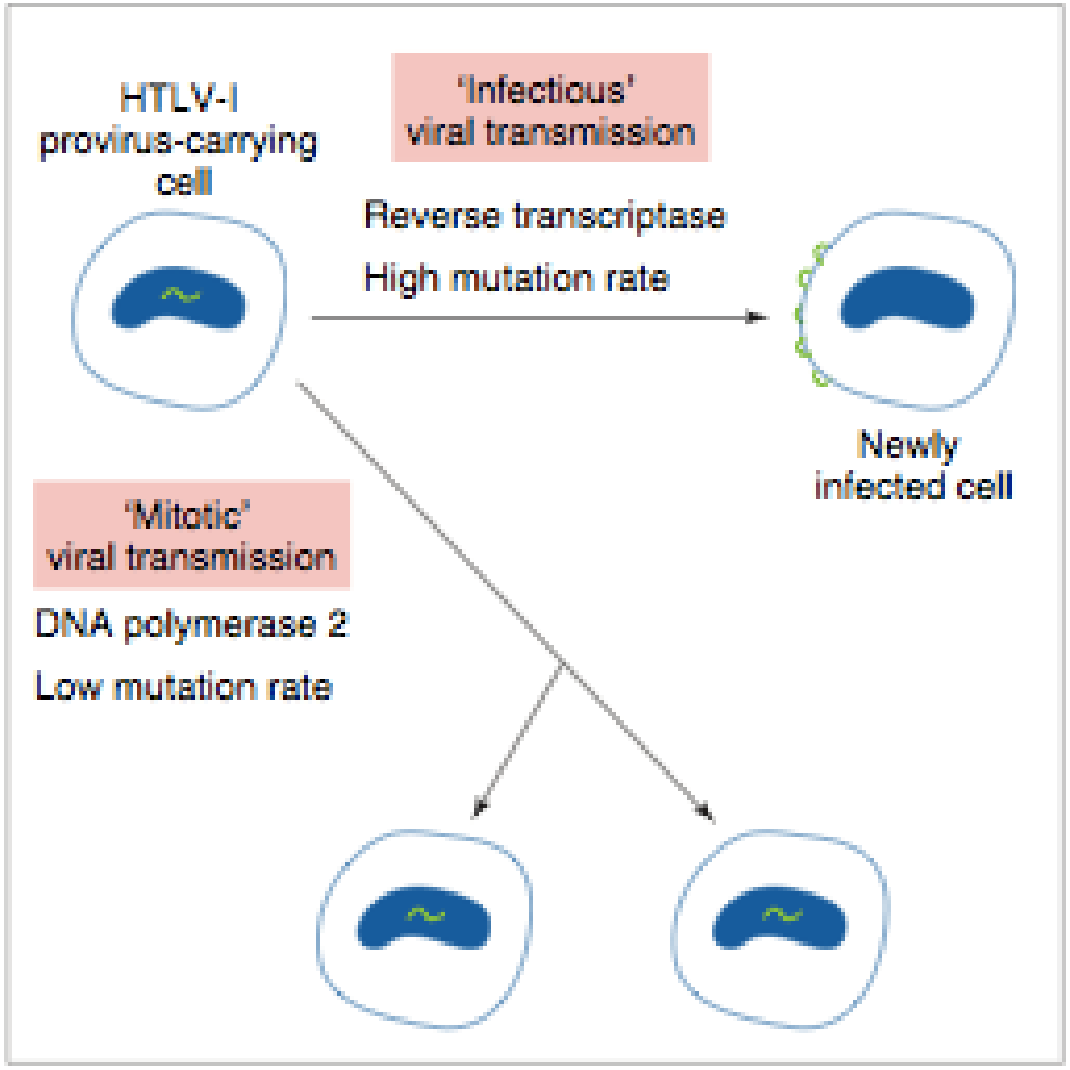
Genome: Multiple spliced RNAs for regulatory and accessory proteins



Pathogenesis:

- **Asymptomatic in majority of individuals**
- **5-8% lifetime risk of developing types of disease:**
  - **Adult T cell leukemia**
    - Clonal malignancy of $CD4^+$ T cells.
    - Long latency; Immune deficiency
    - Tax and HBZ needed for transformation
  - **Inflammatory syndromes**
    - HTLV-I associated myelopathy/Tropical spastic paraparesis
    - Uveitis
    - Arthropathy

Infectious vs. Mitotic transmission of HTLV-1



Increased Cytokine/Chemokine Production in plasma from ATL patients

Concentration in culture supernatant (pg/ml)	**ATL Patient**	**Uninfected**
IL-12p40	130	36
IL-6	2800	17
IL-1β	162	---
TNF-α	600	---
IP10	130	---
MCP-1	770	150
MIP-1α	450	90
IL-8	8500	420

- **Many cytokines such as IL-4, IL-5, IL-7 and type 1 interferons are not expressed in blood of infected patients**

Dysregulated Cytokine/Chemokine Production plasma from ME/CFS patients

CYTOKINES/ CHEMOKINES	Patient N = 156	Control N=140	P value	FUNCTION IN INFLAMMATION
IL-8	1067	11.1	<0.0001	RNase L and CMV activated
IL-13	28	86	<0.0001	Inhibits inflammatory cytokine production
MIP-1β	1840	157	<0.0001	Elevated in Neurodegenerative disease
TNF-α	109	12.8	<0.0001	Stimulates chronic inflammation
MCP-1	468	421	0.003	Elevated in chronic inflammatory diseases
IL-7	21.1	82	<0.0001	Stimulates proliferation of B and T lymphocytes and NK cells
IFN-α	35	60	<0.0001	Stimulates macrophages and NK cells to elicit an anti-viral response
IL-6	271	29	<0.0001	Stimulates chronic inflammation
MIP-1α	673	91	0.0062	Elevated in Neurodegenerative disease
GM-CSF	108	166	<0.0001	Stimulates proliferation of B and T lymphocytes and NK cells

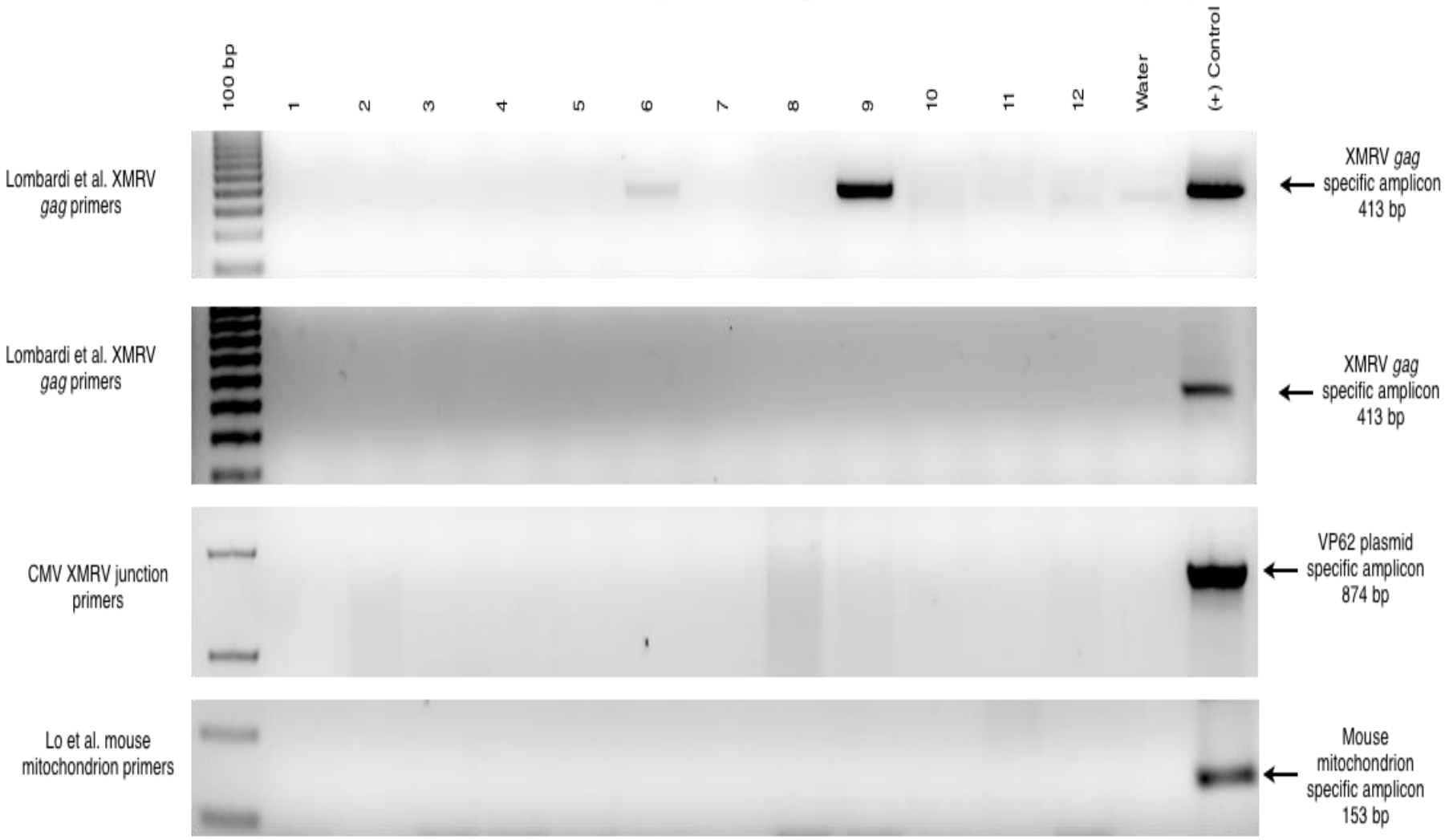
3 B-Cell Lines Derived Directly From CFS Patients' PBMCs

- **CFS patient PBMCs were cultured; 3 samples developed into immortalized cell lines**
- **All three showed high CD20+ expression and two showed high CD23+ expression.**
- **All three showed strong similarity to B cells seen in patients.**

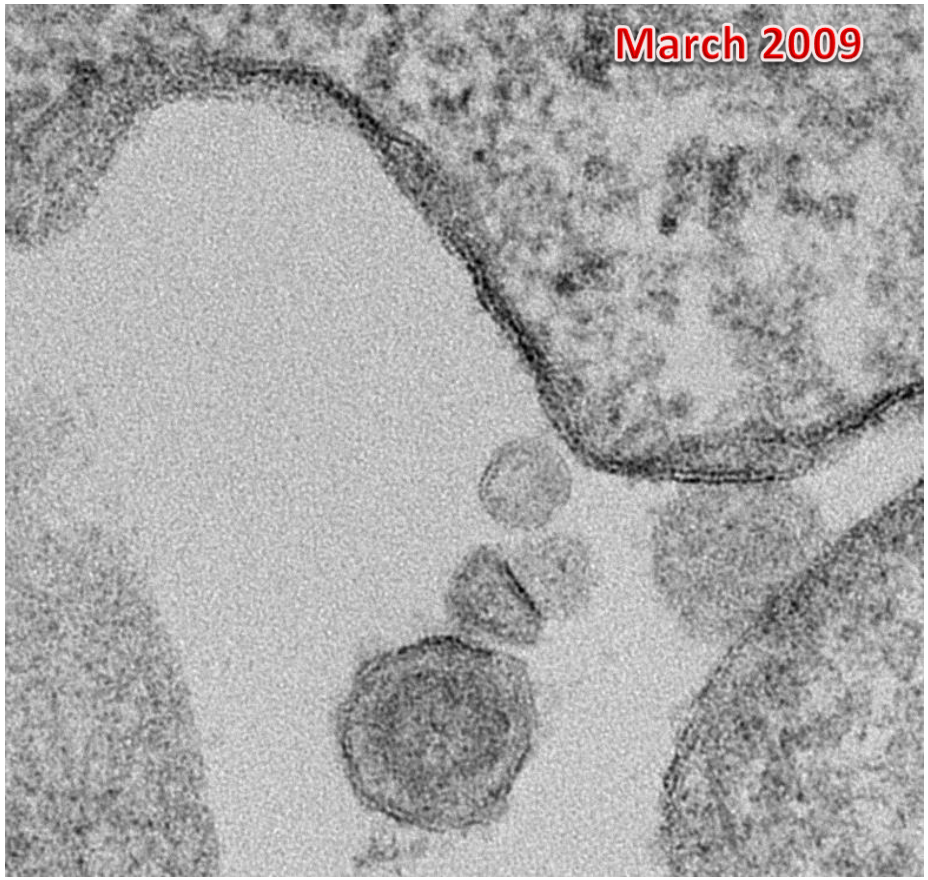
Marker	MCL	WPI 1125	WPI 1186	WPI 1143
CD5	+	+	+	+
CD23	-	-	+	+
CD19	+	+	+	+
CD20	+	+	+	+
FMC7	+	+	-	-
CD3	-	-	-	-
CD4	-	-	-	-
CD7	+	-	-	-
CD8	-	-	-	-
CD10	-	-	-	-
CD38	+	+	+	+
CD45	+	+	+	+
CD56	-	-	-	-
CD122	-	-	-	-
HLA-DR	+	+	+	+
Lambda	+	+	-	-
Kappa	+	+	+	+

These Cell lines were developed from CFS patients. One, (1125) developed MCL; one (1186) was developed from a bone marrow biopsy, 3rd a CLL

Extended PBMC cell culture without manipulation shows XMRV *gag* infection (DNA) in samples negative for XMRV *gag* RNA



Electron Micrograph of gamma retrovirus isolated from ME/CFS patients blood cells



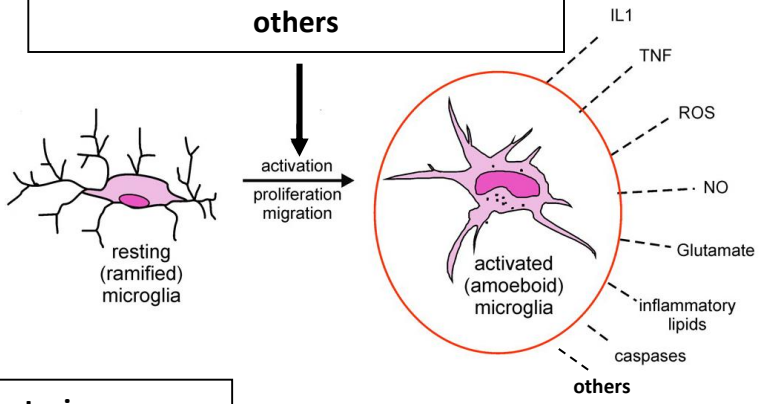
Taken together, these data demonstrate the first direct isolation of infectious XMRV from humans and implicate a role for XMRV infection in the pathogenesis of CFS."

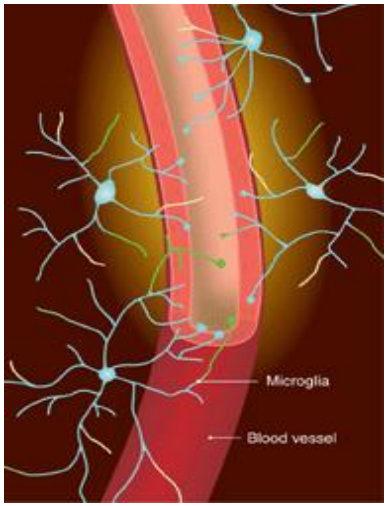
The original abstract of the *Science* article which was published on October, 8, 2009

Plague CH 11 p183

Microglia Activation in Neurodegeneration

Stimuli
Loss of cell:cell communication, matrix breakdown, infections, vascular damage, others





Fetler, L and S Amigorena, Science 2005, 309:392

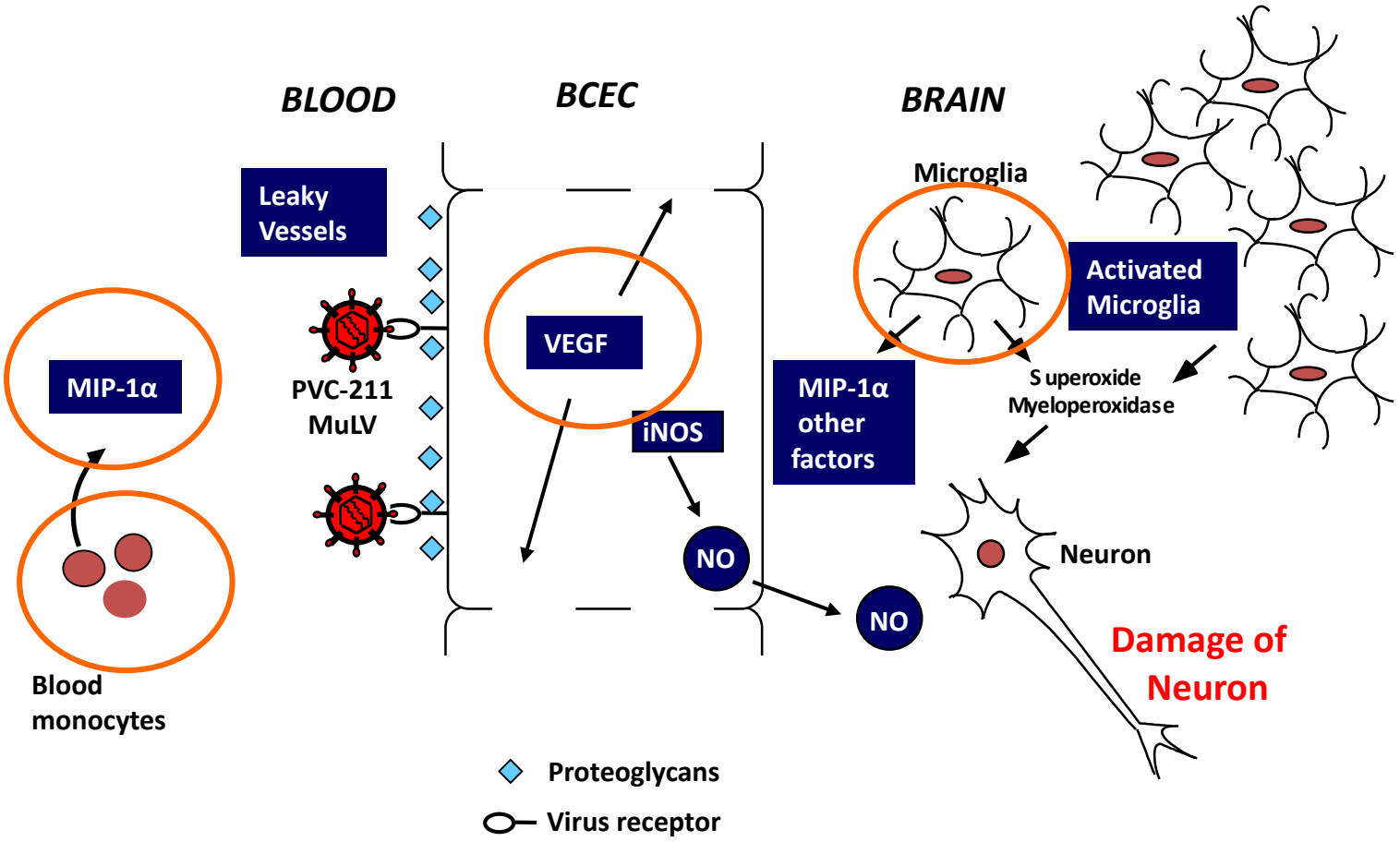
Homeostasis
Phagocytosis of debris, neurotrophins

Damage
Neurotoxins, phagocytosis of normal neurons, apoptosis

Neurodegenerative disorders

- **Parkinson's disease**
- **Alzheimer's disease**
- **Multiple sclerosis**

Model for the Induction of Neurodegeneration by one strain of MLV in an animal model



these immune pathways see in ASD and Other Chronic neurological diseases

Two important lessons learned from studying MuLVs

- **While insertional mutagenesis by MuLVs can result in transformation of cells and the development of leukemias and lymphomas, the envelope proteins encoded by these viruses can also have profound biological effects.**
  So it's important to study the biological effects of the XMRV envelope protein.

- **MuLVs can be expressed in the CNS, triggering an inflammatory response that can cause severe neurological damage. Since similar inflammatory responses are associated with ME/CFS, XMRV could be playing a role.**

XMRV Controversy

- What happened?
- What did we learn?
- Where do we go from here?

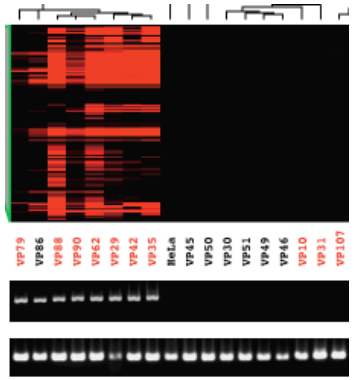
OPEN ACCESS Freely available online PLoS PATHOGENS

Identification of a Novel Gammaretrovirus in Prostate Tumors of Patients Homozygous for R462Q *RNASEL* Variant

Anatoly Urisman[1☯], Ross J. Molinaro[2,3☯], Nicole Fischer[4☯], Sarah J. Plummer[2], Graham Casey[2], Eric A. Klein[5], Krishnamurthy Malathi[2], Cristina Magi-Galluzzi[6], Raymond R. Tubbs[6], Don Ganem[4,7,8], Robert H. Silverman[2*], Joseph L. DeRisi[1,8*]

Prostate Cancer Tissue

DNA → **Virochip** →



Microarray

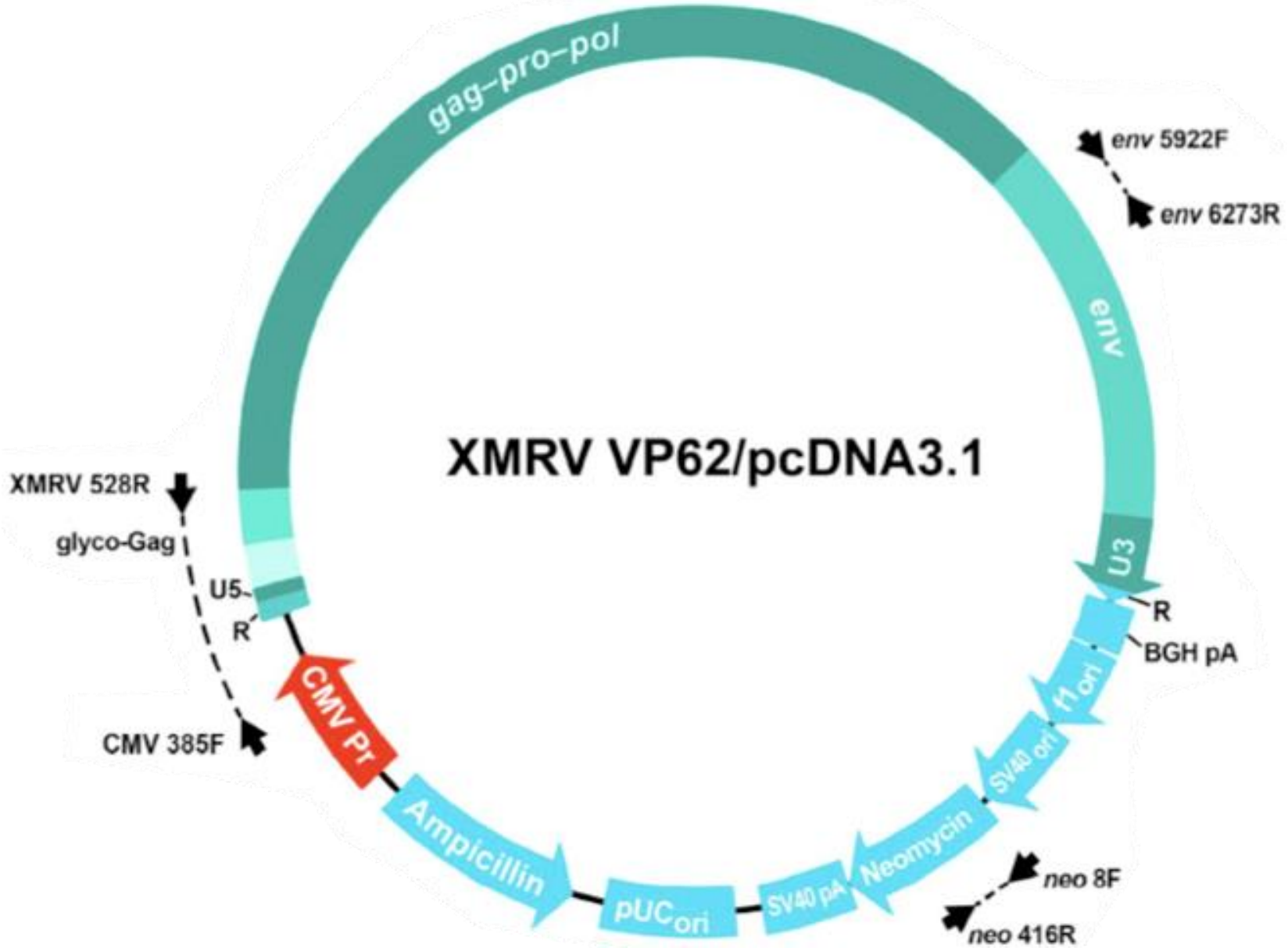
RT- PCR

Lots of risk factors, but there is a genetic predisposition to prostate cancer. For maybe about 10 percent of prostate cancer, there are folks predisposed to it.

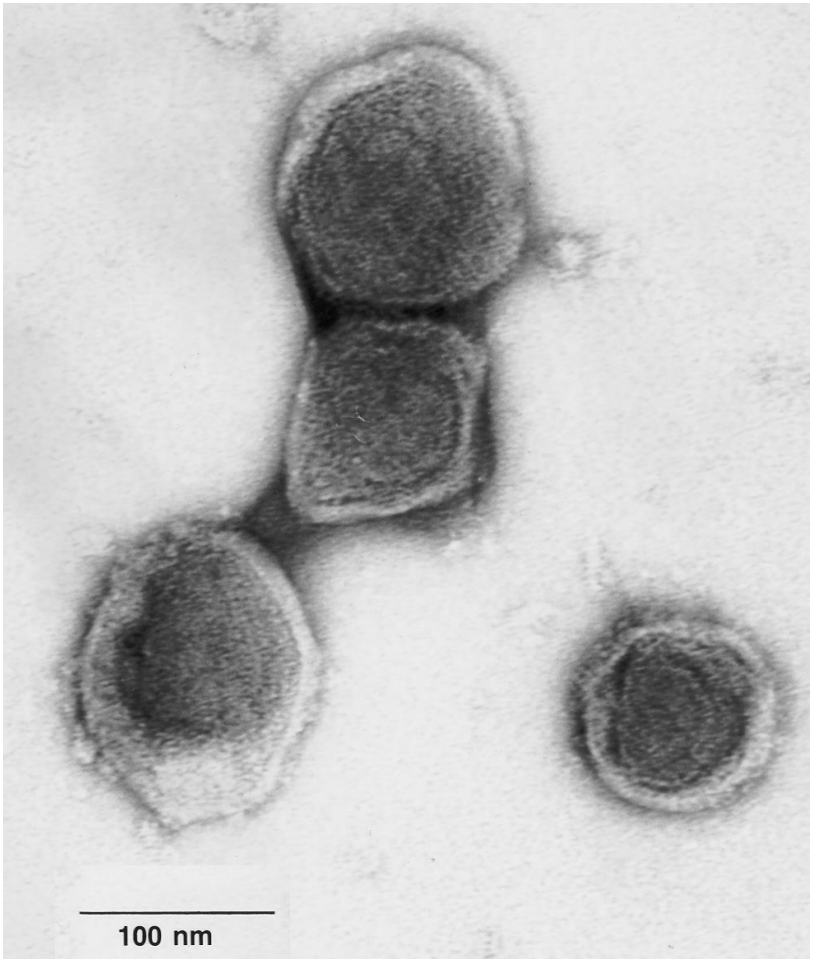
And the first gene that was mapped in association studies for this, early-onset prostate cancer, was this gene called RNASEL. What is that? It's an antiviral defense enzyme. So, we're sitting around and thinking, "Why would men who have this mutation—a defect in an antiviral defense system—get prostate cancer? It doesn't make sense,--unless, maybe, there's a virus?" So, we put tumors—on our array . . . And what I'm telling you from the signal is that men who have a mutation in this antiviral defense enzyme, and have a tumor, often have—40 percent of the time—a signature which reveals a new retrovirus. OK, that's pretty wild. What is it?

So, we clone the whole virus

Schematic of Plasmid containing XMRV/VP62



Electron Micrograph of XMRV virions produced from Silverman molecular clone “VP62”

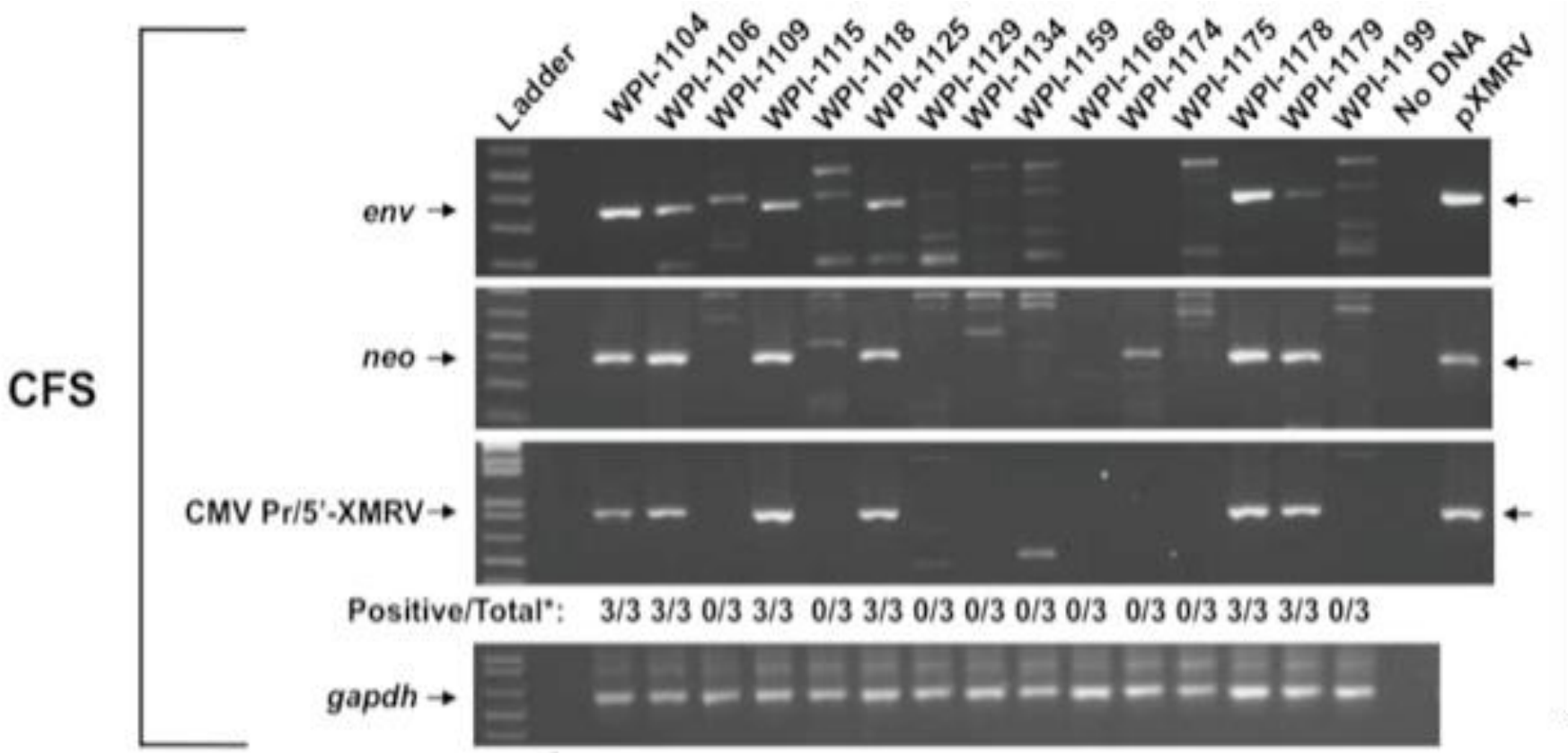


It’s a classic gamma retrovirus, but it’s totally new. Nobody’s ever seen it before. Its closest relative is, in fact, from mice, and so we would call this a xentotropic retrovirus, because it’s infecting a species other than mice.

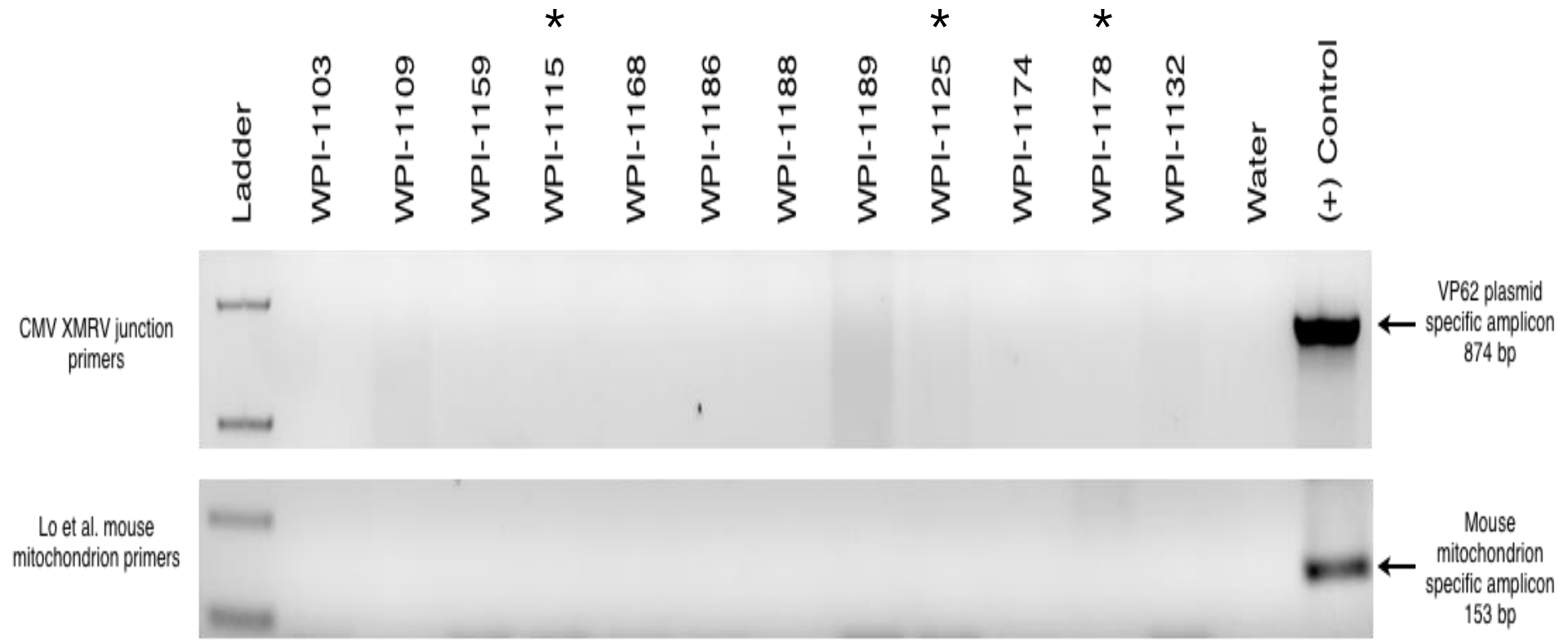
Dr. Joseph De Risi: Hunting the Next Killer Virus –
February 2006 – Monterey, CA – TED Talks

Plague Chapter 4 p41

Six WPI DNA Samples shown in Fig. 1 of the original study analyzed by the Silverman Lab in 2009 contained VP-62 plasmid

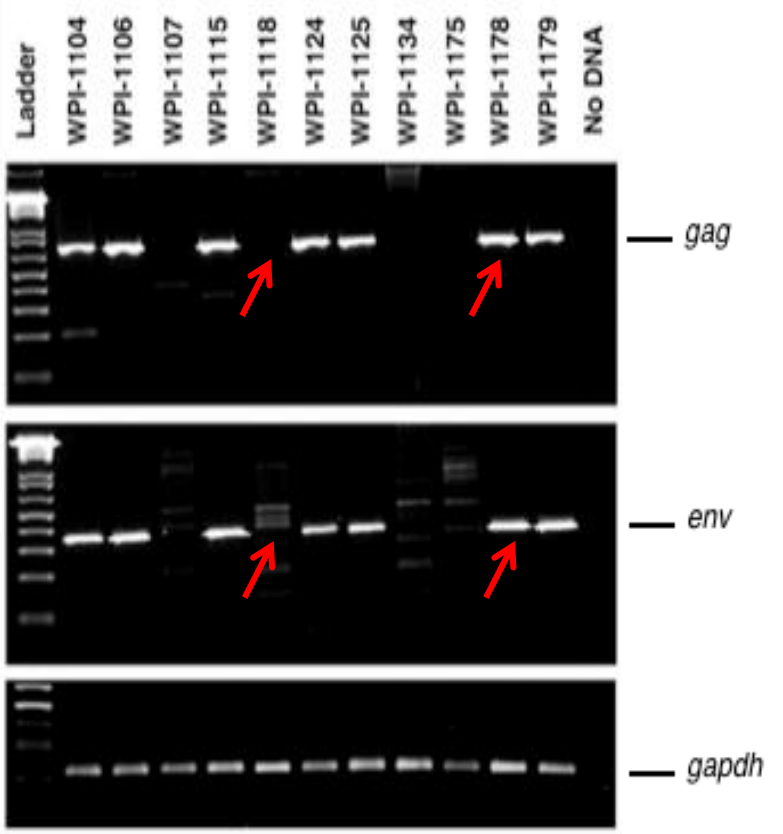


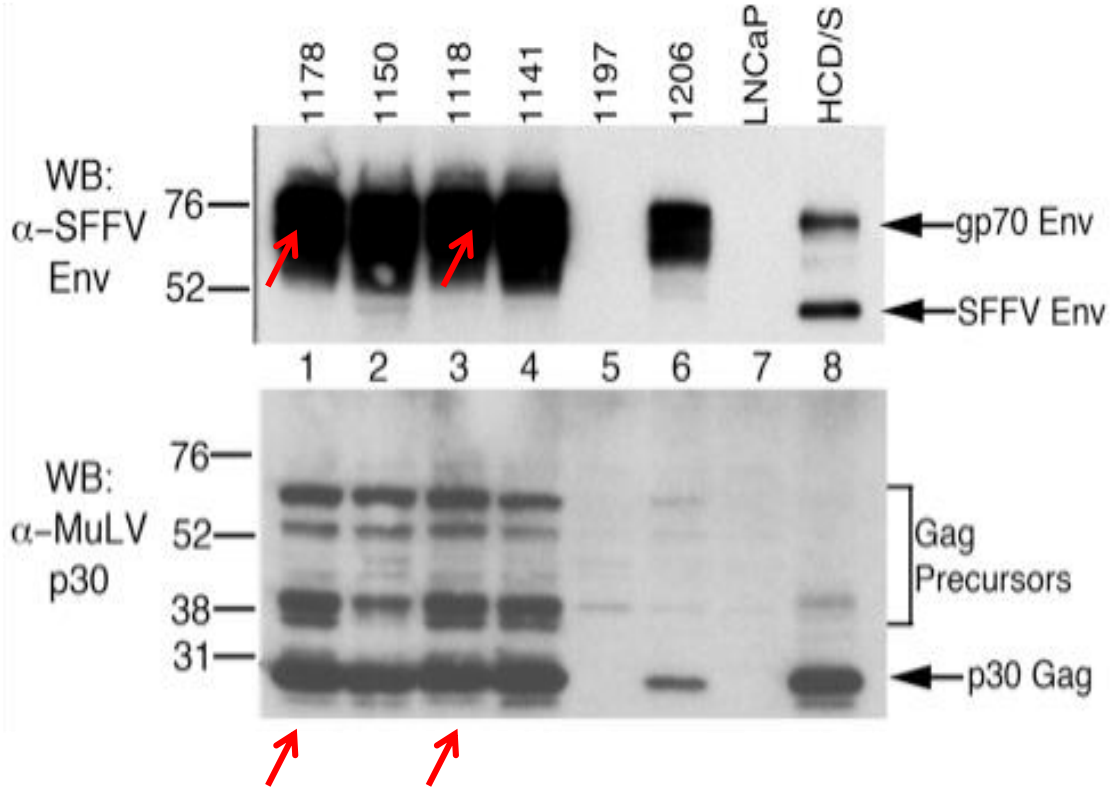
Original DNA Samples were negative for XMRV plasmid



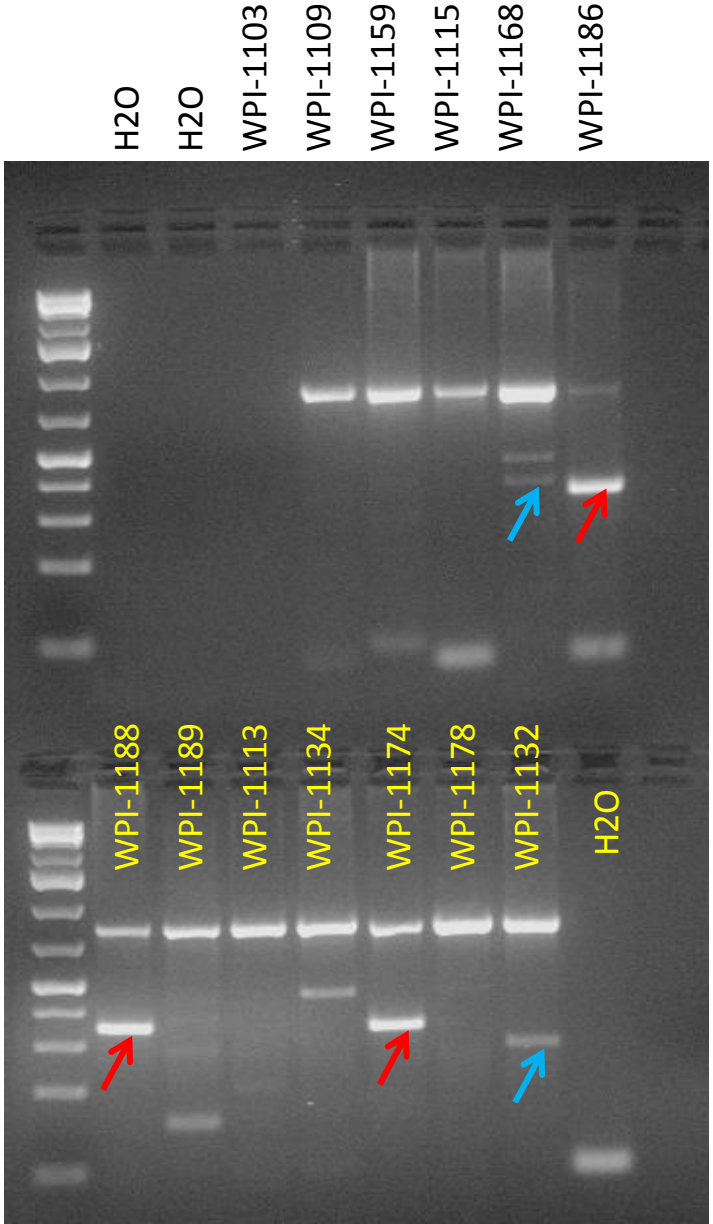
- **Pitfall: Choose your collaborators wisely!!**

Cell-Free Transmission of XMRV from PCR-negative CFS Patients' Plasma to LNCaP cells





Independent Reanalysis of archival samples used in Original Study Detected XMRV gag without plasmid or mouse contamination



PCR performed with USB HotStart-IT FideliTaq Master Mix

94°C 2 min
45 cycles:
94°C 30 sec, 54.8°C 30 sec, 72°C , 30 sec
72°C 3 min.

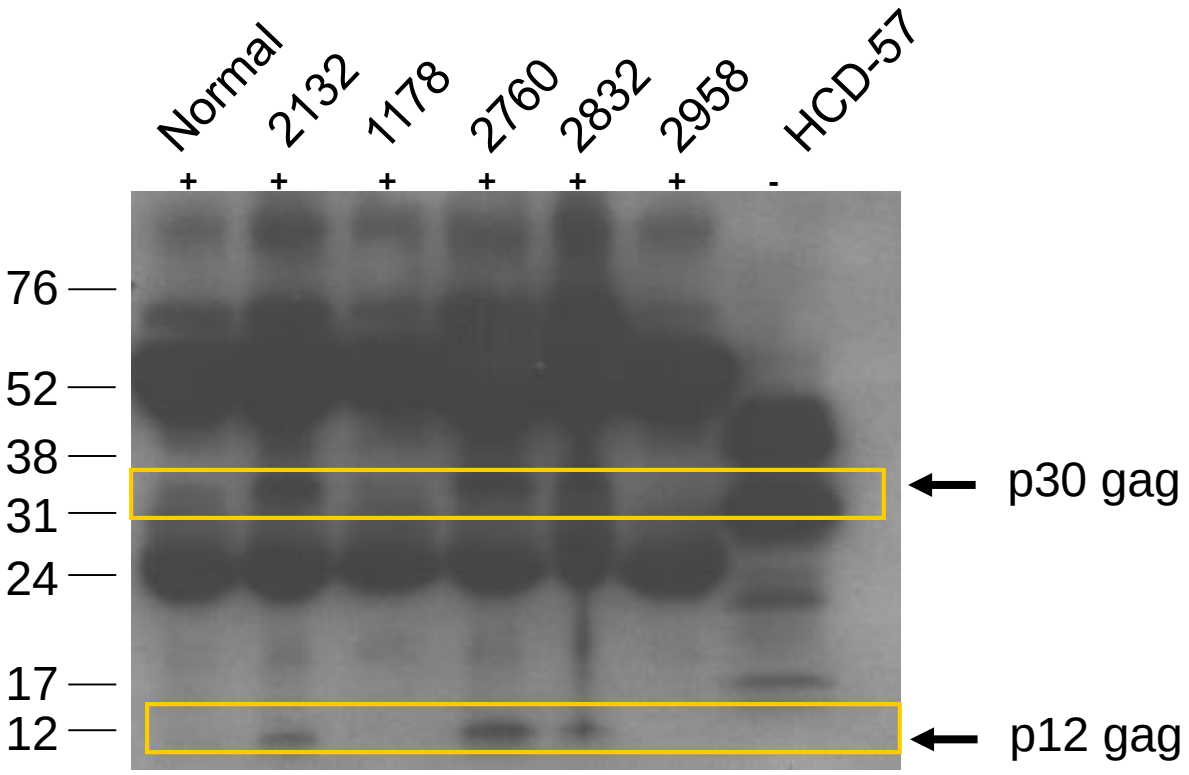
All three are negative for IAP and negative for CMV385F/XMRV528R primers for VP62 junction fragment

Sequencing of bands:

↗ Non-specific (Human DNA)
↗ XMRV Gag

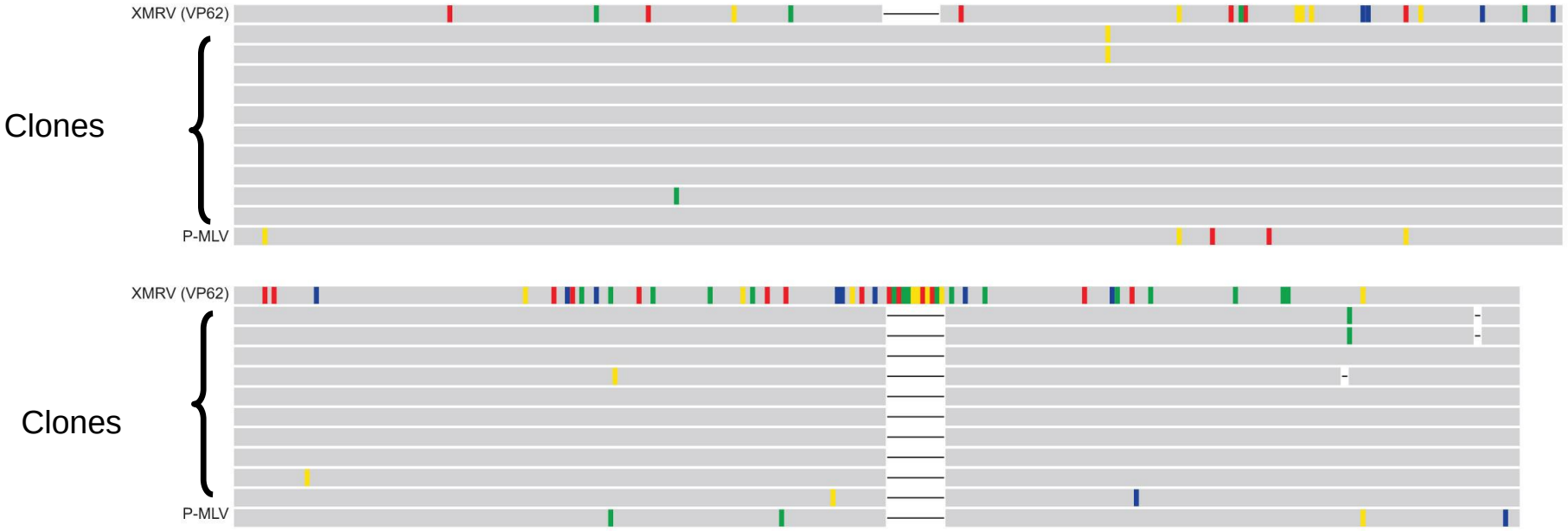
Direct Isolation of XMRV Protein From Plasma of CFS Patients By Immunoprecipitation with Anti-X-MLV Antibodies

IP: Goat anti-X-MLV (BALB-V2)



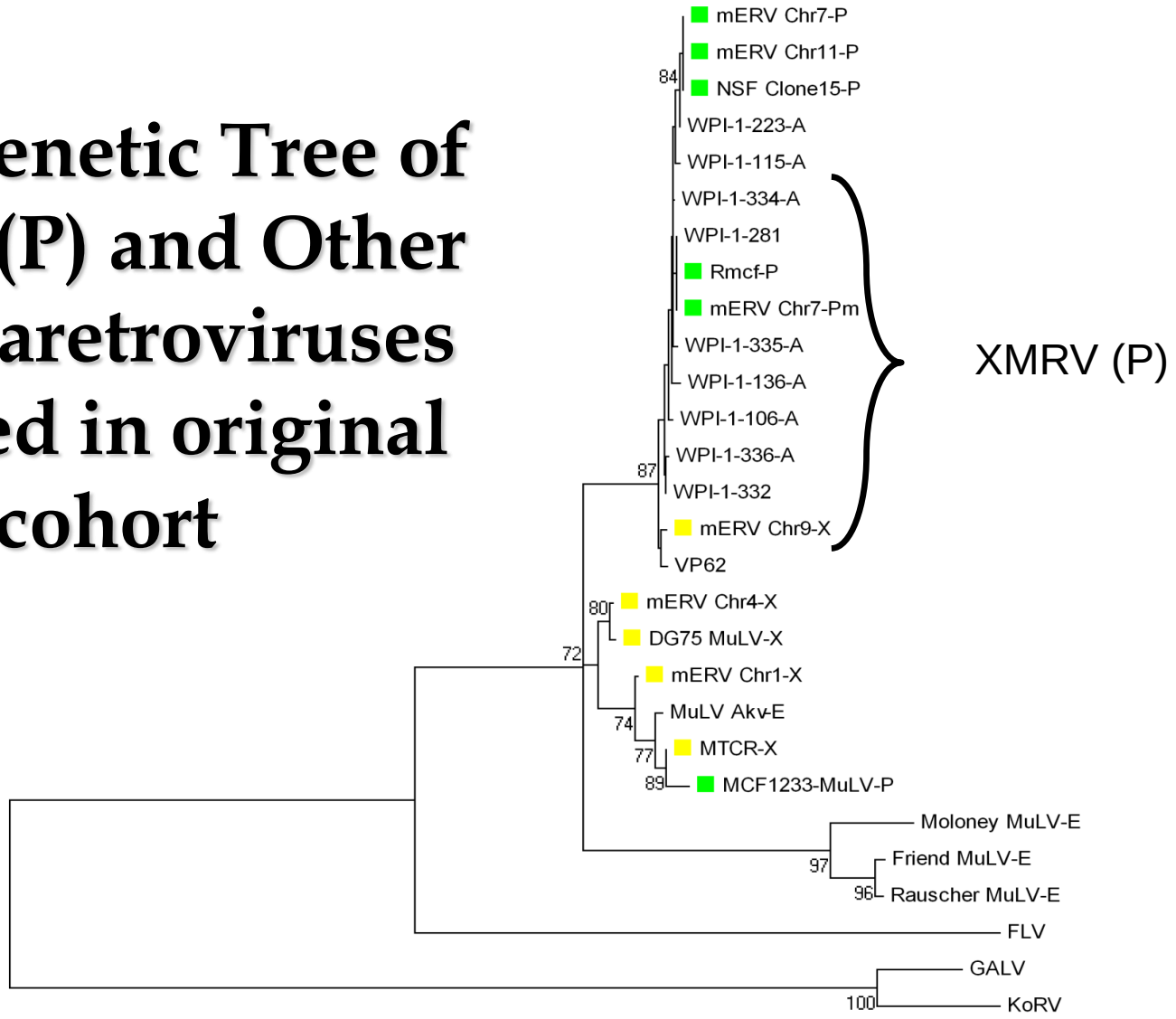
Blot: Goat anti-R-MuLV Gag

Clones of XMRV Env SU Similar to Polytropic XMRVs

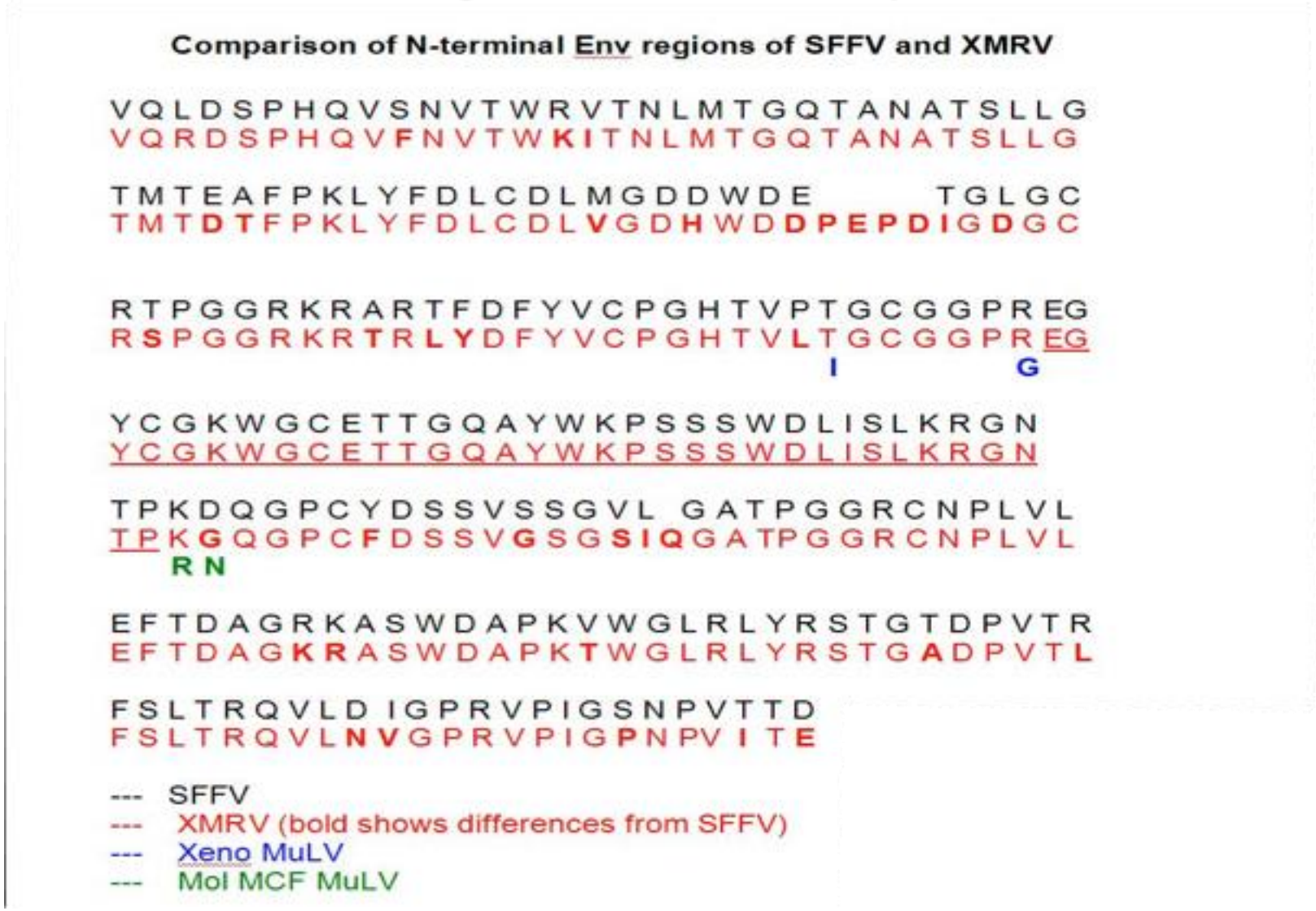


- The main XMRV/ in this patient is unlikely to be VP-62

Phylogenetic Tree of XMRV(P) and Other Gammaretroviruses detected in original cohort

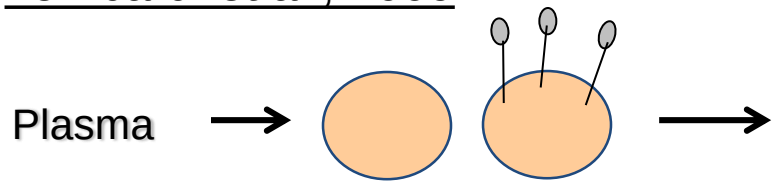


N-Terminus of SFFV ENV allows recognition of most potential XMRVs using monoclonal antibody 7C10



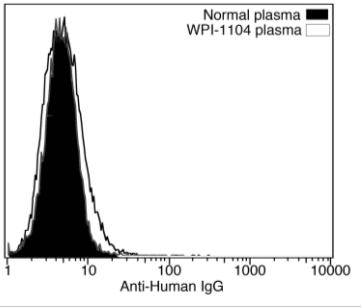
Assay used to Detect Anti-XMRV/HGRV Antibodies

Lombardi et al., 2009

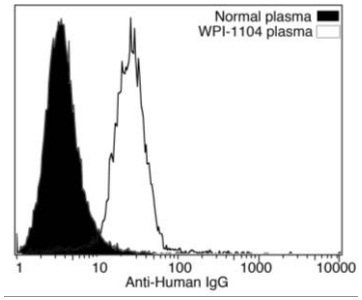


FACS analysis of Murine Cell Line
+/- SFFV Env

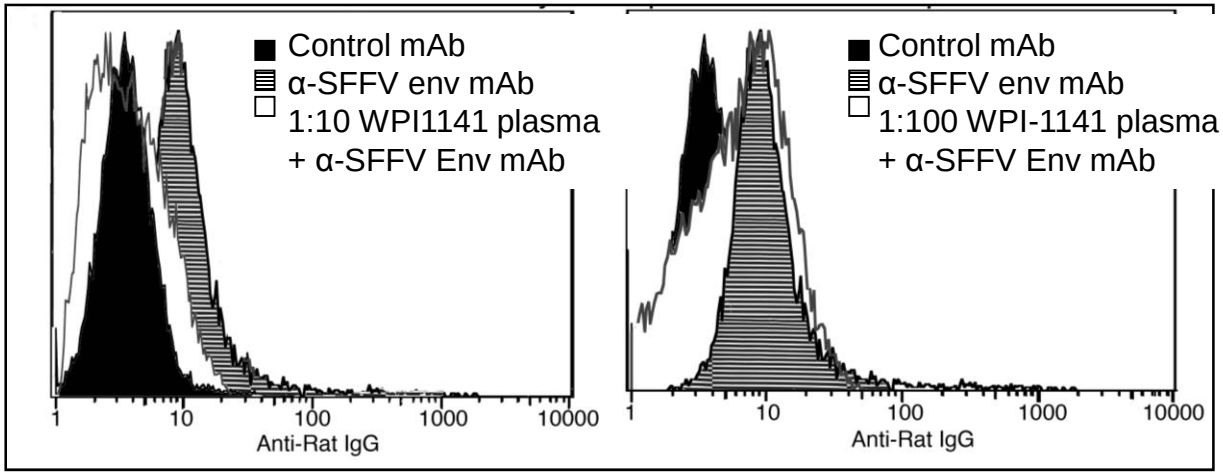
- SFFV Env



+ SFFV Env

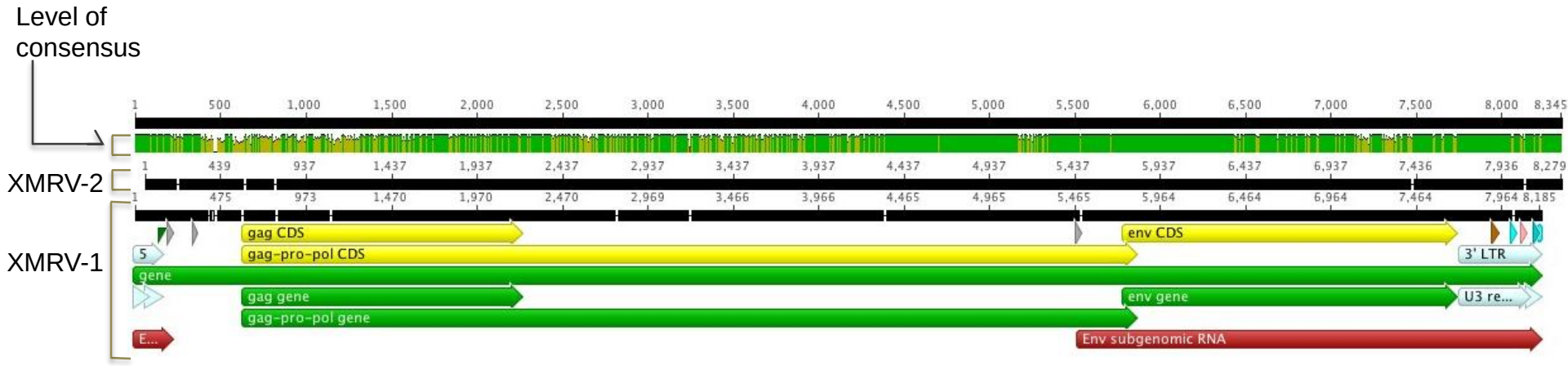


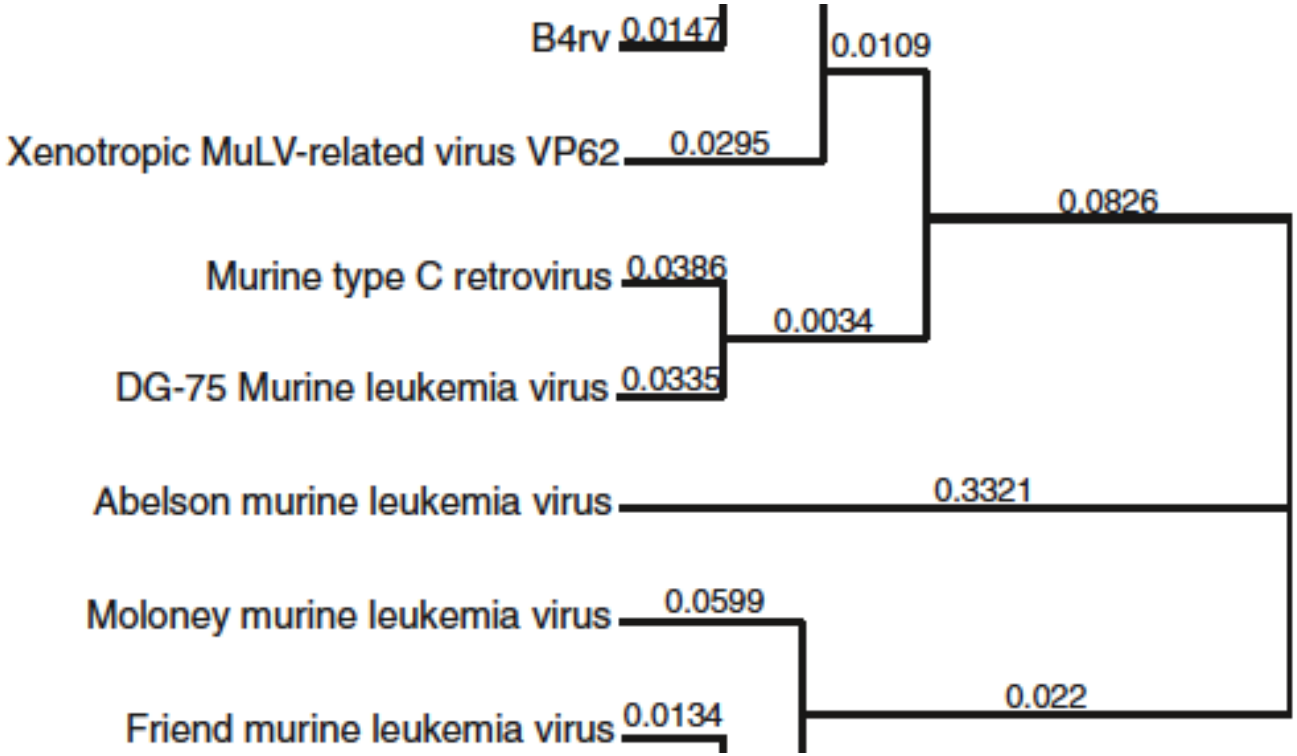
Plasma from CFS patients block binding of SFFV Env rat mAb to the B cell line expressing SFFV Env, demonstrating specificity



An ANTIBODY POSITVE RESULT DOES NOT NECESSARILY SHOW THE PRESENCE OF A REPLCIATION COMEPTENT RETROVIRUS

Sequence and phylogenetic analysis of a novel xenotropic XMRV-like MLV B4rv,





93% overall homology

Gary Owens lab
November 10, 2009
Published 2013

RESEARCH PAPER

Cancer Biology & Therapy 12:7, 617-628; October 1, 2011; © 2011 Landes Bioscience

Frequent detection of infectious xenotropic murine leukemia virus (XMLV) in human cultures established from mouse xenografts

Yu-An Zhang,[1] Anirban Maitra,[2] Jer-Tsong Hsieh,[3] Charles M. Rudin,[4] Craig D. Peacock,[4] Collins Karikari,[2] Rolf A. Brekken,[1] Victor Stastny,[1] Boning Gao,[1] Luc Girard,[1] Ignacio Wistuba,[5] Eugene Frenkel,[6] John D. Minna[1] and Adi F. Gazdar[1,*]

[1]Hamon Center for Therapeutic Oncology Research; [3]Department of Urology; [6]Division of Hematology-Oncology; The University of Texas Southwestern Medical Center at Dallas; Dallas, TX USA; [2]Departments of Pathology and Oncology; The Sol Goldman Pancreatic Cancer Research Center; [4]Department of Oncology; Johns Hopkins University School of Medicine; Baltimore, MD USA; [5]Department of Pathology; MD Anderson Cancer Center; Houston, TX USA

Table 1. Identification of xenotropic murine leukemia viruses (XMLV) and MLV-related viruses in xenograft cell lines

Table 3. Frequent detection of murine leukemia virus (MLV) contamination of non-xenograft human cultures

Characterization of murine leukemia viruses (MLV) detected in human non-xenograft cultures in xenograft culture laboratories

The Name Game and the Immaculate Recombination

How many have we created, John? How many retroviruses are out there Judy Mikovits asking a question to Dr. John Coffin at the Ottawa IACFS ME/CFS meeting 23 September 2011

Plague Chap 17 p 284

Antibodies to XMRV ENV Reproducibly Detected in Human Population

TABLE 3 Equivalent levels of XMRV sequences and anti-XMRV antibodies in CFS (chronic fatigue syndrome) patients and matched controls

			CFS/ME cases ($n = 147$)		Controls ($n = 146$)	
Lab site	Analysis	Sample	Total studied	No. positive (%)	Total studied	No. positive (%)
CDC	RT-PCR	Plasma	147	0 (0.0)	146	0 (0.0)
FDA	RT-PCR	Plasma	121[a]	0 (0.0)	110[a]	0 (0.0)
	PCR	PBMC	121[a]	0 (0.0)	111[a]	0 (0.0)
Mikovits, Ruscetti, and Hanson	PCR of cultured PBMC	PBMC	117[b]	0 (0.0)	126[b]	0 (0.0)
Mikovits and Ruscetti	Serology	Plasma	147	9 (6.1)	146	9 (6.2)

[a] Numbers represent all samples available for analysis at that site.

[b] Fifty samples (30 cases; 20 controls) were unable to be assayed because at least one of two aliquots from each set of subject PBMC did not grow in tissue culture.

Identification of additional retrovirus isolated 15 years ago in ME/CFS

Partial molecular cloning of the JHK retrovirus using gammaretrovirus consensus PCR primers

Brian D Halligan[1], Hai-Yuan Sun[2], Vladimir M Kushnaryov[2] & Sidney E Grossberg*[2]

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Future Virology

The JHK virus (JHKV) was previously described as a type C retrovirus that has some distinctive ultrastructural features and replicates constitutively in a human B-lymphoblastoid cell line, JHK-3. In order to facilitate the cloning of sequences

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http://www.retrovirology.com/content/10/1/34

RETROVIROLOGY

RESEARCH Open Access

Xenotropic MLV envelope proteins induce tumor cells to secrete factors that promote the formation of immature blood vessels

Meera Murgai[1], James Thomas[2], Olga Cherepanova[1], Krista Delviks-Frankenberry[4], Paul Deeble[3], Vinay K Pathak[4], David Rekosh[5] and Gary Owens[1*]

ENV proteins from both viruses impact tumor pathogenesis (change microvasculature)

Similarities to Vascular Pathologies seen in ME/CFS

These Microvasculature aberrations caused solely by XMRV ENV protein

Although it is highly unlikely that either XMRV VP62 or B4Rv themselves infect humans and are pathogenic, the results suggest that xenograft approaches commonly used in these studies of human cancer promote the evolution of novel retroviruses with pathogenic properties.
Similar retroviruses may have evolved to infect humans!

Chapter 21 : The Rediscovery of XMRV

JVI
Journals.ASM.org

Generation of Multiple Replication-Competent Retroviruses through Recombination between PreXMRV-1 and PreXMRV-2

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- Are two RCRs made by passing human prostate tissue through mouse; XMRV, BRV4 (second recombinant infectious virus occurring in human cells)
- Additional XMRV-like viruses may exist

- They do not have to be the exact sequence of XMRV (VP62)

Whether we fail to see the clever virus which does not kill its host, but has learned to live with it
When a disease takes so much from a patient but stops just short of death,
how does the medical community respond?
Will the scientific community have the courage to answer the question of whether these diseases
Might have been of their own creation" ????"

CONCLUSION

Taken together these data suggest there are
additional human gamma retroviruses which may be involved in the Pathogenesis of neuroimmune disease and cancer!

"The question, which urgently needs to be answered is whether the plague feared by Coffin and Stoye has already arrived, but we do not Recognize it…
THEY SEE WHAT THEY WANT TO SEE AND THAT'S THE ***REAL PLAGUE"***

Plague Chapter 21 p382

Thank You

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Here we are not afraid to follow the truth wherever it may lead,
Nor tolerate error so long as freedom is left to combat it
Thomas Jefferson