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Connecting the Cancer Community

1st International Workshop on XMRV

September 7/8, 2010

Virus Biology
Host Response
Prostate

Chronic Fatigue Syndrome
Assay Development
Cancer Epidemiology

NATIONAL INSTITUTES OF HEALTH

NATIONAL CANCER INSTITUTE

"*Curiouser and Curiouser*" - the XMRV Controversy Continues

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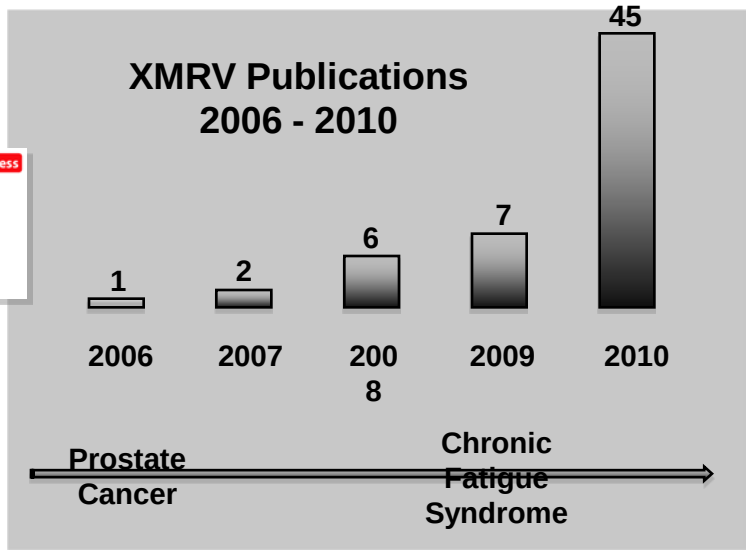
Lack of Detection of XMRV in Seminal Plasma from HIV-1 Infected Men in The Netherlands

Marion Cornelissen[1], Fokla Zorgdrager[1], Petra Blom[2], Suzanne Jurriaans[2], Sjoerd Repping[3], Elisabeth van Leeuwen[3], Margreet Bakker[1], Ben Berkhout[1], Antoinette C. van der Kuyl[1*]

RESEARCH — Open Access

Failure to detect Xenotropic murine leukaemia virus-related virus in Chinese patients with chronic fatigue syndrome

Ping Hong[1,2], Jinming Li[1,2*], Yongzhe Li[3*]



SHORT REPORT — Open Access

No evidence for XMRV association in pediatric idiopathic diseases in France

Eric Jeziorski[1,2], Vincent Foulongne[3], Catherine Ludwig[2], Djamel Louhaem[4], Gilles Chiocchia[5], Michel Segondy[3], Michel Rodière[2], Marc Sitbon[1], Valérie Courgnaud[1*]

Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors

Shyh-Ching Lo[a,1], Natalia Pripuzova[a], Bingjie Li[a], Anthony L. Komaroff[b], Guo-Chiuan Hung[a], Richard Wang[c], and Harvey J. Alter[c,1]

Xenotropic Murine Leukemia Virus–related Gammaretrovirus in Respiratory Tract

Nicole Fischer, Claudia Schulz, Kristin Stieler, Oliver Hohn, Christoph Lange, Christian Drosten, and Martin Aepfelbacher

NATURE MEDICINE | NEWS

Chronic controversy continues over mysterious XMRV virus

Elie Dolgin

XMRV & Gammaretrovirus Biology

Gammaretroviruses:

- Simple retroviruses (only *gag, pol*, and *env* genes).
- Widespread in nature, with isolates from mammals, birds, and reptiles.
- Readily give rise to endogenous proviruses.
- Most commonly transmitted vertically (mother to offspring).
- Cause a wide variety of diseases (cancer, neurological, degenerative, immunodeficiency).
- Cancer is usually the result of insertional inactivation of protooncogenes.
- **Infection is lifelong. Unlike HIV, very few replication cycles per host.**

Source: J. Coffin, Tufts University

XMRV & Gammaretrovirus Biology

Xenotropic MLV:

- Inherited as endogenous proviruses (ca 10-20 copies) in all inbred mice.
- Many more proviruses in some wild *Mus Musculus sub*species.
- Some proviruses (e.g., Bxv1-XMV43) are intact and infectious.
- Can infect virtually all mammals, except some species of *Mus*, due to receptor (Xpr1) polymorphism.
- Not directly pathogenic in mice (due to lack of receptor), but LTR is often found in oncogenic recombinant MLVs. Pathogenicity in other species unknown.
- **Common contaminant of human tumor cell lines due to passage through nude mice (which have Bxv1).**
- **Closely related to XMRV, but none is identical, perhaps excluding inbred mice as the origin.**
- Related MLVs cause a variety of diseases (malignant, immunodeficiency, neurological) in mice.

Source: J. Coffin, Tufts University

XMRV and Gammaretrovirus Biology

Polytropic & Modified Polytropic MLV

- Inherited as endogenous proviruses (ca 10-20 copies each) in all inbred mice.
- Also found in wild *Mus Musculus* subspecies.
- **No infectious virus found to date.**
- Can infect virtually all mammals, using both forms of the Xpr1 Receptor.
- Not directly pathogenic in mice, but *env* gene often found in oncogenic recombinant MLVs.
- Pathogenicity in other species unknown.
- Both types derived independently from XMV, probably in response to Xpr1 mutation.
- Unlike XMV, PMV and MPMV proviruses have suffered significant mutations mediated by mouse APOBEC3.

Source: J. Coffin, Tufts University

XMRV *vs* MLV-like DNA Sequences

XMRV (Urisman et al., Lombardi et al., others):

- Isolated as infectious virus from prostate cancer, CFS patients.
- Isolates are very similar in sequence.
- They form a distinct subclade among XMV proviruses.

MLV-like sequences (Lo et al.)

- PCR amplified using MLV gag and env primers.
- Found preferentially in CFS plasma.
- No infectious virus yet identified.
- Only fragmentary, bulk, sequences available at present.

Until a relationship is further clarified, important to consider these as distinct, unrelated phenomena.

Source: J. Coffin, Tufts University

Precautions Moving Forward

- XMRV may be important in the etiology of CFS and prostate cancer, but:
  - Conflicting reports on the association of either disease with the virus
  - Lack of insight into pathogeninc mechanisms
- Some studies may be complicated by minute traces of mouse DNA; others by viruses derived from lab strain mice.
- One mouse cell contains over 100 PMV and XMV proviruses. Sensitive PCR assays can detect the provirus in 10 fg of mouse DNA.
- **Xenotropic MLVs derived from nude mice have inadvertently spread through many labs.**
- While the major XMRV studies published to date appear to be well controlled for these problems, extraordinary caution is necessary.

Source: J. Coffin, Tufts University

XMRV, CFS and Antiretrovirals

- Like other MLVs, XMRV is sensitive to inhibition by a few antiretrovirals licensed to treat HIV infection.

- Web-based reports suggest significant off-label use of some of these compounds in CFS patients, with anecdotal results posted.

- While there could be value in well-controlled randomized trials of these agents, uncontrolled administration of antivirals inadvisable, since:

  - Still unknown whether XMRV is causal.
  - Drugs can only work by blocking replication cycles, not repairing damage already caused by virus replication.
  - Disease lacks objective biomarkers to monitor treatment.
  - Appropriate assay to monitor effects on virus replication unavailable.
  - Results of such studies will never be accepted by third-party payers/regulators.
  - Will keep treatment out of reach of the large majority of those suffering from CFS.

Source: J. Coffin, Tufts University

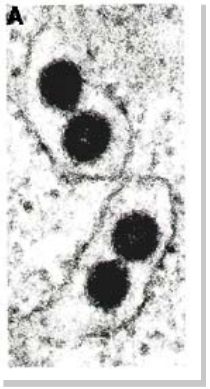
XMRV Elements in Mouse Genomes

- **Highly specific PCR-based assay was developed to screen for XMRV-specific sequences in mouse genomes:**
  - 75 wild-derived and inbred mouse strains tested.
  - 13 strains (so far) seem to harbor XMRV-like sequences.
- **SGA-based assay developed to distinguish MLVs based on their *gag* leader region:**
  - Fragments from 7 strains analyzed.
  - Two strains (so far) have sequences with the gag-leader deletion.
- **Genomic hybridization blots on mouse genomic DNA with XMRV-specific probes:**
  - 10 mouse strains examined.
  - 6 strains (so far) have the same insertion.

Source: O. Cingoz, Tufts University

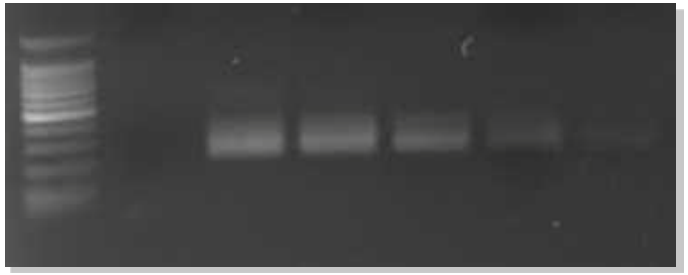
Mouse Intracisternal A Particles (IAP)

- Endogenous retroviral elements of mice.
- Deletion in *env* results in defective particles that accumulate in the cisternae of Golgi.
- Estimated ~1000 copies/haploid genome.
- Form the basis of a highly sensitive assay for detecting minute amounts of mouse DNA.



Coffin JM *et al.* *Retroviruses*. 1997

Mouse DNA Content



← Amplified IAP LTR

Source: O. Cingoz, Tufts University

Recapitulating XMRV Infection

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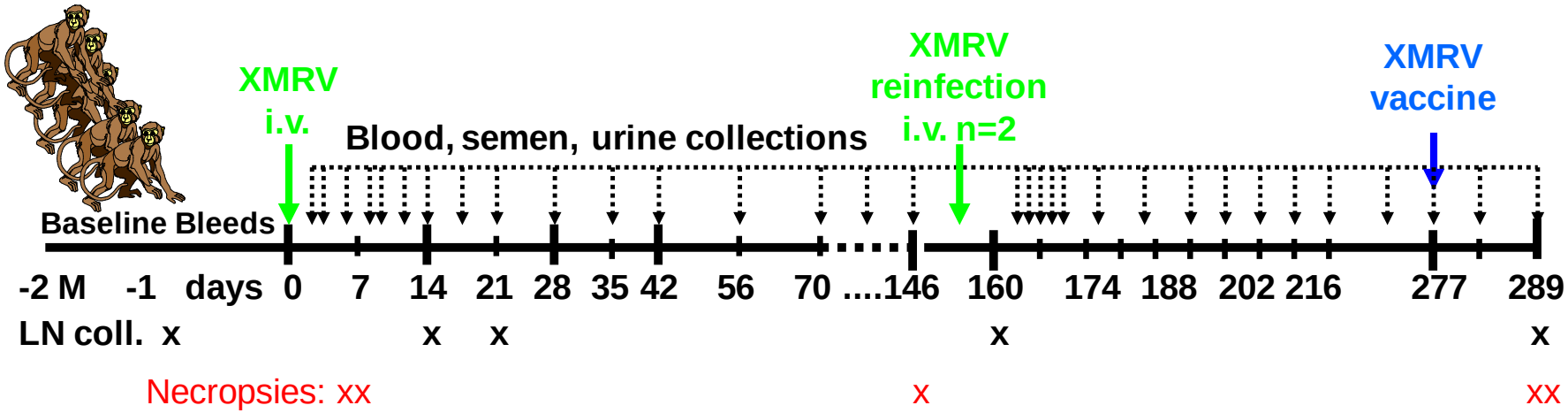
Questions

Can XMRV replicate in a model close to man, i.e. rhesus macaque?

If so, what are the viral kinetics, dissemination and antiviral immune responses?

Animal Protocol



Source: F. Villinger, Emory University

Recapitulating XMRV Infection

- Rhesus macaques susceptible to XMRV infection, resulting into a chronic persistent infection even though the viremia appears transient with low viral loads within the first 10-21 days post infection IV followed by undetectable viral loads.
- Infected BM derived cells appear to be of lymphocytic lineage (T and NK cells).
- The lineage of cell positive for XMRV infection/replication in lymphoid organs are CD4+ T cells. In other organs, such as those of reproductive tract, liver, pancreas, etc. appear to be either epithelial or interstitial cells. In lung most infected cells appear to be alveolar macrophages.
- Primarily foci of infection in epithelial cells in organs such as prostate, seminal vesicle (and epididymis) during early and chronic infection.
- Chronic infection remains completely disseminated in select animals with extensive numbers of FISH and IHC-positive cells even in monkeys exhibiting negative plasma viral loads, suggesting low level of virus release.

Source: F. Villinger, Emory University

Recapitulating XMRV Infection

Mus pahari
(Shrew mouse)

- *Mus pahari* fibroblast cells support XMRV replication.
- XMRV proviral DNA found mainly in blood, spleen, and brain, suggesting that XMRV is lympho- and neurotropic in *Mus pahari*.
- A subset of infected mice showed panleukopenia or pancytopenia.
- XMRV infection rapidly elicits XMRV-specific humoral immune response.
- G-to-A hypermutations in spleen-derived XMRV sequences suggest APOBEC3-medaited block of XMRV replication *in vivo*.
- *Mus pahari* model can be used to study pathogenicity of chronic XMRV infection, and evaluate anti-XMRV strategies.

Source: Y. Ikeda, Mayo Clinic

Vaccine Development – Feline Leukemia Virus *vs* XMRV

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- Gammaretrovirus; first isolated from domestic cats in 1964.
- Both endogenous and exogenous
- Infection of domestic cats world-wide
- Common clinical entity and cause of disease in domestic pet cats
- Incidence significantly reduced due to screening and vaccination

FeLV-associated disease	XMRV-associated disease
Immune suppression	Immune suppression
Disease of CNS	Disease of CNS
Polyarthritis	Painful joints
Induction of cancer	Induction of cancer

Source: E. Sparger, UC Davis

FELV Vaccine Efficacy

Decreased incidence of FeLV Infection over the past 25 years

Current whole inactivated virus	44 – 100%
Recombinant SU p45	87%
Subunit vaccine	55 – 63%
Canarypox vaccine vectored	78 - 100%
DNA vaccine (experimental)	80 – 100%

Pathogenesis and immune correlates for XMRV must be characterized to inform vaccine design and use of novel technologies.

Source: E. Sparger, UC Davis

XMRV *vs* MLV-like DNA Sequences

- Study supports the earlier finding of MLV-related gene sequences in blood of many patients with CFS.
- The viral sequences can also be detected in a small fraction of volunteer healthy blood donors.
- Differing from the reported finding of near genetic identity of all XMRVs in patients with CFS, prostate cancer and in blood donors, analysis of the viral gene sequences revealed a more genetically diverse group of MLV-like viruses. Viral gene sequences more closely related to those of polytropic MLVs.
- Study suggests that MLV-related viruses are infecting some people. Disease association and possibility of blood transmission of these otherwise well-known MLV-like retroviruses in human warrant further studies.

Until the relationship between XMRV & MLV-like sequences is further clarified, these should be considered as distinct, unrelated phenomena.

Source: S.-C. Lo, CBER/FDA

XMRV *vs* MLV-like DNA Sequences

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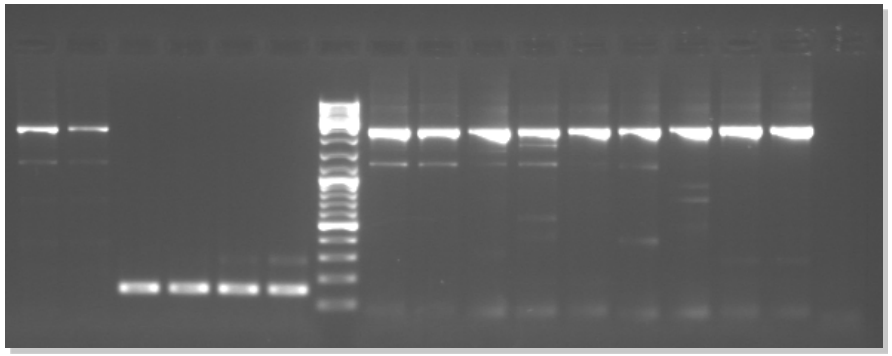
Main sources of contamination:

- Contamination by PCR amplicons
- Contamination by MLVs or viral vectors studied in the laboratories
- Contamination by mouse DNA - mouse DNA genome contains endogenously many closely related proviruses of MLVs or mERVs.

CFS Patients **Healthy Donors**

Mouse mtDNA amplicon -



Source: S.-C. Lo, CBER/FDA

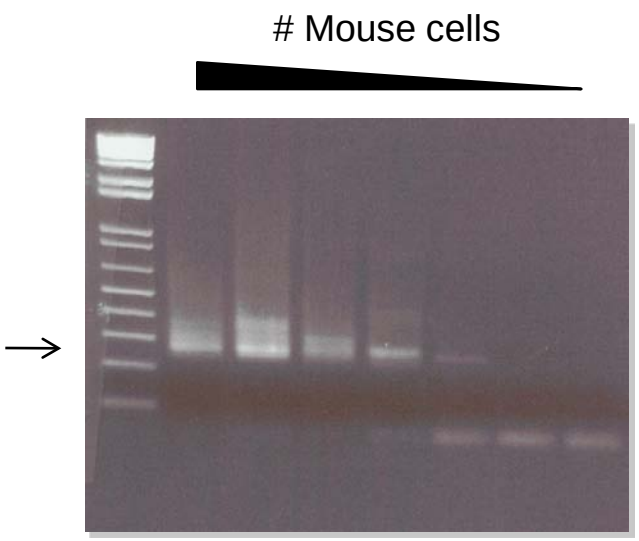
XMRV Single-Copy Detection Assays - NCI

X-SCA – Quantitative PCR assay capable of detecting a single pelletable virion in plasma or XMRV DNA in whole blood or PBMCs

X-SGS – Single genome sequencing assay for sequencing large genomic fragments from single virions in plasma or individual DNA molecules in whole blood or PBMCs

Source: M. Kearney, NCI

XMRV Single-Copy Detection Assays - NCI



PCR amplification of IAP DNA from mouse genomic DNA

- Results from a blinded panel of spiked patient samples showed X-SCA detects XMRV virions and proviruses in blood products with high sensitivity.
- XMRV & mouse endogenous MLVs highly genetically related - important to make a clear distinction between them when evaluating XMRV prevalence in relevant cohorts.
- X-SCA and X-SGS together discriminate between XMRV and mouse endogenous MLVs with 100% accuracy.

Source: M. Kearney, NCI

XMRV Serological Assay Development - Abbott

XMRV antigens

- *E. coli* expressed recombinant p15E, p70 and p30 proteins
- Mammalian expressed gp70 protein

Assay formats

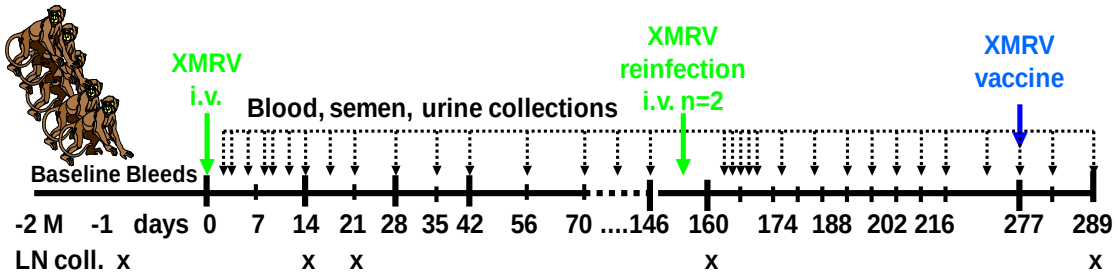
- Indirect (anti-human) assay format
- Direct (double antigen sandwich) assay format

Performance evaluation

- Positive samples
  - Western blot positive serial bleeds from 3 XMRV-infected macaques
  - Goat polyclonal antibodies: anti-Friend MuLV and anti-Env of Rauscher MuLV
  - Rat monoclonal antibody to MuLV *gag* p30
- Negative samples
  - 2262 Blood donor specimens (presumed negative for XMRV; non-reactive to other known bloodborne pathogens)

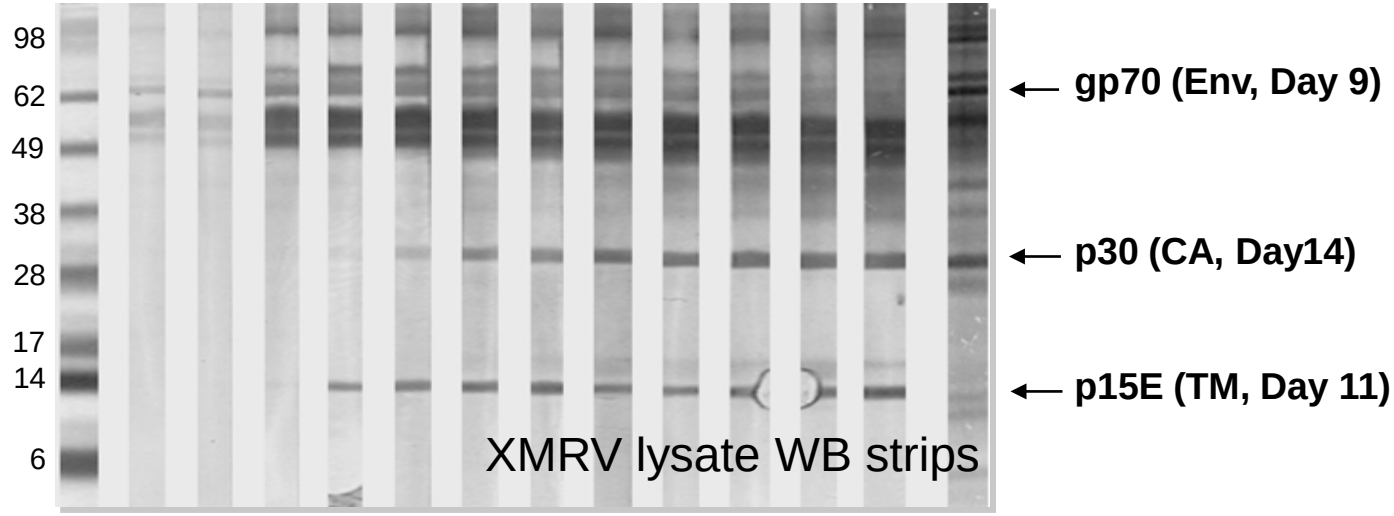
Source: X. Qiu, Abbott

Seroconversion Pattern in XMRV-Infected Macaque RII-10



	0	4	9	11	14	18	28	35	42	56	73	93	gel
XMRV RNA	-	+	+	+	-	-	-						
XMRV DNA	-	-	+	+	+	+	+	-	-				

Day PI



XMRV lysate WB strips

Source: X. Qiu, Abbott

XMRV Serological Assay Development - NCI

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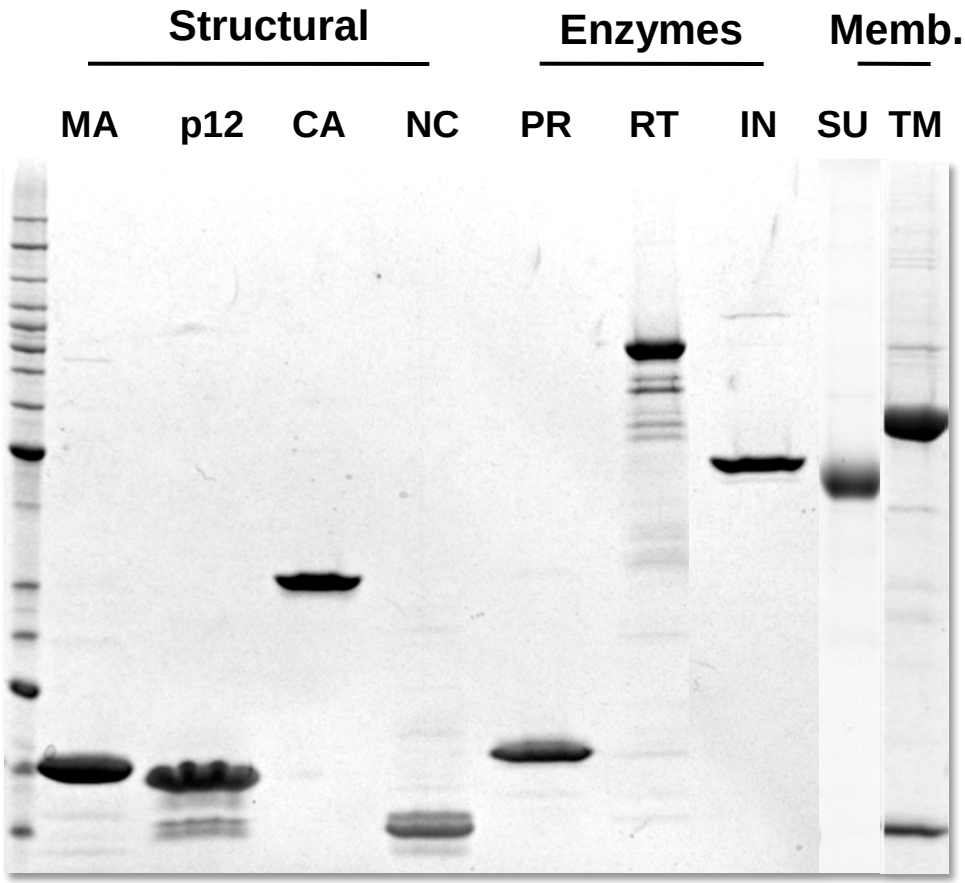
Large Scale XMRV Antigen Protein Production

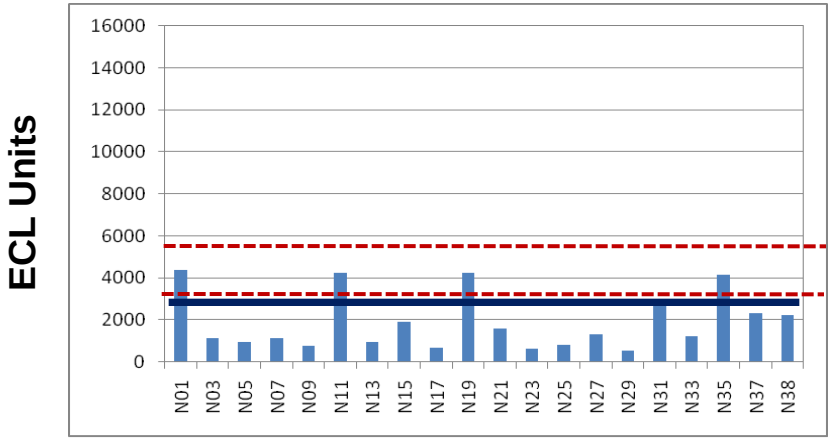


Source: R. Bagni, NCI

XMRV Serological Assay Development – Lo *et al*, PNAS

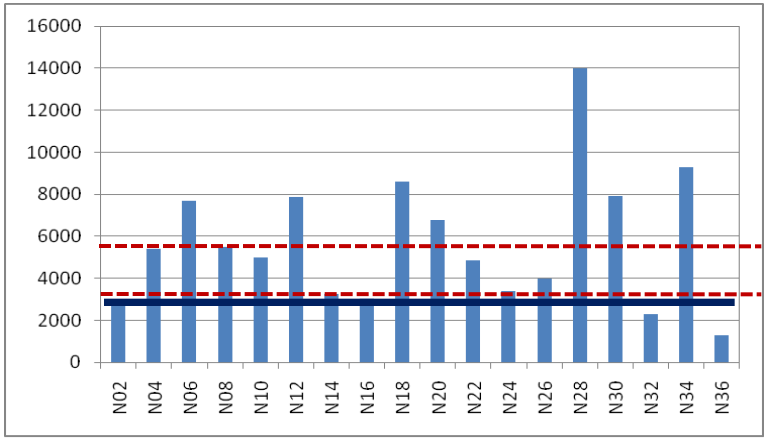
	CA	TM	SU
NA Detected	15/18	15/18	15/18
NA Not Tested	5/20	4/20	4/20

SU



Nucleic Acid: Not Tested

SU



Nucleic Acid: Detected

Source: R. Bagni, NCI

Cell Culture Analysis of XMRV Infection - NCI

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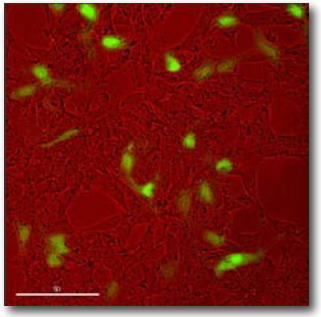
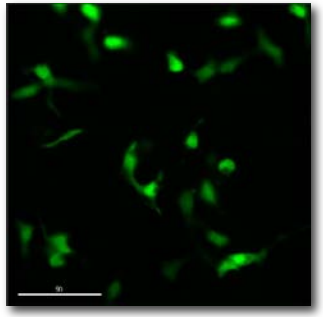
- Lombardi *et al.* - recovery of infectious XMRV from blood of human subjects
- **D**etectors of **E**xogenous **R**etroviral **S**equence **E**lements (DERSE) indicator cells developed, using XMRV-susceptible HEK293T and LNCaP line
- DERSE cells permit detection of infectious XMRV from patient samples in *one week* (vs. 3+ weeks by other methods)
- Under optimization to detect XMRV in EDTA- and heparin-containing samples
- Available for distribution via NIH AIDS Reagent Repository



3 Days Post-Infection



Source: K. Lee, NCI

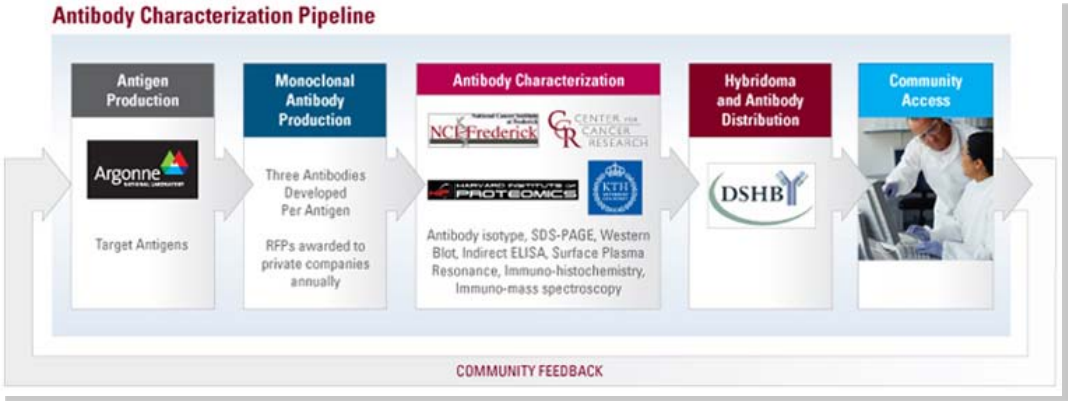
NIH AIDS Research and Reference Reagent Program

https://www.aidsreagent.org

64 DNA clones deposited:

- 20 *E. coli* expression clones (His_6 and His_6-MBP)
- 10 Baculovirus clones (His_6-MBP)
- 2 Baculovirus secreted clones
- 32 Analogous Entry (Gateway) clones

Antibody production (underway)



Source: R. Bagni, NCI

Retrovirus Epidemiology Donor Study (REDS) II Panel Study Phases

***Phase I* - Analytical Panels**

Evaluate performance of XMRV nucleic acid assays

***Phase II* - Pilot Clinical Studies**

Whole Blood *vs* PBMC

Timing of sample preparation

***Phase III* - Clinical Sensitivity/Specificity Panel**

Assay performance on pedigreed clinical samples

***Phase IV* - Blood Donor Clinical Panel**

Preliminary donor prevalence of XMRV nucleic acids

Initiate donor seroprevalence studies

Source: G. Simmons, BSRI, San Francisco

Phase 1 – Analytical Panels

Performance panels

- Compare limit of detection & accuracy of viral loads of current assays; standardize performance of future XMRV detection assays for blood cells and plasma
- Whole blood panel - spiked with XMRV-positive cells
- Plasma panel - spiked with supernatant containing XMRV

22Rv1 cells

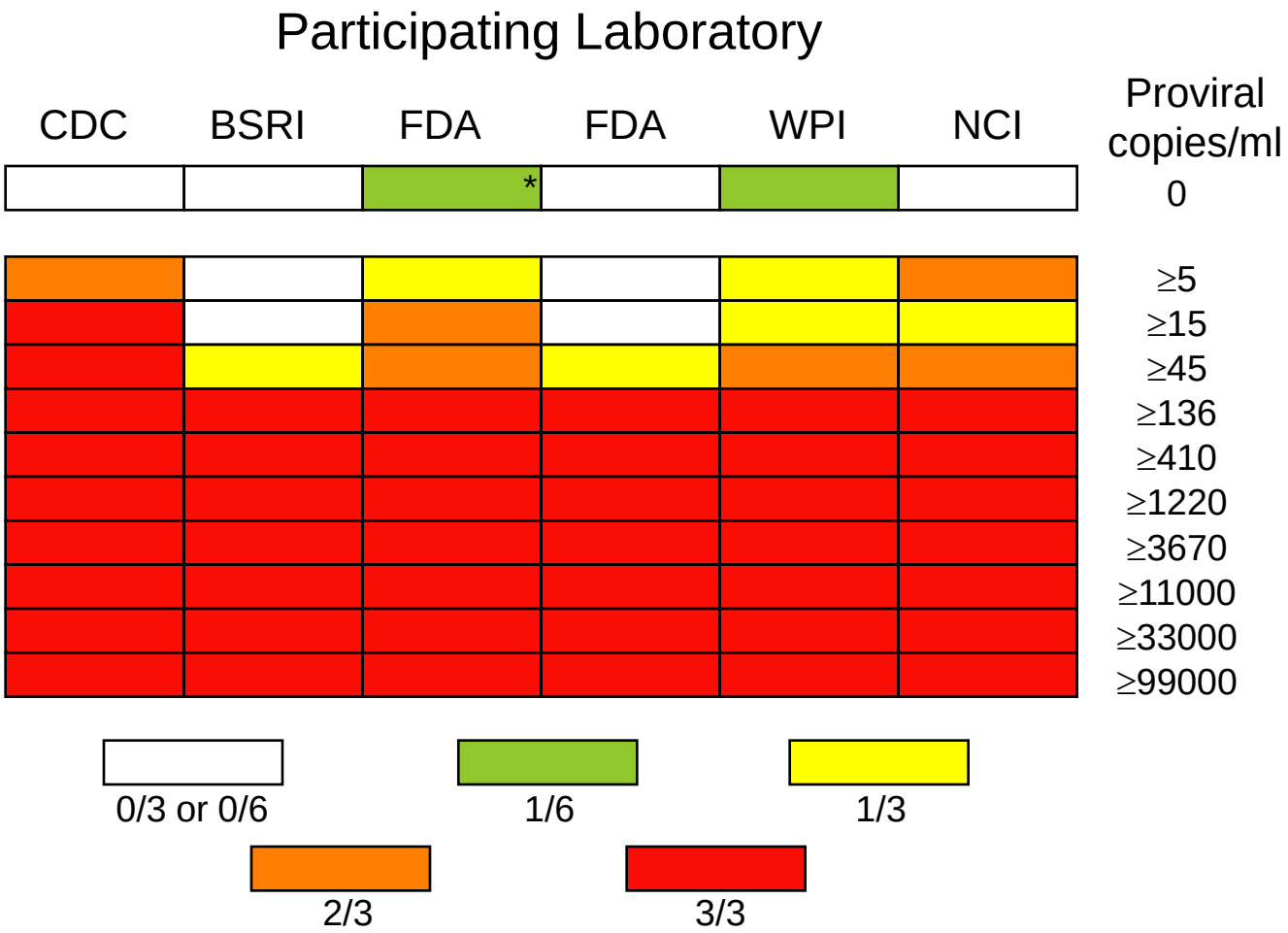
- Human prostate cell line chronically infected with XMRV
- Contain at least 10 XMRV proviral copies

Virus supernatant

- Supernatant from cultured 22Rv1 cells
- Viral load ~5 x 10^9 RNA copies/ml

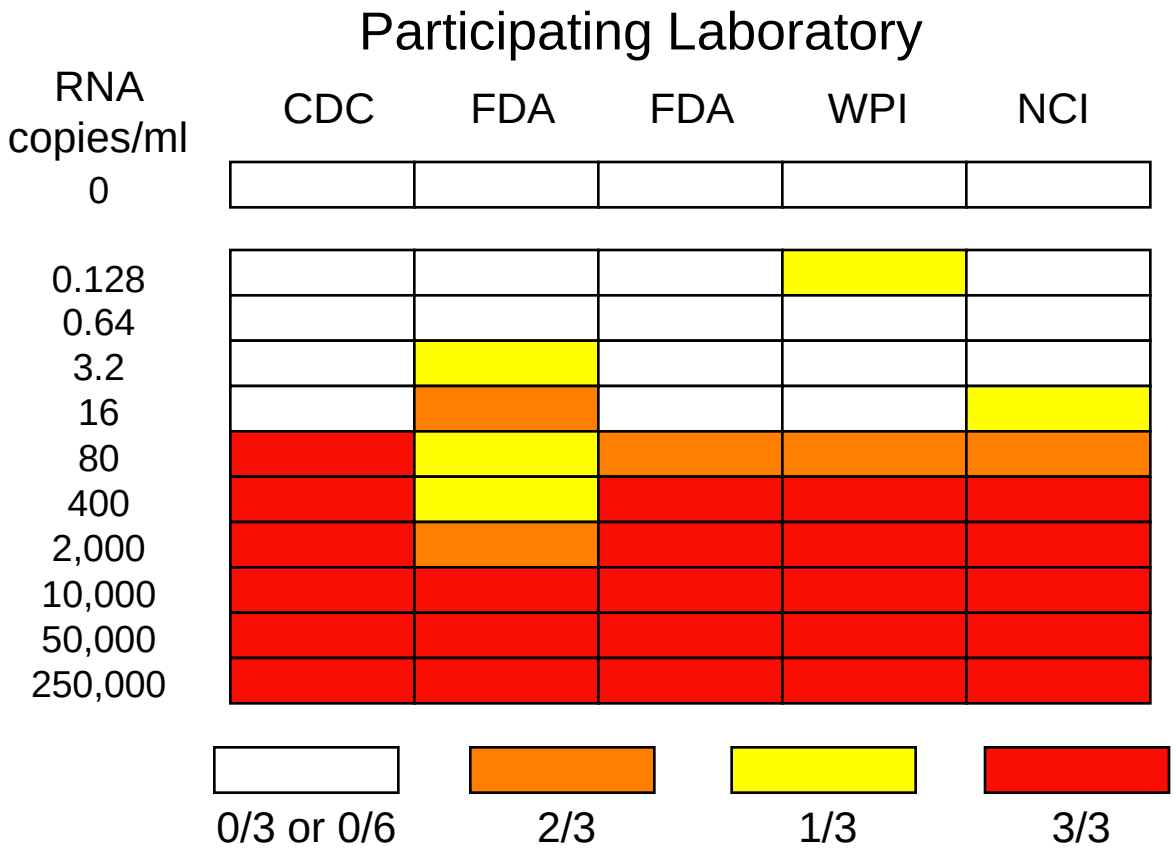
Source: G. Simmons, BSRI, San Francisco

Analytical Panel Results: Whole Blood



Source: G. Simmons, BSRI, San Francisco

Analytical Panel Results: Plasma



Source: G. Simmons, BSRI, San Francisco

Phase II Pilot Studies: Considerations

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Whole Blood *vs* PBMC *vs* Plasma

Lombardi et al. study performed on isolated PBMC and plasma

Large scale studies for prevalence facilitated by using WB or plasma

Donor-recipient and other repositories mainly comprised of frozen WB and/or plasma

Timing of Processing

Processing and freezing of donor samples to be included in the blood donor clinical panel (phase IV) vary from 2-4 days due to requirements for completion of ID testing. Available donor repositories include frozen WB and plasma samples processed 1-3 days post-phlebotomy.

Studies with other cell-associated viruses (HERVs, HTLV, herpesviruses and anelloviruses), demonstrate that levels of viral nucleic acid in plasma and whole blood vary with time from collection to processing and frozen storage.

Source: G. Simmons, BSRI, San Francisco

Phase II Pilot Studies: Setup

XMRV-positive samples

WPI have collected blood from four CFS patients previously identified as XMRV positive in the Lombardi et al study (by PCR, serology and culture)

Samples separated into tubes & processed immediately, or left at 4°C for 2 or 4 days

Each sample processed into PBMC, WB and plasma.

Analysis

Panels distributed by WPI to CDC and NCI for testing; WPI retained one panel for testing. One set of the panel was distributed to BSRI to keep and use for follow-up work as needed.

Source: G. Simmons, BSRI, San Francisco

XMRV Protocol Website - NCI

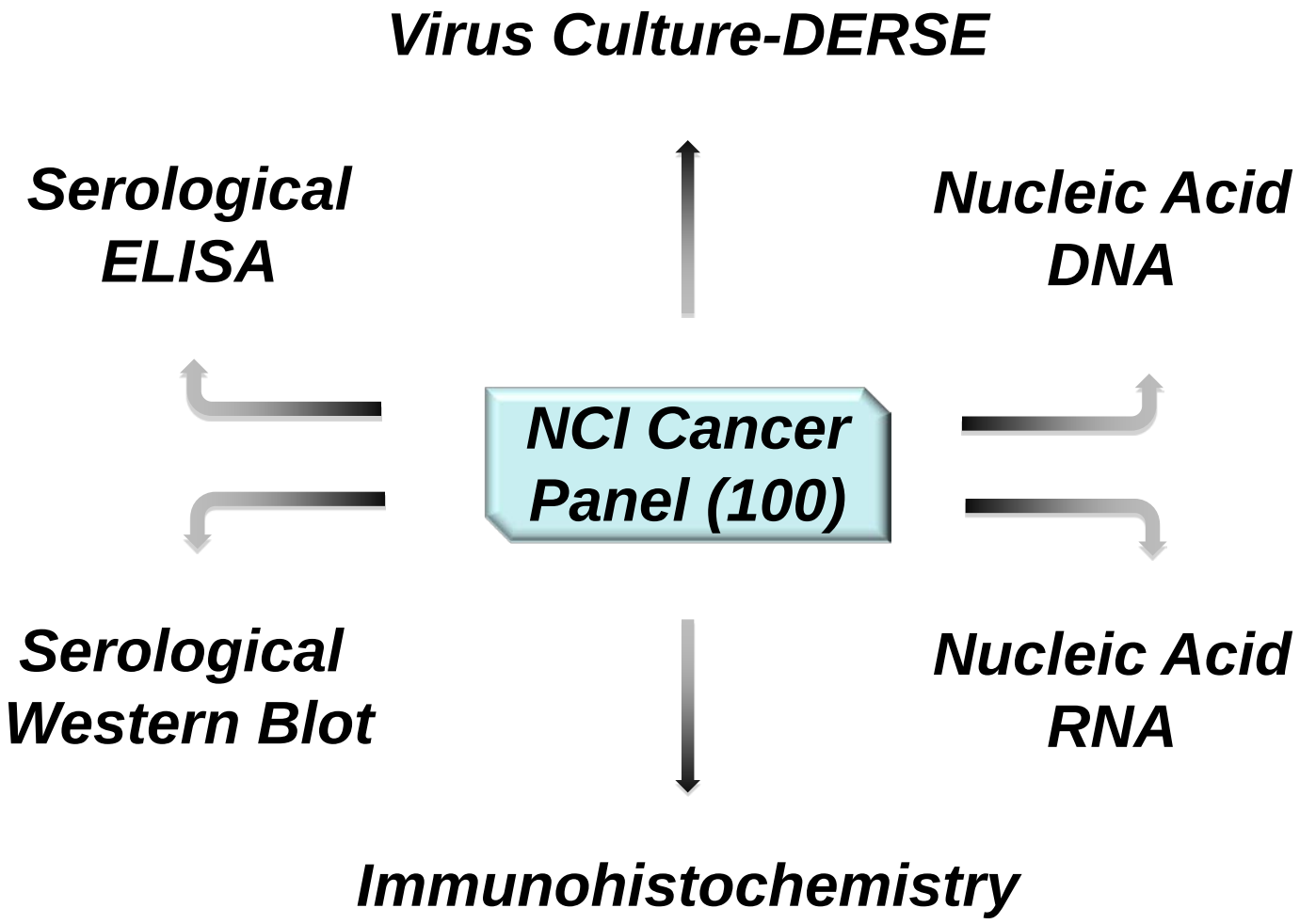
Researchers encouraged to deposit “raw” protocols for:

- Nucleic acid detection (RNA and DNA)
- Serological detection (ELISA and Western blot)
- Detection of mouse DNA (mtDNA, IAP)
- Immunohistochemistry
- Virus culture

Goal: Encourage dialog between researchers with disparate results.
Compare both assay protocols and assay reagents.
Establish uniform assay parameters.
Promote sharing of reagents.

Source: S. Le Grice, NCI

Evaluation of XMRV Assays - NCI



Source: S. Le Grice, NCI