

Update of Blood XMRV Working Group Activities

December 17, 2010 at 1:00 PM (Eastern time)

Hosted by the CFIDS Association of America



Graham Simmons, PhD
Blood Systems Research Institute.



Michael Busch, MD, PhD
Blood Systems Research Institute.




Steve Kleinman, BSc, MD
University of British Columbia.


Program Overview

- Brief orientation to webinar by Kim McCleary
- Introduction of all speakers
- Introductory comments by moderator, Dr. Steven Kleinman
- Presentation by Dr. Graham Simmons
- Presentation by Dr. Michael Busch
- Question and answer session based on questions submitted in advance


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Thank you for joining us today

- 20th CFIDS Association webinar of 2010 series; fourth in the series on XMRV-related topics (recordings posted at www.youtube.com/solvecfs)
- All speakers are in different locations
- 438 people preregistered to participate
- Recording/slides will be posted online within a day or two
- The speakers are not able to address questions about symptoms, test results or therapy

Graham Simmons, PhD



- Associate Investigator, Blood Systems Research Institute
- Adjunct Associate Professor, University of California-San Francisco
- Earned PhD in molecular virology at Institute of Cancer Research, University of London
- Key areas of interest:
 - Emerging Viruses: Chikungunya, SARS-CoV, HIV, HCV, Ebolavirus
 - Blood Transfusion
 - Viral Entry
 - Viral Receptors
 - Glycoproteins

Michael Busch, MD, PhD



- Director, Blood Systems Research Institute
- Senior Vice President, Blood Systems, Inc.
- Professor of Laboratory Medicine, University of California-San Francisco
- Earned MD at University of Southern California
- Earned PhD in experimental pathology at USC
- Dozens of professional appointments, including past member of BPAC
- Key areas of interest:
 - Transfusion associated viral infections
 - Laboratory assays and blood screening protocols for infectious agents
 - Immunological and other consequences of transfusion

Steven Kleinman, BSc, MD



- Clinical Professor of Pathology and Laboratory Medicine, University of British Columbia
- Senior Medical Advisor to AABB
- Earned Bachelor of Science at Massachusetts Institute of Technology
- Earned MD at University of California-San Diego
- Dozens of professional appointments, including former chair of AABB Committee on Transfusion Transmitted Diseases
- Author of more than 100 publications on blood transfusion, blood banking, blood donors, HIV and Hepatitis C
- Member of the HHS Blood XMRV Scientific Working Group and the AABB Interagency XMRV Task Force

Blood XMRV Scientific Research Working Group - Update

Graham Simmons, PhD

Associate Investigator

Blood Systems Research Institute

XMRV Presence in PBMC and plasma

■ **Lymphocyte infection**

- White blood cells isolated from patients with chronic fatigue syndrome (CFS) were activated and cultured for 42 days
- XMRV infection was demonstrated in white blood cells

■ **Culture of virus from plasma**

- XMRV could be directly isolated from the plasma of patients with CFS by culture with a target cell line

Lombardi et al (2009) *Science*, 585:326

The presence of a virus in plasma and white blood cells raises the possibility that the virus could be transmitted by blood transfusion.

Response to XMRV

- **Does XMRV pose a threat to the safety of the U.S. blood supply?**
- **HHS facilitated the establishment and coordination of collaborative groups**
- **HHS/NHLBI Blood XMRV Scientific Research Working Group first convened in December 2009**

Blood XMRV Scientific Research Working Group

- **Mission - design and co-ordinate research studies to evaluate whether XMRV poses a threat to blood safety**
- **Working Group includes representatives from transfusion medicine, retrovirology, and CFS scientific communities, as well as representatives from key Federal Agencies including HHS, FDA, CDC and NIH**
- **Evaluation of blood safety risks includes several steps:**
 - Evaluate XMRV detection assays
 - Establish prevalence of XMRV in blood donors
 - Determine if XMRV is transfusion-transmitted
 - If XMRV is transfusion transmitted, what is the clinical impact on recipients of blood transfusions?

Blood XMRV Scientific Research Working Group

HHS

Jerry Holmberg - Co-chair

NIH

NHLBI - Simone Glynn – Chair

CC - Harvey Alter

NCI - Francis Ruscetti

NCI – John Coffin

OD - Dennis Mangan

CDC

OID/NCEZID - Stephan Monroe

OID/NCEZID - William Bower

OID/NCHHSTP -Michael Hendry

FDA

CBER - Jay Epstein

CBER - Diane Gubernot

CBER – Hira Nakhasi

AABB

Steven Kleinman

ABC

Celso Bianco

ARC

Roger Dodd

Susan Stramer

BSRI

Michael Busch

Leslie Tobler

CFIDS

Suzanne Vernon

Harvard Medical School

Anthony Komaroff

MVRBC

Louis Katz

Central Lab

BSRI - Graham Simmons

NAT Lab Investigators

CDC - Bill Switzer/Walid Heneine

FDA - Indira Hewlett

FDA - Shyh-Ching Lo

NCI - Mary Kearney

WPI - Judy Mikovits

Funding for currently launched studies via NHLBI REDS-II Program

Participating Labs - Discordant results

Pub. date	Journal or conference	Authors	Samples	Assays	Results
Oct 2009	Science	Lombardi et al (WPI)	PBMC Plasma	Viral culture Serology (RT-)PCR	68/101 CFS 8/218 controls
Jul 2010	Retrovirol	Switzer et al (CDC)	PBMC	PCR Serology	0/51 CFS 0/56 controls
Jul 2010	ICEID abstract	Gao et al (Gen-Probe)	Plasma	TMA	0/1435 blood donors
Aug 2010	PNAS	Lo et al (FDA-Lo)	PBMC Whole blood plasma	PCR	32/37 CFS 3/44 controls
Nov 2010	Transfusion	Tang et al (FDA-Hewlett)	Plasma PBMC	(RT-)PCR	0/268 HIV+ Uganda/Camer oon

Detecting a virus or an immunological reaction to a virus

- **Viral culture – Direct isolation and amplification of infectious virus from plasma or cells in a laboratory cell line**
- **Nucleic Acid Testing (NAT) – Direct detection of viral genetic material (RNA or DNA) in plasma, cells or blood using amplification methods**
 - Polymerase chain reaction (PCR)
 - Transcription-mediated amplification (TMA)
- **Serology – The presence of specific antibodies in plasma or serum directed against a virus is evidence of a past or present infection**
 - EIA – Enzyme ImmunoAssay
 - Western blot

Pros and Cons of detection methods

- **Viral culture – Direct isolation and amplification of infectious virus from plasma or cells in a laboratory cell line**
- **Nucleic Acid Testing (NAT) – Direct detection of viral genetic material (RNA or DNA) in plasma, cells or blood using amplification methods**
 - Polymerase chain reaction (PCR)
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- **Serology – The presence of specific antibodies in plasma or serum directed against a virus is evidence of a past or present infection**
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Nucleic Acid Testing (NAT) - Glossary

- **PCR – Amplification of viral DNA sequence using highly specific primers yielding product of a certain size**
 - Specific amplification confirmed by sequencing product
- **Reverse transcription PCR (RT-PCR) – The conversion of viral RNA to DNA followed by PCR**
- **Nested PCR – A second round of PCR performed on product of the first round**
 - Very sensitive
 - Highly prone to contamination
- **Quantitative PCR (qPCR) or real-time PCR – PCR amplification is followed in real-time by the detection of product using a fluorescent probe**
- **Transcription-mediated amplification (TMA) – Amplification of viral RNA or DNA by rounds of in vitro transcription**

Blood XMRV Scientific Research Working Group

Study Phases

- **Phase I - Analytical Panels**
 - Evaluate performance of XMRV NAT assays

- **Phase II - Pilot Clinical Studies**
 - Sample type:
 - Plasma
 - Whole Blood versus Peripheral Blood Mononuclear Cells
 - Timing of sample preparation

- **Phase III - Clinical Sensitivity/Specificity Panel**
 - NAT and serological assay performance on pedigreed clinical samples

- **Phase IV - Blood Donor Clinical Panel**
 - Initial estimation of the proportion of blood donors who have XMRV nucleic acid and/or antibodies in their blood - prevalence studies

Phase I - Analytical Panels

- **Analytical performance panels**
 - Comparison of Limit Of Detections and 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
 - Knouf et al (2010) J Virol 83:7353 (PMID:19403664)
- **Virus supernatant**
 - Supernatant from cultured 22Rv1 cells
 - Approximate Viral Load of 5×10^9 RNA copies/ml

Phase II - Pilot Studies

- **Facilitate labs with divergent results to test clinically validated samples**
- **Plasma versus Whole Blood versus Peripheral Blood Mononuclear Cells**
 - Original Lombardi et al. study performed on isolated Peripheral Blood Mononuclear Cells and plasma
 - Large scale studies for prevalence facilitated by using whole blood or plasma
 - Donor-recipient and other repositories are mainly comprised of frozen whole blood and/or plasma
- **Timing of Processing**
 - Processing and freezing of samples for study varied from 2-4 days due to requirements for completion of infectious disease (ID) testing. Other frozen whole blood and plasma repositories were prepared 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

Phase III - Clinical Sensitivity and Specificity

- **The ability of participant assays to effectively detect XMRV positive and negative clinical samples will be examined**
 - To attempt to overcome the issues of clinical variation and specificity, larger numbers of pedigreed clinical XMRV-positive and -negative samples are being assembled into a coded panel to be distributed to all labs
- **Positives**
 - 30 clinical samples will be collected by WPI and Harvard. All will be from patients reported by multiple assays as XMRV/MRV+ in the Lombardi et al. or Lo et al. studies. Method and timing of processing will be determined based on pilot study data
- **Negatives**
 - PCR detection assays are prone to detect the amplification of non-specific human derived genomic sequences
 - Thus, a larger group of 10 pedigreed negatives are required in order to introduce variability
 - Being pedigreed negative by PCR at WPI and CDC labs, and for serology at WPI, CDC and NCI labs (Ruscetti/Bagni)

Phase IV - Clinical Panel for Donor Prevalence

- **Blinded panels consisting of approximately 300 blood donor samples, as well as confirmed XMRV/MRV-positive and negative analytical standards and clinical samples (from phase III) will be created for whole blood and plasma**

- **Plan would be to distribute blinded panels to at least 4 of the participating laboratories for nucleic acid & serological testing**

- **Test results will be analyzed:**
 - Correlation between whole blood and plasma testing will be determined for each lab and between labs

 - Preliminary XMRV prevalence in blood donors (proportion of donors positive for XMRV nucleic acids and/or antibodies) will be estimated based on compiled results from each lab

Phase I - Analytical Panel Production

Whole blood

**Whole Blood (WB) unit from
pedigreed (NAT and serology)
negative donor**

**22Rv1 cells spiked into WB to yield
9,900 22Rv1 cells per ml of WB**

Three-fold dilutions in fresh WB

**0.5 ml aliquots to give 15 panels
with three replicates at each
dilution, plus 6 negative controls**

Plasma

**Plasma components from two
pedigreed (NAT and serology)
negative donors**

**22Rv1 supernatant spiked to give
approximately 250,000 RNA copies
per ml of plasma**

Five-fold dilutions in fresh plasma

**0.5 ml aliquots to give 15 panels
with three replicates at each
dilution, plus 6 negative controls**







Phase I Analytical Panel - Results

Whole Blood Panel

Proviral copies/ml	CDC	FDA(H)	FDA(L)	GP	NCI	WPI
0						
≥5						
≥15						
≥45						
≥136						
≥410						
≥1,220						
≥3,670						
≥11,000						
≥33,000						
≥99,000						

Plasma Panel

Viral RNA copies/ml	CDC	FDA(H)	FDA(L)	GP	NCI	WPI
0						
0.128						
0.64						
3.2						
16						
80						
400						
2,000						
10,000						
50,000						
250,000						

-  0/3 (for dilution series) or 0/6 (for negatives) replicates called positive
-  1/12 negative controls called positive (panels run twice - once each by two different operators)
-  1/6 negative controls called positive (identified as non-specific band of human genomic origin by sequencing subsequent to decoding of results)
-  1/3 replicates called positive
-  2/3 replicates called positive
-  3/3 replicates called positive

Conclusions and Limitations

- **XMRV NAT detection assays were highly sensitive**
 - Five assays (CDC, FDA-Lo, NCI, WPI, Gen-Probe) for whole blood and all six assays for plasma were found to have no substantial differences in terms of sensitivity
 - The overall similarity of results suggests sensitivity of assays cannot explain differences in XMRV detection in clinical samples reported by the participating laboratories

- **Caveats**
 - XMRV isolate with which 22Rv1 cells are infected may not adequately represent the diversity of XMRV clinical isolates
 - Use of two units of plasma as diluent for preparation of the spiked plasma panel may have compromised the accurate dilution of virus

Phase IIa - Setup

■ **XMRV-positive samples**

- WPI collected blood from four subjects (mostly with CFS) previously identified as XMRV positive in the Lombardi et al. study (by PCR, serology and/or culture)
- Specimens separated into tubes and were processed immediately, or left at 4°C for 2 or 4 days
- Each specimen was processed into replicate peripheral blood mononuclear cells, whole blood, and plasma samples that were frozen for panel preparation

■ **Analysis**

- Unblinded panels were distributed to WPI and CDC and a blinded panel to NCI for testing

Phase II Subject Characteristics

- **Four Females**
- **26-53 Years of age**
- **Three were diagnosed with CFS over 20 years ago, one is a family member of a long-time CFS patient**

Subject	XMRV PCR positive	XMRV Seropositive	XMRV culture positive	CFS patient	Family member	Anti- retroviral drugs
1	5/5 (nested)	5/5	3/5	No	Yes	No
2	1/1 (single)	1/1	4/4	Yes	No	No
3	0/1 (single)	1/4	4/4	Yes	No	No
4	10/10 (nested)	3/10	9/10	Yes	No	Since 05/10

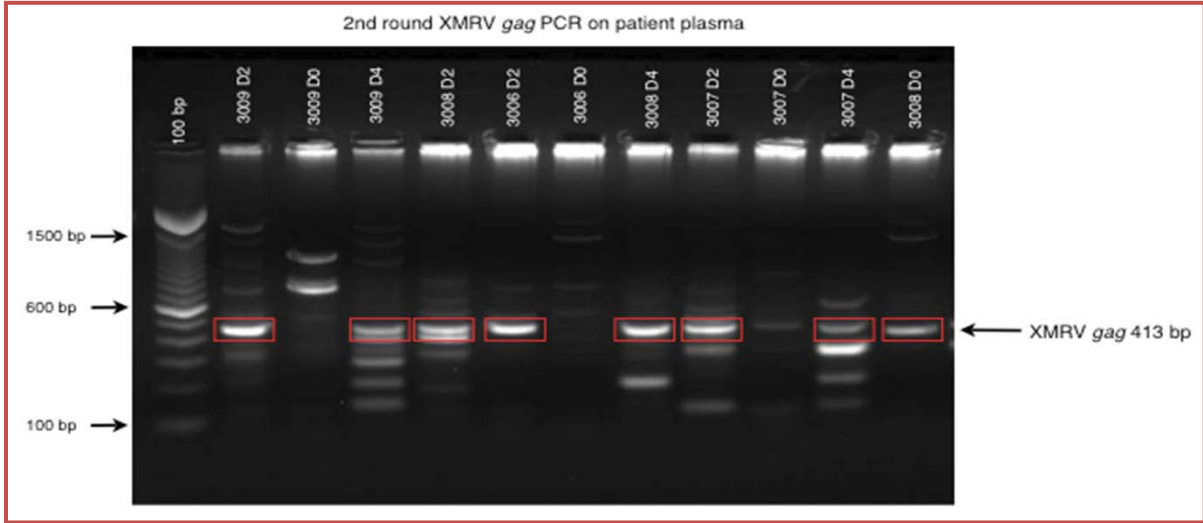
Phase IIa - CDC Results

Subject		Plasma					Whole blood DNA/RNA test				PBL DNA/RNA test				
		WB	N XGAG PCR	Seq	qRT-PCR (pro)	Seq	qRT-PCR (int)	β actin	N Xpol PCR	N XGAG PCR	qRT-PCR (pro)	β actin	N Xpol PCR	N XGAG PCR	qRT-PCR (pro)
1	- D0	neg	Neg		BD		BD	pos	neg	neg	BD	pos	neg	neg	BD
	- D2	neg	Neg		Pos	XMRV/MuLV	Pos	pos	neg	neg	BD	pos	neg	neg	BD
2	- D0	neg	Neg		BD		BD	pos	neg	neg	BD	pos	neg	neg	BD
	- D2	neg	Neg		Pos	XMRV/MuLV	Pos	pos	neg	neg	BD	pos	neg	neg	BD
	- D4	neg	Neg		Pos	XMRV/MuLV	Pos	pos	neg	neg	BD	pos	neg	neg	BD
3	- D0	neg	Neg		BD		BD	pos	neg	neg	BD	pos	neg	neg	BD
	- D2	neg	Pos	XMRV	Pos	XMRV/MuLV	Pos	pos	neg	neg	BD	pos	neg	neg	BD
	- D4	neg	Pos	XMRV	Pos	XMRV/MuLV	Pos	pos	neg	neg	BD	pos	neg	neg	BD
4	- D0	neg	Neg		BD		BD	pos	neg	neg	BD	pos	neg	neg	BD
	- D2	neg	Pos	XMRV	Pos	XMRV/MuLV	Pos	pos	neg	neg	BD	pos	neg	neg	BD
	- D4	neg	Pos	XMRV	Pos	XMRV/MuLV	Pos	pos	neg	neg	BD	pos	neg	neg	BD

Plasma was ultracentrifuge pelleted prior to nucleic acid extraction

All PCR positive samples tested negative for mouse mitochondrial DNA

Phase IIa - WPI Plasma Results



Subject	Nested RT-PCR for XMRV gag		
	Day 0	Day 2	Day 4
1	Negative	Positive	Not tested
2	Positive	Positive	Positive
3	Negative	Positive	Positive
4	Negative	Positive	Positive

Plasma processed using Qiagen Viral RNA kit

*Whole blood samples were all negative for DNA
PBMC were not tested*

Phase IIa – NCI/DRP results

- **Single-Copy quantitative PCR assay for XMRV gag**
 - Plasma spiked with internal control virus and ultracentrifuged prior to nucleic acid extraction using guanidinium isothiocyanate and PCR was performed +/- RT step
 - Whole blood and Peripheral Blood Mononuclear Cells extracted for DNA
- **All samples (Plasma, Whole blood and Peripheral Blood Mononuclear Cells) and all time-points (Day 0, 2, 4) were negative**
 - Plasma internal controls for pelleting of virus and RT step were all in range
 - Whole blood and Peripheral Blood Mononuclear Cells genomic DNA controls in range

Phase IIa - Conclusions and Limitations

- **Two out of three labs detected XMRV in clinical samples**
 - Plasma out-performed whole blood in both labs, and Peripheral Blood Mononuclear Cells in the one lab that tested Peripheral Blood Mononuclear Cells.
 - Day 2 and day 4 samples out-performed day 0 plasma samples in both labs
- **Caveats**
 - Panel was distributed mostly unblinded
 - Small sample size
 - Third laboratory failed to detect virus despite sensitive assay

Phase IIb - Setup

■ XMRV-positive samples

- Blood collected into EDTA tubes by an independent phlebotomist at patients home or work from the **same four subjects** (one patient had independently initiated anti-retroviral therapy)
- The pedigreed negative subject used for the analytical panel and phase IIa panel was bled by the same phlebotomist as a control
- Phlebotomist directly supplied the samples to BSRI for processing
- Samples separated into tubes and were processed the same day, or left at 4°C for 2 days
- Each sample was processed into Peripheral Blood Mononuclear Cells, whole blood, and plasma

■ Analysis

- Blinded panels were distributed by BSRI to WPI, CDC, NCI and Gen-Probe for testing. Two sets of the panel were retained by BSRI to distribute for follow-up work as needed
- Results were reported back to BSRI and decoded

Phase IIb – NCI/DRP results

- **Single-Copy quantitative PCR assay for XMRV gag**
 - Plasma spiked with internal control virus and either extracted directly or ultracentrifuged prior to nucleic acid extraction using guanidinium isothiocyanate and used +/- RT step
 - Peripheral Blood Mononuclear Cells extracted for DNA and RNA and used +/- RT step

- **All Plasma and Peripheral Blood Mononuclear Cell samples at both time points (Day 0, 2) were negative**
 - Plasma internal controls for pelleting of virus and RT step were all in range

Phase IIb – CDC results

- **Multiple Assays for XMRV and generic MLV**
 - Plasma ultracentrifuged prior to nucleic acid extraction
 - Plasma assayed with nested RT-PCR for XMRV gag and envelope and quantitative RT-PCR for MLV gag and integrase
 - Peripheral Blood Mononuclear Cells assayed with nested PCR for XMRV polymerase and quantitative PCR for MLV protease

- **All Plasma and Peripheral Blood Mononuclear Cell samples at both time points (Day 0, 2) were negative**
 - Plasma RNA controls in range
 - Peripheral Blood Mononuclear Cells genomic DNA controls in range

Phase IIb – Gen-Probe results

- **Target Capture/Transcription-Mediated Amplification (TMA)/chemiluminescent detection**
 - **Platform:** TIGRIS System
 - High throughput, fully automated NAT System
 - Currently used routinely world-wide for NAT blood screening
 - **Assay design:** duplex assay that targets conserved sequences in two separate regions of XMRV genome
 - Ability to detect MRV-like sequences is not known; new assay to detect a broad range of MRV sequences is under development
 - Each reaction includes an internal control which validates assay steps (target capture, amplification and detection)
 - **Sample volumes:** 500 µl plasma or 100 µl Peripheral Blood Mononuclear Cells

- **All Plasma and Peripheral Blood Mononuclear Cell samples at both time points (Day 0, 2) were negative**

Phase IIb – WPI results

- **Nested RT-PCR for MRV gag followed by sequencing of positive bands to confirm specificity**
 - RNA extracted directly from plasma
 - Total nucleic acid extracted from Peripheral Blood Mononuclear Cells (PBMC) and RT step performed

- **Whole Blood has not been completed**

Sample Type	Day of Processing	Subject 1	Subject 2	Subject 3	Subject 4	Pedigreed Negative
PBMC	0	+	Negative	Negative	Negative	+ ^a
	2	Negative	+	+	Negative	Negative
Plasma	0	Negative	Negative	Negative	Negative	Negative
	2	Negative	Negative	Negative	Negative	Negative

Negative – Negative by nested PCR/sequencing

+ - Band of correct band size, sequences as XMRV/MRV

a. - Investigation following decoding of results determined that there was a procedural error during PBMC sample extraction involving reuse of needles (employed to lyse cells and shear DNA) on sequential PBMC cell pellets.

Summary of Phase IIb Serology Results

Subject ID (description)	NCI/Ruscetti	CDC
Subject 1 - Plasma - day 0	+	Negative
Subject 1 - Plasma - day 2	Negative	Negative
Subject 2 - Plasma - day 0	Negative	Negative
Subject 2 - Plasma - day 2	Negative	Negative
Subject 3 - Plasma - day 0	+	Negative
Subject 3 - Plasma - day 2	+	Negative
Subject 4 - Plasma - day 0	+	Negative
Subject 4 - Plasma - day 2	+	Negative
Pedigreed negative - Plasma - day 0	Negative	Negative
Pedigreed negative - Plasma - day 2	+	Negative

NCI – Flow cytometry on cells expressing spleen focus-forming virus env (Lombardi et al, 2009)

CDC – Western blot for multiple MLVs (Switzer et al, 2010)

Summary of Phase IIb NAT and Antibody Results

Subject ID (description)	NCI	G-P	CDC	WPI	NCI* Ab	CDC Ab
Subject 1 - Plasma - day 0	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 1 - Plasma - day 2	Negative	Non reactive	Negative	Negative	Negative	Negative
Subject 1 - PBMC - day 0	Negative	Non reactive	Negative	Positive		
Subject 1 - PBMC - day 2	Negative	Non reactive	Negative	Negative		
Subject 2 - Plasma - day 0	Negative	Non reactive	Negative	Negative	Negative	Negative
Subject 2 - Plasma - day 2	Negative	Non reactive	Negative	Negative	Negative	Negative
Subject 2 - PBMC - day 0	Negative	Non reactive	Negative	Negative		
Subject 2 - PBMC - day 2	Negative	Non reactive	Negative	Positive		
Subject 3 - Plasma - day 0	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 3 - Plasma - day 2	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 3 - PBMC - day 0	Negative	Non reactive	Negative	Negative		
Subject 3 - PBMC - day 2	Negative	Non reactive	Negative	Positive		
Subject 4 - Plasma - day 0	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 4 - Plasma - day 2	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 4 - PBMC - day 0	Negative	Non reactive	Negative	Negative		
Subject 4 - PBMC - day 2	Negative	Non reactive	Negative	Negative		
Pedigreed Negative - Plasma - day 0	Negative	Non reactive	Negative	Negative	Negative	Negative
Pedigreed Negative - Plasma - day 2	Negative	Non reactive	Negative	Negative	Positive	Negative
Pedigreed Negative - PBMC - day 0	Negative	Non reactive	Negative	Positive		
Pedigreed Negative - PBMC - day 2	Negative	Non reactive	Negative	Negative		

* Ruscetti

Conclusions from Phase IIb

- In this round of testing, only one out of four labs detected XMRV in clinical samples and only in Peripheral Blood Mononuclear Cells
- Ultracentrifugation or direct extraction of plasma did not seem to make a difference in detection by NCI lab
- Sensitive NAT assay from a diagnostic company was unable to detect XMRV/MRV

Notes on Phase II pilot

- **Pilot designed to validate sample type and national repositories**
- **Not designed or performed as a clinical evaluation or longitudinal study**
- **Impossible to draw any conclusions from this extremely small study**
 - Loss of plasma positivity for all samples
 - False positivity in PCR due to processing issues
 - False positivity in serology

Conclusions

- **Based on Phase II findings, no clear advantage to delayed processing**
- **Include serology in parallel with NAT in future studies**
- **Continue with collection of Phase III panel**
 - Include more positive and negative samples and differently sourced samples
 - Peripheral Blood Mononuclear Cells, whole blood, and plasma will be used, with a standard next day processing protocol

Additional Acknowledgements

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SAIC/NCI

Rachel Bagni

OBRR, FDA

Shixing Tang

Jiangqin Zhao in.

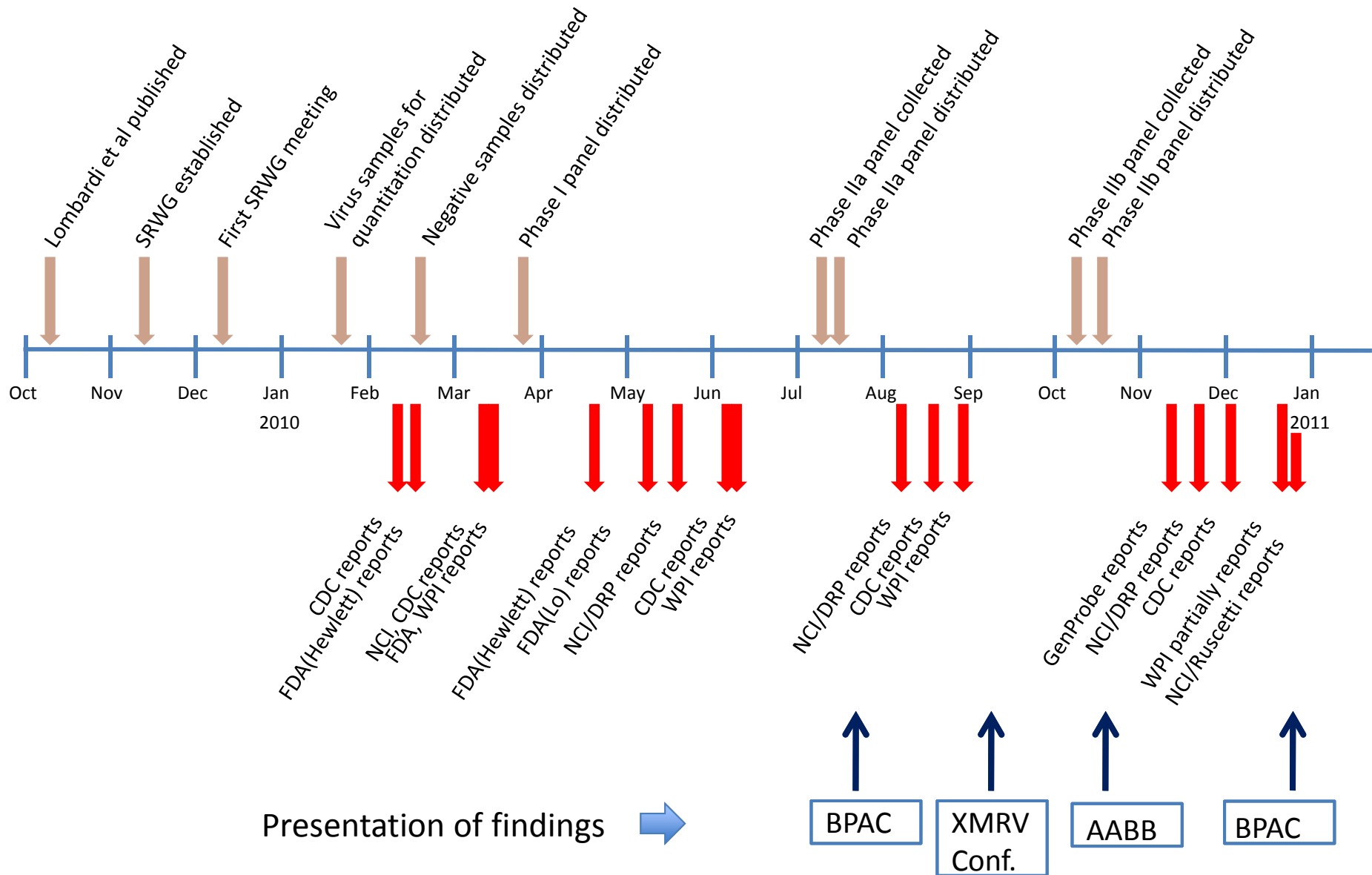
WPI

Max Pfof

Cassandra Puccinelli

Kathryn Hagen

Blood XMRV Scientific Research Working Group Activities



Retrospective and Prospective U.S. Donor MRV Surveillance and Transmission Studies

*Michael Busch, MD, PhD
Director, Blood Systems Research Institute
Senior Vice President, Blood Systems
Professor of Laboratory Medicine, UCSF*

Proposed Retrospective Study

- **Blood donor prevalence over four decades using total of 10,000 samples from 5 NHLBI repositories:**

- Transfusion Transmitted Viruses Study (TTVS)
- Transfusion Safety Study (TSS)
- Retrovirus Epidemiology in Donors Study (REDS) General Leukocyte and Plasma Repository (GLPR)
- Viral Activation by Transfusion Study (VATS)
- REDS Allogeneic Donor and Recipient Repository (RADAR)

- **Transfusion-Transmission using TTVS, VATS and RADAR repositories which contain linked donation and recipient samples**

- Rates of transfusion-transmission and correlations of transmission with viral and serologic findings in XMRV/MLV+ donations
- Effect of routine blood filtration (leukoreduction) and blood component storage period on transmission
- Limited data on mortality and morbidity

- **Utilize Abbott and Gen-Probe high-throughput screening assays for serology and NAT**

NHLBI BioLINCC repositories that would be accessed for the proposed studies

Study Name	Time Frame	Population	Specimen Type	Number of Specimens	Major Agents Studied
TTVS	1974 - 79	Donor - Recip	Serum	5,655 donat'ns 1533 recip'ts	HCV, HBV
TSS	1984-85	Donations	Serum	201,212 donat'ns	HIV, HTLV
REDS GLPR	1994-95	Donations	Plasma; Frozen Whole Blood	147,915 donat'ns	HBV, CMV, HHV-8
VATS	1995 - 99	Donor - Recip	Plasma; Frozen Whole Blood	3,864 donat'ns 531 recip'ts	HIV, CMV, HBV, HCV, HGV, HTLV
REDS: RADAR	1999 - 2003	Donor - Recip	Plasma; Frozen Whole Blood	13,201 donat'ns 3,574 recip'ts	Parvovirus B19

Aim 1. Prevalence and demographic correlates of MLV-related viruses in US blood donors

- The prevalence of XMRV and other MLV-related viruses will be determined using 2000 representative donations from each of five donor repositories dating back to the late 1970s
 - DNA/RNA screening - high-throughput assay systems for XMRV/MLV nucleic acids (e.g., Gen-Probe NAT assay on the TIGRIS platform)
 - Antibody screening – high throughput systems for HIV antibodies (e.g., Abbott antibody assays on the ARCHITECT system)

- *Assuming prevalence rates of 0.2% to 7%, we would identify 20 to 700 blood donors with viremia (NAT +) and/or evidence of XMRV/MLV exposure (antibody +), which will allow us to establish temporal and demographic trends of infection in the US donor population over the past 4 decades*

Aim 2. Transfusion-transmission rates & correlates of transmission of XMRV/MLVs

- Identification of XMRV/MLV-reactive donors within three existing donor/recipient repositories (TTVS, VATS and RADAR) will allow us to establish the rates of transfusion-transmission of these viruses, as well as investigate correlates of transmission, including:
 - levels of viral RNA in serum/plasma, DNA/RNA in whole blood-derived leukocytes, and viral antibodies in serum/plasma of XMRV/MLV-reactive donations
 - impact of filtration (leukoreduction) and storage of the transfused XMRV/MLV-reactive blood products
 - effects of underlying disease and immune status of exposed recipients on rate of acquisition of infection
- *With 11000 donor exposures to enrolled recipients and donor prevalence rates between 0.2 and 7%, we could identify 20-500 XMRV/MLV-reactive transfused components*
 - *characterize overall transmission rates*
 - *if there is sufficient transmission, examine donor, component and recipient correlates of infection*

Aim 3. Virological parameters and clinical outcomes of XMRV/MLV infections in recipients

- If infected recipients identified in Aim 2 and we have sequential samples and/or clinical outcome data, we will study dynamics and consequences of acute XMRV/MLV infections:
 - Viral loads, sequence evolution, presence of culturable virus and humoral immune responses will all be studied using serial post-transfusion/infection samples
 - Data on survival and selected clinical outcomes of recipients who enrolled in these studies may allow for a preliminary assessment of the clinical consequences of TT-XMRV/MLV infections in diverse recipient populations (i.e., elective surgery vs HIV-infected patients with clinical AIDS)

- *Data on viral dynamics and clinical impact of acute infection in recipients will further guide decisions on need for and approach to screening the blood supply, as well as contribute to broader understanding of diagnosis and pathogenesis, of XMRV/MLV*

Prospective IND Trial: How and When?

- **Prospective screening of 20,000+ blood donors for XMRV/MLVs**
 - Parallel NAT (e.g., Gen-Probe/Novartis TIGRIS) and antibodies (e.g., Abbott Architect) assays performed prospectively in donor screening labs under FDA Investigational New Drug (IND) exemption
- **Interdiction of XMRV/MLV-reactive blood components with deferral of donors**
 - Enroll donors into follow-up study with immunologic and virologic assessments (in parallel with CFS patients)
 - Lookback investigations to determine XMRV status of recipients of previous units from XMRV+ repeat donors
- **What are the criteria to justify a prospective trial?**

FDA posed the following question to BPAC for a vote:

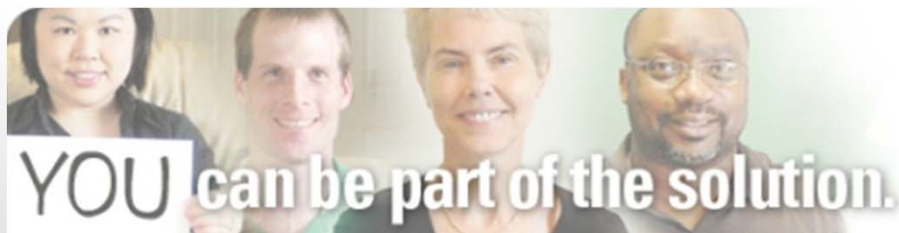
- Do the scientific data support asking donors about a medical history and/or diagnosis of CFS as a basis for indefinite deferral?

FDA posed the following question to BPAC for a vote:

- Do the scientific data support asking donors about a medical history and/or diagnosis of CFS as a basis for indefinite deferral?
- 9 voted “yes” and 4 voted “no.” This vote reflects opinion on the issue of whether asking a question was better than using educational materials to elicit donor disclosure. All BPAC members have indicated that they agree with the indefinite deferral of CFS patients based on all evidence that it will promote donor and recipient safety.

For more information

- CFIDS Association website: www.cfids.org
- XMRV: <http://www.cfids.org/xmrv/default.asp>
- MLV: <http://www.cfids.org/mlv/default.asp>
- Webinar recordings: <http://www.youtube.com/solvecfs>
- CFIDSLink – monthly e-newsletter:
<http://www.cfids.org/development/checkemail.aspx>



The CFIDS Association of America

Our Mission:

For CFS to be widely understood, diagnosable, curable and preventable.

Our Strategy:

To stimulate research aimed at the early detection, objective diagnosis and effective treatment of CFS through expanded public, private and commercial investment.

Our Core Values:

To lead with integrity, innovation and purpose.