

# The CFIDS Association of America

*Working to make CFS widely understood, diagnosable, curable and preventable*

## Follow-Up FAQs to the Study by Lo, Alter et al.

### September 2010 CFIDSLink

*(Updated most recently on September 6, 2010)*

In the days following the August 23, 2010 publication in the *Proceedings of the National Academy of Sciences (PNAS)* of, "[Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors](#)" by Shyh-Ching Lo, Harvey J. Alter and colleagues at the Food and Drug Administration, National Institutes of Health and Harvard Medical School, we collected frequently asked questions (FAQs). We requested answers from a range of experts familiar with this study, the emerging field of research into XMRV and other murine leukemia retroviruses, blood safety issues and the related media coverage. Here are their replies, in their own words.

#### FAQs ABOUT THE LO/ALTER STUDY

Q: Do you consider the *PNAS* study to have been designed as either a validation or replication study of the Lombardi et al. study?

A: The *PNAS* study was stimulated by the *Science* publication and by the negative studies that followed. We tested our cohort of CFS patient samples and controls without bias. That is, we did not have an expectation in either direction, but simply wanted to know whether or not we could find XMRV in our samples. As it turned out, we did not find XMRV, but rather, closely related polytropic murine leukemia viruses (MLVs). The strong association of the MLVs with CFS identified in our patient population was similar to the strong association of XMRV and CFS reported by Lombardi et al. However, the role of MLVs in the CFS disease process is still not clear. – **Harvey J. Alter, MD, MACP, Chief, Clinical Studies & Associate Director for Research, Department of Transfusion Medicine, National Institutes of Health and Shyh-Ching Lo, MD, PhD, Medical Officer, Division of Cellular and Gene Therapies, Federal Drug Administration**

Q: Were the samples tested under blinded conditions?

A: We did not specifically blind and mix the two sample groups (CFS and blood donors). However, they were studied in parallel by polymerase chain reaction (PCR). As shown in the figures of the paper, those samples with positive amplicons of the predicted sizes were all sequenced; no sample was considered positive unless sequencing confirmed PCR reactivity. – **H.J. Alter, MD, MACP and S-C Lo, MD, PhD**

Q: The FDA has posted a [Q&A](#) on its website, stating that Dr. Lo tested 34 CFS patient samples from CDC and that there were "no XMRV-positive" results. Did he test for the broader group of MLVs? Will these results be published when all 82 samples from the CDC's negative study have been tested?

A: The CDC samples were tested for the broader group of MLVs. While we did not find any of the 34 CDC CFS samples to sequence as MLV, there were three samples that were indeterminate in that they had weak PCR-positive signal, but could not be confirmed by sequencing. These samples are

now being further amplified and cloned for resequencing. CDC and FDA/NIH will continue to work to understand the disparities, but there is no current plan to publish the results of this sample exchange. It was not critically designed to be a study for publication, but larger coded panel testing conducted by NHLBI in which both CDC and FDA, as well as other labs, are participating will be published when the data are complete. – **H.J. Alter, MD, MACP and S-C Lo, MD, PhD**

Q: Do the three variants of MLVs found in the CFS patients from your practice correlate to any particular CFS symptoms, type of onset, age, illness severity, etc.?

A: Not that we can tell, but the total numbers are small. We need much larger studies to answer this important question. — **Anthony L. Komaroff, MD, The Simcox/Clifford/Higby Professor of Medicine, Harvard Medical School; Editor in Chief, Harvard Health Publications, Harvard Medical School; and Senior Physician, Brigham and Women’s Hospital**

Q: Eight of the 25 patients from your clinic provided fresh blood samples earlier this year for testing by Dr. Lo. How were those people selected? Are you getting samples from the other 17 so that they can be tested too?

A: These eight patients live in the Boston area and were available to have the blood drawn on a specific day, with short notice. We are attempting to get samples from the other individuals as well. – **A.L. Komaroff, MD**

Q: You’ve been at this a long time and have seen evidence that associates several different infectious agents with CFS. What are your thoughts on this particular study?

A: Based on my experience talking to the patients, and examining them, I think that the most likely explanation of their illness in most of the patients with CFS is that they are suffering from some kind of chronic infection. I think it is very plausible that the infection is of a type that cannot be fully cleared by the immune system, although that has not been proven. I think it is very plausible that the key symptoms of CFS are caused by the immune system’s attack on the infectious agent(s) that may be involved, although that has not been proven. Finally, since symptoms are experienced in the brain, I think it is very plausible that the immune system molecules that may cause the symptoms are either produced in the brain (because the infection is there) or reach the brain through the circulation, although that has not been proven.

This study found strong evidence that a group of retroviruses that were first discovered to infect mice may also be infecting many patients with CFS, and also a few healthy blood donors. However, other studies—both published and unpublished—have not found that. All of the laboratories that have studied this question need to work together to try to understand why they have gotten different results. The PCR techniques that were the basis of most of these studies are very tricky: they can be falsely positive, and they can be falsely negative. Dr. Lo’s laboratory took great pains to rule out various types of falsely positive results, as explained in our paper. We also proposed some

reasons why other studies might have obtained falsely negative results, but that is just speculation.

In summary, our study does not and should not settle the question as to whether mouse retroviruses may be associated with CFS. It is one study, one piece of evidence. Scientific conclusions require multiple studies, and multiple types of evidence. More work needs to be done, particularly among those laboratories already engaged in the study of this question, to understand why their results are different. Even if it is concluded that these viruses are often present in patients with CFS, that will not prove that the viruses are a cause of CFS. So we are a long way from the finish line in getting solid answers to these important questions. – **A.L. Komaroff, MD**

Q: There isn't any information included in the article about 12 patients whose samples were sent to Dr. Lo in the early 1990s. By which criteria were they diagnosed? Were there any particular clinical features about these patients that made them different from other patients you were seeing at the time or have since in the years since?

A: I do not recall the particulars of such patients as that was almost 20 years ago. However, my clinic then saw only CFS and they are pretty much the same as what I see today from 26 states and 7 countries. I have a poster presentation at the International XMRV Workshop on September 7-8 regarding XMRV/MLV/HGRV detection in my practice done on 47 consecutive patients from October 2009 to December 2009. Those 47 patients are very well characterized including the percentage of overlap diagnoses (FM, MCS, Lyme, IBS, mold-illness), KPS, age, sex and geographic distribution. The data, I think, are extraordinary, but I cannot talk about it until after the 8th. – **Paul R. Cheney, MD, PhD, The Cheney Clinic**

A: All patients whose samples I sent for study at that time met the 1988 Holmes criteria for CFS. I have always felt that activity limitation to the imaginary 50% level was crucial to the diagnosis. – **David S. Bell, MD, FAAP, Lyndonville, N.Y.**

Q: In a Q&A with *PNAS* editor-in-chief Randy Schekman [published in \*The Scientist\*](#), he says, "It looks like [Alter's] done as good a job as he could without actually show[ing] that the sequences were contiguous with human DNA. That I trust will be what he does next." What kind of evidence is Schekman referring to?

A: He was referring to the third reviewer's request that the team demonstrate that DNA from a murine leukemia virus has integrated into the host's (human) DNA. This would provide stronger evidence for infection with a retrovirus. Different retroviruses target different integration sites, and evidence of the region of the host's genome targeted by the virus would also strengthen this work. As the authors have said, these are early studies and there is still much work to do. – **Susan L. Stramer, PhD, Executive Scientific Officer, Scientific Support Office, American Red Cross**

## FAQs ABOUT MLVs and XMRV

Q: How many MLVs have been identified?

A: There are many different murine leukemia viruses (abbreviated to either MuLV or MLV), existing as either infectious viruses or endogenous sequences integrated into the germlines of many wild and laboratory strains of mice. MLVs are divided into four subgroups based on cellular receptor usage and hence tropism for murine and other mammalian cells. The four types of murine viruses are: ecotropic (infect only rodent cells); xenotropic (non infectious for rodent cells but infectious for cells of other species); polytropic (infectious for rodent and non-rodent cells) and amphotropic (infectious for rodent and non-rodent cells but through a different cell receptor than the polytropic subgroup). As an added complexity, many of these viruses can and do recombine in dually infected hosts to give rise to hybrid viruses. Indeed, it appears that XMRV is a chimera (hybrid) between xenotropic and polytropic viruses. – **Steven H. Kleinman, BSc, MD, Clinical Professor, Pathology and Laboratory Medicine and Centre for Blood Research, University of British Columbia and Graham Simmons, PhD and Eric Delwart, PhD, Blood Systems Research Institute**

Q: Are the four variants described in the *PNAS* paper entirely new, or is this simply the first time their sequences have been identified in humans?

A: None of the (incomplete) viral sequences described in the *PNAS* paper are identical to any published mouse virus, however they are very closely related to endogenous forms of polytropic MLV. – **S.H. Kleinman, BSc, MD, G. Simmons, PhD and E. Delwart, PhD.**

Q: What does it mean that three of the MLVs identified in the *PNAS* paper are about 96% "homologous" to XMRV?

A: For comparison purposes, it is useful to know that the entire DNA content of the human and chimpanzee genomes differ by only about 1%, which is less genetic variation than the 4% observed between the MLVs and XMRV. It is unknown if this 4% variation in the MLVs and XMRV will lead to different effects in the infected host. – **S.H. Kleinman, BSc, MD, G. Simmons, PhD and E. Delwart, PhD.**

Q: As presented at the Cold Spring Harbor Laboratory (CSHL) conference on Retroviruses (May 2010) and reported in the *Wall Street Journal's* Health Blog, the Whittemore Peterson Institute (WPI) has found *gag* sequences that reflect greater variability amplified from the PBMCs from CFS patients than was originally reported for XMRV. Do the variants you have identified match the four MLV variants described in the *PNAS* paper, or do they represent even greater diversity within this family of gammaretroviruses?

A: The variants presented at CSHL were exactly the variants described in the Lo et al. publication. The

abstract was submitted in late February/ early March by our collaborator Dr. Kathryn S. Jones and she reported that the majority of patients reported by Lombardi et al. had both xenotropic and polytropic MLVs. This is consistent with the antibody and serology data presented in Lombardi et al. which suggested wider sequence variation as XMRV VP 62-specific PCR negative patients shown in Figure 1 [in the original *Science* paper] indeed harbored XMRV-related viruses that could be detected by the antibodies that recognized all known xenotropic, polytropic and ecotropic retroviruses.

It is unfortunate that no matter how much data we presented and published (Mikovits et al., *Virulence*, July 30, 2010) suggesting more sequence variation as a valid scientific explanation for negative studies both in CFS and prostate cancer, our data were met not with critical evaluation but with unsupported allegations of contamination.

We congratulate Dr. Alter and sincerely appreciate his statements defending our work and stating definitively that his work confirmed the data in Lombardi et al. and putting to rest any allegations that there was ever contamination in our work. We had done every possible control and never was there any evidence of contamination. Hopefully now that the association of a human gamma retrovirus family with CFS has been firmly established, the scientific community can work together to move the research forward to understand the role of these viruses in disease and develop treatment protocols as soon as possible. – **Judy A. Mikovits, PhD, Director of Research, Whittemore Peterson Institute**

Q: Some of the media reports have referred to murine leukemia viruses (MLVs) as “cancer-causing agents.” This description refers to some MLVs being cancer-causing in mice, but what does it mean for humans?

A: The MLV family of viruses has never been shown to cause cancer in humans. There are lots of animal viruses that cause tumors in other species. These viruses are also related to feline leukemia virus and porcine leukemia virus. Similarly, it is often possible to cure tumors in mice and rats, but the same agents are ineffective for human tumors, so it is both inaccurate and misleading to suggest that these viruses cause human tumors. – **Harvey G. Klein, MD, Chief, Department of Transfusion Medicine, NIH Clinical Center**

A: Human T-lymphotropic virus (HTLV) was the first human virus linked to cancer, followed by hepatitis B virus (HBV) and hepatocellular carcinoma. Mouse viruses while having the capacity to infect human cells, have not been associated with any human cancers. – **Susan L. Stramer, PhD, Executive Scientific Officer, Scientific Support Office, American Red Cross**

Q: Many of the news reports have been accompanied by pictures of field mice or laboratory mice, like this one from [CNN](#). Does this study (or any of the other papers) suggest or prove that XMRV and/or MLVs sequences in humans come through direct contact with mice or other rodents?

A: XMRV and viral sequences reported by Lo et al. are closely related to some murine leukemia

viruses making it highly likely that the viral ancestors emerged from mice prior to infecting humans. However, it is unknown how or when that occurred, or whether there are intermediate hosts between mice and humans. There is no suggestion that these viruses are spread by insects that bite mice, this route of transmission occurs with other some other viruses, like West Nile Virus for instance, but not with retroviruses such as XMRV. – **Robert H. Silverman, PhD, Mal and Lea Bank Chair and Professor, Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic**

## FAQs ABOUT TESTING FOR AND NAMING THESE AGENTS

Q: Will the assays being used in the [XMRV Scientific Research Working Group REDS-II Panel Study](#) be able to detect XMRV, the MLVs described by Lo et al., and a wider variety of MLVs that might be associated with CFS?

A: The assays employed by the labs participating in the REDS-II Panel Study employ PCR primers targeting multiple genetic regions of XMRV and MLV variants. The labs include the Lo lab at FDA and the Whittemore Peterson Institute lab that have reported high rates of detection of XMRV and MLV-related viruses in CFS patients, as well as labs that have reported absent or low rates of XMRV/MLV virus detection in clinical populations (Switzer (CDC), Simmons (Blood Systems Research Institute), and Hewlett (FDA) labs) and one lab that has not previously evaluated clinical samples for XMRV/MLV but which has extensive published experience with very sensitive assays for HIV (National Cancer Institute lab), and is using an assay which has been shown to detect and distinguish both types of viruses. – **Simone Glynn MD, MSc, MPH, Branch Chief, Transfusion Medicine and Cellular Therapeutics Branch, National Heart, Lung and Blood Institute and Jerry A. Holmberg, PhD, Senior Advisor for Blood Policy, Department of Health and Human Services, both responding on behalf of the DHHS XMRV Scientific Research Working Group**

Q: If geographic restrictions are a source of the discordant data on XMRV in CFS, will the [REDS-II Phase IV](#) study of donor prevalence using samples from the Reno/Tahoe region be sufficient to identify MLVs strains that might be more prevalent in other geographic locations (as suggested in the PNAS paper)?

A: The Phase IV study is currently restricted to donations from the Reno/Tahoe region so it cannot address the prevalence of XMRV/MLV variants in other blood donor populations from other geographic settings. Other studies are in the planning stages to both broaden the geographic representation of US donors and to investigate archived donor samples from the past four decades to establish temporal, geographic and other demographic correlates of infection/exposure to these viruses in donor populations. – **S. Glynn, MD, MSc, MPH and J.A. Holmberg, PhD responding on behalf of the DHHS XMRV Scientific Research Working Group**

Q: Will the XMRV Scientific Research Working Group [REDS-II study](#) be adequate on its own to determine whether XMRV and other MLVs pose a threat to the blood supply?

A: Not on its own, but it will certainly be easier to prove transmission transfusion than to prove disease causation. If indeed 4-8% of normal blood donors are infected with MLVs and the rate of random blood recipients is similar and if infection is a fairly frequent event after transfusion, then the available repository would be expected to hold enough positive units to estimate penetration of the blood supply by MLVs with adequate confidence levels.

A variety of studies and observations will be needed to prove disease causation, particularly if the disease has a long incubation period. At the moment, though, we do not know of any significant diseases that appear to be transfusion transmitted for which there is not an identified agent. Some forms of post-transfusion mortality and/or immune modulation still have not been adequately explained. The first of these may be associated with “older” blood components; infectious agents have not generally been suspected in either of these outcomes.

So, additional studies may be needed to prove transfusion transmission (depending on the extent to which it may occur) and certainly other studies are needed to prove disease causality. Frozen repositories, like the National Heart, Lung and Blood Institute (NHLBI) repository, may be helpful if assays can successfully recover MLVs by nucleic acid testing from frozen cells and plasma collected from suitable samples. – **Roger Y. Dodd, PhD, Vice President, Research & Development, American Red Cross; Louis M. Katz, MD, Executive Vice President, Medical Affairs, Mississippi Valley Regional Blood Center; and Susan L. Stramer, PhD, Executive Scientific Officer, Scientific Support Office, American Red Cross**

Q: There have been several different tests marketed for XMRV and now one is available that also tests for MLVs. Are any of these diagnostic tests approved by the FDA?

A: No; to our knowledge there are currently no FDA-approved tests for XMRV for diagnosis or other indications. – **Indira Hewlett, PhD, Chief, Laboratory of Molecular Virology, Division of Emerging and Transfusion Diseases, Office of Blood Research and Review, Center for Biologics Evaluation and Research, FDA**

Q: Why don't blood collection centers use the tests being marketed to physicians and patients for XMRV and other MLVs to test the blood supply?

A: In our lab at the Mississippi Valley Blood Centers we need a level of testing throughput to handle up to 1000 tests in a shift. That's not possible with the technology being used by labs offering clinical tests. Also, the tests currently on the market have not yet been validated for appropriate performance characteristics ([sensitivity, specificity, positive and negative predictive values](#)) for use by blood collection centers. Obviously, the question also assumes these murine

leukemia viruses both cause human disease and are transfusion transmitted pathogens, i.e., that we should be testing blood donors, but that is the more difficult part of this whole discussion. – **Louis M. Katz, MD, Executive Vice President, Medical Affairs, Mississippi Valley Regional Blood Center**

Q: Reports on the Aug. 25 and 26 [Wall Street Journal Health Blog](#) indicate that some scientists have shortened XMRV to “X” and polytropic MLVs to “P.” [Another group](#) has suggested renaming the assortment of viruses found in the *Science* and *PNAS* papers to “HGRV for Human Gamma Retro Virus” and the illnesses caused by these infections to “HGRAD for Human Gamma Retrovirus Associated Disease.” How do viruses get named?

A: There is an International Committee on the Taxonomy of Viruses (ICTV) of the Virology Division of the International Union of Microbiological Societies whose purposes are to develop an internationally agreed upon taxonomy and nomenclature for viruses; to maintain an index of virus names; and to communicate the proceedings of the committee to the international community of virologists. An update of this work is usually published at three-year intervals. However, the most recent published full report was in 2005 and contains [an authoritative database](#) (ICTVdB) containing taxonomic information for 1,950 virus species. The website database was last updated on 7/20/2010. Anyone can submit information to the Virology Subcommittee on taxonomy for consideration in the database and it doesn't stop informal use of any terms. However, until several items are resolved, it tends to confuse rather than educate, and clutters up the literature. – **F. Blaine Hollinger, MD, Professor of Medicine, Molecular Virology & Epidemiology and Director, Eugene B. Casey Hepatitis Research Center, Baylor College of Medicine.**

## FAQs GENERATED BY MEDIA COVERAGE OF THE LO/ALTER STUDY

Q: Some people have been surprised that the *PNAS* study's finding of 6.8% positives among healthy blood donors has not received more attention from the press or the public. Could this be due in part to the fact that there are 68 infectious agents (including XMRV) tracked by the AABB as being potentially problematic for transfusion safety?

A: The [August 2009 Supplement to Transfusion](#) described 68 agents that were known to be transfusion transmitted, or those with the potential to be transfusion transmitted, all without an effective intervention (e.g., donor and/or donation screening or methods of reduction/inactivation). These agents were selected for inclusion by a group of experts from the AABB Transfusion Transmitted Disease (TTD) Committee; this group also prioritized the risk of these agents to cause harm in transfusion recipients in the U.S. and Canada based on a specific set of criteria. The selection of agents and prioritization process may have yielded different results if examined by a different group of experts. The list of 68 agents was published prior to the findings of Lombardi et al., and thus XMRV or related mouse-derived retroviruses were not included (although an [XMRV fact sheet](#) has since been prepared by the TTD Committee). The agents that received the highest priority for further

consideration of an intervention were all known transfusion transmitted agents causing diseases that are difficult to treat or untreatable and have high rates of mortality and morbidity. These included dengue virus, the parasite *Babesia* and the prion variant Creutzfeldt–Jakob disease (vCJD). Agents such as XMRV/MLV would have been prioritized in a different category, versus the three highest priority agents, in that agents such as XMRV/MLV are of public concern, but the science as of yet does not establish their role in any significant transfusion transmitted disease. Sophisticated molecular techniques have identified many new viral agents for which, as of yet, have not yet been identified to cause any human disease even though some of these agents have high rates of prevalence in the donor population and have been shown to be transfusion-transmitted. While the prevalence of up to 6.8% for a known disease-causing agent would be alarming, further work is needed to establish the incidence/prevalence of XMRV/MLV in the donor population, to determine if in fact the agent(s) is transfusion-transmitted and the consequences in transfused recipients. This will involve the completion of multiple studies that are in various stages of planning and execution.

As a further note, as referenced in [Voisset, et al.](#) (*Microbiology and Molecular Biology Reviews*, March 2008) estimates from the genome sequencing project suggest that endogenous retroviruses (ERVs) now comprise some 8% of human DNA, representing around 4,000 proviruses and thousands more solitary long terminal repeats (LTRs). Therefore, XMRV/MLVs may not be the only group of agents that emerge as related to, or associated with disease syndromes that lack a known etiologic cause. Like XMRV/MLVs, any newly recognized agent(s) may, or may not have any causal relationship to any known disease state. – **Susan L. Stramer, PhD, Executive Scientific Officer, Scientific Support Office, American Red Cross** and **Roger Y. Dodd, PhD, Vice President, Research & Development, American Red Cross**

Q: Many of the headlines for media stories reporting on this study (and others) used the term “chronic fatigue,” even if the writer used “chronic fatigue syndrome” in the article, as was the case with the [article you wrote](#) for the *New York Times* about the PNAS study. Who writes headlines?

A: I understand the unhappiness with the use of "chronic fatigue" instead of the full name. In U.S. mainstream media, reporters generally don't write the headlines – the copy-desk does, often hours after the story has been edited. Newspapers tend not to like too many acronyms; since it gets repetitive to use "chronic fatigue syndrome" over and over again, there's a tendency to use "chronic fatigue" as the shorthand instead of CFS. Different publications have different style guides, and these should include the point that "chronic fatigue" is not an appropriate shorthand for "chronic fatigue syndrome," but these style guides change slowly. – **David Tuller, contributing writer to the *New York Times***

Q: How many reporters participated in the [Aug. 23, 2010 telebriefing](#) and how many times was the recording accessed during the week it was available to the public?

A: We had about 70 mainstream and CFS blog and advocacy publications participate, as well as private organizations, academic institutions, disease associations and research and investment

groups. We are unable to obtain information about how many times the line was accessed by the public and/or press while it was live. – **Kelli Carrington, MA, Office of Communications, Patient Recruitment, and Public Liaison, NIH Clinical Center**

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