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Dr. Bruce Alberts
Editor-in-Chief
Ms. Monica Bradford
Executive Editor Science
1200 New York Avenue, NW
Washington, DC 20005

Re: *Lombardi et al.*

Dear Dr. Alberts and Ms. Bradford:

As the corresponding author of the *Lombardi et al.* study I want to express my deepest concern about the proposed issuance of your editorial expression of concern regarding our XMRV findings and its association with chronic fatigue syndrome. This is especially so in light of the gross disregard for the integrity of the scientific process by the apparent willful breach of your embargo by one of the authors or their collaborators. This has resulted in the apparent public knowledge of the contents of your request that we retract our seminal paper. I would respectfully ask that you focus on the following key facts and reconsider your position. We share your deep concern over the number of negative non-replication studies in this new area of research. However, the publication of your editorial expression of concern over the validity of *Lombardi et al.* findings are premature and would have a disastrous impact on the future of this field of science. Please do not proceed down a path that could be detrimental to the scientific exploration of human retroviruses in infectious disease, cancer, and, therefore, the future health of millions around the world.

First, the title and substance of the *Lombardi et al.* study **Detection of an Infectious retrovirus, XMRV, in blood cells of patients with Chronic Fatigue Syndrome** is accurate, and not one reported study has been able to show why it is not. Using four different methods including PCR (of cultured and co-cultured cells), detection of human gammaretroviral (HGRV) viral proteins (culture and co-culture detected by Western Blot and flow cytometry), anti-gammaretrovirus Env antibodies in human serum (competed by 7C10 rat monoclonal antibody), and virus isolation from primary cell and co-cultures, we reported evidence of human gammaretrovirus infection in at least 67 out of 101 CFS patients. In addition, we reported that 3.7% of the control population had evidence of infection.

Second, this significant study was conducted over eight months and conducted in

five different laboratories. It resulted in the first isolation of a human gammaretrovirus from the blood of humans and concluded that this virus may be a contributing factor in the pathogenesis of CFS. Electron micrographs of gammaretroviruses isolated from patients cells were shown in the *Lombardi et al.* study and support these conclusions. These electron micrographs do not show VP62 plasmid contamination. In addition, we have maintained the viral isolates of five patients from which the electron micrographs were derived. Moreover, data presented in *Lombardi et al.* suggested additional strains of gammaretroviruses and viral Gag proteins could be directly immunoprecipitated from the blood of patients supporting a finding that additional strains of HGRV were isolated. In fact, subsequent work by Jones et al. presented at Cold Spring Harbors supports the presence of more than one strain of HGRV. The original manuscript submitted to Science discussed DG75, a human B cell line, from which a MLV-related virus was fully sequenced. This raises the possibility of many viruses originating from recombination events as human tissue has been passed through mice for more than five decades. PCR would not have detected a DG75 isolate but the rat monoclonal Env antibody used in these studies can detect DG75 isolates. This raises the question of how many gammaretroviruses are circulating in the human population with the potential of contributing to human disease.

Third, this study showed that human gammaretrovirus was transmissible, and a more recent study has confirmed these data in an animal model and has shown that there could be different routes of entry and difference in blood reservoirs between acute and chronic infection.

Fourth, this study conducted the following tests to insure that the reported data were not as a result of contamination, including the detection of a human antibody response to the virus, the screening of all reagents and cell lines for any evidence of gammaretrovirus contamination, human or otherwise. The antibodies used to detect viral proteins in *Lombardi et al.* were rat monoclonal and goat polyclonal antisera, all of which were negative for murine contamination; all reagents used in PCR and tissue culture were lot tested for contamination. In addition, it was clearly stated in the *Supplemental Methods* which taq enzyme manufactures were shown to be contamination free. None of the negative papers, which demonstrated contamination, used the enzymes or antibody reagents used and recommended by *Lombardi et al.*

All samples and controls were processed in the exact same way and placed in a clean lab free from any other cell line. Only five human cell lines were grown in the WPI laboratories during the time these studies were conducted: Raji, SupT1, HFF, LNCaP and HSB2 and all were shown at the initiation of and throughout these studies to be free of XMRV/VP62 and all were used as negative control tested weekly by every method (including pelleting of supernatant over glycerol for virus isolation).

No murine cell line was grown in the WPI labs prior to the submission of the *Lombardi et al.* manuscript. The murine BAF cell line was cultured and used after the July 22, 2009 NCI closed meeting on XMRV during which all of the data of *Lombardi et al.* was shown not only to one of the reviewers of the original manuscript but also to

John Coffin who wrote the accompanying commentary. We were requested at that time to run the mouse mitochondrial assay to show absence of mouse contamination. We conducted this assay on samples from all 101 patients in *Lombardi et al.* and published these data in the subsequent Virulence addenda, a copy of which is attached hereto. While we have been advised by you that *Paprotka et al.* suggest a recombinant origin of an XMRV, it says nothing about the human gammaretroviruses detected and isolated from patient samples in *Lombardi et al.* They cannot have any data to support the conclusion "that laboratory contamination with XMRV produced by a cell line (22Rv1) derived from these early xenograft experiments is the most likely explanation for detection of the virus in patient samples." In fact, the authors of this paper know full well that this explanation cannot explain XMRV integration in human tissue, *in situ* hybridization, or antibodies reported in prostate cancer or CFS patients. Furthermore, all strains of wild rodents have not been examined and other examples of ancestral XMRV can be found. Neither 22Rv1 nor any of the cell lines reported to be contaminated with XMRV or cell lines growing the VP62 infectious molecularly cloned virus was in the laboratories where the patient cells were isolated. This can also not in any way explain the Env antibodies demonstrated in patient plasma in *Lombardi et al.* The reactivity demonstrated to Spleen Focus Forming Virus (SFFV) Env was competed by the rat monoclonal antibody which detects all known xenotropic, polytropic and ecotropic MLVs. This again suggests that we have, in fact, detected more than one strain of human gamma retroviruses in these patient samples. Clearly data presented in *Lombardi et al.* where samples were PCR negative but Western blot positive, using the 7C10 antibody, further support the notion of a family of gammaretroviruses. These data must be appreciated as a complete body of evidence and not in the context of individual pieces, such as PCR amplification using primers designed to an arbitrary reference strain.

All of these data led Harvey Alter, in the NIH State of the Knowledge Workshop (April 2011), to draw the conclusion that there existed no evidence of contamination in 'either the Mikovits or Lo labs'.

The authors are aware of ten negative CFS papers listed in PubMed on the subject of XMRV. Most of the negative studies failed to find any evidence of XMRV in any sample type. This would suggest that the methods and materials used in the non-replication studies are insufficient to use when attempting to detect human gammaretrovirus in the blood of human samples. The methods, processes, and materials of *Lombardi et al.* need to be followed precisely. The Alter and Lo study is the only study which has attempted a partial replication of the methods and materials of the Lombardi study, which confirmed evidence of MLV related viruses. Studies using multiple different methods are not replication studies, and studies optimized to detect murine gammaretroviruses and not human gammaretroviruses must be seriously questioned. See, Virulence attachment.

Scientific research of human gammaretroviruses is in its infancy. Studies of XMRV and macaques are beginning to reveal information concerning the life cycle of the virus, multiple tissue reservoirs, and a description of factors that induce viral activation. These studies are critical to understanding gammaretroviruses in human

disease. Other human studies such as the one by *Fischer et al.*, reported the detection of XMRV in the respiratory tract of immunocompromised individuals pointing to the potential for gammaretroviruses to be more easily transmitted than all other known retroviruses. WPI researchers are contributing to the development of more accurate clinical testing methods with others in the blood working group. Without the participation of Drs. Alter, Lo and the WPI, who have proven gamma retroviral detection methods, it may be impossible to discover whether or not gammaretroviruses are a threat to human transfusion and transplantation medicine.

In summary, human retroviruses are not known to infect individuals according to their sex or age therefore there can be no excuse as to why it would be acceptable to study the viruses in cancer but not in those with infectious neuro-immune diseases. They create lifelong infection in their hosts by integrating into the genome of their victims. Thirty years of murine gammaretroviral research provide compelling evidence that these viruses cause immune deficiencies, neurological disease and cancer in mammals and are therefore possible contributors to human neuro-immune diseases such as CFS. However, good scientific work is difficult and takes time. These ongoing studies deserve to receive a fair and impartial evaluation in the peer-review process. The critical question which remains is not simply whether gammaretroviruses play a role in CFS or cancer but in how many other human diseases? Therefore, we feel this is an extremely premature action which is not in the best interest of the scientific community or human health and again we respectfully request that you allow the scientific process to run its course unhindered by bias.

Thank you for your thoughtful consideration of this matter.

Sincerely yours,



Dr. Judy A Mikovits
Director of Research
Whittemore Peterson Institute