WHITTEMORE PETERSON
INSTITUTE FOR NEURO-IMMUNE DISEASE

May 27, 2011

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 President and Founding Director of the Whittemore Peterson Institute for Neuro-Immune Disease (for purposes of this letter, the affiliates and the institute are referenced as the "WPI"), I was provided a copy of the notice sent by you yesterday to Dr. Judy Mikovits.

I know that the issuance of an Editorial Expression of Concern ("EEC") is a serious matter and was not a step that was taken lightly by you or your organization. I hope that this letter will encourage you to withdraw the issuance of the EEC based on additional information. In addition, I hope that you realize that the publication of such a notice could have major scientific and potentially financial impact on the future viability of the WPI and its mission/research.

Your conclusions as to why you are issuing this EEC are based on "at least 10 studies conducted by other investigators and published elsewhere [that] have reported a failure to detect XMRV in independent populations of CFS patients." Not one of the 10 referenced studies challenging the results of our research can ethically claim to be a replication study using the methods, processes, or materials of Lombardi *et al.* The only claim that any of the previously published studies are even partial replications of the Lombardi study is limited to the Lo, Alter study. This study confirmed the findings of a retroviral association to those with CFS, as did the Lombardi study. The negative studies are PCR centric and have many flaws. For your use, I have attached a very simple exhibit reviewing the methods used in several studies as a tool to compare these studies to the Lombardi study. I want to assure you that there is no data to suggest that our research results are invalid.

We have not had the chance to review the embargoed Paprotka *et al.* report, but the theory of the origin of XMRV from the 22RV1 cell line is not relevant to the Lombardi study. In addition any claim of contamination in the Lombardi study would have to convincingly explain other relevant data such as:

1. How only 3.7% of the healthy controls were 'contaminated' in Lombardi, or
2. How the positive CFS patient samples in Lombardi were contaminated with the 22Rrv1 cell line when the 22Rv1 cell line has never been present in the WPI labs, and, in fact, the Lombardi paper was submitted to *Science* five months before the 22Rv1 cell line was published to express XMRV, or
3. How patients have anti-bodies to multiple XMRV (human gamma retrovirus) proteins.

The WPI researchers did not use laboratory reagents that "were widely contaminated with the virus" and definitively ruled out contamination. To be an appropriate basis for asking the co-authors to voluntarily retract their paper, one would have to have shown that all of the co-authors' work was a result of contamination, which has not been proven, even though others have discovered contamination in their own work.

As to the second report referenced by you, authored by K. Knox *et al.*, I am obligated to report that the first author, Dr. Konstance Knox, is not unbiased, impartial, or independent with respect to the WPI. She and her laboratory, Wisconsin Viral Research Group, were former contractors and left under difficult circumstances. Her conduct was the subject of an internal review of serious matters related to ethical violations; misuse of proprietary information; improper use, retention and destruction of patient samples and data; and disagreements over the ownership of intellectual property.

In addition, the substance of the reported results of Knox *et al.* may have the potential of being inaccurate because these authors should have no way of knowing patient identities or specific XMRV results from the Lombardi study. Dr. Mikovits was responsible for maintaining the human assurance portions of the Lombardi study and has not shared the identity of the patients or their results with Dr. Peterson in spite of his repeated attempts to obtain this private information. On the other hand, if the authors do not claim such an association with the Lombardi study, then the study is simply another negative, non-replication study. Dr. Knox and her co-authors have never requested nor had independent access to the materials necessary to do a replication study.

In light of these facts, we respectfully request that *Science* 1) hold off on the publication of the Knox study and the EEC, and 2) afford us the opportunity to provide you with additional data supporting our conclusions.

Sincerely yours,

Annette Whittemore
President

A Comparison of Methods for the Detection and Association of XMRV in Chronic Fatigue Syndrome					
	Lombardi, Mikovits et al.	Lo, Alter et al.	Satterfield, Switzer et al.	Shin, Singh et al.	Erlwein, McClure et al.
Patient and Control Selection					
Met CDC/CCC Criteria	Yes/Yes	Yes/NR	Yes/NR	Yes/Yes	Yes/NR
Exclusion of Contact Controls	Yes	Yes	NR	No	NP
Physician diagnosed CFS Confirmation	Yes	Yes	26 of 31[1]	Yes	Yes
Self-selection into study	No	No	Yes	No	No
Number of Patients/Controls	101/218	37/44	45/42	100/200	186/0
Patients display NK cell dysfunction	Yes	NR	NR	NR	NR
Patients display elevated inflammatory cytokines	Yes	NR	NR	NR	NR
Sample Processing					
Isolation of PBMC not PBL	Yes	Yes	No	No	No
Evaluated multiple time points	Yes	Yes	No	No	No
Use of lab where mouse work was conducted	No	No	NR	NR	NR
Use of ACK lysis buffer	No	No	NR	Yes	NR
Isolation of Virus	Yes	NP	NP	NP	NP

	Lombardi, Mikovits et al.	Lo, Alter et al.	Satterfield, Switzer et al.	Shin, Singh et al.	Erlwein, McClure et al.
Culturing of Samples					
Culture of Primary Cells	Yes	NP	NP	NP	NP
Sodium Heparin Blood Tubes used for Culture	Yes	NP	NP	No	NP
Isolation of Virus	Yes	NP	NP	NP	NP
Serology as described in Lombardi et al. BAF cell. Competed with mAb.	Yes	NP	No	No	NP
Western Blot of Cultured Primary Cells	Yes	NP	NP	No	NP
Western Blot of Co-cultured Cells	Yes	NP	NP	Yes[2]	NP
PCR on co-cultured cells	Yes	NP	NP	No	NP
PCR Detection					
Gag primers as described in Lombardi et al. Single round.	Yes	Yes	No[3]	NP	No
Env primers as described in Lombardi et al. Single round.	Yes	No[4]	NP	NP	NP
Nested PCR for *gag* using primers as described in Lombardi et al.	Yes	Yes	No	No	No
Amplification methods as described in Lombardi et al.	Yes	Yes[5]	No	No	No
Positive Control used	Split samples	Spiked sample	VP62 plasmid	XMRV Plasmid	VP62 plasmid

	Lombardi, Mikovits et al.	Lo, Alter et al.	Satterfield, Switzer et al.	Shin, Singh et al.	Erlwein, McClure et al.
Contamination Control[6]	Yes	Yes	Yes	No	No
PCR of isolated Lymphocytes	Yes	Yes	Yes	Yes	No
Use of Taq that was reported to be contamination free (USB)	Yes	No	No	No	No
NP = Not performed NR = Not Reported					

Note 1. Subjects self reported that they were diagnosed by a physician. No physician report was reported.

Note 2. Co-cultures must be conducted without the use of EDTA, which can potentially inhibit virus/receptor binding and replication.

Note 3. For nested PCR of gag, Satterfield used the single round primers described in Lombardi et al. for the first round of their nested PCR but used different second round primers. They did not use the same first or second round nested primers described in Lombardi et al.

Note 4. Lo et al. performed semi-nested PCR using the Lombardi et al. env primer as their first round.

Note 5. Lo et al., are the same as Lombardi et al. with the exception of a 1 degree difference on annealing temperature.

Note 6. Contamination control for Lombardi et al. were sequencing, phylogenetic analysis, PCR assay for mouse mitochondrial DNA, and reagent screening for MLV contamination. Lo et al. were same as Lombardi et al., but added PCR assay for mouse mitochondrial DNA. Shin et al. use IAP PCR for mouse sequences. Also of importance is that the laboratory of Lombardi et al. has never used the cell line 22Rv1.

Nested primers described in Satterfield et al.
GagOF ATCAGTTAACCTACCCGAGTCGGAC
GagOR GCCGCCTCTTCTTCATTGTTCTC
GagIF GGGGACGAGAGACAGAGACA
GagOR CAGAGGAGGAAGGTTGTGCT

Single round PCR primers described by Lombardi et al.
For gag
419F (5'- ATCAGTTAACCTACCCGAGTCGGAC-3')
1154R (5'- GCCGCCTCTTCTTCATTGTTCTC-3')
For *env*,
5922F (5'- GCTAATGCTACCTCCCTCCTGG-3')
6273R (5'-GGAGCCCACTGAGGAATCAAAACAGG-3')

Nested PCR primers for env (Urisman et al.)
GAG-O-F primer (5'-CGCGTCTGATTTGTTTTGTT)
GAG-O-R primer (5'-CCGCCTCTTCTTCATTGTTC)
GAG-I-F primer (5'-TCTCGAGATCATGGGACAGA)
GAG-I-R primer (5'-AGAGGGTAAGGGCAGGGTAA)

Nested Primers used by Lo et al.
419F (5′-ATCAGTTAACCTACCCGAGTCGGAC-3′)
1154R (5′-GCCGCCTCTTCTTCATTGTTCTC-3′)
GAG-I-F (5′-TCTCGAGATCATGGGACAGA-3′)
GAG-I-R primer (5′- AGAGGGTAAGGGCAGGGTAA-3′)
NP116 (5′-CATGGGACAGACCGTAACTACC-3′)
NP117 (5′-GCAGATCGGGACGGAGGTTG-3′)

PCR conditions as described by Urisman et al. (Lombardi et al.)
First Round, 2 min 94 °C; [30 sec 94 °C, 30 sec 58 °C, 45 sec 72 °C] x 30 cycles; 7 min 72 °C.
Second Round, 2 min 94 °C; [30 sec 94 °C, 30 sec 60 °C, 30 sec 72 °C] x 30 cycles; 7 min 72 °C.

PCR conditions described in Lo et al.
First Round, 4 min at 94 °C (1min at 94 °C, 1 min at 57 °C, 1 min at 72 °C) × 40 cycles and 10min at 72 °C.
Second Round, 4 min at 94 °C (1 min at 94 °C, 1 min 57 °C, 1 min 72 °C) × 45 cycles and 10 min 72 °C.

PCR conditions as described in Satterfield et al.
First and Second Round, 40 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 45 s for both primary and nested DNA PCR

PCR conditions as described in Erlwein et al.

Reaction conditions were one cycle of 94°C, 8 minutes, 35 cycles of 94°C 30 seconds, 55°C 30 seconds, 72°C 30 seconds and one cycle of 72°C, 7 minutes.