



Nijmegen Institute for
Infection, Inflammation
& Immunity

CFS & XMRV

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CFS & XMRV

- CFS until October 2009
- XMRV in CFS initial findings
- XMRV in prostate cancer
- XMRV in CFS?
- MLV in CFS
- The sad end...



Chronic fatigue syndrome

- Chronic incapacitating fatigue
- Longer than 6 months
- Physically unexplained
- Accompanying Symptoms
 - myalgia/ arthralgia/ concentration disturbances/ anomia/ memory problems / sleep disorders



A case of the CFS is defined by

- 1] clinically evaluated unexplained/relapsing chronic fatigue that is new or definite onset, is not the result of ongoing exertion, is not substantially alleviated by rest and results in substantial reduction in previous levels of occupational, educational, social or personal activities
- 2] the occurrence of 4 of the following symptoms, all of which must have persisted or recurred during ≥ 6 consecutive months and must not have predated the fatigue:



CDC criteria (Fukuda et al 1994)

- self-reported impairment in short-term memory or concentration severe enough to cause substantial reduction in previous levels of occupational, educational, social, or personal activities;
- sore throat;
- tender cervical or axillary lymph nodes;
- muscle pain, multi-joint pain without joint swelling or redness;
- headaches of a new type, pattern, or severity;
- unrefreshing sleep;
- postexertional malaise lasting more than 24 hours.



Reasons for a negative medical view on CFS:

- information from outdated psychiatry books
- 'vague complaints'
- difficulty to diagnose and treat
- irritation about selfdiagnosis "ME"
- strong physical attributions
- militant patient organisations



CFS, a microbial pathogenesis?

- 1940 chronic brucellosis
- 1970 chronic toxoplasmosis
- 1987 chronic EBV infectie
- 1988 chronic enterovirus infection
- 2000 Mycoplasma fermentans

None of these etiologic agents has been proven to play a role in the chronicity



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Chronic fatigue syndrome

Many studies searching for etiology and pathogenesis of CFS are suffering from poor methodology (especially regarding controls)

ESCMID Online Lecture Library



Chronic fatigue syndrome

Good controls:

- Adequate matching
- Blinding
- Careful pre-analytic handling
- Control for actual stress-effects, external factors → Simultaneous neighbourhood controls (Nijmegen) / twins (Seattle)



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Chronic fatigue syndrome

Distinguish

- Predisposing factors
- Precipitating factors
- Perpetuating factors

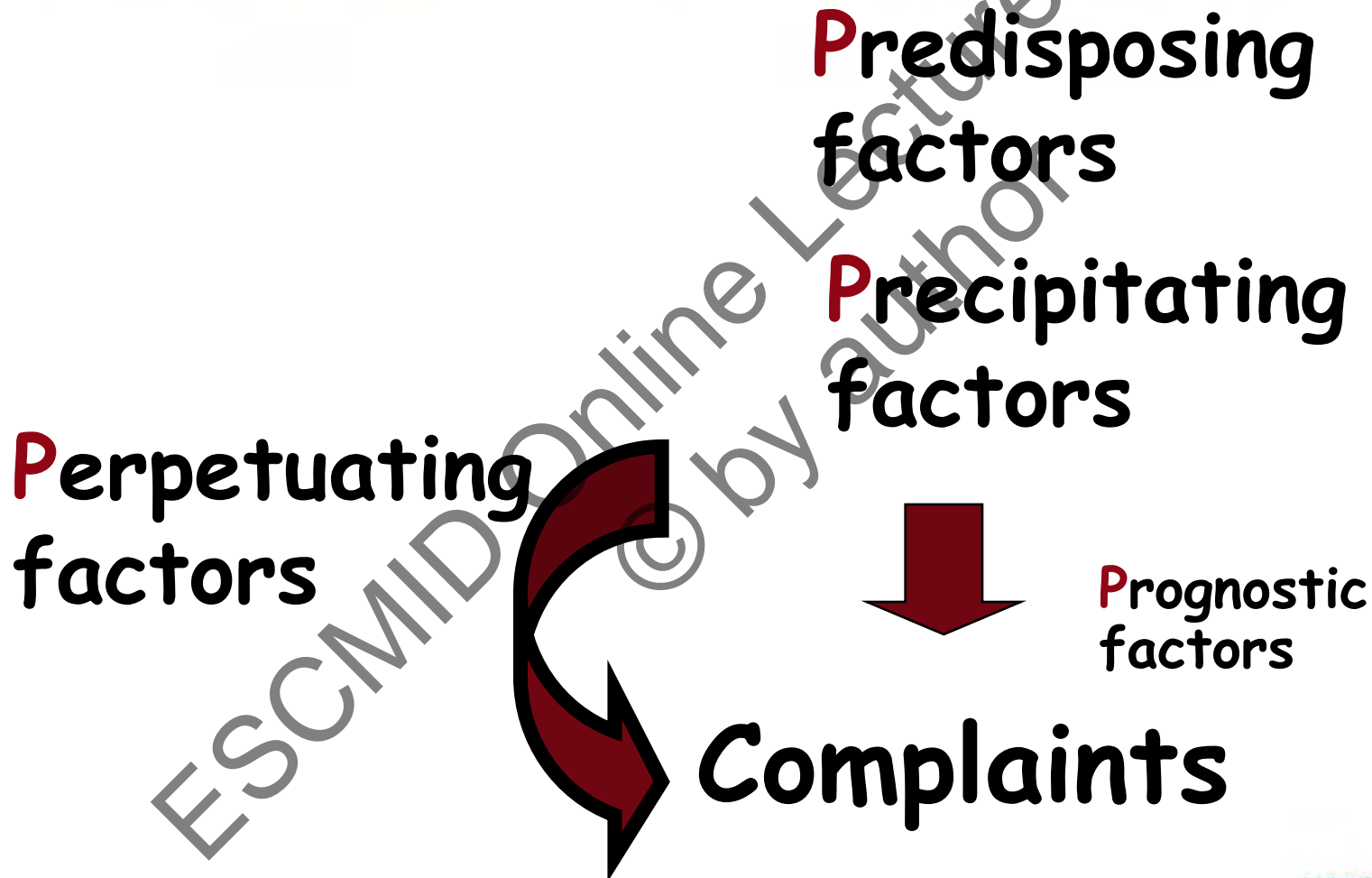
- Prognostic factors

Van der Meer &
Bleijenberg in Cohen &
Powderly 2010

Bleijenberg & van der Meer
in Harrison's Text Book
2011



CFS pathogenesis





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Chronisch vermoeidheids syndroom

? *Precipitating factors:*

- Infection (not necessarily persistent)
- Intoxication
- Operation
- Anaesthesia
- Delivery
- Traumatic events



CFS, perpetuating factors

- Immunologic factors?
- Hormonal factors?
- Metabolic factors?
- Factors in nervous system?
- Psychological factors



Model CFS

**Sense of
Control**

Fatigue

**Somatic
attributions**

**Physical
activity**

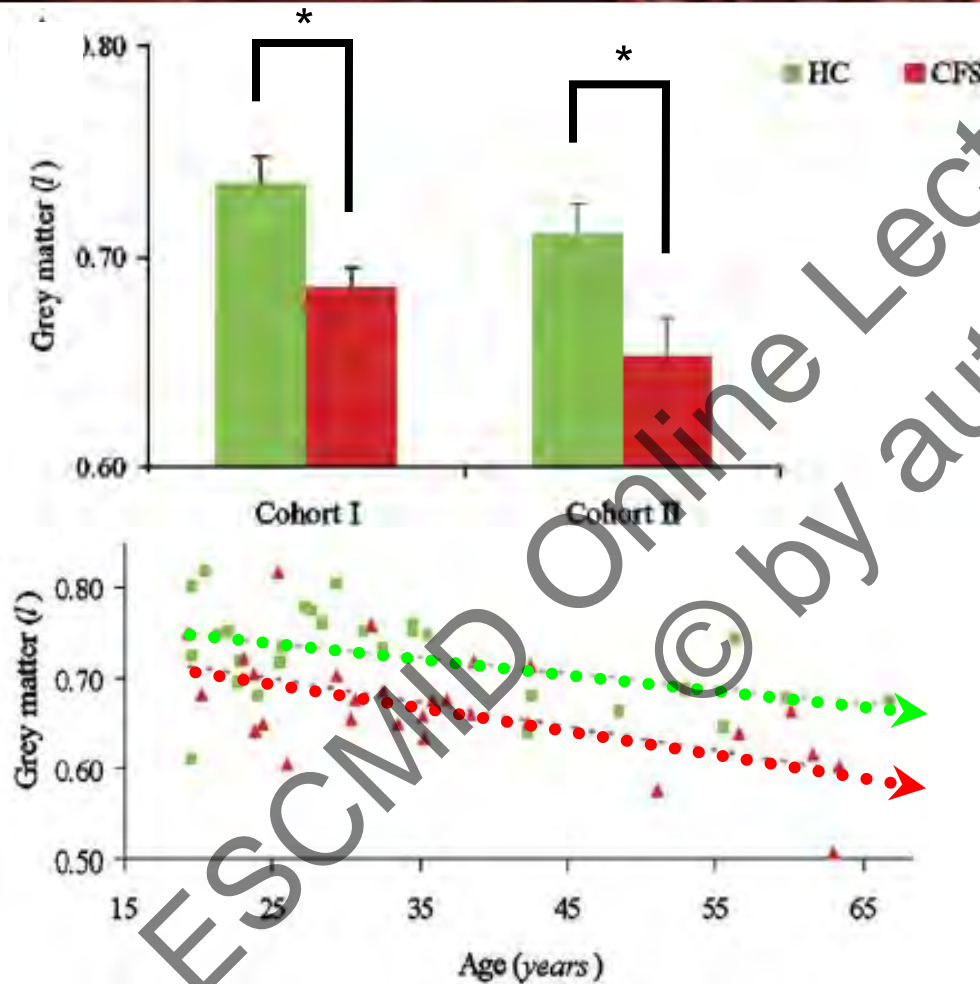
Impairment

**Focus
on symptoms**

Vercoulen et al, 1998



Brain structure



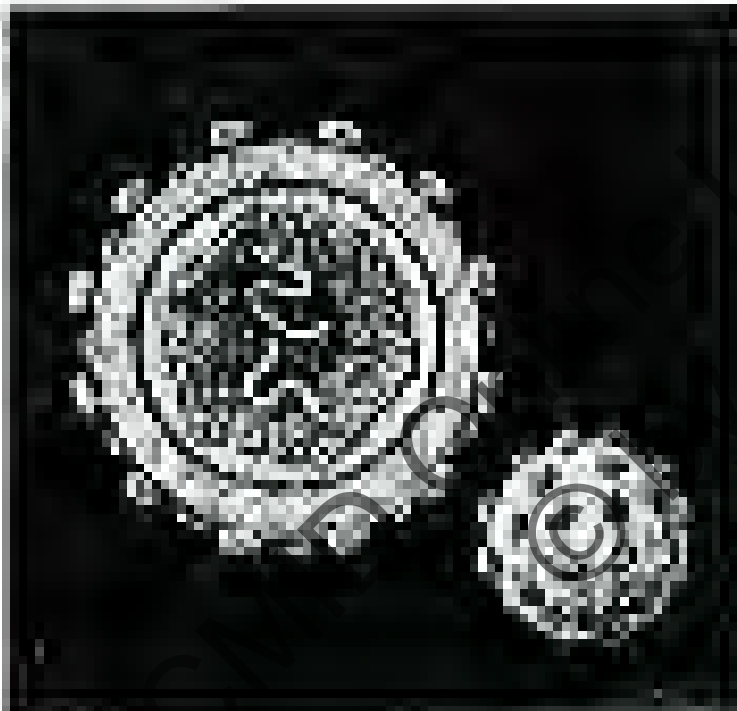
Grey matter

De Lange et al
Neuroimage 2005



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XMRV



ESC



Detection of an Infectious Retrovirus, XMRV, in Blood Cells of Patients with Chronic Fatigue Syndrome

Vincent C. Lombardi,^{1*} Francis W. Ruscetti,^{2*} Jaydip Das Gupta,³ Max A. Plost,¹
Kathryn S. Hagen,³ Daniel L. Peterson,¹ Sandra K. Ruscetti,⁴ Rachel K. Bagni,⁵
Cari Petrow-Sadowski,⁶ Bert Gold,² Michael Dean,² Robert H. Silverman,³ Judy A. Mikovits^{2†}

Chronic fatigue syndrome (CFS) is a debilitating disease of unknown etiology that is estimated to affect 17 million people worldwide. Studying peripheral blood mononuclear cells (PBMCs) from CFS patients, we identified DNA from a human gammaretrovirus, xenotropic murine leukemia virus–related virus (XMRV), in 68 of 101 patients (67%) as compared to 8 of 218 (3.7%) healthy controls. Cell culture experiments revealed that patient-derived XMRV is infectious and that both cell-associated and cell-free transmission of the virus are possible. Secondary viral infections were established in uninfected primary lymphocytes and indicator cell lines after their exposure to activated PBMCs, B cells, T cells, or plasma derived from CFS patients. These findings raise the possibility that XMRV may be a contributing factor in the pathogenesis of CFS.



CFS & XMRV

Doubts relation between XMRV & CFS

(Oct 2009):

- Unclear clinical description of the cohort
 - No 2nd cohort
 - Limited investigations in 2nd lab
 - Controversial virus in prostate cancer
- >100 letters to Science (4 published not until May 2010)

Failure to Detect the Novel Retrovirus XMRV in Chronic Fatigue Syndrome

Otto Erlwein¹, Steve K...¹

Groom et al. *Retrovirology* 2010, 7:10
<http://www.retrovirology.com/content/7/1/10>



RETROVIROLOGY

er², Simon

Campus, Norfolk
rk, Denmark Hill
m

RESEARCH

Open Access

Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome

Harriet CT Groom
John W Gow³, F

BMJ

RESEARCH

Prevalence of xenotropic murine leukaemia virus-related virus in patients with chronic fatigue syndrome in the Netherlands: retrospective analysis of samples from an established cohort

Frank J M van Kuppeveld, associate professor experimental virology,^{1,5,6} Arjan S de Jong, microbiologist,^{1,5} Kjerstin H Lanke, research technician,^{1,5,6} Gerald W Verhaegh, senior research technician,^{1,5,6} Willem J G Melchers, molecular medical microbiologist,^{1,5,6} Caroline M A Swarink, medical microbiologist,^{1,5,6} Mihai G Netea,^{4,5,7} professor experimental internal medicine,^{1,5} Jos W M van der Meer, professor internal medicine,^{1,5} professor clinical virology,^{1,5}



A cautionary tale of virus and disease

Robin A Weiss*

Abstract

The recent identification of the gammaretrovirus XMRV and a second gammaretrovirus of a different subtype in chronic fatigue syndrome has aroused much interest, not least among sufferers. However, it remains highly controversial whether the detection of these viruses represents true infection or laboratory artifacts.

A year ago, Lombardi *et al.* [1] reported in *Science* the detection of a retrovirus in 67% of persons suffering from chronic fatigue syndrome (CFS) compared with a 3.7% infection rate in healthy controls. The virus is known as xenotropic murine related retrovirus (XMRV) because its sequence is closely related to, but distinct from, those of well known strains of xenotropic murine leukemia viruses (XMLVs). In CFS blood samples, XMRV was readily detected by PCR amplification of the viral genome,

(first developed against HIV) in the hope of clearing infection and their symptoms. Blood banks would have to consider whether to screen donations for the implicated retroviruses. But before such steps could be justified, it will be essential to perform truly blinded tests on cases and proper controls in several laboratories. Profoundly disappointing as this would be for patients, without such additional studies, laboratory artifacts cannot be ruled out; also, with the signal exceptions of HIV and human T-lymphotrophic virus, the history of retroviral associations with human disease is not encouraging.

Mouse gammaretroviruses and human disease

XMRV is a gammaretrovirus closely related but not identical to other XMLV strains. These viruses have the curious property that they can infect foreign cells, such as human cells, in culture, but do not re-infect murine cells [7,8]. The term xenotropic was coined by Jay Levy in



Next:

- CDC: CFS cohort: No XMRV! Retrovirology. 2010;7:57.
- Tufts: American CFS cohort: no XMRV
- Seattle: Twins: no XMRV
- China: Virol. J. 2010; 7, 224. no XMRV
- Japan: Retrovirology 2011; 8,20 : no XMRV
- Schutzer Ann Neurol 2011: 69: 735: no XMRV

And more to follow!



In the meantime...

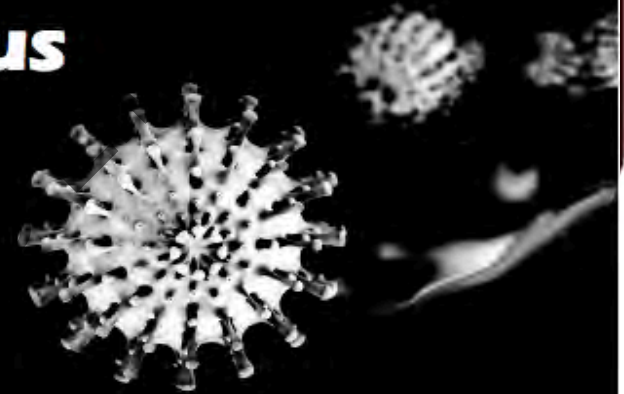
- Doctors in the US and Europe started prescribing anti-retroviral drugs to CFS patients!
- Proponents were very proactive on the internet and in the media
- Those who were not able to confirm the XMRV data were depicted as bad/poor scientists



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New HIV-like Virus in the Blood Supply

Up to 20 Million Could Be Infected



FDA and NIH research recently uncovered a new family of retroviruses in 7% of healthy blood donor samples.* This could mean that 20 million Americans are already infected. These viruses were also detected in an astonishing 87% of Chronic Fatigue Syndrome patient samples.

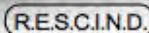
Similar to HIV, this infection is likely to be transmitted through blood.**

Chronic Fatigue Syndrome, also known as Myalgic Encephalomyelitis or ME/CFS, is a serious and sometimes fatal neuroimmune disease that can be as disabling as chemotherapy or late-stage AIDS. ME/CFS afflicts more than 1 million Americans.

Will you or your child be next?

**Stop the Suffering
We Need More ME/CFS Research Now**

ESCO



* Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. Proc. Natl. Acad. Sci., 2010
** "...it would be foolish to think it is not transmitted by blood." Dr. Jerry Holmberg, Department of Health and Human Services, Senior Advisor for Blood Policy, May 2010



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And then...

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^{a,1}

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Contributed by Harvey J. Alter, May 25, 2010 (sent for review March 23, 2010)

Chronic fatigue syndrome (CFS) is a serious systemic illness of unknown cause. A recent study identified DNA from a xenotropic murine leukemia virus-related virus (XMRV) in peripheral blood

infections (11). Whole-blood, peripheral blood mononuclear cell (PBMC), and plasma samples from 37 CFS patients in the mycoplasma studies were maintained in frozen storage at -80°C .

67% in CFS versus 6.8% in controls



More questions than answers!

- Not XMRV but polytropic mouseviruses en γ -retrovirussen (no cross detection!)
- No complete virus (envelope) shown
- Quantitative differences
- Contamination? (mice are everywhere \rightarrow reagents!
1 mouse cell contains >100 provirus)
J.Coffin: 1 drop of mouse blood in my swimming pool \sim
1 provirus of MLV/ml
- Controls (Md) & cases (Mass) differently handled
- No blinding!



- Knox et al. No evidence of murinelike gammaretroviruses in CFS patients previously identified as XMRVinfected
- Paprotka et al. Recombinant origin of the retrovirus XMRV.
- Alberts B. Editorial expression of concern.

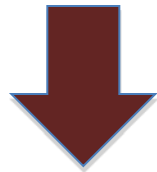
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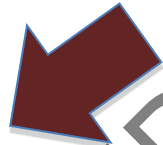
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Unique recombinant virus

CWR22 (progenitor prostate tumour cell
line)



passage through mice



CWR222Rv1



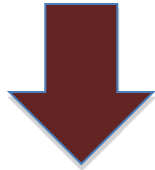
CWR-R1



Unique recombinant virus

CWR22 (progenitor prostate tumour cell
line)

XMRV negative



passage through mice

~1995



preXMRV-1 preXMRV-2

CWR222Rv1

XMRV

CWR-R1

XMRV



REPORTS

Failure to Confirm XMRV/MLVs in the Blood of Patients with Chronic Fatigue Syndrome: A Multi-Laboratory Study

Graham Simmons,¹ Simone A. Glynn,² Anthony L. Komaroff,³ Judy A. Mikovits,⁴ Leslie H. Tobler,¹ John Hackett Jr.,⁵ Ning Tang,⁵ William M. Switzer,⁶ Walid Heneine,⁶ Indira K. Hewlett,⁷ Jiangqin Zhao,⁷ Shyh-Ching Lo,⁸ Harvey J. Alter,⁹ Jeffrey M. Linnen,¹⁰ Kui Gao,¹⁰ John M. Coffin,¹¹ Mary F. Kearney,¹² Francis W. Ruscetti,¹² Max A. Pfost,⁴ James Bethel,¹³ Steven Kleinman,¹⁴ Jerry A. Holmberg,¹⁵ Michael P. Busch,^{1*} for the Blood XMRV Scientific Research Working Group (SRWG)†

Murine leukemia viruses (MLVs), including xenotropic-MLV-related virus (XMRV), have been controversially linked to chronic fatigue syndrome (CFS). To explore this issue in greater depth, we compiled coded replicate samples of blood from 15 subjects previously reported to be XMRV/MLV-positive (14 with CFS) and from 15 healthy donors previously determined to be negative for the viruses. These samples were distributed in a blinded fashion to nine laboratories, which performed assays designed to detect XMRV/MLV nucleic acid, virus replication, and antibody. Only two laboratories reported evidence of XMRV/MLVs; however, replicate sample results showed disagreement, and reactivity was similar among CFS subjects and negative controls. These results

We report a multi-laboratory study in which we analyzed the same blood samples from these blood donors and patients (2) or P-MLV who previously reported to be aliquoted into 96-well coded panels. We performed 10 different assays, including 10 different assays (table 1) to detect XMRV/MLVs (16). We previously found that CFS participants from 10 different laboratories using different amplification methods (e.g., culture assay, nested PCR, and Western blot) reported

NEWS

Researcher who linked chronic fatigue syndrome to mouse virus is arrested

Nigel Hawkes

London

Judy Mikovits, the researcher who led the team that linked a mouse virus with chronic fatigue syndrome, has been arrested at her home in Ventura, California.

The arrest follows a lawsuit filed against her by her former employer, the Whittemore-Peterson Institute for Neuro-Immune Disease in Reno, Nevada, which alleges that after she had been fired by the institute in September she had removed laboratory

has chronic fatigue syndrome. Dr Mikovits was sacked from her post as research director after refusing to hand over cell lines to another scientist at the institute. She argued that the cell lines were for use in a project funded by the US National Institutes of Health and that it would be wrong to use them for another purpose without her knowledge and consent.

The institute then took legal action against her, alleging "wilful,



XMRV

Not detectable in

- in CFS
- in prostate
- in HIV infected patients
- in immunocompromised patients
- in lymphoma
- in live human vaccines
- in transfusion blood
- in animal workers exposed to mice

Contamination of cell lines and commercially available reagents (e.g., RT PCR kits)



D-11-04069

PII requested

11CMT4069vanKuppeveldXMRVvic NL
Online First June 21, passing pages June 17

XMRV and CFS—the sad end of a story

Scientific papers on chronic fatigue syndrome (CFS) often evoke much debate and emotional reactions, as exemplified by the recent discussions in *The Lancet* on the PACE trial.¹ Also, the potential role of a retrovirus in CFS kindled a fierce controversy which has recently culminated. In 2009, in *Science*, Lombardi and colleagues² described the occurrence of the xenotropic murine leukaemia virus (MLV)-related virus (XMRV), a gammaretrovirus, in white blood cells in 67% of patients with CFS and in 3.7% of healthy controls. This finding was remarkable because infection of human beings with

prostate-carcinoma cell-line 22Rv1.³ Together, these studies raised huge scepticism about the role of XMRV or MLV-like viruses in CFS and reminded of previous examples of alleged human retroviruses implicated in cancer and other chronic diseases that were later shown to be laboratory contaminants (so-called tumour viruses).⁴

Now, three new publications seem to provide the final nail in the coffin of the XMRV-CFS story.⁵⁻⁷ Two of these studies—one of which was published in *Science*—fail to detect XMRV or related MLVs, either as infectious virus or viral sequences, as well as antibodies against these



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