Table. Published Studies on XMRV and pMLV Findings in Human Diseases and the General Population[1]

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
Prostate Cancer Studies				
Urisman A, Molinaro R, Fischer N, Plummer S, Casey G, Klein E, Malathi K, Magi-Galluzzi C, Tubbs R, Ganem D, Silverman R, DeRisi J. Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q *RNASEL* variant. PLoS Pathogens 2006;2:e25.	Prostate cancer (PCA) patients with familial tumors US	86 PCA tissue samples including: 20 *RNASEL* R462Q-homozygous cases 14 heterozygous cases 52 homozygous wild-type cases	 8/20 (40%) 0/14 (0%) 1/52 (1.9%)	Nested *gag* and *pol* RT-PCR (reverse transcriptase - polymerase chain reaction)
Fischer N, Hellwinkel O, Schulz C, Chun F, Huland H, Aepflelbacher M, Schlomm T. Prevalence of human gammaretrovirus XMRV in sporadic prostate cancer. J Clin Virol 2008; 43:277-83.	Non-familial PCA patients Germany	105 PCA tissue samples (from 87 patients with non-familial PCA) 70 tissue sample controls from healthy prostate tissue	1/105 (0.95%) 1/70 (1.4%)	Nested *gag* RT-PCR
Hohn O, Krause H, Barbarotto P, Niederstadt L, Beimforde N, Denner J, Miller K, Kurth R, Bannert N. Lack of evidence for xenotropic murine leukemia virus-related virus (XMRV) in German prostate cancer patients.	PCA patients Germany	589 PCA tissue samples 589 PCA tumor samples 146 PCA serum samples tested by PCR and for	0/589 (0%) 0/589 (0%) 0/146 (0%)	DNA/RNA *gag* PCR Nested RT-PCR *gag* and *env* Ab by

[1] Table adapted from Gubernot D and Hewlett I, FDA Blood Products Advisory Committee Meeting, December 2010

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Retrovirology 2009;6:92. doi:10.1186/1742-4690-6-92.		antibodies by EIA (enzyme immunoassay)		2 separate EIAs each using recombinant XMRV antigens (Ag) detected using either goat anti-human or goat anti-human MLV p30
Schlaberg R, Choe D, Brown K, Harshwardhan MT, Singh I. XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. Proc Nat Acad Sci USA 2009;106:16351-6.	PCA patients US	233 PCA tissue samples by PCR and IHC (immunohistochemistry) 101 benign tissue controls by PCR and IHC	14/233 (6.2%) by PCR 54/233 (23%) by IHC 2/101 (2%) by PCR 4/101 (4%) by IHC	Tissue, DNA quantitative *integrase* PCR XMRV-specific IHC stain
Arnold R, Makarova N, Osunkoya A, Supplah S, Scott T, Johnson N, Bhosle S, Liotta D, Hunter E, Marshall F, Ly H, Molinaro R, Blackwell J, Petros J. XMRV infection in patients with prostate cancer: novel serologic assay and correlation with PCR and FISH. Urology 2010;75:755-61.	PCA patients US	40 PCA plasma samples tested for neutralizing XMRV antibodies: 20 *RNASEL* QQ 20 *RNASEL* RQ or RR	8/20 (40%) 3/20 (15%) results were concordant in all 7 samples adequate to perform all 3 assays (PCR, IHC and fluorescence *in-situ* hybridization, FISH)	Serological assay (neutralizing antibodies to HIV virions pseudotyped with XMRV *env*) IHC FISH Nested PCR

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Aloia A. Sfanos K, Isaacs Wheng Q, Maldarelli F, DeMarzo A, Rein A. XMRV: A new virus in prostate cancer? Cancer Research 2010;70:10028-33.	PCA patients North America	161 tumor-derived samples (PCR) 596 tumor tissue samples (IHC) 452 benign prostatic tissue samples (IHC)	0/161 (0%) 0/596 (0%) 0/452 (0%)	Real-time PCR IHC (MLV30, MLV70) assays
Danielson BP, Ayala GE, Kimata JT. Detection of xenotropic murine leukemia virus-related virus in normal and tumor tissue of patients from the southern United States with prostate cancer is dependent on specific polymerase chain reaction conditions. J Infect Dis 2010;202:1470-7.	PCA patients Southern US	144 PCA prostatic tissue samples (in 57/144 normal tissue was available as well)	32/144 (22%) proviral DNA was detected in both normal and malignant tissue	RT-PCR Nested PCR *env*
Martinez-Fierro M, Leach RJ, Gomez-guerra LS, Garza-Guajardo R, Johnson-Pais T, Beuten J, Morales-Rodrigues I, Hernandez-Ordonez M, Calderon-Cardenas G, Ortiz-Lopez R, Rivas-Estilla A, Ancer-Rodriguez J, Rojas-Martinez A. Identification of viral infections in the prostate and evaluation of their association with cancer. BMC Cancer 2010;10:326.	PCA patients Mexico	55 PCA prostate tissue 75 controls	0/55 (0%) 1/75 (1.3%)	Nested RT-PCR

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Verhaegh GW, de Jong AS, Smit FP, Jannink S, Melchers W, Schalken J. Prevalence of human xenotropic murine leukemia virus-related gammaretrovirus (XMRV) in Dutch prostate cancer patients. Prostate 2011;71:415-20.	PCA patients Netherlands	74 PCA prostate tissue samples: 23 low-grade, 25 high-grade, 20 castration-resistant, 6 metastatic	3/74 (4.1%) Repeat testing on total nucleic acid from these 3 positive samples was only performed using a single independent sample from one patient	Real-time *integrase* PCR
Sabunciyan S, Mandelberg N, Rabkin CS, Yolken R, Viscidi R. No difference in antibody titers against xenotropic MLV-related virus in prostate cancer cases and cancer-free controls. Mol Cell Probes 2011. doi:10.1016/J.MCP.2011.01.005.	PCA patients US	200 PCA samples 200 non-cancer samples	No differences in the distribution of immunoreactivity comparing PCA to non-cancer serum samples (numbers not provided) (no XMRV-positive controls were used)	Recombinant *env* and *gag* EIAs
Sakuma T, Hue S, Squillace KA, Tonne JM, Blackburn PR, Ohmine S, Thatava T, Towers GJ, Ikeda Y. No evidence of XMRV in prostate cancer cohorts in the midwestern United States. Retrovirology 2011; 8:23. doi:10.1186/1742-4690-8-23.	PCA patients US (Mayo clinic biospecimen core)	110 prostate tissue from PCA patients (Gleason scores >4) 159 sera from PCA patients tested for neutralizing antibody (Nab)	5/110 (4.5%) 1/40 (2.5%) with high Gleason score (8-10) and 4/70 with intermediate Gleason scores (5-7) 0/159 (0%)	Real-time PCR (TaqMan qPCR; Invitrogen) *gag* Nested PCR XMRV/MLV *gag* IHC WB and Ab neutralization

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		40 benign/normal prostate tissue 201 sera from age-matched controls tested for NAb	1/40 (2.5%) 0/201 (0%) No statistical link between the presence of proviral DNA, PCA, PCA grade and the *RNASEL* R462Q mutation. Amplified sequences were nearly identical to endogenous MLV sequences; samples were also positive for mt DNA suggesting sample contamination with mouse DNA	Mitochondrial (mt) DNA PCR
Switzer WM, Jia H, Zheng H, Tang S, Heneine W. No Association of xenotropic murine leukemia virus-related viruses with prostate cancer. PLoS ONE 2011;6:e19065. doi:10.1371/journal.pone.0019065	PCA patients US	165 PCA patients including those with severe, moderate and poorly differentiated tumors of which 9.3% were homozygous (QQ) for the R462Q *RNASEL* mutation	3/162 (1.9%) XMRV DNA PCR positive with undetectable mouse DNA of which: 0/3 homozygous for the QQ mutation, and 0/3 RT PCR pos 0/162 (0%) Ab pos	PCR and RT PCR; mouse-specific PCR test to exclude contamination WB (western blot) to XMRV and related MLVs

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Akgul B, Pfister D, Knuchel R, Heidenreich A, Wieland U, Pfister H. No evidence for a role of xenotropic murine leukaemia virus-related virus and BK virus in prostate cancer of German patients. Med Microbiol Immunol 2011. doi:10:1007/s00430-011-0215-0.	Biopsy-proven prostate cancer patients undergoing radical prostatectomies; >50% of patients had a locally advanced tumor Germany	Paraffin-embedded prostate cancer tissue samples from 85 consecutive patients	0/85 (0%) XMRV positive 1/85 (1.2%) BK virus positive	XMRV and BK quantitative PCR (qPCR) of DNA extracts; detection of 5-500,000 XMRV copies/200 ng human DNA
Lee D, Gupta JD, Gaughan C, Steffen I, Tang N, Luk K-C, Qiu X, Urisman A, Fischer N, Molinaro R, Broz M, Schochetman G, Klein EA, Ganem D, DeRisi JL, Simmons G, Hackett Jr J, Silverman RH, Chiu CY. In-depth investigation of archival and prospectively collected samples reveals no evidence for XMRV infection in prostate cancer. PLOS One 2012;7:e44954.	39 prospectively identified PCA patients	RNA from PC tissue from prospectively identified patients	0/39 (0%) positive for XMRV 0/39 (0%) positive for XMRV *gag*, *pol* or *env* sequences	Virochip RT-PCR in 3 seperate laboratories
		PC tissue from 19 prospectively identified patients	0/19(0%) positive for XMRV DNA	FISH
		Plasma from 39 patients	0/39 (0%) reactive for p15E Ab 2/39 (5%) reactive for gp70 Ab (both NR for p30)	ChLIA
	Archival tissue and RNA samples from	RNA from original tissue block from	0/1 positive for XMRV	Virochip

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	the Urisman et al. 2006 study US	Urisman et al. 2006 Tissue from original tissue block from Urisman et al. 2006 21 archived RNA aliquots (14 samples from Urisman et al. 2006), 6 originally found XMRV positive, 8 negative	0/1 (0%) positive for XMRV 6 of 6 (100%) of the original positives were positive for XMRV, 0 of 8 (0%) of the original negatives were positive	FISH Virochip Sequencing of 3 isolates showed them to be identical
Chronic Fatigue Syndrome Studies				
Lombardi VC, Ruscetti FW, Das Gupta J, Pfost MA, Hagen KS, Peterson DL, Ruscetti SK, Bagni RK, Petrow-Sadowski C, Gold B, Dean M, Silverman RH, Mikovits JA. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science 2009:326;585-9.	Chronic fatigue syndrome (CFS) patients using the Fukuda (CDC 1994) and Canadian Consensus definitions US	101 CFS samples (peripheral blood mononuclear cells, PBMCs, and serum)	68/101 (67%) *gag* by nested PCR 7/11 (64%) *gag* and *env* by a second nested PCR 1/11 (9%) *gag* (but not *env)* by a second nested PCR 19/30 (63%) MLV (murine leukemia virus) antigens 9/18 (50%) antibodies to SFFV (spleen focus-forming virus) *env* proteins	Nested PCR on PBMC DNA IFC (intracellular flow cytometry) with Ab (antibody) to MLV p30 *gag* WB using spleen focus-forming virus (SFFV) antigens

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
		218 controls	8/218 (3.7%) *gag* by nested PCR 0/16 (0%) MLV antigens 0/7 (0%) antibodies to SFFV *env* proteins	
Erlwein O, Kaye S, McClure MO, Weber J, Wills G, Collier D, Wessely S, Cleare, A. Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome. PLoS ONE 2010;5:e8519.	CFS (Fukuda) patients United Kingdom	186 CFS samples	0/186 (0%)	Nested PCR for XMRV/ MLV Assay controls used DNA extracted from whole blood
Groom HC, Boucherit VC, Makinson K, Randal E, Bapista S, Hagan S, Gow JW, Mattes FM, Breuer J, Kerr JR, Stoye JP, Bishop KN. Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome. Retrovirology 2010;7:10.	CFS (Fukuda) patients United Kingdom	170 CFS samples 395 controls (including 157 blood donors and patient samples)	0/170 (0%) PCR 1/170 (0%) NAb XMRV/MLV 0/157 (0%) blood donors by DNA and/or RNA by PCR 22/157 (14%) Nab in blood donors (21/22 positive samples were tested and found to have non-specific viral neutralization properties)	Real-time PCR and RT-PCR for 2 XMRV *env* sequences NAb XMRV/MLV

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			0/12 (0%) patient samples for XMRV/MLV NAb 3/226 (1.3%) patient samples had weak XMRV/MLV NAb Conclusion: no specific XMRV neutralization from either cohort.	
Van Kuppeveld FJM, de Jong AS, Lanke KH, Verhaegh GW, Melchers WJG, Swanink CMA, Bleijenberg G, Netea MG, Galama JMD, van der Meer JWM. 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. BMJ 2010;340:c1018.	CFS (Fukuda) patients Netherlands	32 CFS samples 43 controls A matched case-control study DMSO (dimethyl sulfoxide)-frozen PBMCs	0/32 (0%) 0/43 (0%)	Real-time PCR *integrase* gene; samples were copy-transcribed by reverse transcriptase to assure testing of total nucleic acids
Switzer W, Jia H, Hohn O, Zheng H, Tang S, Shankar A, Bannert N, Simmons G, Hendry M, Falkenberg VR, Reeves WC, Heneine W. Absence of evidence of xenotropic murine leukemia virus-related virus infection in persons with chronic fatigue syndrome and healthy controls in the United States.	CFS (Fukuda) patients, blood donors and general population US	51 CFS samples 56 healthy controls 41 blood donor controls	0/51 (0%) PCR 0/51 (0%) Ab 0/56 (0%) PCR 0/53 (0%) Ab 0/41 (0%) PCR	Nested PCR for XMRV *gag* and *pol* WB, EIA for recombinant XMRV *gag* and *env* Ab

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Retrovirology 2010;7:57.				
Hong P, Li J, Li Y. Failure to detect xenotropic murine leukaemia virus-related virus in Chinese patients with chronic fatigue syndrome. Virology Journal 2010;7:224.	CFS (Fukuda) patients China	65 CFS samples (PBMCs and plasma) 85 blood donors (65 healthy; 20 with HBV, HCV, HIV and/or HTLV)	0/65 (0%) 0/85 (0%)	RT-PCR
Lo SC, Pripuzova N, Li B, Komaroff AL, Hung GC, Wang R, Alter HJ. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. Proc Nat Acad Sci USA 2010;107:15874-9.	CFS patients collected in the 1990s all meeting the 1988 CDC criteria with 21/37 meeting that 1994 CDC criteria (Fukuda) US	41 PBMC samples from 37 CFS patients (4 patients were sampled twice collected 2 years apart) 44 blood donor controls	32/37 (86.5%) patients with MLV-like *gag* sequences detected of which 21/41 (51.2%) samples were positive after the first round of PCR; 42% of samples also had detectable MLV RNA in plasma; 1 patient also had *env* sequences detected 7/8 (87.5%) *gag* positive patients tested positive nearly 15 years later 3/44 (6.8%) MLV-like sequences; 1/44 (2.3%) was positive after the first round of PCR; 1 *gag* positive donor also	Nested PCR for XMRV/MLV *gag* and *env* using primers described by Lombardi et al. and in-house developed primers XMRV/ML RT-PCR Mouse-specific mtDNA PCR

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			had *env* sequences detected DNA from each amplicon from patients and controls extracted from gels and sequenced to predictable size; all sequences more closely related to polytropic endogenous MLVs than either XMRV or ecotropic MLVs	
Hohn O, Strohschein K, Brandt AU, Seeher S, Klein S, Kurth R, Paul F, Meisel C, Scheibenbogen C, Bannert N. No evidence for XMRV in German CFS and MS patients with fatigue despite the ability of the virus to infect human blood cells *in vitro.* PLoS ONE 2010; 5:e15632.	CFS (Fukuda) and multiple sclerosis (MS) patients with high fatigue scores Germany	39 CFS PBMC samples 112 PBMC samples from MS patients with fatigue 30 healthy donor PBMC	0/36 (0%) Ab 0/39 (0%) DNA from cultured PBMCs 0/13 (0%) DNA using alternate primers 0/10 (0%) PBMC infected LNCaP cells on co-cultivation 0/112 (0%) Ab 0/50 (0%) DNA from cultured PBMCs 0/27 (0%) Ab	*gag* and *env* Ab by 2 separate EIAs each using recombinant XMRV antigens (Ag) detected using either goat anti-human or goat anti-human MLV p30 Nested PCR on DNA from cultured and activated PBMCs Integrity verified by GAPDH sequence amplification

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		samples	0/20 (0%) DNA using alternate primers 0/30 (0%) DNA from cultured PBMCs	Mouse-specific mtDNA PCR Nested PCR with alternate primers used by Urisman et al. LNCaP cells co-cultivated with PBMC, tested for XMRV DNA and RT
Schutzer SE, Rounds MA, Natelson BH, Ecker DJ, Eshoo MW. Analysis of cerebrospinal fluid from chronic fatigue patients for multiple human ubiquitous viruses and xenotropic murine leukemia-related virus. Annals of Neurology 2011;69:A9-A13. doi:10.1002/ana.22389.	CFS (Fukuda) patients US	43 CFS samples, cerebral spinal fluid (CSF)	0/10 (0%) *gag* sequences 0/43 *gag* or *env* sequences by RT-PCR or after co-cultivation with LNCaP cells in pools of 20 and 23 samples	XMRV RT-PCR
Satterfield BC, Garcia RA, Jia H, Tang S, Zheng H, Switzer WM. Serologic and PCR testing of persons with chronic fatigue syndrome in the United States shows no association with xenotropic or polytropic murine leukemia virus-related virus. Retrovirology 2011;8:12.	CFS (Fukuda) patients US	45 CFS samples	0/45 (0%) XMRV *pol* sequences 0/39 (0%) XMRV *pol* and *gag* sequences 0/39 (0%) XMRV *gag* RNA	Buffy coat DNA, real-time PCR for XMRV *pol* sequences Buffy coat DNA, nested PCR for

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doi:10.1186/1742-4690-8-12.		42 control samples	0/39 (0%) XMRV Abs 0/42 (0%) XMRV *pol* sequences 0/9 (0%) XMRV *pol* and *gag* sequences 0/9 (0%) XMRV *gag* RNA 0/9 (0%) XMRV antibodies	XMRV *pol* and *gag* sequences Plasma samples, RT-PCR WB
Erlwein O, Robinson MJ, Kaye S, Wills G, Izui S, Wessely S, Weber J, Cleare A, Collier D, McClure MO. Investigation into the presence of and serological response to XMRV in CFS patients. PLoS ONE 2011:6:e17592. doi:10.1371/journal.pone.0017592.	CFS (Fukuda) patients United Kingdom	130 CFS patients (Erlwein 2010) 30 healthy controls	0/48 (0%) XMRV *gag* and *env* DNA sequences 0/130 (0%) Ab to MLV *env* protein 4/130 (3%) elevated signals in an antigen (Ag) capture assay for MLV (anti-Rauscher Ab) 20/130 (15%) elevated signals in an Ag capture assay for MLV (goat anti-NZB Ab) 0/30 (0%) Ab to MLV *env* protein	DNA from EDTA (ethylenediamine-tetraacetic acid) whole blood, nested PCR EIA to a related mouse retrovirus, New Zealand Black (NZB) gp70 *env* Antigen capture assays based upon anti-MLV antibodies

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			1/30 (3%) with elevated signals in an Ag capture assay for MLV Note: there were no coincident reactives or any considered to be specific	
Shin CH, Bateman L, Schlaberg R, Bunker AM, Leonard, CJ, Hughen RW, Light AR, Light KC, Singh IR. Absence of XMRV and other MLV-related viruses in patients with chronic fatigue syndrome. J. Virol. 2011;85:7195-202. doi:10.1128/JVI.00693-11.	CFS (Fukuda) patients US (Salt Lake City UT)	100 CFS patient samples (whole blood) 14 CFS patient samples (whole blood) having previously tested positive for XMRV at Whittemore Peterson Institute (WPI) or VIPDx (Reno, NV) 31 CFS patient samples (plasma infectivity) 200 samples from healthy controls (whole blood) 35 samples from healthy controls (plasma	0/100 (0%) PCR positive to any sequence 0/14 (0%) PCR positive to any sequence 0/31 (0%) infectious virus cultured 0/200 (0%) PCR positive to any sequence 0/35 (0%) infectious virus cultured	Quantitative real-time and nested PCR for 4 different XMRV/MLV sequences (LTR, *gag*, *pol* and *env*) XMRV recombinant gp70 EIA and WB Infectivity in plasma on culture with LNCaP cells A DNA extraction robot that previously handled XMRV-infected cell cultures

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		infectivity) All tested blindly	Distribution of reactivity to *env* proteins in EIA was identical for CFS and control populations; no reactivity could be confirmed by WB. Discrepancies with prior studies (WPI) were due to the presence of trace amounts of mouse DNA in the Taq polymerase used in the previous studies (WPI).	subsequently contaminated DNA extracts from patient samples leading to XMRV false-positive findings.
Knox K, Carrigan D, Simmons G, Teque F, Zhou Y, Hackett Jr J, Qiu X, Luk K-C, Schochetman G, Knox A, Kogelnik AM, Levy JA. No evidence of murine-like gammaretroviruses in CFS patients previously identified as XMRV-infected. Science 2011;333:94-7. doi:10.1126/science.1204963.	CFS (Fukuda) patients US	61 CFS patient samples in two groups: P1 (41) and P2 (29) of which 9 were in both populations; 43 patients, or 26 in each group (with 9 common to both) previously tested positive for XMRV at WPI or VIPDx (Reno, NV) All tested blindly	0/61 (0%) PCR positive to any sequence 0/29 (0%) RT-PCR positive to any sequence 0/29 (0%) infectious virus cultured 0/60 (0%) antibody reactivity confirmed; one sample was weakly gp70 reactive but unconfirmed by WB	XMRV/MLV nested PCR for *gag* and *env* sequences from peripheral blood leukocytes RT-PCR on plasma Viral culture and co-culture from PBMCs Ab to XMRV recombinant p15E and gp70 by two

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				separate direct-sandwich, particle-based chemiluminescent immunoassays (CMIAs) (Abbott ARCHITECT) and WB
Simmons G, Glynn SA, Komaroff AL, Mikovits JA, Tobler LH, Hackett J Jr, Tang N, Switzer WM, Heneine W, Hewlett IK, Zhao J, Lo SC, Alter HJ, Linnen JM, Gao K, Coffin JM, Kearney MF, Ruscetti FW, Pfost MA, Bethel J, Kleinman S, Holmberg JA, Busch MP; for the blood XMRV scientific research working group (SRWG). Failure to confirm XMRV/MLV in the blood of patients with chronic fatigue syndrome: A multi-laboratory study. Science 2011;334:814-7. Published on line ahead of print 22 September 2011. doi:10.1126/1213841.	CFS (Fukuda) patients and one relative of a CFS patient all reported to be XMRV/MLV positive in either from prior WPI or Lo et al. studies. Healthy negative controls	15 whole blood/PBMC/ plasma samples tested in replicates of 1-3 15 whole blood/PBMC/ plasma samples known XMRV negative by NAT, serology and culture in multiple laboratories tested in	0/15 (0%) except 1/10 WPI subjects with positive NAT in PBMCs at WPI; 3/10 WPI subjects with positive culture at NCI/Ruscetti; 3/10 and 5/10 WPI subjects with positive serology at WPI and NCI/Ruscetti, respectively; 2/5 and 5/5 Lo et al. subjects with positive serology at NCI/Ruscetti and WPI, respectively 0/15 (0%) except 2/15 with positive NAT/plasma at WPI; 6/15 with positive culture at NCI/Ruscetti and 8/15 and 6/15 with positive serology at NCI/Ruscetti and WPI,	Highly sensitive methods modeled after prior studies demonstrating XMRV positive findings XMRV/MLV Ab: 5 different assays Nucleic acids: 11 different NAT assays Virus following culture: 3 different methods

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		replicates of 1-3 5 XMRV-containing 22Rv1 cells spiked into whole blood/PBMC/ plasma samples	respectively 5/5 (100%) spiked positive controls were correctly identified except 3/5 with negative results in NAT/plasma and 4/5 with negative results in NAT/PBMC both at WPI	
	Positive controls Blinded samples sent to 9 different laboratories including WPI, NCI/Ruscetti, and Lo et al. that previously generated XMRV positive findings in patients and controls US			
Steffen I, Tyrrell DL, Stein E, Mentalvo L, Lee TH, Zhou Y, Lu K, Switzer WM, Tang S, Jia H, Hockman D, Santer DM, Logan M, Landi A, Law J, Houghton M, Simmons G. No evidence for XMRV nucleic acids, infectious virus or anti-XMRV antibodies in Canadian patients with chronic fatigue syndrome. PLoS ONE 2011;6(11):e27870. doi:10.1371/journal.pone.0027870.	CFS patients Patients met the Canadian consensus criteria and/or the Fukuda criteria for ME/CFS; all with current clinical symptoms in at least two categories:	58 whole blood/plasma samples 57 whole blood/plasma samples	0/58 (0%) for all markers 0/57 (0%) for all markers	WB for XMRV *env* and *gag* from infected DU145 prostate cells Nested RT-PCR and qRT-PCR (nested RT-PCR sensitivity 5 copies/reaction or < 120 copies/mL;

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	autonomic, neuroendocrine and immune dysfunction. Healthy controls Canada			qRT-PCR sensitivity $<10^3$ copies/mL of plasma or whole blood or infectious virus/mL plasma) Co-culture of plasma in DERSE indicator cells (detectors of exogeneous sequence elements), which are modified LNCaP cells susceptible to XMRV infection
Cool M, Bouchard N, Massé G, Laganière B, Dumont A, Hanna Z, Phaneuf D, Morisset R, Jolicoeur P. No detectable XMRV in subjects with chronic fatigue syndrome from Quebec. Virology. 2011;420:66-72.	CFS patients Quebec, Canada	72 PBMC samples from 72 patients tested for DNA 62 sera samples for Ab detection 50 sera samples for Ag detection	0/72 (0%) 0/62 (0%) 0/50 (0%)	PCR directed to *gag* and *env* regions WB using XMRV Ags to probe for Ab IFA using *gag* anti-p30 to probe for XMRV Ags

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		9 plasma samples for viremia 113 PBMCs/plasma samples for infectivity	0/9 (0%)	XMRV RT-PCR Co-culture of patient PBMCs with LNCaP carcinoma cells; plasma inoculated into LNCaP cells and supernatant tested for XMRV by RT-PCR
Sullivan FP, Allander T, Lindau C, Fahlander K, Jacks A, Evengård B, Pedersen NL, Andersson B. No xenotropic murine leukemia virus-related virus (XMRV) detected in Swedish monozygotic twins discordant for chronic fatigue. Journal of General and Molecular Virology 2011;3:63-8.	Monozygotic twins, discordant for CFS: Affected twin Control twin Sweden	47 DNA samples from whole blood 47 DNA samples from whole blood	0/47 (0%) 0/47 (0%)	Nested PCR for XMRV as described by Lombardi et al.; sensitivity 1-5 copies/reaction
Ali MA, Dale JK, Kozak CA, Goldbach-Mansky R, Miller FW, Straus SE, Cohen JI. Xenotropic murine leukemia virus-related virus is not associated with chronic fatigue syndrome in patients from different areas of the US in the 1990s. Virology	CFS patients fulfilling the CDC case definition	61 PBMC and serum samples	9/61 (15%) *integrase* qPCR positive; mean DNA copy number was 21/µg cellular DNA; rates of low level DNA positivity were not significantly different than zero findings in the controls; also negative	Real-time XMRV *integrase* qPCR (20 copies/µg cellular DNA) Real-time XMRV *env* qPCR (less

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
Journal 2011;8:450.			findings by all other assays; hence, non-specific amplification or false positivity; 5 CFS patient samples yielded *gag* sequences identical to endogeneous MLVs (at rates no different than controls)	sensitive than above) Amplification of proviral DNA after activation of PBMCs with PHA and IL-2
	Control patients with chronic inflammatory diseases including rheumatoid arthritis, Behcet's disease, systemic lupus erythematosus, cryopyrin-associated periodic syndromes	97 PBMC and serum samples	0/97 (0%)	Nested PCR for MLV-related viruses using Invitrogen Plaltinum Taq polymerase Ab to XMRV gp70 antigens by immune precipitation using XMRV-infected or mock-infected ferret cells and patient serum
	Healthy controls US; collected between 1993-2007	50 PBMC and serum samples	0/50 (0%); however, 2 yielded *gag* sequences identical to endogeneous MLVs	

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
Alter HJ, Mikovits JA, Switzer WM, Ruscetti FW, Lo S-C, Klimas N, Komaroff AL, Montoya JG, Bateman L, Levine S, Peterson D, Levin B, Hanson MR, Genfi A, Bhat M, Zheng MQ, Wang R, Li B, Hung G-C, Lee, LL, Sameroff S, Heneine W, Coffin J, Hornig M, Lipkin WI. A multicenter blinded analysis indicates no association between chronic fatigue syndrome/myalgic encephalomyelitis and either xenotropic murine leukemia virus-related virus or polytropic murine leukemia virus. mBio 2012;3:e00266-12.	CFS/ME patients diagnosed using Fukuda and Canadian consensus criteria Controls were healthy, matched by age, sex and location US; collected 2011	147 EDTA patient blood samples, separated to plasma and PBMC aliquots 147 EDTA control blood samples, separated to plasma and PBMC aliquots	0/147 (0%) viral nucleic acid or viral culture positive 9/147 (6%) antibody reactive to cells expressing SFFV *env* Ag 0/146 (0%) viral nucleic acid or viral culture 9/146 (6%) antibody reactive to cells expressing SFFV *env* Ag	RT-PCR, on PBMC or viral culture on stimulated PBMC and serologic testing (flow cytometry using an antiglobulin assay to detect antibodies reactive with BaF3ER cells expressing SFFV *env* Ag). All assays were those routinely used by the investigators participating in the study, and that were those that were previously published
Other Diseases/Populations				
Moles JP, Hadi JC, Guilhou JJ. High prevalence of an IgG response against murine leukemia virus (MLV) in patients with psoriasis. Virus Res 2003;94:97-101.	Psoriasis patients; used MLV antigens to explore the association of MLV-like human endogenous retroviruses	49 serum samples from patients with active psoriasis 47 control serum samples (16 from medical staff, 31 from	45/49 (91%) total MLV Ab 42/49 (86%) MLV IgG 25/47 (53%) MLV Ab 4/47 (8%) MLV IgG	WB; MLV antigen purified from MLV-infected cells in culture

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
	(HERVs) with psoriasis France	transfusion center)		
Henrich TJ, Li JZ, Felsenstein D, Kotton CN, Plenge RM, Pereyra F, Marty FM, Lin NH, Grazioso P, Chochiere DM, Eggers D, Kuritzkes DR, Tsibris AMN. Xenotropic murine leukemia virus-related virus prevalence in patients with chronic fatigue syndrome or chronic immunomodulatory conditions. J Infect Dis 2010;202;1478-81.	Outpatients or repository samples from patients with various illnesses including CFS patients US (Boston, MA)	PBMC samples (may be cryopreserved) from: 32 CFS patients 43 HIV positive patients 97 rheumatoid arthritis (RA) patients 26 transplant recipients 95 controls patients (age and gender matched to the RA patients)	 0/32 (0%) 0/43 (0%) 0/97 (0%) 0/26 (0%) 0/95 (0%)	PCR (Qiagen), 3 primer-probe sets
Fischer N, Schulz C, Stieler K, Hohn O, Lange C, Drosten C, Aepfelbacher M. Xenotropic murine leukemia virus-related gammaretrovirus in respiratory tract. Emerg Infect Dis 2010;16:1000-2. doi: 10.3201/eid 1606.100066.	Patients with respiratory tract infections/disease Germany	75 swab/sputum samples from patients with respiratory tract infection (RTI) who had recent air travel 31 bronchoalveolar lavage (BAL) samples from patients with RTI	3/75 (2.3%) 1/31 (3.2%)	*gag* nested RT-PCR; confirmation of some samples by *gag* RT-PCR

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
		and chronic obstructive pulmonary disease (COPD) 161 BAL/tracheal secretion samples from severely immuno-suppressed patients with an RTI 62 BAL or throat swab samples from healthy controls	 16/161 (9.9%) 2/62 (3.2%)	
Kunstman KJ, Bhattacharya T, Flaherty J, Phair JP, Wolinsky SM. Absence of xenotropic murine leukemia virus-related virus in blood cells of men at risk for and infected with HIV. AIDS 2010;24:1784-5.	HIV positive men US	996 samples from the Chicago Multicenter AIDS Cohort Study (MACS): 562 HIV-positive 434 at-risk, HIV-negative individuals	 0/562 (0%) 0/434 (0%)	qPCR for *gag* sequences on DNA extracted from PBMC
Cornelissen M, Zorgdrager F, Blom P, Jurriaans S, Repping S, van Leeuwen E, Bakker M, Berkhout B, van der Kuyl AC. Lack of detection of XMRV in seminal plasma from HIV-1 infected men in the Netherlands. PLoS ONE	HIV-1 positive patients Netherlands	93 seminal plasma samples from 54 HIV-1 infected men	0/93 (0%)	*gag* nested PCR

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
2010;5:e12040.				
Jeziorski E, Foulongne V, Ludwig C, Louhaem D, Chiocchia G, Segondy M, Rodiere M, Sitbon, Courgnaud V. No evidence for XMRV association in pediatric idiopathic diseases in France. Retrovirology 2010;7:63.	Pediatric patients with idiopathic infectious and respiratory diseases, and adults with spondyloarthritis (SpA) France	72 samples from 62 children with hematologic, neurologic or inflammatory pathologies 80 nasopharyngeal aspirates from children with nasopharyngeal pathologies 19 samples from adult SpA adult patients	0/72 (0%) 0/80 (0%) 0/19 (0%)	*env* nested PCR for XMRV and MLV
Barnes E, Flanagan P, Brown A, Robinson N, Brown H, McClure M, Oxenius A, Collier J, Weber J, Gunthard HF, Hirshel B, Fidler S, Phillips R, Frater J. Failure to detect xenotropic murine leukemia virus-related virus in blood of individuals at high risk of blood-borne viral infections. J Infect Dis 2010:202;1482-5.	HIV-1 positive patients (acute and chronic) and HCV patients United Kingdom and Switzerland	133 samples from HIV chronic infections 101 samples from HIV acute infections 67 samples from HCV chronic infections	0/133 (0%) 0/101 (0%) 0/67 (0%)	*gag* and *env* PCR and RT-PCR ELISPOT (enzyme-linked immunosorbent spot) PBMC
Satterfield BC, Garcia RA, Gurrieri F, Schwartz CE. PCR and serology find no association between xenotropic murine leukemia virus-related virus (XMRV)	Autism disorder (AD) and autism spectrum disorder	25 blood samples from AD children born to CFS mothers (SC)	0/25 (0%)	*pol* real-time PCR in two separate assays for MLV and XMRV with

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
and autism. Mol Autism 2010;1:14.	(ASD) patients US (South Carolina, SC) samples from Italy tested in the US	20 mixed controls including family members of the children tested, fibromyalgia patients and chronic Lyme disease patients (SC) 48 AD samples (SC) 96 Italian ASD samples 61 ASD samples (SC) 184 healthy controls comprised of male and female college students	0/20 (0%) 0/48 (0%) 0/96 (0%) 0/61 (0%) 0/184 (0%)	10-25 times increased sensitivity as described by Lombardi et al., 2009 WB (performed at CDC)
Maric R, Pedersen FS, Moeller-Larsen A, Bahrami S, Brudek T, Petersen T, Christensen T. Absence of xenotropic murine leukaemia virus-related virus in Danish patients with multiple sclerosis. J Clin Virol 2010;49:227-8.	MS patients Denmark	50 MS patient PBMC samples	0/50 (0%)	*gag* and *env* XMRV PCR as described in Lombardi et al., 2009
Luczkowiak J, Sierra O, Gonzalez-Martin JJ, Herrero-Beaumont G, Delgado R. No xenotropic murine leukemia virus-related virus detected in	Fibromyalgia patients	15 fibromyalgia patient samples 10 blood donor samples	0/15 (0%) 0/10 (0%)	*gag* and *env* XMRV and MLV nested PCR (QIAamp DNA mini kit)

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
fibromyalgia patients. Emerg Infect Dis 2011;17:314-5.	Spain			
Lintas C, Guidi F, Manzi B, Mancini A, Curatolo P, Persico AM. Lack of infection with XMRV or other MLV-related viruses in blood, post-mortem brains and paternal gametes of Autistic individuals. PLoS ONE 2011;6:e16609.	ASD patients Italy	102 ASD PBMC patient samples 97 PBMC samples from controls 20 ASD patients, post-mortem brain samples 17 controls, post-mortem brain samples (age and gender matched) 25 semen fractions from 9 fathers of ASD patients 85 semen fractions of 25 infertile individuals and 7 fertile controls, all semen samples	0/102 (0%) 3/97 (3.7%) 0/20 (0%) 0/17 (0%) 0/25 (0%) 0/85 (0%)	Nested *gag* XMRV and MLV PCR
Tang S, Zhao J, Viswanath VR, Nyambi PN,Redd AD, Dastyar A, Spacek LA, Quinn TC, Wang X, Wood O, Gaddam D, Devadas K, Hewlett IK. Absence of	HIV-1 positive individuals	199 plasma samples from HIV positive patients	0/199 (0%)	*gag* and *env* RT-PCR on plasma and nested real-time PCR on PBMC

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
detectable XMRV in plasma or PBMC of human immunodeficiency virus type-1 infected blood donors and individuals in Africa. Transfusion 2011;51:463-8. doi:10.1111/j.1537-2995.2010.02932.x.	Cameroon/Uganda	19 PBMC samples from HIV positive blood donors 50 culture supernatants from PBMC cultures of HIV-positive blood donors	0/19 (0%) 0/50 (0%)	samples; also qPCR for PBMC samples
Gray ER, Garson JA, Breuer J, Edwards S, Kellam P, Pillay D, Towers GJ. No evidence of XMRV or relative retroviruses in a London HIV-1 positive patient cohort. PLoS ONE 2011;6:e18096.	HIV-1 positive patients London, England	540 samples from HIV-1 positive subjects (20%, or 108, never have received any anti-retroviral therapy)	0/540 (0%)	Taqman real-time PCR to XMRV and MLVs with sensitivity of 5 copies/mL
Balada E, Castro-Marrero J, Felip L, Vilardell-Tarres M, Ordi-Ros J. Xenotropic murine leukemia virus-related virus (XMRV) in patients with systemic lupus erythematosus. J Clin Immunol 2011;31:584-7. doi:10.1007/s10875-011-9535-5.	Patients with systemic lupus erythematosus (SLE) and healthy controls in Barcelona, Spain	95 SLE patients of varying activity with ≥4 American College of Rheumatology criteria, (including 45 with high fatigue severity scores) and 50 healthy controls	0/145 (0%)	PCR for proviral DNA extracted from whole blood
Waugh EM, Jarrett RF, Shield L, Montgomery D, Dean RTG, Mitchell A, Greaves MF, Gallagher A. The retrovirus XMRV is not directly involved in the pathogenesis of common types of lymphoid malignancy. Cancer Epidemiology, Biomarkers &	507 patients with lymphoid malignancies, patients with benign lymph adenopathy or other malignancies	286 lymphoid tissue samples (212 lymphomas, 58 benign lymphadenopathy, 16 other malignancy)	0/286 (0%)	Single round, real-time *gag*, *pol* and *env* qPCR using primers and probes from conserved XMRV and MLV sequences; 16

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
Prevention, American Association for Cancer Research 2011. doi:10.1158/1055-9965.	Samples obtained between 1990 and 2009 UK	221 blood or bone marrow samples (64 leukemia, 92 lymphoma and 65 other childhood malignancies)	0/221 (0%)	copies *gag* XMRV DNA detected/µg human DNA in 6 replicates Closed tubes and all sample preparation, DNA extraction and PCR performed in a laboratory that never handled known XMRV or MLV cells lines or samples
Maggi F, Focosi D, Lanini L, Sbranti S, Mazzetti P, Macera L, Davini S, De Donno M, Mariotti ML, Antonelli G, Scatena F, Pistello M. Xenotropic murine leukemia virus-related virus (XMRV) is not found in peripheral blood cells from treatment-naïve HIV+ patients. Clinical Microbiology and Infection 2011. doi:10.111/j.1469-0691.2011.03580x.	HIV-infected patients not yet treated with anti-retroviral drugs; viral loads range from 21-5.6 million copies/mL Italy	124 samples of whole blood and plasma	0/124 (0%)	Nested PCR and single-step TaqMan real-time PCR, both directed to XMRV *gag* according to Lombardi et al.; 100 DNA copies/mL sensitivity of the real-time PCR, which was at least one 10-fold dilution higher than nested PCR

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
Arredondo M, Hackett J Jr, de Bethencourt RF, Trevino A, Escudero D, Collado A, Qiu X, Swanson P, Soriano V, de Mendoza C. Prevalence of XMRV infection in different risk populations in Spain. AIDS Research and Human Retroviruses. doi:10.1089/AID.2011.0149.	Individuals with retroviral infections, chronic viral hepatitis, autoimmune diseases, prostate cancer, CFS and blood donors Spain	1103 plasma and PBMC samples from patients with: 437 CFS/fibromyalgia 69 prostate cancer 149 HIV-1 infection 31 HTLV-I and/or HTLV-II infection 81 chronic hepatitis B 72 chronic hepatitis C 18 autoimmune diseases 246 blood donors	3/1103 (0.3%) p15E reactive (2 HTLV-I and 1 HCV); none was DNA positive 15/1103 (1.4%) gp70 reactive (6 CFS/fibromyalgia, 4 blood donors, 2 HIV-1, 1 prostate cancer, 1 HBV and 1 HCV); none was DNA positive 4/1103 (3.6%) *gag* PCR positive; none confirmed or was Ab reactive	Ab to XMRV recombinant p15E and gp70 by two separate direct-sandwich, particle-based CMIAs (Abbott ARCHITECT) *gag* and *env* PCR
Brooks J, Lycett-Lambert K, Caminiti K, Merks H, McMillan R, Sandstrom P. No evidence of cross-species transmission of mouse retroviruses to animal workers exposed to mice. Transfusion 2012;52:317-25. doi:10.1111/j.1537-2995.2011.03463.x.	Animal handlers at a Health Canada animal facility recruited to investigate human infection with simian foamy virus and other	43 serum and PBMC samples from: Animal handlers of which 36 reported working with mice	0/43 (0%) Ab positive 1/43 (2.3%) DNA positive with both sets of nested PCR primers in 1 of 3 reactions; 12 subsequent nested reactions were all negative; the initial positive reactive product was	XMRV/MLV gag nested PCR using published primer sets (round 1 of those of Urisman et al. using conditions described by Lombardi et al. (10

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
	retroviruses Canada		sequenced and shown to be identical to the MLV positive control	copy/reaction sensitivity); round 2 used primers NP116 and NP117 described by Oaks et al. (1 copy/reaction sensitivity in 1 of 3 reactions) Primary prostate cells and the 22Rv1 cell line were used to prepare lysates for WB Intracisternal A-type particles (IAP) PCR to check for mouse DNA contamination
Gingaras C, Danielson B, Vigil K, Vey E, Arduino RC, Kimata JT. Absence of XMRV in peripheral blood mononuclear cells of ARV-treatment naïve HIV-1 infected and HIV-1/HCV co-infected individuals and blood donors. PLoS ONE 2012;7(2):e31398.	HIV-1 infected, treatment naïve patients, including some who were co-infected with HCV; wide range of viral loads and T-cell	93 PBMCs and serum samples from HIV-1 infected patients	0/93 (0%) DNA positive by all 3 PCR assays 5/8 (62.5%) *gag* Ab reactive 0/8 (0%) *env* Ab reactive	3 sensitive PCR assays that were previously characterized: *gag* and *env* (non-nested) and *env* (nested)

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
doi:10.1371/journal.pone.0031398.	counts Blood donor controls US (Texas)	86 PBMCs and serum samples from HIV-1 and HCV infected patients 54 PBMC and serum samples	1/86 (1.2%) *gag* PCR positive 0/86 by both *env* PCR assays 7/15 (46.7%) *gag* Ab reactive 1/15 (6.7%) *env* Ab reactive 0/54 (0%) DNA positive by all 3 PCR assays 6/12 (50.0%) *gag* Ab reactive 0/12 (0.0%) *env* Ab reactive XMRV not associated with HIV-1 infected or HIV-1/HCV co-infected patients; unable to verify isolated antibody reactivity as XMRV specific	IAP PCR to screen samples for mouse contamination WB using XMRV grown in LNCaP cells
Blood Donor Studies				
Qiu X, Swanson P, Das Gupta J, Onlamoon N, Silverman R, Villinger F, Devare S, Schochetman G, Hackett Jr. J. XMRV: examination of viral kinetics, tissue tropism, and serological markers of infection.	Blood donors US	2851 US blood donor serum samples (numbers not given in the printed abstract)	3/2851 (0.1%) antibody positive to all three XMRV antigens; reported in abstract as preliminary data (the raw data of were not given in the abstract)	Ab to XMRV recombinant p15E, p30 and gp70 by three separate direct-sandwich, particle-based

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
Paper #151, presented at 17th CROI 2010. Abstract accessed at http://www.retroconference.org/2010/Abstracts/39393.htm				CMIAs (Abbott ARCHITECT)
Gao K, Norton KC, Knight JL, Stramer SL, Dodd RY, Linnen JM. Development of a high throughput research assay for the detection of xenotropic murine leukemia virus-related virus (XMRV) nucleic acids. 2010 International Conference on Emerging Infectious Diseases, Atlanta GA abstract book page 142.	Blood donors US	425 US plasma samples from blood donors reported in abstract; 1435 in presentation 44 HIV-1 positive samples (not in abstract)	0/1435 (0%) 0/44 (0%)	Transcription-mediated amplification (TMA) for RNA
Furuta RA, Miyazawa T, Sugiyama T, Kuratsune H, Ikeda Y, Sato E, Misawa N, Nakatomi Y, Sakuma R, Yasui K, Yamaguti K, Hirayama F. No association of xenotropic murine leukemia-related virus with prostate cancer or chronic fatigue syndrome in Japan. Retrovirology 2011;8:20. doi:10.1186/1742-4690-8-20.	PCA patients, CFS patients and blood donors Japan	67 plasma and PBMC samples (PCA patients) 100 EDTA whole blood samples (CFS patients) 500 blood donor samples	2/67 (3%) anti-*gag* 0/2 (0%) PCR/RT-PCR 2/100 (2%) anti-*gag* 0/2 (0%) PCR/RT-PCR 8/500 (1.6%) anti-*gag*	WB PCR/RT-PCR
Qiu X, Swanson P, Luk KC, Tu B, Villinger F, Das Gupta J, Silverman RH, Klein EA, Devare S, Schochetman G, Hackett Jr. J. Characterization of antibodies elicited by XMRV infection and development of immunoassays	Routine blood donors US	880 serum and/or plasma samples 397 serum and/or plasma samples	1/880 (0.1%) p15E reactive* 3/397 (0.8%) gp70 reactive, 1 of which was gp70 WB reactive*	Ab to XMRV recombinant p15E, p30 and gp70 by three separate direct-sandwich, particle-based

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
useful for epidemiologic studies. Retrovirology 2011;7:68-84.		985 serum and/or plasma samples	8/985 (0.9%) p30 reactive, 2 of which were p30 WB* *All reactive samples were tested for Abs to all 3 antigens; no sample had reactivity to more than 1 Ab, thus no sample was considered positive for XMRV infection	CMIAs (Abbott ARCHITECT); reactivity to 3 Ags required for confirmation Further Ab confirmation by WB using XMRV lysate or recombinant gp70
Mi Z, Lu Y Zhang S, An X, Wang X, Chen B, Wang Q, Tong Y. Absence of xenotropic murine leukemia virus-relaed virus in blood donors in China. Transfusion 2012;52:326-31. doi:10.1111/j.1537-2995.2011.03267.x.	Routine blood donors China	391 PBMC and plasma samples	0/391 (0%)	Nested *gag* (425 bp) and *env* (350 bp) RT-PCR for RNA from PBMCs and plasma (sensitivity of 1 copy *gag* plasmid DNA); *env* qPCR for genomic DNA from PBMCs Co-culture of plasma in LNCaP cells; extracted RNA detection by nested RT-PCR

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
Qiu X, Swanson P, Tang N, Leckie W, Devare SG, Schochetman G and Hackett Jr. J. Seroprevalence of xenotropic murine leukemia virus-related virus in normal and retrovirus-infected blood donors. Transfusion 2012;52:307-316-306. doi:10.1111/j.1537-2995.2011.03395.x.	Routine blood donors US HIV-1 infected blood donors Cameroon HTLV-I infected blood donors Japan HTLV-uninfected blood donors Japan STD diagnostic patients US	1000 plasma samples 100 plasma samples 486 plasma samples 156 plasma samples 311 plasma samples	0/1000 (0%) confirmed (3 with isolated gp70 reactivity) 0/100 (0%) confirmed (4 with isolated p15E or gp70 reactivity) 0/486 (0%) confirmed (4 with isolated gp70 reactivity; 20 with isolated p15E reactivity) 0/156 (0%) confirmed (1 with isolated p15E reactivity) 0/311 (0%) (2 with isolated gp70 reactivity)	Ab to XMRV recombinant p15E, p30 and gp70 by three separate direct-sandwich, particle-based CMIAs (Abbott ARCHITECT); p30 used for samples with reactivity to either p15E or gp70; reactivity to 3 Ags required for confirmation Further Ab confirmation by WB using XMRV lysate or recombinant gp70 *pol* and *env* RT-PCR (*m*2000 Abbott Molecular)
Dodd RY, Hackett Jr. J, Linnen JM, Dorsey K, Wu Y, Zou S, Qiu X, Swanson P, Schochetman, Gao K, Carrick JM, Krysztof DE, SL Stramer.	Routine blood donors (6 geographic US regions)	13,399 and 1435 plasma samples	122/13,399 (0.9%) isolated Ab reactivity; 0 confirmed 0/122 (0%) isolated Ab	Ab to XMRV recombinant p15E, p30 and gp70 by three separate

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
Xenotropic murine leukemia virus-related virus (XMRV) does not pose a risk to blood recipient safety. Transfusion 2012;52:298-306. doi:10.1111/j.1537-2995.2011.03450.x.			reactive were RNA reactive 0/1435 (0%) RNA reactive	direct-sandwich, particle-based CMIAs (Abbott ARCHITECT); p30 used for samples with reactivity to either p15E or gp70; reactivity to 3 Ags required for confirmation Gen-Probe TMA (TIGRIS) for XMRV RNA Assays validated by Simmons et al. SRWG
	HTLV confirmed-positive blood donors	97 plasma samples	0/97 (0%) RNA reactive	
	Donor-recipient repository US	3741 sera samples (donors)	25/3741 (0.7%) isolated Ab reactivity; 0 confirmed; RNA testing not performed	
		830 plasma samples (109 recipients)	21/830 (2.5%) isolated Ab reactivity; 0 confirmed 0/830 (0%) RNA reactive	
Tang S, Zhao J, Haleyur Giri Setty MK, Devadas K, Gaddam D, Viswanath R, Wood O, Zhang P, Hewlett IK. Absence of detectable XMRV and other MLV-related viruses in healthy blood donors in the United States. PLoS ONE 2011;6:e27391. doi:10.137/journal.	Blood donors from the NIH Blood Bank, the same blood bank from which donors had previously been reported to harbor pMLV sequences in 6.8% of the donors tested	110 plasma samples 71 PBMC samples	0/110 (0%) 0/71 (0%)	Nested PCR and RT-PCR (sensitivity of nested PCR of 10 and 1 copies plasmid DNA in first and second round) using previously described primers

Study	Population	Samples	Results (samples positive/samples tested; %)	Described Assays/Methods
	US			(Simmons et al.) Co-culture of plasma with DERSE indicator cells, which are modified LNCaP cells susceptible to XMRV infection

Abbreviations: APOBEC3F/G – apolipoprotein B mRNA-editing enzyme catalytic polypeptides 3F and G; **Ab** – antibody; **AD** – autism disorder; **Ag** – antigen; **ASD** – autism spectrum disorder; **BAL** – bronchoalveolar lavage; **bp** – base pairs; **cDNA** – complementary DNA; **CFS** – chronic fatigue syndrome; **CMIA** – chemiluminescent immunoassay; **COPD** – chronic obstructive pulmonary disease; **CSF** – cerebral spinal fluid; **DERSE** indicator cells – detectors of exogenous sequence elements; **DMSO** – dimethyl sulfoxide; **EDTA** – ethylenediaminetetraacetic acid; **EIA** – enzyme immunoassay; **ELISPOT** – enzyme-linked immunosorbent spot; **FISH** – fluorescence *in situ* hybridization; **GAPDH** – glyceraldehyde-3-phosphase dehydrogenase; **HERV** – human endogenous retrovirus; **IAP** – intracisternal A-type particle; **IFA** – indirect fluorescent antibody; **IFN** – interferon; **IgG** – Immunoglobulin G; **IHC** – immunohistochemistry; **MLV/MuLV** – murine leukemia virus; **MLRV –** murine leukemia virus-related virus; **MS** – multiple sclerosis; **mt** – mitochondrial; **NAb** – neutralizing antibody; **NZB** – New Zealand Black retrovirus; **PBMC/PBMNCs** – peripheral blood mononuclear cells; **PCA** – prostate cancer; **PCR** – polymerase chain reaction; **qPCR** – quantitative PCR; **RA** – rheumatoid arthritis; **RTI** – respiratory tract infections; **RT PCR** – reverse transcriptase PCR; **SpA** – spondyloarthritis; **SFFV** – spleen focus-forming virus; **SLE** – systemic lupus erythematosus; **SNPs -** single nucleotide polymorphisms; **SpA** – spondyloarthritis; **SRWG** – NHLBI-sponsored scientific research working group; **STD** – sexually transmitted disease; **TMA** – transcription mediated amplification; **VSV** – vesicular stomatitis virus; **WB** – western blot; **WPI** – Whittemore Peterson Institute; **XMRV** – xenotropic murine leukemia virus-related virus.

Additional Reading - Technical Issues

Bacich DJ, Sobek KM, Cummings JL, Atwood AA. False negative results from using common PCR reagents. BMC Research Notes 2011;4:457. doi:10.1186/1756-0500-4-457. Carry over contamination can be prevented by incorporating uracil instead of thymine into the PCR product, then treating with uracil-DNA-glycosylase (UNG) prior to initiating subsequent PCR reactions, thereby degrading contaminating PCR products while leaving non-uracil (target DNA) intact. The use of UNG can block amplification and minute levels of UNG contamination may lead to false-negative PCR results. The authors suggest that this may be a reason for discrepant results between laboratories attempting to amplify MLV-related viruses including XMRV.

Baliji S, Liu Q, Kozak CA. Common inbred strains of laboratory mice that are susceptible to infection by mouse xenotropic gammaretroviruses and the human-derived retrovirus XMRV. J Virol 2010:84:12841-9. Laboratory mice vary widely in their proviral contents and in their virus expression patterns. This study screened inbred strains for sequence and functional variants of the XPR1 receptor. The study found several strains with *Xpr1sxv* lack the active *Bxv1* provirus or other endogenous XMLVs and may provide a useful model system to evaluate the *in vivo* spread of these gammaretroviruses and their disease potential in their natural host.

Cingöz O, Coffin JM. Endogenous murine leukemia viruses: relationship to XMRV and related sequences detected in human DNA samples. Advances in Virology 2011; doi:10.1155/2011/940210. The review article describes the relationship between the various human and mouse isolates of mouse-derive gammaretroviruses and discusses the potential complications associated with the detection of MLV-like sequences from clinical samples. There is considerable discussion of the possible sources of such isolates and the potential for misinterpretation of results due to contamination.

Cingöz O, Paprotka T, Delviks-Frankenberry KA, Wildt S, Hu WS, Pathak VK, Coffin JM. Characterization, mapping and distribution of the two XMRV parental proviruses. J Virol doi:10.1128/JVI.06022-11. It was shown that both XMRV proviruses described by Paprotka et al. (above PreXMRV-1 and PreXMRV-2) were found in only three of 48 strains of laboratory mice examined but none in wild strains of mice (46 strains examined) consistent with the finding that the recombination event could have only occurred in the laboratory. Further, no laboratory mouse strain could harbor XMRV replication due to the lack of the required receptor (Xpr1) in laboratory mice, indicating that the xenografted human tumor cells were required for XMRV propagation.

Del Prete GQ, Kearney MF, Spindler J, Wiegand A, Chertova E, Roser JD, Estes JD, Hao XP, Trubey CM, Lara A, Lee KE, Chaipan C, Bess Jr JW, Nagashima K, Keele BF, Pung R, Smedley J, Pathak VK, Kewal-Ramani VN, Coffin JM, Lifson JD. Restricted replication of xenotropic murine leukemia virus-related virus in pigtailed macaques. J Virol 2012. doi:10.1128/JVI.06886-11.

Following IV challenge of two male pigtailed macaques with >10^{10} XMRV RNA copies, viral replication was limited with transient plasma viremia peaking at 2200 copies/mL and undetectable by 4 weeks. XMRV DNA remained detectable to 119 days with extensive G- to A- hypermutation suggestive of APOBEC-mediated viral restriction. No cellular immune responses nor spread to the prostate were noted; type I interferon was transiently detected. Antibody was detected by 2 weeks and remained detectable. Both animals remained healthy. Therefore, XMRV replication was limited in pigtail macaques and due to similar anti-retroviral innate immune mechanisms in humans, XMRV infection, if it occurred in humans, would be expected to be similarly limited.

Desai R, Neuberger J. Safety of solid-organ transplantation from donors with chronic fatigue syndrome. Transplantation 2011;91:e51-2. Twenty-five recipients of organs from 10 deceased donors with CFS in the UK followed (lookback study); none of 18 recipients with follow-up data available met the CDC criteria for having CFS. Authors conclude that at the present time there is no justifiable reason to exclude donors with CFS from organ donation.

Dey A, Mantri CK, Pandhare-Dash J, Liu B, Pratap S, Dash C. Downregulation of APOBEC3G by xenotropic murine leukemia-virus related virus (XMRV) in prostate cancer cells. Virology Journal 2011;8:531. doi:10.1186/1743-422X-8-531. The presence of APOBEC3G (A3G) is demonstrated in prostate epithelial cell lines (LNCaP and DU145) by western blot and mass spectrometry. This is in contrast to the findings of Paprotka et al. 2010 (above). XMRV produced from A3G-expressing cells are capable of replication. The mechanism is believed to be due to down regulation of A3G in XMRV-infected LNCaP and DU145 cells. This is a novel mechanism by which retroviruses can counteract the antiviral effects of A3G. The results described in earlier reports on the absence of A3G in prostate cell lines may be due to the sensitivity differences in tests used.

Dong B, Kim S, Hong S, Das Gupta J, Malathi K, Klein EA, Ganem D, DeRisi JL, Chow SA, Silverman RH. An infectious retrovirus susceptible to an IFN antiviral pathway from human prostate tumors. Proc Nat Acad Sci 2007;104:1655-60. This study constructed a full-length XMRV genome from prostate tissue RNA and showed that the molecular viral clone is replication-competent. XMRV provirus integration sites were mapped in DNA isolated from human prostate tumor tissue to genes for two transcription factors (NFATc3 and CREB5) and to a gene encoding a suppressor of androgen receptor transactivation (APPBP2/PAT1/ARA67). The study demonstrates that XMRV is a virus that has infected humans and is susceptible to inhibition by IFN and its downstream effector, RNase L.

Erlwein O, Robinson MJ, Dustan S, Weber J, Kaye S, McClure MO. DNA extraction columns contaminated with murine sequences. PLoS ONE 2011;6:e23484. Discovered eluates from naïve DNA purification columns, when subjected to PCR with primers designed to detect genomic mouse DNA occasionally give rise to amplification products that include XMRV and MLVs.

Garson JA, Kellam P, Towers GJ. Analysis of XMRV integration sites from human prostate cancer tissues suggests PCR contamination rather than genuine human infection. Retrovirology 2011;8:13. A BLAST search on the 14 integration sites of XMRV in human prostate cancer tissue found two that were identical to the published integration sites of experimentally infected DU145 cells suggestive of laboratory contamination.

Hadrovava R, de Marco A, Ulbrich P, Stokrova J, Dolezal M, Pichova I, Rumi T, Briggs JAG, Rumlova M. *In vitro* assembly of virus-like particles of a gammaretrovirus, the murine leukemia virus XMRV. J Virol 2012. doi:10.1128/JVI.05564.11. Using an in vitro assembly system of capsid-nucleocapsid protein (CANC), the formation of XMRV-like particles were studied. Unlike other retroviruses, XMRV capsid and CANC do not assemble into tubular particles characteristic of mature assembly and instead have deletions that result in the assembly of immature-like spherical particles. However, below the disordered N-terminal capsid layer, the C terminus assembles a typical immature lattice linked by rod-like densities with nucleoprotein.

Haleyur Giri Setty MK, Devadas K, Ragupathy V, Ravichandran V, Tang S, Wood O, Gaddam DS, Lee S, Hewlett IK. XMRV: usage of receptors and potential co-receptors. Virology Journal 2011;8:423. doi:10.11.1186/1743-422X-8-423. Different receptors for XMRV infection were studied by infecting cells containing CD4 and various chemokine receptors in comparison to XMRV replication levels in cells expressing XPR. Cell culture supernatants were tested for XMRV replication by real-time quantitative PCR. Levels of XMRV replication varied in different cell lines with high levels of replication in some without XPRI and no replication in some with XPRI indicating the possibility receptors other than XPRI for XMRV.

Hue S, Gray ER, Gall A, Katzourakis A, Tan CP, Houldcroft CJ, McLaren S, Pillay D, Futreal A, Garson JA, Pybus OG, Kellam P, Towers GJ. Disease-associated XMRV sequences are consistent with laboratory contamination. Retrovirology 2010;7:111. This study demonstrated that Taqman PCR primers previously described as XMRV-specific can amplify common murine endogenous viral sequences from mice, suggesting that mouse DNA can contaminate patient samples and confound specific XMRV detection. In addition, the genetic distance among *env* and *pol* sequences from the persistently XMRV-infected prostate cell line, 22Rv1, exceeds those of patient-associated sequences, suggesting laboratory contamination versus human infectious transmission. They propose that XMRV might not be a genuine human pathogen and the XMRV from the 22Rv1 cell line is the genetic ancestor of all subsequent isolates from prostate or CFS patients.

Katzourakis A, Hué S, Kellam P, Greg J, Towers GJ. Phylogenetic analysis of MLV sequences from longitudinally sampled chronic fatigue syndrome patients suggests PCR contamination rather than viral evolution. J. Virol. 2011 85:10909-10913. The article's abstract provides the clearest summary: XMRV has been amplified from human prostate cancer and CFS patient samples. Other studies failed to replicate these findings and suggested PCR contamination with a prostate cancer cell line, 22Rv1, as a likely source.

MLV-like sequences have also been detected in CFS patients in longitudinal samples 15 years apart. Here, we tested whether sequence data from these samples are consistent with viral evolution. Our phylogenetic analyses strongly reject a model of within-patient evolution and demonstrate that the sequences from the first and second time points represent distinct endogenous murine retroviruses, suggesting contamination.

Kearney MF, Spindler J, Wiegand A, Shao W, Anderson EM, Maldarelli F, Ruscetti FW, Mellors JW, Hughes SH, LeGrice SFJ, Coffin JM. Multiple sources of contamination in samples from patients reported to have XMRV infection. PLoS ONE 2012;7(2): e30889. doi:10.1371/journal.pone.0030889. This paper investigated the possibility that XMRV or MLV detection in patient samples reported by Lombardi et al. in Science in 2009 was the result of laboratory contamination by XMRV and/or mouse DNA including endogenous MLVs. Any virus whose sequence is closely related to the recombinant XMRV virus would have arisen from laboratory contamination by XMRV or its descendents. Plasma samples from 4 CFS patients who were previously reported to be infected with XMRV and from 5 healthy, XMRV-uninfected controls, as well as supernatants from cultures reported to contain XMRV isolated from 9 clinical samples from 8 patients, were tested for XMRV and MLV by qPCR and single-genome sequence analysis. Results indicate 3 sources of contamination giving rise to XMRV false positivity in human samples: mouse genomic DNA (including endogenous MLVs) contamination of plasma samples from CFS patients, XMRV contamination of plasma samples from healthy controls, and infectious XMRV contamination (either virus or nucleic acid) in cultures used for viral isolation. Single-genome sequences (n=89) from CFS patient plasma were indistinguishable from endogenous MLVs that are distinct from XMRV. XMRV sequences were instead detected in 2 of 5 healthy controls. Single-genome sequences (n=234) from 9 culture supernatants, from clinical samples reported as XMRV positive, were indistinguishable from XMRV sequences obtained from 22Rv1 and XMRV-contaminated 293T cell lines.

Lin Z, Puetter A, Coco J, Xu G, Strong MJ, Wang X, Fewell C, Baddoo M, Taylor C, Flemington EK. Detection of murine leukemia virus in the Epstein-Barr virus-positive human B-cell JY using a computational RNA-seq based detection pipeline, PARSES. J Virol 2012. doi:10.1128/JVI.06717-11. Multiple cell lines harbor exogenous agents such as human tumor viruses, EBV or human papillomavirus. High-throughput sequence analysis (pipeline for analysis of RNA-seq exogenous sequences, PARSES) was used to look for ectopic viruses within 2 EBV-positive cell lines, Akata and JY. JY was found to contain MLVs in which highly active transcription and APOBEC3G-dependent DNA editing occurred. Three other cell lines commonly used for EBV were also found to be contaminated with MLVs.

Makarova N, Zhao C, Zhang Y, Bhosie S, Suppiah S, Rhea J, Kozyr N, Arnold RS, Ly H, Molinaro RJ, Parslow TG, Hunter E, Liotta D, Petros J, Blackwell JL. Antibody responses against xenotropic murine leukemia virus-related virus envelope in a murine model. PLoS ONE 2011;6:e18272. doi:10.1371/journal.pone.0018272. Mice were vaccinated with a combination of recombinant vectors

expressing XMRV *gag* and *env* genes and virus-like particles that had the size and morphology of infectious XMRV to study immunogenicity *in vivo*. Immunization elicited *env*-specific binding and neutralizing antibodies; peak titers for EIA-binding antibodies and neutralizing antibodies were 1:1024 and 1:464. Titers were not sustained and persisted for less than three weeks after immunization.

Mendoza R, Vaughan AE, Miller AD. The left half of XMRV is present in an endogenous retrovirus of NIH/3T3 Swiss mouse cells. J Virol 2011. doi:10.1128/JVI.051137-11. XMRV exhibits 94% overall sequence identity to known mouse retroviral sequences while the existing XMRV sequences are 99.8% identical. The origin of XMRV was investigated in nude mice to determine if XMRV in 22Rv1 cells originated from the mice. mERV-XL (XMRV-left half) was isolated in NIH/3T3cells, which was virtually identical (99.9%) to the same region of XMRV from 22Rv1 cells, and 99.9% identical to PreXMRV-2 (Paprotka et al.). The authors conclude that because NIH/3T3 cell are in such widespread use, DNA from these cells is an obvious source of contamination that could lead to detection of XMRV.

Mo F, Wyatt AW, Wu C, Lapuk AV, Marra MA, Gleave ME, Volik SV, Collins CC. Next generation sequencing of prostate tumours provides independent evidence of XMRV contamination. J Clin Microbiol 2011. doi:10.1128/JCM.06170-11. Next generation sequencing methods were used to interrogate the entire genomes and RNA transcriptomes for signatures related to XMRV from nine human prostate tumors (6 primary and 3 metastatic), three prostate-derived murine xenografts (positive controls) and one benign tissue sample from a pelvic lymph node. All non-human genomes were mapped to a database containing 3932 viral and 1387 microbial genomes followed by filtered reads mapping specifically to MLVs. As expected, the xenograft tumor transcriptomes yielded thousands of MLV reads. Two primary human tumors also showed enrichment of 5 MLVs, including XMRV, in their transcriptomes; however, no XMRV DNA sequences could be identified and the two positive human tumors also enriched mouse mtDNA suggesting contamination.

Oakes B, Tai AK, Cingoz O, Henefield MH, Levine S, Coffin JM, Huber BT. Contamination of human DNA samples with mouse DNA can lead to false detection of XMRV-like sequences. Retrovirology 2010;7:109. All samples that tested positive for XMRV and/or MLV DNA were also positive for the highly abundant IAP long terminal repeat sequences and most were positive for murine mitochondrial cytochrome oxidase sequences. No contamination was detected in negative control samples.

Onlamoon N, Das Gupta J, Sharma P, Rogers K, Suppiah S, Rhea J, Molinaro RJ, Gaughan C, Dong B, Klein EA, Qiu X, Devare S, Schochetman G, Hackett Jr. J, Silverman RH, Villinger F. Infection, viral dissemination and antibody responses of *Rhesus* macaques exposed to the human gammaretrovirus XMRV. J Virol 2011;85:4547-57. doi: 10.1128/JVI.02411-10. This study demonstrates the rhesus macaque is an appropriate animal model for studying the long-term kinetics of XMRV infection. Findings include that XMRV

was infectious and established persistently replicative infection in several tissues and organs even though circulation of free virus was minimal or below detection. The presence of XMRV in the lower reproductive tract in both male and female monkeys is consistent with the potential for sexual transmission. The rhesus macaque model also demonstrated that XMRV can infect lymphoid cells. However, the macaques received a very high intravenous viral dose outside of the physiologic range in the absence of subsequent antigen-specific cellular responses, a robust antibody response or any pathology.

Paprotka T, Delviks-Frankenberry KA, Cingöz O, Martinez A, Kung H-J, Tepper CG, Hu W-S, Fivash Jr MJ, Coffin JM, Pathak VK. Recombinant origin of the retrovirus XMRV. Science 2011;333:97-101. Published on-line ahead of print, 31 May 2011. doi:10.1126/science.1205292. The authors propose that XMRV originated as the result of a laboratory recombination event involving two mouse proviruses that occurred during the serial passage of a human prostate cancer xenograft (CWR22) in nude mice in the 1990s. When aligned, these two proviruses are identical to the sequence of XMRV. Thus, the authors concluded that XMRV is not a real human pathogen, and the association of XMRV with human disease is due to contamination of human samples with virus originating from the recombination event.

Paprotka T, Venkatachari NJ, Chaipan C, Burdick R, Delviks-Frankenberry KA, Hu WS, Pathak VK. Inhibition of xenotropic murine leukemia virus-related virus by APOBEC3 proteins and antiviral drugs. J Virol 2010;84:5719-29. XMRV evades replication suppression by intracellular defense mechanisms including the APOBEC3G (A3G) and APOBEC3F (A3F) proteins, which are potent inhibitors of MLV replication and are expressed in human CD4+ T and B cells. The *APOBEC3* genes (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3) provide defense against retroviruses by encoding cytidine deaminases that deaminate cytidine to uridine resulting in massive hypermutation. Expression of A3G and A3F in XMRV-producing T cells results in their virion incorporation, inhibition of replication and hypermutation of viral DNA and thus XMRV cannot establish a productive infection. Other retroviruses such as HIV have accessory proteins that destroy A3G and A3F; however, XMRV lacks such accessory proteins. Prostate cancer cell lines LNCaP and DU145 exhibited reduced A3F activity, whereas A3G expression in 22Rv1, LNCaP and DU145 was nearly undetectable. Thus, XMRV may be able to establish infection in prostate cells due to low levels of A3G/A3F expression. RT and *integrase* inhibitors, used for treatment of HIV-1 infection, also inhibit XMRV replication.

Ravichandran V, Major EO, Ibe C, Monaco MC, Haleyur Giri Setty MK, Hewlett IK. Susceptibility of human primary neuronal cells to xenotropic murine leukemia virus-related virus (XMRV) infection. Virology Journal 2011;8:443. doi:10.1186/1743-422X-8-443. Human primary progenitor neuronal cells supported XMRV replication at levels higher than immortalized T-cells or DU145 cells (human prostate carcinoma) but less than that observed in LNCaP cells (human prostate adenocarcinoma).

Robinson MJ, Erlwein OW, Kaye S, Weber J, Cingoz O, Patel A, Walker MM, Kim WJ, Uiprasertkul M, Coffin JM, McClure MO. Mouse DNA contamination in human tissue tested for XMRV. Retrovirology 2010;7:108. XMRV-like sequences were found in 4.8% of prostate cancer patients from the UK, Korea and Thailand. However, these were also positive, as were 21.5% of XMRV-negative cases, for intracisternal A-type particle (IAP) sequences, and many, but not all were positive for mouse mitochondrial (mt) DNA sequences. These results show that contamination with mouse DNA is widespread and detectable by the highly sensitive IAP assay.

Rusmevichientong A, Das Gupta J, Elias PS, Silverman RH, Chow SA. Analysis of single nucleotide polymorphisms in XMRVC patient-derived integration sites reveals contamination from cell lines acutely infected by XMRV. J Virol; doi:10.1128/JVI.05624-11. The 14 published single nucleotide polymorphisms (SNPs) for XMRV were compared to those from cell lines infected with XMRV; two SNPs in the imputed human integration sites matched the SNPs in DU145 and 22Rv1 indicating contamination events in 3 of 9 prostate cancer patients studied.

Sakuma T, Tonne JM, Malcolm JA, Thatava T, Ohmine S, Peng KW, Ikeda Y. Long-term infection and vertical transmission of a gammaretrovirus in a foreign host species. PLoS ONE 7:e29682. doi:10.1371/journal.pone.0029682. The study monitored the long-term consequences of XMRV infection and possible vertical transmission in a permissive foreign host, wild-permissive mice. One year following infection, XMRV-infected mice showed no noticeable pathology with proviral DNA detected in 3 of 8 mice. Specific antibodies to gp70 (*env*) and p30 (capsid) were present, but decreased gradually, during the 1-year study. No viremia, humoral immune responses nor proviral DNA could be detected in any of 9 offspring from infected mothers with the exception of one offspring mouse testing XMRV DNA positive but absent viremia or an antibody response. Amplified sequences showed several mutations including one amino acid deletion in the *env* receptor binding domain; this deletion was shown to impair viral infectivity. The authors conclude that XMRV infection in mice results in long-term asymptomatic infection with a low rate of viral replication, low incidence of vertical transmission and limited evolution in a new host. Thus, trans-species gammaretroviral transmission does not occur frequently due to potent retroviral restriction factors such as APOBEC3s and sustained adaptive immunity in a new host.

Sato E, Furuta RA, Miyazawa T. An endogenous murine leukemia viral genome contaminant in a commercial RT-PCR kit is amplified using standard primers for XMRV. Retrovirology 2010;7:110. The study found that the hot-start enzyme mixture of the one-step RT-PCR kit from Invitrogen were contaminated with endogenous MLV sequences derived from the hybridoma cell line from which the monoclonal antibody used in the hot-start reaction mixture was derived. These MLV sequences could be amplified using standard XMRV primers.

Sfanos KS, Afoia AL, Hicks JL, Esopi DM, Steranka JP, Wei S, Sanchez-Martinez SS, Yegnasubramanian S, Burns KH, Rein A, De Marco AM. Identification of replication competent murine gammaretroviruses in commonly used prostate cancer cell lines. PLoS

ONE 2011;6:e20874. Using a combination of broadly reactive MLV antisera and PCR, 58 prostate and other cell lines were investigated to determine if they contain XMRV or MLV-related viruses (such as the XMRV-producing cell line 22Rv1). Two additional cell lines were found to contain replication competent gammaretroviruses. Prostate cancer cell lines may have a propensity for infection with MLVs possibly due to their establishment by xenograft passage in immunocompromised mice.

Smith RA. Contamination of clinical samples with MLV-encoding nucleic acids; implications for XMRV and other candidate human retroviruses. Retrovirology 2010;7:112. This was the editorial that accompanied the four Retrovirology publications detailing the likely role of XMRV contamination from murine sources in the findings of XMRV in human samples.

Tang N, Frank A, Leckie G, Hackett Jr J, Simmons G, Busch M, Abravaya K. Development of sensitive single-round *pol* or *env* RT-PCR assays to screen for XMRV in multiple sample types. J Virol Methods 2011. doi:10.1016/j.jviromet.2011.10.010. The development of a single-round, RT-PCR assay for XMRV *pol* integrase and *env* detection is described using the automated *m*2000 (Abbott Molecular). Whole blood or plasma samples from the SRWG were tested blindly to assess the performance of the assay; the estimated viral RNA detection limit for both targets with 0.4mL plasma was 29-60 copies/mL; performance was comparable to the other assays evaluated for whole blood by the SRWG. No XMRV was detected in samples from: 196 routine blood donors (EDTA plasma), 214 HIV-1 positive patients from Cameroon, Uganda or Thailand (EDTA plasma), 20 formalin-fixed, paraffin-embedded prostate cancer patients, 400 urine pellets from prostate cancer patients, 166 urine pellets from non-prostate cancer patients and 135 cervical swabs from females with either normal or abnormal cytologies. Only two of the 400 urine pellets gave isolated reactive results (one isolated *pol* RT-PCR positive and one *env* RT-PCR positive from different samples).

Tuke PW, Tettmar KI, Tamuri A, Tedder RS. PCR master mixes harbor murine DNA sequences. Caveat Emptor! PLoS One 2011;6:e19953. Report of contamination of Invitrogen Platinum Taq PCR Master Mix with mouse DNA sequences due to its inclusion of mouse monoclonal antibody-derived reagents.

Williams DK, Galvin TA, Ma H, Khan AS. Investigation of xenotropic murine leukemia virus-related virus (XMRV) in human and other cell lines. Biologicals 2011;39:378-83. XMRV contamination was investigated in cell lines commercially available from ATCC that are commonly used in research and vaccine development including MRC-5 (human diploid fetal lung), Vero (African green monkey kidney), HEK-293 (human embryonic kidney), MDCK (canine kidney), HeLa (human cervical carcinoma), A549 (human lung carcinoma), LNCaP (human prostate carcinoma), Raji (human Burkitt's lymphoma), Mv1Lu (mink lung) and Cf2Th (canine thymus). Nested PCR assays were optimized for *gag* and *env* with sensitivity of <10 copies in the equivalent of $1.8x10^5$ cells of human DNA. XMRV contamination was not found in any cell line although DNA sequences of cellular origin were amplified after the first round of PCR.

Wolff D, Gerritzen A. Presence of murine leukemia virus (MLV)-related virus gene sequences in a commercial RT-PCR reagent. Clin Lab 2011;57:631-34. This study identified false-positive XMRV results due to contamination of Superscript III Platinum One-Step Quantitative RT-PCR System (Invitrogen). A real-time PCR assay was developed targeting the 380-bp fragment of the XMRV *gag* gene using reagents from Invitrogen versus those of QIAGEN. Samples tested included three randomly selected nucleic acid extracts from routine diagnostic blood samples, triplicate water (negative) controls and one positive control (VP62 isolate of XMRV). All samples were positive for the XMRV VP62 using the Invitrogen reagents in an initial run and then again when the one positive control and 20 replicates of the water controls were run. Positive results were then consistently obtained from three subsequent runs of 10 water replicates each. All PCR-positive products had the predicted size of 380 bp; the positive control and five water controls were sequenced with sequences identical to VP62. In contrast, the initial run using the QIAGEN reagents were as expected; in the three following QIAGEN runs, 70 of 70 water controls were negative.

Zhang YA, Maitra A, Hsieh JT, Rudin CM, Peacock C, Karikari C, Brekken RA, Stastny V, Gao B, Girard L, Wistuba I, Frenkel E, Minna JD, Gazdar AF. Detection of infectious xenotropic murine leukemia viruses (XMLV) occur in human cultures established from mouse xenografts. Cancer Biology & Therapy 2011;12:1-12. Six of 23 (26%) mouse DNA-free xenograft cultures were strongly positive for MLVs and their sequences had >99% homology to known MLV strains. Of 78 non-xenograft derived cell lines maintained in the same xenograft culture-containing facilities, 13 (17%) were positive for MLVs; none of 50 cultures were MLV-positive if maintained in xenograft-culture free facilities.

Zheng HQ, Jia H, Shankar A, Heneine W, Switzer WM. Detection of murine leukemia virus or mouse DNA in commercial RT-PCR reagents and human DNAs. PLoS ONE 2011;6:e29050. The study reports that based on findings of mouse DNA contamination of one PCR reagent (Platinum Taq Master Mix), which produces false-positive MLV results, further reagents were investigated. PCR reagents including recombinant-derived RT from six different companies were contaminated with low levels of MLV *pol* DNA sequences but not *gag* or mouse DNA sequences. Sequence and phylogenetic analysis showed a high degree of relatedness to Moloney MLV, suggesting residual contamination with an RT-containing plasmid. Contamination with mouse DNA and MLV sequences were also shown in commercially available human DNAs from leukocytes, brain tissues and cell lines. Thus, careful prescreening of commercial specimens and diagnostic reagents is required to avoid false-positive MLV PCR results.

Zhou Y, Steffen I, Montalvo L, Lee TH, Zemel R, Switzer WM, Tang S, Jia H, Heneine W, Winkelman V, Tailor CS, Ikeda Y, Simmons G. Development and application of high-throughput microneutralization assay: lack of xenotropic murine leukemia virus-related virus and/or murine leukemia virus in blood donors. Transfusion 2012;52:332-42. doi:10.111/j.1537-2995.2011.03519.x. Microneutralization assays have been used as a surrogate for detection of anti-MLV. A high-throughput microneutralization assay was

developed using retroviral vectors pseudotyped with XMRV-specific envelopes. Pseudotype infection was neutralized by sera from both macaques and mice challenged with XMRV but not pre-immune sera. Although 23/354 (6.5%) plasma samples from blood donors from the Reno/Tahoe area were able to moderately neutralize (>50%) XMRV envelope-mediated infection; control and other MLV-envelopes were poorly or not at all neutralized; all remaining samples were negative or only demonstrated weak neutralization (<30%). None of the 23 neutralizing donor samples (or 26 other selected donors with no or weak [<30%] neutralization results) showed any evidence of a serologic response to XMRV by WB testing using XMRV-infected DU145 prostate cells. Whole blood samples were tested by qRT-PCR using *integrase* or *gag* primer sets; all 354 donors were negative; the 23 donors demonstrating moderate XMRV pseudotype neutralization were also RNA negative when tested by RT-PCR using generic MLV primers. The 23 seroreactive donor samples were also negative when cultured in the DERSE indicator cell line.

Special Issue of Advances in Virology, Sept 5, 2011

Khan AS, McClure M, Kubo Y, Jolicoeur P. Xenotropic and other murine leukemia virus-related viruses in humans; editorial. This editorial summarizes the 13 original articles published in the special edition. http://www.hindawi.com/journals/av/2011/si.xmlv/. The 13 articles are listed below. The Special Issue provides the current thinking and recent research results of studies on XMRV and other murine leukemia virus-related sequences in humans. Consensus opinion is that XMRV detected in normal and disease human populations represents laboratory contamination. XMRV has been shown to be a laboratory-derived recombinant that occurred during serial passages of a patient's prostate cancer cells in nude mice.

1. Rein A. Murine leukemia viruses: objects and organisms. Special issue of Advances in Virology, Sept 5, 2011. Article ID 403419, 14 pages. This paper reviews the murine retrovirus genomic structure, viral structural proteins, and virus replication.
2. Kozak CA. Naturally occurring polymorphisms of the mouse gammaretrovirus receptors CAT-1 and XPR1 alter virus tropism and pathogenicity. Special issue of Advances in Virology, Sept 5, 2011. Article ID 975801, 16 pages. This paper provides a detailed review of murine retrovirus cell entry and different receptor usage by different types of murine retroviruses compared to XMRV.
3. Blomberg J, Sheikholvaezin A, Elfaitouri A, Blomberg F, Sjosten A, Ulfstedt JM, Pipkorn R, Kallander C, Ohrmalm C, Sperber G. Phylogeny-directed search for murine leukemia virus-like retroviruses in vertebrate genomes and in patients suffering from myalgic encephalomyelitis/chronic fatigue syndrome and prostate cancer. Special issue of Advances in Virology, Sept 5, 2011. Article ID 341294, 20 pages. This review discusses the phylogenetic analysis of murine retroviruses and other retroviruses. Murine leukemia virus-like retroviruses (MLLVs) are widespread as ERVs among vertebrates especially mice and contain three major MLLV groups of which group 3 contains all MLVs which have so far been detected in humans. The review discusses murine retrovirus pathogenesis and a mouse model for transmission of lymphoma by breast milk. They conclude that all reports associating XMRV/human mouse retrovirus-like retroviruses (HMRVs) with prostate

cancer and in ME/CFS are likely due to laboratory contamination as documented by the mounting published evidence along with the absence of an easily measurable immune response and obvious lack of transmission routes.

4. Chakraborty J, Okonta H, Bagalb H, Duggan J. MoMuLV-ts-1: a unique mouse model of retrovirus-induced lymphoma transmitted by breast milk. Special issue of Advances in Virology, Sept 5, 2011. Article ID 813651, 16 pages. This paper presents a mouse model for perinatal transmission by breast milk of lymphoma by a temperature-sensitive mutant of Moloney murine leukemia virus.
5. Kang DE, Lee MC, Das Gupta J, Klein EA, Silverman RH. XMRV discovery and prostate cancer-related research. Special issue of Advances in Virology, Sept 5, 2011. Article ID 432837, 10 pages. This paper reviews the discovery, research and current status of XMRC findings in prostate cancer patients.
6. Tang S, Hewlett IK. Testing strategies for detection of xenotropic murine leukemia virus-related virus infection. Special issue of Advances in Virology, Sept 5, 2011. Article ID 281425, 5 pages. This paper describes and summarizes the various testing methods used to date for the detection of XMRV and MLV-like viruses including the fact that these methods are not yet validated and fully evaluated due to the lack of well-characterized reference materials.
7. Cingöz O, Coffin JM. Endogenous murine leukemia viruses: relationship to XMRV and related sequences detected in human DNA samples. Special issue of Advances in Virology, Sept 5, 2011. Article ID 940210, 10 pages. The paper provides a detailed review of the controversies related to the identification of XMRV in human clinical samples. The sequence similarity between XMRV and MLVs was consistent with an origin of XMRV from one of more MLVs present as endogenous proviruses in mouse genomes. The relationship of human and mouse viral isolates and potential complications associated with the detection of MLV-like sequences from clinical samples due to contamination that misidentified the virus as a novel human retrovirus are presented.
8. Oakes B, Qiu X, Levine S, Hackett Jr. J, Huber BT. Failure to detect XMRV-specific antibodies in the plasma of CFS patients using highly sensitive chemiluminescence immunoassays. Special issue of Advances in Virology, Sept 5, 2011. Article ID 854540, 5 pages. This paper reviews the absence of XMRV antibodies in CFS patients and healthy controls using two novel sensitive immunoassays. Samples from 112 individuals with CFS classified by the CDC criteria with the majority completely disabled (all of which have been previously published as XMRV negative for nucleic acids), and 36 healthy controls were tested blindly using the ARCHITECT p15E and gp70 CMIAs. Two gp70-reactive samples of 72 tested (2.8%) were unconfirmed since they tested nonreactive by p15E and negative by WBs containing whole viral lysate or recombinant gp70 proteins; both samples were from a single healthy control who triggered a false-positive antibody signal. With the serologic data added to the original negative nucleic acid results for this population, XMRV infection can be unequivocally excluded.
9. Spindler J, Hackett Jr. J, Qiu X, Wiegand A, Boltz VF, Swanson P, Bream JH, Jacobson LP, Li X, Rinaldo CR, Wolinsky SM, Coffin JM, Kearney MF, Mellors JW. Prevalence of XMRV nucleic acid and antibody in HIV-1-infected men and in men at risk for HIV-1 infection. Special issue of Advances in Virology, Sept 5, 2011. Article ID 268214, 6 pages. This paper reports

the absence of XMRV infection in 332 HIV-1 infected men or men at high risk for HIV-1 infection due to male to male sex from the Multicenter AIDS Cohort Study (MACS). PBMC and plasma samples were tested using sensitive PCR assays for XMRV RNA and proviral DNA using a single copy qPCR assay, and for antibodies using the ARCHITECT p15E transmembrane and gp70 *env* CMIAs. Of the 332 samples tested, 9 (5 HIV-1 seroposistive, 4 HIV-1 seronegative) were weakly reactive for p15E (1) or gp70 (8); none had antibody reactive to both and none were p30 CMIA reactive. None of the 9 was positive for XMRV RNA in plasma or XMRV DNA in PBMC samples.

10. Delviks-Frankenberry KA, Chaipan C, Bagni R, Wyvill K, Yarchoan R, Pathak VK. Lack of detection of xenotropic murine leukemia virus-related virus in HIV-1 lymphoma patients. Special issue of Advances in Virology, Sept 5, 2011. Article ID 797820, 4 pages. This paper demonstrates the absence of XMRV in PBMC and plasma samples from 26 HIV-1-infected lymphoma patients and 10 healthy controls using PCR and immunoassays. Real-time qPCR used primers that were specific for a unique 24-nucleotide gap in XMRV *gag*. Antibody testing directly against XMRV capsid and transmembrane proteins was done by ELISA developed at NCI-Frederick. Two subjects did have slight antibody reactivity to the transmembrane proteins. Neither of the two was reactive in WB using XMRV viral lysates obtained from the 22Rv1 cell line.
11. Robinson MJ, Tuke PW, Erlwein O, Tettmar KI, Kaye S, Naresh KN, Patel A, Walker MM, Kimura T, Gopalakrishnan G, Tedder RS, McClure MO. No evidence of XMRV or MuLV sequences in prostate cancer, diffuse large B-cell lymphoma, or the UK blood donor population. Special issue of Advances in Virology, Sept 5, 2011. Article ID 782353, 6 pages. This paper demonstrates the absence of XMRV and MLV proviral DNA sequences in prostate cancer samples from diverse populations (55 fresh biopsies-UK and formalin-fixed, paraffin-embedded-25 India, 16 Japan) and B-cell lymphoma patients (10 formalin-fixed, paraffin-embedded-UK) using XMRV-specific LTR or MLV generic *gag*-like sequences by nested PCR. Mouse DNA contamination was excluded by inclusion of IAP PCR analysis. DNA was extracted from whole blood taken from 540 UK blood donors and XMRV/MLV tested (all negative) and RNA was XMRV/MLV tested from 600 UK donors and 400 plasma minipools (all negative).
12. Kearney MF, Lee K, Bagni RK, Wiegand A, Spindler J, Maldarelli F, Pinto PA, Linehan WM, Vocke CD, Delviks-Frankenberry KA, deVere White RW, Del Prete GQ, Mellors JW, Lifson JD, Kewal Ramani VN, Pathak VK, Coffin JM, Le Grice SFJ. Nucleic acid, antibody, and virus culture methods to detect xenotropic MLV-related virus in human blood samples. Special issue of Advances in Virology, Sept 5, 2011. Article ID 272193, 12 pages. This paper reports on the use of highly sensitive methods developed at the NCI-Frederick to detect XMRV nucleic acids, antibodies and replication-competent virus. XMRV was not detected in plasma or tissue samples from 134 prostate cancer patients including: 108 patients diagnosed at the UC-Davis from 2006-2010 who were tested for XMRV RNA and antibodies to capsid and transmembrane proteins in plasma, and 26 patients who were diagnosed at the NIH Clinical Cancer Center and tested for RNA in plasma and DNA in whole blood. In the second cohort, proviral DNA was also included for 19/26 who had radical prostatectomies, antibody testing for

22/26, and viral rescue for 12/26. The results demonstrated that while the assays were sensitive for XMRV, there was no evidence of XMRV in the blood of patients with prostate cancer.

13. Sharma P, Rogers KA, Suppiah S, Molinaro RJ, Onlamoon N, Hackett Jr. J, Schochetman G, Klein EA, Silverman RH, Villinger F. Sexual transmission of XMRV: a potential infection route. Special issue of Advances in Virology, Sept 5, 2011. Article ID 965689, 5 pages. This paper describes XMRV infection in the reproductive tissues of *Rhesus* monkeys indicating the possibility of an animal model for further investigations of virus transmission. The detailed analysis of XMRV infection, viral dissemination and antibody responses in 9 macaques (4 males, 1 female following IV inoculation of 3.6×10^6 $TCID_{50}$ and 4 controls) is described by Onlamoon et al. 2011; J Virol). All 5 inoculated macaques had XMRV signal in the reproductive tract that was readily detectable by staining of formalin-fixed, paraffin-embedded tissues using an XMRV-cross-reactive antibody and FISH. In males, XMRV was found in the acini of the prostate during acute but not chronic infection, and protein production was detected throughout infection in the interstitial and epithelial cells of the seminal vesicles, epididymis and testes. The cervix and vagina were XMRV *gag* positive in females.

Review Articles, News Articles and Commentaries

Alberts B. Editorial Expression of Concern. Science 2011;333:335. Published on line ahead of print 31 May 2011. doi:10.1126/science.1208542. The Editor-in-Chief of Science is now seriously questioning the validity of the study by Lombardi et al., published in Science in Oct. 2009 due to the high number of published negative findings. The Expression of Concern will be attached to Science's 23 October 2009 publication by Lombardi et al.

Alberts B. Retraction. Science 2011;334:1636. The Editor-in-Chief of Science has issued an editorial retraction of Lombardi et al. (Science 2009;326:585-9) due to the inability of multiple laboratories to reproduce the study findings, including those of the original authors as well as questions of quality control related to a number of specific reported experiments, and finally, an overall loss in confidence in the validity of the conclusions.

Callaway EC. Fighting for a cause. Nature 2011;471:282-5. This feature provides a detailed chronology of the research efforts related at the attempts to find a link between XMRV and CFS including comments from the individuals who have been most visible in the research. The controversies are discussed highlighting the second finding of polytropic endogenous retroviral sequences, which did not confirm XMRV in CFS patients (source by some explained as "mouse DNA, which is chock-full of virus sequences, probably contaminated their samples"). Also referenced is the series of subsequent publications attributing the findings of both Lombardi et al.

and Lo et al. to contamination from multiple possible sources and the finding that XMRV originated in the 1990s as a recombination event during the passage of prostate tumor cells in mice that resulted in the 22Rv1 cell line, known to be XMRV-infected.

Cohen J, Enserink M. NewsFocus: False positive. Science 2011;333:1694-1701. The subtitle of the commentary provides the scope of the article: "a report in *Science* 2 years ago that linked a mouse retrovirus, XMRV, to chronic fatigue syndrome astonished scientists and patients alike. But the theory soon began to take hits, and now, to all but a few researchers, it has completely unraveled." This commentary provides in-depth details regarding the chronology of events since October 2009 as expressed by those most visibly involved in XMRV research and its possible association with CFS.

Delviks-Frankenberry K, Cingöz O, Coffin JM, Pathak. Recombinant origin, contamination, and de-discovery of XMRV. Current Opinions in Virology 2012;2:1-9. This excellent and well-documented review provides detailed information on the history of XMRV from its original recognition through the determination of its origins as a laboratory artifact and frequent contaminant of samples, cultures and reagents. The authors note in their abstract: "The initial association of XMRV with chronic fatigue syndrome and prostate cancer, while providing much hope and optimism, have now been discredited and/or retracted following overwhelming evidence that (1) numerous patient cohorts from around the word are XMRV-negative, (2) the initial reports of XMRV-positive patients were due to contamination with mouse DNA, XMRV plasmid DNA, or virus from the 22R*v 1* cell line and (3) XMRV is a laboratory-derived virus generated in the mid 1990s through recombination during passage of a prostate tumor xenograft in immuno-compromised mice. While these developments are disappointing to scientists and patients, they provide a valuable road-map of potential pitfalls to the would-be microbe hunters."

Dodd RY. Chronic fatigue syndrome, XMRV and blood safety. Future Microbiology 2011;6:385-9. A brief review of the issues surrounding XMRV, MLVs, and CFS and their relationship to blood safety. The author points out that little is known about the relationship between these viruses and blood safety and further data are required for decision making. However, the decision to defer donors with a diagnosis of CFS should be considered independent of XMRV and is defensible on the grounds that an infectious etiology cannot be excluded.

European Centre for Disease Prevention and Control. Risk assessment on xenotropic murine leukemia virus-related virus (XMRV) and its implications for blood donation. Stockholm. ECDC; 2011. ISBN 978-92-9193-325-9. doi:10.2900/17643. ECDC was asked to assess the epidemiological profile, scientific evidence of the link between the presence of XMRV and CFS, presence of XMRV in blood and risk of transfusion transmission, and value of introducing a deferral and/or testing for XMRV in the EU. The expert panel reviewing the above concluded that a causal relationship cannot be established, and reported association is more likely an artifact caused by contamination of cell cultures, PCR reagents or samples themselves. An assessment of the virus' epidemiology among

humans is neither relevant nor possible. Neither blood donation screening nor donor deferral is supported based on no suggestion of transfusion transmission.

Groom HCT, Bishop KN. The tale of xenotropic leukemia virus-related virus. J Gen Virol 2012. doi:10.1099/vir.0.041038-0. This comprehensive review follows the development of scientific knowledge about XMRV, from its recognition in tissue from prostate cancers in 2006, through its purported association with CFS in 2009, and finally to its definition as a laboratory artifact 2 years later. Although early studies were convincing, the prostate cancer studies were not entirely replicable. The virus received relatively little attention prior to the 2009 report suggesting an association between XMRV and CFS. It is noted that this report generated considerable interest among, and public pressure from, CFS patients and their advocates despite a large number of studies that failed to support the original observations. Ultimately, published studies strongly suggested that the data appear to be explainable by several types of contamination with mouse nucleic acids, viral clones or virus replicating in cell cultures. Two critical studies were published; the first demonstrating the origin of XMRV as a recombinant of two sequences found in mice used to passage tumor cells, and the second showing that in controlled conditions, laboratories originally reporting positive findings among CFS patients were unable to reliably detect the virus. These studies, along with all of the other negative findings and the evidence of contamination, led to retraction of the key papers. Thus, XMRV and related mouse-derived viruses cannot be considered to be human pathogens, based on current knowledge.

Hauser SL, Johnston SC. Extraordinary claims require extraordinary evidence. Annals of Neurology 2011;69:A9-A10. Editorial of the XMRV initial findings and what is required to refute or support disease association.

Kaiser J. No meeting of minds on XMRV's role in chronic fatigue, cancer. Science 2011;329:1454. The "news of the week" followed the "1st International Workshop on XMRV, 7-8 September 2010, Bethesda MD" and summarized the controversial aspects of the meeting, including quotes such as, the field remains mired in "a zone of chaos" and "we don't have agreement on almost anything." Issues surfaced regarding the potential of contamination from endogenous mouse retrovirus DNA and whether (or not) CFS patients should be treated experimentally with anti-retroviral drugs. The NIAID study led by W. Ian Lipkin was referenced which is to include blinded blood samples from 150 CFS patients from four parts of the US and 150 healthy controls sent to the FDA, CDC and WPI (Mikovits) for testing. As of the end of 2011, the NIAID study is still pending with sample collected forecasted to be completed during the first quarter of 2012.

Kaiser J. Studies point to possible contamination in XMRV findings. Science 2011;331:17. The "news of the week" continues to discuss the controversies regarding XMRV and a potential link to CFS following the publication of a series of negative studies.

Karafin MS, Stramer SL. The scientific method at work: xenotropic murine leukemia virus-related virus is neither a cause of chronic fatigue syndrome nor a threat to the blood supply. Transfusion 2012;52:222-5. Commentary reviewing the literature and use of the scientific method to initially describing XMRV as an agent of potential human disease association, and a possible threat to the national blood supply, to a laboratory contaminant without a current threat to humans.

Kenyon JC, Lever AM. XMRV, prostate cancer and chronic fatigue syndrome. British Medical Bulletin 2011;98:61-74. doi:10.1093/bmb/dr010. This review searches the NIH library of medicine database for papers on XMRV and highlights the explosion of publications since 2009, most of which cover basic science or technical issues, and most without evidence of confirmed XMRV infection and refuting the studies demonstrating any positive findings.

Klein HG, Dodd RY, Hollinger FB, Katz LM, Kleinman S, McCleary KK, Silverman RH, Stramer SL; the AABB Interorganizational Task Force on XMRV. Xenotropic murine leukemia virus-related virus (XMRV) and blood transfusion: report of the AABB interorganizational XMRV task force. Transfusion 2011;51:654-61. In the context of a potential infectious origin for CFS and the uncertainty at the time regarding blood safety with respect to XMRV, the AABB provided information and advice to their membership for the management of presenting blood donors with a history of CFS.

Lo S, Pripuzova N, Li B, Komaroff AL, Hung GC, Wang R, Alter HJ. Retraction for Lo et al. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. Proc Nat Acad Sci USA 2011;109:346. The retraction included a statement supporting the reproducibility of the findings in the authors' laboratories, but MLV-positive samples were of insufficient volume to confirm in independent laboratories, only one other laboratory has found a similar association, neither antibody, virus by cultures nor integration sites in the human genome have been found for XMRV/MLVs, and recall of patients previously testing MLV positive that were included in the NHLBI panel (Simmons et al.) tested negative. Thus the authors' conclusions are "that the association of murine gamma retroviruses with CFS has not withstood the test of time or of independent verification and that this association is now tenuous. Therefore, we retract the conclusions in our article."

Robinson MJ, Erlwein O, McClure MO. Xenotropic murine leukemia virus-related virus (XMRV) does not cause chronic fatigue. Trends in Microbiology 2011. doi:10.1016/j.tim.2011.08.005. This review careful explores the limitations of the published studies linking XMRV to prostate cancer or to CFS. It also reviews how the published literature supports the concept that the virus arose as a recombination event in mice and has no natural reservoir in humans.

Sfanos KS, Aloia A, de Marzo AM, Rein A. XMRV and prostate cancer – a 'final' perspective. Nature Reviews Urology 2012. doi:10.1038/nrurol.2011.225. Review summarizing the recent literature and has now been defined as a laboratory artifact. "Thus, there is no reason to believe that it has any role in the etiology of prostate cancer or other diseases."

Silverman RH, Das Gupta J, Lombardi VC, Ruscetti FW, Pfost MA, Hagen KS, Peterson DL, Ruscetti SK, Bagni RK, Petrow-Sadowski C, Gold B, Dean M, Mikovits JA. Partial Retraction. Science 2011;334;176. Published on line ahead of print 22 September 2011;10.1126/science.1212182; 334:176. Some of the PBMC DNA preparations were contaminated with XMRV plasmid DNA and therefore several figures and a table have been retracted from Lombardi et al. 2009.

Simmons G, Glynn SA, Holmberg JA, Coffin JM, Hewlett IK, Lo SC, Mikovits JA, Switzer WM, Linnen JM, Busch MP for the Blood XMRV Scientific Research Working Group (SRWG). The blood xenotropic murine leukemia virus-related virus scientific research working group: mission, progress, and plans. Transfusion 2011;51:643-53. This article reviews the formation of the NHLBI-sponsored scientific research working group (SRWG), provides an extensive literature review of the lack of evidence of increased rates of cancer following transfusion and includes the Phase I analytic panel results generated by the SRWG participating laboratories. This included the successful detection of XMRV DNA and RNA from spiked whole blood and plasma samples by all SRWG laboratories.

Urisman A, Molinaro R, Fischer N, Plummer S, Casey G, Klein E, Malathi K, Magi-Galluzzi C, Tubbs R, Ganem D, Silverman R, DeRisi J. Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q *RNASEL* variant. PLoS Pathogens 2006;2:e25. Retraction. The retraction states that "due to the publication of a recent study, "In-depth investigation of archival and prospectively collected samples reveals no evidence for XMRV infection in prostate cancer" (http://dx.plos.org/10.137...) and others in the field, the association of XMRV with prostate cancer has now been thoroughly refuted. Although the original finding of a novel gammaretrovirus, XMRV, with the use of a pan-viral detection microarray is valid, and sequencing and phylogenetic characterization of the virus still stands, the editors agree that it is clear that XMRV found in this study is laboratory-derived and there is no association of XMRV with prostate cancer. As a result the paper was retracted from PLOS Pathogens on September 18th, 2012."

Van Kuppeveld, FJ, Van der Meer, JK. XMRV and CFS-the sad end of a story. Lancet 2011; Epub ahead of print June 21, 2011. doi:10:1016/S0140-6736(11)60899-4. This commentary summarizes several of the most critical publications refuting the role of XMRV as a human pathogen.

Weiss RA. A cautionary tale of virus and disease. BMC Biology 2010;8:124. This "opinion" piece emphasizes the careful analysis and pitfalls for claiming that newly discovered retroviruses are responsible for human diseases for which no etiologic agent has been

identified. Such linkages of retroviruses to human disease began in 1972 after the discovery of reverse transcriptase; the first report involved a human RNA tumor virus (retrovirus) isolated from a pediatric rhabdomyosarcoma cell line. It took 2 years to disprove that this was a human virus and instead was a previously unknown xenotropic retrovirus of cats. "Since then there have been a long succession of human "rumor" viruses promulgated as the cause of chronic human disease; diseases with unknown cause will reamin susceptible to rumor viruses as long as no other etiology is established." Weiss states that it was erroneous to describe the findings of MLVs as confirmation of XMRV in human samples. The implications of confirmation of such findings have impact to patients who would or have started treatment and to blood centers that would have to consider implementing blood donation screening. Based on the reported high XMRV viral loads in PBMCs, that practically every cell in stimulated PBMC cultures expresses viral *env* antigens and that western blots show higher levels of *env* expression indicates "that a higher proportion of PBMCs could be infected in CFS than is seen in any other retroviral infection of humans or animals, yet only 9 of 18 of these highly antigen-positive patients expressed antibody." Weiss reviews the other putative retroviruses that have been disproven as causes of human disease and opportunities for contamination within laboratories due to reagents, samples, equipment and less stringent contamination control practices in non-virological laboratories. And finally, that it does not make sense that the XMRV/MLV "positive findings segregate according to the laboratory performing the tests."