Xenotropic Murine Leukemia Virus-Related Virus (XMRV) and other Polytropic Murine Leukemia Viruses (pMLV)

Disease Agent*:

- Xenotropic murine leukemia virus-related virus (XMRV)
- and other related, Polytropic Murine Leukemia Viruses (pMLV)

*** Extensive work has shown that there is no valid evidence that XMRV and pMLVs infect humans, or are associated with human disease. The original published manuscripts linking XMRV with prostate cancer and XMRV or pLMVs with Chronic Fatigue Syndrome (CFS) have been retracted. Nevertheless, these viruses do exist and information about their properties and the earlier observations are provided for reference.**

Disease Agent Characteristics:

- Family: *Retroviridae*; Subfamily: *Orthoretrovirinae*; Genus: *Gammaretrovirus*; Species: Xenotropic murine leukemia virus-related virus (XMRV).
- Virion morphology and size: Virions have a complex construction that consists of an envelope and a nucleocapsid. Virions are spherical to pleomorphic measuring 80-100 nm in diameter. Virions have a buoyant density in sucrose of 1.15-1.17 g cm^{-3}.
- Nucleic acid: The genome is a dimer of linear, positive-sense, single-stranded RNA, 8300 nucleotides long.
- Physicochemical properties: As enveloped retroviruses, the virions should be susceptible to heat, detergents and many disinfectants such as 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde and ethanol.

Disease Name:

- No confirmed human disease associations

Priority Level:

- Scientific/Epidemiologic evidence regarding blood safety: Theoretical; transmission from transfusion has not been documented in humans, although pathogenic retroviruses (*i.e.*, HIV and HTLV) are clearly transfusion transmitted.
- Public perception and/or regulatory concern regarding blood safety: Absent based on the latest scientific findings indicating that the virus is a laboratory contaminant resulting from a recombination event. The previous priority of Moderate was based on three publications (all now retracted) associating XMRV and related pMLVs with CFS and/or prostate cancer; see Table that accompanies this fact sheet, "Published Studies on XMRV and pMLV Findings in Human Diseases and the General Population."
- Public concern regarding disease agent: Low based at least partly on lack of familiarity with a virus that is potentially linked to human disease; however, is higher in groups with diseases potentially associated with XMRV.

Background:

- XMRV originated from the recombination of two mouse endogenous retroviruses during the passing of a prostate tumor xenograft (CWR22) in mice, generating laboratory-derived cell lines that are XMRV-infected; its finding in human samples is due to contamination.
- A diverse range of mammalian species are susceptible to infection by gammaretroviruses. These retroviruses have genomes that contain only *gag*, *pro*, *pol*, and *env* genes. They include murine leukemia virus, feline leukemia virus, koala retrovirus, and gibbon ape leukemia virus that cause leukemia and other syndromes in their host species.
- Evidence of human infection with gammaretroviruses was lacking until 2006 when genome sequences of a previously undescribed gammaretrovirus, XMRV, were detected in a cohort of US men with localized prostate cancer undergoing radical prostatectomy. The hypothesis was that these men harbored a homozygous mutation of the *RNASEL* gene (R462Q) that impaired the function of the ribonuclease L antiviral enzyme hypothetically rendering patients unusually susceptible to the oncogenic potential of the virus. However, a subsequent study found XMRV DNA in 14/233 (6%) and proteins in 54/233 (23% by immunohistochemical staining), prostate cancers irrespective of the RNase polymorphism. Studies in 833 Irish and German subjects with prostate cancer, including 139 with the RNase L mutations, found XMRV in only one patient. Correspondingly, no antibodies were detected among 146 patients from these cohorts.
- Additional conflicting studies have subsequently been published (see Table).
- In 2009, the presence of XMRV in CFS patients was reported from a single US study. XMRV was demonstrated by one or more methods in 68/101 (67%) of CFS patients as compared to 8/218 (3.7%) of healthy controls. These patients did not have the *RNASEL* polymorphism. Secondary infections in tissue culture could be established from PBMC, B and T cells and plasma of patients. The study concluded, "(T)hese findings raise the possibility that XMRV may be a contributing factor in the pathogenesis of CFS."
- As of October 2012, numerous additional studies attempting to identify XMRV in CFS patients have been published and all have been negative (see Table).
- Investigators at the NIH and FDA found sequences, distinct from, but related to XMRV (96.6% homology), phylogenetically clustered with pMLVs, in 32/37 (86.5%) patients from a well-characterized CFS cohort and 3/44 (6.8%) controls. Seven of 8 positive patients with new samples collected 15 years after the index sample collection remained pMLV positive by PCR. These data were interpreted as indicating that the range of viruses to be investigated for an association with CFS may need to be broadened (see Table).
- Reasons for widely discordant findings were not clear but the following points were discussed:

- sample or laboratory reagent contamination,
- differences in the cohorts studied or selection of patients from cohorts for testing,
- variable assay procedures and controls,
- differences in XMRV prevalence in different populations,
- strain and sequence variation, and
- other varying properties of XMRV or pMLVs.

To date, all studies linking XMRV or pMLVs to human disease have been refuted. The most enduring legacy of the controversy that surrounded this agent may be the graphic reminder it provided of the self-correcting nature of science done properly. The generation of a recombinant virus, even if non-pathogenic is of concern.

Common Human Exposure Routes:

- Unknown; there are no data on transmission between humans. However, a macaque model has shown XMRV infectivity by intravenous inoculation resulting in brief viremia and dissemination to multiple tissues including lymphoid tissue, the GI mucosa, macrophages in the lungs, and reproductive tissue.

Likelihood of Secondary Transmission:

- Unknown

At-Risk Populations:

- Unknown, but XMRV/pMLV studies in populations at high risk for infection with other blood-borne pathogens have been negative to date (see Table).

Vector and Reservoir Involved:

- Unknown

Blood Phase:

- The initial published XMRV-CFS study recovered virus in addition to genetic sequences from plasma and activated PBMC in a permissive indicator cell line (LNCaP). XMRV *gag* and *env* proteins could also be detected in activated T and B cells grown in culture. Subsequent studies have primarily used only PCR-based testing; however, some have used serology, and another has used culture of PHA-activated PBMC as well as co-cultures of PBMC in LNCaP cells, but all have been negative. PCR detection of XMRV in blood was short-lived (4-14 days) in the primate model; seroconversion occurred at 11-14 days.

Survival/Persistence in Blood Products:

- Unknown

Transmission by Blood Transfusion:

- Unknown

Cases/Frequency in Population:

- In the initial CFS study, 8/218 (3.7%) of healthy controls harbored viral DNA *gag* sequences in PBMC; however, the expression pattern of viral genes in the infected controls appeared to differ from those among the CFS population so the relevance of the observation was considered unknown.
- The NIH/FDA study found pMLV sequences in 3/44 (6.8%) blood donors. All other studies have found negative results in blood donors (see Table).
- XMRV and pMLVs have been shown not to infect humans.

Incubation Period:

- Unknown

Likelihood of Clinical Disease:

- Unknown

Primary Disease Symptoms:

- If causal relationships had been confirmed, symptoms would have been those of the associated diseases.
  - Many prostate cancers are asymptomatic, but symptoms of urinary obstruction and metastatic spread occur with advancing disease.
  - CFS (also called, more descriptively, Myalgic Encephalomyelitis, ME) is characterized by new onset, unexplained, persistent or recurrent fatigue, post-exertional malaise and/or fatigue, myalgia, sleep dysfunction, and neurological/cognitive impairment with immune, autonomic and/or neuroendocrine manifestations of 6 months duration or longer (3 months in children). Symptoms are not caused by ongoing exertion, are not relieved by rest, and result in a substantial reduction of previous levels of occupational, educational, social, or personal activities. Co-morbid conditions, such as fibromyalgia syndrome and irritable bowel syndrome may overlap with CFS. The clinical case definition includes a list of exclusionary conditions.

Severity of Clinical Disease:

- The original cohort of prostate cancer patients harboring the homozygous mutation in the *RNASEL* gene had localized prostate cancer. This association has not been reproducible and the original findings retracted.
- CFS produces very significant disability with substantial disruption of activities of daily living among those meeting strict case definitions.

Mortality:

- Unknown

Chronic Carriage:

- Chronicity is a feature of infection by the *Retroviridae*.

Treatment Available/Efficacious:

- Unknown. *In vitro* data suggest XMRV is sensitive to a subset of licensed anti-retroviral drugs approved for the treatment of HIV. There are no clinical trial data.

Agent-Specific Screening Question(s):

- No specific question is in use for blood donors and is not currently recommended by the FDA or AABB.
- No XMRV-specific question is feasible in the absence of any established risk factors for XMRV infection and the experimental nature and limited availability of diagnostic tests. Questions related to XMRV or pMLVs are not needed due to the absence of human infection.
- No sensitive and specific question for CFS has been validated.
    - The rate at which potential donors carrying a medical diagnosis of CFS present to donor centers is unknown, but should be low in light of the associated disability.
- Individual collectors of blood and cellular therapy products should accept or defer donors with a history of CFS based on their clinical judgment of the donor's health status.

Laboratory Test(s) Available:

- No FDA-licensed blood donor-screening test exists.
- Standards for the diagnosis of XMRV infection have not been established.
- Research assays include a variety of PCR systems, cell culture, flow cytometry-based immunoassay, chemiluminescent immunoassay and immunohistochemical analyses.
    - Prototype automated serological assays demonstrated 100% sensitivity by detecting western blot-positive serial bleeds from XMRV-infected animals in the macaque study and ≥99.5% specificity among healthy blood donors.
    - A series of papers in December 2010 and subsequent to that conclude that contamination of PCR reactions may be an important source of the positive results reported to date (see Table). In two studies, the amplified sequences were accompanied by evidence of contamination of the specimens and some controls with mouse DNA, and in a third study mouse contamination of PCR reagents was the apparent source of contamination. All these sequences appear related to endogenous MLVs. In a fourth paper, sequences thought to be unique to XMRV were shown to be present in a much broader range of pMLVs, and patient-derived sequences were shown to be closely related to XMRV from a widely used prostate cancer cell line, further suggesting contamination as a source of positive samples.

Currently Recommended Donor Deferral Period:

- No FDA Guidance or AABB Standard exists regarding XMRV infection. None is needed since neither XMRV nor pMLVs infect humans.
- Current practice per FDA Guidance and AABB Standards is to accept donors who are healthy at the time of donation.
    - CFS advocacy organizations and the National Cancer Institute have historically discouraged blood donation by CFS patients.
- As of January 2011, donors who provide a history of CFS are indefinitely deferred in the UK, Australia, New Zealand and by the Canadian Blood Services. A specific question about CFS resulting in donor deferral is included in the donor history in parts of Belgium.
- AABB Association Bulletin #10-03 (July 2010) recommended that prospective donors be provided with pre-donation information about CFS and asked to self-defer if they have ever had a medical diagnosis of CFS. This was an interim recommendation pending clarification of issues surrounding the theoretical transmission of XMRV or pMLVs to transfusion recipients and concerns about the safety of donation among patients with CFS. Model educational materials were provided to AABB members. In a December 2010 meeting of the FDA's Blood Products Advisory Committee, there was no consensus about the role of XMRV or related viruses in the pathogenesis of human disease, but a committee recommendation was made calling for a direct donor question about a history of CFS, with an affirmative answer constituting grounds for an indefinite deferral. An updated AABB Association Bulletin #12-05 (November 2012) states that individual collectors of blood and cellular therapy products should accept or defer donors with a history of CFS based on their clinical judgment of the donor's health status.
- Blood collection facilities should follow established SOPs regarding donors with cancer.

Impact on Blood Availability:

- Agent-specific screening question(s): Not applicable.
- Laboratory test(s) available: Not applicable

Impact on Blood Safety:

- Agent-specific screening question(s): Not applicable
- Laboratory test(s) available: Not applicable

Leukoreduction Efficacy:

- The initial studies in CFS suggested that there was a plasma viremia, so leukoreduction would have been unlikely to be completely effective.

Pathogen Reduction Efficacy for Plasma Derivatives:

- No specific data are available but presumed to be robust as the agent is an enveloped virus that should be sensitive to many measures used in the fractionation process.

Other Prevention Measures:

- Unknown, but preliminary data using the Cerus™ Intercept system for platelets and S-303 for red blood cells have demonstrated a 4-$\log_{10}$ reduction in XMRV titer assayed in a permissive prostate cancer cell line.

Suggested Reading:

1. Carruthers BM, Jain AK, De Meirleir KL, Peterson DL, Klimas NKG, Lerner AM, Bested AC, Flor-Henry P, Joshi P, Powles ACP, Sherkey JA, van de Sande MI. Myalgic encephalomyelitis/chronic fatigue syndrome: clinical working case definition, diagnostic and treatment protocols. J Chron Fatigue Syndr 2003;11:7-115.
2. Coffin JM and Stoye JP. Perspectives: a new virus for old diseases? Science 2009;326:530-1.
3. 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 Natl Acad Sci USA 2007;104:1655-60.
4. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A, and the International Chronic Fatigue Syndrome Study Group. The chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann Intern Med 1994; 121:953-9.
5. Kaiser J. Chronic fatigue syndrome. Studies point to possible contamination in XMRV findings. Science 2011;331:17.
6. Kearney M, Maldarelli F. Current Status of Xenotropic Murine Leukemia Virus-Related Retrovirus in Chronic Fatigue Syndrome and Prostate Cancer: Reach for a Scorecard, Not a Prescription Pad. J Infect Dis 2010;202:1463-66.
7. Klein HG, Dodd RY, Hollinger FB, Katz LM, Kleinman S, McClearly KK, Silverman RH, Stramer SL for the AABB Interorganizational Task Force on XMRV. Xenotropic murine leukemia virus-related virus (XMRV) and blood transfusion: report of the AABB interoganizational XMRV task force. Transfusion 2011;51:654-61.
8. 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.
9. Lo SC, Pripuzova N, Li B, Komaroff AL, Hung GC, Wang R, Alter HF. Detection jof MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. Proc Natl Acad Sci USA 2010;107:15874-9.
10. Menedez-Arias L. Evidence and controversies on the role of XMRV in prostate cancer and chronic fatigue syndrome. Rev Med Virol 2011;21:3-17.
11. Mikovits JA, Lombardi VC, Pfost MA, Hagen KS, Ruscetti FW. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Virulence 2010;1:386-90.
12. Onlamoon N, Das Gupta J, Sharma P, Rogers K, Suppiah S, Rhea J, Molinaro R, Gaughan C, Dong B, Klein EA, Qiu X, Devare S, Schochetman G, Hackett J Jr, 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.
13. Qiu X, Swanson P, Luk KC, Tu B, Villinger F, Das Gupta J, Silverman R, Klein E, Schochetman G, Hackett J. Characterization of antibodies elicited by XMRV infection and development of immunoassays useful for epidemiologic studies. Retrovirology 2010;7:68.
14. Rusmevichientong A, Chow SA. Biology and pathophysiology of the new human retrovirus XMRV and its association with human disease. Immunol Res 2010;48:27-39.
15. Schlaberg R, Choe DJ, Brown KR, Thaker HM, Singh IR. XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. Proc Natl Acad Sci USA 2009;106:16351-6.
16. Silverman RH, Nguyen C, Weight CJ, Klein EA. The human retrovirus XMRV in prostate cancer and chronic fatigue syndrome. Nature Reviews Urology 2010;7:392-402.
17. Simmons G, Glynn SA, Holmberg J, Coffin J, Hewlett I, Lo S-C, Mikovits JA, Switzer WM, Linnen JM, Busch MP for the Blood XMRV Scientific Research Working Group. The Blood Xenotropic Murine Leukemia Virus-Related Virus Scientific Research Working Group: mission, progress and plans. Transfusion 2011;51:643-53.
18. Smith RA, Gottlieb GS, Miller AD. Susceptibility of the human retrovirus XMRV to antiretroviral inhibitors. Retrovirology 2010;7:70.
19. Smith RA. Contamination of clinical specimens with MLV-encoding nucleic acids: implications for XMRV and other candidate human retroviruses. Retrovirology 2010;7:112.
20. The Universal Virus Database, v3. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/ Accessed November 4, 2009.
21. Urisman A, Molinaro RJ, Fischer N, Plummer SJ, Casey G, Klein EA, Malathi K, Magi-Galluzzi C, Tubbs RR, Ganem D, Silverman RH, DeRisi JL. Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q *RNASEL* variant. PLoS Pathog 2006;2:e25.
22. Weiss RA. A cautionary tale of virus and disease. BMC Biology 2010; 8:124.
23. Editorial. Retraction and the "de-discovery" of XMRV. Lancet Infectious Diseases 2012; 12:817.