Randomized Clinical Trial to Evaluate the Efficacy and Safety of Valganciclovir in a Subset of Patients With Chronic Fatigue Syndrome

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There is no known treatment for chronic fatigue syndrome (CFS). Little is known about its pathogenesis. Human herpesvirus 6 (HHV-6) and Epstein–Barr virus (EBV) have been proposed as infectious triggers. Thirty CFS patients with elevated IgG antibody titers against HHV-6 and EBV were randomized 2:1 to receive valganciclovir (VGCV) or placebo for 6 months in a double-blind, placebo-controlled trial. Clinical endpoints aimed at measuring physical and mental fatigue included the Multidimensional Fatigue Inventory (MFI-20) and Fatigue Severity Scale (FSS) scores, self-reported cognitive function, and physician-determined responder status. Biological endpoints included monocyte and neutrophil counts and cytokine levels. VGCV patients experienced a greater improvement by MFI-20 at 9 months from baseline compared to placebo patients but this difference was not statistically significant. However, statistically significant differences in trajectories between groups were observed in MFI-20 mental fatigue subscore (P = 0.039), FSS score (P = 0.006), and cognitive function (P = 0.025). VGCV patients experienced these improvements within the first 3 months and maintained that benefit over the remaining 9 months. Patients in the VGCV arm were 7.4 times more likely to be classified as responders (P = 0.029). In the VGCV arm, monocyte counts decreased (P < 0.001), neutrophil counts increased (P = 0.037) and cytokines were more likely to evolve towards a Th1-profile (P < 0.001). Viral IgG antibody titers did not differ between arms. VGCV may have clinical benefit in a subset of CFS patients independent of placebo effect, possibly mediated by immunomodulation and/or antiviral effect. Further investigation with longer treatment duration and a larger sample size is warranted. J. Med. Virol. 85:2101-2109, 2013. © 2013 The Authors. Journal of Medical Virology published by Wiley Periodicals, Inc.
INTRODUCTION

Chronic fatigue syndrome (CFS)—a chronic, complex, and incapacitating illness of unknown etiology—is characterized by profound physical fatigue that is not improved by bed rest and can be significantly worsened by physical or mental activity [Fukuda et al., 1994]. Little is known about its pathogenesis and diagnosis is based on clinical symptoms as opposed to an objective biomarker [Klimas et al., 2012]. Treatment regimens are divergent, often deemed controversial, and usually not supported by randomized, placebo-controlled clinical trials [Straus et al., 1998].

Infection has been long suspected to be a trigger of CFS, as many patients recall the onset of their syndrome as an “influenza-like” illness, and outbreaks of CFS have been reported in community and hospital settings [Briggs and Levine, 1994; Levine, 1994; Kerr et al., 2002; Hickie et al., 2006; Komaroff, 2006; Katz et al., 2009; Komaroff and Cho, 2011]. An aberrant immune response against periodic reactivation of or re-infection with an infectious agent(s) has been proposed as a mechanism responsible for the perpetuation and fluctuation of the CFS symptoms [Tobi et al., 1982; Patnaik et al., 1995; Buchwald et al., 1996; Komaroff and Cho, 2011]. Thus, long-term and pathogen-directed interventions have been attempted in subsets of CFS patients who meet certain laboratory markers for a given organism with the hope to significantly ameliorate or end suffering [Kogelnik et al., 2006; Lerner et al., 2007].

It has been postulated that elevated IgG antibody titers against human herpesvirus 6 (HHV-6) and EBV could be interpreted as indicators of their periodic reactivation or re-infection in CFS patients [Straus et al., 1985; Buchwald et al., 1996] and that valganciclovir (VGCV) would suppress their reactivation or treat re-infection [Lerner et al., 2002; Montoya, 2007]. In an open-label study, a significant clinical improvement in this subset of CFS patients was observed following 6 months of VGCV treatment [Kogelnik et al., 2006]. However, it was not possible to conclude whether an antiviral, immunomodulatory, or placebo effect of the drug mediated this benefit. In this study, the efficacy and safety of VGCV in this subset of CFS patients when compared to placebo was examined and biological endpoints that could identify potential mechanisms of action were explored.

METHODS

Study Protocol and Patients

The evaluation of valganciclovir in longstanding viral exposure (EVOLVE) study was an investigator-initiated, randomized, double-blind, placebo-controlled, clinical trial to evaluate the efficacy and safety of VGCV in CFS patients who have elevated IgG antibody titers against HHV-6 and EBV. The Stanford University Institutional Review Board (IRB) approved the study. Patients were consented and enrolled between May 2007 and July 2007. Data collection was completed in April 2008.

Patients were eligible for inclusion if they (1) were 18 years of age or older, (2) met the CFS case definition established in 1994 [Fukuda et al., 1994], (3) had suspected viral onset (“influenza-like” or viral illness diagnosed by a physician) of CFS, and (4) had elevated antibody titers that fit one of the following schema (i) HHV-6 IgG ≥ 1:640, EBV VCA IgG ≥ 1:640, and EBV EA IgG ≥ 1:160 or (ii) HHV-6 IgG ≥ 1:320, EBV VCA IgG ≥ 1:1,280 and EBV EA IgG ≥ 1:160 (for further information see Supplementary Material Document 1). Figure 1 describes the process for patient referral, screening, enrolment, and allocation into the study. One hundred fifty-five patients were referred to the study. Of the 155, 110 were excluded because the initial screening revealed low antibody titers. Fifteen additional patients were excluded because of low antibody titers on repeat testing (five patients), exclusionary comorbidities (3), conflicting medication (3), patients declined to participate (2), study was full (2; Fig. 1). The three patients with exclusionary comorbidities had the diagnosis of: uncontrolled hypothyroidism, uncontrolled major depression, and hepatitis C.

Thirty patients were enrolled and randomized in a 2:1 manner to be treated with either VGCV (20 patients) or placebo (10 patients).

 Patients were given VGCV or placebo based on their assignment for 6 months and followed for 6 additional months. Patients and investigators were blinded for a total of 9 months from the start of randomization and until data were collected and locked onto three CDs. The packaging of VGCV and placebo was performed by Roche at their headquarters (Basel, Switzerland) and sent to the Stanford Pharmacy. VGCV or identical-appearing placebo was initiated at a dose of 900 mg (two 450 mg tablets) twice daily for 21 days followed by 900 mg once daily to complete 6 months.

Clinical Endpoints

In the absence of widely-accepted endpoints for randomized clinical trials involving CFS patients, the Multidimensional Fatigue Inventory (MFI-20, for further information see Supplementary Material Document 2) score change at 9 months from baseline was chosen as the primary outcome of interest because investigators at the Centers for Disease Control and Prevention (CDC) had validated previously the MFI-20 as an instrument that identifies CFS in a reproducible manner [Gentile et al., 2003; Reeves et al., 2005; Lin et al., 2009]. The MFI-20
Fig. 1. Screening, enrolment, and allocation of patients in a randomized clinical trial to evaluate the efficacy and safety of valganciclovir in patients with chronic fatigue syndrome and elevated antibody titers against human herpes virus 6 (HHV-6) and Epstein–Barr virus (EBV).

score assesses general fatigue, physical fatigue, mental fatigue, reduced motivation, and reduced activity. Higher values indicate increased severity. The MFI-20 mental fatigue subscore uses data already collected in the overall MFI-20 questionnaire but primarily assesses cognitive, rather than physical fatigue. The following additional (i.e., secondary) clinical measurements were considered: CDC CFS Symptom Inventory (CDC CFS SI), Fatigue Severity Scale (FSS, for further information see Supplementary Material Document 3), Hamilton Rating Scale for Depression (HAM-D), self-reported physical functioning and cognitive functioning scores assessed by study personnel during the study visit on a scale from 1% to 100%, the Paced Auditory Serial Addition Test (PASAT) and the Sleep Assessment Questionnaire (SAQ). These additional endpoints were chosen because they have been used in CFS studies. In this study, their use for the first time in a randomized clinical trial is reported. Clinical endpoints were collected at weeks 0, 1, 2, 3, 4, 8, 12, 24, and 48.

Finally, prior to unblinding, all subjects were classified as a responder or non-responder by a formal committee (comprised of four members of the
study team) who reviewed the entire profile of clinical symptoms as scored by the MFI-20 scores for each subject before unblinding (physician-determined responder status).

Biological Endpoints

**Monocyte and neutrophil counts.** Absolute monocyte and neutrophil counts were measured as part of routine complete blood cell counts performed in the Stanford Anatomic Pathology & Clinical Laboratories and collected at weeks 0, 1, 2, 3, 4, 8, 12, 16, 20, 24, 25, 36, and 48.

**Cytokines.** Serum cytokine levels were obtained using a 37-multiplex array on the Luminex 200 IS system (Affymetrix, Santa Clara, CA) performed at the Stanford Human Immune Monitoring Center and following the manufacturer's instructions. Serum samples for the cytokine assay was collected at 0, 1, 2, 3, 4, 8, 12, 16, 20, 24, 36, and 48.

**Viral IgG antibody titers.** IgG antibodies titers against EBV and HHV-6 were measured by immunofluorescence assay (IFA) at focus diagnostics (Cypress, CA). IgG antibody titers against HHV-6 were also analyzed by IFA at the laboratory of one of the authors (L.F.). Serum to measure viral IgG antibody titers was collected at weeks 0 and 24.

Adherence to Study Protocol, and Safety

Adherence was reinforced at each visit by direct questioning and pill counts. Additionally, serum samples were obtained from all subjects to measure ganciclovir levels at weeks 3, 12, and 24 (VGCV is the pro-drug of ganciclovir). To assess the safety of the drug, complete blood cell counts with leukocyte differential, renal function tests, and liver function tests were performed per protocol. In addition, safety issues were assessed at each visit.

Statistical Analysis

Differences in baseline and clinical patient characteristics between the VCGV and placebo arms were evaluated by the Fisher’s exact test or a Student’s t-test as appropriate. As many secondary outcomes were considered corresponding to many hypotheses tested, secondary analyses from this study serve a hypothesis-generating purpose. As such, analyses are considered exploratory, and P-values are descriptive. All tests were two-sided and performed at the 0.05 level of significance.

To address the primary hypothesis that VGCV clinically improves physical and mental fatigue in a subset of CFS patients, an intent-to-treat analysis and two statistical methods were used. Analysis of variance (ANOVA) methods were used to determine whether improvement in the VGCV arm occurred at 9 months in the clinical and biological endpoints. Mixed-effects linear (MEL) regression methods were used to address the trajectories of these endpoints measured over time differed by treatment group status and included a subject-specific random intercept term to account for the correlation of measurements within a subject over time. Two models of this type were considered: the first assumed a linear relation between the outcome and time, and the second categorized time into four clinically meaningful time periods—baseline, induction period (first 3 weeks on 900 mg twice daily), maintenance period (last 21 weeks on 900 mg daily), and post-treatment follow-up—and allowed the outcome behavior to be nonlinear over time when appropriate.

RESULTS

Demographics and Clinical Parameters of the Patients at Baseline

Demographic and baseline clinical characteristics by treatment assignment are shown in Table I. Differences among these baseline characteristics were not statistically different between the VGCV and placebo arms with the exception of smoking history. The VGCV arm experienced a higher proportion of individuals with history of smoking but only three were current smokers; no one in the placebo arm was a current or former smoker.

Adherence of Subjects to Treatment Assignment

In the VGCV arm, all of the subjects had detectable ganciclovir levels after 3 weeks of treatment (mean = 5.08 μg/ml, SD = 3.47), 18 had detectable levels at week 12 (mean = 2.34 μg/ml, SD = 2.46), and 13 at week 24 (mean = 1.10 μg/ml, SD = 1.62). Unexpectedly, three subjects in the placebo arm had low but detectable levels ranging from 0.2 to 0.3 μg/ml; the remaining seven had undetectable levels (<0.1 μg/ml) at all time points.

Clinical Outcomes

In Tables II and III, relevant changes in clinical outcomes for the VGCV and placebo arms are reported. The MFI-20 total score (primary outcome) revealed a greater improvement in the VGCV arm (−6.15 [SD 12.06]) relative to the placebo arm (−1.10 [SD 5.90]) at 9 months compared to baseline, however, this difference in improvement was not statistically significant (Table II) by ANOVA or MEL regression model (P = 0.114; Fig. 2A). Statistically significant differences between arms were observed in the trajectories of MFI-20 mental fatigue subscore, FSS score and self-reported cognitive function (Fig. 2B–D) in the MEL regression model.

Trajectory of MFI-20 mental fatigue subscore by the MEL regression model indicated larger improvements in the VGCV arm compared to the placebo arm (P = 0.039) despite different but not statistically significant baselines (Fig. 2B; for the
Likelihood of being a responder by the study team (physician-determined responder status prior to unblinding) was assessed by analysis of variance (ANOVA).

**TABLE II. Primary Outcome and Physician-Determined Responder Status Prior to Unblinding**

| Outcome                              | Valganciclovir          | Placebo          | P-value |
|--------------------------------------|-------------------------|-----------------|---------|
| Primary outcome:                     |                         |                 |         |
| change in MFI-20 total score at 9 months* | -6.15 (SD 12.06)  | -1.10 (SD 5.90) | 0.224   |
| Likelihood of being classified as a responder | 7.4                     | 1                | 0.029   |

*M-Values correspond to either Fisher’s exact test or a t-test as appropriate.

MFI-20 mental fatigue subscore of all patients, see Supplementary Material Fig. 1. The benefit in the VGCV arm was observed within the first 3 months post-randomization. The VGCV arm had slightly larger decreases in fatigue as measured by the FSS score than the placebo arm (P = 0.006; Fig. 2C). In the MFI-20 mental fatigue subscore and FSS scores, lower values indicate decreased severity. The VGCV arm also experienced greater increases in self-reported cognitive functioning score over time compared to the placebo arm (1.72 points vs. 0.59 per month, respectively; \( P = 0.025 \); Fig. 2D). VGCV patients were 7.4 times more likely to be classified as a responder than placebo patients (\( P = 0.029 \)).

Of interest, patients in the VGCV and placebo arms appear to have experienced initial worsening of their CFS symptoms within the first 2 months of the initiation of the trial (Fig. 2A–D). This initial worsening was followed by a significant improvement (reflected in the MFI-20 mental fatigue subscore, FSS score, and self-reported cognitive function) in patients in the VGCV arm but was not observed in patients in the placebo arm (Fig. 2B–D).

Based on MEL regression analysis of other clinical scores including CDC CFS SI, HAM-D score, mean self-reported physical functioning assessment, PASAT, or SAQ, the VGCV arm did not have a greater improvement than the placebo arm.

**Biological Outcomes**

**Monocyte and neutrophil counts.** Patients in the VGCV arm experienced a statistically significant decrease in their monocyte counts (\( P < 0.001 \)) followed by a transient increase during the post-treatment follow-up period and an increase in absolute neutrophil counts (\( P = 0.037 \); Fig. 3 A,B).

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Cytokines. Baseline cytokine levels were largely comparable between arms with the exception of two. Baseline cytokine levels for tumor necrosis factor (TNF)-α and interleukin (IL)-17F were higher in the VGCV arm than the placebo arm by factors of 1.30 and 1.42, respectively (\(P = 0.03\) and \(P = 0.04\), respectively). This study interest, however, was whether changes in cytokine levels over the 9-month period differed between the arms. Cytokines that significantly varied over time by treatment status (\(P < 0.05\)) are shown in (see Supplementary Table I). Among the 37 cytokines, two important families or groupings of cytokines have been previously identified as relevant in other studies of immune function in CFS patients [Broderick et al., 2010; Brenu et al., 2011]. Therefore, the impact of VGCV on trajectories of the Th1 (IL-2, IL-12, IFN-γ)- and Th2 (IL-4, IL-5, IL-6, IL-10, IL-13)-associated cytokines over time was assessed. Levels for each “family” (Th1-associated vs. Th2-associated) were derived by summing the levels of the cytokines within each “family.” Th1 and Th2 were not significantly different between the treatment arms at baseline (\(P = 0.361\) and \(P = 0.127\), respectively). A 2.52-fold increase over a 9-month period in Th1-type cytokines in the VGCV arm and a 1.48-fold decrease in the placebo arm (\(P < 0.001\)) was found. No significant difference was observed for Th-2 type cytokines.

Viral IgG antibody titers. Differences for antibody levels between baseline and 6 months measurements (ANOVA) and in their trajectories (MEL regression model) over a 6-month period were not found.

Safety

VGCV was well-tolerated and was not discontinued due to hematologic or hepatic adverse events. Two patients were diagnosed with cancer during the study period: in the VGCV arm, one patient was removed from the study at week 16 due to the diagnosis of ovarian cancer; in the placebo arm, one patient was diagnosed with breast cancer at week 36. These two serious adverse events were deemed unrelated to VGCV.

DISCUSSION

Antiviral therapy for patients with CFS is widely viewed as unnecessary and is unsupported by clinical trials with rigorous study designs. Straus et al. [1988] reported that acyclovir lacked efficacy for the
MEL regression analysis evaluated the effect of outcomes at a specific time point (9 months), whereas investigated the effect of treatment on change in contradictory, they are not. The primary endpoint regression analysis. While these findings may seem trajectories of MFI-20 and FSS scores by MEL were not found, compelling differences were found in month 9 compared to baseline assessed by ANOVA (i.e., change in MFI-20 at month 9 compared to baseline assessed by ANOVA) were not found, compelling differences were found in the trajectories of MFI-20 and FSS scores by MEL regression analysis. While these findings may seem contradictory, they are not. The primary endpoint investigated the effect of treatment on change in outcomes at a specific time point (9 months), whereas MEL regression analysis evaluated the effect of treatment on an entire trajectory of the particular outcome over time. CFS is characterized by a highly fluctuating clinical course; symptoms vary significantly on a daily, weekly, and monthly basis. Therefore, MEL regression models that make use of all data points appear to be more suitable to determine drug effect over placebo in CFS patients.

Patients in the VGCV and placebo arms experienced an initial worsening of their symptoms that has been previously reported by the Stanford CFS group and in a recent clinical trial [Kogelnik et al., 2006; Fluge et al., 2011]. It is possible that the worsening in the placebo arm was due to placebo effect and/or the additional physical/emotional load of frequent visits to the clinical research center. In the VGCV arm, in addition to the factors present in the placebo group, a drug effect may have taken place as well. The pathogenesis of this initial worsening is unclear but it may resemble a Jarisch–Herxheimer-like reaction that has been observed during the initial treatment of certain infections and may be mediated by an immune response to transiently increased circulating microbial antigen(s) [Bryceson, 1976].

In addition, these results suggest possible mechanisms for the clinical benefit observed in the VGCV arm. Monocytopenia, neutrophilia, and differences in Th1-related cytokines over time were associated with the use of VGCV. In immunocompromised patients, ganciclovir (the active drug in VGCV) is a commonly used antiviral against herpesviruses and appears to work by interfering with viral DNA chain elongation [Montoya, 2007; Razonable, 2011]. In immunocompromised patients, ganciclovir frequently contributes to leukopenia and neutrophilia and it is not known to cause monocytopenia or neutrophilia. Monocytes are known to be targeted by HHV-6 [Kondo et al., 2002; Janelle and Flamand, 2006] and can be infected by EBV [Savard et al., 2000; Tugizov et al., 2007; Walling et al., 2007]. Thus, it is possible that in CFS patients HHV-6 and EBV are circulating in peripheral blood within monocytes. Since monocytes are transformed into macrophages in tissues, including the central nervous system, by lowering monocytes in peripheral blood, VGCV may be indirectly decreasing the viral HHV-6/EBV burden in the tissues of CFS patients. In addition, by decreasing influx of infected monocytes (with the capacity of triggering inflammation) into affected tissues, VGCV may be contributing towards the restoration of a more effective and healthier local immune response.” The neutrophilic response observed in the VGCV arm was unexpected but was also validated by the increase of ENA-78, a known neutrophil chemoattractant [Liu et al., 2009]. The antiviral role of neutrophils is becoming increasingly appreciated [Butler et al., 2011] and should not come as a surprise given the tendency of several viruses to cause leukopenia. The trend towards a Th1 cytokine profile in the VGCV arm would reverse the Th2 predominance that has been reported by.

Fig. 3. A: Mean monocyte count in the valganciclovir treatment and placebo (control) arms. B: Mean neutrophil count in the valganciclovir treatment and control arms.
Broderick et al. in CFS patients [Broderick et al., 2010; Brenu et al., 2011]. Significant declines in the HHV-6 or EBV antibody titers at 6 months were not observed, suggesting that their decline requires longer periods of VGCV administration and/or that VGCV primarily works through immunomodulatory properties in CFS patients. Alternatively, it is possible that HHV-6 and EBV antigens were circulating forming immune complexes resulting in artificially depressed levels of antibodies; thus, when VGCV reduced load of circulating Ag, antibody levels may have risen because less antibodies were bound in the immune complexes.

A study by Fluge et al. [2011] suggested that use of rituximab was associated with significant clinical benefit. Monocytopenia in this study and depletion of B cells in theirs, would suggest that excesses or abnormalities in antigen presentation might be a key underlying mechanism in CFS.

This study has several limitations including the small sample size and testing of numerous exploratory hypotheses. However, the randomized, double-blind, placebo-controlled, study design permits that these findings be worthy of further exploration. A limitation, against a stronger VGCV benefit, was that three patients in the placebo arm had detectable ganciclovir levels. Upon thorough and careful questioning, these patients reported no usage of VGCV or ganciclovir outside the study setting.

Findings in this study suggest that clinical trials using longer courses of VGCV and a larger sample size are warranted. They also suggest that outcomes be analyzed by MEL regression models (or similar methods) and that MFI-20 scores/subscores and the FSS score be used among clinical endpoints. Results in this study also support the view that CFS is a real disease that necessitates sound translational research and that can be amenable to medical interventions.

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Registration, investigational new drug application, and role of F. Hoffmann–La Roche Ltd: EVOLVE was registered at ClinicalTrials.gov under identifier #NCT00478465. Authorization to use VGCV was provided under the United States Food and Drug Administration Investigational New Drug Application #77,331 (J.G.M.). F. Hoffmann–La Roche (Roche; Basel, Switzerland) provided financial support for the study as well as VGCV and its matching placebo, approved the study design proposed by the investigators, and performed randomization. Stanford investigators and authors independently executed the study; collected, locked, and analyzed the data; and wrote the manuscript. Adverse events were reported to and investigated by the Stanford IRB and Roche within 24 hr. Prior to unblinding, data were locked and recorded onto three CDs, which are currently kept at Stanford (2 CDs) and Roche (1 CD). The study was conducted in full conformance with the “Declaration of Helsinki,” the “Guidelines for Good Clinical Practice” harmonized Tripartite Guideline (1997), and local laws.

REFERENCES

Brenu EW, van Driel ML, Staines DR, Ashton KJ, Ramos SB, Keane J, Klimas NG, Marshall-Gradishnik SM. 2011. Immunological abnormalities as potential biomarkers in chronic fatigue syndrome/myalgic encephalomyelitis. J Transl Med 9:81–90.
Briggs NC, Levine PH. 1994. A comparative review of systemic and neurological symptomatology in 12 outbreaks collectively described as chronic fatigue syndrome, epidemic enmyasthenia, and myalgic encephalomyelitis. Clin Infect Dis 18:S32–S42.
Broderick G, Fuite J, Kreitz A, Vernon SD, Klimas N, Fletcher MA. 2010. A formal analysis of cytokine networks in chronic fatigue syndrome. Brain Behav Immun 24:1209–1217.
Bryceson AD. 1976. Clinical pathology of the Jarisch–Herxheimer reaction. J Infect Dis 133:696–704.
Buchwald D, Ashley RL, Pearlman T, Kith P, Komaroff AL. 1996. Viral serologies in patients with chronic fatigue and chronic fatigue syndrome. J Med Virol 50:25–30.
Butler LM, Jeffery HC, Wheat RL, Rae PC, Townsend K, Alkharsah KR, Schulz TF, Nash GB, Blackbourn DJ. 2011. Kaposis sarcoma-associated herpesvirus infection of endothelial cells inhibits neutrophil recruitment through an interleukin-6-dependent mechanism: A new paradigm for viral immune evasion. J Virol 85:7321–7332.
Fluge Ø, Bruland O, Risa K, Storstein A, Kristoffersen EK, Storstein A, Kristoffersen EK, Sapkota D, Hess H, Dahl O, Nyland H, Mella O. 2011. Benefit from B-lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. PLoS ONE 6:e26358.
Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. 1994. The chronic fatigue syndrome: A comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med 121:953–959.
Gentile S, Delaroziere JC, Favre F, Sambuc R, San Marco JL. 2003. Validation of the French ‘multidimensional fatigue inventory’ (MFI 20). Eur J Cancer Care (Engl) 12:58–64.

Hickie I, Davenport T, Wakefield D, Vollmer-Conna U, Cameron B, Vernon SD, Reeves WC, Lloyd A. 2006. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: Prospective cohort study. BMJ 333:575–581.

Janelle ME, Flamand L. 2006. Phenotypic alterations and survival of monocytes following infection by human herpesvirus-6. Arch Virol 151:1603–1614.

Katz BZ, Shiraishi Y, Mears CJ, Binns HJ, Taylor R. 2009. Chronic fatigue syndrome after infectious mononucleosis in adolescents. Pediatrics 124:189–193.

Kerr JR, Bracewell J, Laing I, Matthey DL, Bernstein RM, Bruce IN, Tyrrell DA. 2002. Chronic fatigue syndrome and arthralgia following parvovirus B19 infection. J Rheumatol 29:595–602.

Klimas NG, Broderick G, Fletcher MA. 2012. Biomarkers for chronic fatigue. Brain Behav Immun 26:1202–1210.

Komaroff AL. 2006. Is human herpesvirus-6 a trigger for chronic fatigue syndrome? J Clin Virol 37:S39–S46.

Komaroff AL, Cho TA. 2011. Role of infection and neurologic dysfunction in chronic fatigue syndrome. Semin Neurol 31:325–337.

Kondo K, Kondo T, Shimada K, Amo K, Miyagawa H, Yamanishi K. 2002. Strong interaction between human herpesvirus 6 and peripheral blood monocytes/macrophages during acute infection. J Med Virol 67:364–369.

Lerner AM, Beqaj SH, Deeter RG, Dworkin HJ, Zervos M, Chang CH, Fitzgerald JT, Goldstein J, O’Neill W. 2002. A six-month trial of valganciclovir in the Epstein–Barr virus subset of chronic fatigue syndrome: Improvement in left ventricular function. Drugs Today 38:549–561.

Lerner AM, Beqaj SH, Fitzgerald JT. 2007. Valacyclovir treatment in Epstein–Barr virus subset chronic fatigue syndrome: Thirty-six months follow-up. In Vivo 21:707–713.

Lerner AM, Beqaj SH, Fitzgerald JT, Gill K, Gill C, Edington J. 2010. Subset-directed antiviral treatment of 142 herpesvirus patients with chronic fatigue syndrome. Virus Adapt Treat 2:47–57.

Levine PH. 1994. Summary and perspective: Epidemiology of chronic fatigue syndrome. Clin Infect Dis 18:557–560.

Lin JM, Brimmer DJ, Maloney EM, Nyarko E, Behue R, Reeves WC. 2009. Further validation of the multidimensional fatigue inventory in a US adult population sample. Popul Health Metr 7:18–30.

Liu GN, Shi HZ, Xie ZH, Shen HH, Huang HQ, Deng JM, Liang QL, Wu YB. 2009. Epithelial neutrophil-activating peptide-78 recruits neutrophils into pleural effusion. Eur Respir J 34:184–190.

Montoya JG. 2007. Improving the tools in the fight against cytomegalovirus or strengthening David to defeat Goliath. Curr Opin Infect Dis 20:397–398.

Patnaik M, Komaroff AL, Conley E, Ojo-Amaize EA, Peter JB. 1995. Prevalence of IgM antibodies to human herpesvirus 6 early antigen (p41/p38) in patients with chronic fatigue syndrome. J Infect Dis 172:1364–1367.

Razonable RR. 2011. Antiviral drugs for viruses other than human immunodeficiency virus. Mayo Clin Proc 86:1009–1026.

Reeves WC, Wagner D, Nisenbaum R, Jones JF, Gurbaxani B, Solomon L, Papanicolaou DA, Unger ER, Vernon SD, Heim C. 2005. Chronic fatigue syndrome—A clinically empirical approach to its definition and study. BMC Med 3:19–28.

Savard M, Belanger C, Tardif M, Gourde P, Flamand L, Gosselin J. 2000. Infection of primary human monocytes by Epstein–Barr virus. J Virol 74:2612–2619.

Straus SE, Tosato G, Armstrong G, Lawley T, Preble OT, Henle W, Davey R, Pearson G, Epstein J, Brus I, Blaese RM. 1985. Persisting illness and fatigue in adults with evidence of Epstein–Barr virus infection. Ann Intern Med 102:7–16.

Straus SE, Dale JK, Tobi M, Lawley T, Preble O, Blaese RM, Hallahan C, Henle W. 1988. Acyclovir treatment of the chronic fatigue syndrome. Lack of efficacy in a placebo-controlled trial. New Engl J Med 319:1692–1698.

Tobi M, Morag A, Ravid Z, Chowers I, Feldman-Weiss V, Michaeli Y, Ben-Chetrit E, Shalit M, Knobler H. 1982. Prolonged atypical illness associated with serological evidence of persistent Epstein–Barr virus infection. Lancet 1:61–64.

Tugizov S, Herrera R, Veluppillai P, Greenspan J, Greenspan D, Palefsky JM. 2007. Epstein–Barr virus infection of Langerhans cell precursors as a mechanism of oral epithelial entry, persistence, and reactivation. J Virol 81:7249–7268.