Evaluation of Approved Antivirals for Inhibition of Xenotropic Murine Leukemia-Related Virus (XMRV) in Cell-Based Assays

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Abstract

Xenotropic murine leukemia-related virus (XMRV), a retrovirus discovered in 2006, has been controversially associated with human prostate cancer and chronic fatigue syndrome (CFS). XMRV nucleic acids or proteins are found in 25% of prostate cancers and in 68% of chronic fatigue syndrome patients, and in less than 4-5% of normal controls, suggesting an association between the virus and human disease. To date there is no effective treatment for CFS. Twenty three drugs approved for use in humans were evaluated against XMRV replication in vitro. Drugs used to treat HIV-1 infection, as well as compounds used to treat other virus infections, were evaluated. Published literature indicates little similarity between HIV-1 and XMRV proteins. 28% homology at the amino acid level of protease, 17% homology with RT, and 14% homology with integrase; making it difficult to predict which anti-HIV agents may be effective against XMRV. An in vitro assay utilizing PG-4 cells infected with XMRV collected from 22Rv1 human prostate cancer cells was developed to measure inhibition of virus replication. Several drugs from each major class of antiviral agents, nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTI, NRTI and NNRTI), integrase inhibitors (I), and protease inhibitors (PI) were evaluated. Efficacy and toxicity data for the approved antiviral agents will be reported, as well as evaluation of combined antiviral agents in the anti-XMRV assay.

Anti-XMRV Cytotoxicity Assay Method

Cell Preparation: PG-4 cells (feline astrocytes, ATCC catalog #CRL-2303) cultured for less than 15 passages in McCoy’s 5a medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 Unit penicillin and 100 µM streptomycin at 1 x 10^5 cells per ml, and added to flat bottom microtiter plates in a volume of 150 µl for overnight incubation at 37°C/5% CO2.

Compound Dilution: Medium was removed from the cell monolayer and 100 µl of ZK concentrations of compound-containing PG-4 cell culture medium was transferred to the 96-well microtiter plate.

Virus Preparation: The XMRV virus was collected from the cell-free supernatant of 22Rv1 human prostate cancer cells (ATCC catalog #CRL-2505). A pelleted aliquot of virus was removed from the freezer (-40°C) and allowed to thaw in a biological safety cabinet. The virus was diluted into PG-4 cell culture medium such that the amount of virus added to each well in a volume of 100 µl is the amount determined to yield 85 to 95% cell killing at six days post infection.

XTT-Based Evaluation of Efficacy and Toxicity: Inhibition of virus induced cytopathic effects (CPE) was quantified by measuring the reduction of the tetrazolium dye XTT. XTT solution was prepared daily as a stock of 1 mgw, in PBS with 0.6 µg/ml of PBS added. Following 4 hours incubation at 37°C, the microtiter plates were read at 450 nm (650 nm reference wavelength) with a Molecular Devices SpectroMax Plus 384 well plate format spectrophotometer.

Data Analysis: Raw data was collected from the Softmax Pro 4.6 software and imported into a Microsoft Excel spreadsheet for four parameter curve fitting analysis. Using Microsoft Excel, EC₅₀ and TC₅₀ (50% and 90% inhibition of virus replication), TC₅₀ and TC₉₀ (90% and 90% reduction in cell viability) and a therapeutic index (TI, TC₅₀/EC₅₀ and TC₉₀/EC₉₀) were calculated.

Summary

- Xenotropic murine leukemia-related retrovirus (XMRV) is a gammaretrovirus that may be associated with prostate cancer and/or chronic fatigue syndrome.
- ImQuest BioSciences has standardized an in vitro PG-4 cell-based cytotoxicity assay for the evaluation of antiviral compounds versus XMRV.
- Of the 23 antiviral compounds evaluated for efficacy versus XMRV, FDA-approved anti-HIV drugs AZT, tenofovir disoproxil fumarate (TDF) and raltegravir (RAL) were potent inhibitors of XMRV.
- Anti-XMRV combination therapy evaluations determined AZT, TDF or RBV in combination with RAL yielded synergistic interactions. Other combinations were additive to moderately synergistic.
- If XMRV is determined to be a causative agent of human disease, anti-HIV drugs may be useful in inhibiting virus replication.

References