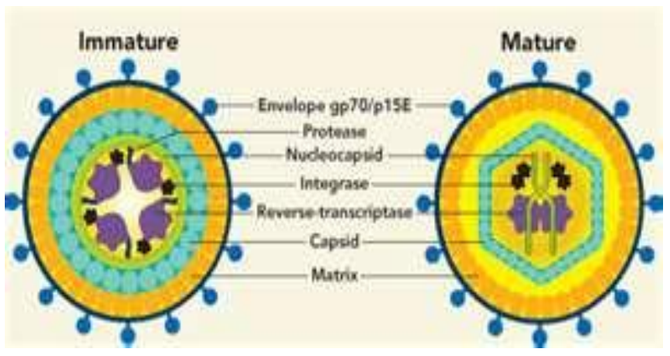
Evaluation of Approved Antivirals for Inhibition of Xenotropic Murine Leukemia-Related Virus (XMRV) in Cell-Based Assays

Tracy L. Hartman and Robert W. Buckheit, Jr. ImQuest BioSciences, Frederick, MD, USA

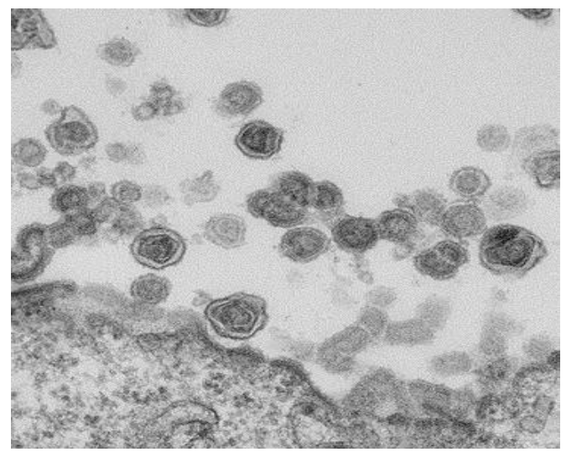
Abstract

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 acid or proteins are found in 27% of prostate cancers and in 68% of chronic fatigue syndrome patients, and in less than 4–6% 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 antiretroviral agents: nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTI, NtRTI and NNRTI), integrase inhibitors (II), 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.

XMRV Virion

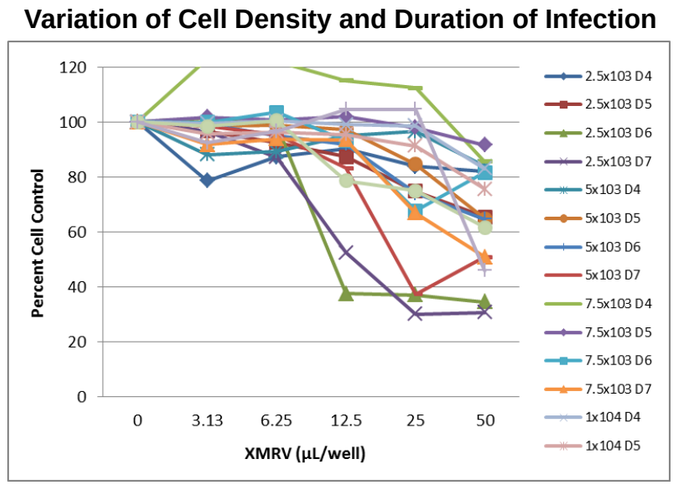


Electron Micrograph of XMRV



OPTIMIZATION OF ASSAY CONDITIONS
Cell Type: PG-4 Versus DU145
Cell Density
Virus Collection from 22Rv1 Cells
Length of Infection
Virus Titer
Assay Endpoint: XTT, NR, RT Incorporation

Variation of Cell Density and Duration of Infection



Anti-XMRV Cytoprotection Assay Method

Cell Preparation: PG-4 cells (feline astrocytes; ATCC catalog #CRL-2032) cultured for less than 15 passages were resuspended in McCoy's 5A medium supplemented with 10% heat inactivated fetal bovine serum, 2 mmol/L L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin at 1 x 10^5 cells per mL and added to flat bottom microtiter plates in a volume of 100 µl for overnight incubation at 37ºC/5% CO_2.

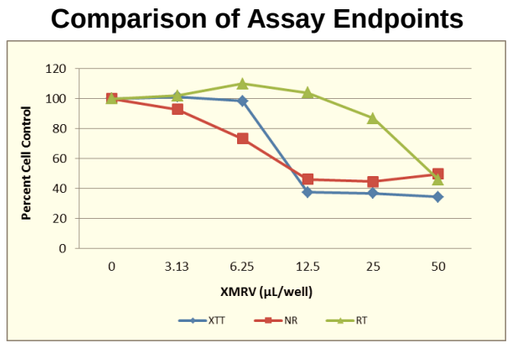
Compound Dilution: Medium was removed from the cell monolayer and 100 µL of 2X concentrations of compound-containing PG-4 cell culture medium was transferred to the 96-well microtiter plate.

Virus Preparation: The XMRV virus was collected from the cell-free supernatant of 22Rv1 human prostate cancer cells (ATCC catalog #CRL-2505). A pretitered aliquot of virus was removed from the freezer (-80ºC) 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 mg/mL in PBS with 0.6 µg per mL of PMS added. Following 4 hours incubation at 37°C, the microtiter plates were read at 450 nm (650 nm reference wavelength) with a Molecular Devices SpectraMax Plus 384 96 well plate format spectrophotometer.

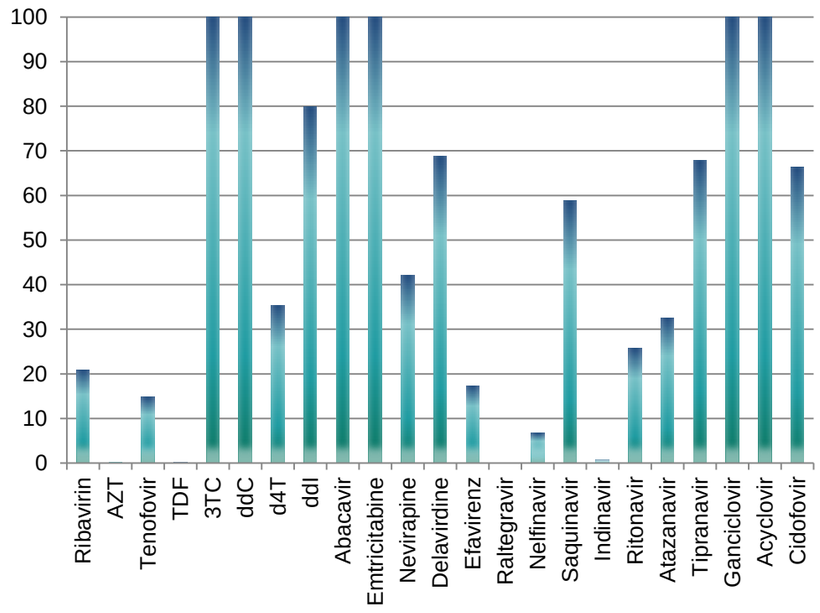
Data Analysis: Raw data was collected from the Softmax Pro 4.6 software and imported into a Microsoft Excel XLfit4 spreadsheet for four parameter curve fit analysis. Using Microsoft Excel, EC_{50} and EC_{90} (50% and 90% inhibition of virus replication), TC_{50} and TC_{90} (50% and 90% reduction in cell viability) and a therapeutic index (TI, TC_{50}/EC_{50} and TC_{90}/EC_{90}) were calculated.

Comparison of Assay Endpoints

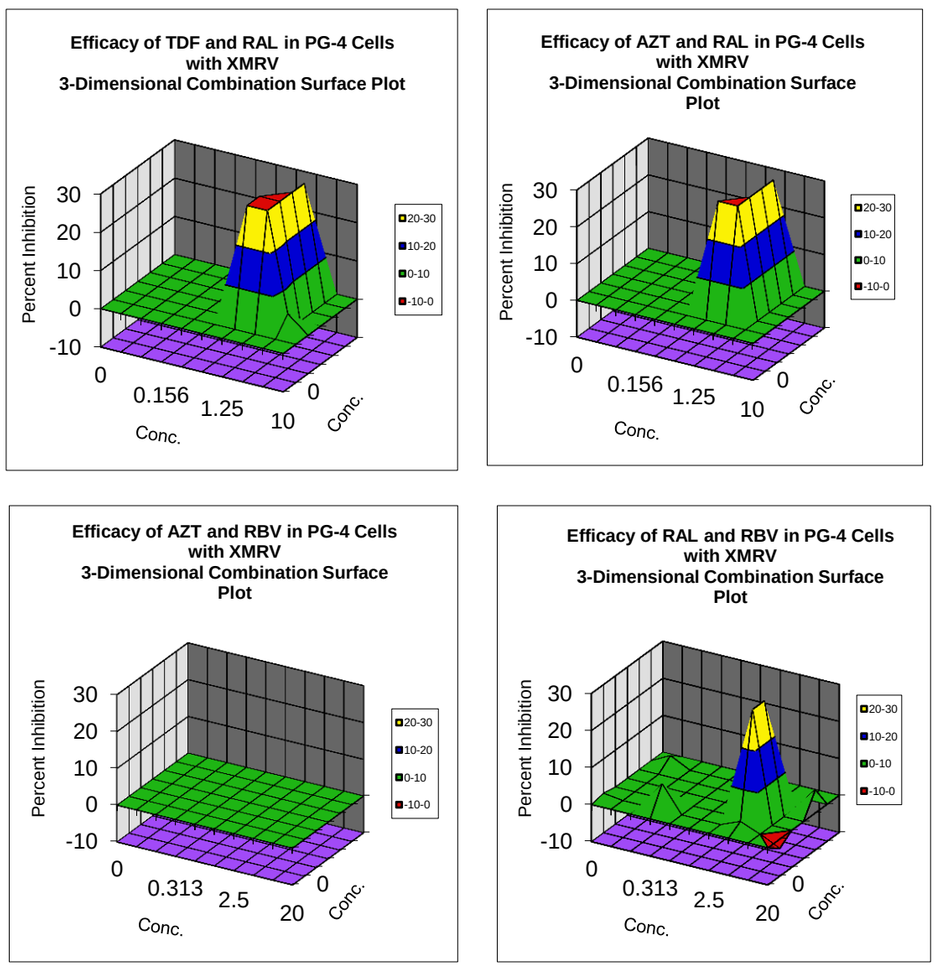


Antiviral Activity of Known HIV Inhibitors Versus XMRV

Compound	EC50 (µM)
Ribavirin	20.9
AZT	0.2
Tenofovir	14.8
TDF	0.3
3TC	>100
ddC	>100
d4T	35.4
ddI	79.9
Abacavir	>100
Emtricitabine	>100
Nevirapine	42.2
Delavirdine	68.9
Efavirenz	17.4
Raltegravir	0.01
Nelfinavir	6.8
Saquinavir	58.8
Indinavir	0.8
Ritonavir	25.8
Atazanavir	32.6
Tipranavir	67.9
Ganciclovir	>100
Acyclovir	>100
Cidofovir	66.3



Combination Therapy Evaluations



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 cytoprotection 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, tenfovir 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

Urisman, A. et al. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog. 2: e25. 2006.

Schlaberg, R. et al. XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially in high-grade tumors. PNAS 106: 16351-6. 2009.

Hohn, O. et al. Lack of evidence of xenotropic murine leukemia-related virus (XMRV) in German prostate cancer patients. Retrovir 6: 92. 2009.

Lombardi, V. et al. Detection of an infectiious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science 326: 585-9. 2009.

Erlwein, O. et al. Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome. PLoS ONE 5: e8519. 2010.

Singh, I. et al. Raltegravir is apotent inhibitor of XMRV, a virus implicated in prostate cancer and chronic fatigue syndrome. PLoS ONE 5(4): e9948. 2010.

Li, Z. et al. PG-4 cell plaque assay for xenotropic murine leukemia virus. J Virol M 81: 47-53. 1999.

Knouf, E. et al. Multiple integrated copies and high level production of the human retrovirus XMRV from 22Rv1 Prostate Carcinoma Cells. J Virol 83(14): 7353-6. 2009