

# XMRV: Virological, immunological and clinical correlations in a patient with Chronic Lymphocytic Leukemia

Abstract #109

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## Background

XMRV has recently been identified in patients with prostate cancer and Chronic Fatigue Syndrome (CFS). CFS patients have an increased incidence of lymphoproliferative malignancy compared to the normal population. While the incidence rate of non-Hodgkin's lymphoma is 0.02% in the United States, nearly 5% of CFS patients developed the disease. To address this, we identified several XMRV infected CFS patients who subsequently developed Chronic Lymphocytic Leukemia (CLL) and Mantle Cell Lymphoma (MCL). Treatment of XMRV associated neoplasia has not been previously reported. However, HTLV-1 associated T-cell lymphoma/leukemia responds to zidovudine (AZT) and IFN  $\alpha$ . In addition, multiple human tumor cell lines including breast cancer show growth inhibition and apoptosis when exposed to AZT. Several groups have reported inhibition of XMRV by FDA approved antiretrovirals including AZT, raltegravir, and tenofovir in cell culture. Our study investigated XMRV associated malignancy other than prostate cancer, including CLL and MCL and the effect of antiretroviral treatment on the various parameters of a patient with CFS and CLL.

## Methods

•Peripheral blood mononuclear cells were isolated, and the CLL cells shown to be infected by intracellular staining of antibodies to XMRV Gag and Env and infectious virus isolated from blood by methods developed in our lab (Lombardi et. Al. Science 2009).

•Cytokine profiles were determined on plasma by multiplex analysis of 30 cytokines, chemokines, and growth factors on a Luminex platform.

•Immune cell phenotyping was performed by multiparameter flow cytometry on an LSR2 flow cytometer.

•Absolute lymphocyte counts (ALC) were determined on a LH 750 Coulter Analyzer with a 200 cell manual differential.

•Trisomy 12 percentages were determined by FISH (Quest Diagnostics) and multiplied by the ALC to determine absolute numbers.

•CD19 counts were determined by flow cytometry (Quest Diagnostics)

## Results

•CFS patients who subsequently developed  $\gamma\delta$  T cell clonalities and cancer were tested for XMRV infection (Table 1).

Cytokine signature of patients with developed  $\gamma\delta$  T cell clonalities and cancer (Figure 1)

•B Cells developed from patients with CLL or MCL express XMRV and these cell lines produce infectious XMRV (Figure 2A,B).

•By flow cytometry, the immunophenotype was compatible with MCL and CLL respectively (i.e. CD5+CD20+CD23-FMC7+) and (CD5+ CD20+ CD23+FMC7). (Table 2)

•A patient with a 3 year history of untreated CLL and symptoms consistent with CFS was identified as having XMRV plasma viremia by the methods of Lombardi et al. (not shown) and his lymphocytes were positive for XMRV as measured by the DERSE assay. (Figure 2B). The first 150 days of treatment showed a stable ALC and improvement in the CD19 cell count and in symptoms of CFS. At day 100 the trisomy 12 clone was reduced but subsequently has been variable (Figure 5). Studies are ongoing to confirm the trisomy 12 result and the mechanisms involved.

•The infectious XMRV viral load and the inflammatory cytokine-chemokine signature improved in parallel with the ALC and trisomy 12 clone following initiation of antiretroviral therapy (Figures 3, 4 and 5 respectively).

ID#	XMRV status	Clonal TCR $\gamma$	Lymphoma/cancer
1103	positive	positive	MCL
1109	positive	negative	Thymoma
1118	positive	negative	myelodysplasia
1125	positive	Positive + IGH	MCL
1186	positive	positive	Lymphoma
1199	positive	positive	Previous Lymphoma
1150	positive	positive	Lymphoma
1320	Not tested	Not tested	Thymoma
1321	Not tested	Not tested	MCL
1174	positive	positive	Thymoma
1205	positive	Not tested	lymphoma
1172	positive	positive	MCL
1135	positive	positive	suspicious
1204	positive	Positive + IGH	suspicious
1113	positive	positive	CLL
1322	Not tested	Not tested	MCL
1181	positive	Not tested	CLL
1188	positive	positive	CLL
1189	positive	positive	MCL
1190	positive	positive	suspicious

Table 1. CFS patients who subsequently developed  $\gamma\delta$  T cell rearrangements and cancer.

## Results Continued

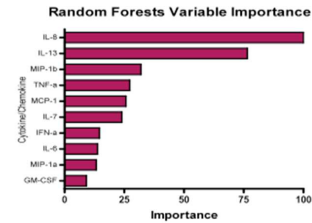


Figure 1. Random Forest generated Cytokine and Chemokine pattern consistent with XMRV infection. Red bars indicated relative importance of each cytokine to delineate CFS related XMRV infection. The 10 most significant are shown on a panel of 25 different cytokines and chemokines measured on a Luminex platform.

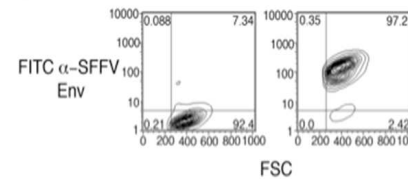


Figure 2A. Flow cytometry analysis of B cells from CLL and MCL patients. Cells are visualized by intracellular staining with FITC conjugated anti SFFV envelope antibodies.

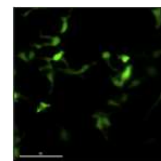


Figure 2B (above) Infection was transferred from patient derived cell lines to DERSE indicator cell line, in which GFP is expressed by reverse transcriptase activity. Table 2 (right) surface marker phenotype of cell lines derived from CFS patients who subsequently developed CLL and MCL

Marker	Mino	WPI-1282	WPI-2119	WPI-2767
CD5	+	+	+	(low)
CD23	-	-	+	+
CD19	+	+	+	+
CD20	+	+	+	+
FMC7	+	+	+	+
CD3	-	-	-	-
CD4	-	-	-	-
CD7	-	-	-	-
CD8	-	-	-	-
CD10	-	-	-	-
CD38	+	+	+	+
CD45	+	+	+	+
CD56	-	-	-	-
CD122	-	-	-	-
HLA-DR	+	+	+	+
Lambda	+	+	+	(low)

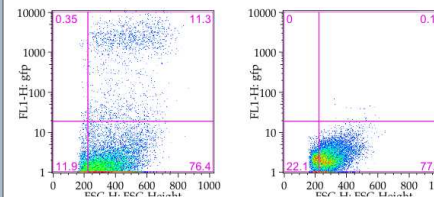


Figure 3. Flow cytometry analysis of virus infected indicator cells pre and post antiretroviral treatment.

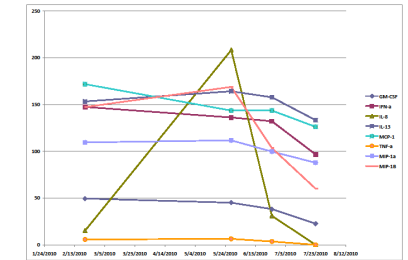


Figure 4. Time course showing the resolution of inflammatory cytokine and chemokine profile following antiretroviral therapy

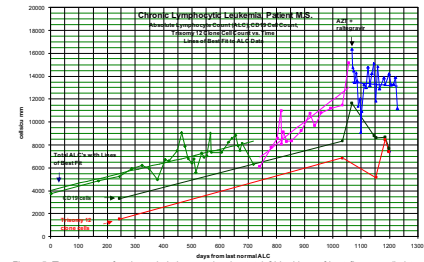


Figure 5. Time course of antiretroviral therapy showing total CLL. Lines of best fit are applied to data to estimate doubling times.

## Conclusions

A patient with CFS and CLL with adverse prognostic factors was shown to have XMRV in plasma and CLL cells. Within the first 150 days of treatment with AZT and raltegravir, he showed multiple benefits simultaneous with disappearance of infectious XMRV. These findings suggest that XMRV is etiological for both the CLL and CFS and that virus-directed treatment was beneficial in this patient. Further CLL patients should be studied especially as CLL has been statistically associated with an increased risk for other neoplasia. Questions to be answered are what neoplasms are associated with XMRV, will existing antiretrovirals have antineoplastic activity in these neoplasms and what is the optimal combination of antiretroviral drugs.

## Author Information

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