XMRV: Virological, immunological and clinical correlations in patients with Chronic Lymphocytic Leukemia and Mantle Cell Lymphoma

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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 does not respond to zidovudine (AZT) and IFN α. 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 additional XMRV associated malignancy, including 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 heparinized plasma by multiplex analysis of 30 cytokines, chemokines, and growth factors on a Luminex platform.

•Immune cell phenotyping was performed by multi-parameter flow cytometry on an LSR2 flow cytometer.

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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 circulating clonal populations of $\gamma\delta$ T cells 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 are positive for XMRV as measured by the DERSE assay. (Figure 2B)

•Inflammatory cytokine/chemokine signature, Infectious XMRV viral load, and Tumor markers improve and/or return to normal following initiation of antiretroviral therapy (Figure 4 , 5. and 6 respectively).

•Decrease in Infectious XMRV viral load flowing 3 months of antiretroviral therapy in CLL patient as measured by the DERSE assay (Figure 3)

ID#	XMRV status	Clonal TCRγ	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

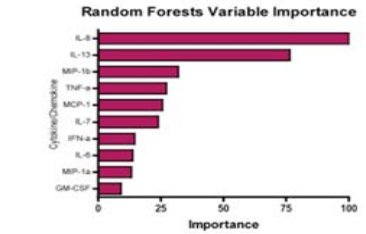

Figure 1. Random Forest generated Cytokine and Chemokine pattern consistent with XMRV infection. Red bars indicated relative importance of each cytokine to delineate CFS related XMRV infection. The 10 most significant are shown from a panel of 25 different cytokines and chemokines measured on a Luminex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

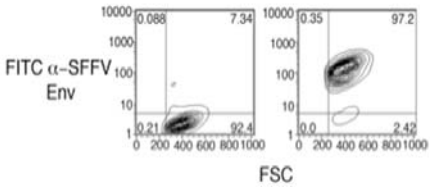

Figure 2A. Flow cytometry analysis of B cell line developed from CLL and MCL patients . Cells are visualized by intracellular staining with FITC conjugated anti SFFV envelope antibodies.

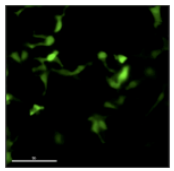

Figure 2B (above) Infection was transferred from patient derived cell lines to DERSE indicator cell line, in which GFP is expressed by reverse transcriptase activity.

Table 2 (right) surface marker phenotype of cell lines from derived from CFS patients who subsequently developed CLL and MCL.

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

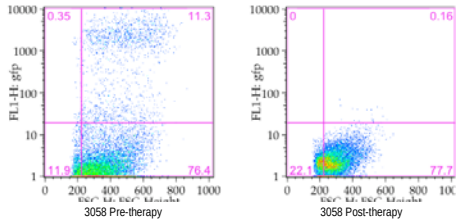

Figure 3. Flow cytometry analysis of virus infected pre and post antiretroviral treatment.

Results Continued

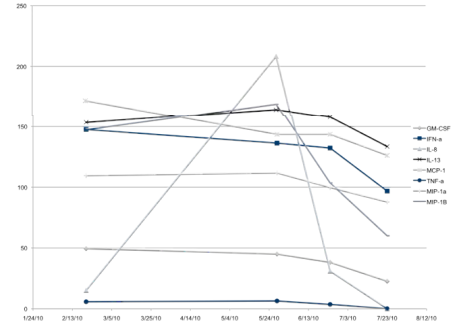

Figure 4. Time course showing the resolution of inflammatory cytokine and chemokine profile following antiretroviral therapy

Figure 5. Time course of antiretroviral therapy showing total CLL numbers decrease

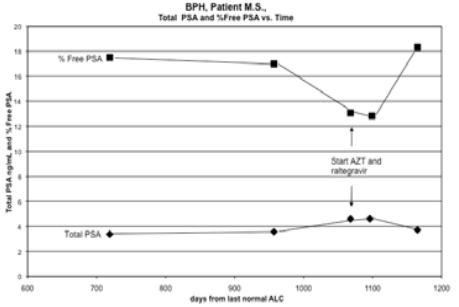

Figure 6. Time course showing the decrease in total PSA following antiretroviral therapy

Conclusions

◆These data associate XMRV with MCL and CLL for the first time.
◆XMRV could be detected in the tumor cells isolated from patients
◆MCL and CLL cell lines developed from patients express XMRV
◆ Decrease in infectious XMRV, inflammatory cytokine profile, and tumor markers correlate with clinical improvement following antiretroviral therapy.