

# **IDENTIFICATION OF NOVEL BIOMARKERS IN CENTRAL NERVOUS SYSTEM (CNS) LEUKEMIA**

**Abstract**

**TESSY XAVIER**

**Amrita Vishwa Vidyapeetham University**

**September 2014**

Acute lymphoblastic leukemia accounts for significant percentage of all leukemias. Approximately 3% of patients were diagnosed with central nervous system (CNS) leukemia at presentation and many develop CNS leukemia later, if CNS directed therapy is not administered. Therefore, prophylactic chemotherapy and cranial irradiation is the norms for preventing and treating CNS leukemia. The grievous side effects of CNS directed prophylactic therapy necessitates the identification of biomarkers specific to CNS leukemia which may facilitates to prevent or predict the progress of the disease and in the adept analysis and designing of better treatment options. In pursuit of a novel biomarker for CNS leukemia, we have adopted the body fluid based far-western clinical proteomics approach based on the possible anticipated interaction between molecules of cerebrospinal fluid (CSF) with proteins of lymphoblastic origin. Though there is less information regarding the role of molecular interaction in leukemic cell infiltration to CNS, the reported CCR7-CC119 interaction in T-ALL and over expression of adhesion promoting molecules such as ICAM-1, RAC2 and AEP in B-ALL provides a positive hint for studying the importance of molecular interaction to identify a biomarker. The 2D profiled B cell lymphoblastic proteins from JM-1 cells were allowed to interact with the biotinylated CSF samples from the same ALL patients at different time points like presentation, remission and relapse in a sequential manner. A comparison of sequential CSF sample reactivity to lymphoblastic proteins at different stages of the disease (diagnosis-remission-relapse-remission) between patients who are heterogeneous for age, sex, cytogenetics, blasts percentage etc. revealed a consistent pattern with respect to the appearance/disappearance of CSF reactivity to certain lymphoblastic proteins. The proteins that showed consistent pattern of reactivity to CSF molecules were PFDN5 $\alpha$ , CIP29, ECH1 and PRDX6. These identified molecules have been shown to have potential involvement in tumorigenesis and in particular proteins like PFDN5 $\alpha$  and CIP29 has direct involvement in leukemia. The protein PFDN5 $\alpha$  showed low reactivity to CSF with CNS leukemia is more reactive towards remission CSF samples on 2D blots. On the contrary,

CIP29, ECH1 and PRDX6 are more reactive to presentation/relapse with CNS involvement CSF samples and less reactive when the patient is in remission. The CSF reactivity to these identified proteins was further validated using purified proteins. Among these different proteins, only PFDN5 $\alpha$  showed a consistent CSF reactivity pattern similar to that seen on 2D far-western blot. Quantification and further appraisal of CSF reactivity to PFDN5 $\alpha$  by ELISA confirms the results on 2D and 1D platforms and showed the usefulness of this methodology for prognostic assessment of patient's clinical status. Based on the results obtained from the current study with limited number of CNS leukemia patients, the CSF reactivity value of 0.05 towards PFDN5 $\alpha$  was selected as cut off value to indicate the prognosis. Noteworthy that irrespective of age, sex and the blast levels in the patients, remission samples with good response to therapy and clinically normal status gives a reactivity value of >0.05 and in all the CNS diseased samples, the value is <0.05. Based on our results, CSF reactivity >0.05 indicates a positive prognosis and <0.05 is an indicator for high risk for developing CNS disease. Although the results are promising towards a prognostic biomarker, additional analysis using more patient samples is required to refine the cut off value to substantiate further the significance of this finding. In conclusion, the consistency of CSF reactivity to PFDN5 $\alpha$  at different time points like presentation, remission and relapse between ALL patients has immense potential to serve as prognostic marker for the prediction of CNS involvement during times at which the patients may not show any infiltration as judged by the current CSF cytology analysis but succumb to disease later on. In such situation, an early warning by way of low reactivity to PFDN5 $\alpha$  is of immense help for the patient to undergo therapy in a risk-based judicious way compared to general prophylactic intrathecal CNS therapy which is given to all patients irrespective of CNS leukemia. Thus, this work has identified an important molecule that has reported role in controlling the oncogene c-Myc expression in leukemia and show differential reactivity during CNS leukemia and remission. Moreover, its low reactivity even when no infiltration of lymphoblasts was seen into the CNS is indicative of the ensuing pathological changes that could lead to development of CNS leukemia.