



## 2007 Research Grant Program Winning Abstract

### A New Prognostic Factor and Potential Treatment of Chronic Lymphocytic Leukemia (CLL)

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Chronic lymphocytic leukemia (CLL) is characterized by accumulation of quiescent B-lymphocytes in the peripheral blood. Maintenance of CLL B cells in G<sub>0</sub> is believed to be dependent on active mechanisms. We have cloned a serine protease, dipeptidyl peptidase 2 (DPP2), which is essential for the survival of quiescent mammalian cells. We have designed an assay where introduction of a small molecule inhibitor into the intact cell blocks DPP2. If the cell is in G<sub>0</sub>, this treatment induces apoptosis, which is detected by BD Annexin V APC staining intensity on a BD FACSCalibur or BD LSR II. Using this assay system, we have analyzed 60 random B-CLL samples and have observed that only 60% were sensitive to apoptosis induction (S-CLL). We further found that CLL cases resistant to apoptosis (R-CLL) expressed higher levels of ZAP-70 mRNA and exhibited a less favorable course of the disease than S-CLL. In a more recent screen of a pilot cohort of molecularly characterized CLL patients (obtained from DFCI, Harvard Medical School) we determined that R-CLL carried unmutated BCR heavy chain immunoglobulin genes (IgVH) and expressed ZAP-70 protein. On the other hand, the frequency of IgVH mutations was higher and ZAP-70 expression was lower in S-CLL. We now propose to verify these results in a larger cohort of 200 CLL samples (again available to us from DFCI), allowing extensive statistical analysis. As there is significant variability in assessing ZAP-70 expression between laboratories, we are planning to repeat this measurement by means of the BD ZAP-70 intracellular staining kit.

Confirmation of our pilot results will lead to the development of a new, inexpensive prognostic kit for CLL by testing for susceptibility to DPP2 inhibition-induced apoptosis. This kit could be readily used in a clinical lab and would replace the cumbersome and expensive analysis for IgVH mutational status that is currently the gold standard for CLL prognosis.

We will further investigate the underlying molecular mechanism that leads to differential ability of the two CLL subsets to undergo apoptosis in response to DPP2 inhibition. Our working hypothesis is that they reside in slightly different stages of cell cycle arrest. It is well documented that quiescence is maintained in B cells by a tonic BCR signal, resulting in a stochastic equilibrium between triggering (phosphorylation of Syk) and negative regulation (dephosphorylation and/or degradation of Syk). According to this model, the majority of B cells from patients with S-CLL rests in G<sub>0</sub> through the tonic kinase activity of Syk, and, thus, is susceptible to DPP2-inhibition induced apoptosis. On the other hand, we propose that R-CLL have exited G<sub>0</sub> and entered an early G<sub>1</sub> stage due to the additional tonic kinase activity of ZAP-70. We will directly test this hypothesis by examining the constitutive phosphorylation level of Syk and ZAP-70 in the two CLL subsets. For this purpose, the CLL B cells will have to be highly purified, as T cells express ZAP-70. This will be achieved by negative selection, using the BD IMag human B lymphocyte enrichment set. Protein levels of Syk and ZAP-70 and their phosphorylated forms will be assessed by Western blotting and probing with kinase and



phosphorylation-specific BD antibodies, respectively. In addition, the BD CBA will be used, because this assay requires minimal protein and allows quantitative measurements. Phospho- and total ZAP-70 and Syk CBA Flex Sets are available from BD to perform these experiments.

Our preliminary results fit this scenario; namely, we found that p27Kip1, a cell cycle inhibitor, is upregulated in the S-CLL samples, while c-Myc, indicative of cellular activation, is increased in the RCLL.

To confirm these data, we will analyze the mRNA and protein levels and phosphorylation status of the following proteins in the two CLL subsets: DPP2, AKT, p27Kip1, c-Myc, cyclin D1 and D2. For this purpose, antibodies available from BD will be conjugated to beads, using the BD Functional Bead Conjugation Buffer Set. In addition, since no markers are available to distinguish G0 from early G1 in B cells, we will test whether R-CLL samples have a comparatively more activated phenotype than S-CLL, as defined by expression of the following cell surface markers: CD23, CD25, CD69, and CD71 (all BD cell surface markers).

If the kinase activity of ZAP70 is responsible for the R-CLL phenotype that we have observed, then interfering with ZAP-70 expression may render these cells sensitive to DPP2 inhibition induced apoptosis. In this regard, the finding that the Hsp90 chaperone plays an important role in the stabilization of ZAP-70 is of special interest. Thus, we will investigate whether inhibition of Hsp-90 will restore susceptibility to apoptosis in R-CLL. In addition, ZAP-70 specific kinase inhibitors will be tested for phenotype reversal of R-CLL into SCLL.

All treated cells will be tested for changes in expression of the various activation markers, defined above. If our model is correct, this would allow the design of new treatment strategies for the more aggressive CLL subset that has worse prognosis. BD Biosciences products are indispensable for the successful execution of the proposed analyses.

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