P138

HIGH CD54 ADHESION MOLECULE EXPRESSION PREDICTS A SHORT PROGRESSION FREE SURVIVAL IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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CD54/intercellular adhesion molecule-1 (ICAM-1) is generallly found at lower levels in B-cell chronic lymphocytic leukemia (B-CLL) and its higher expression has been already associated with marked organomegaly and poor prognosis (Hjalmar, 2002). Furthermore, it has been clearly demonstrated that ZAP-70$^+$ CLL shows a rapid disease progression and an inferior overall survival (Crespo, 2003; Del Principe, 2006). The primary aims of our research were: 1) to correlate CD54 with other recent biologic prognostic factors, 2) to determine progression-free survival (PFS) upon CD54 expression; 3) whether CD54 could predict varied outcome within ZAP-70$^+$ and ZAP-70 negative subgroups; and finally, 4) to test the independent value of CD54 as prognosticator. Therefore, we investigated 202 pts, median age 65 years (range 37-87), 105 males and 97 females. With regard to modified Rai stages, 64 pts had a low stage, 132 an intermediate stage and 6 a high stage. CD54 was determined by multicolor flow cytometry fixing a cut-off value of 30%. CD54$^+$ B-CLL pts were 131/202 (65%). CD54>30% was associated with an intermediate/high Rai stage (p=0.008), with marked lymphadenopathy and/or splenomegaly (p=0.00001) and with beta-2 microglobulin >2.2 mg/dL (p=0.0006). Also, high CD54 and IgVH unmutated status (<2%) were correlated (25/29; p=0.001). Furthermore, significant associations were found either between higher CD54 and higher ZAP-70 (59/79; p=0.01) or between higher CD54 and higher soluble CD23 (sCD23) levels (48/59; p=0.002). A significant shorter PFS was observed in CD54$^+$ pts (22% *vs.* 72% at 14 years; p<0.00001, Figure 1) as well as in ZAP-70$^+$ pts (15% *vs.* 63% at 10 years; p<0.00001). To further explore the prognostic impact of CD54, we investigated its expression within ZAP-70$^+$ (79 pts) and ZAP-70 negative (123 pts) subsets. As a matter of fact, CD54$^+$ pts showed a shorter PFS both within the ZAP-70$^+$ subset (13% *vs.* 22% at 10 years; p=0.03) and within the ZAP-70 negative subset (42% *vs.* 91% at 14 years, p=0.0001). In multivariate analysis of PFS both ZAP-70 (p=0.0001) and CD54 (p=0.004) resulted to be independent prognostic factors. Therefore, CD54, determined by flow cytometry, could be considered as a promising prognostic parameter in B-CLL. Moreover, since the ZAP-70 negative subgroup consists of a large and a heterogeneous population presenting variable outcome, CD54 should be used to identify B-CLL progressive pts, particularly those with important organomegaly.

Chronic Lymphocytic Leukemia (II)

P139

MOLECULAR AND TRANSCRIPTIONAL CHARACTERIZATION OF 17P LOSS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Distinct genetic abnormalities such as TP53 deletion at 17p13.1 have been identified as having adverse prognostic relevance in B-cell chronic lymphocytic leukemia (B-CLL), and conventional cytogenetic studies have shown that TP53 deletion in B-CLL is mainly associated with the loss of 17p due to complex chromosomal rearrangements. We used an integrative genomic approach to investigate the significance of 17p loss in a subset of B-CLLs carrying a TP53 monoallelic deletion detected by Fluorescence in-situ hybridization (FISH). A panel of 71 untreated Binet A B-CLLs (18 carrying 17p-) was characterized for the most recurrent genomic aberrations and for the major prognostic markers. The genomic profile of chromosome 17p was investigated with GeneChip Human Mapping 50K Xba arrays in 12/18 17p- B-CLLs. Inferred copy numbers were derived from a Hidden Markov Model (HMM) based algorithm implemented in CNAT 4.0.1 software (Affymetrix). FISH probes covering a region of approximately 6 Mb in 17p11.2-p12 was selected to validate the array results. The transcriptional profiles of the 60 B-CLLs (7 carrying 17p-) have been generated on Affymetrix GeneChip U133A arrays. The identified transcriptional fingerprints of the 17p- cases was validated on an independent dataset of 100 B-CLL cases (Haslinger *et al*., 2004) using a Multi-class Prediction Analysis. Polymerase chain reaction was used to define the mutational status of the TP53. Genome-wide DNA analysis of TP53-deleted samples showed 17p loss in 11/12 cases, with breakpoints scattered along the 17p11.2 region. FISH analysis confirmed these findings and revealed 17p loss in a small fraction of leukemic cells in the remaining TP53-deleted case. In addition, FISH indicated 17p loss in the 6/18 cases not investigated by SNP. Mutations in exons 2 to 11 of the remaining TP53 allele were found in 9/12 17p- B-CLLs. Gene expression profiling of 60 B-CLLs, identified 40 differentially expressed genes in 17p- versus 17p normal samples, 35 of which were down-regulated in 17p- tumors: the majority (30/35) of these transcripts, including putative tumor suppressor genes (GABARAP, GPS2 and OVCA1) mapped to 17p, indicating a remarkable gene dosage effect. Our data provide evidence that 17p loss may play an additional pathogenetic role in B-CLL, and suggest that the concomitant loss of multiple tumor suppressor genes could be responsible for the highly adverse prognostic relevance associated with TP53 loss.

P140

ANALYSIS OF B-CELL RECEPTOR SIGNAL TRANSDUCTION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA BY PHOSPHOSPECIFIC FLOW-CYTOMETRY

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B-cell chronic lymphocytic leukemia (B-CLL) is a clonal lymphoproliferative disease characterized by the expansion of mature CD5+ B-cells. Recent studies indicated that variability in clinical outcomes observed in CLL patients is related to differences in the ability to transduce B-cell receptor (BCR)-mediate signals. The BCR signal transduction routes include, in the initial phases, Syk tyrosin kinase and BCR prolonged stimulation has been associated to activation of kinases includ-