

ZAP70 Methylation as a Prognostic Marker in CLL

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Problems persist with the harmonisation of ZAP70 expression analysis by flow cytometry. Herein we report the prognostic value of an assay that establishes *ZAP70* methylation as a prognostic marker of equal value to ZAP70 expression analysis by FACS or *IgV_H* sequencing. Pyrosequencing based methylation analysis of the CpG locus +334bp to the ZAP70 transcription start site (*C334*) was performed in triplicate on 303 cases from the UK LRF CLL4 trial and 249 cases from the Royal Bournemouth Hospital.

Stratification into *ZAP70* hypermethylated and hypomethylated cases, using a single cut off for *C334* methylation was comparable to stratification by ZAP70 expression in predicting *IgV_H* status. In the 249 Bournemouth cases, *ZAP70* methylation was superior to CD38 and equal to *IgV_H* and ZAP70 in predicting overall and treatment free, survival. In the 303 LRF CLL4 trial cases, both *ZAP70* methylation and ZAP70 expression significantly predicted progression free survival but only *ZAP70* methylation and not ZAP70 expression significantly predicted overall survival. Methylation status was stable in 35/37 (94.6 ± 7.3%) cases analysed over intervals ranging from 8-128 (mean 50 ± 28) months.

This data shows that *ZAP70* methylation is equal or superior to *IgV_H* status and ZAP70 expression status in predicting prognosis in two large patient series. The measurement of *C334* methylation by pyrosequencing represents a robust, reproducible, non subjective, fully quantitative assay with major advantages over ZAP70 expression analysis by flow cytometry. Furthermore the assay is ideally suited to large retrospective studies using archived samples or in clinical trials.