Evolution and Impact of Subclonal Mutations in Chronic Lymphocytic Leukemia

Reviewed by Reem Alsolami and Anna Schuh

Despite significant advances in our understanding of the biology of leukaemogenesis, the underlying causes of disease relapse and progression remain unclear. Chronic lymphocytic leukaemia (CLL) is characterized by considerable biological and clinical heterogeneity, a chronic relapsing course and the development of chemotherapy resistance. Recently, next generation sequencing technology has enabled sensitive detection of diverse combinations of somatic mutations in CLL that evolve in a hierarchical pattern (Schuh et al). Therefore, it provides an ideal model to examine the process of clonal evolution.

The aim of the current study (Landau et al) was to further examine the significance of genetic alterations in CLL using integrative analysis of whole exome sequencing (WES) and copy number change data.

Total 160 CLL patients were selected for this study. All were diagnosed according to WHO criteria and sub-classified based on the established prognostic risk factors. Genome-wide SNP array was used to analyse 111 samples out the 160 cases to identify somatic copy number aberrations (sCNAs). All 160 tumour normal pairs were sequenced by WEStechnology and CNAs were called when possible. Additional second time points from 18/160 cases were included in the sequencing analysis. For the 149 cases, where both CNAs and WES data were available either from SNP array or from WES alone, integrative analysis of sCNAs and somatic single nucleotide variation (sSNV) was performed using algorithm called ABSOLUTE. This algorithm was used to measure the purity, and calculate the ploidy, and was applied to infer the cancer cell fraction (CCF) and calculate the allele frequency of sSNV in WES data. Each Clonal (> 0.5) and subclonal sSNV(<0.5) was classified based on probability that its CCF was >0.95 .

A total of 2,444 non synonymous and 837 synonymous mutations in protein-coding sequences were identified by the WES, with an average of 15.3(2–53)non synonymous mutations per patient. WES identified 20 predominant clones as candidate CLL driver genes including *SF3B1*, *TP53*,

NOTCH1,*MYD88, ATM, ZMYM3,* and *DDX3X*, which were previously reported. To infer temporal ordering of the recurrent sSNVs and sCNAs, the authors suggested that early events are the predominant clonal mutations affecting a specific gene or chromosomal lesion, while the later event are the common locus-specific subclonal mutations that represent additional mutations. Accordingly, three early driver mutations were identified including *MYD88*, trisomy 12, and hemizygousdel(13q) . In the longitudinal analysis of the 18 cases, clustering analysis of CCF distribution of each genomic abnormality over the two time point showed clear clonal evolution involving subclones with driver mutations in 10/12 CLLpatients treated with chemotherapy, and 1/6 in untreated patients. The presence of subclonal drivers mutations showed an adverseimpacts on clinical outcome and thus might be considered as an independent poor prognostic factor of relapse and disease progression.

In conclusion, the current study has performed a comprehensive analysis of subclonal and clonal mutations present in the CLL coding regions.The integrative analysis of WES and CNAs in 149 patients provides a cost-effective strategy to overcome the small sample size of the published multi-time point analysis and confirms that relapse might in fact be due to a disruption of the subclonal equilibrium. In addition, the potential hastening of the evolutionary process with treatment provides a mechanistic justification for the empirical practice of “watch and wait” as a CLL treatment paradigm.However, these results need to be confirmed within clinical trials and only whole genome sequencing using PCR-free protocols will allow the precise definition of the subclonal composition from variant allele frequencies of exonic and intronic mutations.

Landau DA, Carter SL, Stojanov P, McKenna A, Stevenson K, Lawrence MS, Sougnez C, Stewart C, Sivachenko A, Wang L, Wan Y, Zhang W, Shukla SA, Vartanov A, Fernandes SM, Saksena G, Cibulskis K, Tesar B, Gabriel S, Hacohen N, Meyerson M, Lander ES, Neuberg D, Brown JR, Getz G, Wu CJ.*Cell* 2013;152(4): 714-726.

Schuh A, Becq J, Humphray S, Alexa A, Burns A, Clifford R, Feller SM, Grocock R, Henderson S, Khrebtukova I, Kingsbury Z, Luo S, McBride D, Murray L, Menju T, Timbs A, Ross M, Taylor J, Bentley D. Blood. 2012 Nov 15;120(20):4191-6. doi: 10.1182/blood-2012-05-433540.Epub 2012 Aug 22. PubMed PMID: 22915640.