Deep sequencing identifies genetic heterogeneity and recurrent convergent evolution in chronic lymphocytic leukemia (Ojha *et al*. 2015)

Reviewed by Reem Alsolami and Anna Schuh

Chronic lymphocytic leukaemia (CLL) is characterised by a heterogeneous clinical course ranging from indolent to rapid disease progression. Whilst important progress has been made in the treatment of CLL, the disease remains incurable and the molecular basis of chemo-refractoriness and relapse remains elusive. The advent of next generation sequencing (NGS) and array has revealed the extent of acquired genetic heterogeneity of CLL and increasing evidence suggests that this at least partly reflects the clinical variability (Knight *et al*., 2012; Schuh *et al*., 2012). Moreover, NGS of sequential samples has identified different patterns of mutational behaviour that change with treatment over time (Schuh *et al*., 2012);leading to disruption of the subclonal equilibrium and may result in disease relapse and poor outcome (Landau *et al*., 2013). The current study expands on these initial observations of clonal evolution in CLL.

The aim of this paper (Ojha *et al*., 2015) was to study the clonal evolution of CLL using samples obtained from patients at comparable clinical stages that were undergoing the same treatment. The treatment option was “PCR” regimen, consisting of pentostatin(2 mg/m2), cyclophosphamide (600 mg/m2), and rituximab (375 mg/m2); given for a maximum of 6 cycles. A total number of 30 sequential tumour samples were collected from 12 PCR enrolled patients and analysed with 12 matched germline samples. For all 12 patients, two time-points samples were analysed and 4 patients had additional 1 to 2 longitudinal samples. The samples were collected at two or more of the following time-points: before PCR trial (five samples from five patients), PCR baseline (12 samples from 12 patients) and relapse samples (13 samples from 10 patients).

The study has used WES and aCGH to analyse all the samples, and 5 samples out of 30 were further analysed using targeted semiconductor-sequencing of 24 CLL putative genes, including *DDX3X*, *NOTCH1*, and *SF3B1*. WES data was used to estimate the clonal architecture of the sequential samples using all somatic mutations (intronic and exonic), while the aCGH data was used to

distinguish the mutations located in regions with and without copy number aberrations (CNAs). 2% of mutations were found in regions with CNAs and were excluded from the clustering analysis for not having enough information to correct the allele frequency. The study has identified total of 136 somatic nonsynonymous single-nucleotide variations and 20 indels in 143 genes. 26 nonsynonymous mutations were detected in the 6 unmutated IGHV cases, and 18 in the 6 mutated cases.

The clonal architecture of most investigated cases has changed in response to chemotherapy. The study has identified 2 major patterns of clonal architecture through the course of the disease: 4 out of 12 patients showed linear evolution characterized by the existence of a unique initial clone that acquired additional mutations over time, and remaining patients showed multibranching evolution with 2 or more subclones fluctuating over time. Two cases were further analysed by deep sequencing identifying significant genetic heterogeneity, with several driver mutations found in small subclones. Previous studies showed that mutations in *NOTCH1* can be acquired through the course of the disease (Fabbri *et al*., 2011; Villamor *et al*., 2013), while others showed that *SF3B1* mutations detected at subclonal levels might increase with disease progression (Rossi *et al*., 2013; Schwaederlé *et al*., 2013). Moreover, the later analysis in the current study showed evidence of convergent evolution, in which additional genetic lesions have evolved affecting the same genes or copy number abnormality in different subclones, including mutations in *NOTCH1*, *SF3B1*, *DDX3X*, and del (11q23). The original subclone went undetected by WES and aCGH after therapy, but the coexistence of two distinct subclones was confirmed by deep sequencing.

In conclusion, FISH and microarray based studies demonstrated that the genetic makeup of CLL is reshaped during disease progression (Stilgenbauer *et al*., 2007; Grubor *et al*., 2015) . The study of clonal evolution using NGS overcomes some limitations associated with the conventional techniques. The current study has used WES data to identify clonal architecture in 12 CLL patients, and has studied the subclonal evolution in two cases using targeted deep sequencing approach. The study has tried to overcome the heterogeneity of CLL cohort by investigating comparable CLL stages;

however, the comparable time-points were limited. The study shows evidence of convergent evolution as a recurrent event in the CLL genome. In concordance with our observation (Schuh *et al*., 2012; Ojha *et al*., 2015), most relapse cases were associated with clonal competition, but fewer relapsed cases showed stable clonal dynamics (Ojha *et al*., 2015). NGS technology has enabled more sensitive detection of somatic mutations in cancer, allowing comprehensive analysis of clonal and subclonal evolution and their roles in cancer progression including CLL. However, challenges remain to NGS being applied to determine the most appropriate treatment option for patients depending on individual genomic landscape.

References

Fabbri, G., Rasi, S., Rossi, D., Trifonov, V., Khiabanian, H., Ma, J., … Gaidano, G. (2011). Analysis of the chronic lymphocytic leukemia coding genome: role of NOTCH1 mutational activation. *The Journal of Experimental Medicine*, *208*(7), 1389–1401. http://doi.org/10.1084/jem.20110921

Grubor, V., Krasnitz, A., Troge, J. E., Meth, J. L., Lakshmi, B., Kendall, J. T., … Esposito, D. (2015). Novel genomic alterations and clonal evolution in chronic lymphocytic leukemia revealed by representational oligonucleotide microarray analysis (ROMA), *113*(6), 1294–1304. http://doi.org/10.1182/blood-2008-04-158865.An

Knight, S. J. L., Yau, C., Clifford, R., Timbs, a T., Sadighi Akha, E., Dréau, H. M., … Schuh, a. (2012). Quantification of subclonal distributions of recurrent genomic aberrations in paired pre-treatment and relapse samples from patients with B-cell chronic lymphocytic leukemia. *Leukemia*, *26*(7), 1564–1575. http://doi.org/10.1038/leu.2012.13

Landau, D. a., Carter, S. L., Stojanov, P., McKenna, A., Stevenson, K., Lawrence, M. S., … Wu, C. J. (2013). Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell*, *152*(4), 714–726. http://doi.org/10.1016/j.cell.2013.01.019

Ojha, J., Ayres, J., Secreto, C., Tschumper, R., Rabe, K., Dyke, D. Van, … Braggio, E. (2015). Deep sequencing identi fi es genetic heterogeneity and recurrent convergent evolution in chronic lymphocytic leukemia, *125*(3), 492–499. http://doi.org/10.1182/blood-2014-06-580563.Raw

Rossi, D., Rasi, S., Spina, V., Bruscaggin, A., Monti, S., Ciardullo, C., … Foa, R. (2013). CME Article Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia, *121*(8), 1403–1412. http://doi.org/10.1182/blood-2012-09-458265.The

Schuh, A., Becq, J., Humphray, S., Alexa, A., Burns, A., Clifford, R., … Mcbride, D. (2012). Monitoring chronic lymphocytic leukemia progression by whole genome sequencing reveals heterogeneous clonal evolution patterns Monitoring chronic lymphocytic leukemia progression by whole genome sequencing reveals heterogeneous clonal evolution patterns, *120*(20), 4191–4196. http://doi.org/10.1182/blood-2012-05-433540

Schwaederlé, M., Ghia, E., Rassenti, L. Z., Obara, M., Dell'Aquila, M. L., Fecteau, J. F., & Kipps, T. J. (2013). Subclonal evolution involving SF3B1 mutations in chronic lymphocytic leukemia. *Leukemia*, *27*(5), 1214–7. http://doi.org/10.1038/leu.2013.22

Stilgenbauer, S., Sander, S., Bullinger, L., Benner, A., Leupolt, E., Winkler, D., … Döhner, H. (2007). Clonal evolution in chronic lymphocytic leukemia: Acquisition of high-risk genomic aberrations associated with unmutated VH, resistance to therapy, and short survival. *Haematologica*, *92*(9), 1242–1245. http://doi.org/10.3324/haematol.10720

Villamor, N., Conde, L., Martínez-Trillos, a, Cazorla, M., Navarro, a, Beà, S., … López-Guillermo, a. (2013). NOTCH1 mutations identify a genetic subgroup of chronic lymphocytic leukemia patients with high risk of transformation and poor outcome. *Leukemia*, *27*(5), 1100–6. http://doi.org/10.1038/leu.2012.357