Commentary

Dealing with complexity: prognostic implications of karyotype and mutations in chronic lymphocytic leukemia

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Over the last 40 years, much progress has been made in defining and understanding the genetic factors underlying the heterogeneity in the clinical course of patients with chronic lymphocytic leukemia (CLL). The presence of acquired clonal genetic abnormalities in CLL has been recognized since the early 1980s. However, correlation of chromosomal alterations with prognosis was hampered by the low mitotic rate of CLL cells, which results in only 40–50% of CLL samples having detectable chromosomal abnormalities using conventional karyotyping. Over the last 25 years, two technologic innovations, interphase FISH and stimulated karyotyping, have allowed investigation into the effect of these genetic changes on disease biology, prognosis, and response to therapy. Testing for the presence of known chromosome abnormalities with prognostic significance in CLL is now considered standard of care (1) and is most commonly done via employment of CLL FISH panels. However, the time consuming, technical nature of the culturing step has largely limited stimulated karyotype testing to referral centers.

Two landmark studies provided the foundation for our understanding of the prognostic impact of acquired genetic changes in CLL. Juliusson *et al*. (2) demonstrated inferior outcomes for patients with trisomy 12, deletion 13q14, and deletion 14q32, as detected by conventional G-banding on stimulated CLL cells. The same study reported a significantly worse outcome for those with complex karyotype containing >3 chromosome abnormalities. Döhner *et al*. (3) reported that over 80% of 325 examined CLL cases had at least one abnormality detected by FISH, of which deletion 11q, deletion 13q, trisomy 12, deletion 17p, and deletion 6q were the most common. Importantly, deletion 17p was associated with the shortest overall survival from time of diagnosis compared to all other groups studied. Multiple other studies, including those conducted at our institution, have subsequently replicated these findings (4,5). It is now known that the minimal deleted regions of these chromosomal alterations correspond to tumor suppressor genes such as ATM (11q), BIRC3 (11q) TP53 (17p), mir15a/16 (13q), thus providing a biologic rationale for their impact on prognosis (6)

Of the known chromosome abnormalities in CLL, deletion 17p portends the poorest prognosis. This abnormality accounts for as many as 30-40% of patients with chemorefractory disease (7,8) despite deletion 17p being identified in fewer than 15% of CLL patients at diagnosis. Deletion 17p can also be a progression event acquired after chemotherapy (9) and there is evidence that suggests that these patients have an inferior outcome compared to those with *de novo* deletion 17p (10). Complex karyotype is often but not always associated with deletion 17p (11). CLL patients with complex karyotype have been shown to have inferior outcomes after treatment with chemo-immunotherapy (12), reduced intensity conditioning allogeneic stem cell transplant (13) and the BTK inhibitor ibrutinib (14). There is a synergistic effect of complex karyotype with deletion 17p, with these patients having a worse outcome than those with deletion 17p alone (15).

In recent years, next generation DNA sequencing technologies have revealed the genomic landscape of CLL

(16,17). Over 200 recurrent mutations have been identified to date, with most of these being uncommon events occurring in less than 5% of cases. Importantly, relatively few mutations, 5–20, occur in each individual CLL patient, similar to the mutational load seen in acute leukemias and fewer than is seen with diffuse large B cell lymphomas or epithelial derived malignancies. Recurrent mutations in ATM, NOTCH1, SF3B1, TP53, and BIRC3 were the most common events in multiple studies. However, while no unifying mutation has been found in the majority of CLL, multiple mutations altering cellular pathways involving the cell cycle, DNA damage response, B cell receptor or Toll-like receptor signaling and NfKB signaling are known to be associated with CLL. The most common recurrent mutation, found in the spliceosome component SF3B1, is present in no more than ~20% of CLL cases. The biologic function and prognostic implications of CLL associated mutations remains an area of active investigation.

It is now clear that deletion 17p, complex karyotype and TP53 mutations represent overlapping groups of patients with poor prognosis. In as many as 80% of CLL cases with TP53 mutations, the remaining allele is lost via deletion of 17p (70%) or acquired loss of heterozygosity (10%). TP53 mutations appear to influence prognosis independent of the presence of deletion 17p or additional chromosomal abnormalities (18). Longitudinal genetic profiling suggests that acquisition of TP53 mutation is in most cases the earliest event that precedes loss of 17p and cytogenetic complexity (19). In addition, small subclones containing TP53 mutations may be present; these also appear to be a negative prognostic indicator (20). These associations of deletion 17p, complex karyotype and TP53 mutations with poor outcomes in CLL patients have obvious parallels in multiple other hematologic malignancies, including AML, MDS, mantle cell lymphoma and follicular lymphoma. In the July 21, 2016 issue of *Blood*, Herling *et al*. presented the first prospective, comprehensive prognostic analysis of chromosomal aberrations and gene mutations in conjunction with clinical outcomes in CLL patients treated with chlorambucil-based chemo-immunotherapy (21). Data was obtained from a cohort of 161 CLL patients within the CLL11 phase 3 trial, who had been treated with Chlorambucil (Clb), Clb plus Rituximab (Clb-R) or Clb plus Obinutuzumab (Clb-G) (22). Using pre-treatment stimulated peripheral whole blood, they utilized metaphase karyotyping and next generation sequencing (NGS) of an 85-gene panel to determine the prognostic value of complex karyotypes and distinct somatic mutations on clinical outcome.

Analysis of this data resulted in multiple significant findings. First, the authors identified an increased frequency of KRAS mutations in patients who did not respond to Clb-based chemoimmunotherapy, particularly Clb-rituximab. Herling *et al*. also identified mutations in POT1 as an independent prognostic factor associated with a statistically significant decrease in overall survival. Importantly, none of the patients with POT1 mutations had complex karyotypes, deletion 17p or p53 mutations, suggesting that POT1 mutations may represent a new adverse risk group. Additionally, for the first time in a prospective treatment setting for CLL, complex karyotype was identified as an independent prognostic factor associated with decreased overall survival. This association was even stronger when both complex karyotype and mutant TP53 were present. Lastly, the authors identified mutations in six genes that have not been previously described in CLL, highlighting the potential value of incorporating NGS into large prospective trials.

The data presented in Herling *et al*. raise important questions that will need to be addressed in future studies. For example, it is not yet known whether the adverse impact of KRAS and POT1 mutations on outcomes following Chl-based chemo-immunotherapy will be seen with other chemo-immunotherapy based regimens, or with targeted therapies such as BTK inhibitors, PI3K inhibitors or BCL2 inhibitors. In addition, these findings will need to be validated in larger studies and those that include younger age groups, since the patients treated in CLL11 were elderly.

Collectively, Herling *et al*. have demonstrated the value of incorporating chromosomal banding analysis and NGS into large clinical trials in order to identify novel prognostic factors that can correlate with clinical outcomes and responses to therapy. Ultimately, these findings may be added to the armamentarium of risk stratification elements in patients with CLL. Improved genomic technologies had been instrumental in identifying genetic and epigenetic events in CLL. Moving forward, the challenge is to define which events are truly drivers of disease pathogenesis or response to therapy. In addition, it is also important to investigate the effect of combinations of various mutations and chromosomal alterations on disease biology. The ultimate goal must be to identify those patients who may respond poorly to chemo-immunotherapy and should therefore receive targeted therapies or referral for clinical trials with novel agents.

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Footnote

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