their translation modulated by CTORC2. It will be an important avenue of research to determine the RNAs that are specifically targeted, likely by carrying out RNA-Seq of polysomal fractions or similar strategies.

Leveraging their new-found biochemical insights, the authors explored the effects of inhibiting CDK9 with Atuveciclib in in vivo, ex vivo, and in vitro models of AML. In these cases, they compared its activity alone with that of the cornerstone of AML therapy, cytarabine. The combination of cytarabine and Atuveciclib yielded the most robust responses. Atuveciclib is currently being tested in a phase 1 clinical trial in advanced leukemia patients, suggesting the insights gamered here could be rapidly translated into the clinic (ClinicalTrials.gov, #NCT02345382). These studies also provide important insights as to why targeting mTORC1/2 in the clinic has yielded limited clinical efficacy.[4,6]

Many exciting questions arise, and new areas of exploration will emerge based on these elegant studies. For instance, CDK9 inhibition alters the association of specific ribosomal proteins with polysomes. Could this mean that CDK9 plays some role in the formation of specialized ribosomes (ie, ribosomes that are optimized for the translation of a certain subset of transcripts)? Oftentimes, factors that play roles in multiple steps of RNA metabolism can coordinate the protein expression of subsets of transcripts that act in the same biochemical pathways, in this way modulating RNA regulons.[7] Could CDK9 affect the production of groups of RNAs at the transcription, translation, and perhaps other levels, such as splicing or nuclear export? In this way, could CDK9 be a central node in an RNA regulon that supports malignancy? From the perspective of mTOR components, it will be interesting to understand how many other kinases can coopt these factors and what variety of processes these can function in.

These studies further highlight the importance of RNA processing in AML and other cancers. Although the conventional view has been that dysregulated transcription and signaling are the drivers of cancer, it is clear that dysregulated RNA processing in its many forms (eg, RNA trafficking, translation, stability, splicing, etc) contributes to the oncogenic phenotype and is targetable in malignancies.[8,9] AML has already been characterized to have dysregulated RNA processing, including elevated export of RNAs that support malignancy, increased translation, and dysregulated splicing, and these processes can be targeted in patients corresponding to clinical benefit.[8-10] The report by Beauchamp et al provides novel insights into the mechanisms that can dysregulate translation and transcription in AML and provides means to exploit these to identify next-generation strategies to target these processes in the clinic.

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LYMPHOID NEOPLASIA

Comment on Baliakas et al, page 1205

Intricacies of CLL cytogenetic complexity

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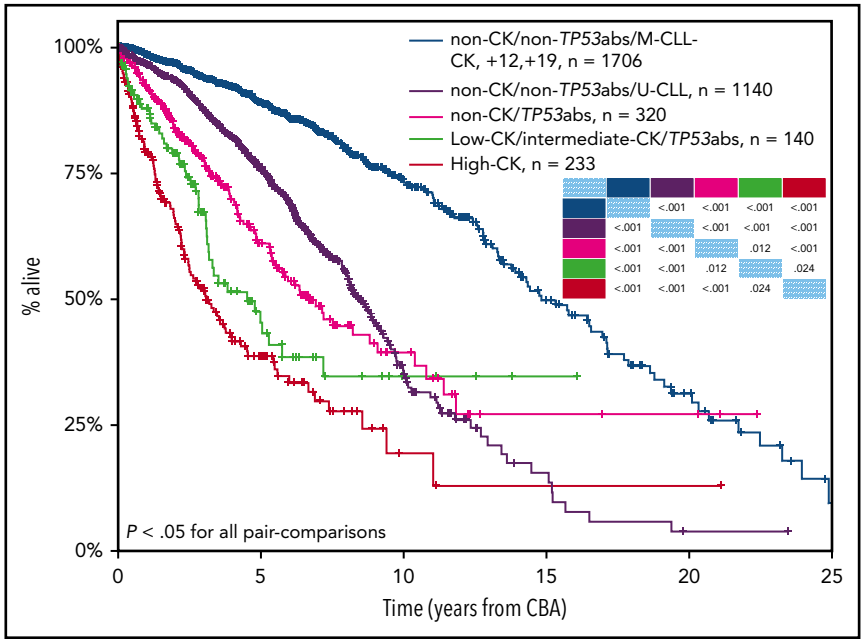
In this issue of *Blood*, Baliakas et al, on behalf of the European Research Initiative on CLL, present the largest retrospective analysis of the prognostic significance of karyotypic complexity, assessed by chromosome banding analysis (CBA) performed on stimulated cultures, in patients with chronic lymphocytic leukemia (CLL) and monoclonal B-cell lymphocytosis (MBL).[1] Their cohort contains >5000 CLL patients and ~400 patients with MBL from 17 European institutions. Based on their results, the authors demonstrate prognostic heterogeneity among CLL cases that are karyotypically complex. They propose a hierarchical prognostic model that integrates complex karyotype, *TP53* aberrations, and immunoglobulin heavy chain variable region gene (*IGHV*) somatic mutation status.

The landmark study published in 2000 by Döhner and coworkers demonstrated that 80% of CLL cases could be risk stratified based on the presence of 4 recurrent abnormalities detectable by fluorescence in situ hybridization (FISH) analysis performed on interphase (nondividing) nuclei: del(13)(q14.1), trisomy 12, del(11)(q22-23), and del(17)(p13.1).[2] Patients with del(13q) as the sole abnormality had a good prognosis. Those with trisomy 12 or who lacked FISH-detectable abnormalities had an intermediate prognosis, and those with del(11q) had a relatively poor prognosis.



Kaplan-Meier curves based on a hierarchical model for OS incorporating complex karyotype (CK), TP53abs (deletion of chromosome 17p and/or TP53 mutations), and somatic hypermutation status of the IGH genes. High-CK (≥5 aberrations, red line) exhibits the shortest OS followed by cases with TP53abs and 3 or 4 aberrations (low CK and intermediate CK, respectively, low CK/intermediate CK/TP53abs, green line), non-CK cases with TP53abs (non-CK/TP53abs, purple line), and non-CK/non-TP53abs cases with unmutated IGHV genes (non-CK/non-TP53abs/U-CLL, black line). Patients with the longest OS are those with non-CK/TP53abs and mutated IGHV genes (M-CLL) as well as patients with CK and +12,+19 (non-CK/non-TP53abs/M-CLL-CK, +12,+19, blue line). *P* values for all pair comparisons are provided in the table, where the colored cells indicate the respective subgroups based on the color of each Kaplan-Meier curve. See Figure 4 in the article by Baliakas et al that begins on page 1205.

Patients with del(17)(p13.1), the site of the *TP53* tumor suppressor gene, had a particularly poor prognosis characterized by rapid disease progression, treatment resistance, and poor survival. Based on these results, and on the high yield and relative ease of performing FISH analysis on interphase nuclei compared with CBA performed on metaphases, FISH analysis using a panel of probes to the common recurrent abnormalities has become part of the routine clinical evaluation at the time of CLL diagnosis. However, a significant limitation of FISH is that it detects only those abnormalities to which the probes are directed; it cannot detect new abnormalities or karyotypic complexity.

Karyotypic complexity is defined by the International System for Human Cytogenetic Nomenclature as the presence of ≥3 numerical or structural abnormalities in a karyotype; unbalanced or balanced translocations are considered a single abnormality.[3] Under standard culture conditions, most CLL cells, which are in the G0/G1 phase of the cell cycle, fail to divide. Newer methods, using different combinations of cytokines, CpG oligodeoxynucleotides, and mitogens, stimulate CLL cells to divide in culture and yield analyzable metaphases in up to 90% of samples.[4,5] Karyotypic complexity is associated with shorter treatment-free and overall survival (OS).[4-7]

Consistent with previous studies, the authors found that karyotypic complexity is generally associated with poor outcome. However, because their cohort was so large, the authors could assess the prognostic impact of the number and types of abnormalities in karyotypically complex cases (see figure). A major finding is that patients with ≥5 abnormalities exhibit a uniformly dismal prognosis regardless of clinical stage, *TP53* aberration (deletion of the short arm of chromosome 17 and/or *TP53* mutation), or *IGHV* somatic mutation status. The genomic abnormalities in these cases involve all chromosomes, presumably representing underlying genomic instability. In contrast, patients with 3 or 4 cytogenetic abnormalities (low or intermediate cytogenetic complexity, respectively) had better outcomes, with poor outcomes only in the presence of *TP53* aberrations. The abnormalities in these cases tended to be the typical recurrent CLL abnormalities.

The authors described several additional important findings. First, not all patients with complex karyotypes have a poor prognosis. Cases with +12 and +19, in addition to other numerical and structural abnormalities, demonstrate distinctive clinicopathologic features and an indolent clinical course. Second, karyotypic complexity can occur early in the disease course in the absence of previous treatment. The vast majority of samples in this study were collected within 1 year of diagnosis before treatment, and karyotypic complexity was also identified in a small subset of patients with MBL. Finally, ~5% of patients with either isolated del(13q) or lacking FISH abnormalities (so-called FISH-normal cases) are karyotypically complex and have a significantly shorter OS than patients with the same FISH findings, but without karyotypic complexity. The vast majority of these cases lack *TP53* aberrations. Thus, CBA identifies a subset of high-risk patients who would otherwise be considered to have a good prognosis.

The latest International Workshop on Chronic Lymphocytic Leukemia (iwCLL) guidelines indicate that genetic risk stratification by FISH analysis for the common recurrent abnormalities and screening for *TP53* mutation should be performed in all patients before treatment.[8] The guidelines recognize that karyotypic complexity likely has adverse prognostic significance and consider stimulated CBA "desirable" in prospective clinical trials. The results of the current retrospective study have refined the prognostic impact of karyotypic complexity in patients with CLL, and have identified prognostically distinct subsets within the larger category of karyotypically complex CLL. The findings support the inclusion of stimulated CBA in prospective clinical trials to determine its potential contribution to routine clinical practice.

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