Questions still remain, however, about the applicability of these results. (1) Is the 60-mg/m² daunorubicin dose in the control arm adequate for comparison? Although there have not been randomized clinical trials comparing daunorubicin 60 mg/m² to daunorubicin 90 mg/m², it appears that higher doses of daunorubicin (90 mg/m²) are more effective than lower doses (45 mg/m²) in patients with favorable and intermediate cytogenetics and those ≤65 years of age. However, higher doses of daunorubicin may have led to even further increases in toxicity in the control arm in this older group of patients (60-75 years). (2) Although the response rates were higher with CPX-351, why did this not translate to an OS benefit? One of the main reasons for the dearth of therapies approved for AML is the difficulty of showing a significant OS benefit for experimental approaches, in part because of variable approaches to the treatment of minimal residual disease (ie, consolidation, maintenance, and stem cell transplant strategies). In addition, the cross-over design further adds to the complexity of the OS analysis in this study. Improving OS is the holy grail of clinical cancer research and AML in particular; however, there are challenges when considering OS as an end point in therapeutic trials. Although allogeneic stem cell transplantation is a goal for most patients with nonfavorable risk disease, there are a panoply of conditioning regimens (myeloablative, reduced intensity, and nonmyeloablative) at each institution that may impact overall outcome. Additionally, there is a lack of consistent consolidation/maintenance therapeutic approaches, and the ultimate choice of regimen (and how many cycles) may also impact overall outcome. How do we circumvent these biases to determine whether an experimental therapy is effective in AML patients? One approach would be to design phase 3 trials with identical postremission therapeutic strategies in each arm, clearly delineating which patients are to undergo stem cell transplantation, what type of consolidation therapy is to be administered, how many cycles, and at what doses. Another approach may be to determine alternative end points that may appear to be surrogates of OS. Lancet et al’s secondary end point of EFS represents a promising evaluation that may mitigate some of the biases seen with clinical studies in AML.

Will CPX-351 become the new “standard” regimen for older adults presenting with AML? Time will tell, and we eagerly await the phase 3 comparison of CPX-351 vs 7+3 in older patients. To date, there has been considerable variability in response rates and duration for diverse novel agents including tipifarnib, clofarabine, and gemtuzumab in older AML patients. We can remain optimistic with Lancet et al’s findings and other investigational approaches demonstrating potential benefit in elderly AML and secondary AML. We hope that these confirmatory trials will ultimately improve outcomes and provide us with additional therapeutic options for our patients. Moreover, this study represents a superb template to explore the combination of CPX-351 with mechanistically distinct modalities such as inhibitors of DNA damage response pathways, cyclin-dependent kinase inhibitors, tyrosine kinase inhibitors, and immunotherapeutic strategies, so that the salutary effects of CPX-351 could be extended to other stages of AML where effective therapy is lacking. From Lancet et al’s work, there is reason for excitement for the future of AML therapies.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Comment on Stilgenbauer et al, page 3247

Predicting treatment outcomes in CLL

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In this issue of Blood, Stilgenbauer and colleagues report on the prognostic and predictive value of gene mutations assessed prospectively in patients with chronic lymphocytic leukemia (CLL) treated with first-line chemoimmunotherapy.

Heterogeneity in the clinical course of the disease is one of the hallmarks of CLL. The median survival can be <3 years for patients in high-risk subgroups and >25 years in low-risk patients. Understanding the biologic basis for this clinical variability and the development of prognostic markers to dissect the heterogeneity have been areas of intense investigation over the past decades. Established prognostic biomarkers include the mutation status of the expressed immunoglobulin gene variable region (IGHV), chromosomal abnormalities assessed by fluorescence in situ hybridization, and expression of CD38, CD49d, and ZAP-70. These markers can predict the pace of disease...
progression in addition to overall survival. As newer and more effective treatment options emerge, there is a pressing need to identify biomarkers that can predict how individual patients respond to a specific treatment. In this issue of *Blood*, Stilgenbauer et al present their analysis on the interaction of somatic mutations and clinical outcomes in a large cohort of prospectively studied patients treated with standard therapy. This study may set an example how to obtain the data necessary to tailor treatment to distinct subgroups of patients with CLL.

The CLL 8 trial of the German CLL study group randomized patients to either fludarabine plus cyclophosphamide (FC) or FC with rituximab (FCR) and established the superiority of chemoimmunotherapy. Remarkably, of the 817 patients enrolled into the treatment trial, more than 600 could be included in the biomarker analysis with a median follow-up of 70 months. Several acquired somatic mutations have been identified in CLL using next-generation sequencing techniques. The most common, found in 5% to 15% of patients, affect *SF3B1, TP53,* and *NOTCH1.* In the CLL 8 study cohort studied here, at least 1 mutation was identified in 35% of patients, affecting *SF3B1* in 18.4%, *TP53* in 11.5%, and *NOTCH1* in 10%. Mutations in *TP53* and *SF3B1,* unmutated *IGHV* (which identifies the more progressive subtype of CLL), 11q deletion, and 17p deletion were associated with shorter progression-free survival (PFS), whereas *TP53* mutations, unmutated *IGHV,* and 17p deletion were associated with inferior overall survival, consistent with previous observations.

Interestingly, in patients carrying mutated *NOTCH1,* there was no benefit from the addition of rituximab to FC (see figure). Whereas the rate of minimal residual disease—negative remissions in most subgroups was twice as high in FCR-treated patients compared with FC-treated patients, there was no difference in patients with *NOTCH1* mutations (50% vs 46.2%). Further, patients with *NOTCH1* mutations were the only subgroup that did not demonstrate an improvement in PFS from the addition of rituximab—albeit the difference for patients with mutated *TP53* was minimal (median PFS 12.1 months for FC and 15.4 months for FCR).

*NOTCH1* was among the first genes identified as recurrently mutated in CLL. *NOTCH1* is a ligand-activated transcription factor that regulates downstream pathways important for cellular growth and plays a key role in T-cell acute lymphoblastic leukemia. Most of the mutations found in CLL are frameshift mutations that lead to a truncated constitutively active protein. Although the role of activated *NOTCH1* in the pathobiology of CLL remains to be defined, more rapid disease progression and inferior survival in patients with *NOTCH1* mutations have been reported. Consistent with a postulated role in driving disease progression is the increasing prevalence of *NOTCH1* mutations in chemotherapy-refractory patients and in patients with Richter transformation.

As the observation that mutations in *NOTCH1* may predict a lack of benefit from rituximab awaits confirmation, it will also be important to investigate whether mutated *NOTCH1* affects the treatment outcome with other anti-CD20 antibodies or monoclonal antibodies in general. Uncovering the mechanism of how *NOTCH1* mutations influence response to rituximab will also require further study; in the CLL 8 trial there was no association with lower CD20 expression, more advanced disease or absolute lymphocyte count. If confirmed, this raises the intriguing possibility that a better understanding of the molecular pathways downstream of *NOTCH1* could uncover novel mechanisms of resistance to antibody therapy. From a therapeutic standpoint, patients with *NOTCH1* mutations might benefit from tailored approaches including agents that inhibit *NOTCH1* activation or kinase inhibitors that target B-cell receptor signaling. The latter is suggested by the observation that *NOTCH1* mutations, trisomy 12, and a specific B-cell receptor configuration (referred to as subset 8) appear to cooperate in Richter transformation.

In summary, 17p deletion and *TP53* mutations predicted a particularly poor outcome with chemoimmunotherapy, mutated *NOTCH1* was associated with no benefit from the addition of rituximab to chemotherapy, and *SF3B1* mutations, although neutral in regard to treatment response, were associated with more rapid disease progression in this prospective cohort of patients treated according to standard criteria. Whether newer treatments can overcome the negative impact of these mutations remains to be determined, but emerging data with novel agents are promising, and enrollment of patients into clinical trials that aim to address these fundamental translational questions will be critical.

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Comment on Duckworth et al, page 3277

**Anergy: the CLL cell limbo**

**Federico Caligaris-Cappio**

In this issue of *Blood*, Duckworth et al find that in chronic lymphocytic leukemia (CLL), malignant cell anergy is associated with failure of inducing PRDM1 (BLIMP1), a critical regulator of differentiation into plasma cells, and that epigenetic modifications account for such failure. These findings link two major problems of CLL cells, the anergic response to B-cell receptor (BCR) stimulation and the incapacity to differentiate.

In normal B cells, the engagement of BCR induces either proliferation and then differentiation into antibody-producing cells or a reversible lethargic state named anergy, a sort of limbo that B cells enter when they encounter an antigen (usually an autoantigen), in the absence of the costimulation provided by T cells. Because the engagement of BCR has a key role in the pathogenesis of CLL, the question becomes if and how CLL leukemic cells differ from normal B cells when their BCR is stimulated. There are two main differences. First, the proliferating CLL B cell fails to undergo plasma cell differentiation. Hence, no antibody is produced to neutralize the triggering antigen whose stimulating activity may proceed unabated and favors clonal expansion. Second, although normal anergic B cells are short-lived and prone to apoptosis (thus preventing the development of dangerous autoreactive cells), CLL cells are not, as they are uniformly protected by the overexpression of the antiapoptotic protein BCL2. Duckworth et al, using different stimuli such as interleukin-21 (IL-21) and cytosine guanine dinucleotide-oligodeoxynucleotides that robustly induce differentiation into plasma cells and having the expression of PRDM1 as readout are able to show that: (1) the reduced differentiation capacity of anergic CLL cells is independent of the signaling pathway; (2) at variance with normal B cells the costimulation of anergic CLL cells does not overcome the differentiation hurdle; and (3) the reduced capacity of inducing PRDM1 is also reflected by a block in immunoglobulin secretion (see figure). Of interest, they also observe the reversing of CLL cell anergy by appropriate in vitro culture associated with the ability to induce expression of PRDM1 upon adequate stimuli.

BCR-induced cell proliferation tends to be associated with unmutated (U) IgVH gene status and anergy with mutated (M) IgVH gene status. Anergic CLL B cells can be identified by their molecular signature.

Considering that the mutational status of IgVH genes is an important prognostic determinant with U-CLL bearing a worse prognosis, the modality of response to BCR stimulation with the sequence Ag stimulation⇒proliferation is considered dangerous, whereas the sequence Ag stimulation⇒anergy is regarded as more advantageous and has been taken to partly explain the more indolent clinical behavior...
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