responses, an argument can be made for using imatinib first and switch patients who are lagging behind early on. This is an attractive approach, but one without data that confirms that patients who fall behind on their response can catch up (in long-term outcome) after such intervention. Then there is the argument of what we would use if we start with a second-generation TKI and the patient develops resistance to it. This argument reminds me of a family anecdote when my father wanted to buy all the balloons from a balloon vendor for my infant brother many years ago. The balloon vendor would not sell them to my father because he would not have anything else to sell if he did. In cancer, using our best therapy first gives us the best long-term outcome, even if the patients who do not have an adequate response might be more difficult to treat. Despite all these arguments for and against, we have to be realistic that circumstances will exist that will mandate the use of one agent or the other, because of our medical expertise and interpretation of the data, or because of peripheral factors such as economics. Based on results such as the ones presented by Radich et al, second-generation TKI might give us the best overall and would be preferred whenever possible. For patients for whom these options are not available or preferred, proper monitoring, suitable management of adverse events, and adequate dose optimization are of increasing relevance to offer each patient the opportunity for the best long-term outcome. With optimal management, our goal today ought to be that no patient should die of CML. And we should aim higher, to cure all patients with CML. If we are to accomplish this, continued research is needed and all patients should be included in clinical trials that help us understand the biology and optimal management of CML.

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Lymphoid Neoplasia

Comment on Xu-Monette et al, page 3986

TP53 mutations and rituximab-CHOP

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In this issue of Blood, Xu-Monette and colleagues report the results of TP53 mutational profiles in a cohort of 506 de novo diffuse large B-cell lymphoma patients treated with rituximab-CHOP, and they concluded that TP53 mutation was an independent adverse factor for survival.1

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma and is characterized by an aggressive clinical course. However, DLBCL exhibits considerable heterogeneity in terms of clinical, morphologic, molecular, and cytogenetic features. Gene expression profiling (GEP) studies have identified 2 primary molecular subgroups of DLBCL: germinal center B (GCB) cells and activated B cells (ABCs). The GCB subgroup shows better survival than the ABC subgroup, independent of the international prognostic index (IPI). In the past decade, the introduction of the humanized monoclonal anti-CD20 antibody rituximab (R) to the combination chemotherapy of cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone (CHOP) has clearly improved the outcome of DLBCL.3 The improvement is mostly observed in patients with a low or intermediate risk based on the IPI and in the GCB subtype.4 The search for new biologic markers in neoplastic diseases is of major interest because they can provide a better understanding of the biology of the disease. They can also suggest the probability of treatment failure. In relapsed DLBCL patients, among those with early relapse and prior treatment with rituximab, the response rate of salvage therapy is only 46%, with a 25% 3-year progression-free survival (PFS).5 It is clear that a biomarker could be affected by new treatment,6 but conducting studies for designing more targeted treatment is essential. However, such a marker needs to be validated in a large cohort of patients. It should also be an independent parameter from clinical data and be much easier and less expensive to collect.

The choice for this study was the TP53 tumor suppressor gene, which plays an important role in the regulation of the cell cycle, cell proliferation, apoptosis, and genomic integrity. The p53 protein mediates cell-cycle arrest when cells experience stressful challenges, such as DNA damage, hypoxia, or oncogene activation, whereas mutant p53 protein results in cell-cycle dysregulation, genomic instability, and the uncontrolled proliferation of damaged cells (see figure). The presence of TP53 mutations has been associated with drug resistance, poor response to treatment, and short survival in several cancers, including DLBCL.7

In DLBCL, patients treated with CHOP, the same group described that TP53 mutations were an adverse prognostic factor for survival but was restricted to patients with GCB-DLBCL.8 The focus of this new study.
was also TP53 but in a larger cohort of patients treated with rituximab and CHOP. To overcome some of the limitations of the technical aspects, they used gene sequencing microarray data from DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissues, gene expression data from RNA extracted from FFPE tissues, fluorescence in situ hybridization results for TP53 gene deletion, and immunohistochemical staining. Bioinformatics and database support were widely used for the analysis. The incidence of TP53 mutations was 21%, and there was no difference between the 2 subgroups. GCB-DLBCL had a significantly better survival than ABC-DLBCL. There was a significantly better survival for patients with wild-type (WT) TP53 compared with mutated (MUT) TP53 for both subtypes, GCB- and ABC-DLBCL. Further stratification can be achieved according to WT TP53 and MUT TP53. The data were validated in this study with sophisticated technology and bioinformatics, and IHC could be standardized for clinical study. This biomarker would mostly be interesting for the GCB subtype with better prognosis, in which the response to treatment can be related to the type of chemotherapy. In the relapsed DLBCL patients from the Collaborative trial in Relapsed Aggressive Lymphoma (CORAL) study,9 51% of the patients had the GCB subtype, and 49% had the ABC subtype according to the Hans algorithm.10 Patients with GCB DLBCL who were treated with rituximab, dexamethasone, aracytine, and cisplatin (R-DHAP) had a better PFS than patients with non-GCB DLBCL (3-year PFS = 52% vs 32%, respectively, P = .01). Patients treated with rituximab, ifosfamide, carboplatin, and etoposide (R-ICE) had a poor PFS, with no significant difference between GCB and non-GCB types. Multivariate analysis showed an independent prognostic impact of the following parameters: GCB/non-GCB interaction with treatment (P = .04), prior rituximab exposure (P = .0052) and secondary IPI (P = .039). The incidence of the GCB subtype in relapsed patients suggests that other parameters in addition to an IPI score of more than 2 may play a role. TP53 mutations were found in 26% of de novo GCB-DLBCL patients and should be considered as a key factor. In addition, fluorescence in situ hybridization has demonstrated that the survival of DLBCL patients is influenced by 8q24/MYC overexpression. Although most current treatments rely on the dose modulation of cytotoxic drugs, targeted therapy that may act independently of the DNA damage pathway is becoming available and should be evaluated according to the tumor molecular profile.

The present study is a great example of how we can make progress in studying a large cohort of patients through a collaborative effort. It demonstrates that the introduction of rituximab to chemotherapy did not change the
prognostic value of TP53 mutation. However, it also shows that translation to more accessible procedures is possible in future clinical trials.

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REFERENCES


Comment on Ambrosio et al, page 4072

Monitoring granule traffic in megakaryocytes

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Heritable granule deficiencies have enabled the identification of proteins involved in dense granule biogenesis. Yet how these proteins are organized into pathways to identify how a maturing dense granule acquires dense granule biogenesis. They first develop a set of markers capable of identifying different components of the dense granule trafficking apparatus. They identify dense granules using the marker LAMP2 as well as the dense granule transporters MRP4 and VMAT2. Rab7 is used as a marker for multi-vesicular bodies (MVBs) and late endosomes (see “MVB/late endosome” in figure). They find that Rab7 associates with immature granules expressing dense granule markers. The MEG-01 model also enables more dynamic measurements to interrogate granule trafficking. The authors use live cell imaging and pulse-chase experiments with several fluid phase markers to demonstrate the involvement of the late endocytic pathway in dense granule biogenesis (see “Endocytic pathway” in figure). Based on these findings, they conclude that dense granules derive from MVB/late endosomes.

But if dense granules have an MVB/late endosomal origin, how do they acquire the transporters necessary for achieving such high concentrations of cargo? To address this question, Ambrosio et al use the serotonin transporter, VMAT2, as a model protein to evaluate how a maturing dense granule acquires such molecular pumps. They show that the adaptor protein AP-3, which localizes to an early endosomal compartment, recognizes a tyrosine- and dileucine-based sorting motif on the cytoplasmic tail of VMAT2 (see “AP-3” in figure). They also demonstrate a role for Rab32 and Rab38 in transferring VMAT2 and MRP4, the putative adrenal nucleotide transporter, to maturing dense granules (see “Rab32/38” in figure). Further evidence that Rab32/38 associate with immature dense granules that subsequently lose these Rab proteins and concentrate ADP is obtained by isolation and characterization of dense granules at different stages of development. Immature dense granules have higher concentrations of Rab32/38, while more mature dense granules lose their Rab32/38 and concentration ADP.

The major breakthrough of this work by Ambrosio and colleagues is the establishment
TP53 mutations and rituximab-CHOP

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