

MYC protein overexpression had poor prognostic impact when BCL2 protein was coexpressed.⁸

The study by Horn et al¹ took advantage of a large series of elderly patients (age 61–80 years) homogeneously treated in a prospective trial with CHOP or rituximab plus CHOP (R-CHOP) to analyze the prognostic impact of MYC, BCL2, and BCL6 at both the gene level and the protein level. First, the description of the distribution of the oncogenic events and the deregulation of the protein expression is of great interest. MYC translocation and MYC protein overexpression (>40%) were detected in 8.8% and 31.8% of the cases, respectively. MYC translocation was associated in double-hit or triple-hit aberrations with BCL2 and/or BCL6 rearrangements in 60% of these cases. MYC overexpression occurred independently of MYC translocation in 30% of the cases. In terms of cell of origin, MYC and BCL2 rearrangements were more frequently observed in GCB-DLBCL and BCL6 translocation was observed more frequently in non-GCB-DLBCL, whereas at the protein level, no significant difference with respect to MYC overexpression was noted between GCB- and non-GCB-DLBCLs. Second, and more importantly for clinical practice, the survival analyses showed that, taken individually, MYC translocations in the whole group of patients as well as MYC, BCL2, and BCL6 protein overexpression in the R-CHOP group were associated with an adverse prognosis, independently of the IPI score (see figure). Moreover, the pattern associating MYChigh, BCL2high, BCL6low, and MYC rearrangement was highly predictive of the prognosis, independently of the IPI score.

Why is this report significant in routine practice for DLBCL patients? The pivotal importance of this report is that the authors identified in elderly patients treated with R-CHOP a worse group within the IPI high-risk group (3–5 adverse prognostic parameters) with 3-year event-free survival and OS of only 15.6% and 41.6%, respectively. Whether these results will also be true in young patients (younger than age 60 years) in whom intensity of treatment may be different still has to be proven.

Regarding the physiopathology of these oncogenic events, why should MYC be diagnostic in BL but prognostic in DLBCL?

One possible explanation is that these diseases are molecularly distinct as reflected by gene expression profiling.¹⁰ Moreover, by using small interfering RNA against MYC, it was shown that MYC target genes modulate a completely different and unique set of genes in BL compared with DLBCL, with the nuclear factor κ B pathway being one distinguishing set of affected genes.¹⁰ This may explain the profound negative prognostic significance of MYC expression in DLBCL.

In addition to the fact that DLBCL is described in the World Health Organization classification with 18 subentities, choice of treatment is still based on clinical features only. Clearly, because of the recent identification of GCB-like and ABC-like DLBCL subtypes as well as the outstanding analysis reported by Horn et al¹ describing the major impact of MYC, BCL2, and BCL6, we can argue that the classification of DLBCL is changing. New entities with clinical relevance are emerging. In the near future, this will have a major impact on defining the most appropriate treatment to propose to patients with DLBCL. The number of ongoing clinical trials attests to the search for novel targeted agents tailored toward these specific molecules or pathways.

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

● ● ● CLINICAL TRIALS & OBSERVATIONS

Comment on Jourdan et al, page 2213

MRD in AML: time for redefinition of CR?

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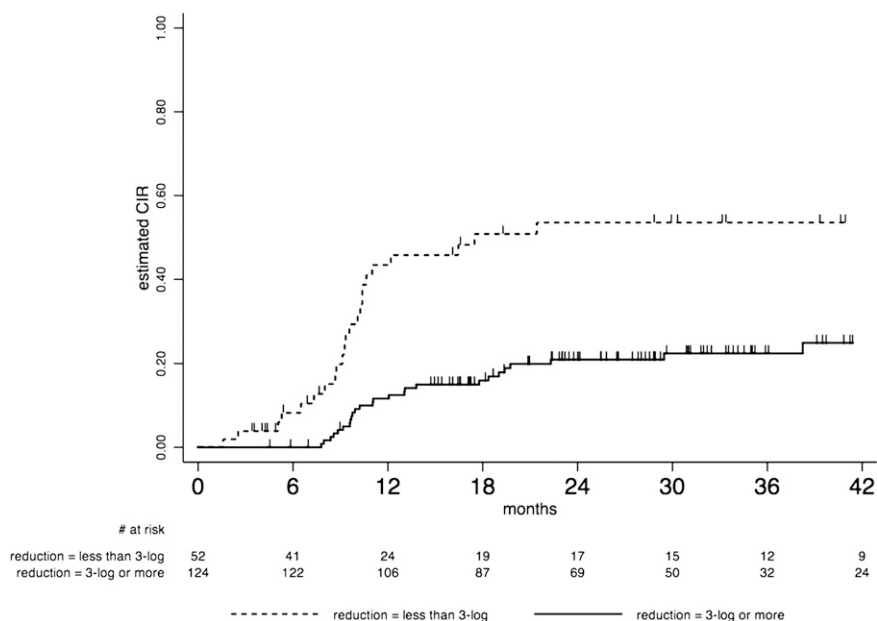
In this issue of *Blood*, Jourdan and colleagues from the French AML Intergroup demonstrate the prognostic value of minimal residual disease (MRD) in adult patients with core binding factor (CBF) acute myeloid leukemia (AML).¹

Currently, the most important prognostic factors for AML are based on cytogenetics and molecular abnormalities, which are assessed at diagnosis.² Although these factors have been shown to be of utmost importance in risk stratification, the treatment outcome of patients within the thus-defined risk groups is still highly variable. New prognostic factors that, apart from diagnosis parameters, may include treatment and response related factors are needed.

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MRD, defined as the persistence of leukemic cells after chemotherapy at numbers below the sensitivity detection level of routine morphology, represents the sum of the effect of all relevant cellular resistance mechanisms, pharmacokinetic resistance, dosage and compliance, and other unknown factors affecting the effectiveness of treatment. Relapses still are a major cause of dismal outcome in AML treatment and are generally thought to be the result



Outcome by "Minimal Residual Disease response" after the first consolidation course in CBF AML cumulative incidence of relapse (CIR): At 36 months CIR was estimated at 22% (95% CI, 16-32) in patients who achieved a 3 log MRD reduction vs 54% (95% CI, 39-69) in those who did not. See figure 5A in the article by Jourdan et al that begins on page 2213.

of outgrowth of these persisting leukemic cells.

Many studies have shown that MRD cell frequency after different cycles of therapy offers a highly independent prognostic factor, both in adult and childhood AML. In adult AML, these data are mostly derived from retrospective correlative studies.

In this prospective study, patients with CBF-AML carrying the t(8;21) or inv(16)/t(16;16) chromosomal abnormality, characterized by the presence of *RUNX1-RUNX1T1* or *CBFB-MYH11* fusion transcripts, respectively, were monitored for MRD. CBF-AML has a favorable prognosis with a cure rate of 65% with chemotherapy alone.² Frequent receptor tyrosine kinase mutations are present in CBF-AML, where especially mutations in *KIT* and *FLT3* have been associated with a worse outcome. In the present study, *KIT*, *FLT3*, and N/K-RAS gene mutations were examined at diagnosis.¹ Patients were randomly allocated either to an intensive induction or a standard treatment arm. Subsequently, when a complete remission (CR) was achieved, patients received 3 postremission cycles consisting of high-dose ARA-C. After each consolidation course, the levels of MRD were monitored for *RUNX1-RUNX1T1* or *CBFB-MYH11* transcripts by real-time quantitative polymerase chain reaction. It was planned by

protocol to perform an allogeneic transplant in patients who did not achieve at least a 3-log MRD reduction. However only 12 of 52 patients, who based on MRD level classified for an allogeneic transplant, actually received one. Reasons for that are not mentioned by the investigators, but most likely are due to reluctance of individual physicians to transplant these good-risk patients who, at least for inv(16) AML, usually have a rather good prognosis if transplanted in second remission. The clinical outcome of the study confirmed the good prognosis of CBF-AML, but intensified induction was not associated with a better survival. Striking were the findings associated with the MRD monitoring: although higher WBC, RTK gene mutations, and a <3-log MRD reduction after the first consolidation cycle individually were associated with a higher risk of relapse, MRD response remained the only significant prognostic factor in multivariate analysis. In patients who achieved 3-log MRD reduction vs patients who did not, the 3-year cumulative incidence of relapse was 22% and 54%, respectively, and 3-year relapse-free survival was 73% and 44%, respectively. The same applied for the absolute 0.1% MRD level.

This is in line with recent observations by the UK MRC trial group, who also concluded that MRD monitoring by quantitative reverse-

transcription polymerase chain reaction in CBF-AML at different time points identified patients at high risk for relapse.³

Clearly, a new definition of CR is emerging in CBF-AML. How this should be implemented and whether preemptive therapeutic intervention would be of benefit is not established given the fact that the majority of patients can be rescued after relapse.

In a recent ELN recommendation, a patient-specific application of allogeneic hematopoietic stem cell transplantation (HSCT) in patients with AML in first CR was proposed integrating the risk for relapse and nonrelapse mortality. The recommendation aims for a disease-free survival benefit of at least 10% for the individual patient as compared with consolidation by a nonallogeneic HSCT approach.⁴ It could be argued that a patient with CBF-AML and a <3-log reduction of MRD level associated with a relapse risk of around 50% should be offered an allogeneic HSCT if the estimated transplant-related mortality is 10% to 15%.

Can we extrapolate to AML groups other than the CBF-AML? The German Austrian AML study group showed that NPM1(mut) transcript levels were significantly associated with prognosis after each treatment cycle.⁵ Mutations in *FLT3*, *WT1*, and *CEBP α* offer other molecular markers potentially useful for MRD detection. However, robust data in prospective studies are currently lacking.

Another valuable method to monitor MRD is by flow cytometry that relies on the expression of "leukemia-associated immunophenotypes" defined as the presence of a combination of antigens and/or other flow-cytometric abnormalities that are absent in normal cells. It is widely applicable (in >90% of AML), quick, and relatively cheap, but usually less sensitive than molecular MRD.

Studies showing the prognostic value of flow-cytometric MRD were mostly performed in a single-institute setting, resulting in well-known potential pitfalls such as bias in patient groups and subjective judgment.

The next logical and obvious step would be to perform the studies in a prospective and preferably multicenter way. In childhood AML, such a recent study showed that after

initial induction chemotherapy, MRD was detected in one-third of patients without morphologic evidence of disease, which in turn was highly correlated with relapse and an independent predictor of outcome.⁶ In adult AML, prospective studies are about to be published. However, based on the existent data, many AML trial groups are in the process of implementing MRD monitoring (flow cytometry and molecular) in new clinical trials.

Fine-tuning of techniques and merging of flow and molecular genetic assays may ultimately bring us closer to the final goal of real individualized risk assessment and therapy in patients with AML.

MRD is at the edge to offer a new definition for CR and is possibly useful as a surrogate end point for outcome of studies investigating new drugs in AML.

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

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experimental systems that model the outcome in humans. More than a decade of AAV research in rodents and dogs failed to elicit the anticapsid response that was ultimately observed in humans. Thus, before testing whether they could generate an AAV that would evade the anticapsid response, Martino et al developed a model that would mimic the response in humans. To do this, they immunized mice with a known immune epitope from the AAV2 capsid, isolated CD8+ T cells, and expanded them ex vivo by repeat stimulation with the antigen. This established a pool of CD8+ T cells with specificity against the AAV capsid. When these anticapsid T cells were transferred into mice that were injected 24 hours prior with an AAV2 vector expressing factor IX, they killed the hepatocytes harboring the vector, and this resulted in diminished factor IX expression and an elevation in transaminases in the serum. This was similar to the outcome observed in the earlier human trial of AAV2 and in 1 of the patients in the recent AAV8 trial.^{2,3}

With a suitable model established for studying the anticapsid response, the authors evaluated the immune evasive potential of a novel AAV2 vector variant they previously generated,⁷ which harbors mutations in 3 different tyrosine residues that are normally exposed on the vector's surface. In contrast to what was observed with the wild-type AAV2 vector, when they transferred anticapsid CD8+ T cells into mice injected with the AAV2 variant vector, there was no transaminitis, and factor IX expression was similar to the levels in mice that did not receive the anticapsid T cells. This is a promising achievement because it was performed in a system that models some of the hallmarks of the patients' response to therapy. It is important to note that the anticapsid response that occurred in patients was subdued by transient immunosuppression.³ A therapy that does not require any immune modulation would obviously be preferable, but clinical adoption of their AAV2 variant will initially be warranted mostly in patients where immunosuppression is contraindicated. It also remains to be determined whether the tyrosine mutations can be introduced into the capsids of other AAV vector serotypes and improve immune evasion without impacting the efficiency of gene transfer.

● ● ● GENE THERAPY

Comment on Martino et al, page 2224

Stealth gene therapy

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In this issue of *Blood*, Martino et al¹ report on a novel adeno-associated viral (AAV) vector, which overcomes one of the last remaining impediments for liver gene therapy, the anticapsid immune response.

Just over 7 years ago, the first successful gene therapy for hemophilia appeared to be at hand, until an unexpected immune response against the vector capsid led to clearance of corrected liver cells and a loss of the replacement factor IX gene.² Since that trial, much has been learned, and in 2011, it was reported that intravenous injection of an AAV8 vector encoding factor IX was able to achieve sustained factor IX expression in 6 individuals with hemophilia B.³ This stunning achievement, long-lasting correction of a genetic disease from a single drug injection, is nearly unprecedented in medicine and will likely change the course of hemophilia therapy.⁴ However, although the trial was largely successful, the anticapsid

immune response did occur at the most corrective vector dose, and transient immunosuppression with prednisolone was used to prevent the immune system from eliminating hepatocytes harboring the replacement factor IX gene. Thus, improved strategies are still needed to circumvent the anticapsid immune response before AAV can become an off-the-shelf drug for hemophilia B. In Martino et al, High, Herzog, Mingozzi, and colleagues, who pioneered the use of AAV for hemophilia gene therapy,^{2,5,6} describe an innovative modification to the AAV capsid that demonstrates the potential to avoid immune-mediated clearance.

One of the challenges of studying the anti-AAV capsid response has been the lack of