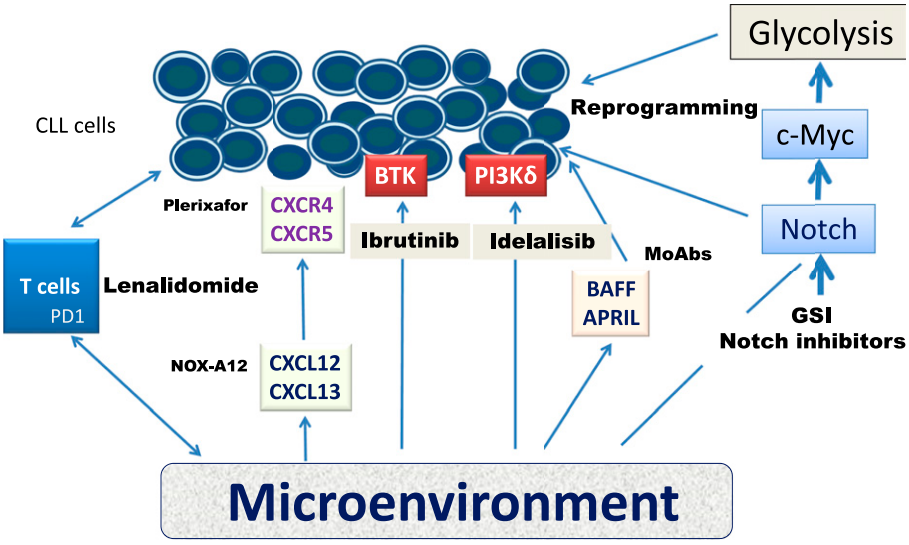

Schematic representation of the microenvironment and agents with the potential of targeting specific CLL pathways. APRIL, a proliferation-inducing ligand; BAFF, B cell–activating factor of the TNF family; BTK, Bruton tyrosine kinase; PD1, programmed death 1 receptor; PI3Kδ, phosphoinositide 3-kinase δ.

of c-Myc and its target gene *cyclinb1*, but also in the abolishment of the glycolytic shift. As a result, a decrease in glycolytic enzymes and extracellular acidification rate were observed. Furthermore, this inhibition conveyed a synergistic effect on fludarabine-induced cell death on CLL cells under stromal contact.

In short, besides well-known physiopathological connections between the microenvironment and CLL cells, it appears that the microenvironment has an important role in shifting glycolysis in CLL cells, and that this effect is at least mediated in part by the Notch-c-Myc axis.

From the clinical perspective, CLL treatment is rapidly moving from cytotoxic agents, which in most cases are given in combination with an anti-CD20 monoclonal antibody (MoAb), to noncytotoxic compounds targeting specific CLL pathways revolving around the microenvironment (see figure). Some of these agents (eg, ibrutinib, idelalisib) have entered into clinical practice, have shown remarkable effectiveness, and are changing CLL treatment algorithms.[6] Now, to the plethora of microenvironment-governed chemokines that operate in a complex and the intertwined network of different pathways, an old and well-known actor in the physiopathology of cancer, the so-called Warburg effect, does apply for a prominent role in CLL biology and its therapy. In fact, reprogramming glycolytic metabolism is again being considered a target for cancer treatment. There is no reason why CLL should be an exception to this appealing approach. In the exciting era of precision medicine for CLL, the study reported by Jitschin et al paves the road for further studies on CLL metabolism and its potential implications in the treatment of this still incurable disease.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● MYELOID NEOPLASIA

Comment on Martelli et al, page 3455, and El Hajj et al, page 3447

ATRA and ATO team up against NPM1

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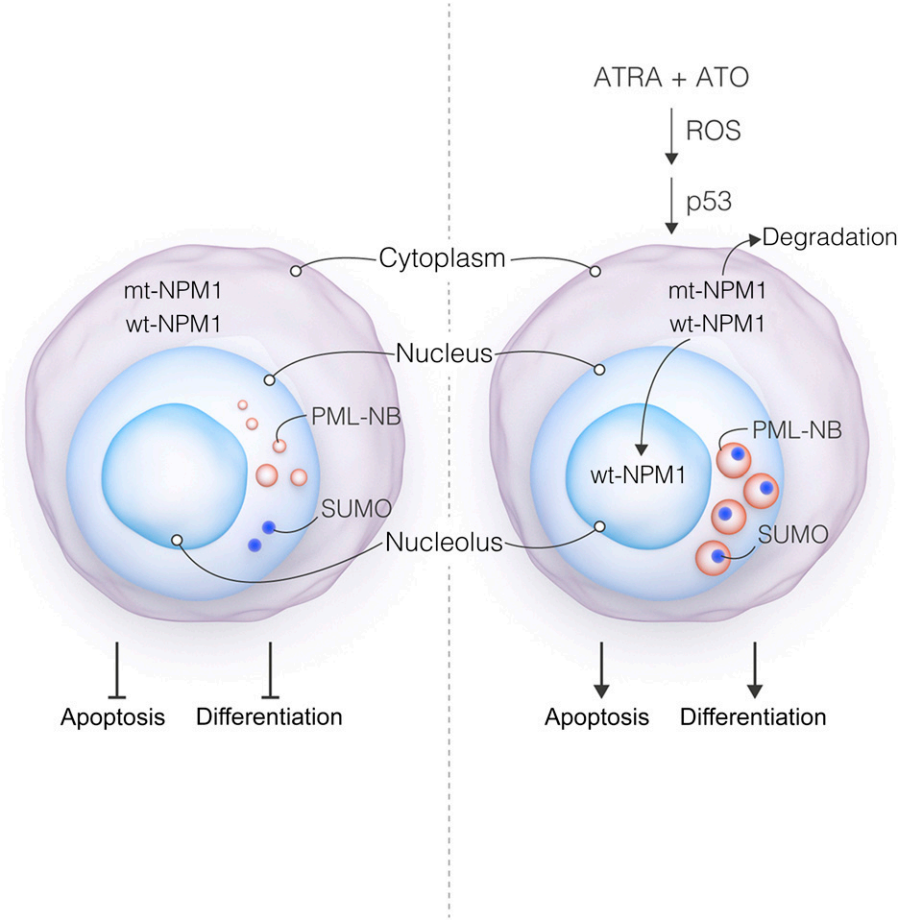
In this issue of *Blood*, Martelli et al[1] and El Hajj et al[2] independently report that nucleophosmin-1 (NPM1)-mutant leukemia is particularly vulnerable to a novel strategy combining all-*trans* retinoic acid (ATRA) with arsenic trioxide (ATO). The era of targeted therapy has seen some of its greatest successes in the hematologic arena (eg, breakpoint cluster region [BCR]/Abelson [ABL] kinase inhibitors in chronic myeloblastic leukemia and ATRA in acute promyelocytic leukemia [APL]). Moreover, addition of ATO, an agent that induces oxidative stress and interferes with protein translation, to ATRA sharply increases APL cell killing to the extent that cures in this disease are no longer unrealistic.[3] A theoretical (and practical) basis for translating ATRA/ATO-based strategies to non-APL acute myelocytic leukemia (AML) is currently lacking.

The central question addressed in the 2 studies is whether the ATRA/ATO strategy might be appropriate for non-APL leukemias, and particularly for leukemia characterized by another mutant oncoprotein, NPM1. *NPM1* is a gene encoding a nucleolar shuttling protein that is frequently mutated in AML (30%) and which has been implicated in leukemogenesis. Although it carries a favorable prognosis, this feature is overcome by the presence of FMS-like tyrosine kinase-3 internal tandem duplication mutations. Moreover, despite initial responses to induction chemotherapy, relapses often occur in NPM1-mutant AML, and cures remain elusive, justifying the search for novel



Proposed model of ATRA/ATO actions in NPM1-mutant cells. Mutant NPM1 leads to delocalization of both mutant (mt) and wild-type (wt) NPM1 (ie, from their normal nucleolar distribution to the cytoplasm). NPM1 mutation also results in disorganization of PML nuclear bodies (PML-NB), manifested by small size, heterogeneous appearance, nucleoplasmic localization, and dissociation from SUMO-1. These events, through mechanisms that have yet to be determined, disrupt both cell differentiation and apoptosis, contributing to leukemogenesis. Exposure of mutant NPM1-expressing cells to ATRA + ATO triggers pronounced oxidative stress and p53 activation, culminating in mutant NPM1 degradation. The loss of mutant NPM1 leads in turn to re-localization of wt NPM1 to the nucleolus, accompanied by reversal of PML-NB disorganization (eg, reflected by larger size, more homogeneous appearance, and restoration of the SUMO-1 association). These events culminate in leukemic cell differentiation and/or cell death. Professional illustration by Luk Cox, Somersault18:24.

approaches. Although the mechanism of action (MOA) of NPM1 is not known with certainty, its deregulation leads to delocalization of mutant NPM1 from the nucleolus associated with disorganization of promyelocytic leukemia (PML) nuclear bodies. Interestingly, in APL cells, correction of PML disorganization accompanied by PML-retinoic acid receptor-α (PML-RAR-α) degradation and differentiation induction occurs with ATO treatment.[4] Although pharmacologic inhibitors of NPM1 function (eg, deguelin) have been described,[5] their clinical relevance remains to be established. Notably, previous studies have shown that genetic or pharmacologic NPM1 inhibition sensitizes mutant NPM1 leukemia cells to ATRA.[6]

Anecdotal evidence from the clinic indicating that NPM1 mutations may render AML patients susceptible to ATRA raised the possibility that a mutant oncoprotein-targeting strategy involving ATRA (possibly in combination with ATO) could be a viable alternative to specific pharmacologic NPM1 inhibitors. However, the mechanism(s) by which such a strategy might operate in this setting are unknown. The results of the 2 studies provide a theoretical foundation for applying this approach to mutant NPM1. Specifically, the authors report that exposure of NPM1-mutant AML cells to ATRA and ATO induces selective proteasomal degradation of mutant NPM1 protein accompanied by nucleolar redistribution of wild-type NPM1, reversal of the characteristic disorganization of PML bodies, and pronounced apoptosis and/or differentiation. Significantly, these events did not occur in non–NPM1-mutant leukemias, were dependent upon oxidative stress induction and p53 activation and, importantly, were also observed in primary NPM1-mutant AML blasts (see figure). Of note, El Hajj et al[2] treated 5 elderly patients ineligible for chemotherapy with the ATRA/ATO regimen on a compassionate basis, and reductions in blasts and/or other hematologic improvements were observed in some of these patients. Collectively, these findings argue that, in addition to its established role in APL, the ATRA/ATO strategy may represent a viable option in NPM1-mutant AML and raise the possibility that analogous MOAs may be operative.

The findings presented in these studies could have implications well beyond NPM1-mutant AML and could be applicable to other leukemias (and possibly other hematologic malignancies) characterized by diverse mutant oncoproteins. They also highlight fundamental differences between approaches that directly inhibit specific mutant oncoproteins (driver mutations) vs those targeting so-called "orthogonal" processes required to circumvent neoplastic cell oncogenic stress.[7] For example, a major success in leukemia therapy involved the introduction of inhibitors that specifically disrupt the function of the primary leukemogenic BCR/ABL kinase. More recently, specific IDH1/2 kinase inhibitors (eg, AG-221) have shown highly promising results in IDH2-mutant AML.[8] Unfortunately, for many mutant oncoproteins, including transcription factors or proteins whose functions are not clearly defined, development of specific inhibitors can be problematic. However, such proteins may be vulnerable to agents that nonspecifically disrupt stress-related pathways required for oncoprotein maintenance. In this context, mutant oncoproteins are highly dependent upon chaperone proteins (eg, HSP90) to maintain stability, prompting the development of HSP90 antagonists in this setting.[9] For various reasons, including host toxicity, a clear role for HSP90 antagonists has not yet been established in AML or other malignancies. Conversely, ATO induces oxidative stress in neoplastic cells[10] and also interferes with the translation of mutant oncoproteins.[11] It appears that, as in the case of PML-RARα APL, the mutant NPM1 protein may be particularly vulnerable to this approach and that its resulting degradation and redistribution may circumvent the need for specific NPM1

inhibitors. The possibility also exists that other mutant oncoproteins implicated in leukemogenesis, and for which specific inhibitors are not yet available, could represent additional targets for this strategy.

There are, however, several questions and caveats that could clearly determine the potential of this approach. For example, it remains to be established whether the ATRA/ATO strategy targets mutant NPM1-expressing leukemia-initiating cells (stem cells) which, at least theoretically, could be responsible for disease relapse. In addition, although both cultured and primary NPM1-mutant AML cells were very susceptible to this regimen, it is less certain whether the degree of cell killing can approximate that observed in the case of APL cells. Finally, although the ATRA/ATO regimen showed some activity in patients with mutant NPM1 AML, it is generally recognized that survival benefits in this (or other) disease(s) absent objective complete responses are unlikely. In this regard, the finding that ATRA/ATO enhanced the activity of an active anti-leukemic agent (eg, daunorubicin) in mutant NPM1 AML is noteworthy and raises the possibility of a more effective future approach. Alternatively, combining the ATRA/ATO strategy with other targeted therapies, including those directed against NPM1 itself, may provide opportunities for cure in this disease, as observed in some patients with APL. Finally, extrapolation of this strategy to other AML subtypes that display different oncogenic mutant proteins represents a promising possibility and one that clearly deserves further investigation.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Gebhart et al, page 3477

Lupus anticoagulant, thrombosis, and death

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In this issue of *Blood*, Gebhart et al report a prospective observational cohort study evaluating 151 patients with persistently positive lupus anticoagulant (LA) for a median period of 8.2 years. They observed increased mortality in LA-positive patients, mainly due to new thrombotic events.[1]

A 32-year-old woman was referred to the hematology clinic after she was found to have persistently positive LA tests. In such a case, 3 distinct scenarios may prompt very different responses by the hematologist. In scenario 1, the patient has no symptoms or signs of antiphospholipid syndrome (APS) other than a positive LA. In scenario 2, she has livedo reticularis and thrombocytopenia that cannot be diagnosed as a definite APS. In scenario 3, she has already been diagnosed with APS due to thrombotic events and/or pregnancy complications. What are the consequences of having a positive LA for this patient? How should the hematologist manage these 3 situations?

Unfortunately, only a few prospective studies have addressed these questions. Antiphospholipid antibodies (aPLAs) are a group of immunoglobulins that can bind to phospholipid-protein complexes. Three major antibody groups (LAs, anticardiolipin antibodies [aCLs], and anti-β2-glycoprotein I [anti-β2GPI] antibodies) are thought to be involved in the pathogenesis of APS and are accepted as being part of the serological criteria for its diagnosis.[2] Appropriate laboratory identification of aPLAs is critical because the clinical criteria for APS (thrombosis and recurrent pregnancy complications) are very common. Although the prevalence of aPLAs in healthy populations has been reported to be as high as 5% in some studies,[3] these high rates are due to very liberal cutoff definitions. In a large multicenter case-control study, the prevalence of LAs, aCLs, and anti-β2GPI antibodies in 628 healthy women was found to be 0.63%, 0.96%, and 0.95%, respectively, when the cutoff level was defined as above the 99th percentile of normal.[4] All aPLAs are thought to be associated with the clinical findings of APS, but prospective studies have shown both thrombosis and pregnancy morbidity to be more strongly associated with LAs than with aCLs or anti-β2GPI antibodies.[4,5] In a prospective study involving 82 newly diagnosed immune thrombocytopenia patients, 31 patients were found to have aPLAs at the time of diagnosis (as in scenario 2). Fourteen patients in the aPLA-positive group developed thrombosis after 5 years of follow-up, and patients with LAs showed a significantly high risk for thrombosis (relative risk, 7.15).[6] Which