including T315I mutated, BCR-ABL positive, therapy-refractory B-ALL and CML samples. Thus, LDK shows promise for a variety of hematologic malignancies.

Ideally, anticancer therapeutics should specifically target unwanted proliferative cells, with few off-target effects. LDK activity appears to be highly specific. In cell culture, LDK, compared with an AKT inhibitor, showed equivalent toxicity toward malignant lymphoblasts. But unlike the AKT drug, LDK was not nearly as lethal toward mature T cells in peripheral blood at the lymphoblast-lethal dose. LDK appears to work in 2 ways: by inhibition of the PI3K/AKT/mTOR pathway and by cell-cycle delay in G2/M. Interestingly, cell-cycle effects appeared to be relatively specific for lymphoblasts, because cell-cycle defects were not observed in LDK-treated zebrafish embryos during development, before the emergence of lymphoblasts. Moreover, because the original screen was performed in whole animals, LDK was preselected for its lack of general toxicity. This tolerability was confirmed in mammals where injection or oral administration produced reasonably long-lasting serum levels of drug, but had no obvious organ toxicity in a variety of assays. Thus LDK appears to be a highly effective antileukemic agent, with few off-target effects.

The data presented by Ridges and colleagues demonstrate that zebrafish are an exceptional cancer and drug discovery model. Previously it has been shown that zebrafish get cancer,[2] that human oncogenes cause malignancy in zebrafish,[2] that novel oncogenes can be discovered by mutagenesis,[6] and that known human cancer therapeutics can be effective in fish.[7] High throughput means of transplanting, injecting, and screening embryos are rapidly developing.[3,8] Here, Ridges et al show that novel human anticancer therapeutics can be discovered de novo using small molecule screening in zebrafish.

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

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MYELOID NEOPLASIA

Comment on Ribeiro et al, page 5824

# Mutant *DNMT3A*: teaming up to transform

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The report by Ribeiro et al in this issue of *Blood* confirms the evolving data that *DNMT3A* mutations represent another common alteration in adult acute myeloid leukemia (AML) and are an important modulator of outcome.[1]

Within the past 2 years, the invention of next-generation sequencing (NGS) has revealed a plethora of previously undescribed genetic abnormalities affecting several different pathways, including mutations in *IDH1* and *IDH2*,[2] and *DNMT3A*[3,4] as well as numerous less common or even patient-specific abnormalities. Clarification of the prevalence and the prognostic impact of these changes has become a critical issue in how to identify the driver lesions and in deciding which factors should be added to the set of molecular abnormalities routinely tested.

In a large and well-characterized population of adult patients with AML, Ribeiro et al investigated the prevalence and prognostic impact of *DNMT3A* mutations. In this series, 96 of 415 patients (23%) carried a mutation of the gene, which ranks it among the most common changes in adult AML. *DNMT3A* mutations were predominantly found in patients with French-American-British (FAB) M4 and M5 morphology and were significantly associated with increased white cell counts at diagnosis. More importantly and in agreement with most studies published so far, patients with *DNMT3A* mutations were a median 10 years older than patients with wt-*DNMT3A*. *DNMT3A* mutations were mostly found in patients with cytogenetically normal (CN)–AML and occurred together with certain abnormalities enriched in this group, most importantly *NPM1* and *FLT3-ITD*. Although *DNMT3A* mutations were not associated with a specific mRNA gene expression profile (GEP), the authors could show an enrichment of *DNMT3A* mutant samples in a cluster associated with a specific methylation pattern; however, this cluster was mostly characterized by *NPM1* mutations.

When analyzing the reported data for *DNMT3A* in adult AML (see table),[1,3,5-10] several interesting aspects become evident. The mutation is found in approximately 15% to 25% of series reported from Europe and the United States[1,3,5,9]; however, the prevalence is somewhat lower in the 2 large unselected studies from Asia (7% and 14%),[8,10] potentially indicating an effect of ethnic background. Given the association with CN-AML observed in all studies, it is not astonishing that the highest prevalence was reported in the 2 series focusing on CN-AML (29%–36%).[6,7] Most reports agree in certain clinical aspects (increased patient age, high WBC) and all confirmed the association with FAB M4/M5-morphology. The important association with increased patient age is also evident in most series, which is in line with the very low prevalence in pediatric AML.[11] In addition, the majority of studies could confirm the association with outcome, indicating that patients with *DNMT3A* mutations have a significantly shorter overall and disease-free survival. This appears not to be an effect of a decreased rate of complete remission, but reflects an increase in disease recurrence. On the biologic side, the correlation with other molecular changes (*NPM1*, *FLT3-ITD*) and the mutual exclusive mutational spectrum with genes directly involved in the regulation of DNA methylation (ie, *TET2* and *ASXL1*) further strengthens the importance of epigenetics as a key molecular pathway for leukemic transformation (see figure). Because the majority of these mutations are associated with inferior outcome,

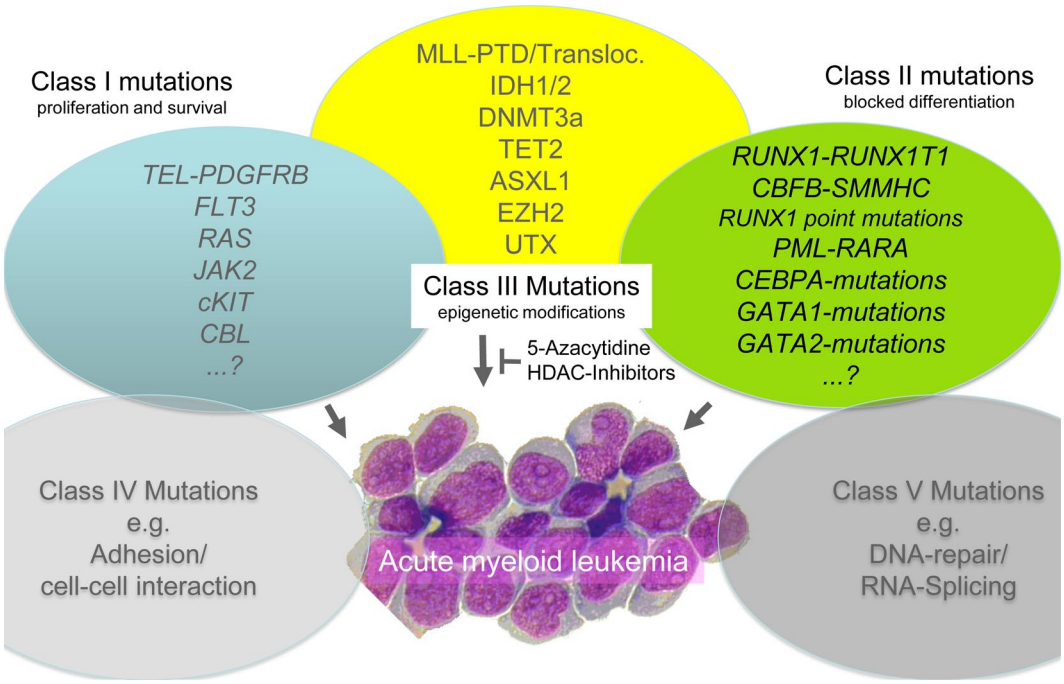
**Table 1. Published clinical studies on DNMT3A mutations**

| | Ley[3] | Thol[9] | Ribeiro[1] | Patel[5] | Shen[10] | Hou[8] | Marcucci[6] | Renneville[7] |
|---|---|---|---|---|---|---|---|---|
| Patients analyzed (N) | 281 | 489 | 415 | 398 | 1141 | 500 | 415 | 123 |
| *DNMT3A* mut (N) | 62 | 87 | 96 | 89 | 75 | 70 | 148 | 36 |
| *DNMT3A* mut (%) | 22 | 18 | 23 | 22 | 7 | 14 | 36 | 29 |
| AML population (all adult) | all | < 60/de novo | < 60 | < 60 | de novo | de novo | CN/de novo | CN, < 60/de novo |
| *DNMT3A* region analyzed | complete | E15–23 | E11–23 | complete | complete | E2–23 | E15–23 | E8–9/E11–23 |
| Median age, y (mut/wt) | 53/48* | 52/45*** | 51/41*** | ng | 54/38*** | 61/49*** | 61/62 | 48/47 |
| Median WBC (Gpt/l) (mut/wt) | 59/39*** | 38/17** | 53/23*** | ng | 38/7*** | 32/16*** | 43/22*** | 13/11 |
| Median BM blasts (%) (mut/wt) | 70/70 | 78/70* | 68/68 | ng | 78/65*** | ng | 58/57 | ng |
| FAB | M4/M5 | M4/M5 | M4/M5 | M4/M5 | M4/M5 | M4/M5 | M4/M5 | M4/M5 |
| Median CR rate (%) (mut/wt) | ng | 71/76 | 89/79 | ng | 46/62* | ns | ns | 90/80 |
| Median OS (mo mut/wt) | 12/41*** | 21/40* | 12/24 | 14/21 | $P < .001$ | 15/38 | $P = .07$ | 23/45* |
| Median DFS/RFS (mo mut/wt) | ng | $P = .2$ | ng | ng | $P < .001$ | 8/15 | $P = .03$ | ng |
| CN-AML (%) | 72*** | 82*** | 75*** | ng | ng | 51 | only CN | only CN |
| *NPM1* mut (%)† | 60 | 64 | 76 | 64 | | 54 | 75* | 67 |
| *FLT3-ITD* (%)† | 41 | 39 | 41 | ng | | 43 | 44* | 19 |
| *CEBPA* mut (%)† | ng | ng | 3 | 7 | | 4 | 5*** | 0 |
| *MLL-PTD* (%)† | ng | ng | ng | 6 | | 9 | 6 | ng |
| *TET2* mut (%)† | ng | ng | ng | 7 | | 9 | 22 | ng |

ng indicates not given. *$P < .05$; **$P < .01$; and ***$P < .001$. †For DNMT3A mutation patients.

the use of targeted treatment options (eg, demethylating agents such as 5-azacytidine or decitabine) specifically within this group of patients will be an important question for future studies. Despite the considerable number of studies reported, the impact of the location of the mutations within the *DNMT3A* gene is another open question. Approximately two-thirds of the mutations affect the catalytic domain, whereas the other 30% are distributed over the rest of the protein, clustering mostly in the zink finger and proline-tryptophane-tryptophane-proline (PWWP) domains.[3] Because these types of mutations show considerable functional differences, their biologic consequences and potential differences in outcome observed in some studies[6] but not confirmed in others,[3] remain to be further clarified, which may become even more important in the context of targeted treatment strategies.

Although these data shed important light on the biology of AML, the individual patient picture might actually be more obscure. Because NGS data of several AML patients indicate that typically 8 to 12 somatic mutations can be found per genome in CN-AML,[2,3] several additional pathways might be affected (eg, DNA repair or cellular migration). Thus, the current scheme of least 3 major pathways affected in adult AML, that is, proliferation, differentiation and epigenetic modification, might need further extension in the near future (see figure).




**Molecular pathogenesis of AML.**

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Debrincat et al, page 5850

# Death regulates platelet birth and life

**Karin M. Hoffmeister** HARVARD MEDICAL SCHOOL

In this issue of *Blood*, Debrincat et al show that Mcl-1 and Bcl-xL coordinately regulate megakaryocyte survival and ultimately affect platelet life-span.[1]

In recent years it has become apparent that platelet survival depends on the interplay between proteins of the Bcl-2 family, which are critical regulators of the "intrinsic" apoptosis pathway. The Bcl-2 family contains both prosurvival and proapoptotic members, and the balance between these competing systems regulates the apoptotic switch. The prosurvival family consists of Bcl-2, Bcl-$x_L$, Mcl-1, A1, and Bcl-w. Prosurvival proteins maintain cellular viability by restraining proapoptotic Bak and Bax, which represent the effector arm of the intrinsic pathway. Once activated, Bak and Bax damage mitochondria, triggering a cascade of events that ultimately leads to activation of caspase-9, the so-called "initiator" caspase that triggers the apoptotic cascade.[2]

Bcl-$x_L$, a member of the Bcl-2 family of prosurvival proteins, is clearly essential for platelet survival, both in vitro and in vivo (see figure). Its role is to control the activity of Bak, and to a lesser extent Bax.[3] Mice lacking Bcl-$x_L$ exhibit a shortened platelet life span of only 24 hours, compared with 5 days in their wild-type counterparts[3] More recently, mice specifically lacking Bcl-$x_L$ in the megakaryocyte lineage have been generated.[4] These animals had platelet counts reduced to less than 5% of those of wild-type mice, due in part to a platelet life span reduced to only 5 hours. The study has also shown that mature megakaryocytes depend on the function of Bcl-$x_L$ to efficiently produce platelets, although Bcl-$x_L$ loss alone does not impair the growth or maturation of megakaryocytes, indicating that apoptotic factors may be redundant.[4]

Debrincat et al add Mcl-1 to the repertoire of prosurvival Bcl-2 family members affecting platelet formation and survival. They propose that the combination of both Bcl-$x_L$ and Mcl-1 is essential for the viability of the megakaryocyte lineage. Using an elegant system in which Mcl-1, alone or in combination with Bcl-XL, is specifically deleted in mouse megakaryocytes, they convincingly demonstrate that Mcl-1 is dispensable for normal megakaryocytopoiesis and platelet life span, even when stressed to produce new platelets after platelet ablation.[1] However, the role of Mcl-1 in platelet life span remains unclear, as circulating platelets do not contain Mcl-1. It is likely that Mcl-1 affects the sensitivity of megakaryoctes to undergo apoptosis, as the BH3 mimetic compound ABT-737, which binds to and inhibits the prosurvival proteins, Bcl-2, Bcl-$x_L$ and Bcl-w, was toxic to megakaryocytes when applied in mice deficient in Mcl-1.

Further, mice with megakaryocyte-specific deletion of Bcl-$x_L$ and Mcl-1 (Bcl-$x^{Pf4\Delta/Pf4\Delta}$ Mcl-$1^{Pf4\Delta/Pf4\Delta}$ mice) die in preweaning stages and Bcl-$x^{Pf4\Delta/Pf4\Delta}$ Mcl-$1^{+/Pf4\Delta}$ survivors have low platelet counts (< 5% of wild-type levels) with increased size and fewer megakaryocyte numbers than those of mice with the single Bcl-xL deletion. The low megakaryocyte numbers may result from developing megakaryocytes being prone to apoptosis. Interestingly, megakaryocyte-specific deletion of Bcl-$x_L$ and Mcl-1 produces hemorrhage and lethality. Specifically, Bcl-$xL^{Pf4\Delta/Pf4\Delta}$ Mcl-$1^{Pf4\Delta/Pf4\Delta}$ embryos possess aberrant connections between the blood and lymphatic vascular networks, resulting in blood-filled lymphatic vessels. They also exhibit focal hemorrhages, consistent with a failure of hemostasis. However, the embryonic liver morphology appears normal, with no apparent signs of bleeding. It is intriguing that other organs but not the liver have impaired vessel-lymphatic networks. Are platelets not necessary for proper establishment of the liver morphology? What other signals keep the liver vessel and lymphatic systems intact? It would be of particular interest to investigate whether the double Bcl-$xL^{Pf4\Delta/Pf4\Delta}$ Mcl-$1^{Pf4\Delta/Pf4\Delta}$ deficient embryos are able to establish an early bone marrow function.

The work by Debrincat et al opens new investigative avenues and raises interesting questions. For example, why are mature megakaryocytes in particular dependent on Bcl-$x_L$ and Mcl-1? Why do mature platelets lack Mcl-1, but express Bcl-$x_L$? How does the megakaryocyte "sort and package" its apoptotic machinery while producing platelets? Why does only a certain subset of megakaryocytes have an increased sensitivity toward apoptotic events?

The same group has previously shown that Bak-deficient mice exhibit an almost doubling of platelet life span in vivo.[3] The extended life span of $Bak^{-/-}$ $Bax^{-/-}$ platelets in vivo raises interesting questions relevant to platelet circulation. Why do $Bak^{-/-}$ $Bax^{-/-}$ platelets not circulate beyond 10 days? By what means are $Bak^{-/-}$ $Bax^{-/-}$ platelets removed from the circulation? The observations indicate that other clearance mechanisms regulate platelet life span.

Glycan modifications on platelet surface proteins are becoming increasingly recognized