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MYELOID NEOPLASIA

Comment on Man et al, page 2002

PLK4, a potential target against AML

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In this issue of *Blood*, Man et al investigate the therapeutic potential of Polo-like kinase 4 (PLK4) in *TP53* mutated and wild-type acute myeloid leukemia (AML) cell lines, patient samples, and mouse models.[1] PLK4 is a member of the PLK family of 5 serine-threonine kinases involved in cell cycle regulation;[2] the family includes the more famous PLK1 that has been investigated in several clinical trials in AML and in other cancer diagnoses. The PLK1 inhibitor volasertib progressed up to a phase 3 study, where it was given as a combination treatment with cytarabine in patients with refractory AML.[3] Unfortunately, the addition of volasertib did not improve the overall survival but did result in worse adverse effects compared with the single-arm treatment with cytarabine. Although the clinical trial did not conclude if the adverse reactions were due to on- or off-target effects, several off-target proteins to volasertib have been identified that could explain some of the adverse effects;[4] thus, the 3-dimensional molecular structure is an important factor to take into consideration as it could result in off-target binding. Importantly, there are still ongoing clinical trials of volasertib and other PLK1 inhibitors as well as PLK4 inhibitors.

The PLK family has a C-terminal polo-box domain that is representative of the family and an N-terminal kinase domain that contains the adenosine triphosphate (ATP) pocket, where ATP-competitive small molecules can bind and inactivate PLKs. Because the kinase domains of PLK1, PLK2, and PLK3 are structurally similar, selectivity may be an issue for PLK1 inhibitors. As PLK2 and PLK3 are believed to have tumor suppressor effects, PLK1 inhibitors may induce unwanted effects by targeting PLK2 and PLK3. PLK4, on the other hand, has a structurally different kinase domain, and small inhibitory molecules targeting PLK4 may be more selective, at least within the PLK family. Thus, PLK4 may have an advantage over PLK1.

In the current study, Man et al investigate both PLK4 inhibition by small inhibitory molecules, such as CFI-400945,[5] and knockdown of PLK4 by short hairpin RNA and find that both inhibition and knockdown result in reduced proliferation by different mechanisms, depending on the *TP53* status of the AML cells. As CFI-400945 has been shown to inhibit Aurora B (another serine-threonine kinase with a key role in the cell cycle) as well,[6] the knockdown by RNA interference corroborates the suggestion that the effects are due to PLK4 and not Aurora B inhibition. Moreover, the PLK4 expression was found to be higher in AML cell lines and patient samples with mutated *TP53* compared to AML cells with wild-type *TP53*. In accordance with previous studies,[7,8] PLK4 expression was also significantly higher in samples of patients with AML compared with bone marrow from healthy donors, which is of importance for future clinical trials.

By comparing *TP53* wild-type and mutated AML cell lines, patient samples, and mouse models, including patient-derived xenografts, the authors show that inhibition of PLK4 in *TP53* mutated AML cells remodels histone methylation and activates the immune response via the cyclic GMP-AMP synthase-stimulator of interferon genes pathway. More important, in both mutated and wild-type *TP53* AML cells, inhibition of PLK4 involves a novel PLK4/protein arginine methyltransferase 5/enhancer of zeste homolog 2/trimethylation of histone H3 at lysine 27 axis that could be the driver of the antileukemic effect that results in DNA damage, apoptosis, and reduced proliferation.

In *TP53* wild-type cells, PLK4 inhibition significantly suppresses cell growth during initial exposure, resulting in more apoptotic cells compared with the mutant *TP53* cells. However, DNA damage is induced in both p53 wild-type and mutated AML cells, whereas senescence is only detected in *TP53* mutated AML cells. Prolonged inhibition of PLK4 by both small-molecule inhibitors and RNA interference reduced cell growth of mutated AML samples. More important, the small molecule CFI-400945 displayed cytotoxicity in *TP53* mutated AML cells but not in normal cells.

Using a *TP53* mutant AML mouse model, the authors show that PLK4 inhibition by small-molecule inhibitor CFI-400945 is more effective than a combination of cytarabine and doxorubicin (see Figure 5B in the article by Man et al). Similarly, PLK4 inhibition results in suppressed leukemic burden in *TP53* wild-type AML mice and in patient-derived xenografts (Figure 5D in the article), where mice have been engrafted with primary *TP53* mutated AML cells. Last, the combination of PLK4 inhibitor, CFI-400945, with an anti-CD47 antibody that inhibits the "don't-eat-me" signal in macrophages, revealed a synergistic effect. The blockade of this signal could enhance macrophage-mediated phagocytosis, as suggested by the prolonged survival compared with single treatment (see Figure 5H in the article by Man et al). This reasoning was used in a phase 1b clinical trial in *TP53* mutated AML, where the combination of an anti-CD47 monoclonal antibody, magrogliab, with azacititidne was investigated.[9]

PLK4 inhibition by CFI-400945 was previously studied in a phase 1 trial in advanced solid tumors,[10] and there are currently 2 clinical trials with the PLK4

inhibitor, CFI-400945, in patients with AML. One recently opened trial (NCT04730258) includes patients with AML, myelodysplastic syndrome, and chronic myelomonocytic leukemia; and it is a combination treatment with or without azacitidine or decitabine. The other ongoing clinical trial (NCT03187288) is investigating CFI-400945 fumarate in patients with relapsed or refractory AML or myelodysplastic syndrome. Thus, the data from these studies plus the work presented here by Man et al should better define the role of PLK4 as a potential cancer therapy target, especially in *TP53* mutated AML.

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

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RED CELLS IRON, AND ERYTHROPOIESIS

Comment on Baro et al, page 2016

Parasite hijacks red cell membrane proteins

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In this issue of *Blood*, Baro et al explored the role of CD44, a glycoprotein expressed on the surface of human red cells, in regulating malarial parasite invasion of red cells.[1] Using CRISPR/Cas9 genome editing, they abrogated the surface expression of CD44 in erythroid cells and found that the lack of CD44 had little effect on in vitro erythropoiesis and on the enucleation of orthochromatic erythroblasts. Interestingly, however, they observed that the rate of *Plasmodium falciparum* invasion was reduced in these CD44-null culture-derived red cells, validating CD44 as an important host factor for parasite invasion of red cells. Molecularly, 2 previously well-characterized parasite invasion ligands, erythrocyte-binding antigen 175 (EBA-175) and EBA-140, identified as the ligands for glycophorin A (GYPA) and glycophorin C (GYPC), respectively, were shown to be binding partners for CD44, and, finally, the authors demonstrated that EBA-175–induced phosphorylation of erythrocyte cytoskeletal proteins following invasion is CD44 dependent. These findings imply that CD44 is a coreceptor during invasion of human erythrocytes, which, by stimulating CD44-dependent phosphorylation of host cytoskeletal proteins, alters host cell deformability and facilitates parasite entry (see figure).

Malaria due to the parasite *P falciparum* is a leading cause of morbidity and mortality in the developing world, with ≈600 000 deaths annually, despite implementation of various control strategies. The clinical symptoms of malaria occur following invasion of red cells by the malarial parasite. During the ≈48 hours of intraerythrocytic development, the parasites replicate in exponential cycles and induce significant morphologic, structural, and functional changes in infected red cells, which account for various clinical manifestations, including anemia. As asexual replication is critical to disease pathogenesis, major efforts in development of new interventional strategies are focused on developing detailed molecular and mechanistic understanding of the invasion process. The present study represents a significant contribution to such efforts.

Plasmodium falciparum follows a multistep process for successful invasion of red cells, the first stage of intraerythrocytic parasite development. Amazingly, this multistep process that requires the coordinated execution of diverse events occurs during a period of ≈2 minutes. The process requires numerous ligand-receptor interactions and a complex machinery engaging several distinct cytoskeletal proteins and signaling interventions.[2-5] Distinct features of the invasion process include the apical reorientation of the merozoite, host cell deformation, the formation of a moving junction, and the creation and maintenance of a vacuole that surrounds the intracellular organism in which the intracellular parasite proliferates. The driving force for internalization is powered by the parasite's actinomyosin motor. Like other invasive parasites of