

Improved survival in ETP and near-ETP ALL using risk-adapted therapy based on MRD. As reported by Wood et al, patients with ETP and near-ETP ALL achieved 5-year event-free survival equivalent to not-ETP ALL using this approach. EFS, event-free survival; SD, standard deviation.

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MYELOID NEOPLASIA

Comment on *Miller et al*, page 2079

# PPM1D inhibition may allow us to WIP leukemia

**Bridget K. Marcellino** | Icahn School of Medicine at Mount Sinai

**In this issue of *Blood*, Miller et al examine the role of increased activity of protein phosphatase Mg<sup>2+</sup>/Mn<sup>2+</sup> dependent 1D (PPM1D), also known as wild-type induced phosphatase 1 (WIP1), on chemotherapeutic resistance of hematopoietic stem cells (HSCs).<sup>1</sup> Chemotherapeutic resistance of the malignant clones is a common characteristic and is a serious hurdle to our efforts to cure patients with leukemia.<sup>2</sup> Elucidating the mechanisms by which leukemic cells evade chemotherapy and/or immunotherapy is important in developing strategies to prevent resistance.**

PPM1D is a serine-threonine phosphatase that negatively regulates TP53 and the DNA damage response pathway, as well as

other pathways including NF-κB, ATM, and PI3K/AKT.<sup>3-6</sup> Truncating mutations of PPM1D in patients with acute myeloid

leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasm lead to loss of the C-terminal degradation domain, resulting in increased levels of PPM1D (see figure).<sup>7,8</sup> These mutations have been shown to drive clonal hematopoiesis in the presence of certain chemotherapeutics, and approximately 20% of therapy-related MDS or AML harbor a *PPM1D* mutation.<sup>7</sup> In addition, PPM1D overexpression without a *PPM1D* mutation is common in many cancer subtypes, including leukemia, making PPM1D an enticing therapeutic target.<sup>9</sup>

Miller et al, as well as other groups, have previously shown that *PPM1D* mutations in hematopoietic cells induce resistance to apoptosis and chemotherapeutic agents.<sup>7,10</sup> In this issue, Miller et al provide further evidence that PPM1D is a crucial regulator of HSC fitness and that its absence leads to greater sensitivity of malignant cells to chemotherapeutic agents. They developed conditional mouse models of PPM1D truncation (gain of PPM1D) and deletion and used them to evaluate the effects of PPM1D gain and loss, respectively, on the fitness of normal and malignant HSCs. Competitive transplant assays revealed that in normal murine HSCs, PPM1D gain conferred a

competitive advantage that was increased in the presence of cytotoxic stress (cisplatin or radiation), whereas PPM1D loss elicited a competitive disadvantage (see figure). They found similar results when they assayed mouse leukemia cells with the fusion MLL-AF9 and either PPM1D gain or loss: the fitness of the leukemia HSC was heightened with PPM1D gain and reduced with PPM1D loss.

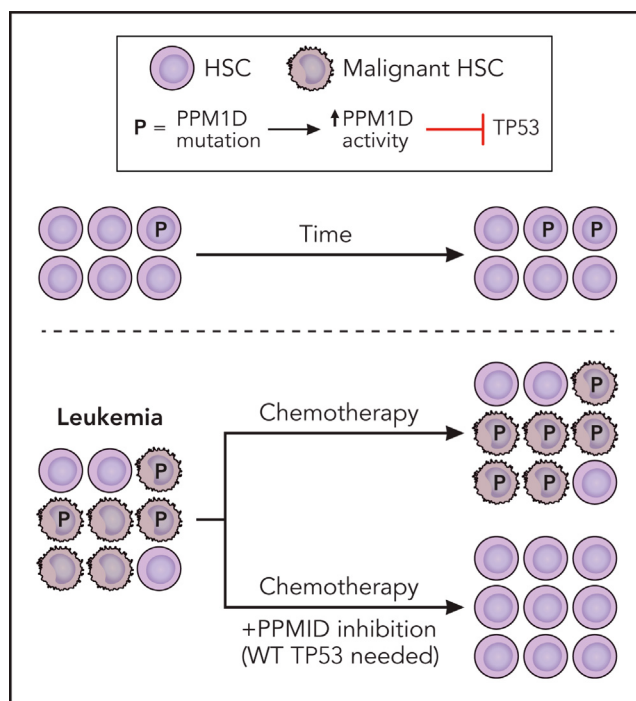
The group went on to show through an elegant series of experiments that PPM1D could serve as a therapeutic target in myeloid malignancies. PPM1D loss or treatment with PPM1D inhibitor sensitized murine leukemic HSCs to chemotherapy (see figure). In vitro, PPM1D inhibition was found to be synergistic with a broad array of commonly used chemotherapeutics, including daunorubicin, cytarabine, decitabine, platinum salts, topoisomerase inhibitors, and radiation. The sensitization of daunorubicin and cytarabine with PPM1D inhibition was corroborated in leukemia cells from xenograft models derived from patients with AML. In vivo, MLL-AF9 leukemia xenografts had significantly increased survival when a PPM1D inhibitor was administered in conjunction with doxorubicin and cytarabine.

The actions of PPM1D inhibition are hypothesized to involve multiple pathways due to the numerous known substrates of this phosphatase. The authors, however, clearly demonstrate that TP53 is crucial for the sensitizing of malignant cells to PPM1D inhibition. When compared with knockdown of other modulators of the DNA damage response pathway, inflammation, and the p38 pathway, loss of TP53 instigated the greatest resistance.

With PPM1D mutations and amplifications present across myeloid malignancies, the clinical implications of these findings have the potential to be far reaching. In addition, the authors begin to address the applicability of this approach to solid malignancies. Using a cell line screen, they show that the majority of TP53 wild-type solid tumor cell lines exemplified enhanced sensitivity to daunorubicin with the addition of a PPM1D inhibitor.

It is important to note, however, that PPM1D inhibition on its own did not have significant activity against leukemia cells but rather worked in synergy with chemotherapeutics or radiation (see figure). The question therefore arises as to which therapies would be optimal to combine with PPM1D inhibition. The authors make a compelling argument for DNA-damaging treatments such as chemotherapeutics and radiation, but could this extend to other approaches? Given that PPM1D modulates the immune system, could it serve as a sensitizer for immunotherapeutics? As PPM1D interacts directly with MDM2, could it synergize with MDM2 inhibitors?

Important work lies ahead to translate the compelling findings presented by Miller et al. Although it is reassuring that PPM1D loss did not cause significant hematologic or nonhematologic toxicity in mice, further testing in primary cells will be crucial to understand the specificity of PPM1D inhibition to malignant vs normal HSCs, ensuring that a potential therapeutic window exists. The authors' finding that PPM1D expression level was associated with response and corroboration of PPM1D as a biomarker for sensitivity to PPM1D inhibition could allow us to determine which patients would derive most benefit from this sensitizer. Furthermore, a greater understanding of the downstream events of



Truncating mutations of PPM1D found in myeloid malignancies lead to loss of the C-terminal degradation domain, resulting in increased levels of PPM1D. Increased PPM1D activity results in inhibition of TP53 that enhances the fitness of both normal and malignant HSCs. Leukemia HSCs that have high PPM1D activity are resistant to chemotherapy. Although PPM1D inhibition alone does not have significant activity against leukemia cells, it is synergistic with chemotherapy. Professional illustration by Patrick Lane, ScEYence Studios.

PPM1D inhibition will enable us to optimally choose the best combinatorial partners for PPM1D inhibition. On a final note, there is lack of a clinical-grade PPM1D inhibitor, which prohibits clinical translation of these important findings. It is therefore essential that novel PPM1D-targeting agents are developed so the potential of this approach can be further explored.

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

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## THROMBOSIS AND HEMOSTASIS

Comment on Lafoux et al, page 2092

# Hemostasis in arenavirus infection

Swati Sharma and Silvio Antoniak | University of North Carolina at Chapel Hill

**In this issue of *Blood*, Lafoux and colleagues<sup>1</sup> present a comprehensive study of mammarenavirus infection in cynomolgus macaques, a model for viral hemorrhagic fevers, analyzing the changes in hemostatic and platelet activation markers. Importantly, viral hemorrhagic fever infections are almost always associated with the activation of coagulation cascade and platelets, which can be due to the pathogen itself or can be a protective defensive host response to maintain hemostasis.<sup>2,3</sup> For instance, fibrin entrapment of bacteria is thought to be due to the host's response, but viral infections may also enhance fibrin depositions.<sup>2</sup> Although the virus itself might be too small to be entrapped in a fibrin network, viruses can interact with platelets. Platelets mediate viral clearance by engulfing them for destruction and thus limiting systemic viremia.<sup>2,3</sup>**

Lafoux et al were able to identify hemostatic and platelet activation markers and correlate them with the hemorrhagic fever infection stages. The study compared the disease progression of the old-world

arenavirus such as Lassa virus (LASV, using the arenavirus [AV] and Josiah strains), which is most prevalent in West Africa, with the new-world arenaviruses Machupo virus (MACV, the Carvallo strain) and

Guanarito virus (GTOV, the INH-95551 strain), both endemic in South America.<sup>1</sup> Importantly, the cynomolgus macaque model recapitulates all reported hematologic features including thrombocytopenia, leukopenia, platelet dysfunction, clotting time prolongation, high plasminogen activator inhibitor-1 (reduced fibrinolysis), and hemorrhagic complications. Strain-specific features observed in human patients were confirmed in the presented study, showing distinct differences between the AV and Josiah LASV strains. Importantly, the study revealed that arenavirus infection can cause cerebral and cerebellar hemorrhages. These brain hemorrhages are severe and can lead to death by infarction and compression of the central nervous system. The authors found that the increased vascular permeability and lung edema can contribute to terminal shock syndrome by severely effecting fluid distribution and tissue hypoxia, with resultant multiorgan failure, as observed in severe human cases. Similar to human patients, the animals exhibited prolonged clotting times but limited signs of disseminated intravascular coagulopathy (DIC). Interestingly, the authors measured a downregulation of fibrinolysis, potentially suggesting that the host body tries to accommodate fluid loss during bleeding by stabilizing hemostatic plugs. This could point to a role of coagulation activation as protective wound healing response. In addition, the authors reported the occurrence of a soluble factor in plasma directed against procoagulant proteins in animals infected with the LASV Josiah strain. They also found that the observed thrombocytopenia was not a result of DIC or inflammation-dependent reduced platelet production but suggested that there is a so-far unknown factor induced by the virus. Identification of this unknown infection-associated factor has the potential to be of great therapeutic benefit.

Animals fatally infected with LASV Josiah, MACV, and GTOV had hemorrhages in the lungs, gastrointestinal tract, and intercranial space. The authors observed that tissue factor (TF) expression was elevated in monocytes and the vessel wall, which correlated with fibrin deposition.<sup>1</sup> Lafoux et al concluded that vascular leakage is not connected to viral infection of endothelial cells (ECs).<sup>1</sup> Overall, the authors suggested that all signs including the expression of TF on monocytes suggest a sepsislike phenotype.