

donor-derived AML or MDS,<sup>9</sup> but it does raise the question of whether donors should be screened for CH. Much larger studies are required to determine whether such a strategy is advisable and whether the presence of sporadic CH in donors represents a more significant risk to recipients than factors such as donor age, sex, ABO compatibility, and cytomegalovirus status.

Another important observation was the larger mutant clone size in recipients vs donors in the cases of donor-engrafted CH. This supports the premise that mutant HSCs were imparted with an additional growth advantage by peri-/posttransplant factors. Peritransplant factors include possible enrichment of harvests with or preferential engraftment of mutant HSCs and the impact of pretransplant irradiation or peritransplant inflammation or infection on CH behavior. Studies in mouse models have shown that HSCs with mutations in CH genes outcompete wild-type HSCs early after transplantation and that this can be enhanced by inflammation.<sup>10</sup> The possibility that the recipient offers a more favorable environment for the expansion of CH beyond the peritransplant period is more difficult to investigate and would require the study of multiple time points, while being compounded by any lasting impact of peritransplant events.

Boettcher et al also provide interesting insights into the clonal architecture of CH by sequencing individual colony-forming units (CFUs), arising from single hematopoietic stem or progenitor cells, to show that CH mutations identified in bulk granulocyte DNA can be derived from a single or multiple independent clones. With 1 exception, the proportion of mutation-positive CFUs correlated well with variant allele fraction in granulocytes. In addition, the authors show that CH mutations were consistently present in myeloid cells, but were not always present in B and T cells. These findings help interpret other studies of CH that used whole blood or granulocyte DNA.

Finally, by measuring telomere length in donor and recipient CFUs, the authors demonstrate 20 years of additional hematopoietic aging in the latter. Intriguingly, within individuals with CH, telomere length was not consistently different between CFUs with and without CH mutations. The authors speculate that different

mutations might have distinct requirements for telomerase activity or might activate alternative mechanisms of telomere maintenance. Furthermore, this variability may help explain differences in the risk of malignant progression associated with distinct mutations.

In the future, prospective longitudinal studies or retrospective clonal phylogenetic deconvolution will be required to build on the insights provided by the Boettcher et al study by providing more granular detail on the dynamics of CH driven by different mutations in the context of sibling and unrelated donor allogeneic HSCT. This would enhance decision making and donor choice for allogeneic HSCT and help quantify the risks associated with individual CH clones or mutations.

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## LYMPHOID NEOPLASIA

Comment on De Dominici et al, page 1560

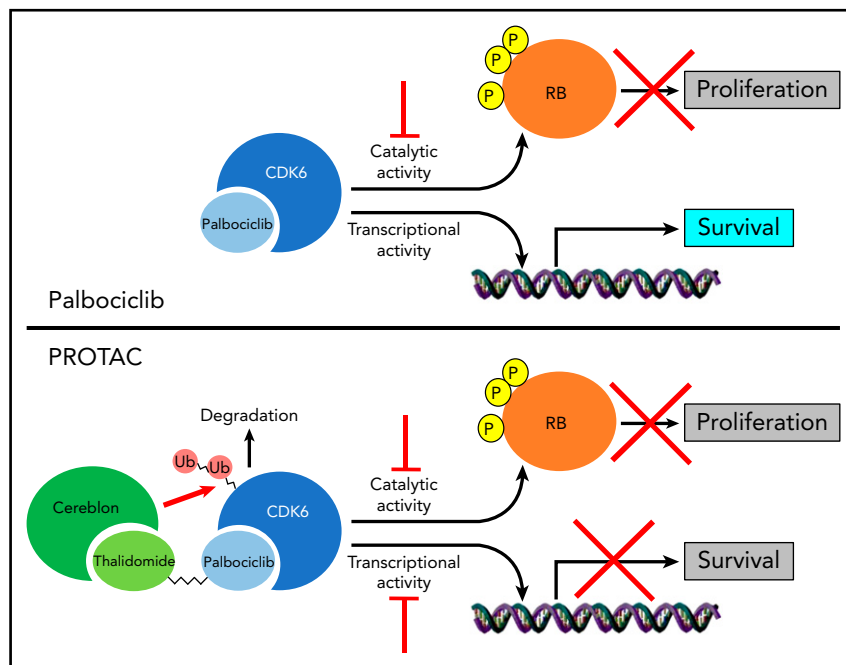
# CDK6 degradation hits Ph<sup>+</sup> ALL hard

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**In this issue of *Blood*, De Dominici et al have identified and tested cyclin-dependent kinase 6 (CDK6)-selective proteolysis-targeting chimeras (PROTACs) that suppressed Philadelphia chromosome-positive (Ph<sup>+</sup>) acute lymphoblastic leukemia (ALL) ex vivo and in mice more effectively than approved kinase inhibitors of CDK4/6.<sup>1</sup>**

PROTACs represent a new paradigm in pharmacology with the potential to be as transformative for cancer treatment as targeted kinase inhibitors, therapeutic antibodies, or immunotherapies.<sup>2</sup> PROTACs are bifunctional molecules that use 1 arm to bind a protein target and the other to bind an E3 ubiquitin ligase. The ligase

then labels the target with a polyubiquitin chain, thereby marking it for degradation by the cell's disposal machinery: the proteasome. It is the fundamentally different pharmacology of PROTACs that may be the key to its success. For conventional drugs, a high systemic drug exposure is needed to ensure sufficient



Inhibition of CDK6 activity with the kinase inhibitor palbociclib inhibits proliferation of Ph<sup>+</sup> ALL cells, but does not interfere with the functions of CDK6 as a transcriptional regulator. In contrast, targeted degradation of CDK6 with a bifunctional PROTAC degrader removes both catalytic and transcriptional CDK6 functions. RB, retinoblastoma tumor suppressor, Ub, ubiquitin. See the visual abstract in the online version of the article by De Dominici et al that begins on page 1560.

occupancy of drug binding sites *in vivo*. In contrast, PROTACs act catalytically and iterative, meaning that after inducing degradation of its target, the PROTAC molecule can be reused for further rounds of target binding and degradation. Therefore, the efficiency of PROTACs may be less susceptible to resistance development that is caused by increased target expression or mutations of the target protein. Impressive progress was made: in only 5 years, PROTACs to >50 distinct target proteins, including many key oncogenes in hematological disorders, were developed and successfully validated in proof-of-concept studies in cell culture and animal models.<sup>3</sup> The broad investment of the biotechnology and pharmaceutical industries has resulted in rapid PROTAC drug development and the initiation of the first clinical trial of a PROTAC degrader targeting the androgen receptor in patients with prostate cancer in March 2019.<sup>4</sup>

De Dominici et al have developed a PROTAC targeting CDK6 in Ph<sup>+</sup> ALL. CDK6 was previously validated as a drug target in Ph<sup>+</sup> ALL that may be exploited in cases of drug resistance.<sup>5</sup> CDKs are key cell-cycle regulators. CDK4 and CDK6 are ubiquitously expressed, bind D-type

cyclins, and drive proliferation by relieving the transcriptional repression of E2F-dependent genes by phosphorylating the retinoblastoma protein. This drives cells through the G1 phase into the S phase of the cell cycle. The partially overlapping and redundant physiologic roles of CDK4 and CDK6 were studied in great detail and unexpectedly identified a kinase activity-independent role of CDK6 as a transcriptional regulator mediating growth-promoting functions.<sup>6</sup> Thereby, targeted degradation of CDK6 by means of a PROTAC may be able to kill 2 birds with 1 stone through blocking functions of CDK6 that are dependent on its catalytic activity, as well as its independent functions (see figure). Those cannot be targeted with conventional drugs, including the potent Food and Drug Administration–approved small molecule CDK4/6 kinase inhibitors, palbociclib, ribociclib, and abemaciclib, which are approved for the treatment of patients with hormone receptor–positive breast cancer.<sup>7</sup> Neutropenia, observed in approximately two thirds of these patients and caused by concomitant CDK4 and CDK6 inhibition in hematopoietic progenitors, and CDK4/6 inhibitor resistance due to compensatory increases in CDK6 expression are some of the shortcomings of palbociclib treatment.

Hence, selective ablation of CDK6 expression with a PROTAC strategy would target both the kinase activity-dependent and -independent functions of CDK6 and possibly diminish adverse events and resistance development (see figure).

To provide a solid basis for a possible therapeutic application of a CDK6 PROTAC in Ph<sup>+</sup> ALL, De Dominici et al first compared a genetic CDK6 knockdown with palbociclib treatment side by side. In Ph<sup>+</sup> ALL cell lines *ex vivo* and a mouse xenograft model, CDK6 knockdown induced more apoptosis, stronger reduction in leukemic burden, and marked longer survival when compared with palbociclib treatment. Analysis of gene expression profiles identified candidate genes that were differentially regulated by CDK6 silencing and palbociclib treatment. Importantly, several of these genes also correlated with CDK6 expression in samples from Ph<sup>+</sup> ALL patients. The authors then synthesized and carefully evaluated several derivatives of palbociclib linked to either cereblon or VHL, the most commonly used E3 ligases for PROTAC approaches. YX-2-107 was developed and resulted in rapid degradation of CDK6, but surprisingly not CDK4, and inhibition of CDK6 signaling, in line with previous reports on other CDK6 PROTACs.<sup>8-10</sup> Selective CDK6 degradation was also observed in normal hematopoietic progenitors, but did not change the cell-cycle distribution of these cells. For a possible clinical translation, the authors demonstrated excellent bioavailability and plasma stability of YX-2-107, which contributed to strong inhibition of leukemic burden in mouse xenograft experiments with Ph<sup>+</sup> ALL cell lines, as well as comparable or superior effects in patient-derived xenografts with sensitive and TKI-resistant patient cells when compared with palbociclib treatment. Importantly, YX-2-107 did not perturb normal mouse hematopoiesis. Apart from Ph<sup>+</sup> ALL, other diseases with CDK6 dependence, such as other subtypes of B-cell ALL, acute myeloid leukemia, multiple myeloma, and mantle cell lymphoma, may benefit from the superior activity CDK6 PROTACs as compared with palbociclib.

Given that palbociclib is equally potent in inhibiting CDK4 and CDK6 kinase activity, a striking molecular finding was that YX-2-107, which contains palbociclib as a targeting moiety, efficiently degraded CDK6, whereas CDK4 protein levels

remained unchanged. This further supports prior evidence that PROTACs may have increased selectivity as compared with their parent drug. Therefore, besides the “added value” of PROTACs to degrade its target on top of its inhibition, it may also improve the therapeutic window by being more selective with less adverse events caused through inhibition of off-targets. If this would turn out to be a general feature of PROTACs, drug candidates who have failed in clinical trials due to safety concerns may be revived and have a second life as a PROTAC.

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## RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Jung et al, page 1588

# Disease and mutation: correlations coming to fruition

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**In this issue of *Blood*, Jung et al<sup>1</sup> provide a comprehensive study of the manifestations and clinical course of patients with a particular subtype of Fanconi anemia, FA-B, characterizing the associated genetic variations in the corresponding *FANCB* gene and their mutational effects and drawing sophisticated genotype-phenotype correlations.**

From the beginning of the era of molecular genetics, genotype-phenotype analysis has raised hope of better understanding the clinical presentation of a genetic disease, including predicting the natural history and prognosis and, ideally, enabling intervention by precision medicine. In most instances, the results have not lived up to expectations. Mutational diversity still limits our ability to make valid predictions of the course of

monogenetic diseases. Classical Mendelian genetics has fostered the illusion that a pathogenic mutation in a specific gene causes monomorphic disruption of gene function and a consistent phenotype. In reality, Mendelian traits can present in many ways with variations in the manifestations of the disease.

Genotype-phenotype correlations often have limitations or are ambiguous. On

the clinical side, this may be due to using data extracted from case reports rather than full medical review of patients, inclusion of patients not representative of the entire range of abnormalities, and/or the limited number of patients affected by rare diseases. On the genetic side, the mutational effects of missense, splice, or other variants have not always been studied sufficiently, introducing uncertainty. Regarding inheritance, patients with recessive disorders often have a different pathogenic variant on either allele. Even if either one has been characterized satisfactorily, it is rarely clear what the contribution of each is or their effect in combination. Patients with consanguineous parents carrying a homozygous pathogenic variant in a single gene will have considerable and partly homozygous sequence variation in other genes, making it hard to determine the phenotypic effect.

This is also the situation for the genetic disease Fanconi anemia, which is usually inherited as a recessive trait, apart from 3 reported patients with a dominant-negative de novo mutation in *RAD51/FANCR*, associated with a Fanconi anemia-like syndrome.<sup>2</sup> Fanconi anemia is a heterogeneous disease, with 22 complementation groups and underlying genes reported to date.<sup>3</sup> A major function of the Fanconi anemia/BRCA pathway is sensing, removal and repair of DNA interstrand crosslinks. Defects therein result in a disorder with typical yet variable multisystemic congenital malformations.<sup>4</sup> Progressive bone marrow failure with pancytopenia typically presents in the first decade of life.<sup>5</sup> Patients experience a high risk of malignancies at an early age, most commonly acute myeloid leukemia and squamous cell carcinoma of the oropharynx and upper gastrointestinal tract and external female genitalia.

Genotype-phenotype analysis in Fanconi anemia patients has been performed in different ways, according to the mutated Fanconi anemia gene (or complementation group), the location of the defect in the pathway, or type of pathogenic variant.<sup>6,7</sup> A clear example of a genotype-phenotype relationship in Fanconi anemia patients is the infantile cancer phenotype due to mutations in *BRCA2/FANCD1* or *PALB2/FANCN*. Another correlation is the overlap between Fanconi anemia manifestations and specific sets of abnormalities (associations) such as VACTERL-H (vertebral, anal, cardiac, trachea-esophageal fistula, esophageal atresia, renal, upper limb, and hydrocephalus) and PHENOS