

NOTCH1 is hypomethylated and over-expressed in primary MCL,⁶ but its role has not been explored in this disease. The findings of Kridel and colleagues demonstrate the power of next-generation sequencing to expand our understanding of tumor pathogenesis, and pave the way for future studies examining the prognostic and therapeutic implications for *NOTCH1* signaling in MCL. Recent studies suggest that *NOTCH1* mutations can have context-specific oncogenic or tumor-suppressor roles in human cancers,⁷ so the precise contribution of *NOTCH1* mutations to the pathogenesis of MCL and other B-cell malignancies remains an interesting and open area for future investigation.

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● ● ● MYELOID NEOPLASIA

Comment on Rucker et al, page 2114

Unveiling the complexity of CK+ AML

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In acute myeloid leukemia (AML), cytogenetic abnormalities are present in more than half of patients and these chromosomal alterations are critical factors that determine response to chemotherapy and survival.^{1,2}

Historically, metaphase analysis has stratified AML patients into 1 of 3 cytogenetic categories (ie, favorable, intermediate, and adverse),² although recently a new category (monosomal karyotype [MK]), associated with dismal prognosis, has been described.^{3,4} Unfortunately, the molecular mechanisms predisposing the genetic instability observed in patients with complex and monosomal karyotype have remained elusive.

TP53, the most commonly mutated tumor-suppressor gene in human cancers, is the master regulator of cell cycle, playing a major role in determining the fate of cells that contain damaged DNA. Upon DNA damage, the tumor protein p53 can delay cell-cycle progression, allowing cells to either repair their damaged DNA or initiate programmed cell death. In the absence of the normal activated p53 protein, either because of loss of tumor-suppressor function mutations and/or deletion of genetic material, cells containing damaged DNA can survive and proliferate, which contributes to malignant transformation. Al-

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though most *TP53* missense mutations cause loss of p53-dependent tumor-suppression function, hotspot mutations located at 4 codons (175, 248, 249, and 273) may also acquire gain-of-function properties to promote tumorigenesis. In fact, these gain-of-function p53 mutations have been associated with genetic instability, leading to recurrent interchromosomal translocation, and defective G2/M checkpoint through inactivation of the Mre11/ATM-dependent DNA damage responses.^{5,6}

In an attempt to better understand the molecular mechanisms associated with complex chromosomal rearrangements, Rucker et al, on behalf of the German-Austrian AML Study Group (AML-SG), determined the molecular and genomic profile of *TP53* in a large group of AML patients with complex karyotype and the clinical and prognostic features associated with these alterations.⁷ Their findings suggest that abnormalities in *TP53* are commonly found in AML with complex and monosomal karyotype and negatively impact the outcome

of these patients, independent of other variables. Using a combined approach to detect both DNA copy number abnormalities as well as the mutational status of *TP53*, the authors established that nearly 70% of AML patients with a complex karyotype have a biallelic inactivation of *TP53*.⁷ AML patients with mono- or biallelic *TP53* alterations were older, which is consistent with the higher frequency of complex and monosomal karyotypes in older patients.⁴ In addition, these patients presented with unique features, such as high degree of genetic complexity and correlation with specific genetic alterations, such as 5/5q- and concomitant -5/5q- and -7/7q-. Interestingly, although a positive correlation between cytogenetically defined MK+ AML and *TP53* alteration was noted (> 85% of cases), a correlation could not be established when MK was determined using more sensitive methods (DNA copy loss array data). Clinically, abnormalities in *TP53* were independently associated with chemoresistance, measured by lower complete remission rates and higher rates of refractory disease, and dismal median and 3-year overall survival rates that did not seem to be improved by allogeneic hematopoietic stem cell transplantation (alloHSCT); albeit the sample size was small.

The results presented by Rucker et al are extremely significant as they begin to unveil the molecular mechanisms of leukemogenesis in AML patients with complex karyotype. However, several questions remain in light of this report. First, what are the molecular leukemogenic mechanisms in the nearly 30% of AML patients with complex karyotype and lack of *TP53* alterations? A recent report suggests that amplifications of the 1q chromosome region, which contains the potent p53 inhibitor *MDM4*, were identified in nearly 20% of patients with leukemic transformation after chronic-phase myeloproliferative neoplasms.⁸ As expected, these amplifications were always mutually exclusive from *TP53* mutations. Thus, could other downstream (*ATM*) or upstream (*MDM2*) regulators of the p53-signaling pathway also be involved? These questions have not been fully explored to date. Next, could differences in the baseline clinical characteristics, chemosensitivity, and prognosis be uncovered in patients with hotspot mutations (codons 175, 248, 249, and 273) who also have gain-of-function properties? Finally, and likely most importantly, how can the dismal

outcome of these patients be improved? Nearly 90% of *TP53*-altered patients also had a cytogenetically defined MK + AML, suggesting that MK may be a surrogate for *TP53* abnormalities. Although an initial retrospective study conducted at the Fred Hutchinson Cancer Research Center suggested that alloHSCT might improve the dismal outcome of patients with MK + AML,⁹ novel therapeutic approaches likely will need to be evaluated in this subset of very-high-risk patients as alloHSCT resulted in a limited survival improvement in larger subsequent analyses.¹⁰

In summary, abnormalities in *TP53* are common in this subgroup of patients with AML and play a pivotal role on the inferior outcomes observed in these patients.

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both coding and noncoding regions. In addition, several hundred candidate mutations have been described in patients with VWD.⁵ There is a strong impetus to connect these underlying genetic defects to disease pathophysiology and clinical phenotype; doing so holds promise for more precise diagnostics and targeted therapeutics. However, distinguishing between causative mutations and neutral sequence variants remains challenging.⁶

Until now, investigators have addressed this challenge largely through the study of patients with clinical and laboratory evidence of VWD. Bellissimo and colleagues have taken a different approach. They analyzed the coding DNA sequences and corresponding standard VWF clinical laboratory tests in 184 healthy individuals with a negative bleeding history recruited from 7 centers across the US.¹ The study population included 118 whites and 66 blacks. Sequence analysis revealed 21 new variations, highlighting the polymorphic nature of the *VWF* gene. Remarkably, an additional 14 sequence variants previously implicated as pathogenic mutations, primarily in type 1 VWD, were identified in the study. Individuals carrying these alleles had a negative bleeding history and all but 1 had normal VWF clinical laboratory testing, suggesting that many of these putative disease-causing mutations are likely to be benign sequence variations, although incomplete penetrance remains an alternative explanation. Analysis of healthy black subjects proved particularly revealing. Thirteen hitherto undescribed variants and 10 sequence variations previously classified as pathogenic occurred exclusively in this cohort, likely reflecting the greater genetic diversity observed in individuals of African origin⁷ and underrepresentation of this group in previous studies of *VWF* genetics.

The current study represents the first analysis of *VWF* genetic variation in a large number of ethnically diverse healthy controls and exposes a potential pitfall of genetic testing in type 1 VWD and other genetically heterogeneous disorders, namely the uncertainty inherent in classifying a given variant as pathogenic. From the ongoing 1000 Genomes Project and the International HapMap Consortium, in which genomic variation in major population groups from Europe, East Asia, South Asia, West Africa, and the Americas are

● ● ● THROMBOSIS & HEMOSTASIS

Comment on Bellissimo et al, page 2135

VWF sequence variants: innocent until proven guilty

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William James, the 19th century American philosopher, famously asserted, “To study the abnormal is the best way of understanding the normal.” In this issue of *Blood*, using a distinctly non-Jamesian approach, Bellissimo and colleagues shed new light on our understanding of normal genetic variation in von Willebrand factor (*VWF*) through the study of ethnically diverse healthy controls and, in doing so, exonerate a number of benign sequence variants previously regarded as pathogenic mutations.¹

Since the *VWF* gene was first characterized,² tremendous strides have been made in our understanding of the pathophysiology and molecular genetics of type 1 von Willebrand disease (VWD), a common inherited bleeding disorder caused by quantitative deficiency of the VWF protein. The *VWF* gene extends over 178 kilobases on chromosome 12 and is composed of 52 exons, encoding a large precursor protein of 2813 amino acids. The precursor protein is synthesized in megakaryocytes and endothelial cells and un-

dergoes a series of posttranslational modifications to produce the 2050 amino acid VWF monomer. Monomers dimerize and further polymerize via disulfide bonds to form large multimers ranging from 0.5 to 10 million Daltons in size. The mature VWF multimers stabilize factor VIII and mediate platelet adhesion at sites of vascular injury, functions carried out by conserved structural domains.³

The *VWF* gene is highly polymorphic⁴ with a number of single nucleotide substitutions, insertions, and deletions reported in