validate that the observed *BAX* mutations impede venetoclax-induced cell death. Four of 5 variants tested endowed cells with the capacity to resist venetoclax. Therefore, the clonal expansion of BAX mutants in the myeloid compartment can be attributed, at least in part, to increased fitness because of the loss of BAX proapoptotic function.

In their final experiment, the authors perform a longitudinal study of 2 patients who received 7 years of continuous venetoclax treatment. Both patients showed steadily increasing allelic frequencies of *BAX* mutations over time. The authors then leveraged single-cell sequencing to show that *BAX* mutations present in a single patient are mutually exclusive at the single cell level, representing 2 independent clonal expansions within the same healthy tissue. This finding is a powerful testimony both to the remarkable genetic diversity that fuels clonal mosaicism and to the strong selection pressure exerted by venetocalx on normal hematopoietic cells. Interestingly, when multiple BAX-mutated clones are observed, the dominant clone was found to be carrying co-occurring clonal hematopoiesis mutations (DNMT3A in one patient and ASXL1 in another patient), suggesting increased fitness. Thus, the strong venetoclax selection pressure in healthy tissue drives BAX convergent clonal evolution, potentially cooperating with canonical clonal hematopoiesis mutations.

Overall, the study by Blombery et al provides robust evidence for the presence of clonal evolution in healthy tissues receiving targeted therapy with venetoclax. This exciting observation raises interesting questions. First, given that the observed *BAX* mutations are predicted to be loss-of-function, is biallelic loss required for functional impact? The authors infer loss of heterozygosity in high variant allele fraction samples, supporting homozygous *BAX* loss. Biallelic loss was also the model used in vitro. However, other cases were compatible with heterozygous loss, suggesting that it may be sufficient to impart a fitness advantage. Further studies are needed to definitively link gene dosage with resistance phenotypes. Second, perhaps one of the most exciting observations in this work is the diverging disruption of *BCL2* vs *BAX* in the malignant lymphoid vs the normal myeloid compartments, respectively. What lineage-intrinsic dependencies in the apoptosis pathway drive this observation remains to be determined and may also aid in therapeutic development of apoptosis targeting across blood cancers.

More broadly, this study shows that a strong targeted therapy can select for resistant mutations not only in malignant populations but also in normal tissue clonal mosaicism. As somatic evolution occurs in both normal and malignant cells,[2–6,8] the exquisitely focused selection pressure of targeted therapies is likely to impact both. Future studies will reveal if this observation is unique to venetoclax or will be found with other targeted interventions.

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

REFERENCES

1. Blombery P, Lew T, Dengler M, et al. Clonal hematopoiesis, myeloid disorders, and BAX-mutated myelopoiesis in patients receiving venetoclax for CLL. *Blood.* 2022;139(8):1198-1207.
2. Kakiuchi N, Ogawa S. Clonal expansion in non-cancer tissues. *Nat Rev Cancer.* 2021;21(4):239-256.
3. Yizhak K, Aguet F, Kim J, et al. RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. *Science.* 2019;364(6444):eaaw0726.
4. Mustjoki S, Young NS. Somatic mutations in "benign" disease. *N Engl J Med.* 2021;384(21):2039-2052.
5. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med.* 2014;371(26):2488-2498.
6. Bolton KL, Ptashkin RN, Gao T, et al. Cancer therapy shapes the fitness landscape of clonal hematopoiesis. *Nat Genet.* 2020;52(11):1219-1226.
7. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2016;374(4):311-322.
8. Blombery P, Anderson MA, Gong JN, et al. Acquisition of the recurrent Gly101Val mutation in BCL2 confers resistance to venetoclax in patients with progressive chronic lymphocytic leukemia. *Cancer Discov.* 2019;9(3):342-353.
9. Carney DA, Westerman DA, Tam CS, et al. Therapy-related myelodysplastic syndrome and acute myeloid leukemia following fludarabine combination chemotherapy. *Leukemia.* 2010;24(12):2056-2062.

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MYELOID NEOPLASIA

Comment on Yang et al, page 1208

And the germline beat (AML) goes on

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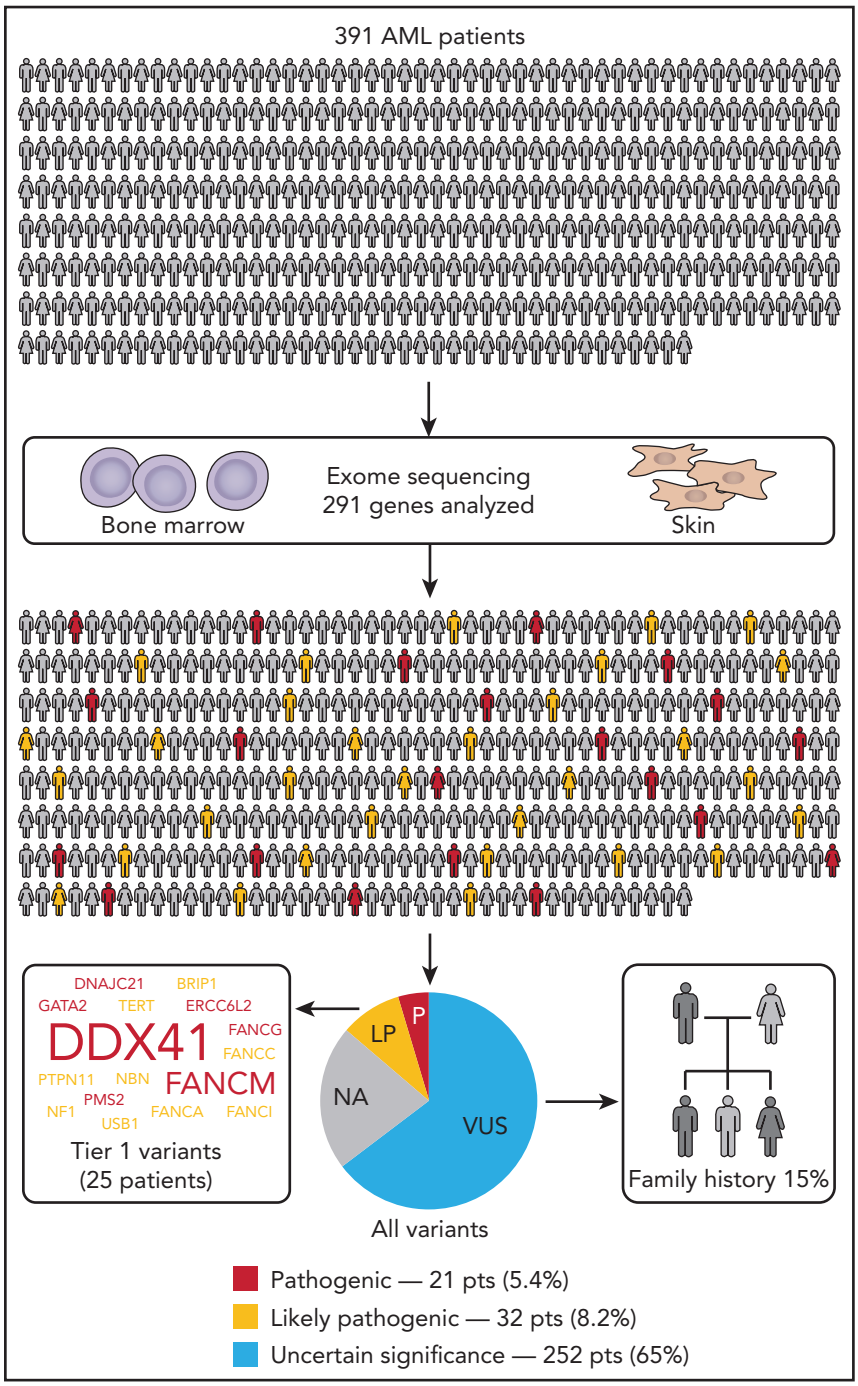
In this issue of *Blood*, Yang et al[1] report on the germline screening of 391 patients from the BEAT acute myeloid leukemia (AML) study, finding that 13.6% of patients unselected by family history have pathogenic or likely pathogenic variants of known cancer-predisposition genes.

Germline predisposition to hematological malignancies (HMs) is a topic of increasing interest to the research community and increasing concern to clinicians, particularly with regard to the selection of hematopoietic stem cell donors from family members.[2] Over the past 2 decades, family studies of the germline predisposition to myeloid neoplasms have increased our understanding of the genes known to underlie this predisposition. The *2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia* included a new category for myeloid neoplasms with germline predisposition.[3-5] Despite these advances, genetic analysis of well-curated families who are predisposed to myeloid malignancy identifies known or potential predisposition candidates at ~50%, suggesting that additional predisposition genes are yet to be identified.[5] Likewise, the frequency of germline predisposition mutations in patients in a typical leukemia



Germline screening for HM-predisposition variants in the Beat AML cohort identified 13.6% of patients with AML with P/LP variants classified according to American College of Medical Genetics and Genomics guidelines. Family history was also assessed independent of genomic findings, with 15% of assessed individuals positive for a family history of HMs. LP, likely pathogenic; NA, patients without variants in the other categories; P, pathogenic; pts, patients; VUS, variant of uncertain significance.

clinic is not known. Because most genomic sequence data in the latter context come from tumor sequencing at leukemia presentation, a range of recommendations have emerged for identification and prioritization of potential germline variants from tumor-only sequencing.[1,6] It is also important to remember that tumor panels are not a replacement for dedicated germline screening.

In their study, Yang and colleagues tackled the frequency question by performing tumor/normal tissue sequencing of 391 patients from the BEAT AML cohort.[1] Casting their net wide, they performed exome sequencing of skin fibroblasts and used a set of 291 genes associated with cancer predisposition, curated from guidelines and the literature. Variants were selected and prioritized based on parameters including variant allele frequency (>40%), population frequency (Minor Allele Frequency <1.0%), and predicted pathogenicity (eg, Combined Annotation–Dependent Depletion >15). This presented the investigators with the unenviable task of classifying 1547 unique variants from 228 genes, utilizing the American College of Medical Genetics and Genomics guidelines.[7] They identified pathogenic or likely pathogenic (P/LP) germline variants in 13.6% of patients with AML (see figure). They then looked at this group in greater detail to identify clinical and phenotypic criteria (other than family history) with which to prioritize individuals for germline screening. There was no statistical association with AML subtype, sex, or age. Notably, no patients in this group fell into the favorable category based on European LeukemiaNet stratification.[8] There were some exceptions to the above findings for patients with variants, in particular clinically actionable genes (found in 6.4% of patients). For example, *DDX41* was the most recurrently mutated clinically actionable gene (7 patients; 1.8%); as seen in previous studies,[9] P/LP variants were more prevalent in older individuals and expressed a marked male bias (6:1 males/females). Other familiar familial genes included singleton cases with germline P/LP variants in *GATA2* and *TERT* and the RAS pathway genes *PTPN11* and *NF1*.

Moving beyond this small set of known myeloid-predisposition genes, an interesting pattern emerged within the remaining P/LP variants. Recognizing genes in a similar functional category, 22 patients were found to have variants in DNA damage response (DDR) genes, including 8 patients with P/LP variants in *CHEK2* and other patients with variants in Fanconi anemia (FA) pathway genes (*FANCM*, *FANCA*, *BRIP1*, *FANCC*, *FANCG*, and *FANCI*) and other DDR genes. FA genes are well known for their increased risk of myeloid leukemia with an autosomal-recessive inheritance associated with FA.[2] However, the potential for autosomal-dominant predisposition to myeloid malignancies due to heterozygous variants is an intriguing, but not well-understood, possibility. Segregation

studies to look for high penetrance for myeloid malignancy predisposition for these genes may be difficult because variants in some of these genes also predispose to breast cancer and a range of solid tumor malignancies.[2] These families may present with heterogeneous pan-cancer phenotypes, complicating segregation studies. Alternative evidence for risk of myeloid predisposition for some genes or alleles in the DDR pathway could be more amenable to careful case-control studies, because, collectively, they are identified more frequently than very rare myeloid predisposition gene mutations, such as in *RUNX1*, which was not identified as germline in any patients in this study. Understanding the role of DDR pathway mutations in HMs could lead to the extension of specific treatment approaches that are efficacious for this subgroup, such as PARP inhibitors, which, for unselected AML, have limited activity, at least in the single-agent trial setting.[10]

This study also highlights 1 of the principal challenges for clinical laboratories today. Of the 1547 variants curated, 252 patients (65%) had ≥1 variant classified as a variant of uncertain significance (VUS). Attempting to understand whether a VUS is vicious (truly pathogenic), vexing (unable to be resolved), or veiled (truly benign) often requires extensive follow-up, including de novo/segregation studies or functional studies.

Finally, this study made clear that routine germline genetic profiling of patients with leukemia could overcome some of the deficits of incomplete family history (due to biology [ie, incomplete penetrance] or missing information). Although, overall, their clinical information showed that 15% of individuals had a known family history of HMs, these overlapped, but did not intersect completely, with individuals who had P/LP germline variants in clinically actionable genes. As the field continues to move toward precision medicine in the diverse fields of diagnosis, prognosis, treatment, and monitoring for AML, this study adds to the accumulating evidence that including prospective assessment of the germline genetics in patients is an important consideration.

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REFERENCES

1. Yang F, Long N, Anekpuritanang T, et al. Identification and prioritization of myeloid malignancy germline variants in a large cohort of adult patients with AML. *Blood.* 2022;139(8):1208-1221.
2. Tawana K, Brown A, Churpek J. Integrating germline variant assessment into routine clinical practice for myelodysplastic syndrome and acute myeloid leukaemia: current strategies and challenges. *Br J Haematol.* In press.
3. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391-2405 [published correction appears in Blood. 2016;128(3):462–463].
4. Brown AL, Hahn CN, Scott HS. Secondary leukemia in patients with germline transcription factor mutations (RUNX1, GATA2, CEBPA). *Blood.* 2020;136(1):24-35.
5. Rio-Machin A, Vulliamy T, Hug N, et al. The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. *Nat Commun.* 2020;11(1):1044.
6. Brown AL, Hiwase DK. What's germane in the germline? Finding clinically relevant germline variants in myeloid neoplasms from tumor only screening. *Leuk Res.* 2020;96:106431.
7. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-423.
8. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 2017;129(4):424-447.
9. Sébert M, Passet M, Raimbault A, et al. Germline DDX41 mutations define a significant entity within adult MDS/AML patients. *Blood.* 2019;134(17):1441-1444.
10. Fritz C, Portwood SM, Przespolewski A, Wang ES. PARP goes the weasel! Emerging role of PARP inhibitors in acute leukemias. *Blood Rev.* 2021;45:100696.

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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Chiang et al, page 1222

Clearing NETs with T-series resolvins

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In this issue of *Blood*, Chiang et al[1] report that the T-series of resolvins (RvTs) reduce the formation of neutrophil extracellular traps (NETs) and enhance NET clearance by macrophages, thereby identifying a novel mechanism for the resolution of infections and coagulopathies.

Neutrophils play a key role in host defense against invading pathogens and are rapidly deployed to sites of infection or injury. However, many of the defense mechanisms they use to destroy microorganisms are potentially deleterious to the host. Among these mechanisms is release of NETs (also known as NETosis), which consist of a nucleic acid scaffold decorated with histones and granular proteins to entrap and kill bacteria, viruses, and fungi.[2] Because the effects of NET components are not restricted to microbes, excessive or uncontrolled NET formation can inflict damage to the surrounding tissue, thus maintaining a pro-inflammatory and pro-thrombotic environment that underlies various pathologies, including autoimmune diseases,[3] severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–associated coagulopathies, and acute respiratory distress syndrome (ARDS).[4] To ensure timely resolution of acute inflammation, neutrophils need to be disarmed and removed from the affected sites. Resolution of inflammation is an active process governed by specialized pro-resolving lipid mediators (lipoxins, resolvins, protectins, and maresins), proteins (eg, annexin A1), and gaseous mediators (eg, hydrogen sulfite and carbon monoxide), which predominantly act on phagocytes and other immune cells.[5-7]

By analyzing lipid profiles in resolution exudates, the authors previously identified a new series of resolvins that originate from a central intermediate of