subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell.* 2020;37(4):551-568.

2. Schmitt A, Xu W, Bucher P, et al. Dimethyl fumarate induces ferroptosis and impairs NF-κB/STAT3 signaling in DLBCL. *Blood.* 2021; 138(10):871-884.
3. Stockwell BR, Jiang X, Gu W. Emerging mechanisms and disease relevance of ferroptosis. *Trends Cell Biol.* 2020;30(6): 478-490.
4. Milhollen MA, Traore T, Adams-Duffy J, et al. MLN4924, a NEDD8-activating enzyme inhibitor, is active in diffuse large B-cell lymphoma models: rationale for treatment of NF-κB-dependent lymphoma. *Blood.* 2010; 116(9):1515-1523.
5. Yang Y, Shaffer AL III, Emre NC, et al. Exploiting synthetic lethality for the therapy of ABC diffuse large B cell lymphoma. *Cancer Cell.* 2012;21(6):723-737.
6. Mills EA, Ogrodnik MA, Plave A, Mao-Draayer Y. Emerging understanding of the mechanism of action for dimethyl fumarate in the treatment of multiple sclerosis. *Front Neurol.* 2018;9:5.
7. Wang W, Green M, Choi JE, et al. $CD8^+$ T cells regulate tumour ferroptosis during cancer immunotherapy. *Nature.* 2019; 569(7755):270-274.
8. Efimova I, Catanzaro E, Van der Meeren L, et al. Vaccination with early ferroptotic cancer cells induces efficient antitumor immunity. *J Immunother Cancer.* 2020;8(2):e001369.
9. Kornberg MD, Bhargava P, Kim PM, et al. Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity. *Science.* 2018;360(6387):449-453.
10. Chen K, Wu S, Ye S, et al. Dimethyl fumarate induces metabolic crisie to suppress pancreatic carcinoma. *Front Pharmacol.* 2021;12:617714.

DOI 10.1182/blood.2021011933

TRANSPLANTATION

Comment on Reilly et al, page 898

The journey of a thousand miles begins with 1 step

Luca Malcovati | University of Pavia and IRCCS S Matteo Hospital Foundation

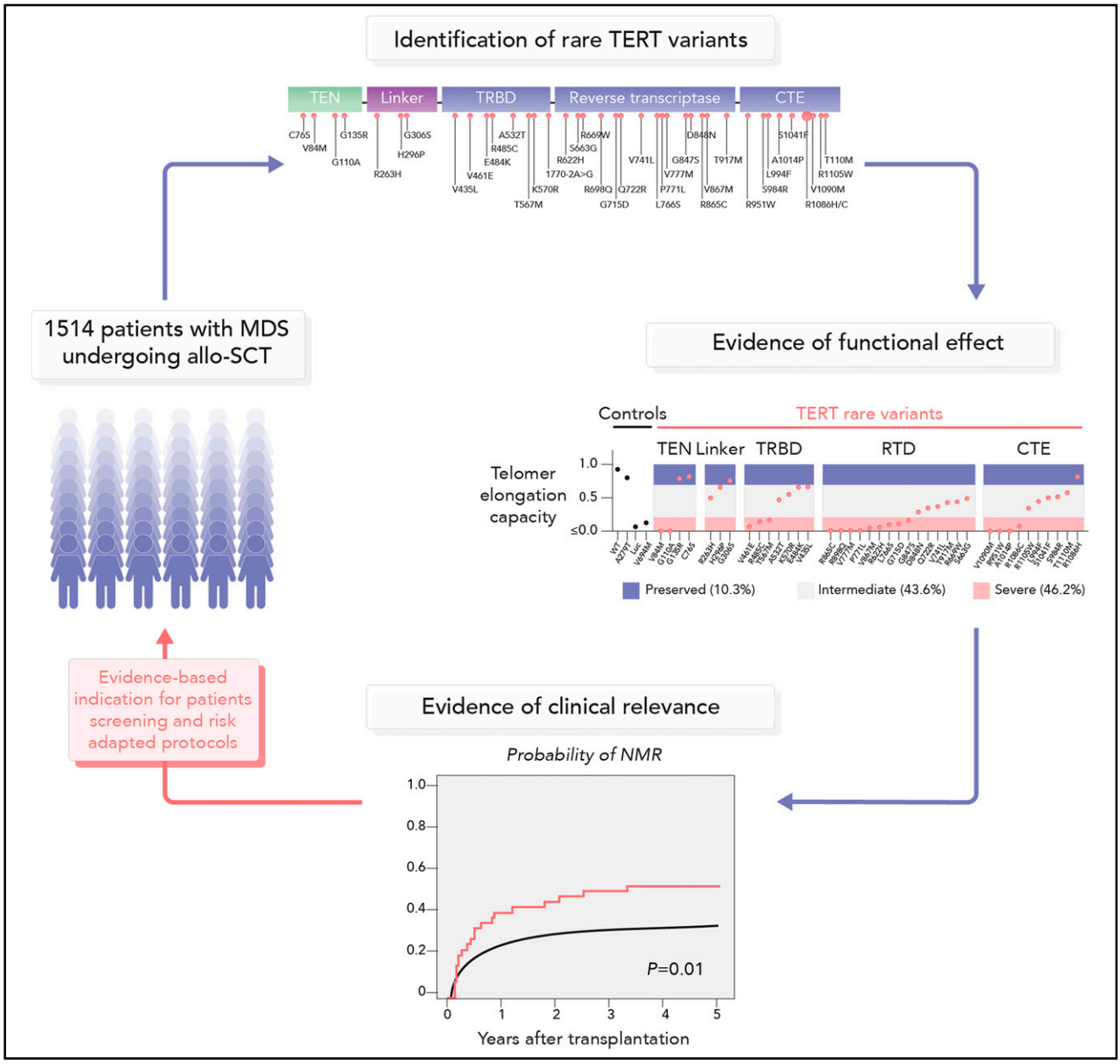
In this issue of *Blood*, Reilly et al analyze *TERT* rare variants in a large cohort of patients with myelodysplastic syndrome (MDS) enrolled in the Center for International Blood and Marrow Transplant Research (CIBMTR) database. They cloned all variants, quantified their impact on telomere elongation in cell-based assays, and proved their association with inferior posttransplant outcome.[1]

This study has implications for both clinical and translational research. The pathophysiology of MDS has remained largely obscure for a long time. High-throughput DNA sequencing has identified the key somatic mutation drivers of this type of malignancy, including splicing factors, DNA methylation, chromatin modification, transcription regulation, DNA repair, and signal transduction.[2] In 2016, the World Health Organization classification added germline predisposition to myeloid neoplasms.[3] This change has helped reveal the relatively well-hidden world of germline mutations in apparently sporadic hematologic cancers associated with a spectrum of underlying conditions. However, the relative contribution of the germline genetic component to the pathophysiology of MDS, as well as its clinical implications, still needs to be elucidated. Indeed, some disorders seem to have high penetrance and strong clinical expressivity that raise clinical suspicion, whereas others, such as germline *DDX41* mutations, do not.[4] In addition, even within those mutations associated with organ dysfunction, clinical expression may be extremely variable, which results in late onset or incidental diagnosis in adulthood.[5]

Reilly et al analyzed a large cohort of 1514 MDS patients in the CIBMTR repository and research database who had banked whole peripheral blood DNA, as well as a separate cohort of 401 adult patients with non-Hodgkin lymphoma who had been treated with autologous stem cell transplantation as a control group. In a previous study on the same CIBMTR cohort, the authors reported compound heterozygous mutations in the Shwachman-Diamond syndrome–associated *SBDS* gene in young adults with MDS that were associated with a remarkably shorter posttransplant survival.[6] More recently, using the same cohort, the authors measured relative telomere length in blood samples from pretransplant recipients and found a significant association with inferior survival because of a high risk of nonrelapse mortality (NRM).[7] On the basis of this information, Reilly et al analyzed *TERT* rare variants in the CIBMTR cohort and found a non-negligible prevalence of 2.7% in patients with no clinical diagnosis of telomere biology disorder. In line with their previous findings, functionally relevant *TERT* mutations proved to be clinically relevant, significantly affecting posttransplant outcome, mainly as a consequence of an increased incidence of NRM. Overall, these results concur to sustain systematic genetic screening of germline variants in patients who are candidates for allogeneic stem cell transplantation to guide risk-adapted approaches and inform donor selection.

Although next-generation sequencing (NGS) technologies have helped pave the way to integrating somatic mutation analysis into the diagnosis process, disease classification, and risk assessment, they have also fostered a harmful feeling of having a solution to every (diagnostic) problem within arm's reach. Indeed, not all that glitters is gold, and the study by Reilly et al nicely illustrates the huge effort and the translational research methodology that must be applied to generate a robust basis of evidence for interpreting NGS test results in our clinical practice. In fact, high-throughput genome sequencing outputs a plethora of data (variants) of uncertain significance, of which we do not yet know the actual relevance for protein function and, consequently, their clinical value.

Standards and guidelines for interpreting sequence variants have been developed to provide guidance for interpreting genetic tests by integrating a spectrum of information sources, including population data, computational data, functional data, and segregation data, and by adopting of standard terminology.[8] In addition, the Clinical Genome Resource (ClinGen; https://clinicalgenome.org), a large collaborative effort funded by the National Institutes of Health, has been promoted



Workflow for the study by Reilly et al. By analyzing a cohort of 1514 MDS patients enrolled in the CIBMTR database, the authors identified rare TERT variants, quantified their functional impact on telomere elongation, and proved their clinical relevance, eventually laying the foundations for evidence-based indication for patients screening and risk-adapted protocols. CTE, C-terminal extension [domain]; NMR, nonrelapse mortality; RTD, reverse transcriptase domain; SCT, stem cell transplantation; TEN, telomerase essential N-terminal [domain]; TRBD, telomerase RNA-binding domain.

with the aim of building a central resource that defines the clinical relevance of genes and variants for use in precision medicine and research.[9] Furthermore, a ClinGen Myeloid Malignancy Variant Curation Expert Panel has been created collaboratively with the American Society of Hematology to make gene- and disease-specific modifications for inherited myeloid malignancies and to incorporate strength adjustments of existing rules, including calculation of minor allele frequency thresholds, identification of functional domains and mutational hotspots, establishment of functional assay thresholds, and characterization of phenotype-specific guidelines.[10]

Despite these efforts, in many cases, the classification of germline variants remains challenging, mostly because of the lack of appropriate functional studies and cell models. Classification becomes even more challenging when applying these analyses to genetic defects with mild or even absent clinical expressivity or when analyzing newly identified genes for which the available evidence is scanty. Thus, the study by Reilly et al provides a paradigm of the approach that has to be adopted to generate the evidence required for interpretation of germline variants (see figure). After identifying *TERT* rare mutations, they undertook the huge effort of cloning each single variant and quantifying its impact on telomere elongation in in vitro assays. This allowed them to identify variants that exhibited severe or intermediate impaired or preserved capacity to

elongate telomeres, which generated the evidence required to apply the analysis of these variants in the clinical setting.

In summary, this study adds a relevant contribution to the field by providing further evidence of the as yet underestimated effect of germline predisposition to apparently sporadic myeloid neoplasms with myelodysplasia, and by moving a step forward on the long journey toward accumulating the evidence required for clinical implementation of germline testing in myeloid malignancies. With this aim in mind, collaborative efforts and international networking are warranted more than ever before, because the set of genes of potential interest is large (and continually increasing), genes that confer predisposition to myeloid cancer are (almost invariably) rare, which compels large international cohorts to reach adequate sample size and, last but not least, consensus-based standardized variant curation is essential for making recommendations for clinical care.

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

REFERENCES

1. Reilly CR, Myllymäki M, Redd R, et al. The clinical and functional effects of *TERT* variants in myelodysplastic syndrome. *Blood*. 2021; 138(10):898-911.
2. Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. *Blood*. 2013;122(25):4021-4034.
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.
4. 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.
5. Kraft IL, Godley LA. Identifying potential germline variants from sequencing hematopoietic malignancies. *Blood*. 2020; 136(22):2498-2506.
6. Lindsley RC, Saber W, Mar BG, et al. Prognostic mutations in myelodysplastic syndrome after stem-cell transplantation. *N Engl J Med*. 2017;376(6):536-547.
7. Myllymäki M, Redd R, Reilly CR, et al. Short telomere length predicts nonrelapse mortality after stem cell transplantation for myelodysplastic syndrome. *Blood*. 2020; 136(26):3070-3081.
8. 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):405-424.
9. Rehm HL, Berg JS, Brooks LD, et al; ClinGen. ClinGen–the Clinical Genome Resource. *N Engl J Med*. 2015;372(23):2235-2242.
10. Luo X, Feurstein S, Mohan S, et al. ClinGen myeloid malignancy variant curation expert panel recommendations for germline RUNX1 variants. *Blood Adv*. 2019;3(20):2962-2979.

DOI 10.1182/blood.2021012304