in hospitalized patients, including in those requiring supplemental oxygen, but not in those requiring ICU level care, may reflect better management of patients over time, but also highlight the significance of early introduction of various anti–COVID-19 therapies to prevent clinical deterioration to ICU level care. The findings that corticosteroids increased both secondary infections and death rates in patients with CLL and COVID-19 are intriguing. In the RECOVERY trial,[8] the use of dexamethasone improved survival in patients hospitalized with COVID-19 who received respiratory support. Perhaps the impaired immune reactions in patients with CLL moderate the hyperinflammatory reactions to COVID-19, thus turning corticosteroids beneficial effects to somewhat redundant in this frail population.

After the acute phase of SARS-CoV-2 infection, seroconversion is noted in nearly all immunocompetent subjects, whereas it was documented in only 60% of the patients with CLL.[1] Failure to produce protective antibodies may put seronegative patients at risk for reinfection with SARS-CoV-2 and consequently justifies vaccinating all patients with CLL who have recovered from COVID-19. Likewise, patients with CLL may develop persistent COVID-19 infection, as result of their inability to effectively eradicate the virus. In such cases, prolonged shedding of infectious SARS-CoV-2 virus and within-host genomic evolution may eventually lead to emergence of new virus variants.[9] Antibody-mediated response to COVID-19 vaccines in patients with CLL is also impaired and impacted by disease activity and anti-CLL treatment.[10] High rates of humoral responses to COVID-19 vaccine were observed in patients with CLL in remission after treatment. Treatment-naive patients had a lower rate of response to vaccination, whereas only a minority of patients on active treatment at the time of vaccination responded.[10] Recently, administration of a third messenger RNA COVID-19 vaccine dose to solid-organ transplant recipients was shown to significantly improve humoral response to the vaccine, as 44% of seronegative patients became seropositive after a third dose.[11] Given the high risk of severe COVID-19 disease and impaired antibody-mediated immune response to the SARS-CoV-2 virus and its vaccine in patients with CLL, a booster dose may be justified in patients with CLL who fail to achieve seropositivity after 2 vaccine doses.

In summary, Roeker et al report on the recent outcome of patients with CLL and COVID-19, showing decreased risk of death, and provide hints regarding the efficacy of specific treatments in these patients. The gathering data call for early application of antiviral drugs, mAbs, and convalescent plasma as well as improved vaccination strategy, to improve the odds for patients with CLL confronting COVID-19. As the global COVID-19 pandemic is ongoing and seems far from resolution, especially as the virus continues to mutate, well-designed, large-scale prospective studies, on the clinical course, outcomes, the efficacy of specific therapeutics, and vaccination timing and schedule, in patients with CLL and COVID-19, are still warranted.

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

REFERENCES

1. Roeker LE, Eyre TA, Thompson MC, et al. COVID-19 in patients with CLL: improved survival outcomes and update on management strategies. *Blood.* 2021;138(18):1768-1773.
2. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. *Blood.* 2015;126(5):573-581.
3. Scarfò L, Chatzikonstantinou T, Rigolin GM, et al. COVID-19 severity and mortality in patients with chronic lymphocytic leukemia: a joint study by ERIC, the European Research Initiative on CLL, and CLL Campus. *Leukemia.* 2020;34(9):2354-2363.
4. Vijenthira A, Gong I, Fox TA, et al. Outcomes of adult and pediatric patients with hematologic malignancies and COVID-19: a systematic review and meta-analysis of 1847 patients [published online ahead of print 17 December 2020]. *Blood.* doi:10.1182/blood.2020008824.
5. Beigel JH, Tomashek KM, Dodd LE, et al; ACTT-1 Study Group Members. Remdesivir for the treatment of Covid-19 - final report. *N Engl J Med.* 2020;383(19):1813-1826.
6. Simonovich VA, Burgos Pratx LD, Scibona P, et al; PlasmAr Study Group. A randomized trial of convalescent plasma in Covid-19 severe pneumonia. *N Engl J Med.* 2021; 384(7):619-629.
7. Libster R, Pérez Marc G, Wappner D, et al; Fundación INFANT–COVID-19 Group. Early high-titer plasma therapy to prevent severe Covid-19 in older adults. *N Engl J Med.* 2021;384(7):610-618.
8. Horby P, Lim WS, Emberson JR, et al; RECOVERY Collaborative Group. Dexamethasone in hospitalized patients with Covid-19. *N Engl J Med.* 2021;384(8): 693-704.
9. Avanzato VA, Matson MJ, Seifert SN, et al. Case study: prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised individual with cancer. *Cell.* 2020;183(7):1901-1912.e9.
10. Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood.* 2021;137(23):3165-3173.
11. Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three doses of an mRNA Covid-19 vaccine in solid-organ transplant recipients [published online ahead of print 23 June 2021]. *N Engl J Med.* 2021;NEJMc2108861.

DOI 10.1182/blood.2021013286

MYELOID NEOPLASIA

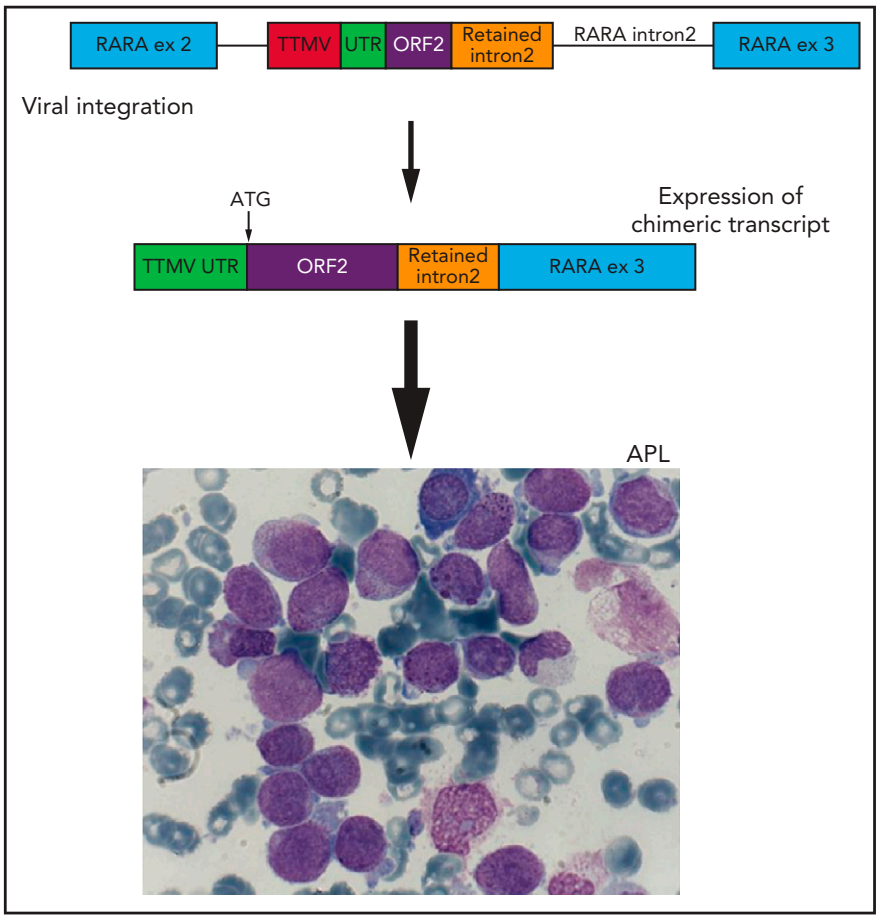
Comment on Astolfi et al, page 1773

A viral cause of APL

Rachel E. Rau[1] | Texas Children's Hospital

In this issue of *Blood*, Astolfi et al report the first known cases of acute promyelocytic leukemia (APL) caused by integration of the ubiquitous, seemingly nonpathogenic torque teno mini virus (TTMV) into the *RARA* locus.[1]

APL accounts for 5% to 10% of pediatric and adult acute myeloid leukemia (AML) and is characterized by specific clinical and biologic features and is managed by a unique therapeutic approach. Indeed, compared with other AML subtypes, it seems APL diagnosis, biology, and therapy are well defined with little left to be worked out. The diagnosis and management of APL often proceeds down a relatively predictable path beginning with the patient presenting with a variably high white blood cell count with promyelocytic blasts, typically with characteristic intense azurophilic granules and obvious Auer rods. Diagnosis is facilitated by the



Schematic representation of viral integration of a portion of the TTMV gene into intron 2 of the *RARA* gene. The integration includes TTMV open reading frame 2 (ORF2) and an upstream untranslated region (UTR) adjacent to a short sequence of retained *RARA* intron 2, in-frame with exon (ex) 3. This integration results in the transcription of a chimeric fusion messenger RNA with a start codon (ATG) at the initiation of TTMV ORF2. This chimeric fusion transcript results in an APL-like gene expression signature and ultimately the development of acute promyelocytic leukemia (APL). Adapted from Figure 1 in the article by Astolfi et al that begins on page 1773.

fact that APL is, by in large, a genetically uniform disease characterized by the pathognomonic *PML/RARA* (promyelocytic leukemia gene/retinoic acid receptor α) fusion gene resulting from the t(15;17)(q22;q12) translocation.[2] Importantly, APL is exquisitely sensitive to all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), making it highly curable in most patients without need for intensified chemotherapy.[3,4]

Of course, APL still poses challenges, including significant disseminated intravascular coagulopathy at presentation that can lead to the patient's demise from a catastrophic hemorrhage. Because of this risk, physicians suspecting APL must initiate ATRA therapy almost immediately, long before genetic confirmation of the disease. Additionally, patients can suffer life-threatening differentiation syndrome, particularly after ATRA initiation. Beyond these clinical challenges in the early disease course, diagnosis itself can prove surprisingly elusive in some cases that by all clinical and morphologic features fit the definition of APL. Occasionally, the *PML/RARA* fusion results from a cryptic translocation, missed by all standard cytogenetic assays. Rarely, RARA fusions involving other genes can drive the disease and, in fewer cases still, fusions involving other members of the retinoic acid receptor family have been identified as drivers of APL.[5] Astolfi et al report an even more unexpected genetic cause of APL.

The authors report the case of a 6-year-old girl with clinical and morphologic features of APL without evidence of t(15;17) or the *PML/RARA* fusion. To investigate the genetic cause of her disease after its unfortunate relapse, the authors conducted whole-transcriptome sequencing on a leukemic sample. This analysis identified an in-frame viral insertion of a portion of the TTMV into intron 2 of the *RARA* gene, within the region of *RARA* involved in the *PML/RARA* fusion. This integration resulted in transcription of a chimeric *TTMV/RARA* fusion gene (see figure). Based on this finding, the patient was treated with ATRA/ATO, which gradually decreased the fusion transcript and ultimately resulted in a complete remission. Going further, the authors interrogated retrospective whole-transcriptome sequencing data, identifying a similar integration of a portion of the TTMV gene into *RARA* intron 2 in a sample from a 3-year-old with normal karyotype AML. These chimeric fusions were also associated with an APL gene expression signature, and when expressed in a human cell line conferred sensitivity to ATRA, supporting a pathogenic role in the genesis of APL.[1]

Although viral infections are known drivers of 12% to 16% of cancers, this is the first description of a viral cause of APL.[6] Additionally, this is the first direct evidence of TTMV, a nonpathogenic member of the *Anelloviridae* family of nonenveloped, circular single-stranded DNA viruses, as a cause of cancer. The identification of viral integration causing APL leads one to wonder if viral-driven APL outbreaks are possible, as there are reports of clusters of APL in the literature.[7] However, the cases reported in these clusters were characterized by presence of the *PML/RARA* fusion, thus genetically distinct from the *TTMV/RARA* fusion cases reported by Astolfi et al. Furthermore, TTMV is an omnipresent virus, likely acquired by all humans during infancy and persisting throughout life[8]; thus, it is unlikely to be a prominent cause of periodic APL outbreaks. There is evidence suggesting TTMV is held in check by the intact immune system, raising the question of whether increased viral load in an immunocompromised host could facilitate the integration of a portion of the virus into the *RARA* gene.[8] In the 2 cases presented by Astolfi et al, neither child had an identified preexisting immune deficit before their leukemia diagnosis, though this cannot be ruled out.

Last, this fascinating report raises the question of whether TTMV viral integration is a recurrent cause of APL. To fully define the frequency of TTMV/RARA fusions, sequencing of many additional APL samples lacking *PML/RARA* fusions will be necessary. Given that sensitivity to ATRA and ATO is likely dependent on the presence of the *PML/RARA* fusion,[9] precisely defining the genetic basis of each APL case is of clinical value. Therefore, if future work establishes *TTMV/RARA* fusion as a recurrent cause of APL, development of clinical assays for its detection could be warranted.

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

REFERENCES

1. Astolfi A, Masetti R, Indio V, et al. Torque teno mini virus as a cause of childhood acute promyelocytic leukemia lacking PML/RARA fusion. *Blood*. 2021;138(18):1773-1777.
2. 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.
3. Lo-Coco F, Avvisati G, Vignetti M, et al; Gruppo Italiano Malattie Ematologiche dell'Adulto; German-Austrian Acute Myeloid Leukemia Study Group; Study Alliance Leukemia. Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N Engl J Med*. 2013;369(2):111-121.
4. Strocchio L, Gurnari C, Santoro N, et al. Arsenic trioxide and all-trans retinoic acid treatment for childhood acute promyelocytic leukaemia. *Br J Haematol*. 2019;185(2):360-363.
5. Conneely SE, Rau RE. The genomics of acute myeloid leukemia in children. *Cancer Metastasis Rev*. 2020;39(1):189-209.
6. Zapatka M, Borozan I, Brewer DS, et al. The landscape of viral associations in human cancers. *Nat Genet*. 2020;52(3):320-330.
7. Brunner AM, Kim PG, Sadrzadeh H, et al. Clustered incidence of adult acute promyelocytic leukemia. *Leuk Res*. 2018;74:47-50.
8. Kaczorowska J, van der Hoek L. Human anelloviruses: diverse, omnipresent and commensal members of the virome. *FEMS Microbiol Rev*. 2020;44(3):305-313.
9. de Thé H, Pandolfi PP, Chen Z. Acute promyelocytic leukemia: a paradigm for oncoprotein-targeted cure. *Cancer Cell*. 2017;32(5):552-560.

DOI 10.1182/blood.2021013630