Pharmacyclics, and TG Therapeutics and consulting relationships with AbbVie, ADC Therapeutics, Astra-Zeneca/Acerta, Bayer, Celgene, Genentech, Gilead Sciences, Inc, Janssen/Pharmacyclics, Juno Therapeutics, Kite, Portola, Sanofi-Genzyme, Seattle Genetics, Spectrum, TG Therapeutics, Verastem. J.O.A. reports consulting relationships with Conatus IDMC, Samus Therapeutics, Oncology Analytics, and Ascentage; he is a member of the Board of Directors for Tesaro Bio, Inc. ■

REFERENCES

1. van Rhee F, Voorhees P, Dispenzieri A, et al. International, evidence-based consensus treatment guidelines for idiopathic multicentric Castleman disease. *Blood*. 2018;132(20):2115-2124.

2. Keller AR, Hochholzer L, Castleman B. Hyaline-vascular and plasma-cell types of giant lymph node hyperplasia of the mediastinum and other locations. *Cancer*. 1972; 29(3):670-683.

3. Fajgenbaum DC, Uldrick TS, Bagg A, et al. International, evidence-based consensus diagnostic criteria for HHV-8-negative/ idiopathic multicentric Castleman disease. *Blood*. 2017;129(12): 1646-1657.

4. Dispenzieri A, Armitage JO, Loe MJ, et al. The clinical spectrum of Castleman's disease. *Am J Hematol*. 2012;87(11):997-1002.

5. Fajgenbaum DC, van Rhee F, Nabel CS. HHV-8-negative, idiopathic multicentric Castleman disease: novel insights into biology, pathogenesis, and therapy. *Blood*. 2014;123(19): 2924-2933.

6. van Rhee F, Wong RS, Munshi N, et al. Siltuximab for multicentric Castleman's disease: a randomised, double-blind, placebo-controlled trial. *Lancet Oncol*. 2014;15(9): 966-974.

7. Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, Kakehi T. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. *Blood*. 2008;112(10): 3959-3964.

8. Le RQ, Li L, Yuan W, et al. FDA approval summary: tocilizumab for treatment of chimeric antigen receptor T cell-induced severe or life-threatening cytokine release syndrome. *Oncologist*. 2018;23(8): 943-947.

9. Iwaki N, Fajgenbaum DC, Nabel CS, et al. Clinicopathologic analysis of TAFRO syndrome demonstrates a distinct subtype of HHV-8-negative multicentric Castleman disease. *Am J Hematol*. 2016; 91(2):220-226.

Comment on Chapman et al, page 2154

Knick-knack PADIMAC

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In this issue of *Blood*, Chapman et al[1] have derived a 7-gene expression signature that predicts whether a newly diagnosed myeloma patient will respond to treatment with bortezomib when autologous stem cell transplant (ASCT) is not considered as part of the upfront therapy.

The goal of the phase 2 PADIMAC (Bortezomib, Adriamycin, and Dexamethasone Therapy for Previously Untreated Patients with Multiple Myeloma: Impact of Minimal Residual Disease in Patients with Deferred ASCT) trial is to provide a reliable estimate of the 2-year progression-free survival (PFS) in patients achieving very good partial response (VGPR) or better to induction therapy with bortezomib, adriamycin, and dexamethasone. RNA was isolated and sequenced from patients in the trial from CD138+ sorted cells from bone marrow aspirates. To ensure the robustness of the data set, the team performed several quality control checks on the sequencing data for expected myeloma characteristics including expression of key translocation partner oncogenes, presence of expressed mutations, and fusion genes between immunoglobulin and *MMSET*.

The 44 patients in the study were classified by response, where those with a VGPR or better and who were progression-free at
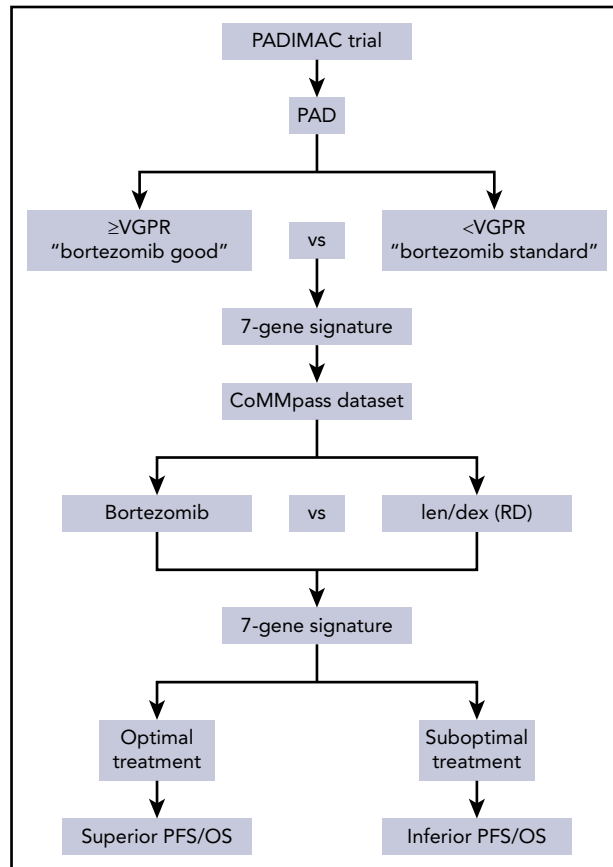
Overview of the process to generate and validate a RNA-seq gene signature to determine optimal treatment. CoMMpass, Clinical Outcomes in Multiple Myeloma to Personal Assessment of Genetic Profile; OS, overall survival; PAD, bortezomib, adriamycin, and dexamethasone.

1 year without ASCT were classified as "bortezomib-good"; the remaining patients were "bortezomib-standard" (see figure). Comparison of the 2 groups identified an optimal 7-gene signature, consisting of *EMC9, FAM171B, PLEK, MYO9B, RCN3, FLNB,* and *KIF1C*. Three of these proteins are associated with the endoplasmic reticulum, 3 associated with actin filaments, and probably most important, 3 (EMC9, MYO9B, and KIF1C) are positively associated with proliferation.

To ensure that the signature was robust, the authors used it to determine its predictive power in a separate data set, namely a subset of the CoMMpass data set. This data set consisted of previously untreated patients who did not proceed to ASCT, who were either treated with bortezomib (without an immunomodulatory drug, n = 147), with lenalidomide and dexamethasone (RD) (n = 40), or both (n = 208). The 7-gene signature was used to identify patients within the CoMMpass study who would benefit from bortezomib-based therapy, and indeed, those who were assigned to the "bortezomib-good" group had a better PFS.

The most important finding of the group was to show that patients predicted to do well with bortezomib actually have an inferior PFS when treated with RD and vice versa. Therefore, in future prospective studies, it may be important to assign treatment regimens based on their gene expression profiles at diagnosis. To test this hypothesis, the authors used the data set to determine which patients should have received either bortezomib or RD, based on the 7-gene signature, and compared the outcome of patients who received the correct treatment. This showed that those with the correct predicted treatment had a superior PFS and overall survival.

Gene expression profiling has been used in myeloma for more than a decade to determine high-risk subgroups, translocation subtypes, proliferation rates, and prognostic subgroups.[2-6] It is increasingly important when treating patients to be able to identify the most effective, cost-efficient regimen with the fewest toxicities. In this regard, precision medicine has been used to identify subgroups of patients with genomic abnormalities to receive treatments directed against the identified abnormality. Here, however, the authors have shown using RNA-sequencing

that it is possible to predict which treatment will result in a superior response when considering novel agents that are not targeted against specific genomic abnormalities. This methodology could be used for other drug combinations to further identify optimal treatments for patients, including those undergoing transplants.

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

REFERENCES

1. Chapman MA, Sive J, Ambrose J, et al. RNA-seq of newly diagnosed patients in the PADIMAC study leads to a bortezomib/lenalidomide decision signature. *Blood*. 2018;132(20):2154-2165.

2. Shaughnessy JD Jr. Global gene expression profiling in the study of multiple myeloma. *Int J Hematol*. 2003;77(3):213-225.

3. Stein CK, Pawlyn C, Chavan S, et al. The varied distribution and impact of RAS codon and other key DNA alterations across the translocation cyclin D subgroups in multiple myeloma. *Oncotarget*. 2017;8(17): 27854-27867.

4. Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy J Jr. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. *Blood*. 2005;106(1): 296-303.

5. Broyl A, Hose D, Lokhorst H, et al. Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients. *Blood*. 2010;116(14): 2543-2553.

6. Walker BA, Leone PE, Jenner MW, et al. Integration of global SNP-based mapping and expression arrays reveals key regions, mechanisms, and genes important in the pathogenesis of multiple myeloma. *Blood*. 2006;108(5): 1733-1743.

PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Ueki et al, page 2183

Charcot-Leyden crystals: solving an enigma

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In this issue of *Blood*, Ueki et al elegantly demonstrate the active formation of Charcot-Leyden crystals (CLCs) during eosinophil cytolysis.[1] After confirming the association of CLC deposition with eosinophilic inflammation and eosinophil plasma membrane disruption in tissue sections by light and electron microscopy, Ueki et al used a combination of sophisticated imaging techniques, including immunofluorescent time-lapse photography, to follow the course of CLC formation in vitro in response to a variety of stimuli that induce eosinophil extracellular trap death (EETosis). The association of CLC with the disintegration of eosinophils was proposed as early as the 1940s,[2] and agents, such as Aerosol MA, which disrupt the integrity of eosinophils, were subsequently shown to promote CLC formation in vitro in the surrounding media.[3] Ueki et al provide the first definitive evidence that CLC formation is energy-dependent and closely tied to the process of EETosis (see figure).

Although the presence of CLC in tissues was first described in the late 1800s, the mechanism of formation and function of these crystals are only beginning to be understood. Charcot-Leyden protein (galectin-10) composes up to 10% of the total protein in the eosinophil, and although galectin-10 has been demonstrated in T cells,[4] CLC formation appears to be restricted to human eosinophils and basophils.[5] Moreover, tissue deposition of CLC has been described exclusively in tissues and body fluids at sites of eosinophilic inflammation. Recent studies have begun to shed light on the functional roles of Charcot-Leyden protein in eosinophilic inflammation. Initially thought to be a lysophospholipase on the basis of gel chromatography studies, the observed increase in lysophospholipase