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.* ■

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**PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS**

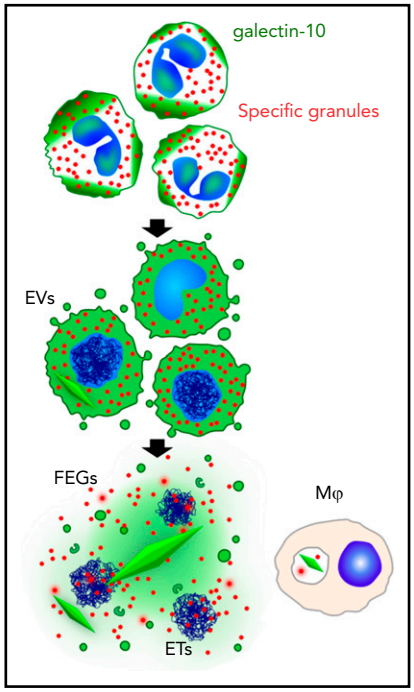
*Comment on Ueki et al, page 2183*

# Charcot-Leyden crystals: solving an enigma

**Amy D. Klion** | National Institutes of Health

**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




EETosis mediates galectin-10 crystallization. The graphic shows the temporal course of galectin-10 crystallization in tissues. During stimuli-elicited EETosis, loss of regulated intracellular localization of galectin-10 occasionally causes galectin-10 crystallization in the cytoplasm before cell lysis. Galectin-10 is released by plasma membrane disintegration, which may result in extracellular crystallization by increasing local concentrations. Some galectin-10 is also budded from the plasma membrane within enveloped EVs. Thus, FEG, ETs, and galectin-10–containing EV were associated with varied sizes of CLC. Tissue macrophages can also take up galectin-10 and/or small CLC. ET, extracellular trap; EV, extracellular vesicle; FEG, free extracellular granule. See supplemental Figure 8 in the article by Ueki et al that begins on page 2183.

activity was subsequently shown to be due to binding of galectin-10 to a lysophospholipase inhibitor.[6] Galectin-10 has also been implicated in eosinophil lineage development[7] and, more recently, in eosinophil regulation of T-cell proliferation.[8] The role, if any, of crystal formation in these scenarios is unknown, however.

Extracellular crystals, including calcium oxalate and monosodium urate crystals, can deposit in tissues and trigger an inflammatory response resulting in tissue damage. They have also been shown to induce regulated cell death (necroptosis) in a caspase-independent manner that involves receptor-interacting serine-threonine kinase 3 and mixed lineage kinase domain-like pseudokinase in vitro and in vivo.[9] These data suggest that Charcot-Leyden crystals may contribute to eosinophil-induced tissue damage in a manner that is distinct from that of the secondary eosinophil granules, previously shown by Ueki et al to be associated with eosinophil extracellular DNA traps.[10]

In summary, the work of Ueki et al clearly demonstrates that CLC formation is an active, energy-dependent process that is associated with eosinophil cytolysis. Moreover, the energy dependence of this process supports the hypothesis that CLC formation is not merely a by-product of eosinophil destruction, and provides a first and essential step in understanding the function of CLC in eosinophilic inflammation. Whether the primary role of CLC is cytotoxic or is more intricately involved in immunoregulatory processes remains to be elucidated.

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

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**TRANSPLANTATION**

*Comment on Radojcic et al, page 2188*

# Int"Dll"igent control of T-cell pathology in GVHD

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**In this issue of *Blood*, Radojcic et al achieved almost total prevention of chronic graft-versus-host-disease (cGVHD) in 2 complementary mouse models of bone marrow transplantation by inhibition of Notch Delta-like ligands 1 and 4 (Dll1 and Dll4) signaling, raising hope for a new therapeutic intervention in humans.[1]**

GVHD remains a major complication after allogeneic hematopoietic bone marrow transplantation. Acute GVHD (aGVHD), usually treated with glucocorticoids, is frequently followed by the devastating chronic form of this disease for which treatment options are limited. What if a limited number of antibody injections following bone marrow transplantation could mitigate or even prevent both aGVHD and cGVHD? This vision has gained support by data presented in 3 studies that identified Notch signaling as a highly promising target for dampening excessive T- and B-cell activity.[1-3]