progenitors, which reside within a population of cells co-expressing the surface markers CD43 (leukosialin), CD41a (GPIIIa, a progenitor and megakaryocytic antigen), and CD235a (glycophorin A, an erythrocyte antigen). Now, work published in this issue of Blood extends previous studies by showing that single human ESC-derived CD41+/CD235a+ cells can give rise to primitive erythroblasts and megakaryocytes in culture, thus defining a human primitive MEP. Gene expression analysis showed that the differentiation of this bipotential progenitor into single lineages is accompanied by signature changes in the expression of key transcription factors that have been previously implicated in definitive erythrocyte and megakaryocyte production. This is the first report of primitive human MEPs and fits well with the original identification of the same progenitor in mouse embryos by Tober et al. Together, these studies provide new concepts on the origins of mammalian blood (see figure). Specifically, yolk sac primitive hematopoiesis is not restricted to erythrocytes, as posited in older models. Rather, the process produces at least 2 cell types (erythroid and megakaryocytic) that are intimately linked by a common bipotential progenitor. More generally, the current study illustrates the utility of ESCs as a model system for human hematopoiesis, particularly for investigating early embryonic events.

As for all interesting studies, new questions arise. Why are platelets produced in early embryos? NF-E2–null embryos, which exhibit low (but not absent) platelet levels, do not bleed until they experience birth trauma. However, platelets express many potent signaling molecules that may exert currently unrecognized functions in development of the vasculature or other embryonic tissues. There are also important issues of therapeutic relevance. For example, it may be possible to use human ESCs or related induced pluripotent stem cells (iPSCs) to generate platelets and erythrocytes for transfusion therapies. For this strategy to succeed, it is necessary to develop new culture methods that optimize the purity and yield of megakaryocytes, platelets, and erythrocytes from human ESCs or iPSCs. It may also be important to control the production of primitive versus definitive erythromegakaryocytic cells and to better understand the functional differences between these related but distinct lineages.

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Comment on Reece et al, page 1489

Finishing off fibrils

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Bortezomib shows high activity in light chain amyloidosis. Responses occur rapidly and are seen in cardiac, renal, and hepatic disease. Toxicity in this fragile population is significant.

In this issue of Blood, Reece and colleagues report a phase 1/2 study of bortezomib in the treatment of immunoglobulin light chain amyloidosis (AL). The authors report hematologic responses in 15 of 30 evaluable patients, 20% of which were complete, achieved at a median of 1.2 months. The activity of bortezomib, producing hematologic responses in AL, should not be surprising, given the high activity of the agent in multiple myeloma, the neoplastic counterpart of the clonal plasma cell dyscrasia AL. Virtually all chemotherapeutic stratagems for AL have been derived from the treatment of multiple myeloma and have included melphalan–based therapies, dexamethasone, thalidomide, and lenalidomide. High-dose therapy with autologous stem cell replacement is widely used for the treatment of AL, but this therapy is unsuitable for high-risk patients with the disease. The high prevalence of cardiomyopathy makes high-dose therapy excessively toxic for many patients with AL, necessitating the use of alternatives. In a retrospective report of bortezomib, combined with dexamethasone in 90%, hematologic response was achieved in 71% of patients including complete response in 25%. An organ response was observed in 30% of patients. In this issue of Blood, the first prospective study of bortezomib is reported.

What are the strengths of this study? In the current paper, dexamethasone was not administered with bortezomib. One can, therefore, confidently conclude that bortezomib alone is responsible for the reported benefit. A second strength is the high frequency of rapidly achieved hematologic responses occurring at virtually all dose levels and both schedules of bortezomib, suggesting that the maximum tolerated dose may not be the same as the lowest effective dose. Rapid response may reduce the organ damage the toxic light chains produce over time.

What are the weaknesses of this paper? The results may not be generalizable, since the median time from AL diagnosis to study enrollment was 32 months. In an unselected amyloidosis population, 70% will have succumbed to the disease at this time point, and the remaining patients have a much better prognosis than the oft-quoted 12 to 18 months median survival. Patients with New York Heart Association class III and class IV heart failure, found in a quarter of patients with amyloidosis, were excluded from this protocol. The impact of excluding these patients, since cardiomyopathy is the leading cause of death in
amyloidosis, may make it difficult to generalize the outcomes to an unselected population of patients with AL. The failure to report cardiac biomarkers, troponin, and NT-pro-BNP is a weakness, since it is difficult to assess the severity of cardiomyopathy in enrolled patients. Moreover, NT-pro-BNP has been used to measure cardiac response so estimates of cardiac benefit in a subsequent report may be inaccurate. This manuscript fails to report organ response, admittedly an often delayed end point, but hematologic responses that do not ultimately produce organ responses may not be of value to this patient population.

What are the unanswered and new questions this report generates? Will the drug be of use in patients with moderate amyloid cardiomyopathy since bortezomib can disrupt the ubiquitin–proteasome system predisposing to cardiac failure?1,5,6 Fifteen percent of patients with AL have peripheral and autonomic neuropathy; can the agent be used safely in this subgroup? Now that bortezomib is established to have activity in amyloidosis, will the responses be durable when administered to a broader cohort of patients, or will we see rapid, perhaps deep responses that are short-lived?7 Will bortezomib in combination with stem cell transplantation be effective for multiple myeloma?8,9 Provide even higher response rates in AL patients? Will there be an opportunity to combine bortezomib with stem cell transplantation? Two such variations would be to use bortezomib induction either in an attempt to improve organ function and render patients suitable for high-dose therapy, or to reduce the risk of fatal complications associated with high-dose therapy. Alternatively, risk-adapted stem cell transplantation with posttransplantation adjunct thalidomide has been shown to be beneficial,7 and the potential of bortezomib to suppress residual disease after stem cell transplantation in the hope of achieving a complete response may be achievable in a significant proportion of patients.

Amyloidosis is a difficult disorder to treat. The dropout rates in studies are generally high. In this protocol, 12 of the 30 patients discontinued therapy and grade 3 to 4 adverse events were seen in 50%. Nonetheless, the authors are to be congratulated for a meticulously conducted study in a single-agent setting in a devastating disorder.

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A<br>lthough experimental evidence supports the role of the immune system in controlling tumor development and progression. Immunologic interventions including allogeneic hematopoietic stem cell transplantation, donor lymphocyte infusion, adoptive transfer of antigen-specific cytotoxic T lymphocytes (CTLs) and tumor/peptide vaccination have all shown clinical activity that supports the ability of the immune system to impact tumor growth and prevent disease recurrence. Conversely, tumor escape from immune recognition by down-regulation of MHC molecules and direct inhibition of the function of tumor-specific CTLs has also been extensively demonstrated. Indeed, the tumor environment exploits multiple factors that cooperate to inactivate immune responses, such as production of inhibitory cytokines (TGF-β), aberrant expression of proapoptotic T-cell ligands (Fas-ligand), production of tryptophan catabolic enzyme, indoleamine 2,3-dioxygenase (IDO), and accumulation of regulatory T cells.1

Programmed cell death 1 (PD-1) receptor is rapidly up-regulated in T lymphocytes responding to viral infections, but under most circumstances it is quickly down-regulated upon removal of the antigen. By contrast, during chronic infections such as HIV2 and HCV, virus-specific CD8+ T lymphocytes show sustained expression of PD-1 which triggers T-cell dysfunction or exhaustion on interaction with antigen-presenting cells expressing PD ligand 1 (PD-L1).3 Previous reports demonstrated that the PD-1–PD-L1 pathway may also serve as a novel immune escape mechanism in mouse models of solid tumors.4 In this issue of Blood, 2 independent groups confirm the contribution of the PD-1 signaling pathway to immune tumor escape in 2 hematologic malignancies, chronic myelogenous leukemia (CML) and acute myelogenous leukemia (AML). Mumprecht and colleagues show that tumor-specific CTLs express high levels of PD-1 and have impaired function in a mouse model of CML.5 Importantly, these experiments confirm not only that PD-1 expression is a marker of T-cell exhaustion, but also that PD-L1 expressed by tumor cells contributes to T-lymphocyte dysfunction. Zhang and colleagues obtained similar results in a mouse model of AML, in which leukemic cells once again expressed PD-L1 in vivo, suggesting a direct pathogenetic role of

**Immunobiology**


**Blocking PD-1 in cancer immunotherapy**

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The PD-1 pathway is emerging as an important tumor-evasion mechanism. In this issue of Blood, 3 independent groups report that PD-1 is highly expressed by tumor-specific cytotoxic T lymphocytes in hematologic and nonhematologic malignancies, and is associated with impaired T-cell function.
Finishing off fibrils

Morie Gertz