current study is the first to use this combination
to guide clinical management of patients with
suspected HIT in real time. The main take-
home messages from this impressive study are
that a negative PaGIA in a patient with low/
intermediate 4Ts score alone is insufficient to exclude HIT.

However, this study provides data with
just 1 rapid assay. This assay is not available
everywhere. In addition, during the study,
a worldwide shortage ensued, complicating
the study. In real-world use, the consequences
could be more drastic. Finally, the reproducibility
of different batches of PaGIA has been
questioned.3 Accordingly, readers of Blood are
referred elsewhere for studies assessing additional
rapid assays for utility in HIT;4 as well as comparative studies evaluating several rapid
HIT assays in parallel (eg, Vianello et al).6 This
study limitation is summarized in the figure.

In conclusion, there are failures in both
pretest probability assessment (eg, the
4Ts score) and laboratory rapid assays, but
concurrent failures in both are likely to be
difficult. Also, there remains ongoing
problems of what to do with low 4Ts scores
with positive rapid assays and the dangers of
overcalling HIT, which to a large extent will
still likely remain, despite the study findings
and the authors’ expert guidance (refer to study
algorithm). Finally, additional caution is
indicated due to laboratory difficulties in
terms of test performance as evidenced by
interlaboratory test variation (eg, Smock et al).7

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REFERENCES
1. Linkins LA, Bates SM, Lee AY, Heddle NM, Wang G,
Warkentin TE. Combination of 4Ts score and PF4/H-PaGIA for
diagnosis and management of heparin-induced thrombocytopenia:
2. Cuker A. Clinical and laboratory diagnosis of heparin-
induced thrombocytopenia: an integrated approach. Semin
3. Cuker A, Gimotty PA, Crowther MA, Warkentin TE.
Predictive value of the 4Ts scoring system for heparin-
induced thrombocytopenia: a systematic review and
Expert Probability (HEP) Score: a novel pre-test probability model
for heparin-induced thrombocytopenia based on broad
5. Schneider S, Colucci G, Suter I, Barizzi G, Lamml B,
Alberio L. Variability of anti-PF4/heparin antibody results
obtained by the rapid testing system ID-4/H-PaGIA.
three different immunoassays in the diagnosis of heparin-induced
7. Smock KJ, Ledford-Kraemer MR, Meijer P, Hsu P,
Van Cott EM. Proficiency testing results for heparin-
induced thrombocytopenia in north america. Semin

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Palladini et al, page 612

Upfront CyBorD in AL amyloidosis

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In this issue of Blood, Palladini et al report on the outcome of a large series of 230
patients with systemic immunoglobulin (Ig) amyloid light chain (AL) amyloidosis
treated frontline with cyclophosphamide, bortezomib, and dexamethasone
(CyBorD) at 2 referral centers.1

Systemic Ig AL amyloidosis is a plasma cell
disorder characterized by the production
of monoclonal light chains, usually of A type,
that misfold and result in amyloid aggregates.
Organ damage in this disease is mediated
both by organ deposition of amyloid aggregates
and by direct cytotoxicity of circulating
amyloidogenic light chains. Prognosis depends

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mainly on the presence and degree of cardiac involvement, measured by cardiac biomarkers, as well as on the disease burden, measured by the free light-chain amount. Accordingly, the choice of treatment will have to take into account both factors, ideally in a risk-adapted approach. Among the available treatment options, high-dose melphalan followed by stem cell transplant has demonstrated its ability to obtain high rates of hematologic and organ responses and, most importantly, long-term responses in this setting. However, a high transplant-related morbidity and mortality makes this treatment option not suitable for a significant proportion of patients. For nontransplant candidates, oral melphalan and dexamethasone has been considered the standard of care, but has shown to be less effective in patients with severe cardiac involvement mostly due to a high early death rate. Thus, any survival improvement in this disease requires an early detection of cardiac involvement and also rapid, deep, and durable reduction of the AL burden in order to avoid further progression of cardiac damage, and hopefully allow the recovery of organ function. In this sense, bortezomib showed its ability to obtain rapid and good-quality responses in previously treated patients, raising the possibility of further improving outcomes by upfront use of this drug and also by adding an alkylating agent and dexamethasone.

In 2012, 2 small retrospective studies reported high rates of hematologic response and excellent early outcomes using the combination CyBorD upfront or at relapse. In the first study, Mikhael et al reported 94% hematologic responses, including a complete response (CR) rate of 71% with a median duration of 22 months. In the second study, Venner et al reported 81% hematologic responses, including a CR rate of 42%, and interestingly, an estimated 2-year overall survival of 94% in the group of patients with Mayo stage III, suggesting that this treatment might overcome the poor prognosis associated to advanced-stage disease. Based on these exciting results, CyBorD was adopted as the new standard of care at many institutions. However, the retrospective design of these studies, the small number of patients included, possible selection bias, and short follow-up might explain why other groups have not been able to replicate the reported response rates. For example, a French study conducted in patients with Mayo Clinic stage III treated with CyBorD reported a 1-year mortality rate of 40% while on therapy. Moreover, 2 case-controlled series could not demonstrate a survival improvement by adding bortezomib to an alkylating agent plus dexamethasone, due to the high rate of early deaths in AL amyloidosis.

Herein, Palladini et al report their experience in 230 patients with AL amyloidosis treated with upfront CyBorD at the National Amyloidosis Centre in the United Kingdom and at the Amyloidosis Research and Treatment Center in Pavia, Italy, between 2006 and 2013. In this large series, the overall hematologic response rate was 60%, including CR in 23% of patients. After a median follow-up of 25 months for living patients, cardiac and renal responses were obtained only in 17% and 25% of them, respectively. Regarding patients with advanced cardiac disease, those with NT-proBNP over 8500 ng/L (stage IIIb) had lower hematologic response rates (42%, CR 14%) and poorer survival (median of 7 months) due to the inability of CyBorD to reduce the early mortality in this high-risk population (see figure).

In conclusion, it seems that noncardiac patients with AL amyloidosis have an excellent outcome with high chances of benefit irrespective of the treatment approach. Also, patients with low-risk cardiac disease (stage II and IIIa) may benefit provided that they achieve at least a partial response, whereas high-risk cardiac patients (stage IIIb) still have a poor prognosis. Conclusions should be made with caution from retrospective studies. Unfortunately, it is not uncommon that promising results from small phase 2 trials or trials with short follow-up are not reproduced in larger series with adequate follow-up and particularly in real clinical practice. And finally, despite the contributions to our understanding of the treatment of AL amyloidosis made by Palladini et al, there are a number of unanswered questions regarding this treatment approach: Could CyBorD increase the number of younger patients eligible for autologous transplant? Would responses to CyBorD have an equivalent duration to those obtained with high-dose melphalan and, consequently, should these patients be or not be consolidated with high-dose melphalan? Should patients in CR after CyBorD proceed to autologous transplant? Future trials with enough follow-ups are needed to answer these questions.

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REFERENCE

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Comment on Yi et al, page 620

Wipping p53 into subservience in B-cell development

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In this issue of Blood, Yi et al reveal an important role for the protein phosphatase Wip1 (PPM1D) in the regulation of B-cell homeostasis.1 Mice deficient in the Wip1 gene display increased apoptosis in the pre–B-cell compartment and a reduction in peripheral B-cell numbers, a phenotype exacerbated with age and upon serial transplantations of bone marrow (BM) cells.1 Even though Wip1 has the ability to modulate multiple signaling pathways in the cell, the restoration of B-cell numbers upon deletion of the p53 gene1 suggests that an autoregulatory loop between p53 and Wip1 is of importance to maintain normal production of B lymphocytes.

The development of mature B lymphocytes from hematopoietic stem cells in the BM is a complex process in which proliferation and expansion need to be coordinated with DNA recombination events as well as the selection of functional progenitor cells (see figure). In order to better understand the molecular interplay underlying the homeostatic expansion of B-lymphoid cells, Yi and colleagues explored the functional role of the p53-activated serine/threonine protein phosphatase Wip12 in the formation and expansion of B-lymphoid cells.3 Wip1 was originally identified as a p53 target gene,2 and subsequent experiments revealed that this protein acts in an autoregulatory loop to reduce the functional activity of p53.3 The current report reveals that in the absence of Wip1, the formation of the pre–B-cell compartment is impaired.1 The early pre–B-cell stage represents one of the major windows for cell proliferation in early B-cell development, as coordinated interleukin-7 and pre–B-cell receptor signaling drives the proliferation of cells carrying a functional immunoglobulin (Ig) heavy-chain gene,4 and it is therefore reasonable that targeting of this developmental stage impairs the formation of mature B-lineage cells. p53 can block cell-cycle progression by induction of p21 expression; however, deletion of p21 could not rescue B-cell development in Wip1-deficient mice, arguing against the idea that the loss of pre–B cells would be due to a disruption of cell-cycle progression.1

Analysis of the cell-cycle status of defined B-cell compartments supported this notion, because no significant differences in G1–S–G2 composition could be detected. Rather, the phenotype appeared to be related to increased apoptosis in the pre–B-cell compartment, a phenotype that could be rescued by loss of p53 function, indicating a need for harnessing p53 to control apoptosis in early B-cell development.

p53 is activated by DNA damage, and it is tempting to speculate that Ig rearrangements, known to induce double-strand breaks and a DNA damage response, would result in increased p53 activity that needs to be modulated by Wip1. However, while a CDK/cyclin–mediated degradation of RAG-2 restricts Ig recombination to the G1 phase of the cell cycle,5 Wip1 has been suggested to act mainly in G2 phase, where the protein is able to potently release a cell-cycle block.6 Furthermore, DNA damage generated during G1 phase would likely result in sustained p21 expression and a cell-cycle block at the G1–S transition. Hence, the p53 response modulated by Wip1 is unlikely to be related to the Ig recombination process per se but rather to other DNA-damaging events occurring during the replication process. The reduction in pre–B-cell numbers in Wip1-deficient mice could not be compensated for by peripheral expansion of mature B cells because the reduction in cell numbers was consistent in blood, lymph nodes, and spleen,4 possibly indicating an additional need of modulated p53 activity in peripheral cells. Even though Wip1 deficiency results in impaired T-cell development7 and an expanded peripheral pool of neutrophils8 that could potentially impact the peripheral expansion of the mature B-cell compartments, Yi et al use BM chimera experiments to demonstrate that Wip1 deficiency impacts B-cell development in a cell-autonomous manner.1 The impact of reduced Wip1 activity was further exacerbated upon serial transplantation or aging, suggesting an involvement of immature long-lived progenitor compartments. In line with this notion, the exacerbated phenotype was associated with reduced numbers of pre–pro– and pro–B cells that was not observed to the same extent in young adult mice.1 In further support of a role for Wip1 in early progenitors, recently published data suggest that regulation of p53 activity by Wip1 impacts
Upfront CyBorD in AL amyloidosis

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