this disease and treatment outcomes is largely based on small retrospective case series. Sayed and colleagues should be commended for reporting a large series of patients diagnosed after 2002 and systematically followed with measurements of FLCs and markers of organ damage. Their findings offer the opportunity to highlight 2 important points: (1) the vital importance of early diagnosis and (2) the high efficiency of bortezomib-based therapy in preserving and rescuing renal function. They also report that almost one-fifth of patients were already on renal replacement therapy at diagnosis and 38% had severe or end-stage renal failure (CKD stage 4 or 5) that led to dialysis in a median time of 2.7 years, compared with 9.0 years for patients with moderately impaired renal dysfunction (CKD stage 2 or 3). This is in agreement with recent observations in 2 relatively large LCDD patient populations, with 16% to 23% of patients already on dialysis at diagnosis and mean serum creatinine concentration at diagnosis of 2.6 to 3.8 mg/dL.6,7 These findings demonstrate that even now, advanced renal dysfunction is common at diagnosis, with a major impact on renal outcomes. In fact, although chemotherapy can rescue patients even in advanced (CKD stage 4) renal failure, those treated in earlier stages had a substantially better renal survival.1,6,7

Early diagnosis is challenging for hematologists because up to 63%6 of patients with LCDD do not have a serum monoclonal whole immunoglobulin. A monoclonal protein in serum or urine is detected by electrophoresis/immunofixation in only ~65% of cases,1,7 possibly because the κ FLC tends to aggregate and does not form a discrete, detectable electrophoretic band (see figure, panel B). On the other hand, the FLC ratio is abnormal in all patients with LCDD,1,6,7 suggesting that serum FLC measurement should be performed in the diagnostic workup of adults presenting with renal disease, independent of the presence of a serum or urine monoclonal protein. This may facilitate early diagnosis and prompt therapy. Diagnosis relies on renal biopsy and close collaboration between nephrologists and hematologists is essential. Sayed and coworkers elected to use the FLC-based criteria for hematologic response validated in AL amyloidosis8 in their LCDD population, and they found that the outcome of patients who achieved VGPR or CR was characterized by an improvement of glomerular filtration rate in most patients, significantly diverging from the progressive decrease in renal function observed in subjects obtaining less than VGPR (see figure, panel A), thus validating the use of the hematologic response criteria developed for AL amyloidosis also in LCDD. Although the number of patients is small, a biomarker-based assessment of response of organs other than the kidney, analogous to that used in AL amyloidosis, seems applicable also in LCDD. For instance, the 2 patients with heart involvement who improved had a pronounced decrease in NT-proBNP, which is recognized as an indicator of cardiac response in AL amyloidosis.8,9 Although this study was not powered for comparison of treatment regimens, it confirms several previous observations that high-dose melphalan followed by autologous stem cell transplantation (HDM/ASCT) is well tolerated (also in patients with advanced kidney damage) and very effective. It also highlights that bortezomib treatment is indeed a targeted treatment of LCDD, providing rapid, profound (complete response in 8 of 9 patients) and sustained hematologic responses that translate into prolonged renal survival (as demonstrated by the need for dialysis). Similar findings have been reported by French investigators,7 who also showed that hematologic response rates were similar after HDM/ASCT and bortezomib-based regimens. These findings suggest that the latter can be considered as front-line therapy, particularly in patients with severe renal failure. This is a new era for diseases caused by monoclonal LC deposition/aggregation; we now have effective therapies that can completely change the natural history of these conditions, making more acute the need for early diagnosis through improved awareness and wider use of the FLC assay in adults presenting with renal disease.

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Comment on Blatt et al, page 2832

CD30-targeting drugs: cure for mastocytosis?

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New promising targets and targeted drugs for treatment of aggressive forms of systemic mastocytosis are few; in this issue of Blood, Blatt et al provide evidence that aberrant Ki-1 antigen (CD30) expression on neoplastic mast cells may serve as a therapeutic target of brentuximab-vedotin alone or in combination with KIT-targeting drugs.
CD30 is an established therapeutic target of the CD30-targeting antibody-drug conjugate brentuximab-vedotin in patients with Hodgkin lymphoma\(^2\) and anaplastic large cell lymphoma.\(^3\) Recent reports have also demonstrated brentuximab-vedotin efficacy in relapsed/refractory diffuse large B-cell lymphoma\(^4\) and primary effusion lymphoma.\(^5\) Valent and colleagues have recently shown that CD30 is aberrantly expressed in the cytoplasm of neoplastic mast cells in patients with advanced systemic mastocytosis (SM).\(^6\) In the current study, the authors asked whether CD30 is expressed on the surface of neoplastic mast cells in advanced systemic mastocytosis and whether this surface structure may serve as a therapeutic target in mastocytosis. The authors found that CD30 is expressed on the surface of neoplastic mast cells in 3/25 patients (12%) with indolent SM (ISM), 4/7 patients (57%) with aggressive SM (ASM) and 4/7 patients (57%) with mast cell leukemia (MCL). The immature RAS-transformed human mast cell line MCV1.1 also expressed cell surface CD30, whereas the KIT-transformed human mast cell line HMC-1.2 expressed no detectable CD30. In most patients, CD30 expression in mast cells was confirmed by immunohistochemistry on bone marrow sections. Using flow cytometric analysis, the authors demonstrated a correlation between the type of systemic mastocytosis and the surface CD30 expression on mast cells. CD30 levels on mast cells in patients with advanced disease (ASM and MCL) were higher than in ISM patients (median CD30 MFI: ASM/MCL 4.24 vs ISM 1.88, \(P < .05\)).

Further experiments showed downregulation of cell surface CD30 expression and CD30 messenger RNA levels by mitogen-activated protein kinase kinase (MEK) inhibitors PD032509 and RDEA119 in CD30\(^+\) mast cell lines, suggesting that expression of CD30 in neoplastic mast cells is regulated by a RAS-MEK–dependent signaling pathway. The authors then proceeded to measure serum levels of soluble CD30 (sCD30) in mast cell patients. Results revealed an increase in serum levels of sCD30 in advanced SM compared with ISM. The highest levels of sCD30 were measured in patients with ASM or MCL, with a median of 129.0 ng/mL, compared with a median of 21.0 ng/mL in ISM patients.

Prior reports demonstrated that CD30–targeting antibody-drug conjugate brentuximab-vedotin inhibits the growth of CD30\(^+\) lymphoma cells.\(^7,8\) In the current study, Blatt et al showed that brentuximab-vedotin inhibited proliferation of neoplastic mast cells. Lower 50% inhibitory concentration values were obtained in CD30\(^+\) mast cell lines MCPV-1.1 (10 \(\mu\)g/mL) compared with CD30\(^+\) HMC-1.2 cells (\(>50 \mu\)g/mL). Brentuximab-vedotin produced a G2/M cell cycle arrest in CD30\(^+\) cell lines MCPV-1.1 and C2 and at high concentrations in CD30 low-expressing HMC-1.1 cells. In contrast, brentuximab-vedotin did not induce a cell-cycle arrest in CD30\(^+\) cell lines HMC-1.2 and MCPV-1.4. In addition, brentuximab-vedotin produced apoptosis in all CD30\(^+\) mast cell lines tested as well as in primary neoplastic mast cells in patients with CD30\(^+\) SM, but not in neoplastic mast cells in patients with CD30\(^-\) SM. The concentrations of brentuximab-vedotin required to inhibit proliferation in primary neoplastic mast cells and CD30\(^+\) mast cell lines corresponded well with drug concentrations that can be reached in patients treated with this drug.\(^9\) By contrast, the CD30\(^+\) mast cell lines that were examined showed only a weak response or did not respond at all. Furthermore, the authors confirmed the growth-inhibitory effect of brentuximab-vedotin in an in vivo xenotransplantation assays showing that brentuximab-vedotin suppressed engraftment of CD30\(^+\) MCPV-1.1 cells in nonobese-severe combined immunodeficiency interleukin-2R\(^\alpha\)-null mice.

Patients with advanced systemic mastocytosis not only suffer from the consequences of mast cell proliferation in various organs, but also from symptoms caused by the mediator release from activated neoplastic mast cells. The authors tested whether brentuximab-vedotin treatment triggered histamine release, which is important for assessment of future potential for severe reactions during therapy. Brentuximab-vedotin was found to downregulate anti-immunoglobulin E–induced histamine release in CD30\(^+\) mast cells, whereas no effect of brentuximab-vedotin on histamine release was seen in CD30\(^-\) mast cells. Also, no substantial effects of brentuximab-vedotin on immunoglobulin E–mediated upregulation of CD63 or CD203c on basophils or CD30\(^+\) mast cell lines were seen.

Most patients with ASM or MCL show clinically meaningful and sometimes even complete responses to KIT D816V–targeting drug midostaurin (PKC412).\(^10\) However, responses are usually short-lived. Therefore, there is a need for novel potent, targeted drugs that can elicit synergistic growth-inhibitory effects when combined with PKC412. In this study, Blatt et al demonstrated that brentuximab-vedotin and midostaurin (PKC412) produced synergistic growth-inhibitory effects in CD30\(^+\) mast cell line MCPV-1.1. Based on these data, it seems tempting to propose a clinical trial exploring anti-neoplastic effects of the drug combination PKC412 and brentuximab-vedotin in advanced systemic mastocytosis.

Overall, this is a very exciting study that points to the Ki-1 antigen (CD30) as a promising new drug target for patients with advanced systemic mastocytosis. The data presented in this study show that antineoplastic effects of brentuximab-vedotin are largely dependent on the surface expression of CD30, suggesting the need for routine testing for CD30 surface expression on neoplastic mast cells by flow cytometry. In the future, CD30 expression on mast cells may serve as a potentially valuable screening tool, prognostic marker, and therapeutic target in advanced forms of mast cell disease that currently represent a treatment challenge with poor prognosis.

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

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Comment on Harbort et al, page 2842

DNA damage signals inhibit neutrophil function

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In this issue of Blood, Harbort et al identify a novel role for DNA damage responses in the regulation of cytokine production and cell death of activated neutrophils. They show that reactive oxygen species (ROS) generated by stimulated neutrophils trigger DNA damage signaling, which suppresses proinflammatory functions (see figure). Their work reveals new insights into the control of innate immunity and inflammation.

Damage to DNA is caused by genotoxic agents, such as irradiation and chemotherapies, and during normal biologic processes, including DNA replication, transcription, telomere maintenance, and antigen–receptor assembly in lymphocytes. Regardless of the mechanism of injury, DNA breaks activate conserved signaling pathways that are initiated by the DNA damage sensors ATM and ATR (AT and RAD3-related). These serine/threonine kinases phosphorylate hundreds of proteins to coordinate DNA repair, cell-cycle checkpoints, and cell-death pathways. DNA damage responses also regulate cell-type–specific programs, including cell survival and differentiation. In their study, Harbort et al show that DNA damage signaling is activated by ROS in neutrophils and represses proinflammatory functions. In response to invading pathogens, neutrophils secrete proinflammatory cytokines, which recruit additional neutrophils and other immune cells. Stimulation of neutrophils also triggers a burst of ROS that contribute directly to microbial destruction and function as signaling molecules. Defects in the generation of reactive species, such as those found in CGD, result in recurrent infections, poor clearance of pathogens, and hyperinflammation.

Harbort et al show that the generation of ROS in neutrophils is required for suppressing cytokine production and limiting neutrophil lifespan (see figure). This repression of neutrophil function depends on ROS-mediated activation of DNA damage-response signaling. ATM and ATR function synergistically to inhibit neutrophil function. Loss of ROS or of DNA damage signaling increases cytokine production and prolongs survival of activated neutrophils. Thus, ROS and ATM are necessary to inactivate neutrophil responses and limit inflammation.

ROS are known to cause oxidative damage to DNA but can also directly oxidate and activate ATM without DNA damage intermediates. The histone H2AX is phosphorylated (γH2AX) at sites of DNA damage and Harbort et al find that γH2AX is generated in neutrophils following the oxidative burst but is absent in ROS scavenged cells. These findings suggest that ROS directly damages DNA in activated neutrophils and that activation of ATM occurs in response to this injury. However, H2AX is phosphorylated by ATM and thus, the decreased generation of γH2AX may be a consequence of reduced ATM activity in the absence of ROS. Further work is necessary to determine if DNA is directly damaged by ROS and to define the precise mechanism of ATM activation in stimulated neutrophils.

Model of the ROS- and ataxia-telangiectasia mutated (ATM)–mediated repression of neutrophil inflammatory responses. Following stimulation, neutrophils generate a burst of ROS that activates ATM-dependent DNA damage signaling to suppress cytokine production and induce apoptosis. Deficiency in ROS production or in ATM disables this inhibitory circuit, resulting in overproduction of cytokines, enhanced survival of activated neutrophils, and hyperinflammation. The MRN complex is composed of Mre11/Rad50/Nbs1 proteins. "P" represents phosphorylation of ATM, which is the active form of the kinase. AT, ataxia-telangiectasia; CGD, chronic granulomatous disease. See supplemental Figure 12 in the article by Harbort et al, available on the Blood Web site.
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