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respectively) and frequency of grade 3-4 adverse events (40% and 39%, respectively). In contrast, 4 cycles of ABVD was associated with a considerably higher rate of early treatment termination (18%) and grade 3-4 toxicity (65%). With regard to bleomycin-induced lung toxicity, 0% and 1.5% cases were reported among patients receiving 2 cycles of AVD and ABVD, respectively, whereas 7 cases (10%) were reported among patients receiving 4 cycles ABVD, 3 of which were fatal.

In applying this data to the care of older patients with HL, it is important to note that most patients included in this analysis had good performance status (most with Eastern Cooperative Oncology Group grade 0 or 1) and were fairly young in age (median age, 64-66 years). Furthermore, as mentioned by the authors, comprehensive geriatric assessments were not included in the HD10 or HD13 studies, therefore the impact of factors such as functional status, fall risk, and social support on treatment toxicity within these studies is not known. In the retrospective analysis by Evens et al, age 70 (or greater) and loss of activities of daily living were the most important adverse prognostic factors in this patient population.[5] Ongoing and future prospective studies will determine whether these factors predict for treatment toxicity and need to be considered when deciding upon treatment courses. Current studies for elderly patients incorporating novel agents for HL, such as the ongoing study with sequential brentuximab vedotin and AVD (clinicaltrials.gov #NCT01476410) may obviate the need for bleomycin in this population. For now, though, this analysis from the GHSG provides us with a framework for using bleomycin in older patients (see figure). At the very least, we should aim to use no more than 2 cycles of chemotherapy with bleomycin for older HL patients. In early-stage disease, this is accomplished by using radiation consolidation to shorten the course of chemotherapy. In advanced-stage disease, it is appropriate to treat as per the response-adjusted therapy for HL study, in which patients with PET-2–negative scans had bleomycin removed after 2 cycles of ABVD with no adverse impact on tumor control.[9] As the authors mention, known risk factors for bleomycin toxicity, such as underlying lung disease, renal insufficiency, pulmonary radiation, and the tobacco history could not be assessed in their analysis due to the small numbers; however, the use of bleomycin for patients with these comorbities should likely be avoided altogether. Ongoing and future prospective studies, which incorporate comprehensive geriatric assessments and novel HL agents, are likely to facilitate the development of risk-adapted treatment approaches for older patients with HL and hopefully improve outcomes for this group.

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● ● ● MYELOID NEOPLASIA

Comment on Zhou et al, page 2219

NEDD8 and HDACs: promising cotargets in AML

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In this issue of *Blood*, Zhou et al have identified synergistic in vitro and in vivo anti–acute myeloid leukemia (AML) activity of the lysine neddylation inhibitor pevonedistat and pan–histone deacetylase (HDAC) inhibitor (pan-HDACI) belinostat and examine the mechanisms responsible for this synergistic activity.[1]

Protein lysine neddylation is a posttranslational modification in which the ubiquitin-like molecule NEDD8 (neural precursor cell expressed, developmentally downregulated 8) is covalently linked to several cellular proteins, including the E3 ubiquitin ligases known as cullin-ring E3 ligases (CRLs).[2] Neddylation activates CRLs, which in turn ubiquitylate and degrade, via the proteasome, a variety of their substrate-protein targets, including CDT1 (chromatin-licensing and DNA replication factor), CDKN1B (cyclin-dependent kinase inhibitor 1B) (p27), IκBα (nuclear factor of κ light polypeptide gene enhancer in B cells inhibitor, α), and NRF2 (nuclear factor, erythroid 2–like 2).[2,3] The first step leading to CRL neddylation is catalyzed by the NEDD8 activating enzyme (NAE), and pevonedistat (MLN4924) has been developed as an irreversible inhibitor of the

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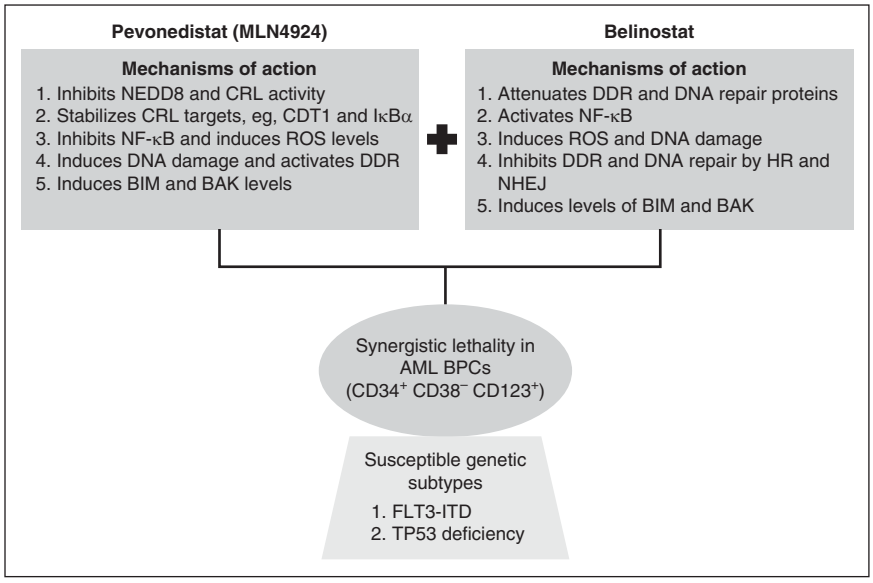

Mechanistic interactions creating synergy between pevonedistat and belinostat against AML blast progenitor cells (BPCs).

NAE.[2,4] Treatment of cells with pevonedistat inhibits neddylation and CRL activity, leading to stabilization and accumulation of the CRL substrate-proteins noted before. This has been shown to cause nuclear factor–κB (NF-κB) inhibition, reactive oxygen species (ROS) accumulation, DNA re-replication, DNA damage, as well as in vitro and in vivo lethality in AML cells.[4,5] Notably, treatment with pevonedistat simultaneously induces DNA damage and DNA damage response (DDR) but compromises DNA repair, thereby sensitizing transformed cells to DNA-damaging agents.[3,4,6] Based on its promising preclinical, in vitro, and in vivo anti-AML activity, phase 1 clinical trials of pevonedistat were conducted in patients with relapsed/refractory AML or myelodysplastic syndrome.[7] Although hepatotoxicity was dose-limiting, complete and partial remissions were observed at or below the maximal tolerated dose of pevonedistat.[7] Collectively, these findings underscored the potential of developing rational combinations of pevonedistat with agents that would increase its anti-AML efficacy and exert synergistic lethality against AML. In this issue of *Blood*, Zhou et al chose the class I and II HDACI belinostat to fulfill this role.[1] HDACs are commonly overexpressed in cancer and AML cells.[8] HDACs are also recruited by oncogenic fusion proteins in AML (eg, AML1-ETO and PML-RARα) to repress target genes involved in differentiation and apoptosis of AML cells.[8] Over the past decade, several preclinical studies have shown that pan–HDACIs, such as belinostat, induce ROS, DNA damage, differentiation, and apoptosis in AML cells.[8] However, despite their promising preclinical anti-AML efficacy, single-agent clinical activity of HDACI in AML has been quite modest and disappointing.[8,9] Yet, HDACI did display significant clinical activity against cutaneous T-cell or peripheral T-cell lymphoma and are therefore approved as a therapy for these clinical entities.[8]

In the studies reported in this issue of *Blood*, Zhou et al examined the preclinical activity of a combination of pevonedistat and belinostat.[1] They demonstrated that, compared with treatment with each agent alone, combined therapy with pevonedistat and belinostat exerts in vitro synergistic lethality against a variety of cultured AML cell types with diverse genetic backgrounds, including the presence of FLT3-ITD and MLL-AF4 (as in MV4-11 cells), as well as the deficiency of wild-type TP53 (see figure). The combination was also synergistically lethal against patient-derived primary AML cells. Furthermore, cotreatment with pevonedistat and belinostat significantly improved the survival of immune-depleted mice engrafted with MV4-11 cells. What might be the basis of the potent anti-AML activity of this combination? The authors demonstrate multiple mechanisms that may be involved. First, whereas pan-HDACI, such as belinostat, activates prosurvival activity of NF-κB, cotreatment with pevonedistat inhibits NF-κB, thereby potentiating HDACI-mediated lethality in AML cells. Second, although pevonedistat treatment promotes DNA damage, activates DDR, and induces activity of the homologous recombination (HR) repair–related proteins, cotreatment with belinostat attenuated the levels of proteins involved in the DDR and DNA repair through HR and nonhomologous end-joining mechanisms. Consistent with this, belinostat treatment inhibited the DNA repair foci in the nucleus, thereby markedly increasing the single- and double-strand DNA damage and cell death. Third, although pevonedistat stabilized the DNA re-replication licensing factor CDT1 and activated the intra-S phase checkpoint, thereby promoting chromosome decondensation and elongation,[2–4] cotreatment with belinostat led to chromosome pulverization and increased lethality. How do these mechanistic interactions upstream between combination partners markedly reduce the threshold for apoptosis? It was demonstrated that cotreatment with pevonedistat and belinostat is associated with induction of BH3 domain-only proteins BIM and NOXA, as well as the multidomain proapoptotic protein BAK. These alterations are likely to be the final trigger for the ensuing caspase-dependent AML cell death.[4]

It is noteworthy that, compared with the normal $CD34^+$ progenitor cells, the combination of pevonedistat and belinostat was clearly more lethal against the AML blast progenitor cells, especially the leukemia-initiating cells (LICs) characterized by a $CD34^+$ $CD38^-$ $CD123^+$ phenotype.[10] With respect to any novel targeted therapy, it is important to determine whether the effectiveness of the therapy is limited to specific genetic subtypes and/or molecular pathway dependencies, which would increase the chances for identifying patient populations that would benefit the most from the novel therapy. Therefore, it is also significant that the lethal effect after cotreatment with pevonedistat and belinostat were observed on LICs from AML of relatively diverse genetic backgrounds. However, whether the combination also undermined the functional capacity of the LICs to initiate AML in the relevant mouse models was not investigated.

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Going forward, the promising preclinical anti-AML efficacy of the cotreatment with pevonedistat and belinostat demonstrated by Zhou et al, coupled with the documented single-agent clinical activity of pevonedistat in AML, creates a strong rationale to further evaluate the efficacy of the combination in a phase 2 trial in patients with AML. Importantly, to determine the predictors of response or resistance to the combination, studies involving the genetic profiling by whole-exome DNA- and RNA-sequencing, as well as evaluation of selected biomarkers of the on-target effects of pevonedistat and belinostat in the AML cell samples, must be incorporated in the trial. This would more rationally guide the future clinical development of the combination in the therapy of AML.

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Comment on Jin et al, page 2249

Antibodies are back for thymic attack in cGVHD

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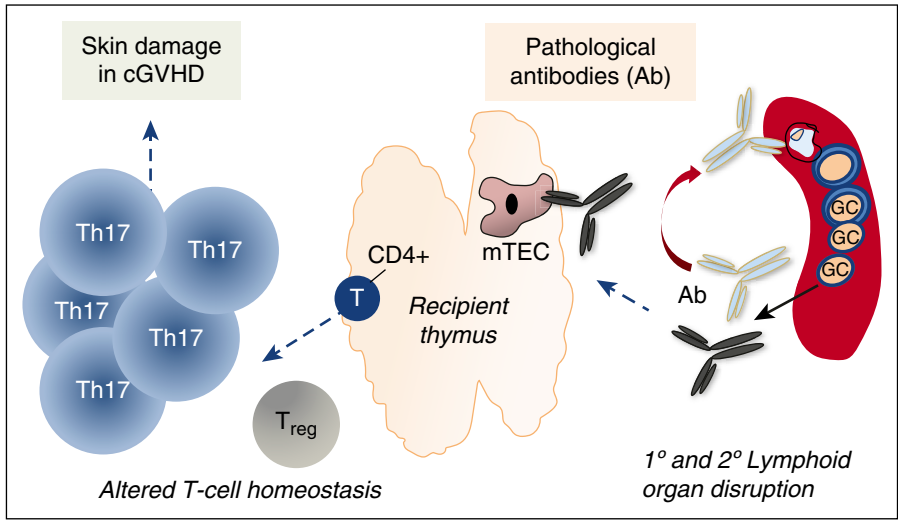
In this issue of *Blood*, Jin et al uncover how antibodies contribute to B- and T-cell pathology in sclerodermatous chronic graft-versus-host disease (cGVHD).[1]

Given that cGVHD patients are often cured of their cancer or other primary blood/marrow disease by allogeneic hematopoietic cell transplantation (HCT), the morbidity and sometimes lethality of cGVHD is especially tragic. For many long-term HCT survivors, cGVHD remains an untreatable, relentlessly morbid condition. Effective prophylaxis and treatment of cGVHD has been significantly hampered by the lack of understanding of the pathophysiology of cGVHD.

Studies in murine models continue to improve our understanding of the immunopathologic mechanisms of cGVHD, much as they did in acute GVHD. Animal studies, including some by the authors of this current paper and others, have confirmed that disease-mediating lymphocytes arise in recipients of allogeneic donor transplants and that these cells are capable of causing autoimmune disease in syngeneic animals. Insidious development of pleiotropic autoimmune disease manifestations in murine models and in patients after allogeneic HCT, but not autologous HCT, suggest that alloreactivity incites autoreactivity.[2,3] Separating the distinct cGVHD events that result in ongoing broad reactivity to nonpolymorphic antigens and recipient tissues from specific immunologic reactions to malignant cells will be pivotal for developing more active and specific cGVHD treatments.

Elegant experiments in murine models have substantiated specific roles for B- and T-cell subsets in cGVHD development.[4] Several studies suggested a role for B cells in cGVHD, and a seminal paper by Bruce Blazar's group used transgenic mice either incapable of producing B cells or having B cells that cannot release immunoglobulin G (IgG) to



Working model of how donor-derived antibodies may help perpetuate cGVHD immune pathology. GCs are required for production of disease-inciting alloantibody early in the disease process. Due to altered T- and B-cell homeostasis, aberrant B cells develop over time that produce antibodies directed against primary (1°) and secondary (2°) lymphoid organs. Lymphoid-organ damage perpetuates loss of immune tolerance and the promotion of cGVHD of the skin. T_{reg}, regulatory T cell.

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