

dose escalation. It was chosen to avoid treatment of patients well below the effective dose, however, with an increased risk of unanticipated toxicities. The study included patients with at least one prior antimyeloma treatment (bortezomib permitted if it had been effective) and adequate bone marrow and organ function. The primary end point was to determine the maximum tolerated dose (MTD) of romidepsin/bortezomib/dexamethasone; secondary end points were efficacy variables and survival. The study protocol foresaw the “conventional” administration of bortezomib/dexamethasone: bortezomib 1.3 mg/m² as an intravenous push on cycle days 1, 4, 8, and 11 along with dexamethasone 20 mg on every bortezomib day and the day after. Dose escalation was restricted to romidepsin. The starting dose of 8 mg/m² intravenously on days 1, 8, and 15 was to be increased in 2-mg increments. A cycle length of 28 days was chosen and romidepsin maintenance was available for responding subjects on days 1 and 8 of every 4-week cycle. In all, 25 patients with a median of 2 prior lines of therapy (range, 1–3) were enrolled in the study. Pretreatment intensity appeared moderate with only half of the patients having received prior autologous transplant and IMiDs, respectively; 24% had a history of bortezomib exposure. When 2 subjects receiving the HDAC inhibitor at 12 mg/m² experienced dose-limiting toxicities (DLTs), the MTD turned out to be romidepsin 10 mg/m² on days 1, 8, and 15 along with bortezomib/dexamethasone. This dose is well below that (14 mg/m²) used when romidepsin is given as a single agent in cutaneous T-cell lymphomas. This was the first indication approved by the US Food and Drug administration in 2009.

Thrombocytopenia is an overlapping toxicity between bortezomib and romidepsin and had been anticipated by the investigators. Severe (Common Terminology Criteria for Adverse Events [CTC AE] grade 3 and higher) thrombocytopenia at MTD level was 58%. Even with this level of toxicity, there was 1 fatal hemorrhage that was attributable to disease progression but not to study drug treatment, and no other bleeding episodes were reported. Incidence of nonfebrile neutropenia (CTC AE grade 3 and higher) at MTD was 36%. The main nonhematologic toxicity was bortezomib-induced peripheral neuropathy (PNP) in 76% of patients. Incidence of clinically relevant PNP without improvement

after interruption of bortezomib was 20%. Incidences of all other severe nonhematologic toxicity except hyponatremia and fatigue were below 10%. Overall response rate (ORR) according to the International Myeloma Working Group uniform response criteria (partial response [PR] or better) was 60%, including a very good PR plus complete response rate of 36%, which is quite impressive for this setting. Response rates with single-agent HDAC inhibitors, as mentioned earlier, were far below that,^{3,7} as were those seen in the SUMMIT trial: single-agent bortezomib induced an ORR of 35%.⁸

Considering results from those 3 studies, the activity reported by Harrison et al suggests a synergism of romidepsin and bortezomib in myeloma.¹ And indeed, very recently Kikuchi and colleagues in this journal reported on a clear synergistic effect of bortezomib and romidepsin in vitro.⁹ Furthermore, Kikuchi et al demonstrated that bortezomib transcriptionally down-regulates class I HDACs while romidepsin seems able to inhibit both class I and II enzymes and suggested HDAC inhibition may be important in the efficacy of bortezomib in multiple myeloma. They proposed to further investigate bortezomib–HDAC combinations.⁹

While several clinical trials are still ongoing, the study by Harrison and colleagues is the first fully published trial supporting this combinatorial approach: the synergism that had previously been observed in vitro translated to superior response rates in the clinical setting.¹ The 7-month duration of response,

however, was disappointing. This observation leads to the question on how to dose-modify the regimen (bortezomib weekly or subcutaneously) to allow for a prolonged administration of this effective regimen.

Conflict-of-interest disclosure: The author has received honoraria from Millennium, Janssen (Germany), and Celgene (Germany). ■

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● ● ● CLINICAL TRIALS

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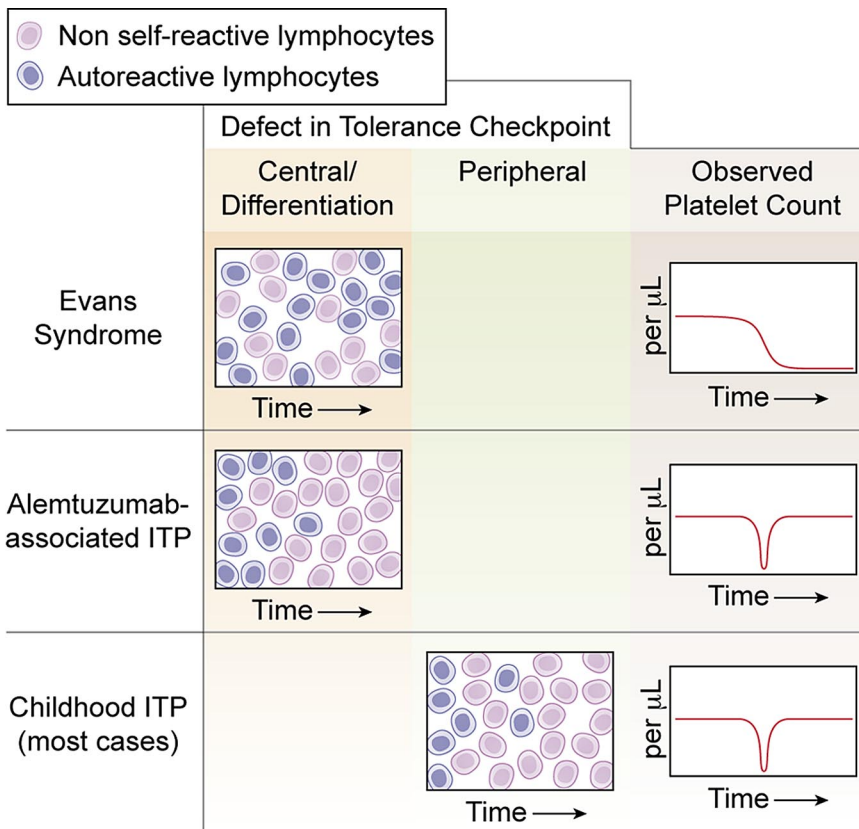
ITP: Tolerance Lost

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Despite a seemingly unifying phenotype comprising a reduced platelet count and mucocutaneous bleeding in many newly identified cases, immune thrombocytopenia (ITP) is a disorder of diverse pathogenesis. In this issue of *Blood*, Cuker and colleagues describe a little-recognized form of secondary ITP that occurred in a minority of patients receiving the anti-CD52 monoclonal antibody alemtuzumab.¹ Their observations may provide insight into a key concept of immunity—tolerance—and supply indirect evidence for previously advanced hypotheses regarding the acquisition of autoimmunity across diverse forms of the disorder.

ITP classically has been defined as arising from autoantibody-mediated accelerated platelet destruction,² but in recent years additional mechanisms for thrombocytopenia,

including cell-mediated autoimmunity³ and insufficient platelet production,⁴ have been identified. In addition, longstanding recognition of distinct clinical presentations has



Defects in lymphocyte immune tolerance checkpoints may lead to inappropriate recognition of platelet self-antigens, causing immune thrombocytopenia (ITP). In Evans syndrome, a central defect resulting in persistence of an autoreactive lymphocyte clone produces ITP and chronic thrombocytopenia. Alemtuzumab-associated ITP may be due to a central defect in immune tolerance that develops during initial recovery of the lymphocyte pool and resolves as further reconstitution of B and T lymphocytes occurs, concurrent with improvement in the platelet count. In most cases of childhood ITP, an acquired loss of peripheral tolerance due to of immune stimulation by an exogenous precipitant (viral antigen, immunization) typically resolves once exposure to the antigen dissipates, leading to spontaneous remission of ITP. Conceptualized platelet count trends are representative of the potential for platelet count recovery after limited or no intervening medical therapy; because of inter-individual disease-modifying factors, remissions may not occur in every patient. Pink-shaded lymphocytes represent non self-reactive species; purple-shaded lymphocytes represent those with autoreactive potential. Professional illustration by Kenneth X. Probst.

fueled speculation that the molecular and immunologic underpinnings of ITP probably vary considerably among patients. One feature, the rate of spontaneous remission, undoubtedly distinguishes certain patients from others; children with acute ITP experience relapse-free recovery of the platelet count at a strikingly high rate, frequently without any treatment,⁵ whereas new-onset ITP typically becomes chronic in adult patients even if an initial response to immunomodulation has occurred.⁶ Although these observations have provided some clues about potential underlying immunologic aberrancies, the triggers and mechanisms responsible for inciting the autoimmunity have remained largely elusive.

In recognition of these widely diverse natural histories and patterns of responsiveness to treatment, Cines and colleagues have proposed a unifying hypothesis built on the con-

cept of immune tolerance.⁷ Tolerance comprises the mechanisms necessary for ensuring that B and T lymphocytes, which generally are charged with responding to foreign pathogenic invaders, do not recognize self-antigens. By mechanisms including clonal deletion, receptor editing, anergy, activation-induced cell death, and others,⁷⁻¹⁰ autoreactive lymphocytes are deleted from the immune repertoire.

According to this model, a defect at a “tolerance checkpoint” during lymphocyte development and maturation may both lead to platelet-directed autoimmunity and define the clinical manifestations of the disorder. Proposed defects in tolerance may occur early in development or during lymphopoiesis in the bone marrow (central defects); in B-cell maturation leading to abnormal repertoires (differentiation); or in mature lymphocytes as a consequence of antigenic stimulation (peripheral

defects).⁷ Peripheral defects are posited to be more likely to incur platelet-specific autoimmunity and be durably responsive to immunosuppression or elimination of a stimulatory antigen, whereas central defects are more likely to lead to relapse after therapy, because of a high frequency of autoreactivity among the primary immune repertoire.⁷

The observations by Cuker et al may provide further evidence of such a hypothesis. In their report, 2.8% of patients (6 of 216) with multiple sclerosis who were treated with 12 or 24mg/d of alemtuzumab developed ITP a median of 10.5 months after the last exposure to the drug.¹ The disorder was fatal in 1 subject (because of intracranial hemorrhage), severe in 4, and mild in 1. Of the 5 patients who received initial therapy, all responded within a median of 2.5 days. Relapses occurred in 2, requiring additional treatment, but all remained in remission subsequently (median duration of follow-up, 34 months).

This pattern led the authors to postulate a loss of platelet-specific tolerance at a central checkpoint in lymphocyte development, occurring during initial immune reconstitution after administration of alemtuzumab and leading to recognition of platelet self-antigens. The autoreactivity dissipated presumably through the deletion of the self-reactive lymphocyte clone(s) during subsequent expansion of the lymphocyte repertoire (see figure). The significant delay in the onset of thrombocytopenia after last exposure to the drug would appear to be consistent with their hypothesis, as would the rapid responsiveness to first-line therapy and the long-lasting remissions.

Cuker and colleagues distinguished this clinical pattern from Evans syndrome, which typically has little potential to remit durably after initial immunomodulation,¹¹ presumably because of an enduring defect in a central tolerance checkpoint. In contrast, the clinical course of the alemtuzumab-treated patients is reminiscent of the usual pattern of spontaneous remission in acute childhood ITP, although in that disorder a peripheral perturbation in tolerance because of transient stimulation by an antigen is more likely.⁵

Although tantalizing, this hypothesis is limited. If a singular mechanism (a defect in a central tolerance checkpoint allowing for growth of an autoreactive lymphocyte clone) was causative, why were there any differences in the clinical manifestations among the patients? Why was the severity of thrombocytopenia not

uniform? Why, if further immune recovery led to deletion of the autoreactive species, did thrombocytopenia persist in 2 of the patients, requiring additional immunomodulation? Why were platelet-bound antibodies detectable in only 3 of 5 patients, and why was their appearance and disappearance coincident with the onset and resolution of thrombocytopenia in even fewer patients? Even in this well-defined group of individuals, whose ITP almost certainly was because of a shared pathogenic immunologic mechanism, undeniable heterogeneity suggests the presence of additional disease-modifying factors.

Nonetheless, some conclusions are possible. Alemtuzumab-associated ITP is highly immunoresponsive and has a favorable prognosis, which may direct management away from splenectomy, even though severe thrombocytopenia is expected and relapses after first-line treatment may occur. Implications of these observations for commoner presentations of ITP, however, are less certain. Truly individualized treatment of adult primary ITP, for instance, which exhibits widely ranging variances in clinical features at presentation, in responsiveness to treatment, and in rates of spontaneous remission,⁶ continues to be elusive. Clinical heterogeneity almost certainly indicates inter-individual differences in response to a shared pathogenic mechanism, different pathogenic mechanisms, or both.

Improved outcomes encompassing increased numbers of durable remissions and reduced untoward iatrogenic effects are within our grasp as the understanding of the complex role of the immune system—and a failure to

maintain tolerance of self-antigens—continues to emerge.

Conflict-of-interest disclosure: The author has received research support from Biogen IDEC and Baxter Healthcare and consultancy fees from Biogen IDEC, Baxter Healthcare, Bayer Healthcare, and Amgen. ■

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mized clinical efficacy of these drugs and some patients now enjoy durable remissions, including a minor subset that is cured from the disease.² However, most patients relapse, some with high-risk genetic features relapse after only a short period of disease control.³ Improved strategies are needed.

Based on the seminal work done in triple negative breast cancer and ovarian cancers,^{4,5} Neri et al propose that inhibiting PARP may provide promise in the armamentarium against myeloma.¹ The rationale for PARP inhibition is based on the observation that PARP1, a DNA damage repair enzyme, represents one of the last chances of cancer cells to undergo DNA repair and thus survive (see figure). Breast cancer patients with BRCA associated mutations (genotype) exhibit a large degree of genomic instability, the consequence of it genomic complexity on the form of DNA gains and losses (genotype–phenotype).⁶ This is particularly common in patients with triple negative breast cancer, where array-based comparative genomic hybridization analysis shows a high degree of genomic variance from normal (the so-called “BRCAness”).⁷ By inhibiting the repair activity of PARP, a safety net for DNA repair, investigators have been able to increase sensitivity of cancer cells to DNA-damaging agents. In contrast to solid tumors where PARP inhibition leads to sensitization of cells to cytotoxic agents, Neri et al show that bortezomib renders myeloma sensitive to PARP inhibition.¹

Neri and colleagues show PARP inhibition, after exposure to bortezomib, enhances the activity of both drugs. The key to their proposal is that bortezomib causes a BRCAness state, by virtue of the disruption of protein metabolism required for DNA repair. While ubiquitination is normally thought as focused on protein degradation, many non-degradation functions exist; including mediation of DNA repair mechanisms. This was achieved by diminishing the pool of ubiquitin and abrogation of H2AX polyubiquitylation, a necessary step in DNA repair. PARP inhibition with ABT-888 alone did not cause cell death, but when cells were pretreated with bortezomib PARP inhibition caused a greater degree of cytotoxicity than either drug alone. Normal cells can repair DNA breaks by the homologous recombination or the base excision repair mechanism. Cells that are deficient in homologous recombination as a consequence of BRCA mutations, or a BRCA state

● ● ● LYMPHOID NEOPLASIA

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Innovation in myeloma treatments PARP excellence!

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In this issue of *Blood*, Neri and colleagues report a promising treatment of myeloma using a combination of bortezomib plus inhibition of the enzyme PARP1 (REF).¹ This is welcome news, as new avenues for the treatment of the disease are needed.

Over the past decade we have seen new treatments available for patients; including proteasome inhibitors and the so-called

immunomodulatory drugs. Combination strategies, often in conjunction with autologous stem cell transplantation, have maxi-