relapsed disease (minimal residual disease [MRD]) in one and overt relapse in the other) post–allogeneic hematopoietic stem cell transplantation (alloHSCT). One patient who had TKI-resistant MRD received an autologous product, whereas the post-HSCT patients received donor-derived T-cell products. To ensure selective targeting of the mutant sequence, all products were stimulated with 9-mer peptide pools spanning the junctional region of BCR-ABL–only. As expected, in all patients, there was an increase in the frequency of bone marrow (BM) T cells specific for BCR-ABL–postfusion. This correlated with a deepening of the molecular response in the 2 patients with MRD and a complete hematologic response (66%–2%) BM blasts) in the patient with overt relapse. Both patients treated with donor–derived products had previously relapsed post–donor lymphocyte infusions, and one of them has now maintained a major molecular response for over 4 years postinfusion. Remarkably, none of the 3 reported patients developed any infusion–related adverse events, including graft–versus-host disease, cytokine release syndrome, or neurological toxicity.

Although impressive, this study remains a proof-of-concept case series that will need to be replicated in confirmatory trials to establish safety and then efficacy. One of the limitations of targeting small, highly selected amino acid sequences is that they are typically only immunogenic in the context of specific HLA types, so the feasibility of applying this strategy broadly to patients that bear the 

\[ ^{190} \text{BCR-ABL} \]

translocation will need to be tested in patients with diverse HLA haplotypes. Because all patients received T cells concurrently with TKIs, it remains to be seen whether BCR-ABL–specific T cells will be capable of inducing single-agent responses similar to that observed with CD19 CAR T cells. The authors propose using this therapy in combination with TKIs for patients unfit for high-dose chemotherapy or alloHSCT, and it will be interesting to see whether this combination will engender TKI– or T-cell–resistant clones.

In summary, the elegant work by Comoli et al has given us a teaser to the beneficial in vivo effects of infusing BCR-ABL neantigen–specific T cells in patients with Ph \(^{2} \) ALL. In concert with the recent success of adoptively transferred neantigen–specific T cells in a patient with colon cancer, this report highlights the emerging successes and limited toxicities associated with T-cell products selectively targeting cancer-specific mutants.

Conflict-of-interest disclosure: H.E.H. has received research support from Cell Medica and Celgene and is a founder of ViraCyte. P.L. declares no competing financial interests.

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DOI 10.1182/blood-2016-12-757336
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Hematopoiesis and Stem Cells

Neutrophils set the bone marrow on fire

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Neutrophils are immune defenders. In this issue of Blood, Kawano et al show that, by producing prostaglandin E \(_{2} \) (PGE\(_{2} \)), they also regulate hematopoietic stem cell mobilization and cause fever.\(^{1} \)

The bone marrow (BM) is home to hematopoietic stem/progenitor cells (HSCs/HPCs) and their descendants. Continued production of all blood lineages requires a structured organization and complex interactions among the many cell types that reside in the BM. Early studies identified osteoblastic cells lining the endosteal surface as important components of the hematopoietic niche, whereas macrophages and other cells of hematopoietic origin have only been recognized as regulators of the BM niche in recent years.\(^{2} \)

Sympathetic nerves and mesenchymal progenitors are also regulators of the HSC/HPC niche. Current efforts focus on understanding how all of these very different cellular elements coordinate to preserve BM homeostasis.

Transient reorganization of the cellular niche network may be important when the BM needs to increase the supply of immune cells in response to a stress. For example, during acute infections endothelial cells respond by producing the cytokine granulocyte colony-stimulating factor (G-CSF).\(^{3} \) On one hand, this cytokine accelerates production of neutrophils (ie, granulopoiesis), the genuine antimicrobial defenders; but G-CSF also initiates a cascade of events within the BM that disrupts niche activity and leads to the release of HSCs/HPCs into the bloodstream. Hematologists appreciated the therapeutic potential of using G-CSF to expand and push HSCs/HPCs out of the BM for their efficient collection from blood. Indeed, blood obtained from G-CSF–treated donors is now the preferred source of HSCs/HPCs for transplantation therapy. A surprising side effect of G-CSF treatment is the appearance of low-grade fever and bone pain that responds to nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the prostaglandin synthesis pathway. How G-CSF caused fever was unknown, until now.

While studying the mechanism of fever in experimental models of G-CSF mobilization,
Kawano et al identified a previously unknown molecular and cellular circuitry in the BM that induces the production of PGE$_2$. This lipid not only caused fever, but also limited the inhibitory effect of G-CSF on the hematopoietic niche.

The authors reasoned that if G-CSF treatment caused a rise in temperature that could be prevented by NSAIDs, activation of the arachidonic acid cascade and production of pyrogenic PGE$_2$ might be involved in the arachidonic acid cascade and production of PGE$_2$, and suggested that generation of fever and mobilization were antagonistic effects. The response to this puzzle came, again, after revisiting earlier work showing that PGE$_2$ mobilizes HSCs/HPCs during stress. The study thus provides a system that better integrates regulatory network within the marrow.

These findings, however, were at odds with the exaggerated mobilization of HSCs/HPCs in mice unable to synthesize PGE$_2$, and suggested that generation of fever and mobilization were antagonistic effects. The response to this puzzle came, again, after revisiting earlier work showing that PGE$_2$ does not trigger PGE$_2$ production by acting directly on hematopoietic cells. How, then, did G-CSF prevent, mobilization of HSCs/HPCs into blood, and these effects required its expression in the hematopoietic compartment. The authors found, however, that G-CSF did not trigger PGE$_2$ production by acting directly on hematopoietic cells. How, then, did G-CSF control prostaglandin production?

To search for possible mechanisms, the authors revisited their own seminal work from a decade earlier demonstrating that G-CSF stimulates the catecholaminergic tone in the BM. They therefore predicted that catecholamines might be intermediaries needed for PGE$_2$ production. Indeed, treatment with a β3-adrenergic agonist stimulated prostaglandin release by neutrophils, and to a lesser extent by macrophages, in a process that depended on the β3-adrenergic receptor. The authors went on to demonstrate that if neutrophils were depleted before G-CSF treatment, or if the cells that produce catecholamines were eliminated, then fever disappeared from treated mice.

These findings, however, were at odds with the exaggerated mobilization of HSCs/HPCs in mice unable to synthesize PGE$_2$, and suggested that generation of fever and mobilization were antagonistic effects. The response to this puzzle came, again, after revisiting earlier work showing that PGE$_2$ improves HSC/HPC retention by stimulating osteoblastic cell function through production of osteopontin, an HSC/HPC retention molecule. In agreement with these observations, Kawano et al found that, indeed, PGE$_2$ acts locally in the BM by dramatically increasing osteopontin on preosteoblastic cells, through the prostaglandin receptor EP4. Altogether, the data presented in this article uncover a signaling network elicited by G-CSF that stimulates catecholamine production, which acts on β3-adrenergic receptors on neutrophils to induce production of PGE$_2$. This lipid acts locally on niche cells to limit HSC/HPC release, and systemically on the hypothalamus to induce fever (see figure).

The findings by Kawano et al are important because they provide formal demonstration that neutrophils, which are the most abundant cells in the BM, are bona fide regulators of the HSC/HPC niche. Previous studies had demonstrated that neutrophils can induce local inhibition of niches or control granulopoiesis from extramedullary tissues. The present study, however, is the first to identify a neutrophil-derived product that positively regulates niche cells. Although less abundant, monocytes and macrophages respond in a similar way, thereby reinforcing the notion that innate immune cells form an important regulatory network within the marrow.

Different from other niche cells, however, neutrophils and other myeloid cells appear to provide a system that better integrates environmental signals for finer regulation of HSCs/HPCs during stress. The study thus raises the question of whether (and if so how) innate immunity regulates stem cell niches in the context of pathological stress (eg, infection or leukemia).

Finally, it is intriguing that experimental depletion of neutrophils caused mild but...
significant reductions in body temperature after G-CSF treatment. Because neutrophils are distributed in many more organs than the BM, and can themselves produce not only PGE₂ but also catecholamines,⁸ it would seem that neutrophils are superbly positioned to regulate core processes, as illustrated here for body temperature and stem cell niches. Clearly, neutrophils are much more than immune defenders.

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

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DOI 10.1182/blood-2016-11-751867 © 2017 by The American Society of Hematology

Comment on Walter et al, page 598

HSP90, a chaperone that can make you SYK

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In this issue of Blood, Walter et al demonstrate an association between heat shock protein 90 (HSP90) and spleen tyrosine kinase (SYK) as a critical upstream component in tonic BCR signaling of Burkitt lymphoma (BL). Integrity of this HSP90-SYK complex appears to be critical for maintenance of tonic BCR signaling, which keeps BL cells alive. Consequently, the authors propose HSP90 as a novel therapeutic target in BL.¹

Activating of B-cell receptor (BCR) signaling, in the presence or absence of antigenic stimulation (“activated” or “tonic” BCR signaling), is critical for the survival and proliferation of normal B cells. It is also an important and targetable pathway in several B-cell malignancies, as documented by the clinical success of Bruton’s tyrosine kinase (BTK) and phosphatidylinositol 3-kinase δ (PI3Kδ) inhibitors.¹ Walter et al demonstrate an association between heat shock protein 90 (HSP90) and spleen tyrosine kinase (SYK) as a critical upstream component in tonic BCR signaling of Burkitt lymphoma (BL). Integrity of this HSP90-SYK complex appears to be critical for maintenance of tonic BCR signaling, which keeps BL cells alive. Consequently, the authors propose HSP90 as a novel therapeutic target in BL.¹

HSP90 is a member within the class of molecular chaperones, which collectively ensure the proper folding of proteins in order to prevent misfolding, protein aggregation, and their ubiquitination and proteasomal degradation. Signal transduction proteins are well-characterized HSP90 substrates, which require HSP90 for maturation and conformational maintenance. Furthermore, many oncogenic proteins, including tyrosine-kinase receptors, transcription factors, cell-cycle regulatory proteins, antiapoptotic proteins, and telomerase, are HSP90 client proteins. Consequently, the genetic or pharmacologic inhibition of HSP90 disrupts many cellular signaling networks that depend upon these molecules. Small molecule HSP90 inhibitors, such as the orally bioavailable, small molecule HSP90 inhibitor AT13387, selectively bind to HSP90, thereby inhibiting its chaperone function and promoting the degradation of proteins that may be involved in tumor cell proliferation and survival. Given the importance of HSP90 for the stability and function of various oncogenic proteins, HSP90 inhibitors have been under clinical development for a while, with mixed results, and some have been promising in selected solid (ALK-rearranged non–small cell lung cancer, HER2+ breast cancer) and hematological (multiple myeloma) malignancies.

By screening the in vitro sensitivity of BL cell lines in response to a panel of agents that included cytotoxic drugs, signaling inhibitors, and AT13387, the investigators noted striking activity of AT13387 to induce apoptosis of BL cells. Stable isotope labeling with amino acids in cell culture (SILAC)-based phosphoproteomic analyses demonstrated that HSP90 targeting downregulated BCR signaling, inhibiting the activation of the BCR-related kinases LYN, SYK, and BTK. Interestingly, SYK inhibition interfered with BL cell survival in a fashion that was similar to HSP90 inhibition, whereas neither LYN downregulation nor BTK inhibition with ibrutinib had any comparable effects. Based on these observations, the authors studied in more detail the interactions between HSP90 and SYK and report their importance for tonic BCR signaling, and the requirement for phosphorylation of tyrosine Y197 resides on HSP90 for its function. The authors conclude that HSP90 “chaperones” SYK, as a prerequisite for intact BCR signaling in BL, and on the flipside, they build the therapeutic concept that HSP90 inhibition can promote BL apoptosis through destabilization of SYK (see figure).

The study expands previous work by the same group of investigators in which they characterized the importance of tonic BCR signaling in BL through phosphoproteomic approaches.³ HSP90 inhibition has previously been shown to be effective in reducing chronic lymphocytic leukemia (CLL) cell and activated B-cell diffuse large B-cell lymphoma⁵ cell survival...
Neutrophils set the bone marrow on fire

Andrés Hidalgo