A day (or 5) in a neutrophil's life
Scott I. Simon and Min-Ho Kim
synergistic combination of phosphodiesterase inhibitors and adenosine receptor agonists in myeloma. Subsequent studies demonstrated that combinations of inhibitors of PDE2, PDE3, and PDE7—along with A2A adenosine agonists—acted synergistically in myeloma. Interestingly, while synergistic in myeloma and lymphoma cell lines, this combination was minimally cytotoxic to leukemia or solid tumor cell lines. Furthermore, the combination was preferentially cytotoxic to primary myeloma cells versus normal hematopoietic cells, suggesting a therapeutic window.

The high-throughput nature of this study and the large number of combinations analyzed with statistical rigor has advanced the field of drug repositioning. Using their unbiased high-throughput approach, the authors have identified combinations that are active in myeloma. Using traditional drug-discovery approaches, it is highly unlikely that the combination of PDE inhibitors and adenosine analogues would have been tested in combination with each other or with dexamethasone. As the authors indicate in their article, their screening platform also identified additional synergistic combinations active in myeloma that are also being pursued by this group.

The results from this study also open several opportunities for additional investigation. The authors have demonstrated that the combination of PDE inhibitors and adenosine agonists increases cyclic AMP (cAMP) above either agent alone, but additional studies will help clarify whether this increase in cAMP is functionally important for the cytotoxicity of the combination. Moreover, it is unclear why increasing cAMP is preferentially cytotoxic to myeloma and lymphoma cell lines and primary samples. Additional studies that answer these questions would be very interesting and provide further rationale to advance this combination into clinical trial. As part of these additional mechanistic studies, subpopulations of myeloma patients most likely to benefit from this combination could be identified.

The prior toxicology and pharmacology of PDE inhibitors and adenosine analogues suggest that this combination could be rapidly advanced into clinical trial for patients with myeloma. The rationale for this type of clinical study would be enhanced by demonstrating activity in myeloma xenografts. Such animal studies would help address the potential toxicity of this combination.

Finally, this article highlights the general applicability of a high-throughput approach to identify novel combinations. As an extension of this study, one could test the library compiled by Rickles et al, in a similar matrix screen, to identify unexpected synergistic combinations that are active in malignancies other than myeloma. Alternatively, as part of the development of a novel agent for the treatment of myeloma or other hematologic malignancies, one could conduct a focused screening effort to identify agents that act synergistically with the new agent. Rather than testing combinations manually, this rapid high-throughput approach can be used to increase the number of combinations tested.

Thus, in summary, Rickles et al have described a novel and unexpected combination of drugs that act synergistically in myeloma. Furthermore, they offer a path into the future for high-throughput combination drug discovery.

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

A day (or 5) in a neutrophil’s life

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In this issue of Blood, Pillay and colleagues apply a novel technique to reliably measure the half-life of circulating neutrophils that does not rely on ex vivo isolation and labeling before reintroduction to the circulation.1

Neutrophils are the most common immune cell in blood and function as professional phagocytes, highly sensitive to the molecular scent of microbes that initiate an innate program of vascular adhesion, transendothelial migration, and killing at the site of infection. This property renders neutrophils notoriously hard to isolate from blood and study in a pristinely unactivated state. Hematologic lore indicates that they circulate a mere 8 to 12 hours before exiting the blood stream to either phagocytose foreign invaders or be engulfed themselves by host macrophages.24 By incorporating 3H2O into the drinking water of humans and mice, Pillay and colleagues effectively labeled neutrophils as the heavy water was incorporated as 3H-labeled adenosine in the DNA of cells produced in the bone marrow. A mathematical model was applied to balance the books between the dynamics of 3H-adenosine in neutrophils and its appearance in serum and urine as degraded cells released it. Estimation of neutrophil half-life is based on the assumption that the rate at which neutrophils enter the blood from bone marrow equals the rate at which they are lost from the circulation, which is reasonable given that the subjects studied also showed no sign of immunologic challenge. The authors arrived at a neutrophil lifetime of 5.4 days in the circulation of humans and .75 days in mice. The latter estimate is on par with reported measures using in vivo labeling techniques in mice, but the duration in humans is approximately 10-fold longer than previously measured using ex vivo labeling techniques in human blood. Such a prolonged lifetime has only been detected for neutrophils called to battle in inflamed tissue, where they are activated by cytokines such as granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor...
factor, various interleukins, and bacterial lipopolysaccharides that delay apoptosis.3,5,6 The findings of Pillay et al suggest that the neutrophil’s lifespan is not quite as fleeting as once thought and provide impetus to further examine its interactions with other long-lived partners such as dendritic cells, monocytes, and lymphocytes.

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The main function of the lymphatic vessels is to return excess tissue fluid to the blood circulation. Other functions include antigen monitoring via the sieving function of the lymph nodes, and dietary lipid transport in chylomicrons via the intestinal lymph vessels. For the lymphatic vascular system to operate efficiently, the lymphatic vessels must separate from the blood vessels during development and the connections with the blood vessel lumens remain unidirectional at the sites where lymph drains into blood at the subclavian veins. Prior to reports in this and other recent issues of Blood, the mechanisms behind this separation and the reason for the leakage of blood into lymphatic vessels in several mouse mutant models were unknown. Now, it seems that the mystery has largely been resolved. The new evidence does not indicate a role for circulating endothelial progenitor cells as first suspected, but instead uncovers an important function for platelets, which are recruited to prevent the entry of blood into lymphatic vessels.

A schematic view of the new concepts of lymphatic-blood vascular separation is shown in the figure. In developing embryos, platelets aggregate and form a clot at sites where paired lymph sacs and lymphatic vessels emerging from them connect with their parental anterior cardinal veins. According to the recent results of Dontscho Kerjaschki, platelet aggregation at these sites is triggered by the O-glycosylated mucoprotein podoplanin, named after its main expression site in kidney podocytes and which was found to be specifically expressed in lymphatic, but not blood vascular, endothelial cells.2 Some of the earliest findings on the failure of lymphatic-blood vascular separation dealt with mice carrying homozygous mutations of the gene encoding the Syk tyrosine kinase or its adaptor protein SLP-76 (Lcp2).3 Selective Syk and SLP-76 expression in hematopoietic cells suggested initially that the mutations could compromise a rare cell population that was believed to contribute to at least some of the endothelial cells involved in lymphangiogenesis.4 However, in this issue of Blood, Bertozzi et al instead propose that the Syk protein of platelets is critical in ensuring lymphatic-blood vascular separation.5 Additional recent findings indicate that the deletion of Syk also induces an excess of tissue leukocytes that produce sufficient amount of the lymphangiogenic factors vascular endothelial growth factor (VEGF)–C and –D to stimulate lymphatic hyperproliferation, eventually resulting in anastomoses between the developing lymphatic and blood vessels elsewhere in the developing embryo.6 A somewhat similar phenotype also occurs in mice deficient in 2 suppressors of phosphorylation of the mitogen-activated protein kinase–extracellular

Comment on Bertozzi et al, page 661

Inside bloody lymphatics

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Lymphatic vessels were missed by many pathologists for centuries because they do not contain red blood cells. Yet the reason for this has been illuminated only now in several articles in Blood.