Comment on Roubinian et al, page 1003

A rose is a rose is a rose, or not

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In this issue of Blood, Roubinian et al provide important evidence to confirm, and refute, a long-standing maxim in clinical medicine that a 1-unit transfusion of red blood cells (RBCs) should yield a posttransfusion hemoglobin increment of 1 g/dL. Although true, in general, this rule was not always accurate, and deviations could be misleading. They evaluated many single-unit transfusion outcomes in stable adult patients by mining electronic health records (EHRs) and linked blood donor data. This approach was not only pragmatic, but it also points the way to future studies.

This work fits with recent efforts to improve the quality, safety, and efficacy of blood transfusions. These efforts are significantly supported by the National Institutes of Health National Heart, Lung, and Blood Institute (NHLBI), which sponsored symposia and workshops on this topic. These meetings encouraged the study of questions such as “What’s in the bag?” “How can we make better products?” “How can we build better donors?” “How do we know if it works?” These issues are also being studied in the NHLBI-supported Recipient Epidemiology and Donor Evaluation Study III (REDS-III) and REDS-IV-P programs.

To provide context for these efforts, one can think of blood products metaphorically as pharmaceuticals. This analogy is straightforward regarding hemophilia A, in which patients were historically treated with plasma or cryoprecipitate (ie, blood products), whereas current treatment uses recombinant factor VIII, which is considered a drug. Although this metaphor may be less concrete in the context of RBC transfusions, it may become relevant in the not too distant future. Indeed, with the advent of patient blood management, multiple drugs (eg, iron, tranexamic acid, erythropoietin) can supplement transfusions or render them unnecessary altogether.

If one accepts the pharmaceutical metaphor, then some pharmacology concepts become heuristically useful, including active ingredients, purity, stability, dosage, volume of distribution, pharmacokinetics/pharmacodynamics, indications, effectiveness, and adverse outcomes. These are relevant to the current contribution, particularly quality and efficacy. But RBC quality is not easy to define. Thus, US Food and Drug Administration criteria for licensing purposes include quantifying spontaneous hemolysis in vitro and posttransfusion recovery of radioabeled RBCs in vivo, both at the end of storage; however, neither readily correlates with desirable outcomes in transfused patients. More clinically relevant criteria could include improving tissue perfusion and/or oxygenation or preventing or ameliorating end-organ damage, but these are not widely accepted or applied. Finally, as in the article by Roubinian et al, posttransfusion hemoglobin increment is clearly relevant in ameliorating anemia and/or increasing reserve (eg, for ongoing or anticipated bleeding). Indeed, although hemoglobin increment can be a surrogate for overall RBC transfusion quality, in some settings (eg, chronic transfusion for hemoglobinopathies), one can argue that hemoglobin increment actually is quality.

Using the pharmaceutical metaphor, some of the current findings are not unexpected, and they fit with pharmacologic concepts. For example, an increased dose (eg, RBC units from male donors) yields an increased response in the recipient. Similarly, a decreased dose (eg, irradiated donor units) yields a decreased response in the recipient; the latter is also expected when washed or frozen/thawed RBCs are used. In addition, a smaller volume of distribution (eg, in female recipients) yields an increased response, and the opposite is seen with increasing body mass index; similar results were found in a REDS-III-supported study. In contrast, some results in the Roubinian et al study were somewhat surprising or potentially controversial. For example, although a univariate analysis did not identify a correlation between hemoglobin increment and storage age, the authors did find smaller increments at 24 and 48 hours posttransfusion when storage age was >35 days; these results are similar to those in a prospective, randomized clinical trial and a large epidemiologic study. The article by Roubinian et al also identified novel and, as yet, unanswered questions. For example, hemoglobin increments were lower in RhD-negative individuals, whether they were donors or recipients. It is unknown whether this relates to the function of RhD, inventory control issues, or some other cause.

The validity of the Roubinian et al results is supported by the strengths of their approach. These include studying large numbers of transfusions, evaluating single-unit transfusions in otherwise stable patients with appropriately timed pre- and posttransfusion hemoglobin determinations (including both inpatients and outpatients), and linking donor databases with recipient EHRs. Indeed, the latter pragmatic approach, which does
not require consent, will be exploited further in observational studies in REDS-IV-P. In addition, their results will help inform the design of future prospective clinical trials. The authors also acknowledged several weaknesses in their study, including using only stable adult patients, using blood products from only one supplier, and providing relatively little insight regarding variations induced by different manufacturing methods.

Finally, the Roubinian et al study results may prompt a reevaluation of hemoglobin dose. The current standard of care for routine RBC transfusions in adults uses a standard dose (eg, 1 or 2 units) for all adult patients without modification based on donor or recipient characteristics. This differs from the approach used in pediatrics, in which transfusions are based on mL/kg calculations. It also differs from treating adult or pediatric patients with sickle cell disease by exchange transfusion, for which we calculate the number of units required to achieve specific total hematocrit and hemoglobin S levels. Because the hemoglobin dose is knowable for every RBC unit (ie, what’s in the bag), should this information routinely be provided to the ordering physician? If so, would it be useful? For example, that information could be used to dose adults more appropriately. It could also help in decision-making regarding what hemoglobin increment to expect, allowing one to judge transfusion efficacy. For example, when the increment does not meet expectations, that would suggest an underlying pathology (eg, ongoing or new bleeding, a hemolytic transfusion reaction). Although these ideas may seem far-fetched at the moment, misinterpreting an expected hemoglobin increment currently leads to unnecessary clinical investigations and potential patient harm. Indeed, given that Roubinian et al found the different modifiers of hemoglobin increments to be additive, the potential for misinterpretation was evocatively emphasized in Table 6 of their article, in which the expected posttransfusion hemoglobin increment per unit ranged between 0.59 and 1.65 g/dL. Thus, it is hoped that future studies based upon their results will continue to help optimize transfusion therapy.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Goy et al, page 1024

ibrutinib and lenalidomide: when 1+1 = >2

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In this issue of Blood, Goy et al report on the promising activity of a phase 1b trial of the targeted therapy triplet rituximab, ibrutinib, and lenalidomide in patients with relapsed nongenital center diffuse large B-cell lymphoma (DLBCL).1

Approximately 2 of 3 patients with DLBCL, the most common lymphoid cancer, are cured with a chemotherapy combination originally created in 1976 (cyclophosphamide, doxorubicin, vincristine, and prednisone [CHOP]), which was last successfully modified in 1999 (by adding rituximab [R-CHOP]).2 For patients with DLBCL who are not cured by R-CHOP, a second chance for curative therapy is high-dose chemotherapy and autologous stem cell transplantation (ASCT). Patients who are not able to tolerate, do not respond to, or cannot access aggressive approaches such as ASCT or chimeric antigen receptor T-cell therapy have few therapeutic options with the potential for long-term disease control and are often considered palliative.3

DLBCL is a heterogeneous disease, most commonly classified on the basis of the putative cell of origin. The activated B-cell (ABC) subtype of DLBCL is characterized by chronic active B-cell receptor (BCR) signaling and requires NF-kB signaling for survival.4

The use of novel therapies that specifically target aberrant DLBCL biology has great promise, but it has yielded frustratingly few advances relevant to daily...
Patient care to date. The BTK inhibitor ibrutinib is US Food and Drug Administration (FDA) approved for multiple B-cell malignancies and has been studied in DLBCL as a single agent, but responses were transient, with a median progression-free survival of 1.6 months and overall survival of 6.4 months. The immunomodulatory drug lenalidomide is FDA approved for multiple hematologic malignancies and has been studied as a single agent and in combination with rituximab in DLBCL patients, also demonstrating frustratingly short progression-free survival of 2.7 and 2.8 months, respectively. Even more disappointing, both ibrutinib and lenalidomide have failed to significantly improve outcomes in DLBCL when added as single agents to R-CHOP. Why have these drugs with valid targets not resulted in better results?

Considering the many aberrations that cancer cells possess, perhaps their greatest asset is robustness. Drugs such as ibrutinib and lenalidomide have complex mechanisms of action, hitting many targets other than BTK or cereblon alone, including both tumor- and immune-mediated effects. Despite this pleiotropic potential, their potency as single agents (or as “+X” add-ons to standard chemotherapy) is limited because of the way cancer cells use alternate survival mechanisms to bypass their pathway blockade. To realize the true potential of targeted therapies, we must simultaneously target multiple related pathways to prevent escape.

The study of complex networks such as the internet, flight patterns, and cancer biology has found that this robustness is the result of a recurring pattern: a reliance on a few critical parts with redundancies that are difficult to damage simultaneously via nonspecific targeting. However, if critical parts of the network are concurrently targeted, the house of cards can collapse with limited effort.

The combination of ibrutinib and lenalidomide against DLBCL has been extensively studied in vitro, and a synthetic lethality was identified that was based upon interferon signaling in the ABC DLBCL subtype. In addition to anti-lymphoma immune changes, lenalidomide also decreases expression of IRF4, allowing for a modest amount of interferon production, which is toxic to ABC DLBCL. Ibrutinib, via blockade of BTK upstream in the BCR pathway, combines with lenalidomide to completely block IRF4 expression, resulting in an ABC DLBCL–lethal increase in interferon production (see figure). These two targeted agents,
when administered simultaneously, are greater than the sum of their parts.

The multicenter phase 1b trial reported by Goy et al is impressive on the basis of its overall response rate of 38% (44% in evaluable patients), and a median duration of response of 15.9 months. In comparison with results for single agents, the Goy et al trial demonstrates 2 important points. First, these drugs can work very well in ABC DLBCL when used with the right partner, even if activity is only modest when used as a single agent or with the wrong partner (ie, chemotherapy). Second, the benefits of combination therapy, a hallmark of hematologic malignancy treatments for more than 40 years, applies in spades in the era of targeted therapies. Drug approval based upon single-agent activity (or as an addition to chemotherapy) uses the imatinib paradigm of expecting miraculous single-agent results and can miss the full potential of active agents. Additional studies of rituximab-lenalidomide-ibrutinib and other targeted therapy combinations are warranted to improve the outcomes for our patients.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on O’Byrne et al, page 1059

Origins of the human B-cell lineage

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By comparing fetal and adult B-lymphopoiesis, O’Byrne et al,1 in this issue of Blood, identified a pre-pro-B–cell subset that marks the earliest stages of B-cell lineage commitment in utero.

The origins and developmental progression of the B-cell lineage have been studied at substantial resolution for many years in mice.2,3 Many of the phenotypic and functional distinctions that demarcate early stages of B-cell development in mice are also applicable to differentiating B-cell subsets in adult human bone marrow.4 The pre-pro-B–cell subset, marking earliest stages of B-cell differentiation in mice, was discovered and functionally characterized 28 years ago; however, developmental origins of human B-cell development remained elusive.

Although previous studies in humans focused on adult bone marrow,4 O’Byrne et al compared fetal liver and bone marrow to adult bone marrow samples. The authors identified pre-pro-B cells (CD19+CD10−CD34+) as the first committed B-cell precursor subset that lacks T-cell and myeloid potential, adding an

Fetal origin of human B-cell development. Pre-pro-B cells (CD19+CD10−CD34+) originate from the fetal liver and are abundant in fetal bone marrow (BM; shown here, left). In adult bone marrow, pre-pro-B cells are exceedingly rare (right) and differ from their fetal counterparts functionally and transcriptionally. See Figure 2A in the article by O’Byrne et al that begins on page 1059.
important new observation to the emerging picture of human B-lymphopoiesis. Although abundant in fetal tissues, pre-pro-B cells are exceedingly rare in adult bone marrow (see figure) and differ from their fetal counterparts functionally and transcriptionally. Leveraging state-of-the-art technology, including single-cell RNA-seq, ATAC-seq for transcriptome profiling and to survey chromatin accessibility, O’Byrne et al reconstructed developmental trajectories that place the pre-pro-B-cell subsets downstream of early lymphoid progenitors (ELPs; CD19+CD10+CD34+CD127+) and upstream of pro-B cells (CD19+CD10−CD34+). Unlike ELPs, pre-pro-B cells did not retain non-B-lymphoid potential, suggesting that fetal pre-pro-B cells likely represent the earliest committed B-cell precursor. Despite the lack of T-cell and myeloid potential, pre-pro-B cells continued to express genes that are commonly expressed in hematopoietic stem or multipotent progenitor cells, myeloid, or T cells. Surface expression of CD19 represents the main phenotypic distinction between ELP and pre-pro-B cells. Given that CD19 expression requires activity of the PAX5 transcription factor, a central driver of B-cell lineage commitment, it is likely that initiation of B-cell lineage commitment mirrors the onset of PAX5 expression in pre-pro-B cells.

The existence of an even earlier B-lymphoid precursor population cannot be excluded, but it seems unlikely that these rare progenitors would be functionally and phenotypically distinct from ELPs and pre-pro-B cells in a meaningful way. Pre-pro-B cells and pro-B cells functionally overlap in that both subsets give rise to mature B-cell development and actively undergo V(D)J recombination of immunoglobulin V\(_\text{H}\) region genes. Although D-J\(_\text{H}\) rearrangements were detected in both pre-pro-B cells and pro-B cells, rearrangement of V\(_\text{H}\)-to-D-J\(_\text{H}\) joints was specific for pro-B cells.

Studying matched liver and bone marrow samples from the same fetuses, the authors discovered 2 waves of early B-lymphopoiesis. Pre-pro-B cells first emerged in the fetal liver ~7 weeks postconception, which was followed by a proliferative burst of pre-pro-B cells, mainly in fetal bone marrow. At 20 weeks postconception, pre-pro- and pro-B cells occupied nearly half of the hematopoietic progenitor cell pool in fetal bone marrow. The extent of proliferative expansion during these earliest stages of B-cell development was previously unrecognized and raised the possibility that pre-pro-B cells may be vulnerable to malignant transformation.

Adding to the significance of the discovery of this subset as developmental origin of the B-cell lineage, fetal pre-pro-B cells may also represent the cell of origin of acute lymphoblastic leukemia (B-ALL) developing in utero or early in postnatal life. For instance, B-ALL subtypes defined by MLL- or ETV6-RUNX1 rearrangements originate from a pre-malignant clone during fetal development. Like pre-pro-B cells, MLL-rearranged B-ALL clones typically carry immunoglobulin loci with rearranged D-J\(_\text{H}\) but not V\(_\text{H}\)-to-D-J\(_\text{H}\) segments and lack CD10 expression. Both pre-pro-B cells and MLL-rearranged B-ALL share a "mixed lineage" phenotype, although pre-pro-B cells, unlike MLL-rearranged B-ALL, lack myeloid lineage potential. Mirroring abundance of pre-pro-B cells in fetal bone marrow, MLL-rearranged B-ALL arises in utero and represents the vast majority of cases of infant B-ALL. In addition, O’Byrne et al highlight striking similarities in gene expression between fetal pre-pro-B cells and MLL-rearranged infant B-ALL. Based on a series of phenotypic and functional comparisons, the authors draw a fascinating connection between fetal pre-pro-B cells as the origin of human B-cell development and their potential role as cell of origin of MLL-rearranged infant B-ALL.

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Antibiotics can improve CTCL

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In this issue of Blood, Lindahl et al report that aggressive antibiotic treatment inhibits disease activity and lymphocyte proliferation in cutaneous T-cell lymphoma (CTCL). These results are significant because they provide further evidence for a potential link between bacterial infection, activation of the immune system, and CTCL progression as well as a rationale for aggressive antibiotic treatment as adjuvant therapy in CTCL.

In recent years, next-generation sequencing studies have provided new insight into the genetic background of CTCL and identified, among others, STAT signaling as a critical pathway operative in CTCL tumor cells. A key question now is if interaction of tumor cells with their microenvironment can drive STAT signaling and thereby accelerate progression of disease. Biologic rationale for increased STAT signaling induced by bacterial products is most compelling. Patients with advanced stages of CTCL are frequently colonized with *Staphylococcus aureus* (SA), and antiseptic and antibiotic treatment can be helpful in clinical management. Malignant T cells may carry functional T-cell receptors (TCRs) expressing SA enterotoxin-binding Vβ chains, and SA enterotoxins may stimulate malignant T cells in vitro. Furthermore, in a STAT3-dependent mouse model of CTCL, disease progression was critically dependent on bacteria. In vitro studies showed that SA enterotoxins can stimulate a bidirectional cross talk between nonmalignant T cells and malignant CTCL cells that promotes proliferation of the malignant cells that depends on interleukin-2 (IL-2). Combined, these observations suggest that SA and its toxins may accelerate disease progression in CTCL either by directly stimulating tumor cells or by activating nonmalignant T cells, resulting in an inflammatory environment that may drive disease progression (see figure).

Following a dramatic clinical response on treatment with 4 weeks’ IV broad-spectrum antibiotics (carbapenem) in a mycosis fungoides patient (stage IIB) with extensive ulcerating tumors, Lindahl et al treated 8 additional patients with advanced refractory CTCL for 10 days with IV antibiotics (cephalosporins and metronidazole) and subsequent oral treatment for 14 days with combined amoxicillin and clavulanate.

Subjective improvement was observed as early as 10 days after initiation of antibiotic treatment, and in all patients, a marked clinical improvement with a significant decrease in skin disease burden was noted after 2 months. Immunohistochemical staining of skin biopsies taken before and 2 months after initiation of antibiotic treatment demonstrated that clinical improvement was accompanied by a decrease in cell proliferation, expression of interleukin-2 receptor (IL2R)-α, and tyrosine-phosphorylated STAT3 (pY-STAT3). Using TCRβ sequencing, it was found that the dominant TCR clonotype decreased significantly in 5 of 6 patients 60 days after initiation of antibiotic treatment, whereas the fraction of nonmalignant T cells increased following antibiotic therapy. Ex vivo studies showed that SA enterotoxins isolated from CTCL skin lesions could induce expression of pY-STAT3 and of the high-affinity IL2R-α chain in primary malignant cells and nonmalignant T cells. At transcription level, the clinical improvement was accompanied by a normalization of expression profiles with a clear decrease in IL-2 signaling and STAT activation. Importantly, antibiotics at clinically relevant concentrations did not induce apoptosis or affect the viability of malignant T cells in vitro. These observations suggest that SA and its toxins activate STAT3 signaling, increase expression of the IL2R, and stimulate proliferation of tumor cells in CTCL, whereas antibiotic treatment can effectively eradicate this stimulus, normalize the tumor microenvironment, and inhibit disease activity in skin.

Limitations of this study are the relatively small sample size and the short follow-up in the majority of patients. Therefore, it remains uncertain if the dramatic responses that were observed in this case series are a general characteristic in all CTCL patients and how durable these responses will prove to be.

A strength of this study is the translation of a clinical observation to a relatively
small, but well-executed, clinical study combined with translational research that provides insight into the biology of the disease. Clearly, based on these observations, larger trials should be designed to optimize SA treatment protocols in the management of CTCL. Ideally, these studies should be combined with translational research to further explore the interaction of the microbiome with the immune system and malignant T cells. Insight into the cellular interactions, signaling pathways, and cytokines that play a role in the inflammatory microenvironment in CTCL may lead to identification of additional therapeutic targets. Because this study lends further support to the SA/STAT axis as a therapeutic target in CTCL, it will be of interest to explore synergy of antibiotics combined with STAT3 targeting treatment in these trials as well. As in the present study, all patients had advanced stages of disease, and open questions remain if similar interactions between microbiome, nonmalignant and malignant T cells are operative in early stages of CTCL as well and if manipulation of the skin microbiome in these early stages of disease can prevent disease progression.

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