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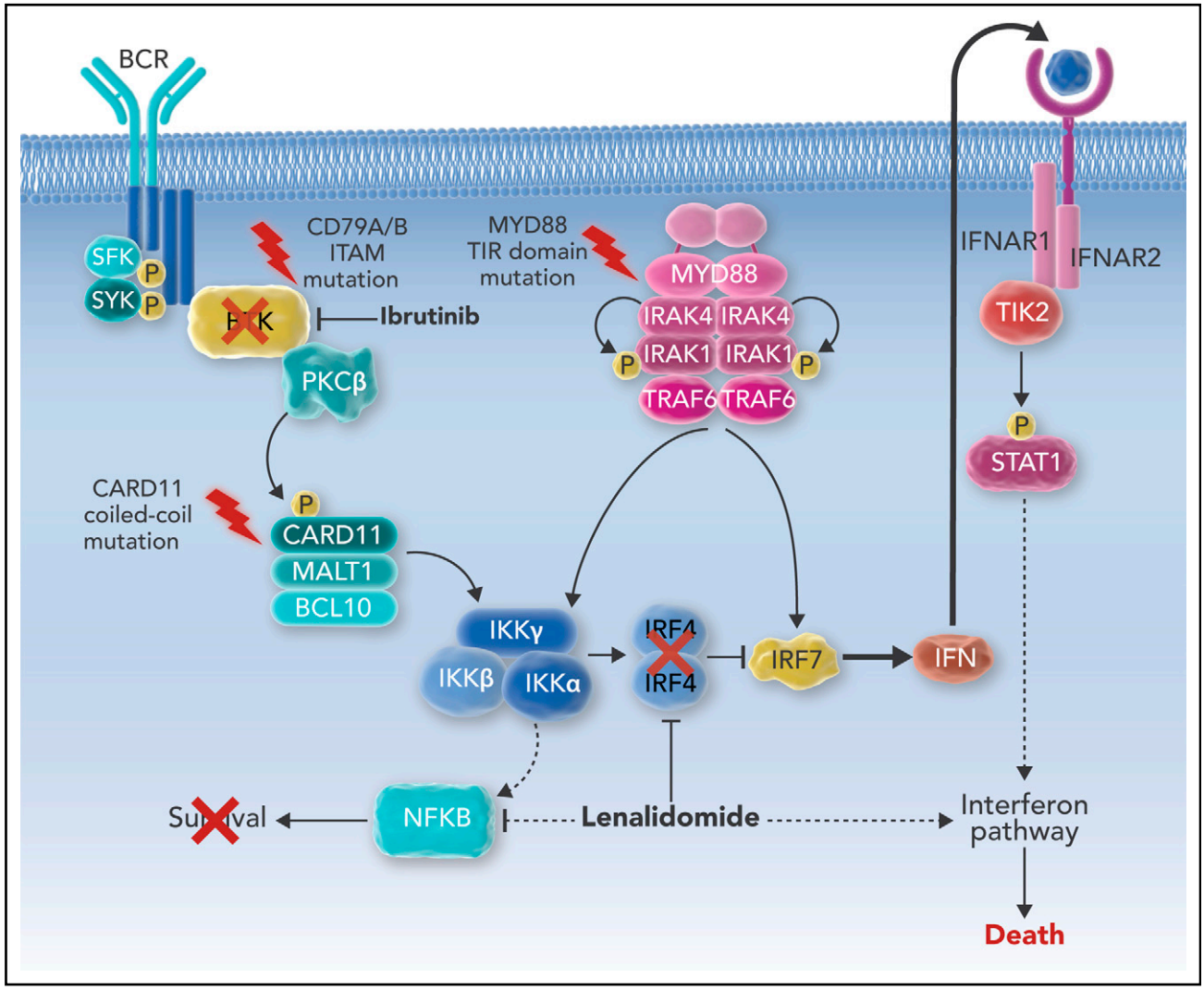
In this issue of *Blood*, Goy et al report on the promising activity of a phase 1b trial of the targeted therapy triplet rituximab, ibrutinib, and lenalidomide in patients with relapsed nongerminal center diffuse large B-cell lymphoma (DLBCL).[1]

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-κB 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



Ibrutinib and lenalidomide cooperate to kill ABC DLBCL via interferon signaling. P, phosphorylation.

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.[5] 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.[6] Even more disappointing, both ibrutinib and lenalidomide have failed to significantly improve outcomes in DLBCL when added as single agents to R-CHOP.[7,8] Why have these drugs with valid targets not resulted in better results?

Considering the many aberrances 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.[9] 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.[10] 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.[11] In addition to antilymphoma 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 add-on 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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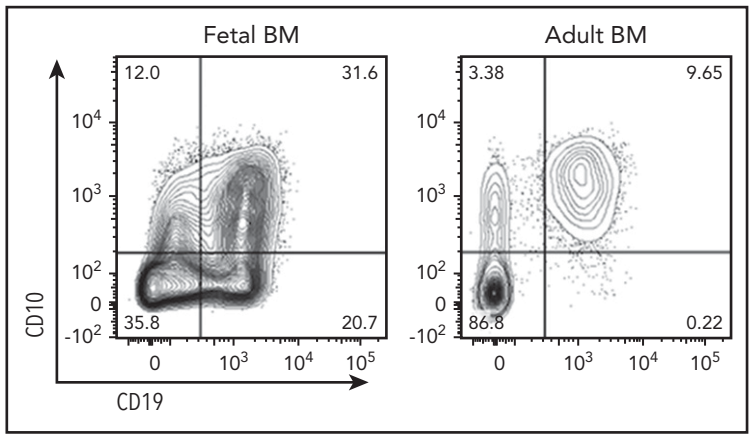
By comparing fetal and adult B-lymphopoiesis, O'Byrne et al,[1] in this issue of *Blood*, identified a pre-pro-B–cell subset that marks the earliest stages of B-cell lineage commitment in utero.

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[5]; 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.