preparing the deformation of the nucleus. This step is followed by further deformation and elongation of the nucleus and, once migration is completed, the nucleus is refolded into a roundish, multilobular shape.[4] Nuclear deformation has been shown to be required for neutrophil migration within tissues with pore cross sections of 2 to 20 μm.[5] This process was significantly impaired in Myo1f-deficient murine neutrophils or HL-60 cells stably expressing EGFP-Myo1f. Altogether, these studies suggest that defective nucleus deformation was the reason for impaired 3D migration and accumulation in vivo in the genetic absence of Myo1f.

Class I myosins are widely expressed members of the myosin superfamily that bind to actin filaments and hydrolyze adenosine triphosphate to produce mechanical force.[6] These proteins bind to membranes by their basic tail homology 1 (TH-1) domains and may be important for membrane-associated functions, such as endocytosis, cell signaling, and cell motility. Myo1f was found to be mainly expressed in neutrophils.[3] Although the neutrophil nucleus may be quite malleable, the nucleus-cytoskeleton connection is essential for 3D migration by transmitting force from the cytoskeleton to the inside of the nucleus.[7] An interesting possibility raised from the studies of Salvermoser and colleagues is that Myo1f may link the cytoskeleton to the nuclear envelope via its TH1 domain to provide a high malleability of the neutrophil nucleus. This possibility clearly deserves further investigation.

Lipopolysaccharide-induced neutrophil extravasation into the lung interstitium and in the bronchoalveolar space was significantly impaired in Myo1f$^{-/-}$ mice as compared with Myo1f$^{+/+}$ mice. In contrast, there was increased neutrophil accumulation within capillaries of the lungs of Myo1f$^{-/-}$ mice. Interestingly, it has been shown that various mediators of inflammation can cause neutrophil sequestration in the lungs and other capillaries during acute inflammation by increasing the stiffness of the neutrophils. Stimulated neutrophils (diameter, 8 μm) are retained in pulmonary capillaries (5.5 μm) as a result of a decreased ability of the cell to deform within the capillary in response to the hydrodynamic forces of the bloodstream.[8] Increased neutrophil stiffness and retention in the lungs are thought to contribute to their migration into the lung parenchyma and airway spaces.[9] It will be very interesting to evaluate the relevance of Myo1f in this context, as activation and retention of neutrophils within capillaries may contribute to lung injury and the systemic inflammatory response syndrome secondary to sepsis. Clearly, Myo1f may contribute not only to the beneficial role of neutrophils to fight infection, but also to the role these cells play in acute and chronic inflammatory diseases not caused by infection.

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

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DOI 10.1182/blood-2018-03-837872

CLINICAL TRIALS AND OBSERVATIONS

Comment on O'Brien et al, page 1910

Ibrutinib: coming of age?

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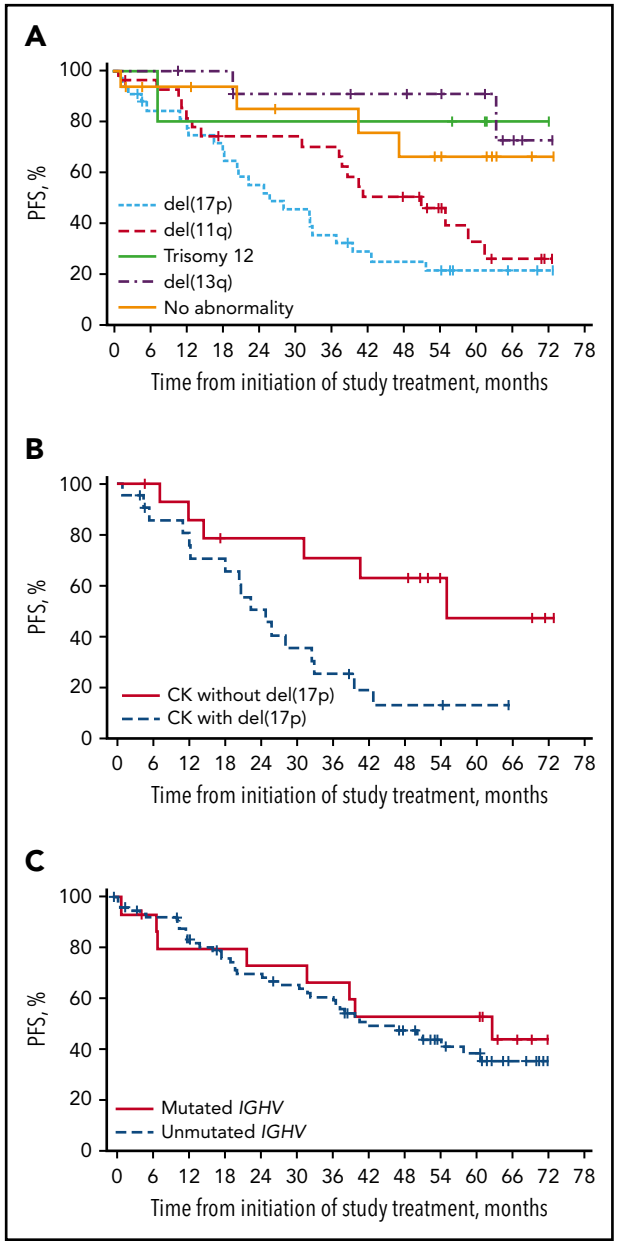
In this issue of *Blood*, O'Brien et al report long-term efficacy and safety of ibrutinib in the first cohort of patients with chronic lymphocytic leukemia (CLL) treated, now with 5-year follow-up, the longest of any CLL cohort to date.[1]

Ibrutinib has come into widespread use since its initial US Food and Drug Administration approval for relapsed CLL 4 years ago, based on the early results of this same study.[2] Although many other studies have confirmed the high efficacy of ibrutinib in CLL, follow-up has remained quite short. Thus, many questions about durability and predictors of response, as well as long-term tolerability, have remained unanswered. In this study of high-risk patients with relapsed/refractory disease, the median progression-free survival (PFS) was reached at a strikingly good 51 months, which compares favorably to that achieved with older regimens in a comparable patient population.[3] The median treatment duration for the relapsed/refractory cohort was 39 months, with 33% discontinuing for disease progression. Interestingly, the PFS curves by cytogenetic abnormality have a distribution similar to that of the classic survival curves of Döhner et al[4] (see figure panel A), with del(17p) remaining highest risk, with a median PFS of 26 months. Complex karyotype has emerged as a predictor of shortened PFS in ibrutinib studies,[5] and in this study, it was associated with a 31-month PFS, driven significantly by co-occurrence with del(17p) (see figure panel B). The extent to which the adverse prognosis of complex karyotype is driven by its association with del(17p) remains unknown, but clearly, this needs to be investigated prospectively in future ibrutinib studies.

Overall, these findings seem aligned with the recently reported 59% 3-year PFS achieved with ibrutinib in the confirmatory RESONATE trial,[6] which randomly assigned patients with relapsed CLL to ibrutinib or ofatumumab. In the study by O'Brien et al,



PFS with ibrutinib in patients with relapsed/refractory CLL by chromosomal abnormalities detected by fluorescence in situ hybridization (A), by presence or absence of del(17p) among those with complex karyotype (CK) (B), and by *IGHV* mutational status (C). See complete Figures 3, 4, and 6 in the article by O'Brien et al that begins on page 1910.

significant predictors of both PFS and overall survival in multivariate analysis included del(17p) and number of prior therapies. Similarly, in RESONATE, biallelic inactivation of TP53 [including del (17p)] and >2 prior therapies were associated with shorter PFS.[6,7] Recent reports from RESONATE and other ibrutinib studies have suggested that ibrutinib may overcome the negative impact of other traditionally high-risk prognostic factors, such as del(11q) or unmutated *IGHV*.[8] With no difference seen at 2- to 3-year follow-up, these reports establish the efficacy of ibrutinib in these prognostic subgroups, but they raise the question of whether long follow-up will be required to truly assess differences. With the 5-year follow-up of O'Brien et al, we see perhaps a hint that these factors may still have adverse impacts, at least in some contexts. For example, the duration of response for del(11q) CLL was reduced at 39 months, with a median PFS of 51 months (see figure panel A). More patients with unmutated *IGHV* discontinued therapy, with disease progression the most common reason, although PFS was not different at present (see figure panel C). Ultimately, the data remain relatively immature among subgroups in which few progression events have occurred, underscoring both the effectiveness of ibrutinib and the length of follow-up required for a comprehensive understanding of its effect within subgroups.

The other notable result of this study is the long follow-up of adverse events (AEs) among these patients intended to remain on continuous ibrutinib therapy. Approximately 20% of treatment discontinuations in both the treatment-naïve and relapsed/refractory cohorts were attributed to AEs over 5-year follow-up, which compares favorably to the 42% discontinuation rate at a median of 17 months in a recent real-world report of ibrutinib use.[9] It is notable, however, that 30% of patients in this study developed grade ≥3 hypertension, and ~10% each had major hemorrhage and atrial fibrillation. Pneumonia of grade ≥3 also seems constant across the 5-year treatment interval. Prior work has suggested a particular susceptibility to invasive fungal infections among ibrutinib-treated patients, and this issue of *Blood* also contains a report from Ghez et al[10] (with commentary) on 33 patients who developed invasive fungal infections during ibrutinib treatment. This updated AE profile may start to close the gap between clinical trials and the higher rates of toxicity reported in recent real-world reports of ibrutinib use[9] and underscores the need for ongoing vigilance throughout ibrutinib therapy.

In the context of this more mature AE profile, it is notable that 45% of the small low-risk treatment-naïve cohort in this study has now discontinued ibrutinib, many apparently after year 4, because only 23% were treated for <4 years. The reasons for this are not fully explained, with 19% attributed to AEs and 6% to disease progression, leaving 20% of unclear etiology, potentially related to low-grade AEs. The observation that the median duration of ibrutinib therapy in this frontline lower-risk cohort was only ~5.5 years is potentially quite useful in counseling patients, if these results in this admittedly small cohort are confirmed. The relatively late timing of discontinuation in this study suggests that longer follow-up of RESONATE-2, which randomly assigned untreated older patients with CLL to ibrutinib or chlorambucil, could have similar findings; at current 24-month follow-up, 79% of patients remain on ibrutinib.[11] Disease outcomes in this small frontline low-risk cohort

are not surprisingly excellent, even at 5 years. However, the key unanswered question, with almost no data, concerns the durability of remission after stopping ibrutinib in a deep but probably not complete remission, as compared with continuing ibrutinib. This durability may ultimately differ based on depth of response, duration of therapy, and CLL prognostic factors, but as yet, it remains unknown. Further follow-up of patients who discontinue without disease progression, as well as systematic investigation of time-limited therapy, including novel likely combination approaches, is clearly warranted given this long-term toxicity and discontinuation data, with the goal of maximizing ibrutinib benefit while minimizing toxicity.

With this 5-year update of single-agent ibrutinib therapy, we have reached a median PFS in patients with relapsed/refractory disease, as well as a median duration on therapy in previously untreated older patients. Both represent a significant step forward in our knowledge of the natural history of ibrutinib therapy, but many questions remain for the future: mature follow-up of larger trials, outcomes in high-risk and/or young patients treated frontline, and outcomes of time-limited or combination therapy, among others. Ibrutinib data are starting to mature, but much opportunity for growth remains.

Conflict-of-interest disclosure: J.R.B. has served as a consultant for Janssen, Pharmacyclics, AstraZeneca, Sun, Redx, Sunesis, Loxo, Gilead, TG Therapeutics, Verastem, and AbbVie and receives research funding from Sun and Gilead. ■

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DOI 10.1182/blood-2018-02-832071

LYMPHOID NEOPLASIA

Comment on Ghez et al, page 1955

Ibrutinib and fungus: an invasive concern

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In this issue of *Blood*, Ghez et al report on 33 patients who developed invasive fungal infections during ibrutinib treatment, the majority of which were invasive aspergillosis, which supports the observation that fungal infections are a potential risk with ibrutinib.[1]

Also in this issue of *Blood*, O'Brien et al[2] report a 5-year experience with ibrutinib which shows continued favorable outcomes and should increase enthusiasm for this agent, but as the associated commentary by Brown[3] points out, it strengthens the need to understand early- and late-occurring toxicities. Through the extended follow-up and review of the non-trial experience, we are gaining additional knowledge regarding risks of ibrutinib, including some that were not recognized in the clinical trial population.

Ibrutinib is a disease-altering therapy for many B-cell malignancies that has been approved for 4 different cancers and chronic graft-versus-host disease. Ibrutinib is highly effective in all categories of patients with chronic lymphocytic leukemia (CLL), the most prevalent adult leukemia, and is better tolerated than other available therapies. This has led to its widespread and increasing use.

However, *Pneumocystis jirovecii* pneumonia and other opportunistic fungal infections have recently been noted in small series and case reports on ibrutinib treated patients.[4,5] This raises concern that ibrutinib may increase the risk for these infections because they are uncommon in CLL patients. Adding to the concern is an alarming observation from an ibrutinib combination study in primary central nervous system lymphoma that 39% of patients developed invasive aspergillosis.[6]

This information prompted Ghez et al to conduct a survey of centers in the French Innovative Leukemia Organization to identify patients who were diagnosed with invasive fungal infections while taking ibrutinib. They found a total of 33 cases: 27 aspergillosis, 4 disseminated cryptococcosis, 1 mucormycosis, and 1 pneumocystis pneumonia. Unsurprisingly, all but 3 of these cases were in CLL patients because CLL is likely to be the most common indication for prescribing ibrutinib. They report a high rate of central nervous system involvement in the patients with aspergillosis (11 of 27). This is consistent