Despite the morbidity and mortality associated with VTE in cancer patients, current clinical practice guidelines do not recommend routine thromboprophylaxis in outpatients with cancer.[5,6] The relatively low overall rate of VTE among breast cancer patients, <1%/year in this study population, does not justify the notion of population-wide thromboprophylaxis given the risk of serious treatment-related adverse events such as major bleeding. A significant challenge is identifying the patients at moderate to high risk for VTE who are most likely to benefit from primary thromboprophylaxis treatment. Because the net benefit of therapies involving treatment-related harm depends on the likelihood of the outcome, a trial enrolling patients without a risk-based sampling strategy or subgroup analysis is unlikely to yield favorable summary results.[7] The Evaluation of AVE5026 in the Prevention of Venous Thromboembolism in Cancer Patients Undergoing Chemotherapy trial demonstrated a large relative effect size (nearly threefold higher VTE risk in placebo vs semuloparin treated), but a small absolute risk reduction in VTE of only 2.2% (3.2% and 1.4% in control and treatment groups, respectively).[8] Absolute risk reductions of this magnitude are not enough to counterbalance potential treatment related harms, assuming a 1% risk of intracranial hemorrhage. In contrast, the Charité Onkologie (CONKO)-004 trial selected patients with advanced pancreatic cancer, a malignancy associated with the highest VTE rates, and demonstrated a clinically significant absolute reduction in VTE risk from 15.1% to 6.4% in control vs treated patients.[9]

Although VTE is a relatively rare event, breast cancer is the most common cancer in women worldwide. Thus, the question of whether to use thromboprophylaxis to reduce breast cancer–related VTE has a substantial clinical impact. The findings by Walker et al suggest that the future of thromboprophylaxis in breast cancer patients should be time-limited treatment of those at highest risk. Selectively applying thromboprophylaxis to those patients at highest risk and, critically, only while those patients are at risk, may limit overtreatment of patients unlikely to benefit while avoiding the majority of adverse outcomes. Recognition of the time-sensitive nature of VTE risk associated with surgery and treatments could be leveraged to improve risk prediction algorithms to better identify candidates for thromboprophylaxis. Future studies are needed to evaluate how these results may be used to more precisely target patients with the best chance of benefiting from new and existing therapies. The current study provides valuable evidence that can both inform this future research and be used for counseling patients about VTE risk. Women with breast cancer undergoing surgery, chemotherapy, or hormonal therapy have a specific time interval during which they are at increased risk of VTE. Discussion of common VTE symptoms should therefore be included, alongside admonitions on neutropenic fevers and more common treatment side effects, as infrequent, although highly morbid, potential complications of therapy.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Walker AJ, West J, Card TR, Crooks C, Kirwan CC, Grainge MJ. When are breast cancer patients at highest risk of venous thromboembolism? A cohort study using English health care data. *Blood*. 2016;127(7):849-857.
2. Chew HK, Wun T, Harvey DJ, Zhou H, White RH. Incidence of venous thromboembolism and the impact on survival in breast cancer patients. *J Clin Oncol*. 2007;25(1):70-76.
3. Khorana AA, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood*. 2008;111(10):4902-4907.
4. Thaler J, Ay C, Pabinger I. Venous thromboembolism in cancer patients - risk scores and recent randomised controlled trials. *Thromb Haemost*. 2012;108(6):1042-1048.
5. Lyman GH, Bohlke K, Khorana AA, et al; American Society of Clinical Oncology. Venous thromboembolism prophylaxis and treatment in patients with cancer: American Society of Clinical Oncology clinical practice guideline update 2014. *J Clin Oncol*. 2015;33(6):654-656.
6. Farge D, Debourdeau P, Beckers M, et al. International clinical practice guidelines for the treatment and prophylaxis of venous thromboembolism in patients with cancer. *J Thromb Haemost*. 2013;11(1):56-70.
7. Kent DM, Lindenauer PK. Aggregating and disaggregating patients in clinical trials and their subgroup analyses. *Ann Intern Med*. 2010;153(1):51-52.
8. Agnelli G, George DJ, Kakkar AK, et al; SAVE-ONCO Investigators. Semuloparin for thromboprophylaxis in patients receiving chemotherapy for cancer. *N Engl J Med*. 2012;366(7):601-609.
9. Pelzer U, Opitz B, Deutschinoff G, et al. Efficacy of prophylactic low-molecular weight heparin for ambulatory patients with advanced pancreatic cancer: outcomes from the CONKO-004 Trial. *J Clin Oncol*. 2015;33(18):2028-2034.

DOI 10.1182/blood-2015-12-683482

● ● ● LYMPHOID NEOPLASIA

Comment on Culjkovic-Kraljacic et al, page 858

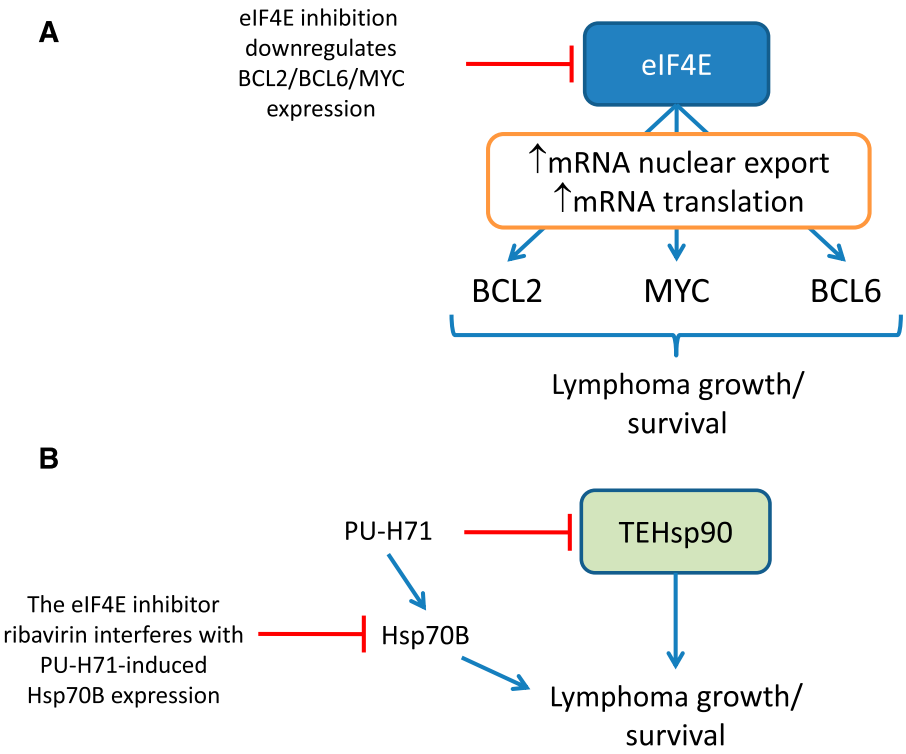
Shooting the messenger (RNA) in B-cell lymphoma

Alison Yeomans and Graham Packham UNIVERSITY OF SOUTHAMPTON

In this issue of *Blood*, Culjkovic-Kraljacic et al demonstrate that targeted inhibition of the messenger RNA (mRNA) translation initiation factor eIF4E is an effective strategy to reduce expression of the MYC, B-cell lymphoma (BCL)2, and BCL6 oncoproteins in aggressive BCLs.[1]

mRNAs are the essential intermediates that convey genetic information from genes to their protein products. Transcription, the process by which DNA is first converted into mRNA, has been studied in depth; mRNA expression profiling has provided remarkable insight into the pathogenesis of hematologic malignancies and small molecule inhibition of epigenetic regulators of transcription remains an important area for drug development. However, it is also clear that mRNAs are not just passive conveyers of genetic information but are themselves subject to tight regulation. mRNAs form dynamic 3-dimensional structures with multiple RNA:RNA and RNA:protein interactions that influence function.[2] mRNAs are also the target for modulatory effects of microRNAs, frequently dysregulated in cancer. Thus, the export of mRNAs from the nucleus to the cytoplasm, mRNA translation, and mRNA degradation are all tightly controlled processes.

In light of the tight regulation normally imposed on mRNA, it is not surprising that alterations in these processes play significant roles in many cancers, including hematologic



Roles of eIF4E in BCLs. (A) eIF4E is required for efficient expression of the oncoproteins BCL2, MYC, and BCL6 in aggressive DLBCL. (B) eIF4E inhibition enhances response to the tumor-enriched Hsp90 (TEHsp90) inhibitor PU-H71 by interfering with feedback induction of Hsp70B.

malignancies. For example, the eukaryotic initiation factor eIF4E is a core component of the translational preinitiation complex and recognizes the m^7G 5′-cap structure of mRNAs. mRNAs encoding oncoproteins are often highly dependent on eIF4E for efficient translation and are therefore particularly sensitive to decreased eIF4E expression/function.[3] eIF4E is frequently overexpressed in B-cell malignancies, especially in more aggressive subtypes,[4] and eIF4E cooperates with MYC to drive B-cell lymphomagenesis in mouse models.[5] Other eukaryotic initiation factors also appear to contribute to dysregulated mRNA translation in lymphoma. For example, eIF4B overexpression in diffuse large BCL (DLBCL) has been linked to altered cell survival and DNA damage response pathways.[6] In light of such findings, targeted inhibition of mRNA translation has become an active area for drug development. The translational elongation inhibitor omacetaxine mepesuccinate (homoharringtonine) is approved for the treatment of chronic myeloid leukemia, and other compounds are in preclinical and clinical development for a wide range of cancer types.[7]

The study by Culjkovic-Kraljacic et al provides important new insight into the role of eIF4E in aggressive BCLs.[1] Their study focused on double- and triple-hit (DH/TH) DLBCL. These tumors are characterized by activating mutations leading to enhanced expression of MYC, and BCL2 and/or BCL6, and are particularly difficult to treat. The authors show that eIF4E was often highly expressed in DLBCL tumor biopsies and that RNA interference–mediated eIF4E knockdown in lymphoma-derived cell lines resulted in decreased expression of MYC, BCL2, and BCL6 (see figure). eIF4E RNA-immunoprecipitation was used to "capture" eIF4E-associated mRNAs and demonstrated directly that eIF4E bound *BCL6*, *BCL2*, and *MYC* mRNAs. eIF4E also interacted with many other mRNAs encoding proteins involved in B-cell receptor signaling, metabolism, and DNA repair, suggesting a broad role for eIF4E in maintaining pro-lymphoma protein expression. Stimulatory effects of eIF4E on expression of its target mRNAs appeared to involve increased mRNA export from the nucleus,[8] perhaps acting in addition to enhanced target mRNA translation. Importantly, the antiviral drug ribavirin, which can act as an m^7G 5′-cap mimic and interfere with binding between eIF4E and its mRNA substrates, also reduced MYC, BCL2, and BCL6 expression in B-cell lines in vitro. Ribavirin also effectively suppressed growth of a xenografted primary DLBCL sample in immunocompromised mice.

Another notable finding revealed by the study was the functional interplay between eIF4E and heat shock proteins. Tumor cells frequently contain relatively high levels of a "stress active" form of Hsp90 termed TEHsp90, which is required to maintain malignant cell viability and proliferation. Hsp90 inhibitors, including PU-H71, have shown promising preclinical activity in B-cell malignancies;[9] however, cellular responses are typically limited by feedback induction of another chaperone, Hsp70B. Culjkovic-Kraljacic et al showed that eIF4E was a TEHsp90 client protein in BCL cell lines and that, in turn, eIF4E was required for PU–H71-induced Hsp70B expression.[1] Moreover, the combination of ribavirin and PU-H71 exerted stronger antilymphoma activity in vivo compared with either drug alone. There may be many mechanisms of crosstalk between PU-H71 and ribavirin, but the study suggests that ribavirin interferes with eIF4E-mediated feedback induction of Hsp70B following TEHsp90 inhibition (see figure).

Overall, this study provides important new insight into the role of eIF4E and mRNA control in B-cell cancers. eIF4E's contribution may be multifunctional, and further studies are required to dissect the relative contribution of effects on nuclear export and mRNA translation. Although the study focused on DH/TH lymphoma, the applicability of these results to other tumors lacking genetic dysregulation of MYC, BCL2, and/or BCL6 also requires further study. It will also be important to investigate whether these functions of eIF4E are modulated by posttranslational modification, eg, via mitogen-activated protein kinase interacting kinase (MNK)-kinase dependent phosphorylation.[10] Moreover, ribavirin may exert its anti-lymphoma effects act via effects on targets, in addition to eIF4E. Regardless, the growing attention that is being focused on mRNA is revealing fascinating insight into lymphoma biology. This promises to be a fruitful area for the discovery of new anticancer drugs.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Culjkovic-Kraljacic B, Fernando TM, Marullo R, et al. Combinatorial targeting of nuclear export and translation of RNA inhibits aggressive B-cell lymphomas. *Blood*. 2016;127(7):858-868.
2. Müller-McNicoll M, Neugebauer KM. How cells get the message: dynamic assembly and function of mRNA-protein complexes. *Nat Rev Genet*. 2013;14(4):275-287.
3. Siddiqui N, Sonenberg N. Signalling to eIF4E in cancer. *Biochem Soc Trans*. 2015;43(5):763-772.
4. Wang S, Rosenwald IB, Hutzler MJ, et al. Expression of the eukaryotic translation initiation factors 4E and 2alpha in non-Hodgkin's lymphomas. *Am J Pathol*. 1999; 155(1):247-255.
5. Ruggero D, Montanaro L, Ma L, et al. The translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in lymphomagenesis. *Nat Med*. 2004;10(5):484-486.
6. Horvilleur E, Sbarrato T, Hill K, et al. A role for eukaryotic initiation factor 4B overexpression in the pathogenesis of diffuse large B-cell lymphoma. *Leukemia*. 2014;28(5):1092-1102.
7. Bhat M, Robichaud N, Hulea L, Sonenberg N, Pelletier J, Topisirovic I. Targeting the translation machinery in cancer. *Nat Rev Drug Discov*. 2015;14(4): 261-278.
8. Osborne MJ, Borden KL. The eukaryotic translation initiation factor eIF4E in the nucleus: taking the road less traveled. *Immunol Rev*. 2015;263(1): 210-223.
9. Trendowski M. PU-H71: an improvement on nature's solutions to oncogenic Hsp90 addiction. *Pharmacol Res*. 2015;99:202-216.
10. Landon AL, Muniandy PA, Shetty AC, et al. MNKs act as a regulatory switch for eIF4E1 and eIF4E3 driven mRNA translation in DLBCL. *Nat Commun*. 2014;5:5413.

DOI 10.1182/blood-2015-12-685156

● ● ● MYELOID NEOPLASIA

Comment on Larrue et al, page 882

Not only TKI! Targeting FLT3-ITD by autophagy

Guido Marcucci and Ling Li CITY OF HOPE MEDICAL CENTER

In this issue of *Blood*, Larrue et al identify the downstream posttranslational regulation of the Fms-related tyrosine kinase 3 (FLT3) internal tandem duplication (ITD) protein.[1] This study adds further to the understanding of beneficial impact of using "dirty" proteasome inhibitor in acute myeloid leukemia (AML). This study also provides a novel antileukemia role of autophagy, since it is a well-known physiological process that controls normal cell homeostasis through protein degradation and turnover of cell organelles.[1]

The extensive cytogenetic and molecular characterization of AML has led to a better understanding of basic mechanisms of leukemogenesis, has defined prognostic subgroups of AML patients, and has provided much-needed treatment guidance for selecting the most appropriate therapies based on genetic lesions present.[2] Although these lesions have been informative, an understanding of how these genetic lesions act in concert to deregulate fundamental mechanisms of normal cell homeostasis and to contribute to leukemic transformation is necessary for a rational design of more active therapies.[3]

Nevertheless, the discovery of recurrent mutated genes encoding proteins with proleukemic activity has created opportunities for designing "smart" targeting therapeutics and led to the concept of personalized medicine for AML.[2] The ITD is a gain-of-function mutation of the FLT3 gene encoding 1 of the receptor tyrosine kinases, and is one of the most common genetic abnormalities in AML.[4] The ITD mutation results in constitutive ligand-independent FLT3 activation, which aberrantly activates a signaling cascade of downstream effectors (ie, mitogen-activated protein kinase, STAT5, PI3K), thereby supporting leukemia cell proliferation and survival.[4] Although patients harboring FLT3-ITD are initially sensitive to chemotherapy, they frequently relapse even after allogeneic stem cell transplantation, consistent with the failure of these approaches to eradicate the so-called leukemia stem cell subpopulation.[4]

Tyrosine kinase inhibitors (TKIs) have been designed to target the aberrantly activated FLT3 receptor and to "shut off" constitutive tyrosine phosphorylation in AML blasts. Although these compounds have shown initial preclinical and early clinical results, a survival benefit from their use in combination with chemotherapy has only recently been reported in FLT3-ITD AML patients.[4] However, this class of compounds may still have limitations related an inherent deficiency of substrate specificity (potentially less problematic with newer generation TKIs), early onset of mutagenesis in the FLT3 kinase domain, and inevitable toxicity.[4,5] In addition, complex regulatory feedback mechanisms that govern the expression of the mutated and/or wild-type alleles and the stability of the corresponding encoded proteins may contribute to early occurrence of TKI resistance in FLT3-ITD AML blasts. Finally, the concurrent presence of other gene mutations (eg, *NPM1*, *DNMT3A, IDH1*, and *IDH2*) not only impacts the prognostic significance of FLT3-ITD, but may also contribute to reduced clinical response of this subset of patients.[2] Thus, understanding the multifaceted biological role of FLT3-ITD in AML is a necessary step to discover how to deactivate completely tyrosine kinase-mediated pro-leukemogenic signals.

The complexity of upstream transcription regulation of genes encoding receptor tyrosine kinases has been dissected, and strategies for inhibiting the expression of *FLT3* mutated alleles have been already suggested and tested to in early clinical trials.[6-8] In this issue, Larrue et al take a different approach and focus on the downstream posttranslational regulation of the FLT3-ITD protein (see figure). The authors demonstrate an antileukemia role of autophagy, a well-known physiological process that controls normal cell homeostasis through protein degradation and turnover of cell organelles.[1] The fine-tuned mechanisms regulating autophagy are complicated and not fully understood, especially in the context of cancers in which contrasting roles of autophagy-dependent cell survival and death have been reported previously.[1] Upon observing proteasome inhibitor-initiated autophagy indicated by the conversion of LC3-I to LC3-II, Larrue et al show that FLT3-ITD molecules become detectable within the autophagosomes and eventually are degraded.[1] Using both genetic and pharmacological tools, they validate the involvement of key autophagy steps in the degradation of the FLT3-ITD protein, which