

Comment on Messling et al, page 245

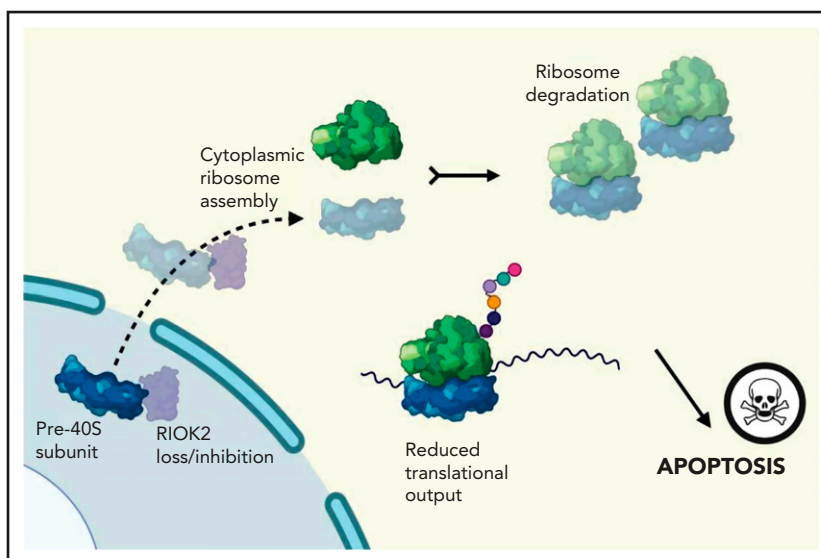
# Road to RIO-kinase 2 for AML therapy

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**In this issue of *Blood*, Messling et al<sup>1</sup> provide proof of concept that targeting the ribosome assembly factor RIO-kinase 2 (RIOK2) provides a potential therapeutic approach in acute myeloid leukemia (AML), a hematologic malignancy in which the overall long-term survival remains poor, and the mainstays of treatment have not changed significantly over the past 20 years.**

The recognition that increased ribosome synthesis is a hallmark of cancer dates to the end of the 19th century, when Pia-nese first reported that enlarged nucleoli were a characteristic feature of malignant tumor cells.<sup>2</sup> More recently, the idea that cancer cells are “addicted” to protein synthesis has stimulated efforts to exploit this as a druggable vulnerability, particularly with the realization that many chemotherapeutics suppress ribosome synthesis, and in turn, translation. However, although the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway that drives protein synthesis and translation initiation is constitutively activated in  $\leq 80\%$  of cases of AML, the results of clinical trials targeting mTOR signaling have been disappointing.

Messling et al set for themselves the important challenge of identifying new approaches to targeting protein synthesis in AML that are independent of the PI3K/AKT/mTOR pathway. Their compelling study provides several lines of evidence that RIOK2 inhibition leads to loss of protein synthesis and apoptosis in AML (see figure). Using a domain-focused, kinome-wide CRISPR screen in murine MLL-AF9-translocated leukemic cells, they identified guide RNAs targeting the gene encoding RIOK2 as being among the most depleted. Consistent with this finding, a proliferation-based screen of human AML cell lines also showed depletion of single-guide RNAs targeting RIOK2,<sup>3</sup> which is highly expressed in AML, compared with healthy bone marrow mononuclear cells.<sup>4</sup>



Loss of the ribosome assembly factor RIOK2 leads to translational stalling and ribosome degradation in leukemic cells. See Figure 6I in the article by Messling et al that begins on page 245.

The RIO (right open reading frame) kinases are a family of protein kinases found in eukaryotes and archaea that function in ribosome biogenesis. While RIOK1 and RIOK2 have been conserved throughout evolution, humans also possess a third RIO kinase, RIOK3. After their preassembly in the nucleolus where the ribosomal RNAs (rRNAs) are transcribed, the precursors of the large 60S and small 40S ribosomal subunits are exported independently to the cytoplasm to complete their maturation. The complex process of ribosome synthesis involves the concerted action of at least 200 assembly factors, including RIOK1 and RIOK2 that have essential roles in sculpting the pre-40S ribosomal subunits in the cytoplasm. RIOK1 converts the pre-40S particle into a translation-competent 40S ribosomal subunit during the final step of cytoplasmic maturation.<sup>5</sup> Because RIOK2 acts earlier in the pathway, loss of RIOK2 function blocks multiple downstream steps in 40S subunit maturation, including the release of inhibitory assembly factors, the recruitment of ribosomal proteins, and the final maturation of the rRNA.<sup>6</sup> Finally, the ATPase function of RIOK2 triggers its release from the decoding center of the ribosome,<sup>7</sup> allowing RIOK1 to bind and initiate the last step of 40S subunit maturation.

Messling et al next demonstrated that RIOK2 is essential for the proliferation of AML cells in vitro. Interestingly, they noted that leukemic cells and fibroblasts have differential sensitivities for RIOK2 loss. The molecular basis for this effect is unexplained, but the differential effect of RIOK2 depletion has also been observed in glioblastoma cancer cell lines vs normal glial cells. Interestingly, this phenomenon is reminiscent of the incompletely understood, tissue-specific phenotypes observed in the ribosomopathies, a group of disorders that are caused by diverse mutations in ribosome assembly factors or the ribosomal proteins themselves.<sup>8</sup>

Using a genetic approach in mice, Messling et al established that RIOK2 and more specifically, its ATPase function, are essential for AML maintenance in vivo. Mass spectrometry and RNA sequencing analysis revealed significant downregulation of protein synthesis, cell cycle arrest, and apoptosis in RIOK2-deficient leukemic cells, but not in fibroblasts, due to the defective translation initiation and

decreased ribosome stability. Finally, Messling et al took advantage of a small-molecule inhibitor of RIOK2 (1-[2-(2-thiazolyl)diazetyl]-2-naphthalenol) that had been found to be effective in delaying prostate cancer growth,<sup>9</sup> to show that pharmacological inhibition of RIOK2 has antileukemic effects, both in vitro and in vivo. Importantly, the RIOK2 inhibitor has no substantial cross-reactivity with RIOK1<sup>9</sup> and only mild cross-reactivity with RIOK3, which is not necessary for the proliferation of AML cells.

This exciting report from Messling et al provides proof of concept that targeting the ribosome assembly machinery may expand the range of therapeutic options in AML. An important caveat is that the RIOK2 inhibitor reduced protein synthesis in vivo for only a short time, promoting a slight, but not significant, increase in the survival of leukemic mice. However, pharmacological optimization of the compound or alternative dose regimens is likely to circumvent this issue in future.

An important concern raised by this study is whether there is merit in developing drugs targeting proteins such as RIOK2 that are encoded by "pan-essential" genes.<sup>10</sup> However, existing clinical trial data already indicate that there is a potential therapeutic window for targeting ribosome assembly in hematologic malignancies. For example, an inhibitor of the ribosome nuclear export receptor exportin-1 (selinexor) is approved for the treatment of diffuse large B-cell lymphoma and multiple myeloma, while the ribosomal DNA transcriptional inhibitor CX5461 has shown responses in myeloma and diffuse large B-cell lymphoma.<sup>11</sup> The study by Messling et al provides considerable optimism that targeting RIOK2 will provide a PI3K/AKT/mTOR-independent route to reduce protein synthesis for AML therapy.

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

## REFERENCES

- Messling J-E, Agger K, Andersen KL, et al. Targeting RIOK2 ATPase activity leads to decreased protein synthesis and cell death in acute myeloid leukemia. *Blood*. 2022; 139(2):245-255.
- Pianese G, Teucher R. Beitrag zur Histologie und Aetiologie des Carcinoms: histologische und experimentelle Untersuchungen. In: Beiträge zur

- pathologischen Anatomie und zur Allgemeinen Pathologie. Supplementhefte, 1. Jena: G Fischer; 1896.
- Tarumoto Y, Lu B, Somerville TDD, et al. LKB1, salt-inducible kinases, and MEF2C are linked dependencies in acute myeloid leukemia. *Mol Cell*. 2018;69(6):1017-1027.e6.
- Tyner JW, Tognon CE, Bottomly D, et al. Functional genomic landscape of acute myeloid leukaemia. *Nature*. 2018;562(7728):526-531.
- Plassart L, Shayan R, Montellese C, et al. The final step of 40S ribosomal subunit maturation is controlled by a dual key lock. *eLife*. 2021;10:e61254.
- Zemp I, Wild T, O'Donohue MF, et al. Distinct cytoplasmic maturation steps of 40S ribosomal subunit precursors require hRio2. *J Cell Biol*. 2009;185(7):1167-1180.
- Ameismeier M, Cheng J, Berninghausen O, Beckmann R. Visualizing late states of

- human 40S ribosomal subunit maturation. *Nature*. 2018;558(7709):249-253.
- Warren AJ. Molecular basis of the human ribosomopathy Shwachman-Diamond syndrome. *Adv Biol Regul*. 2018;67:109-127.
- Mohamed AA, Xavier CP, Sukumar G, et al. Identification of a small molecule that selectively inhibits ERG-positive cancer cell growth. *Cancer Res*. 2018;78(13):3659-3671.
- Chang L, Ruiz P, Ito T, Sellers WR. Targeting pan-essential genes in cancer: challenges and opportunities. *Cancer Cell*. 2021;39(4):466-479.
- Khot A, Brajanovski N, Cameron DP, et al. First-in-human RNA polymerase I transcription inhibitor CX-5461 in patients with advanced hematologic cancers: results of a phase I dose-escalation study. *Cancer Discov*. 2019;9(8):1036-1049.

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## MYELOID NEOPLASIA

Comment on Kemps et al, page 256

# Histiocytoses converge through common pathways

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**In this issue of *Blood*, Kemps et al<sup>1</sup> provide an expanded view of the pathological, genomic, and clinical features of anaplastic lymphoma kinase (ALK)-positive histiocytosis, and demonstrate the clinical efficacy of ALK inhibition, with durable responses in 11 of 11 patients, including 10 with central or peripheral nervous system disease.**

ALK-positive histiocytosis was first recognized by Chan et al,<sup>2</sup> who identified 3 cases of a novel form of histiocytosis presenting in early infancy and associated with systemic involvement of bone marrow, spleen, and liver. The discovery of this condition was perhaps accidental, based on the frequent involvement of ALK in one of the more common forms of T-cell lymphoma in childhood: ALK-positive anaplastic large cell lymphoma. The early reports focused on cases presenting in the pediatric age group, but expanded examination of histiocytic lesions in children and adults showed a broader spectrum of this condition, with cases in adults more often presenting with localized disease, including involvement of skin and soft tissue, breast, and gastrointestinal tract.<sup>3,4</sup> It also emerged that this condition was most often associated with a *KIF5B-ALK* fusion,<sup>5</sup> a finding confirmed in the current report.

The current report in *Blood* is the largest series reported to date and includes 39 confirmed cases. The authors have shown that ALK-positive histiocytosis is clinically heterogeneous. They divide their cases into 3 clinical cohorts. Group 1A includes cases in infants with systemic disease, similar to the original report.<sup>2</sup> Group 1B comprises children and adults with multi-system disease and frequent involvement of the central nervous system. Group 2 includes patients presenting with a single site of involvement, with some variation in age (0-41 years), but mainly presenting in infants and children (20 of 23; 87%). In comparison with prior cases reported in the literature, the current series identified fewer cases in adults, perhaps reflecting the personal interests and expertise of the authors, many of whom are experts in pediatric pathology and hematology.

The classification of histiocytic and dendritic cell neoplasms has evolved in fits