blood® Editorial

Introduction to a review series on RNA therapeutics in hematology

The last 30 years have witnessed an explosion in the successful application of protein-targeted therapeutics (eg, small-molecule inhibitors, monoclonal antibodies) in hemato-oncologic diseases. However, the number of such targets represents a small fraction of the genome. Notwithstanding exceptional examples such as tyrosine kinase inhibitors in chronic myeloid leukemia,[1] most disease targets are not druggable by small-molecule inhibitors.[2-4] By virtue of the functions that different types of RNA exert in the interactome between genes, transcripts, and proteins, RNA-targeting drugs have the potential to broaden the therapeutic arsenal for many diseases.[4,5]

Several types of RNA therapeutics have been developed and have either been approved or are in late-stage clinical testing. Antisense oligonucleotides (ASOs) consist of single-stranded oligonucleotides (12-24 nucleotides) that target a specific messenger RNA through Watson-Crick base pairing, enabling modulation of protein expression.[4-7] ASOs can alter mRNA expression through degradation mediated by enzymes such as RNase H or by ribozymes. ASOs can also downregulate or upregulate target transcripts through nonenzyme degradation pathways, which include exon skipping or inclusion, nonsense-mediated mRNA decay, inhibition or activation of translation, or blockage of microRNA (miRNA) binding to target mRNA.[4-7] Fomivirsen was the first ASO approved by the US Food and Drug Administration (FDA) in 1998 for the treatment of cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome (since discontinued because of the efficacy of highly active antiretroviral therapy).[8] Nusinersen and eteplirsen were the first examples of splice-modulating ASOs approved in 2016 for the treatment of spinal muscular atrophy and Duchenne muscular dystrophy, respectively.[9,10] In 2018, the FDA approved the ASO inotersen for the treatment of polyneuropathy in adults with hereditary transthyretin amyloidosis (ATTRv) (refer to the review article by Adams et al).[11,12]

Andrew Fire and Craig Mello were awarded the Nobel Prize in 2006 for their seminal work on RNA interference (RNAi) in the nematode worm *Caenorhabditis elegans*, published in 1998.[13] RNAi is a naturally occurring phenomenon by which double-stranded DNA induces the degradation of specific RNA targets. It is leveraged by organisms during development and for many cellular processes, including defense against viruses and transposons, by recognizing foreign pathogenic nucleotide sequences.[14]

Short interfering RNAs (siRNAs) consist of short double stranded RNAs (dsRNAs) (20-24 nucleotides) containing 5′-phosphate/3′-hydroxyl endings and two 3′-overhang ribonucleotides on each duplex strand.[4,15-19] The process of siRNA is initiated by the endoribonuclease dicer, which cleaves the dsRNA into guide (antisense) and passenger (sense) strands. The argonaute 2 protein degrades the passenger siRNA strand, and the guide siRNA strand is incorporated into the RNA–induced silencing complex, where it binds and degrades the target mRNA. miRNAs are also key components of endogenous RNAi pathways and are able to either repress or upregulate target genes, the latter through enhancing mRNA stability and/or translation.[4,20]

siRNA has become an invaluable research tool in cell cultures and organisms to interrogate how the silencing of specific genes and the proteins they encode affect normal cellular processes and disease pathobiology. This preclinical work has been foundational to the clinical development of RNAi therapeutics; as of this writing, 4 siRNA drugs have been approved: lumasiran for primary hyperoxaluria type 1,[21] and 3 included in this review series: givosiran for acute hepatic porphyria (AHP)[22,23] and patisiran[24] and vutrisiran for ATTRv.[25,26] The siRNA fitusiran, which targets antithrombin mRNA,[27,28] has completed phase 3 trials in patients with hemophilia with and without inhibitors and is under evaluation for potential regulatory approval. Additional types of RNA therapeutics include CRISPR/Cas–based RNA editing, RNA aptamers (eg, pegaptanib, approved for neovascular (wet) age–related macular degeneration in 2004),[29-31] miRNA antagonists (miRNA mimics and antagomirs, artificial circular RNA sponges),[32] and mRNA vaccines. The last are best exemplified by the FDA-approved BNT162b2 (BioNTech/Pfizer) and mRNA-1273 (Moderna) vaccines, which encode the SARS-CoV-2 spike protein.[33,34] Their safety and efficacy profiles are now well-established, and their success in the COVID-19 pandemic augurs accelerated development of mRNA vaccines for the prevention of certain types of cancer and infectious disease.

This review series on RNA targeting of hematologic disorders focuses on 3 diseases where clinical development of RNA therapeutics has advanced the farthest, either through regulatory approval or late-stage phase 3 trials: AHP, ATTRv, and hemophilia A and B. All 3 diseases remain part of the inventory of a hematology curriculum, but only hemophilia remains primarily tethered to the clinical care of

Submitted 18 September 2023; accepted 18 September 2023. https://doi.org/10.1182/blood.2023020910.

hematologists. AHP and ATTRv typically require management by teams of subspecialists, including gastroenterologists/hepatologists, neurologists, dermatologists, and cardiologists.

In this series, the following articles are included:

- Makiko Yasuda, Siobán Keel, and Manisha Balwani, "RNA interference therapy in acute hepatic porphyrias"
- David Adams, Vincent Algalarrondo, and Andoni Echaniz-Laguna, "Hereditary transthyretin amyloidosis in the era of RNA interference, antisense oligonucleotide, and CRISPR-Cas9 treatments"
- Margaret Ragni and Stephen Chan, "Innovations in RNA therapy for hemophilia"

These reviews touch on several shared themes, including (1) the unmet therapeutic needs in these conditions, (2) the disease-specific biologic rationale for application of RNA therapeutics, (3) drug development from preclinical work to late-stage registrational trials and/or regulatory approval, (4) safety issues, and (5) future directions/outstanding questions.

The clinical benefits of RNA-targeting therapies in AHP, ATTRv, and hemophilia point to the promise of this new class of therapeutics in hematologic diseases. For example, ASOs and siRNAs targeting TMPRSS6, a negative regulator of hepcidin, are being explored in clinical trials of patients with β-thalassemia and polycythemia vera.[35] Other classes of RNA-interfering drugs, such as miR-34a, the first anticancer miRNA inhibitor, demonstrate promise in the preclinical phase of development.[36] Depending on the mode of RNAi, ongoing priorities include optimization of drug delivery (eg, chemical modification, nanocarriers), vigilant monitoring for short- and long-term toxicities such as hepatotoxicity and immunogenicity, and incorporation of relevant biomarkers into trials and clinical practice. Based on the specific disease, physicians will need to determine the best fit of these novel agents alongside conventional therapies.

We hope you enjoy this review series on RNA therapeutics, which provides a glimpse of the ever-expanding armamentarium of these genomic-era medicines, which are expected to be increasingly impactful across a spectrum of nonmalignant and malignant hematologic diseases.

Jason Gotlib
Associate Editor, *Blood*

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

REFERENCES

1. Hochhaus A, Larson RA, Guilhot F, et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. *N Engl J Med*. 2017;376(10):917-927.
2. Dixon SJ, Stockwell BR. Identifying druggable disease-modifying gene products. *Curr Opin Chem Biol*. 2009;13(5-6):549-555.
3. Falese J, Donlic A, Hargrove A. Targeting RNA with small molecules: from fundamental principles towards the clinic. *Chem Soc Rev*. 2021;50(4):2224-2243.
4. Zhu Y, Zhu L, Wang X, Jin H. RNA-based therapeutics: an overview and prospectus. *Cell Death Dis*. 2022;13(7):644.
5. Crooke ST, Witztum JL, Bennett CF, Baker BF. RNA-targeted therapeutics. *Cell Metab*. 2018;27(4):714-739.
6. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics–developing a new class of drugs. *Nat Rev Drug Discov*. 2014;13(10):759-780.
7. Wang F, Zuroske T, Watts JK, Wu F, Liu L, Zhou XH. RNA therapeutics on the rise. *Nat Rev Drug Discov*. 2020;19(7):441-442.
8. Perry CM, Lamb HM. Fomivirsen. *Drugs*. 1999;58(2):375-390.
9. Nakamura A, Takeda S. Exon-skipping therapy for Duchenne muscular dystrophy. *Lancet*. 2011;378(9791):546-547.
10. Corey DR. Nusinersen, an antisense oligonucleotide drug for spinal muscular atrophy. *Nat Neurosci*. 2017;20(4):497-499.
11. Benson MD, Waddington-Cruz M, Berk JL, et al. Inotersen treatment for patients with hereditary transthyretin amyloidosis. *N Engl J Med*. 2018;379(1):22-31.
12. Keam SJ. Inotersen: first global approval. *Drugs*. 2018;78(13):1371-1376.
13. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature*. 1998;391(6669):806-811.
14. Matzke M, Matzke AJ, Kooter JM. RNA: guided gene silencing. *Science*. 2001;293(5532):1080-1083.
15. Kim DH, Rossi JJ. Strategies for silencing human disease using RNA interference. *Nat Rev Genet*. 2007;8(3):173-184.
16. Caplen NJ, Parrish S, Imani F, Fire A, Morgan RA. Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. *Proc Natl Acad Sci U S A*. 2001;98(17):9742-9747.
17. Elbashir SM, Lendeckel W, Tuschl T. RNA interference is mediated by 21- and 22- nucleotide RNAs. *Genes Dev*. 2001;15(2):188-200.
18. Castanotto D, Rossi JJ. The promises and pitfalls of RNA-interference-based therapeutics. *Nature*. 2009;457(7228):426-433.
19. Wilson RC, Doudna JA. Molecular mechanisms of RNA interference. *Annu Rev Biophys*. 2013;42:217-239.
20. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov*. 2017;16(3):203-222.
21. Scott LJ, Keam SJ. Lumasiran: first approval. *Drugs*. 2021;81(2):277-282.
22. Scott LJ. Givosiran: first approval. *Drugs*. 2020;80(3):335-339.
23. Balwani M, Sardh E, Ventura P, et al. Phase 3 trial of RNAi therapeutic givosiran for acute intermittent porphyria. *N Engl J Med*. 2020;382(24):2289-2301.
24. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med*. 2018;379(1):11-21.
25. Adams D, Tournev IL, Taylor MS, et al. Efficacy and safety of vutrisiran for patients with hereditary transthyretin-mediated amyloidosis with polyneuropathy: a randomized clinical trial. *Amyloid*. 2023;30(1):1-9.
26. Keam SJ. Vutrisiran: first approval. *Drugs*. 2022;82(13):1419-1425.
27. Young G, Srivastava A, Kavakli K, et al. Efficacy and safety of fitusiran prophylaxis in people with haemophilia A or haemophilia B with inhibitors (ATLAS-INH): a multicentre, open-label, randomised phase 3 trial. *Lancet*. 2023;401(10386):1427-1437.
28. Srivastava A, Rangarajan S, Kavakli K, et al. Fitusiran prophylaxis in people with severe haemophilia A or haemophilia B without inhibitors (ATLAS-A/B): a multicentre, open-label, randomised, phase 3 trial. *Lancet Haematol*. 2023;10(5):e322-e332.
29. Ng EW, Shima DT, Calias P, Cunningham ET Jr, Guyer DR, Adamis AP. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat Rev Drug Discov*. 2006;5(2):123-132.

30. Zhou J, Rossi J. Aptamers as targeted therapeutics: current potential and challenges. *Nat Rev Drug Discov*. 2017;16(3):181-202.

31. Adachi T, Nakamura Y. Aptamers: a review of their chemical properties and modifications for therapeutic application. *Molecules*. 2019;24(23):4229.

32. Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. *Nature*. 2013;495(7441):384-388.

33. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021;384(5):403-416.

34. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2020;383(27):2603-2615.

35. Ganz T, Nemeth E, Rivella S, et al. TMPRSS6 as a therapeutic target for disorders of erythropoiesis and iron homeostasis. *Adv Ther*. 2023;40(4):1317-1333.

36. Abdelaal AM, Sohal IS, Iyer S, et al. A first-in-class fully modified version of miR-34a with outstanding stability, activity, and anti-tumor efficacy. *Oncogene*. 2023;42(40):2985-2999.