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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Su et al, page 167

MicroRNA immunomodulating therapeutics

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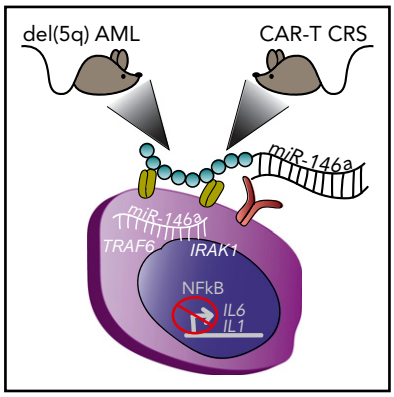
In this issue of *Blood*, Su et al[1] employ an elegant microRNA (miRNA or miR) conjugation strategy to unleash the potential of targeted miRNA therapeutics, and, as a proof of concept, effectively counteract common inflammatory and myeloid disease states.

miRNA comprise a group of small noncoding RNA that negatively regulates target mRNA stability and/or translation in a sequence-directed manner to reduce target protein expression. miRNA are highly conserved across species and involved in a wide range of normal cellular and developmental processes. miRNA exhibit remarkable cell and tissue type specificity, such that perturbations in select miRNA expression directly contribute to disease states, including inflammation and cancer. miRNA expression is predictive and prognostic in hematologic malignancies, and circulating miRNA may serve as a biomarker of minimal residual disease.



Depiction of 2 different mouse xenograft models of myeloid diseases treated with miR-146a conjugate therapy that enters myeloid cells through surface receptors. (Left) Mouse with human del(5q) AML. (Right) Mouse with human lymphoma that developed myeloid-derived CRS upon treatment with CD19 CAR-T cell therapy. Inside the myeloid cell, the delivered miR-146a targets *TRAF6* and *IRAK1*, that block NF-κB–mediated production of inflammatory cytokines *IL1* and *IL6*. del(5q), deletion of chromosome 5q.

A major goal in the field is to leverage miRNA for disease treatment by either blocking or restoring miRNA activity to normal levels, thereby relieving or imposing regulation on specific miRNA targets to counteract disease processes. Specific miRNAs with potential therapeutic benefit have been identified. A major obstacle is how to deliver miRNA-based therapies. Progress has been made in optimizing chemical modifications to nucleic acids that render RNA significantly more stable in vivo. However, bypassing liver uptake and metabolism and directing tissue or cell type specificity remain great challenges to miRNA or RNA interference–based therapeutics, particularly for hematologic diseases and malignancies.

Su et al focused their efforts on miR-146a as a therapeutic regulator to dampen NF-κB–mediated inflammatory signaling. miR-146a is a critical gene lost in del(5q) myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) that contributes to pathogenesis of these malignancies.[2] Critical targets of miR-146a function within the Toll-like receptor (TLR) signaling pathway, including IRAK1 and TRAF6, which are important regulators of NF-κB transcription factor activity.[3] The role of NF-κB in MDS and AML to date is largely context dependent; however, in the context of miR-146a loss, deregulation of IRAK1, TRAF6, and subsequent NF-κB activation leads to inflammatory cytokine production and myeloid malignancy.[4,5] In addition, NF-κB–mediated production and release of interleukin-6 (IL6) from monocytes are important factors contributing to the cytokine release syndrome (CRS) associated with chimeric antigen receptor T-cell therapy (CAR-T) therapy in B-cell lymphoma.[6,7] Remarkably, Su et al demonstrate that in vivo restoration of miR-146a expression, using a novel miR-146a conjugate discussed below, prolongs the survival of mice transplanted with human del(5q) AML cells and in lymphoma-bearing mice significantly reduces cytokine overproduction in response to CD19 CAR-T cell therapy (see figure). In vivo, miR-146a conjugate delivery significantly reduces the NF-κB signaling pathway and inflammatory cytokine production in both model systems. The significance of this work extends beyond the tested models because targeting the NF-κB pathway has potential therapeutic relevance in a variety of malignancies and diseases rooted by inflammation.

How were the in vivo challenges of uptake, specificity, and toxicity overcome in this study? Su and colleagues used a thoughtful miRNA conjugation strategy with impressive specificity for myeloid and, to a lesser extent B-cell, uptake. They selected a scavenger receptor/TLR9-targeting type A specific CpG oligodeoxynucleotide[8] to conjugate to miR-146a. Critically, this type A oligodeoxynucleotide is specific for myeloid cells, but blunted in its ability to trigger a TLR9 immune response.[9] These unique miRNA conjugate features set this study apart from other approaches.

In sum, miR-146a as a therapeutic to dampen inflammatory signaling is a novel

strategy with an exciting future. This contribution by Su et al is a crucial step forward in the development of miRNA therapies targeting myeloid diseases and provides a foundation to build upon to further refine and innovate miRNA conjugate therapeutic strategies for other cell types. This conjugate method offers several advantages, including limited or no cytotoxicity in nonmyeloid cell types, myeloid targeting, and miRNA-mediated immunomodulation. As such, this is an attractive approach for other prospective miRNA or anti-miRNA therapeutics or as a mechanistic tool for dissection of miRNA function in myeloid biology.

Conflict-of-interest disclosure: S.E.M. declares no competing financial interests. ■

REFERENCES

1. Su Y-L, Wang X, Mann M, et al. Myeloid cell-targeted miR-146a mimic inhibits NF-κB-driven inflammation and leukemia progression in vivo. *Blood*. 2020;135(3):167-180.
2. Starczynowski DT, Kuchenbauer F, Argiropoulos B, et al. Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. *Nat Med*. 2010;16(1):49-58.
3. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA*. 2006;103(33):12481-12486.
4. Boldin MP, Taganov KD, Rao DS, et al. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. *J Exp Med*. 2011;208(6):1189-1201.
5. Zhao JL, Rao DS, Boldin MP, Taganov KD, O'Connell RM, Baltimore D. NF-kappaB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. *Proc Natl Acad Sci USA*. 2011;108(22):9184-9189.
6. Norelli M, Camisa B, Barbiera G, et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat Med*. 2018;24(6):739-748.
7. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat Med*. 2018;24(6):731-738.
8. Kortylewski M, Swiderski P, Herrmann A, et al. In vivo delivery of siRNA to immune cells by conjugation to a TLR9 agonist enhances antitumor immune responses. *Nat Biotechnol*. 2009;27(10):925-932.
9. Zhang Q, Hossain DM, Nechaev S, et al. TLR9-mediated siRNA delivery for targeting of normal and malignant human hematopoietic cells in vivo. *Blood*. 2013;121(8):1304-1315.

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THROMBOSIS AND HEMOSTASIS

Comment on Jaffray et al, page 220

Diversifying study design in pediatrics

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In this issue of *Blood*, Jaffray et al report the increased association of percutaneously inserted central catheters (PICCs) with both catheter-associated venous thromboembolism (VTE) and catheter-related blood stream infection compared with tunneled central venous access devices.[1]

Their study is important for 2 reasons. First, the multicenter, prospective, observational cohort study design used by Jaffray et al enabled them to complete a pediatrics study with 1742 unique participants. As detailed in the 2006 commentary by Massicotte et al,[2] performing successful clinical trials with pediatric patients is fraught with challenges unique to this type of cohort. The recently published study by Male et al[3] that reported on the phase 3 trial of rivaroxaban vs standard of care in children with acute venous thrombosis demonstrates that these trials can in fact be completed successfully, but they remain challenging for several reasons. One of the main reasons is that thrombosis in children is increasingly recognized as a rare disease, with significant heterogeneity existing within study cohorts, as is evident in the Jaffray et al study. It is challenging to conduct a randomized trial that is able to recruit sufficient numbers of patients to mitigate this heterogeneity, even with the support of pharmaceutical sponsors. In publishing this study, Jaffray et al demonstrate that novel trial designs can successfully generate robust data with the power to inform clinical practice.

The second point, inextricably linked to the first, is that the number of participants recruited to the Jaffray et al study enabled the application of robust statistical analysis that generated findings with relatively tight confidence intervals for a study investigating thrombosis in children. There is evidence of disproportionate representation across the 2 groups in this study; children with PICC lines in situ contributed 64% of the total study population. In addition, there are differences in characteristics between the 2 groups: children with a PICC in situ tend to be older and less likely to have cancer compared with children with tunneled lines in situ. Nonetheless, the number of participants recruited across the 4 tertiary centers enabled the authors to perform meaningful analyses beginning with univariable analyses followed by a multivariable analysis.

There are some important points to note in the Jaffray study. First, the authors justify their use of Doppler ultrasonography to diagnose 94% of VTEs on the basis of the 2018 American Society of Hematology guidelines for the diagnosis of VTE.[4] The data informing that guideline is essentially derived from adult participants; studies that specifically focus on upper-limb VTE, as seen in the Jaffray study, have much smaller numbers of patients compared with studies that validate Doppler ultrasound for diagnosing lower-limb VTE. This evidence is at odds with the 2002 study by Male et al,[5] which demonstrated significant limitations in the sensitivity of Doppler ultrasound in diagnosing upper-limb VTE in children, with the exception of the jugular vessels. As with many clinical scenarios, the extrapolation of evidence generated from studies in adults needs to be applied very cautiously to pediatric populations because there are significant differences in hemostasis, thrombosis etiology, and anticoagulant response in children compared with adults. Second, Jaffray et al excluded infants younger than 6 months of age from participating in this study. Although their rationale for this exclusion is justified, it does preclude application of these findings to a cohort of pediatric patients who are significant contributors to the workload of pediatric