of attenuating adverse effects such as cytokine release syndrome or neurotoxicity, which is justified by the authors by their desire to minimize toxicity. There is a clear safety signal with this approach: primary grade 1 headache and confusion are confirmatory; however, these CARs were extremely transient and had disappeared in all patients by day 21.

The choice of CD19 as a target is similarly curious for HL. The expression of CD30 on HRS cells is ubiquitous, and the activity of brentuximab vedotin (BV), the antibody drug conjugate (ADC) against CD30, clearly shows the viability of CD30 as a therapeutic target.[6] In contrast, activity was modest in both CD30 CART studies,[4,5] suggesting that although CD30 is an appropriate target for an ADC, it may be less than optimal for immunotherapy. The high and durable response rate of the checkpoint inhibitors pembrolizumab and nivolumab in relapsed HL clearly demonstrate the activity of therapies that activate the TME rather than targeting the HRS cells directly.[7,8] However, the role of $CD19^+$ B cells in the TME is controversial, and although the rich cytokine cross talk in the TME contributes to HRS growth and survival, it is far from clear that $CD19^+$ B cells are the prime driver.

The clinical activity described for the anti-CD19 CART (CART19) in the Svoboda et al study was modest. One patient was taken off study. For 4 patients (3 of whom received bridging therapy and all of whom received conditioning chemotherapy), the response rate was 50% (1 complete response and 1 partial response), with progression at 3 months in the patient who had a complete response. The short duration of progression-free survival, even in responders, shows that this therapy is more a proof of concept than a potential novel therapy. Current approved therapies such as BV or the checkpoint inhibitors have high response rates and long response durations in responding patients. How then, can this therapy be improved upon, and what place does it have as a potential new therapy?

Rational design of CARs for relapsed HL should begin from the ground up. Because toxicity has been mild to date, design of future therapies using lentiviral vectors should be considered, potentially adding a molecular suicide option which could provide a safety valve. To date, neither CD19 nor CD30 has proved to be a clear winner as a therapeutic target for HL CARs. But as the data for the combination of checkpoint inhibitors and BV clearly show, dual CARS targeting both the HRS cells and immune activation of the TME might overcome this. Perhaps CD19 is the ideal target for cotargeting with CD30. However, macrophage or natural killer cell targets should also be considered. The question of the optional target(s) in HL remains to be answered. For CARTs to find a place among therapies for relapsed HL, they will need to do this.

In summary, the article by Svoboda et al provides a proof of concept for the safety and feasibility of a nonviral CART19 in relapsed HL. Toxicity is mild, but with a viral vector and greater CAR persistence, will it increase? Activity of the CART19 in relapsed HL is modest and does not appear to be durable. Although the data for checkpoint blockade plus BV or chemotherapy will set a high bar for therapies in relapsed HL,[9,10] there remains a population of patients who are relapsed or refractory to standard therapies, to BV, and to checkpoint inhibitors. For these young patients who have no viable therapeutic options, these new CARs cannot come down the road soon enough.

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REFERENCES

1. Svoboda J, Rheingold SR, Gill SI, et al. Nonviral RNA chimeric antigen receptor–modified T cells in patients with Hodgkin lymphoma. *Blood*. 2018;132(10):1022-1026.
2. Steidl C, Connors JM, Gascoyne RD. Molecular pathogenesis of Hodgkin's lymphoma: increasing evidence of the importance of the microenvironment. *J Clin Oncol*. 2011;29(14):1812-1826.
3. Marshall NA, Christie LE, Munro LR, et al. Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood*. 2004;103(5):1755-1762.
4. Ramos CA, Ballard B, Liu E, et al. Chimeric T cells for therapy of $CD30^+$ Hodgkin and non-Hodgkin lymphomas [abstract]. *Blood*. 2015;126(23). Abstract 185.
5. Wang CM, Wu ZQ, Wang Y, et al. Autologous T cells expressing CD30 chimeric antigen receptors for relapsed or refractory Hodgkin lymphoma: An open-label phase I trial. *Clin Cancer Res*. 2017;23(5):1156-1166.
6. Younes A, Gopal AK, Smith SE, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. *J Clin Oncol*. 2012;30(18):2183-2189.
7. Younes A, Santoro A, Shipp M, et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. *Lancet Oncol*. 2016;17(9):1283-1294.
8. Chen R, Zinzani PL, Fanale MA, et al. Phase II study of the efficacy and safety of pembrolizumab for relapsed/refractory classic Hodgkin lymphoma. *J Clin Oncol*. 2017;35(19):2125-2132.
9. Diefenbach CS, Hong F, David KA, et al. A phase I study with an expansion cohort of the combination of ipilimumab and nivolumab and brentuximab vedotin in patients with relapsed/refractory Hodgkin lymphoma: A trial of the ECOG-ACRIN Cancer Research Group (E4412 Arms D and E) [abstract]. *Blood*. 2016;128(22). Abstract 1106.
10. Herrera AF, Moskowitz AJ, Bartlett NL, et al. Interim results of brentuximab vedotin in combination with nivolumab in patients with relapsed or refractory Hodgkin lymphoma. *Blood*. 2018;131(11):1183-1194.

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LYMPHOID NEOPLASIA

Comment on Morelli et al, page 1050

A miRaculous new therapy in myeloma?

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In this issue of *Blood*, Morelli et al describe a novel method for targeting cancers with dysregulated c-MYC (MYC), such as multiple myeloma, by inhibiting the micro-RNA 17-92 (miR-17-92) cluster through degradation of its precursor RNA.[1]

Early work by Evan and colleagues initially established that dysregulation of MYC could sensitize cells to apoptosis, providing evidence that overcoming apoptosis is required for cells to survive the inappropriate proliferation induced

during oncogenesis.[2] We now know that MYC dysregulation results in the induction of apoptosis in part through the upregulation of the pro-apoptotic BCL2 family member, BIM (BCL2L11).[3] Cancer cells survive by neutralizing BIM through binding by antiapoptotic members of the BCL2 family including BCL2, BCL-XL (BCL2L1), and MCL1, the latter of which can adapt to changes in BIM levels through stabilization.[4] Additionally, although MYC can induce BIM transcription, expression is also regulated posttranscriptionally by miRs of the miR-17-92 cluster.[5]

Expression of the miR-17-92 cluster is upregulated in a variety of cancers and has been previously associated with poor prognosis in myeloma,[6] a finding verified in this report. The cluster consists of 6 related mIRs encoded from a polycistronic long, noncoding RNA (MIR17HG transcript variant 1, also known as pri-miR-17-92).[5] Given its apparent role in oncogenesis and ability to regulate the expression of proapoptotic molecules such as BIM, the ability to modulate the miR-17-92 cluster to restore apoptotic function makes it an attractive therapeutic target. However, this has been challenging because the 6 miRs in the cluster have overlapping target specificity, decreasing the utility of targeting single miRs.[5] To overcome this issue, the authors took advantage of a novel approach to target nuclear RNAs. Specifically, they designed antisense oligonucleotides against MIR17HG to target the precursor RNA for RNaseH-dependent degradation. The oligonucleotides were modified to block degradation as well as increase binding stability to RNA through the inclusion of locked nucleic acids at the 5′ and 3′ ends. Interestingly, they were also able to demonstrate that these modified oligonucleotides (called LNA gapmeRs) were able to enter and accumulate in cells in a transfection-free process referred to as gymnosis.[7]

The authors went on to test a panel of LNA gapmeRs and focused on 1 (called MIR17PTi for MIR17 precursor transcript inhibitor) that was able to deplete MIR17HG as well as subsequent processed miRs. Importantly, the authors demonstrated that this occurred in an RNaseH-dependent fashion. They next tested the effects of MIR17PTi on a panel of 48 cancer cell lines and 5 nontransformed lines and although sensitivity was observed in most tumor types tested, hematological malignancies, especially myeloma lines, were most sensitive. This result is consistent with previous studies demonstrating that MYC is the most commonly dysregulated gene in myeloma patients and that MYC translocations are found in the vast majority of myeloma cell lines.[8] MIR17PTi was more effective than targeting individual mIRs from the cluster. In contrast, targeting all miRs simultaneously had similar activity as MIR17PTi, demonstrating the need to block the expression of all miRs in the cluster. Importantly, activity was also demonstrated in freshly isolated patient samples and could overcome the protective effects of coculture with bone marrow stromal cells. Additional mechanistic studies confirmed that MYC upregulation sensitized cells to MIR17PTi and that induction of BIM was important for activity. However, loss of BIM had only a partial effect on MIR17PTi activity, suggesting that additional factors or pathways targeted by the cluster are involved in MIR17PTi-induced cell death. In this regard, it is worth noting that the miR-17-92 cluster has also been shown to regulate PTEN/phosphatidylinositol 3-kinase, NF-κB, and p21, which might also play a role in the observed phenotypes.[5] Moreover, derepression of BIM would not be expected to induce cell-cycle arrest or senescence, yet these outcomes were also observed in cells treated with MIR17PTi.

The final studies point to the potential for translation of MIR17PTi. First, the authors demonstrated that MIR17PTi can work in combination with commonly used myeloma drugs such as melphalan, dexamethasone, and bortezomib. Importantly, MIR17PTi was active in blocking tumor growth in vivo in multiple xenograft models. Finally, a potential concern regarding the ability to deliver an oligonucleotide-based therapy was addressed by pharmacokinetic data in *Cynomolgus* monkeys provided and, although no pathology data are shown, the authors reported that toxicity was not observed.

Additional questions remain regarding the use of MIR17PTi. Would this combine with other drugs used in myeloma therapy? The use of IMiDs such as lenalidomide with MIR17PTi was not tested; however, it would be interesting to determine if this combination is active. The direct cellular activity of IMiDs is associated with downregulation of MYC, which could potentially antagonize the effects of MIR17PTi. Similarly, BET domain inhibitors also regulate MYC expression in myeloma[9]; however, previous studies have suggested that the BRD4 inhibitor, JQ1, kills in part by upregulating BIM through inhibition of miR-17-92 expression in hematopoietic tumors.[10] Therefore, additional tests are warranted. Use of MIR17PTi in combinations in vivo will also be of interest. Of course, challenges remain because oligonucleotide approaches for targeting BCL2 showed preclinical promise but were not successful in the clinic; thus, additional pharmacodynamic and toxicity studies beyond the initial pharmacokinetic experiments presented in the current paper are required.

These studies point to a potentially new therapeutic approach in myeloma that exploits a synthetic lethality created by the most common genetic alteration in this disease. It will be interesting to follow the progress of this approach in the clinic because it represents a new twist on targeting undruggable targets. LNA gapmeRs could be used to degrade the RNAs of not only primary transcripts of miR clusters, but also of other noncoding RNAs where a protein target is not available. Additionally, they could be used to target the messenger RNA of proteins where small molecule targeting has been elusive. This could be more efficient than proteolysis targeting chimera approaches because developing oligonucleotides may be easier than the targeting molecules for protein degradation.

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REFERENCES

1. Morelli E, Biamonte L, Federico C, et al. Therapeutic vulnerability of multiple myeloma to MIR17PTi, a first-in-class inhibitor of pri-miR-17-92. *Blood*. 2018;132(10):1050-1063.
2. Evan GI, Wyllie AH, Gilbert CS, et al. Induction of apoptosis in fibroblasts by c-myc protein. *Cell*. 1992;69(1):119-128.
3. Hemann MT, Bric A, Teruya-Feldstein J, et al. Evasion of the p53 tumour surveillance network by tumour-derived MYC mutants. *Nature*. 2005;436(7052):807-811.
4. Conage-Pough JE, Boise LH. Phosphorylation alters Bim-mediated Mcl-1 stabilization and priming. *FEBS J*. 2018;285(14):2626-2640.
5. Mogilyansky E, Rigoutsos I. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ*. 2013;20(12):1603-1614.
6. Gao X, Zhang R, Qu X, et al. MiR-15a, miR-16-1 and miR-17-92 cluster expression are linked to poor prognosis in multiple myeloma. *Leuk Res*. 2012;36(12):1505-1509.

7. Stein CA, Hansen JB, Lai J, et al. Efficient gene silencing by delivery of locked nucleic acid antisense oligonucleotides, unassisted by transfection reagents. *Nucleic Acids Res.* 2010;38(1):e3.

8. Affer M, Chesi M, Chen WG, et al. Promiscuous MYC locus rearrangements hijack enhancers but mostly super-enhancers to dysregulate MYC expression in multiple myeloma. *Leukemia.* 2014;28(8):1725-1735.

9. Delmore JE, Issa GC, Lemieux ME, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell.* 2011;146(6):904-917.

10. Xu Z, Sharp PP, Yao Y, et al. BET inhibition represses miR17-92 to drive BIM-initiated apoptosis of normal and transformed hematopoietic cells. *Leukemia.* 2016;30(7):1531-1541.

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THROMBOSIS AND HEMOSTASIS

Comment on Wu et al, page 1075

ADAMTS-13 in traumatic brain injury?

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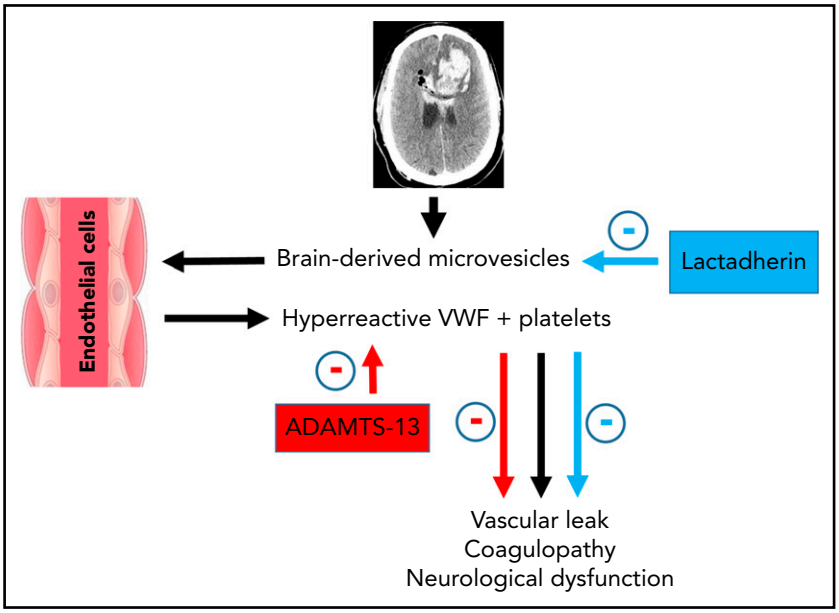
In this issue of *Blood*, Wu et al report that following traumatic brain injury (TBI) von Willebrand Factor (VWF) is released, binds to microvesicles, and results in vascular leakage and coagulopathy, findings that were reversed by a disintegrin and metalloprotease with thrombospondin (ADAMTS-13).[1]

In the United States, TBI is a leading cause of death among children and adults.[2] Each year, ~1.5 million Americans sustain a TBI, with a mortality as high as one-third in patients with severe TBI.[3] Additionally, when coagulopathy accompanies severe TBI, it is an independent predictor of poor prognosis.[4,5] The pathophysiological mechanisms of coagulopathy are poorly defined and not well understood. Both hyper- and hypocoagulable states have been identified, each leading to opposite but deleterious outcomes. A hypercoagulable state can lead to brain ischemia from microthrombosis while hypocoagulopathy can result in hemorrhage with worsening of the brain injury. Coagulopathy had been attributed to release of tissue factor from the injured brain, but it is now understood that the mechanisms are much more complex. The authors previously provided novel insight into this complex pathway and reported that brain-derived microparticles are released from the injured brain into the systemic circulation and induce an early hypercoagulable state that is quickly followed by consumptive coagulopathy.[6]



Overview of the pathway for VWF and brain-derived microparticle-induced vascular leakage and coagulopathy with reversal by ADAMTS-13. Following TBI, mivrovesicles are released from the injured brain and bind to hyperreactive VWF released from endothelial cells. In the presence of activated platelets, VWF-bound microvesicles result in vascular leakage, coagulopathy, and neurological dysfunction. These pathologic processes can be reversed by either ADAMTS-13, which blocks VWF reactivity, or lactadherin, which promotes clearance of the microvesicles.

In the current article, the authors hypothesized that VWF promotes TBI-induced and microvesicle-mediated blood-brain disruption and coagulopathy and that ADAMTS-13 protects endothelial integrity and prevents TBI-induced consumptive coagulopathy (see figure). VWF is an adhesive glycoprotein synthesized in megakaryocytes and endothelial cells and plays a role in hemostasis by recruiting platelets to the site of vessel injury.[7] It is stored in Weibel-Palade bodies in endothelial cells and in α granules of platelets and is released into the circulation following trauma. The activity of VWF is modulated by ADAMTS-13, a metalloprotease that cleaves the large procoagulant VWF multimers once released into the circulation.[8] Although low levels of VWF result in a bleeding diathesis, deficiency of ADAMTS-13 is associated with occlusive diseases such as myocardial infarction and stroke.

The authors investigated the protective effects of exogenously administered ADAMTS-13. To do so, they used their fluid percussive mouse brain injury model to confirm both brain and distant organ (lung) vascular leakage and bleeding as well coagulopathy following injury. They then demonstrated increased activity and adhesive levels of circulating VWF. In vitro, they were able to verify their previous findings that brain-derived microvesicles were responsible for vascular leakage by adding the microvesicle fraction of plasma from brain-injured mice to cultured endothelial cells. To assess the role of VWF, they added blocking antibody, and leakage was reduced. Interestingly, purified VWF in the absence of microvesicles had no effect on leakage.

When recombinant ADAMTS-13 (rADAMTS-13) was administered prior to injury, not only were vascular leakage and coagulopathy