price of ECU, which is still unaffordable for more than half of the world. One might hope that, for once, competition will bring prices down.

With the advent of these new agents, the issue of monotherapy vs combination therapy is here with us. So far, most data are from patients who are already on ECU (or ravulizumab), but with DNC as a single agent, the distal pathway is already largely disabled (see figure, panel D). It seems reasonable that you do not need to use your foot brake when the handbrake is already on. The two brakes are not uniformly equivalent because with DNC and IPT the classic pathway C5 convertase can still be formed (see figure, panel D); however, the patients themselves will prefer one regular regimen rather than two.

A typical adverse event in the management of a patient with PNH is the so-called "breakthrough IVH"; on intravenous ECU, it can occur when massive complement activation (eg, from intercurrent infection) displaces ECU from C5. In a patient who is on an oral or subcutaneous drug (eg, ITP, DNC, APL2), it may occur simply because the patient has skipped a couple of doses. Having looked after patients with PNH for some decades before ECU,[2] when massive hemolytic attacks were not infrequent (and I have never lost a patient from one), I would not be too concerned by an occasional minor breakthrough IVH. However, the proportion of PNH red cells is usually <50% in untreated patients, in whom the total red cell mass is reduced as a result of anemia. In patients who are on complement-blockade therapy, instead, the red cell mass may be normal, and the proportion of PNH red cells may be >90%; thus, the complement-sensitive red cell population is much larger, and, therefore, the threat to life from a hemolytic attack is greater. Three things will be crucial: (1) dosage and pharmacokinetics, as it is not clear that they have been optimized for any of the upstream drugs; (2) very strict adherence to the schedule, demanding as it may be; and (3) one must have a protocol to deal with a sudden hemolytic contingency. An antibody, compared with most small molecules, has the advantage of a long in vivo half-life; for any patient on alternative pathway monotherapy, I recommend always having a dose of ECU on hand for emergency use.

Finally, I recall that when I saw my first patient with PNH I could only offer supportive treatment; I feel humbled because today we have the luxury of keenly debating which of several sophisticated targeted medicines is best. I am grateful for all I have learned from patients with PNH in 5 countries; we owe it to them to use each of these medicines optimally and safely, and we must make them available to all patients who may benefit.

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HEMATOPOIESIS AND STEM CELLS

Comment on Wang et al, page 1939

Upping the antizyme: AZIN1 directs stem cell fate

Luisa Ladel and Catriona H. M. Jamieson | University of California, San Diego

In this issue of *Blood*, Wang et al[1] report that adenosine-to-inosine (A-to-I) RNA editing of antizyme inhibitor 1, *Azin1*, is a novel regulator of hematopoietic cell fate, capable of influencing self-renewal and differentiation at the stem cell level.

Hematopoietic stem cells have served as one of the main experimental models for decades.[2] The study of normal tissue-specific stem cells and the exploration of the capacity of cancer stem cells to self-renew and induce metastasis have been based on the biology of hematopoietic stem cells. Therefore, dissecting the regulatory networks that maintain stem cell function, such as quiescence, self-renewal, and differentiation, as Wang et al elegantly demonstrate, is both timely and impactful.

Enzymatic editing of RNA, and specifically A-to-I editing of *AZIN1*, has been implicated in the progression and therapeutic

resistance of a wide range of malignancies.[3-8] Specifically, increased levels of RNA editing of *AZIN1*, mediated by adenosine deaminase acting on RNA-1 (ADAR1), have been shown to lead to disease progression and higher tumor-initiating potential in hepatocellular carcinoma.[3] Similarly, both ADAR1 expression and *AZIN1* RNA editing levels were found to be significantly elevated in colorectal and gastric cancers compared with healthy mucosa.[4,5] In addition, hyper-editing of *AZIN1* has been demonstrated to be a prognostic factor for survival and metastasis in both colorectal and gastric cancers.[4,5] In non–small cell lung cancers, RNA editing of *AZIN1* accelerated cell proliferation and promoted tumor cell migration.[6] In malignant hematopoiesis, we and others discovered increased ADAR1-mediated editing of *AZIN1* and other transcripts as part of an RNA editing cancer stem cell signature that was associated with disease progression in myeloproliferative neoplasms, chronic myeloid leukemia, and multiple myeloma.[7-10] In addition, deregulated RNA editing via ADAR1 promotes transformation of pre-leukemia stem cells into leukemia stem cells and increases malignant self-renewal capacity.[10] Thus, RNA editing of *AZIN1* has been implicated in conferring "stemness" to pre-malignant progenitor cells[5] and warrants further investigation in its role in stem cell biology.

In the current study, Wang et al find translocation of *Azin1* protein, AZI, from the cytoplasm into the nucleus on editing.[1] The authors identify the nuclear interaction of AZI with DEAD box polypeptide 1, DDX1, as key to the regulatory network governing hematopoietic stem cell maintenance. Moreover, they elucidate the A-to-I editomes for 12 murine adult hematopoietic cell populations, which is an important dataset and resource for future studies. After identifying *Azin1* as a stage-specific editing site exclusive to hematopoietic stem and progenitor cells, the authors reveal the importance of RNA editing and *Azin1* to hematopoietic stem cell regulatory gene expression and protein function through a series of in vitro and in vivo assays. Next, the authors perform immunoprecipitation and mass spectrometry, identifying DDX1 as one of the top 10 specific interacting proteins when comparing edited and nonedited *Azin1*. The interaction between AZI and DDX1 is specific to the nuclear location of edited AZI. Using chromatin immunoprecipitation, the authors show that AZI and DDX1 are involved in the transcriptional regulation of hematopoietic stem cells via genes bound by both proteins simultaneously, such as *Plaur*, *Tlr2*, and *Plxnc1*. In several in vitro knockdown and overexpression experiments, the functional importance of the AZI–DDX1 interaction to the self-renewal capacity of hematopoietic stem cells was shown. The editing of *Azin1*, resulting in nuclear translocation, enables binding to DDX1, which in turn induces further regulatory gene expression, leading to differentiation of hematopoietic stem cells and successful lineage reconstitution. In hematopoietic stem cells with nonedited *Azin1*, AZI remains in the cytoplasm and is therefore unable to bind to DDX1, resulting in failed reconstitution.

The main limitation of this study is the exclusive use of murine model systems, whereas ADAR1-mediated editing is predicated primarily on the presence of primate-specific Alu sequences. Future studies will need to confirm these data in human primary hematopoietic stem and progenitor cells. Moreover, there is no investigation into the effect of differential ADAR1 expression levels on A-to-I editing of *Azin1* or effects of edited *Azin1* on ADAR1 expression levels regarding a possible feedback loop mechanism. Nonetheless, the mechanistic insights into A-to-I edited *Azin1* and its regulatory impact on stem cell function are novel and will facilitate future studies. Additionally, these data may inform the development of novel therapeutics targeting edited *Azin1* or other downstream regulators. Future studies might focus on the downstream regulators the authors touch on in their last figure, such as *Plaur* and others, to investigate potentially druggable targets and to glean further understanding of major regulatory networks that AZI and DDX1 might govern.

Overall, this study demonstrates the vital role of ADAR1-mediated RNA editing of Azin1 in the maintenance of hematopoietic stem cell function, thereby setting the stage for developing RNA editing–targeted therapeutics for stem cell expansion and conversely preventing AZIN1-induced cancer stem cell generation by inhibiting this process when deregulated.

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