

ongoing studies and provides a rich set of new questions to investigate in the ongoing effort to reduce the threat of RT to our CLL patients.

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## MYELOID NEOPLASIA

Comment on Surka et al, page 661

# Targeting cereblon in AML

Steven Grant | Virginia Commonwealth University

**In this issue of *Blood*, Surka et al<sup>1</sup> describe the identification and characterization of the novel cereblon-related E3 ligase modulator (CC-90009). Although cereblon-interacting agents (eg, immunomodulatory drugs; IMiDs) have established activity in multiple myeloma and myelodysplastic syndromes, these findings could have significant implications for the treatment of acute myelogenous leukemia (AML).**

This group reported that CC-90009 coopts the cereblon (CBRN) ring ligase 4, leading to enhanced ubiquitination and subsequent degradation of the translation termination protein GSPT1, culminating in AML cell death. They further demonstrated that GSPT1 depletion plays a significant functional role in the anti-AML activity of CC-90009 and that this agent effectively eradicated AML stem cells in multiple AML patient-derived xenograft models. Mechanistically, the investigators used a genome-wide CRISPR-Cas9 screen

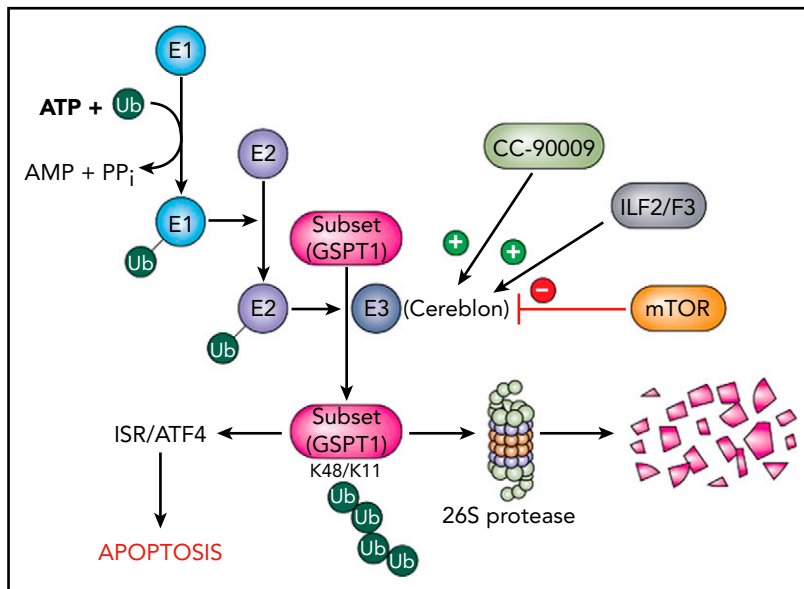
to identify the ILF2/3 heterodimeric complex and the mTOR pathway as key regulators of CC-90009 activity. Finally, they showed that engagement of the ATF4-related integrated stress response (ISR) played a key functional role in antileukemic actions. Given that CC-90009 is currently undergoing phase 1 evaluation in AML (NCT02848001), such insights could have important clinical implications for AML therapy.

This study represents an extension of the investigators' previous article in which

they described the precursor CBRN modulator CC-885, which also targeted GSPT1, as well as multiple other substrates.<sup>2</sup> The clinical development of this agent was hampered by multiple factors, including toxicity, presumably due to lack of specificity, as well as uncertainty regarding the mechanism(s) by which it triggered cell death. In contrast, CC-90009 is highly specific for GSPT1 degradation, raising the possibility that the absence of off-target toxicity could make this agent a superior clinical candidate compared with its predecessor.

CC-90009 is a member of an expanding class of antitumor agents exhibiting the ability to modulate the disposition of critical protein substrates by perturbing degradative pathways. Several of these agents converge upon ubiquitin E3 ligases that are responsible for K48/K11 ubiquitination of proteins, a process that targets them for degradation by the ubiquitin-proteasome system (UPS).<sup>3</sup> However, modulation of the UPS can lead to enhanced degradation or accumulation of proteins, depending upon context. For example, proteasome inhibitors block the degradation of diverse proteins, including IκBα, leading to inactivation of the cytoprotective NF-κB pathway.<sup>4</sup> Alternatively, proteolysis targeting chimeras (PROTACS) selectively eliminate oncogenic proteins by directly targeting them for E3 ubiquitin ligase-mediated degradation.<sup>5</sup> NEDD8 inhibitors act by preventing neddylation and activation of E3 ligases, leading to accumulation of diverse proapoptotic proteins.<sup>6</sup> Interest in CBRN as a therapeutic target was catalyzed by the observation that IMiDs (eg, lenalidomide) act, at least in part, in myeloma cells by modulating CBRN function to increase ubiquitination and degradation of important survival proteins (eg, the zinc-finger transcription factors IKZF1/3).<sup>7</sup> Moreover, the efficacy of IMiDs in del(5q) myelodysplastic syndrome has been attributed to CK1α depletion.<sup>8</sup> However, the basis by which E3 ligase modulators might induce cell death in AML is essentially unknown.

Using a CRISPR-Cas screen, the investigators identified 3 new genetic pathways that influenced the ability of CC-90009 to disrupt cereblon-related events and, by extension, antileukemic activity. First, they found that modulators of RNA alternative splicing, such as ILF2/3, diminished cereblon expression and reduced CC-90009 activity. Second, they discovered that



Schema of cereblon/CC-90009 interactions in the context of the UPS. Ubiquitin (Ub) is activated by a ubiquitin-activating enzyme (E1) and transferred to a ubiquitin-conjugating enzyme (E2) prior to linkage to protein substrates by a ubiquitin ligase (E3) complex, of which cereblon is a key component. The K48/K11 ubiquitination of substrates targets them for degradation by the 26S proteasome. CC-90009 promotes binding of cereblon to the translation termination protein GSPT1, which leads to the selective elimination of this protein. Loss of GSPT1 in AML cells triggers the ISR via ATF4, culminating in cell death. These events are opposed by loss of the alternative splicing modulators ILF2/ILF3, which diminish cereblon expression, as well as by hyperactivation of the mTOR pathway, which blocks GSPT1 degradation.

activation of the mTOR signaling pathway antagonized GSPT1 degradation and attenuated CC-90009-mediated cell killing. Finally, they demonstrated that CC-90009 exposure resulted in induction of various endoplasmic reticulum stress response elements (eg, ATF4) and that genetic ablation of these factors reduced CC-90009 efficacy. Importantly, they made the novel observation that GSPT1 degradation was necessary and sufficient to account for CC-90009 activity. Such findings provide a foundation for understanding the molecular basis for CC-90009 actions. A schematic diagram summarizing these observations and placing the CC-90009/cereblon/GSPT1 link within the context of the protein-degradative pathway is provided (see figure).

Collectively, these findings furnish a theoretical foundation for adding a cereblon modulator, such as CC-90009, to the therapeutic armamentarium for AML treatment, as well as suggest a number of possibilities for optimizing its use in the future. Furthermore, the mechanistic insights described in their article could help to identify biomarkers, allowing individualized CC-90009 treatment. For example, inasmuch as genetic ablation of interleukin-F2/F3 attenuated CC-90009

efficacy, it is conceivable that cells with high basal expression of these RNA alternative splicing genes might be particularly sensitive to this agent. Analogously, cells with high expression of GSPT1 may be addicted to this translation termination protein and, as a consequence, be particularly vulnerable to its loss following cereblon modulation-mediated degradation. Additionally, the observation that hyperactivation of the mTOR pathway attenuated CC-90009 activity by antagonizing GSPT1 degradation suggests that cells in which this pathway is intrinsically activated may display CC-90009 resistance. Answers to these questions await further preclinical investigation; results emerging from ongoing trials of CC-90009 in AML could be informative.

The present observations could also provide insights into rational combination therapies involving cereblon modulators, such as CC-90009, in AML and potentially other malignancies. For example, given that intrinsic activation of the mTOR pathway significantly reduced CC-90009 activity, it would be logical to explore the possibility that mTOR inhibitors, such as rapamycin,<sup>9</sup> might synergize with CC-90009 and/or overcome resistance to this agent. Preclinical studies confirming or

refuting this possibility could easily be conducted.

Finally, the present study, while providing significant information about the mechanism of action of CC-90009, leaves a number of questions to be addressed. For example, the mechanism by which GSPT1 depletion induces the ISR and triggers cell death in AML cells remains to be defined. In addition, it still needs to be determined whether an agent, such as CC-90009, that induces protein degradation by modulating E3 ligase activity and selectively reduces GSPT1 abundance will prove superior to PROTACs, which exhibit similar selectivity. Will the specificity of CC-90009 toward GSPT1 be associated with diminished toxicity in humans? What mechanisms are responsible for intrinsic or acquired resistance to this agent? Given the current degree of interest in modulators of protein degradation in AML and other hematopoietic malignancies, it is very likely that answers to these questions will be forthcoming shortly.

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## TRANSFUSION MEDICINE

Comment on McVey et al, page 690

# Chewing the fat on TRALI

Simon J. Cleary and Mark R. Looney | University of California, San Francisco

**In this issue of *Blood*, McVey et al<sup>1</sup> identify storage-induced disturbances, specifically increased frequency of platelet extracellular vesicles (EVs), and sphingolipid imbalances in EVs, which together predispose to transfusion-related acute lung injury (TRALI).**

Blood transfusions are life-saving interventions, but they occasionally harm recipients by causing a variety of transfusion reactions. In TRALI, the transfused blood product triggers an inflammatory response in the pulmonary vasculature of the recipient. The potentially fatal consequence is acute respiratory failure due to fluid gushing out of the bloodstream and into the lungs through leaks in the endothelial wall.<sup>2</sup>

Recipient-reactive alloantibodies in blood products are a major cause of TRALI, and

reducing the quantity of these pathogenic antibodies in plasma-rich blood products has been a successful strategy for reducing TRALI incidence.<sup>3</sup> However, transfusions continue to cause TRALI, sometimes in the absence of detectable alloantibodies in the blood product. Indeed, in a prospective study on the incidence of TRALI and its risk factors, the presence of alloantibodies explained only about one-half of the identified TRALI cases.<sup>4</sup>

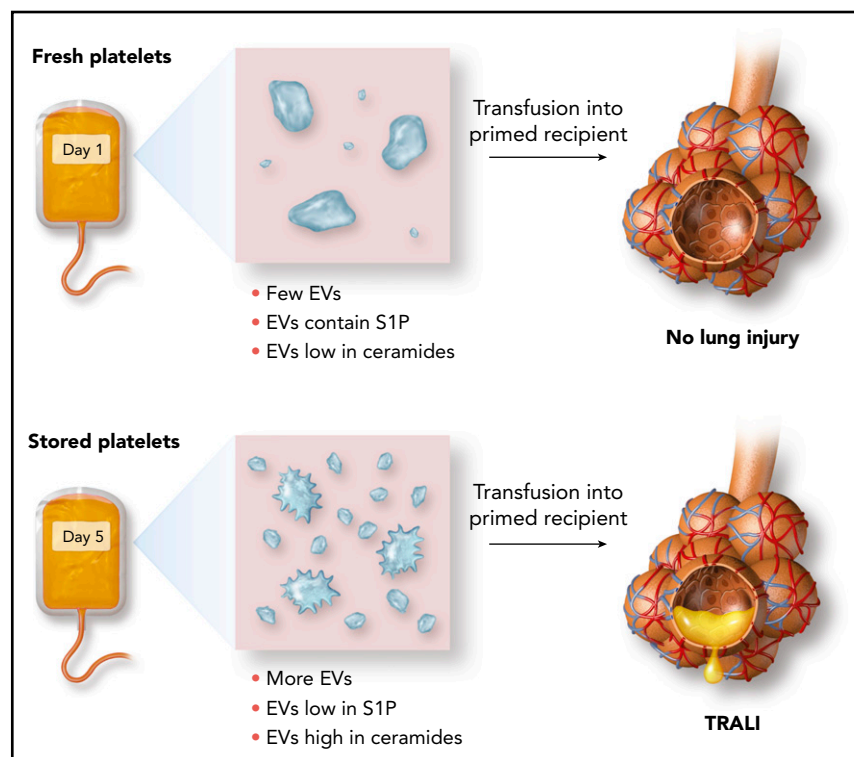
The suspected causative agents of "non-antibody-mediated" TRALI cases are

bioactive lipids, which may be suspended in the plasma or storage medium of cellular blood products in the form of EVs.<sup>5</sup> During storage in blood banks, these fat-laden EVs are released from both red blood cells and platelets. Recent studies have identified that EVs arising from "storage lesions" likely act as shuttles, which traffic bioactive lipids to the pulmonary endothelium to cause TRALI. However, compared with antibody-mediated TRALI, the mechanistic basis of lung injury provoked by EVs and bioactive lipids has been more elusive.

Here, McVey and colleagues identify an EV lipid imbalance pathway through which transfusions of platelets approaching their "use by date" can lead to pulmonary endothelial injury. During storage, the quantity of EVs released from mouse or human platelets increases. Over a period of several days, the balance of sphingolipids contained in these platelet EVs shifts, with an increase in long-chain ceramides and a decrease in sphingosine-1-phosphate (S1P) (see figure). Because ceramides can cause pulmonary endothelial injury and S1P has endothelial barrier protective properties, this switch in the EV "sphingolipid rheostat" is a plausible mechanism for TRALI that results from the transfusion of aged platelets.

Using a 2-hit mouse model of stored platelet-mediated TRALI in combination with studies of human platelets and endothelial cells, McVey et al also demonstrate approaches to prevent this potentially harmful outcome of platelet transfusions. Endothelial injury caused by stored platelets could be reduced by: (a) washing platelets to remove EVs, (b) preventing ceramide-dependent EV production by blocking its production from sphingomyelin by acid sphingomyelinase, and (c) adding exogenous S1P to counteract loss of S1P from aged platelet EVs. Of these approaches, washing of stored platelets is potentially attractive, as washing is occasionally done in clinical practice to mitigate certain severe, nonhemolytic transfusion reactions, and clinical trials are already underway to test whether washing red blood cells can reduce incidence of transfusion reactions.<sup>6</sup> However, platelets are highly sensitive to manipulation, so the difficulty of safely exchanging platelet buffer solutions may make implementation of this strategy challenging.

As demonstrated in this article, platelet-derived signaling moieties can cause lung injury, but platelets can also play



Effect of storage on the release of platelet EVs and their ceramide and S1P content and susceptibility to TRALI.