New precision medicine weapons for targeted treatment of high-risk B-cell precursor acute lymphoblastic leukemia

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In this issue of *Haematologica*, Vervoort *et al.*[1] demonstrate that deletion of *IKZF1* (*IKZF1*-del) in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) promotes resistance to cytarabine (AraC). They further show that inhibition of either cyclin-dependent kinases 8/19 (CDK8/19) or casein kinase 2 (CK2) restores the sensitivity of *IKZF1*-del BCP-ALL to AraC treatment.

BCP-ALL is the most common childhood malignancy. Despite improved outcomes, more children die from BCP-ALL than from any other type of malignancy. In adults, BCP-ALL is an aggressive disease with high mortality and requires very toxic treatment. Advances in targeted chemotherapy along with immunotherapy have greatly improved the overall prognosis of BCP-ALL in both children and adults.[2,3] However, relapsed BCP-ALL remains a clinical challenge, and is associated with high mortality.[4] *IKZF1* encodes IKAROS, a master regulator of hematopoiesis and a tumor suppressor protein.[5,6] Deletion of a single copy of *IKZF1* results in high-risk BCP-ALL, which very often has a gene expression profile that is characteristic of Philadelphia chromosome-like BCP-ALL, which is associated with a high relapse rate and a poor outcome.[7] Resistance to chemotherapy is commonly observed in BCP-ALL with *IKZF1*-del.[8] Several mechanisms have been proposed to explain these observations, along with therapeutic strategies to overcome resistance to particular chemotherapeutics.[9]

To understand the mechanism of chemoresistance in BCP-ALL due to *IKZF1* deletion, Vervoort *et al*. used both a genetic (by CRISPR/Cas9) and pharmacological (administration of iberdomide) approach to target Ikaros. They tested IKAROS-null BCP-ALL cells for sensitivity to common chemotherapeutics and showed that the absence of *IKZF1* is strongly associated with increased resistance to AraC. The causative role of *IKZF1* deletion in increased resistance to AraC was demonstrated both *in vitro* and *in vivo* using patient-derived xenografts and a retrospective analysis of minimal residual disease in patients. Using a functional approach, the authors identified two mechanisms through which loss of Ikaros drives resistance to AraC treatment (Figure 1).

The first mechanism is decreased incorporation of AraC due to lower expression of the solute carrier human equilibrative nucleoside transporter 1 (*hENT1*). The expression of this transporter is critical for AraC influx, at the doses of AraC used for the treatment of BCP-ALL. The authors found that *IKZF1*-del cells have reduced AraCTP incorporation, which can explain the mechanism of resistance to AraC in those cells. Reduced expression of *hENT1* has been associated with AraC sensitivity in both acute myeloid leukemia (AML) and BCP-ALL.

The second mechanism is increased expression of the *Evi1* oncogene. Elevated expression of *Evi1* has been associated with resistance to AraC and poor prognosis in AML. In addition, *Evi1* functions as a regulator of hematopoietic stem cell self-renewal. Although the function of Evi1 has been studied extensively in AML and AML stem cells, alterations in Evi1 have not been associated with BCP-ALL. Based on the data presented by Vervoort *et al.*, it is likely that *Evi1* repression is also important for normal B-cell differentiation and that increased *Evi1* expression can promote stemness and drug resistance in BCP-ALL. Since *IKZF1* is located at chromosome 7p, and *IKZF1* mutations have been observed in pediatric AML, these data suggest a potential mechanism of IKAROS tumor suppressor activity in AML – via repression of *Evi1* expression.

The authors searched for a way to restore sensitivity to AraC in *IKZF1*-null BCP-ALL (Figure 1). They used a CRISPR/Cas9-based loss-of-function screen that targets all human kinases. They identified that the loss of homologous CDK8/19 restores sensitivity of *IKZF1*-null BCP-ALL to AraC. The use of the small molecule inhibitor CCT251921, which specifically targets CDK8 and 19, in combination with AraC, showed a strong synergistic effect in both *IKZF1*-wildtype and *IKZF1*-null BCP-ALL cells. This suggests that this novel

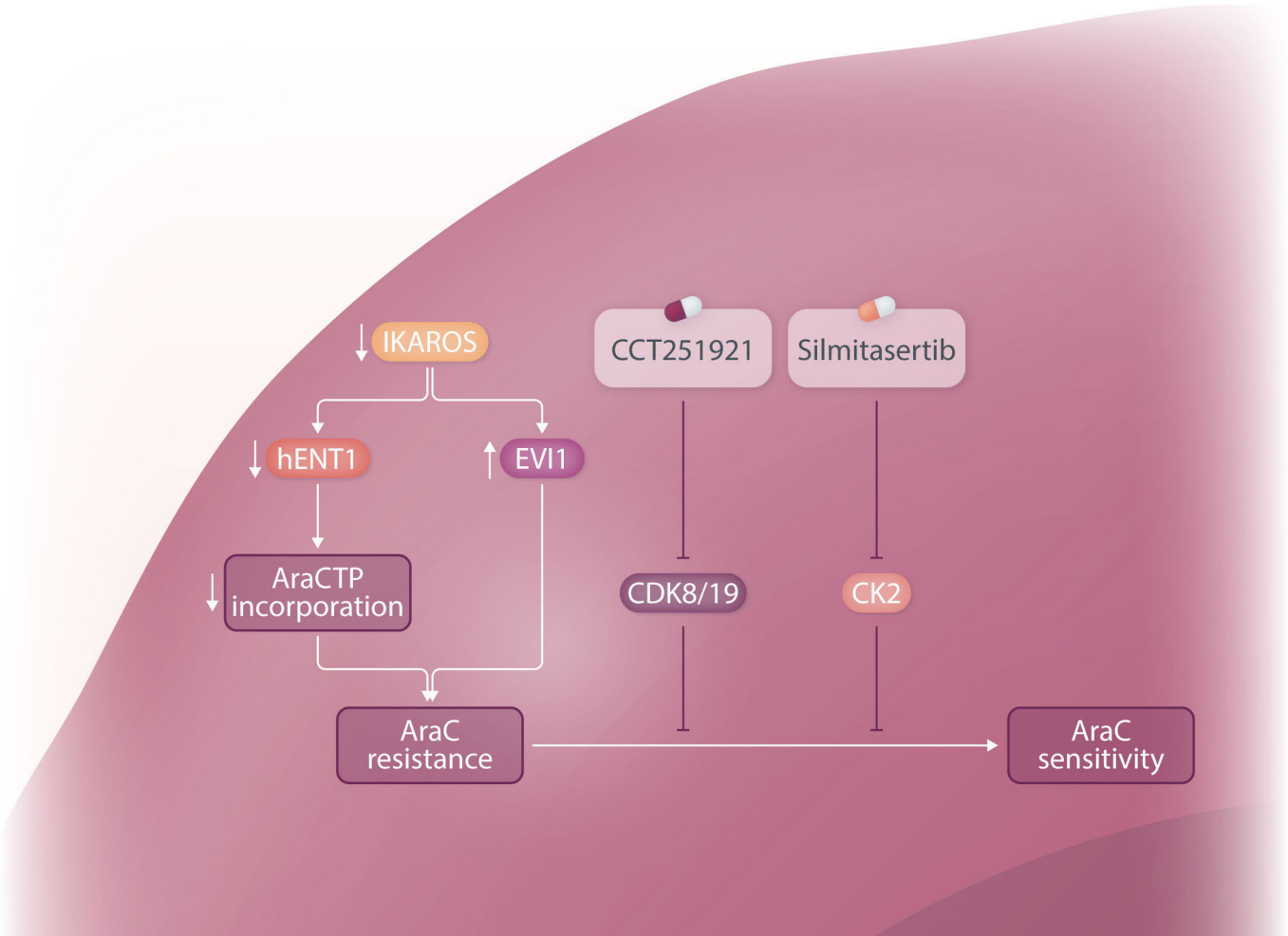

Figure 1. Mechanisms of resistance to cytarabine treatment. Vervoort *et al.* demonstrate that *IKZF1* deletion results in resistance to cytarabine (AraC) via reduced expression of *hENT1* and increased expression of *EVI1*. Treatment with CDK8/19 or CK2 inhibitors restores sensitivity to AraC treatment.

combination can be a potent treatment for different types of high-risk BCP-ALL, regardless of *IKZF1* status. Additional findings showed that loss of CK2 restores sensitivity to AraC in *IKZF1*-null BCP-ALL. Combination treatment with the CK2 inhibitor silmitasertib and AraC showed strong synergistic cytotoxicity in both *IKZF1*-wildtype and *IKZF1*-null BCP-ALL. It has been shown previously that CK2 inhibition can sensitize *IKZF1* haploinsufficient BCP-ALL via enhancement of IKAROS protein function produced from the remaining allele.[10] The data presented in the paper by Vervoort *et al.* suggest that CK2 inhibition can induce chemosensitivity of BCP-ALL cells via other mechanisms. It is intriguing that CK2 enhances Evi1 activity through direct phosphorylation, which could be an additional mechanism of CK2 inhibition in leukemia.

This research has both basic/translational and clinical impacts on the BCP-ALL field in many different ways. First, it identifies a novel mechanism of drug resistance to chemotherapy in high-risk BCP-ALL. As the authors mentioned in the Discussion, awareness that *IKZF1*-del BCP-ALL is resistant to AraC treatment due to *hENT1* repression suggests that the use of different nucleoside analogs (e.g., fludarabine and clofarabine) would be beneficial for patients since these analogs are imported by other transporters. Secondly, the research indicates a potential, novel targeted treatment for BCP-ALL, with the data providing a rationale for preclinical testing of silmitastetib and AraC combination therapy for BCP-ALL. Silmitasertib has been tested in phase I/II trials for other malignancies, which could further accelerate testing of this combination treatment in clinical trials. Thirdly, the role of *Evi1* in the pathogenesis of BCP-ALL is examined, with the results suggesting a potential role of *Evi1* in BCP-ALL drug resistance. Further testing of the utility of Evi1 expression as a biomarker for drug-resistant BCP-ALL is warranted. Finally, the role of *IKZF1*/IKAROS is considered. Most of the research concerning the tumor suppressive role of IKAROS is focused on BCP-ALL,

despite evidence for a role of *IKZF1* in the pathogenesis of AML. The data presented suggest that *IKZF1* can regulate the expression of *Evi1*, a known oncogene in AML, which provides further support for studying the role of *IKZF1* in AML. Overall, the studies by Vervoort *et al*. allow a better understanding of the role of *IKZF1* in the chemoresistance of BCP-ALL and also provide a rationale for preclinical and clinical testing of these novel combination treatments for this disease.

Disclosures

No conflicts of interest to disclose.

Contributions

SD and JS contributed equally.

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