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POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Establishment and Characterization of a Model of Acquired Resistance to DOT1L Inhibition in *KMT2A*-Rearranged Acute Lymphoblastic Leukemia Cells

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Abstract *KMT2A*-rearranged acute lymphoblastic leukemia (ALL) in infants (<1 year of age) is an aggressive malignancy with event-free survival rates of only ~37%. *KMT2A* translocation represents the cytogenetic hallmark of this type of leukemia that is largely driven by inappropriate epigenetic events: oncogenic KMT2A fusion proteins recruit the histone methyltransferase DOT1L which leads to misplaced H3K79 methylation inducing an abnormal transcriptomic landscape that favors leukemia development. Therefore, the development and subsequent (pre-)clinical testing of the specific DOT1L inhibitor pinometostat was anticipated to be key to the successful treatment of *KMT2A*-rearranged acute leukemia. However, although clinical trials in adult *KMT2A*-rearranged acute leukemia patients did report initial responses (including complete remissions), disease progression due to treatment-related resistance appeared unavoidable. Nonetheless, while these observations urge the development of more effective inhibitors of DOT1L, the mechanisms underlying acquired resistance to DOT1L inhibition may well provide valuable insights into the unique biology of *KMT2A*-rearranged leukemia.

To explore this, we provoked and established acquired pinometostat resistance in a pediatric *KMT2A::AFF1*$^{+}$ ALL cell line model that stably became ~35-fold more resistant to DOT1L inhibition. Interestingly, pinometostat was still able to completely inhibit H3K79 di-methylation (H3K79me2) but failed to induce apoptosis, indicating that these cells largely became independent of DOT1L-mediated H3K79 methylation. Yet, shRNA-mediated knock-down of *KMT2A::AFF1* revealed that these cells remained completely dependent on the presence of the KMT2A-fusion protein. In addition, pinometostat-resistant *KMT2A*-rearranged ALL cells proliferate more slowly and appeared to have switched their preference from oxidative phosphorylation to glycolysis to provide energy for metabolic activities, while remaining perfectly viable. As anticipated, pinometostat-resistant cells displayed a slight cross-resistance to most chemotherapeutic agents currently used in ALL treatments but became remarkably more sensitive toward the BCL-2 inhibitor venetoclax.

To further characterize these cells and gain insights into the underlying mechanisms of resistance, we performed RNA-, ATAC-, and ChIP-sequencing analysis of *KMT2A* and *AFF1* as well as of histone marks essential in *KMT2A*-rearranged ALL, including H3K79me2, H3K4 tri-methylation (H3K4me3) and H3K27 acetylation (H3K27ac). This revealed that acquired resistance to DOT1L inhibition leads to a selective loss of KMT2A-fusion driven epigenetic regulation and expression of genes such as *PROM1* (encoding the hematopoietic/ leukemia stem cell marker CD133) and its enhancer *TAPT1*, as well as putative *KMT2A::AFF1* target genes including *RUNX2*, *GNAQ*, *SERPINB1*, *PRSS12*, and *ZC3H12C*. In contrast, however, the expression of (and levels of H3K79 methylation at) other established *KMT2A::AFF1* target genes like *HOXA9*, *HOXA10*, *MEIS1*, and *CDK6* remained unaffected. Concomitantly, pinometostat-resistant *KMT2A*-rearranged ALL cells are characterized by significant enrichment of gene expression of various MYC targets, and specific up-regulation of genes associated with a myeloid immunophenotype, including *CD33*, *CD38*, *LILRB4*, *MPEG1*, *LIMK1*, and *CCL5*.

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Taken together, we here present an *in vitro* model of acquired resistance to DOT1L inhibition in *KMT2A*-rearranged ALL, instantly providing new insights and raising important questions regarding this elusive type of leukemia. For instance, *KMT2A*-rearranged ALL cells readily adapt to prolonged inhibition of H3K79 methylation but remain dependent on the KMT2A fusion, suggesting that recruitment of DOT1L may not be the only oncogenic action exerted by KMT2A fusion proteins. Also, our model demonstrates that under prolonged pressure of DOT1L inhibition, *KMT2A*-rearranged ALL cells seem to initiate a reprogramming process that involves the acquirement (or selection) of myeloid-like characteristics, an ability that may be connected to leukemic lineage switches which are not uncommon in *KMT2A*-rearranged acute leukemias.
Hence, our model represents an important tool to study the complex biology of *KMT2A*-rearranged leukemia, and its existence and availability requires to be shared with the community.

Disclosures Milne: *Dark Blue Therapeutics:* Consultancy, Current equity holder in private company.

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