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POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Differentiation State Plasticity As a Mechanism of BCL2 Inhibitor Resistance in Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is composed of a heterogeneous blast population that exhibits characteristics of normal myeloid cells, including immature, HSC-like cells and differentiated, monocyte-like cells. The balance of immature and differentiated blasts within a patient is important clinically, as immature AML blasts are acutely sensitive to treatment with venetoclax, a BH3 mimetic that blocks the anti-apoptotic activity of BCL2. BCL2-inhibitor-based therapeutic strategies are the standard of care among elderly patients with untreated or relapsed/refractory AML. While these strategies result in a remission rate of nearly 70%, the majority of patients who do achieve remission ultimately relapse. Frequently, the relapsed disease is composed of differentiated, monocyte-like blasts. Given the importance of BCL2 inhibitors to the clinical management of AML, it is critical to understand the mechanisms by which BCL2 inhibitors result in monocytic relapse.

Disease relapse has been traditionally thought to emerge from intrinsically resistant cells that confer a survival advantage under therapeutic pressure. Several studies have suggested that differentiated AML blasts have a survival advantage in the presence of BCL2 inhibition due to a reliance on anti-apoptotic proteins other than BCL2. Immature AML blasts express high levels of *BCL2*, whereas blasts that are primed for lympho-myeloid or granulocytic-monocytic progenitor cell fates express high levels of *MCL-1* and *BCL-XL*, respectively. However, these studies do not explain why patients with dominant, differentiated subclones frequently achieve long-lasting remission with BCL2-inhibitor-based strategies. Therefore, simple selection of differentiated blasts appears to be an insufficient model to describe BCL2 inhibitor resistance. Alternative models of therapeutic resistance have been identified where cancer cells adapt to a resistant phenotype and acquire a survival advantage under therapeutic pressure. In the context of BCL2 inhibitor resistance, it is unclear the extent to which the emergence of differentiated blasts is the result of selective pressure or blast differentiation state plasticity in response to BCL2 inhibition.

In our work, we have investigated the adaptive mechanism of BCL2 inhibition resistance in AML. We have explored this mechanism using an AML cell line model in which immature AML cells continuously generate differentiated AML cells (OCI-AML8227). We used fluorescence-activated cell sorting to isolate immature CD34+/CD38- OCI-AML8227 cells. After 72 hours in liquid culture, we observed that these cells recapitulated a pool of differentiated CD34-/CD38+/CD14+ cells. Furthermore, we used single cell ATAC sequencing to confirm that the unfractionated pool of OCI-AML8227 cells display chromatin accessibility profiles resembling cells across the continuum of myeloid differentiation. To understand whether this model is appropriate to investigate the differentiation-state-specific differences in sensitivity to BLC2 inhibition, we measured the viability of immature CD34+ and differentiated CD34- OCI-AML8227 cells following BCL2 inhibition. Consistent with previous studies, immature CD34+ cells were significantly more sensitive to BCL2 inhibition than differentiated CD34- cells. In addition, BLC2 inhibition of unfractionated OCI-AML8227 cells resulted in an 82% increase in the number of differentiated CD34-/CD38+ cells. While the number of immature CD34+/CD38- cells was reduced following BCL2 inhibition, it was only reduced by 27%. Furthermore, there was no change in the apoptosis marker Annexin V among immature CD34+/CD38- cells. These results support an adaptive resistance model where AML cells acquire resistance through differentiation state plasticity. We will explore this mechanism by following the temporal dynamics of immature and differentiated blasts following BCL2 inhibition. Using single cell barcoding, we will not only be able to trace the trajectories of these populations but also investigate tran-

scriptional adaptation of the immature and differentiated populations. With an understanding of the adaptive mechanism of resistance to BCL2 inhibition, we can nominate alternative targets to prevent the outgrowth of resistance populations and design more effective, rational BLC2-inhibitor-based therapeutic strategies.

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