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MEETING ABSTRACT

A3.1

Palbociclib treatment of *FLT3*-mutant AML cells reveals a kinase-dependent transcriptional regulation of *FLT3* and *PIM1* by CDK6

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Background: Up to 30% of patients with acute myeloid leukemia (AML) have constitutively activating internal tandem duplications (ITDs) of the FLT3 receptor tyrosine kinase (FLT3-ITD) on initial diagnosis, and additional patients may acquire them on relapse. Such mutations are associated with a poor prognosis and a shortened overall survival. FLT3 tyrosine kinase inhibitors (FLT3-TKI) are being developed as targeted therapy for *FLT3*-ITD$^+$ AML; however, their use is complicated: they provide short-term disease control but relapse invariably occurs within months, illustrating the need for additional therapeutic targets. PIM protein kinases are oncogenic targets expressed in AML cells.

Methods: We used human leukemic cells with wildtype *FLT3* and *FLT3*-ITD, respectively. Cell viability was analysed in a high-throughput drug screen. The molecular mechanism was deciphered biochemically, by ChIP experiments and via FACS stainings (*e.g.* PI, annexin V/7-AAD). The clinical relevance was addressed by single agents and combinatorial strategies. We also performed mouse xenograft experiments to test whether palbociclib represses FLT3-mediated leukemia *in vivo* in immune-compromised Rag2$^{-/-}\gamma c^{-/-}$ mice. We further evaluated the effects of palbociclib in a *FLT3*-ITD$^+$ subcutaneous tumor xenograft model. *PIM1* and *FLT3* gene expression was analysed ín tumor tissues. Primary human AML biopsies were used for further validation.

Results: The FDA-approved CDK4/6 kinase inhibitor palbociclib induced apoptosis of *FLT3*-ITD$^+$ AML cells. The drug toxicity was specific for *FLT3*-mutant cells and was ascribed to the transcriptional activity of CDK6 in a kinase-dependent manner: CDK6, but not its functional homolog CDK4, was bound to the promoters of *FLT3* and *PIM1*, factors at two signaling nodes that are critical for survival of the leukemic cells. Dual targeting with palbociclib and PIM1 inhibitors or palbociclib and FLT3 inhibitors resulted in synergistic cytotoxicity.

Discussion: Targeted CDK6 inhibitors harbor the potential to suppress the relapse after remission. Concomitantly targeting two critical signaling nodes in leukemogenesis could represent a therapeutic breakthrough, overcoming/preventing therapy resistance, thereby prolonging therapeutic efficacy.

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