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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Inactivation of DNA Polymerase Theta (PolΘ) Is Synthetic Lethal in DNMT3A Mutated Myeloid Malignancies - Potential Clinical Applications

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Somatic mutations in *DNMT3A* are associated with unfavorable outcome in patients with AML, MPN and CML. *DNMT3A* mutations promote resistance to anthracyclines (including daunorubicin, the component of standard "7+3" induction therapy), interferon alpha, and ABL1 kinase inhibitor imatinib. Thus, malignant clones carrying *DNMT3A* mutations may be difficult to eliminate using standard treatments.

AML, MPN and CML cells harbor oncogenic tyrosine kinase (OTK) such as FLT3(ITD), JAK2(V617F) and BCR-ABL1, respectively. We reported before that elevated levels of formaldehyde generated by altered serine/one-carbon cycle metabolism contributed to accumulation of highly lethal DNA double-strand breaks (DSBs) in OTK-positive cells. To protect malignant cells from DSB-induced apoptosis, OTKs regulate DNA damage response (DDR) mechanisms involving DSBs sensing (ATM and ATR kinases) and repairing (RAD51-mediated homologous recombination = HR, RAD52-mediated transcription associated homologous recombination = TA-HR and single strand annealing = SSA, DNA-PK -mediated non-homologous end-joining = NHEJ, Polθ-dependent microhomology-mediated end-joining = TMEJ) as well as these activating cell cycle checkpoints (CHK1 and CHK2 kinases). Unfortunately, *DNMT3A* mutations caused resistance of OTK-positive cells to numerous DDR inhibitors (DDRis).

DDRis sensitivity screen and synthetic lethal CRISPR/Cas9 screen revealed that OTK-positive cells with *DNMT3A* mutations are uniquely sensitive to the inhibition of DNA polymerase theta (Polθ encoded by *POLQ* gene). This effect was dependent on generation of formaldehyde by serine/one-carbon cycle metabolism. Abrogation of DNMT3A function by CRISPR/Cas9 targeting, Cre-loxP gene deletion, and heterozygous R882H mutation resulted in hypersensitivity of OTK-positive murine AML-like cells and human primary AML cells to Polθ inhibitors (Polθis) due to accumulation of toxic DSBs and activation of cGAS/STING pro-apoptotic pathway. Moreover, simultaneous loss of functional DNMT3A combined with inactivation of Polθ (by CRISPR/Cas9 targeting, insertion of neo-resistance gene, and D2230A+Y2231A polymerase inactive mutant) caused accumulation of DSBs, and reduced OTK-driven clonogenic potential and leukemogenic activity in mice.

Polθ is abundantly overexpressed in OTK-positive DNMT3A-deficient cells due to enhanced *POLQ* mRNA stability and elevated translation of Polθ protein but not due to altered *POLQ* methylation and Polθ protein stability. Polθ is a key element not only in TMEJ of DSBs with limited end-resection, but also in replication fork restart and in single-strand DNA (ssDNA) gap filling. OTK-positive DNMT3A-deficient cells displayed hyperactivity of Polθ-mediated TMEJ and replication fork restart, but not ssDNA gap filling. These effects were accompanied by increased loading of Polθ on DNA damage detected by Polθ foci

formation and chromatin extraction. Moreover, DNMT3A deficiency modulates chromatin architecture at DSBs to limit DNA end-resection thus favoring TMEJ over HR.
Furthermore, we tested the effectiveness of Pol$\theta$is combined with FDA approved drugs (quizartinib, etoposide, cytarabine, azacytidine) against FLT3(ITD)-positive DNMT3A-deficient cells (primary patient cells and cell lines) *in vitro* and *in vivo*. The combination of Pol$\theta$is + quizartinib and Pol$\theta$is + etoposide completely eradicated clonogenic activity of these cells while Pol$\theta$is + cytarabine and Pol$\theta$is + azacytidine exerted modest and weak effects, respectively, when compared to individual compound treatments. These drug combinations were only modestly toxic to normal bone marrow cells. Treatment with Polθi or etoposide reduced the percentage of GFP+ FLT3(ITD)-positive DNMT3A-deficient leukemia cells in peripheral blood of the mice by ~2-fold and prolonged survival time by ~1.5-fold. Remarkably, the combination of Polθi and etoposide eradicated leukemia cells below detectable levels in 6/12 mice with no visible toxicity. Median survival time of the mice will be recorded. Altogether, we discovered that Polθ protects OTK-positive DNMT3A-deficient myeloid malignant cells from the toxic effects of DSBs and identified Polθ as a novel therapeutic target.

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