ELSEVIER

Blood 142 (2023) 2803–2804

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

PRC2-Mediated Apoptosis Evasion Is a Therapeutic Target of MDS/AML Harboring Inv(3)/t(3;3) and -7

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Myelodysplastic syndromes and acute myeloid leukemia (MDS/AML) with both inversion/translocation of chromosome 3 (inv(3)/t(3;3)) and monosomy 7 (-7) is an extremely poor prognostic entity. To explore potential therapeutic target of MDS/AML harboring both inv(3)/t(3;3) and -7, we performed drug screen using YCU-AML1, a high-risk MDS/AML cell line harboring t(3;3) and -7 (Kunimoto et al. Hemasphere 2020), as well as OCI-AML20, another AML cell line with inv(3) and -7, and found that both YCU-AML1 and OCI-AML20 showed high response to EZH2 inhibitors valemetostat and tazemetostat. Previous study has shown that EVI1, an oncogenic transcription factor highly expressed in MDS/AML with inv(3)/t(3;3), directly binds to EZH2 and thereby recruits PRC2 complex to *PTEN* locus, leading to epigenetic silencing of *PTEN* expression and activation of PI3K/AKT/mTOR pathway in leukemia with 3q rearrangement (Yoshimi et al. Blood 2011). Together with the fact that *EZH2* locus is on chromosome 7q, we hypothesized that the survival of MDS/AML cells with inv(3)/t(3;3) and -7 may be highly dependent on residual allelic EZH2-mediated silencing of specific targets which drive cell death. We further validated in colony-forming unit and cell growth assays that YCU-AML1 and OCI-AML20 are both highly sensitive to valemetostat and tazemetostat, whereas FKH-1, Kasumi-3 and SKM-1, MDS/AML cell line with -7, 3q rearrangement, and complex karyotype without chromosome 3 and 7 abnormalities respectively, are resistant to these drugs. Apoptosis analysis revealed that valemetostat and tazemetostat efficiently induced apoptosis in YCU-AML1 and OCI-AML20 but not in FKH-1, Kasumi-3 and SKM-1. To seek molecular basis of EZH2 inhibitor-mediated apoptosis induction, we performed CUT&Tag sequence for H3K27me3 using vehicle or valemetostat-treated cells. Strikingly, promoter region of *GADD45 γ* was the most robustly and significantly decreased annotated peak locus of H3K27me3 in valemetostat-treated OCI-AML20. H3K27me3 peak in *GADD45 γ* locus was also significantly decreased in valemetostat-treated YCU-AML1 but not in FKH-1 and Kasumi-3. As expected, valemetostat treatment induced increased expression of *GADD45 γ* in OCI-AML20. Moreover, transcriptomic analysis also demonstrated *GADD45 γ* as the most robustly upregulated gene in valemetostat-treated YCU-AML1 compared to vehicle-treated cells. GADD45γ is known to be an upstream regulator of stress-activated protein kinases such as p38 and JNK in which signaling pathways activation induce apoptosis upon various cellular stresses. *GADD45 γ* promoter region possessed putative EVI1 binding site predicted by rVista2.0 software, indicating that EVI1 may directly bind and recruit PRC2 complex to *GADD45 γ* locus. We further confirmed phosphorylation of Thr180/Tyr182 (T180/Y182) residues of p38α in valemetostat-treated YCU-AML1 and OCI-AML20 but not in SKM-1 and Kasumi-3. p38 is known to phosphorylate Ser33 (S33) and Ser46 residues of TP53,

leading to apoptosis in lung cancer cells (Yogosawa et al. Cancer Sci. 2018). In line with this finding, valemetostat treatment induced phosphorylation of Ser33 residue of TP53 in YCU-AML1 and OCI-AML20. Phosphorylations of p38α (T180/Y182) and TP53 (S33) were also noted in tazemetostat-treated YCU-AML1 and OCI-AML20, suggesting that EZH2 inhibition directly activates GADD45γ-p38α-TP53 axis leading to apoptosis preferentially in MDS/AML cells with inv(3)/t(3;3) and -7. As a proof of concept, p38MAPK inhibitor SB203580 restored valemetostat-induced colony growth inhibition as well as apoptosis induction in YCU-AML1 and OCI-AML20.

Importantly, valemetostat treatment significantly reduced leukemic burden and improved overall survival in xenotransplant mouse model of YCU-AML1. Moreover, primary MDS/AML patient bone marrow (BM) sample harboring inv(3) and -7 exhibited preferential sensitivity to valemetostat compared to BM samples derived from healthy control or MDS/AML patients with or without -7 in vitro.

Taken together, our study unraveled PRC2-mediated inactivation of GADD45γ-p38α-TP53 axis as a molecular basis for evasion of apoptosis in MDS/AML with inv(3)/t(3;3) and -7, which can be preferentially abrogated by EZH2 inhibition leading to efficient induction of apoptosis in this high-risk MDS/AML.

Disclosures Kunimoto: *Daiichi Sankyo:* Research Funding. **Honma:** *Daiichi Sankyo Co., Ltd.:* Current Employment. **Tsutsumi:** *Daiichi Sankyo Inc.:* Current Employment. **Doki:** *Janssen Pharmaceutical K.K.:* Honoraria; *Novartis Pharma K.K.:* Honoraria. **Cai:** *Imago Biosciences:* Consultancy, Current equity holder in private company. **Levine:** *Zentalis:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Mission Bio:* Membership on an entity's Board of Directors or advisory committees; *Ajax:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Auron:* Membership on an entity's Board of Directors or advisory committees; *Prelude:* Membership on an entity's Board of Directors or advisory committees; *C4 Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Isoplexis:* Membership on an entity's Board of Directors or advisory committees; *Incyte:* Consultancy; *Qiagen:* Membership on an entity's Board of Directors or advisory committees; *Janssen:* Consultancy; *AstraZeneca:* Consultancy, Honoraria; *Novartis:* Consultancy; *Roche:* Honoraria; *Lilly:* Honoraria; *Amgen:* Honoraria. **Nakajima:** *Novartis:* Speakers Bureau; *Takeda:* Research Funding; *Eisai:* Research Funding; *Astellas:* Research Funding; *Pfizer:* Research Funding; *Daiichi Sankyo:* Research Funding, Speakers Bureau.

https://doi.org/10.1182/blood-2023-178436