(PF424) COMBINED INHIBITION OF HSP90 AND CDK9 OVERCOMES VENETOCLAX-RESISTANCE IN AML

Topic: 03. Acute myeloid leukemia - Biology & translational research

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Background

Venetoclax (VEN), combined with chemotherapy or hypomethylating agents, has shown therapeutic benefit against acute myeloid leukemia (AML), yet resistance frequently emerges from the upregulation of BCL-2 family members (BCL-xL, MCL-1), including in *TP53*-mutant AML. The molecular chaperone HSP90 sustains leukemic cell survival by stabilizing multiple oncogenic client proteins involved in apoptotic regulation, thereby contributing to stress adaptation and therapeutic resistance. CDK9 inhibition suppresses RNAPII-dependent transcription of short-lived pro-survival factors, such as MCL-1, uncovering a rational novel strategy to overcome VEN resistance. We previously demonstrated that RNAPII inhibition potentiates HSP90 inhibitor activity in therapy-resistant leukemia (*Vogt et al., Cell Death Dis., 2023*).

Aims

Given that *TP53* inactivation increases the dependence of AML cells on HSP90-mediated chaperone activity (*Carter et al., Blood, 2023*) and since HSP90 stabilizes key BCL-2 family proteins, we hypothesize that combined HSP90 and CDK9 targeting would synergistically disrupt AML survival networks and overcome VEN resistance.

Methods

Ex vivo drug screening was performed across a panel of 33 cell lines and 37 PDX samples from patients with initial or relapsed AML/ALL. VEN resistance was modeled in (HL60 and SKNO-1) AML cells via enforced MCL-1 overexpression or chronic VEN exposure. Mechanistic analyses were conducted using integrated transcriptomic (bulk RNA-seq) and (mass spectrometry-based) proteomic profiling. The combination was further tested on isogenic *TP53*-mutant (R248Q, R273H, +/- or -/-) MOLM13 AML models. Therapeutic efficacy was evaluated *in vivo* using preclinical NSG mouse models inoculated with AML-PDX cells.

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Results

Ex vivo drug screening identified a selective (p = 0.009) synergistic interaction between HSP90 and CDK9 inhibition in AML cells compared to ALL, demonstrating a favorable therapeutic window with minimal impact on healthy PBMCs (Fig. 1A). Notably, the combination demonstrated synergistic activity (Fig. 1B) and enhanced apoptosis induction in VEN-resistant isogenic AML models. Integrated multi-omics profiling revealed that dual inhibition profoundly suppressed key pro-survival pathways, including the downregulation of MCL-1. Importantly, *in vivo* administration of the HSP90i (PU-H71): CDK9i (Enitociclib, Lead Discovery Centre GmbH) combination was well tolerated in preclinical NSG mouse models. In *KMT2A-r* and *NPM1*-mutated relapsed/refractory AML-PDX models, the treatment induced a significant ($p < 0.05$) reduction in leukemia burden and prolonged survival (Fig. 1C). Correlative transcriptomic analyses were performed against *ex vivo* drug sensitivity, revealing a significant ($p < 0.05$) inverse association between *TP53* expression and treatment response, with *TP53*-deficient AML cells exhibiting enhanced sensitivity. Isogenic *TP53*-mutant models (MOLM13 R248Q and R273H) and those with mono-allelic ($TP53^{+/-}$) and bi-allelic ($TP53^{-/-}$) loss exhibited comparable or enhanced synergistic responses compared to respective *TP53*-WT controls. Consistently, *in vivo* evaluation using the *TP53*-null (HL60) AML model demonstrated a significant ($p < 0.05$) reduction in leukemia progression with the combination compared to monotherapies or the vehicle group.

Summary/Conclusion

Combined HSP90 and CDK9 inhibition synergistically overcomes VEN resistance by disrupting key survival networks, particularly in *TP53*-deficient AML, offering a promising therapeutic strategy for high-risk AML.

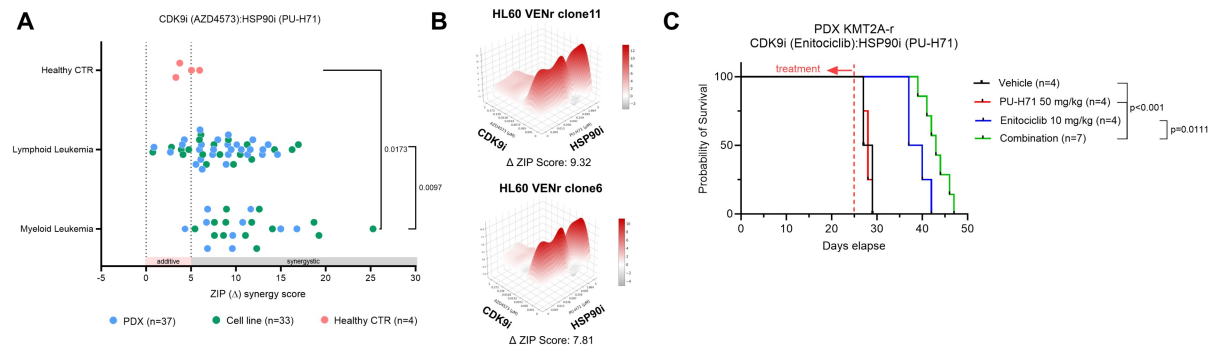


Figure 1: Dual HSP90 and CDK9 inhibition induced a significant synergistic response in AML cell lines and PDX-AML models as compared to ALLs, as well as in venetoclax resistant cells and prolongs survival in AML mouse model. (A) High throughput drug screening was performed on 74 samples, including healthy PBMCs, cell lines and PDX samples. The delta ZIP synergy scores were calculated from 8 × 8 dose response matrices with Synergyfinder software and plotted for each sample. Data points represent individual samples. Statistical analysis was performed using a student's t-test (unpaired, two-tailed). **(B)** Three-dimensional synergy landscapes of venetoclax resistant cells confirm strong combinatorial activity. **(C)** Kaplan-Meier survival curves of NSG AML-PDX model treated with CDK9i and HSP90i. Treatment significantly reduces leukemia burden and prolongs survival in NSG AML-PDX model compared to monotherapy or vehicle control. Survival curves were compared using the log-rank test (Mantel-Cox Test).

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