(PB2674) SCREENING MITOCHONDRIAL-TARGETING COMPOUNDS IN AML IDENTIFIES MITOTAM AS A BROAD VENETOCLAX-SENSITIZER

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

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Background

Venetoclax resistance is a major barrier in acute myeloid leukemia (AML) and is often driven by adaptive programs that preserve mitochondrial bioenergetics and integrity. As venetoclax priming and apoptotic commitment are tightly coupled to mitochondrial state, disrupting mitochondrial fitness, using mitochondria-targeted agents such as MitoTam, or targeting mitochondrial-supporting pathways (e.g., DHODH and SPHK1), may re-sensitize resistant AML.

Aims

To assess single-agent activity and venetoclax-combination effects of mitochondrial-targeting compounds across a molecularly diverse AML cell-line panel.

Methods

Sixteen AML cell line models covering prevalent genetic subtypes were screened, including two in-house venetoclax-resistant models (MV4;11VR and MOLM-13VR) generated by intermittent, stepwise venetoclax escalation and characterized by loss of venetoclax-induced apoptosis with distinct metabolic rewiring (MV4;11VR: glycolytic shift; MOLM-13VR: increased oxidative phosphorylation). Cells were exposed (72h) to MitoTam (TPP-conjugated, mitochondria-targeted tamoxifen derivative), brequinar (DHODH inhibitor) or SK1-In-1 (SPHK1 inhibitor). Combinations with venetoclax were assessed using a mitochondrial metabolic activity readout; interactions were quantified by ZIP (ZIP > +10, synergistic; ZIP < −10, antagonistic). CCLE baseline transcriptomes were integrated with drug-response metrics and correlated pathways were identified by gene set enrichment of ranked gene–response correlations. Mechanistic follow-up of MitoTam used flow cytometry (Annexin V; JC-1 for mitochondrial membrane potential, MMP).

Results

Brequinar and SK1-In-1 showed heterogeneous single-agent activity and variable interactions with venetoclax (ZIP spanning antagonism to synergy). MitoTam showed consistent activity (72h IC50 0.18–1.83μM), with potency maintained in resistant derivatives (MOLM-13VR 0.23μM vs MOLM-13 0.28μM; MV4;11VR 0.56μM vs MV4;11 0.27μM) and high sensitivity in Kasumi-1 (0.18 μM). Time-course analyses showed increasing potency from 24/48/72h: MOLM-13 0.82/0.68/0.28 μM and MOLM-13VR 0.91/0.69/0.23μM; MV4;11 0.60/0.48/0.27μM and MV4;11VR 1.3/0.83/0.56μM. MitoTam yielded uniformly positive ZIP scores with venetoclax across all models (range 2.76–16.37; median 10.82), including MOLM-13VR (13.77) and MV4;11VR (8.59). CCLE baseline transcriptomics revealed that MitoTam resistance tracked with a mitochondrial “fitness”/stress-adaptation phenotype, including stemness, PRC2-linked epigenetic repression programs, and HIF2-associated hypoxia signaling, consistent with adaptive survival states. MitoTam sensitivity aligned with more differentiated myeloid states and oncogenic drivers, including GMP-like programs, MLL-rearranged leukemia signatures, and MYC target, supporting a vulnerability in proliferative, GMP/MLL-driven contexts. Functionally, MitoTam increased Annexin V positivity after 48h in both parental and resistant contexts, and JC-1 revealed a dose-dependent loss of MMP, reaching 90–95% at 5 μM in MV4;11 and Kasumi-1 (P<0.001).

Summary/Conclusion

In a heterogeneous AML panel including acquired venetoclax-resistant models, MitoTam demonstrated time-dependent cytotoxicity and uniformly positive synergy with venetoclax by ZIP scoring. Annexin V and JC-1 assays support mitochondrial dysfunction and apoptosis as associated features. These findings support mitochondrial targeting with MitoTam as a strategy to overcome venetoclax resistance in AML.