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Acute Leukemias

METABOLIC REPROGRAMMING AND DNA REPAIR PLASTICITY FUEL RESISTANCE TO LONG-TERM CHK1/CHK2 INHIBITION IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

A. Marranci[1], T. Salvadori[1|2], A. Padella[3], F. Rea[1|2], A. Ferrari[4], F. Olimpico[1], N. Ndimurwanko[1], F. Greco[1], E. Fonzi[4], C. Baldazzi[5], M.C. Fontana[4], M. Ghetti[4], L. Mcdonnell[1], I. Squiccimarro[1|6], E. Ottalagana[1|6], P. Mastella[1|6], G. Martinelli[4|5], G. Simonetti[4], A. Ghelli Luserna Di Rora'[1]

[1]Fondazione Pisana per la Scienza Onlus; [2]University of Pisa; [3]WellMicro srl; [4]Istituto Romagnolo per lo studio dei Tumori "Dino Amadori"; [5]Dipartimento di Scienze Mediche e Chirurgiche-Università di Bologna; [6]NEST laboratory, Scuola Normale Superiore, Italy

Introduction: DNA damage response (DDR) inhibitors are effective across multiple cancers; however, the long-term consequences of sustained DDR checkpoint blockade, especially in terms of genomic stability and resistance mechanisms, remain poorly understood. Here, we model chronic dual CHK1/CHK2 inhibition in B-acute lymphoblastic leukemia (B-ALL) to define evolutionary trajectories of resistance and uncover actionable vulnerabilities.

Methods: NALM-6 cells were exposed long-term to the CHK1/CHK2 inhibitor PF-0477736, generating stepwise resistant subclones (N6R-PF4, N6R-PF8). We profiled viability, cell cycle, apoptosis, DDR signaling, cytogenetics/FISH, genome-wide copy number (CytoScan HD), whole-exome sequencing, RNA-seq, and DIA proteomics. Seahorse flux, comet assays, and γH2AX foci quantified mitochondrial and genotoxic responses. Combination studies tested ATM inhibition (KU-60019) and metabolic co-targeting with atorvastatin.

Results: Prolonged CHK1/CHK2 inhibition selected for resistant clones restoring copy-number neutrality at the 11q22 (ATM) locus, in contrast to parental cells with heterozygous ATM loss. Resistant cells exhibited impaired G1 checkpoint activation, reduced DDR signaling and apoptosis in response to PF-0477736, and partial cross-resistance to a second CHK1/CHK2 inhibitor. Exome profiling revealed increased mutation burden with enrichment of DDR gene lesions (e.g., TP53BP1, NBN, FANCD2, PMS2) and mismatch-repair-like mutational signatures. Multi-omics and metabolic analysis converged on metabolic rewiring: resistant cells upregulated oxidative phosphorylation and sterol/fatty-acid biosynthesis, with suppression of global protein translation. This metabolic shift could be targeted using atorvastatin (sterol biosynthesis inhibitor) but not with metformin (oxidative phosphorylation inhibitor), indicating selective dependence on mevalonate metabolism. Proteomics showed increased expression of several repair pathways (NER, MMR, NHEJ) alongside reduced BER/HR proteins. These DDR-alterations modified significantly the tolerance to DNA damages in the resistant clone. Indeed, N6R-PF8 cells accumulated significantly less DNA damage and underwent reduced apoptosis following oxidative (H_2O_2) or chemotherapeutic stress (anthracyclines, antimetabolites, vinca alkaloids). Finally, combination treatment demonstrated strong synergy between PF-0477736 and ATM inhibition in N6R-PF8 cells compared to NALM-6 confirming the importance of ATM kinase in the sensitivity to PF-0477736.

Conclusions: Chronic CHK1/CHK2 inhibition promotes genomic instability, highlighting the need for clinical strategies that prevent adaptive escape and avoid aggravating the intrinsic instability of leukemic cells. Our data reveal a tight functional link between metabolic rewiring and DNA repair pathways, suggesting that dual targeting of DDR and metabolic vulnerabilities may be essential to optimize the safe and effective use of DDR inhibitors in leukemia.