

Novel Combination Treatments for AML

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Drug resistance of cancer patients toward chemotherapy remains a major problem in the clinic. Hence, understanding the intrinsic and acquired molecular mechanisms of drug resistance in cancer patients is paramount to identify relevant targets for therapeutic interventions that enhance chemotherapy response, and ultimately improve clinical outcome.

Acute myeloid leukemia (AML) is an aggressive hematological malignancy, with a dismal 5-year overall survival rate of 25%. AML develops through the accumulation of genetic aberrations including a few drivers that in part confer aberrant activity of signaling and DNA damage response (DDR) pathways, which promote proliferation, survival, DNA repair, and eventually resistance toward AML standard therapies [1-6]. Hence, therapeutic inhibition of drug resistance, promoted by the aberrant activity of signaling and DDR pathways, holds great promise to enhance therapy response and improve the overall survival of AML patients.

Currently, elderly or unfit AML patients (>65-75 years) with poor physical performance status, who are not eligible for curative high-dose chemotherapy regimens due to toxicity, will obtain *non-curative* treatment, including low dose chemotherapy regimens (hydroxyurea, cytarabine) or hypomethylating agents (HMA) (azacitidine, decitabine), of which the latter also induce some extent of DNA damage. In contrast, the standard treatment of younger and fit AML patients (<65-75 years) with *curative intention* consists of

high-dose induction chemotherapy comprising a backbone of the anti-metabolic agent cytarabine and a DNA damaging anthracycline such as dauno- or doxorubicin. Successful induction chemotherapy leading to complete remission is followed by consolidation therapy with either additional cycles of chemotherapy or allogeneic stem cell transplantation dependent on their genetic risk stratification. Even though 40% of younger AML patients are cured following standard treatment, the majority will still die of their disease due to relapse or primary resistance toward conventional therapy [5,7].

Aberrant activation of phosphoinositide 3-kinase (PI3K)/protein kinase B (or AKT) signaling is a common theme in cancer. This activation is frequently promoted by mutational activation of PI3K, various receptor tyrosine kinases, intracellular kinases, RAS, as well as dominant-negative mutations of the regulatory phosphatase and tensin homolog (PTEN). In cancer cells, mutational activation of PI3K/AKT signaling confers survival, DNA repair, and ultimately resistance to DNA damaging radiation- and chemotherapy [8]. Activated PI3Ks generate the second messenger phosphatidylinositol 3 phosphate (PIP₃), which directs phosphoinositide-dependent protein kinase-1 (PDK1)- and mTOR complex 2 (MTORC2)-dependent phosphorylation of AKT at its T308 and S473 residues, respectively [9]. AKT itself acts through a plethora of downstream substrates including glycogen synthase 3 (GSK3), forkhead box proteins (FOXO), and mTOR complex 1 (MTORC1), which together facilitate

survival, proliferation, and DNA repair [10]. Strikingly, AKT downstream signaling can also be activated upon radiation- and chemotherapy- induced DNA damage by the DNA-dependent protein kinase (DNA-PK), a DDR master regulator, which promotes repair of therapy-induced DNA double-strand breaks through non-homologous end-joining (NHEJ) [11]. Mechanistically, this highlights AKT as a key regulatory hub, which links the DDR with collateral inhibition of TP53-dependent apoptosis (i.e. through AKT-dependent activation of mouse double minute 2 homolog (MDM2)-dependent degradation of TP53) to facilitate survival in response to DNA damaging therapies [12].

In AML, more than 60% of patients harbor mutations of receptor tyrosine kinases (proto-oncogene KIT, fms-like tyrosine kinase 3 = FLT3), polycomb group protein ASXL1, intracellular kinases (Janus kinase 2 or JAK2), and GTPases such as neuroblastoma RAS viral oncogene homolog (NRAS)/ Kirsten rat sarcoma viral proto-oncogene (KRAS), which all direct constitutive activation of PI3K/AKT signaling, and generally are associated with a poor response to chemotherapy [13-21].

The latter is exemplified by the significantly higher relapse rate of inv(16) AML patients harboring activating mutations of KIT as compared to wild-type KIT [22]. Consistently, Estruch *et al.* recently demonstrated constitutive activity of PI3K/AKT signaling in primary murine inv(16)/KIT^{D816Y} AML cells, which was markedly enhanced by AML chemotherapy through complementary DNA-PK-dependent phosphorylation and activation of AKT [23]. In a series of mechanistic experiments, Estruch *et al.* further demonstrated that therapeutic inhibition of either PI3K or DNA-PK reduced chemotherapy-induced AKT downstream signaling leading to increased DNA damage and apoptosis of inv(16)/KIT^{D816Y} AML cells treated with conventional AML chemotherapy. Consistently, combinatorial treatment, with both chemotherapy and PI3K inhibitor (BKM120) or chemotherapy and DNA-PK inhibitor (NU7026), synergistically inhibited *in vitro* growth and survival of AML cells, and significantly prolonged survival of inv(16)/KIT^{D816Y} AML mice in preclinical trials compared to untreated or single treated mice. Notably, combinatorial treatment with chemotherapy and PI3K inhibitor doubled the survival of inv(16)/KIT^{D816Y} AML mice (mean survival 18 days vs. 36 days, $p=0.0001$). Hence, the study by Estruch *et al.* implicates that DNA repair and cell survival mediated by cooperative mutational and therapy-induced activation of AKT signaling stands out as a key mechanism of resistance toward DNA damaging therapies (Figure 1). In a broader perspective, this has important clinical implications. First, it highlights that AML patients, who exhibit high mutational PI3K/AKT signaling activity might benefit from combinatorial treatment with chemotherapy and inhibitors of DNA-PK, PI3K/AKT, or

inhibitors of upstream PI3K activators such as FLT3, KIT, RAS, and JAK2. Given that both conventional induction chemotherapy and the HMA azacitidine induce double-strand breaks (DSBs) conferring DNA-PK-dependent AKT activation, more than 60% of AML patients receiving curative or non-curative treatments might benefit from such combinatorial therapies. Indeed, this potential therapeutic benefit is supported by two recent studies demonstrating that concurrent treatment with the FLT3 inhibitor gilteritinib enhanced the therapeutic effect of (i) azacitidine in elderly FLT3 mutant AML patients, and (ii) chemotherapy in a FLT3-ITD mutant AML mouse model [24,25]. The efficacy of combinatorial treatment is also highlighted by a recent clinical trial revealing that concomitant treatment of patients with relapsed or refractory chronic lymphatic leukemia with a PI3K inhibitor (idelalisib) and antibody/chemotherapy (i.e. rituximab and bendamustine) doubled median progression-free survival (PFS) compared to treatment with antibody/chemotherapy alone (median PFS 20.8 month vs. 11.1 months, $p<0.0001$) [26]. Consistently, patients with metastatic breast cancer expressing the receptor tyrosine kinase HER2, a designated activator of PI3K/AKT signaling, demonstrated prolonged survival in response to combinatorial treatment with a HER2 inhibitor (tucatinib) and antibody/chemotherapy (trastuzumab/capecitabine) compared to antibody/chemotherapy alone (2-year OS 44.9% vs. 26.6%, $p=0.005$) (27). In the context of the above clinical trials, the study by Estruch *et al.* provides a strong rationale for future clinical trials selecting AML patients exhibiting constitutive mutational PI3K/AKT activation for concurrent treatment with chemotherapy and inhibitors of PI3K/AKT or relevant PI3K upstream activators, in order to boost chemotherapy response.

Although numerous Phase I/II clinical trials have tested PI3K/AKT pathway inhibitors in combination with current standard treatments for cancer patients, none of these inhibitors have been approved for single or combinatorial treatment of AML patients [28]. Moreover, DNA-PK inhibitors have yet to be approved for AML treatment. However, several DNA-PK inhibitors are tested in ongoing phase I/II trials of solid cancers and AML, alone or in combination with DNA damaging radiation- or chemotherapies including CC-115 (NCT01353625), nedisertib (M3814) (NCT031169719), and VX-984 (NCT02644278) [29,30]. If these trials should demonstrate acceptable toxicity as well as therapeutic efficacy, they would indeed provide a strong rationale for future AML trials exploring the efficacy of PI3K and DNA-PK inhibitors in combination with chemotherapy and HMAs.

The study by Estruch *et al.* also suggests that AML patients with the high mutational activity of PI3K/AKT signaling partially exhibit drug resistance through

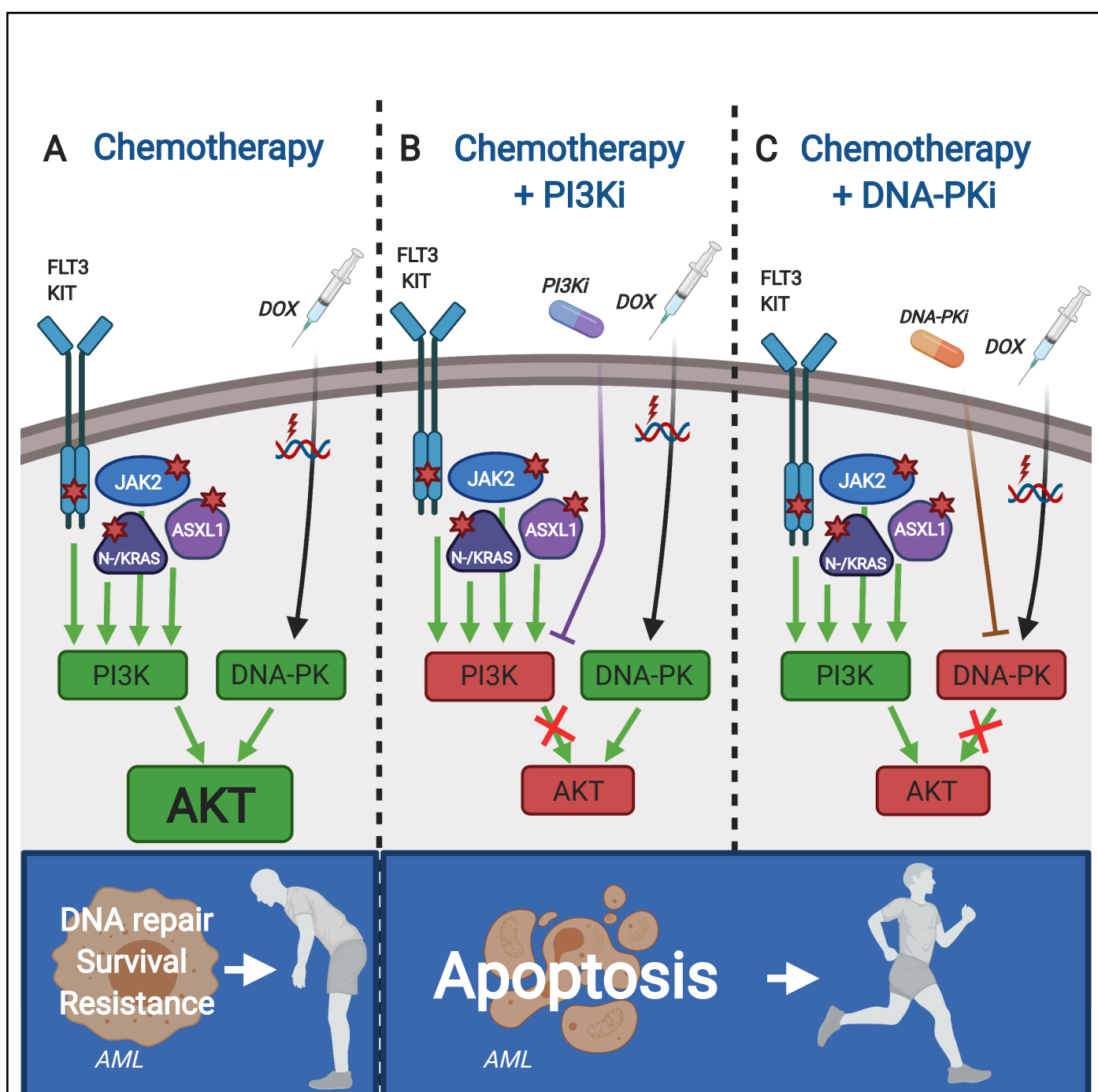


Figure 1: Model for concurrent treatment of AML exhibiting constitutive mutational PI3K/AKT activation with chemotherapy and inhibitors of PI3K/AKT or DNA-PK. A) AML harboring mutations of RTKs (i.e. KIT or FLT3), JAK2, NRAS/KRAS or ASXL1 exhibit constitutive activation of the PI3K/AKT signaling pathway, promoting survival and DNA repair of AML cells. The chemotherapeutic doxorubicin (DOX) used for treatment of AML, induces double strand breaks (DSBs) and activation of a DNA damage response (DDR) partially through DNA-PK-dependent complementary AKT activation of AML cells exhibiting constitutive mutational PI3K/AKT activation, leading to enhancement of DNA repair, survival, and therapy resistance. **B) and C)** Concomitant treatment with chemotherapy and (B) an inhibitor of PI3K/AKT signaling or (C) an inhibitor of DNA-PK, impairs AKT downstream signaling, resulting in enhanced DNA damage and apoptosis of AML cells.

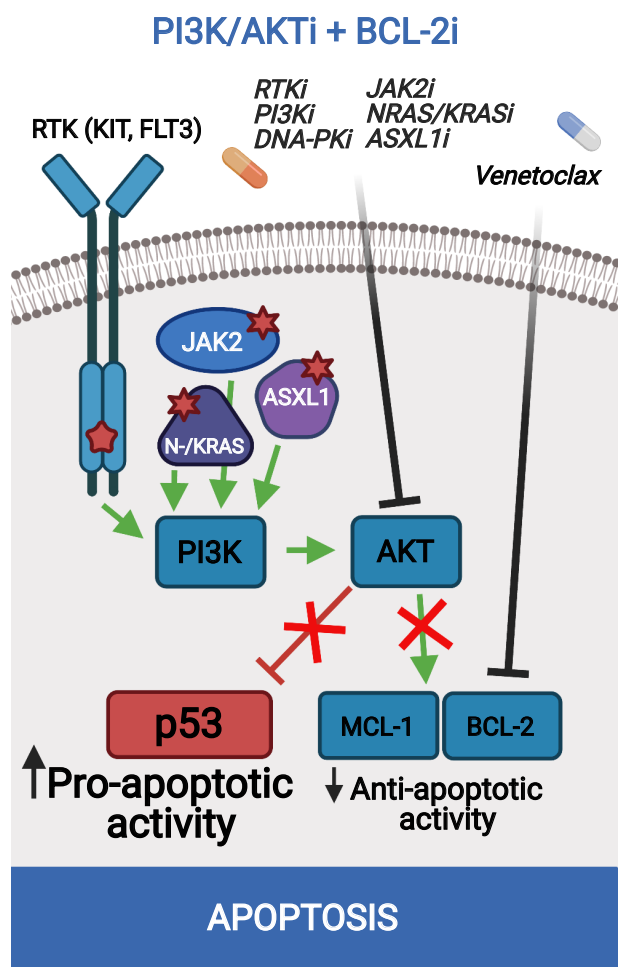


Figure 2: Putative model for concurrent treatment of AML exhibiting constitutive mutational PI3K/AKT activation with inhibitors of PI3K/AKT signaling and the BCL-2 inhibitor venetoclax. AML harboring mutations of RTKs (i.e. KIT or FLT3), JAK2, NRAS/KRAS or ASXL1 exhibit constitutive activation of the PI3K/AKT signaling pathway, which inhibits the tumor suppressor p53 and activates the anti-apoptotic MCL-1 and BCL-2 molecules, promoting survival of AML cells. Therapeutic targeting of PI3K/AKT pathway by PI3Ki or DNA-PKi (or indirectly by inhibitors of either RTKs, JAK2, NRAS/KRAS or ASXL1) in combination with BCL-2 inhibitors such as venetoclax, are predicted to cooperatively activate the intrinsic apoptotic pathway leading to enhanced apoptosis of AML cells.

collateral inhibition of chemotherapy-induced apoptosis. Mechanistically, this inhibition is potentially promoted by (i) PI3K/AKT downstream activation of MDM2-mediated TP53 degradation and by (ii) PI3K/AKT upregulation of the anti-apoptotic BCL2 and MCL1 proteins, via transcriptional regulation by cyclic adenosine monophosphate response element-binding protein (CREB) [12,31,32]. Given that MCL1 expression, promoted by PI3K/AKT activation, can confer resistance to the BCL2 inhibitor venetoclax in AML patients, concomitant therapeutic inhibition of PI3K/AKT signaling and relevant proapoptotic proteins such as BCL2, MCL1, or BCL2L1 (BCL-XL), might indeed cooperatively enhance apoptosis in AML cells. The latter is supported by preclinical PDX trials of AML and solid cancers underpinning the therapeutic efficacy of the

BCL2 inhibitor venetoclax in combination with PI3K/AKT inhibitors [33,34]. Hence, given that some AML patients will not respond or eventually develop resistance to venetoclax/azacitidine treatment [35], there is a strong rationale to explore the therapeutic potential of PI3K/AKT and/or DNA-PK inhibitors in combination with venetoclax (Figure 2), and eventually chemotherapy or HMAs.

In conclusion, preclinical and clinical trials underpin the huge potential of AML treatment modalities combining standard chemotherapy and HMAs with simultaneous targeted inhibition of key signaling hubs, at the boundaries of oncogenic signaling and DDR, to boost therapy response and ultimately overcome therapy resistance in AML patients.

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