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Enhancing the effectiveness of nucleoside analogs with mTORC1 blockers to treat acute myeloid leukemia patients

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Despite continuous advances in the knowledge of the biology of acute myeloid leukemia (AML), the prognosis of AML patients treated with standard chemotherapy is still poor, especially in the elderly (> 60 y). It should be considered that AML mainly develops in the elderly, with a median age at diagnosis of 68 y and a growing incidence over 65 y. AML accounts for about 25% of all adult leukemias in the western world, and it is the second most frequent form of leukemia following chronic lymphocytic leukemia.[1] Given the extremely poor prognosis of AML, there is a need for novel targeted and less toxic therapies, especially for patients who are over 60 y or those who develop resistance to traditional chemotherapeutic drugs. Constitutively active phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling is a common feature of AML patients, where it negatively influences response to therapeutic treatments.[2] A major issue in the efforts to treat AML patients is the inability of current therapies to efficiently target and eradicate leukemia initiating cells (LICs), which are the cells thought to initiate and maintain the leukemic phenotype.[3] In a recent, open-label phase II trial performed by the Italian GIMEMA cooperative group, the efficacy and safety of the drug combination consisting of low-dosage clofarabine with the allosteric mTOR complex 1 (mTORC1) inhibitor temsirolimus (CCI-779, Torisel©) was studied in a group of elderly patients with refractory/relapsed AML.[4] Some encouraging clinical results were seen. Clofarabine is a second-generation purine nucleoside analog that has been synthesized to overcome the limitations and incorporate the best properties of fludarabine and cladribine.[5] Although clofarabine is quite widely used for the treatment of AML patients, surprisingly there were no data in the literature regarding the effects of this drug on signaling pathways of AML cells.

We recently performed a translational study, related to the above reported clinical trial, in which we assessed the therapeutic potential of a combination consisting of clofarabine with temsirolimus (CLO-TOR)[6] in AML cells. The drug combination displayed synergistic cytotoxic effects against a panel of AML cell lines and primary cells from AML patients. Treatment with CLO-TOR induced a G_0/G_1-phase cell cycle arrest, apoptosis and autophagy. Cell cycle arrest was characterized by an induction of $p27^{Kip1}$, which was much stronger when the two drugs were used in combination than as monotherapy. We also observed that the CLO-TOR combination was more effective than either drug alone in dephosphorylating key components of the PI3K/Akt/mTOR pathway, including the translational repressor, 4E-BP1, which mainly regulates oncogenic protein synthesis (**Fig. 1**). 4E-BP1 phosphorylation is usually quite resistant to treatment with rapamycin/rapalogs in AML cells, and this could at least partly explain why this class of drugs only display a limited efficacy in AML.[2] Indeed, we observed that eIF4F complex formation was markedly downregulated by CLO-TOR treatment in AML patient samples, and this suggested that the drug combination efficiently targeted translation of oncogenic proteins. The CLO-TOR combination also affected STAT3 and c-Myc expression in AML cell lines. c-Myc downregulation could be critical for the cytotoxic effects of CLO-TOR, as a decrease in c-Myc levels could result in the inhibition of ribosome synthesis that, in turn, causes proliferative arrest and/or apoptosis. Last but not least, CLO-TOR was pro-apoptotic in an AML patient blast subpopulation ($CD34^+/CD38^-/CD123^+$), which is enriched in putative LICs. Importantly, the combined treatment was more effective than either drug alone in inducing apoptosis in this leukemic cell subset. CLO-TOR was able to downregulate the phosphorylation levels of S6RP at Ser 235/236 and of Akt at Ser 473, implying targeting of both mTORC1 and mTORC2 in the $CD34^+/CD38^-/CD123^+$ subset (**Fig. 1**). How could clofarabine increase the previously reported cytotoxic activity of an mTORC1 inhibitor toward LICs? Indeed, the majority of LICs are quiescent and therefore not sensitive to various chemotherapeutic agents that kill rapidly dividing cells.[2] However, it should be recalled that clofarabine, besides inhibiting ribonucleotide reductase and DNA polymerase, directly targets the mitochondria and induces apoptosis, even in quiescent cells[5] (**Fig. 1**). Over the last few years, the results of two other clinical trials in which chemotherapy was combined with mTORC1 blockers have been

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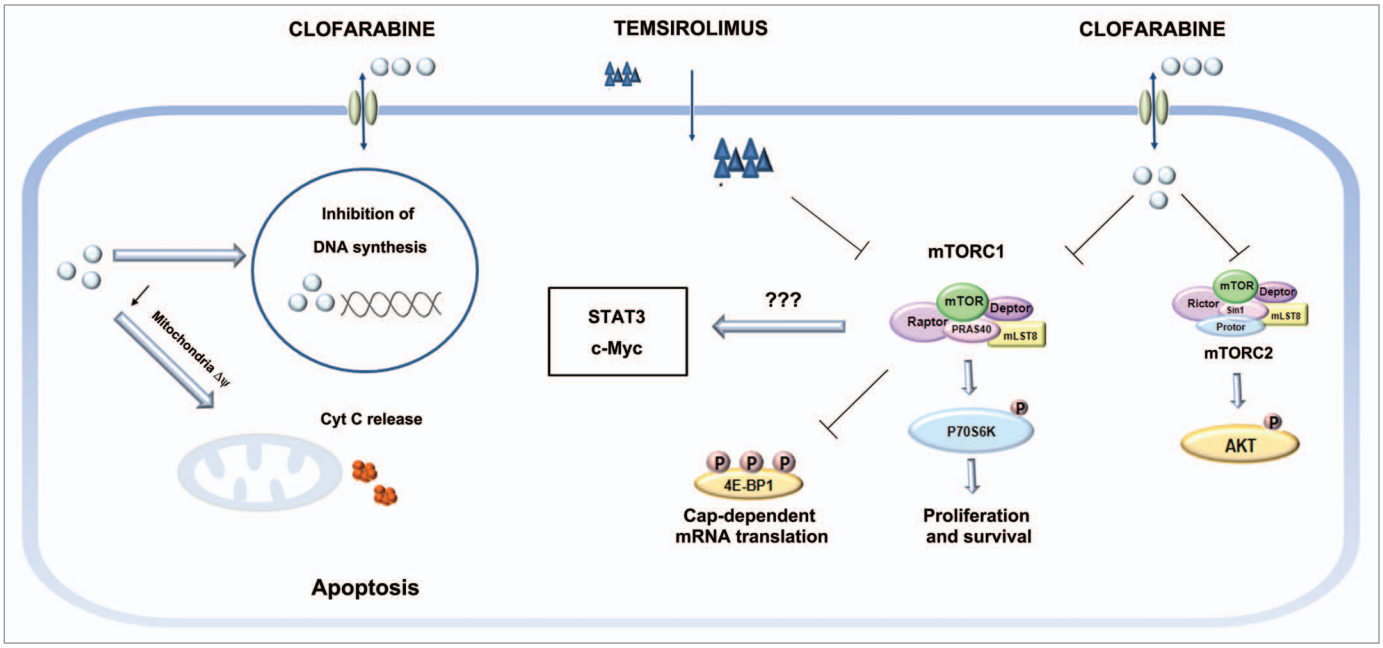

Figure 1. A cartoon highlighting the effects of clofarabine and temsirolimus on AML cells. Arrows indicate activating events, whereas perpendicular lines indicate inhibitory events.

disclosed.[7,8] Although the treatments appeared to be feasible and quite well tolerated by the patients, the clinical results were not satisfactory. In the first study, the synergy between the chemotherapy regimen and sirolimus was not confirmed. Thus, future investigations were planned with different schedules to clarify the clinical and biochemical effects of sirolimus in AML cells.[7] For this reason, we believe that pre-clinical studies are of the utmost importance for a better assessment of the efficacy of combined treatments to be used in vivo in humans. Indeed, our study has unequivocally documented the existence of a synergism between clofarabine and temsirolimus at clinically relevant concentrations.[6] However, our study has also highlighted additional and unexpected effects of clofarabine on AML cell signaling pathways that could certainly help in designing more effective therapeutic protocols, in which clofarabine will be combined with mTORC1 modulators or other signal transduction inhibitors, for treatment of AML patients.

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