# Power out chronic lymphocytic leukemia: unplugging OXPHOS/mTOR pathways to overcome venetoclax resistance

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In this issue of *Haematologica*, Chen *et al.* report their findings on the contribution of metabolic pathways to CD40-induced venetoclax resistance in chronic lymphocytic leukemia (CLL).[1] The BCL-2 inhibitor venetoclax is a widely used first-line treatment in fit patients with CLL. Venetoclax-based treatment has shown efficacy and a favorable safety profile, but emergence of resistance remains an important challenge to overcome. Development of chemoresistance is profoundly shaped by multifaceted interactions occurring within the tumor microenvironment. Indeed, venetoclax efficiently eliminates quiescent circulating CLL cells in the peripheral blood, while it produces a less satisfactory response in proliferation centers, in which the CLL cells are exposed to a myriad of signals.[2] Notably, within the lymph nodes, interactions between CLL and $CD4^+$ T helper cells via CD40-CD40L signaling contribute to enhanced expression of anti-apoptotic proteins (i.e., BCL-XL, MCL-1 and BFL-1) in CLL cells, leading to subsequent resistance to anticancer therapies.[3]

When entering proliferation centers, B-cell receptor engagement induces metabolic reprograming mainly relying on aberrant mitochondrial oxidative phosphorylation (OXPHOS) and increased glycolytic capacity/reserve in CLL cells, thereby promoting CLL cell survival and proliferation.[4-6] Chen *et al.* have already provided additional insights into the metabolism of CLL cells in lymph nodes and resistance to therapy.[7] They demonstrated that B-cell receptor/CD40-engagement boosts energy metabolism in CLL cells, an effect involving enhanced glycolysis, OXPHOS and the tricarboxylic acid cycle. They demonstrated, for the first time, the dependency of B-cell receptor/CD40-induced CLL cells on glutamine, this being the main substrate fueling the tricarboxylic acid cycle.

To further elucidate the mechanisms behind CD40-induced resistance to venetoclax in CLL, in the study published in this issue of *Haematologica*, Chen and colleagues used an *in vitro* model in which CLL cells isolated from the peripheral blood of patients were activated with CD40L in order to mimic lymph node signaling (Figure 1A). Similar to resistant CLL cells arising from long-term selection with venetoclax during therapy or in the laboratory, *in vitro* CD40-activated CLL cells exhibit comparable metabolic characteristics. Here, the strategy was to use inhibitors of different metabolic pathways during CD40 stimulation, and subsequently expose the cells to venetoclax. Thereby, the authors identified OXPHOS as the main driver of CD40-mediated resistance to venetoclax, while neither the inhibition of glycolysis nor that of glutaminolysis counteracted venetoclax resistance. To go further into OXPHOS dismantling, the activity of complexes I, II, III and V of the electron transport chain was independently targeted. The authors reported that only the inhibition of electron transport chain complexes involved in proton pumping and ATP production (complexes I, III and V) led to increased susceptibility to venetoclax (Figure 1B). Inhibitors of electron transport chain complexes I, III and V acted by reducing the two critical players in venetoclax resistance, BCL-XL and MCL-1 proteins, and by downmodulating CD40 signaling through a negative feedback loop.

Chen *et al.* also highlighted that induction of OXPHOS and subsequent venetoclax resistance, following CD40-stimulation of CLL cells, is mediated by mammalian target of rapamycin (mTOR) signaling. As for OXPHOS inhibition, specific targeting of mTOR1/2 decreased the basal oxygen consumption and extracellular acidification rates, and limited the spare respiration capacity, therefore sensitizing CLL cells to venetoclax.

Furthermore, the authors emphasize the crucial role of aberrant *de novo* protein translation in CD40-mediated venetoclax resistance in CLL cells. These results strengthen recent findings demonstrating that B-cell receptor- or Toll-like receptor-engagement leads to the activation of translation initiation in CLL cells. It appears that, rather than a general increase in mRNA translation, enhanced translation

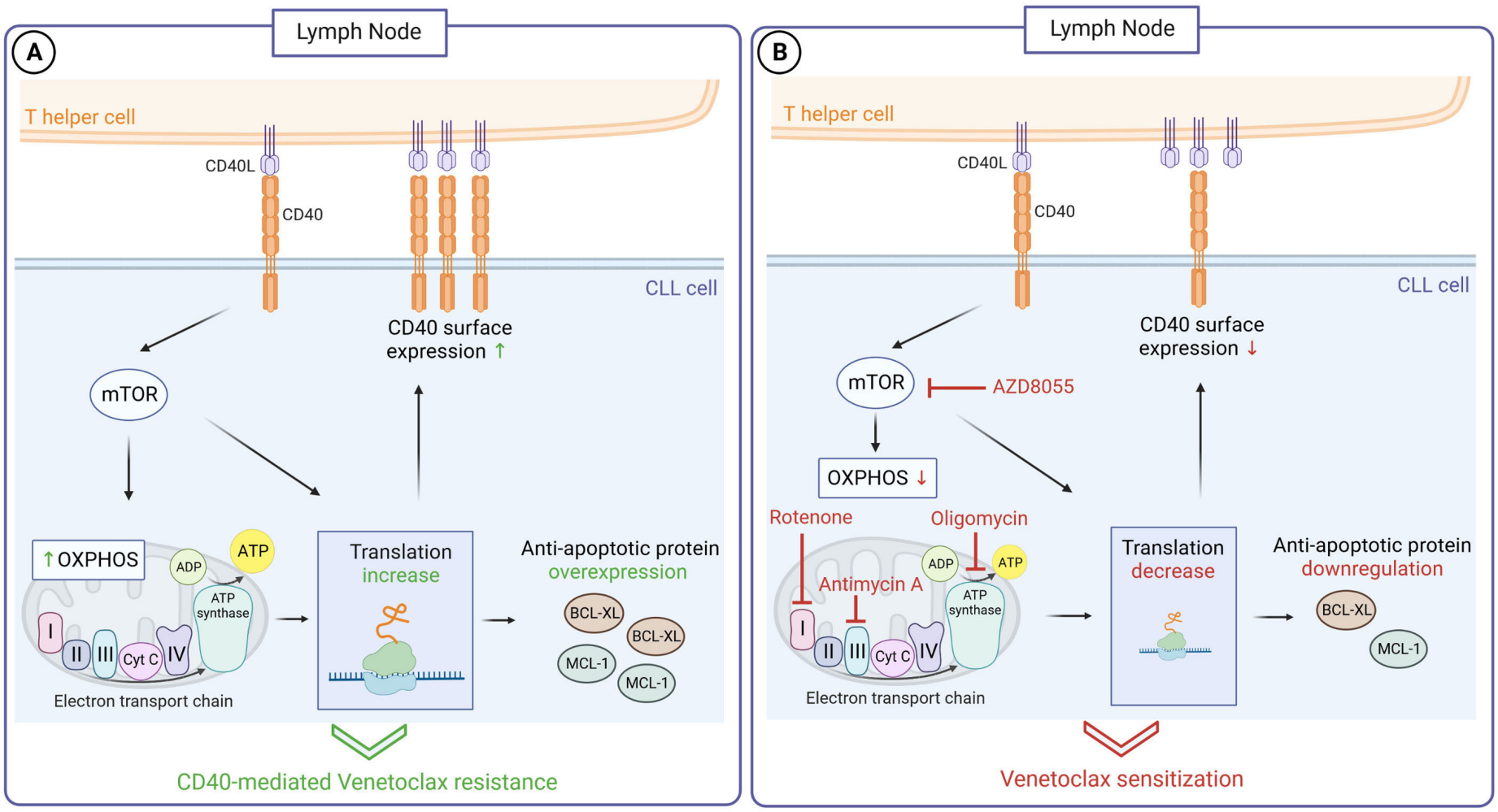


**Figure 1. CD40-mediated venetoclax resistance in chronic lymphocytic leukemia cells involves mTOR and OXPHOS pathways.** (A) Within the lymph node, stimulation of chronic lymphocytic leukemia (CLL) cells by T helper cells, via the CD40-CD40L interaction, leads to increased oxidative phosphorylation (OXPHOS), which is a crucial driver of resistance to venetoclax. During this process, the mammalian target of rapamycin (mTOR) pathway connects OXPHOS with CD40 signaling. Both mTOR and OXPHOS pathways exhibit similar effects by promoting protein translation. Together, these two pathways likely affect the sensitivity of CLL cells to venetoclax by upregulating the expression of the anti-apoptotic proteins BCL-XL and MCL-1, while also over-activating CD40 signaling. (B) mTOR inhibition by AZD8055 decreases OXPHOS in CLL cells. Both mTOR inhibition and direct OXPHOS inhibition by rotenone, antimycin A, or oligomycin lowers all regulators linked with venetoclax sensitivity, including decreased protein translation, downregulation of the anti-apoptotic proteins BCL-XL and MCL-1, and downmodulation of CD40 signaling. Together, inhibition of both OXPHOS and mTOR synergistically counteracts venetoclax resistance in CD40-activated CLL cells.

of specific pathways, particularly MYC and MCL-1, is likely to be essential for CLL cell survival and proliferation.[8,9] Interestingly, Chen *et al.* reported synergistic effects on translation when combining OXPHOS and mTOR inhibitors, converging to an almost complete rescue of CD40-mediated venetoclax resistance.

In their study, Chen and colleagues shed light on the critical role of the tumor microenvironment in conferring resistance to therapies in CLL. They established that CD40-mediated venetoclax resistance in CLL is mediated by mTOR signaling and aberrant OXPHOS metabolism, both mechanisms sharing an elevated translation rate as a common feature. The authors propose that new therapeutic opportunities for CLL patients who are resistant to venetoclax could come from combining OXPHOS and mTOR inhibitors. While mTOR and OXPHOS inhibitors have been approved for clinical use against different types of pathologies,[10] some of them are now being tested in clinical trials for the treatment of resistant malignancies. One notable example is the combination of everolimus, a Food and Drug Administration-approved mTOR inhibitor used in advanced kidney cancer, with rituximab, a chimeric anti-CD20 monoclonal antibody, administered after high-dose consolidative therapy to prevent relapse in lymphoma patients. On the other hand, the OXPHOS inhibitor IACS-010759 has recently been investigated in phase I clinical trials involving patients with relapsed or refractory acute myeloid leukemia, as well as those with advanced, metastatic, or unresectable solid tumors. However, no clinical studies have been conducted on the combination of OXPHOS and mTOR inhibitors, particularly in the context of refractory malignancies. Chen and colleagues aim to address this gap by targeting both mTOR and OXPHOS, with the goal of overcoming resistance mechanisms observed in CLL, emphasizing the significance of addressing translation dysregulation and developing innovative therapeutic strategies.

## Disclosures

*No conflicts of interests to disclose.*

## Contributions

*Both authors contributed equally.*

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