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Auto-Commentary on: "Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells"

Lucie de Beauchamp¹, Pablo Baquero¹, Elodie M. Kuntz², Eyal Gottlieb^{2,3} & G. Vignir Helgason¹

¹Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, College of Medical, Veterinary & Life Sciences, University of Glasgow, G61 1QH, UK. ²Cancer Research UK, Beatson Institute, Garscube Estate, Switchback Road, Glasgow G61 1BD, UK. ³Technion Integrated Cancer Center, Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, 3525433, Israel.

Correspondence: E.G. (e.gottlieb@technion.ac.il) and G.V.H. (Vignir.Helgason@Glasgow.ac.uk)

Abstract:

We have recently uncovered an abnormal increase in mitochondrial oxidative metabolism in therapy-resistant chronic myeloid leukaemia stem cells (LSCs). By simultaneously disrupting mitochondrial respiration and inhibiting BCR-ABL kinase activity using the antibiotic tigecycline and imatinib respectively, we effectively eradicated LSCs and prevented disease relapse in pre-clinical animal models.

Author's comment:

Chronic myeloid leukaemia (CML) arises following reciprocal translocation between chromosomes 9 and 22 (t(9;22)(q34;q11)) in a haematopoietic stem cell (HSC), which results in the expression of the fusion onco-protein BCR-ABL, a constitutively active tyrosine kinase. Although BCR-ABL-specific tyrosine kinase inhibitors (TKIs), including first-generation imatinib, have revolutionised the treatment of the disease, almost two-thirds of CML patients show residual disease after five years on TKI treatment. This is due to the presence of TKI-insensitive leukaemic stem cells (LSCs), which is associated with acquired resistance and disease relapse [1]. In this context, our main aim is to find new therapeutic strategies to target LSCs and achieve therapy-free remission in CML patients.

Recently, colleagues from the University of Glasgow and others have uncovered new molecular pathways involved in the survival of LSCs. These include networks regulated by c-MYC and p53 [2], the histone-lysine N-methyltransferase EZH2 [3] and the peroxisome proliferator-activated receptor gamma (PPAR γ) [4]. However our study is, to our knowledge, the first to use a metabolomic-based approach to characterise normal human HSCs versus CML LSCs in order to find potential selective therapeutic targets [5]. Interestingly, the antibiotic tigecycline, shown to successfully inhibit mitochondrial protein translation in acute myeloid leukaemia (AML) cells [6], proved to be a promising candidate to target upregulated mitochondrial oxidative metabolism in CML LSCs [5].

To identify metabolic vulnerabilities in CML LSCs, we compared the steady-state levels of 70 key metabolites of fatty acid, amino acid, glucose and energy metabolism from CML patients-derived CD34⁺ cells with their normal counterparts. We found higher levels of carnitine and acylcarnitine derivatives and lower levels of free fatty acids in CML CD34⁺ cells, suggesting a potential upregulation of lipolysis and fatty acid oxidation (FAO; Figure 1). Furthermore, both stable isotope-assisted metabolomic analysis, using uniformly ¹³C₁₆-labelled palmitate or ¹³C₁₆-labelled glucose, and functional assays measuring the oxygen consumption rate revealed a clear upregulation of the overall oxidative metabolism in CML CD34⁺ cells compared to their normal counterparts [5]. Interestingly, CML CD34⁺ cells also showed lower lactate levels and increased oxidative and anaplerotic activity compared to the more differentiated CD34⁻ fraction, suggesting that primitive CML cells rely preferentially on oxidative metabolism rather than glycolysis [5]. Similar ¹³C isotopic enrichment experiments revealed that treatment with tigecycline reduced the amount of labelled carbons in tricarboxylic acid (TCA) cycle intermediates, proving that tigecycline can effectively inhibit the oxidative metabolism of LSCs. Our data also shows that treating CML cells with a combination of tigecycline and imatinib almost fully inhibits the colony formation capacity of CML progenitors and long-term stem cells [5]. Interestingly, the drug combination did not elicit the same effect in normal haematopoietic CD34⁺ cells, ruling out the potential for broad cytotoxicity.

Importantly, as mitochondria constitute the main source of reactive oxygen species (ROS) in cells, our results may explain previous studies showing that LSCs present higher levels of ROS and DNA damage than normal HSCs [7]. Our findings indeed support the *in vivo* experiments results obtained by Bolton-Gillespie *et al.*, which report higher levels of ROS in primitive cells compared to the more differentiated compartment within the bone marrow of leukaemic mice. Other studies on cancer stem cells, including in AML, have shown that these primitive cells rely more heavily on oxidative phosphorylation (OXPHOS) to supply their energetic demands compared to bulk tumour cells and normal stem cells, regardless of the low-ROS levels found in the more quiescent AML stem cells [8]. Therefore, elucidating the link between mitochondria, ROS generation and stemness in CML LSCs represent an attractive topic for future investigation. Lastly, using single-cell RNA sequencing coupled with a highly sensitive BCR-ABL detection method, Giustacchini *et al.* elegantly showed in a recent study that primitive BCR-ABL⁺ cells from CML patients presented an overexpression of OXPHOS and FAO-related genes when compared to their bystander BCR-ABL⁻ counterparts [9]. This further suggests that the oxidative phenotype we characterised is specific to LSCs in patients, further highlighting the clinical relevance of our findings.

Using a well-established xenotransplant model of human CML, we also tested the effect of tigecycline treatment alone or in combination with imatinib *in vivo*. The combination treatment drastically reduced the number of the most primitive long-term engrafting cells. In order to investigate whether this combination was also effective in preventing relapse, we treated xenografted CML cells with tigecycline alone or in combination for a period of four weeks, then discontinued the treatment for two or three weeks. Strikingly, mice treated with the drug combination maintained a low number of LSCs after treatment discontinuation, whereas similar levels of LSCs were found in imatinib treated mice and untreated mice. These results reveal that combining tigecycline with imatinib might prevent disease relapse in CML patients with minimal residual disease.

Taken together, these findings make a strong case for further investigation of the use of tigecycline in CML treatment in human, especially so as it has already been approved by the American food and drug administration for antibiotic use. The disappointing results from a recent phase I clinical trial where tigecycline was used as a single agent in relapsed or refractory AML [10] also highlights the relevance of the TKI combination strategy we employed in our study. Tigecycline has incidentally been added to the list of drug candidates in a clinical trial grant, which aims to test promising combinations of novel or repurposed drugs with TKIs. If results from the present study are confirmed in a future trial, tigecycline could represent the first step towards a real cure for CML patients and prove relevant in other diseases driven by OXPHOS-dependent cancer stem cells.

Figure 1: Selective targeting of CML LSCs using tigecycline in combination with TKI

While normal HSCs have low mitochondrial activity, transformation from a HSC to CML LSCs is associated with constitutive BCR-ABL kinase activity and an increase in mitochondrial oxidative metabolism. During the course of glycolysis, glucose is broken down to pyruvate, which can be used to produce lactate. Alternatively, pyruvate can be transferred to the mitochondria, where it is broken down in the TCA cycle. This leads to generation of reducing agents (NADH and FADH₂), which can be used by the electron transport chain (ETC) to generate ATP. Similarly, fatty acids are activated (converted to fatty acyl CoA) in the cytosol and transported to the mitochondrial via the carnitine shuttle, where they undergo oxidation to yield acetyl-CoA for the TCA cycle. Tigecycline treatment inhibits OXPHOS by hindering mitochondrial translation and leads to eradication of CML LSCs, when used in combination with imatinib, a BCR-ABL targeting TKI. PHD, pyruvate dehydrogenase

(transforms pyruvate into acetyl-CoA); PC, pyruvate carboxylate (catalyses anaplerotic reaction that creates oxaloacetate from pyruvate).

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Normal HSCs



CML Stem Cell



OXPHOS

