



# A Permeable Succinate Improved Platelet Mitochondrial Respiration in Paediatric Acute Lymphoblastic Leukaemia in Remission – Case Report

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## Abstract

Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy. In the last decades, the survival rate of paediatric patients diagnosed with ALL has been significantly improved due to standardised treatment protocols based on risk stratification. Platelet mitochondrial dysfunction has been recently reported to occur in most chronic diseases, including malignancies. Permeable succinate (NV118) is a novel mitochondria-targeted compound capable to alleviate disease and drug-induced mitochondrial dysfunction. It is reported here that ex vivo incubation with NV811 elicited an increase in platelet mitochondrial respiration in a paediatric patient with acute lymphoblastic leukaemia in remission.

**Key words:** Platelet; Mitochondria respiration; Cell-permeable succinate; Paediatric acute lymphoblastic leukaemia; Remission.

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## Introduction

Paediatric leukaemia is the most common childhood malignancy. Acute lymphoblastic leukaemia is diagnosed in approximately 80 % of paediatric leukaemia cases.<sup>1</sup>

Due to major advances in diagnosis, risk stratification and improved international treatment protocols, the survival rate of paediatric ALL patients significantly increased up to 80-90 %.<sup>2,3</sup> Mitochondrial dysfunction is nowadays a widely accepted mechanism in the pathogenesis of all age-related chronic diseases.<sup>4,5</sup>

Peripheral platelets represent a convenient source of human mitochondria since they can be easily sampled as compared to other tissues/organs which require invasive biopsy. Recent

studies have revealed that assessment of platelet respiration can provide an overall view of mitochondrial dysfunction and bioenergetic health.<sup>6-8</sup>

In an elegant study, Baaten et al reported the presence of platelet mitochondrial dysfunction in adult patients diagnosed with haematological malignancies undergoing chemotherapy.<sup>(9)</sup> However, the long-lasting effects of chemotherapeutic drugs, after the termination of the standard cures, are well known. Whether platelet mitochondrial function can be further supported in the settings of haematological remission it is not known and has been here addressed in a paediatric case diagnosed with acute lymphoblastic leukaemia (ALL).

Diacetoxymethyl succinate (NV118) is a novel mitochondria-targeted compound, which has been reported to alleviate mitochondrial dysfunction induced by various drugs, such as paracetamol, statins and amiodarone.<sup>10-12</sup>

The present study was aimed at investigating whether NV118, a cell-permeable succinate prodrug, is capable to improve platelet mitochondrial respiration in platelets isolated from a paediatric patient with ALL remission.

## Case history

An 11-year-old male patient, with no previous significant medical history, presented in April 2019 with the following symptoms with insidious onset: fatigue, inappetence, pallor, myalgia. Clinical exam revealed general lymphadenopathies and splenomegaly. Laboratory investigations at the presentation showed the following abnormalities: moderate normocytic normochromic anaemia (Hb = 8.6 g/L, E = 3.410.000/mm<sup>3</sup>), leukocytosis (18,180/mm<sup>3</sup>) with lymphocytosis (15,010/mm<sup>3</sup>), elevated C reactive protein (101 mg/L), elevated erythrocyte sedimentation rate (103 mm/h), elevated lactate dehydrogenase (1176 U/L), increased serum ferritin (553 ng/mL), hyperuricaemia (438 µmol/L) and mild hepatocytolysis. Viral serology testing excluded infectious mononucleosis, cytomegalovirus infection, hepatitis B and C and HIV infection. Bacterial infections were excluded by assessing procalcitonin, which was in normal range and by performing blood and body fluids cultures that remained sterile.

**Table 1:** Immunophenotype and molecular characteristics at ALL onset

<b>Bone marrow aspirate – immunophenotype</b>	<p>45% Blasts with the following phenotype:            CD45var+CD19+cyCD79a+CD10+C-D34var+CD20+/-CD58+/-CD-38dim+/-CD22+CD24+nulTdT+C-D13+CD15+CD65+;            Markers not expressed on blast cell surface:            cyCD3, cyMPO, CD7, smCD+, CD66, smIgKappa, CylgM, CD117, smIgM, smIgLambda, CD9,NG2,CD123,C-D81,CD21.            Conclusion: Immunophenotype compatible with a B cell precursor ALL (common B ALL)</p>
<b>Cytogenetic tests</b>	<b>TEL-AML1 (ETV6/RUNX1) fusion gene</b>

Due to the increased probability of an acute leukaemia diagnosis, the patient underwent a bone marrow aspiration procedure. After bone marrow immunophenotyping through flow cytometry, the diagnosis of acute lymphoblastic leukaemia was confirmed. Furthermore, in accordance with the International BMF–Berlin-Frankfurt-Münster Study Group protocol (ALL IC BFM 2009), cerebral-spinal fluid analysis was performed, which excluded central nervous system leukaemia.<sup>13</sup> Cytogenetic testing was also performed and revealed the presence of the TEL-AML1 (ETV6/RUNX1) fusion gene (Table 1). This patient was included in the medium risk group according to the international protocol risk stratification<sup>13</sup> and treated accordingly. In November 2021, ALL remission was confirmed by minimal residual disease assessment with flow cytometry.

## Experimental protocol and results

The study was approved by the Research Ethics Commission of the "Victor Babeş" University of Medicine and Pharmacy, Timișoara (Nr. 42/20.12.2018).

Venous blood (10 mL) was sampled in K<sub>2</sub>EDTA tubes (BD Vacutainer® Plymouth) by a single venipuncture after obtaining the written informed consent from the parents and immediately transferred to the laboratory for analysis. Platelet isolation was performed according to a previously established protocol.<sup>14</sup> In brief, platelet rich plasma was obtained after a first centrifugation of peripheral blood at 500 g at room temperature and was subjected to a second centrifugation at 4,600 g at room temperature. The platelet pellet was resuspended in own plasma and the number of platelets necessary for high-resolution respirometry studies was counted using a classic haematology analyser. Respiration of isolated platelets was assessed at 37°C using the Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) by means of high-resolution respirometry (HRR). In order to perform HRR studies, 50 million platelets/ml (100 million platelets/2 ml oxygraph chamber) were suspended in the MIR05 buffer (D-Sucrose 110 mM, taurine 20 mM, EGTA 0.5 mM, HEPES 20 mM, MgCl<sub>2</sub> 3 mM, Lactobionic Acid 60 mM, KH<sub>2</sub>PO<sub>4</sub> 10 mM, BSA 1 g/L, pH 7.1 adjusted with KOH 5 M).

Platelet oxygen consumption was allowed to stabilise until a steady state (ROUTINE respiration) was reached. Afterwards, intact platelets were subsequently exposed to either cell-permeable succinate (NV118, 500 µM) or its solvent, dimethyl sulphoxide (DMSO). The non-phosphorylating respiration (or LEAK in HRR) was first assessed by adding oligomycin (1µg/mL), an ATP synthase inhibitor. Subsequently, the maximal non-coupled respiration or the convergent respiratory capacity of the electron transport system (ETS in HRR) was determined after consecutive titrations of FCCP, a classical uncoupler. Further, rotenone, a Complex I inhibitor (2 µM), was added to assess the ETS capacity supported only by Complex II (ETS<sub>CI</sub> in HRR). Finally, mitochondrial respiration was inhibited by the addition antimycin A (1 µg/mL), a complex III inhibitor, allowing for the measurement of residual oxygen consumption (ROX). All values of the respiratory parameters were corrected for ROX. Last but not least, R-L net routine capacity (R-L) was calculated as the difference between ROUTINE respiration and LEAK respiration in order to assess respiratory capacity available for phosphorylation of ADP to ATP, according to refs.<sup>10, 15</sup>

An overlay of the HRR tracings in the presence of DMSO (dark blue) vs NV118 (light blue) is depicted in Figure 1.

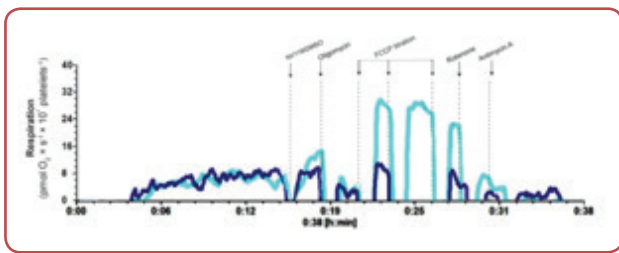


Figure 1: Overlay of a HRR tracings in the absence (dark blue) and presence (light blue) of NV118.

Table 2: Mitochondrial respiratory rates in the presence of DMSO (solvent) and NV118 in platelets harvested from a pediatric patient with ALL in remission.

Respiratory Parameters	ALL + DMSO	ALL + NV118
Routine respiration	7.52	13.24
LEAK respiration	1.74	3.41
ETS capacity	10.02	28.86
ETSII capacity	1.91	6.52
R-L net routine capacity (R-L)	5.79	9.83

Routine: basal respiration; LEAK: non-phosphorylating respiration; ETS capacity: maximal uncoupled respiration of complex I and II; ETSII: maximal uncoupled respiration driven only by complex II;

It is reported here that the cell permeable succinate improved mitochondrial respiration in isolated platelets during ALL remission. As showed in Table 1, NV118 increased routine respiration by 76 % and LEAK respiration by 96 %, as compared with the control (DMSO). The most significant increase elicited by NV118 was found for the maximal non-coupled respiration capacity ETS (187 %) and more important, for ETS<sub>II</sub> (242 %), since succinate is a Complex II substrate. Besides, the R-L net routine capacity noted an increase of 69,9 %, thus demonstrating that the respiratory capacity available for phosphorylation of ADP to ATP is high in this patient.

## Discussion

This is a case of a paediatric patient diagnosed in 2019 with TEL-AML1 positive, medium risk ALL, treated according to the standardised chemotherapy protocol (ALL IC BFM 2009, International BMF–Berlin-Frankfurt-Münster Study Group) and who achieved remission in 2021.<sup>13</sup>

While mitochondrial dysfunction has been reported as a central pathomechanism in various diseases, literature regarding platelet mitochondrial dysfunction in hematologic malignancies is scarce. In a pioneering study, Baaten et al reported the occurrence of platelet mitochondrial dysfunction in 77 adult patients diagnosed with various haematologic malignancies (37 patients with either ALL or AML, 21 patients with multiple myeloma,<sup>15</sup> patients with lymphoma and 4 patients with other types of haematologic malignancies) and chemotherapy-induced thrombocytopenia.<sup>9</sup> Mitochondrial respiration was assessed by means of high-resolution respirometry and revealed a significant decrease in the maximal active respiration after chemotherapy initiation. Whether mitochondrial respiration was altered in these patients due to chemotherapy or the malignant disease itself is a matter of debate; however, a combination of the two factors is most probable.<sup>16</sup>

The presence of platelet mitochondrial dysfunction at the onset (ie, before treatment initiation) of paediatric ALL has been previously reported. Accordingly, in paediatric patients aged 10-11,

newly diagnosed with ALL, a significant decrease in ROUTINE and LEAK respiration as well as in ETS and ETSII capacities was found. Unexpectedly, a significant increase in complex I-supported active respiration was found, which might be a compensatory phenomenon.<sup>14</sup>

An increasing number of pharmacological strategies aimed at supporting/restoring mitochondrial function have emerged in the past decades - comprehensively reviewed in ref.<sup>17</sup> - and several agents have already entered clinical trials.<sup>18</sup> Oxidative phosphorylation and mitochondrial metabolism have recently emerged as potential therapeutic targets in cancer, including the haematological malignancies.<sup>19,20</sup>

The past decades witnessed an increasing interest in characterising novel compounds that may alleviate mitochondrial dysfunction induced by disease or various drugs.<sup>10-12, 21-26</sup> In the line, NV118 is a novel permeable succinate prodrug, which has been reported to support mitochondrial respiration in various cellular models of complex I inhibition determined by rotenone, a classic complex I inhibitor, intoxication or poisoning with carbon monoxide, carbamate, cyanide or drug toxicity (statins, metformin, amiodarone).<sup>10, 12, 21-25, 27</sup> Since certain anticancer drugs cause formation of reactive oxygen species by impairing complex II of the ETS,<sup>28</sup> it might be speculated that supporting complex II function during chemotherapy (using a cell-permeable succinate would be of some benefit in reducing oxidative stress, a hypothesis that needs to be investigated.

Since chemotherapy-related impairment of mitochondrial function has been systematically reported in malignant cells<sup>16</sup>, most probably, it also occurs in the healthy ones yet with long-lasting effects. Therefore, protecting healthy cells is an essential part of cancer therapeutics.<sup>29</sup> Whether cell-permeable succinates are able to improve mitochondrial respiration in patients after chemotherapy has not been investigated so far. In the present study, NV118 increased all examined parameters of mitochondrial respiration in platelets sampled from a paediatric patient with ALL in remission. While it is true that the increase in respiration might be inefficient due to an important increase (by 96 %) in LEAK respiration, ie, nonATP-generating respiration, this might not necessarily be a downside.<sup>15</sup> Last but not least, there is evidence in the literature that high ATP levels might cause cancer drug resistance and it

has been suggested that downregulating the ATP synthesis might be a solution to drug resistant cancers.<sup>30</sup>

## Conclusion

In conclusion, a novel succinate prodrug, NV118, improved mitochondrial respiration in platelets isolated from a paediatric patient with ALL in remission. Whether this effect will be correlated with an improvement in the chemotherapy response and/or survival, remains to be assessed.

## Author Contributions

TL: writing—original draft preparation, investigation, data curation; AMB: investigation, data curation; VFA: formal analysis, visualisation; CB: visualisation, supervision; STA: conceptualisation, supervision; DMM: writing—review and editing, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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## Institutional Review Board Statement

The study was performed with the approval of the Committee of Research Ethics of “Victor Babeş” University of Medicine and Pharmacy, Timisoara, Romania (nr. 42/20.12.2018).

## Informed Consent Statement

Informed consent was obtained from the parents of the paediatric patient participating in the study before blood sampling.



## Data Availability Statement

Data are contained within the article.

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## Conflict of interest

None.

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