Clinical significance of mitochondrial DNA content in acute promyelocytic leukaemia

Pereira-Martins, Diego A.; Coelho-Silva, Juan L.; Weinhauser, Isabel; Franca-Neto, Pedro L.; Silveira, Douglas R.; Ortiz, Cesar; Moreira-Aguiar, Amanda; Lima, Marinus M.; Koury, Luisa C.; de Melo, Raul A.

Published in:
British Journal of Haematology

DOI:
10.1111/bjh.18510

Publication date:
2023

Received: 15 August 2022 | Accepted: 29 September 2022

DOI: 10.1111/bjh.18510

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

SHORT REPORT

Clinical significance of mitochondrial DNA content in acute promyelocytic leukaemia

Diego A. Pereira-Martins[1,2,3,4] | Juan L. Coelho-Silva[1,3,4] | Isabel Weinhäuser[2,3,4] | Pedro L. Franca-Neto[1] | Douglas R. Silveira[5] | César Ortiz[3,4] | Amanda Moreira-Aguiar[1] | Marinus M. Lima[1] | Luisa C. Koury[3,4] | Raul A. de Melo[6] | Ana B. Glória[7] | Evandro M. Fagundes[7] | Bruno K. Lino[8] | Katia Pagnano[8] | Rosane Bittencourt[9] | Elenaide Nunes[10] | Fabiola Traina[3,4] | Lorena Figueiredo-Pontes[3,4] | Armand Keating[11] | Martin S. Tallman[12] | Raul C. Ribeiro[13] | Richard Dilon[14] | Arnold Ganser[15] | Miguel A. Sanz[16,17] | Nancy Berliner[18] | Peter Valk[19] | Bob Löwenberg[19] | Tiziana Ottone[20,21] | Nelida I. Noguera[20,21] | Maria T. Voso[20,21] | Francesca Paoloni[21] | Paola Fazi[22] | Emanuele Ammatuna[2] | Gerwin Huls[2] | Jan Jacob Schuringa[2] | Eduardo M. Rego[3,4,23] | Antonio R. Lucena-Araujo[1]

[1]Department of Genetics, Federal University of Pernambuco, Recife, Brazil
[2]Department of Hematology, Cancer Research Centre Groningen, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands
[3]Department of Medical Imaging, Haematology, and Oncology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil
[4]Center for Cell Based Therapy, São Paulo Research Foundation, Ribeirão Preto, SP, Brazil
[5]Myeloid Leukaemia Genomics and Biology Group, School of Cancer and Pharmaceutical Sciences, King's College London, London, UK
[6]Department of Internal Medicine, University of Pernambuco, Recife, Brazil
[7]Hematology Division, Federal University of Minas Gerais, Belo Horizonte, Brazil
[8]Hematology and Hemotherapy Center, University of Campinas, Campinas, Brazil
[9]Hematology Division, Federal University of Paraná, Curitiba, Brazil
[10]Hematology Division, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[11]Princess Margaret Cancer Centre, Toronto, Ontario, Canada
[12]Leukemia Service, Memorial Sloan-Kettering Cancer Center/Weill Cornell Medical College, New York City, New York, USA
[13]Department of Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA
[14]Department of Medical and Molecular Genetics, King's College London School of Medicine, London, UK
[15]Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany
[16]Department of Hematology, Valencia University Medical School, Valencia, Spain
[17]CIBERONC, Instituto Carlos III, Madrid, Spain
[18]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA
[19]Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands
[20]Department of Biomedicine and Prevention, University of Tor Vergata, Rome, Italy
[21]Santa Lucia Foundation, I.R.C.C.S., Neuro-Oncohematology, Rome, Italy
[22]Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA), GIMEMA Foundation, Rome, Italy
[23]Hematology Division, LIM31, Faculdade de Medicina, University of São Paulo, São Paulo, Brazil

Diego A. Pereira-Martins and Juan L. Coelho-Silva contributed equally for this study.

Correspondence
Antonio R. Lucena-Araujo, Department of Genetics, Federal University of Pernambuco, Av. Prof. Moraes Rego, 1235, Recife, PE 50670-901, Brazil.
Email: araujoarl@gmail.com

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 303479/2018-3; Fundação de Amparo à Pesquisa do Estado de São Paulo, Grant/Award Number: 2013/08135-2, 2015/09228-0, 2016/23191-4 and 2017/23117-1

Summary

Although a growing body of evidence demonstrates that altered mtDNA content (mtDNAc) has clinical implications in several types of solid tumours, its prognostic relevance in acute promyelocytic leukaemia (APL) patients remains largely unknown. Here, we show that patients with higher-than-normal mtDNAc had better outcomes regardless of tumour burden. These results were more evident in patients with low-risk of relapse. The multivariate Cox proportional hazard model demonstrated that high mtDNAc was independently associated with a decreased cumulative incidence of relapse. Altogether, our data highlights the possible role of mitochondrial metabolism in APL patients treated with ATRA.

KEYWORDS
anthracycline-based chemotherapy, ATRA, mtDNA content, oxidative phosphorylation

INTRODUCTION

Being in use in the clinics for more than three decades, all-*trans* retinoic acid (ATRA)-based treatment (in combination with chemotherapy or arsenic trioxide, ATO) represents the first-line therapy for standard-risk acute promyelocytic leukaemia (APL). Consequently, APL remains the most curable subtype of acute myeloid leukaemia (AML) to date. In addition to the degradation of the PML/RARA fusion protein,[1] ATRA modulates the mitochondria-mediated cellular metabolism and promotes a metabolic shift from glycolysis to oxidative phosphorylation.[2] Considering that mitochondrial alterations potentially represent a metabolic vulnerability in leukaemic cells,[3] metabolic changes driven by ATRA could be clinically relevant.

Being an important part of the metabolic machinery, the biosynthesis of mitochondrial DNA (mtDNA) has been associated with increased mitochondrial biogenesis and dependence on oxidative phosphorylation.[4] In addition, studies in solid tumours suggested that alterations in mtDNA content (mtDNAc) could have clinical implications.[5,6] Yet, whether altered mtDNAc has an impact on clinical outcomes of patients with haematological malignancies (in particular APL and non-APL AML) remains largely unknown. Here, we hypothesize that APL patients with high mtDNAc, and consequently a high oxidative phosphorylation-driven metabolism, would be better responder to ATRA-based therapies.[7] To test this hypothesis, we determined the mtDNAc in bone marrow (BM) samples of patients treated with ATRA and anthracycline-based chemotherapy as well as patients treated with the state-of-the-art combination ATRA+ATO.

METHODS

Overall, we enrolled 156 patients treated with ATRA and chemotherapy. Details of treatment protocols are published elsewhere.[8] A second cohort was composed of 22 patients treated according to the APL 0406 protocol.[9] All samples were obtained at diagnosis. For comparison purposes, we included peripheral blood (PB) samples from 293 age- and sex-adjusted healthy volunteers with no history of haematological disorders. Additionally, we analysed $CD34^+$ cells isolated from BM (n = 3), cord blood (n = 9), and apheresis (n = 4) samples from healthy donors. The study adhered to the tenets of the Declaration of Helsinki and informed consent was obtained from all patients or their relatives. The local Research Ethics Board of each participating centre approved the study. mtDNAc was quantified using quantitative real-time polymerase chain reaction and the results were normalized and expressed as fold-change relative to a reference DNA. Details can be found in the Supplemental data. Descriptive analyses (frequency and central tendency and dispersion measurements) were performed for patients' characteristics at baseline. Details of the statistical analysis and clinical endpoints are described in the supplemental data.

RESULTS AND DISCUSSION

First, we compared the mtDNAc between healthy ($CD34^+$ and PB cells) and APL samples. Figure 1A shows that patients with APL had a higher-than-normal mtDNAc. Multiple comparison tests indicated no difference between healthy $CD34^+$ and PB cells (p = 0.956), while the relative mtDNAc was significantly lower in $CD34^+$ cells compared to APL (p = 0.005). Table S1 summarizes the central tendency and dispersion of mtDNAc measurements of all the enrolled subjects. Restricting our analyses to APL, we noticed that the relative mtDNAc of APL patients ranged between 0.1 and 39. To evaluate the impact of mtDNAc on clinical outcomes without seeking an optimal cut-point, we used values of mtDNA higher than the 95th percentile of healthy PB samples (i.e., ≥1.63; Figure 1A, dashed line) to define APL patients with high mtDNAc. Consequently, patients that presented values within the range of normal control samples (<1.63) were classified as normal mtDNAc. To account

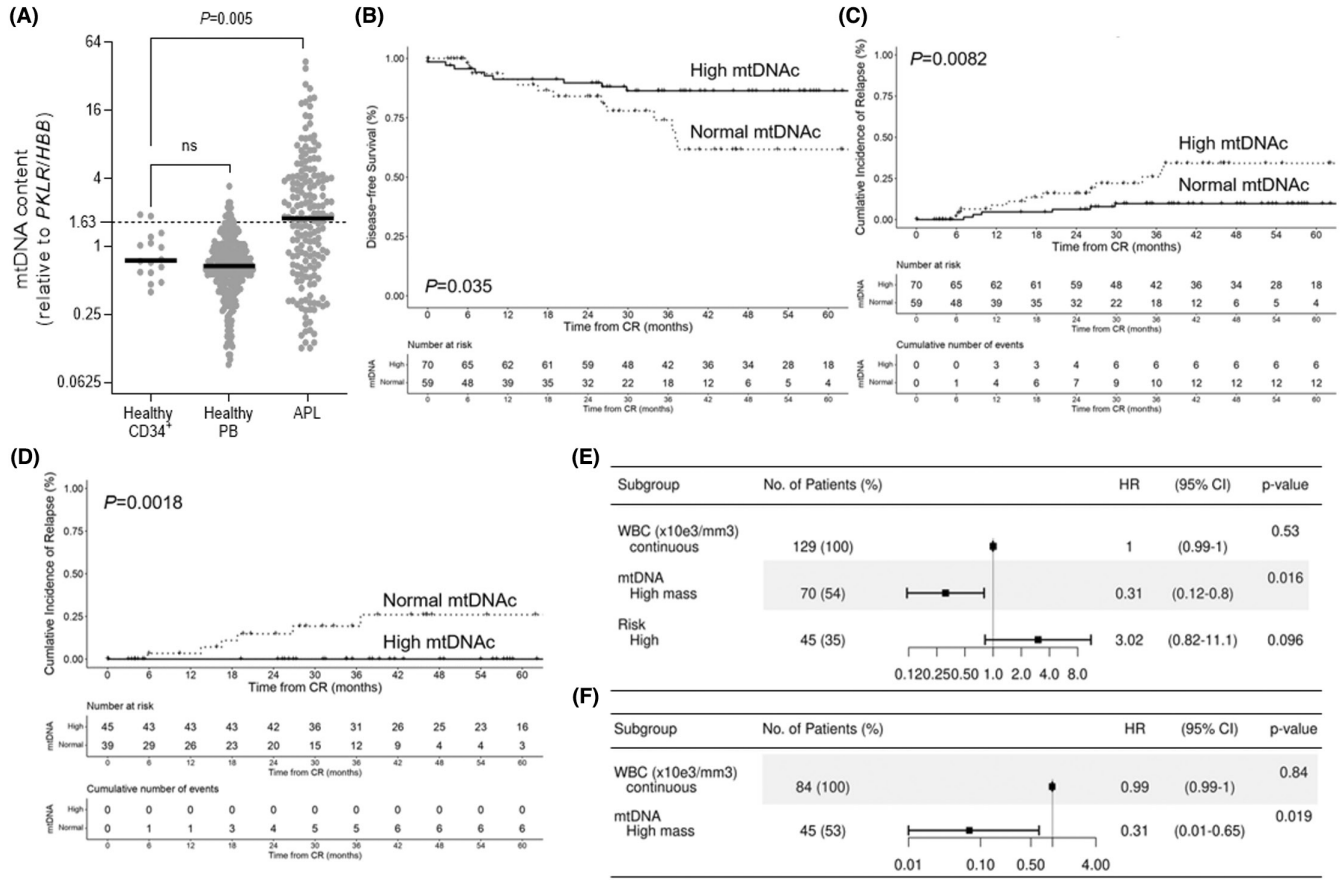

FIGURE 1 Relative quantification of mtDNAc in CD34$^+$ and peripheral blood cells from healthy donors and bone marrow cells from patients with acute promyelocytic leukaemia (APL) treated with ATRA + ATO (A + A) and ATRA + chemotherapy (A + C) (A). The relative quantification of mtDNAc was performed by quantitative real-time polymerase chain reaction relative to a single-copy nuclear gene (*PKLR and HBB*). The horizontal bars represent the median value of mtDNAc (fold-change). Patient survival. The probability of disease-free survival (B) and cumulative incidence of relapse (C) in APL patients treated with A + C according to the mtDNAc (entire cohort). Cumulative incidence of relapse in APL patients with low-risk of relapse (D) treated with A + C according to the mtDNAc. Survival curves were estimated using the Kaplan–Meier method, and the log-rank test was used for comparison. Cumulative incidence curves for non-relapse death and relapse with or without death were constructed to reflect time to relapse and time to non-relapse death as competing risks. Time to relapse and time to non-relapse death was measured from the date of complete remission. Forest plot representing the multivariate Cox proportional hazard model for the cumulative incidence of relapse in APL patients treated with A + C according to the mtDNAc (entire cohort) (E) and in low-risk patients (F). Confounding variables included in the model were mtDNAc (high mass versus normal mass), risk of relapse (high-risk versus low-risk),[10] and white blood cells counts (continuous variable).

for the potential variability in mtDNAc between different sources, we measured the mtDNAc of 22 paired BM and PB samples from APL patients collected at the time of diagnosis. Our analysis showed a strong correlation between BM and PB measured mtDNAc of the same patient (Pearson correlation coefficient, $r = 0.78$, 95% confidence interval, CI: 0.54–0.9; Figure S1), suggesting that mtDNAc detected in BM and PB could be used interchangeably with little bias.

Analysis of baseline characteristics indicated significantly lower mtDNAc in *FLT3*-ITD mutated (median: 1.62, 95%CI: 1.6–4.4) compared to *FLT3*- wild-type patients (median: 2.7, 95%CI: 3.7–6.9) ($p = 0.03$; Figure S1). No other differences were found between patients with normal and high mtDNAc (Table S2). Patient follow-up was last updated in May 2022. All patients treated with the combination ATRA+ATO achieved complete haematological remission with no deaths or relapses until the last follow-up. Consequently, univariate or multivariate regression analyses were performed only for patients treated with ATRA and chemotherapy. With a median follow-up of 40 months (95% CI: 34–47 months), the estimated 5-year overall survival rate was 79% (95% CI: 72–84%). Overall, 129/156 (83%) patients achieved complete haematological remission. mtDNAc had no impact on complete remission ($p = 0.518$) or overall survival ($p = 0.69$). Yet, patients with normal mtDNAc had significantly lower 5-year disease-free survival (DFS) (61%, 95% CI: 46%–82%) compared to patients with high mtDNAc (86%, 95% CI: 78%–95%) ($p = 0.035$; Figure 1B). Considering non-relapse mortality as a competing cause of failure, the 5-year cumulative incidence of relapse (CIR) for patients with normal and high mtDNAc was 35% (95%CI: 16%–49%) and 10% (95%CI: 2%–17%), respectively ($p = 0.0082$; Figure 1C). After stratifying patients into low- and high-risk of relapse,[10] we observed that the prognostic impact of mtDNAc on CIR was applicable to the low-risk group (high mtDNAc: 3%,

95%CI: 1%–7% versus normal mtDNAc: 26%, 95% CI: 5%–43%) (p = 0.0018; Figure 1D), but not to the high-risk group (high mtDNAc: 34%, 95% CI: 12%–51% versus normal mtDNAc: 58%, 95% CI: 14%–80%) (p = 0.41). Finally, the multivariate Cox proportional hazard model indicated that high mtDNAc was independently associated with decreased CIR in the entire cohort (Figure 1F) and in patients assigned to the low-risk group (Figure 1F).

In summary, we show that patients with higher-than-normal mtDNAc had better clinical outcomes (DFS and CIR) regardless of tumour burden. In addition, mtDNAc was useful to sub-stratify low-risk patients and identify those with a higher probability of relapse. Despite our promising data, these findings are only applicable to patients treated with ATRA and chemotherapy due to the limited sample size of patients treated with ATRA+ATO. Yet, it is conceivable that the greater and more sustained antileukemic effect of the combination ATRA+ATO[11] may overcome characteristics at diagnosis associated with an adverse outcome,[12] abrogating the need and the use of mtDNAc as prognostic marker in APL. Therefore, perhaps more important than providing a new prognostic marker for APL, we are interested to determine how to integrate the metabolic profile of leukaemic blasts into clinical decision making. Unfortunately, most studies of the mitochondrial genome in acute leukaemia are related to mtDNA mutations,[13,14] and very few studies have explored these findings in a clinical context.[15,16] Most of our knowledge regarding mtDNAc in cancer comes from studies conducted in solid tumours and, although altered amounts of mtDNAc have been widely reported, there is no consensus on how it affects tumour development. Both high and low mtDNAc have been associated with tumour progression and worse prognosis.[5,6] These inconsistencies may be a result of different types of tumours, tissue sites, and treatments, whereby acute leukaemias are no exception. Similarly in acute leukaemias, unpublished data from our group indicate contrasting results in non-APL AML patients treated with cytotoxic chemotherapy compared to the results presented herein (Pereira-Martins et al., manuscript in progress). Altogether, it is possible that different types of tumours (including APL and non-APL AML) present different bioenergetic requirements. Particularly in non-APL AML, it remains to be explored whether its genetic heterogeneity dictate different metabolic requirements.

AUTHOR CONTRIBUTIONS

D.A.P-M, J.L.C-S, I.W. and P.L.F-N, performed experiments, collected, analysed, and interpreted data; and drafted the manuscript. D.R.S. performed the statistical analyses, interpreted the data, and drafted the manuscript. A.M-A., M.M.L., C.O., performed experiments data; and drafted the manuscript. L.C.K., R.A.M. A.B.G., E.M.F., B.K.L., K.P., R.B., E.N., F.T., L.L.F-P, A.K., M.S.T., R.C.R., R.D., A.G., M.A.S., N.B., P.V., B.L., T.O., N.I.N., M.T.V., F.P., P.F., E.A. G.H., obtained patient samples, updated the clinical data, and drafted the manuscript. D.A.P-M, J.L.C-S, J.J.S., E.M.R, A.R.L-A conceived and designed the study and reviewed the manuscript. A.R.L-A. gave the final approval of the version to be submitted.

ACKNOWLEDGEMENTS

This investigation was supported by *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq; grant # 303479/2018-3) and *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, Grant #2013/08135-2). D.A.P.M. (Grant #2017/23117-1), J.L.C.-S. (Grant #2016/23191-4) and I.W. (Grant #2015/09228-0) received a fellowship from FAPESP. I.W and D.A.P-M were sponsored by the Abel Tasman Talent Program of the Graduate School of Medical Sciences of the University of Groningen/University Medical Center Groningen, The Netherlands.

CONFLICT OF INTEREST

The authors have no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Diego A. Pereira-Martins https://orcid.org/0000-0002-3302-4311
Douglas R. Silveira https://orcid.org/0000-0001-5298-9592
Bruno K. Lino https://orcid.org/0000-0002-6155-3253
Katia Pagnano https://orcid.org/0000-0001-7975-0805
Raul C. Ribeiro https://orcid.org/0000-0002-9956-5647
Richard Dilon https://orcid.org/0000-0001-9333-5296
Tiziana Ottone https://orcid.org/0000-0003-1936-3592
Nelida I. Noguera https://orcid.org/0000-0001-7682-862X
Maria T. Voso https://orcid.org/0000-0002-6164-4761
Eduardo M. Rego https://orcid.org/0000-0003-1567-4086
Antonio R. Lucena-Araujo https://orcid.org/0000-0003-1694-8958

REFERENCES

1. Zhu J, Gianni M, Kopf E, Honore N, Chelbi-Alix M, Koken M, et al. Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor alpha (RARalpha) and oncogenic RARalpha fusion proteins. Proc Natl Acad Sci. 1999;96(26):14807–12. http://www.pnas.org/cgi, https://doi.org/10.1073/pnas.96.26.14807
2. Tourniaire F, Musinovic H, Gouranton E, Astier J, Marcotorchino J, Arreguin A, et al. All-trans retinoic acid induces oxidative phosphorylation and mitochondria biogenesis in adipocytes. J Lipid Res [Internet] 2015;56(6):1100–9. https://linkinghub.elsevier.com/retrieve/pii/S0022227520317223
3. Panina SB, Pei J, Kirienko N V. Mitochondrial metabolism as a target for acute myeloid leukemia treatment. Cancer Metab [Internet] 2021;9(1):17. https://doi.org/10.1186/s40170-021-00253-w. https://cancerandmetabolism.biomedcentral.com/articles
4. Basak NP, Banerjee S. Mitochondrial dependency in progression of acute myeloid leukemia. Mitochondrion [Internet] 2015;21:41–8. https://linkinghub.elsevier.com/retrieve/pii/S1567724915000173
5. He X, Qu F, Zhou F, Zhou X, Chen Y, Guo X, Li J., Huang Q., Yang Y., Lyu Z., Zhang H., Xing J. High leukocyte mtDNA content contributes to poor prognosis through ROS-mediated immunosuppression in hepatocellular carcinoma patients. Oncotarget [Internet] 2016;7(16):22834–45.
6. Dang S, Qu Y, Wei J, Shao Y, Yang Q, Ji M, et al. Low copy number of mitochondrial DNA (mtDNA) predicts worse prognosis in early-stage laryngeal cancer patients. Diagn Pathol [Internet] 2014 5;9:28.

7. Wierenga ATJ, Cunningham A, Erdem A, Lopera NV, Brouwers-Vos AZ, Pruis M, et al. HIF1/2-exerted control over glycolytic gene expression is not functionally relevant for glycolysis in human leukemic stem/progenitor cells. Cancer Metab [Internet]. 2019 27;7(1):11. https://doi.org/10.1186/s40170-019-0206-y. https://cancerandmetabolism.biomedcentral.com/articles/
8. Rego EM, Kim HT, Ruiz-Argüelles GJ, Undurraga MS, Uriarte Mdel R, Jacomo RH, et al. Improving acute promyelocytic leukemia (APL) outcome in developing countries through networking, results of the International Consortium on APL. Blood [Internet] 2013;121(11):1935–43.
9. Lo-Coco F, Avvisati G, Vignetti M, Thiede C, Orlando SM, Iacobelli S,et al. Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. N Engl J Med [Internet] 2013;369(2):111–21.
10. Sanz MA, Lo Coco F, Martín G, Avvisati G, Rayón C, Barbui T, et al. Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood [Internet] 2000;96(4):1247–53.
11. Platzbecker U, Avvisati G, Cicconi L, Thiede C, Paoloni F, Vignetti M et al. Improved outcomes with retinoic acid and arsenic trioxide compared with retinoic acid and chemotherapy in non-high-risk acute promyelocytic leukemia: final results of the randomized Italian-German APL0406 trial. J Clin Oncol [Internet] 2017;35(6):605–12.
12. Cicconi L, Divona M, Ciardi C, Ottone T, Ferrantini A, Lavorgna S, Alfonso V, Paoloni F, Piciocchi A, Avvisati G, Ferrara F, di Bona E, Albano F, Breccia M, Cerqui E, Sborgia M, Kropp MG, Santoro A, Levis A, Sica S, Amadori S, Voso MT, Mandelli F, Lo-Coco F PML-RARα kinetics and impact of FLT3-ITD mutations in newly diagnosed acute promyelocytic leukaemia treated with ATRA and ATO or ATRA and chemotherapy. Leukemia [Internet] 2016;30(10):1987–92.
13. Piccoli C, Ripoli M, Scrima R, Stanziale P, Di Ianni M, Moretti L, et al. MtDNA mutation associated with mitochondrial dysfunction in megakaryoblastic leukaemic cells. Leukemia [Internet] 2008;22(10):1938–41.
14. Yao Y-G, Ogasawara Y, Kajigaya S, Molldrem JJ, Falcão RP, Pintão M-C, et al. Mitochondrial DNA sequence variation in single cells from leukemia patients. Blood [Internet] 2007;109(2):756–62.
15. Silkjaer T, Nørgaard JM, Aggerholm A, Ebbesen LH, Kjeldsen E, Hokland P, Nyvold CG Characterization and prognostic significance of mitochondrial DNA variations in acute myeloid leukemia. Eur J Haematol [Internet] 2013;90(5):385–96.
16. Kang M-G, Kim Y-N, Lee JH, Szardenings M, Baek H-J, Kook H, Kim HR, Shin MG Clinicopathological implications of mitochondrial genome alterations in pediatric acute myeloid leukemia. Ann Lab Med [Internet] 2016; 36(2):101–10.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Pereira-Martins DA, Coelho-Silva JL, Weinhäuser I, Franca-Neto PL, Silveira DR, Ortiz C, et al. Clinical significance of mitochondrial DNA content in acute promyelocytic leukaemia. Br J Haematol. 2022;00:1–5. https://doi.org/10.1111/bjh.18510