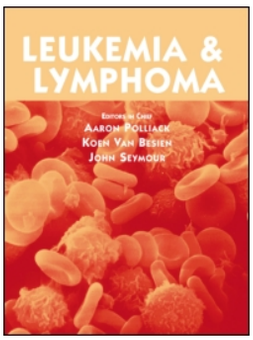

Leukemia & Lymphoma

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ilal20

RUNX1 gene alterations characterized by allelic preference in adult acute myeloid leukemia

Cosimo Cumbo, Giuseppina Tota, Anna De Grassi, Luisa Anelli, Antonella Zagaria, Nicoletta Coccaro, Francesco Tarantini, Crescenzio Francesco Minervini, Elisa Parciante, Luciana Impera, Maria Rosa Conserva, Immacolata Redavid, Anna Mestice, Immacolata Attolico, Ciro Leonardo Pierri, Pellegrino Musto & Francesco Albano

To cite this article: Cosimo Cumbo, Giuseppina Tota, Anna De Grassi, Luisa Anelli, Antonella Zagaria, Nicoletta Coccaro, Francesco Tarantini, Crescenzio Francesco Minervini, Elisa Parciante, Luciana Impera, Maria Rosa Conserva, Immacolata Redavid, Anna Mestice, Immacolata Attolico, Ciro Leonardo Pierri, Pellegrino Musto & Francesco Albano (2021): *RUNX1* gene alterations characterized by allelic preference in adult acute myeloid leukemia, Leukemia & Lymphoma, DOI: 10.1080/10428194.2021.1929960

To link to this article: https://doi.org/10.1080/10428194.2021.1929960

LEUKEMIA & LYMPHOMA
https://doi.org/10.1080/10428194.2021.1929960

Taylor & Francis
Taylor & Francis Group

LETTER TO THE EDITOR

RUNX1 gene alterations characterized by allelic preference in adult acute myeloid leukemia

Cosimo Cumbo[a], Giuseppina Tota[a], Anna De Grassi[b], Luisa Anelli[a], Antonella Zagaria[a], Nicoletta Coccaro[a], Francesco Tarantini[a], Crescenzio Francesco Minervini[a], Elisa Parciante[a], Luciana Impera[a], Maria Rosa Conserva[a], Immacolata Redavid[a], Anna Mestice[a], Immacolata Attolico[a], Ciro Leonardo Pierri[b], Pellegrino Musto[a] and Francesco Albano[a]

[a]Hematology and Stem Cell Transplantation Unit, Department of Emergency and Organ Transplantation (D.E.T.O.), University of Bari "Aldo Moro", Bari, Italy; [b]Department of Biosciences, Biotechnologies and Biopharmaceutics, University "Aldo Moro" of Bari, Bari, Italy

ARTICLE HISTORY Received 27 February 2021; revised 3 May 2021; accepted 7 May 2021

Germline mutations in the *RUNX1* gene define a familial platelet disorder with a predisposition to myeloid malignancy (FPDMM). This autosomal dominant disorder is characterized by variable penetrance of quantitative and/or qualitative platelet defects with a tendency to develop hematological malignancies, especially acute myeloid leukemia (AML) [1,2]. However, the eventual progression to AML is associated with the acquisition of somatic mutations in the remaining wild-type *RUNX1* allele, as well as *GATA2* mutations. Moreover, *RUNX1* gene mutations are found in approximately 4–16% of AML cases, identified as a World Health Organization (WHO) entity with recurrent genetic abnormalities named 'AML with mutated *RUNX1*', associated with worse overall survival. In addition, 1–5% of cases of AML have the t(8;21)(q22;q22.1) resulting in the *RUNX1-RUNX1T1* rearrangement, related to a better prognosis [3]. We describe an AML patient bearing complex *RUNX1* genomic alterations strictly related to leukemia pathogenesis.

A 56-year-old man was referred to our center with pruritus and widespread bruising. He referred a history of mild thrombocytopenia, never considered for further investigations. The blood count revealed mild leukocytosis, anemia, and severe thrombocytopenia. Peripheral blood smear showed 40% myeloid blasts. Subsequent bone marrow (BM) aspirate and biopsy evaluation revealed the presence of trilineage dysplastic features and 30% myeloid blasts. The karyotype was complex: 48, XY,+20,+21[7]/49,XY,+12,+20,+21[12]/46,XY[1]. According to the WHO classification, a diagnosis of AML with myelodysplastic syndrome (MDS) – related changes was made. Familial HLA typing was performed, and a matched sibling donor (brother) was identified. Next-generation sequencing (NGS) analysis with a customized panel, encompassing 26 target genes involved in the pathogenesis of myeloid malignancies [4], identified the presence of the following variants: *NRAS* (NM_002524.5) c.183A>T, p.Gln61His (VAF: 43.85%); *GATA2* (NM_032638.4) c.890A>T, p.Asn297Ile (VAF: 12.53%); *RUNX1* (NM_001754.4) c.-298T>C, p.? (VAF: 63.85%) and c.351 + 2T>C, p.? (VAF: 61.83%). The patient underwent induction therapy and consolidation with CPX-351, achieving complete remission (CR) according to European LeukemiaNet response criteria. He is now in preparation for allogeneic hematopoietic stem cell transplantation. The study was approved by the local ethics committee 'Azienda Ospedaliero Universitaria Policlinico di Bari' No. 624 from 21 May 2010. Written, informed consent was obtained from patient before enrollment in the present study in accordance with the Declaration of Helsinki. His records/information was anonymized and de-identified before analysis.

The triple alterations affecting the *RUNX1* gene (i.e. two single nucleotide variants (SNVs) and a copy number variation (CNV)) were further investigated to better characterize their role in the disease pathogenesis and to describe the novel variant (c.351 + 2T>C) affecting the splicing of its transcripts. The three alterations were validated as somatic or germline on normal CD3+ peripheral T cells isolated from the patient; bidirectional Sanger sequencing and fluorescence *in situ* hybridization approaches were used for the two SNV and trisomy 21, respectively (Supplementary file and Figure 1). Only the c.-298T>C variant was found in CD3+ T cells and so defined 'germline'. Vice versa, the presence of c.351 + 2T>C variant and trisomy 21 was confirmed only in BM cells and so defined as 'somatic'.

CONTACT Francesco Albano francesco.albano@uniba.it Hematology and Stem Cell Transplantation Unit, Department of Emergency and Organ Transplantation (D.E.T.O.), University of Bari "Aldo Moro", P.zza G. Cesare 11, 70124 Bari, Italy

Supplemental data for this article can be accessed here.

The variant g.36421494A>G, c.-298T>C, p.? is a rare (G-allele frequency in European Human samples, gnomAD project: 0.00091) germline mutation in the 5′-UTR of the *RUNX1* transcript variant 1 (NM_001754) (Figure 1(A)) reported in the dbSNP (rs77393863) and MalaCards databases in association with FPDMM (OMIM #601399). To verify whether this variant may be associated with gene expression alterations, absolute quantification of the *RUNX1* transcript variant 1 was performed (Supplementary file 1). The assay showed overexpression of the *RUNX1* transcript variant 1 (1.8-fold change, $p<.0001$) in BM cells of the patient in CR, compared with a pool of 56 healthy control BM samples (Figure 1(C)). The *RUNX1* sequence (30 nucleotides centered on the variant site) was then compared against a database of 1808 DNA motifs for transcription factor binding sites (TFBSs). Despite the large number of screened motifs, only two statistically significant matches were found. The wild-type sequenced only matched to the RUNX2/RUNX3 bipartite motif, while the variant sequence only matched to the interferon regulatory factor IRF4 motif (Figure 1(C)), suggesting that the 5′-UTR variant might cause loss of the former and gain of the latter.

The variant g.36259138A>G, c.351 + 2T>C, p.? is a novel somatic mutation affecting a *RUNX1* splice-site (Figure 1(A)), never before described. It was predicted to affect mRNA splicing by altering the wild-type donor site: HSF software [5] predicted a 90.65% reduction of donor site efficiency, and SPiCe software [6] predicted a 99.9% probability of splicing alterations, causing the loss of exon 4 for the transcript variant 1 (NM_001754) and the retention of intron 4 (for all transcript variants). Both events were experimentally verified and confirmed (Supplementary file 1 and Figure 1(B)).

After translating the variant *RUNX1* splicing isoforms, both the observed events, namely exon skipping and intron retention, resulted in early frameshifts, premature stop codons, and prematurely truncated protein fragments (Figure 2(A)). In particular, the skipping of exon 4 of *RUNX1* transcript variant 1 (NM_001754) generates a 51-amino-acid long protein fragment, i.e. 32 N-terminal-native amino acids and 19 out-of-frame amino acids. By contrast, the full retention of intron 4 in *RUNX1* transcript variant 2 (NM_001001890) and transcript variant 3 (NM_001122607) generates a 134-amino-acid long protein fragment, i.e. 93 N-terminal-native amino acids and 41 out-of-frame amino acids (Figure 2(A)). The crystallized structure of the RUNX1 runt domain showed that the 134-amino-acid long protein fragment lacks vast portions of the runt domain involved in interactions with the TCRα promoter and the Ets1 protein (Figure 2(B)) and with the Cbfβ protein (Figure 2(C)). Thus, the observed loss and replacement of the wild-type RUNX1 runt domain could cause loss of the above-cited RUNX1–DNA and RUNX1–protein interactions.

Our AML case showed a *RUNX1* allele carrying a germline 5′-UTR and somatic splice-site mutations, this latter never having previously been described, together with a CNV event, which explains the VAF 63.85% and 61.83% of the two SNVs. This AML case offers points for considerations about *RUNX1* biology. First, clinical evidence implies that severe inflammation may contribute to the disease severity in FPD/AML patients [7], and several data have documented a connection between *RUNX1* gene deregulation and inflammation, although the mechanisms by which *RUNX1* regulates inflammation are still unclear [8,9]. The 5′-UTR germline variant reported in our case ('first hit', Figure 1(D)) is associated with *RUNX1* overexpression and a predicted reduction of the binding intensity of RUNX3 to DNA. These two observations are highly consistent with a role of this variant in lowering the RUNX3 repressive effect on RUNX1, and this effect was previously demonstrated for another RUNX3 binding site in the same exon [10]. Moreover, in the context of inflammation, it is noteworthy that in our case this variant might generate a binding site for IRF4. The latter is a hematopoietic-specific transcription factor also involved in inflammasome response mechanisms [11] and in controlling the granulocyte-macrophage colony-stimulating factor promotion of arthritis [12]. However, in our case, we can only predict RUNX1 and IRF4 interactions but not verify their possible interplay. Our work describes a novel *RUNX1* mutation, never previously reported, affecting mRNA splicing and resulting in a truncated protein fragment that does not bind the subunit partner CBFβ, generally associated with the RUNX1 DNA-binding domain. This event follows the inactivation of the functions usually performed by *RUNX1* and can be considered a 'second hit' (Figure 1(D)). In this regard, in many predisposition syndromes, the disease progression (AML or MDS) generally occurs through a stepwise process involving the loss of the remaining wild-type allele and acquisition of additional cooperating mutations, whereas others appear to maintain the wild-type allele [13]. Our case showed a mono-allelic double mutation on the *RUNX1* gene at the time of the AML diagnosis, so the 'second hit' consisted of a second gene mutation on the same *RUNX1* allele bearing the first one. The duplication of the same allele in the circumstance of trisomy 21, the 'third hit' (Figure 1(D)), demonstrates the doubly mutated allele's relevance in the pathogenesis of AML, as previously reported [14].

In short, we speculate that in our AML case, systemic inflammation may have been elevated at the time of the first *RUNX1* gene mutation and that an inflammatory BM microenvironment may provide a positive selective pressure for hematopoietic stem-cells that have acquired secondary mutations. Finally, the complex karyotype (including the trisomy 21) which provides evidence of the clonal evolution of our AML case, may be a consequence of the dysregulated *RUNX1* gene activity effects on DNA repair pathways, as previously reported [15].

In conclusion, we report an AML case showing multiple *RUNX1* alterations, that sheds light on the

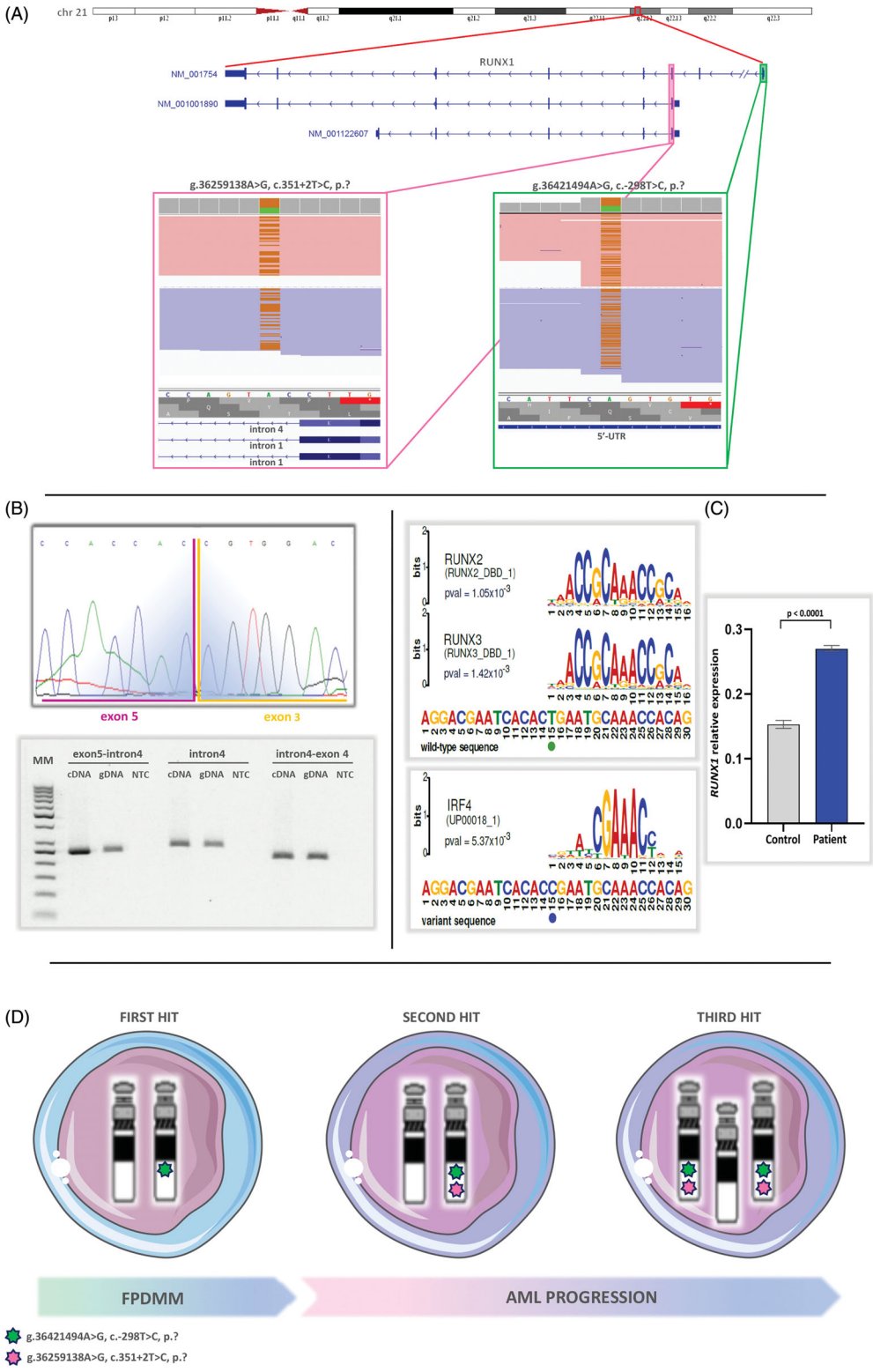

Figure 1. *RUNX1* gene alterations. (A) Two *RUNX1* SNVs detected at the AML onset: a new splice-site variant: g.36259138A>G, c.351+2T>C, p.? (left square) and a 5′-UTR variant: g.36421494A>G, c.-298T>C, p.? (right square). (B) The effects of the splice site variant: exon 4 skipping verified by SS (upper panel) and intron 4 retention verified by three RT-PCR mapping on intron 4 and on its junction with exon 4 and 5 (lower panel). (C) TFBSs matching the 30-nucleotide-long DNA sequence hosting the 5′-UTR variant site (on the left). Outputs of Tomtom software indicating sequence logos of TFBS that showed statistically significant alignments to the wild-type or variant sequences. Transcription factors and statistical significance of matches are indicated, together with the original identifier name of each DNA motif (in brackets). On the right, the *RUNX1* transcript variant 1 expression quantification in control and patient's samples. (D) Timeline of the triple alterations affecting *RUNX1* gene and their association with disease progression. cDNA: complementary DNA, gDNA: genomic DNA (positive control), NTC: no template control, DBD: DNA binding domain, FPDMM: familial platelet disorder with associated myeloid malignancy, AML: acute myeloid leukemia.

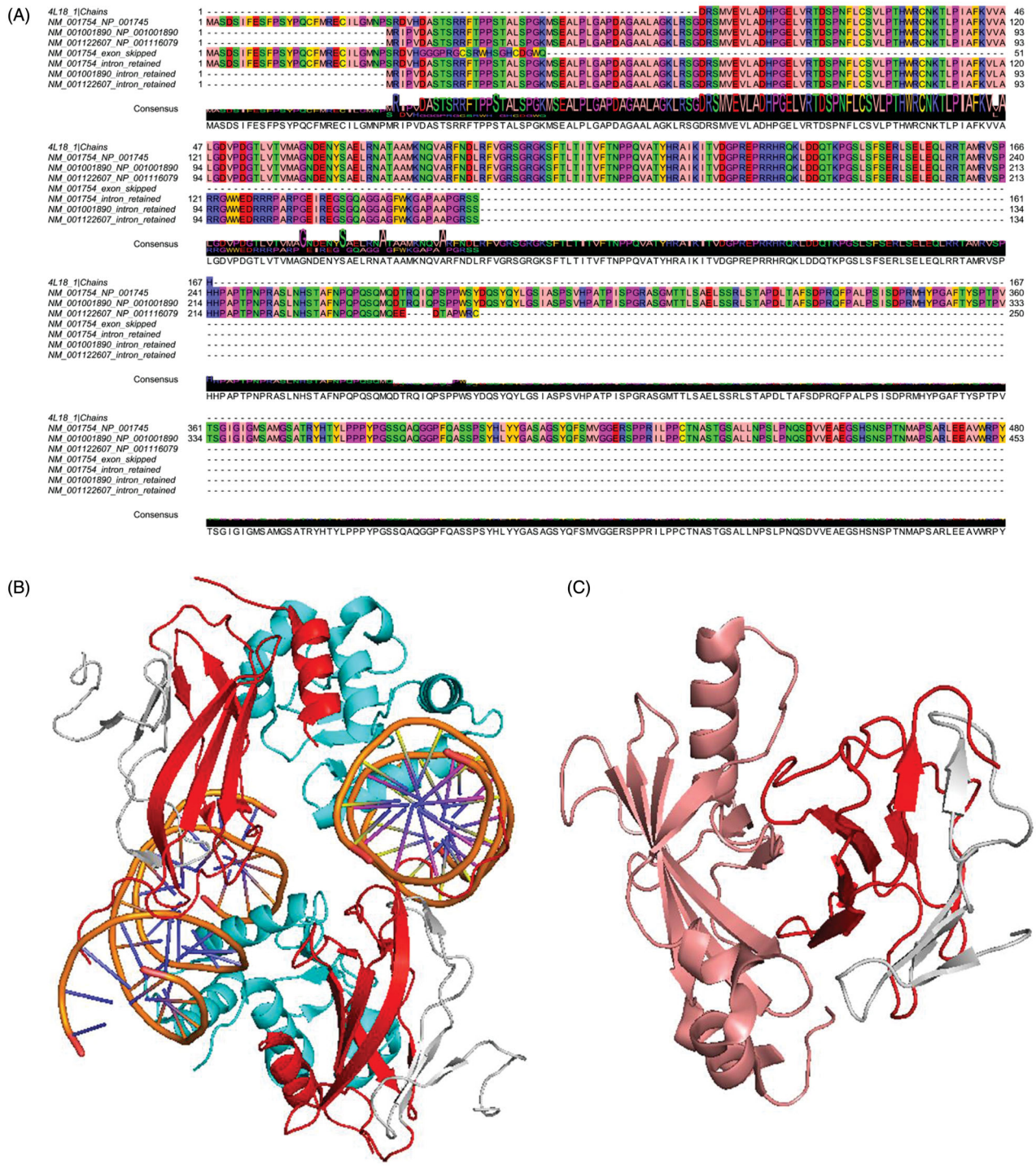

Figure 2. Aberrant protein isoforms deriving from the *RUNX1* splice-site variant lack the runt domain. The sequence of the crystallized *RUNX1* runt domain (4l18.pdb), the three *RUNX1* wild-type protein isoforms and the four aberrant protein isoforms deriving from exon skipping and intron retention are reported (A). The *RUNX1* splice-site variant results in loss of runt domain interactions with the TCRα promoter, with Ets1 and Cbfβ. The crystallized *RUNX1* runt domain is graphically illustrated in cartoon representation (light grey, native amino acids; dark grey, native amino acids lacking in the intron-retained RNA isoforms) as it interacts with the TCRα promoter DNA (ribbon and sticks) and with an Ets1 protein domain (dark grey cartoon) (B), and with a Cbfα protein domain (C) (grey cartoon on the left).

possible biological steps involved in RUNX*1*-mediated leukemogenesis.

Acknowledgements

Health and safety: All mandatory laboratory health and safety procedures have been complied with in the course of conducting any experimental work reported in this paper.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by Associazione Italiana contro le Leucemie (AIL)-BARI.

ORCID

Francesco Albano http://orcid.org/0000-0001-7926-6052

References

[1] Antony-Debré I, Duployez N, Bucci M, et al. Somatic mutations associated with leukemic progression of familial platelet disorder with predisposition to acute myeloid leukemia. Leukemia. 2016;30(4):999–1002.

[2] Brown AL, Arts P, Carmichael CL, et al. RUNX1-mutated families show phenotype heterogeneity and a somatic mutation profile unique to germline predisposed AML. Blood Adv. 2020;4(6):1131–1144.

[3] Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–2405.

[4] Coccaro N, Zagaria A, Orsini P, et al. RARA and RARG gene downregulation associated with EZH2 mutation in acute promyelocytic-like morphology leukemia. Hum Pathol. 2018;80:82–86.

[5] Desmet FO, Hamroun D, Lalande M, et al. Human splicing finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. 2009;37:e67.

[6] Leman R, Gaildrat P, Gac GL, et al. Novel diagnostic tool for prediction of variant spliceogenicity derived from a set of 395 combined in silico/in vitro studies: an international collaborative effort. Nucleic Acids Res. 2018;46(15):7913–7923.

[7] Chin DWL, Sakurai M, Nah GSS, et al. RUNX1 haploinsufficiency results in granulocyte colony-stimulating factor hypersensitivity. Blood Cancer J. 2016;6:379.

[8] Luo MC, Zhou SY, Feng DY, et al. Runt-related transcription factor 1 (RUNX1) binds to p50 in macrophages and enhances TLR4-triggered inflammation and septic shock. J Biol Chem. 2016;291(42):22011–22020.

[9] Nakagawa M, Shimabe M, Watanabe-Okochi N, et al. AML1/RUNX1 functions as a cytoplasmic attenuator of NF-κB signaling in the repression of myeloid tumors. Blood. 2011;118(25):6626–6637.

[10] Spender LC, Whiteman HJ, Karstegl CE, et al. Transcriptional cross-regulation of RUNX1 by RUNX3 in human B cells. Oncogene. 2005;24(11):1873–1881.

[11] McDaniel MM, Kottyan LC, Singh H, et al. Suppression of inflammasome activation by IRF8 and IRF4 in cDCs is critical for T cell priming. Cell Rep. 2020;31:107604.

[12] Achuthan A, Cook AD, Lee MC, et al. Granulocyte macrophage colony-stimulating factor induces CCL17 production via IRF4 to mediate inflammation. J Clin Invest. 2016;126(9):3453–3466.

[13] Klco JM, Mullighan CG. Advances in germline predisposition to acute leukaemias and myeloid neoplasms. Nat Rev Cancer. 2020;21:122–137.

[14] Carraway HE, LaFramboise T. Myeloid neoplasms with germline predisposition: practical considerations and complications in the search for new susceptibility loci. Best Pract Res Clin Haematol. 2020;33(3):101191.

[15] Wang CQ, Krishnan V, Tay LS, et al. Disruption of Runx1 and Runx3 leads to bone marrow failure and leukemia predisposition due to transcriptional and DNA repair defects. Cell Rep. 2014;8(3):767–782.