Pure erythroid leukemia is characterized by biallelic TP53 inactivation and abnormal p53 expression patterns in de novo and secondary cases

Pure erythroid leukemia (PEL) is a rare type of acute myeloid leukemia (AML) characterized by a neoplastic proliferation of immature erythroblasts associated with a complex karyotype and a poor prognosis. To date, the potential differences between de novo and secondary cases (therapy-related or MDS) have not been well explored. Recent studies have shown that TP53 mutations are common in PEL and that p53 overexpression is also frequent. Strong p53 expression shown by immunohistochemistry has become an important clue in the initial workup of PEL. However, we have observed some PEL cases lacking p53 expression despite the presence of TP53 mutations. We conducted the current study to investigate TP53 mutation characteristics and p53 protein expression in PEL and to examine whether secondary PEL cases differ from de novo disease.

We collected 22 cases of PEL, defined by a predominant proliferation of neoplastic erythroblasts that formed sheets in bone marrow, of which 30% or more were pro-normoblasts. The clinical characteristics of our patients are summarized in Table 1. There were 14 men and eight women with a median age of 69 years (range, 37-81). Eleven (50%) patients had a history of chemotherapy for other malignancies (therapy-related), five (23%) had MDS, one (4%) had primary myelofibrosis (PMF), and five (23%) occurred de novo. Among 21 patients with treatment information available, five (24%) did not receive any therapy due to poor performance status or poor response to prior treatments for MDS. The remaining 16 patients received treatments after the diagnosis of PEL (Table 1). Four (25%) patients achieved a complete response with incomplete hematologic recovery; the response in three patients (#3, 12, 20) was transient and one patient (#22) remained in complete response with incomplete hematologic recovery at last follow-up, 4.7 months after diagnosis. None of the patients was eligible for stem cell transplantation. Twenty-one patients had clinical follow-up: 20 had died at last follow-up and one patient (#22) was alive. The median survival time for this entire cohort was 2.8 months (range, 0.2-7.3); 2.3 months (range, 0.2-7.3) for therapy-related, 2.6 months (range, 0.4-4.9) for patients with a history of MDS, and 3.9 months (range, 2.2-5.5) for de novo PEL.

Targeted next generation sequencing (NGS) with panels composed of genes commonly mutated in myeloid neoplasms was performed on bone marrow samples from 20 patients (19 using an 81-gene panel and 1 using a 28-gene panel) at the time of PEL diagnosis as previously described. One case was tested for TP53 mutation using Sanger sequencing. In total, 21 cases were tested and all patients had TP53 mutation(s) (Table 2). A total of 25 TP53 mutations were detected: 18 patients had one TP53 mutation, two patients (#15 and 17) had two mutations, and one (#5) patient had three mutations. Twenty-two (88%) mutations occurred in the DNA binding domain (exons 5-8), including 12 in exon 5, one in exon 6, four in exon 7, and five in exon 8. The remaining three mutations occurred in exon 4, exon 10, and a splice site, respectively. The types of TP53 mutations included 19 (76%) missense, 1 (4%) nonsense, 1 (4%) splice site, and 4 (16%) small deletion. Among the four cases with small deletion mutations, three caused frameshift. The median variant allele frequency (VAF) of TP53 mutations was 35% (range, 1-92.3%). One patient (#13) was not assessed for TP53 mutation, but immunohistochemistry showed strong and diffuse p53 expression, suggestive of TP53 mutations. The detailed TP53 mutational profiles are summarized in Table 2. Among the 16 patients who received treatment, 11 (#3, 4, 7, 8, 11, 15, 17, 18, 20-22) had repeat TP53 mutation analysis by NGS after treatment and all showed persistent TP53 mutations. Among the 20 cases tested by NGS, additional gene mutations were detected in nine (45%) patients (Online Supplementary Table S1), including DNMT3A (n=3; VAF 10.3-29.3%), NRAS (n=2; VAF 5% and 26.6%), TET2 (n=1; VAF <3%), FLT3 (n=1; VAF 1.7%), PRPF40B (n=1; VAF 40.8%), KMT2A (n=1; VAF 15.2%) and GATA2 (n=1; VAF 1.8%). Patient #22 had a history of PMF that was positive for JAK2 V617F (VAF 32%) and negative for TP53. At the time of progression to PEL, JAK2 V617F was detected with a VAF of 1%, and TP53 mutation was acquired (VAF 80.6%). Twenty cases underwent conventional karyotyping at the time of PEL diagnosis and all (100%) had complex karyotypes (Online Supplementary Table S1). Among 19 cases with karyotype data available, 12 (63%) had -5/5q-, 12
LETTER TO THE EDITOR

Table 1. Clinical characteristics of pure erythroid leukemia.

| Case # | Sex | Age (years) | F/U (months) | Treatment | Response | Status at F/U | History |
|--------|-----|-------------|--------------|-----------|----------|--------------|---------|
| 1      | M   | 77          | 2.8          | None      | N/A      | Dead         | Therapy-related (B-ALL and DLBCL) |
| 2      | M   | 66          | 0.2          | None      | N/A      | Dead         | Therapy-related (PCN) |
| 3      | F   | 68          | 7.3          | Decitabine + Venetoclax, 4 cycles Azacitidine + Hu5F9-G4, 1 cycle | Transient CRi, 2.1 months | Dead | Therapy-related (ovarian cancer) |
| 4      | M   | 55          | 1.4          | CLIA + Venetoclax, 1 cycle | No | Dead | Therapy-related (DLBCL) |
| 5      | F   | 70          | 1.7          | Decitabine + Venetoclax, 1 cycle | No | Dead | Therapy-related (PCN) |
| 6      | M   | 48          | 2.3          | Fludarabine + AraC + Idarubicin, 1 cycle | No | Dead | Therapy-related (AML) |
| 7      | F   | 54          | 4.8          | Cytarabine + Daunorubicine, 1 cycle Decitabine + Venetoclax, 2 cycles | No | Dead | Therapy-related (breast cancer) |
| 8      | M   | 66          | 6.3          | Azacitidine, 4 cycles | No | Dead | Therapy-related (PCN) |
| 9      | F   | 81          | 0.8          | None      | N/A      | Dead         | Therapy-related (DLBCL) |
| 10     | M   | 69          | 2.1          | Low dose Cytarabine + Venetoclax, 1 cycle | No | Dead | Therapy-related (lung cancer) |
| 11     | M   | 56          | 4.4          | ASTX660 + ASTX727, 1 cycle | No | Dead | Therapy-related (PCN) |
| 12     | M   | 76          | 4.9          | Sapacitabine, 3 cycles | Transient CRi, 2 months | Dead | MDS |
| 13     | F   | 37          | 0.4          | None      | N/A      | Dead         | MDS |
| 14     | M   | 78          | 2.6          | Low dose Cytarabine + Venetoclax, 2 cycles | No | Dead | MDS |
| 15     | M   | 79          | 3.6          | FF1101 (BET inhibitor), 2 cycles | No | Dead | MDS |
| 16     | M   | 60          | 1.3          | None      | N/A      | Dead         | MDS |
| 17     | F   | 78          | 2.2          | Azacitidine + Nivolumab, 2 cycles | No | Dead | De novo |
| 18     | M   | 59          | 5.5          | Azacitidine, 3 cycles FIA + Venetoclax, 1 cycle | No | Dead | De novo |
| 19     | F   | 78          | N/A          | N/K      | N/K      | N/K          | De novo |
| 20     | M   | 65          | 5.0          | Azacitidine + Venetoclax, 2 cycles | Transient CRi, 2.6 months | Dead | De novo |
| 21     | M   | 72          | 2.8          | Decitabine + Venetoclax, 1 cycle | No | Dead | De novo |
| 22     | F   | 73          | 4.7          | Azacitadine + Venclexa + Magrolimab, 3 cycles | CRi at the last F/U, 3 months | Alive | PMF |

AML: acute myeloid leukemia; B-ALL: B-acute lymphoblastic leukemia; BET: bromodomain and extra-terminal; CLIA: cladribine, idarubicin, and cytarabine; CRi: complete response with incomplete hematologic recovery; DLBCL: diffuse large B-cell lymphoma; FIA: fludarabine, idarubicin, cytarabine; F/U: follow up; MDS: myelodysplastic syndrome; N/A: not applicable; N/K: not known; PCN: plasma cell neoplasm; PMF: primary myelofibrosis.

(63%) had -7/7q-, and nine (47%) had concomitant -5/5q- and -7/7q-. The status of 17p/TP53 was assessed by conventional karyotyping and/or fluorescence in situ hybridization (FISH) in 17 cases: deletion of 17p and/or TP53 was detected in 13 (76%) cases (Table 2). The remaining four patients were negative but three (cases # 5, 15, and 17, Table 2) had more than one TP53 mutation by NGS, raising the possibility that both alleles were affected by TP53 mutations. In one patient (#12), the status of 17p/TP53 was unknown, but the VAF of TP53 mutation was 92.3%, consistent with the loss of wild-type TP53. We performed p53 immunohistochemistry on 21 cases
and correlated the results with TP53 mutation types (Table 2). Sixteen (76%) cases of PEL were strongly and uniformly positive for p53; 15 had missense mutations and one had a deletion mutation but no frameshift (#3). In the remaining five (24%) cases, p53 expression was completely absent in the neoplastic cells (null pattern). In cases negative for p53 expression, three (#6, 16, 21) had TP53 frameshift mutations, one (#18) had a nonsense mutation, and one (#1) had a splice site mutation. Representative cases of PEL with p53 overexpression and completely absence of p53 expression are shown in Figure 1A and B. In this study, two patients (cases #3 and 14) had a single TP53 mutation with a VAF less than 15%. In both cases, erythroblasts formed sheets in the core biopsy and were diffusely and strongly positive for p53 by immunohistochemistry. These findings suggest that most of the erythroblasts had mutated TP53 and the low VAF of TP53 mutation may be due to hemodiluted specimen submitted for molecular analysis. However, we also cannot exclude the possibility that only a subclone of leukemic cells had TP53 mutation.

Table 2. TP53 mutational profiles and p53 protein expression in pure erythroid leukemia.

| Case # | Monosomy 17 or TP53 Deletion (karyotype or FISH)* | Number of TP53 Mutation | Biallelic TP53 Inactivation | TP53 Mutation (Ref: NM_000546.5) | Type of Mutation | VAF % | Exon(s) | IHC-p53 |
|--------|-----------------------------------------------|------------------------|-----------------------------|---------------------------------|-----------------|-------|---------|--------|
| 1      | yes                                          | 1                      | yes                         | c.673-2A>T                     | splice site     | 74.9  | splice site | negative |
| 2      | yes                                          | 1                      | yes                         | c.405G>C p.C135W               | missense        | 42.1  | 5       | positive |
| 3      | yes                                          | 1                      | yes                         | c.534_536del p.H179del         | deletion, no frameshift | 11.9  | 5       | positive |
| 4      | yes                                          | 1                      | yes                         | c.818G>A p.R273H               | missense        | 59.6  | 8       | positive |
| 5      | no                                           | 3                      | likely yes                  | c.715A>G p.N239D c.401T>G p.F134C c.329G>T p.R110L | missense missense missense | 1     | 29.3  4,5,7 | positive |
| 6      | N/K                                          | 1                      | N/K                         | c.501del p.Q167fs              | deletion, frameshift | 62.6  | 5       | negative |
| 7      | N/K                                          | 1                      | N/K                         | c.524G>A p.R175H               | missense        | 37.2  | 5       | positive |
| 8      | yes                                          | 1                      | yes                         | c.377A>C p.Y126S               | missense        | 23.0  | 5       | positive |
| 9      | N/K                                          | 1                      | N/K                         | c.715A>G p.N239D               | missense        | 42.6  | 7       | positive |
| 10     | yes                                          | 1                      | yes                         | c.818G>C p.R273P               | missense        | 27.2  | 8       | positive |
| 11     | yes                                          | 1                      | yes                         | c.488A>G p.Y163C               | missense        | 48.0  | 5       | positive |
| 12     | N/K                                          | 1                      | likely yes                  | c.797G>A p.G266E               | missense        | 92.3  | 8       | positive |
| 13     | N/K                                          | N/D                    | N/K                         | N/D                            | N/D             | N/D   | N/D     | positive |
| 14     | yes                                          | 1                      | yes                         | c.745A>G p.R249G               | missense        | 8.4   | 7       | positive |
| 15     | no                                           | 2                      | likely yes                  | c.434T>G p.L145R c.1010G>C p.R337P | missense missense | 20.4  | 5,10  | N/D     |
| 16     | yes                                          | 1                      | yes                         | c.455del p.P152fs              | deletion, frameshift | 70.1% | 5       | negative |
| 17     | no                                           | 2                      | likely yes                  | c.844C>T p.R282W c.734G>T p.G245V | missense missense | 35.1  | 14.4  7.8 | positive |
| 18     | yes                                          | 1                      | yes                         | c.493C>T p.Q165*               | nonsense        | 39.1  | 5       | negative |
| 19     | no                                           | 1                      | probably no                 | c.476C>T p.A159V               | missense        | 16.0  | 5       | positive |
| 20     | yes                                          | 1                      | yes                         | c.590T>G p.V197G               | missense        | 15.1  | 6       | positive |
| 21     | yes                                          | 1                      | yes                         | c.558del p.D186fs              | deletion, frameshift | 47.1  | 5       | negative |
| 22     | yes                                          | 1                      | yes                         | c.824G>T p.C275F               | missense        | 80.6  | 8       | positive |

IHC: immunohistochemistry; N/D: not done; N/K: not known; VAF: variant allele frequency; FISH: fluorescence in situ hybridization. *Detailed karyotype and FISH findings are listed in the Online Supplementary Table S1.
critical role in the development of PEL. Of note, biallelic TP53 alteration is not specific to PEL and can be seen in other myeloid neoplasms, such as AML and therapy-related MDS. Thus, TP53 mutations alone may not be sufficient to block the differentiation of erythroid lineage and drive pronormoblast proliferation, a pathognomonic feature of PEL. Alterations of other genes (not covered in our mutation panels) or pathways involved in erythroid differentiation likely also play a role in PEL development.

As mutational analysis often takes time, checking p53 expression status by immunohistochemistry has been used as a surrogate to predict the presence of TP53 mutations. One caveat is that TP53 mutations do not always correlate with p53 overexpression. In the current study, approximately one quarter of PEL cases showed a null pattern by immunohistochemistry. In these cases, TP53 mutations were either frameshift, nonsense, or involved a splice site. Of note, the null pattern of p53 expression can usually be distinguished from the “negative” wild-type pattern which often shows variable p53 expression in a subset of cells and the staining intensity ranges from weak to moderate (Figure 1C). In some cases, however, as-

Figure 1. The expression pattern of p53 by immunohistochemistry in pure erythroid leukemia. Immunohistochemistry shows two patterns of p53 expression: complete absence of p53 expression (case #1, upper panel) and uniform and strong overexpression (case #17, middle panel). Of note, in the case with absence of p53 expression in tumor cells (case #1, upper panel), there were scattered reactive cells in the background variably positive for p53, serving as positive controls. A normal bone marrow and its p53 expression by immunohistochemistry is illustrated in the lower panel, in which p53 is variably expressed in a subset of cells with weak to moderate intensity.
essment of p53 using immunohistochemistry can be challenging, especially in cases where PEL is mixed with residual normal hematopoietic cells in the background which have a wild-type p53 staining pattern. Lastly, we suggest that the category of PEL should be preserved, despite the fact that some cases also can be classified as therapy-related AML/MDS or AML with myelodysplasia-related changes (AML-MRC) using the current World Health Organization (WHO) criteria. We believe classifying these cases as something other than PEL does not fully capture the distinctive features of this disease. The rationale for this proposal includes: i) PEL cases, irrespective of their origin (de novo or secondary), share similar clinicopathological features including poor response to treatment, dismal prognosis, complex karyotype, and biallelic TP53 alterations. By contrast, the WHO-defined categories of therapy-related AML/MDS or AML-MRC are highly heterogeneous at the molecular level, and are associated with highly variable prognoses for different patient subsets. We believe that the distinctive clinicopathologic and molecular features of PEL may be obscured when these neoplasms are placed in the therapy-related AML/MDS or AML-MRC WHO categories; ii) the survival of PEL patients with a history of receiving cytotoxic therapy or MDS is similar to de novo PEL patients but is worse than patients with therapy-related AML and AML-MRC, respectively; iii) all PEL cases, whether they are de novo or secondary, share distinctive morphologic features with prominent pronormoblast proliferation. Pronormoblasts have been shown to play an important role in treatment resistance and increased pronormoblasts have contributed to a poorer prognosis in AML patients. By keeping secondary PEL cases in the category of PEL, these cases can be studied together to explore therapeutic strategies targeting the neoplastic pronormoblasts. Of note, the number of de novo PEL cases in our study is relatively small and future studies to include more cases will be valuable.

In summary, we show that PEL is characterized by biallelic TP53 loss-of-function, a complex karyotype, poor response to AML or MDS directed therapy, and a very dismal prognosis. These unique features are the same for de novo or secondary cases of PEL, and therefore we advocate for keeping them under the category of PEL to facilitate further studies and drug discovery. Immunohistochemistry for p53 can be used as a preliminary screening tool to assess TP53; strong p53 expression correlates with missense mutations of TP53 and a null p53 pattern is often associated with frameshift, nonsense, or TP53 mutations involving splice sites.

References

1. Wang W, Wang SA, Medeiros LJ, Khoury JD. Pure erythroid leukemia. Am J Hematol. 2017;92(3):292-296.

2. Reinig EF, Greipp PT, Chiu A, Howard MT, Reichard KK. De novo pure erythroid leukemia: refining the clinicopathologic and molecular features.
cytogenetic characteristics of a rare entity. Mod Pathol. 2018;31(5):705-717.

3. Wang SA, Hasserjian RP. Acute erythroleukemias, acute megakaryoblastic leukemias, and reactive mimics: a guide to a number of perplexing entities. Am J Clin Pathol. 2015;144(1):44-60.

4. Liu W, Hasserjian RP, Hu Y, et al. Pure erythroid leukemia: a reassessment of the entity using the 2008 World Health Organization classification. Mod Pathol. 2011;24(3):375-383.

5. Montalban-Bravo G, Benton CB, Wang SA, et al. More than 1 TP53 abnormality is a dominant characteristic of pure erythroid leukemia. Blood. 2017;129(18):2584-2587.

6. Alexandres C, Basha B, King RL, Howard MT, Reichard KK. p53 immunohistochemistry discriminates between pure erythroid leukemia and reactive erythroid hyperplasia. J Hematop. 2021;14(1):15-22.

7. Fang H, Yabe M, Zhang X, et al. Myelodysplastic syndrome with t(6;9)(p22;q34.1)/DEK-NUP214 better classified as acute myeloid leukemia? A multicenter study of 107 cases. Mod Pathol. 2021;34(6):1143-1152.

8. Tashakori M, Kadia TM, Loghavi S, et al. TP53 copy number and protein expression inform mutation status across risk categories in acute myeloid leukemia. Blood. 2022;140(1):58-72.

9. Bernard E, Nannya Y, Hasserjian RP, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Nat Med. 2020;26(10):1549-1556.

10. Murnyak B, Hortobagyi T. Immunohistochemical correlates of TP53 somatic mutations in cancer. Oncotarget. 2016;7(40):64910-64920.

11. Ruzinova MB, Lee YS, Duncavage EJ, Welch JS. TP53 immunohistochemistry correlates with TP53 mutation status and clearance in decitabine-treated patients with myeloid malignancies. Haematologica. 2019;104(8):e345-e348.

12. Kern W, Haferlach T, Schnittger S, Hiddemann W, Schoch C. Prognosis in therapy-related acute myeloid leukemia and impact of karyotype. J Clin Oncol. 2004;22(12):2510-2511.

13. Montalban-Bravo G, Kanagal-Shamanna R, Class CA, et al. Outcomes of acute myeloid leukemia with myelodysplasia related changes depend on diagnostic criteria and therapy. Am J Hematol. 2020;95(8):e345-e348.

14. Mazzella FM, Smith D, Horn P, et al. Prognostic significance of pronormoblasts in erythrocyte predominant myelodysplastic patients. Am J Hematol. 2006;81(7):484-491.

15. Kowal-Vern A, Cotelingam J, Schumacher HR. The prognostic significance of proerythroblasts in acute erythroleukemia. Am J Clin Pathol. 1992;98(1):34-40.