Emergence of *TP53* mutation during lenalidomide therapy of myelodysplastic syndrome with del(5q) and its subsequent disappearance following salvage therapy with decitabine

According to the International Consensus Classification (ICC), diagnosis of myelodysplastic syndrome (MDS) with del(5q) requires presence of i) del5q, alone or associated with no more than one other cytogenetic abnormality, exclusive of -7/del(7q), ii) <5% bone marrow (BM) and <2% circulating blasts, and iii) the absence of "multi-hit" *TP53*.[1] The latter is defined by the ICC as the presence of i) two or more distinct *TP53* mutations, each with variant allele frequency (VAF) ≥10%, ii) a single *TP53* mutation with VAF ≥50%, or iii) a single *TP53* mutation with VAF ≥10% accompanied by a cytogenetically-apparent del(17p13.1), copy-neutral loss of heterozygosity (LOH) at the *TP53* locus, or, in the absence of loss of heterozygosity (LOH) information, complex karyotype.[1] MDS-del(5q) is uniquely characterized by its relatively indolent clinical course and its sensitivity to treatment with lenalidomide.[2-4]

Patients with MDS-del(5q) are known to harbor multiple somatic mutations with the two most frequent ones being *TP53* (~20% incidence at diagnosis) and *SF3B1* (~18%).[5] These two mutations display significant clustering and are more prevalent in leukemic phase disease, with an estimated 50% incidence.[5] The latter suggests vulnerability or pathogenetic contribution to progression into acute myeloid leukemia (AML). In this regard, we have recently reported on *TP53* VAF >22% as being the most prominent risk factor for leukemic progression and overall survival in MDS-del(5q).[5] These observations were consistent with an earlier report suggesting adverse impact of strong P53 protein expression on cytogenetic response and overall and leukemia-free survival in MDS-del(5q).[6] Consistent with these observations, a 2018 report in *Haematologica* described the emergence of *TP53* mutations and disease progression during lenalidomide therapy, in patients with MDS-del(5q).[7] In the current case report of a patient with MDS-del(5q), we confirm the acquisition of *TP53* mutation during lenalidomide therapy and, in addition, we describe, for the first time, successful salvage therapy with 3-day decitabine, resulting in resolution of transfusion need, cytogenetic remission, and elimination of mutant *TP53* and *SF3B1* clones. Informed consent was obtained from the patient.

Our patient was first diagnosed with MDS-del(5q) at 75 years of age in May 2021. Co-morbidities included history of hemochromatosis (diagnosed in 2008 based on serum ferritin value of 1,553 mcg/L and liver biopsy that showed moderate hemosiderosis but with no documentation of genotyping and subsequently treated with phlebotomy in the remote past), atrial fibrillation, non-ischemic cardiomyopathy, mitral regurgitation, and alcohol use. The earliest complete blood count (CBC) record from January 2012 showed hemoglobin 14.1 g/dL, mean corpuscular volume (MCV) 97.3 FL, platelet count 277x10^9/L and leukocyte count 6.3x10^9/L. Macrocytosis without anemia first appeared in July 2013 (MCV 103.7 FL) and by July 2015, MCV was 107.7 FL and hemoglobin 12.9 g/dL. Subsequently, his anemia worsened progressively until he required two units of packed red cell transfusion 10 days prior to his first visit to our institution in May 2021. At that time, physical examination did not reveal palpable spleen, liver, or lymph nodes; CBC showed hemoglobin 8.9 g/dL, MCV 130.9 FL, leukocyte count 4.2x10^9/L, absolute neutrophil count (ANC) 1.96x10^9/L, platelet count 152x10^9/L, normal serum B12 and folate, serum ferritin 172 mcg/dL, serum erythropoietin level 225 mIU/mL, and no circulating blasts. Bone marrow (BM) examination revealed normocellular marrow with 1% blasts and dysmegakaryopoiesis (monolobated and bilobate forms). Cytogenetic studies revealed isolated 5q deletion in eight (40%) of 20 metaphases (46,XY,del(5)(q13q33)[8]/46,XY[12]). Next-generation sequencing (NGS) of BM samples revealed *DNMT3A* (VAF 23%) and *SF3B1* (VAF 14%) mutations, only (Figures 1 and 2).

Treatment with lenalidomide (10 mg daily) was initiated on June 16, 2021, and the patient became transfusion-independent within 3 months of treatment. Lenalidomide dose was reduced to 7.5 mg/day on May 13, 2022 because of treatment-emergent diarrhea that did not get better and was subsequently attributed to another cause, leading to treatment resumption at 10 mg/day dose (patient remained transfusion-independent during this period). CBC from January 2022 showed hemoglobin 12.8 g/dL, MCV 112.1 FL, leukocyte count 5.2x10^9/L and platelet count 264x10^9/L. By December 2022, the patient had relapsed with transfusion-dependent anemia: hemoglobin 6.0 g/dL, MCV 124.7 FL, leukocyte count 3.2x10^9/L, ANC 0.83x10^9/L, and platelet count 53x10^9/L. BM examination at the time showed normocellular marrow with slightly increased blasts (5%), and persistent dysmegakaryopoiesis (Figures 1 and 2). Cytogenetic studies revealed isolated 5q deletion in 13 (65%) of 20 metaphases (46,XY,del(5)(q13q33)[13]/46,XY[7]). BM NGS revealed clonal expansion of *DN-*

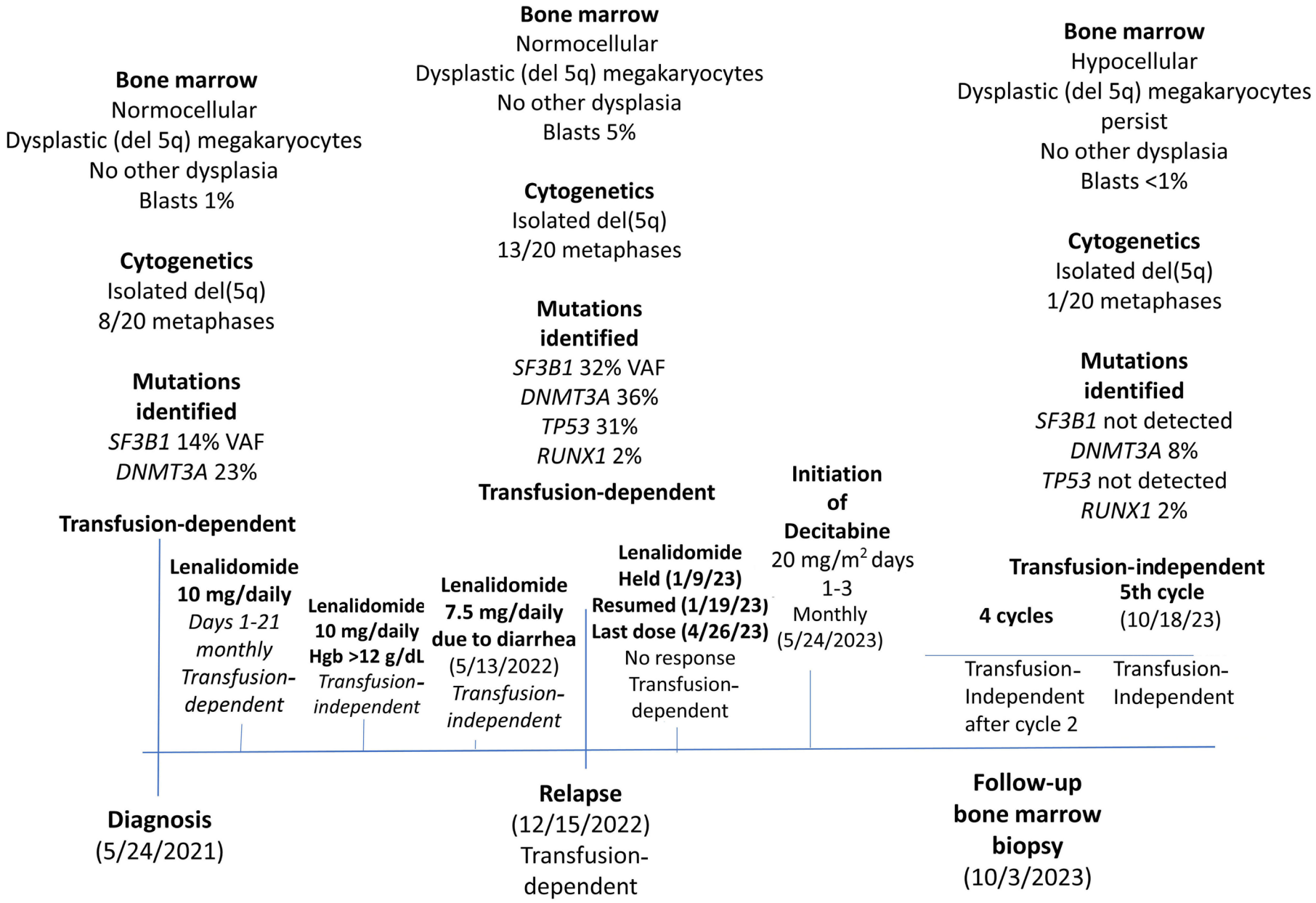

Figure 1. Timeline of disease course and treatment history of a 75-year-old man with myelodysplastic syndromes with del(5q). VAF: variant allele frequency; HgB: hemoglobin B.

MT3A (VAF 36%) and *SF3B1* (VAF 32%) mutations, as well as emergence of two new mutations: *TP53* (VAF 31%), and *RUNX1* (VAF 2%). Lenalidomide was held on January 9, 2023 and resumed on January 19, 2023 without any benefit and treatment discontinued on April 26, 2023. Salvage therapy with lower-dose decitabine (20 mg/m^2 daily x 3 days, per 28-day cycle) was initiated on May 24, 2023, and the patient became transfusion-independent after the second cycle of treatment and has remained transfusion-independent to date (October 18, 2023); the 3-day *versus* the standard 5-day dose schedule for decitabine was chosen based on patient fraility. A follow-up BM examination performed on March 10, 2023, showed hypocellular marrow with <1% blasts and persistence of small hypolobated megakaryocytes, although decreased in number; cytogenetic studies revealed isolated 5q deletion in only one of (5%) of 20 metaphases (46,XY,del(5)(q13q33)[1]/46,XY[19]). BM NGS revealed disappearance of both the *TP53* and *SF3B1* mutant clones and a decrease in mutant allele burden for *DNMT3A* (VAF 8%) while *RUNX1* (VAF 2%) remained unchanged (Figures 1 and 2).

The current case report confirms observations from recent reports that suggest the emergence or clonal expansion of *TP53* mutations during treatment with lenalidomide.[7,8] In a cohort of 24 patients with MDS-del(5q), 18 (75%) had an erythroid response during lenalidomide therapy and five (21%) a complete cytogenetic response;[7] a *TP53* mutation was detected in six (25%) patients at diagnosis and in an additional nine (38%) patients during follow-up, of whom, one also manifested a new *RUNX1* mutation, as was the case in the current report (median duration of exposure to lenalidomide was 11 months).[7] The particular study also showed a correlation between *TP53* clonal evolution and disease progression.[7] In another report of 416 patients with therapy-related myeloid neoplasms, the authors described an association between *TP53* mutations and prior treatment with thalidomide analogs, specifically lenalidomide.[8] In the latter report, the authors were able to provide experimental evidence for lenalidomide-induced selective advantage for *TP53* mutant clones.[2] What is novel in our case report was the successful treatment with 3-day (instead of the standard 5-day) decitabine, culminating not only in resolution of transfusion-need but also in inducing major cytogenetic and molecular re-

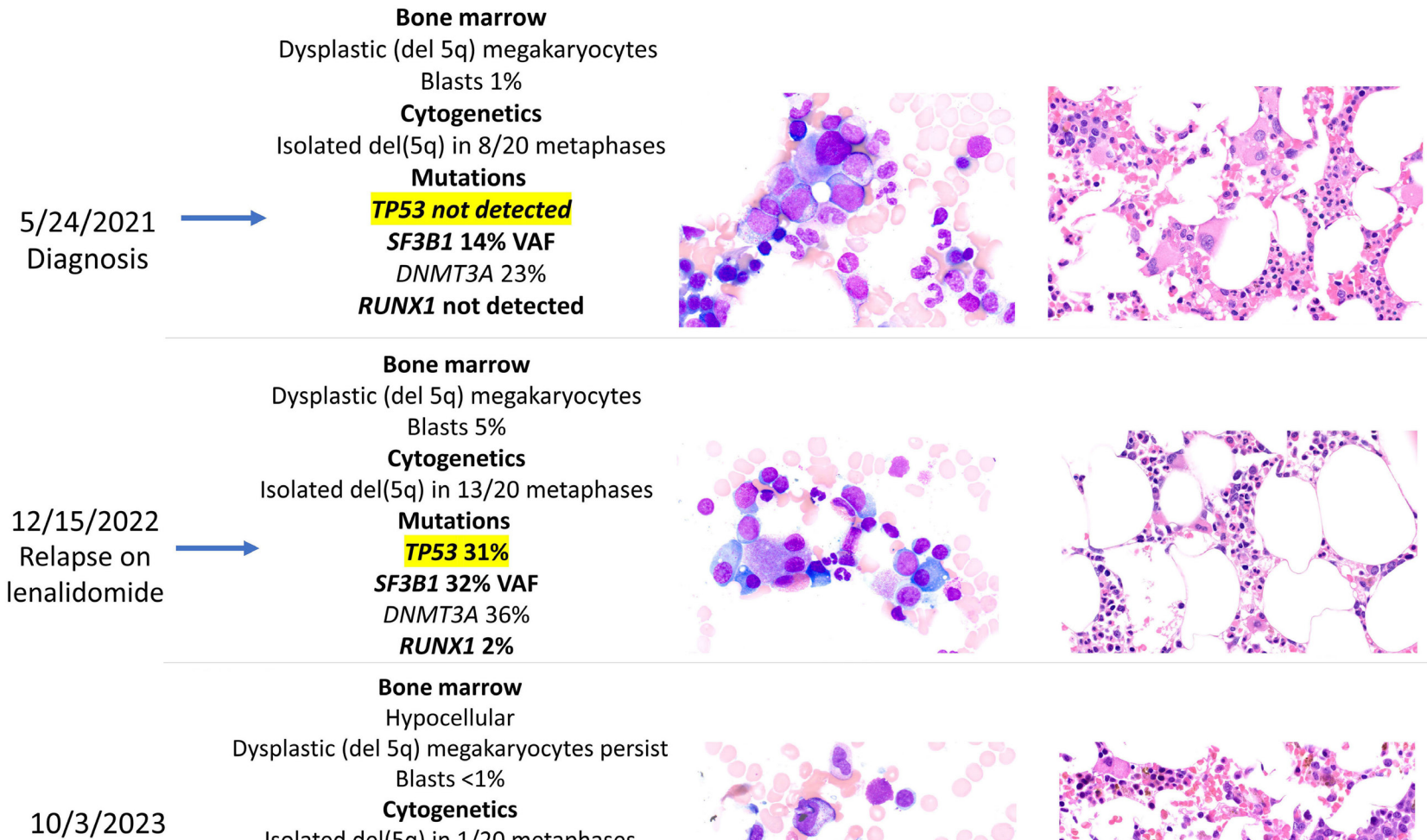

Figure 2. Sequential bone marrow examination. A 75-year-old man with myelodysplastic syndromes with del(5q) diagnosed on May 24, 2021 was treated initially with lenalidomide but lost response by December 15, 2022 and was successfully salvaged with decitabine therapy on March 10, 2023. VAF: variant allele frequency.

mission, including the eradication of the *TP53* mutation; it is difficult, based on a single case report, to comment on whether or not the 3-day, as opposed to the standard 5-day, decitabine dosing schedule made a difference or whether we would have been as successful using azacytidine instead of decitabine.

There are a number of practically important suggestions that can be extrapolated from the current case report, but only after confirmation from additional studies. At the minimum, it is reasonable to recommend NGS in all patients with MDS-del(5q) at diagnosis as well as periodically during treatment with lenalidomide. In the event of treatment-emergent *TP53* mutations or clonal expansion, we would recommend addition or switching to hypomethylating agents and continue monitoring *TP53* VAF. The particular treatment strategy is also relevant in the context of bridging chemotherapy, in preparation for allogeneic stem cell transplant, which is currently the only treatment modality that can secure long-term survival in MDS-del(5q)[9] as well as *TP53*-mutated myeloid neoplasms, especially if not associated with complex karyotype,[10] which would be the case in MDS-del(5q); whether or not treatment success can further be enhanced with the addition of venetoclax to hypomethylating agent therapy is plausible but needs to be studied.

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Contributions

NG and AT co-wrote the paper. NB participated in patient care. KR reviewed bone marrow morphology and genetic studies. All authors reviewed and approved the final draft of the paper.

Data-sharing statement

Please email the corresponding author to obtain original data.

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