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Therapy-related acute myeloid leukemia developing 14 years after allogeneic hematopoietic stem cell transplantation, from a persistent R882H-*DNMT3A* mutated clone of patient origin

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ABSTRACT

Background: Therapy-related acute myeloid leukemia (t-AML) develops in patients with prior exposure to cytotoxic therapies. Selection of a pre-existing *TP53* mutated clone prone to acquire additional mutational events has been suggested as the main pathogenic mechanism of t-AML. Here, we report a unique case of t-AML which developed from a pre-existing *DNMT3A* mutated clone that persisted in the patient for more than 10 years despite treatment with intensive chemotherapy and allogeneic hematopoietic stem cell transplantation (alloHSCT).

Case presentation: A 42-year-old male was diagnosed with AML harboring a normal karyotype and mutations in the *NPM1* (c.863_864ins, p.W288 fs*12), *DNMT3A* (c.2645G > A, p.R882H), and *IDH1* (c.395G > A, p.R132H) genes. He achieved complete remission with intensive chemotherapy and was subsequently submitted to alloHSCT. Eleven years later, he was given chemotherapy and radiotherapy to treat a lung carcinoma. Three years later, t-AML was diagnosed; the disease had arisen from a pre-existing *DNMT3A* mutated patient-origin clone that had subsequently acquired a *TP53* mutation and a complex karyotype. Although a second transplantation was intended, the disease was refractory to induction chemotherapy, and the patient eventually died from disease complications. We retrospectively demonstrated the persistence and post-transplantation latency of the R882H-*DNMT3A* mutation using a real-time PCR allele-specific analysis at different time-points during the observation period.

Discussion and conclusion: The present case highlights the potential clinical implications of a R882H-*DNMT3A* mutated clone that persisted after conventional AML treatment, including alloHSCT. It also reinforces the notion of the importance of cell non-intrinsic factors, such as the hematopoietic-stress induced by chemotherapy and radiotherapy, as drivers of clonal expansion.

1. Background

Therapy-related AML (t-AML) is a heterogeneous myeloid disease that can occur as a late complication in patients with prior exposure to cytotoxic therapies or environmental toxins (Zeichner and Arellano, 2015). Cases that develop in patients treated with topoisomerase II inhibitors usually present abruptly after a short latency period of 2 to 3 years. In contrast, t-AML that develops after treatment with alkylating agents or radiotherapy has a longer latency of 5 to 10 years, is often preceded by a myelodysplastic syndrome (MDS) phase, and typically has aberrations in chromosomes 5 and 7 and/or a complex karyotype (Heuser, 2016). Different models have been suggested to explain the

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pathogenesis of t-AML. One model proposes that cytotoxic therapy has direct effects on the instability of oncogenes and induces somatic mutations in a susceptible target cell, with this leading to clonal expansion of the transformed cells. Nevertheless, there is currently little experimental data to support this model. In contrast, there is growing evidence for an alternative model which suggest that t-AML results from the selection of a pre-existing mutated clone that is treatment-resistant and permissive to genetic instability (Ganser and Heuser, 2017). In support of this, Wong et al. (2015) found a significantly higher frequency of *TP53* mutations in t-AML compared with *de novo* AML and suggest that the selection of a *TP53* mutated clone could be involved in the pathogenesis of t-AML. However, in addition to the *TP53* gene, recent studies have reported that pre-existing treatment-resistant mutations in other genes such as *TET2*, *ASXL1,* or *DNMT3A* could also be related to the development of t-AML (Takahashi et al., 2017; Fabiani et al., 2017). In this sense, the clinical case herein reported provides evidence on the potential implication of a treatment-resistant *DNMT3A* mutated clone in the development of t-AML after allogeneic hematopoietic stem cell transplantation (alloHSCT).

2. Case presentation

In 2001, a 42-year-old Caucasian male, smoker (24 pack-year), with no significant comorbidities, was admitted to hospital due to fatigue and persistent fever. Blood tests revealed the presence of anemia and thrombocytopenia. A bone marrow aspirate displayed 57% blasts cells expressing HLA-DR, CD13, CD33, and CD15, myeloperoxidase (MPO), and strong CD64 co-expression. CD14 and CD34 expression were negative. Dysplasia was observed in at least 50% of the cells in all cell lineages. Cytogenetics analysis showed a normal karyotype. According to the French-American-British classification the patient was diagnosed with acute myelomonocytic leukemia (M4 subtype) with trilineage dysplasia.

The patient was treated with intensive chemotherapy, including cytarabine, idarubicin, and etoposide which led to the achievement of complete remission. One cycle of consolidation chemotherapy (cytarabine plus mitoxantrone) was given before submitting the patient to alloHSCT from his HLA-compatible sister. The conditioning regimen consisted of myeloablative doses of total body irradiation and cyclophosphamide. At day +180, he remained in complete remission with full donor chimerism.

In 2012, the patient was diagnosed with squamous lung carcinoma (p-T3N2M0) and a mutational study of the *EGFR*, *TP53,* and *DNMT3A* genes in the carcinoma cells revealed a pathogenic mutation in the *TP53* gene (c.461G > T, p.G154 V; supplementary: Fig. S1). After a right upper lobectomy, the patient received chemotherapy with 4 cycles of gemcitabine and cisplatin, followed by radiation. The patient remained in complete remission up to his last follow-up visit.

In October 2015, the patient was hospitalized again due to community-acquired pneumonia with concomitant normocytic anemia. A bone marrow aspirate showed minimal erythroid dysplasia without blasts. The anemia was interpreted as secondary to chronic renal failure and treatment with darbepoetin was administered. However, two months later, the patient developed pancytopenia, and a second bone marrow aspirate revealed the presence of 14% blasts cells with multilineage dysplasia. In addition, cytogenetics analysis found a complex karyotype (supplementary: Fig. S2 and S3). Based on the World Health Organization classification (2008) the diagnosis was refractory anemia with excess blasts-2 (RAEB-2), possibly related to the prior cytotoxic treatments (*i.e.*, therapy-related MDS, abbreviated as t-MDS). Since the disease was classified into the very high-risk group by the Revised International Prognostic Scoring System (9.5 points), a second alloHSCT from the same donor was planned.

Intensive chemotherapy with high-dose cytarabine was given to reduce the disease burden prior to transplant. However, after recovering from a period of aplasia, the bone marrow aspirate showed 43% immature cells with dysplasia and proerytroblastic appearance. The blasts cells reacted with antibodies to glycophorin, CD36, CD38, CD71, CD105, CD117, and HLA-DR, lacked myeloid-associated markers, and were negative for anti-MPO. Based on morphology and cytometry studies the diagnosis was compatible with pure erythroid leukemia (*i.e.*, therapy-related AML, abbreviated to t-AML).

Because his disease was refractory to intensive chemotherapy, treatment with azacitidine was initiated. However, the clinical condition of the patient rapidly deteriorated, leading to his death two months later.

3. Cellular origin of the t-AML clone

We first aimed to ascertain if t-AML had a donor or a patient-cell origin. To achieve this objective, an analysis of the chimerism was performed on DNA from cells taken from the patient prior to transplantation, DNA from bone marrow cells at the time of the t-AML diagnosis, and DNA extracted from the donor cells. A Mentype® Chimera® PCR Amplification Kit (Biotype) was used to simultaneously compare 12 polymorphic autosomal short tandem repeat loci (D2S1360, D3S1744, D4S2366, D5S2500, D6S474, D7S1517, D8S1132, D10S2325, D12S391, D18S51, D21S2055, and SE33) next to the gender-specific marker, amelogenin, in all three DNA samples. Of the analyzed loci, 8 were informative and showed an identical profile between the t-AML cells collected in 2015 and the patient's pre-transplant cells taken in 2001, confirming the graft loss as well as the patient-cell origin of the t-AML blasts (supplementary: Fig. S4).

4. Pathogenic origin of the t-AML clone

Next, we aimed to determine if the 2015 t-AML clone was the same as the 2001 AML clone. To genetically characterize both clones, we performed a diagnostic panel on DNA obtained from the samples taken at the time of diagnosis of the 2001 AML and of the 2015 t-AML for some of the most frequently mutated genes in myeloid diseases: *FLT3*, *CEBPA*, *NPM1*, *NRAS*, *KRAS*, *ASXL1*, *DNMT3A*, *IDH1*, *IDH2*, *TP53*, *SRSF2*, *SF3B1*, *U2AF1*, *SETBP1*, *JAK2*, *ETNK1*, *KIT*, *CALR*, *MPL*, *CSF3R*, and *BRAF*. This study detected three pathogenic mutations in the 2001 AML clone: *NPM1* (c.863_864ins, p.W288 fs*12), *IDH1* (c.395G > A, p.R132H), and *DNMT3A* (c.2645G > A p.R882H; supplementary: Fig. S5). The same *DNMT3A* gene mutation (c.2645G > A p.R882H), together with a new pathogenic mutation in the *TP53* gene (c.617delT, p.L206 fs*41; supplementary: Fig. S6) was present in the 2015 t-AML clone. However, no mutations in the *NPM1* and *IDH1* genes were identified in this latter sample. Therefore, we hypothesized that the t-AML clone originated in the persistence of a pre-leukemic *DNMT3A* mutated clone that, 14 years later, had acquired new genetic and chromosomal alterations (Fig. 1).

5. Monitoring of the pathogenic clone

We aimed to retrospectively assess the variant allele frequency (VAF) of the R882H-*DNMT3A* mutated clone during the observation period. First, we designed a real-time allele-specific oligonucleotide PCR (ASO-qPCR) to detect the R882H-*DNMT3A* mutation (supplementary: materials and methods and Fig. S7). For these experiments, the detection limit (DL) was established at the 0.5% dilution (supplementary: Fig. S8).

Next, we analyzed the R882H-*DNMT3A* mutation status at different points disease-evolution timeline, according to the availability of peripheral blood (PB) or bone marrow (BM) DNA samples from the patient: 2001 AML diagnosis (BM), alloHSCT +21 days (PB, donor cells, DC = 99.1%), alloHSCT +90 days (BM, DC = 94.3%), alloHSCT +180 days (PB, DC = 100%), alloHSCT +360 days (PB, DC = 100%), alloHSCT +7 years (PB, DC = 100%), 2015 t-MDS diagnosis (BM, DC = 22%) and 2015 t-AML transformation (BM, DC = 10%). As