Small myeloid subclones are present at diagnosis of multiple myeloma in patients who develop secondary myelodysplastic syndromes

Patients with monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM) have a higher risk of developing myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) than the general population, even without receiving any treatment.[1,2] This risk is even more important in patients with multiple myeloma (MM) receiving myeloma-specific therapies,[3] with a reported prevalence of therapy-related myeloid neoplasms (t-MN) in about 3% of all patients.[4]

Substantial improvements in survival of patients with MM have been observed in the recent years with the emergence of new generation treatments such as proteasome inhibitors, immunomodulators and monoclonal antibodies. As a consequence, there are growing concerns about the risk of developing therapy-related myelodysplastic syndrome (t-MDS) or therapy-related acute myeloid leukemia (t-AML) with newer regimens. Those complications are uncommon but associated with a poor prognosis. The reported median survival after the diagnosis of MDS or AML is 6.7 months.[5]

The risk of t-MN in MM is associated to multiple factors, including age, sex, ethnicity, genetics and prior treatment received.[3] Among MM-specific therapies, alkylating agents and lenalidomide have drawn specific attention. High-dose melphalan used in autograft stem cell transplantation (ASCT) conditioning is thought to induce an accumulation of mutations in myeloid cells leading to t-MN.[6] The 5-year cumulative incidence of MDS and AML after ASCT for MM is 1.0%.[7] And the reported median times from ASCT to t-MDS or t-AML are 5 (range, 1-15) and 4 (range, 1-9) years, respectively.[8] Lenalidomide maintenance has been associated with an increased risk of developing t-MN.[9,10] Those patients developing t-MN after being exposed to lenalidomide have an increased incidence of *TP53* alterations in myeloid cells.[11] This suggests that lenalidomide might provide a selective advantage to *TP53* mutant myeloid sub-clones. Finally, the role of clonal hematopoiesis of indeterminate potential (CHIP) and pre-existing dysplastic hematopoiesis is still unclear.[12,13]

In this work, we hypothesized that myelodysplastic subclones might be already present at the time of MM diagnosis, even years before developing a t-MDS. We analyzed a case series of five patients with MM who developed t-MDS in our Institution between January 2017 to March 2020 and for which we had serial bone marrow samples. This study has been approved by the institutional review board (IRB) of Lille University Hospital and the Ethics Committee Nord Ouest IV (protocol # ECH23/02) in accordance with the Declaration of Helsinki.

Median age at diagnosis of MM was 69 years (range, 57-75), Table 1. Two patients had high-risk cytogenetic features per International Myeloma Working Group criteria. Median time between MM diagnosis and MDS diagnosis was 5 years (range, 3-8) and median prior lines of treatment was three (range, 1-4). Four of five patients received an ASCT and all patients received immunomodulatory drugs, specifically lenalidomide. Different treatment exposures are depicted in Table 1 and Figure 2. At the time of t-MDS, patients had a bone marrow evaluation with cytology, myeloid next generation sequencing (NGS) panel sequencing and conventional karyotypes. Of note, all patients had no or minimal plasma cell infiltration at that time point. We observed in all five cases the presence of both a monosomal karyotype and a *TP53* mutation in their myeloid clones. Three patients had a complex karyotype. Other genomic mutations involved *DNMT3A, TET2, RUNX1,*

Table 1. Characteristics of patients.

Characteristics	At diagnosis of MM N=5
Age in years, median (range)	69 (57-75)
ISS stage III, %	60
High-risk cytogenetics, %	40
	Between MM and MDS diagnoses N=5
Time in years, median (range)	5 (3-8)
N of lines of treatment, median (range)	3 (1-4)
ASCT, %	80
Alkylating agents, %	80
Lenalidomide, %	100
	At diagnosis of MDS N=5
Age in years, median (range)	76 (64-78)
R-IPSS intermediate or high, %	100
Complex karyotype, %	60
Monosomal karyotpe, %	100
TP53 mutation, %	100
	Survival from MDS diagnosis
Median OS in months (range)	21 (1-38)

MM: multiple myeloma; MDS: myelodysplastic syndrome; ISS: International Staging System; ASCT: autograft stem cell transplantation; R-IPSS: Revised International Prognostic Scoring System; OS: overall survival.

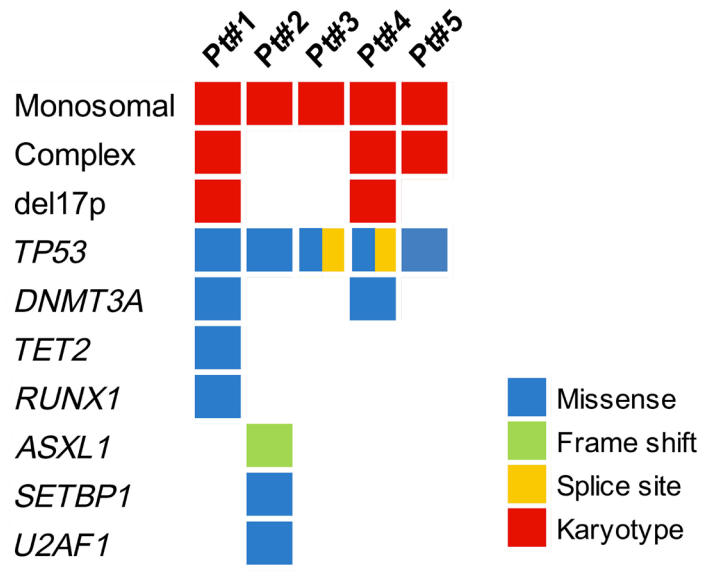

Figure 1. Genomic alterations at diagnosis of therapy-related myelodysplastic syndrome. Genomic alterations were assessed at the time of therapy-related myelodysplastic syndrome diagnosis by conventional karyotype and targeted next generation sequencing in myeloid cells. Pt: patient.

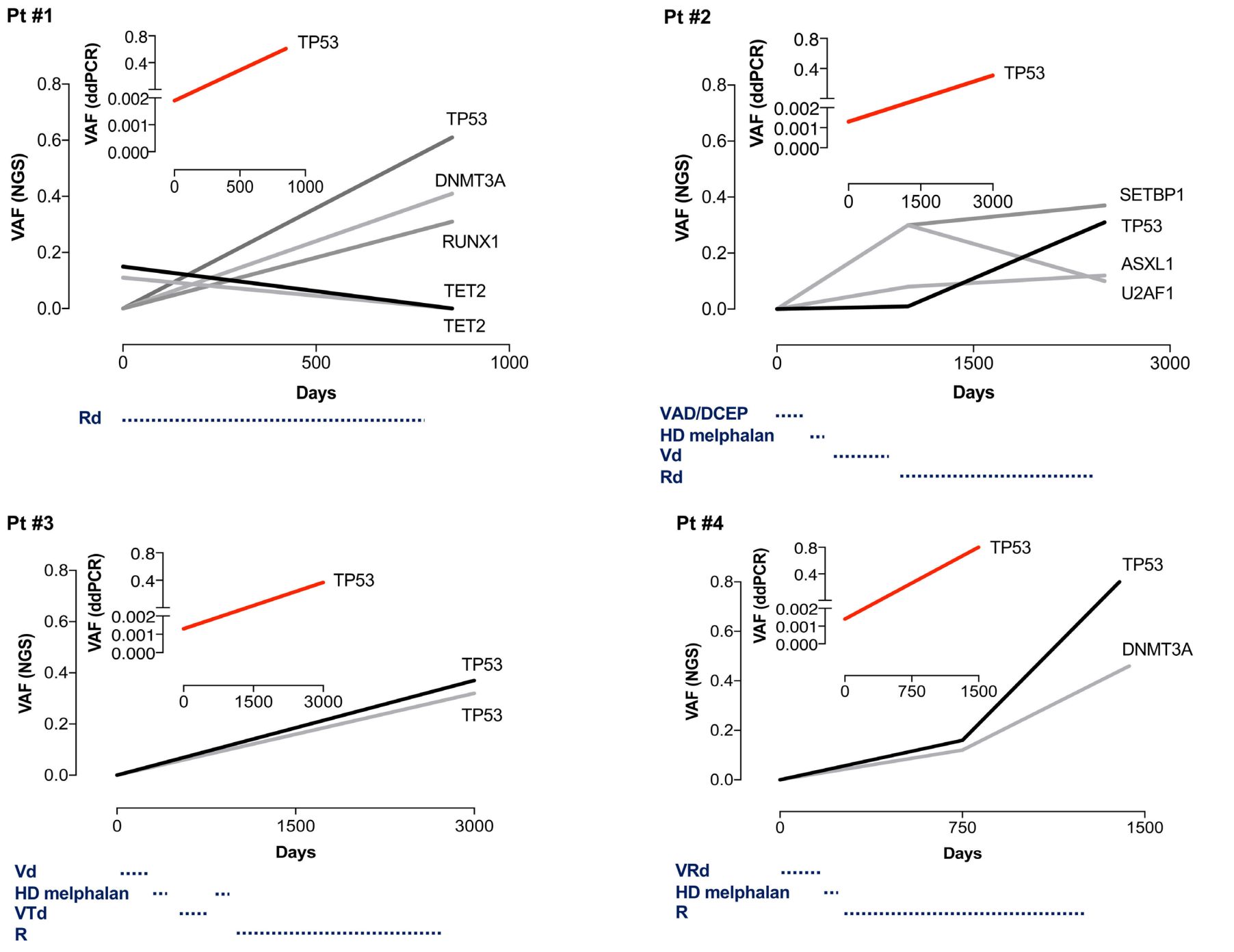

Figure 2. Clonal evolution of myeloid subclones during multiple myeloma treatments. Genomic alterations were evaluated on bone marrow samples, by a myeloid targeted next generation sequencing (NGS) panel, at the time of therapy-related myelodysplastic syndrome (t-MDS) diagnosis and at the time of multiple myeloma (MM) diagnosis. The NGS panel covers 90 myeloid genes. Libraries were prepared using the Twist BioScience® kit and sequenced on a NovaSeq6000 for a median depth of 3,600X. Raw data were analyzed using MuTect2, Vardict, GATK and GnomAD. Droplet digital polymerase chain reaction (ddPCR) for each *TP53* mutations were also performed on bone marrow samples at the time of MM and t-MDS diagnoses. Clonal evolution are depicted by variant allelic fractions (VAF). Rd: lenalidomide-dexamethasone; VAD/DCEP: vincristine-adriamycine-dexamethasone/dexamethasone-cyclophosphamide-etoposide-cisplatin; HD: high dose; Vd: bortezomib-dexamethasone; VTd: bortezomib-thalidomide-dexamethasone; R: lenalidomide; VRd: bortezomib-lenalidomide-dexamethasone; Pt: patient.

ASXL1, SETBP1 (Figure 1). In all cases, the diagnosis of MDS happened after a prolonged exposure to lenalidomide. The size of MDS clones were high with a median cancer cell fractions of *TP53* mutations of 37% (range, 30-82). In order to capture the evolution of the t-MDS clones, we analyzed bone marrow samples at diagnosis of MM and at intermediate time points when available by NGS and digital droplet polymerase chain reaction (ddPCR) for *TP53* mutations (except for 1 patient due to a lack of material). No mutations were detectable by NGS sequencing with a limit of detection of 1%. But all patients had a detectable *TP53* subclones (with allelic fraction between 0.14% to 0.20%) at the time of MM diagnosis by ddPCR, between 3 to 8 years before being diagnosed with t-MDS (Figure 2). In all four cases with sequential samples we observed a clonal evolution of the t-MDS subclones during MM treatments. Interestingly, in one case with multiple samples (Pt#2), the selection of the *TP53* subclone happened during lenalidomide treatment and not during exposure to three prior lines of treatment.

This work suggests that tiny myeloid subclones with *TP53* mutations are present in patients with MM, years before developing t-MDS. The concept of acquisition or selection of pre-existing mutations is essential to better understand the processes that drive myeloid transformation during treatments. Our data indicate that *TP53* mutant subclones were pre-existing and not induced by MM therapies. In a large study over 10,000 individuals, patients who had received previous cancer treatment were more likely to have CHIP compared with treatment-naive patients, likely reflecting the selection pressure induced by therapies.[6] Evidences suggest that mutations are selected differentially based on prior treatment exposures. Mutations in *ASXL1* are enriched in current or former smokers, whereas cancer therapy with radiation, platinum and topoisomerase II inhibitors preferentially selects for clones with mutations in DNA damage response (DDR) genes (*TP53, PPM1D, CHEK2*). This indicates that DDR and non-DDR clones have distinct evolutionary trajectories with different competitive fitness. This is illustrated in our case series in Pt#1 where a *TP53/DNMT3A* subclone emerges during treatment with lenalidomide while a *TET2* subclone is eliminated (Figure 2). In the case of Pt#2, an *ASXL1* subclone is initially selected during treatment with cytotoxic agents and proteasome inhibitor but disappears during treatment with lenalidomide, while a *TP53* subclone emerges during lenalidomide exposure (Figure 2). It was recently reported that lenalidomide provides a selective advantage to *TP53* mutant clones in hematopoietic stem cells.[11] In patients with t-MN, *TP53* mutations are significantly associated with prior exposure to immunomodulatory drugs, specifically lenalidomide. Interestingly, pomalidomide does not provide an equivalent level of selection of *TP53* subclones due to difference in CK1α degradation and p53-dependent apoptosis.[11]

Our data demonstrate the presence of tiny *TP53* myeloid subclones, in patients with MM diagnosis, up to 8 years before developing t-MDS. Those *TP53* subclones are expanded during MM-specific treatments, particularly by lenalidomide exposure, and drive transformation to myeloid neoplasm. Altogether, theses data suggest the potential for exploring alternative therapeutic strategies in patients with MM and the presence of *TP53* myeloid subclones in order to mitigate the risk of developing t-MN, especially due to long term exposure to lenalidomide.

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Contributions

GE, EF, CP, ND and SM developed the concept and methodology, and acquired data. All authors analyzed the data. All authors wrote, edited and reviewed the manuscript.

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Data-sharing statement

Genomic data from the targeted NGS panel and the ddPCR have not been deposited for public access.

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