Emergence and evolution of *TP53* mutations are key features of disease progression in myelodysplastic patients with lower-risk del(5q) treated with lenalidomide

The cytogenetic anomaly of isolated del(5q) characterizes a subgroup of patients with lower-risk myelodysplastic syndrome (MDS). In the 2016 World Health Organization classification, one additional karyotypic abnormality (except -7 or del7q) can be present in such patients.[1] Median survival is around 6 years and the cumulative incidence of progression towards acute myeloblastic leukemia is 15–20% at 5 years in untreated patients.[2]

The specific efficacy of lenalidomide as a treatment for anemia in these patients was first described in 2006[3] and confirmed by a phase III trial.[4] Lenalidomide enables about 50% of patients to become independent of red blood cell transfusions. Approximately 20% of treated patients achieve a complete cytogenetic response although the median response duration is only 2 years and 40% of treated patients still progress to acute myeloblastic leukemia by 5 years.[4] This confirms previous data showing that at least some resistant driver del(5q) subclones are not eradicated during lenalidomide treatment.[5]

TP53 mutations occur in lower-risk del(5q) MDS at a frequency of approximately 20%[6] and, as in other hematologic malignancies, are associated with a poor prognosis.[6,7] Progression of MDS into secondary acute myeloblastic leukemia has been reported to be linked to clonal evolution, which can be related to the emergence or variations of genomic anomalies.[8] These observations, supported by recent studies,[9-11] led us to address the appearance and evolution of *TP53*-mutated subclones in lower-risk del(5q) MDS patients along the course of the disease. We, therefore, retrospectively screened for *TP53* mutations in a cohort of such MDS patients who received lenalidomide in the course of their treatment.

These patients had been diagnosed by cytogenetics as having the del(5q) anomaly, were treated at one point by lenalidomide and had had at last two blood or bone marrow samples taken during their follow-up at the discretion of their physicians. Two of them, with 5% of blasts at disease discovery, were considered to have low-risk MDS with excess blasts associated with del(5q). To investigate for the appearance or evolution of *TP53*

Table 1. Mutations details.

#	*TP53* mutations VAF at diagnosisVAF(s)	*TP53* mutations at follow-up	*TP53* mutations DNA	Type	Protein
1		24%/29%	c.524G>A	SNV	p.R175H
			c.844C>T		p.R82W
2	29%	20%	c.818G>A	SNV	p.R273H
4		43%	c.818G>A	SNV	p.R273H
6		16%/14%	c.314G>T		
			c743G>A		
			c.818G>A		
			c.833C>G		
			c.614A>G	SNV	p.G105V
			p.R248Q		
			p.R273H		
			p.P278R		
			p.Y205C		
7		21%	c.844C>T	SNV	p.R282W
10		4%	c.818G>A	SNV	p.R273H
11	36%	31%	c.830G>A	SNV	p.C277Y
12	3%	45%	c.725G>T	SNV	p.C242F
			c.920-1G>A	Splice	p. ?(splice)
13	25%	25%	c.343C>G	SNV	p.H115D
15		100%	c.821T>C	SNV	p.V271A
18		33%	c.711G>A	SNV	p.N237I
19	54%	55%	c.584T>A	SNV	p.I195N
21		49%	c.413C>T	SNV	p.A138V
22		55%/20%	c.421T>G	SNV	p.C141G
			c.711G>T		p.M237I
23	14%	6%/32%	c.743G>A		p.R248Q
			c.817C>T	SNV	p.R273C
			c.844C>T		p.R282W

SNV = single nucleotide variant. Note that use of a broader sequencing panel could have revealed other anomalies.

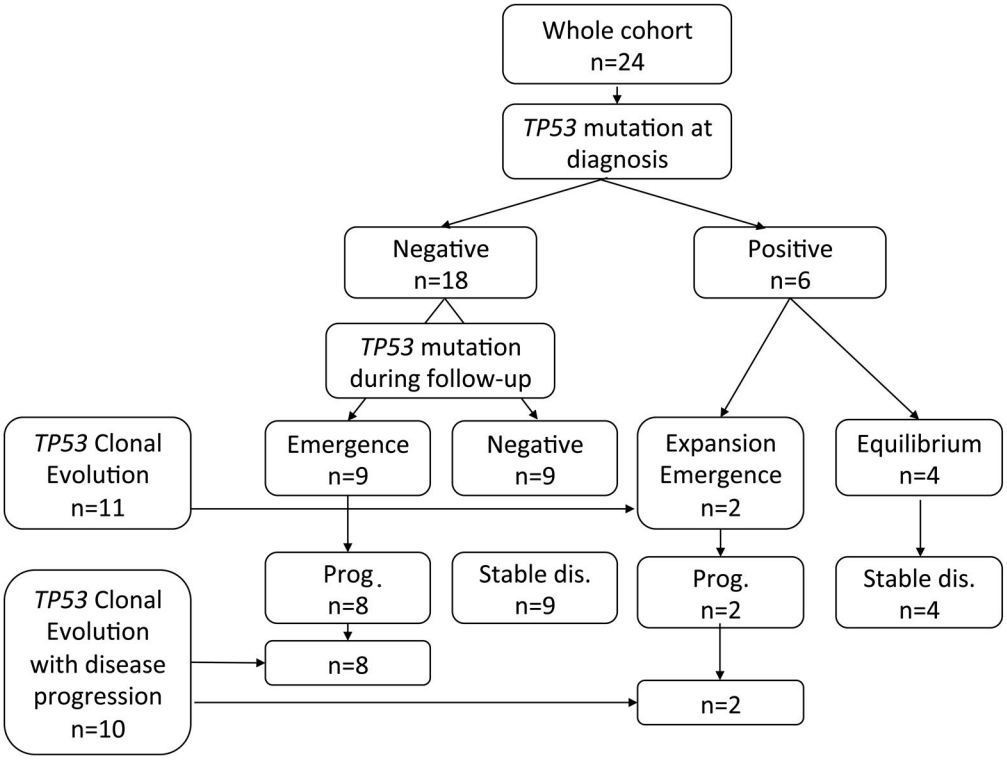

Figure 1. Diagram of the patients' evolution and *TP53* status over the follow-up period. Prog.: progression; dis.: disease.

mutations, both the diagnostic sample and the most recent sample (at first progression or last follow-up after initiation of lenalidomide) were first tested for each patient. If one of these samples was positive, *TP53* mutation was investigated in historical stored samples. The study was approved by the Institutional Board of Nantes University Hospital. All patients were followed until death or survival as of January 2017. Erythroid and cytogenetic responses were checked for all cases according to the International Working Group 2006 criteria.[12] Disease progression was defined by an increase of the International Prognostic Scoring System (IPSS) score over 1.

Lenalidomide dispensing data were collected from the relevant hospitals' pharmacies, allowing calculation of the cumulative doses of lenalidomide actually delivered to the patients.

Genomic DNA was extracted from cytogenetics or dried pellets. *TP53* (exons 4-11) mutations were analyzed by deep-targeted sequencing according to the IRON-II study network recommendations.[13] The *TP53* mutations detected were compared to those in TP53-dedicated databases (*http://p53.iarc.fr and http://p53.free.fr/ Database/p53_cancer/all_cancer.html*) and only mutations with deleterious functional impact scores were retained.

TP53 clonal evolution was defined either as expansion of a clone or as emergence of a new *TP53*-mutated subclone undetectable in previous samples (Table 1).

A Fisher exact test was used for comparisons of binary variables and a Mann-Whitney test for comparisons of medians. The cumulative incidence of progression was analyzed using the time between diagnosis and progression taking into account the competitive risk of death from another cause. The Gray test was used to compare the impact of various factors on cumulative incidence of progression. All statistical tests were performed with Stata/IC 11.1 software (StataCorp, College Station, TX, USA).

The cohort comprised 20 women and 4 men, with a median age of 71 years (range, 51-79). Eleven patients had an IPSS score of 0, another 11 had an IPSS score of 0.5 and two had an IPSS score of 1 at diagnosis (*Online Supplementary Table S1*). Patients were offered lenalidomide therapy based on the severity of their anemia, comorbidities and non-availability of other therapeutic options, such as allogeneic stem cell transplantation. The median follow-up was 74 months (range, 16–207). Lenalidomide treatment (median duration, 25 months; range, 0.06–71) resulted in an erythroid response in 18 of the 24 patients (75%) with a median duration of 31 months (range, 3–92). Five patients (21%) achieved a complete cytogenetic response.

Ninety-four samples from the 24 patients were analyzed, i.e. between two and nine samples per patient (median 3). A *TP53* mutation was detected at diagnosis in six patients (25%). *TP53* clonal evolution was observed in ten patients (Figure 1) and in another one a mutation of *RUNX1* appeared. Of the six patients positive at diagnosis (25%), four remained stable and the clone grew in size in two with an additional *TP53* subclone emerging during treatment in one of them. In nine of the 18 patients negative at diagnosis, *TP53* mutations emerged during the course of the disease. Finally, in nine patients (37.5%), *TP53* mutations were not detected at any time.

Eleven of the 24 patients experienced disease progression (45.8%), with a median time from diagnosis of 61 months (range, 22–171). Of note, ten of these 11 patients had or acquired *TP53* clonal evolution (expansion or

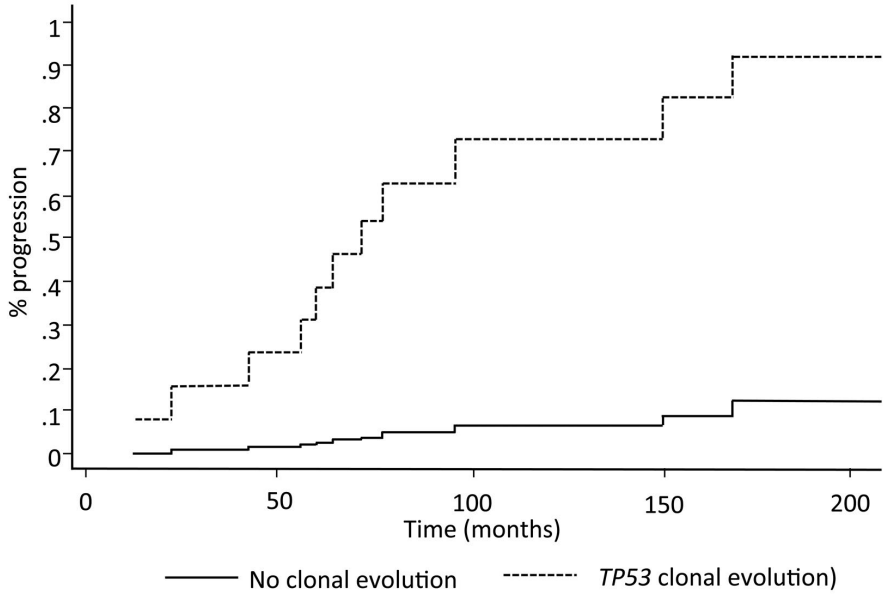

Figure 2. Cumulative incidences of progression in 24 patients according to *TP53* clonal evolution during follow-up. *P*=0.009 using the Gray test; death not caused by progression is analyzed as a competing risk.

emergence) the last one being the one with a *RUNX1* mutation. The cumulative incidence of progression at 10 years was 77.3% (95 CI: 48.8-96.21%) in patients who underwent clonal evolution *versus* 7.7% (95 CI: 1.1-43.3%) in patients who did not (*P*=0.009) (Figure 2). By contrast, the cumulative incidence of progression at 10 years was similar in patients with (58.3%; 95 CI: 15.7-98%) or without (52.7%; 95 CI: 26%-84%) *TP53* mutation at diagnosis (*P*=0.661).

The erythroid response rate according to the International Working Group 2006 criteria[12] was 100% in the group with *TP53* clonal evolution, which was higher than the 54% observed in the group without *TP53* clonal evolution (*P*=0.037). This former group also had a significantly longer median duration of exposure to lenalidomide than the other group (22 *versus* 3 months, *P*=0.02). At the time of disease progression, complex karyotype and/or monosomy 7, classical features of leukemic transformation, were found in eight cases out of 11. These karyotypic clonal evolutions were always associated with or preceded by *TP53* clonal evolution, except in one patient. Karyotypic clonal evolution could be detected in only one patient 14 months before progression, whereas *TP53* clonal evolution was detected in four patients before progression. The median time from *TP53* clonal evolution to progression was 18 months (range, 10-44).

The median duration of exposure to lenalidomide was 11 months for the whole cohort without any significant difference depending on whether the patients had disease progression or not (17 *versus* 7.5 months, respectively; *P*=0.2). However, a statistically significantly greater exposure to lenalidomide (cumulative dose) was observed in patients with progressive disease (2870 mg *versus* 1120 mg respectively; *P*=0.036) (Figure 2B). This exposure was also greater in patients with *TP53* clonal evolution compared to those without (3710 mg *versus* 630 mg, respectively; *P*=0.004) (Figure 2A). In one patient, variations in dosage were associated with changes in the size and type of the *TP53* mutation.

This longitudinal study analyzed a large number of consecutive samples from lower-risk del(5q) MDS patients treated with lenalidomide, and confirmed the heterogeneity and clonal evolution of this disease. Focusing on clonal evolution of *TP53*, some evidence was obtained of an association of *TP53* clonal evolution and disease progression with a possible link to therapy, as in other hematologic malignancies.[8,14,15]

The backtracking strategy used allowed us to identify several patterns of *TP53* clonal evolution or equilibrium, consistent with previous reports.[2]*TP53* mutations at diagnosis have been shown to be predictive of disease progression in lower-risk del(5q) MDS.[6] At variance, we observed stable disease in four of six cases with *TP53* mutation at diagnosis. Indeed, disease progression mostly occurred in patients with *TP53* mutations emerging before or at the time of progression, as recently reported by Scharenberg *et al.*[9] The absence of a mutation at diagnosis was in these cases concluded after deep sequencing at a validated threshold of 1%, which does not exclude the presence of smaller subclones at a lower frequency. Finally, the cumulative incidence of progression was shown here to be significantly increased when *TP53* clonal evolution occurred, whatever the mutational status at diagnosis.

TP53 mutations may be acquired by the del(5q) founding clone over the course of the disease or be present very early in selected subclones over time with linear or branched evolution patterns, respectively. Here, the *TP53* mutations detected were mainly transitions, a mutational pattern in favor of an aging-induced process as described elsewhere.[15] In the latter study, new deeper digital sequencing proved effective to validate the detection, long before the evolution to therapy-related acute myeloblastic leukemia, of minor subclones with a variant allele frequency as small as 0.1%.

In summary, this study suggests that disease progression of lower-risk del(5q) MDS is mostly related to the evolution of pre-existing or emerging subclones carrying a *TP53* mutation. Monitoring *TP53* clonal evolution could thus predict disease progression better in these patients than the mere detection of *TP53* mutations at diagnosis. This could have important practical implications for pre-emptively modifying patients' management. Such decisions could slow down the natural history of

the disease while maintaining both erythroid response for a better quality of life and clonal equilibrium to reduce, it is to be hoped, the likelihood of disease progression.

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