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

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To the Editor,

The cytogenetic anomaly of isolated del(5q) characterizes a subgroup of patients with lower risk myelodysplastic syndrome (MDS). In the 2016 WHO classification, one additional karyotypic abnormality (except -7 or del7q) can be present in such patients[1]. Median survival is around 6 years and cumulative incidence of progression towards acute myeloblastic leukemia (AML) 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 allows ~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 AML at 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 ~20%[6] and, as in other hematological malignancies, are associated with poor prognosis[6,7]. Progression of MDS into secondary AML 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 thus 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 benefited from at last 2 blood or bone marrow samplings during follow-up as per the discretion of their physician. Two of them, with 5% of blasts at disease discovery were considered low-risk MDS-EB associated to del(5q). To investigate for the appearance or evolution of *TP53* mutations, both diagnosis and the most

recent sample (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 on 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 (ER) and Cytogenetic Responses (CyR) were checked for all cases according to the IWG 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 to calculate 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 *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 binary variable comparisons and a Mann-Whitney test for comparisons of medians. Cumulative incidence of progression (CIP) was analyzed using the time between diagnosis and progression taking into account the competitive risk of death from another cause. Gray's test was used to compare the impact of various factors on CIP. All statistical tests were performed with the Stata/IC 11.1 software (StataCorp, College Station, TX).

The cohort comprised 20 women and 4 men, of a median age of 71 years (range 51-79). Eleven patients had an IPSS of 0, another 11 had an IPSS of 0.5 and 2 an IPSS of 1 at diagnosis (Supplemental Table 1). Patients were offered to receive lenalidomide therapy

based on the severity of their anemia, co-morbidities 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 ER in 18 of the 24 patients (75%) with a median duration of 31 months (range, 3–92). Five patients (21%) achieved a complete CyR.

Ninety-four samples from the 24 patients were analyzed, *i.e*. between 2 and 9 samples per patient (median 3). A *TP53* mutation was detected at diagnosis for 6 patients (25%). *TP53* clonal evolution was observed for 10 patients (Figure 1) and in another one a mutation of *RUNX1* appeared[1]. Of the 6 patients positive at diagnosis (25%), 4 remained stable, the clone grew in size for 2 with an additional *TP53* subclone emerging during treatment in one of them. For 9 of the 18 patients negative at diagnosis, *TP53* mutations emerged during the course of the disease. Finally, for 9 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, 10 of these 11 patients had or acquired a *TP53* clonal evolution (expansion or emergence) the last one being that with *RUNX1* mutation. CIP at 10 years was 77.3% (95 CI, 48.8-96.21%) in patients who underwent *TP53* clonal evolution *vs.* 7.7% (95 CI, 1.1-43.3%) in patients who did not (p=0.009, Figure 2). By contrast, CIP at 10 years was similar between patients with (58.3%; 95 CI, 15.7-98%) or without (52.7%; 95 CI, 26%-84%) *TP53* mutation at diagnosis (p=0.661).

ER according to the IWG 2006 criteria[12] was 100% in the group with *TP53* clonal evolution, higher than the 54% observed in the group without (p=0.037). This former group also had a significantly higher median duration of exposure to lenalidomide than the other group (22 *vs. 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 8 cases out of 11. These

[1] This was observed as the patient entered another NGS study where only this mutation was observed, in the absence of *TP53* anomaly, thus possibly explaining the outcome.

karyotyping clonal evolutions were always associated with or preceded by *TP53* clonal evolution, except for one patient. Karyotypic clonal evolution could be detected in only one patient 14 months before progression, whereas *TP53* clonal evolution was detected in 4 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 whether the patients had disease progression or not (17 *vs. 7.5* months respectively; p = 0.2). However, a statistically significantly higher exposure to lenalidomide (cumulative dose) was observed in patients with progressive disease (2870 mg *vs* 1120 mg respectively; p = 0.036, Fig 2B). This exposure was also higher in patients with *TP53* clonal evolution compared to those without (3710mg versus 630mg respectively; p = 0.004, Figure 2A). In one patient, variations in dosage were associated with changes in the size and type of *TP53* mutation.

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

The backtracking strategy used allowed to identify several patterns of *TP53* clonal evolution or equilibrium, consistent with previous reports[14]. *TP53* mutations at diagnosis have been shown to be predictive of disease progression in lower-risk del(5q) MDS[6]. At variance, we observed a 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 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,

CIP 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 disease course or be present very early in selected subclones over time as respectively linear or branched evolution patterns. Here, the *TP53* mutations detected were mainly transitions, a mutational pattern in favor of an ageing-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 TR-AML, of minor subclones with a VAF 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 better predict disease progression in these patients than the mere detection of *TP53* mutations at diagnosis. This could have important practical implications in preemptively modifying patients' management. Such decisions could slow down the natural history of the disease while maintaining both ER for a better quality of life and clonal equilibrium to hopefully reduce the likelihood of disease progression.

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Conflict of Interest

The authors declare no COI disclosure in relation with this study.

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Table 1. Mutations details

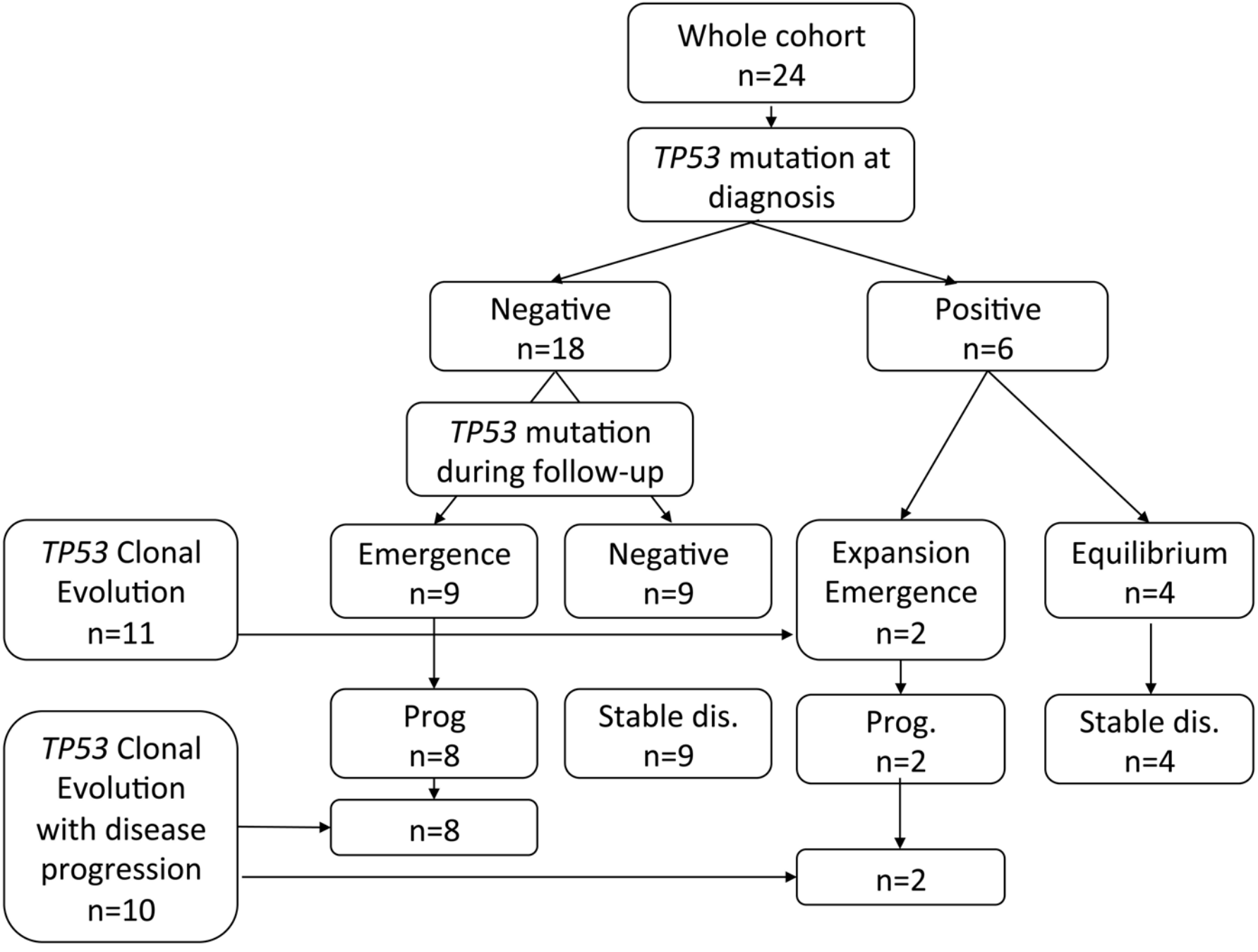
#	*TP53* mutations VAF at diagnosis	*TP53* mutations VAF(s) at follow-up	*TP53* Mutations		
			DNA	Type	Protein
1		24%/29%	c.524G>A c.844C>T	SNV	p.R175H p.R82W
2	29%	20%	c.818G>A	SNV	p.R273H
4		43%	c.818G>A	SNV	p.R273H
6		16%/14%	c.314G>T c743G>A c.818G>A c.833C>G c.614A>G	SNV	p.G105V p.R248Q p.R273H 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 c.920-1G>A	SNV Splice	p.C242F 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 c.711G>T	SNV	p.C141G p.M237I
23	14%	6%/32%	c.743G>A c.817C>T c.844C>T	SNV	p.R248Q p.R273C p.R282W

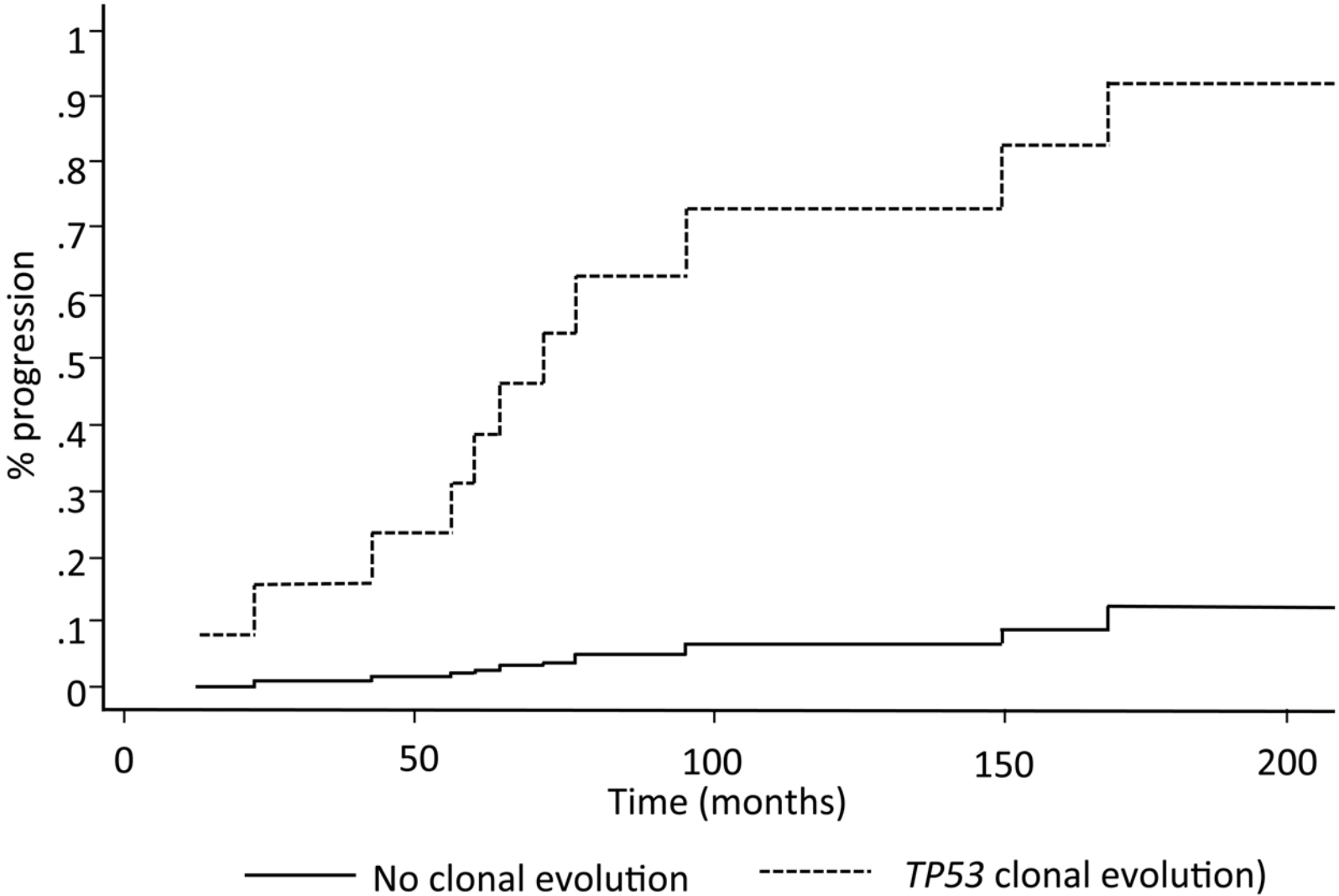
SNV = single nucleotide variant. Note that use of a broader sequencing panel could have disclosed other anomalies.

Figure legends.

Figure 1. Diagram of patients' evolution and *TP53* status over the follow-up period.

Figure 2. Cumulative incidences of progression according to *TP53* clonal evolution during follow-up (n=24, p=0.009). Gray's test, death not caused by progression is analyzed as competitive risk.


Whole cohort
n=24
TP53 mutation at diagnosis
Negative
n=18
Positive
n=6
TP53 mutation during follow-up
TP53 Clonal Evolution
n=11
Emergence
n=9
Negative
n=9
Expansion Emergence
n=2
Equilibrium
n=4
Prog
n=8
Stable dis.
n=9
Prog.
n=2
Stable dis.
n=4
TP53 Clonal Evolution with disease progression
n=10
n=8
n=2


% progression
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1
0
50
100
150
200
Time (months)
No clonal evolution
TP53 clonal evolution)

Supplemental Table 1. Patients details

#	Age (y)	Sex	IPSSR	Karyotype	*TP53* mut at diagnosis	*TP53* mut At follow-up	Progression	Alive
1	70	F	3	46,XX,del(5)(q13q32)	No	Yes	AML	No
2	51	F	3.5	46,XX,del(5)(q21q35)	Yes	Yes	No	Yes
3	75	F	3.5	FISH del(5q)	No	No	No	No
4	77	F	3.5	46,XX,del(5)(q14q34)	No	Yes	MDS-EB I	Yes
5	61	F	2.5	46,XX,del(5)(q14q34)	No	No	No	Yes
6	73	M	3	46,XX,del(5)(q15q35)	No	Yes	No	Yes
7	77	F	3.5	46,XX,del(5)(q13q34)	No	Yes	MDS-EB II	No
8	74	F	2	46,XX,del(5)(q14q34)	No	No	No	No
9	73	F	1	46,XX,del(5)(q14q34)	No	No	No	Yes
10	71	M	2	46,XY,del(5)(q14q34)	No	Yes	MDS-EB II	No
11	79	F	2.5	46,XX,del(5)(q14q34)	Yes	Yes	No	Yes
12	61	F	4.5	47,XX,del(5)(q15q35), +8	Yes	Yes	MDS-EB II / AML	No
13	71	F	4.5	46,XX,del(5)(q14q34), +8	Yes	Yes	No	No
14	62	F	3	46,XX,del(5)(q13q35)	No	No	No	Yes
15	63	M	4	46,XY,del(5)(q14q34)	No	Yes	AML	No
16	64	F	2.5	46,XX,del(5)(q22q35)	No	No	No	No
17	76	F	3.5	46,XX,del(5)(q15q35), t(9;12)	No	No	AML	No
18	58	F	3	46,XX,del(5)(q14q34)	No	Yes	MDS-EB II	No
19	78	F	3	46,XX,del(5)(q13q24)	Yes	Yes	No	No
20	76	F	3	46,XX,del(5)(q12q31)	No	No	No	Yes
21	58	F	1	46,XX,del(5)(q14q35)	No	Yes	AML	Yes
22	55	M	2	46,XY,del(5)(q14q35), del(20)(q11q13)	Yes	Yes	AML	Yes
23	62	M	2	46,XY,del(5)(q21q35)	No	Yes	MDS-EB II / AML	No
24	75	F	1	46,XX,del(5)(q12q34)	No	No	No	No

Supplemental Figure 1. Evolution of the 11 patients for whom *TP53* appeared during therapy.