Burton, were involved in management decisions and collected data; Peter Baker, Sarah Harper, James Beavis analysed blood samples and collected data. All authors approved the final version.

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Distinct mutational pattern of myelodysplastic syndromes with and without 5q– treated with lenalidomide

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders characterised by ineffective haematopoiesis leading to peripheral blood cytopenias and an increased risk of transformation to acute myeloid leukaemia (AML) (Haferlach *et al.*, 2014; Makishima *et al.*, 2017). One of the most common cytogenetic alterations is the deletion of the long arm of chromosome 5q [del(5q)], which can be found isolated or with other alterations (10–30% of patients with MDS). Lenalidomide (LEN) has been approved for the treatment of patients with del(5q) low-risk MDS and transfusion dependence. Almost 50% of patients with del(5q) will show a complete cytogenetic remission and 70% of them will reach transfusion independence (List *et al.*, 2006). LEN has also been approved for MDS non-del(5q) transfusion dependent and resistant to erythropoietin-stimulating agents (Santini *et al.*, 2016), suggesting that other factors besides del(5q) modulate response to LEN (Negoro *et al.*, 2016). Herein, we aimed to define the mutational spectrum of patients with MDS with and without del(5q) and define a signature of mutations influencing response to LEN.

We collected peripheral blood and/or bone marrow samples from patients with MDS treated with LEN from eight institutions at the Josep Carreras Leukaemia Research Institute (on behalf of the MDS Spanish Group and the MDS French Group) according to the institutional ethic committees and the revised Declaration of Helsinki. We collected 74 samples from patients with MDS at diagnosis or treatment-naïve with LEN follow-up treatment of two or more cycles; 32 patients presented with del(5q), while 42 patients did not have del(5q) in their karyotype (Table S1). The World Health Organization (WHO) classification (2017), Revised International Prognostic Scoring System (IPSS-R) and International Working Group response criteria (IWGc) (Cheson *et al.*, 2006; Greenberg *et al.*, 2012; Dolatshad *et al.*, 2015) were used to classify patients. Responders to LEN included patients with complete and partial response, haematological response and cytogenetic response, while non-responders included patients with treatment failure, stable disease or relapse. We combined results of multi amplicon targeted sequencing with Ion Torrent (Thermo Fisher Scientific, Inc.,

Table I. Clinical characteristics of the patients.

Variable	Lenalidomide responders	Lenalidomide non-responders	*P*
n	35	39	
del(5q), *n*	26	6	<0·0001
non-del(5q), *n*	9	33	
WHO subtype (2017), *n*			
MDS with isolated del (5q)	25	5	
MDS-SLD	0	0	
MDS-RS-SLD	4	13	
MDS-MLD	3	10	
MDS-RS-MLD	2	6	
MDS-EB-1	0	3	
MDS-EB-2	1	1	
MDS/MPN	0	1	
Blood counts, median (min–max)			
Haemoglobin, g/l	9·3 (6–12)	8 (5·4–11)	0·0006
Platelets, $\times 10^9$/l	279 (29–1161)	213 (68–495)	0·0343
White blood cells, $\times 10^9$/l	4·2 (2·0–11·6)	3·9 (1·5–55)	
Absolute neutrophil counts, $\times 10^9$/l	1·9 (0·1–10·2)	2·2 (0–10·1)	
BM Blast %	2 (0–15)	2 (0–14)	
Mutations*			
Number of mutations, median (min–max)	1 (0–5)	2 (1–4)	0·0003
SF3B1, %	31	69	
TET2, %	17	83	
DNMT3A, %	60	40	
ASXL1, %	14	86	
JAK2, %	57	43	
TP53, %	17	83	
SRSF2, %	17	83	
CSNK1A1, %	100	0	
	del(5q)	non-del(5q)	*P*
n	32	42	
WHO subtype (2017), *n*			
MDS with isolated del(5q)	28	2	
MDS-SLD	0	0	
MDS-RS-SLD	0	17	
MDS-MLD	4	9	
MDS-RS-MLD	0	8	
MDS-EB-1	0	3	
MDS-EB-2	0	3	
MDS/MPN	0	1	
Blood counts, median (min–max)			
Haemoglobin, g/l	9·3 (6·0–12)	8·0 (5·7–10·1)	0·0008
Platelets, $\times 10^9$/l	286 (97–1161)	218(29–494)	0·0523
White blood cells, $\times 10^9$/l	4·3 (1·5–55)	3·7 (1·5–11·6)	
Absolute neutrophil counts, $\times 10^9$/l	1·96 (0–5)	1·9 (0·14–10·19)	
BM Blast %	2 (0–4)	2 (0–15)	
Mutations*			
Number of mutations, median (min–max)	1 (0–5)	2 (1–4)	<0·0001
SF3B1, %	8	92	
TET2, %	11	89	
DNMT3A, %	40	60	
ASXL1, %	14	86	
JAK2, %	57	43	
TP53, %	50	50	
SRSF2, %	0	100	
CSNK1A1, %	100	0	

Patients without any mutation: responders: $n = 10$; del(5q) $n = 10$. MDS-SLD, MDS with single lineage dysplasia; MDS-MLD, MDS with multilineage dysplasia; MDS-EB, MDS with excess blasts; RS, ring sideroblasts.

*% corresponds to the distribution of the mutation between the two groups.

Waltham, MA, USA) (28 cases) and captured-based targeted sequencing with MiSeq (Illumina, San Diego, CA, USA) (46 cases). Amplicon and capture custom panels included 39 and 82 most recurrently mutated genes in MDS, respectively (Table S2). Capture libraries were generated using the KAPA Library Preparation Kit (Kapa Biosystems, Wilmington, MA, USA), enriched with the SeqCap EZ capture chemistry (Roche, Basel, Switzerland) and sequenced on MiSeq sequencers following a 150 base pairs (bp) paired-end reads Illumina standard protocol. Average coverage per gene was 777×. Reads were aligned against human genome build 19 (hg19) using Burrows-Wheeler Aligner (BWA) 0.7.12 and post-alignment was performed using Genome Analysis Toolkit (GATK) 3.4.46 software package. Libraries for the amplicon-based panel were prepared with Ampliseq (Thermo Fisher Scientific, Inc.) and sequenced in an ion torrent proton sequencer according to the manufacturer's instructions. Average coverage per genes was 567×. Primary bioinformatic analysis [SAMtools 1.2 (http://www.htslib.org/), VarScan 2.4.0 (http://dkoboldt.github.io/varscan/), and ANNOtate VARiation (https://doc-openbio.readthedocs.io/projects/annovar/en/latest/)] was performed and followed by an in-house protocol (Ibáñez *et al.*, 2016). Variants at highly variable regions, with low coverage (<100×), or a minor allele frequency >1% according to available population databases [Exome Aggregation Consortium (ExAC), Exome Variant Server, 1000 Genomes Project] were filtered out. Mutations were called when the variant allelic frequency (VAF) was >5%. Continuous variable comparisons were performed with Wilcoxon signed-rank tests, while Fisher's exact test was used to compare variables. Survival curves were calculated using the Kaplan–Meier method and log-rank test were used for comparisons. Two-sided *P* values < 0·05 were considered as statistically significant.

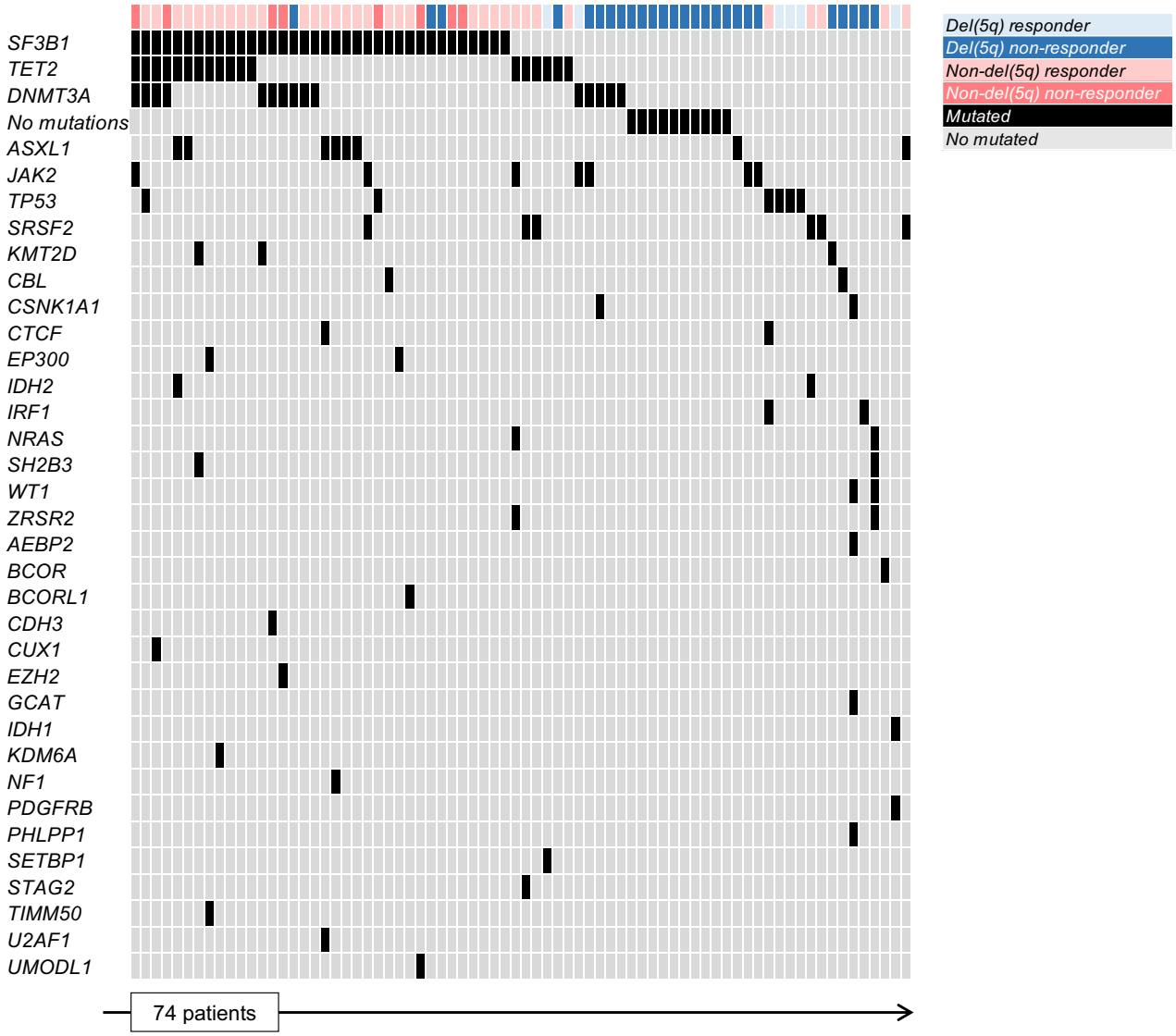

Fig 1. Mutational distribution of the 74 patients.

Patients were grouped according to the presence or absence of del(5q) and according to their response to LEN. Patients with non-del(5q) were more anaemic, thrombocytopenic, and more mutated than del(5q) ($P = 0{\cdot}0008$; $P = 0{\cdot}0523$; $P < 0{\cdot}0001$, respectively). Non-responder patients had more anaemia, lower platelet counts, and higher number of mutations than responder patients ($P = 0{\cdot}0006$; $P = 0{\cdot}0343$; $P < 0{\cdot}0003$, respectively). Patients were also classified according to the WHO 2017. As expected, responders were mainly classified in MDS with isolated del(5q) (71%), while non-responders were enriched in MDS with ring sideroblasts and with single lineage dysplasia (MDS-RS-SLD; 33%), and MDS with multilineage dysplasia (MDS-MLD; 26%; Table 1).

To assess if LEN response correlated with del(5q), we first compared responders *versus* non-responders and then we sub-classified each group according to the presence or absence of del(5q). The LEN median (min–max) treatment duration was 12 (3–45) months, and the median (min–max) overall follow-up was 5 (1–20) years. LEN response was achieved in 47% of patients. Overall, LEN response was significantly higher in del(5q) than in non-del(5q) patients, at 74% (26/35) vs. 15% (six of 39) ($P < 0{\cdot}0001$).

We identified a total of 147 mutations (non-synonymous single nucleotide variants and small indels; Table S3), del(5q) showed a lower median number of mutations than non-del (5q) (1 vs. 2; $P < 0{\cdot}0001$), the same results were obtained when we compared LEN responders *versus* non-responders (1 vs. 2; $P = 0{\cdot}0003$). Ten patients with del(5q) and responders to LEN did not have any mutation. We then explored the mutational distribution of our cohort (Table 1). Splicing factor 3b subunit 1 (*SF3B1*) and tet methylcytosine dioxygenase 2 (*TET2*) were overrepresented in non-del(5q) and non-responders, while patients with DNA methyltransferase 3 alpha

(*DNMT3A*) mutations were overrepresented in del(5q) and LEN responders. Similarly to previous studies (Chesnais *et al.*, 2016) reported a better response to LEN in patients with del(5q) and *DNMT3A* mutations. ASXL transcriptional regulator 1 (*ASXL1*) was found to be mutated in only one patient with del(5q), while it was found in 8% of non-del (5q) non-responders. Tumour protein p53 (*TP53*) was mutated in three del(5q) patients and three non-del(5q) patients, all but one non-del(5q) were non-responders to LEN. Serine and arginine-rich splicing factor 2 (*SRSF2*) was only mutated in non-del(5q) and non-responders (83%). Casein kinase 1 alpha 1 (*CSNK1A1*) was only found in del (5q) and responders (Fig 1).

Although no significant differences were found in overall survival (OS), patients with non-del(5q) showed a longer OS than del(5q) patients (64 vs. 50 months), probably due to the high percentage of normal karyotypes and *SF3B1* mutations in non-del(5q) patients. LEN responders with del(5q) showed a better OS than non-responders (median OS 133 months vs. not reached; $P = 0{\cdot}1361$), while non-del(5q) patients seemed to not benefit from LEN treatment (68 vs. 64 months).

In conclusion, del(5q) and non-del(5q) treated with LEN have a different mutational profile. While, *DNMT3A* mutations were very frequent in del(5q) and predicted a better response to LEN, *TP53* mutations were observed in both groups of patients and predicted poor response to LEN. In contrast, *SF3B1* and *TET2* were mainly detected in non-del (5q) and correlated with refractoriness to LEN.

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Author contributions

The project was conceptualised by Vera Adema and Francesc Sole. Vera Adema analysed sequencing data, collected clinical information, designed the study, and wrote the paper. Laura Palomo helped with sequencing analysis, clinical data collection and helped in manuscript preparation. Data performing, analysis, clinical information collection was done by Andrea Toma, Olivier Kosmider, Francisco Fuster-Tormo, Rocío Benito, Rocío Salgado, Esperanza Such, María José Larrayoz, Jesus Maria Hernandez-Sanchez, Paolo Maietta, Alexander Neef, Michaela Fontenay, Mariam Ibañez, Maria Diez Campelo, Sara Alvarez. Jaroslaw P. Maciejewski and Pierre Fenaux, provided patients and important help on the manuscript. All co-authors revised the paper.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Conventional cytogenetics of patients included in the study.

Table SII. Miseq and Ion Torrent gene panel.

Table SIII. Mutational spectrum of patients included in the study.

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No increased risk of cataract in adult patients with primary immune thrombocytopenia treated with eltrombopag. A French nationwide nested case-control study

Eltrombopag, a thrombopoietin receptor agonist, has been approved since 2010 in Europe for the treatment of primary immune thrombocytopenia (ITP). In preclinical studies, eltrombopag has been associated with a dose and time-dependent increased incidence of cataract in young mice and rats, but not in dogs (European Medicine Agency, 2010). Clinical trials have not demonstrated an increased risk of cataract as compared with placebo (Cooper *et al.*, 2011). During the extension study, 9·3% (28/302) of the patients developed or had worsening cataract over a median duration of