Fluorescence in situ hybridization (FISH) of TP53 for the detection of chromosome 17 abnormalities in myelodysplastic syndromes

Journal:	*Leukemia and Lymphoma*
Manuscript ID:	GLAL-2014-1330.R2
Manuscript Type:	Original Article - Research
Date Submitted by the Author:	23-Feb-2015

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Keywords:	Myeloid Leukemias and Dysplasias < Neoplasia, Cytogenetics < Neoplasia, Molecular genetics < Neoplasia

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Fluorescence *in situ* hybridization (FISH) of *TP53* for the detection of chromosome 17 abnormalities in myelodysplastic syndromes

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ABSTRACT

Conventional G-banding cytogenetics (CC) detects chromosome 17 (chr17) abnormalities in 2% of patients with *de novo* myelodysplastic syndromes (MDS). We used CC and fluorescence *in situ* hybridization (FISH) (LSI p53/17p13.1) to assess deletion of 17p in 531 patients with *de novo* MDS from the Spanish Group of Hematological Cytogenetics. FISH detected –17 or 17p abnormalities in 13 cases (2.6%) in whom no 17p abnormalities were revealed by CC: 9% of patients with a normal karyotype, 0% in non-informative cytogenetics, 50% of patients with a chr17 abnormality without loss of 17p and in 4.7% of cases with an abnormal karyotype not involving chr17. Our results suggest that applying FISH of 17p13 to identify the number of copies of the *TP53* gene could be beneficial in patients with a complex karyotype. We recommend using FISH of 17p13 in young patients with a normal karyotype or non-informative cytogenetics, and always in isolated del(17p)

Keywords:
Myelodysplastic syndromes
Cytogenetics
FISH
Chromosome 17

Word Count: 2963

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Abbreviations:

A17, abnormal karyotype with chr17 abnormalities other than i(17q), –17, del(17p), or add(17p)

AML, acute myeloid leukemia

AOUT17, abnormal karyotype with no chr17 abnormalities

AZA, azacitidine

CC, conventional G-banding cytogenetics

CEP17, chromosome centromeric probe of chr17

chr, chromosome

CMML, chronic myelomonocytic leukemia

del, deletion

FAB, French–American–British

FISH, fluorescence *in situ* hybridization

GCECGH, Grupo Cooperativo Español de Citogenética Hematológica (Spanish Group of Hematological Cytogenetics)

GESMD, Grupo Español de Síndromes Mielodisplásicos (Spanish Group for MDS)

IPSS, International Prognostic Scoring System

ISCN, International System for Human Cytogenetic Nomenclature

MDS, myelodysplastic syndromes

N1–9, normal karyotype based on 1–9 metaphases

N10–19, normal karyotypebased on 10–19 metaphases

N20, normal karyotype based on ≥20 metaphases

NM, patients with non-informative cytogenetics or no metaphases

RA, refractory anemia

RAEB, refractory anemia with excess blasts

RAEB-t, refractory anemia with excess blasts in transformation

RARS, refractory anemia with ringed sideroblasts

R-IPSS, revised International Prognostic Scoring System

WHO, World Health Organization

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1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders, characterized by inefficient hematopoiesis, peripheral blood cytopenias, and an increased risk of progression to acute myeloid leukemia (AML) [1–3]. Both the prognosis and the clinical course of MDS are highly variable, and several scoring systems have been developed to assess prognosis [4–7].

Chromosomal abnormalities in bone marrow cells are found in 40–60% of MDS patients [8], while, using conventional G-banding cytogenetics(CC), alterations of chromosome 17 (chr17) are detected in 2% of patients with *de novo* MDS [9]. The most common abnormalities detected by CC are deletion of [(del)]5q, monosomy 7, del(7q), gain of chromosome 8 [add(8)], del(11q), del(12p), isochromosome 17q(i17q), and del(20q)[10].

Detection of cytogenetic abnormalities is integral to the prognostic evaluation of patients with MDS. The International Prognostic Scoring System (IPSS), which categorizes cytogenetic abnormalities as low risk, intermediate risk, or high risk, has become the gold standard for risk assessment in *de novo* MDS [4]. Chromosome (chr) 17 abnormalities are included in the intermediate-risk group if they are not present in a complex karyotype. The revised IPSS(R-IPSS) classification for cytogenetic abnormalities [11] involves five groups proposed by Schanz et al [12], and includes i(17q) as a single anomaly in the intermediate cytogenetic risk group.

Recently, our group published a cooperative study that compared the prognostic impact of chr17 abnormalities (*n*=88) with that of other karyotypic abnormalities (*n*=1070) in patients with primary MDS [9]. In agreement with the R-IPSS classification, this study confirmed the intermediate prognostic impact ofi(17q) [11,12].It also demonstrated that different types of chr17 abnormalities confer different prognoses: patients with monosomy 17 (–17) as part of a complex karyotype were found to havethe poorest prognosis. Interestingly, it also demonstrated that chr17 abnormalities are more commonly associated with complex karyotypes than are non-chr17 abnormalities. Consequently, patients with chr17 abnormalities are often associated with higher-risk MDS, and therefore could potentially benefit from active therapies – for example,

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hypomethylating agents, which have demonstrated efficacy in this setting in patients ineligible for allogeneic stem cell transplantation. Azacitidine (AZA), in particular, has demonstrated a survival benefit compared with conventional care regimens [13–15]. Thus, detection of any chr17 abnormalitiesat diagnosis is important, as it enables more accurate prediction of the patient's prognosis and the selection of appropriate treatment options.

Although CC is the gold standard technique for clinical karyotyping, it is associated with some limitations: cells must be divided from the neoplastic clone,and the sensitivity of the technique is relatively low. Fluorescence *in situ* hybridization (FISH) is a commonlyused technique that identifies specific abnormalities, does not require cell division, and can be easily quantified.Previous studies demonstrated the value of FISH in detectingchr5 and 7 abnormalities in patients with MDS[16,17].The main advantage of FISH versus CC is the higher sensitivity, with respect to the number of cells analyzed (CC analyzes only 20 metaphases). However, FISH only provides information relating to the specific region to which the probe hybridizes; it yields no information about other chromosomes.

The objective of the present study was to apply FISH of 17p to a large cohort of patients with*de novo* MDS,in whom CC had failed to detect del(17p).

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2. Materials and methods

2.1. Patients

Patients from hospitals belonging to the Grupo Cooperativo Español de Citogenética Hematológica (GCECGH; Spanish Group of Hematological Cytogenetics) were divided into two groups according to their cytogenetic profile (Group A or B depending onwhetherornot they showedlossofthe short armof chr17).

Group A (n = 501) included patients in whom CC had failed to detect the following specific chr17 abnormalities: i(17q), –17, del(17p), or add(17p). Group B (n = 30) included patients with i(17q), –17, del(17p) or add(17p) detected by CC (alterations that involve the loss of 17p). Group A was subsequently divided into six subgroups: normal karyotype based on ≥20 metaphases (N20); normal

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karyotype based on 10–19 metaphases (N10–19);normal karyotype based on 1–9 metaphases (N1–9); abnormal karyotype with no chr17 abnormalities (AOUT17); abnormal karyotype with chr17 abnormalities that not involve loss of ofthe short armof chr17, therefore other than i(17q), –17, del(17p), or add(17p) (A17); and patients with non-informative cytogenetics or no metaphases (NM). Our group defines cytogenetic study non informative, in those cases with less than 10 normal metaphases.

Patients were classified according to French–American–British (FAB) (*n*=527) and/or World Health Organization (WHO) 2008 (*n*=423) criteria. The study was approved by the ethics committee of Hospital Universitari Germans Trias i Pujol, Badalona. All patients provided written informed consent.

2.2. CC and FISH analysis of TP53

Prior to treatment, CC and FISH were performed on bone marrow samples at individual laboratories with short-term, non-stimulated cultures, following standard procedures. Karyotypes were defined according to the International System for Human Cytogenetic Nomenclature(ISCN) 2009 [18].

FISH analyses were carried out on fixed cells. The probe set consisted of LSI p53/17p13.1 (Ref: 32-190008, Vysis®) with a chromosome centromeric probe of chr17 (CEP17; a control probe; Ref: 32-112017, Vysis®) to detect –17 or del(17p). The number of interphase nuclei analyzed ranged from 100 to 400. Interphase FISH cut-off percentages varied between centers. The median cut-off values for –17and del(17p) were 10% and 5%, respectively.

3. Theory

In patients with MDS, chromosomal abnormalities have been found in up to 40–60% of cases [8]. By CC, alterations of chr17 are detected in 2% of patients with *de novo* MDS [9]. CC has become the gold standard for

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karyotyping, but has limitations. FISH, for which cell division is not required, is a highly sensitive technique that enables examination of a large number of cells.

Patients with *de novo* MDS and chr17 abnormalities typically have a poor prognosis and poor outcomes [9]. Thus, it is important to detect chr17 abnormalities at the time of diagnosis of MDS patients, in order to ensure that the most appropriate treatment options are selected.

We hypothesized that FISH of *TP53* could potentially identify del(17p) in some MDS patients in whom CC had failed to detect this abnormality.

To date, few studies have been published that describe the benefits of applying 17p FISH at the time of diagnosis of patients with *de novo* MDS. Those studies that have been published did not exclusively analyze del(17p) in patients with MDS.To our knowledge, the present study included the largest cohort of patients with *de novo* MDS in whom FISH has been applied to detect del(17p).

4. Results

In total, 531 patients with *de novo* MDS from 18 hospitals belonging to the GCECGH were included in the study. Tables 1A and 1B show the diagnosis of these patients according to FAB and/or WHO 2008 criteria. Using CC, 501 patients were found to have no i(17q), –17, del(17p), or add(17p) abnormalities (Group A), while 30 patients had abnormalities of these types (Group B).
Table 2 shows the results of FISH analysis of 17p13.1(*TP53*) in the six Group A subgroups. In 13 patients (2.6%), FISH detected –17 or 17p abnormalities that were not detected by CC. In patients with a normal karyotype by CC, del(17p) was detected in 0.9% of cases. FISH did not detect del(17p) in any of the 58 patients within conclusive CC analyses, but did detect del(17p) in 50% of patients classified by CC as having an abnormal karyotype involving a chr17 abnormality other than i(17q), –17, del17p, or add(17p) (subgroup A17). These patients defined as A17 have an abnormal karyotype with chr17 aberration but notinvolve loss of the short arm of chr17. We could expect that all the patients did no present *TP53* deletion by FISH but our results show a *TP53* deletion in a 50% of the cases. This could be explained because most of these patients

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presented a complex karyotype and the karyotype could be by an error in interpretation.

In patients classified by CC as having an abnormal karyotype not involving chr17 (subgroup AOUT17), FISH detected del(17p) in 4.7% of cases.

Table 3 shows the characteristics of those patients in our study with del(17p) that was detected by FISH alone.

FISH revealed deletion of *TP53* in two patients with normal karyotype.Taking into account that FISH revealed just 25% and 27% of cells with deletion of TP53, we could suspect that the clone with deletion of 17p was not detected by CC due to low grow of tumoral cells than by a cryptic deletion. In seven cases with complex karyotype without a deletion of 17p by CC, FISH revealed *TP53* deletion. These discrepancies could be explained by an error in the interpretation of the karyotype due to the poor morphology of the chromosomes and by the difficulty to define complex karyotypes. Furthermore, in two case with 5q- as a single anomaly detected by CC, FISH revealed deletion of *TP53* in 33% and 79% of cells. In those cases will could argue that both patients presented a cryptic deletion of 17p.

In Group B, the loss of 17p13.1 was confirmed by FISH in 25 patients (83.3%). However, FISH failed to confirm del(17p) in five patients (16.7%)(Supplementary Appendix Table A1).

5. Discussion

To date, few studies have been published that demonstrate the benefits of applying FISH of *TP53* during the diagnosis of patients with *de novo* MDS. To our knowledge, our study cohort is the largest *de novo* MDS population that has been analyzed by FISH of 17p or *TP53*. Table 4 summarizes the main previously published studies in which FISH wasused to detect del(17p)in patients with MDS. It should be noted that none of these studies analyzed del(17p) exclusively.

Overall, 2.6% of patients in our study were found by FISH to have chr17 abnormalities that were not detected by CC. In the majority of these cases, CC had indicated abnormal karyotypes. Of the 220 patients with a normal karyotype

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by CC (analysis of 20 metaphases), FISH of *TP53* detected del(17p) in only two patients (0.9%). Furthermore, in cases where CC was inconclusive or no metaphases were obtained, FISH of 17p detected no *TP53* deletions. Our group is coordinating an international study among MDS patients with normal karyotype studied by SNP arrays (unpublished data, IWG-PM, MDS Foundation). In those cases with normal cytogenetics and deletion of TP53 by FISH, the application of SNP arrays demonstrated a normal karyotype that could be explained by the low number of cells carrying deletion of TP53 (25 and 27% of cells by FISH). The findings could suggest that the not detection of del17p by cytogenetics could be due by the number of cells analyzed.

Thus, our findings suggest that FISH of *TP53* may complement CC in the detection of chr17 abnormalities in patients with MDS, particularly in those patients with other karyotype abnormalities. This may be valuable in helping to identify patients with a poor prognosis, who could benefit from active treatment options, including hypomethylating agents.

Our findings suggest that FISH of 17p is likely to be of little benefit in diagnosing patients with *de novo* MDS who are classified by CC as having a normal karyotype (or with no metaphases). Therefore, we would only recommend the use of FISH of 17p in the diagnosis of young patients, in whom detection of del(17p) could lead to more appropriate treatment decisions.

A number of recent studies have demonstrated the value of FISH analysis of specific abnormalities that are of particular prognostic/diagnostic importance, such as del(5q) and chr7 abnormalities. In a study of 716 MDS patients, Mallo et al. reported that FISH detected del(5q) in 6% of patients who did not appear to have a del(5q) abnormality when analyzed by CC [16]. Similarly, Adema et al. reported that, when FISH of 7q31 was performed in 773 MDS patients in whom CC failed to detect any chr7 abnormalities, just over 5% of the patients did indeed have chr7 abnormalities [17]. In both of the above studies, the authors recommended that FISH analysis should be mandatory in specific circumstances for patients in whom CC has detected no abnormalities, or for whom insufficient metaphases are available. Our own previous data suggest that FISH is also valuable in some cases to verify the presence of specific karyotypic abnormalities detected by CC. In a study carried out on behalf of the Grupo Español de Síndromes Mielodisplásicos (GESMD; Spanish Group for

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MDS), we identified 10 patients with del(17p) according to CC, who appeared to have a better prognosis than expected. Subsequent FISH failed to verify del(17p) in any of these 10 patients [9].

Other studies have demonstrated the potential benefit of using a panel of FISH probes to screen MDS/AML patients classified by CC as having normal karyotypes. In one such study, Rigolin et al. analyzed 101 patients with MDS and a normal karyotype by CC, using FISH to detect –5/del(5q), –7/del(7q), add(8), and del(17p)) [19]. FISH identified abnormalities in 18 patients, including del(17p) in one patient (1.0%). Similarly, Cuneo et al. used a panel of eight FISH probes to screen 27 patients with AML secondary to MDS. Cryptic abnormalities were detected in eight patients, including del(17p13)(*TP53*) in one patient [20]. These observations, as well as the findings of the present study, demonstrate the value of FISH during the diagnosis of MDS, enabling the detection of abnormalities that are associated with an increased rate of progression to AML and a worse prognosis.

Few published studies have assessed the use of FISH specifically to detect chr17 aberrations during the diagnosis of patients with *de novo* MDS (Table 4). However, several small studies have highlighted the importance of chr17 abnormalities in the pathogenesis of the disease. In a study of patients with MDS (n=11) or AML (n=6) and chr17 abnormalities [15 with unbalanced translocations, one with –17, and one with i(17q)], FISH identified *TP53* deletion in 14 of the 16 patients (88%) to whom the technique was applied [21]. All but one of these patients had p53 mutations and/or overexpression. Similarly, in a study of 43 patients with del(5q) MDS (n=26)/AML (n=17), FISH confirmed chr17 abnormalities in 10 patients (23%), all of whom had complex karyotypes [22]. Moreover, *TP53* mutations were detected in 17% and 53% of patients with isolated del(5q) or complex karyotype, respectively, and were found to be associated with shorter survival. These findings suggest that *TP53* plays a pathogenic role in a proportion of patients with MDS, and that FISH analysis would be valuable to identify such individuals.

In addition to being of value in identifying chr17 abnormalities in elderly patients with MDS, FISH may be an important tool in the assessment of cases of pediatric MDS. Although pediatric MDS is relatively rare, accounting for less than 10% of childhood hematologic diseases [23], where it does occur it is often

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associated with chromosomal abnormalities. Using CC, chromosomal abnormalities are detected in more than 50% of *de novo* pediatric MDS cases, while the detection rate is higher in secondary pediatric MDS. In a study of pediatric MDS carried out by Silveira et al., FISH detected del(17p) in 18 of the 19 patients (95%) [24]. By contrast, CC detected chr17 abnormalities in only six patients. These findings suggest that routine application of chr17 FISH should be considered in cases of *de novo* pediatric MDS.

While we did not evaluate the prognostic impact of *TP53* mutations in patients with MDS, we believe that it may be important to address this aspect in future studies. Using CC, Bejar et al. analyzed 439 patients with MDS, and found *TP53* mutations and chr17 abnormalities in 7.5% and 24.2% of patients, respectively [25]. Furthermore, *TP53* mutations were associated with the occurrence of del(17p) (p<0.001). The authors also observed that *TP53* mutations were associated with markers of poor prognosis, such as complex karyotypes, thrombocytopenia, and a high proportion of bone marrow blasts, and were found mainly in patients with intermediate-2-/high-risk IPSS cytogenetics (79%). After adjusting for IPSS risk group, *TP53* mutations were associated with shorter survival, and can therefore be considered an independent predictor of survival.

In the present study, –17 or 17p abnormalities were detected only by FISH, and not by CC, in 13 patients (2.6%). In patients with CC-detected chr17 abnormalities that did not involve loss of 17p (subgroup A17), FISH detected del(17p) in 50% of cases. In these patients, FISH enabled the patients' prognosis to be better defined, by determining the type of chr17 aberration present [9].

FISH detected del(17p) in 4.7% of patients who, according to CC, had an abnormal karyotypewithout chr17 abnormalities. By CC, these patients presented an abnormal karyotype without evidence of loss of *TP53* (no deletions in the short arm of chr17);by FISH, however, loss of *TP53* was detected. In patients in whom CC detected an abnormal karyotype and –17, FISH failed to confirm –17 or loss of *TP53*. In view of its association with a poor prognosis in patients with a complex karyotype [9], detection of –17 is of particular importance. Thus, FISH of 17p could be of great benefit in these

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patients, enabling detection of new cases of –17 not detected by CC, and confirming –17 cases indicated by CC.

6. Conclusions

The prognostic impact of chr17 abnormalities in patients with primary MDS is well established. Thus, the identification of the *TP53* deletion does impact on outcome because adding FISH for the detection of *TP53* deletion to CC enables more accurate prediction of the patient's prognosis and the selection of appropriate treatment options.

The findings of our study demonstrate that FISH provides important prognostic information in *de novo* MDS. On the basis of the results obtained, we recommend that FISH of 17p13 should be performed: in all patients with *de novo* MDS and a complex karyotype (with or without involvement of chr17), in order to identify the number of copies of *TP53;* and in young patients with a normal karyotype or non-informative cytogenetics, to better predict their prognosis and make more appropriate treatment decisions. Furthermore, we recommend that isolated del(17p) detected by CC should always be verified by FISH.

Acknowledgements

Financial support. This work was supported in part by a grant from the *Instituto de Salud Carlos III, Ministerio de Economía y Competitividad*, *Spain (PI 11/02010 and PI/14/00013);* by the *Red Temática de Investigación Cooperativaen Cáncer (RTICC, FEDER) (*RD12/0036/0044*); Sociedad Española Hematología y Hemoterapia; 2014 SGR225 (GRE) Generalitat de Catalunya, by José Carreras Leukämie-Stiftung , Ref. AR 14/34; economical support from Fundació Internacional Josep Carreras and from Celgene Spain. The research leading to this invention has received funding from "la Caixa" Foundation.*

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Contributions. JS performed FISH studies, collected and analyzed data, and wrote the manuscript. VM, XG and TG helped to write the manuscript. RL and ET helped FISH studies. SF collected clinical information on the subjects.VA, IM, EL, AB, IB, MLMR, BB, RC, MTB and TG provided cytogenetic, FISH and clinical data. GS helped write the manuscript. FS designed the overall project and helped write the manuscript. All authors gave the final approval to the manuscript. Medical writing support was provided by Sandra Lee Lewis of the Investigator-Initiated Research Writing Group (part of the KnowledgePoint360 Group), and was funded by Celgene.

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References

[1] Nimer SD. Myelodysplastic syndromes. Blood 2008;111:4841–51.

[2] Lewis SM, Verwilghen RL. Dyserytropoiesis and dyserythropoietic anaemia. Br J Haematology 1972;23:1–4.

[3] Catenacci DV, Shiller GJ. Myelodysplasic syndromes: a comprehensive review. Blood Rev 2005;19:301–19.

[4] Greenberg P, Cox C, Le Beau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079–88.

[5] Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, et al.Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 2007;25:3503–10.

[6] Sanz GF, Sanz MA, Vallespí T, Cañizo MC, Torrabadella M, García S, et al. Two regression models and a scoring system for predicting survival and planning treatment in myelodysplastic syndromes: a multivariate analysis of prognostic factors in 370 patients. Blood 1989;74:395–408.

[7] Germing U, Strupp C, Kuendgen A, Isa S, Knipp S, Hildebrandt B, et al. Prospective validation of the WHO proposals for the classification of myelodysplastic syndromes. Haematologica 2006;91:1596–604.

[8] Sole F, Luno E, Sanzo C, Espinet B, Sanz GF, Cervera J, et al. Identification of novel cytogenetic markers with prognostic significance in a series of 968 patients with primary myelodysplastic syndromes. Haematologica 2005;90:1168–78.

[9] Sánchez-Castro J, Marco-Betés V, Gómez-Arbonés X, Arenillas L, Valcarcel D, Vallespí T, et al. Characterization and prognostic implication of 17 chromosome abnormalities in myelodysplatic syndrome. Leuk Res 2013;37:769–76.

[10] Haase D, Germing U, Schanz J, Pfeilstöcker M, Nösslinger T, Hildebrandt B, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood 2007;110:4385–95.

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[11] Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised International Prognostic Scoring System (IPSS-R) for myelodysplastic syndromes. Blood 2012;120:2454–65.

[12] Schanz J, Tüchler H, Solé F, Mallo M, Luño E, Cervera J, et al. A new, comprehensive cytogenetic scoring system for primary myelodysplastic syndromes and oligoblastic AML following MDS derived from an international database merge. J ClinOncol 2012;30:820–9.

[13] Itzykson R, Thépot S, Eclache V, Quesnel B, Dreyfus F, Beyne-Rauzy O, et al. Prognostic significance of monosomal karyotype in higher risk myelodysplastic syndrome treated with azacitidine. Leukemia 2011;25:1207–9.

[14] Itzykson R, Thépot S, Quesnel B, Dreyfus F, Beyne-Rauzy O, Turlure P, et al. Prognostic factors for response and overall survival in 282 patients with higher-risk myelodysplastic syndromes treated with azacitidine. Blood 2011;117:403–11.

[15] Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol 2002;20:2429–40.

[16] Mallo M, Arenillas L, Espinet B, Salido M, Hernández JM, Lumbreras E, et al. Fluorescence in situ hybridization improves the detection of 5q31 deletion in myelodysplastic syndromes without cytogenetic evidence of 5q–. Haematologica 2008;93;1001–8.

[17] Ademà V, Hernández JM, Abáigar M, Lumbreras E, Such E, Calull A, et al. Application of FISH 7q in MDS patients without monosomy 7 or 7q deletion by conventional G-banding cytogenetics: does -7/7q- detection by FISH have prognostic value? Leuk Res 2013;37:416–21.

[18] Shaffer LG, Slovak ML, Campbell LJ, editors. ISCN (2009): an international system for human cytogenetic nomenclature, Basel: S. Karger; 2009.

[19] Rigolin GM, Bigoni R, Milani R, Cavazzini F, Roberti MG. Clinical importance of interphase cytogenetics detecting occult chromosome lesions in myelodysplastic syndromes with normal karyotype. Leukemia 2001;15:1841–7.

For Peer Review Only

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[20] Cuneo A, Bigoni R, Cavazzini F, Bardi A, Roberti MG, Agostini P, et al. Incidence and significance of cryptic chromosome aberrations detected by fluorescence in situ hybridization in acute myeloid leucemia with normal karyotype. Leukemia 2002;16:1745–51.

[21] Soenen V, Preudhomme C, Roumiere C, Daudignon A, Luclaï J, Fenaux P. 17p deletion in acute myeloid leukemia and myelodysplastic syndrome. analysis of breakpoints and deleted segments by fluorescence in situ. Blood 1998;91;1008–15.

[22] Sebaa A, Ades L, Baran-Marzack F, Mozziconacci MJ, Penther D, Dobbelstein S, et al. Incidence of 17p deletions and TP53 mutation in myelodysplastic syndrome and acute myeloid leukemia with 5q deletion. Genes Chromosomes Cancer 2012;51:1086–92.

[23] National Cancer Institute at the National Institutes of Health. Childhood acute myeloid leukemia/other myeloid malignancies treatment (PDQ). Available at: http://www.cancer.gov/cancertopics/pdq/treatment/childAML/HealthProfessional. Accessed 21 March 2014.

[24] Silveira CG, Oliveira FM, Valera ET, Ikoma MR, Borgonovo T, Cavalli IJ, et al. New recurrent deletions in the PPAR gamma and TP53 genes are associated with childhood myelodysplastic syndrome. Leuk Res 2009;33:19–27.

[25] Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 2011;364:2496–506.

For Peer Review Only

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Table I

Diagnosis of study population.

A. Diagnosis of study population by FAB criteria.

RA	122
RARS	155
CMML	0
RAEB	124
RAEB-t	1
MDS-U	125

B. Diagnosis of study population by WHO 2008 criteria.

RA	17
RARS	64
RCUD	7
RCMD	126
RCMD-RS	17
RAEB-1	68
RAEB-2	43
MDS/MPD CMML	3
MDS/ MPD no CMML	2
5q- Syndrome	33
MDS-U	43

FAB, French–American–British; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; CMML, chronic myelomonocytic leukemia; RAEB, refractory anemia with excess blasts; RAEB-t,refractory anemia with excess blasts in transformation; MDS-U, MDS unclassifiable; WHO, World Health Organization; RCUD, refractory cytopenia with unilineage dysplasia; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, refractory cytopenia with multilineage dysplasia with ringed sideroblasts; RAEB-1, refractory anemia with excess blasts type 1; RAEB-2, refractory anemia with excess blasts type 2; MDS/MPD CMML, myelodysplastic/myeloproliferative disease type chronic myelomonocytic leukemia; MDS/MPD no CMML,

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myelodysplastic/myeloproliferative disease no chronic myelomonocytic leukemia.

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Table II

Results of FISH analysis of 17p13.1 (*TP53*) in Group A.

Results of CC analysis	Patients (*N* = 531)	Results of FISH analysis of 17p , *n* (%)
N20	220	2 (0.9)
N (10–19)	27	0
N (1–9)	20	0
NM	58	0
A17	6	3 (50.0)
AOUT 17	170	8 (4.7)
Group A (total)	501	13 (2.6%)

FISH, fluorescence *in situ* hybridization; Group A, patients without i(17q), –17, del17p or add(17p) detected by CC; CC, conventional G-banding cytogenetics; N20, 20 normal metaphases; N (10–19), 10–19 normal metaphases; N (1–9), 1–9 normal metaphases; NM, patients with non-informative cytogenetics or no metaphases; A17, abnormal karyotype with chr17 aberration but not i(17q), –17, del(17p) or add(17p); AOUT17, abnormal karyotype without chr17 affected; del, deletion.

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Table III. Characteristics of patients with del(17p) detected by FISH alone.

Subgroup	FAB	WHO	Karyotype by CC	% aberrant nuclei (FISH)
AOUT17	RA	5q-syndrome	46,XY,del(5)(q31)[2]/46,XY [27]	79.2
AOUT17	MDS-U	RCMD	47,XY,der(3)t(3;?)(q11;?),del(5)(q12qter),+mar;	91.7
N20	MDS-U	RCMD	46,XY[20]	25.0
AOUT17	RAEB	RAEB-II	43,XX,add(1)(q34),del(4)(q22),-10,-13,-15[5]/idem -11,+mar[5]/46,XX[10]	30.0
AOUT17	RA	5q-Syndrome	46,XX,del(5)(q13q33)[11]/46,XX[9]	33.0
N20	RA	RCMD	46,XY[20]	27.0
AOUT17	MDS-U	MDS-U	46,XY,add(6)(p22)[8]/46,XY[12] nucish(TP53x1)[12/100]	12.0
AOUT17	MDS-U	MDS-U	46,XX,der(5)t(5;14;13)(q14;q24;q21), t(12;13)(q23;q21)[8]/46,XX[10] nucish (TP53x1)[12/100]	12.0
A17	RARS	RCMD	46,XY,del(5)(q31q34),der(16;17)(q23;q21)[8]/45,XY,der(2)t(2;5)(p?;q12), der(16)t(16;17)(q23;q21),-20[8] nucish(TP53x1)[70/100]	70.0
A17	RAEB	RAEB-I	43,X,-Y,del(4)(q?),-5, -7,t(12;?)(q22;?),t(17;?)(p11;?)[20]. nucish(TP53x1)[30/100]	30.0
AOUT17	MDS-U	RAEB-II	45,X,-X,t(X;1;20)(q21;p22;?),del(5)(q13q33),add(7)(p11),r(20)[15] nucish(TP53x1)[25/100]	25.0
AOUT17	RAEB	RCMD	45,XY,-7[7]/46,XY[12]	-
A17	RA	MDS-U	45,XY,-16,t(17;20)(q11;q11)[15]/ 47,XY,sl,+del(8)(p11),+13 [3]/ 47,XY,sdl1,-18,+19 [2]	59.5

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Table IV

Previous studies in MDS that used FISH of 17p or *TP53*.

Authors	*N*	Characteristics	Region analyzed/probe	Results of FISH of 17p, *n* (%)
Soenen V et al. (1998)	17	MDS/AML 15 patients with translocations of chr17, 1 monosomy, 1 i(17q)	3 YACs + D17Z1 (Vysis) + D5Z2 (Omcor)	p53 deletion observed in 14 of 16 patients assessed
Rigolin GM **et al. (2001)**	101	MDS with normal karyotype	Probes Vysis Downers Grove, IL, USA for 5q31 band, 7q31 band, a chromosome- 8-centromeric probe and 17p13 band.	One patient found to have del(17)(p13).
Cuneo A et al. (2002)	82	Normal karyotype: Group A: 55 AML *de novo* Group B: 27 (21 elderly AML + 6 AML after MDS)	17p13/p53 PSC-P1 probe 144G9 isolated by J Landegent (Department of Hematology, AZ Leiden, The Netherlands)	Group A: No deletions found. Group B: 1 case with del17p/13/*TP53*
Silveira CG et al. (2009)	19	Pediatric MDS patients	PPARgamma and *TP53* genes. RP11-275J11(3p25.1) and RP11-89D11 (17p13.1)	Deletion of *TP53* found in 18 of 19 patients
Sebaa A et al. (2012)	43	26 MDS and 17 AML with del(5q)	EGR1 (Abbott Molecular, des Plaines, IL)	Patients with isolated del(5q) or one additional abnormality (*N* = 20): no 17p deletions found. Patients with complex karyotype (*n* = 23): 17p deletion indicated by CC in 15 cases, and confirmed by FISH in 10

MDS, myelodysplastic syndromes; FISH, fluorescence *in situ* hybridization; AML, acute myeloid leukemia; chr, chromosome; del, deletion; CC, conventional G-banding cytogenetics.

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Supplementary Appendix

Table A1

Results of CC and FISH analyses in Group B with loss of 17p by CC.

FAB	WHO	Karyotype by CC	% CG	Result of FISH analysis	% aberrant nuclei by FISH
RAEB	RAEB-2	45,XY,-7,der(5)t(5;?)(q31;?),t(9;17)(p13;p11)[2]/46,XY,t(9;17)(p13;p11)[1]/46,XY[1]	75.0	+	80.0
MDS-U	RAEB-1	46,XY,i(17)(q10)[7]	100.0	+	Deletion
MDS-U	RCMD	45,XY,del(7)(q22),-14,-17,+mar[8]/46,XY[3]	72.7	+	Monosomy
MDS-U	RAEB-2	45,XX,-4,del(5)(q13q33),-7, i(17)(q10)[5]/46,XX[6]	45.5	+	Deletion
RAEB	RAEB-2	43,XX,-5,der(6)t(5;6)(q31;q25),-12,der(13)t(12;13)(q11;p22),der(13)t(13;17)(p12;q11),-17,-18,del(20)(q11),-21.-22,-22,+4mar[17]/46,XX[3]	85.0	+	Monosomy and not deletion 56.0%
RAEB	RCMD	47,XX,del(5)(q14q34),del(7)(q22),add(17p13),del(18)(p11p13),+mar[15]/46,XX[3]. nucish(TP53x1)[35/100]	83.3	+	35.0
RAEB	RAEB-1	46,XY,i(17)(q10)[4]/46,XY[12].nucish(*TP53*x1)[30/100]	25.0	+	20.0
RAEB	RAEB-1	45,XY,del(5)(q13q33),der(9)t(9;12)(p22;q13),-12,i(17)(q10)[9]/44,XY,sl,add(8)(p22),-18[2]/ 46,XY[2]	84.6	+	Deletion 72.0%
RAEB	RAEB-2	46,XY,del(5)(q13q33),i(17)(q10)[2]/45,sl,-9[13]/44,sld1,-7,dic(7;20),-20[2]/near-tetra,sld2[3]/46,XY[2]	90.9	+	Deletion 60.0%
RAEB	-	50,XX,+1,del(5)(q13q33),+6,+8,der(17)t(17;20)(p13;p11),+18[25]	78.1	+	Deletion 44.0%
RAEB	-	46,XY,i(17)(q10)[26]/ 46,XY[4]	86.7	+	Deletion 62.5%
RAEB	-	46,XY,del(12)(p12),i(17)(q10)[30]	100.0	+	Deletion 84.5%
-	-	52,XX,+der(1),del(5)(q13q33),+8,del(9)(p23),+11,+13,+14,del(17)(p11),+20[11]/ 46,XX[24]	31.4	+	46.0%
MDS-U	-	46,XY,i(17)(q10)[26]/ 46,XY[4]	86.7	+	84.0%
RAEB	-	45,X,-Y[21]/ 43,XY,-5,-7,-8,add(15)(p13),16,-17,add(17)(p13),+mar[21]/ 46,XY[8]	84.0	+	Monosomy 73.5%
RAEB	-	44,XY,-5,-7,i(10)(q10),-13,der(16)(q),i(17)(q10),-18,+2mar[22]/ 46,XY[15]	59.5	+	Deletion 13,5%
MDS-U	-	46,XX,del(17)(p13)[10]/ 46,XX[20]	33.3	-	

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RA	-	46,XX,i(17)(q10)[2]/ 46,XXX,-9,i(17)(q10)[2]/ 47,XX,+2,i(17)(q10)[2]/ 46,XX[34]	15.0	+	Deletion 89.5%
RA	-	46,XX,i(17)(q10)[30]	100.0	+	Deletion 41.5
RA	-	46,XY,i(17)(q10) [1]/ 46,XY [29]	3.5	-	
MDS-U	-	45,XX,-4,-5,-12,-17,+3mar [4]/ 46,XX [26]	13.3	+	Deletion 46.0%
RA	-	46,XX,-3,del(5)(q13q33),-6,del(7)(q?),add(11)(p15),-13,-17,add(21)(p13),+4mar [9]/ 46,XX [41]	18.0	+	Deletion 47.0%
RAEB	-	43,XY,add(1q),-4,-5,-6,-7,i(8)(q10),add(12)(p13),-16,-17,+3mar [35]/ 46,XY [15]	70.0	+	Deletion 47.5%
RAEB	-	44,XY,-5,-7,del(9)(q?),add(17)(p13),del(20)(q12) [17]46,XY [3]	85.0	+	Deletion 71.5%
RAEB	-	45,XY,del(5)(q13q32),add(12)(p13),-15,der(17)(t(15;17)(q10;p10) [50]	100.0	+	Deletion 82.5%
MDS-U	-	43,XY,der(5),-7,add(11)(p15),add(12)(p13),-13,-16,-17,-17,+2mar/ [16]46,XY [4]	80.0	-	Other alterations
RAEB	-	42,XY,t(1;7)(q32;q32),-9,inv(9)(p13q13),-10,-12,-15,-17,+3mar [4]/46,XY [16]	20.0	+	Deletion
MDS-U	-	44,XX,del(3)(q21),del(5)(q13q32),-6,add(11)(q23),der(12)(q?),-14,add(15)(q25),-16,add(17)(p13),+mar (94%) 46,XX (6%)	94.0	-	Other alterations
RAEB	-	44,XY,del(5)(q13q23),del(7)(q22),-17,-18,add(19)(p13),-20,add(21)(q22),-22,+2mar [50]	100.0	-	Other alterations
RAEB	-	44,XX,der(3)(q?),del(5)(q13q33),-6,-7,-13,i(17)(q10),add(18)(q23),-19,del(20)(q12),+2mar [49]/46,XX [1]	98.0	+	Deletion 61.0%

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Fluorescence *in situ* hybridization (FISH) of *TP53* for the detection of chromosome 17 abnormalities in myelodysplastic syndromes

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ABSTRACT

Conventional G-banding cytogenetics (CC) detects chromosome 17 (chr17) abnormalities in 2% of patients with *de novo* myelodysplastic syndromes (MDS). We used CC and fluorescence *in situ* hybridization (FISH) (LSI p53/17p13.1) to assess deletion of 17p in 531 patients with *de novo* MDS from the Spanish Group of Hematologicalfor Clinical Cytogeneticsall of them studied by conventional cytogenetics. FISH detected –17 or 17p abnormalities in 13 cases (2.6%) in whom no 17p abnormalities were revealed by CCFISH detected –17 or 17p abnormalities in 13 cases (2.6%) in whom CC had failed:FISH detected -17 or 17p- abnormalities in 13 cases in whom CC had failed0.9% of patients with a normal karyotype, 0% in non-informative cytogenetics, 50% of patients with a chr17 abnormality without loss of 17p and in 4.7% of cases with an abnormal karyotype not involving chr17. Our results suggest that applying FISH of 17p13 to identify the number of copies of the *TP53* gene could be beneficial in patients with a complex karyotype.On the basis of our findings,W we recommend using FISH of 17p13 in young patients with a normal karyotype or non-informative cytogenetics, and always in cases of isolated del(17p).

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Keywords:
Myelodysplastic syndromes
Cytogenetics
FISH
Chromosome 17

Word Count: 2950867

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Abbreviations:

A17, abnormal karyotype with chr17 abnormalities other than i(17q), –17, del(17p), or add(17p)

AML, acute myeloid leukemia

AOUT17, abnormal karyotype with no chr17 abnormalities

AZA, azacitidine

CC, conventional G-banding cytogenetics

CEP17, chromosome centromeric probe of chr17

chr, chromosome

CMML, chronic myelomonocytic leukemia

del, deletion

FAB, French–American–British

FISH, fluorescence *in situ* hybridization

GCECGH, Grupo Cooperativo Español de Citogenética Hematológica (Spanish Group of Hematological Cytogenetics)

GESMD, Grupo Español de Síndromes Mielodisplásicos (Spanish Group for MDS)

IPSS, International Prognostic Scoring System

ISCN, International System for Human Cytogenetic Nomenclature

MDS, myelodysplastic syndromes

N1–9, normal karyotype based on 1–9 metaphases

N10–19, normal karyotypebased on 10–19 metaphases

N20, normal karyotype based on ≥20 metaphases

NM, patients with non-informative cytogenetics or no metaphases

RA, refractory anemia

RAEB, refractory anemia with excess blasts

RAEB-t, refractory anemia with excess blasts in transformation

RARS, refractory anemia with ringed sideroblasts

R-IPSS, revised International Prognostic Scoring System

WHO, World Health Organization

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1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders, characterized by inefficient hematopoiesis, peripheral blood cytopenias, and an increased risk of progression to acute myeloid leukemia (AML) [1–3]. Both the prognosis and the clinical course of MDS are highly variable, and several scoring systems have been developed to assess prognosis [4–7].

Chromosomal abnormalities in bone marrow cells are found in 40–60% of MDS patients [8], while, using conventional G-banding cytogenetics(CC), alterations of chromosome 17 (chr17) are detected in 2% of patients with *de novo* MDS [9]. The most common abnormalities detected by CC are deletion of [(del)]5q, monosomy 7, del(7q), gain of chromosome 8 [add(8)], del(11q), del(12p), isochromosome 17q(i17q), and del(20q)[10].

Detection of cytogenetic abnormalities is integral to the prognostic evaluation of patients with MDS. The International Prognostic Scoring System (IPSS), which categorizes cytogenetic abnormalities as low risk, intermediate risk, or high risk, has become the gold standard for risk assessment in *de novo* MDS [4]. Chromosome (chr)17 abnormalitiesare included inthe intermediate-risk group if they are not present in a complex karyotype.The revised IPSS(R-IPSS) classification for cytogenetic abnormalities [11]involves five groups proposed by Schanz et al[12], and includes i(17q) as a single anomaly in the intermediate cytogenetic risk group.

Recently, our group published a cooperative study that compared the prognostic impact of chr17 abnormalities (*n*=88) with that of other karyotypic abnormalities (*n*=1070) in patients with primary MDS [9]. In agreement with the R-IPSS classification, this study confirmed the intermediate prognostic impact ofi(17q) [11,12].It also demonstrated that different types of chr17 abnormalities confer different prognoses: patients with monosomy 17 (–17) as part of a complex karyotype were found to havethe poorest prognosis. Interestingly, it also demonstrated that chr17 abnormalities are more commonly associated with complex karyotypes than are non-chr17 abnormalities. Consequently, patients with chr17 abnormalities are often associated with higher-risk MDS, and therefore could potentially benefit from active therapies – for example,

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hypomethylating agents, which have demonstrated efficacy in this setting in patients ineligible for allogeneic stem cell transplantation. Azacitidine (AZA), in particular, has demonstrated a survival benefit compared with conventional care regimens [13–15]. Thus, detection of any chr17 abnormalitiesat diagnosis is important, as it enables more accurate prediction of the patient's prognosis and the selection of appropriate treatment options.

Although CC is the gold standard technique for clinical karyotyping, it is associated with some limitations: cells must be divided from the neoplastic clone,and the sensitivity of the technique is relatively low. Fluorescence *in situ* hybridization (FISH) is a commonlyused technique that identifies specific abnormalities, does not require cell division, and can be easily quantified.Previous studies demonstrated the value of FISH in detectingchr5 and 7 abnormalities in patients with MDS[16,17].The main advantage of FISH versus CC is the higher sensitivity, with respect to the number of cells analyzed (CC analyzes only 20 metaphases). However, FISH only provides information relating to the specific region to which the probe hybridizes; it yields no information about other chromosomes.

The objective of the present study was to apply FISH of 17p to a large cohort of patients with*de novo* MDS,in whom CC had failed to detect del(17p).

2. Materials and methods

2.1. Patients

Patients from hospitals belonging to the Grupo Cooperativo Español de Citogenética Hematológica (GCECGH; Spanish Group of Hematological Cytogenetics) were divided into two groups according to their cytogenetic profile (Group A or B depending onwhetherornot they showedlossofthe short armof chr17).

Group A (*n* = 501) included patients in whom CC had failed to detect the following specific chr17 abnormalities: i(17q), –17, del(17p), or add(17p). Group B (*n* = 30) included patients with i(17q), –17, del(17p) or add(17p) detected by CC (alterations that involve the loss of 17p). Group A was subsequently divided into six subgroups: normal karyotype based on ≥20 metaphases (N20); normal

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karyotype based on 10–19 metaphases (N10–19);normal karyotype based on 1–9 metaphases (N1–9); abnormal karyotype with no chr17 abnormalities (AOUT17); abnormal karyotype with chr17 abnormalities that not involve loss of ofthe short armof chr17, therefore other than i(17q), –17, del(17p), or add(17p) (A17); and patients with non-informative cytogenetics or no metaphases (NM). Our group defines cytogenetic study non informative, in those cases with less than 10 normal metaphases.

Patients were classified according to French–American–British (FAB) (*n*=527) and/or World Health Organization (WHO) 2008 (*n*=423) criteria. The study was approved by the ethics committee of Hospital Universitari Germans Trias i Pujol, Badalona. All patients provided written informed consent.

2.2. CC and FISH analysis of TP53

Prior to treatment, CC and FISH were performed on bone marrow samples at individual laboratories with short-term, non-stimulated cultures, following standard procedures. Karyotypes were defined according to the International System for Human Cytogenetic Nomenclature(ISCN) 2009 [18].

FISH analyses were carried out on fixed cells. The probe set consisted of LSI p53/17p13.1 (Ref: 32-190008, Vysis®) with a chromosome centromeric probe of chr17 (CEP17; a control probe; Ref: 32-112017, Vysis®) to detect –17 or del(17p). The number of interphase nuclei analyzed ranged from 100 to 400. Interphase FISH cut-off percentages varied between centers. The median cut-off values for –17and del(17p) were 10% and 5%, respectively.

3. Theory

In patients with MDS, chromosomal abnormalities have been found in up to 40–60% of cases [8]. By CC, alterations of chr17 are detected in 2% of patients with *de novo* MDS [9]. CC has become the gold standard for

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karyotyping, but has limitations. FISH, for which cell division is not required, is a highly sensitive technique that enables examination of a large number of cells.

Patients with *de novo* MDS and chr17 abnormalities typically have a poor prognosis and poor outcomes [9]. Thus, it is important to detect chr17 abnormalities at the time of diagnosis of MDS patients, in order to ensure that the most appropriate treatment options are selected.

We hypothesized that FISH of *TP53* could potentially identify del(17p) in some MDS patients in whom CC had failed to detect this abnormality.

To date, few studies have been published that describe the benefits of applying 17p FISH at the time of diagnosis of patients with *de novo* MDS. Those studies that have been published did not exclusively analyze del(17p) in patients with MDS.To our knowledge, the present study included the largest cohort of patients with *de novo* MDS in whom FISH has been applied to detect del(17p).

4. Results

In total, 531 patients with *de novo* MDS from 18 hospitals belonging to the GCECGH were included in the study. Tables 1A and 1B show the diagnosis of these patients according to FAB and/or WHO 2008 criteria. Using CC, 501 patients were found to have no i(17q), –17, del(17p), or add(17p) abnormalities (Group A), while 30 patients had abnormalities of these types (Group B). Table 2 shows the results of FISH analysis of 17p13.1(*TP53*) in the six Group A subgroups. In 13 patients (2.6%), FISH detected –17 or 17p abnormalities that were not detected by CC. In patients with a normal karyotype by CC, del(17p) was detected in 0.9% of cases. FISH did not detect del(17p) in any of the 58 patients within conclusive CC analyses, but did detect del(17p) in 50% of patients classified by CC as having an abnormal karyotype involving a chr17 abnormality other than i(17q), –17, del17p, or add(17p) (subgroup A17). These patients defined as A17 have an abnormal karyotype with chr17 aberration but notinvolve loss of the short arm of chr17. We could expect that all the patients did no present *TP53* deletion by FISH but our results show a *TP53* deletion in a 50% of the cases. This could be explained because most of these patients

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presented a complex karyotype and the karyotype could be by an error in interpretation.

In patients classified by CC as having an abnormal karyotype not involving chr17 (subgroup AOUT17), FISH detected del(17p) in 4.7% of cases.

Table 3 shows the characteristics of those patients in our study with del(17p) that was detected by FISH alone..

FISH revealed deletion of *TP53* in two patients with normal karyotype.Taking into account that FISH revealed just 25% and 27% of cells with deletion of TP53, we could suspect that the clone with deletion of 17p was not detected by CC due to low grow of tumoral cells than by a criptic deletion. In seven cases with complex karyotype without a deletion of 17p by CC, FISH revealed a *TP53* deletion. These discrepancies could be explained by an error in the interpretation of the karyotype due to the poor morphology of the chromosomes and by the difficulty to define complex karyotypes. Furthermore, iIn two case with 5q- as a single anomaly detected by CC, FISH revealed deletion of *TP53* in 33% and 79% of cells. In those cases will could argue that both patients presented a criptic deletion of 17p.

In Group B, the loss of 17p13.1 was confirmed by FISH in 25 patients (83.3%). However, FISH failed to confirm del(17p) in five patients (16.7%)(Supplementary Appendix Table A1).

5. Discussion

To date, few studies have been published that demonstrate the benefits of applying FISH of *TP53* during the diagnosis of patients with *de novo* MDS. To our knowledge, our study cohort is the largest *de novo* MDS population that has been analyzed by FISH of 17p or *TP53*. Table 4 summarizes the main previously published studies in which FISH wasused to detect del(17p)in patients with MDS. It should be noted that none of these studiesanalyzeddel(17p) exclusively.

Overall, 2.6% of patients in our study were found by FISH to have chr17 abnormalities that were not detected by CC. In the majority of these cases, CC

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had indicated abnormal karyotypes. Of the 220 patients with a normal karyotype by CC (analysis of 20 metaphases), FISH of *TP53* detected del(17p) in only two patients (0.9%). Furthermore, in cases where CC was inconclusive or no metaphases were obtained, FISH of 17p detected no *TP53* deletions. Our group is coordinating an international study among MDS patients with normal karyotype studied by SNP arrays (unpublished data, IWG-PM, MDS Foundation). In those cases with normal cytogenetics and deletion of TP53 by FISH, the application of SNP arrays demonstrated a normal karyotype that could be explained by the low number of cells carrying deletion of TP53 (25 and 27% of cells by FISH). The findings could suggest that the not detection of del17p by cytogenetics could be due by the number of cells analyzed.

Thus, our findings suggest that FISH of *TP53* may complement CC in the detection of chr17 abnormalities in patients with MDS, particularly in those patients with other karyotype abnormalities. This may be valuable in helping to identify patients with a poor prognosis, who could benefit from active treatment options, including hypomethylating agents.

Our findings suggest that FISH of 17p is likely to be of little benefit in diagnosing patients with *de novo* MDS who are classified by CC as having a normal karyotype (or with no metaphases). Therefore, we would only recommend the use of FISH of 17p in the diagnosis of young patients, in whom detection of del(17p) could lead to more appropriate treatment decisions.

A number of recent studies have demonstrated the value of FISH analysis of specific abnormalities that are of particular prognostic/diagnostic importance, such as del(5q) and chr7 abnormalities. In a study of 716 MDS patients, Mallo et al. reported that FISH detected del(5q) in 6% of patients who did not appear to have a del(5q) abnormality when analyzed by CC [16]. Similarly, Adema et al. reported that, when FISH of 7q31 was performed in 773 MDS patients in whom CC failed to detect any chr7 abnormalities, just over 5% of the patients did indeed have chr7 abnormalities [17]. In both of the above studies, the authors recommended that FISH analysis should be mandatory in specific circumstances for patients in whom CC has detected no abnormalities, or for whom insufficient metaphases are available. Our own previous data suggest that FISH is also valuable in some cases to verify the presence of specific karyotypic abnormalities detected by CC. In a study carried out on behalf of the

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Grupo Español de Síndromes Mielodisplásicos (GESMD; Spanish Group for MDS), we identified 10 patients with del(17p) according to CC, who appeared to have a better prognosis than expected. Subsequent FISH failed to verify del(17p) in any of these 10 patients [9].

Other studies have demonstrated the potential benefit of using a panel of FISH probes to screen MDS/AML patients classified by CC as having normal karyotypes. In one such study, Rigolin et al. analyzed 101 patients with MDS and a normal karyotype by CC, using FISH to detect –5/del(5q), –7/del(7q), add(8), and del(17p)) [19]. FISH identified abnormalities in 18 patients, including del(17p) in one patient (1.0%). Similarly, Cuneo et al. used a panel of eight FISH probes to screen 27 patients with AML secondary to MDS. Cryptic abnormalities were detected in eight patients, including del(17p13)(*TP53*) in one patient [20]. These observations, as well as the findings of the present study, demonstrate the value of FISH during the diagnosis of MDS, enabling the detection of abnormalities that are associated with an increased rate of progression to AML and a worse prognosis.

Few published studies have assessed the use of FISH specifically to detect chr17 aberrations during the diagnosis of patients with *de novo* MDS (Table 4). However, several small studies have highlighted the importance of chr17 abnormalities in the pathogenesis of the disease. In a study of patients with MDS (*n*=11) or AML (*n*=6) and chr17 abnormalities [15 with unbalanced translocations, one with –17, and one with i(17q)], FISH identified *TP53* deletion in 14 of the 16 patients (88%) to whom the technique was applied [21]. All but one of these patients had p53 mutations and/or overexpression. Similarly, in a study of 43 patients with del(5q) MDS (*n*=26)/AML (*n*=17), FISH confirmed chr17 abnormalities in 10 patients (23%), all of whom had complex karyotypes [22]. Moreover, *TP53* mutations were detected in 17% and 53% of patients with isolated del(5q) or complex karyotype, respectively, and were found to be associated with shorter survival. These findings suggest that *TP53* plays a pathogenic role in a proportion of patients with MDS, and that FISH analysis would be valuable to identify such individuals.

In addition to being of value in identifying chr17 abnormalities in elderly patients with MDS, FISH may be an important tool in the assessment of cases of pediatric MDS. Although pediatric MDS is relatively rare, accounting for less

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than 10% of childhood hematologic diseases [23], where it does occur it is often associated with chromosomal abnormalities. Using CC, chromosomal abnormalities are detected in more than 50% of *de novo* pediatric MDS cases, while the detection rate is higher in secondary pediatric MDS. In a study of pediatric MDS carried out by Silveira et al., FISH detected del(17p) in 18 of the 19 patients (95%) [24]. By contrast, CC detected chr17 abnormalities in only six patients. These findings suggest that routine application of chr17 FISH should be considered in cases of *de novo* pediatric MDS.

While we did not evaluate the prognostic impact of *TP53* mutations in patients with MDS, we believe that it may be important to address this aspect in future studies. Using CC, Bejar et al. analyzed 439 patients with MDS, and found *TP53* mutations and chr17 abnormalities in 7.5% and 24.2% of patients, respectively [25]. Furthermore, *TP53* mutations were associated with the occurrence of del(17p) (p<0.001). The authors also observed that *TP53* mutations were associated with markers of poor prognosis, such as complex karyotypes, thrombocytopenia, and a high proportion of bone marrow blasts, and were found mainly in patients with intermediate-2-/high-risk IPSS cytogenetics (79%). After adjusting for IPSS risk group, *TP53* mutations were associated with shorter survival, and can therefore be considered an independent predictor of survival.

In the present study, –17 or 17p abnormalities were detected only by FISH, and not by CC, in 13 patients (2.6%). In patients with CC-detected chr17 abnormalities that did not involve loss of 17p (subgroup A17), FISH detected del(17p) in 50% of cases. In these patients, FISH enabled the patients' prognosis to be better defined, by determining the type of chr17 aberration present [9].

FISH detected del(17p) in 4.7% of patients who, according to CC, had an abnormal karyotypewithout chr17 abnormalities. By CC, these patients presented an abnormal karyotype without evidence of loss of *TP53* (no deletions in the short arm of chr17);by FISH, however, loss of *TP53* was detected. In patients in whom CC detected an abnormal karyotype and –17, FISH failed to confirm –17 or loss of *TP53*. In view of its association with a poor prognosis in patients with a complex karyotype [9], detection of –17 is of particular importance. Thus, FISH of 17p could be of great benefit in these

patients, enabling detection of new cases of –17 not detected by CC, and confirming –17 cases indicated by CC.

6. Conclusions

The prognostic impact of chr17 abnormalities in patients with primary MDS is well established. Thus, the identification of the *TP53* deletion does impact on outcome because adding FISH for the detection of *TP53* deletion to CC enables more accurate prediction of the patient's prognosis and the selection of appropriate treatment options.

~~It is known the prognostic impact of chr17 abnormalities in patients with primary MDS. Thus, the identification of the TP53 deletion does impact on outcome because add FISH of TP53 to CC enables more accurate prediction of the patient's prognosis and the selection of appropriate treatment options".~~

The findings of our study demonstrate ~~the value of~~that FISH provides important prognostic information in ~~diagnosing~~ *de novo* MDS. On the basis of the results obtained, we recommend that FISH of 17p13 should be performed: in all patients with *de novo* MDS and a complex karyotype (with or without involvement of chr17), in order to identify the number of copies of *TP53;* and in young patients with a normal karyotype or non-informative cytogenetics, to better predict their prognosis and make more appropriate treatment decisions. Furthermore, we recommend that isolated del(17p) detected by CC should always be verified by FISH.

Acknowledgements

Financial support. This work was supported in part by a grant from the *Instituto de Salud Carlos III, Ministerio de Economía y Competitividad*, *Spain (PI 11/02010 and PI/14/00013);* by the *Red Temática de Investigación Cooperativaen Cáncer (RTICC, FEDER)* (RD12/0036/0044*); Sociedad Española Hematología y Hemoterapia; 2014 SGR225 (GRE) Generalitat de*

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Catalunya, by José Carreras Leukämie-Stiftung , Ref. AR 14/34; economical support from Fundació Internacional Josep Carreras and from Celgene Spain. The research leading to this invention has received funding from “la Caixa” Foundation.

Contributions. JS performed FISH studies, collected and analyzed data, and wrote the manuscript. VM, XG and TG helped to write the manuscript. RL and ET helped FISH studies. SF collected clinical information on the subjects.VA, IM, EL, AB, IB, MLMR, BB, RC, MTB and TG provided cytogenetic, FISH and clinical data. GS helped write the manuscript. FS designed the overall project and helped write the manuscript. All authors gave the final approval to the manuscript. Medical writing support was provided by Sandra Lee Lewis of the Investigator-Initiated Research Writing Group (part of the KnowledgePoint360 Group), and was funded by Celgene.

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References

[1] Nimer SD. Myelodysplastic syndromes. Blood 2008;111:4841–51.

[2] Lewis SM, Verwilghen RL. Dyserytropoiesis and dyserythropoietic anaemia. Br J Haematology 1972;23:1–4.

[3] Catenacci DV, Shiller GJ. Myelodysplasic syndromes: a comprehensive review. Blood Rev 2005;19:301–19.

[4] Greenberg P, Cox C, Le Beau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079–88.

[5] Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, et al.Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 2007;25:3503–10.

[6] Sanz GF, Sanz MA, Vallespí T, Cañizo MC, Torrabadella M, García S, et al. Two regression models and a scoring system for predicting survival and planning treatment in myelodysplastic syndromes: a multivariate analysis of prognostic factors in 370 patients. Blood 1989;74:395–408.

[7] Germing U, Strupp C, Kuendgen A, Isa S, Knipp S, Hildebrandt B, et al. Prospective validation of the WHO proposals for the classification of myelodysplastic syndromes. Haematologica 2006;91:1596–604.

[8] Sole F, Luno E, Sanzo C, Espinet B, Sanz GF, Cervera J, et al. Identification of novel cytogenetic markers with prognostic significance in a series of 968 patients with primary myelodysplastic syndromes. Haematologica 2005;90:1168–78.

[9] Sánchez-Castro J, Marco-Betés V, Gómez-Arbonés X, Arenillas L, Valcarcel D, Vallespí T, et al. Characterization and prognostic implication of 17 chromosome abnormalities in myelodysplatic syndrome. Leuk Res 2013;37:769–76.

[10] Haase D, Germing U, Schanz J, Pfeilstöcker M, Nösslinger T, Hildebrandt B, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood 2007;110:4385–95.

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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

[11] Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised International Prognostic Scoring System (IPSS-R) for myelodysplastic syndromes. Blood 2012;120:2454–65.

[12] Schanz J, Tüchler H, Solé F, Mallo M, Luño E, Cervera J, et al. A new, comprehensive cytogenetic scoring system for primary myelodysplastic syndromes and oligoblastic AML following MDS derived from an international database merge. J ClinOncol 2012;30:820–9.

[13] Itzykson R, Thépot S, Eclache V, Quesnel B, Dreyfus F, Beyne-Rauzy O, et al. Prognostic significance of monosomal karyotype in higher risk myelodysplastic syndrome treated with azacitidine. Leukemia 2011;25:1207–9.

[14] Itzykson R, Thépot S, Quesnel B, Dreyfus F, Beyne-Rauzy O, Turlure P, et al. Prognostic factors for response and overall survival in 282 patients with higher-risk myelodysplastic syndromes treated with azacitidine. Blood 2011;117:403–11.

[15] Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol 2002;20:2429–40.

[16] Mallo M, Arenillas L, Espinet B, Salido M, Hernández JM, Lumbreras E, et al. Fluorescence in situ hybridization improves the detection of 5q31 deletion in myelodysplastic syndromes without cytogenetic evidence of 5q–. Haematologica 2008;93;1001–8.

[17] Ademà V, Hernández JM, Abáigar M, Lumbreras E, Such E, Calull A, et al. Application of FISH 7q in MDS patients without monosomy 7 or 7q deletion by conventional G-banding cytogenetics: does -7/7q- detection by FISH have prognostic value? Leuk Res 2013;37:416–21.

[18] Shaffer LG, Slovak ML, Campbell LJ, editors. ISCN (2009): an international system for human cytogenetic nomenclature, Basel: S. Karger; 2009.

[19] Rigolin GM, Bigoni R, Milani R, Cavazzini F, Roberti MG. Clinical importance of interphase cytogenetics detecting occult chromosome lesions in myelodysplastic syndromes with normal karyotype. Leukemia 2001;15:1841–7.

For Peer Review Only

[20] Cuneo A, Bigoni R, Cavazzini F, Bardi A, Roberti MG, Agostini P, et al. Incidence and significance of cryptic chromosome aberrations detected by fluorescence in situ hybridization in acute myeloid leucemia with normal karyotype. Leukemia 2002;16:1745–51.

[21] Soenen V, Preudhomme C, Roumiere C, Daudignon A, Luclaï J, Fenaux P. 17p deletion in acute myeloid leukemia and myelodysplastic syndrome. analysis of breakpoints and deleted segments by fluorescence in situ. Blood 1998;91;1008–15.

[22] Sebaa A, Ades L, Baran-Marzack F, Mozziconacci MJ, Penther D, Dobbelstein S, et al. Incidence of 17p deletions and TP53 mutation in myelodysplastic syndrome and acute myeloid leukemia with 5q deletion. Genes Chromosomes Cancer 2012;51:1086–92.

[23] National Cancer Institute at the National Institutes of Health. Childhood acute myeloid leukemia/other myeloid malignancies treatment (PDQ). Available at: http://www.cancer.gov/cancertopics/pdq/treatment/childAML/HealthProfessional. Accessed 21 March 2014.

[24] Silveira CG, Oliveira FM, Valera ET, Ikoma MR, Borgonovo T, Cavalli IJ, et al. New recurrent deletions in the PPAR gamma and TP53 genes are associated with childhood myelodysplastic syndrome. Leuk Res 2009;33:19–27.

[25] Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 2011;364:2496–506.

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