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Title page: Lenalidomide treatment of Myelodysplastic Syndromes with chromosome 5q deletion. Results from the National Registry of the Italian Drug Agency

Running title: Lenalidomide in 5q-: an Italian registry

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ABSTRACT

Objective: The most typical cytogenetic aberration in myelodysplastic syndromes is del(5q), which, when isolated, is associated with refractory anemia and good prognosis. Based on high rates of erythroid response and transfusion independence, Lenalidomide (LEN) became the standard treatment. This multi-centre study was designed to supplement Italian Registry data on LEN by addressing prescription, administration appropriateness, hematological and cytogenetic responses and disease evolution.

Methods: MORE study was an observational, non-interventional, multi-centre, retrospective and prospective study. Cases were recruited from 45 Hematological Centres throughout Italy. Data were collected from the Italian National Registry for Lenalidomide administration and supplemented by a MORE data form.

Results: Data from 190/213 patients were analysed. 149 had been diagnosed by conventional cytogenetics (GROUP A) and 41 only by FISH (GROUP B). Overall erythroid response was obtained in 92.8% of cases. Overall cytogenetic remission was achieved in 22.6% of cases. Disease progression occurred in 15.6% of cases. Clonal cytogenetic evolution characterized progression to AML but not to higher risk MDS.

Conclusions: Erythroid response to Lenalidomide was similar in MDS with isolated del(5q) and with del(5q) plus one anomaly. Progression to AML or higher risk MDS showed different cytogenetic features.

ClinicalTrials.gov: NCT01347944

Key words: del(5q); Lenalidomide; MDS; Registry Study

Introduction

The most typical cytogenetic aberration in *de novo* myelodysplastic syndromes (MDS) is isolated del(5q), an interstitial deletion within the chromosome 5 long arm, which is the hallmark of refractory anemia, macrocytosis, normal or high platelet count, erythroid hypoplasia, non-lobulated megakaryocytes, and relatively good prognosis. LEN was recognized as efficacious therapy as it suppresses del(5q) clonal cells and acts on the erythroid compartment to improve hemoglobin levels, leading to an 83% erythroid response rate and durable transfusion independence (1). Cytogenetic response rates were much higher in patients with isolated del(5q) than in patients with del(5q) in complex karyotypes (2). The European MDS 004 study confirmed these data, recommending 10 mg LEN every 21 days in 28-day cycles (3).

After LEN was approved by the Food and Drug Administration (USA) and by the European Medicines Agency, administration under the Italian National Health Service was limited to patients with del(5q), whether isolated or not, and low or intermediate-1 risk MDS (LOW or INT-1 according to the IPSS score) (4). They were enrolled in the Italian National Drug Agency Registry (AIFA, Agenzia Italiana Farmaco), which monitors administration of drugs that are still under investigation.

The present multi-centre study was designed to supplement Registry data on LEN by addressing prescription and administration appropriateness, hematological and cytogenetic responses and disease evolution.

Methods

Setting: Inclusion Criteria for the AIFA LEN Registry were: LOW or INT-1 MDS; transfusion-dependent anemia (at least 2 units of packed RBCs in 8 weeks prior to starting LEN treatment); 5q deletion, whether isolated or associated with other chromosomal abnormalities.

Study design: This observational, non-interventional, multi-centre, retrospective/prospective cohort study was registered as MORE (ClinicalTrials.gov NCT01347944) and designed as follows: collection of retrospective data on patients that had been enrolled in the Registry from Oct.31st 2008 to May 20th 2010; collection of prospective data as patients were recruited to the study from May 21st 2010 to June 13th 2012 (Supplementary Figure 1); integration of both databases using a new MORE data form. All subjects in the Registry, who had undergone at least one cycle of LEN, were included in the MORE study.

The present study included 45 Italian Hematological Centres: 22 from northern Italy, 11 middle and 12 southern (Supplementary Figure 2). The Ethics Committee of each participating centre approved the study.

All data were independently collected from AIFA Registry through CINECA, a non-profit Inter-university Consortium for data collection, processing and statistical analysis. Data were monitored by CRO (MeDePha, Via Aosta 4/A, 20155, Milano), a data management centre that organized, and was responsible for, inter-centre contacts, checked patient electronic Continuous Reinforcement Schedules (e-CRFs) and replied to e-Queries. A Scientific Steering Committee focused on centre compliance with procedures and checked data analysis.

Patients were divided into Group A, who underwent conventional cytogenetic testing, and Group B, who underwent only FISH (Table 1). Two groups were analyzed separately according to LEN dosage: 10 mg/day *vs* 5 mg/day (Supplementary Table1).

The primary study objectives were to determine prescription and administration appropriateness and to assess clinical, hematological and cytogenetic responses to LEN in-depth, according to International Working Group criteria (5).

Secondary objectives were to evaluate diagnostic approaches; to monitor cytogenetic and hematological changes during the course of disease; to identify subgroups with significant prognostic features and monitor LEN safety and tolerability.

Data analysis was conducted at predetermined time-points: after 4-6 cycles of treatment; after 8-12 cycles; at last follow-up and/or at the end of treatment. Only the cases with complete information at the three time-points entered both univariate and multivariate analysis. A total of 9 clinical variables were considered: bone marrow blasts, MCV, hemoglobin level, neutrophil count, ferritin levels, platelet count, Abnormal Localization of Immature Precursors (ALIP), megakaryocytic dysplasia and bone marrow fibrosis (Supplementary Table 2). For safety and toxicity assessment, frequency of patients with any adverse events such as neutropenia, thrombocytopenia or infections, early withdrawal, hospitalizations were reported.

Statistical Analysis: Descriptive statistics (mean, SD, median, range and inter-quartile range) were calculated for all continuous variables. Frequency distributions were calculated for categorical variables (disease, risk category, hematological and cytogenetic profiles over time, response to LEN, disease progression). The t-test for continuous variables and the chi-square test for categorical variables were used to analyze inter-group differences (unless otherwise stated, all p-values are intended as two-tailed). Kaplan-Meier survival curves were calculated using time-to-event variables and the log-rank test for group comparison. Univariate logistic

regression analyses evaluated some baseline variables and prognostic factors including blast and platelet counts, cytogenetic complexity at diagnosis, erythroid and/or cytogenetic response, to identify the prognostic factors associated with evolution to AML or higher risk MDS. Significant variables in univariate analysis (at alpha 0.1) were analysed in multivariate logistic regression analysis.

The LOCF (Last Observation Carried Forward) method was applied to handle missing data.

IT infrastructure and software: The web-based system for data collection was the CINECA AXMR ® (Advanced Extended Multicentre Research) technology, that was designed to manage clinical research processes. Data management (DB freezing, intermediate tables, views, and materialized views in support of the analysis) was carried out using PL/SQL Developer (Oracle Corporation database), which is based on the PL/SQL (Procedural Language/Structured Query Language) program.

Data analysis was performed using PL/SQL Developer and R open source software, which is specific for statistical calculations and charting.

Funding Source

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Results

Study size, grouping and bias: 213 patients were included in the registry during the study period (56 up to May 20, 2010 who provided retrospective data and 134 afterwards who yielded both prospective and retrospective data).

190/213 patients (M:F 60:130) were eligible for the study as they satisfied all inclusion criteria. At inclusion in the registry 56 patients had been pre-treated with LEN, and the previous cycles were added by MORE integration. Starting doses of LEN were extremely heterogeneous; however we were able to distinguish two groups: 59% of patients received 10 mg daily (in 21-day cycles or continuously) and 27% of patients were given 5 mg (in 21-day cycles or continuously) (Supplementary Table 1). Moreover 128/190 cases (62%) stopped LEN treatment during the observation period (Supplementary Table 3). Patients who had undergone only FISH testing (Group B) (41 patients, median age 71, 10 pre-treated) were analyzed separately from those who underwent a full cytogenetic evaluation (Group A) (149 patients, median age 75, 46 pre-treated). On a total of 18.1% of patients with non-isolated 5q-, 1.3% had a complex karyotype, 16.8% one additional chromosomal abnormality. No significant inter-group (A *vs* B) differences emerged in patient demographics, disease and risk categories, or median time from diagnosis to LEN treatment (Table1).

Erythroid response: the complete erythroid response rate was 74.6% in group A and 78.6% in group B after 4-6 cycles, rising to 85.8% and 88.9%, respectively after 8-12 cycles. The partial response rate was 11.5% in group A and 10.7% in group B after 4-6 cycles, falling to 6.2% and 7.4% respectively after 8-12 cycles as patients achieved complete response (Figures 1, Supplementary Table 4). In univariate analysis number of cycles >6, platelet count >100.000 and ALIP were significant both for complete and partial response; Hb and blast count resulted significant only for overall response ($p<0.05$) (Supplementary Tables 5-6.). Only the duration of therapy reached the statistical significance in multivariate analysis ($p<0.001$) (Supplementary Table 7-8). No differences in overall and complete responses emerged in two treatment schedules.

Cytogenetic response: Cytogenetic response was analyzed and monitored only in patients who had undergone conventional cytogenetics for 5q- diagnosis (Group A). After 4-6 cycles the complete response rate was 7.8% while the partial response rate was 2.4%. After 8-12 cycles the complete response rate rose to 13% and the partial to 9.6% (Figure 2, Supplementary Table 4). Starting dosage at 10 mg LEN daily ($p<0.001$) and erythroid dysplasia ($p<0.05$) significantly correlated with overall cytogenetic response. Only the starting dosage retains the statistical significance in multivariate analysis (Table 3). None of these factors resulted significant for complete cytogenetic response in univariate analysis (data not shown). Within the 10 mg Group 60% of cases showed cytogenetic response at 24 months therapy (figure 2C). However dosage did not impact both Leukemia Free Survival (LFS) and Overall Survival (OS) (Supplementary Figure 6).

Disease progression: During the 44 months observation period (range: 0.5-237), disease progression occurred in 15.6% of cases: 18/190 cases (9.5%) developed AML (Table 2) and 12 cases (6.1%) progressed to higher risk MDS (Table 3). We analysed separately cases developing AML and those progressing to higher risk MDS. Both subgroups were negative for any cytogenetic response to LEN treatment (Table 2). In 7/13 Group A patients, AML diagnosis corresponded to clonal cytogenetic progression and acquisition of a complex karyotype, while complex cytogenetics never appeared in the subgroup evolving to higher risk MDS; in the last group, the del(5q) karyotype of diagnosis remained unchanged. In univariate analysis cytogenetic and erythroid response did not influence progression to AML or higher risk MDS (Supplementary Figure 3,4). Significant differences emerged in time to progression (AML median 25 months, range 7-89; MDS median 54 months, range 15-102; p 0.028) and number of LEN cycles (median: 7.5 for AML and 19.5 for higher risk MDS, p 0.010). Other clinical and biological variables assessed in the present study include blast and platelet counts, cytogenetic complexity at diagnosis, erythroid and/or cytogenetic response and time from MDS diagnosis and inclusion in the Registry.

The only factor which achieved significance in our study was a blast count of >5% (p. 0.010) in both univariate analysis (Supplementary Figure 5) and multivariate analysis ($p<0.05$) (Supplementary Table 9).

LEN Safety and Toxicity. During treatment most patients had slight to moderate neutropenia (75%; grade 3-4 59%) and thrombocytopenia (62%; grade 3-4 21%). Grade 3-4 Neutropenia led to drug discontinuation in fewer than 50% of cases and was treated with G-CSF in fewer than 10%. The incidences of neutropenia and thrombocytopenia were greater in the first 6 months of treatment. Infections (21%) were mostly upper respiratory tract infections. During the observation period 13 patients (31.7%) were hospitalized because of infection. Five patients with platelet counts of >100.000/mmc and hemoglobin >10 g/dl in the early phase of treatment had deep venous thrombosis leading to LEN withdrawal. In one patient with HCV-related cirrhosis, liver toxicity led to early withdrawal after only 1 LEN cycle.

Discussion

As far as we know this is the first study on LEN use in the framework of a national registry, in which Hematology Units throughout Italy participated. Italian hematologists correctly selected patients and managed LEN administration. MORE integration data indicated a high response rate, supporting appropriate diagnosis and monitoring.

High erythroid response rates confirmed LEN had exerted its well-known effect in del(5q) MDS (1-3,6-8). This effect has been emphasized in a heterogeneous cohort of 716 MDS patients with 71% overall erythroid response after 3 cycles of LEN; 83% of responders had MDS with del(5q) (9). The drop of hemoglobin level we observed at the last follow-up was likely due to the relative high number of LEN suspension (98 cases) or disease progression (30 cases). Hematological response was independent of cytogenetic remission, as demonstrated by the lower cytogenetic response rate compared to the erythroid, similarly to previous published series (3,10).

Overall cytogenetic response in this study was very low compared to the previous reported data. Indeed major limitations of this study were treatment schedules that were heterogeneous for duration, continuous vs intermittent administration and individual dosage adjustments. No significant differences were found considering overall response after 6 cycles ($p=0.8$). Instead LEN dosage, similarly to that reported in pivotal European MDS-004 (3), influenced cytogenetic response as the 10 mg initial dosage predicted cytogenetic response. Notably in the group of patients treated by long-standing 10 mg dosage, cytogenetic response increased to 60%.

The impact of 5q- plus another anomaly on the erythroid response rate in low and intermediate-1 risk MDS has been debated. A retrospective cytogenetic multi-centre Spanish study found the erythroid response differed significantly if one aberration in addition to the 5q- was present, but the IPSS risk category was not evaluated (11). In the present study, a good response rate was observed even when an additional chromosomal change accompanied del(5q), thus emphasizing similarities between isolated del(5q) and del(5q) plus one additional anomaly, as recently recognized by the WHO 2016 (12). Remarkably, in our series all cases belonged to LOW or INT1 IPSS category. This may be relevant considering that around 43% of LOW and INT1 MDS without del(5q) responded to LEN (13). Consequently, LEN appears optimal treatment for LOW and INT1 MDS with isolated del(5q), or del(5q) plus one more change.

Overall evolution rate (15,6%) in this Registry study was lower than the rate of 25,4% in the MDS-004 European study (3), even though they had comparable observation time (44 months *vs* 35.5 months in the LEN 5 mg group and 36.9 months in the LEN 10 mg group) and baseline cytogenetics. In his update of 148 cases, including 16,9% with one more change and 8,1% with complex 5q-, List et al found AML evolution in 28.6% of cases five years after treatment initiation (14). Median time of progression was not reached for patients with isolated 5q-, while it was 4,1 years for patients with additional cytogenetic abnormalities. A retrospective study, reported no significant difference in the AML evolution rate in 125 untreated and 295 treated cases with isolated del(5q) (15). AML evolution was observed in 12.6% of 381 internationally recruited untreated cases with hematological and cytogenetic features similar to those in our study, namely low-int1 risk MDS, a low proportion of complex karyotypes (4.2%) a low proportion of 5q- plus another aberration (14.2%), a median observation time of 49.8 months (16).

In this study, cases developing AML and those progressing to higher risk MDS were both negative for any cytogenetic response to LEN treatment. Clonal progression and development of a complex karyotype in 7/13 cases corresponded to AML development, while complex cytogenetics and new cytogenetic aberrations never appeared when MDS evolved towards a higher risk category. Although this observation needs to be confirmed in larger series of cases, our cytogenetic findings suggest that different biological features underlie disease progression to either worsening MDS or AML.

Unfortunately, biological samples were not available to evaluate molecular prognostic factors predictive of lower cytogenetic response or disease evolution, such as p53 expression or mutations (17,18), TET2, ASXL1, RUNX1 and CSNK1A1 mutations (19, 20), or the expression of cereblon, whose reduction correlates with LEN resistance (21).

In conclusion, this "real life" Registry showed that LEN treatment successfully achieved a high erythroid response rate and reduced transfusion dependence in Low-Int1 risk MDS with del(5q), both as sole anomaly or associated with one change. The leukemic evolution rate was similar to that observed in other multi-centre studies. Cytogenetic results emphasized biological differences in cases with evolution to AML or to higher risk MDS. International efforts should be made to investigate predictive biological markers in large-scale clinical studies.

Contributors

FA, AR, VDB, AC, CM collected, assembled, analyzed, and interpreted the data. CM, SC, ST conceived and designed the study. FA, AR, VDB, CM wrote the Article. All Authors provided final approval.

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References

1) List A, Kurtin S, Roe DJ, Buresh A, Mahadevan D, Fuchs D, Rimsza L, Heaton R, Knight R, Zeldis JB. Efficacy of lenalidomide in myelodysplastic syndromes. N Engl J Med 2005; 352:549-57.

2) List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E, Powell B, Greenberg P, Thomas D, Stone R, Reeder C, Wride K, Patin J, Schmidt M, Zeldis J, Knight R. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 2006; 355:1456-65.

3) Fenaux P, Giagounidis A, Selleslag D, Beyne-Rauzy O, Mufti G, Mittelman M, Muus P, te Boekhorst P, Sanz G, del Can˜izo C, Guerci-Bresler A, Nilsson L, Platzbecker U, Lu¨bbert M, Quesnel B, Cazzola M, Ganser A, Bowen D, Schlegelberger B, Aul C, Knight R, Francis J, Fu T, Hellstro¨m-Lindberg E, for the MDS-004 Lenalidomide del5q Study Groupet al. A randomized phase 3 study of lenalidomide versus placebo in RBC transfusion dependent patients with low/Intermediate-1-risk myelodysplastic syndromes with del 5q. Blood 2011; prepublished online. Blood. 2011;118:3765-76.

4) Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, Sanz M, Vallespi T, Hamblin T, Oscier D, Ohyashiki K, Toyama K, Aul C, Mufti G, Bennett J. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997; 89:2079-88.

5) Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, Pinto A, Beran M, de Witte TM, Stone RM, Mittelman M, Sanz GF, Gore SD, Schiffer CA, Kantarjian H. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006;108:419-25.

6) Giagounidis AA. Lenalidomide for del(5q) and non-del(5q) myelodysplastic syndromes. Semin Hematol. 2012; 49:312-22.

7) Le Bras F, Sebert M, Kelaidi C, Lamyc T, Dreyfus F, Delaunaye J Banos A, Blanc M, Vey N, Schmidti A Visanicaj S, Eclache V, Turlurel P, Beyne-Rauzy O, Guerci A, Delmer A, de Bottonp S, Rea D, Fenaux P, Adès L. Treatment by Lenalidomide in lower risk myelodysplastic syndrome with 5q deletion the GFM experience. Leuk Res 2011; 35:1444-8.

8) Giagounidis A. Mufti GJ, Fenaux P, Germing U, List A, MacBeth KJ. Lenalidomide as a disease-modifying agent in patients with del(5q) myelodysplastic syndromes: linking mechanism of action to clinical outcomes. Ann Hemat. 2014; 93:1-11

9) Zeidan AM, Gore SD, McNally DL, BSPharm MS, Baer MR, Hendrick F, Mahmoud D and Davidoff AJ. Lenalidomide performance in the real world: patterns of utilization of effectiveness in a medicare population with Myelodysplastic Syndromes. Cancer 2013; 119: 3870-3878.

10) Schuler E, Giagounidis A, Haase D, Shirneshan K, Büsche G, Platzbecker U, Nolte F, Götze K, Schlenk RF, Ganser A, Letsch A, Braulke F, Lübbert M, Bug G, Schafhausen P, Bacher U, Gattermann N, Wulfert M, Haas R, Germing U. Results of a multicenter prospective phase II trial investigating the safety and efficacy of lenalidomide in patients with myelodysplastic syndromes with isolated del(5q) (LE-MON 5). Leukemia 2015; 30:1580-2.

11) Mallo M, Del Rey M, Ibáñez M, Calasanz J, Arenillas L, Larrayoz MJ, Pedro C, Jerez A, Maciejewski J, Costa D, Nomdedeu M, Diez-Campelo M, Lumbreras E, Martınez TG, Marugan I, Such E, Cervera J, Cigudosa JC, Alvarez S, Florensa L, Hernandez JM, Sol F. Response to lenalidomide in myelodysplastic syndromes with del(5q): influence of cytogenetics and mutations. Br J Haematol 2013; 162:74-86.

12) Bennett JM. Changes in the Updated 2016: WHO Classification of the Myelodysplastic Syndromes and Related Myeloid Neoplasm; Clin Lymph Myeloma Leuk. 2016;16:607-9.

13) Raza A, Reeves JA, Feldman EJ, Dewald GW, Bennett JM, Deeg HJ, Dreisbach L, Schiffer CA, Stone RM, Greenberg PL, Curtin PT, Klimek VM, Shammo JM, Thomas D, Knight RD, Schmidt M, Wride K, Zeldis JB, List AF. Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and

intermediate-1 risk myelodysplastic syndromes with karyotypes other than deletion 5q. Blood 2008; 111:86-93.

14) List AF, Bennett JM, Sekeres MA AF, kikne B, Fu T, Shammo JM, Nimer SD, Knight RD, Giagounidis A, on behalf of the MDS-003 Study Investigators. Extended survival and reduced risk of AML progression in erythroid-responsive lenalidomide-treated patients with lower-risk del(5q) MDS. Leukemia 2014;28:1033-40.

15) Kuendgen A, Lauseker M, List AF, Fenaux P, Giagounidis AA, Brandenburg NA, Backstrom J, Glasmacher A, Hasford J, Germing U, on behalf of the International Working Group on MDS with del(5q) et al. Lenalidomide does not increase AML progression risk in RBC transfusion-dependent patients with Low- or Intermediate-1-risk MDS with del(5q): a comparative analysis. Leukemia 2013;27:1072-9.

16) Germing U, Lauseker M, Hildebrandt B, Symeonidis A, Cermak J, Fenaux P, Kelaidi P, Pfeilstocker M, Nosslinger P, Sekeres M, Maciejewski J, Haase D, Schanz J, Seymour J, Kenealy M, Weide R, Lubbert M, Platzbecker U, Valent P, Gotze P, Stauder R, Blum S, Kreuzer K-A, Schlenk R, Ganser A, Hofmann W-K, Aul C, Krieger O, Kundgen A, Haas R, Hasford J, Giagounidis A. Survival, prognostic factors and rates of leukemic transformation in 381 untreated patients with MDS and del(5q): a multicenter study. Leukemia 2012; 26: 1286-92.

17) Jädersten M, Saft L, Smith A, Kulasekararaj A, Pomplun S, Gohring G, Hedlund A, Hast R, Schlegelberger B, Porwit A, Hellstrom-Lindberg E, Mufti GJ. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. J Clin Oncol 2011 May 20;29:1971-9.

18) Haferlach C, Dicker F, Herholz H, Schnittger S, Kern W, Haferlach T. Mutations of the TP53 gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype. Leukemia 2008; 22:1539-41.

19) Scharenberg C, Giai V, Pellagatti, Saft L, Dimitriou M, Jansson M, Jädersten M, Grandien A, Douagi I, Neuberg DS, LeBlanc K, Boultwood J, Karimi M, Jacobsen SEW, Woll PS, Hellström-Lindberg E. Progression in patients with low- and intermediate1-risk del(5q) myelodysplastic syndromes is predicted by a limited subset of mutations. Haematologica 2017; 102:498-508.

20) List A, Ebert BL, Fenaux P. A decade of progress in myelodysplastic syndrome with chromosome 5q deletion. Leukemia. 2018 Jan 30. [Epub ahead of print]

21) Jonasova A, Bokorova R, Polak J, Vostry M, Kostecka A, Hajkova H, Neuwirtova R, Siskova M, Sponerova D, Cermak J, Mikulenkova D, Cervinek L, Brezinova J, Michalova K, Fuchs O. High level of full-length cereblon mRNA in lower risk myelodysplastic syndrome with isolated 5q deletion is implicated in the efficacy of lenalidomide. Eur J Haematol. 2015 Jul;95(1):27-34.

Legends

Figure 1. Time to development of complete (a) and overall erythroid response (b) in all patients (group A and B). Time to complete erythroid response, only Group A (5q isolated vs 5q not isolated) (c) since treatment begin.

Figure 2. Time to development of complete (a) and overall cytogenetic response (b) in Group A (data are referred to first 24 months of treatment); cytogenetic response by treatment group for the first 48 months of treatment (c).

Supplementary Figure 1: Schematic representation of the observational time of the study including retrospective and prospective data collection.

Supplementary Figure 2: Representation of patients enrolled into the AIFA Registry. Colors are distributed by Italian Regions.

Supplementary Figure 3: Univariate analysis for AML evolution and MDS evolution based on erythroid response (p not significant).

Supplementary Figure 4: Univariate analysis for AML evolution (a) and MDS evolution (b) based on cytogenetic response (p not significant).

Supplementary Figure 5: Univariate analysis for AML evolution (a) and MDS evolution (b) based on blast count (p=0.010). The red line is the frequency of evolution linked to bone marrow-blast at diagnosis. Lower histograms refer to non-evolved patients. Upper histograms refer to the evolved patients.

Supplementary Figure 6. AML free survival (a) and Overall survival (b) based on dosing.

	Group A 149 patients	**Group B** 41 patients
Gender		
Female	105 (70,5%)	25 (61,0%)
Male	44 (29,5%)	16 (39,0%)
Age		
Median	75	71
Range	[38 ; 95]	[41 ; 87]
FAB Classification		
Refractory Anaemia	105 (70.5%)	24 (58,5%)
Refractory Anaemia with ringed sideroblasts	2 (1.3%)	0
Refractory Anaemia with excess of blasts	17 (11.4%)	3 (7.3%)
Missing	25 (16,8%)	14 (34.1%)
WHO Classification		
Refractory Anaemia	9 (6%)	3 (7.3%)
Refractory Anaemia with excess of blasts-1	15 (10.1%)	3 (7.3%)
Refractory Anaemia with excess of blasts-2	1 (0.7%)	0
Refractory cytopenia with multilineage dysplasia	25 (16.8%)	5 (12.2%)
MDS-5q	79 (53%)	26 (63.4%)
MDS-Unclassificable	3 (2%)	0
Missing	17 (11.4%)	4 (9.8%)
IPSS Classification		
LOW	69 (46.3%)	20 (48.8%)
INTERMEDIATE -1	80 (53.7%)	21 (51.2%)
WPSS Classification		
VERY LOW	7 (4.7%)	2 (4.9%)
LOW	51 (34.2%)	12 (29.3%)
INTERMEDIATE	16 (10.7%)	2 (4.9%)
HIGH	8 (5.4%)	1 (2.4%)
MISSING	67 (45%)	24 (58.5%)
Karyotype (only for Group A)		
5q isolated	122 (81.9%)	NA
5q not isolated	25 (16.8%)	NA
Complex	2 (1.3%)	NA
LEN cycles (median)		
	12 [5;24]	13 [4;34]

Table 1: Demographics, MDS classification according to IPSS, WPSS, FAB and WHO 2008 and cytogenetic characteristics of patients.

UPN	A/B	S/A	Diagnosis	Diagnosis (evolution)	Time from diagnosis to registry inclusion (months)		Registry observation period (months)	N° cycles	PLT	K (diagnosis)	K (evolution)	Cy Resp	Erythroid Resp
					Morph	Cy							
49259	B	M/65	RAEB1	AML	46	46	42	30 (7)	220000	/	Complex	/	Complete
50266	A	F/80	AR	AML	9	7	7	1	232000	Isolated 5q-	Complex	No	No
51081	A	F/65	RAEB 1	AML	60	0,42	6	4 (1)	88000	Isolated 5q-	Complex	No	No
52149	A	F/63	AR	AML	48	48	19	7 (1)	208000	Isolated 5q-	Isolated 5q-	No	Complete
52961	A	F/74	AR	AML	2	1	41	27	737000	Isolated 5q-	Isolated 5q-	No	Complete
54021	A	F/82	RAEB 1	AML	7	7	27	5	130000	5q- + 1 abn	Complex	No	No
59751	A	F/64	AR	AML	2	2	8	7	695000	Isolated 5q-	Isolated 5q-	No	No
80601	A	F/71	AR	AML	20	20	35	14	358000	Isolated 5q-	Isolated 5q-	No	No
81684	A	F/72	AR	AML	13	13	25	16	473000	Isolated 5q-	Complex	No	No
82594	B	M/73	AR	AML	2	1	8	8	17100	/	/	/	No
82814	B	M/66	AR	AML	2	0,25	2	2	76000	/	/	/	No
106412	A	F/68	AR	AML	0	0	14	14	587000	Isolated 5q-	Complex	No	Complete
122064	A	M/79	RAEB1	AML	2	2	10	6	159000	5q- + 1 abn	Complex	No	No
140846	A	F/71	AR	AML	3	0,5	11	11	20600	Isolated 5q-	Isolated 5q-	No	No
158370	B	F/85	AR	AML	0,5	0,5	4	4	22100	/	/	/	No
171549	B	M/72	AR	AML	4	1	2	2	8000	/	Complex	/	No
114677	A	F/76	AR	AML	18	15	31	9	78000	Isolated 5q-	Complex	No	No
159635	A	F/75	AR	AML	24	1	10	10	21400	Isolated 5q-	Isolated 5q-	No	Complete
48876	A	M/72	AR	RAEB1	10	42	33	35 (2)	122000	Isolated 5q-	Abnormal without 5q-	No	Complete
49105	A	F/66	RAEB1	RAEB2	29	7	14	8	154000	Isolated 5q-	Isolated 5q-	No	No
49570	A	M/67	AR	RAEB1	36	6	40	33 (2)	298000	Isolated 5q-	Isolated 5q-	No	Complete
49634	A	F/61	AR	RAEB2	26	19	20	6	237000	Isolated 5q-	Isolated 5q-	No	Complete
49974	B	F/61	AR	RAEB1	60	41	39	56 (17)	87000	/	/	/	Complete
50185	A	F/74	AR	RAEB1	54	27	31	29 (1)	55000	5q- + 1 abn	5q- + 1 abn	No	Complete
50368	A	M/58	AR	RAEB2	12	8	31	23 (1)	45000	Isolated 5q-	5q- + 1 abn	No	Complete
51948	B	F/71	RAEB1	RAEB2	6	35	7	4	201000	/	Isolated 5q-	/	No
71655	A	M/67	AR	RAEB1	84	25	12	17 (5)	121000	Isolated 5q-	Isolated 5q-	No	No
132971	A	M/89	AR	RAEB2	36	8	26	22	243000	Isolated 5q-	Isolated 5q-	No	Complete
145855	B	F/76	AR	RAEB2	24	2	8	7	157000	/	/	/	No
157011	A	F/68	AR	RAEB1	53	14	17	17	88000	5q- + 1 abn	5q- + 1 abn	No	Complete

Table 2: Description of cases progressed to AML or to higher risk MDS (in brackets the number of cycles before the enrollment in the registry).

UNIVARIATE ANALYSIS		
	OR (95% CI)	**P-value**
Blasts	1.10 [0.93 - 1.29]	0.261
Granulocytic dysplasia	0.56 [0.25 - 1.27]	0.164
Erythroid dysplasia	5.35 [1.08 – 26.4]	**0.039**
Megakaryocytic dysplasia	2.77 [0.93 - 8.24]	0.066
Platelets(≥100.000)	0.58 [0.20 - 1.67]	0.312
ALIP	0.67 [0.06 - 7.55]	0. 749
MCV	0.98 [0.94 - 1.01]	0.184
Bone marrow fibrosis	0.41 [0.07 - 2.23]	0.302
Hb (g/dl)	0.98 [0.80 - 1.20]	0.871
Karyotype (5q- isolated)	2.04 [0.65 - 6.35]	0.219
IPSS (Low risk)	0.81 [0.37 - 1.76]	0.597
Initial dosage (5mg)[§]	0.14 [0.05 - 0.41]	**<0.001**
Nr. Cycles (≥6)[§§]	0.90 [0.28 - 2.92]	0.863
MULTIVARIATE ANALYSIS		
	OR (95% CI)	**P-value**
Erythroid dysplasia	4.85 [0.82 -28.63]	0.081
Megakaryocytic dysplasia	2.19 [0.66 - 7.24]	0.198
Initial dosage (5mg)[§]	0.12 [0.04 - 0.37]	**<0.001**

[§] Reference 10 mg (either continuous or for 21 days)

[§§] Reference <6 cycles

Table 3: Factors associated with cytogenetic response (any response, both complete and partial) in group A.