Lenalidomide with or without Erythropoietin in transfusion dependent erythropoiesis-stimulating agent-refractory lower risk MDS without 5q deletion

Running title: Lenalidomide in transfused non del 5q low risk MDS

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ABSTRACT

After failure of erythropoiesis-stimulating agents (ESA), Lenalidomide (LEN) yields red blood cells (RBC) transfusion independence (TI) in 20-30% of lower risk non-del5q MDS. Several observations suggest an additive effect of ESA and LEN in this situation. We performed a randomized phase III study in 131 RBC transfusion-dependent (TD, median transfusion requirement 6 RBC units/8weeks) lower risk ESA-refractory non-del5q MDS. Patients received LEN alone, 10mg/day, 21 days/4 weeks (L arm) or LEN (same schedule) + erythropoietin (EPO) beta, 60,000U/week (LE arm).

In an intent-to-treat (ITT) analysis, erythroid response (HI-E, IWG 2006 criteria) after 4 treatment cycles (primary endpoint) was 23.1% (95% CI: 13.5-35.2) in the L arm and 39.4% (95% CI: 27.6-52.2) in the LE arm (p=0.044), while RBC transfusion independence (TI) was reached in 13.8 and 24.2% of the patients in the L and LE arms, respectively (p=0.13). Median response duration was 18.1 and 15.1 months in the L and LE arms, respectively (p=0.47). Side effects were moderate and similar in the 2 arms. Low baseline serum EPO level and a G polymorphism of CRBN gene predicted HI-E.

Combining LEN and EPO significantly improves erythroid response over LEN alone in lower risk non-del5q MDS patients with anemia resistant to ESA.
INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders characterized by ineffective hematopoiesis leading to blood cytopenias and a variable risk of evolution to acute myeloid leukemia (AML)\(^{(1)}\).

In International Prognostic Scoring System (IPSS)\(^{(2),(3)}\) Low or Intermediate-1 risk patients (“lower risk” MDS), the risk of AML progression is relatively low, survival generally prolonged, and treatment of anemia is the main therapeutic challenge in most cases. In the absence of isolated chromosomal 5q deletion (del5q), erythropoiesis-stimulating agents (ESA) give 30 to 50% erythroid responses, with a median response duration of about 2 years\(^{(4),(5),(6),(7),(8)}\).

Second line treatments after relapse or resistance to ESA include hypomethylating agents, yielding response rates between 25 to 35%\(^{(9)}\), while various other agents are currently being evaluated, including lenalidomide (LEN).

LEN, a thalidomide analogue, yields 60 to 70% erythroid response (HI-E) and transfusion independence (TI) in lower risk MDS patients with del5q\(^{(10),(11),(12)}\), and is approved in this situation in many countries. In lower risk MDS patients without del5q resistant to ESA alone, LEN yields lower TI rates of 20% to 30%\(^{(10),(13),(14),(15)}\). However, our recent clinical experience suggested that the response rate to LEN could be increased by the addition of ESA\(^{(14)}\).

To analyze more precisely this finding, we designed a phase III prospective open label randomized trial comparing LEN alone and LEN combined to erythropoietin (EPO) as second
line treatment in non del5q RBC transfusion-dependent lower risk MDS patients with primary or secondary resistance to ESA.

**METHODS**

**Patients**

Main inclusion criteria were: (i) MDS according to World Health Organisation (WHO) 2008 criteria\(^{(16)}\), with Low or Intermediate-1 IPSS and ECOG status ≤2, (ii) no del5q based on conventional chromosomal or (if less than 20 mitoses were obtained) FISH analysis, (iii) RBC transfusion dependence (TD) ≥4 packed RBC units during the 8 weeks before inclusion (administered at hemoglobin levels <9g/dl), (iv) no erythroid response to epoetin (≥60,000 Units/week) or darbepoetin (≥250 µg/week) administered during at least 12 consecutive weeks (i.e. a sufficient treatment duration to assess response according to most studies and recommendations)\(^{(17)}\) or relapse after an initial response, both defined according to International Working Group (IWG) 2006 criteria\(^{(18)}\), (v) platelet count >50G/L and absolute neutrophil count (ANC) >0.5G/L, and (vi) written informed consent.

Patients receiving chelation therapy were eligible if chelation was continued at similar dosage, but onset of chelation therapy was not allowed during the trial.

The study was approved by an ethics committee (CPP Ile-de-France X) and registered at clinicaltrials.gov (NCT01718379), EudraCT number 2008-008262-12.

**Study design**

This was a two-parallel arms open multicenter phase III clinical trial. Randomization between the two arms (1:1) was centralized and performed at the Unité de Recherche Clinique, Hôpital Cochin (Paris), using computerized lists based on permutation blocks stratified by centers. Patients were randomized between the LEN (L) arm: LEN alone 10 mg/day for 21 days every
28 days, and the LEN plus EPO (LE) arm: same LEN schedule combined to epoetin beta (60,000 units/week) (Figure 1). Celgene (Summit, NJ, USA) and Roche (Basel, Switzerland) provided Lenalidomide and epoetin beta, respectively, for the study, along with a research grant, but were not involved in data analysis and reporting.

Blood counts were monitored weekly during the first four months of treatment, and every two weeks thereafter. The LEN dose was reduced to 5mg/day if ANC decreased below 1G/L and/or platelets below 50G/L, with further dose reductions to 5mg every other day and 5mg twice a week. The use of G-CSF was not allowed in case of neutropenia, due to its potential effect on the erythroid lineage when combined to an ESA (5).

Responders after 4 cycles were planned to continue the same treatment until relapse. A new bone marrow aspirate and cytogenetic analysis were performed after 4 cycles, and then every 6 months in responders.

**Assessment of response and safety**

The primary endpoint chosen for the trial was HI-E according to IWG 2006 criteria (18) after 4 treatment cycles, *i.e.* reduction of RBC transfusions of at least 4 units during the 8 weeks prior to evaluation compared to the pretreatment period, for transfusions performed at hemoglobin levels <9g/dL.

Secondary endpoints were TI, defined by the absence of RBC transfusion during the 8 weeks before evaluation, HI-E duration, time to progression to higher risk MDS or AML, and treatment safety. Adverse events were assessed using NCI-CTC version 3.0.

All responses were blindly reviewed by an independent committee (composed of Dr V. Santini, Firenze, Italy, and Dr A. Giagounidis, Dusseldorf, Germany).

**CRBN polymorphism**

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Genetic studies were performed on marrow genomic DNA collected at inclusion. *Cereblon* (*CRBN*) gene A>G polymorphism located in the 5’UTR was analyzed, as previously described(19).

**Sample size justification**

Sample size computation was based on the primary endpoint, assuming a response rate of 30% and 57% in the control and experimental arms, respectively, based on previous findings with LEN alone and LEN combined to an ESA(13),(14). With a type I and type II error rates fixed at 0.05 and 0.20, respectively, a minimum of 63 patients had to be enrolled in each randomized arm, based on a two-sided chi-square test with continuity correction.

**Statistical analysis**

Summary statistics were reported as median [interquartile range, IQR] for continuous variables and percentages otherwise. Analysis was based on the intent-to-treat (ITT) principle, including all patients allocated to their randomized arm whatever the treatment actually administered, except in case of consent withdrawal. All patients without response evaluation at 4 courses (primary endpoint) were considered as failures; such a simple imputation was used in all the manuscript unless specified in the text. Reasons for patient discontinuation that could be informative about treatment effectiveness were tabulated. Sensitivity analyses to such an assumption were then performed, assuming failure in one arm and response in the other arm. The response rate was estimated with 95% confidence interval (95%CI), compared between the randomized arms by the Chi-square test. Multivariate logistic regression was then performed to assess the prognostic set of baseline characteristics of the patients. Treatment-by-covariates interactions were tested using the Gail and Simon statistics(20). Time to failure data (response duration) were analyzed using Kaplan-Meier estimate and log-rank test.
All statistical tests were two-sided, with p-values of 0.05 or less denoting statistical significance. All analyses were performed on SAS 9.3 (SAS Inc, Cary, NC) and R version 3.0.2 (2013-09-05) ([http://www.R-project.org/](http://www.R-project.org/)) packages.

**RESULTS**

**Baseline patient characteristics**

132 patients from 34 French centers were included from July 2010 to June 2012, 66 patients per treatment arm. One patient withdrew consent before the onset of treatment, and the ITT analysis was therefore performed in the 131 remaining patients. Baseline characteristics of the two arms are summarized in Table 1.

Median age at inclusion was 73 years [IQR: 64-77] and M/F ratio was 2. According to WHO classification, there were 57 (43.5%) refractory anemia with ring sideroblasts (RARS), 24 (18.3%) refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS), 22 (16.7%) refractory anemia with excess blasts type 1 (RAEB1), 15 (11.5%) refractory cytopenia with multilineage dysplasia (RCMD), and 13 (9.9%) unclassified MDS (MDS-U).

According to classical IPSS, 57 (43.5%) and 74 (56.5%) patients had Low and Intermediate-1 risk, respectively. The median number of RBC units administered during the 8 weeks before inclusion in this series was quite high, i.e. 6 units [IQR 4-8]. Most (n=110, 84.0%) patients had favorable cytogenetics according to classical IPSS, while no patient had unfavorable cytogenetics. Median serum EPO level was 225.5 U/l [IQR: 69-498.5]. Eighty-one (61.8%) of the patients had primary resistance to ESA, and 50 (38.1%) had relapsed after an erythroid response (Table 1).

Twenty-one patients (16.0%), 11 in the L arm and 10 in the LE arm, were also refractory to azacitidine administered after ESA failure, and were included after a wash-out period of at least 4 weeks.
least 3 months. At inclusion, 62 (47.3%) patients were receiving iron chelation with deferasirox.

_Treatment courses and observed responses_

A total of 32 patients did not receive the 4 planned courses, 16 in each randomized arm, due to adverse events (n=21), early death (n=4), investigator’s or patient’s decision (n=5), suicide (n=1), loss-to follow-up (n=1). One patient (LE arm) did not receive any treatment because of early death, 10 patients received only one course (5 in each randomized arm), 10 patients received two courses (3 in the L arm and 7 in the LE arm) and 11 patients received three courses (8 in the L arm and 3 in the LE arm) (Figure 2).

Among the 99 (75.6%) patients who received at least 4 cycles of treatment, 49 patients in the L arm and 50 patients in the LE arm, HI-E after 4 cycles was observed in 15 (30.6%) patients in the L arm and 26 (52%) patients in the LE arm, while TI was achieved in 25 patients, including 9 (18.3%) and 16 (32%) in the L and LE arm, respectively.

**ITT analyses**

Based on the ITT analysis, HI-E after 4 cycles was 23.1% (15/65) in the L arm (95%CI: 13.5-35.2) and 39.4% (26/66) in the LE arm (95%CI: 27.6-52.2; p=0.044 by the Chi-square test). Sensitivity analyses mostly reached similar conclusions (Table 2). TI was achieved in 13.8% (9/65) and 24.2% (16/66) of the patients in the L and LE arms, respectively (p=0.13 by the Chi-square test). The median time to erythroid response was estimated at 4 months in both arms. In patients who achieved transfusion independence, the mean peak increase in hemoglobin level compared to baseline was 1.8 g/dL (range 0.5-2.5) and 2.6 g/dL (range 0.5-8.2) in the arm L and LE, respectively, with no significant difference between the two arms.
Seven (33%) of the 21 patients with baseline cytogenetic abnormalities achieved HI-E after 4 cycles, but none of them achieved cytogenetic response.

Among the 58 non-responders after the 4th cycle, who therefore discontinued treatment, late responses were observed in 2 patients (one in each arm), 4 and 6 weeks after evaluation in the L and LE arm, respectively. However, they were not included as responders for subsequent analyses.

Relapse after erythroid response occurred in 7 of the 15 responders in the L arm and in 13 of the 26 responders in the LE arm. Median response duration was estimated at 18.1 months (95%CI: 7.6-NA) in the L arm, and 15.1 months (95%CI: 10.5-NA) in the LE arm (p=0.64 by the log-rank test) (Figure 3). Fourteen patients (7 in each arm) were still responders at the end of the study (i.e. at 2 years), 12 of them (5 in L arm and 7 in LE arm) being TI. Median TI duration was 18.5 months (95%CI: 12.69-NA) in the L arm, and 11 months (95%CI: 6.07-NA) in the LE arm (p=0.31 by the log-rank test).

Prognostic factors of response

None of the following pretreatment factors: gender, age, WHO classification, cytogenetics (according to classical IPSS), classical IPSS, type of ESA resistance (primary or secondary) had any significant impact on HI-E in the two treatment arms (Table 3). By contrast, baseline serum EPO level below 100 UI/l (OR= 3.3, 95%CI: 1.35-7.9; p=0.0087) and presence of the G allele at CRBN rs1672753 (OR= 2.6, 95%CI: 1.09-6.3; p=0.032) were associated with a better response rate (Table 3). When considered jointly with the randomization arm into a multivariate logistic model based on the 99 patients who were tested for CRBN rs1672753 polymorphism, both the treatment arm (OR= 5.0, 95%CI: 1.64-15.1; p=0.0047) and baseline serum EPO level (OR= 4.05, 95%CI: 1.3-12.6; p= 0.016) were found to add predictive information to each other (Table 4).
We then searched for treatment-by-covariates interaction to assess whether the difference in HI-E response rate after 4 cycles between the 2 randomized arms depended on baseline patient characteristics. No evidence of any difference in the benefit of LE was found according to those characteristics except for RBC transfusion requirement, where the benefit of LE was restricted to patients who had received, over the 8 weeks prior to inclusion, 4 RBC units (OR= 6.6, 95%CI: 2.08-21.4) compared to those who had received >4 RBC units (OR=1.32, 95%CI: 0.87-2.01) (p=0.010 by the Gail and Simon test) (Figure 4). Thus, in the 39 patients who had received only 4 RBC units in the 8 weeks before inclusion (18 patients in L arm and 21 patients in LE arm), the TI rate was significantly higher in the LE arm (10/21 patients, 47.6%) than in the L arm (3/16 patients, 18.7%) (p=0.04).

Of note also that for karyotype, the benefit of LE appeared to be restricted to patients with favorable cytogenetics (representing 84% of the patients), with borderline significance (P=0.056 by the Gail and Simon test) (Figure 4).

Finally, in the 50 patients with serum EPO levels ≥ 100 U/L there were 5/24 (21%) erythroid responses in the L arm and 10/26 (38%) in the LE arm (p=0.10).

**Toxicity**

Hematological and non hematological toxicity was comparable in the two treatment arms. Before the end of the fourth cycle, grade 3 and 4 non hematological adverse events (AEs) were observed in 25% of the patients, including deep vein thrombosis (DVT) in 4 patients (3 in the L arm and 1 in the LE arm) (Table 4), while grade 3 or 4 myelosuppression occurred in 25% of the cases and included neutropenia in 21% and 18% of patients in the L and LE arm, respectively, and thrombocytopenia in 3% and 7% of patients, respectively, with no difference between the two arms (Table 5).
During the first 4 cycles, the dose of LEN was reduced in 31% and 36% of the patients in the L and LE arm, respectively, and the mean daily dose of LEN administered was 8.4 mg in both arms. After the 4th cycle, LEN doses had to be reduced in 50% and 57% of responders in the L and LE arm respectively, and the mean daily doses of LEN administered were 8.5 mg in arm L and 8.1 mg in arm LE.

**DISCUSSION**

In this randomized study, LEN combined with EPO induced a significantly higher erythroid response rate than LEN alone in ESA-resistant RBC TD lower risk MDS without del5q. The estimated HI-E response rate among all the population was 39.4% in the LE arm compared to 23.1% in the L arm, and these figures reached 52% and 30.6%, respectively, in patients who received at least 4 cycles of treatment. However, RBC-TI rates were not significantly different between the 2 treatment arms (24.2% in the L arm versus 13.8 % in the LE arm, p=0.13).

All patients included in the study could be considered resistant to ESA, combined or not to G-CSF or were in relapse after an initial response. Indeed, although some responses to ESA (+/- G-CSF) may occur after more than 12 weeks, this is relatively rare, and most treatment guidelines use a 12 weeks period of treatment with at least 60,000 units of EPO or 250 µg of darbepoetin per week to define ESA resistance\(^6,21\).

The rationale for combining an ESA to LEN treatment was based on the hypothesis that LEN could restore sensitivity of MDS erythroid progenitors to EPO. Biological studies have demonstrated that LEN increases the expansion of normal mature erythroid progenitors of CFU-E type in the presence of EPO\(^22\). The Cereblon protein, encoded by the \(CRBN\) gene, is the molecular target of LEN and a substrate receptor of CRBN-CRL4 E3 ubiquitin ligase complex. Recent evidence shows that LEN binding to Cereblon induces the proteosomal
degradation of casein kinase 1A, a protein encoded by CSNK1A1 located in 5q33.1 in myeloid cells with a del5q, while Cereblon targets in non del5q MDS remain to be determined\(^{(23)}\). Thus, the mechanism of action of LEN is still unclear in these cells. However, LEN may directly improve EPO receptor signaling in non del5q cells by inhibiting the protein tyrosine phosphatase CD45, leading to the reversal of CD45-induced inhibition of EPO-R/STAT5 signaling, and also by improving the membrane lipid raft integrity, and thus the EPO receptor platform\(^{(24),(25)}\). A clinical benefit of LEN combined to ESA in lower risk MDS without del5q resistant to ESA alone had been observed in a French non randomized compassionate program, where 36% and 55% of the patients who received LEN alone and LEN combined with an ESA, respectively, achieved HI-E\(^{(14)}\). In another report from Moffitt’s center\(^{(26)}\), 4 of 19 (21%) lower risk MDS patients resistant to an ESA alone and to LEN alone responded to a LEN+ESA combination.

Our response rate in the L arm was apparently somewhat lower than that obtained by Raza et al.\(^{(13)}\) in a similar population (where RBC TI was achieved in 26% of the cases, compared to 13.9% in our hands). However, in Raza et al’s study\(^{(13)}\), ESA resistance was not documented in all patients (contrary to the present study). In that study\(^{(13)}\), patients who fulfilled our inclusion criteria (at least 4 RBC concentrates administered for Hb<9g/dL in the 8 weeks preceding study treatment) had a TI rate of 17%, similar to our results in the L arm. In another recent study using LEN alone (MDS 005 study)\(^{(15)}\) in a similar population, so far presented only in abstract form, RBC-TI was achieved in 26.7% of the patients. There was no RBC minimal transfusion requirement in Raza et al. study\(^{(13)}\) and a minimal transfusion requirement of 1.5 RBC units/28 days in MDS-005 study\(^{(15)}\), compared to 4 units/8 weeks in the present study, possibly explaining the higher transfusion burden we observed.

In previous studies of lower risk MDS patients without del5q treated with LEN\(^{(13),(14),(26)}\), TD<2 units/month, platelets>150G/L, shorter duration of MDS, lower LDH level, favorable
karyotype\(^\text{(13)}\), lower serum EPO level\(^\text{(26)}\), and more favorable WHO classification\(^\text{(14)}\) were associated with higher HI-E. In our study, baseline EPO level was the only conventional factor predicting HI-E, and it was the only prognostic factor in multivariate analysis. We also confirmed the predictive value for HI-E of a G polymorphism in the 5’ UTR of the \(CRBN\) gene\(^\text{(19)}\) in univariate analysis. The predictive value of this G polymorphism should however be confirmed in an external cohort. In addition, reasons for this possible impact of \(CRBN\) rs1672753 on response to LEN, with or without EPO, in MDS cells remain to be identified. We also found that only patients with favorable karyotype according to conventional IPSS seemed to benefit from the addition of EPO to LEN. However, this cytogenetic category represented 84% of the patients included. The absence of cytogenetic responses after 4 months in patients with baseline cytogenetic abnormalities in our study may support a different mechanism of action of the drug in MDS without del5q and MDS with del5q where LEN often leads to cytogenetic response\(^\text{(27)}\).

Response duration and TI duration did not significantly differ between the 2 arms (median duration of 18.1 versus 14.6 months, and 18.5 and 11 months in the L and LE arm, respectively). By comparison, median TI duration with LEN alone was 10 months in Raza et al.’s study\(^\text{(13)}\), 8.2 months in Santini et al.'s study\(^\text{(15)}\).

Side effects were similar in the 2 arms. Severe neutropenia and thrombocytopenia with LEN are less frequent and less profound in non del5q than in del5q patients. In Raza et al’ s study\(^\text{(13)}\), the incidence of grade 3 or 4 neutropenia and thrombocytopenia was 25% and 20% respectively, with little difference observed between patients receiving the 10 mg daily and 21-day lenalidomide schedule. The incidence of grades 3 or 4 neutropenia and thrombocytopenia was similar in our study, 24% and 25% respectively, and those cytopenias were the major reasons for early treatment discontinuation. Still, about 20% of the patients had early protocol discontinuation due to side effects, mainly cytopenias, and the dose of
LEN had to be reduced in 31% and 36% of the patients in the L and LE arm, respectively, also due to cytopenias. These results suggest that a daily dose of LEN of 10mg during 3 weeks every 4 weeks may be too high in a substantial proportion of the patients. A lower starting dose may have to be considered in future studies, in order to avoid early discontinuation by many patients. Management recommendations, based to some extent on those published for LEN in lower risk MDS with del 5q\(^{(28)}\) could also be useful. In MDS 005 study\(^{(27)}\), grade 3-4 neutropenia and thrombocytopenia were reported in 62% and 36% of the patients, respectively.

In our study, non hematological grade 3 and 4 toxicity was rare and included DVT in 3% of patients. Of note, the addition of high dose EPO did not increase the incidence of thrombotic events.

Our current results with LEN and EPO appear at least equivalent to those reported in lower risk MDS without del5q resistant to ESA treated with hypomethylating agents, in our experience\(^{(29)}\) and in a Nordic study\(^{(30)}\). Interestingly, we found in lower risk MDS without del5q resistant to ESA that the addition of EPO did not improve the response rate to AZA\(^{(29)}\) (manuscript in preparation). Other treatments in this situation have yielded only modest results. Immunosuppressive treatment with anti-thymocyte globulin with or without cyclosporine or with alemtuzumab can yield response rates i.e. TI up to 30 to 40%, but in highly selected patient populations\(^{(31),(32)}\). Among innovative agents, the glutathione-S-transferase P1-1 (GSTP1-1) inhibitor Ezatiostat and the MAP kinase inhibitor ARRY 614 both gave a 29% erythroid response rate in heavily pretreated lower risk MDS without del5q\(^{(33),(34)}\), while early results using drugs with TGF beta pathway ligand traps (ACE 011 and ACE 536) appear promising\(^{(15),(35)}\).
A possible weakness of our work is that we chose HI-E according to IWG 2006 criteria, but not RBC-TI, as primary study endpoint, and that the patient number was not powered on the RBC-TI endpoint. Achieving TI, and not only a significant reduction of transfusion requirement, may indeed be considered as a better endpoint from a clinical perspective, and most recent clinical studies in lower risk MDS have chosen TI as primary endpoint, an endpoint that may also be required by health agencies like FDA and EMA \(^{(13),(27)}\). There was indeed no significant difference in TI rates in the whole patient series between the L and the LE arm. However, in this very heavily transfusion dependent population, when we restricted the analysis to patients who had received only 4 RBC units/8 weeks before inclusion, TI rates were significantly higher in the LE arm than in the L arm (47% versus 16%, \(p=0.04\)).

In conclusion, our prospective phase III randomized study shows that a LEN plus EPO combination can yield higher erythroid response rates than LEN alone in lower risk MDS without del 5q resistant to ESA. This did not translate into a significant improvement of RBC-TI rate, possibly due to the insufficient size of our patient population or to the high transfusion burden in our patients, as TI rate differences were significant between the 2 treatment arms when the analysis was restricted to patients who did not receive more than 4 RBC units in the 8 weeks before inclusion. Tolerance of this treatment association was generally acceptable, although combinations using a lower initial dose of LEN may have to be explored to avoid early treatment discontinuation in a substantial proportion of the patients.

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AUTHORSHIP CONTRIBUTIONS

AT performed clinical research, analyzed data, and wrote the paper

OK performed biological research, and wrote the paper

SC analyzed data and wrote the paper

AR, JD, AS, CR, OBR, AB, AGB, SW, DC, KL, BDR, DB, CG, BS, LS, VS, RP, BG, PCM, BC, CS, RB, LL, EW, GT, KB, FG, ALT, SC, KM, SN, CS, FI, EG, CP: performed research, and wrote the paper

MF, PF, FD: designed research, analyzed data, and wrote the paper

DISCLOSURES OF CONFLICTS OF INTEREST

PF has received honoraria and research funding from Celgene Corporation.

FD has received honoraria from Celgene and Novartis.

No competing financial interest are to declare for the others authors.
REFERENCES


FIGURE LEGENDS

Figure 1: GFM-LENEPO 2008 study

Treatment plan

(registered at clinicaltrials.gov (NCT01718379), EudraCT number 2008-008262-12)

Figure 2: CONSORT 2010 flow diagram of the GFM-LENEPO 2008 study

Figure 3: Response duration according to the randomization arm

Reported figures are the number of exposed patients in each randomized arm over time

L, Lenalidomide; LE, Lenalidomide + Erythropoietin.

Figure 4: Search for treatment-by-subset interactions

This forest plot displays the estimated effect of randomized arm on response rate (LE vs L) in patient subsets, using the odds ratio (OR) as the measure of effect. The heterogeneity in OR across the subsets are tested by the Gail and Simon test.
Table 1: Baseline Patient Characteristics overall and according to the randomization arm

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Randomized Treatment arms</th>
<th>All (n=131)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L arm (n=65)</td>
<td>LE arm (n=66)</td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>73 [64-77]</td>
<td>73.5 [68-76]</td>
</tr>
<tr>
<td>M/F</td>
<td>1.6</td>
<td>2.6</td>
</tr>
<tr>
<td>WHO classification, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RARS</td>
<td>25 (39.7)</td>
<td>32 (48.8)</td>
</tr>
<tr>
<td>RCMD-RS</td>
<td>16 (25.4)</td>
<td>8 (12.1)</td>
</tr>
<tr>
<td>RAEB1</td>
<td>7 (11.1)</td>
<td>15 (22.7)</td>
</tr>
<tr>
<td>RCMD</td>
<td>6 (9.2)</td>
<td>9 (13.6)</td>
</tr>
<tr>
<td>MDS-U</td>
<td>11 (16.9)</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>Cytogenetics (IPSS), N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>56 (86.1)</td>
<td>54 (81.8)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>9 (14.1)</td>
<td>12 (18.8)</td>
</tr>
<tr>
<td>IPSS, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>30 (46.1)</td>
<td>27 (40.9)</td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>35 (54.7)</td>
<td>39 (60)</td>
</tr>
<tr>
<td>Type of EPO resistance, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary resistance</td>
<td>39 (60)</td>
<td>42 (63.6)</td>
</tr>
<tr>
<td>Serum EPO level, U/L median [IQR]</td>
<td>Relapse</td>
<td>26 (40)</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>134 [69-306]</td>
<td>185 [72.5-498.5]</td>
</tr>
<tr>
<td>Number of RBC units transfused/8wk, median [IQR]</td>
<td>6 [4-8]</td>
<td>6 [4-8]</td>
</tr>
<tr>
<td>Genetic characteristics</td>
<td>L arm (n=52)</td>
<td>LE arm (n=47)</td>
</tr>
<tr>
<td>CRBN rs1672753 polymorphism, N (%)</td>
<td>A/A</td>
<td>35 (67.3)</td>
</tr>
<tr>
<td>G/A or G/G</td>
<td>17 (32.7)</td>
<td>13 (27.6)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; M/F, male/female; WHO, world health organisation; RARS, refractory anemia with ring sideroblasts; RCMD-RS, refractory cytopenia with multilineage dysplasia; RS, ring sideroblasts; RAEB, refractory anemia with excess blasts; MDS-U, MDS unclassified; IPSS, international prognostic scoring system; EPO, erythropoietin; RBC, red blood cells; CRBN, cereblon gene.
Table 2: Erythroid response according to IWG 2006 criteria (primary endpoint), in the two randomized arms

<table>
<thead>
<tr>
<th></th>
<th>L Arm (N=65)</th>
<th>LE Arm (N=66)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intent-to-treat analysis, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assuming missing outcomes as failures: Primary analysis</td>
<td>15 (23.1)</td>
<td>26 (39.4)</td>
<td>0.044</td>
</tr>
<tr>
<td>Sensitivity analyses, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assuming missing outcomes as failures in the L arm and responses in the LE arm</td>
<td>15 (23.1)</td>
<td>42 (63.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Assuming missing outcomes as responses in the L arm and failures in the LE arm</td>
<td>31 (47.7)</td>
<td>26 (39.4)</td>
<td>0.38</td>
</tr>
<tr>
<td>Assessed responses, N (%)</td>
<td>49 (75.4)</td>
<td>50 (75.8)</td>
<td>N=99</td>
</tr>
<tr>
<td>Reasons for non-observed outcomes*, N (%)</td>
<td>16 (24.6)</td>
<td>16 (24.2)</td>
<td>N=32</td>
</tr>
<tr>
<td>Adverse events</td>
<td>12</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>Progression/death</td>
<td>1 (death after 2 cycles)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Investigator’s decision</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient’s decision</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Suicide</td>
<td>1 (after 2 cycles)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Loss to follow-up</td>
<td>0</td>
<td>1 (after 2 cycles)</td>
<td>1</td>
</tr>
</tbody>
</table>

*All due to treatment discontinuation before the end of the 4th course; L, Lenalidomide; LE, Lenalidomide + Erythropoietin.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of patients</th>
<th>Number of HI-E response</th>
<th>% HI-E</th>
<th>OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>43</td>
<td>10</td>
<td>23.3</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>88</td>
<td>31</td>
<td>35.3</td>
<td>1.8 (0.78-4.12)</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70 years</td>
<td>45</td>
<td>12</td>
<td>26.7</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>≥70 years</td>
<td>86</td>
<td>29</td>
<td>33.7</td>
<td>1.4 (0.63-3.11)</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>WHO classification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RARS</td>
<td>57</td>
<td>17</td>
<td>29.8</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>RCMD-RS</td>
<td>24</td>
<td>5</td>
<td>20.8</td>
<td>0.6 (0.20-1.93)</td>
<td>0.41</td>
</tr>
<tr>
<td>RAEB1</td>
<td>22</td>
<td>8</td>
<td>36.4</td>
<td>1.3 (0.48-3.79)</td>
<td>0.58</td>
</tr>
<tr>
<td>RCMD</td>
<td>15</td>
<td>8</td>
<td>53.3</td>
<td>2.7 (0.84-8.60)</td>
<td>0.095</td>
</tr>
<tr>
<td>MDS-U</td>
<td>13</td>
<td>3</td>
<td>23.1</td>
<td>0.7 (0.17-2.89)</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Cytogenetics (IPSS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>21</td>
<td>7</td>
<td>33.3</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>110</td>
<td>34</td>
<td>30.9</td>
<td>0.89 (0.33-2.42)</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Risk (IPSS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>74</td>
<td>25</td>
<td>33.8</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>55</td>
<td>16</td>
<td>29.1</td>
<td>0.80 (0.38-1.71)</td>
<td>0.57</td>
</tr>
</tbody>
</table>
### Type of EPO resistance

<table>
<thead>
<tr>
<th></th>
<th>50</th>
<th>18</th>
<th>36.0</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>81</td>
<td>23</td>
<td>28.4</td>
<td>1.42 (0.67-3.01)</td>
</tr>
</tbody>
</table>

### Serum EPO level

<table>
<thead>
<tr>
<th></th>
<th>70</th>
<th>15</th>
<th>21.4</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100U/L</td>
<td>34</td>
<td>16</td>
<td>47.1</td>
<td>3.3 (1.35-7.9)</td>
</tr>
<tr>
<td>&lt; 100U/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### CRBN rs1672753 SNP

<table>
<thead>
<tr>
<th></th>
<th>69</th>
<th>23</th>
<th>38.3</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>30</td>
<td>17</td>
<td>56.7</td>
<td>2.6 (1.09-6.3)</td>
</tr>
<tr>
<td>G/A or G/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RARS, refractory anemia with ring sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RS, ring sideroblasts; RAEB, refractory anemia with excess blasts; MDS-U, MDS unclassified; IPSS, international prognostic scoring system; EPO, erythropoietin; CRBN, cereblon gene.
Toma et al.

Table 4: Multivariate analysis

<table>
<thead>
<tr>
<th>Patients tested for CRBN rs1672753 polymorphism (N=99)</th>
<th>IWG 2006 responses OR (IC95%)</th>
<th>Multivariate analysis p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE versus L treatment</td>
<td>5.0 [1.64-15.1]</td>
<td>0.0047</td>
</tr>
<tr>
<td>M/F</td>
<td>1.79 [0.78 ; 4.12]</td>
<td>NS</td>
</tr>
<tr>
<td>IPSS Low versus Intermediate-1</td>
<td>0.80 [0.38 ; 1.71]</td>
<td>NS</td>
</tr>
<tr>
<td>Type of EPO resistance: primary versus relapse</td>
<td>1.42 [0.67 ; 3.01]</td>
<td>NS</td>
</tr>
<tr>
<td>Serum EPO level &lt; 100 U/L versus ≥ 100 U/L</td>
<td>4.05 [1.3-12.6]</td>
<td>0.016</td>
</tr>
<tr>
<td>CRBN A/A versus G/A or G/G</td>
<td>0.38 [0.16 ; 0.92]</td>
<td>0.026</td>
</tr>
</tbody>
</table>

CRBN, cereblon gene; LE, Lenalidomide + Erythropoietin; L, Lenalidomide
Table 5: Grade 3 and 4 adverse events during the first four cycles

<table>
<thead>
<tr>
<th>Non hematological grade 3-4 AEs before the end of the 4th cycle (number of patients)</th>
<th>L Arm N=65</th>
<th>LE Arm N=66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep vein thrombosis</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Acute coronary syndrome</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Bowel obstruction</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Rash</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Renal failure</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Gout</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Other metabolic disorders</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

| Hematological grade 3-4 AEs before the end of the 4th cycle (number of patients) |
|---|---|
| Neutropenia | 14 | 12 |
| Thrombocytopenia | 2 | 5 |

AE, adverse event; L, Lenalidomide; LE, Lenalidomide + Erythropoietin.
**INCLUSION CRITERIA**

- Lower risk MDS  
  Low and Int-1 IPSS  
- Without del 5q  
- Transfusion dependence  
  ≥ 4 RBC units/8 weeks  
  before randomization  
- ESA failure  
  ≥ 12 consecutive weeks  
  ≥ 60,000 UI or 250µg/week  
  or relapse after ESA response

**RANDOMIZATION**

**Arm LEN**  
LEN 10 mg/day  
21 days every 28 days

**x 4 cycles of 28 days**

**Arm LEN + EPO**  
LEN 10 mg/d  
21 days every 28 days  
+  
EPO beta  
60,000 U/Week

**RESPONDERS (IWG 2006)**

LEN 10 mg/day  
21 days every 28 days

Until relapse

LEN 10 mg/day  
21 days every 28 days  
+  
EPO beta  
60,000 U/Week

Toma et al. - Figure 1: GFM LENEPO 2008 study – Schema and treatment plan
Patients enrolled (n=132)

Allocation

Randomly assigned to LEN alone treatment (n=66)
- Received allocated intervention (n=65)
- Did not receive allocated intervention (n=1, consent withdrawal)

Randomly assigned to LEN+EPO treatment (n=66)
- Received allocated intervention (n=66)
- Did not receive allocated intervention (n=0)

Induction treatment (4 cycles)

Discontinued intervention (n=16)
- Adverse events (n=12)
- Patient's or investigator's decision (n=2)
- Unknown reason (n=2)

Discontinued intervention (n=16)
- Adverse events (n=9)
- Patient's or investigator's decision (n=3)
- Death (n=3)
- Unknown reason (n=1)

Evaluation after 4 cycles

Received 4 cycles of LEN alone (n=49)

Received 4 cycles of LEN+EPO (n=50)