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# Lenalidomide treatment in lower risk myelodysplastic syndromes—The experience of a Czech hematology center. (Positive effect of erythropoietin ± prednisone addition to lenalidomide in refractory or relapsed patients)

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ABSTRACT

Lenalidomide therapy represents meaningful progress in the treatment of anemic patients with myelodysplastic syndromes with del(5q). We present our initial lenalidomide experience and the positive effect of combining erythropoietin and steroids with lenalidomide in refractory and relapsed patients. We treated by lenalidomide 55 (42 female; 13 male; median age 69) chronically transfused lower risk MDS patients with del(5q) (45) and non-del(5q) (10). Response, meaning transfusion independence (TI) lasting ≥ eight weeks, was achieved in 38 (90%) of analyzed patients with del(5q), of whom three achieved TI only by adding erythropoietin ± prednisone. Another five patients responded well to this combination when their anemia relapsed later during the treatment. In the non-del(5q) group only one patient with RARS-T reached TI. Cytogenetic response was reached in 64% (32% complete, 32% partial response). The *TP53* mutation was detected in 7 (18%) patients; four patients progressed to higher grade MDS or acute myeloid leukemia (AML). All seven RAEB-1 patients cleared bone marrow blasts during lenalidomide treatment and reached complete remission (CR); however, three later progressed to higher grade MDS or AML. Lenalidomide represents effective treatment for del(5q) group and combination with prednisone and erythropoietin may be used for non-responders or therapy failures.

## 1. Introduction

The most common cytogenetic abnormality of MDS is the deletion of the long arm of chromosome 5 (del(5q)), which occurs in nearly 30% of MDS patients [1]. The group of patients with this aberration is relatively heterogeneous with regard to clinical manifestation, although this depends on other factors such as bone marrow myeloblast count, additional cytogenetic aberrations, and the presence of mutations and cytopenias (especially thrombocytopenia) [2–5]. A special categorization is reserved among the subgroups of MDS for patients with isolated del(5q) without increased blasts. In this group, patients are predominantly those with 5q- syndrome, which, with regard to survival, is one of the most prognosticly favorable syndromes of MDS [6,7]. Unfortunately, within a few years the majority of these patients develop transfusion dependency, along with all its negative consequences, such as the organ siderosis, deterioration of quality of life, worsening morbidity, and decrease overall survival (OS). For these patients, the primary therapeutic aims consist of reaching a normalized blood count and eliminating transfusion dependency. In recent years, new therapeutic approach, immunomodulation therapy, represented by lenalidomide have been instituted in MDS treatment [8,9]. Lenalidomide have a significant effect, specifically in MDS patients with del(5q)

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[10,11]. Lenalidomide treatment leads to normalized blood count and, in some patients, to the minimization (or, less often, the disappearance) of the pathological clone, which is detectable by standard cytogenetic examination. Response to lenalidomide therapy occurs in about 60–70% lower-risk MDS patients with del(5q) and in about 90% of patients that meet the criteria of typical 5q- syndrome [11,12]. Particular caution needs to be taken with patients who have other cytogenetic aberrations, higher blast counts, the *TP53* mutation, and thrombocytopenia. These patients are at greater risk of earlier disease progression, and thus it is necessary, in their cases, to consider more aggressive therapy [13,14,3]. However, only about 25–27% of non-del (5q) patients treated by lenalidomide reach red blood cell transfusion independence (RBC-TI) [15,16]. The response rate in these non-del(5q) patients could be increased by combining EPO with lenalidomide. A randomized phase III study in 131 RBC transfusion-dependent (RBC-TD) lower-risk erythropoietin (EPO)-refractory non-del(5q) MDS patients who were treated by lenalidomide alone or in combination with EPO showed positive effect of adding EPO [17]. RBC-TI was reached in 13.8 and 24.2% of the patients in the lenalidomide versus lenalidomide + EPO arm. Low baseline serum EPO level and a G polymorphism of CRBN gene in this study predicted erythroid response. Nevertheless, additional clinical research is further required to determine who might be the potential lenalidomide responder within the non-del(5q) group. In our recent study, we have analyzed the mRNA level of Cereblon (CRBN) in MDS patients with del(5q), non-del (5q) MDS patients, and controls and found statistically significant higher levels in del(5q) patients, as well as amongst the lenalidomide responders [18].

In our work, we herein present one Czech University center's experience with lenalidomide treatment in lower risk del(5q) and non-del (5q) MDS patients and point to some specific clinical observations, namely the positive effect of the erythropoietin and prednisone addition to lenalidomide therapy in refractory and relapsed patients.

## 2. Patients and methods

Since 2007 we have provided lenalidomide treatment for 55 (42F/13M) MDS patients, median age 69 (range 51–83 years). The patients' characteristics are summarized in the Table 1. All patients belong to the International Prognostic Scoring System (IPSS) low or intermediate group 1. They represent two groups: 45 patients with del(5q) [36 cases (82%) with isolated del(5q), nine cases (18%) with del(5q) and one additional cytogenetic aberration] with classic sex distribution 39F/6M, and 10 non-del(5q) patients (3F/7M). Six out of these 10 patients were treated with lenalidomide when accepted into MDS 005 trial, which was approved by the Institutional Review Board and conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients prior to enrollment.

WHO 2008 (World Health Organization) diagnoses are listed in Table 1. All patients were examined by conventional G-banding and by FISH with appropriate Vysis locus-specific DNA probes (Abbott Molecular). Six out of nine patients with a single additional aberration to del (5q) had an unrelated clone with trisomy of chromosome 8 and represent a specific group. We have repeatedly collected bone marrow samples for cytogenetic analysis and for other studies every six months of therapy, then at 12 months, and then every other 12 months thereafter. Thirty-nine patients (70%) were tested for *TP53* mutations before lenalidomide treatment. In order to determine the mutational status of the *TP53* gene, we analyzed the patients' DNA isolated from the bone marrow mononuclear cells, and applied the method of new generation sequencing (454 GS Junior, Roche).

All studied MDS patients were red blood cell transfusion dependent (RBC-TD), with a median hemoglobin (Hb) level 80 g/l (51–100) at the beginning of treatment. RBC-TD was defined as the requirement of at least two transfusions per month for at least eight weeks prior to the initiation of therapy. Forty-four patients received erythropoietin (EPO) prior to lenalidomide treatment (34 del(5q)), 10 non-del(5q) with just one intermittent responder. One of our patients also presented an interesting case, which following the diagnosis of RAEB2 with isolated del (5q) was treated by azacitidine (18 cycles, 7-day regime, 75 mg/m$^2$/day) and reached bone marrow complete response with just transient transfusion independency (TI). This patient was switched to lenalidomide upon RBC-TD and reached until now more than 2 years lasting TI without any signs of disease progression.

**Table 1**
Characteristics of lenalidomide treated patients.

| Patients characteristics (No = 55) | Value |
|---|---|
| **Age (years)** | |
| median | 69 |
| range | 51–83 |
| **Sex** | |
| F | 42 |
| M | 13 |
| **WHO 2008 in 5q- group** | |
| Isolated del(5q) (true 5q- syndrome) | 28 |
| RCMD | 9 |
| RAEB 1 | 7 |
| MDS/MPN-U | 1 |
| **WHO 2008 in non 5q- group** | |
| RCMD | 7 |
| RARS | 1 |
| RARS-T | 2 |
| **IPSS (both groups)** | |
| ISPP low | 36 |
| IPSS int I | 16 |
| Unclassified | 3 |
| **Cytogenetic findings in 5q- group** | |
| Isolated del(5q) | 36 |
| two unrelated clones with del(5q) and +8 | 6 |
| del(5q),del(20q) | 1 |
| del(5q)der(19)t(1,19) | 1 |
| del(5q), t(2;11) | 1 |
| **Cytogenetic findings in non 5q- group** | |
| Normal karyotype | 10 |
| ***TP53*mutation (39 tested)** | |
| 5q- group | 7 (18%) |
| Non 5q- group | 0 |
| **Previous treatment** | |
| EPO | 44 (80%) |
| CyA + prednisone | 1 |
| Azacitidine | 1 |
| **Transfusion dependency** | 55 (100%) |
| **Hb**g/l (median, range) | 80 (51–100) |
| **Thrombocytopenia**($< 100 \times 10^9$/l) | 1 |
| **Time from diagnosis to lenalidomide start (months)** | |
| Median (range) | 15 (2–199) |

Abbreviations: CyA, cyclosporine, EPO, erythropoietin, Hb, hemoglobin F, female, IPSS, MDS/MPD-U, myelodysplastic/myeloproliferative neoplasm, M, male, unclassifiable international prognostic scoring system, RARS, refractory anemia with ring sideroblasts, RAEB-1, refractory anemia with excess of blasts, RARS-T refractory anemia with ring sideroblasts and thrombocytosis, RCMD, refractory cytopenia with multilineage dysplasia.

The median time from diagnosis to the beginning of lenalidomide therapy was 15 months, with a range of 2–199 months. Lenalidomide was administered at a dose of 5 mg from the beginning of treatment in 26 patients, and at a dose of 10 mg in 29 patients (including all non-del (5q) patients). The median duration of lenalidomide therapy was 15.5 months, with a range of 2–70 months.

Nine patients, six with del(5q) and three non-del(5q), were treated by EPO or by EPO plus prednisone in addition to lenalidomide when the initial response to lenalidomide was inadequate. In seven patients, all with del(5q), EPO ± prednisone was administered in addition to the lenalidomide therapy when their anemia relapsed during the lenalidomide treatment. Prednisone was added to EPO only when the response to EPO was not satisfactory within two months. All these patients received EPO before lenalidomide initiation, and all were primarily refractory patients. Median serum EPO level of these patients before

lenalidomide treatment was 300 mU/ml (range 197–774).EPO added to lenalidomide was administered at a dose of 40–80,000 units/week. Prednisone was started with a dose of 20 mg/day. The dose was decreased to 5 mg/day in all responding patients. None of these refractory or relapsed patients were candidates for bone marrow transplantation.

The comparison of the overall survival (OS) from the date of the first lenalidomide treatment of each group was evaluated using the Kaplan-Meier estimation. P-values $< 0.05$ were considered as statistically significant. The Log-rank test was used to compare the survival distributions between each patient group. Analyses were conducted using the GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com).

## 3. Results

In regards to response, we evaluated 52 patients (42 del(5q) and 10 non-del(5q)) with known RBC-TD. In the del(5q) group, we evaluated 42 patients who had been treated with lenalidomide longer than three months, of whom 38 (90.4%) reached RBC-TI. From these 38 del(5q) responders, two patients reached TI only after adding EPO and one patient after EPO plus prednisone. The characteristics of these EPO ± prednisone treated patients are summarized in Table 2. Three del(5q) patients did not respond even after EPO or EPO + prednisone. Among four del(5q) non-responders, there were two patients with the *TP53* mutation, and two patients with del(5q) and one additional cytogenetic aberration der(19)(q21.2;p13.3) and t(2;11)(p21;q23). On the other hand, all patients with two cytogenetically unrelated clones (del(5q) and trisomy 8) belonged among well responding patients and showed features very similar to patients with low risk MDS with isolated del(5q) as reported by Neuwirtova et al. [19]. There was only one responder diagnosed with RARS-T, out of the 10 non-del(5q) patients. Once again, this patient reached TI with lenalidomide but experienced a further increase of Hb ($> 90$ g/l) by combining the lenalidomide with EPO.

Response to lenalidomide treatment in all del(5q) patients was well demonstrated by increased erythropoiesis in the bone marrow. Response, as previously described in the literature, usually began at 2–8 weeks of therapy (median 4 weeks). Responses (RBC-TI) are detailed in the Table 3. The median duration of hematologic response (RBC-TI) was 35 months (3–79).

In terms of cytogenetic response in the del(5q) group, we evaluated consecutive bone marrow samples in 34 patients (75%) with ≥3 samples per patient in 23 of them (51%). Cytogenetic response was reached in altogether 64% of patients. Eleven (32%) patients achieved complete cytogenetic response (CCyR), but only in four of these patients did the response last longer throughout the course of the disease. Partial cytogenetic response (the shrinkage of pathological clones by more than 50%) was documented in 11 (32%) patients. Stable cytogenetic results were documented in six patients and an increase of the clone size was noted in two patients. However, all these patients are good clinical responders with TI. Four patients (12%) developed additional cytogenetic abnormalities, including complex karyotypes (≥3 aberrations) in three cases (two of them progressed). The median time to cytogenetic response was 12 months (range 6–23 months).

In terms of the dose/response relationship, we did not observe any correlative evidence between the cytogenetic response and the drug dosage. From 22 responding patients, 11 were treated with 5 mg daily and another 11 with 10 mg daily. CyCR was reached in six patients on 5 mg and in five patients on 10 mg of lenalidomide. Thanks to repeated BM collections we have documented 10 cytogenetic relapses amongst the patients who achieved cytogenetic response (CyCR + CyPR, 22 cases). Nine out of these 10 patients had stable complete hematological

**Table 2**
Basic characteristics and responses of lenalidomide plus EPO ± prednisone treated patients.

| No | Age | Sex | WHO 2008 | Cytogenetics | EPO level | Lenalidomide dose | EPO only | EPO + Prednisone | Response |
|---|---|---|---|---|---|---|---|---|---|
| **A/responders** | | | | | | | | | |
| Refractory or unsatisfactory responding to lenalidomide | | | | | | | | | |
| 1 | 82 | F | RAEB I | del(5q) | 55 | 10 mg | yes | no | RBC-TI |
| 2 | 68 | F | Isol.5q- | del(5q) | 300 | 5 mg | yes | no | RBC-TI |
| 3 | 65 | F | RARS-T | 46XX | 287 | 10 mg | yes | no | RBC-TI |
| 4 | 83 | F | Isol.5q- | del(5q) | 120 | 10 mg | no | yes | RBC-TI |
| Relapsed (transfusion dependency) | | | | | | | | | |
| 5 | 74 | F | Isol.5q- | del(5q), | 255 | 5 mg | yes | no | RBC-TI |
| 6 | 75 | F | Isol.5q- | del(5q) | 380 | 10 mg | yes | no | RBC-TI |
| 7 | 79 | F | Isol.5q- | del(5q) | 774 | 10 mg | no | yes | RBC-TI |
| 8 | 72 | F | Isol.5q- | del(5q) | 300 | 5 mg | no | yes | RBC-TI |
| 9 | 78 | F | RCMD | del(5q), +8 | 203 | 5 mg | no | yes | RBC-TI |
| **B/non-responders** | | | | | | | | | |
| Refractory or unsatisfactory responding to lenalidomide | | | | | | | | | |
| 10 | 71 | F | Isol.5q- | del(5q) | 600 | 10 mg | no | yes | no |
| 11 | 68 | F | MDS/MPD | del(5q) | 60 | 10 mg | no | yes | no |
| 12 | 60 | M | RCMD | 46XY | 769 | 10 mg | no | yes | no |
| 13 | 60 | M | RCMD | 46XY | 258 | 10 mg | no | yes | no |
| Relapsed (transfusion dependency) | | | | | | | | | |
| 14 | 74 | F | RCMD | del(5q),del(20q) | 456 | 10 mg | no | yes | no |
| 15 | 77 | F | RCMD | del(5q), +8 | ND | 10 mg | no | yes | no |
| 16 | 72 | M | RCMD | del(5q),t(2;11) | 400 | 10 mg | no | yes | no |

A/Patients responding to lenalidomide and EPO alone, or EPO plus prednisone.
B/Patients non-responding to lenalidomide and EPO alone, or EPO plus prednisone.
We divide our responders and non-responders each into two groups:
Refractory or unsatisfactory responding patients: those who were treated by lenalidomide at least 3–4 months were still transfusion dependent or did not increased hemoglobin (Hb) above 90 g/l.
Relapsed patients: those who reached RBC-TI but became RBC-TD, or had Hb level ≤ 80 g/l at least in 2 consecutive samples, later during the course of lenalidomide treatment.
Patients, who were treated by 5 mg, did not tolerate higher dose of lenalidomide due to thrombocytopenia or neutropenia.
EPO only: patients treated by lenalidomide plus EPO.
EPO + prednisone: patients treated by lenalidomide plus EPO and prednisone.
Abbreviation: EPO, erythropoietin, ND, not done, RBC-TD, red blood cell transfusion dependence, RBC-TI, red blood cell transfusion independence.
EPO level units: mU/ml.

**Table 3**
Erythroid response evaluation in relation to lenalidomide dosage.

| Variable (No,%) | Lenalidomide 10 mg | Lenalidomide 5 mg | All patients |
|---|---|---|---|
| **A/5q- group** | No = 19 | No = 23 | No = 42 |
| Transfusion independence (TI) | 17 (89%) | 21 (91%) | 38 (90%) |
| Time to TI, median (range) (weeks) | 4,2 (2–18) | 4,4 (3–20) | 4,3 (2–20) |
| Hb (g/l) baseline, median (range) | 78 (60–104) | 79 (51–97) | 80 (51–104) |
| Hb (g/l) first response, median (range) | 96 (90–120) | 95 (92–125) | 95 (90–125) |
| **B/non5q- group** | No = 10 | No = 0 | No = 10 |
| Transfusion independence (TI) | 1 (10%) | none | 1 (10%) |
| Time to TI (weeks) | 8 | | 8 |
| Hb (g/l) baseline, median (range) | 79 (51–88) | | 79 (51–88) |
| Hb (g/l) first response | 90 (single responder) | | 90 (single responder) |

response without any clinical signs of progression, had lasting complete TI at the time of the BM examination, and were on a stable lenalidomide dosage. The *TP53* mutation was detected in seven patients (18%), two of who carried two mutations simultaneously. The median of variant allele frequency (VAF) of detected mutations was 14.7% with a range of 1.1–44.2%. Four of these seven *TP53* mutation positive patients progressed to the higher risk MDS during the lenalidomide treatment.

The relapse of anemia (TD or decrease of Hb below 90 g/l) in patients still treated by lenalidomide was observed in seven del(5q) patients. All of these patients were further treated with a combination of lenalidomide and EPO ± prednisone. Five of these patients responded: three with just EPO, two to EPO + prednisone. We saw a positive effect of combining lenalidomide with EPO ± prednisone in nine out of 16 patients (four patients with initial insufficient response to lenalidomide and five patients with relapse of anemia). Moreover, two of our well-responding patients had an acute attack of polymyalgia rheumatica during the course of lenalidomide, both accompanied by a profound decrease of Hb and requirement of RBC transfusions. Both reacted promptly to a high dose of prednisone with an increase of hemoglobin and restoring TI.

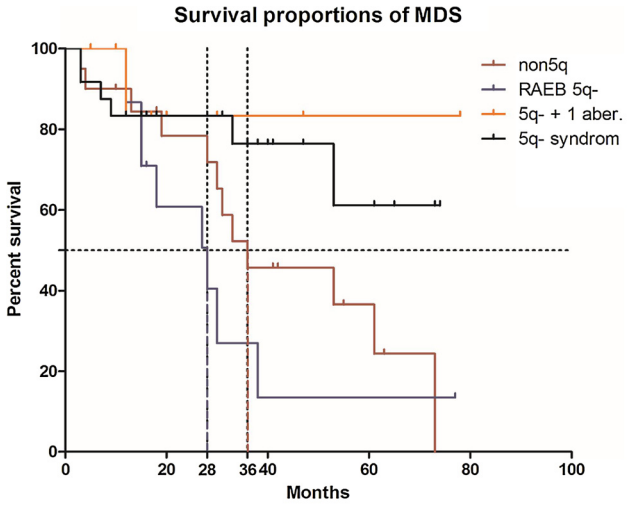
Lenalidomide was interrupted/discontinued in six well-responding and transfusion independent patients with del(5q) (4 with CyCR, 2 with CyPR). One of them relapsed after 12 months and responded well after resumption of lenalidomide treatment. Median duration of TI in this group is 38 months (12–54).

Interestingly, six patients with del(5q) (13%) but none of the non-del(5q) patients progressed to higher risk MDS or AML. These del(5q) patients' characteristics at the time of diagnosis, before lenalidomide treatment, were noted as follows: three patients with RAEB 1 and three patients with *TP53* mutation. Only one patient is currently alive and is on the azacitidine treatment. Consequent treatment of the six progressing patients included subcutaneous azacitidine (3 patients), allogeneic stem cell transplantation (1 patient), low dose of cytosine arabinoside (1 patient), and best supportive care (1 patient).

Nine del(5q) patients were no longer alive at the time of analysis; of these, five died from disease progression and transformation to acute leukemia (AML), while the other four died as a result of comorbidity. Four patients from the non-del(5q) group also died as a consequence of comorbidity.

In terms of median overall survival; we separate our patients into four subgroups. Median OS since the start of lenalidomide treatment was not reached in low risk MDS patients with isolated del(5q) (patients with features of 5q- syndrome) and in the group of patients with del(5q) and one additional aberration and was reached at 36 months for non-del(5q) patients and 28 months for RAEB1 (all with del(5q)). The median OS, much like the median of response duration (35 months, range 3–79), is influenced by a relatively recent treatment initiation. Regardless, we present the OS curves for various groups in Fig. 1.

Concerning adverse effects (we have evaluated all 55 patients together), the most frequent were hematological, with such as




**Legend**
Lenalidomide treated patients are divided into four groups: 10 non5q- patients, 7 RAEB 1 patients, 9 patients with del(5q) plus 1 cytogenetic aberration, and 28 low risk MDS patients with isolated del(5q) (5q- syndrome). P-value for Log-rank (Mantel-Cox) Test = 0.04; P-value for Logrank test for trend = 0.03.

**Fig. 1.** Patients' survival proportions represented as Kaplan Meyer curves. Lenalidomide treated patients are divided into four groups: 10 non5q- patients, 7 RAEB 1 patients, 9 patients with del(5q) plus 1 cytogenetic aberration, and 28 low risk MDS patients with isolated del(5q) (5q- syndrome). P-value for Log-rank (Mantel-Cox) Test = 0.04; P-value for Logrank test for trend = 0.03.

neutropenia occurring in 94% (in 70,6% toxicity grade 3,4), and thrombocytopenia in 88% (23,5% with grade 3,4). Later, throughout the course of therapy, we observed a gradual increase of neutrophils and thrombocytes. Nevertheless, hematologic toxicity was the primary reason for the reduction of the initial dosage from 10 mg to 5 mg per day, and in some cases even to 2.5 mg daily. Non-hematological toxicity occurred in 70% of patients, typically of grades 1 or 2 and well manageable. Most frequent were dermatological side effects, which usually presented as transient reactions such as dry skin, itching, and rashes. Fourteen percent of patients experienced gastrointestinal symptomatology (nausea, obstipation, diarrhea) not exceeding grade 2 toxicity. Unlike previous reports, we observed a higher number of thrombotic events. Five patients (9%) experienced venous thrombosis of the lower extremities. None were on steroid therapy at the time of thrombosis. One received lenalidomide plus EPO. All continued on lenalidomide treatment with anticoagulation therapy after the short lenalidomide discontinuation. We never terminated the therapy for reasons relating to adverse side effects.

## 4. Discussion

The introduction of immunomodulation therapy, represented by

lenalidomide, has been of great benefit for treating anemia in MDS patients. This treatment's high efficacy is, however, specifically tied to a smaller subgroup of MDS: patients with the del(5q). As stated above, previous large studies demonstrated a response in the form of transfusion independence in 60–70% of patients [10,11]. We observe an even higher response rate reaching 90% in our set of 42 evaluated patients with del(5q). This is likely because we predominantly treat patients with 'true' 5q- syndrome (63% of our del(5q) patients) with superior response to lenalidomide. Furthermore, for those patients not responding to lenalidomide we were able to improve the response significantly by the addition of EPO, or EPO plus prednisone. This combination improved further the clinical results and lead to a better response in eight del(5q) patients (19%) and one out of ten non-del(5q) MDS patients. The positive effect of the EPO addition to lenalidomide therapy was previously reported in non-del(5q) patients by Toma et al. [17]. Possible mechanisms are explored in Basiorka et al. [20], which suggest that lenalidomide is potentially involved in the up-regulation of expression and stability of the EPO receptor (EpoR) and in enhancing the JAK2-STAT 5 signaling pathway. Lenalidomide has E3 ubiquitin ligase inhibitory effects that extend to RNF41. The inhibition of RNF41 auto-ubiquitination promotes membrane accumulation of signaling competent JAK2/EpoR complexes that augment EPO responsiveness. It is important to note that those patients who improved their Hb levels after combining EPO with lenalidomide were EPO-refractory before the lenalidomide treatment, much like our group of patients.

The additional positive effect of administering steroids together with lenalidomide is explained by Narla et al. [21]. Corticosteroids increase the proliferation of erythroid progenitor cells *in vitro* and improve burst-forming units-erythroid (BFU-E) colony formation, whereas lenalidomide specifically increases colony-forming unit-erythroid (CFU-E) [21,22]. The author's findings indicate that dexamethasone and lenalidomide promote different stages of erythropoiesis and thus the combination of steroids plus lenalidomide might possess a synergistic clinical effect. Narla et al. described the effects of dexamethasone and lenalidomide, individually and in combination, on the cell differentiation of primary human bone marrow progenitors *in vitro*. Both agents promoted erythropoiesis by increasing the absolute number of erythroid cells produced from normal $CD34^+$ cells. Authors also found that lenalidomide with dexamethasone promotes erythropoiesis in *RPS14*- and *RPS19*-deficient cells and suggested that lenalidomide and dexamethasone, individually or in combination, have the potential to increase red blood cell production in erythroid disorders of ribosome dysfunction, such as MDS with del(5q).

Among the excellent responders to lenalidomide in our set of patients is a specific group with two cytogenetically unrelated clones with del(5q) and trisomy 8, that was previously described by us in Neuwirtova et al. [19]. This group has striking similarities to real 5q-syndrome. We even observed in one patient the disappearance of both clones (del(5q) and +8). On the other hand, two patients with different additional aberrations in one clone with del(5q) were refractory to the lenalidomide therapy.

Even patients with the RAEB 1 subtype reached surprisingly positive results. All seven patients were very good responders in terms of reaching transfusion independence and all cleared the bone marrow blasts; but later, during the course of therapy, three patients relapsed and their disease progressed. Interestingly, the decrease of bone marrow blasts was not always accompanied by cytogenetic response. We thus suggest that these patients should be carefully and more frequently monitored, and have regular bone marrow examinations to detect early progression to a more advanced stage of disease.

We found just one responder in the non-del(5q) group with a WHO 2008 diagnosis RARS-T, a disease rather similar in terms of peripheral blood findings and OS to 5q- syndrome. We know of only a few case reports in the literature with positive responses to lenalidomide in these patients [23]. Our one RARS-T patient reached TI after lenalidomide and also showed further increase of hemoglobin following the addition of EPO therapy.

Our results support previous studies that document the high sensitivity of cells with 5q deletion to lenalidomide and the relatively high percentage of cytogenetic response. Cytogenetic response was recorded in 64% of patients. In comparison to larger studies, however, we report a lower percentage of complete cytogenetic response (11% in our study compared to 25% in MDS 004) supporting the possibility that lenalidomide may not completely eliminate the malignant clone/s. As of now, this can be explained by a shorter duration of therapy, given the recent authorization of lenalidomide in the Czech Republic and the use of generally lower doses of lenalidomide (the prevailing dose is 5 mg, even 2.5 mg in some long-term schedules). On the other hand, the dosing does not affect the number of hematological responses and leads to excellent drug tolerance without any further discontinuations of treatment because of side effects. The very good profile of adverse side effects from lenalidomide thus contributes to the likability of this oral medication. Although hematological toxicity is higher in the first few months of treatment, in most cases it gradually disappears and does not indicate more serious problems in lenalidomide therapy. Furthermore, the Sekeres' study demonstrates a positive correlation of the depth of thrombocytopenia and neutropenia in the first cycles of treatment with the quality of response [24]. The only exception in terms of side effects in our group is strikingly higher occurrence of deep vein thrombotic events.

It remains unclear why patients with del(5q) have such a high sensitivity to lenalidomide and how to identify the proper responders from non- del(5q) group. Our recent work demonstrates a connection between the specific sensitivity to lenalidomide and the mRNA levels of CRBN, a protein that is part of ubiquitin ligase E and the binding site for immunomodulation drugs, and whose expression, for reasons yet unknown, is significantly higher in patients with 5q- syndrome [18]. CRBN is a primary target of immunomodulating drugs in MM and MDS and is required for the activity of these drugs [25–27]. In our previous study, we also analyzed several del(5q) patients treated by lenalidomide and found high levels of CRBN mRNA in both the bone marrow and the peripheral blood mononuclear cells of the responders. Moreover, we found that patients who stopped responding to lenalidomide and whose disease progressed showed a sudden decrease of mRNA CRBN expression [18]. The new analysis of a larger group of low risk MDS lenalidomide-treated patients is just being prepared for publication. Our work also indicate that patients with del(5q) especially those carrying mutations in *TP53* gene progress to high risk MDS or MDS/AML. Interestingly, our other work recently showed that preceding isolated single del(5q) state represents rather strong positive predictor of longer overall survival and response duration in high risk MDS patients treated with azacitidine [28]. This implies that progression of del(5q) might be a result of different pathogenetic mechanisms as compared to those without del(5q) and that acquired resistance to lenalidomide might be involved in this process.

In conclusion, data from our set of MDS patients with del(5q) and transfusion dependence confirm a high efficacy of lenalidomide and a very good tolerance of this drug. Inadequate response or the relapse of anemia during lenalidomide treatment can be alleviated by combining lenalidomide with EPO or EPO plus steroids. We plan to confirm these clinical observations by means of a clinical study with a larger patient cohort, conducted in cooperation with other hematology centers in the Czech Republic.

## Conflicts of interests

None.

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