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Unimpaired terminal erythroid differentiation and preserved enucleation capacity in myelodysplastic 5q(del) clones: a single cell study

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Background
Anemia characterizes myelodysplastic syndromes (MDS), such as the rare 5q- syndrome, but its mechanism remains unclear. In particular, data are lacking on the terminal phase of differentiation of erythroid cells (enucleation) in MDS.

Design and Methods
We used a previously published culture model for the in vitro generation of mature red blood cells (RBC) from human hematopoietic progenitor cells to study the pathophysiology of 5q- syndrome. Our model enables analysis of cell proliferation and differentiation at a single cell level and determination of the enucleation capacity of erythroid precursors.

Results
The erythroid commitment of 5q(del) clones was not altered and their terminal differentiation capacity was preserved since they achieved final erythroid maturation (enucleation stage). The drop in RBC production was secondary to the decrease in the erythroid progenitor cell pool and to impaired proliferative capacity. The RPS14 gene haploinsufficiency was related to defective erythroid proliferation but not to differentiation capacity.

Conclusions
The 5q- syndrome should be considered as a quantitative rather than qualitative bone marrow defect. This observation might open the way to new therapeutic concepts.

Key words: myelodysplastic syndrome, 5q- syndrome, erythroid differentiation, enucleation.


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ABSTRACT

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**Introduction**

The mechanism of acquired alterations of erythropoiesis in myelodysplastic syndromes (MDS) has been the subject of countless publications. However, no study so far has addressed the terminal phase of erythroid cell differentiation, namely, i.e. enucleation. Because there is no culture model for enucleation, only the proliferation and commitment phases have undergone scrutiny.

Anemia is the main feature of MDS. MDS are thought to be due to acquired clonal anomalies of hematopoietic stem cells (HSC), in particular to a defect in HSC differentiation. This is the rationale that underpins current treatment. However, the lack of a complete in vitro model for erythropoiesis that includes enucleation means that the pathophysiology of anemia remains unclear. In addition, the relatively mild anemia observed in some patients with a high percentage of abnormal clones in their bone marrow remains a paradox.

5q- syndrome is a rare syndrome characterized by an isolated del(5q) cytogenetic abnormality, macrocytic anemia, fewer than 5% of marrow and blood blast cells, and a favorable clinical outcome. One treatment option is by lenalidomide, a drug that has received FDA approval for the treatment of MDS in patients with an interstitial deletion of the long arm of chromosome 5. It effectively reduces red blood cell (RBC) requirements. The 5q deletion was the first to be associated with a distinct clinical phenotype in cases of malignancy. Recently, a defect in the function of a ribosomal protein subunit (RPS14) has been implicated in 5q- syndrome. The RPS14 gene is located in the deleted region. Its partial inactivation in normal hematopoietic progenitors gives rise to a phenotype that matches 5q-syndrome. RPS14 deficiency affects erythroid differentiation.

Earlier papers by our team have described an in vitro model of erythropoiesis in which mature RBC are generated from human progenitor cells. This model can be used to analyze cell proliferation and differentiation in a homogeneous erythroid population in culture, and to measure the enucleation capacity of erythroid precursors. In the present study, we use it to investigate whether RBC production is altered in patients with 5q-syndrome and whether enucleation (enucleation capacity) is impaired. By furthering our understanding of anemia in 5q-deleted MDS, we may be able to design novel treatment strategies.

**Design and Methods**

**Patients**

Patients were classified according to the French-American-British (FAB) and WHO classification. Five patients with 5q- syndrome (fewer than 5% blasts in the marrow and a single chromosome abnormality, namely, a 5q deletion), of median age 82 years (range 72-96), entered the study (Table 1). Median hemoglobin was 9.2 g/L (7.5-9.4). At bone marrow sampling, four out of the five patients required transfusions. Patients were classified as having either a low (0) or intermediate (1-0.5) prognosis according to the International Prognostic Score System. All were heterozygous for the 5q deletion and had the same breakpoint region. The percentage of 5q deleted clones was 79% (54-81) by standard karyotyping of whole bone marrow and 96% (91-98) in CD34+ cells by fluorescence in situ hybridization (FISH) analysis. Normal control bone marrow samples were obtained from 6 healthy individuals of median age 83 years (range 71-86). Both patients and control subjects gave their informed consent. The study followed the guidelines of the ethical committee for research at Saint Antoine Hospital.

**Cell culture**

CD34+ cells were isolated by supermagnetic microbead selection using Mini-MACS columns (Miltenyi Biotech, Bergisch Glodbach, Germany). Cell purity was 92±6%. The cells were plated in a liquid culture medium based on IMDM-glutamax (Iscove modified Dulbecco’s medium, Biochrom, Berlin, Germany) and heparinized human plasma. The expansion procedure was a modification11 of our published 3-step technique. In the first step (days 0-8), 10/mL CD34+ cells were cultured in the presence of 10-6 M hydrocortisone (HC, Sigma), 100 ng/mL SCF (kindly provided by Amgen, Thousand Oaks, CA, USA), 5 ng/mL IL-3 (R&D Systems, Abingdon, UK) and 3 IU/mL Epo (Eprex, kindly provided by Janssen-Cilag, Issy-les-Moulineaux, France). On day 4, one volume of cell culture was diluted in four volumes of fresh medium containing HC, SCF IL-3 and Epo. In the second step (days 8-11), the cells were resuspended at 3x105/mL in fresh medium supplemented with SCF and Epo. In the third step (up to day 18), the cells were cultured in fresh medium in the presence of Epo alone. Cell counts were adjusted to 1x106 and 5x106 cells/mL on days 11 and 15, respectively. The cultures were maintained at

<table>
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<td>5q-</td>
<td>Yes</td>
<td>46XXdel(5)(q13q34)[22]/46XX[11]</td>
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</table>

37°C in 5% CO₂ in air. Results are expressed as expansion rates after plating. Cells were stained with May-Grünwald-Giemsa (MGG, Sigma) for morphological analyses. They were then spotted on slides. Cytological observations were evaluated by microscopical analysis on at least 300 cells/slide.

**Semisolid culture assays**

BFU-E progenitors were assayed in methylcellulose cultures as previously described. The cultures were incubated at 37°C in 5% CO₂ in air, and colonies were scored on day 14.

**Limiting dilution assay (LDA)**

To study erythroid differentiation at a clonal level, we performed a limiting dilution assay starting from CD34⁺ MDS cells. The cells were seeded on day 0 in 96-well microtiter plates in a final volume of 100 µL per well. Dilution steps were 1/200 cells/well with 10 to 240 replicates per dilution. Culture media and cytokines were as described in the cell culture section. Medium was changed from day 7 to day 18 in line with cell amplification. On day 11, frequencies were calculated by Poisson statistics. On day 14, before the enucleation stage, karyotyping was performed using half of the cells from each well, the remaining cells being maintained in culture until day 18 to measure RBC content. The abnormal karyotypes identified at diagnosis in patients with 5q(del) were detected by FISH analysis of interphase nuclei. Thus, at the end of culture, it was possible with 5q(del) were identified at diagnosis in patients from each well, the remaining cells being maintained in culture until day 18 to measure RBC content. The abnormal karyotypes identified at diagnosis in patients with 5q(del) were detected by FISH analysis of interphase nuclei. Thus, at the end of culture, it was possible to correlate the level of differentiation with the karyotype and to calculate the number of RBC produced by one 5q(del) CD34⁺ cell as compared to one normal CD34⁺ cell.

**Conventional and molecular cytogenetic analyses at diagnosis**

Conventional chromosome analyses were performed on unstimulated bone marrow aspirates after culture for 24 hours in RPMI 1640 supplemented with fetal calf serum. Chromosomes were GTG and RHG banded and at least 20 metaphases were analyzed using standard procedures. The karyotypes were described according to the International System for Human Cytogenetic Nomenclature.

Molecular cytogenetic (FISH) analysis of metaphases was performed using the LSI EGR-1/D5S721, D5S23 Dual Color Probe (Vysis, Downers Grove, IL, USA). Hybridization procedures were carried out according to manufacturer guidelines.

**Molecular cytogenetic analysis of cultured LDA cells**

Slide preparations of pooled, cultured LDA cells were obtained using standard procedures and adjusted for the limited number of cells. FISH analysis of interphase nuclei was performed using the LSI EGR-1/D5S721, D5S23 Dual Color Probe (Vysis, Downers Grove, IL, USA). Hybridization procedures were carried out according to manufacturer guidelines. The signals were analyzed with the Genikon System (Nikon Europe B.V., The Netherlands). Whereas normal cells display four distinct signals (2 orange, 2 green), deleted cells show only 1 orange and 2 green signals. The number of nuclei examined depended on the number of available cells, but whenever possible at least 200 nuclei were analyzed per pooled LDA cell.

**Measurement of RPS14 expression**

RBC were collected on days 0, 4, 8, 11 and 15 from bulk erythroid cultures of CD34⁺ cells from 5q(del) patients and normal donors. Depending on the number of cells, total RNA was extracted using either the Trizol method (Invitrogen, Paisley, Scotland) or an extraction kit (Ambion RNAqueous-4 PCR kit) according to manufacturer guidelines. DNase-treated RNA (1 µg) was transcribed into cDNA using 200 units of SuperScript II reverse transcriptase (Invitrogen) and 150 ng of random primers (Invitrogen), and the resulting cDNA was aliquoted to avoid repeated freeze/thaw cycles. Real-time PCR amplification was performed in an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The RPS14 gene was amplified to establish a relative gene expression pattern. β₂-microglobulin gene was used as reference. All primer sequences and probes were from PE Applied Biosystems. PCR runs were performed in duplicate using Taqman Master Mix (Applied Biosystems) with 10 ng of cDNA and 300 nM primers in a final reaction volume of 20 µL. After incubation for 2 min at 50°C, AmpliTaq Gold was activated by incubation for 10 min at 95°C. A total of 40 amplification cycles were run with an annealing temperature of 60°C. Calibration curves were established to check that the PCR efficiencies were similar for RPS14 and β₂-microglobulin. The relative expression of the RPS14 gene in patient and normal samples was calculated according to the formula: fold expression = 2⁻ΔΔCt where ΔΔCt = (CtRPS14 − Ctβ2m) on day of culture − (CtRPS14 − Ctβ2m) on day 0 of culture. This calculation defines the expression of a target gene relative to a reference gene and is suited to testing physiological changes in gene expression levels. If 2⁻ΔΔCt > 1, RPS14 is upregulated at a given time of culture and if 2⁻ΔΔCt < 1, it is downregulated. In the LDA analysis, the higher the ∆Ct (CtRPS14 − Ctβ2m), the lower is RPS14 gene expression.

**Statistical analyses**

Values are expressed as the median with the range. Differences between groups were analyzed with the Mann-Whitney test, p-values of less than or equal to 0.05 being considered to be significant.

**Results**

**Overall capacity of 5q(del) bone marrow cells to generate mature RBC in vitro**

To evaluate whether bone marrow progenitor cell populations from 5q(del) patients behave like normal controls with regard to in vitro capacity to generate mature enucleated RBC, we applied the three-step protocol described under Methods. CD34⁺ cell proliferation and erythroid commitment were first induced with SCF, IL-3, Epo and hydrocortisone for 8 days. Erythroid proliferation was then amplified with SCF and Epo for 3
days. Finally, the cells were maintained until terminal maturation in the presence of Epo alone up to day 18. The fold increase rate of CD34+ cells from 5q(del) patients was 33-fold lower than that of controls throughout culture. On day 18, median fold expansion was 1,265 (range 515-1398) versus 38,962 (range 27,738-43,305) (p<0.005) (Figure 1A). Erythroid commitment, on the other hand, was as high in patients as in controls. By day 8, 91% (range 87-99) of cells were proerythroblasts/basophilic erythroblasts in patients and 98% (range 96-99) in controls (p=0.1). Erythroid cells in both patients and controls continued to proliferate/differentiate and achieved complete maturation into enucleated RBC (67% (range 45-76) versus 45% (range 31-51) on day 15, and 86% (range 86-90) versus 86% (range 80-88) on day 18 (p=0.46 on day 18)) (Figure 1B).

In MDS patients, CD34+ cells is a mixed population of malignant and non-malignant cells. Among this population, from the expansion rates and enucleation levels, we calculated that, on day 18, CD34+ cells from patients generated 32 times fewer RBC than normal CD34+ bone marrow cells (1,088 (range 413-1,248) vs 35,043 (range 24,410-34,644) RBC/CD34+ cell, respectively) (p<0.005). This decrease in RBC generation in 5q(del) patients could be partly related to the impaired clonogenic capacity of the bone marrow samples at the time of collection as the median number of BFU-E was 17 (range 9-100) colonies/10^4 cells in patients versus 755 (range 420-1110) colonies/10^4 cells in controls (p<0.005).

FISH analysis during culture showed that the proportion of 5q(del) clones decreased steadily over time. On day 15, 56 to 92% of the cells did not express 5q(del). As 5q- clones are outgrown by their non-del (5q) counterparts in bulk culture, the vast majority of RBC present on day 18 presumably derived from the residual non-deleted clones that were seeded. Interestingly, the observed deficit in proliferation was correlated with the residual percentage of deleted clones (r^2= 0.96). In the absence of 5q(del) clones, proliferation rate reached normal levels.

To determine the contribution of non-deleted and 5q-deleted clones to defective erythropoiesis in 5q(del) patients, we performed the following experiments.

**Capacity of 5q(del) clones to achieve full maturation into RBC**

We first investigated whether RBC generated in vitro in patients originated from non-deleted clones [denoted NDC] and/or from pathological, i.e. 5q(del), clones [denoted DC]. The data were compared to the RBC generating capacity of CD34+ clones from normal controls [denoted HC, for Healthy Clones].

To study erythroid differentiation at a clonal level and discover whether in vitro maturation is related to the presence of 5q(del), we performed a LDA starting with CD34+ cells. On day 18, DC and HC generated enucleated RBC to similar extents (median level: 52% (range 1-75) versus 62% (range 3-90), respectively, p=0.7) (Figure 2). Account taken of proliferation levels and enucleation rates, this implies that one CD34+ DC cell generated 88 times fewer RBC than one CD34+ HC cell (1 DC cell generated 2.02±1.01x10^4 RBC and 1 HC cell 1.77±0.196x10^4 RBC). We then investigated whether diminished erythroid production by DC was related to a maturation defect, i.e. impaired enucleation, or was due to impaired clonal proliferation. We focused on differentiation as from day 15. We calculated the number of RBC generated by one erythroblast from the number of erythroblast cells on day 15 and of enucleated cells on day 18, after correction for cell amplification. Using LDA, we selected a 100% pure 5q(del) DC population and compared it to HC. No impairment in DC ability to
achieve final maturation was observed. A single erythroblast generated 0.54 RBC and 0.53 RBC when derived from DC and HC, respectively. We concluded that the decreased RBC production was not due to a diminished intrinsic incapacity to expel the nucleus.

**Non-deleted erythropoiesis in 5q(del) patients**

To determine whether the capacity to generate RBC was impaired in non-deleted clones in the bone marrow of 5q(del) patients, we selected a 100% cytogenetically non-deleted population (NDC) by LDA and assessed its erythroid competence. On day 18, one CD34+ NDC cell generated $3.7 \times 10^5$ RBC as compared to $1.77 \times 10^6$ for one CD34+ HC cell and $2.02 \times 10^4$ for one CD34+ DC cell. Overall, residual non-deleted clones in MDS patients generated 18 times more RBC than DC, but 8 times fewer RBC than HC.

**RPS14 gene expression in erythroid cultures and its correlation with cell proliferation rate and terminal differentiation**

We compared the kinetics of RPS14 expression during culture of CD34+ cells derived from 5q(del) patients (n=3) and controls (n=3) using real-time quantitative RT-PCR (see protocol in cell culture section) (Figure 3A). RPS14 expression diminished gradually in control cells whereas marked underexpression was noted throughout culture in cells from 5q(del) patients. Mean gene expression was several fold higher in controls than patients (12.65 versus 1.09 on day 4, 3.45 versus 0.012 on day 8, 0.47 versus 0.06 on day 11, 0.36 versus 0.845 on day 15). We then correlated the kinetics of RPS14 with the cell proliferation rate. In normal controls, RPS14 is highly expressed between day 0 and day 8, during the proliferation phase, and it is underexpressed afterwards from day 8 to day 15, during the differentiation phase. In cells from 5q(del) patients, RPS14 underexpression throughout culture was correlated with a low cell proliferation rate (Figure 3B).

We also studied RPS14 gene expression at the single cell level in a LDA. We compared expression in 5q(del) clones (over 99% 5q(del) clones by FISH analysis) and normal clones (HC) on day 8 and related this expression to stage of enucleation on day 18. Whereas gene expression in pathological clones was at least half that in normal clones on day 8, enucleation rates were similar on day 18 (Figure 4) ($\Delta\text{Ct} = 11.27$ versus 5.91, respectively).

**Lenalidomide action in 5q(del) syndrome patients**

We studied lenalidomide action in 2 patients at treatment initiation and after 4 and 10 months of treatment.
As the patients were becoming transfusion independent at 4 months, the percentage of 5q(del) clones decreased, and the BFU-E and amplification capacity increased. On pooling the data (Figure 5A-B), we found an *ex vivo* correlation between the percentage of 5q(del) clones, BFU-E numbers, and amplification capacity.

**Discussion**

In the absence of a tool to study the terminal phase of enucleation, data on defective erythropoiesis in MDS has remained incomplete.

The *in vitro* model of erythropoiesis we have developed allows the differential analysis of cell proliferation, commitment and enucleation, and the monitoring of healthy and pathological clones at the single cell level by the use of molecular markers such as the 5q deletion detected by FISH analysis. Our model can thus be used to analyze the erythroid lineage from the early stage of CD34+ progenitors to the late stage of differentiation into enucleated RBC. We established that: (i) erythroid commitment of the pathological clones in patients with 5q- syndrome was not impaired; (ii) terminal differentiation capacity was preserved since final erythroid maturation was achieved including the stage of enucleation; (iii) the drop in RBC production was secondary to a decrease in the erythroid progenitor cell pool and to impaired proliferative capacity.

Our results differ somewhat from those of Span et al. describing abrogation of enhanced proliferation of MDS progenitors by increased apoptosis. However, the two studies are not entirely comparable. Our study focused on the 5q- syndrome (IPSS score <1) whereas the Span et al. study concerned high-risk MDS patients (IPSS ≥1.5); only one patient (with sideroblastic anemia) was low risk. The starting cell population was also different (CD34+ cells versus bone marrow mononuclear cells) as were culture conditions. We engaged CD34+ cells into the erythroid lineage and analyzed burst-forming unit erythroid colonies whereas Span et al. used a cocktail of cytokines without erythropoietin and correlated their results with the clonogenic capacity associated with an apoptosis score.

Our focus was on erythroid cell line proliferation and differentiation, and on the faithful expression of CD34 on MDS cells. We cannot, however, rule out that CD34 is expressed in more terminally differentiated erythroid cells in 5q(del) patients, leading to an apparent decrease...
in proliferative capacity per cell. However, in a study of 2 patients responding to lenalidomide, we found a direct correlation between the percentage of 5q(del) clones and CD34+ cell proliferation capacity. With fewer than 5% 5q(del) clones, proliferation returned to normal. The same was true for BFU-E content; BFU-E numbers normalized with the elimination of 5q(del) clones.

A large number of pathophysiological mechanisms have been proposed for MDS, ranging from abnormalities of the medullar stroma, to autoimmune mechanisms. The major defects are nevertheless attributed to disorders of cellular proliferation/differentiation. Our results on terminal differentiation are novel but those on cell proliferation agree with those of many other studies. The primary target of 5q deletions is known to be a lympho-myeloid HSC. 5q(del) stem cells are CD34+ CD38- but inefficient in reconstituting hematopoiesis. Such ineffective erythropoiesis has been correlated with an increase in the intramedullary Fas-dependent apoptosis of differentiated cells. Interestingly, our CD34+ progenitors were able to proliferate in the presence of human plasma whereas impaired proliferation has been observed in a serum-free medium despite optimal concentrations of SCF, Flt-3 ligand, thrombopoietin, IL-3, G-CSF, GM-CSF and Epo. Our results do not confirm the impaired cell differentiation due to excess apoptosis that was observed by analysis of membrane phenotype in a recent murine model of MDS. The study, however, did not address enucleation. They nevertheless agree with regard to progenitor deficiency (27-fold less in our study versus 10-fold less) and proliferative capacity which was much reduced (53- versus 30-fold decrease).

Haploinsufficiency of the gene encoding RPS14 is thought to cause the characteristic hematologic phenotype defining the 5q- syndrome, namely blockade of erythroid differentiation. Our observations challenge this view. We show that complete differentiation to the enucleated state is possible. Basal RPS14 expression was relatively low even in healthy individuals, but was much reduced in 5q(del) patients. We compared the kinetics of RPS14 expression in erythroid cells derived from progenitor cells obtained from patients with 5q-syndrome and in healthy controls. We found that in controls, RPS14 expression was strongly expressed during the proliferation phase and very much underexpressed during the terminal differentiation phase. It therefore suggests a role of RPS14 during the proliferation stage and not the differentiation one. Indeed, in patients, RPS14 was underexpressed throughout culture, the proliferation rate was very much decreased and the ultimate stage of differentiation into RBC was reached. The kinetics of RPS14 expression support our hypothesis that anemia in 5q- syndrome is not linked to disorders of cellular proliferation/differentiation but rather is due to a defect in the terminal differentiation of erythroid lineages. This explains why 5q(del) clones reconstituted hematopoeisis in a LDA under our culture conditions.

That the enucleation capacity of 5q(del) clones does not depend on RPS14 gene expression was confirmed by single cell analysis in a LDA. Our findings thus suggest that the RPS14 gene is involved in the proliferation of erythroid precursors rather than in a true process of terminal differentiation, i.e. enucleation. They are supported by those of Ebert et al. who attributed 5q(del) syndrome to haploinsufficiency of the RPS14 gene. However, unlike these authors who associated the lack of differentiation into RBC with RPS14 underexpression, on the basis of decreased expression of antigenic markers (CD71 or glycophorin A), we did not observe impaired differentiation since the erythroid cells derived from progenitor cells obtained from 5q(del) patients reached the final stage of differentiation (i.e. enucleation) under our culture conditions.

Recent work on Blackfan Diamond anemia has revealed an impact on erythropoiesis similar to the one we observe. A quarter of patients with Blackfan Diamond anemia present a mutation of the RPS19 gene responsible for an anomaly of ribosomal synthesis. The authors reported cell cycle blockade with excess apoptosis and much impaired cell proliferation, but no effect on terminal erythroid differentiation. The findings of this study might support novel treatment strategies for 5q- syndrome. Lenalidomide is able to suppress 5q(del) clones, as indicated by the frequently observed cytogenetic remission in treated patients.

However, lenalidomide might increase the risk of progression to acute myeloid leukemia through a mechanism of selective pressure. Due to this reason, in 2008 the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMEA) recommended the refusal of marketing authorization for lenalidomide, intended for treatment of transfusion-dependent patients with MDS associated with del(5q) and with a low to intermediate risk of progressing to leukemia or death. An alternative therapeutic strategy might be aimed at reducing apoptosis and restoring proliferation of 5q(del) clones rather than at suppressing them. For example, inhibition of FADD-dependent caspase-8 activation might be used to restore peripheral RBC counts.

As we have shown, once they expand, 5q(del) clones will differentiate into RBC. Corticosteroids is a treatment option in Blackfan Diamond anemia and it is of interest to note that in our in vitro culture conditions corticosteroids increase the proliferation of erythroid progenitor cells. In fact, the combination of corticosteroids and lenalidomide has been proposed. In our study, we assumed that non-deleted clones in MDS patients were residual normal clones.

However, their capacity to generate RBC was lower than in controls because of impaired cell proliferation. The reason for this impairment is unclear. Direct contact inhibition can be ruled out since the analysis was performed at the single cell level. MDS is a clonal disease based on markers that are not acquired. However, cytogenetic abnormalities are acquired and, therefore, do not necessarily define the size of the initial neoplastic clone. Thus, cytogenetically normal cultures from MDS CD34+ cells may not arise from residual normal stem cells. This might explain the partial defect we noted in erythroid expansion. One could also argue that five times difference in expansion in an 18 day culture is relatively minor. Moreover, a CD34+ cell from a MDS patient is different from a healthy CD34+ cell control. MDS CD34+ progenitors are heterogeneous with differ-
ent expansion capacities and it could explain this small difference. A careful phenotypic analysis of the early progenitor compartments would have been helpful such as the CD34+/CD38- subpopulation.

On the basis of the above findings, it is unlikely that the decreased erythroid maturation and subsequent anemia in 5q- syndrome could be caused by a specific blockade of late differentiation. The anemia is more likely to be the consequence of the proliferative defect of both pathological and residual non-deleted clones in the patient’s bone marrow, their enucleation capacity remaining unchanged.

In conclusion, our erythroid model is valuable for the erythropoietic analysis of hematological malignancies. We chose to apply it to the 5q- syndrome, but it could also be applied to other MDS with a karyotypic marker. Our study revealed that the 5q- syndrome is a quantitative rather than merely qualitative bone marrow defect. This observation might open the way to new therapeutic concepts.

Authorship and Disclosures

LG, IK, CM, CdW: performed the experiments; Laurent Garderet: collected samples; LG, IK, NCG, HL, LD: analyzed results, make the figures, wrote the paper; LG, IK, NCG, MCG, HL, LD: analyzed results; LG, IK, MCG, HL, LD: designed the research.

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