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Myelodysplastic syndrome with isolated 5q deletion (5q- syndrome). A clonal stem cell disorder characterized by defective ribosome biogenesis

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In 1974 Herman van den Berghe *et al.*[1] reported a distinct hematologic disorder associated with acquired deletion of the long arm of chromosome 5 [del(5q)]. This novel nosological entity was described in more detail one year later by Sokal, van den Berghe, and coworkers.[2] Patients with del(5q) had macrocytic anemia with oval macrocytes, normal to slightly reduced white blood cell counts, and normal to elevated platelet counts. With respect to the bone marrow, there was erythroid hypoplasia but "*the most striking abnormality concerned the megakaryocytes and especially their nuclei, which were generally small, round or oval, and nonlobulated*".[2] These morphological abnormalities are illustrated in Figure 1. Until that time, the only specific chromosomal abnormality in hematologic disorders was the Philadelphia chromosome associated with chronic myeloid leukemia.[3,4] Sokal *et al.*[2] concluded that del(5q) represented a novel specific chromosomal abnormality associated with refractory anemia, although they had no explanation to connect the abnormal chromosome 5 with the hematologic manifestations.

The 5q- syndrome

Subsequent studies showed that a chromosome 5q deletion can be found in different myeloid disorders, and underscored the need to define the 5q- syndrome properly. Boultwood and Wainscoat[5] proposed the following simple definition of the 5q- syndrome: primary myelodysplastic syndrome (MDS) with del(5q) as the sole karyotypic abnormality and without excess of blasts. In their experience, patients with the 5q- syndrome so defined had macrocytic anemia, a normal or

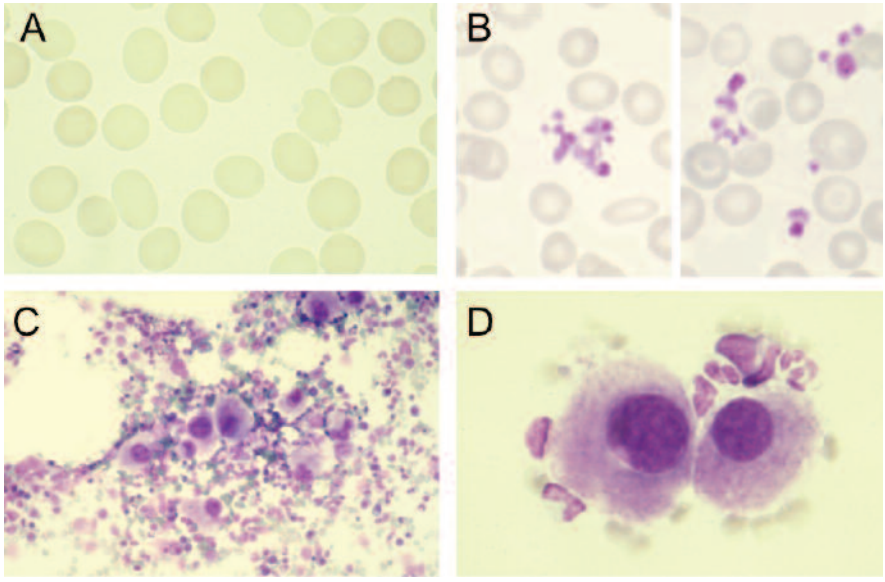

Figure 1. Peripheral blood smear and bone marrow aspirate from a patient with 5q- syndrome. (A) Oval macrocytes. (B) Numerous platelets. (C) and (D) Megakaryocytes with round, non-lobulated nuclei. *Courtesy of Rosangela Invernizzi.*

increased platelet count, hypolobular megakaryocytes, and a low risk of transformation to acute myeloid leukemia. By studying these patients, Boultwood and co-workers[6] found that the common deleted region of the 5q- syndrome was the approximately 1.5-megabase interval at 5q31-q32 flanked by D5S413 and the *GLRA1* gene. This region is distinct from that of the 5q deletion at 5q31 of malignant myeloid disorders such as acute myeloid leukemia or therapy-related MDS.[7,8]

In 2001 the World Health Organization (WHO) published a new classification for hematopoietic and lymphoid neoplasms that recognized the MDS with isolated del(5q) – the 5q- syndrome – as a unique, narrowly defined entity.[9] According to the WHO classification, additional cytogenetic abnormalities or 5% or more blasts in the blood or marrow excludes the diagnosis of 5q- syndrome. Indeed, a subsequent study showed that within MDS patients with del(5q), those with excess of blasts and those with an additional chromosomal abnormality have a significantly shorter overall survival than patients with isolated del(5q).[10] In this issue of the journal, Wang *et al.*[11] report on studies of genome-wide analysis of copy number changes and loss of heterozygosity in patients with MDS with del(5q). Their findings show a clear distinction between patients with 5q- syndrome and other MDS patients with del(5q). Unlike these latter, 5q- syndrome patients had no additional copy number changes, a finding that may indicate relatively genetic stability. These observations further support the definition of a separate entity of MDS with isolated 5q deletion that has been proposed both by Boultwood and Wainscoat[5] and the WHO classification.[9]

Since isolated del(5q) is associated with good prognosis in primary MDS, proper recognition of this chromosomal abnormality is of fundamental importance. In this issue of the journal, Mallo *et al.*[12] report findings of a study showing that fluorescence *in situ* hybridization (FISH) improves the detection of deletion 5q31-q32 in patients with MDS without cytogenetic evidence of del(5q). They correctly conclude that FISH of 5q31 should be performed in cases of a suspected 5q- syndrome in which the cytogenetic study has shown no metaphases.

The 5q- syndrome as a clonal stem cell disorder

The molecular basis for the 5q- syndrome has been the subject of extensive investigation for decades,[5] but major advances have been made only recently. There is no question that the 5q- syndrome is a clonal disorder.[13] It is, however, unclear how a clonal proliferation of hematopoietic stem cells can occur in a MDS. In myeloproliferative disorders, a somatic gain-of-function mutation of *JAK2* provides hematopoietic cells with less propensity to apoptosis and a growth advantage determining clonal proliferation (Figure 2A).[14] By contrast, in paroxysmal nocturnal hemoglobinuria (PNH) the clonal expansion of the PNH clone depends on the existence of one or more additional external environmental factors that damage normal hematopoietic stem cells and spare the PNH cells, thus exerting a selective pressure in favor of these latter (Figure 2B).[15–17] Since myelodysplastic clones are defective with respect to both differentiation and maturation, it is unlikely that clonal myelodysplastic stem cells can have a growth advantage over normal hematopoietic stem cells. Thus, the most likely model for clonal proliferation of myelodysplastic stem cells is that of conditional selection:[17] as in PNH, this dual pathogenesis would involve both the existence of stem cells with a somatic mutation and a failure of normal bone marrow. This latter may reasonably involve autoimmune mechanisms.

Haploinsufficiency of genes mapping to chromosome 5q31-q32 and aberrant ribosome biogenesis

The mechanisms responsible for failure of normal bone marrow in MDS patients are currently unknown. By contrast, recent observations indicate that haploin-

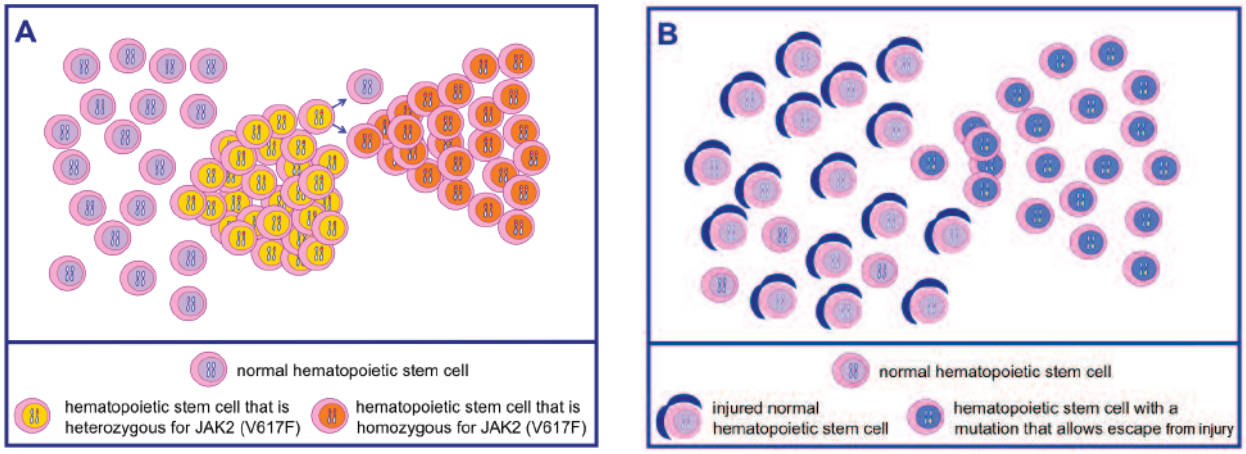

Figure 2. Two different models of clonal stem cell proliferation. (A) Growth advantage of mutant hematopoietic cells. In polycythemia vera, *JAK2* (V617F) occurs in a hematopoietic stem cell and causes a selective expansion of its myeloid-lineage cell progeny, giving rise to a clone that is heterozygous for the mutation. In some patients, as a second step, a mitotic recombination occurs in a hematopoietic cell that is heterozygous for *JAK2* (V617F). The daughter cell that is homozygous for the mutation gives rise to a subclone that expands and may progressively replace the heterozygous clone. (B) Conditional selection of clonal hematopoietic cells. In PNH, two events are required for clonal expansion of PNH cells.[17] One is the existence of a hematopoietic stem cell carrying a somatic mutation of the *PIG-A* gene; the other is a condition of cellular selection likely due to failure of normal bone marrow. This latter might involve autoimmune mechanisms. The dual pathogenesis of model (B) is more likely to be responsible for the clonal proliferation of hematopoietic cells that occurs in MDS, e.g., in the 5q- syndrome.

sufficiency for one or more of the genes mapping to the common deleted region at 5q31-q32 (a dosage effect resulting from the loss of a single allele of a gene) is likely the pathophysiological basis of the 5q- syndrome.[18] Candidate genes showing haploinsufficiency included the tumor suppressor gene *SPARC* and *RPS14*, this latter encoding a component of the 40S ribosomal subunit. Germline mutations in other genes controlling ribosome biogenesis – *RPS19* and *RPS24* – have been found in patients with Diamond-Blackfan anemia, a congenital disorder characterized by erythroid hypoplasia.[19,20] In a very elegant study, Ebert *et al.*[21] recently found that partial loss of function of RPS14 phenocopies the 5q- syndrome in normal hematopoietic progenitor cells, and that forced expression of RPS14 rescues the disease phenotype in bone marrow cells from patients with 5q- syndrome. Their observations suggest that defective erythropoiesis in the 5q- syndrome is caused by a defect in ribosomal protein function. In another recent study, Pellagatti *et al.*[22] indeed found that patients with the 5q- syndrome have defective expression of genes involved in ribosome biogenesis and in the control of translation, suggesting that the 5q- syndrome represents a disorder of aberrant ribosome biogenesis. This abnormality cannot, however, explain the growth advantage of 5q- hematopoietic cells. Haploinsufficiency of the *SPARC* gene, encoding a protein with antiadhesive properties,[23] might result in increased adhesiveness of 5q- cells to their bone marrow niche, but experimental evidence supporting this hypothesis is lacking. We, therefore, believe that a dual pathogenesis model likely operates also in patients with 5q- syndrome (Figure 2B). Were this to be true, 5q- cells would rescue the patient from bone marrow aplasia as cells carrying a mutant *PIG-A* do in PNH.

Lenalidomide treatment of myelodysplastic syndrome with del(5q): benefits and risks

Patients with the typical 5q- syndrome have a relatively good prognosis with a low risk of leukemic evolution. However, their anemia tends to worsen with time. Many of these patients have elevated serum erythropoietin levels, as do other patients with erythroid hypoplasia and a reduced rate of erythropoietin utilization,[24] and do not, therefore, respond to recombinant human erythropoietin.[25] Thus, until recently regular red cell transfusions and iron chelation[26] represented the standard treatment for severely anemic patients with 5q- syndrome.

In December 2005 the US Food and Drug Administration (FDA) approved the use of lenalidomide "*for the treatment of patients with transfusion-dependent anemia due to low- or intermediate-1-risk myelodysplastic syndromes associated with a deletion 5q cytogenetic abnormality with or without additional cytogenetic abnormalities*". Studies by List *et al.*[27,28] had shown that lenalidomide is indeed able to induce a cytogenetic remission and to abolish transfusion requirement in a substantial portion of patients with MDS and del(5q). The drug was not developed for the treatment of this condition, and its mechanism of action is unclear. Nonetheless, lenalidomide inhibits growth of del(5q) erythroid progenitors *in vitro*,[23] and likely inhibits del(5q) hematopoietic cells *in vivo*, at least in those patients who achieve cytogenetic remissions.[28] It still remains to be established why treatment results in a quick recovery of red cell production while it is associated with long-lasting neutropenia and thrombocytopenia in many cases. We proposed that these divergent effects may be consistent with an anti-cytokine activity of the drug, which would favor erythropoiesis while inhibiting granulocytopoiesis and megakaryocytopoiesis.[29]

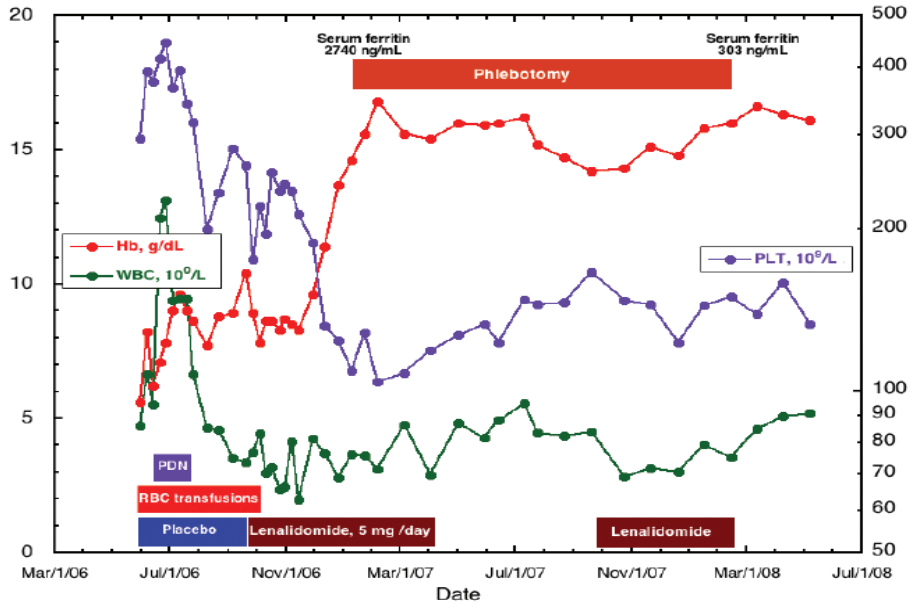

Figure 3. Response to lenalidomide in a 40-year old man with 5q- syndrome. This man developed severe anemia, a regular need for blood transfusion, and transfusion iron overload. His serum erythropoietin concentration was greater than 1000 mU/mL and he had no compatible family donor. This patient was enrolled in the CC-5013-MDS-004 study (ClinicalTrials.gov Identifier: NCT00179621) in 2006, and initially received placebo. A few weeks later, he developed severe autoimmune hemolytic anemia (which may occur in the 5q- syndrome[10]), and required prednisone (PDN) treatment; he developed leukocytosis and thrombocytosis, and then responded to PDN treatment with regression of immune hemolysis and a reduction of transfusion requirements. After 16 weeks of placebo treatment, this patient received lenalidomide, 5 mg/day; subcutaneous administration of granulocyte colony-stimulating factor was required to treat neutropenia during the first weeks of therapy. A normal hemoglobin (Hb) level was achieved after 14 weeks, and a peak of 16.8 g/dL after 20 weeks: at this point, with a complete cytogenetic response, as determined by FISH of chromosome 5q31, lenalidomide treatment was discontinued, and phlebotomy therapy was started. Reappearance of 5q- cells and a slight decline in Hb level induced us to restart lenalidomide, and this resulted in a second complete cytogenetic response as determined by FISH and in successful completion of the phlebotomy program: in about 1 year, serum ferritin concentration decreased from 2740 ng/mL to 303 ng/mL. Lenalidomide administration was then discontinued again, and peripheral blood counts are fully normal at the time of writing this report (June 2008). This young man, who was unable to work, is currently enjoying a normal life.

Following a request by the author, Celgene Corporation, sponsor of the CC-5013-MDS-004 study, approved the presentation of this case report for this perspective article.

In a commentary[29] to the first study on the use of lenalidomide in patients with MDS,[27] we concluded that, although this treatment was promising, its feasibility and adverse effects needed to be defined more precisely in prospective studies. An European, multicenter, randomized, double-blind, placebo-controlled, three-arm study is currently evaluating the efficacy and safety of two doses of lenalidomide versus placebo in transfusion-dependent subjects with low- or intermediate-1-risk MDS associated with del(5q) (http://www.clinicaltrials.gov/ct/show/NCT00179621). The outcome of one of the patients enrolled in this study is reported in Figure 3 to illustrate the remarkable efficacy of lenalidomide in some patients with 5q- syndrome. It should be noted, however, that lenalidomide treatment of patients with MDS and del(5q) is challenging for clinicians and requires considerable hematologic know-how, especially because it may be associated with long-lasting grade 3-4 neutropenia and/or thrombocytopenia.[28]

Lenalidomide was designated as an orphan medicinal product in MDS by the European Medicines Agency (EMEA) on March 8, 2004. On January 24, 2008, the EMEA Committee for Medicinal Products for Human Use (CHMP) adopted a negative opinion, recommending the refusal of marketing authorization for lenalidomide, intended for the treatment of anemia due to MDS, more specifically 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.[30] Following the applicant's request for a re-examination of the opinion, the CHMP confirmed the refusal of the marketing authorization on May 30, 2008. The CHMP concluded that the safety of lenalidomide was difficult to assess, and that, in particular, it was difficult to determine whether treatment with this drug increased the risk of progression to acute myeloid leukemia. In conclusion, the CHMP was of the opinion that the benefits of lenalidomide in the treatment of anemia of MDS with del(5q) did not outweigh its potential risks.

Since lenalidomide in unlikely to be mutagenic, a potential mechanism determining leukemic evolution might be selective pressure. Considering the model reported in Figure 2B and assuming that lenalidomide suppresses the 5q- clone (as *in vitro*[23] and *in vivo*[28] observations suggest) and allows restoration of normal hematopoiesis, a prerequisite for response is that normal residual hematopoietic cells are present in the patient's bone marrow. The absence of a sufficient number of such stem cells would involve development of marrow aplasia with severe pancytopenia following

the suppression of the 5q- clone that sustained blood cell production. Moreover, should a more abnormal subclone pre-exist and be unresponsive to lenalidomide, this subclone might emerge and lead to a more aggressive hematologic disorder.

As European hematologists who take care of patients with MDS we are experiencing a problematical situation. Almost none of the drugs used in the treatment of patients with MDS (including erythropoiesis-stimulating agents) have an approved indication for these disorders in Europe. With few effective therapeutic options available, it is not easy for us to renounce a drug that can provide results such as those illustrated in Figure 3. On the other hand, the concerns of the EMEA CHMP are fully understandable, as our main duty as physicians is *primum non nocere*.

Celgene Corporation informed EMEA that it will continue to make lenalidomide available for patients included in clinical trials or compassionate use programs. The CHMP made the following statement for patients currently receiving lenalidomide: "*If you are in a clinical trial or compassionate use program and need more information about your treatment, contact the doctor who is giving it to you*".[30] As a doctor, I have no certainties, some hopes, and many doubts on this subject now, and find it extremely difficult to provide patients with accurate information about lenalidomide treatment. I believe it is imperative for us to identify those patients who could benefit from treatment with a low risk of adverse effects: transfusion-dependent patients with a typical 5q- syndrome[5] likely fit into this category. By contrast, the efficacy of lenalidomide in MDS patients with del(5q) who have pancytopenia (neutropenia and thrombocytopenia in addition to anemia – a feature of patients with long-lasting disease), excess of blasts or additional chromosomal aberrations is questionable. More importantly, adverse effects are more likely to occur in these latter cases, mainly because few normal residual stem cells may be present. We hope that results of the ongoing multicenter, placebo-controlled study (NCT00179621) may help to clarify, at least in part, the current uncertainties on the use of lenalidomide in MDS patients with del(5q).

The author reported no potential conflicts of interest, in particular no financial relationship with pharmaceutical companies selling drugs employed in the treatment of myelodysplastic syndromes.

Key words: myelodysplastic syndrome, 5q- syndrome, deletion 5q, clonality, RPS14, ribosome biogenesis, lenalidomide.

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Molecular pathophysiology of Philadelphia-negative myeloproliferative disorders: beyond *JAK2* and *MPL* mutations

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The classic Philadelphia-chromosome negative chronic myeloproliferative disorders (MPDs), recently renamed as myeloproliferative neoplasms, that include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), had been largely neglected among hematologic neoplasms until early 2005 when the first recurrent molecular abnormality was described, consisting of a G>T point mutation at nt1849 in *JAK2* and resulting in a valine to phenylalanine substitution at residue 617 (V617F).[1-4] Then, the discovery of mutations in *MPL*, represented by a W>L or W>K shift at codon 515,[5] and of variable molecular abnormalities (point mutation, insertion, deletion) in *JAK2* exon 12[6] was also reported. Almost all patients with PV have a somatic genetic defect in *JAK2* that is represented by the V617F allele in 90-95% of cases and by abnormalities in exon 12 in roughly 2%, while they are spared by *MPL* mutations; on the other hand, only 60% of patients with ET or PMF harbor the *JAK2*V617F mutation and 3-7% exhibit the *MPL*W515L/K mutation. While other infrequent mutations in *MPL* can also occur,[7] exon 12 abnormalities have not yet been reported in PMF or ET patients. The presence of any of these molecular abnormalities, that point to a clonal myeloproliferation, stands as a major diagnostic criterion in the revised classification of myeloid neoplasms of the World Health Organization.[8] They are *gain-of-function* abnormalities that conferred growth-factor independence to cells transduced with mutant allele and induced a myeloproliferative disease when expressed in murine transplant models.[9]

It was unexpected to find a single mutated allele associated with more than one disease, notwithstanding the fact that the different MPDs are strictly related to each other and show substantial phenotypic mimicry. There are possible explanations for this. One is that the unique clinical phenotypes mirror the stem/progenitor cell level at which the mutational event occurred. However, this does not seem to be the case, since the V617F allele has been found in myelo-lymphoid progenitors in patients with PV or PMF, as well as in flow cytometry purified populations of hematopoietic stem cells and committed progenitors. Similar observations have been reported for *MPL*W515L/K mutations.[10] In only a minority of ET cases a restriction of V617F allele to megakaryocytic lineage was described, although it was unclear whether a very low burden of mutated granulocytes might have meant the abnormal genotype went undetected. Another possibility is that different diseases are caused by variable *dosage* of mutant V617F allele (information about *MPL*W515L/K mutation in this regard is scarse) that in turn influences the level of activation of the JAK/STAT signaling pathway. Indeed, there are substantial differences in the median burden of V617F allele in peripheral blood granulocytes, with the highest level being found in PV and the lowest in ET patients.[11,12] Furthermore, homozygosity for the *JAK2*V617F mutation, that originates from mitotic recombination of the short arm of chromosome 9, is present in approximately 30% of PV or PMF patients as opposed to 2-4% of ET. Variable proportions of wild-type, heterozygous and homozygous progenitors are present in most patients with PV, while homozygous progenitors are reported as being rare in ET; mutated erythroid progenitors are more sensitive to erythropoietin than normal ones, and most erythropoietin independent erythroid colonies (EEC) are made up of homozygous progenitors. Conceivably, duplication of mutant allele is expected to result in a higher level of JAK2/STAT activation than in cells harboring one mutant and one wild-type allele, possibly because of the loss of competition between normal and mutated allele and/or impaired interaction of mutant JAK2 with cellular regulators such as the suppressor of cytokine signaling-3 (SOCS3).[13] A correlation between