What lies beyond del(5q) in myelodysplastic syndrome?

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Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell malignancies characterized by ineffective differentiation of one or more bone marrow cell lineages. Much of the phenotypic variability is likely explained by the diverse set of genetic abnormalities responsible for the development and progression of these disorders. However, current clinical decision-making for MDS is based on diagnostic and prognostic criteria that do not include any molecular genetic information. In fact, the only subtype of MDS to be defined by a genetic abnormality is the group with isolated deletion of chromosome 5q [del(5q)].[1] Just under 50% of patients with *de novo* MDS will be found to have cytogenetic abnormalities, of which del(5q) is the most common. In the 10% of cases with the del(5q) as a sole abnormality, this lesion is associated with a more favorable prognosis.[2] In another 5-10% of cases, del(5q) is found as part of a complex karyotype (3 or more cytogenetic abnormalities): the prognosis in these cases is poorer.

Substantial effort has gone into understanding how the del(5q) abnormality contributes to the pathogenesis of MDS.[3] Early studies tried and failed to find recurrently mutated genes in the remaining intact genes that mapped to the commonly deleted region of chromosome 5. Instead, it was discovered that haploinsufficiency of several genes located in this region were capable of generating the clinical phenotype seen in patients with MDS. Loss of one *RPS14* allele for example, can recapitulate the dyserythropoiesis seen in MDS patients with del(5q). Loss of one copy of the microRNA miR-145 and miR146 may confer a clonal advantage and contribute to preserved or increased platelet counts observed in patients with the 5q-minus syndrome.[4] Haploinsufficiency of several other genes of the commonly deleted region, including *HSPA9*, *CTNNA1*, and *EGR1*, may also cooperate to promote the development of disease. Finally, loss of the more proximal *APC* gene and the more distal *NPM1* gene may play a role in higher risk MDS cases with more adverse outcomes since this group tends to have larger 5q deletions that extend well beyond the commonly deleted region.[3]

Identification of these disease-related haploinsufficient genes has informed our biological understanding of del(5q) MDS. Loss of ribosomal protein genes, such as *RPS14*, has been shown to increase levels of p53, primarily in erythroblasts, and promote their apoptosis – both elements observed in patients with isolated del(5q) MDS. If p53 activity checks the growth of more primitive del(5q) disease cells, there may be a selective pressure for them to mutate or lose the *TP53* gene. In fact, the del(5q) abnormality and *TP53* mutations have been found to co-exist more often than their base occurrence rates alone would predict, indicating a likely synergy between these lesions.[5] More importantly, this is not restricted to those patients with complex karyotypes in whom *TP53* mutations are most common. Even patients with isolated del(5q) and presumed lower risk MDS appear more likely to harbor a concurrent, and often subclonal, *TP53* mutation. When this occurs, patients may have poorer response to therapy and a higher than predicted risk of transformation to acute myeloid leukemia (AML).[6]

This variable relationship between del(5q) and somatic mutations highlights how complex the molecular pathophysiology of MDS actually is. Patients that we group together based on a shared cytogenetic finding may actually have little in common if we were to examine the full range of molecular abnormalities they contain. We now know that recurrent mutations in over 40 genes can occur in patients with MDS in a wide variety of combinatorial and subclonal relationships.[7] Understanding how these mutations cooperate could provide mechanistic insight into the pathophysiology of MDS and help us better classify, risk stratify and, treat these patients.

In their article, Fernandez-Mercado *et al.* describe the application of targeted next-generation sequencing in patients with MDS and del(5q).[8] They designed a panel of 25 frequently mutated myeloid malignancy genes (*ASXL1*, *ATRX*, *CBL*, *CBLB*, *CBLC*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *MPL*, *NPM1*, *NRAS*, *PDGFRA*, *RUNX1*, *SF3B1*, *SRSF2*, *TET2*, *TP53*, *U2AF1*, *WT1*, *ZRSR2*), covering a total of 46 kilobases.

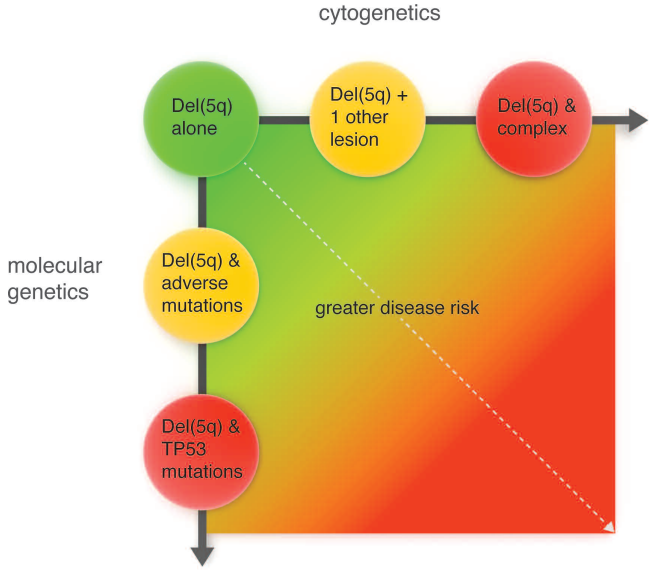

Figure 1. New dimension to the spectrum of genetic abnormalities identified in del(5q) MDS and their impact on disease risk. Increasing chromosomal complexity is recognized as an adverse risk factor in del(5q) MDS. Fernandez-Mercado *et al.*[8] demonstrate the molecular genetic heterogeneity of this group which include additional mutations in many genes, including *TP53* which has been associated with a very poor prognosis. This may lead us to reconsider how we assess MDS patients we previously put into discrete risk categories based on cytogenetics alone.

This panel was used to study samples from 43 patients with del(5q) MDS [22 with del(5q) syndrome, 9 with refractory anemia and del(5q) plus additional alterations, 11 with refractory anemia with excess of blasts and 1 with chronic myelomonocytic leukemia in transformation]. Thirty-three patients were also studied by single nucleotide polymorphism arrays. In total 29 non-synonymous alterations, distributed over ten genes were found. The most frequently mutated genes were *TP53* (7 cases), *ASXL1* (6 cases), and *TET2* (5 cases). Only six mutations were detected at a frequency less than 20%, which may have been missed by traditional Sanger sequencing.

The first conclusion that can be drawn from these results is that the spectrum of mutations in patients with del(5q) abnormalities is not dramatically different from that in patients with other forms of MDS.[7,9] This is not surprising given data that del(5q) is not necessarily a primary pathogenic abnormality and may instead be acquired after other disease-initiating mutations.[5] The second finding is that patients with more clinically advanced disease tend to have a greater number of mutations, a pattern that is seen in patients without del(5q) as well.

Interestingly, *TP53* mutations were particularly concentrated in del(5q) MDS patients with additional chromosomal abnormalities [4.5% for del(5q) alone *versus* 41.7% for complex karyotype with del(5q)]. *TP53* mutations have been described in patients with MDS and AML patients with complex karyotypes that often include del(5q). However, they have also been identified in as many as 17% of patients with low-risk MDS with isolated del(5q) in whom they have been associated with resistance or relapse during lenalidomide treatment.[10,11] This highly adverse mutation may partially explain why patients with multiple chromosomal abnormalities have a worse prognosis and lower likelihood of response to lenalidomide, but may also help to refine risk prediction in patients presumed to have lower risk disease.

MDS risk stratification is routinely performed with models such as the International Prognostic Scoring System-Revised (IPSS-R)[12] which considers cytogenetic abnormalities, percentage of bone marrow blasts, and the severity of cytopenias as risk factors. Nevertheless, the IPSS-R does not include molecular genetic criteria. More than half of MDS patients present with a normal karyotype when analyzed with conventional G-banding cytogenetics and two-thirds fall into the 'good risk' cytogenetic category which includes those with isolated del(5q). For these cases, new biomarkers are needed to refine the risk stratification.[13]

Next-generation sequencing platforms sequence many DNA strands in parallel enabling greater coverage at less cost while providing quantitative information about mutation abundance. This allows for sequencing of many more genes, including those that make rare contributions to a particular phenotype.[14,15] The greater sensitivity and digital nature of next-generation sequencing also provide information about the clonal architecture of the disease. In their study Fernandez-Mercado *et al.* assessed clonal architecture according to the proportion of mutant sequencing reads identified. They assume that mutations only occur once during clonal evolution and therefore they can establish mutation timing. For example, relative clonality was established between a *DNMT3A* mutation (44% abundance) and a presumably subclonal *JAK2* mutation (7%). In another case, 80% of cells appeared to carry an *SF3B1* mutation, 20% an *ASXL1* mutation, and 10% a *TET2* mutation. This highlights the potential complexity associated with interpreting molecular genetic tests. Additional studies will be needed to determine whether subclonal complexity and order of acquisition affect clinically meaningful endpoints. However, even low abundance clones defined by adverse mutations such as those in *TP53* may be harbingers of aggressive disease and important to detect at the time of diagnosis. This is particularly true in patients perceived as having lower risk disease or if treatments actually select for the growth of the adverse clone.

In summary, the report by Fernandez-Mercado *et al.* represents an important, focused look into the genetic makeup of MDS with del(5q).[8] More will be learned as additional patients are examined and as more agnostic approaches, such as exome and whole genome techniques, are applied. Next-generation sequencing is rapidly moving from research laboratories into clinical settings. For MDS patients, the identification of gene mutations in a wide set of genes will provide relevant information for diagnosis, accurate risk stratification, assessment of therapy, development of minimal residual disease strategies, characterization of progression mechanisms and identification of molecular targets. Despite its complexity, we welcome this new era of molecular genetic medicine.

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The role of antibiotic stewardship in limiting antibacterial resistance among hematology patients

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Haematologica has published European guidelines for empirical and targeted antibacterial therapy for febrile neutropenic patients in the era of emerging resistance (ECIL-4). Indeed, collateral damage by broad-spectrum antibiotic therapy includes selection of multidrug resistant pathogens, and increased predisposition to infection by fungi and *Clostridium difficile*. Antibiotic resistance has become a major public health concern, with fears expressed that we will soon run out of antibiotics.[1]

This is critically important for the management of hematologic cancer patients who receive consecutive courses of immunosuppressive treatments, resulting in varying degrees and durations of neutropenia. During immunosuppressive therapy, many patients will develop fever and are prescribed antibiotics for prevention or treatment of infection.

Several recent studies have shown an increasing prevalence of multidrug-resistance among Gram-negative pathogens in hematology patients. In one recent prospective observational study, the only independent risk factors for the acquisition of multiresistant pathogens were prior antibiotic exposure (OR 3.57; 95%CI: 1.63-7.80) and urinary catheterization (OR 2.41; 95%CI: 1.01-5.74).[2]

The use of broad-spectrum antibiotics also is a well-known risk factor for invasive fungal infection in hematology patients. Chronic disseminated candidiasis in patients with acute leukemia and/or bone marrow transplantation has been independently associated with the use of quinolone antibiotics in particular,[3] and a recent observational study showed that 92% of patients with candidemia had received broad-spectrum antibiotics.[4] Important risk factors for *C. difficile*-associated disease in hematology patients include the number and duration of antibiotics received, with particular risk attached to certain classes, such as cephalosporins.[5]

For all these reasons, it is becoming more and more necessary to optimize antibiotic use in hematology patients, and to deploy antimicrobial stewardship strategies that have shown benefit in other categories of patients. Key components of stewardship include: i) de-escalation of broad empirical regimens once the pathogen is identified; ii) dose optimization in critically-ill patients;[6] and iii) the long tradition of prudent antibiotic use in Northern European countries which is reflected in their low resistance rates, as shown in, for example, the European Antimicrobial Resistance Surveillance Network EARS-Net.[7]

Antimicrobial stewardship aims to limit the unnecessary use of broad-spectrum antibiotics and involves a continuous effort by healthcare institutions to optimize antimicrobial use in hospitalized patients. Its targets are to improve outcomes, ensure cost-effective therapy, and to reduce adverse effects of antimicrobial use, including resistance.[8] Control of infection is closely related to antimicrobial stewardship programs, as it aims to prevent the spread of the resistant organisms, when these are selected locally or introduced *via* patient transfers. The successful implementation of antimicrobial stewardship and infection control programs complement each other in limiting the number of infections caused by multidrug-resistant organisms.

Infection control strategies found their way into hematology many years ago, and guidelines in this field are published elsewhere.[9] The most important measures in hematology are: i) enforcement of hand hygiene by using alcohol-based hand-rubs; ii) standard barrier precautions; iii) enforcement of isolation criteria for patients colonized or