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Improving treatment outcomes by understanding cytogenetics in myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) represent a heterogeneous group of blood disorders that are generally characterised by refractory anaemia (RA) or cytopenia, hypercellular bone marrow, and ineffective haematopoiesis. Most cases of MDS have an increased risk of transformation to acute myeloid leukaemia (AML). The prognosis of patients with MDS varies considerably, reflecting the underlying heterogeneity within this class of disorders. Recent advances in our understanding of some of the cytogenetic characteristics of MDS are helping to better classify patients and predict clinical outcomes. Cytogenetic analysis has therefore become an essential part of the standard work-up of patients with MDS. Familiarity with certain cytogenetic abnormalities and how they affect prognosis and treatment decisions will lead to better management of patients with MDS.

Classification of MDS

The French-American-British (FAB) system was introduced in 1982, and quickly became a standard tool for the classification of MDS (Table 1).[1] However, several limitations of the FAB system have been identified, leading to heterogeneity in outcomes within certain subtypes. The more recently developed World Health Organization (WHO) classification system is similar to the FAB system, but attempts to overcome the limitations of the FAB system by refining the definition of some subtypes of MDS.[2] The primary difference between the 2 systems is that the WHO system refines the morphological diagnosis by including uni- or multilineage involvement and defining different levels of blast excess between 5% and 20%. It also includes cytogenetics as a mandatory component of the diagnostic work-up of MDS.

The WHO system more precisely defines the FAB categories of RA and RA with ringed sideroblasts (RARS) by accounting for the number of dysplastic cell lineages: RA and RARS according to WHO have dysplastic features confined to their erythropoiesis, while refractory cytopenia with multilineage dysplasia (RCMD) and RCMD with ringed sideroblasts (RS) show dysplasia in at least 1 additional myeloid cell line (Table 1).[2] Compared with patients with dysplasia restricted to the erythroid lineage, those with multilineage dysplasias have a worse prognosis. The WHO system also breaks down the FAB category of RA with excess blasts (RAEB) into 2 subtypes: RAEB-1 (bone marrow blasts 5-9%) and RAEB-2 (bone marrow blasts 10-19%). This change was made to account for the more favourable prognosis of patients with fewer blasts.

In the WHO system, the threshold blast percentage for the diagnosis of AML was reduced from 30% to 20%, thereby eliminating the category RAEB in transformation (RAEB-t).[2] Unlike the FAB system, the WHO system does not include chronic myelomonocytic leukaemia in the MDS classification. Instead, this disease is reclassified as a myelodysplastic/myeloproliferative disease. Lastly, the WHO system introduced 2 new categories: unclassified MDS (MDS-U), and MDS with 5q- syndrome. The latter addition addresses the unique prognosis of patients with isolated del 5q and specific clinical characteristics. The WHO system has been shown to successfully stratify patients into subtypes with distinct clinical outcomes, based on overall and leukaemia-free survival.[3]

Prognosis of MDS

The International Prognostic Scoring System (IPSS) was introduced in 1997 as a means to predict clinical out-

Table 1. Overview of FAB and WHO classification systems for MDS, highlighting key differences between the 2 systems. (Data from Bennett JM, *et al.*[1] and Vardiman JW, *et al.*[2])

FAB	*WHO*
RA, blasts <1% in blood, <5% in BM	*RA*
–	*RCMD*, ≥10% dysplastic cells in ≥2 myeloid lineages
RARS, blasts <1% in blood, <5% in BM, >15% RS	*RARS*
–	*RCMD-RS*, same as RCMD with >15% RS
RAEB, blasts <5% in blood, 5-20% in BM	*RAEB-1*, bone marrow blasts 5–9%
–	*RAEB-2*, bone marrow blasts 10–19%
CMML, blasts <5% in blood, 5-20% in BM, with peripheral monocytes	(Moved to myeloproliferative diseases)
RAEB-t, blasts ≥5% in blood, 21-30% in BM, optional Auer rods	(Moved to acute myeloid leukaemia)
–	*MDS* with isolated del 5q
–	*MDS-U*

Abbreviations: BM, bone marrow; CMML, chronic myelomonocytic leukaemia; MDS, myelodysplastic syndromes; MDS-U, unclassified MDS; RA, refractory anaemia; RAEB, RA with excess blasts; RAEB-t, RAEB in transformation; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RS, ringed sideroblasts.

Table 2. The WHO-classification-based Prognostic Scoring System. (Data from Malcovati L, *et al.*[5])

Prognostic factor	*Score value*				*Sum score*
	+0	+1	+2	+3	
WHO category	RA, RARS, del 5q	RCMD, RCMD-RS	RAEB-1	RAEB-2	_____
Karyotype*	Good	Intermediate	Poor	–	_____
Transfusion requirement°	No	Regular	–	–	_____
				Total score	_____

Total score	*Risk group*	*Median survival (months)*
0	Very low	136
1	Low	63
2	Intermediate	44
3-4	High	19
5-6	Very high	8

**As defined in the IPSS: Good = normal karyotype or isolated -Y, isolated del 5q, or isolated del 20q; Intermediate = other abnormalities; Poor = complex karyotype (≥3 abnormalities) or chromosome 7 abnormalities.[3] °At least 1 packed red cell concentrate every 8 weeks. Abbreviations: RA, refractory anaemia; RAEB, RA with excess blasts; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts.*

comes, such as leukaemic transformation and mortality, more precisely.[4] The IPSS is based on 3 prognostic factors: blast percentage in the bone marrow, karyotype, and the number and degree of cytopenias. Blast percentage is divided into 4 categories (<5%, 5-10%, 11-20%, and 21-30%); because the IPSS was based on the FAB system, patients with 21-30% blasts were included. Karyotypes are categorized as Good (normal karyotype or isolated -Y, del 5q, or del 20q), Intermediate (other chromosomal abnormalities), or Poor (complex karyotype consisting of 3 or more abnormalities, or chromosome 7 abnormalities). Based on these prognostic factors, the IPSS stratifies patients into 4 distinct risk groups (Low, Int-1, Int-2, and High) with regard to leukaemic transformation and mortality.[4] Of the patients in the clinic, two-thirds have Low- or Int-1-risk disease, and one-third have Int-2- or High-risk disease.[4]

Recently, Malcovati *et al.* developed a new prognostic scoring system based on the WHO classification system (Table 2).[5] The WHO-classification-based Prognostic Scoring System (WPSS) addresses additional variables not included in the IPSS that have been shown to affect prognosis, such as transfusion requirement.[3] The WPSS

stratifies patients into 5 risk groups based on 3 main prognostic factors (WHO category, karyotype, and transfusion requirement) identified by uni- and multivariate analysis of a learning cohort of 467 patients.[5] When validated in an independent cohort of 620 patients, the 5 risk groups were found to have significantly different risks of leukaemic transformation ($p<0.0001$) and overall survival ($p<0.0001$); median survival ranged from 136 months in very low risk patients to 8 months in very high risk patients. The probability of developing leukaemia was 7% at 10 years in the very low risk group compared with 50% at 8 months in the very high risk group. Based on this report, it appears that the WPSS is a simple way to enhance the prognostic ability of the WHO system with regard to leukaemic transformation and overall survival in patients with MDS, and may therefore be of value in making treatment decisions.

Cytogenetics influence prognosis of MDS

Cytogenetic abnormalities are found in approximately 50% of patients with *de novo* MDS.[6] The most common abnormality is interstitial deletion of the long arm of chromosome 5 (del 5q). Other frequently encountered abnormalities include deletion or monosomy of chromosome 7, trisomy 8, and multiple abnormalities.[6]

del5q

Among patients with MDS and chromosomal abnormalities, approximately 20-30% have del 5q, including about 7% with the 5q- syndrome. The WHO system defines the 5q- syndrome as *de novo* MDS with an isolated cytogenetic abnormality resulting in deletion between bands q31 and q33 of chromosome 5, and blast counts in blood and bone marrow <5%.[2,7,8] Patients with additional chromosomal abnormalities or blast counts >5% are not considered to have 5q- syndrome. The 5q- syndrome is typically associated with macrocytic anaemia with normal or elevated platelet counts, a reduced neutrophil count, hypoplastic erythropoiesis, and megakaryocytes that often have hypolobulated nuclei.

Compared with most other patients with MDS, those with 5q- syndrome have an excellent prognosis (median survival >5 years). Compared with the general age-matched population, however, 5q- syndrome still carries a significant mortality risk. In a retrospective study of patients with MDS and del 5q, the median survival of 60 patients with 5q- syndrome who did not undergo intensive therapy or stem-cell transplantation was 107 months, which was considerably lower than the life expectancy of the general population with the same median age (approximately 244 months for women, 188 months for men).[9,10]

It is now recognised that outcomes vary considerably among patients with del 5q, and other factors have been shown to influence the prognosis of these patients. In the aforementioned retrospective study of patients with MDS and del 5q, the median survival seen in patients with 5q- syndrome (107 months; n=60) was significantly greater than the median survival in patients with del 5q plus 1 additional chromosomal abnormality (47 months; n=14) or increased blast count (23 months; n=12).[9,10] In a study of 25 patients with MDS and complex chromosomal abnormalities including del 5q, the median survival was between 7 and 8 months, regardless of blast count.[11]

These observations were confirmed in a recent review of 2,124 patients with MDS treated at 8 treatment centres in Germany and Austria (Figure 1).[6] Patients with isolated del 5q had a longer median survival than those with a normal karyotype (69 months versus 49 months). However, those with del 5q plus 1 other chromosomal abnormality had a median survival that was similar to those with a normal karyotype (47.7 months), and those with del 5q plus 2 or more additional abnormalities had a median survival of 7.4 months. These findings highlight the fact that prognosis varies considerably among patients with del 5q, depending on the presence of other cytogenetic abnormalities.

Other cytogenetic abnormalities

While some common cytogenetic abnormalities, such as del 5q, have been shown to affect prognosis, less is known about rare abnormalities and unique combinations of abnormalities. Solé et al. characterized the incidence and prognostic influence of various chromosomal abnormalities in a series of 640 patients with primary MDS.[12] Cytogenetic abnormalities were found in 51% of the patients. Single, double, and complex abnormalities were found in 29%, 8%, and 14% of the patients, respectively. Among those with cytogenetic abnormalities, the most common types were del 5q (23%), monosomy 7 or del 7q (21%), and trisomy 8 (17%). Other common abnormalities included del 11q, del 12p, involvement of 12q, involvement of 13q, isochromosome 17q, del 20q, trisomy 21, monosomy 21, and loss of sex chromosomes. Univariate analysis indicated that the presence and number of

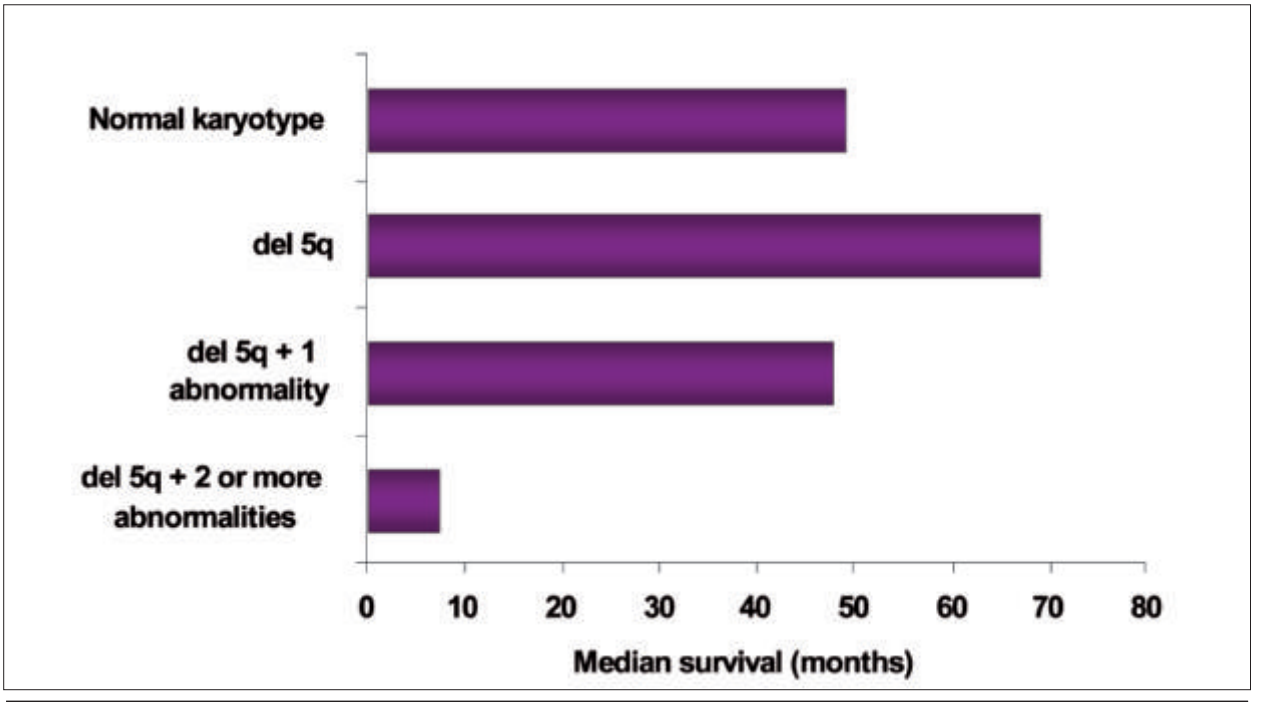

Figure 1. Median survival according to cytogenetic characteristics based on a database of 2,124 patients with myelodysplastic syndromes treated at 8 centres in Germany and Austria. (Data from Haase D, *et al.*[6])

abnormalities predicted mortality and leukaemic transformation. Certain isolated cytogenetic abnormalities were associated with clinical outcomes, although the number of patients in these subgroups was small. For example, compared with patients with a normal karyotype, those with isolated del 12p had a similar prognosis, whereas those with isolated 1q involvement had a poor prognosis. In the aforementioned database of 2,124 patients treated in Germany and Austria, Haase et al. reported the incidence of various cytogenetic abnormalities (Figure 2).[6] Of the 2,072 patients who underwent cytogenetic analysis, 52% had at least 1 chromosomal abnormality. The median survival in patients with normal karyotype was 49 months compared with 7.5 months in patients with complex chromosomal abnormalities. Among patients with chromosomal abnormalities, the prognosis was influenced by the presence of additional abnormalities and in different ways, depending on the type of cytogenetic abnormality. For example, patients with isolated trisomy 8 had a similar median survival as those with trisomy 8 plus 1 additional abnormality (21.6 months versus 23.3 months). Those with trisomy 8 plus 2 or more abnormalities had a median survival of 11.7 months. Further research is needed to better understand how various cytogenetic abnormalities and combinations of abnormalities influence clinical outcomes in patients with MDS.

Cytogenetics affect treatment decisions

The efficacy of some treatment approaches may be influenced by the presence of cytogenetic abnormalities. For example, the hypomethylating agent decitabine has been shown to be more effective in patients with high-risk karyotype compared with those with an intermediate-risk karyotype, according to the IPSS.[13] Azacytidine may be especially effective in patients with MDS and chromosome 7 abnormalities.[14] More recently, researchers have analysed treatment outcomes in specific subtypes of MDS based on cytogenetic features, such as del 5q. Various approaches have been used to treat patients with 5q- syndrome, but have met with little success.[9] Treatment with pyridoxine, steroids, or danazol is generally ineffective in these patients. Given that endogenous erythropoietin levels are usually highly elevated at presentation (mean 1,000 U/L),[10] erythropoietin treatment has proven ineffective in most patients with del 5q. The combination of all-trans-retinoic acid and tocopherol-alpha produced a modest response rate of 17% in one study of 29 patients with isolated del 5q and Low- or Int-1-risk MDS; 30% of patients discontinued treatment due to adverse events.[15] More promising results were found in 2 small preliminary studies of low-dose cytarabine, which reported response rates of 57% and 100%.[16,17] Allogeneic stem-cell transplantation has also been used in patients with isolated del 5q.[18] How-

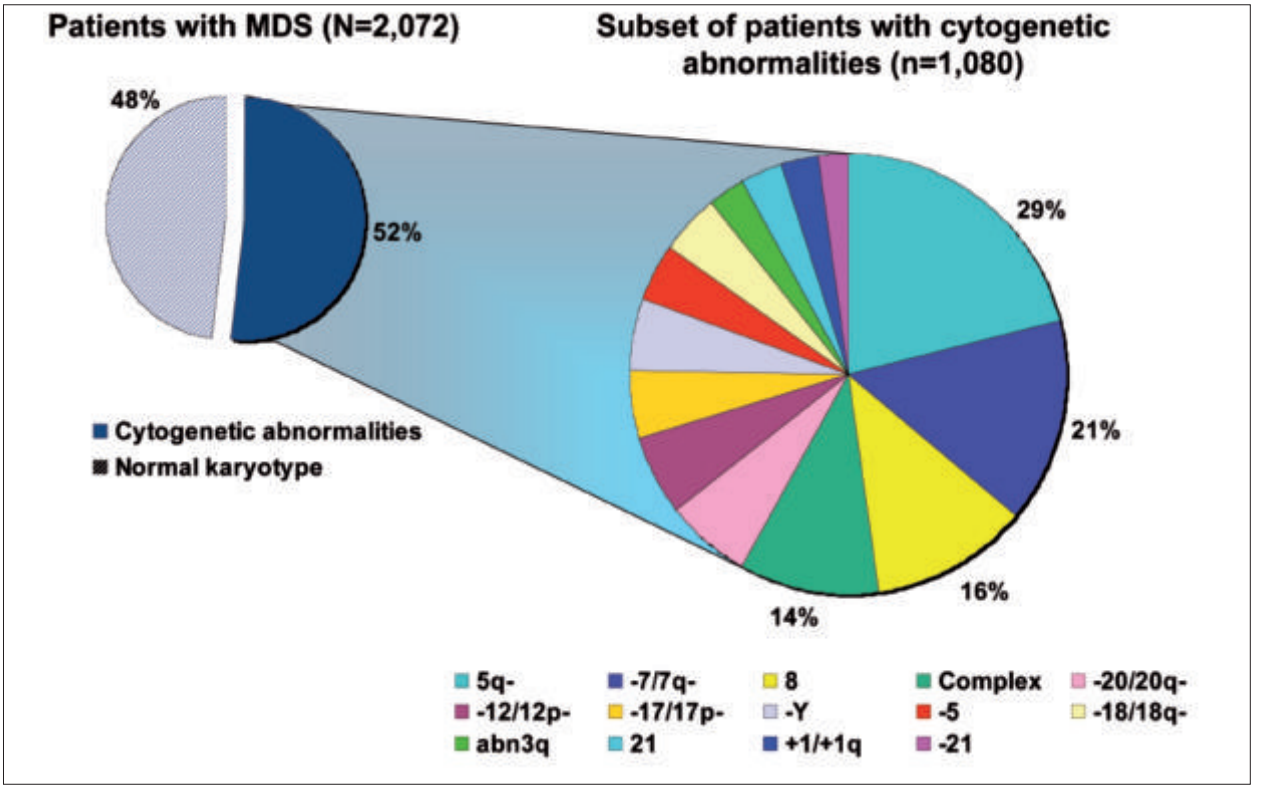

Figure 2. Frequency of cytogenetic abnormalities in patients with myelodysplastic syndromes (MDS). Results are from a database of 2,124 patients treated at 8 centres in Germany and Austria, 2,072 of whom underwent cytogenetic analysis. (Data from Haase D, *et al.*[6])

ever, given the relatively good prognosis for patients with del 5q and the intensity of transplantation, this approach should be reserved for carefully selected patients.

One of the most promising approaches to the treatment of patients with del 5q is lenalidomide. Lenalidomide is an antineoplastic and anti-angiogenic immunomodulatory drug that has been shown to affect a broad range of ligand-induced processes relevant to MDS. It reduces angiogenesis, cell adhesion, tumour progression, and production of pro-inflammatory cytokines, as well as enhances immune response.[19,20] In an initial phase I/II study of lenalidomide in patients with MDS, a preponderance of responding patients had del 5q.[19] Of the 20 patients with chromosomal abnormalities, 10 achieved a complete cytogenetic remission by week 16 of treatment with lenalidomide, 9 of whom had del 5q. Responses were reported to occur more rapidly in patients with del 5q compared with those with other chromosomal abnormalities or a normal karyotype (8.0 weeks versus 11.2 weeks). These results suggested that response to lenalidomide may depend on karyotype, and led to a larger phase II study in 148 patients with transfusion-dependent MDS and del 5q with or without additional chromosomal abnormalities.[21] Of these patients, 67% achieved transfusion independence by week 24. Notably, response rates were similar in patients with isolated del 5q, del 5q plus 1 additional chromosomal abnormality, and del 5q plus 2 or more abnormalities (69%, 52%, and 67%, respectively). The cytogenetic response rate was 71% in patients with isolated del 5q, which was similar in patients with 1 or multiple additional chromosomal abnormalities (65%, 75%, respectively). These data suggest that lenalidomide is active in patients with MDS and del 5q, regardless of the presence or absence of additional chromosomal abnormalities. Based on this evidence, lenalidomide was approved in the USA for use in patients with transfusion-dependent Low- or Int-1-risk MDS with del 5q with or without additional chromosomal abnormalities.

The experience with lenalidomide illustrates how the cytogenetic characteristics of MDS can affect not only prognosis but also treatment outcomes. Understanding the relationship between del 5q and other cytogenetic abnormalities may lead to further improvements in treatment strategies. For example, del 5q has been found in very early haematopoietic precursor cells, whereas

other cytogenetic abnormalities, such as trisomy 8, typically occur later in the MDS transformation process.[9,22] This suggests that del 5q may be a key early event in the development of MDS, which later leads to further chromosomal abnormalities. Therefore, achieving a complete cytogenetic remission in patients with del 5q could impact the course of the disease. Further research is needed to continue to explore the relationship between various cytogenetic abnormalities and the efficacy of treatment.

Conclusions

Cytogenetic characteristics can influence prognosis and treatment outcomes in patients with MDS. Accordingly, certain cytogenetic characteristics have been incorporated into the WHO classification and WPSS to improve prognostic assessment in these patients. Cytogenetic analysis is therefore an essential part of the work-up for patients with MDS. The most common chromosomal abnormality in MDS is del 5q, but the prognostic value of del 5q is influenced by other factors, such as blast count and the presence of additional chromosomal abnormalities. The presence or absence of cytogenetic abnormalities can affect the efficacy of certain treatments. For example, lenalidomide is particularly effective in patients with del 5q with or without other chromosomal abnormalities. Further study is needed to investigate how del 5q and other chromosomal abnormalities and combinations of abnormalities affect clinical outcomes in MDS.

References

1. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51:189-99.
2. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms Blood 2002;100:2292-302.
3. Malcovati L, Della Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol 2005;23:7594-603.
4. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079-88.
5. Malcovati L, Germing U, Kuendgen A, Della Porta MG, Invernizzi R, Giagounidis A, et al. A WHO classification-based prognostic scoring system (WPSS) for predicting survival in myelodysplastic syndromes [abstract 788]. Blood 2005;106: 232a-3a
6. Haase D, Steidl C, Schanz J, Schabla R, Pfeilstocker M, Nosslinger T, et al. Correlation of cytogenetic findings with morphology, clinical course and prognosis in 2124 patients with MDS [abstract 787]. Blood 2005;106;232a.
7. Boultwood J, Fidler C, Strickson AJ, Watkins F, Kostrzewa M, Jaju RJ, et al. Transcription mapping of the 5q- syndrome critical region: cloning of two novel genes and sequencing, expression, and mapping of a further six novel cDNAs. Genomics 2000;66:26-34.
8. Horrigan SK, Arbieva ZH, Xie HY, Kravarusic J, Fulton NC, Naik H, et al. Delineation of a minimal interval and identification of 9 candidates for a tumor suppressor gene in malignant myeloid disorders on 5q31. Blood 2000;95:2372-7.
9. Giagounidis AA, Germing U, Wainscoat JS, Boultwood J, Aul C. The 5q- syndrome. Hematology 2004;9:271-7.
10. Giagounidis AA, Germing U, Haase S, Hildebrandt B, Schlegelberger B, Schoch C, et al. Clinical, morphological, cytogenetic, and prognostic features of patients with myelodysplastic syndromes and del(5q) including band q31. Leukemia 2004;18:113-9.
11. Giagounidis AA, Germing U, Strupp C, Hildebrandt B, Heinsch M, Aul C. Prognosis of patients with del(5q) MDS and complex karyotype and the possible role of lenalidomide in this patient subgroup. Ann Hematol 2005;84:569-71.
12. Solé F, Espinet B, Sanz GF, Cervera J, Calasanz MJ, Luno E, et al. Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary myelodysplastic syndromes. Grupo Cooperativo Espanol de Citogenetica Hematologica. Br J Haematol 2000;108:346-56.
13. Hackanson B, Robbel C, Wijermans P, Lübbert M. *In vivo* effects of decitabine in myelodysplasia and acute myeloid leukemia: review of cytogenetic and molecular studies. Ann Hematol 2005;84 Suppl 13:32-8.
14. Raj KK, John AM, Ho A, Thomas NSB, Mufti GJ. Early and sustained response to azacytidine in high-risk MDS patients with monosomy 7 correlates with increased apoptosis and not CDKN2B demethylation [abstract 2530]. Blood 2005;106:710a-711a.
15. Giagounidis AA, Haase S, Germing U, Schlegelberger B, Wilkens L, Busche G, et al. Treatment of myelodysplastic syndrome with isolated del(5q) including bands q31-q33 with a combination of all-trans-retinoic acid and tocopherol-alpha: a phase II study. Ann Hematol 2005;84:389-94.
16. Giagounidis AAN, Haase S, Germing U, Hildebrandt B, Heinsch M, Aivado M, et al. Low-dose cytarabine in the treatment of patients with the 5q- syndrome. Onkologie 2002;25:66.
17. Juneja HS, Jodhani M, Gardner FH, Trevarthen D, Schottstedt M. Low-dose ARA-C consistently induces hematologic responses in the clinical 5q- syndrome. Am J Hematol 1994;46:338-42.
18. Stewart B, Verdugo M, Guthrie KA, Appelbaum F, Deeg HJ. Outcome following haematopoietic cell transplantation in patients with myelodysplasia and del (5q) karyotypes. Br J Haematol 2003;123:879-85.
19. Bartlett JB, Dredge K, Dalgleish AG. The evolution of thalidomide and its IMiDs® derivatives as anticancer agents. Nat Rev Cancer 2004;4:314-22.
20. List A, Kurtin S, Roe DJ, Buresh A, Mahadevan D, Fuchs D, et al. Efficacy of lenalidomide in myelodysplastic syndromes. N Engl J Med 2005;352:549-57.
21. List AF, Dewald G, Bennett J, Giagounidis AA, Raza A, Feldman E, et al. Hematologic and cytogenetic (CTG) response to lenalidomide (CC-5013) in patients with transfusion-dependent (TD) myelodysplastic syndrome (MDS) and chromosome 5q31.1 deletion: results of the multicenter MDS-003 study [abstract 5]. J Clin Oncol 2005;23:2s.
22. Nilsson L, Astrand-Grundström I, Anderson K, Arvidsson I, Hokland P, Bryder D, et al. Involvement and functional impairment of the $CD34^+CD38^-Thy\text{-}1^+$ hematopoietic stem cell pool in myelodysplastic syndromes with trisomy 8. Blood 2002;100;259-67.