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# Involvement of deleted chromosome 5 in complex chromosomal aberrations in newly diagnosed myelodysplastic syndromes (MDS) is correlated with extremely adverse prognosis

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ABSTRACT

MDS with complex chromosomal aberrations (CCA) are characterized by short survival and a high rate of transformation to AML. A comprehensive genome-wide analysis of bone-marrow cells of 157 adults with newly diagnosed MDS and CCA revealed a large spectrum of nonrandom genomic changes related to the advanced stages of MDS. Chromosome shattering, probably resulting from chromothripsis, was found in 47% of patients. Deleted chromosome 5 was unstable and often involved in different types of cryptic unbalanced rearrangements. No true monosomy 5 was observed. Patients with CCA involving deleted chromosome 5 had an extremely poor prognosis (median overall survival, 2 months).



## 1. Introduction

Clonal chromosomal changes are detected in the malignant bone-marrow cells of approximately 50–60% of adults with primary myelodysplastic syndromes (MDS) at the time of diagnosis and in up to 80% of patients with secondary MDS, using conventional cytogenetic techniques. The interstitial deletion of the long arm of chromosome 5—del(5q)—is the most common aberration, accounting for roughly 30% of abnormal MDS karyotypes, and occurs as either the sole abnormality or in combination with other aberrations [1,2]. The extent of the 5q deletion varies in individual patients, but chromosome band 5q31 is deleted in most. Many studies have identified two different commonly deleted regions (CDRs) at 5q. The proximal 5q31.1–q31.2 region is possibly associated with a high risk of MDS/acute myelogenous leukemia (AML). Nevertheless, rare cases of myeloid malignancies with atypical deletions of 5q that do not include the 5q31 region have also been described [3]. The distal CDR in the 5q32–5q33 bands is thought to be involved in the pathogenesis of 5q− syndrome, which has a good prognosis [4]. However, the vast majority of MDS patients (>95%) with del(5q) have large deletions that encompass both defined CDRs and other segments of the chromosome [5,6]. Many candidate genes, whose haploinsufficiency could lead to malignant transformation, have been identified in these regions [7,8]. The direct relationship between the pathogenesis of MDS and the *RPS14* gene, which encodes a ribosomal protein that affects the maturation of erythroid progenitor cells, has already been demonstrated [9]. Apart from del(5q), other specific chromosomal aberrations that correlate closely with the prognosis of MDS have been detected, including monosomy 7/del(7q), trisomy 8, del(20q), and rearrangements of 11q [1,10]. Complex karyotypes with multiple chromosomal changes are seen in approximately 20% of patients with newly diagnosed MDS and are associated with a poor prognosis and high risk

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of transformation to AML [11–13]. It is not clear whether the complex karyotypes in MDS arise by a gradual acquisition of genetic changes in individual cells during the clonal evolution or by extensive chromosome fragmentation and reorganization at a single event (chromothripsis), as was recently suggested for several types of malignant diseases [14].

Standard chromosomal analysis with G-banding has limited resolution (4 Mb). Today, more-sensitive molecular cytogenetic techniques should be used to precisely define the origin of individual chromosomal rearrangements in complex chromosomal aberrations (CCA) and to identify cryptic abnormalities and genomic lesions. A combination of molecular cytogenetic techniques (fluorescence in situ hybridization [FISH], multicolor FISH [mFISH], multicolor banding [mBAND], and array-based assays) was used in this study to analyze bone-marrow samples from MDS patients with CCA to evaluate the involvement of specific chromosomes and/or chromosomal regions, to establish the exact locations and frequencies of chromosomal breakpoints, and to identify unbalanced genomic changes that could be related to disease progression.

## 2. Patients and methods

### 2.1. Patients

A comprehensive retrospective molecular cytogenetic analysis was performed of fixed bone-marrow cells from 157 patients with CCA (≥3 aberrations; exact definition and scoring system for counting aberrations see in the online Supplementary material) identified with conventional G-banding techniques at the diagnosis of MDS (80 men, 77 women). All patients provided written informed consent to the use of their samples for research purposes. The diagnoses, according to the WHO classification [10], were as follows: refractory anemia, 10 patients; refractory cytopenia with multilineage dysplasia, 14 patients; refractory anemia with excess blast-1, 28 patients; refractory anemia with excess blast-2, 74 patients; AML with myelodysplasia-related changes, 24 patients; and MDS unclassified, seven patients. Thirteen patients underwent stem-cell transplantation (SCT); 11 of them died (median overall survival [OS] after SCT was 22 months, range 7–112 months). Only eight patients are currently living, six of them 2–12 months after the diagnosis of their disease and two of them 16 and 22 months after SCT. One hundred forty-nine patients died, and the median OS of the whole cohort was 4 months (range, 1–43 months).

### 2.2. Conventional cytogenetic analysis

For the cytogenetic analysis, unstimulated bone-marrow cells were cultured for 24 h in RPMI 1640 medium containing 10% fetal calf serum. Chromosomal spreads were prepared according to standard techniques and G-banded with Wright–Giemsa stain. Whenever possible, at least 20 metaphases were evaluated. The karyotypes were described according to the ISCN (2013) [15].

### 2.3. FISH

FISH analyses were performed using commercially available probes. The CCA and breakpoints on the affected chromosomes were studied with mFISH and mBAND methods, using the 24*X*Cyte and the *X*Cyte color kits and an *ISIS* computer analysis system (MetaSystems, Altlussheim, Germany). Interphase FISH with a DNA probe specific for the 5q31 region was performed using the Vysis LSI EGR1/D5S23, D5S721 Dual Color Probe (Abbott, Des Plaines, IL). Chromosomal abnormalities identified with microarray assays were confirmed by FISH with bacterial artificial chromosome (BAC) probes (BlueGnome, Cambridge, UK).

### 2.4. Microarrays and data analysis

Microarray assays were performed retrospectively on the same fixed material that had been analyzed with conventional cytogenetic techniques and/or FISH, which had been stored in fixative at −25 °C. The cell suspensions from 119 patients were available. BlueGnome CytoChip Focus Haematology ($n = 57$), CytoChip Cancer SNP 180K ($n = 55$) (BlueGnome, Cambridge, UK) or Illumina Human CytoSNP-12 arrays (Illumina, San Diego, CA) were used. Details are given in the Supplementary material.

### 2.5. Statistical analysis

The Kaplan–Meier product limit method was used to estimate the probability of OS. The statistical comparison of OS in different groups was based on the long-rank test [16,17]. OS was measured as the period from diagnosis to death or last follow-up. Thirteen patients treated with SCT were excluded from the survival analysis.

## 3. Results

In the period 2002–2012, bone-marrow samples from 870 adults with newly diagnosed MDS were examined and 157 patients (18%) with CCA were identified (Table 1).

**Table 1**
Characteristics of the 157 patients with MDS and complex chromosomal aberrations (CCA).

| Characteristics | Whole cohort | *Group 1* *n* (%) | *Group 2* *n* (%) | *Group 3* *n* (%) |
|---|---|---|---|---|
| Number of patients | $n = 157$ | 80 | 67 | 10 |
| Gender (male/female) | 80/77 | 50/30 | 24/43 | 6/4 |
| Median age at diagnosis (years) | 67.0 | 66.5 | 70.0 | 58.5 |
| Median OS time (months) | 4.0 | 2.0 | 5.0 | 9.0 |
| WHO category | | | | |
| RA | 10 | 3 (30.0) | 5 (50.0) | 2 (20.0) |
| RCMD | 14 | 4 (28.6) | 8 (57.1) | 2 (14.3) |
| RAEB-1 | 28 | 14 (50.0) | 12 (42.9) | 2 (7.1) |
| RAEB-2 | 74 | 44 (59.5) | 27 (36.5) | 3 (4.0) |
| AML with MDS-related changes | 24 | 13 (54.2) | 11 (45.8) | 0 (0.0) |
| MDS-US | 7 | 3 (42.85) | 3 (42.85) | 1 (14.3) |
| Primary or secondary MDS | | | | |
| De novo | 139 | 72 (51.8) | 60 (43.2) | 7 (5.0) |
| Treatment-related | 18 | 8 (44.4) | 7 (38.9) | 3 (16.7) |
| Number of karyotypic abnormalities | | | | |
| 3 abnormalities | 3 | 0 (0.0) | 1 (33.3) | 2 (66.7) |
| 4–5 abnormalities | 14 | 1 (7.1) | 10 (71.5) | 3 (21.4) |
| >5 abnormalities | 140 | 79 (56.4) | 56 (40.0) | 5 (3.6) |
| Modal chromosome number | | | | |
| Hypodiploid (<46 chromosomes) | 105 | 63 (60.0) | 40 (38.1) | 2 (1.9) |
| Hyperdiploid (>46 chromosomes) | 33 | 8 (24.2) | 20 (60.6) | 5 (15.2) |
| Pseudodiploid (46 chromosomes) | 19 | 8 (42.1) | 7 (36.8) | 4 (21.1) |

*Abbreviations*: AML, acute myeloid leukemia; *Group 1*, patients with deleted chromosome 5 involved in CCA; *Group 2*, patients with del(5q) and CCA not involving chromosome 5; *Group 3*, patients with CCA with no deletion of chromosome 5; MDS, myelodysplastic syndromes; MDS-U, MDS unclassified; OS, overall survival; pseudodiploid, 46 chromosomes with structural aberrations; RA, refractory anemia; RAEB, refractory anemia with excess of blasts; RCMD, refractory cytopenia with multilineage dysplasia; WHO, World Health Organization classification.