Myelodysplastic syndromes with a deletion 5q display a characteristic immunophenotypic profile suitable for diagnostics and response monitoring

by Uta Oelschlaegel, Theresia M Westers, Brigitte Mohr, Michael Kramer, Stefani Parmentier, Katja Sockel, Christian Thiede, Martin Bornhäuser, Gerhard Ehninger, Arjan A. van de Loosdrecht, and Uwe Platzbecker

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Myelodysplastic syndromes with a deletion 5q display a characteristic immunophenotypic profile suitable for diagnostics and response monitoring

Running head: Immunophenotypic profile in del(5q) MDS

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Acknowledgments
We appreciated the excellent technical assistance of Claudia Klotsche, Cathleen Rüger, Catrin Theuser, and Susann Helas. Mutational analyses for mTP53 were performed by Dr. A. Kohlmann and colleagues at the MLL Munich Leukemia Laboratory (Munich; Germany).
Patients with myelodysplastic syndromes (MDS) harbouring a clonal deletion of the long arm of chromosome 5 [del(5q)] display characteristic cytomorphologic features.\textsuperscript{1,2} Flow cytometry (FCM) is currently considered complementary to the microscopic evaluation of bone marrow (BM) smears and karyotyping and has been referred as a supplementary method in otherwise non-informative cases.\textsuperscript{3} FCM-scoring-systems (FCSS and Ogata-score)\textsuperscript{4,5} have been developed evolving from an analysis of progenitors and myelomonocytic cells. The European LeukemiaNet (ELN)-iMDS-FCM working group has successfully made efforts in order to foster standardization arguing for the inclusion of FCM in the diagnostic work up of patients with a suspicion of MDS.\textsuperscript{3,6} It is however still unknown whether cytogenetic subgroups are associated with a distinct FCM profile thus allowing classification and disease monitoring. The aim of this two-center-study was to compare the immunophenotype of different hematopoietic cell lineages in MDS patients with and without del(5q).

We investigated 156 untreated MDS patients within a training cohort, 38 patients showed a del(5q), including 26 patients with del(5q) as a single abnormality or with one additional aberration. An additional “validation cohort” consisted of 64 MDS [22 with del(5q), 12 showing del(5q) as single abnormality or with one additional aberration]. A standardized lyse-stain-wash procedure, using 8-color or 4-color staining (Dresden or Amsterdam laboratory, respectively), was performed. This allowed for a comprehensive FCM analysis according to FCSS and Ogata-score.\textsuperscript{4,5} We incorporated additional antigens, as CD10, its decrease in granulopoiesis described as loss of synchronicity in relation to other maturation antigens\textsuperscript{7}, CD36/CD71 expression on granulopoiesis,
reported to correlate with IPSS\textsuperscript{8}, and diminished (dim) expression CD71 on nucleated red cells (NRC), known as dyserythropoietic feature.\textsuperscript{9,10} For gating strategy and cut-offs refer to the Online Supplementary Appendix and Tables S1-S2.

Initially, we compared the whole immunophenotype in MDS patients with isolated del(5q) or one additional cytogenetic abnormality (n=26) vs. normal karyotype (n=88) as part of the training cohort. Among the distinct immunophenotype of del(5q) MDS (Table 1) the increased myeloid progenitor cells (myPC) in 81\% vs. only 36\% of normal karyotype (NK) patients was one of the hallmarks even in the lower risk groups and irrespective of cytomorphologically assessed blast count: <5\% blasts [del(5q)=15 and NK=57 patients] with myPC of 3.2±2.8\% vs. 1.3±1.4\% (\(P=0.003\)) and ≥5\% [del(5q)=11 and NK=31 patients] with 8.2±8.8\% vs. 5.0±7.0\% (\(P=0.024\)). The CD45-MFI-ratio, a feature associated with maturity within the progenitor cell compartment, was normal-to-low in each del(5q) patient.\textsuperscript{4} Granulopoiesis presented with an abnormally low sideward scatter (SSC)-ratio in almost all del(5q) patients reflecting hypogranularity. Interestingly, this is known to be a rather uncommon cytomorphological feature in del(5q) MDS.\textsuperscript{1,2}

Notably, CD71 and CD10 expression were significantly more often normal compared to NK patients, pronouncing the maturity of granulopoiesis which is concordant with the low rate of dysgranulopoiesis evaluated cytomorphologically.\textsuperscript{11} The percentage of CD71\textsuperscript{dim} NRC was distinctly higher in the del(5q) cohort. The entire immunophenotypic pattern of MDS with del(5q) as a single aberration or with only one additional abnormality provides further evidence for a clonal multilineage involvement which is supported by other studies.\textsuperscript{12}
Second, we aimed at building a robust model by comparing all del(5q) samples of the training cohort [isolated del(5q) and del(5q) within complex aberrant karyotypes] with all non-del(5q) samples (normal; abnormal karyotypes). By incorporating 14 parameters, which appeared to best characterize the MDS with del(5q) and assigning one point each (Table 1), a score ≥8 allowed to distinguish del(5q) from non-del(5q) MDS with a remarkably high sensitivity and specificity (Table 2). Thus both del(5q) subgroups, namely MDS with del(5q) as single aberration or with one additional abnormality as well as those patients with del(5q) as part of a complex aberrant karyotype could be equally well characterized with the above mentioned score. Next, a logistic regression analysis was performed in order to weight single parameters and to simplify the model (Online Supplementary Appendix). The final 5-parameter-del(5q)-score (Figure 1A) includes characteristics of myPC (percentage and CD45-MFI-ratio), granulocytes (SSC-ratio and CD71 expression), and was further refined by adding female gender, known to be associated with del(5q).\textsuperscript{11} The normal-to-low CD45-MFI-ratio is mandatory and received the highest ranking. Even the presence of all four further variables in parallel was not enough to characterize del(5q) MDS without an appropriate CD45-MFI-ratio. A 5-parameter-del(5q)-score of ≥15.0 was considered typical for the presence of del(5q) and could predict del(5q) in 95% of MDS harboring this abnormality. The robustness of the del(5q)-score was proven in a validation cohort (Table 2 and Figure 1B; Online Supplementary Table S3A-B). In non-del(5q) MDS (normal and abnormal karyotype), considering the whole data set, the del(5q)-score was comparably low (mean: 11.5 vs. 11.0), with 25 patients comprising a score <10.0, never being present in del(5q) MDS. Some of the mentioned variables are part of the Ogata-score\textsuperscript{5} accentuating the
importance of these features in MDS. Remarkably, the addition of two parameters (CD71 and gender) and the differential weighting further refined the Ogata-score and allowed for a clear separation of del(5q) and non-del(5q) MDS as well as non-clonal cytopenias and healthy BM (Table 2).

Finally, we tested in 18 MDS patients (71 measurements) whether the proposed 5-parameter-del(5q)-score could be used for monitoring response to lenalidomide and/or azacitidine. The del(5q)-specific-profile was lost (mean score=13.0) in all del(5q) patients achieving a complete cytogenetic response (cCR) after a lenalidomide-based therapy (n=13 measurements), including normal myPC and SSC-ratio (12/13 patients), whereas in most measurements in MDS without cCR (45/58 patients) a characteristic high del(5q)-score (mean=16.5; Figure 1C-D) was present. A more complex aberrant karyotype as an expression of a higher grade of genetic instability might result in a different immunophenotype. However, a typical del(5q)-score reflected patients with a complex karyotype in a similar way as MDS with isolated del(5q). Namely, during all measurements in del(5q) MDS with complex karyotype, receiving azacitidine and not achieving a cCR, a typical high del(5q)-score was detectable. Additionally, only one patient without cCR but a non-typical low del(5q)-score lower than the proposed threshold of 15.0 presented with a complex karyotype, both arguing for del(5q) as an early cytogenetic abnormality which drives the overall immunophenotype. In contrast, in del(5q) patients without a complete response to therapy but a non-typical low del(5q)-score this expression profile seems to be associated with the presence of a TP53 mutation. In 12/13 measurements with this non-typical low score (mean score=13.0; range=3.5-14.5) a TP53 mutation and/or a chromosome 17p deletion was present,
whereas only 1/23 investigations in patients with wtTP53 had a non-typical low del(5q)-score ($P=0.009$). This observation hints at a possible link between TP53 mutations and concomitant alterations of the immunophenotypic profile. Thus, the del(5q)-score can be used as a reliable complementary tool for cytogenetic response in wtTP53 patients. TP53 mutations in MDS with isolated del(5q) have been reported in up to 20% of lower risk MDS.\textsuperscript{15} A mutational screening is recommended in patients with isolated del(5q) because of their potential resistance to lenalidomide and higher rate of AML evolution in TP53 mutated cases. From our data we hypothesize, that especially patients with isolated del(5q) but a non-typical low del(5q)-score might be suspicious of harboring a TP53 mutation and should subsequently be referred to mutational screening.

In summary, we demonstrate a strong association of a common cytogenetic abnormality with an immunophenotypic profile in MDS, emphasizing the role of FCM as reproducible and fast tool for diagnostics and monitoring of response to therapeutic interventions. Additionally, this contributes to a better understanding of the biology of del(5q) vs. other MDS and emphasizes the uniqueness of this cytogenetically based MDS subgroup. Besides cytomorphology, FCM including the here proposed 5-parameter-del(5q)-score should be incorporated as part of the MDS diagnostics. It might also be used to speed up further cytogenetic and molecular diagnostics as well as to be confirmatory especially in cases where cytogenetic evaluation is inconclusive with results around the cut-off percentage for this method or fails. Response monitoring in the context of immunomodulatory drugs might be possible using an easy-to-perform 4-color (CD45/CD34/CD19/CD71) immunophenotypic approach, which should be further substantiated in prospective studies.
References


Table 1. Immunophenotypic differences in MDS with isolated del(5q) or one additional abnormality vs. MDS with normal karyotype (as a part of the training cohort).

<table>
<thead>
<tr>
<th>Feature</th>
<th>MDS patients</th>
<th>14-parameter score† (thresholds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>del(5q) (n=26)</td>
<td>NK (n=88)</td>
</tr>
<tr>
<td>MyPC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% myPC</td>
<td>(mean±SD)</td>
<td>5.3±6.5</td>
</tr>
<tr>
<td>&gt;2%</td>
<td>(% pts.)</td>
<td>81</td>
</tr>
<tr>
<td>aberrant CD7</td>
<td>(% pts.)</td>
<td>12</td>
</tr>
<tr>
<td>CD45-MFI-ratio (lympho vs. myPC)</td>
<td>(mean±SD)</td>
<td>4.5±0.8</td>
</tr>
<tr>
<td>LyPC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% lyPC</td>
<td>(mean±SD)</td>
<td>3.7±8.5</td>
</tr>
<tr>
<td>&lt;5%</td>
<td>(% pts.)</td>
<td>88</td>
</tr>
<tr>
<td>Granulopoiesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSC-ratio (granulo vs. lympho)</td>
<td>(mean±SD)</td>
<td>5.3±0.9</td>
</tr>
<tr>
<td>&lt;6.0</td>
<td>(% pts.)</td>
<td>92</td>
</tr>
<tr>
<td>CD10 (%)</td>
<td>(mean±SD)</td>
<td>37±13.7</td>
</tr>
<tr>
<td>&gt;25%</td>
<td>(% pts.)</td>
<td>85</td>
</tr>
<tr>
<td>CD36 (%)</td>
<td>(mean±SD)</td>
<td>10.1±10.0</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>(% pts.)</td>
<td>31</td>
</tr>
<tr>
<td>CD71 (%)</td>
<td>(mean±SD)</td>
<td>11.7±5.8</td>
</tr>
<tr>
<td>≤20%</td>
<td>(% pts.)</td>
<td>92</td>
</tr>
<tr>
<td>CD15 (MFI)</td>
<td>(mean±SD)</td>
<td>3661±1811</td>
</tr>
<tr>
<td>Monopoiesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD56 (%)</td>
<td>(mean±SD)</td>
<td>8.0±8.4</td>
</tr>
<tr>
<td>&lt;20%</td>
<td>(% pts.)</td>
<td>92</td>
</tr>
<tr>
<td>NRC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% NRC</td>
<td>(mean±SD)</td>
<td>3.9±5.4</td>
</tr>
<tr>
<td>≤8%</td>
<td>(% pts.)</td>
<td>85</td>
</tr>
<tr>
<td>CD71dim (of all CD71) (%)</td>
<td>(mean±SD)</td>
<td>10±11</td>
</tr>
<tr>
<td>Ogata-score</td>
<td>(mean±SD)</td>
<td>3±1</td>
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<tr>
<td>Female gender</td>
<td>(% pts.)</td>
<td>77</td>
</tr>
<tr>
<td>IPSS-R</td>
<td>(mean±SD)</td>
<td>3.5±1.0</td>
</tr>
</tbody>
</table>

† Besides the here described 14-parameter all other detected MDS related immunophenotypic changes in the antigen pattern according to FCSS and Ogata score, e.g. abnormal CD13/CD16/CD11b expression in the granulopoiesis or changes of the MFI of myeloid antigen expression on myPC, were present in a comparable number of del(5q) and NK patients (data not shown). ‡All those features are characteristics of normal bone marrow as well as of del(5q) MDS, meanwhile all other FCM characteristics represent MDS related abnormalities. *This threshold is valid for the investigations performed on the FACS Canto II. NK, normal karyotype; pts., patients; myPC, myeloid progenitors; lyPC, lymphoid progenitors; SSC, side scatter; MFI, mean fluorescence intensity; granulo, granulopoiesis; lympho, lymphopoiesis; NRC, nucleated red cells; dim, diminished i.e. weak expression; ns, not significant.
Table 2. Sensitivity and specificity of immunophenotypic profile (training and validation cohort) analysing del(5q) vs. non-del(5q) patients as well as non-clonal cytopenias and healthy bone marrow.

<table>
<thead>
<tr>
<th></th>
<th>training cohort</th>
<th>validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no.</td>
<td>score (mean±SD)</td>
</tr>
<tr>
<td>del(5q) MDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 parameters†</td>
<td>38</td>
<td>8±2</td>
</tr>
<tr>
<td>5 parameter‡</td>
<td>38</td>
<td>16.5±1.5</td>
</tr>
<tr>
<td>non-del(5q) MDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 parameters†</td>
<td>118</td>
<td>6±2</td>
</tr>
<tr>
<td>5 parameter‡</td>
<td>118</td>
<td>11.5±3.5</td>
</tr>
<tr>
<td>non-clonal cytopenias</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 parameters†</td>
<td>88</td>
<td>5±1</td>
</tr>
<tr>
<td>5 parameter‡</td>
<td>88</td>
<td>11.5±2.5</td>
</tr>
<tr>
<td>healthy bone marrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 parameters†</td>
<td>50</td>
<td>5±1</td>
</tr>
<tr>
<td>5 parameter‡</td>
<td>50</td>
<td>12.0±2.0</td>
</tr>
</tbody>
</table>

†All 14 parameters (Table 1A) were included and a threshold of at least 8 was set. ‡The final 5-parameter-del(5q)-score was established after logistic regression analysis including weighting and choosing of the most informative parameters. A threshold of at least 15.0 was applied. §Four patients have been excluded because of an incomplete data set. no., patient numbers; SD, standard deviation; BM, bone marrow.
Figure Legend

Figure 1. 5-parameter-del(5q)-score. (A) The del(5q)-score is significantly higher ($P<0.001$) in del(5q) MDS (grey rectangles) vs. non-del(5q) MDS, as well as healthy BM donors (hBM) and non-clonal cytopenias (white rectangles). (B) Composition of the final 5-parameter-del(5q)-score. (C-D) show monitoring of lenalidomide response in two representative MDS patients with isolated del(5q). In (C) the typical del(5q)-score before treatment and in partial CR is present; the loss of the del(5q) immunophenotype later on predicts cCR reliably at all appropriate investigation points. In (D) data of a patient who achieved an intermittent cCR only are shown; already 10 months after the start of the treatment FCM could detect the reappearance of the disease with the presence of a typical del(5q)-score. At this time point FCM analysis substantiated the cytogenetic result with metaphase FISH analysis detecting only 1/16 metaphases positive for del(5q) and interphase FISH was negative. In the following samples the del(5q)-score increased further reaching the maximum of 18.0 seven month ahead of the full cytogenetic and hematological relapse (month 30).
### Table A

<table>
<thead>
<tr>
<th>Feature</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45-MFI-ratio (lymho vs. myPC) ≤7.0</td>
<td>10</td>
</tr>
<tr>
<td>myPC &gt;2.0%</td>
<td>3</td>
</tr>
<tr>
<td>SSC-ratio (granulo vs. lymho) &lt;6.0</td>
<td>2</td>
</tr>
<tr>
<td>CD71 (granulo) ≤20%</td>
<td>1.5</td>
</tr>
<tr>
<td>gender</td>
<td>1.5</td>
</tr>
</tbody>
</table>

### Diagram B

- **x-axis**: therapy with lenalidomide (month)
- **y-axis**: del(5q) score
- **Legend**:
  - MDS validation cohort
  - hBM non-clonal cytopenias

### Diagram C

- **x-axis**: therapy with lenalidomide (month)
- **y-axis**: del(5q) score
- **Threshold**: (≥15.0) of del(5q) FCM score; FCM score without
- **Note**: complete cytogenetic response (cCR), MFI, mean fluorescence intensity; myPC, myeloid progenitors; SSC, side scatter; lymho, lymphopoiesis; granulo, granulopoiesis.
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² VU University Medical Center Amsterdam, Amsterdam, The Netherlands

Methods

Patients

The “training cohort” consisted of 156 untreated MDS, 38 patients showed a del(5q), including 26 patients with del(5q) as an isolated aberration or with one additional aberration. The “validation cohort” consisted of 64 MDS [22 with del(5q), 12 showing isolated del(5q) or one additional aberration]. In this cohort 22 MDS [(11 with del(5q)] were diagnosed at the Department of Hematology (VU University Medical Center Amsterdam). Non-clonal cytopenias and healthy age-matched BM samples served as controls (Online Supplementary Table 1).

In 18 MDS patients with del(5q), 71 FCM investigations at diagnosis and during the disease course were performed. Within these patients were 10 MDS with del(5q) as a single abnormality and 1 patient with one additional aberration (trisomy 8) included, 7 receiving lenalidomide and 3 azacitidine, a combined treatment within the AZALE trial¹, or an induction therapy with daunorubine plus cytarabine. The further 8 MDS patients showed del(5q) as part of a complex aberrant karyotype and were treated with azacitidine alone (7 patients) or received a combined treatment within the AZALE trial.
This study was performed according to the principles of the Declaration of Helsinki and has received votes from the institutional review boards.

**Flow cytometric immunophenotyping**

FCM was performed on a FACSCanto II equipped with 3 lasers using FACS-DiVa software (BD Biosciences, San Jose, CA). Instrument set up including fluorescence amplification and compensation was fixed automatically applying FACS-DiVa compensation set up. Flow cytometer performance was checked using CS&T beads (BD Biosciences). In each sample 200,000 events were registered. In the VU University Medical Center, Amsterdam, measurements were performed on a FACSCalibur (BD Biosciences).

In both laboratories the applied Boolean gating strategy consisted of the exclusion of doublets and debris, followed by gating of the main populations according to their side scatter (SSC) and CD45 expression. For progenitors with subsequent separation in myeloid (myPC, CD34+CD19-) and lymphoid (lyPC, CD34+CD19+) progenitors CD34 expression and for monocytes CD33/CD36 expression and subsequent backgating to SSC/CD45 was added. Nucleated red cells (NRC) were characterized using CD45, CD235a, and CD71 expression.

We applied published thresholds for abnormal antigen expression as myPC >2.0%, lyPC <5.0%, SSC ratio - granulocytes vs. lymphocytes <6.0. Subsequent cut-offs for abnormal expression, mean and 2 standard deviations (SD), have been determined after assessing healthy BM, myPC: CD7 >15.0%, CD45-MFI-ratio (lymphocytes vs. myPC) ≥7.0; granulopoiesis: CD10 ≤25.0%, CD36 >10.0%, CD71 >20.0%;
monopoiesis: CD56 >20.0%; NRC: percentage >8.0, CD71^{dim} >10% of all CD71+ NRC).
The threshold for abnormal mean fluorescence intensity (MFI) was set at mean and 2 SD compared to normal BM (e.g. CD15 on granulocytes >5300 in the Dresden and >698 in the Amsterdam lab).

Cytogenetics and molecular genetics
Chromosome preparation, G-banding technique, and karyotyping have been done according to routine cytogenetic procedures. In cases of questionable chromosome morphology results were confirmed by spectral karyotyping (SKY; Applied spectral Imaging, Edingen-Neckarhausen, Germany) or single color FISH with commercial DNA probes. According to the recent cytogenetic classification by Schanz and coworkers MDS presenting with a deletion (5q) were subdivided in MDS with del(5q) as single aberration or with one additional abnormality and in MDS with del(5q) as part of a complex aberrant phenotype.
Evaluation of molecular mutations of TP53 gene was performed in unselected BM cells using a PCR (exons 4-9) and a DNA based dHPLC (WAVE) analysis by the Munich Leukemia Laboratory, Germany.

Statistical analyses
Data are presented as mean ± SD or as percent patients positive for the analyzed parameter. Non-parametric Mann-Whitney test comparing continuous variables (e.g. SSC ratio) as well as Chi-Square or Fisher’s exact test analyzing contingency tables (e.g. % patients with/without increased myPC in del(5q) MDS vs. MDS with normal
karyotype) were performed in the basic assessment of possible differences in the antigen pattern of MDS with del(5q) vs. MDS with NK. As a next step, the most discriminatory immunophenotypic features were evaluated and their discriminatory power has been weighted. Thus, the optimal classifier was constructed using a best subset logistic regression approach with initially potentially influential variables in the training cohort (n=14). The model with the optimal Bayesian information criterion was chosen. The construction of the classifier was performed in the training cohort as well. Receiver operating characteristic analysis (ROC including Youden-Index) have been applied in the following to estimate sensitivity and specificity of del(5q) profile. Therefore, statistical analyses were performed with the statistical computing environment R (a language and environment for computing, R Core Team, Vienna, 2013, version 2.15.1; http://wwwR-project.org) or GraphPad Prism (version 4.0.3, GraphPad Software Inc., San Diego, CA).
Supplementary Tables

**Supplementary Table S1.** Patient characteristics in the training and validation cohort.

<table>
<thead>
<tr>
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<th>MDS training cohort</th>
<th>validation cohort</th>
<th>cytopenias</th>
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<tbody>
<tr>
<td>patients (no.)</td>
<td>156</td>
<td>64†</td>
<td>88</td>
</tr>
<tr>
<td>del(5q) isolated‡</td>
<td>26</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>complex</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>88</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>other karyotypes</td>
<td>30</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>age (years) (median; range)</td>
<td>69 (19-84)</td>
<td>72 (26-89)</td>
<td>66 (18-87)</td>
</tr>
<tr>
<td>gender (f/m)</td>
<td>56/100</td>
<td>23/41</td>
<td>39/49</td>
</tr>
<tr>
<td>IPSS (median; range)</td>
<td>0.5 (0.0-3.5)</td>
<td>0.5 (0.0-3.0)</td>
<td></td>
</tr>
<tr>
<td>IPSS-R (median; range)</td>
<td>3.0 (0.0-10.0)</td>
<td>3.0 (1.0-8.5)</td>
<td></td>
</tr>
</tbody>
</table>

†In this cohort 22 MDS [11 with del(5q)] were diagnosed and investigated with flow cytometry at the Department of Hematology of the VU University Medical Center Amsterdam. ‡This includes MDS with del(5q) as a single abnormality (19 in the training and 9 in the validation cohort) or with one additional abnormality. *There were no significant differences in gender or age between MDS and non-clonal cytopenias or healthy BM controls. BM, bone marrow; NK, normal karyotype; f, female; m, male.

**Supplementary Table S2A.** 8-color antibody panel used at Technical University Dresden.

<table>
<thead>
<tr>
<th>FITC</th>
<th>PE</th>
<th>PerCP5.5</th>
<th>PE-cy7</th>
<th>APC</th>
<th>APC-H7</th>
<th>PacBlue</th>
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<td>CD2</td>
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<td>CD10</td>
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<td>CD19</td>
<td>CD45</td>
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<td>(L138)</td>
<td>(8G12)</td>
<td>(HI10a)</td>
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<td>(L243)</td>
<td>(HD37)</td>
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<td>CD123</td>
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<td>(UCHT1)</td>
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<td>eBioscience</td>
<td>BC</td>
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<td>Biolegend</td>
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<td>HLA DR</td>
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<td></td>
<td>(104D2)</td>
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<td>(266)</td>
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CD number, clone, and supplier were provided for each antibody. BD: Becton Dickinson; BC: Beckman Coulter.
Supplementary Table S2B. 4-color panel used at Amsterdam Cancer Center.

<table>
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<th>PE-cy7</th>
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</thead>
<tbody>
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<td>CD45 (2D1)</td>
<td>CD11b (D12)</td>
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<td>BD</td>
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<tr>
<td>CD34 (8G12)</td>
<td>CD11b (D12)</td>
<td>CD45</td>
<td>HLA-DR (L243 (G46-6))</td>
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<td>BD</td>
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<td>BD</td>
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</tr>
<tr>
<td>CD36 (CLB-IVC7)</td>
<td>CD33 (P67.6)</td>
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<td>CD14 (MoP9)</td>
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<tr>
<td>Sanquin</td>
<td>BD</td>
<td>BD</td>
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<tr>
<td>CD36 10.1</td>
<td>CD64 (10.1)</td>
<td>CD45</td>
<td>CD14</td>
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<td>DAKO</td>
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<tr>
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<td>CD34 (8G12)</td>
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</tr>
<tr>
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<td>CD117 (104D2)</td>
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<td>CD13+CD33 (WM15+P67.6)</td>
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CD number, clone, and supplier were provided for each antibody. BD, Becton Dickinson.
Supplementary Table S3A. Logistic regression analysis with estimates and significances from the analysis of the final 5-parameter-del(5q)-score.

|                | Estimate | Std. Error | z value | Pr(>|z|) |
|----------------|----------|------------|---------|----------|
| (Intercept)    | -23.623  | 1314.094   | -0.018  | 0.986    |
| % myPC         | 2.591    | 0.607      | 4.266   | <0.001   |
| CD45 ratio     | 18.028   | 1314.093   | 0.014   | 0.989    |
| SSC ratio      | 2.070    | 0.552      | 3.749   | <0.001   |
| % CD71 gran    | 1.477    | 0.627      | 2.355   | 0.019    |
| female gender  | 1.594    | 0.532      | 2.995   | 0.003    |

Supplementary Table S3B. ROC table of the validation set from the analysis of the final 5-parameter-del(5q)-score.

<table>
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<th>cut-off</th>
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</tbody>
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†ROC curve: AUC=0.946; P<0.001.

References


