Feedback Signals in Myelodysplastic Syndromes: Increased Self-Renewal of the Malignant Clone Suppresses Normal Hematopoiesis

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Index

Supplemental Methods ............................................................................................................................ 2
  Mathematical modeling of normal hematopoiesis and MDS ............................................................... 2
  Derivation of the mathematical model ................................................................................................. 3
  Model of hematopoiesis in presence of dysplastic cells ...................................................................... 5
  Derivation of the feedback signal in presence of dysplastic cells ........................................................ 6
  Immunophenotypic analysis ................................................................................................................ 7

Supplemental Figures .............................................................................................................................. 8
  Figure 1: Simulation of MDS-development with alternative parameters. ............................................ 9
  Figure 2: Detection of CD34 + cells in MDS-bone marrow and normal controls.................................. 9
  Figure 3: Fold expansion of CB-derived CD34 + cells. ................................................................. 9
  Figure 4: Immunophenotypic analysis upon co-culture with MSCs. .................................................. 10
  Figure 5: MDS serum stimulates proliferation of BM-derived CD34 + cells. ..................................... 11
  Figure 6: Analysis of cytokine concentration in serum samples. ....................................................... 11

Supplemental Tables ............................................................................................................................. 12
  Table 1: Clinical data for serum samples.......................................................................................... 12
  Table 2: Patient data for bone marrow samples used in CD34 FACS analysis................................. 14

Supplemental References ..................................................................................................................... 15
Supplemental Methods

Mathematical modeling of normal hematopoiesis and MDS

The mathematical model developed in this study describes the interaction of physiological hematopoiesis and MDS cells. It is based on a previously proposed model of the hematopoietic system [1] that was extended to describe leukemia dynamics [2]. According to the classical understanding, hematopoiesis is assumed to be a multi-step process [3,4] in which cells move through an ordered sequence of different maturation stages (compartments). The following maturation stages are considered: (1) long-term repopulating stem cells (LT-HSCs), (2) short-term repopulating stem cells (ST-HSCs), (3) multi-potent progenitor cells (MPPs), (4) committed progenitor cells (CPCs), (5) precursors and (6) mature cells. For simplicity, we take only one hematopoietic lineage into account. We assume that daughter cells arising from division can either belong to the same type as the mother cell (self-renewal) or to a more mature cell type (differentiation). Time dynamics of each compartment are governed by cell gain due to self-renewal and by cell loss due to differentiation or apoptosis. The following cell properties are considered [1]: (1) proliferation rate, describing how many cell divisions take place per unit of time. We assume that mature blood cells and the most differentiated dysplastic cells are post-mitotic. (2) Fraction of self-renewal, describing which fraction of daughter cells belongs to the same maturation stage as their mother cell. (3) Apoptosis rate, describing which fraction of a cell population dies per unit of time.

It is further assumed that physiological hematopoiesis is regulated by feedback signals. In contrast to previous models, we consider two different feedback signals:

(1) The signal for proliferation is inversely correlated with the number of mature cells - resulting in higher proliferation upon cytopenia. This signal is motivated by the regulation of hematopoiesis by cytokines such as G-CSF or EPO, which increase if there is a shortage of mature cells [5,6]. For simplicity, we assume that this signal acts on all mitotic cells although some cell lineages and maturation stages are probably more sensitive than others. It is well known that many cytokines act on more than one cell type, e.g. G-CSF acts on the myeloid lineage and on HSC at the same time [6].

(2) The signal regulating self-renewal is dependent on the number of stem and progenitor cells in the marrow niche and acts on the cells residing in the niche. This signal is motivated by the fact that primitive cells require an appropriate microenvironment to maintain stemness [4,7]. We assume that if cells are outcompeted from this microenvironment, they can no longer perform self-renewal. For this reason, self-renewal rates decrease if the number of cells in the niche increases. For simplicity, we assume that the three most primitive cell types of each lineage are located in the bone marrow niche.

It is assumed that the MDS clone is maintained by cells with stem cell-like properties. It is known that MDS cells possess variable potential to differentiate into mature cells [8,9]. For simplicity, we assume in this model that no mature cells are produced by MDS cells (this does not account for the alternative model presented in Figure S1). Therefore, we consider five compartments of aberrant cells (MDS-LT-HSCs, MDS-ST-HSCs, MDS-MPPs, MDS-CPCs, and dysplastic progenitors). This maturation process is also regulated by the feedback signals mentioned above. We have tested various parameters for
the MDS clone. The results – and mathematical analysis [2] – demonstrate that the MDS-LT-HSC must reveal higher self-renewal potential than LT-HSCs for sustained disease progression. For different parameter values, qualitative behavior is very similar. Cell numbers and self-renewal rates for each compartment are chosen such that they adopt the same steady state behavior as described in our previous work [1]. For numerical simulations, we have used the ODE solver ode23t from Matlab (MathWorks, Natick, MA).

Derivation of the mathematical model

We use ordinary differential equations (ODEs) to describe interaction of hematopoietic and dysplastic cells. Time evolution of each maturation stage (compartment) is described by one ODE. We denote the concentration of healthy cells in maturation stage \( i \) at time \( t \) as \( c_i(t), \) \((i=1,\ldots,6)\). For example, \( c_1(t) \) denotes the number of LT-HSCs at time \( t \), \( c_6(t) \) the number of mature cells. Cell concentrations in the dysplastic cell line are denoted as \( \hat{c}_j(t), \) \( j=1,\ldots,5 \).

The proliferation rate of hematopoietic cells in stage \( i \) at time \( t \) is denoted as \( p_i(t) \), that of dysplastic cells in stage \( j \) as \( \hat{p}_j(t) \). Mature cells and dysplastic progenitors are considered to be post-mitotic:
\[
p_6(t) = 0 \quad \text{and} \quad \hat{p}_5(t) = 0
\]
(this does not account for the alternative model in Figure 1 in Text S1). The fraction of self-renewal of hematopoietic cells in stage \( i \) at time \( t \) is denoted as \( a_i(t) \) that of dysplastic cells in stage \( j \) as \( \hat{a}_j(t) \). Analogously, the apoptosis rate of hematopoietic cells in stage \( i \) at time \( t \) is denoted as \( d_i(t) \) and that of dysplastic cells in stage \( j \) as \( \hat{d}_j(t) \). For simplicity, we assume that immature cells do not commit apoptosis, i.e., \( d_i(t) = 0 \) for \( i=1,\ldots,5 \) and that mature cells die at a constant positive rate \( d_6(t) = d > 0 \). In analogy, we set \( \hat{d}_i(t) = \ldots = \hat{d}_4(t) = 0 \) for all \( t \) and \( \hat{d}_5(t) = \hat{d}_5 > 0 \) (this does not account for the alternative model in Figure 1 in Text S1).

At time \( t \), the flux to mitosis in hematopoietic compartment \( i \) \((i=1,\ldots,5)\) equals \( p_i(t)c_i(t) \). This amount of cells disappears from compartment \( i \) and gives rise to \( 2p_i(t)c_i(t) \) daughter cells, \( 2a_i(t)p_i(t)c_i(t) \) of which stay in compartment \( i \) and \( 2(1-a_i(t))p_i(t)c_i(t) \) move on to compartment \( i+1 \).

We assume that proliferation and self-renewal change over time due to the feedback signals \( s_1 \) and \( s_2 \). As derived from a quasi-steady-state approximation in our previous work [1], \( s_1 \) has the form
\[
s_1(t) = \frac{1}{1 + k \cdot c_6(t)}.
\]
The positive constant \( k \) depends on the rates of cytokine production and
elimination. In analogy to our previous study [1], we assume that \( p_i(t) \) (for \( i = 1,...,5 \)) is given by
\[ p_i(t) = p_{i,\text{max}} \cdot s_i(t), \]
where \( p_{i,\text{max}} \) is the maximal proliferation rate of cells in stage \( i \). This regulation is a negative feedback: if there is a shortage of mature cells (i.e., \( c_6(t) \) is small), proliferation rates increase and if mature cell counts are high, proliferation rates decrease.

We further assume that the number of primitive cells in BM niche regulates the fraction of self-renewal of the primitive cells. If it is assumed that self-renewal is regulated by the concentration \( s_2(t) \) of signaling factors produced at constant rates and degraded by immature cells, we obtain
\[ s_2(t) = \frac{1}{1 + l \cdot \sum_{i=1}^{3} c_i(t)} \]
where \( l \) is a positive constant. In analogy to proliferation rates, we set
\[ a_i(t) = a_{i,\text{max}} \cdot s_2(t), \]
for cells in the stages \( i = 1,...,3 \), where \( a_{i,\text{max}} \) is the maximal self-renewal fraction of cells in stage \( i \). Self-renewal of more mature cell types is supposed to be constant and denoted by \( a_i(t) \equiv a_i, \ i = 4,5 \). Consequently, self-renewal increases if there is a shortage of primitive cells in the BM niche. Taking into account that mature cells are post-mitotic and that there exists no influx due to differentiation to the most primitive stage, we obtain for the hematopoietic lineage:

\[
\frac{d}{dt} c_1(t) = (2a_{1,\text{max}}s_2(t) - 1)p_{1,\text{max}}s_1(t)c_1(t)
\]
\[
\frac{d}{dt} c_2(t) = 2(1-a_{1,\text{max}}s_2(t))p_{1,\text{max}}s_1(t)c_1(t) + (2a_{2,\text{max}}s_2(t) - 1)p_{2,\text{max}}s_1(t)c_2(t)
\]
\[
\frac{d}{dt} c_3(t) = 2(1-a_{2,\text{max}}s_2(t))p_{2,\text{max}}s_1(t)c_2(t) + (2a_{3,\text{max}}s_2(t) - 1)p_{3,\text{max}}s_1(t)c_3(t)
\]
\[
\frac{d}{dt} c_4(t) = 2(1-a_{3,\text{max}}s_2(t))p_{3,\text{max}}s_1(t)c_3(t) + (2a_{4} - 1)p_{4,\text{max}}s_1(t)c_4(t)
\]
\[
\frac{d}{dt} c_5(t) = 2(1-a_{4})p_{4,\text{max}}s_1(t)c_4(t) + (2a_{5} - 1)p_{5,\text{max}}s_1(t)c_5(t)
\]
\[
\frac{d}{dt} c_6(t) = 2(1-a_{5})p_{5,\text{max}}s_1(t)c_5(t) - d_6c_6(t)
\]
\[ s_1(t) = \frac{1}{1 + k \cdot c_6(t)} \]
\[ s_2(t) = \frac{1}{1 + l \cdot \sum_{i=1}^{3} c_i(t)} \]
The values of $k = 1.28 \cdot 10^{-9}$ and $l = 7.56 \cdot 10^{-9}$ were chosen such that the physiological equilibrium of mature cells is of the order of $10^9$ cells / L of blood and such that proliferation rates could increase about 3 to 4 fold under regenerative pressure [10]. Parameters for the hematopoietic system were chosen as previously described [1,11].

**Model of hematopoiesis in presence of dysplastic cells**

We assume that, similar to the hematopoietic system, all dysplastic cells depended on the signal $s_1$ and that the three most primitive dysplastic cell types depend on the signal for self-renewal $s_2$. Including modulation of $s_2$ by primitive MDS-cells leads to the following expression for $s_2$:

$$s_2(t) = \frac{1}{1 + l \left( \sum_{i=1}^{3} c_i(t) + \sum_{j=1}^{3} \hat{c}_j(t) \right)}.$$

The positive constant $l$ depends on production or elimination of signal molecules. This expression is motivated below.

For the interaction of healthy and dysplastic cells, the following ODE system is obtained:

\[
\begin{align*}
\frac{d}{dt} c_1(t) &= \left(2a_{1,\text{max}}s_2(t) - 1\right)p_{1,\text{max}}s_1(t)c_1(t) \\
\frac{d}{dt} c_2(t) &= 2\left(1 - a_{1,\text{max}}s_2(t)\right)p_{1,\text{max}}s_1(t)c_1(t) + \left(2a_{2,\text{max}}s_2(t) - 1\right)p_{2,\text{max}}s_1(t)c_2(t) \\
\frac{d}{dt} c_3(t) &= 2\left(1 - a_{2,\text{max}}s_2(t)\right)p_{2,\text{max}}s_1(t)c_2(t) + \left(2a_{3,\text{max}}s_2(t) - 1\right)p_{3,\text{max}}s_1(t)c_3(t) \\
\frac{d}{dt} c_4(t) &= 2\left(1 - a_{3,\text{max}}s_2(t)\right)p_{3,\text{max}}s_1(t)c_3(t) + \left(2a_{4} - 1\right)p_{4,\text{max}}s_1(t)c_4(t) \\
\frac{d}{dt} c_5(t) &= 2\left(1 - a_{4}\right)p_{4,\text{max}}s_1(t)c_4(t) + \left(2a_{5} - 1\right)p_{5,\text{max}}s_1(t)c_5(t) \\
\frac{d}{dt} c_6(t) &= 2\left(1 - a_{5}\right)p_{5,\text{max}}s_1(t)c_5(t) - d_6c_6(t) \\
\frac{d}{dt} \hat{c}_1(t) &= \left(2\hat{a}_{1,\text{max}}s_2(t) - 1\right)p_{1,\text{max}}s_1(t)\hat{c}_1(t) \\
\frac{d}{dt} \hat{c}_2(t) &= 2\left(1 - \hat{a}_{1,\text{max}}s_2(t)\right)p_{1,\text{max}}s_1(t)\hat{c}_1(t) + \left(2\hat{a}_{2,\text{max}}s_2(t) - 1\right)p_{2,\text{max}}s_1(t)\hat{c}_2(t) \\
\frac{d}{dt} \hat{c}_3(t) &= 2\left(1 - \hat{a}_{2,\text{max}}s_2(t)\right)p_{2,\text{max}}s_1(t)\hat{c}_2(t) + \left(2\hat{a}_{3,\text{max}}s_2(t) - 1\right)p_{3,\text{max}}s_1(t)\hat{c}_3(t) \\
\frac{d}{dt} \hat{c}_4(t) &= 2\left(1 - \hat{a}_{3,\text{max}}s_2(t)\right)p_{3,\text{max}}s_1(t)\hat{c}_3(t) + \left(2\hat{a}_{4} - 1\right)p_{4,\text{max}}s_1(t)\hat{c}_4(t)
\end{align*}
\]
\[
\frac{d}{dt} \hat{c}_s(t) = 2\left(1 - \hat{a}_s\right) \hat{p}_{4,\text{max}} s_1(t) \hat{c}_s(t) - \hat{a}_s \hat{c}_s(t)
\]

\[
s_1(t) = \frac{1}{1 + k \cdot c(t)}
\]

\[
s_2(t) = \frac{1}{1 + l \cdot \left(\sum_{i=1}^{3} c_i(t) + \sum_{j=1}^{3} \hat{c}_j(t)\right)}
\]

By setting \(\hat{c}_j(t) = 0\), for \(j = 1, \ldots, 3\), the system describing healthy hematopoiesis is obtained.

**Derivation of the feedback signal in presence of dysplastic cells**

The concentration of the signal regulating self-renewal at time \(t\) is denoted by \(S(t)\). We assume that self-renewal is the default pathway with signal production at constant rate \(\alpha_s\). Degradation of this signal is assumed to be proportional to the cells in the bone marrow niche at a rate \(\beta_s\). We further assume a cell independent degradation at rate \(\gamma_s\). In analogy, we assume that dysplastic cells secrete an inhibitor \(I\) proportional to their concentrations at a rate \(\alpha_j\) and that this inhibitor is degraded at a constant rate \(\gamma_j\). The complex of inhibitor and signal molecules, denoted by \(IS\), is supposed to be formed at a rate \(k_1\). After formation of the complex, the signal molecule is degraded at a rate \(k_2\). For simplicity, backward reactions are neglected. Dynamics of signal and inhibitor are described by the following ODE system:

\[
\frac{d}{dt} I(t) = \alpha_j \sum_{j=1}^{3} \hat{c}_j(t) - \gamma_j \cdot I(t) - I(t) \cdot S(t) \cdot k_1 + IS(t) \cdot k_2
\]

\[
\frac{d}{dt} IS(t) = k_1 \cdot I(t) \cdot S(t) - k_2 \cdot IS(t)
\]

\[
\frac{d}{dt} S(t) = \alpha_s - \beta_s \left(\sum_{i=1}^{3} c_i(t) + \sum_{j=1}^{3} \hat{c}_j(t)\right) S(t) - \gamma_s S(t) - k_1 \cdot I(t) \cdot S(t)
\]

We assume that molecular dynamics are fast in comparison to cell proliferation and differentiation, i.e. they are in a quasi-steady-state. We denote steady state concentrations of \(I\), \(S\) and \(IS\) in the above system as \(\overline{I}\), \(\overline{S}\) and \(\overline{IS}\). We express them as a function of the level of hematopoietic and dysplastic cells. It holds:

\[
\overline{I} = \frac{\alpha_j \sum_{j=1}^{3} \hat{c}_j(t)}{\gamma_j} \quad \text{and}
\]
\[ S = \frac{\alpha_s}{\beta_s \left( \sum_{i=1}^{3} c_i(t) + \sum_{j=1}^{3} \hat{c}_j(t) \right) + \gamma_s + k_1 \frac{\alpha_i \sum_{j=1}^{3} \hat{c}_j(t)}{\gamma_i}}. \]

Renormalization with respect to its maximal possible value yields

\[ s(t) := \frac{\gamma_s \bar{S}}{\alpha_s} = \frac{1}{\beta_s \left( \sum_{i=1}^{3} c_i(t) + \sum_{j=1}^{3} \hat{c}_j(t) \right) + 1 + \frac{k_1 \cdot \alpha_i}{\gamma_s \cdot \gamma_i} \sum_{j=1}^{3} \hat{c}_j(t)}, \]

which is between zero and one. If we assume that production rates of the inhibitor are small, we obtain

\[ s(t) \approx s_2(t) \]

which is used in the model.

**Immunophenotypic analysis**

Cells were washed in PBS, stained with CD34-allophycocyanin (APC; Becton Dickinson, San Jose, CA, USA [BD], clone 8G12), CD133-phycoerythrin (PE, Miltenyi Biotec, Bergisch Gladbach, clone AC141) and CD45-V500 (BD, clone HI30) in a dilution of 1:200 and analyzed using a FACS Canto II (BD) running FACS Diva software (BD). Further analysis was performed using WinMDI software (WinMDI 2.8; The Scripps Institute, San Diego, CA, USA). Discrimination between MSC and HPC was possible by forward scatter, side scatter, propidium iodide (PI) staining and CFSE-staining. Immunophenotypic data of MDS-BM samples was obtained from 52 routinely screened MDS and 13 healthy control samples from the University Hospital Düsseldorf. The samples were stained with CD34-PE (BD, clone 8G12) and CD45-APC (BD, clone 2D1) in a dilution of 1:200 and analyzed using a FACS Calibur (BD) and FCS Express V3 software (De Novo Software, Los Angeles, CA, USA). The percentages of cells positive for CD34 were extracted and analyzed. Relevant patient data are shown in Table 2 in Text S1.
### Supplemental Figures

#### Figure A

| Bone Marrow Niche | Bone Marrow | Peripheral Blood |
|-------------------|-------------|------------------|
| Normal Hematopoiesis | LT-HSC | MPP | CPC | Precursors | Mature Cells |
| Proliferation | > 50d | > 10d | > 5d | > 2d | > 2d |
| Self-Renewal | < 70% | < 65% | < 65% | = 45% | = 40% |
| Half-Life | 10d | |

MDS Development

| MDS-LT-HSC | MDS-ST-HSC | MDS-MPP | MDS-CPC | Dysplastic BM Precursors | Dysplastic PB Precursors |
|-----------|------------|---------|---------|-------------------------|-------------------------|
| Proliferation | > 25d | > 5d | > 2.5d | > 1d | > 1d |
| Self-Renewal | < 90% | < 65% | < 65% | = 45% | = 40% |
| Half-Life | 0.66d | |

#### Figure B

- LT-HSC (c₁)
- ST-HSC (c₂)
- MPP (c₃)
- CPC (c₄)
- Precursors (c₅)
- Mature Cells (c₆)
- MDS-LT-HSC (c₇)
- MDS-ST-HSC (c₈)
- MDS-MPP (c₉)
- MDS-CPC (c₁₀)
- Dysplastic BM Precursors (c₁₁)
- Dysplastic PB Precursors (c₁₂)

#### Figures C, D, E, F

Legend on next page
Figure 1: Simulation of MDS-development with alternative parameters.

(A) In comparison to parameters of Figure 1, we used slightly different parameters for this alternative simulation: 1) MDS cells can progress to a dysplastic mature stage in peripheral blood which then also feeds back on proliferation, i.e. $S_1 = \frac{1}{(1+k(c_0+e_0))}$; 2) proliferation rate of MDS cells of all differentiation stages is much higher than in normal hematopoiesis; and 3) dysplastic progenitors and dysplastic mature cells both undergo apoptosis at a much shorter half-life as compared to normal mature cells. This is in accordance with higher apoptosis rates observed in MDS patient bone marrow and peripheral blood [12,13,14]. The corresponding parameters are indicated for each cell type.

(B) Simulated MDS development over 60 years. (C) A sharp decline of mature cells is achieved after about 20 years and this would correspond to clinical manifestation of MDS. (D) Simulated cellular composition in the BM at the relevant time frame. Bone marrow becomes hypocellular due to high apoptosis rates of dysplastic cells. (E) Corresponding signal intensities for self-renewal and proliferation are presented. Self-renewal decays due to the accumulation of malignant cells in the BM-niche; proliferation is activated due to ineffective hematopoiesis. (F) The percentage of apoptotic cells in the bone marrow is presented. It increases in the course of simulated MDS development. For simulations, $k$ and $I$ were chosen as for the model presented above.

Figure 2: Detection of CD34$^+$ cells in MDS-bone marrow and normal controls.

(A) Example of presentation of CD34 measurement in healthy bone marrow, low-risk MDS and high risk MDS. The percentage of positive cells is depicted. (B) 52 MDS and 13 healthy BM-samples were analyzed with regard to CD34 and CD45 expression. High percentages of CD34$^+$ cells in BM samples correlate with bad disease prognosis. Relevant patient data are provided in Table 2 in Text S1. Error bars represent SEM (*p < 0.05, **p < 0.01).

Figure 3: Fold expansion of CB-derived CD34$^+$ cells.

Cells were expanded for 13 days in medium supplemented with SCF, TPO and FGF either without or with stromal support. Mean expansion rates were 60-fold and 242-fold, respectively.
Figure 4: Immunophenotypic analysis upon co-culture with MSCs.

(A) CD34⁺ CB-HPCs were stained with CFSE and subsequently cultivated for seven days in vitro on a confluent layer of MSCs. Culture medium was additionally supplemented with 10% serum from healthy controls (upper panel) or 10% serum from MDS-patients (lower panel). Flow cytometric analysis was used to monitor cell division history by residual CFSE-staining (dotted line in histogram indicates five cell divisions). Expression of CD34, CD45 and CD133 was analyzed in relation to the number of cell divisions. Representative histograms and density plots are demonstrated. (B) Analysis of mean fluorescence intensities after five cell divisions normalized to the control samples. No significant differences were detected. Error bars represent SD.
Figure 5: MDS serum stimulates proliferation of BM-derived CD34⁺ cells.

CD34⁺ HPCs from bone marrow were stained with CFSE and subsequently cultivated for four days in vitro in culture medium supplemented with 10% of individual patient or control serum. Mean fluorescence intensities after five cell divisions were normalized to control samples. Error bars represent SD (*p < 0.05, **p < 0.01, ***p < 0.001).

Figure 6: Analysis of cytokine concentration in serum samples.

Concentrations of SCF, bFGF and TPO in MDS-serum and healthy control serum samples were detected using RayBio Human ELISA Kits. Although there are tendencies to a higher amount of bFGF and TPO in MDS-serum samples, these differences were not statistically significant. Error bars represent SD.
## Supplemental Tables

### Table 1: Clinical data for serum samples.

| ID | sex | age | MDS-subtype | leukocytes [µL] | erythroc. [1x10^6/µL] | thromboc. [x1000/µL] | HB [g/dL] | BM-cellularity |
|----|-----|-----|-------------|----------------|-----------------------|-----------------------|----------|----------------|
| 1  | m   | 51  | RCMD        | n.d.           | n.d.                  | n.d.                  | n.d.     | n.d.           |
| 2  | m   | 73  | RCMD        | 4000           | n.d.                  | 410                   | 9.2      | n.d.           |
| 3  | f   | 66  | RCMD        | 2500           | 4.98                  | 83                    | 12.4     | n.d.           |
| 4  | m   | 81  | RCMD        | 5600           | 3.54                  | 101                   | 10.6     | n.d.           |
| 5  | m   | 75  | RCMD        | 2400           | 2.31                  | 87                    | 9        | n.d.           |
| 6  | m   | 69  | RAEB I      | 3100           | 2.53                  | 51                    | 10.5     | normocellular  |
| 7  | m   | 76  | del(5q)     | 5500           | 4.00                  | 165                   | 13.2     | hypocellular   |
| 8  | m   | 74  | RCMD        | 2800           | 2.25                  | 391                   | 6.7      | normocellular  |
| 9  | m   | 67  | RCMD        | 6100           | 3.42                  | 338                   | 11.7     | hypercellular  |
| 10 | f   | 70  | del(5q)     | 6000           | 4.77                  | 117                   | 14.4     | normocellular  |
| 11 | f   | 83  | RCMD        | 3700           | 2.97                  | 22                    | 9.7      | hypocellular   |
| 12 | m   | 44  | CMMML I     | 5700           | 5.30                  | 30                    | 16       | hypocellular   |
| 13 | m   | 53  | RAEB II     | 2500           | 3.69                  | 40                    | 10.7     | hypocellular   |
| 14 | m   | 73  | sAML        | 1800           | 3.13                  | 12                    | 9.1      | normocellular  |
| 15 | f   | 85  | del(5q)     | 9700           | 3.00                  | 397                   | 10       | hypercellular  |
| 16 | m   | 76  | RAEB II     | 2700           | 3.28                  | 80                    | 10       | hypercellular  |
| 17 | f   | 72  | del(5q)     | 2270           | 2.27                  | 77                    | 6.1      | hypercellular  |
| 18 | m   | 49  | RCMD        | 9300           | 2.85                  | 88                    | 8        | hypercellular  |
| 19 | f   | 71  | del(5q)     | 4700           | 2.86                  | 55                    | 10.4     | normocellular  |
| 20 | m   | 60  | RCMD        | 3100           | 2.98                  | 133                   | 10.5     | n.d.           |
| 21 | m   | 71  | RAEB I      | 2600           | 3.73                  | 268                   | 13.1     | n.d.           |
| 22 | m   | 57  | RCMD        | 18100          | 2.43                  | 73                    | 7.3      | hypercellular  |
| 23 | f   | 61  | RCMD        | 3900           | 2.69                  | 262                   | 8.4      | normocellular  |
| 24 | f   | 61  | RCMD        | 16400          | 2.96                  | 344                   | 8.7      | hypocellular   |
| 25 | m   | 67  | sAML        | 13600          | 2.99                  | 12                    | 8.8      | hypercellular  |
| 26 | f   | 92  | RCMD-RS     | 6200           | 1.66                  | 505                   | 5.7      | hypercellular  |
| 27 | m   | 64  | RCMD        | 10200          | 2.37                  | 283                   | 9.8      | hypercellular  |
| 28 | f   | 68  | RAEB I      | 800            | 3.42                  | 13                    | 9.8      | n.d.           |
| 29 | f   | 71  | CMMML I     | 23800          | 4.66                  | 115                   | 13.8     | normocellular  |
| 30 | m   | 71  | RAEB II     | 1700           | 3.88                  | 103                   | 10.8     | n.d.           |
| 31 | m   | 77  | sAML        | 43700          | 2.77                  | 31                    | 7.7      | n.d.           |
| 32 | f   | 64  | RCMD        | 3800           | 2.95                  | 162                   | 9.5      | n.d.           |
| 33 | m   | 70  | CMMML I     | 4600           | 4.06                  | 57                    | 12.1     | normocellular  |
| 34 | m   | 57  | CMMML I     | 4300           | 4.61                  | 144                   | 14.1     | hypercellular  |
| 35 | m   | 77  | CMMML I     | 5800           | 3.25                  | 102                   | 8.5      | hypercellular  |
| 36 | f   | 73  | CMMML I     | 3800           | 4.48                  | 80                    | 12.5     | n.d.           |
| 37 | f   | 59  | RCMD        | 1900           | 1.85                  | 387                   | 7.2      | hypercellular  |
| 38 | f   | 83  | RCMD-RS     | 2000           | 2.47                  | 265                   | 8.4      | normocellular  |
| 39 | m   | 74  | RCMD        | 3000           | 3.79                  | 18                    | 10.6     | hypercellular  |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 40 | m | 77 | CMML I | 10300 | 5.76 | 192 | hypercellular |
| 41 | f | 65 | CMML I | 1800 | 3.54 | 74 | 10.7 | n.d. |
| 42 | m | 60 | del(5q) | 14600 | 2.31 | 734 | 7.3 | n.d. |
| 43 | m | 58 | RAEB I | 3200 | 4.68 | 231 | 13.9 | hypercellular |
| 44 | m | 62 | RCMD | 1900 | 2.42 | 236 | 6.9 | hypercellular |
| 45 | f | 54 | RAEB I | 800 | 3.19 | 22 | 10.4 | n.d. |
| 46 | m | 73 | RCMD | 2500 | 3.03 | 22 | 8.5 | normocellular |
| 47 | m | 76 | RCMD | 1200 | 2.97 | 36 | 9.1 | normocellular |
| 48 | f | 80 | CMML I | 184000 | 2.74 | 37 | 8.2 | n.d. |
| 49 | f | 75 | RCMD | 11000 | 2.19 | 420 | 7.9 | hypercellular |
| 50 | m | 72 | CMML I | 5600 | 3.69 | 62 | 13.7 | hypercellular |
| 51 | f | 68 | RAEB I | 2200 | 2.81 | 100 | 10.2 | hypocellular |
| 52 | f | 77 | RAEB II | 2600 | 3.09 | 90 | 9 | hypercellular |
| 53 | m | 74 | RCMD-RS | 2690 | 2.69 | 374 | 9.5 | normocellular |
| 54 | m | 68 | RCMD-RS | 4100 | 3.39 | 263 | 9.5 | n.d. |
| 55 | f | 65 | sAML | 1500 | 2.70 | 102 | 8.7 | n.d. |
| 56 | m | 76 | RCMD | 5800 | 3.06 | 10 | 9.9 | hypercellular |
| 57 | m | 81 | RCMD | 12800 | 3.35 | 364 | 9 | hypercellular |

Data from healthy controls

|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| H1 | m | 46 | healthy | 7100 | 5.2 | 269 | 16.2 | n.d. |
| H2 | m | 61 | healthy | 7400 | 4.17 | 180 | 13.9 | n.d. |
| H3 | m | 71 | healthy | 8200 | 4.87 | 219 | 15.7 | n.d. |
| H4 | f | 73 | healthy | 5000 | 3.92 | 378 | 11.9 | n.d. |
| H5 | m | 61 | healthy | 7400 | 3.71 | 464 | 12.1 | n.d. |
| H6 | f | 74 | healthy | 5600 | 4.7 | 199 | 14.3 | n.d. |
| H7 | f | 68 | healthy | 4100 | 4.5 | 313 | 14.4 | n.d. |
| H8 | f | 34 | healthy | 4600 | 5 | 250 | 14.8 | n.d. |
| H9 | f | 40 | healthy | 9900 | 4.4 | 216 | 13.2 | n.d. |
| H10 | f | 48 | healthy | 9400 | 2.9 | 335 | 8.2 | n.d. |
| H11 | f | 51 | healthy | n.d. | 3.5 | 953 | 9.9 | n.d. |
| H12 | f | 43 | healthy | 7400 | 5 | 279 | 14.8 | n.d. |

CMML (chronic myelomonocytic leukemia); del(5q) (chromosomal deletion 5q); RAEB (refractory anemia with excess of blasts); RCMD (refractory cytopenia with multilineal dysplasia); RCMD-RS (with ringed sideroblasts); sAML (secondary acute myeloid leukemia), n.d. (not determined); all patients were treated without involvement of erythropoietin.
Table 2: Patient data for bone marrow samples used in CD34 FACS analysis.

| ID | sex | age | MDS-subtype | leukocytes [µL] | erythroc. [1x10^6/µL] | thromboc. [x1000/µL] | HB [g/dL] | BM-cellularity |
|----|-----|-----|-------------|----------------|------------------------|-------------------------|----------|----------------|
| F1 | m   | 76  | RCMD        | 3600           | 2.06                   | 355                     | 8.5      | n.d.           |
| F2 | m   | 69  | RAEB II     | 3200           | 3.52                   | 175                     | 10.0     | n.d.           |
| F3 | f   | 69  | RCMD        | 4800           | 2.00                   | 402                     | 7.2      | n.d.           |
| F4 | m   | 76  | del(5q)     | 5500           | 4.00                   | 165                     | 13.2     | hypocellular   |
| F5 | f   | 71  | RAEB II     | 1800           | 3.03                   | 17                      | 7.6      | n.d.           |
| F6 | m   | 70  | RCMD        | 1400           | 2.84                   | 51                      | 8.1      | n.d.           |
| F7 | m   | 53  | RCMD        | 5300           | 3.06                   | 192                     | 9.0      | n.d.           |
| F8 | m   | 62  | CMML II     | 16700          | 3.39                   | 87                      | 10.2     | n.d.           |
| F9 | f   | 67  | RAEB II     | 1600           | 2.57                   | 80                      | 9.5      | n.d.           |
| F10| m   | 79  | RAEB II     | 1800           | 3.23                   | 215                     | 9.5      | n.d.           |
| F11 | f  | 49  | RAEB II     | 6900           | 3.47                   | 160                     | 10.6     | n.d.           |
| F12| m   | 64  | RAEB II     | 900            | 3.86                   | 149                     | 11.0     | n.d.           |
| F13| m   | 76  | RAEB II     | 1000           | 3.38                   | 64                      | 8.9      | n.d.           |
| F14| f   | 65  | RAEB II     | 2400           | 3.20                   | 50                      | 11.0     | n.d.           |
| F15| m   | 75  | RAEB II     | 1300           | 2.78                   | 23                      | 10.4     | n.d.           |
| F16| m   | 73  | sAML        | 1800           | 3.13                   | 12                      | 9.1      | normocellular  |
| F17| m   | 65  | RCMD        | 1900           | 2.87                   | 113                     | 8.6      | n.d.           |
| F18| f   | 68  | RCMD        | 1700           | 3.45                   | 24                      | 12.7     | n.d.           |
| F19| m   | 71  | RCMD        | 3200           | 3.38                   | 52                      | 10.7     | n.d.           |
| F20| m   | 78  | RCMD        | 3300           | 3.62                   | 70                      | 9.0      | n.d.           |
| F21| m   | 62  | RCMD        | 1900           | 2.42                   | 236                     | 6.9      | hypercellular  |
| F22| f   | 72  | RAEB II     | 6100           | 2.89                   | 33                      | 8.8      | n.d.           |
| F23| m   | 79  | RCMD        | 2800           | 2.59                   | 306                     | 8.0      | n.d.           |
| F24| m   | 53  | RAEB II     | 2500           | 3.69                   | 40                      | 10.7     | hypocellular   |
| F25| m   | 68  | RCMD-RS     | 4100           | 3.39                   | 263                     | 9.5      | n.d.           |
| F26| f   | 65  | CMML I      | 1800           | 3.54                   | 74                      | 10.7     | n.d.           |
| F27| f   | 76  | CMML II     | 9400           | 2.80                   | 198                     | 9.8      | n.d.           |
| F28| m   | 64  | RCMD        | 10200          | 2.37                   | 283                     | 9.8      | hypercellular  |
| F29| f   | 42  | RARS        | 3300           | 2.75                   | 468                     | 9.8      | n.d.           |
| F30| m   | 49  | RCMD        | 9300           | 2.85                   | 88                      | 8.0      | hypercellular  |
| F31| f   | 71  | RCMD        | 4200           | 2.69                   | 97                      | 8.7      | n.d.           |
| F32| m   | 38  | RAEB II     | 2500           | 2.96                   | 50                      | 9.3      | n.d.           |
| F33| m   | 51  | RAEB II     | 2400           | 3.86                   | 32                      | 11.3     | n.d.           |
| F34| m   | 65  | RCMD        | 6600           | 2.98                   | 302                     | 9.9      | n.d.           |
| F35| f   | 60  | RCMD        | 2700           | 3.25                   | 407                     | 10.1     | n.d.           |
| F36| m   | 62  | RAEB II     | 4100           | 3.11                   | 30                      | 8.8      | n.d.           |
| F37| m   | 59  | RCMD        | 4800           | 3.06                   | 159                     | 11.4     | n.d.           |
| F38| m   | 69  | RAEB I      | 3100           | 2.53                   | 51                      | 10.5     | normocellular  |
| F39| m   | 63  | RARS        | 3700           | 2.50                   | 248                     | 7.4      | n.d.           |
| F40| m   | 60  | del(5q)     | 14600          | 2.31                   | 734                     | 7.3      | n.d.           |
| F41| m   | 71  | RAEB I      | 2000           | 2.88                   | 86                      | 9.2      | n.d.           |
| F42| f   | 73  | RCMD        | 1600           | 3.02                   | 20                      | 9.9      | n.d.           |
| F43| m   | 70  | RCMD        | 10400          | 4.71                   | 148                     | 13.6     | n.d.           |
| F44| f   | 68  | RCMD        | 4900           | 3.27                   | 158                     | 10.0     | n.d.           |
| F45| m   | 74  | RCMD        | 1100           | 2.56                   | 33                      | 9.8      | n.d.           |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| **F46** | m | 47 | CMML I | 5000 | 3.65 | 69 | 10.5 | n.d. |
| **F47** | f | 66 | RCMD | 3800 | 2.31 | 119 | 7.1 | n.d. |
| **F48** | m | 69 | RCMD | 10800 | 3.01 | 13 | 8.8 | n.d. |
| **F49** | m | 70 | RCMD | 1500 | 3.41 | 47 | 10.3 | n.d. |
| **F50** | m | 72 | CMML I | 5600 | 3.69 | 62 | 13.7 | hypercellular |
| **F51** | m | 83 | del(5q) | 2600 | 3.48 | 81 | 10.1 | n.d. |
| **F52** | m | 75 | RCMD | 2800 | 2.25 | 391 | 6.7 | normocellular |

**Data from healthy controls:**

|   |   |   |   |   |   |
|---|---|---|---|---|---|
| **HF1** | f | 41 | healthy | 6000 | 4.22 | 158 | 13.1 | n.d. |
| **HF2** | m | 55 | healthy | 5600 | 3.51 | 90 | 10.6 | n.d. |
| **HF3** | n.d. | 22 | healthy | 11200 | 3.73 | 438 | 10.5 | n.d. |
| **HF4** | f | 82 | healthy | n.d. | n.d. | n.d. | n.d. | n.d. |
| **HF5** | f | 59 | healthy | 3200 | 2.94 | 35 | 9.7 | n.d. |
| **HF6** | m | 22 | healthy | 9600 | 3.91 | 165 | 13.1 | n.d. |
| **HF7** | f | 36 | healthy | 7000 | 3.24 | 234 | 9.4 | n.d. |
| **HF8** | m | 71 | healthy | 11800 | 3.28 | 44 | 9 | n.d. |
| **HF9** | m | 75 | healthy | n.d. | n.d. | n.d. | n.d. | n.d. |
| **HF10** | m | 71 | healthy | 4000 | 2.97 | 88 | 8.6 | n.d. |
| **HF11** | m | 81 | healthy | 4200 | 3.18 | 47 | 9.9 | n.d. |
| **HF12** | f | 53 | healthy | 3300 | 4.73 | 143 | 12.4 | n.d. |
| **HF13** | f | 63 | healthy | 2100 | 3.76 | 54 | 9.9 | n.d. |

CMML (chronic myelomonocytic leukemia); del(5q) (chromosomal deletion 5q); RAEB (refractory anemia with excess of blasts); RCMD (refractory cytopenia with multilinear dysplasia); RCMD-RS (with ringed sideroblasts); sAML (secondary acute myeloid leukemia), n.d. (not determined).

### Supplemental References

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