Proliferative activity is disturbed in myeloproliferative neoplasms (MPN), myelodysplastic syndrome (MDS), and MDS/MPN diseases. Differences between MDS and MDS/MPN

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Abstract
The proliferation marker Ki-67 is widely used within the field of diagnostic histopathology as a prognostic marker for solid cancers. However, Ki-67 is hardly used for prognostic and diagnostic purposes in flow cytometric analyses of hematologic neoplasms. In the present study, we investigated to what extent the proliferative activity, as determined by Ki-67 expression, is disturbed in myeloproliferative neoplasms (MPN), myelodysplastic syndrome (MDS), and MDS/MPN diseases. Bone marrow aspirates from 74 patients suffering from MPN, MDS, or MDS/MPN, and aspirates from 50 non-malignant cases were analyzed by flow cytometry for Ki-67 expression in the erythro-, myelo-, and monopoiesis. Ki-67 expression was used to investigate the proliferative activity during the various maturation steps within these hematopoietic cell lineages. In the MPN patient cohort, the proliferative activity of all cell lineages is significantly higher during almost all maturation stages compared to those of the benign control cohort. In the MDS and MDS/MPN cohort, a significantly lower proliferative activity is observed in the early maturation stages. In the MDS/MPN patient cohort, increased proliferative activity is seen in the later stages of the maturation. MDS and MDS/MPN display a distinct pattern in the proliferating fraction of maturing hematopoietic cells. This could become of added value in order to classify these malignancies based on their biological background and behavior, as well as in gaining a better understanding into the pathobiology of these malignancies.

KEYWORDS
flow cytometry, MDS, MPN, proliferation

1 | INTRODUCTION

Myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), and MDS/MPN overlap disorders are the three major categories of malignant myeloid bone marrow (BM) disorders. These conditions are known to affect blood and BM cell composition, are characterized by an increase in cell number, but are also known to originate from different mechanisms (Tanaka & Bejar, 2019). The
surprisingly, the Ki-67 marker is only rarely used. This limited use of Ki-67 can be explained by the limited number of additional parameters that could be analyzed by flow cytometry (two to four parameters) at the time Ki-67 was initially studied in myeloid BM malignancies, which did at that time not allow the distinction between the individual hematopoietic cell populations.

With the introduction of 10 to 12-parameter flow cytometry and the increasing knowledge about cell surface marker expression of hematopoietic cells, we now are able to determine the Ki-67 proliferative index at the level of maturing cell populations, which could entail novel applications of Ki-67 in myeloid BM malignancies.

Together with the fact that Ki-67 is a diagnostic and prognostic marker in histopathology, flow cytometric analysis of Ki-67 could be promising for prognostic and diagnostic purposes in hematological malignancies. Not only can it be combined with multiple phenotypic parameters, it has also been described as being more time-efficient than the semi-quantitative immunohistological analysis of cell proliferation (Pagnucco, Giambanco, & Gervasi, 2006).

Our group has recently described and validated a multiparameter flow cytometric Ki-67 assay for the objective determination of the proliferative activity in the diverse hematopoietic cell lineages in normal BM. Reference Ki-67 values were established in the different hematopoietic cell lineages, while also its expression was quantified during the maturation process of the myelo-, mono-, and erythropoiesis (Supplemental Figure S1) (Nies et al., 2018).

The objective of the present study was to investigate the Ki-67 proliferative index in MDS, MPN, and MDS/MPN, with special emphasis on the maturation process of the different hematopoietic cell lineages. We wondered to what extent the overlapping disorders would exhibit proliferative characteristics of MDS on the one hand and/or MPN on the other.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Seventy-eight patients diagnosed with MPN, MDS, or MDS/MPN (Table 1) and fifty individuals with non-malignant hip lesions (also used in our earlier study) were included in this study (Nies et al., 2018). For only 26 of the 36 MDS patients and 6 of the 10 MDS/MPN patients, data for the erythropoiesis were available. For MPN, data of the erythropoiesis was available for all patients. Data of the myelo- and monopoiesis was available for all patients.

Leftover materials from BM samples drawn for routine diagnostic purposes at the Zuyderland Medical Center, were analyzed. Data was acquired after informed consent in accordance with the Declaration of Helsinki, while also approval for this study was obtained from the Medical Ethical Committee of the Zuyderland Medical Center and Hogeschool Zuyd (METC Z registration nr. 15-N-201). Patients with ongoing radio- and/or chemotherapy were excluded from analysis.
| Patient characteristic | Number of cases positive | Age (median, range) | Gender (M/F) |
|------------------------|--------------------------|---------------------|--------------|
| Non-malignant          | 50                       | 66, 55–77           | 23/27        |
| MPN                    | 32                       | 71, 40–82           | 19/18        |
| **Subdiagnosis**       |                          |                     |              |
| ET                     | 16/32                    | 70, 59–82           | 7/9          |
| PV                     | 7/32                     | 70, 64–81           | 4/3          |
| CML                    | 9/32                     | 63, 40–74           | 6/3          |
| **ET diagnostic criteria** |                      |                     |              |
| Platelet count ≥450 x 10^9/L | 16/16                  |                     |              |
| JAK2/V617F             | 15/16                    |                     |              |
| CALR                   | 1/16                     |                     |              |
| **PV diagnostic criteria** |                      |                     |              |
| Elevated Hb            | 3/7                      |                     |              |
| Elevated Ht            | 1/7                      |                     |              |
| JAK2/V617F             | 7/7                      |                     |              |
| **CML diagnostic criteria** |                      |                     |              |
| WBC >10 x 10^9/L       | 9/9                      |                     |              |
| BCR-ABL                | 7/9                      |                     |              |
| Immature myeloid cells present | 3/9                   |                     |              |
| MDS                    | 36                       | 76, 54–92           | 24/12        |
| **Subdiagnosis**       |                          |                     |              |
| MDS Sq-                | 1/36                     | 60                  | 0/1          |
| SLD                    | 2/36                     | 75                  | 2/0          |
| SLD-RS                 | 5/36                     | 69, 58–78           | 2/3          |
| MLD                    | 8/36                     | 79, 74–85           | 5/2          |
| MLD-RS                 | 3/36                     | 81, 69–92           | 2/1          |
| EB-1                   | 8/36                     | 78, 54–83           | 7/1          |
| EB-2                   | 9/36                     | 70, 59–82           | 6/3          |
| **Diagnostic criteria** |                          |                     |              |
| Cytopenia              | 36/36                    |                     |              |
| Myelodysplasia         | 36/36                    |                     |              |
| Ringed sideroblasts (≥15%) | 8/36                |                     |              |
| Blasts 5–9%            | 8/36                     |                     |              |
| Blasts 10–19%          | 8/36                     |                     |              |
| Sq-deletion            | 1/36                     |                     |              |
| MDS/MPN                | 10                       | 74, 61–88           | 7/3          |
| **Subdiagnosis**       |                          |                     |              |
| CMML                   | 8/10                     | 63, 61–88           | 5/3          |
| Atypical CML           | 1/10                     | 74                  | 1/0          |
| MDS/MPN-RS-T           | 1/10                     | 86                  | 1/0          |
| **Diagnostic criteria** |                          |                     |              |
| Cytopenia              | 10/10                    |                     |              |
| Myelodysplasia         | 10/10                    |                     |              |
| Blasts <20%            | 10/10                    |                     |              |
| Monocytosis blood (≥1 x 10^9/L) | 8/10                |                     |              |
| WBC >10 x 10^9/L       | 6/10                     |                     |              |
| Ringed sideroblasts (≥15%) | 1/10                   |                     |              |
| Platelet count ≥450 x 10^9/L | 1/10               |                     |              |

Abbreviations: aCML, atypical chronic myeloid leukemia; CALR, Calreticulin; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; EB, excess blast; ET, essential thrombocythemia; JAK, Janus Kinase 2; MDS/MPN-RS-T, MDS/MPN with ringed sideroblasts and thrombocytosis; MDS, myelodysplastic syndrome; MLD, multilineage dysplasia; MLD-RS, multilineage dysplasia with ringed sideroblasts; MPN, myeloproliferative neoplasm; PV, polycythemia vera; SLD, single lineage dysplasia; SLD-RS, single lineage dysplasia with ringed sideroblasts.
2.2 Immunocytochemical staining protocols

Immunophenotypic analysis was performed using 10-color flow cytometry, as described previously (Nies et al., 2018). Four different antibody panels were used to determine the Ki-67 positive fraction during the erythro-, myelo-, and monopoiesis. The antibody panels of the different tubes are shown in Table 2. Antibody characteristics can be found in our previous publication (Nies et al., 2018). Analysis of these tubes was performed using the Infinicyt v1.7 software package (Cytognos SL, Salamanca, Spain) as described previously (Nies et al., 2018).

In short, 50 μl of BM sample (diluted to a white blood cell count <20 × 10⁹/L) were incubated with the antibodies for extracellular surface staining. After incubation for 15 min at room temperature in the dark, cells were washed with 4 ml of phosphate buffered saline (PBS) pH 7.4 and centrifuged for 5 min at 300 g.

Cells were fixed and permeabilized for intracellular and intranuclear staining of Ki-67 and IgG1 isotype control using the Fix&Perm buffer set (Nordic-MUBio, Susteren, The Netherlands) according to the manufacturer’s protocol. After intracellular staining with Ki-67-FITC (DAKO A/S, Glostrup, Denmark; diluted 1:30) for 15 min, the cells were washed in 4 ml PBS, centrifuged for 5 min at 300 g, and resuspended in 0.5 ml of PBS. The samples were analyzed within 2 hours after immunolabeling.

2.3 Flow cytometry

The Navios Flow Cytometer in combination with the Navios Tetra software (Beckman Coulter) were used for data collection. The instrument setup was performed according to standard procedures and verification of the optical alignment and fluidics system of the Navios Flow Cytometer was performed using Flow-Check Pro Fluorospheres (Beckman Coulter). Flow-Set Pro Fluorospheres (Beckman Coulter) were used for establishment of the compensation for each fluorochrome and was performed weekly.

A minimum of 500,000 relevant events were measured per tube. Relevant events were determined by excluding debris with the FSC-Area versus SSC-Area plot without excluding erythroblasts.

2.4 Data analysis and gating strategy

Classification of the various hematopoietic cell lineages was performed according to the gating strategy described in Supplemental Figure S2 (Nies et al., 2018). Briefly, single cells were gated by excluding debris and doublets.

Subsequently, the cell populations of the myelopoiesis (neutrophilic granulopoiesis), monopoiesis, and erythropoiesis were gated. The myelopoiesis was gated by selecting the granulocytes in a SSC versus CD45 plot, thereby excluding basophils and other cell populations. Then, the myeloid cells were selected, while monocytes and eosinophils were excluded. The maturation stages of the myelopoiesis were determined based on expression of CD13 and CD11b. Monocytes were selected in the SSC versus CD45 plot by back gating CD14 positive cells and nonmyeloid cells were excluded. Erythroblasts were selected by gating the CD45 negative and CD45 dim cells, followed by gating based on CD33 expression (Machherndl-Spandl et al., 2013). The erythroblasts were then separated from other myeloid cells, lymphoid cells, and platelets. Blasts were selected based on CD34 positivity and by selection of the blast region in the SSC versus CD45 plot.

Since Ki-67 shows a dynamic staining pattern, gradually increasing in staining intensity when going from S phase to G2-M phase, the Ki-67 positive fraction was determined for all relevant populations based on the threshold set by the use of the IgG1 isotype control (Supplemental Figure S3). Besides, the Ki-67 negative population of matured cells is used as an internal biological control and an additional tool for setting the threshold. The maturation pathways were drawn for both the Ki-67 negative cells as well as the Ki-67 positive cells in each cell lineage (Nies et al., 2018). The maturation pathways were all drawn by the use of the “Draw Maturation” function in the Infinicyt v1.7 software package.

2.5 Statistical analysis

Statistical analysis was performed by use of the GraphPad Prism 5.0 software package (GraphPad Software, San Diego, CA). Normality was tested using the Kolmogorov–Smirnov test. In case of a normal distribution, the Independent T-test was used for further statistical analysis. If data followed a skewed distribution, the data was subjected to non-parametric analysis.

Table 2 presents the panels of antibodies used in this study to determine the Ki-67 positive fraction during the maturation process of the erythro-, myelo-, and monopoiesis.

| Fluorochrome | FITC | PE | ECD | PC5.5 | PE-Cy7 | APC | APC-A700 | APC-A750 | PB | KO |
|--------------|------|----|-----|-------|--------|-----|----------|----------|----|----|
| Panel 1      | IgG1 |     |     | CD13  | CD117  | CD34|          |          |     |     |
| Panel 2      | Ki-67| CD14| CD64| CD13  | CD117  | CD34|          |          |     |     |
| Panel 3      | Ki-67| CD105| CD123| CD33  | CD117  | CD71|          |          |     |     |
| Panel 4      | Ki-67| CD33|     | CD117 | CD36   |     |          |          |     |     |

Abbreviations: APC, allophycocyanin; APC-A700, allophycocyanin A700; APC-A750, allophycocyanin A750; ECD, electron-coupled dye; FITC, fluorescein isothiocyanate; KO, krome orange; PB, pacific blue; PC5.5, peridinin chlorophyll protein complex5.5; PE, phycoerythrin; PE-Cy7, phycoerythrin-cyanine7.
the Mann–Whitney U test for statistical analysis. Significance levels were indicated as $p < 0.05$, $p < 0.01$, and $p < 0.001$.

3 | RESULTS

3.1 | Patient characteristics

Table 1 presents the relevant characteristics for all patient cohorts. Routine diagnosis was based on (a) morphological criteria obtained from blood cytology and BM aspiration cytology, (b) flow cytometric immunophenotyping, (c) morphological criteria from BM biopsies, and (d) genetic parameters in specific cases.

All four patient cohorts (non-malignant, MDS, MPN, MDS/MPN) have a similar age range. The non-malignant cohort consists of 50 cases with a mean age of 66 years and an age range between 55 and 77 years. The MPN cohort consists of 32 cases with a median age of 71 years (range 40–82). The MDS cohort comprises of 36 cases with a median age of 76 years (range 54–92). The MDS/MPN cohort comprises of 10 patients with a median age of 74 years (range 61–88). The majority of these latter cases concerned CMML (8 out of 10).

3.2 | Ki-67 expression during normal erythro-, myelo-, and monopoiesis

All analyzed cell populations show an exponential increase in cell number throughout the maturation. The different morphological phenotypes that correspond to the various maturation stages of the erythro-, myelo-, and monopoiesis are indicated in Supplemental Figure S1.

Generally, the erythropoiesis shows the highest proliferating fraction, starting at 100% and ending at 30% proliferating cells. The myelopoiesis shows an intermediate proliferating fraction, starting at a proliferative activity of 50% of the cells and ending at 0–10%. The...
monopoiesis shows the lowest proliferative activity, which starts at 30% proliferating monocytic cells and ends at 0–10% cells.

### 3.3  |  Ki-67 expression in MPN, MDS, and MDS/MPN patients compared to the healthy cohort

To investigate the Ki-67 expression during maturation in myeloproliferative disorders in more detail, the Ki-67 expression in MPN, MDS, and MDS/MPN patients were compared to those of non-malignant BM (Figure 1). This comparison was done for the different stages of erythropoiesis, myelopoiesis, and monopoiesis. Stage 0 depicts the Ki-67 positive fraction of the CD34 positive blast cells.

While a significantly decreased Ki-67 positive fraction is observed during the early stages of the erythropoiesis, a significantly elevated Ki-67 positive fraction is seen during the later stages in MPN patients compared to healthy BM. The Ki-67 positive fraction of monocytes is also significantly increased in MPN patients. PV and CML mainly affect the erythropoiesis and myelopoiesis, respectively. Therefore, we also assessed the different subtypes of MPN separately (Figure 2).

A significantly lower Ki-67 positive fraction is observed in MDS patients in the early maturation stages, while only a slight decrease in the Ki-67 positive fraction of CD34 positive blast cells is seen. This significant decrease is most pronounced in the early stages of the erythropoiesis, since the fraction of proliferating cells in these stages in the healthy erythropoiesis is almost 100%. The MDS patient cohort shows a high variation in the Ki-67 positive fraction in all assessed cell lineages.

Interestingly, in the MDS/MPN patient cohort, a combination of a significant increase in the Ki-67 positive fraction in the later stages and the decrease in the early stages of maturation is seen.

The significant increase of proliferation is observed during the whole myelopoiesis and monopoiesis of MPN and MDS/MPN patients. This increase of the proliferation is also seen in the CD34 positive blast cell compartment of MPN patients. The significantly reduced Ki-67 positive fraction observed in the MDS patient group in

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**FIGURE 2**  Ki-67 expression in PV, ET, and CML. The Ki-67 positive fraction during the erythropoiesis, myelopoiesis, and monopoiesis of PV, ET, and CML patients, respectively. These fractions are compared with the fractions in healthy BM. The solid line depicts the mean Ki-67 positive fraction. The area surrounding the solid line indicates the standard deviation. For the myelopoiesis and monopoiesis, the Ki-67 positive fraction of the CD34 positive blast cells is used as Stage 0 (one dot = p < 0.05; two dots = p < 0.01; three dots = p < 0.001). It can be seen that a higher proliferative fraction is observed in PV and CML patients as compared to the whole MPN patient cohort. Furthermore, a significantly higher Ki-67 positive fraction is observed in the erythropoiesis of PV patients. This same pattern is also observed in the myelopoiesis of CML patients. However, a significantly higher proliferative fraction is also observed in non-affected lineages in PV and CML patients. Besides, a significantly higher proliferative fraction is observed in the erythro- and monopoiesis of ET patients. BM, bone marrow; CML, chronic myeloid leukemia; ET, essential thrombocythemia; MLD, multilineage dysplasia; MPN, myeloproliferative neoplasm; PV, polycythemia vera.
the early maturation stages of all assessed lineages is consistent with the decreased Ki-67 positive fraction seen in the erythropoiesis of the MDS/MPN patient cohort.

4 | DISCUSSION

This study shows that flow cytometric analysis of the Ki-67 positive fraction in combination with an appropriate fixation and permeabilization procedure, can detect a significant disturbance of proliferative activity in the BM of patients with an MPN, MDS, or MDS/MPN. This results in disease specific patterns in the individual hematopoietic cell lineages for each type of myeloid malignancy. This is particularly evident during the process of erythropoiesis. The MPN patients show a higher proliferative activity during the later maturation stages. A lower proliferative activity is found during the early maturation stages in MDS patients. The observed changes in the proliferative activity of MPN and MDS patients are concomitantly observed in the MDS/MPN overlap syndrome (Figure 3).

4.1 | Characteristics of the Ki-67 expression pattern in malignancies compared to non-malignant BM

Common patterns in Ki-67 expression are observed when comparing the different cell lineages of the patient BM and those of the non-malignant BM. The highest Ki-67 positive fraction is observed in the erythropoiesis, followed by the myelopoiesis and monopoiesis. Although common patterns are observed between cell lineages of patient BM compared to non-malignant BM, differences are observed in the early and late stages of the patient BM.

4.2 | The Ki-67 expression pattern in MPN patients

A higher proliferative activity is observed during the later maturation stages in each of the assessed cell lineages in patients with MPN compared to the fraction in the non-malignant BM. The MPN patient group investigated in this study consists of the subtypes PV, ET, and CML (Figure 2), all of which showed this phenomenon mainly in the erythro-, megakaryo-, and myelopoiesis, respectively. Surprisingly, significantly higher proliferative activities are seen in the hematopoietic cell lineages that are not primarily affected in these different subtypes of MPN. For example, in CML, one would not expect the observed higher proliferative activity in the erythro- and monopoiesis. This phenomenon can be partly explained by the findings of Lubberich et al. (2018), who show that the serum of myelofibrosis, PV and ET patients contains higher levels of cytokines, such as granulocyte-macrophage-colony stimulating factor, that stimulate proliferation of hematopoietic cells (Lubberich et al., 2018; Padron et al., 2013). The suggestion that malignant cells secrete cytokines that stimulate non-malignant cells to proliferate may apply to all different subtypes of MPN.

In PV and CML patients, a significantly higher proliferative fraction as compared to the non-malignant BM was observed during the erythropoiesis and myelopoiesis, respectively. This finding is consistent with the known proliferative phenotype of these malignancies, which is mainly caused by somatic mutations (Lubberich et al., 2018; Padron et al., 2013). A specific somatic mutation in ET and PV patients is the JAK2/V617 mutation, which encodes for the JAK2 protein (Zaleskas et al., 2006). In CML patients, the specific fusion product BCR-ABL1 is commonly encountered (Modi et al., 2007). Both the JAK2/V617 mutation and the BCR-ABL1 gene stimulate abnormal cell signaling, which ultimately results in elevated levels of proliferation (Modi et al., 2007). In our series, we found these specific mutations in nearly all MPN patients.

The known increase in the proliferative activity by somatic mutations, such as JAK2/V617F and BCR-ABL1 in MPN is in line with an increase in the Ki-67 positive fraction observed in this study. This finding further validates the use of Ki-67 as a marker for proliferation in flow cytometric analysis of myeloid BM malignancies.

The increased Ki-67 positive fraction in the later maturation stages of the erythropoiesis in MPN is also observed in the MDS/MPN overlapping syndrome, which confirms that this syndrome shows features of MPN in terms of proliferation. CD34 positive blast cells also display an elevated Ki-67 positive fraction. MPN originates from transformed HSCs which harbor the earlier mentioned somatic mutations (Zacharaki et al., 2018). These cells induce a self-reinforcing malignant BM niche that favors the malignant stem cells over the normal HSCs (Mead & Mullally, 2017).

4.3 | The Ki-67 expression pattern in MDS patients

Flow cytometric analysis of the Ki-67 expression pattern of the erythropoiesis can be used to discriminate between MDS and MDS/MPN. The MDS patient group shows a significantly lower proliferating fraction in the early maturation stages in each of the assessed lineages, compared to the non-malignant cohort. A slight decrease in the proliferating fraction was seen in the CD34 positive blast cell compartment. This decrease in the proliferative fraction in MDS patients is controversial to the general assumption that a malignancy is characterized by uncontrolled proliferation as well as the morphological finding of cell-rich BM in MDS patients (Matarraz et al., 2012). Matarraz et al. (2012) also observed the downregulation of the proliferative activity in advanced and high-risk MDS patients. This decreased proliferative activity could be related to the accumulation of secondary genetic aberrations, an inhibitory cytokine response by the dysplastic hematopoietic BM precursors and alterations in the BM microenvironment (Abe-Suzuki et al., 2014; Riether, Schürch, & Ochsenbein, 2014). One of these alterations is the induction of quiescence in dysplastic and malignant cells by the CXCL12-CXCR4 axis. The main function of the CXCL12 protein is to attract blast cells, subsequently inducing
from aberrancies in the HSCs. This finding is in accordance with findings in the literature showing that this MDS/MPN entity is characterized by the abnormal proliferation of hematopoietic cells.

FIGURE 3 Summary of basic characteristics and Ki-67 expression of myeloid BM malignancies. Disease specific patterns of morphological and proliferative aberrancies in the spectrum of MPN and MDS. Upregulated Ki-67 expression, especially in the later stages of the maturation, is observed in MPN. This finding correlates with the known mutational background of MPN (Zacharakis, Ghazanfari, Li, Lim, & Scheding, 2018). MDS is characterized by morphological aberrancies that lead to ineffective hematopoiesis. This is reflected by an aberrantly downregulated Ki-67 expression. The MDS/MPN overlap syndrome is characterized by a typical Ki-67 expression pattern, which allows its distinction from MDS and MPN based on its biological behavior. MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm.

4.4 The Ki-67 expression pattern in MDS/MPN patients

The MDS/MPN patient cohort shows a decrease in the proliferating cell fraction only during the early erythropoiesis, while a significant increase is observed in the late phases of erythropoiesis. The increased proliferative activity of the late erythropoiesis in MPN on the one hand and the decreased proliferative activity of early erythropoiesis in MDS patients on the other are therefore concomitantly observed in the MDS/MPN overlap syndrome (Figure 3). Also, during the whole myelo- and monopoiesis in our MDS/MPN patients an increased proliferative activity is seen. Since the major part of the MDS/MPN patients analyzed in the present study suffer from CMML (8 out of 10), this observation is in accordance with findings in the literature showing that this MDS/MPN entity is characterized by the accumulation of monocytic cells and possibly also myeloid cells (Patnaik & Tefferi, 2016; Solary & Itzykson, 2017). However, no changes in the proliferating fraction of the CD34 positive blast cell compartment are seen.

The most frequent somatic mutations that are reported in CMML are the TET2, ASXL1 and/or SRSF2 mutations. TET2 is a gene involved in self-renewal of HSCs, lineage commitment and cell line differentiation (Ko et al., 2011). Loss-of-function mutations in this gene abrogate these processes and induce myelodysplasia. Loss of ASXL1 leads to an increase in apoptotic and proliferating cells, but also reduced HSC and progenitor populations (Wang et al., 2014). Mutations in the SRSF2 gene lead to growth arrest, early senescence and apoptosis in hematopoietic cells (Aujla, Linder, Iragavarapu, Karass, & Liu, 2018). These mutations combined could explain the distinct pattern of proliferative aberrancies seen in the MDS/MPN cohort.

The overlap in the mutational background of all myeloid malignancies, including MPN, MDS, and MDS/MPN, creates challenges for characterization of these malignancies based on this background. Analyzing the proliferative behavior of maturing cell lineages could become of added value to differentiate between MDS and MDS/MPN based on their biological behavior and provide more insight into the pathobiology of these malignancies.

4.5 Differences in Ki-67 expression patterns between MDS and MDS/MPN

This study shows that MDS and MDS/MPN show distinct patterns in terms of the Ki-67 proliferative index in maturing hematopoietic cells. MDS and MDS/MPN display a decreased Ki-67 proliferative index in the early stages of the erythropoiesis, while the increase in the Ki-67 proliferative index in the last stages of the erythropoiesis is exclusive to MDS/MPN. Besides, the increases in the Ki-67 proliferative index during the myelo- and monopoiesis are specific for MDS/MPN. The opposite is observed for MDS, in which a decrease in the Ki-67 proliferative index is seen during the early stages of the myelo- and monopoiesis.

4.6 Conclusion

While MPN patients show an increase in the proliferating fraction in maturing hematopoietic cells, MDS patients display a decrease in the proliferating fraction of these cells. The MDS/MPN overlap patient group show combined proliferation features of MPN and MDS. MDS and MDS/MPN therefore show a distinct pattern in the proliferating fraction of maturing hematopoietic cells. This could become of added value in order to classify these malignancies based on their biological background. Furthermore, analyzing the proliferative behavior of individual hematopoietic cell populations during maturation could lead to new insights into the role of somatic mutations in the pathogenesis of these malignancies.
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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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