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IMPROVING PROGNOSTICATION FOR PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Improving prognostication for patients with myelodysplastic syndromes

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To my family, past and present members

Popular science summary of the thesis

Myelodysplastic syndromes (MDS) are a group of blood cancers that affects around 400 persons in Sweden each year. Typically, the disease is diagnosed in individuals around 70 to 75 years old. At diagnosis patients will be either asymptomatic or have symptoms related to abnormal blood counts such as fatigue (low red blood cell [RBC] counts), increased bleeding tendency (low platelet counts) or infections (low white blood cell [WBC] counts). During the disease course some MDS can transform to a more aggressive type of blood cancer called acute myeloid leukemia (AML), which is a disease classically associated with short survival. However, there are several subtypes of MDS, and the prognosis varies both within and between subtypes.

Several tools have been developed during the last two decades to understand the heterogeneity of MDS and to estimate life expectancy at the time of diagnosis as well as to guide therapeutic decision-making. Hence, MDS are usually divided in i) higher-risk MDS associated with an increased AML transformation rate and shorter overall survival (OS), and inversely ii) lower-risk MDS. The use of DNA analyses at diagnosis has increased exponentially during the last decade and it has generated evidence of the prognostic impact of specific genetic markers in MDS. However, these markers have not yet been included in prognostic scoring systems.

Hence, in ***study I*** we wanted to develop a novel prognostic score combining clinical variables and novel genetic markers. In an international cohort of 2,957 MDS patients we first showed that specific gene mutations (alterations of the DNA) such as *TP53* multi-hit (*TP53*^{multi,} i.e., more than one mutation), *MLL-Partial Tandem Duplication (PTD, i.e., a part of the gene is duplicated)* and *FLT3* mutations strongly predicted dismal outcome. In contrast, mutations of the *SF3B1 gene* were associated with favorable prognosis, but this effect was significantly influenced by co-occurring gene mutations (co-mutations). Next, we built the International Prognostic Scoring System Molecular (IPSS-M) score via a mathematical model of 22 variables (blood counts, percentage of bone marrow malignant cells, chromosome abnormalities and genetic markers). It resulted in a unique score for individual patients, and also assigned each case to one of the 6 IPSS-M risk categories. When compared to the score in use at that time (IPSS-R, Revised International Prognostic Scoring System), the IPSS-M score significantly improved outcome prediction. A web- and app-based calculator was also made available for clinical use and was very well received by the international MDS community.

MDS with ring sideroblasts (MDS with RS) is a good example of the heterogeneity of MDS. This subtype of MDS characterized by iron accumulation in the mitochondria – a structure producing energy in cells – has a strong association with mutations of the *SF3B1* gene and it typically behaves more like a benign disease. However, RS are sometimes found in more

aggressive MDS subtypes. In ***study 2***, based on a local cohort of 129 patients with MDS and RS (MDS^{RS+}), we reported first that most of MDS^{RS+} cases were found to have a mutation in *SF3B1, SRSF2* genes or $TP53^{multi}$ mutations, but that these three subgroups exhibited an important variability in outcomes. Analysis of gene expression through RNA sequencing confirmed the differences between *SF3B1, SRSF2* and $TP53^{multi}$ – mutated MDS with RS. An unbiased digital algorithm analysis used to discover hidden gene expression patterns additionally found three gene expression groups predicting OS independently of IPSS-M score. Interestingly, these three distinct gene expression groups were shown to be ultimately defined by the underlying composition of the bone marrow cells. Hence, the fraction of a particular subpopulation of bone marrow cells was found to predict prognosis independently of current prognostic scoring system.

Like in all human beings, patients and patients' characteristics change over time. Thus, the course of MDS, driven by the natural evolution of the disease and treatments, is also variable. Although the clinical management of patients always takes into consideration the dynamic of the disease, current prognostic tools do not. Therefore, in **study III**, we wanted to assess whether red blood cells (also called erythrocytes [E]) transfusion dependency over time improves estimation of prognosis in MDS. We comprehensively collected E-transfusion data during the disease course in a cohort of 677 Swedish patients. First, we observed that specific variables such as $TP53^{multi}$, and higher percentage of malignant cells in the bone marrow (blasts) predicted shorter time to first transfusion event. In contrast, higher hemoglobin level and a specific comutation pattern of *SF3B1* ($SF3B1^{-alpha}$) were associated with a longer time to first E-transfusion. Next, we found that E-transfusion state at 8 months (transfusion dependent or not) after diagnosis predicted OS independently of IPSS-M, and a new model (model 2) based on E-transfusion state at 8 months and IPSS-M improved prognostic estimation compared to IPSS-M only (model 1). Finally, our dynamic mathematical model showed that individual trajectories of transfusion patterns during the early disease course can be used to foresee both OS and future transfusion requirements.

Thus, in this thesis, we first show that integration of comprehensive genetic data and clinical characteristics greatly improves prognosis estimation in MDS, and we propose that the novel IPSS-M prognostic score is used in clinical practice to provide further guidance in clinical decision-making. We also provide evidence that the heterogeneity of outcome in MDS cannot be explained by genetic profiling only and that studies of gene expression and integration of dynamic parameters can contribute to a better understanding of the clinical course and improve management of patients. In general, this thesis advocates for the need of a holistic approach of the disease to deepen our understanding of underlying mechanisms and ultimately improve patient care. Enormous efforts are currently put in the field of precision medicine in cancer. Future research combining multiple advanced technologies will hopefully result in truly personalized treatments and improve survival and quality of life of patients with MDS.

Abstract

Background and aims

MDS constitute a heterogenous group of myeloid malignancies mainly characterized by dysfunctional hematopoiesis. Although cytopenia, dysplastic features and evidence of clonality are essential criteria for the diagnosis of all MDS, the several subtypes of the disease have a highly variable prognosis. The increasing quality and accessibility of DNA sequencing techniques have enabled huge advances for molecular characterization of the disease, and the prognostic impact of specific molecular markers in MDS is now well established. Several prognostic scoring systems have been developed during the last two decades but none of these tools accounted for the effect of molecular markers on outcome. MDS with RS is easily recognizable by the intra-cellular presence of iron-loaded mitochondria and this subtype reflects the heterogeneity of MDS. Hence, while RS are classically associated with *SF3B1* mutations and an indolent disease course, RS are sometimes found in aggressive subtypes of MDS or AML. Patients and diseases change over time, and evolution patterns themselves can tell us something about disease biology and outcome. Clinicians account for these variations in practice, but current prognostic models do not. This may partly explain remaining discrepancies between observed and predicted prognosis. Hence, in this thesis we aimed to i) develop a novel prognostic score including molecular markers to refine prognosis prediction at diagnosis, ii) study the prognostic impact of combined gene mutation and gene expression in MDS with RS and iii) assess whether changes in erythrocyte (E) transfusion patterns during the early disease course can refine outcome prediction.

Methods

Study I – an international cohort of 2957 patients with MDS, MDS/myeloproliferative neoplasms (MPN) were retrospectively collected. DNA sequencing with a panel of 152 genes known to be involved in myeloid malignancies was performed on all samples. Clinical data, cytogenetic and molecular features were retrieved and their association with outcomes was studied. A Cox multivariable model was used to estimate relative weights of selected explanatory variables. The score was validated on an independent cohort of 754 Japanese patients with MDS. ***Study II*** – A total of 129 patients with MDS and RS (MDS^{RS+}) was assembled. All samples underwent DNA sequencing according to study I and thereafter RNA sequencing of CD34 sorted bone marrow mononuclear cells. Supervised/unsupervised clustering analysis and digital sorting were performed. A Cox multivariable model was used to assess association between clinical and genomic/transcriptomic patterns and outcome. ***Study III*** – a cohort of 677 Swedish patients was gathered from study I. We collected complete information on administered E-transfusions through the nationwide SCANDAT3-S database. Cox regression analyses were used to assess associations between clinical, molecular and transfusion data, and

outcome. A Markov multistate model was used to assess association between changes in transfusion patterns and outcome.

Results

Study I – *TP53*^{multi}, *MLL-PTD* and *FLT3* mutations were shown to be predictive of dismal outcome. In contrast, *SF3B1* mutations were associated with favorable prognosis, however this effect was significantly influenced by the co-mutation patterns. A total of 22 variables (clinical, cytogenetic, and molecular markers) were used to build the IPSS-M score, each of them carrying a specific mathematic weight according to their individual impacts on endpoints. The calculation of the IPSS-M resulted in a unique score for individual patients and assigned each case to one of the 6 IPSS-M risk categories. When compared to the IPSS-R, the IPSS-M score clearly improved outcome prediction and led to the restratification of 46% of patients. The IPSS-M is validated both in MDS/MPN with WBC count below 13x10^{9}/L and in therapy related MDS (t-MDS). ***Study II*** – Most (~90%) MDS^{RS+} cases were found to have a mutation in *SF3B1, SRSF2* or *TP53*^{multi}. Overall, *TP53*^{multi} and splice factors mutations were mutually exclusive, and *SF3B1* and SRSF2 mutations cooccurred in only 3% of the patients. The three genetic subgroups were shown to have very different outcomes. Supervised transcriptome analysis confirmed the distinction between *SF3B1-, SRSF2-* and *TP53*^{multi}-mutated MDS with RS. Unsupervised clustering analysis found three transcriptomic groups, each with distinct erythroid/megakaryocytic progenitor fraction, which predicted OS independently of IPSS-M. ***Study III*** – Whereas *TP53*^{multi}, poor cytogenetic and higher bone marrow blasts predicted shorter time to first E-transfusion event, higher hemoglobin level and *SF3B1*^{alpha} only were associated with longer time to first E-transfusion event. Next, E-transfusion state at 8 months after diagnosis was shown to be a strong predictor of OS independently of IPSS-M. Our model based on E-transfusion state at 8 months and IPSS-M (model 2) improved significantly prognostic prediction compared to IPSS-M only (model 1). Finally, a multistate model revealed that individual transfusion trajectories during the early disease course impacted both future transfusion requirement and OS.

Conclusion

This thesis provides evidence that integration of genomic data to clinical characteristics improves greatly prognostication in MDS and we suggest that the novel IPSS-M prognostic score is implemented in clinical practice to provide further guidance in therapeutic decision-making. Our work also indicates that the heterogeneity of outcome in MDS cannot be explain by genetic profiling only and that studies of gene expression and integration of dynamic parameters among other techniques will contribute to a better understanding of the clinical course. In general, this thesis advocates for the need of a holistic approach of the disease to deepen our understanding of underlying mechanisms and ultimately to

improve the care of patients with MDS. Enormous efforts are currently put in the field of precision medicine in cancer. Future integrative multiomics studies will hopefully improve individualized care to increase survival and quality of life of patients with MDS.

List of scientific papers

I. Molecular International Prognostic Scoring System for Myelodysplastic Syndromes. Bernard E, Tuechler H, Greenberg PL, Hasserjian RP, Arango Ossa JE, Nannya Y, Devlin SM, **Creignou M**, Pinel P, Monnier L, Gundem G, Medina-Martinez JS, Domenico D, Jädersten J, Germing U, Sanz G, van de Loosdrecht AA, Kosmider O, Follo MY, Thol F, Zamora L, Pinheiro RF, Pellagatti A, Elias HK, Haase D, Ganster C, Ades L, Tobiasson M, Palomo L, Della Porta MG, Takaori-Kondo A, Ishikawa T, Chiba S, Kasahara S, Miyazaki Y, Viale A, Huberman K, Fenaux P, Belickova M, Savona MR, Klimek VM, Santos FPS, Boultwood J, Kotsianidis I, Santini V, Sole F, Platzbecker U, Heuser M, Valent P, Ohyashiki K, Finelli C, Voso MT, Shih LY, Fontenay M, Jansen JH, Cervera J, Gattermann N, Ebert BL, Bejar R, Malcovati L, Cazzola M, Ogawa S, Hellström-Lindberg E, and Papaemmanuil E. *NEJM Evid* 2022;1(7). doi: 10.1056/EVIDoa2200008

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Scientific papers not included in the thesis

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Implication of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Bernard E, Nannya Y, Hasserjian RP, Devlin SM, Tuechler H, Medina-Martinez JS, Yoshizato T, Shiozawa Y, Saiki R, Malcovati L, Levine MF, Arango JE, Zhou Y, Solé F, Cargo CA, Haase D, **Creignou M**, Germing U, Zhang Y, Gundem G, Sarian A, van de Loosdrecht AA, Jädersten M, Tobiasson M, Kosmider O, Follo MY, Thol F, Pinheiro RF, Santini V, Kotsianidis I, Boultwood J, Santos FPS, Schanz J, Kasahara S, Ishikawa T, Tsurumi H, Takaori-Kondo A, Kiguchi T, Polprasert C, Bennett JM, Klimek VM, Savona MR, Belickova M, Ganster C, Palomo L, Sanz G, Ades L, Della Porta MG, Smith AG, Werner Y, Patel M, Viale A, Vanness K, Neuberg DS, Stevenson KE, Menghrajani K, Bolton KL, Fenaux P, Pellagatti A, Platzbecker U, Heuser M, Valent P, Chiba S, Miyazaki Y, Finelli C, Voso MT, Shih LY, Fontenay M, Jansen JH, Cervera J, Atsuta Y, Gattermann N, Ebert BL, Bejar R, Greenberg PL, Cazzola M, Hellström-Lindberg E, Ogawa S, Papaemmanuil E. *Nat Med.* 2020;26(10):1549-1556. doi: 10.1038/s41591-020-1008-z

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List of abbreviations

aCML	Atypical chronic myeloid leukemia
AML	Acute myeloid leukemia
AML-t	AML transformation
AUC	Area under the receiver operating characteristic curve
BFU	Burst forming unit
BM	Bone marrow
C-index	Concordance index
CCUS	Clonal cytopenia of unknown significance
CFU	Colony forming unit
CH	Clonal hematopoiesis
CHIP	Clonal hematopoiesis of indeterminate potential
CHRS	Clonal hematopoiesis risk score
CI	Confidence interval
CMML	Chronic myelomonocytic leukemia
CMP	Common myeloid progenitor
CPSS	CMML-specific Prognostic Scoring System
CPSS-mol	CPSS molecular
DAG	Direct acyclic graph
Del	Deletion
DNA	Deoxyribonucleic acid
EMA	European Medicines Agency
EMK	Erythroid/megakaryocytic
ESA	Erythropoiesis stimulating agent
FAP	Fibroblast activating protein
FDA	United States Food Drug Administration
GCSF	Granulocyte-colony stimulating factor
GDF11	Growth differentiation factor 11
GMP	Granulocyte-macrophage progenitor
HMA	Hypomethylating agent
HR	Hazard ratio
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
HSPC	Hematopoietic stem and progenitor cell

HU	Hydroxyurea
ICC	International consensus classification
IL-3	Interleukin 3
IMP	Immature progenitors
INT	Intermediate cluster
IPSC	Induced pluripotent stem cell
IPSS	International Prognostic Scoring System
IPSS-M	International Prognostic Scoring System Molecular
IPSS-R	Revised International Prognostic Scoring System
IWG	International Working Group
KB	Karolinska Institutet MDS biobank
LFS	Leukemia-free survival
MDS	Myelodysplastic syndromes
MDS 5q-	MDS with deletion of the long arm of chromosome 5
MDS-(RS)-MLD	MDS (with ring sideroblasts) and multiple lineage dysplasia
MDS-(RS)-SLD	MDS (with ring sideroblasts) and single lineage dysplasia
MDS-EB	MDS with excess blasts
MDS-IB	MDS with increases blasts
MDS-NOS	MDS not otherwise specified
MDS^{RS+}	MDS with ring sideroblasts irrespectively of classifications
MDS-U	MDS-unclassifiable
MDS/MPN-RS-T	MDS/MPN with ring sideroblasts and thrombocytosis
MDS/MPN-U	MDS/MPN unclassifiable
MDSC	Myeloid-derived suppressor cell
MEP	Megakaryocyte/erythroid progenitors
MLP	Multi-lymphoid progenitor
MN	Myeloid neoplasm
MNC	Mononuclear cell
MPN	Myeloproliferative neoplasms
MPP	Multipotent progenitor
mRNA	Messenger RNA
MSC	Mesenchymal stem cell
MSM	Markov multistate model
NB	National Swedish MDS biobank
NBM	Normal bone marrow

NGS	Next generation sequencing
NMD	Nonsense-mediated mRNA decay
OS	Overall Survival
OS	Overall survival
PB	Peripheral blood
PRC2	Polycomb repressive complex 2
PTD	Partial Tandem Duplication
RARS	Refractory anemia with ring sideroblasts
RBC	Red blood cell
RCMD-RS	Refractory Cytopenia with Multilinear Dysplasia and Ring Sideroblasts
RNA	Ribonucleic acid
RPS	Ribosomal protein S
RS	Ring sideroblasts
s-MDS	Secondary MDS
SCF	Stem cell factor
SNP	Single Nucleotide Polymorphism
t-MDS	Therapy related MDS
TD1	First transfusion dependency state
TDx	Second (or more) transfusion dependency state
TF1	First transfusion independency state
TFx	Second (or more) transfusion independency state
TGF-β	Transforming growth factor β
VAF	Variant allele frequency
VEXAS	Vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic
WBC	White blood cell
WHO	World health organization
WPSS	WHO classification-based Prognostic System

1 Introduction

Myelodysplastic syndromes (MDS) are a heterogenous group of hematological malignancies characterized by ineffective hematopoiesis.[1–3] All MDS subtypes initiate from mutations at the stem cell level, followed by expansion of the mutated clone. The ineffective production of mature blood cells results in low blood counts and determines largely the symptoms of the disease. Nonetheless, cytopenia are necessary but not sufficient for the diagnosis of MDS; additional prerequisites are the presence of dysplastic features in the bone marrow and evidence of clonality.[1–3] Apart from initiating genetic events, interaction between the types of clonal events, epigenetic changes, and interplay with the microenvironment lead to diverse clinical courses and make each disease as unique as patients themselves.

In fact, while the recent classifications of MDS define up to 19 subtypes of MDS or mixed MDS/myeloproliferative neoplasms (MPN) there is an even greater variability in outcome both within and between the several MDS subtypes.[1–3] Although MDS in some cases will be indolent during the whole disease course, other MDS subtypes will behave aggressively and rapidly transform to acute myeloid leukemia (AML). Prognostic scoring systems have been developed over the years to guide therapeutic decision-making. The International Prognostic Scoring System (IPSS) published in 1997 was based on clinical parameters and cytogenetic abnormalities and became broadly used in the MDS clinical community.[4] Five years later, its revised version (IPSS-R, 2012) improved prognostication by accounting for the severity of cytopenia and blasts increase.[5] While the systematic use of DNA sequencing in clinical practice offers novel and invaluable insights both into disease biology and outcome, it took a long time before gene mutations were included in risk scores.[6–10]

Despite great improvements in prognostication during the last decade, clinicians still regularly experience discrepancies between predicted and observed outcome. The complexity of the disease beyond gene mutations and the lack of prognostic tools accounting for time-varying disease and patients characteristics are few of the potential reasons for these inconsistencies. MDS with ring sideroblasts (RS) reflects the granularity within subtypes of the disease; while this entity characterized by iron-loaded mitochondria in erythroid precursors is typically associated with *SF3B1* mutations and an indolent disease course, some cases behave unexpectedly aggressively.[2,3,11–13] Overall, studies refining prognostication of patients with MDS are warranted.

In this thesis, a literature review will first summarize key findings on clinical aspects and pathophysiology of MDS with a particular focus on MDS with RS and related dyserythropoiesis. I will next describe the methods used in the thesis before summarizing

and discussing the results of each study. Finally, after concluding remarks, I will touch upon future perspectives of research in the field.

2 Literature review

2.1 Myelodysplastic syndromes

2.1.1 Epidemiology and risk factors

In Sweden, population-based estimates report an incidence of MDS of 3 cases/100000 inhabitants with a male/female ratio of 1.4 which is comparable to what has been reported in the United States during the last decade.[14–16] However, with a median age at diagnosis of 75 years old, MDS is mainly a disease of the elderly and looking at groups of patients younger than 70 years old or over 80 years old, the incidence increases from 3 to 10 folds.[15,17,18]

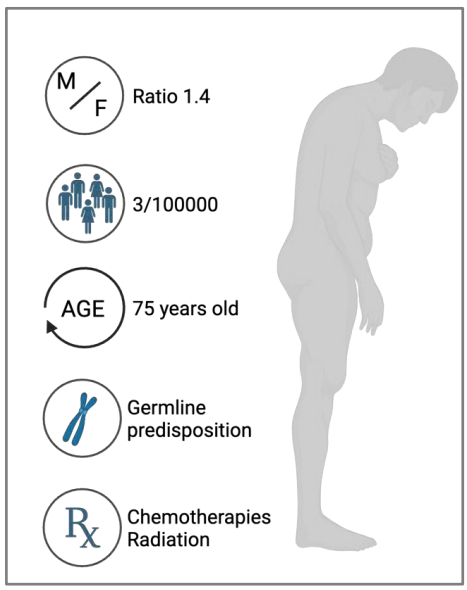


Figure 1 – Main facts on MDS epidemiology. M: male; F: female

While most MDS are considered as "*de novo*", MDS evolving after exposure to chemotherapy and/or radiation define the class of therapy-related MDS (t-MDS) and are associated with poorer outcome.[19–26] For many years, MDS was not considered a hereditary disease apart from sporadic cases of the youth related to rare congenital bone marrow failure syndromes. However, improvement in genetic sequencing and studies of families with hereditary patterns for blood cancers and/or MDS/AML have unraveled several novel germlines predisposition syndromes.[27–34] Hence, genes such as *GATA2, ETV6, DDX41, SAMD9, and SAMD9L, RUNX1, CEBPA, TP53* among others show association with familial myeloid malignancies.[35–45] Future studies will probably discover several other predisposition syndromes to hematological malignancies and cancer in general.

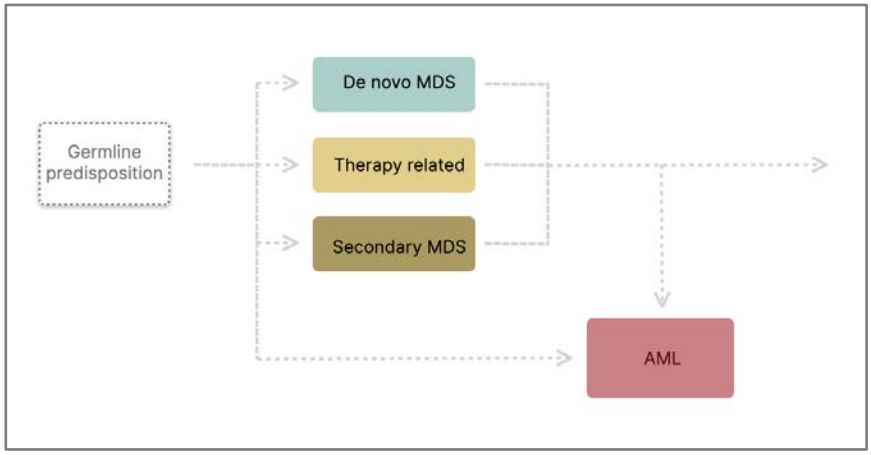


Figure 2 – Different origins of MDS: germline versus somatic and de novo versus secondary (s-MDS) or therapy-related MDS (t-MDS)

2.1.2 Pathogenesis

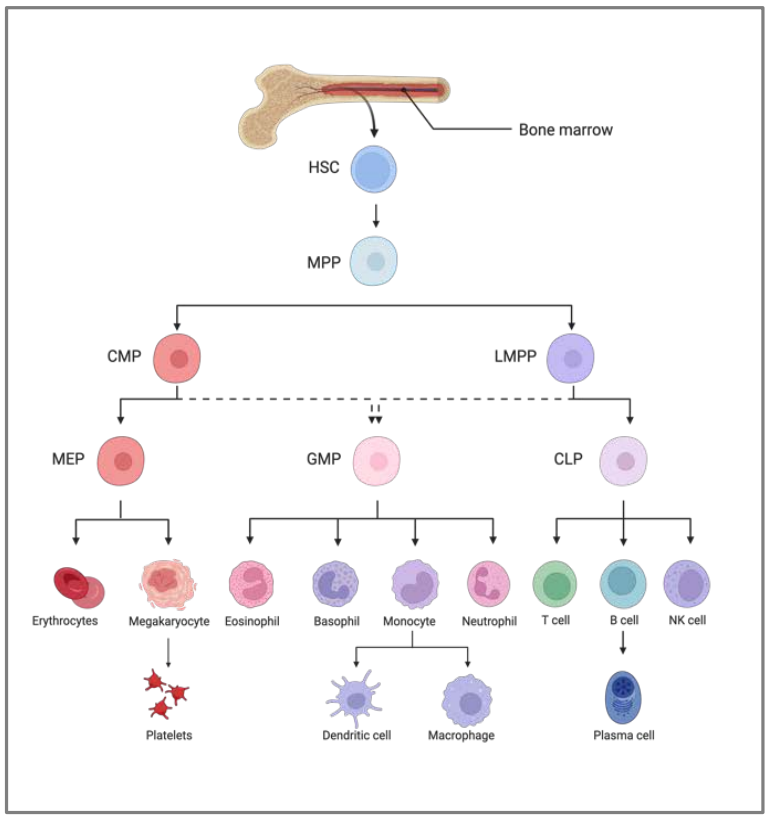
2.1.2.1 Normal hematopoiesis

Inefficient hematopoiesis is a common feature of MDS. MDS is clinically characterized by cytopenia, and morphologically by bone marrow cells with abnormal shape and content (i.e., dysplasia) and/or increased amount of immature clonal myeloid progenitors called blasts. The first description of MDS – at that time called "a preleukemic acute human leukemia" – was made in 1953 by Block *et al.*[46]

Unraveled in the late 19th century, adult hematopoiesis is the process of producing mature and functional blood cells in the bone marrow (BM).

However, the discovery of the self-renewal capacity of multipotent hematopoietic stem cells (HSC) and the ability of HSC to differentiate to all types of mature blood cells – occurred only a hundred years later.[46–49] Until recently, hematopoiesis has been classically represented as a stepwise and hierarchic process where the originating multipotent HSC first gives rise to oligopotent progenitors from which arise unipotent progenitors before differentiation to mature blood cells. During the past two decades, the scientific community has put enormous efforts into immunophenotypical and single-cell characterization of hematopoietic stem and progenitor cells (HSPC) and their progeny. Hence, the originating HSC can be categorized into three distinct subsets based on their self-renewal capacity and their duration of repopulation: long-term HSC, intermediate HSC, and short-term HSC.[50] Thereafter, HSCs are thought to successively differentiate

through different steps of progenitors to mature blood cells.[51] Nonetheless, the discovery of additional surface markers and the recent rise of single cell assays and RNA sequencing techniques have challenged the traditional "tree-like" representation of human hematopoiesis.[52–63] Recent works reported indeed that multipotent progenitors were able to generate mature blood cells without going through the step of oligopotent progenitors, and that lineage commitment was observed also in cell subtypes supposed to be oligopotent. Thus, new models of hematopoiesis suggest a more continuous differentiation process in which the destiny of undifferentiated HSPC varies upon demand (Figure 3).[64,65]



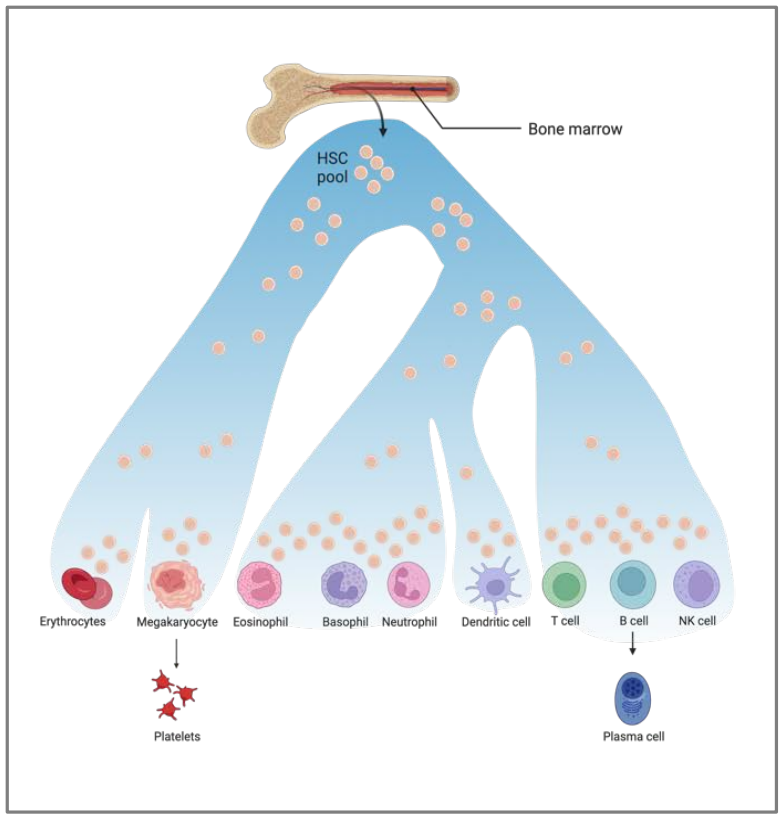


Figure 3 – Schematic representation of "stepwise" hematopoiesis (top) vs "continuous" hematopoiesis (bottom)

HSC are however by themselves not sufficient for producing mature blood cells; a supportive microenvironment and hematopoietic growth factors are also required.[66]

One of the first studies pointing towards the importance of the bone marrow niche showed that the concentration of colony forming units (CFU) in the femoral bone of mice increased towards the bone surface.[67] Later, mesenchymal "stromal" cells, localized with HSC around blood vessels, were found to play a major role in sustaining hematopoietic cells ex-vivo. Recent works on mesenchymal stem cells (MSC) has also demonstrated that MSC expressing nestin, CXCL12, and fibroblast activating protein (FAP) were essential contributors to HSC maintenance and expansion.[68–72] Evidence reviewed by, among others, Ding *et al*. suggested that hematopoietic stem cells and their downstream progenitors have distinct niches in the bone marrow.[73]

As a part of the perivascular niche, endothelial cells play an essential role in HSC maintenance.[57] Hence, Yao and colleagues showed that deletion of gp130 cytokine receptor in endothelial cells resulted in a decrease in the number of HSC.[74] Later studies

demonstrated that endothelial cells promoted HSC maintenance and expansion in culture.[75,76] Macrophages were reported to affect the niche indirectly by acting on perivascular stromal cells to regulate CXCL12 release.[77,78]

Osteoblasts and their production of cytokines were also shown to be necessary to maintain hematopoiesis but rather through indirect interactions than via cell-to-cell contact.[79–82]

The sympathetic nervous system seems to regulate the bone marrow niche as well through several mechanisms leading to circadian oscillation of CXCL12 production and HSC retention[83,84]. Also, Yamakasi et *al.* suggested that non-myelinating Schwann cells affected the niche by regulation of TGF-β (Transforming growth factor β) activation.[85]

Nonetheless, even though a lot of knowledge has been gathered during the last decades, many underlying mechanisms of hematopoiesis remain unknown.

2.1.2.2 Clonal expansion, CHIP and CCUS, driver mutations

Clonal expansion

As in most stem cells, a substantial number of somatic mutations occur in HSC during a lifetime.[86–90] Most of these alterations will neither have significant functional consequences, nor lead to clonal expansion; they are classically called "passenger mutations". However, other genetic abnormalities called "driver mutations" have the potential to not only initiate clonal expansion but also to enhance the growth advantage of the clone, and therefore enable a positive selection of clonal versus non-clonal cells (Figure 4).[91–96] In the 2000's, studies provided evidence of founding genetic events occurring at the HSC level in MDS.[97,98] Since then the genetic landscape of MDS has been extensively characterized by targeted sequencing.[6,10,99] Hence, functional groups have been described: genes involved in DNA methylation (*TET2, DNMT3A, IDH1/2*) and in chromatin modification (ASXL1, EZH2) known as epigenetic modifiers, splicing factor genes (*SF3B1, SRSF2, ZRSR2, U2AF1*), genes belonging to the cohesin family *(STAG2, RAD21, SMC1A, SMC3),* genes involved in transcription (*RUNX1, BCOR, BCORL1, ETV6),* receptor/kinases genes *(JAK2, MPL),* genes involved in the RAS-pathway (*NRAS, KRAS, CBL, PTPN11*), or genes taking part of DNA repair (*BRCC3, ATM*). Underlying molecular mechanisms of MDS will be discussed more extensively in a separate section.

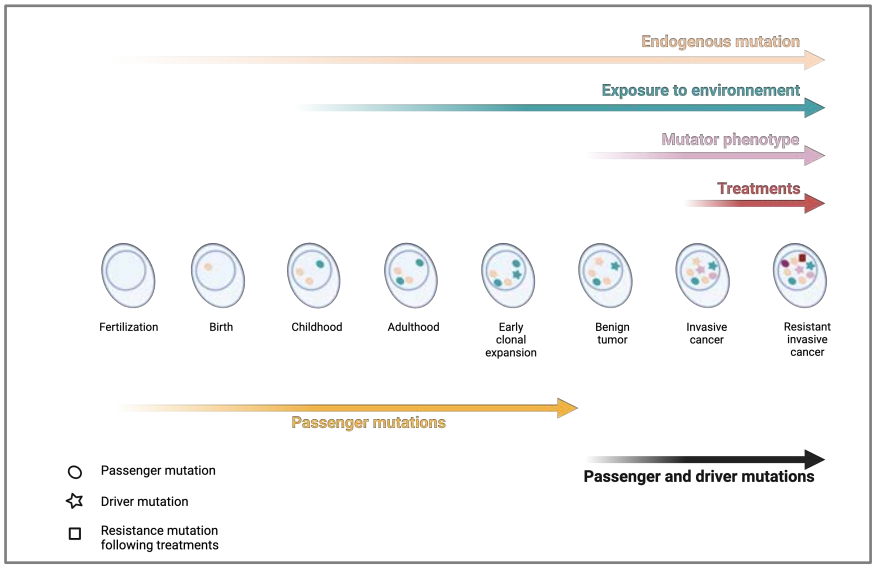


Figure 4 – Schematic representation of clonal evolution with acquisition of passenger and driver mutations through life and factors influencing the course of the clone over time.

CHIP/CCUS

Acquiring a driver somatic mutation is necessary but not always sufficient to develop an overt malignant disease. Hence, clonal hematopoiesis (CH), first described in 1996 by Busque *et al.* is characterized by a clonal expansion of somatically mutated hematopoietic cells in cases that don't otherwise fulfill diagnostic criteria for MDS or any other disorder.[100–104] The presence or absence of cytopenia discriminates clonal hematopoiesis of indeterminate potential (CHIP) from clonal cytopenia of undetermined significance (CCUS). Clonal hematopoiesis is overrepresented in populations previously exposed to chemotherapies and prior treatments impact the molecular profile of CHIP/CCUS.[105,106] Early studies reported that 0.5 to 1% of individuals with CHIP will be diagnosed with MDS later on.[100,105,107] Moreover, it was found that the presence of multiples mutations, mutations with a higher variant allele frequency (VAF) and spliceosome gene mutations were highly predictive of further progression to myeloid neoplasms.[100,103,108] A recent publication from Weeks *and al.* performed whole genome sequencing on 438,890 individuals without known hematological malignancy.[109] Mutations in splicing factor genes (*SRSF2, SF3B1, ZRSR2*), AML-like genes (*IDH1, IDH2, RUNX1*) and the *TP53* gene were considered as high-risk mutations with regards to the risk of developing a myeloid neoplasms (MN) over time. Individuals with CCUS had overall an increased risk of developing MN compared to those with CHIP. Several other variables were associated with a higher risk of MN and a risk score (Clonal Hematopoiesis Risk Score [CHRS]) was established based on 8 variables (age >65years, CCUS, RBC distribution width>15%, mean corpuscular volume >100fL, high-risk mutations, single *DNMT3A* mutation, number of mutations, VAF ≥0.2 %). Interestingly, the

cumulative incidence of MN at 10 years varied dramatically between lower-risk CHRS and higher-risk CHRS from 0.7% to 52%, respectively.

Despite recent advances in the field, a lot remains unknown about CHIP/CCUS and the triggers of progression to overt malignant disease require further investigation. Apart from increasing the risk of MN, it is established that clonal hematopoiesis is associated with a higher risk of cardiovascular diseases and atherosclerosis,[110–112] and an increased incidence of other cancers.[113,114]

Hence, while the acquisition of a somatic driver mutation is one of the key events of the underlying mechanisms in MDS, other factors such as occurrence of additional mutations, epigenetic regulation, microenvironment, immune surveillance must be considered.

2.1.2.3 Molecular mechanisms

Genetic events

Epigenetic modulators

Epigenetics modulators play a major role in regulating gene function. Interestingly, genes belonging to the epigenetic regulator group or chromatin binding family are very frequently mutated in MDS.[6,7,10] Hence, both loss-of-function mutations in *TET2* as well as *IDH1/IDH2* mutations were reported to lead to the disruption of the catalytic function of *TET2* and resulted in a hypermethylated state and impaired hematopoietic differentiation.[115,116] *DNMT3A* encodes an enzyme transferring methyl groups to Cytosine-Guanine structures in DNA. *DNMT3A*-mutant HSC were shown to have a competitive advantage in comparison with wild type HSC.[117,118] Loss of function mutations in *ASXL1* and *EZH2* resulted through different mechanisms to inhibition of the polycomb repressive complex 2 (PRC2) which plays a role in gene silencing.[119,120] *EP300* encodes a lysin acetyltransferase that plays a role as a transcription factor and chromatin modifier. While it seemed to enhance leukemogenesis in AML-ETO1 models[121,122], it was reported to have rather a suppressor effect for transformation to AML in MDS.[123–125]

Splicing factors mutations

SF3B1, SRSF2, U2AF1 and *ZRSR2* are the most frequently mutated splicing factors genes. Next generation targeted sequencing (NGS) and RNA sequencing studies have shed light on their role in the pathophysiology of MDS. Splicing factors mutations have been shown to result in aberrant binding of the splice site[99,126–129] and misplicing of mRNAs of key genes such as *ABCB7* and *ERFE* for *SF3B1*-mutated MDS (described more extensively in the section of MDS with RS)[128,130–132] or *EZH2* for *SRSF2*-mutants.[127,129,131] More recently, induced pluripotent stem cells (IPSC) studies have unraveled further downstream effect of misplicing. As an example, Wheeler *et al.* discovered that *U2AF1* and *SRSF2* mutants both

led to a dysregulation of the *GNAS* gene with preferential usage of a long isoform resulting in activation of the ERK/MAPK pathway.[133] Other groups have used *SF3B1*-mutant IPSC lines and showed i) links between *ABCB7* expression level and the percentage of RS[134], ii) the role of the TEA domain transcription factor family as a transcription regulator[135] , (iii) and a dysregulation of the MAP3K7-p38-GATA1 pathway.[136]

TP53 mutations

TP53 is the most mutated gene in cancer overall and *TP53* mutations are also frequently found in myeloid malignancies and MDS.[6,7,10] The overexpression of the p53 protein has been quiet extensively studied in MDS with deletion (del) of the long arm of chromosome 5 (5q) where the haploinsufficiency of ribosomal protein S14 (*rps14)* leads to increased binding of riboproteins to *MDM2* resulting in p53 stabilization and accumulation which induces cell cycle arrest and apoptosis.[137–140] Although lenalidomide was shown to be able to counteract dyserythropoiesis and anemia in del(5q) MDS, occurrence of *TP53* mutation was associated with treatment resistance and transformation to AML[141–147] Later studies in MDS in general showed that *TP53* mutation was linked to chromosome abnormalities and chromotripsis.[148,149] Since then association between *TP53* and complex karyotype is well recognized and the mutational burden as well as the allelic status are known to play a major role in disease biology and prognosis of *TP53*-mutant MDS.[10,150]

Transcriptome analyses

Beyond aberrant splicing, gene expression has been shown to be impacted in MDS. Hence, interferon-stimulating genes were reported upregulated in CD34-positive bone marrow mononuclear cells (MNC) and bone marrow microenvironment of MDS patients.[127,151–153] In MDS with RS, heme pathway genes were upregulated. In del(5q) MDS, genes related to the long arm of chromosome 5 were downregulated but the *HIST1* gene cluster was upregulated. Moreover, the first attempt of a gene expression-based classification by Shiosawa *et al.* suggested two main groups: a first "EMK"-cluster which exhibited a higher expression of genes related to erythroid/megakaryocytic (EMK) lineages, and a second "IMP"-cluster associated with an increased expression of genes related to immature precursors (IMP) instead. The IMP subgroup was associated with upregulation of signaling pathways and downregulation of DNA repair genes compared to the EMK group. The IMP profile also predicted a poor prognosis and an increased risk of AML transformation.[131] Finally, a recent publication of our group (also part of this thesis) looked deeper into the transcriptomic profile of MDS with RS irrespectively of their morphological class and shed some light on the heterogeneity of outcomes of MDS with RS. Results of the study are detailed in another section.[154]

2.1.2.4 Microenvironment

As in normal hematopoiesis, MDS stem cells depend on a supportive bone marrow niche to survive and expand. Hence, numerous studies suggested that the MDS-niche is also affected in MDS, and it may provide a favorable environment for the clone to thrive.

Mesenchymal stem cells (MSC) are important elements of the bone marrow niche. In MDS, there is evidence suggesting that MSC provide beneficial conditions for clonal expansion and play a role in maintaining the disease. Several studies showed that the growth potential of MDS-MSC was affected.[155–157] However, whether MDS-MSC retain the same differentiation potential as normal MSC is still debated. Similarly, there are contradictory studies regarding the potential of MDS-MSC to support HSPC. Nonetheless, the deregulation of the MDS-MSC immunomodulatory functions is well established.[158–163] Raaijmakers and colleagues also reported that dysfunction of bone progenitors had a role in the pathogenesis of MDS.[164]

Myeloid-derived suppressor cells (MDSC) are another type of innate immune cells that are closely related to neutrophils and monocytes but are expanded in pathological conditions such as cancer, inflammation or other stress. In MDS, MDSC have been reported to contribute to disease expansion particularly through immunosuppressive effect in the microenvironement.[165,166]

Several studies have supported the notion of a MDS bone marrow niche in a hyperinflammatory state. Hence, activation of the Toll-like-receptor 4 signaling pathway with increased alarmin S100A8 and S100A9 have been reported in MDS models.[167–169] Moreover, CCL3 overexpression has been observed in MDS and activation of NF-KB signaling pathway in MDS-MSC was shown to negatively impact hematopoiesis.[168]

Immune surveillance is a key process to counteract cancer genesis and expansion. In MDS, several studies reported that the innate immune system is skewed towards a pro-inflammatory profile. Hence, in MDS specifically, the NLRP3 inflammasome and its constituents was reported to lead to a powerful caspase-1 overactivation which in turn contributed to cell death.[170–173] There is also evidence of dysfunctional immune cells in MDS. While lower levels of regulatory T-cells have been described in low-risk MDS, this cell population seemed to be increased in high-risk MDS.[174–176] Moreover, natural killer cells have not only been reported to be fewer in the peripheral blood of MDS patients but also dysfunctional.[177–181]

While recent advances in single cell sequencing techniques have shed some light on novel components of the niche, the complexity of the micro-environment in MDS is challenging and the numerous interactions between the structures of the niche remain poorly understood overall.

2.1.3 Diagnosis

2.1.3.1 Signs and symptoms

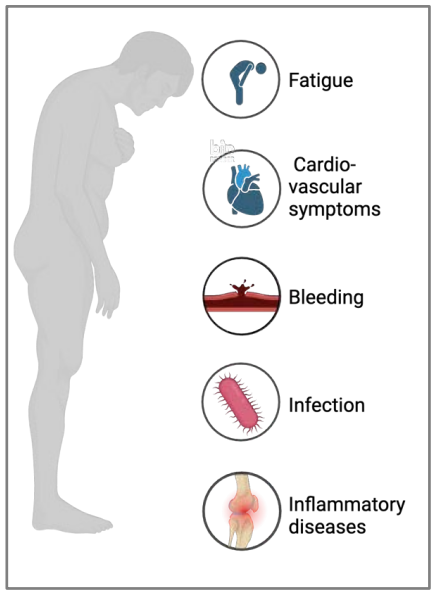


Figure 5 – Main symptoms of MDS

The symptomatology of MDS is largely driven by the type and the severity of cytopenia. Hence, about 56% of patients with MDS and 41% of patients with MDS/MPN were reported to have a hemoglobin value below 10g/dL at diagnosis.[15] Anemia commonly causes shortness of breath, fatigue, dizziness and sometimes cardio-vascular symptoms. Neutropenia with neutrophils below $0.8x10^9$/L is observed in about 20 % of MDS cases at diagnosis and is associated with increased occurrence of infections.[15] Finally, about 16% of MDS patients have severe thrombocytopenia at diagnosis leading to a higher propensity for bleeding.[15,182] Interestingly, a smaller subset of patients also suffers from auto-inflammatory symptoms, but underlying mechanisms have largely remained unexplained.[175,183–188] However, the recent discovery of VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome shed some light on this singular clinical phenotype. VEXAS syndrome is specifically linked to somatic mutations in the *UBA1* gene and is associated with treatment refractory inflammatory diseases as well as cytopenia or overt MDS. Although the incidence of VEXAS seems higher in male, there are a few reports on VEXAS in female patients.[189–194]

2.1.3.2 Diagnostic criteria

The diagnosis of MDS is sometimes challenging. A confirmation of the diagnosis requires repeated assessments and is based on several criteria.[1–3]

- Cytopenia, and other causes of cytopenia should be excluded first
- Significant dysplasia in more than 10% of the erythroid precursors, granulocytes or megakaryocytes
- Cytogenetic abnormalities
- Gene mutations

2.1.3.3 Classifications

Based on these criteria, a confirmed MDS diagnosis is then categorized in one of the MDS-subclasses. While the former World Health Organization (WHO) 2016 classification of myeloid neoplasms was internationally accepted, two competitive revisions were published simultaneously in 2022, and yet, the international MDS community has not reached a consensus on a unique revised MDS classification.[1–3] For this reason, all three classifications will be summarized here.

WHO 2016

The WHO 2016 classification divides MDS in seven main MDS subtypes and four main MDS/MPN classes.[1] One of the principal novelties at the time of publication was the distinction for the first time of a MDS subtype based on molecular finding i.e., *SF3B1* mutation in MDS with RS (Table 1).

WHO classification of MDS 2016	
MDS	**MDS/MPN**
MDS with single lineage dysplastia, **MDS-SLD**	Chronic Myelomonocytic Leukemia **CMML** **- CMML-0** **- CMML-1** **- CMML-2**
MDS with multiple lineage dysplasia, **MDS-MLD**	Atypical Chronic Myeloid Leukemia, BCR-Abl -, **aCML**
MDS with ring sideroblast and single lineage dysplasia, **MDS-RS-SLD**	MDS/MPN with ring sideroblast and thrombocytosis, **MDS/MPN-RS-T**
MDS with ring sideroblast and multiple lineage dysplasia, **MDS-RS-MLD**	MDS/MPN unclassifiable, **MDS/MPN-U**
MDS with excess blast, **MDS-EB** **- MDS-EB1** **- MDS-EB2**	
MDS with isolated del(5q), **MDS 5q-**	
MDS-unclassifiable, **MDS-U**	

Table 1 – World Health Organization classification of MDS 2016

WHO 2022 and ICC 2022

In both the WHO 2022 and the International Consensus Classification (ICC) 2022 classifications, cytogenetic and molecular MDS- or AML-defining events gain importance compared to the previous WHO 2016 classification.[2,3]

While the WHO 2022 classification discerns genetically defined-MDS from morphologically defined-MDS, it keeps the blasts threshold of 20% to distinguish between MDS and AML. The former atypical chronic myeloid leukemia subtype is now replaced with MDS/MPN with neutrophilia. In CMML previously divided in three sub-classes according to blasts count, the subclass CMML-0 is eliminated. The WHO 2022 classification also stresses the importance of biallelic T*P53* mutation and makes it a separate entity (Table 2).

WHO classification of MDS 2022	
MDS	**MDS/MPN**
MDS with defining genetic abnormalities - MDS with low blast en isolated Del(5q) - MDS with low blast and *SF3B1* mutation - MDS with biallelic *TP53* inactivation	Chronic Myelomonocytic Leukemia
MDS, morphologically defined - MDS with low blasts - MDS hypoplastic - MDS with increased blasts • MDS-IB1 • MDS-IB2 • MDS with fibrosis	MDS/MPN with neutrophilia
	MDS/MPN with *SF3B1* mutation and thrombocytosis
	MDS/MPN not otherwise specified

Table 2 – World Health Organization classification of MDS 2022. Del: deletion; IB: increased blasts

Whereas clonal cytopenia are not part of WHO 2022, it enters the ICC 2022 classification as a stand-alone category in both MDS and MDS/MPN. The ICC 2022 differs mainly by lowering the AML-defining blast threshold to 10%. Hence, it creates a large class of MDS/AML and stresses the importance of MDS-defining cytogenetic and molecular features. As in the WHO 2022, the presence of a *TP53* mutation becomes an important classifier. Finally, the ICC 2022 creates a separate group of pediatric- and/or germline mutation-associated disorders (Table 3).

ICC 2022		
MDS	MDS/MPN	Pediatric and/or germline mutation associated disorders
Clonal cytopenia of undetermined significance	Clonal cytopenia with monocytosis of undetermined significance	Juvenile myelomonocytic leukemia
Myelodysplastic syndrome with mutated *SF3B1*	Clonal monocytosis of undetermined significance	Juvenile myelomonocytic leukemia-like neoplasms
Myelodysplastic syndrome with del(5q)	Chronic myelomonocytic leukemia	Noonan syndrome-associated myeloproliferative disorder
Myelodysplastic syndrome with mutated *TP53*	Atypical chronic myeloid leukemia	Refractory cytopenia of childhood
Myelodysplastic syndrome, not otherwise specified (MDS, NOS) • MDS, NOS without dysplasia • MDS, NOS with single lineage dysplasia • MDS, NOS with multilineage dysplasia	Myelodysplastic/myeloproliferative neoplasm with thrombocytosis and *SF3B1* mutation	Hematologic neoplasms with germline predisposition
Myelodysplastic syndrome with excess blasts	Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis, not otherwise specified	
Myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) • MDS/AML with mutated *TP53* • MDS/AML with myelodysplasia-related gene mutations • MDS/AML with myelodysplasia-related cytogenetic abnormalities • MDS/AML, not otherwise specified	Myelodysplastic/myeloproliferative neoplasm, not otherwise specified	

Table 3 – The International Consensus Classification (ICC) of MDS 2022. NOS: not otherwise specified

2.1.3.4 Cytogenetic and molecular features

Chromosomal aberrations

Chromosomal aberrations occur in about 50% of patients with MDS. Loss or gain of larger parts of, or a whole chromosome – also called unbalanced cytogenetic abnormalities – are clearly over-represented in MDS where balanced chromosomal aberrations occur in less than 2% of patients[195]. The most frequent cytogenetic abnormalities in MDS are del(5q), complex karyotype (three or more than three chromosomal aberrations), monosomy 7 or del(7q), trisomy 8 and del(20q). Although several studies since the 1980's showed that specific chromosomal anomalies have a prognostic impact in MDS[195–199], validation in a larger cohort came twenty years later with the International Prognostic Scoring System and its revised version confirming that complex karyotype, deletion or loss of chromosome 7 and inversion, translocation or deletion of chromosome 3 had a detrimental effect on prognosis.[4,5] Despite advances in molecular characterization of MDS, cytogenetic features and their clinical significance remain, and chromosomal abnormalities are part of the recent IPSS-M score.[10]

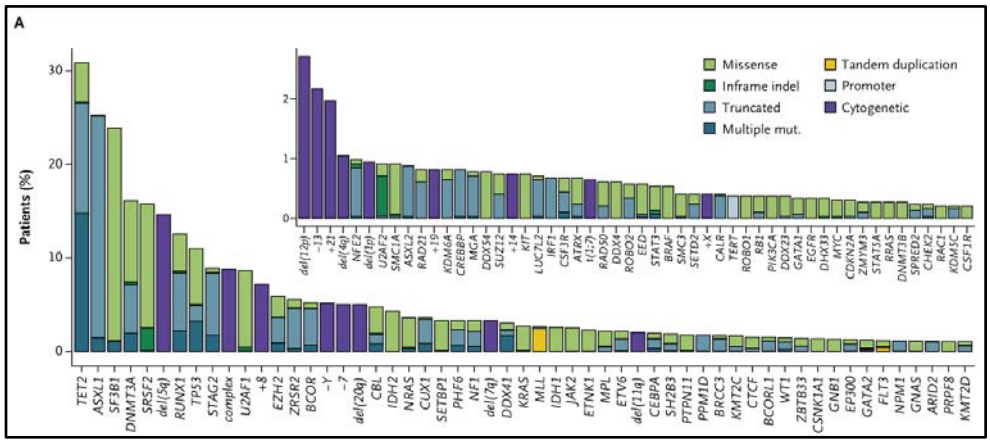


Figure 6 – Frequency of mutated genes and cytogenetic aberrations in the international MDS cohort of 2957 patients. The colors correspond to the type of alteration. Mut.: mutations. Adapted from figure 1, NEJM Evid., Bernard et al., Molecular International Prognostic Scoring System for Myelodysplastic Syndromes. Volume 1, Page 7. Copyright © (2022) Massachusetts Medical Society. Reprinted with permission.

Exploration of molecular patterns in cancer diseases took off two decades ago with the increased availability and efficiency of genetic sequencing techniques.[86,200] Several studies confirmed the presence of distinct genetic mutations in MDS and related myeloid neoplasms.[13,201–203] The first comprehensive molecular profiling of MDS were published only a few years ago by Papaemmanuil *et al.* and Haferlach *et al.* in large cohorts of 738 patients and 844 patients, respectively.[6,7]

Oncogenic mutations are reported in up to 90% of MDS patients. Pathological variants occur in genes involved in the RNA splicing machinery, chromatin modification, DNA methylation, hematopoietic transcription factors and kinase signaling pathways as mentioned above. Whether the driver mutation is clonal or sub-clonal does not seem to impact prognosis. However, the number of oncogenic mutations was reported to affect overall survival (OS). Interestingly, even though numerous gene mutations were associated with outcome, only *SF3B1* mutations were predictive of better OS, an effect that was mainly influenced by the patterns of comutation. In contrast, studies confirmed a clear deleterious effect of TP*53* ^{multi}, *MLL-PTD, FLT3* mutations.[6–10,150]

Apart from their prognostic value, mutations play a substantial role in MDS diagnosis as per ICC 2022 and WHO 2022 classifications. Additionally, as noted in prior section, the hereditary pattern of the disease has probably been underestimated for years and germline mutations in specific genes have been shown to increase the risk of developing MDS or related myeloid neoplasms.

2.1.4 Prognosis

Several types of complications occur in MDS – commonly resulting from cytopenia – and impact significantly both survival and quality of life.

Neutropenia-related infections are recurrent and are reported to be the main cause of deaths in about 20-40% of deceased cases. Similarly, reports suggest bleeding complications as the main or contributing cause in 10% and 20% of deaths, respectively. Moreover, it has been reported that anemia is associated with cardiac hypertrophy and cardiac mortality in MDS patients.[182,204–208]

Secondary hemochromatosis resulting from chronic blood transfusion is also an important concern as it causes multiple organ damages over time and is associated with poor prognosis both in lower-risk MDS and in the setting of allogeneic hematopoietic stem cell transplantation (HSCT).[209–213]

Foremost, the prognosis of MDS is largely driven by the risk of transformation to AML. Secondary AML, such as post-MDS AML, is associated with shorter OS compared with de novo AML. [214] In the Swedish MDS registry, about 13% of patients were reported to have transformed to AML within the first year after MDS diagnosis.[15] However, the transformation rate varies greatly between and within the different subtypes of MDS.[214,215] Several prognostic tools have been developed since the late 1990's in order to estimate the risk of transformation to AML and OS and guide clinicians' treatment decision-making.[4,5,216–223]

2.1.4.1 Prognostic scoring systems

IPSS and IPSS-R scoring systems

The International Prognostic Scoring System was established in 1997 and is based on the number of cytopenia, the percentage of blasts and specific chromosomal aberrations. It assigns the disease in one of the four risk categories based on the estimated risk of AML transformation and death: low, intermediate-low, intermediate-high, and high.[4]

Its revised version, the IPSS-R, allows a larger granularity in the severity of cytopenia, the percentage of blasts and cytogenetic aberrations. The IPSS-R score distinguishes 5 risk categories based on the estimated risk of AML transformation and death: very-low, low, intermediate, high, and very-high (Table 4 and 5).[5]

	Points						
Prognostic variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good	—	Good	—	Intermediate	Poor	Very poor
BM blast, %	≤ 2	—	> 2%- < 5%	—	5%-10%	> 10%	—
Hemoglobin	≥ 10	—	8- < 10	< 8	—	—	—
Platelets	≥ 100	50-< 100	< 50	—	—	—	—
ANC	≥ 0.8	< 0.8	—	—	—	—	—

Risk category	Risk score
Very low	≤ 1.5
Low	> 1.5-3
Intermediate	> 3-4.5
High	> 4.5-6
Very high	> 6

Table 4 – IPSS-R constitutive variables and risk categories. BM: bone marrow, ANC: absolute neutrophil count. Cytogenetic risk groups: very-good, -Y, del(11q); good, normal karyotype, del(5q), del(12p), del (20q), double including del(5q); Intermediate, del(7q), +8, +19, i(17q), any other single or double independent clone; poor, -7, inv(3)/t(3q)/del(3q), double including -7/del(7q), complex 3 abnormalities; very-poor, complex > 3 abnormalities.

	N. Of patients	Very low	Low	Intermediate	High	Very high
Patients, %	7012	19	38	20	13	10
Survival Medians, years (95% CI), *P* < .001		8.8 (7.8-9.9)	5.3 (5.1-5.7)	3.0 (2.7-3.3)	1.6 (1.5-1.7)	0.8 (0.7-0.8)
Hazard ratio (95% CI)		0.5 (0.46-0.59)	1.0 (0.93-1.1)	2.0 (1.8-2.1)	3.2 (2.9-3.5)	8.0 (7.2-8.8)
Patients, %	6485	19	37	20	13	11
AML/25% Median time(years) to 25% AML evolution (95% CIs), *P* < .001		NR (14.5-NR)	10.8 (9.2-NR)	3.2 (2.8-4.4)	1.4 (1.1-1.7)	0.73 (0.7-0.9)
Hazard ratio (95% CI)		0.5 (0.4-0.6)	1.0 (0.9-1.2)	3.0 (2.7-3.5)	6.2 (5.4-7.2)	12.7 (10.6-15.2)

Table 5 – Summary of outcomes (survival and AML transformation) across IPSS-R risk categories. CI: confidence interval; P: p-value; AML: acute myeloid leukemia; NR: not reached

IPSS-M

The IPSS-M score was developed based on an international cohort of 2957 patients with MDS and non-proliferative MDS/MPN.[10] A total of 22 variables (hemoglobin, platelets, bone marrow blasts percentage, karyotype, gene mutations) are computed to a unique continuous score according to which the disease is assigned to one of the six risk categories (very-low, low, moderate-low, moderate-high, high, and very-high risk). Details about the IPSS-M score are found in a separate section (Table 6, Study I).

	IPSS-M Risk Category					
Characteristic	**Very Low**	**Low**	**Moderate Low**	**Moderate High**	**High**	**Very High**
Patients — No. (%)	381 (14)	889 (33)	302 (11)	281 (11)	379 (14)	469 (17)
Risk score	≤−1.5	>−1.5 to −0.5	>−0.5 to 0	>0 to 0.5	>0.5 to 1.5	>1.5
Hazard ratio (95% CI)†	0.51 (0.39–0.67)	1.0 (Reference)	1.5 (1.2–1.8)	2.5 (2.1–3.1)	3.7 (3.1–4.4)	7.1 (6.0–8.3)
Median LFS (25–75% range) — yr‡	9.7 (5.0–17.4)	5.9 (2.6–12.0)	4.5 (1.6–6.9)	2.3 (0.91–4.7)	1.5 (0.80–2.8)	0.76 (0.33–1.5)
Median OS (25–75% range) — yr	10.6 (5.1–17.4)	6.0 (3.0–12.8)	4.6 (2.0–7.4)	2.8 (1.2–5.5)	1.7 (1.0–3.4)	1.0 (0.5–1.8)
AML-t — %						
By 1 yr	0.0	1.7	4.9	9.5	14.3	28.2
By 2 yr	1.2	3.4	8.8	14.0	21.2	38.6
By 4 yr	2.8	5.1	11.4	18.9	29.2	42.8
Death without AML — %						
By 1 yr	2.2	8.5	12.0	18.0	19.3	30.6
By 2 yr	7.0	16.2	19.8	31.1	39.8	45.6
By 4 yr	15.9	29.5	33.6	51.1	54.2	51.3

Table 6 –Estimated outcomes (overall survival, leukemia-free survival and AML-transformation) in the IPSS-M risk categories. CI: confidence interval; LFS: leukemia-free survival; OS: overall survival; AML-t: transformation to acute myeloid leukemia; yr: year. From NEJM Evid., Bernard et al., Molecular International Prognostic Scoring System for Myelodysplastic Syndromes. Volume 1, Page 7. Copyright © (2022) Massachusetts Medical Society. Reprinted with permission.

2.1.4.2 CMML-specific prognostic scores

As most myeloproliferative CMML (WBC ≥13x10^9/L) are excluded from MDS prognostic tools, specific risk scoring systems have been developed.

CPSS score

The calculation of the CMML-specific prognostic scoring system (CPSS), developed on a cohort of 558 CMML patients, is based on the percentage of bone marrow blasts, WBC <13x10^9/L or ≥13x10^9/L, karyotype and transfusion dependency. It discriminates patients into 4 risk groups according to OS and risk of AML transformation (Table 7 and 8).[220]

Prognostic variable	Points		
	0	1	2
Blasts (%)	<10 % in BM and < 5 % in PB	10-19 % in BM or 5-19 % in PB	
White blood cell count	<13 x 10^9/L	≥ 13 x 10^9/L	
Karyotype	Low risk	Intermediate	High risk
Transfusion dependency	No	Yes	

Table 7– CPSS score constitutive variables. BM: bone marrow, PB: peripheral blood; CMML-specific karyotype risk groups: low risk, normal and isolated –Y; intermediate risk, other abnormalities; and high risk, trisomy 8, complex karyotype (≥3 abnormalities), and abnormalities of chromosome 7. Transfusion dependency was defined as having at least 1 red blood cell transfusion every 8 weeks over a period of 4 months.

Risk group	Total score
Low	0
Intermediate-1	1
Intermediate-2	2-3
High	4-5

Table 8 – CPSS score risk categories.

CPSS-mol

Advances in molecular profiling of CMML resulted in the integration of mutational patterns in novel prognostic tools. The CPSS-mol was developed on a cohort of 218 CMML patients. The CPSS-mol score is calculated based on genetic categories (combination of cytogenetic risk groups and gene mutations), bone marrow blasts percentage, WBC count and transfusion dependency, and assigns the disease in one of the four CPSS-mol risk groups (low, intermediate-1, intermediate-2 and high risk) (Table 9 and 10).[218]

	CPSS cytogenetic risk group	*ASXL1*	*NRAS*	*RUNX1*	*SETBP1*
Score of each variable					
0	Low	Unmutated	Unmutated	Unmutated	Unmutated
1	Intermediate	Mutated	Mutated	NA	Mutated
2	High	NA	NA	Mutated	NA
Genetic risk group	**Score**				
Low	0				
Intermediate-1	1				
Intermediate-2	2				
High	≥3				

Table 9 – CPSS-mol genetic groups based on previously validated CPSS karyotype risk categories and presence of mutations in *ASXL1, NRAS, RUNX1* or *SETBP1 genes;* NA: not applicable.

	Genetic risk group	BM blasts	WBC count	RBC transfusion dependence
Variable score				
0	Low	< 5 %	< $13x10^9$/L	No
1	Intermediate-1	≥ 5 %	≥ $13x10^9$/L	Yes
2	Intermediate-2	NA	NA	NA
3	High	NA	NA	NA
CPSS-Mol risk group	**Score**			
Low	0			
Intermediate-1	1			
Intermediate-2	2			
High	≥4			

Table 10 – CPSS-mol score, its constitutive variables and resulting risk groups. BM: bone marrow; WBC: white blood cells; RBC: red blood cells; NA: not applicable.

2.1.5 Treatment

2.1.5.1 Higher-risk MDS

In higher-risk MDS, therapy mainly aims to reduce the risk of transformation to AML, decrease the disease burden and improve blood counts. As HSCT is currently the only potentially curative option, it should be considered early on in patients who are eligible (≤ 75 years old and no or minor comorbidities).[224,225]

Hypomethylating agents (HMA) are widely used as first line treatment either as a bridging to HSCT or to delay disease progression in palliative settings.[226,227] Azacytidine or

Decitabine alone are relatively well tolerated and easy-to-handle HMA. However, about 50% of MDS patients do not benefit from HMA only. Combination treatments with Bcl-2 inhibitor Venetoclax are currently studied. Results from a phase 1 study of the combination Azacytidine-Venetoclax showed promising results, but response was clearly influenced by the type of prior therapy.[228] A phase 3 study is ongoing (NCT04401748) and yet, Venetoclax is not approved as a part of the standard treatment in MDS in Sweden (Figure 8).

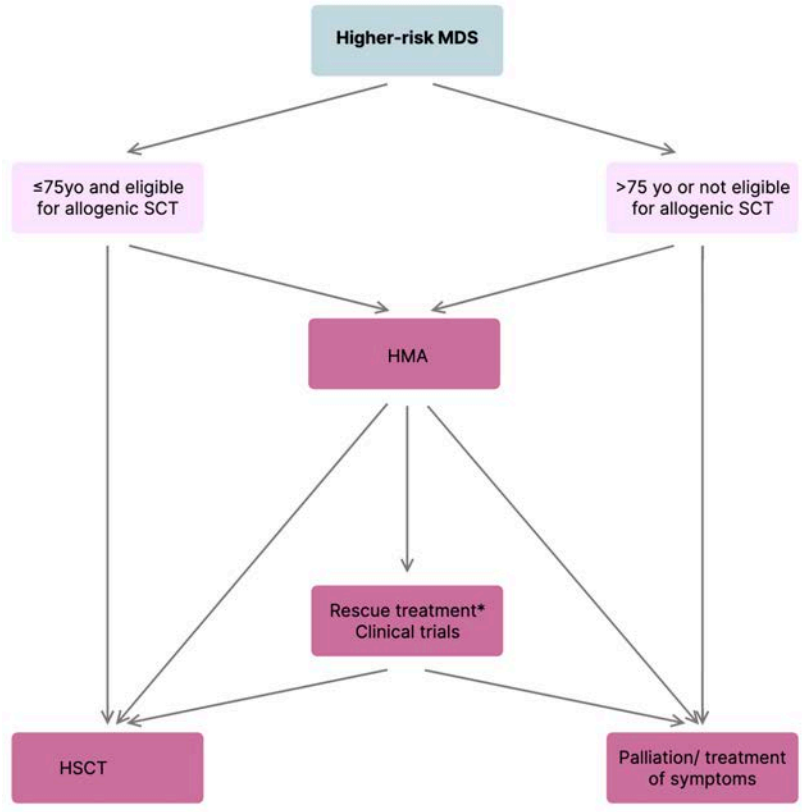


Figure 8 – Treatment algorithm of higher-risk MDS using compounds approved in Sweden. Yo: years old, HSCT: allogeneic stem cell transplantation; HMA: hypomethylating agents; *: AML-like intensive chemotherapy; although BCL-2 inhibitors are not yet formally approved in Sweden for use in MDS, they are sometimes used in clinical practice in patients with failure to HMA single agent; other targeted treatments such as *IDH*-inhibitors, *FLT3* inhibitors.

Several ongoing clinical trials are currently assessing other compounds either as single drug or in combination such as *IDH*-inhibitors, check point inhibitors, anti-CD47 antibodies (NCT04417517, NCT04313881, NCT04900350, NCT05709093, MCL1 inhibitor (NCT05209152), selective inhibitor of nuclear export (NCT05918055), anti-TIM3 antibodies (NCT03946670), splicing modulator (NCT05732103), *EP300* inhibitor

(NCT04068597), NK-cell therapy (NCT05115630), CAR-T cell therapy (NCT03927261), *MDM2*-inhibitor (NCT03940352), novel BCL2-inhibitor (NCT04964518).

Yet, approved therapeutic options in HMA resistant disease are limited and AML-like chemotherapy remains an alternative as a bridging to HSCT.

2.1.5.2 Lower-risk MDS

The main goal of treatment in lower-risk MDS focuses on relieving the symptoms of the disease by improving cytopenia. Erythropoiesis stimulating agents (ESA) with or without Granulocyte Colony Stimulating Factor (GCSF) have been a backbone of the first line therapy of lower-risk MDS for many years.[224,225,229–231] About 50% of patients will respond – at least partially – to ESA, however the treatment effect will eventually fade out after a median time of less than two years.

More recently, a new compound named Luspatercept was approved as a therapeutic option in ESA resistant/refractory patients with MDS-RS. The molecule, also characterized as an erythroid maturating agent is designed as a ligand-trap for the TGF-β superfamily.[232–234] While both the American Federal Drug Agency (FDA) and the European Medicines Agency (EMA) approved a few years ago the use of Luspatercept as a second line treatment in MDS, Luspatercept is not available in Sweden. Results from a phase 3 randomized clinical trial comparing erythropoietin versus Luspatercept were recently published and resulted in FDA approval of Luspatercept as a first line treatment of anemia in MDS (EMA decision is still pending).[235,236]

A few other therapies are available for specific subsets of lower-risk MDS. Hence, Lenalidomide can be an adequate second line treatment in patients with del(5q) MDS, and immunosuppressive regimens might be an appropriate choice in hypoplastic MDS.[237–245]

Treatment with chelating agents is warranted when lifelong RBC transfusions remain the only option after failure of first line therapies (more details about RBC transfusion in MDS in separate section)

Considering both the risk of transplantation related mortality and the low AML transformation rate, the benefit/risk balance of HSCT in lower-risk MDS is ambiguous. Thus, it might only be considered in young and fit patients with high transfusion burden or high-risk genetic features (Figure 9).[225,246]

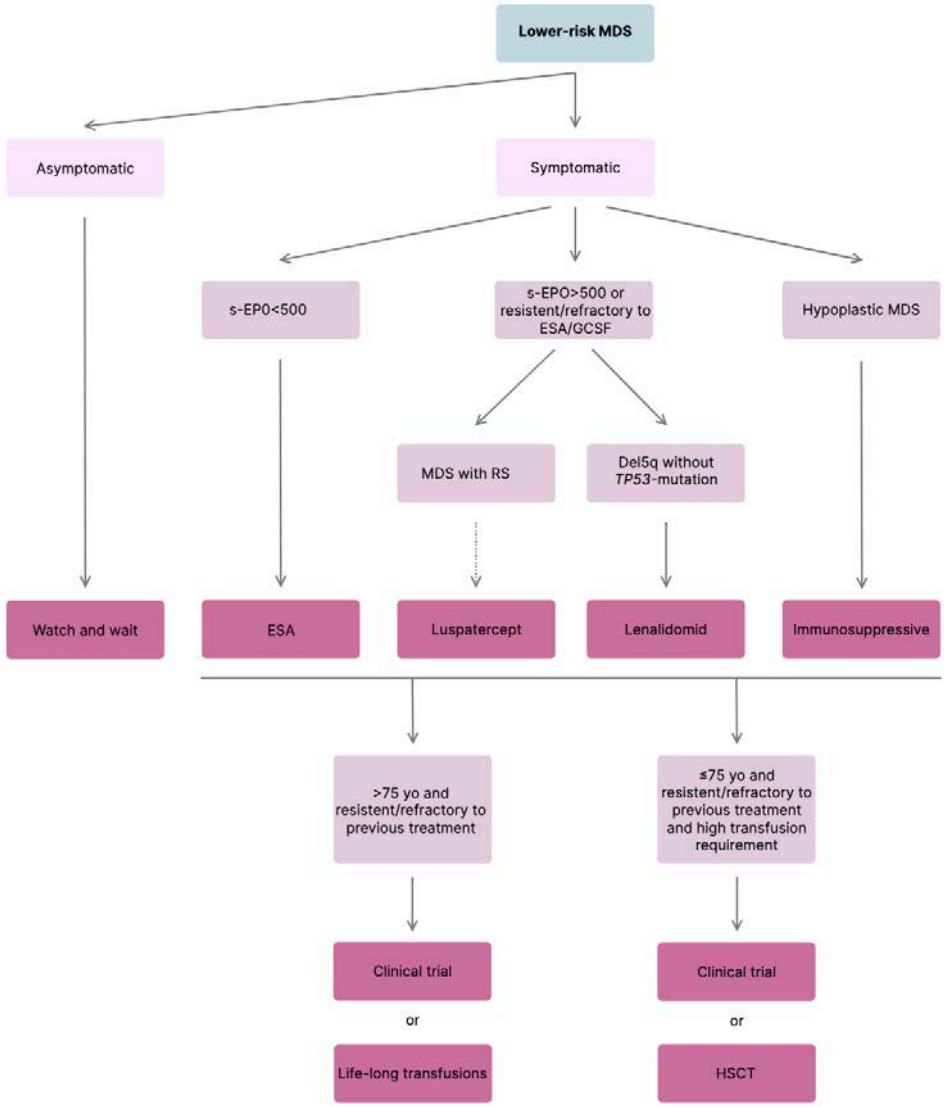


Figure 9 – Algorithm for treatment of lower-risk MDS using compounds that are approved in Sweden. s-EPO: serum erythropoietin; ESA: erythropoiesis stimulating agents, GCSF: granulocyte colony stimulating factor; yo: years old; HSCT: allogeneic stem cell transplantation.

Inclusion in clinical trials is recommended early on in ESA-refractory/resistant patients. A number of drugs are currently being studied such as: IRAK1/4 inhibitors, (NCT05308264, NCT05178342), activator of erythroid pyruvate kinase (NCT05568225 NCT05490446), oral decitabine and cedazuridine (NCT03502668), Activin Receptor IIA ligand trap (NCT04419649), anti-IL1β antibody (NCT05237713), PRMT-5 inhibitor (NCT03573310), anti-

cKit antibody (NCT05903274). With promising results in pre-clinical settings, phase 1 studies of spliceosome modulators showed tolerability issues and no clear efficacy signal.[247–249]

2.1.5.3 CMML

Treatment of CMML is difficult and as in other MDS subtypes, HSCT is the only curative option.[225,250]

Patients with higher risk disease as per CPSS-mol should be considered for HSCT if eligible (younger patients with no or few comorbidities), and bridging therapy, mainly with HMA, is often indicated to decrease the disease burden prior to transplantation.

Treatment with HMA or with AML-like chemotherapy can be considered in patients with higher-risk disease not candidate for HSCT to delay progression to AML and improve survival.[225,250] HMA are routinely used for CMML with low leucocyte counts (<13x 10^9/L) as the response is not as good in proliferative CMML (WBC ≥13x10^9/L).[251–254] Moreover, a recent randomized phase 3 trial by Itzykson *et al.* enrolling myeloproliferative CMML patients with advanced disease showed that decitabine did not improved survival compared to hydroxyurea even though a lower rate of transformation was observed in the decitabine arm.[255]

Hydroxyurea and/or growth factors can be used in patients with symptomatic CMML-0 and lower risk CMML-1. Active surveillance is recommended in patients with CMML-0 and asymptomatic disease (Figure 10).[225,250]

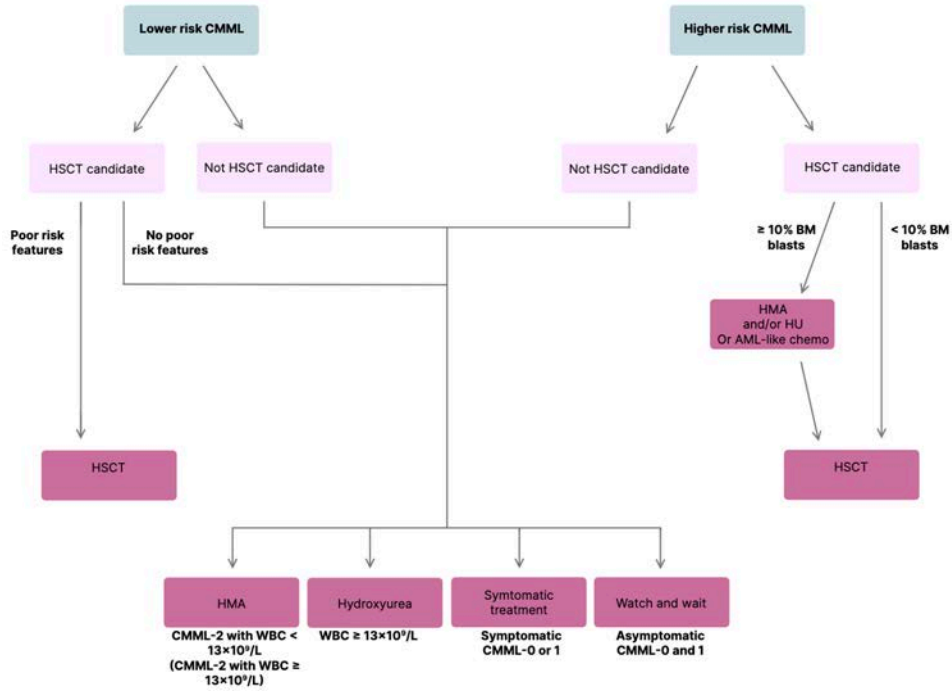


Figure 10 – Algorithm summarizing main treatment options in higher risk versus lower risk CMML as per current prognostic classifications. Poor risk features: poor-risk cytogenetics and/or molecular features, persistent blast increase [>50% or with >15% BM blasts], deep cytopenia [neutrophil counts $<0.3\times10^9$/L; platelet counts $<30\times10^9$/L], transfusion intensity ≥2 units per months for 6 months). SCT: stem cell transplantation candidate, i.e., <75 yo and no or few comorbidities; HSCT: allogeneic stem cell transplantation; BM: bone marrow; HMA: hypomethylating agents; HU: hydroxyurea; AML: acute myeloid leukemia; WBC: white blood cells

2.1.5.4 Red blood cell transfusion in MDS

As mentioned in a previous section, anemia is a common symptom of the disease. Hence, according to several reports about 30-50% of patients are RBC transfusion-dependent at diagnosis.[4,15] Based on studies showing association between a liberal transfusion strategy and a higher quality of life,[256–259] current guidelines recommend to base transfusion thresholds on symptoms rather than on an absolute hemoglobin level.[224,225] However, reports consistently demonstrated that transfusion dependency at diagnosis is associated with shorter survival, and that quality of life seems to be impacted by the cumulative transfusion burden.[220,260–265]

The hemoglobin level at diagnosis is one of the main driver of prognosis overall in the IPSS-M.[10] Additionally, the rescue of the erythropoiesis translating into increased hemoglobin levels is part of the assessment of treatment response (erythroid response, "HI-E" criteria).[266,267] Interestingly, transfusion dependency as a time-varying parameter was shown to have a prognostic value and was for instance part of the WHO classification-based prognostic system (WPSS).[261,268]

Iron overload and resulting organ damages is one of the complications in chronic transfusion-dependent patients. Hence, numerous studies have demonstrated the deleterious impact of high transfusion burden and iron overload on outcomes post HSCT.[269–278] Both retrospective and prospective studies provided support for the favorable effect of chelating therapy on outcomes.[279–285]

2.2 MDS with Ring Sideroblasts

2.2.1 General considerations

MDS with RS is a particular subtype of MDS characterized by ineffective erythropoiesis and iron-loaded mitochondria on microscopy after Prussian blue staining (Perl's reaction). While often associated with *SF3B1* mutations and an indolent disease course, RS are sometimes found in more aggressive MDS and MDS/AML entities.[1,2,286,287]

2.2.2 Normal human erythropoiesis

Erythropoiesis is the process of generating mature functional RBC. In normal condition in adults, all erythropoiesis happens in the bone marrow where it represents between 10 and 30% of the nucleated cells.

The erythropoietic progenitors' compartment is constituted of HSCs which then evolve to Burst Forming Units (BFU), themselves developing to CFU. HSC, BFU and CFU are characterized by their ability to form colonies on media, such as methylcellulose. Although both CFU and BFU already have a lineage commitment, some studies showed that they have a limited self-renewal capacity.[288–290] Erythroid precursors are unipotent

erythroblasts which during differentiation go through morphological changes such as condensation of the nucleus, increase of hemoglobin content as well as decrease of RNA load. *GATA1* and *GATA2* are major players in erythropoiesis. Hence while *GATA2* has a substantial role in lineage commitment, the switch to higher expression level of *GATA1* is essential for erythroid differentiation.[291,292] The control of the erythroid maturation process is closely related to TGF-β ligand family, particularly growth differentiation factor 11 (GDF11) which inhibits erythroid maturation via the Smad2/3 pathway.[293–295] A TGF-β ligand trap (Luspatercept) was developed for the treatment of anemia in MDS and β-thalassemia.[296,297] Overall, erythropoiesis is a meticulously regulated process requiring flexibility to adapt to the demand both in physiological and stress conditions.[298] Hence, interplay between erythropoietin production mechanisms[290,299–304], iron metabolism[305] and growth factors such as stem cell factor (SCF) and interleukin 3 (IL-3)[306] among others are necessary to produce mature RBC. Immunophenotypically, the different erythroid stages can be distinguished by CD34, CD117, CD105, CD36, CD71, CD235 expression on the cell surface (Figure 11).[307,308]

Finally, the erythroid niche and particularly macrophages forming erythroid islands are required in the erythroid differentiation and maturation process.[309–314]

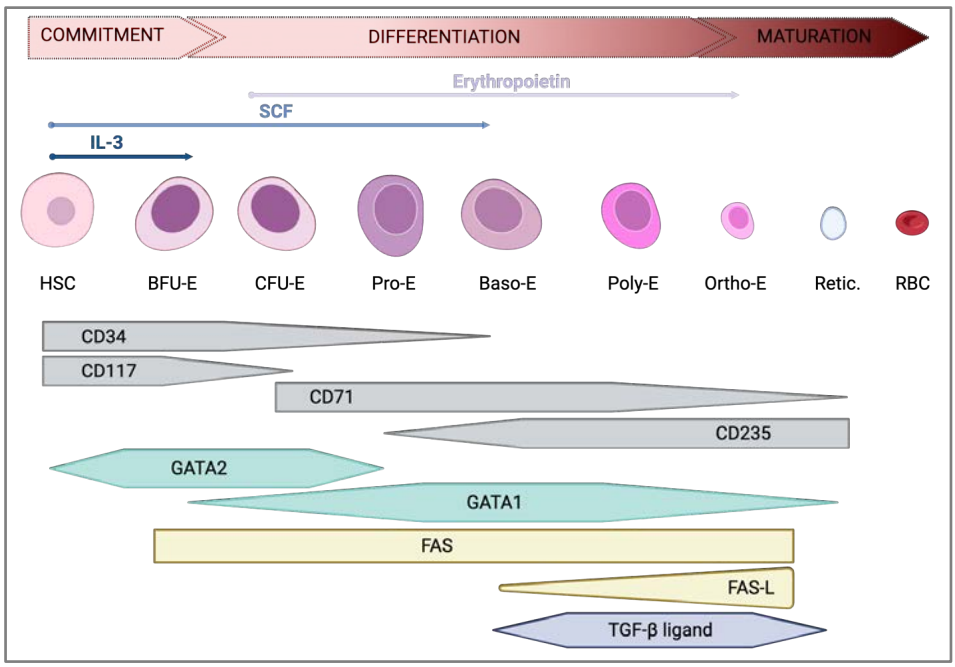


Figure 11 – Schematic representation of human erythropoiesis. SCF: stem cell factor; IL-3: interleukin-3; HSC: hematopoietic stem cell, BFU-E: burst-forming unit of erythroid lineage, CFU-E: colony-forming unit of erythroid lineage; Pro-E: pre-erythroblast; Baso-E: basophilic erythroblast; Poly-E polychromatophilic erythroblast; ortho-E: orthochromatic erythroblast; Retic.: reticulocyte; RBC: red blood cell; CD: cluster of differentiation; FAS-L: FAS-ligand; TGF: transforming growth factor.

2.2.3 Pathogenesis

In a cohort of myeloid neoplasms, Yoshida *et al.* reported in 2011 a high frequency of splicing factor mutations in MDS. Interestingly, about 80% of MDS with RS carried mutations in the *SF3B1* gene.[126] Moreover, aberrant splicing leading to activation of non-sense mediated mRNA decay (NMD) was observed in splicing factor mutants. Further characterizations of *SF3B1* mutations in different cancers were published later and confirmed frequent *SF3B1* mutations in MDS, particularly in MDS with RS where most mutations occurred as heterozygous substitution and in codon 700.[11,13,315] The presence of *SF3B1* mutations in MDS also had a very high positive predictive value for the presence of RS (98%) and there was a significant association between the VAF of *SF3B1* and the percentage of RS. Interestingly, *SF3B1* mutations constituted the dominant clone in MDS with RS but not in MDS without RS. Another experiment with a conditional knock-in *SF3B1* K700E mice managed to reproduce partly the MDS phenotype and resulted in the development of erythroid dysplasia and maturation defect. However, competitive advantage was not observed in transplantation settings.[316]

Terhanchi *et al.* suggested that erythroid progenitors from MDS patients and particularly MDS with RS displayed, due to mitochondrial stress, a spontaneous release of mitochondrial cytochrome-c resulting in activation of caspase 9 and increased apoptosis. Early erythroblasts of MDS with RS exhibiting cytochrome-c release were also reported to have a higher expression of mitochondrial ferritin and a downregulation of erythroid differentiation genes such as *GATA-1*.[317,318]

Moreover, downregulation of mitochondrial genes as well as upregulation of the *ALAS2* gene and downregulation of the iron transporter *ABCB7* gene were described as part of the underlying mechanisms of MDS with ring RS.[127,151,319,320] Interestingly, downregulation of *SF3B1* in erythroid progenitors resulted in a lower expression of *ABCB7* suggesting a link between both genes.[130] Hence, a suggested underlying mechanism is that *SF3B1* mutation and resulting aberrant splicing impact *ABCB7* expression. Downregulation of *ABCB7* results in iron accumulation in mitochondria, mitochondrial stress and increased apoptosis as a downstream effect. Oppositely, induced upregulation of *ABCB7* was reported to have a beneficial effect on growth of erythroid colonies and led to a decreased expression of the gene *FTMT* coding for mitochondrial ferritin.[130,319] A recent study using *SF3B1* mutant IPSC also showed that mitochondrial transporters *TMEM14C* and *ABCB7* were mispliced in *SF3B1*-mutated MDS which resulted in mitochondrial iron accumulation and formation of RS.[134]

2.2.4 Prognosis

MDS with RS is one of the subtypes of MDS with most favorable outcomes. Hence, long before advanced molecular characterization of MDS with RS, the Haferlach group reported

a 2-years OS ranging between 86% and 91 % for RARS (refractory anemia with ring sideroblasts as per WHO 2008 classification) and RCMD-RS (refractory cytopenia with multilinear dysplasia and ring sideroblasts as per WHO 2008 classification), respectively.[321] Studies of outcome of *SF3B1*-mutated patients later confirmed associations with better OS and lower risk to AML, particularly when associated with RS. Nonetheless, there is evidence that this effect is greatly modulated by co-occurring mutations.[10,12,13,315] Hence, while MDS with *SF3B1* and RS is mostly considered as an indolent disease, RS are sometimes found in other MDS subtypes with more aggressive course.[286,287] One of the studies of this thesis aimed to shed light on the heterogeneity of outcome in MDS with RS through integrated genomic/transcriptomic characterization (study II).[154]

3 Research aims

3.1 Overall objective

The objective of this thesis was to study predictive markers of outcome to refine classification and prognostication in MDS.

3.2 Specific aims

Study I: Establish a new International Prognostic Scoring System for MDS including novel molecular markers

Study II: Integrate genomic and transcriptomic analyses with clinical data to understand the heterogeneity of pathophysiology and outcome in MDS with ring sideroblasts

Study III: Assess the prognostic impact of combined clinical, molecular and longitudinal transfusion data and its potential to refine dynamic outcome prediction

4 Materials and methods

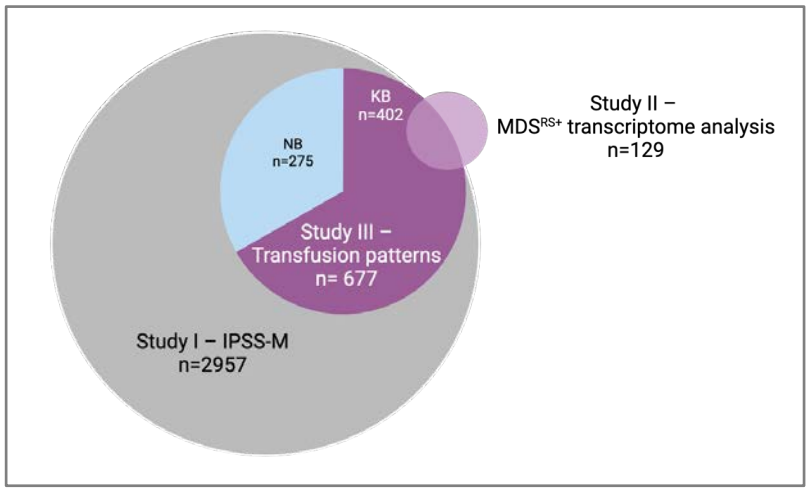


Figure 12 – Patients populations in study I, II and III. NB: National biobank; KB: Karolinska Institutet biobank.

4.1 Study I – A novel prognostic scoring system, IPSS-M

4.1.1 Patients and samples

The training dataset was constituted of a cohort of 2957 patients with MDS included from 24 international centers. Patients with bone marrow blasts percentage ≥20% were excluded. Patients with MDS/MPN were eligible only if they had a WBC count <13x10^9/L. A total of 370 patients with MDS/MPN and 234 patients with secondary/therapy related MDS (s/t-MDS) were included. Peri-diagnostic and previously untreated samples were collected, and DNA was extracted. Clinical data including complete blood counts, treatments, cytogenetic results as well as information on OS and AML transformation were retrieved locally and reviewed centrally. A dataset of 754 patients from Japan was used as validation cohort. All patients consented to sampling and biobanking for the research purpose. The study was approved by local ethical review boards.

4.1.2 DNA sequencing

DNA was extracted as per local standard operating procedures. Targeted DNA sequencing was performed centrally at Memorial Sloan Kettering Cancer Center by paired-end Illumina HiSeq (median coverage of 730× [range, 127–2,480×]). A panel of 152 genes known to be involved in myeloid neoplasms was used as well as 1,118 genome-wide single-nucleotide polymorphism (SNP) probes for copy number analysis. Artifact variants and putative germline variants were filtered out. A VAF threshold of 2% was set to detect putative

oncogenic mutations which were later identified based on reports in commonly used databases (COSMIC, ClinVar, OnkoKB, other in-house databases)

The DNA sequencing of the validation cohort was processed independently from the targeted sequencing of the training cohort.

4.1.3 Statistical methods

We estimated OS and LFS with the Kaplan-Meier method and compared subgroups with the log-rank test. We estimated the rate of AML transformation using cumulative incidence functions where death without transformation was treated as a competing risk. Comparisons between subgroups were performed with the Gray's test. All statistical analyses were made with R version 3.6.1.

4.2 Study II – Combined genome/transcriptome study of MDS with RS

4.2.1 Patients and samples

From an initial cohort of 834 patients diagnosed with myeloid neoplasms (682 MDS, 101 MDS/MPN, 51 AML with myelodysplastic-related changes) at Karolinska University Hospital between 2004 and 2020, we identified 129 patients with MDS and RS (MDS^{RS+}). Bone marrow morphology was reassessed by a pathologist in all cases and a threshold of ≥5% of total nucleated erythroid cells was set to identify MDS^{RS+}. Peri-diagnostic samples from all cases were collected from the biobank. Bone marrow samples from ten healthy volunteers were used as controls. Patient and disease characteristics were retrieved from patient records. All patients consented to bone marrow collection for the study purpose and the study was approved by the ethical review board.

4.2.2 DNA and RNA sequencing and bioinformatic analyses

DNA sequencing was performed in all patients following the same procedure and on the same platform as described in study 1.

RNA was extracted from CD34 positive cells (sorted with double separation on AUTO-MACS® [Miltenyi Biotec, Germany]). However, due to changes in standard operative procedures and freezing solutions over the years in the biobank, several optimization experiments were required to ensure a homogenous RNA quality across all samples. RNA was finally extracted successfully with RNA integrity number higher than 6.5 in all samples. RNA sequencing used a paired-end Novaseq 6000 technology.

We used unsupervised consensus clustering of gene mutations to distinguish subclasses with matching genetic characteristics and similar transcriptome profiles.[322,323]

We used data from single cell RNA sequencing available in public databases as a reference to perform digital sorting (CYBERSORTx) and estimate the composition of HSPC in our RNA

sequencing dataset of CD34+ sorted cells.[324–326] Findings from digital sorting were validated by multiparameter flow cytometry in five cases.

4.2.3 Statistical methods

We estimated OS with the Kaplan-Meier method and compared subgroups with the log-rank test. Cox proportional hazard model was used for the multivariable analysis of OS. All statistical analyses were made with R version 3.6.2.

4.3 Study III – Changes in transfusion patterns inform prognosis

4.3.1 Patients and samples

A cohort of 677 Swedish adult patients with MDS or MDS/MPN was assembled from the large international cohort of the IWG/IPSS-M project to constitute the training cohort. The Swedish dataset contained two sub populations: the Karolinska Institutet MDS biobank (KB, 402 patients diagnosed between 2003 and 2017) and the Swedish National MDS biobank (NB, 275 patients diagnosed between 2013 and 2017). Peri-diagnostic samples were collected from a total of 34 Swedish centers prior to disease modifying treatment and consisted of either BMMNC (KB) or whole blood (NB).

We retrieved clinical data from patients records (KB) or the national MDS registry (NB). We used pre-transfusion blood counts as diagnostic values. Chromosomal abnormalities were sorted according to IPSS-R/M cytogenetic risk categories. IPSS-M very low, low and moderate low categories were classified as lower-risk MDS while IPSS-M moderate high, high and very high were considered higher-risk MDS.[10]

We used the SCANDAT3-S database, with a nationwide coverage since the mid 1990's, to collect complete data on transfusion requirement during the disease course.[327] Erythrocyte transfusion dependency (E-TD) was defined as requirement of any RBC transfusion during the past four months.[328]

Follow-up ranged from diagnosis of MDS until censoring due to death, AML transformation, allogeneic stem cell transplantation, or end of follow-up August 31, 2018, whichever occurred first. All patients consented to bone marrow/blood sample collection for the study purpose and the study was approved by the ethical review board.

4.3.2 DNA sequencing

DNA extraction was done as per standard operating procedures. All samples went through paired-end targeted DNA sequencing at Memorial Sloan Kettering Cancer Center as a part of the International working group (IWG)/IPSS-M project as described in previous section. We classified *TP53*-mutated cases as either *TP53* monoallelic (*TP53*^{mono}) or multi-hit (*TP53*^{multi}).[150] According to their co-mutation patterns, *SF3B1*-mutated cases were further subclassified as *SF3B1*^{alpha}, *SF3B1*^{beta}, or *SF3B1*^{5q}.[10]

4.3.3 Statistical methods

We estimated probabilities of OS at different landmark times by using the Kaplan-Meier method and compared OS between subgroups with the log-rank test. Estimations of the cumulative incidence of first transfusion event and AML-transformation were made via competing risk analyses and comparison within subgroups used the Gray's test.

We developed a multivariable model of OS using Cox proportional hazards model and explanatory variables in the model were selected through Direct Acyclic Graphs (DAG).

Substantial efforts were put into the development of a Markov multistate model (MSM). The MSM model enables patients to navigate between the different states of the disease over time and is thought to reflect the medical journey of a patient more accurately than traditional statistical methods. Hence, we portioned our dataset in consecutive 4 months periods during which patients could be in any of the following states: diagnosed, first transfusion dependency state (TD1), second or more transfusion dependency state (TDx), first transfusion independency state (TF1), second or more transfusion independency state (TFx), AML-transformation, allogeneic stem cell transplantation (SCT) or death (D). The model adjusted for the IPSS-M allowed us to assess the impact of transfusion trajectories on outcome.[329,330]

5 Results and discussion

5.1 Study I – A novel prognostic scoring system, IPSS-M

5.1.1 Results

Overall, at least one molecular aberration was found in 94% of patients with a median of four molecular aberrations per patient. Consistently with previous reports, *TET2*, *ASXL1*, *SF3B1* were by far the most frequently mutated genes and the number of molecular abnormalities correlated with prognosis.[6,7]

Next, we selected a total of 48 genes mutated in more than 1% of the patient population and assessed their association with LFS, OS and AML-transformation. *TP53*^{multi}, mutations in the *FLT3* gene and *KMT2A* (*MLL*) partial tandem duplication (PTD) were the genetic aberrations with the most deleterious impact on outcome. Similarly, mutations in *ASXL1*, *BCOR*, *EZH2*, *NRAS*, *RUNX1*, *STAG2*, and *U2AF1* had also an adverse effect on prognosis.

In contrast, *SF3B1* mutations were associated with a favorable prognosis across all endpoints LFS, OS, AML-transformation (HR [95% CI] 0.64 [0.56-0.73]; HR [95% CI] 0.67 [0.59-0.76]; HR [95% CI] 0.35 [0.26-0.47]). However, this was largely influenced by the type of *SF3B1*-associated mutations. Hence, three co-mutation patterns were identified: *SF3B1*^{5q}: *SF3B1* mutation associated with isolated deletion of the long arm of chromosome 5; *SF3B1*$^{\beta}$, *SF3B1* mutation and oncogenic mutation in any of the following genes: *BCOR*, *BCORL1*, *NRAS*, *RUNX1*, *SRSF2*, or *STAG2*; and *SF3B1*$^{\alpha}$: any other *SF3B1*-mutant. Interestingly, only the *SF3B1*$^{\alpha}$ co-mutation pattern was significantly associated with favorable outcome and the *SF3B1* hotspot mutational type had no impact on prognosis (Figure 13).

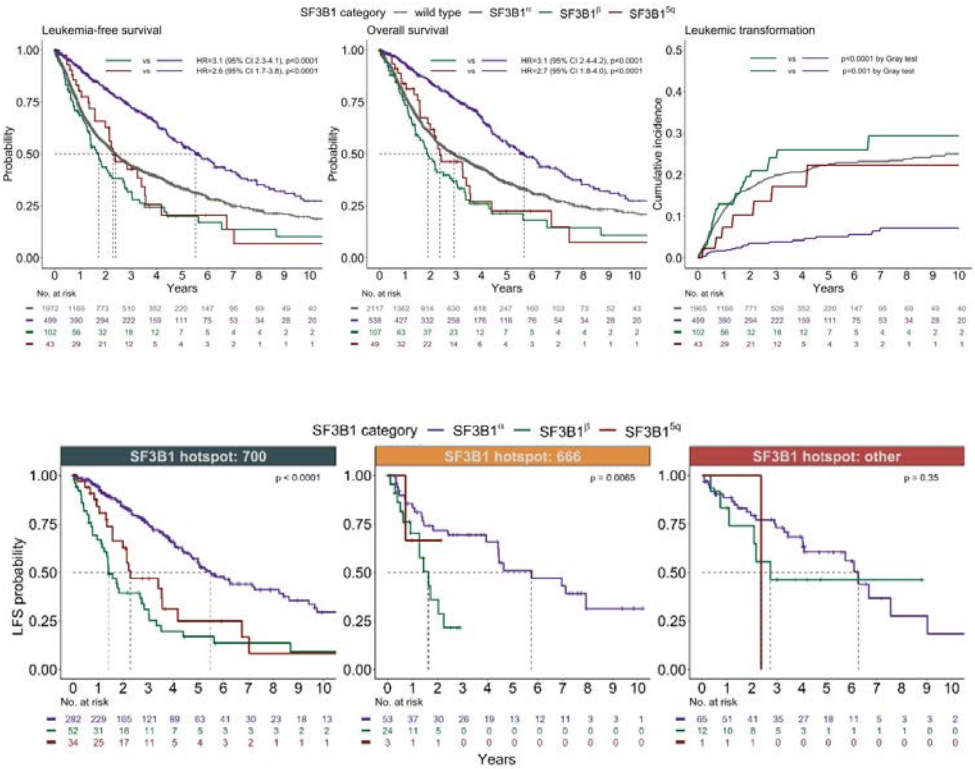


Figure 13 – Top row: Kaplan-Meier curves showing probability of leukemia-free survival, and overall survival as well as the cumulative incidence of AML-transformation of the different SF3B1 co-mutation patterns. Bottom row: Kaplan-Meier curves showing probability of leukemia-free survival of the different SF3B1 co-mutations pattern according to SF3B1 hotpots (700, 666 or others). Adapted from figures S5 and S6, NEJM Evid., Bernard et al., Molecular International Prognostic Scoring System for Myelodysplastic Syndromes. Volume 1, Page 7. Copyright © (2022) Massachusetts Medical Society. Reprinted with permission.

To build the IPSS-M score, an adjusted Cox multivariable model selected a total of 22 variables (level of hemoglobin, platelets count, percentage of bone marrow blasts, cytogenetic risk categories, 17 binary genetic features, and number of mutations in genes not included in the latter) which significantly associated with all three endpoints. A mathematical weight was assigned to each variable according to its impact on prognosis. Hence, in each individual patient, the algorithm computed a unique continuous IPSS-M score and assigned the disease to one of the six IPSS-M risk categories (very low, low, moderate low, moderate high, high and very high risk).

Compared to IPSS-R, IPSS-M improved discrimination (C-index) in both treated and untreated patients across all endpoints. Overall, 46% of the patients changed risk categories from the IPSS-R to the IPSS-M, of these 74% were re-stratified to higher-risk

categories. Interestingly, most reclassified patients (62%) had two or more gene mutations, and this was particularly true in the subset of patients who were upstaged by IPSS-M (82%) (Figure 14). Interestingly, although s/t-MDS were associated with *TP53*^{multi}, complex karyotype and higher-risk IPSS-M categories, 39% were assigned to the very low, low and moderate low risk groups. While *TP53*^{multi} remained the main predictor of outcome across all types of treatment, results suggested that *DDX41* mutations might predict response to HMA.

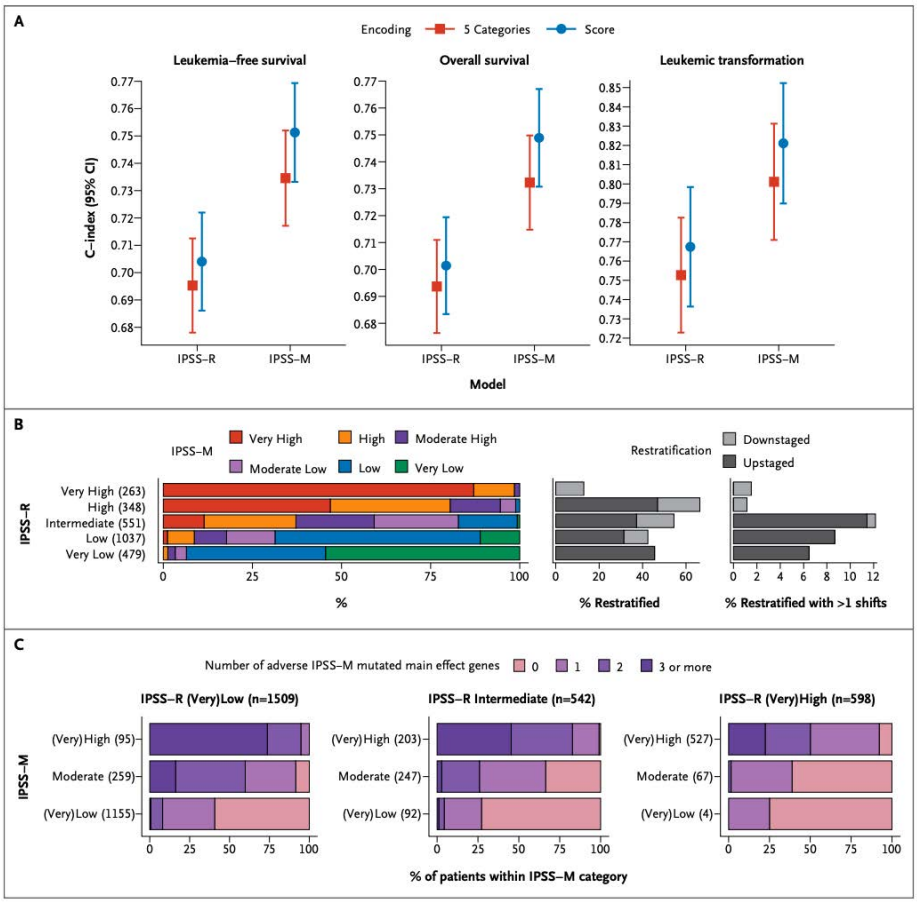


Figure 14– **A.** Comparison of C-index (concordance index, discrimination of the model) between the IPSS-R and the IPSS-M for OS, LFS and AML-transformation. **B.** *Left:* Bar plot representing the IPSS-R categories (y-axis) and within each categories the colors represent the distribution of restratification to IPSS-M risk categories. *Right:* bar plot representing for each IPSS-R categories the proportion of patients down-staged or upstaged with IPSS-M, hence restratified with any shift (left) or more than one shift (right). **C.** Bar plots representing the association between patient restratification (from IPSS-R to IPSS-M) and the number of mutated main effect genes of the IPSS-M. We created simplified risk categories and merged the very low/low categories into (very) low, and very high/high categories into (very) high for both IPSS-R and IPSS-M. The simplified IPSS-R risk categories are represented in facets and the simplified IPSS-M categories in y-axis. CI: confidence interval. From figure 3, NEJM Evid., Bernard et al., Molecular International Prognostic Scoring System for Myelodysplastic Syndromes. Volume 1, Page 7. Copyright © (2022) Massachusetts Medical Society. Reprinted with permission.

The IPSS-M score was further validated in an independent Japanese cohort of 754 patients with MDS and MDS/MPN based on the same inclusion/exclusion criteria as in the discovery cohort. Finally, to enable the clinical implementation of the score, a web- and app-based calculator was developed (https://mds-risk-model.com).

5.1.2 Discussion

As mentioned in previous sections of this thesis, despite the broad use of DNA sequencing analyses in clinical routine and strong evidence of the prognostic impact of specific gene mutations, genetic features were absent from current prognostic scores. Hence, a novel prognostic scoring system including molecular features was warranted in the MDS community. This great international effort successfully resulted in a novel molecular prognostic score improving outcome prediction in MDS.

Although the mutational landscape of this large international cohort of MDS and MDS/MPN patients was consistent with previous reports, novel markers were identified.[6,7] Hence. *TP53*^{multi} previously reported by Bernard *et al.* in 2020, was confirmed as one of the main driver of dismal prognosis in MDS.[150] Moreover, aberrations of *MLL*-PTD and *FLT3*, usually analyzed mainly in AML, were shown to have a deleterious effect also in MDS. These findings stress the importance of *TP53* allelic status and support the implementation of *FLT3* and MLL-PTD testing in clinical routine in MDS. Interestingly, we demonstrated for the first time in a large cohort that specific combinations of gene mutations also impact prognosis. Hence, only the *SF3B1*$^{\alpha}$ comutation pattern was predictive of favorable outcome.

Despite the fact that treatment data were limited in the dataset, we showed that *TP53*^{multi} remained strongly associated with dismal prognosis irrespectively of the type of treatment received. Although the presence of *DDX41* mutations seemed to correlate to a higher rate of transformation to AML, it did not translate into poorer OS. By contrast, it predicted longer OS in patients treated with HMA. Thus, these results suggest that genetic features can inform treatment response as indicated in previous reports[40,45]. However, prospective trials are warranted to identify predictive markers of drug efficacy.

As reported by several studies in the past[23,24,222,331], s/t-MDS have been classically considered as higher-risk disease. In contrast to IPSS-R which only included de novo MDS, the IPSS-M score included 234 patients with s/t-MDS. We showed that even though most of them were associated with higher-risk IPSS-M, 39% were assigned to lower-risk categories. Hence, s/t-MDS is a heterogenous subset of MDS, and further investigation of the underlying disease biology are warranted to better understand the variety of outcome.

Importantly, to facilitate the use of the IPSS-M in clinical practice, we developed a web and app-based calculator. The calculator allows a certain degree of missing data and

adapts therefore to the variety of targeted sequencing panels worldwide. The six IPSS-M categories enable a simplified risk classification and facilitate inclusion in clinical trials. Made available in 2022, the score and its calculator have been very well received by the MDS community.

5.2 Study II – Combined genome/transcriptome study of MDS with RS

5.2.1 Results

A total of 129 MDS^{RS+} were included based on the presence of RS and irrespectively of their morphological classification. The most frequently mutated genes were *SF3B1* (67%), *TET2* (37%), *DNMT3A* (19%), *SRFS2* (15%) and *TP53* (15%). Almost all patients (97.7%) carried one or more oncogenic mutation.

A first unsupervised clustering analysis revealed three subgroups with homogeneous genetic profiles and driven by *SF3B1*, *SRSF2* and *TP53*^{multi} mutations. Patients not harboring mutation in any of the three genes (11%) were assigned to MDS not otherwise specified (NOS). Mutations in *TP53*^{multi}, *SF3B1* and SRSF2 tended to be mutually exclusive. Hence, co-mutations with *SRSF2* or *SF3B1* were never observed in *TP53*^{multi}-mutated cases. Of the four cases where *SRSF2* and *SF3B1* mutations co-occurred, co-mutation on a cellular level was confirmed in two cases while we observed two separate clones (a dominant *SF3B1* clone and a separate *SRSF2* subclone) in the two other cases. Interestingly, co-mutated cases in a single clone versus cases with two different clones displayed gene expression and alternative splicing profiles with *SF3B1*-like, and mixed *SRSF2*/*SF3B1* features, respectively. The *TP53*^{multi} genetic subset was associated with the WHO 2016 morphological subclass MDS-EB2 and the *SF3B1* genetic subset was enriched for MDS-MLD-RS. While clonal hematopoiesis-like molecular profiles (mutations in *TET2*, *ASXL1*, *DNMT3A* and monosomy Y) were frequently observed in *SF3B1* and *SRSF2*-subgroups (53% and 75%, respectively) it was not the case in *TP53*^{multi}-driven cases where there was an enrichment for del(5q) (OR=94.7, P<0.001), del(7q)/monosomy 7 (OR=32.9, P<0.001) or complex karyotype instead (OR=29.9, P<0.001).

We next conducted an unsupervised analysis of the RNA sequencing dataset of CD34 positive BM MNC. Results displayed three distinct clusters. Further differential gene expression analysis showed that cluster 1 was enriched for genes expressed by immature myeloid progenitors (IMP cluster), cluster 2 had an enrichment of genes expressed by erythroblast-megakaryocyte (EMK-cluster) precursors, and in cluster 3 we found genes related to mature myelopoiesis (INT, intermediate cluster). Cases with WHO 2016 subclasses MDS-EB2 and MDS-MLD were frequently observed in the IMP-cluster which was also characterized by higher bone marrow blasts percentage (median 5% vs 1.5%, P=0.002) and lower RS percentage (median 22% vs 40%, P<0.001) compared to the EMK

group. While, as expected, there was a correlation between *SF3B1* mutations and the EMK-cluster, surprisingly, *TP53*^{multi} correlated with the INT-cluster.

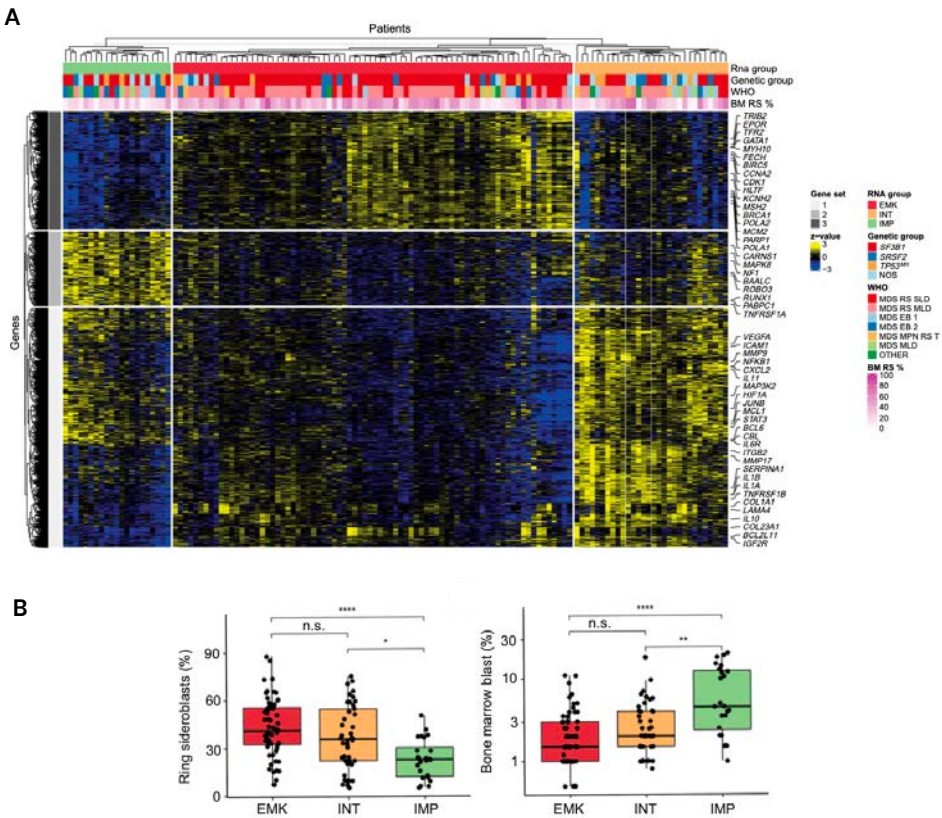


Figure 15 – **A.** Heat map showing the three main transcriptomic groups EMK/INT/IMP and level of expression of differentially expressed genes. Each row of the heat map corresponds to a gene and each column corresponds to a sample. Top rows (from top to bottom) represent transcriptomic groups (Rna groups), genetic groups, WHO classification 2016 (WHO), and percentage (%) of bone marrow (BM) ring sideroblasts (RS). **B.** Box plots representing the percentage of ring sideroblasts (left) and bone marrow blasts (right) across the three transcriptomic groups. EMK: erythroid/megakaryocytic lineage-like; INT: intermediate; IMP: immature progenitor. ****: P-value <0.0001; ***: P-value <0.001; **: P-value <0.01; *: P-value <0.05. Adapted from figure 2, Todisco et al., 2023, *Clin Cancer Res* OF1–OF12, reproduced with permission from *Clinical Cancer Research.*

As none of the unbiased transcriptome profiles were specifically associated to a genetic subset, an unsupervised differential gene expression analysis was performed in previously defined genetic subsets. In *SF3B1*-mutated cases, we observed downregulation of *ABCB7,* DNA/RNA polymerases and phosphatases involved in cell signaling, and upregulation of genes involved in protein translation. In contrast, the *TP53*^{multi} subset displayed upregulation of DNA/RNA polymerases and phosphatases and downregulation of protein translation genes. However, none of these genes were significantly impacted in the *SRSF2*-mutated cases. As expected, a higher number of aberrant splicing events was detected in the splicing factor-driven subsets *SF3B1* and *SRSF2*.

Finally, to assess whether the granularity of transcriptome profiles could be related to the content of HSPC we performed single cell transcriptomic-based digital sorting. We found that the EMK-cluster was associated with a higher MEP-like composition (45%) compared to INT-and IMP-clusters (37% and 13%, respectively), independently of the myeloid/erythroid ratio. A statistical cut point selection model was used to estimate an optimal predictive threshold of MEP content. Hence, MEP ≥ 38% was shown to predict OS independently of IPSS-M.

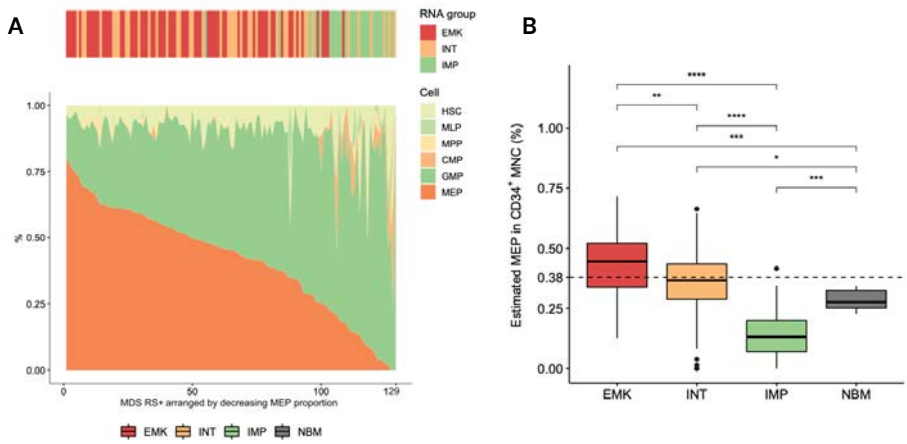


Figure 16 – A. Frequency of the hematopoietic stem and progenitor cell subpopulations in the transcriptomic subsets. B. Box plots showing the estimated MEP fraction in the total CD34+ bone marrow mononuclear cells across the three transcriptomic subsets and in normal control (NBM). EMK: erythroid/megakaryocytic lineage-like; INT: intermediate; IMP: immature progenitor; HSC: hematopoietic stem cell; MLP, multi-lymphoid progenitors; MPP, multipotent progenitors; CMP: common myeloid progenitors; GMP: granulocyte-macrophage progenitors; MEP, megakaryocyte-erythroid progenitors. ****: P-value <0.0001; ***: P-value <0.001; **: P-value <0.01; *: P-value <0.05. Adapted from figure 4, Todisco et al., 2023, *Clin Cancer Res* OF1–OF12, reproduced with permission from *Clinical Cancer Research*.

5.2.2 Discussion

The presence of RS in MDS is highly associated with mutations in the *SF3B1* gene which in several studies have been shown to predict favorable prognosis.[12,13,315] The presence of RS is commonly used as a surrogate marker for *SF3B1* mutation. Hence, in the recent WHO 2022 classification the presence of more than 15% RS can be substituted to *SF3B1* mutation to define the class of MDS with low blasts and *SF3B1* mutation (MDS-*SF3B1*).[3] However, the presence of RS is regularly observed in more aggressive disease subtypes. Hence, we gathered 129 patients with MDS and ≥5% RS and performed unbiased genetic and transcriptomic analyses to improve our understanding of the pathophysiology of MDS

with RS and assess the potential clinical relevance of combined genomic/transcriptomic profiling.

Most (89%) patients had at least on oncogenic mutation and unsupervised analysis of somatic genetic events identified three distinct genetic subsets characterized by *TP53*^{multi}, *SF3B1* or *SRSF2* mutations. The high allelic burden in all three subsets and the fact that mutations were mutually exclusive suggest that *TP53*^{multi}, *SRSF2* and *SF3B1* mutations might act as driver events. In contrast, co-occurring mutations, as previously suggested for *SF3B1*- or *SRSF2*-mutated MDS, would rather modulate the course of the disease.[10,332]

Unsupervised transcriptome analysis confirmed previous findings by Shiosawa *et al.*[333] based on a cohort of 100 patients and revealed EMK-like and IMP-like clusters. Additionally, we also identified a third intermediate (INT) gene expression profile characterized by increased expression of genes related to myeloid progenitors and inflammation. Further investigation by digital sorting suggested that differences between all three clusters were likely related to the underlying HSPC composition. Hence, higher MEP-content correlated to EMK-like signature while IMP-like signature displayed a low MEP-frequency. Interestingly, in multivariable analysis, higher MEP fraction was predictive of OS independently of IPSS-M. These findings are consistent with previous studies and suggest that flow cytometry-based quantification of HSPC subpopulations might refine prognostication in MDS.[334,335] However, prospective studies are warranted to confirm the prognostic impact of the MEP-fraction.

Finally, we showed that OS in MDS^{RS+} was clearly influenced by underlying mutations. Hence, *SF3B1*-mutated MDS^{RS+} had a significantly better prognosis than other genetic subsets. Also, in *SF3B1*-wild type cases, percentage of RS did not significantly impact OS. This findings stress the importance of underlying genetic events rather than percentage of RS; it advocates against current WHO 2022 classification and rather support categorizing *SF3B1*-wt MDS-RS cases in the MDS-NOS group as per the ICC 2022 classification.[2,3]

Overall, study II provides evidence of distinct genetic and transcriptome subsets of MDS^{RS+} and links the heterogeneity of gene expression profiles to the underlying HSPC content. Also, our results open the field for further prospective investigations on the prognostic impact of MEP-fraction in MDS. Finally, this study confirms the clear distinction between *SF3B1*-mutated and *SF3B1*-wild type MDS^{RS+} and provides additional support for *SF3B1*-mutated MDS-RS as a separate entity in novel MDS classifications.

5.3 Study III – Changes in transfusion patterns inform prognosis

5.3.1 Results

With a median number of erythrocyte transfusion per patient of 27, a total of 26,489 units were administered in E-transfusion-dependent patients during follow-up. Interestingly, 80% of changes in E-transfusion states were observed during the first year after diagnosis.

The IPSS-M score was a strong predictor of occurrence of first transfusion event (HR [95% CI] 1.92 [1.69-2,18]). *TP53*^{multi}, poorer cytogenetic groups and higher bone marrow blasts percentage in particular were associated with shorter time to first E-transfusion and in contrast *SF3B1*$^{\alpha}$ and higher hemoglobin level were predictive of longer time to first RBC transfusion (P<0.05).

Kaplan-Meier estimation of OS showed that transfusion dependency at any time points during the first year after diagnosis had a deleterious effect overall, and across IPSS-M lower- and higher-risk groups or genetically defined subsets such as *TP53*^{multi} and *SF3B1*-mutated MDS (P<0.05) (Figure 17).

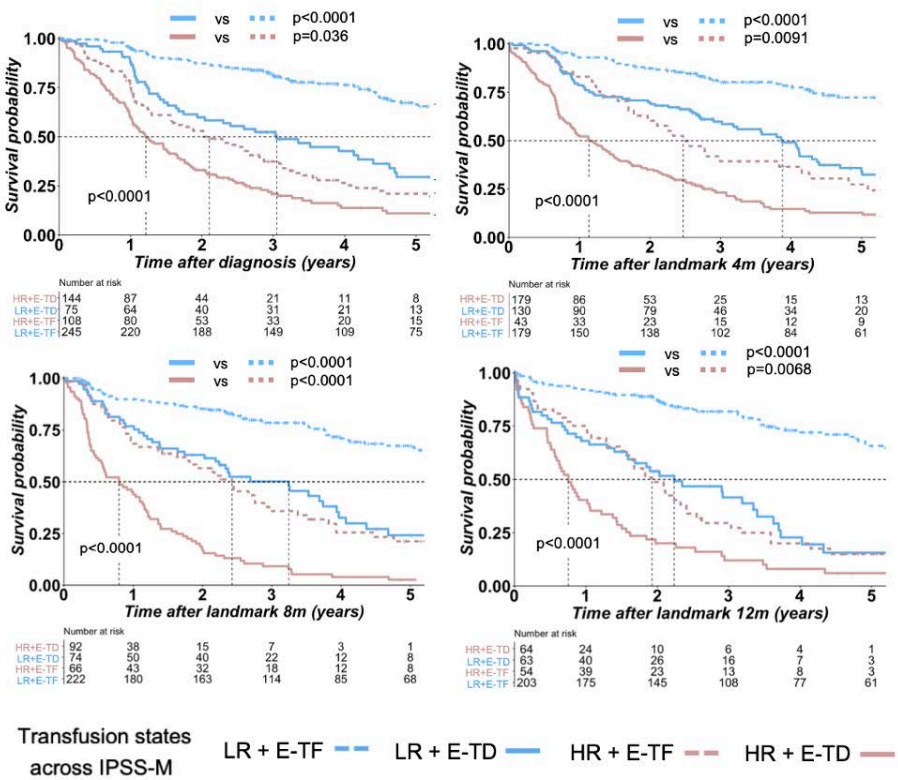
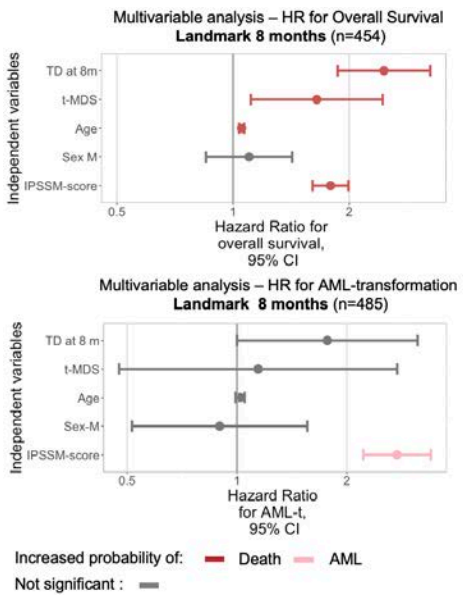


Figure 17 – Kaplan-Meier curves showing survival probability (y-axis) over time (years, x-axis) diagnosis, and landmarks 4-, 8- and 12 months (m) according to E-transfusion state at landmark and IPSS-M lower- versus higher-risk. HR+E-TD: higher-risk IPSS-M and erythrocyte transfusion dependent at landmark; LR+E-TD: lower-risk IPSS-M and erythrocyte transfusion dependent at landmark; HR+E-TF: higher-risk IPSS-M and erythrocyte transfusion-free at landmark; LR+E-TF: lower-risk IPSS-M and erythrocyte transfusion-free at landmark. Subgroups comparison with the log-rank test

The multivariable analysis confirmed these findings and showed that E-transfusion dependency at 8 months predicted OS independently of the IPSS-M score (HR [95%CI] 2.46 [1.87-3.24]). We next demonstrated that the novel predictive model based on the IPSS-M and E-transfusion state at 8 months (model 2) resulted in both improved discrimination (concordance index 75.3 vs 73.7) and overall performance (Brier score 15.4 vs 17.2, P<0.01) compared to the IPSS-M only (model 1) (Figure 18).

A



B

Validation Analyses	Score (95% CI)	
C-index		
Model 1	73.7	
Model 2	75.3	
AUC (CI95%)		
Model 1	81.4 (76.4-86.4)	p=0.007
Model 2	84.9 (80.5-89.3)	
Brier (CI95%)		
Model null	23.9 (22.8-25.0)	p<0.0001
Model 1	17.2 (14.9-19.4)	
Model 2	15.4 (13.3-17.5)	p=0.0023

Figure 18 – **A**. Forest plot showing results of multivariable analyses for overall survival (top) and AML-transformation (bottom) at landmark 8 months (m). TD: transfusion dependent; t-MDS: therapy-related MDS, Sex M: sex male; HR: Hazard ratio; CI: confidence interval. **B**. Table summarizing results of discrimination and overall performance tests between overall survival predictive model 1 (IPSS-M only) and 2 (IPSS-M + erythrocyte transfusion state at 8 months). 95% CI: 95% confidence interval; C-index (concordance index); AUC: area under the receiver operating characteristic curve; Brier: Brier score.

Finally, we used a Markov multistate model to assess whether changes in E-transfusion state over time impacted outcome. We found that early transfusion trajectories matter and can be used to inform both prognosis and future transfusion requirement. Hence, for example, in patients who were permanently E-transfusion dependent during the first 8 months after diagnosis the probability of death was more than doubled compared to patients who never required any transfusion during the same period (56% versus 23%).

5.3.2 Discussion

In lower-risk MDS, anemia is one of the leading symptoms and resistance or refractoriness to ESA results in chronic blood transfusion requirement. However, some lower-risk MDS patient will remain transfusion-free during their whole life. In higher-risk MDS, transfusion requirement or achievement of transfusion-independency is one of the criteria in the treatment-response assessment.[266,267] Hence, we hypothesized that early RBC transfusion patterns, as a surrogate marker for disease biology and treatment response, could add value to the IPSS-M prediction.

Interestingly, we showed for the first time that most changes in E-transfusion requirement occurred during the first twelve months after diagnosis. Although this is probably partly related to the start of therapies, it supported our theory that hemoglobin level and transfusion state at diagnosis do not always reflect the future disease course and that early transfusion patterns may be more informative.

Several studies have already demonstrated the prognostic impact of high cumulative transfusion burden over time in MDS.[262–265,269] However, from a clinical perspective, earlier predictions are more relevant as they can guide changes in treatment strategy and improve management of patients with MDS. A few reports showed that transfusion state at early landmarks after diagnosis was a strong predictor of prognosis, however none of these studies were done in the molecular era.[261,268] To date, no study has assessed whether early individual transfusion trajectories might inform prognosis. First, we confirmed that E-transfusion state at 8 months predicted OS independently of IPSS-M. Next, the prognostic model including both E-transfusion state at 8 months and IPSS-M improved discrimination and overall performance compared to the IPSS-M only. Finally, this is to date the first study to show that individual transfusion trajectories after diagnosis may be used to foresee both OS and future transfusion requirement.

Despite the rarity of well-annotated cohort of MDS patients with complete clinical, molecular and transfusion features, we collaborated with the University of Pavia and gathered an independent cohort of 218 patients with MDS to validate our model. Likely due to underpowered analyses, the comparison of model 1 and model 2 did not reach statistical significance, but model 2 displayed comparable patterns as in the Swedish cohort with improved discrimination and overall performance.

As expected, longitudinal molecular reassessments were not included in this dataset. Thus, while it can be seen as a limitation of our study, it actually mirrors clinical practice where regular NGS monitoring in MDS is not performed. Additionally, on a global level, implementation of diagnostic NGS remains very heterogenous.

Taken together, these results suggest that E-transfusion state during the early disease course predicts outcome in MDS and we propose that it can be used as a complement of the IPSS-M to refine prognostic prediction in dynamic setting. This may be of particular value to support HSCT decision-making in younger lower-risk MDS patients. Moreover, this is the first study showing that individual transfusion trajectories impact OS and future transfusion need. Hence, our results warrant the integration of time-varying patient and disease characteristics in future prognostic scoring systems.

5.4 Ethical considerations

MDS is a hematological malignancy with an overall poor survival, and its management has still many unmet needs such as better prognostication tools and novel therapeutic alternatives. As in all human research, our studies were conducted according to the pillars of medical research. Hence, pre-requisites were:

- to make sure research participants received proper information about the research project, expected benefits and potential risks, and that all could give their consent autonomously.
- to assure that expected benefits overweighted potential risks.
- to warrant equal treatment of all participants.

Informed consent

The research project recruited only adult patients. Consent was obtained from patients by the treating physician after both oral and written information. The research project was approved by the Stockholm Region Ethical Review Board (approval 2017/1090-31/4).

Expected benefits

In the three studies constituting this thesis we aimed to fill some of the knowledge gap in the field by identifying novel prognostic factors, proposing a more performant prognosis scoring system and ultimately offering better guidance for treatment decision-making.

By bringing our results into public domain through scientific publications, we contributed to improve current knowledge in the field of MDS. Hence, these studies can directly benefit participants still alive at the time of publication as well as future patients with MDS.

Potential risks

To reduce potential risk and discomfort for the patients, bone marrow/blood samples for research and routine purposes were collected as often as possible on the same occasion. Storage and tracing of samples were done in compliance with the Swedish biobank legislation.[336] Pseudonymized clinical and genetic data, so called sensitive data, were

processed in all studies and were stored safely as per current European General Data Protection Regulation.[337] Pseudonymized data was kept on secured and approved server, and the protected key was stored separately. As parts of data are nowadays required to be available on public repositories at the time of publication, we amended our ethical application and clarified corresponding informed consent form accordingly. We also followed our institution's procedures and guidelines for the transfer to repositories.

Management of incidental molecular findings was however one of the main ethical concerns we had while conducting the research. Hence, whereas knowledge on mutations of constitutional origin in MDS was limited at the time we designed our studies, the amount of evidence supporting germline predisposition syndromes in myeloid neoplasms increased exponentially thereafter. Even though the DNA sequencing technique we used was panel-based, we found several cases of putative germline mutations.[338] The discovery of germline mutation in myeloid neoplasms has several consequences. It can for instance influence clinical care (type of treatments, the choice of donor and/or conditioning in a transplant setting). Foremost, patients apprehend genetic testing and its potential repercussions differently. Hence, while some patients will perceive it as beneficial, it will be more ambiguous for others.[339–341] Hence, it is important to inform the patient at the time of consent about the risk of incidental findings and according to the patient's choice, report germline mutations for which there is sufficient evidence and specific measures can be offered (early treatment and/or specific follow-up). Thus, although a few germline mutations in myeloid neoplasms are now well-characterized with regard to penetrance and recommended management, several other are not.[342] Finally, the retrospective design of our studies was challenging with regards to this particular matter as most patients were deceased at the time of the analysis. Thus, we developed a stepwise process to manage incidental genetic findings. We amended our ethical application and clarified corresponding informed consent form accordingly.

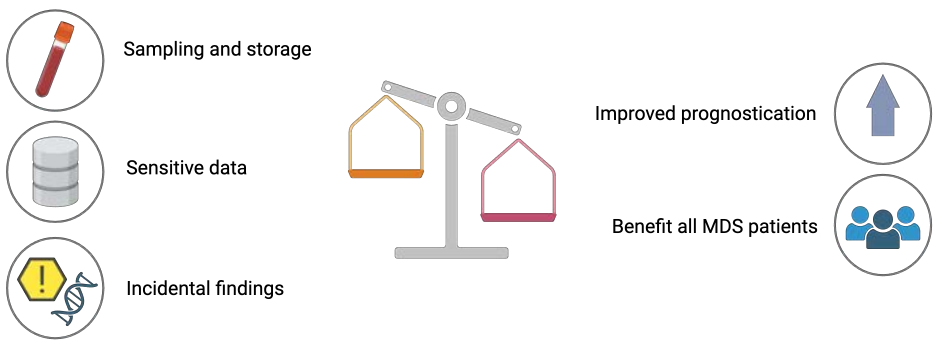


Figure 19 – risk/benefit balance of the research project

6 Conclusions

MDS is a group of hematological malignancies with a great variability with regard to outcome. Apart from initiating genetic events, numerous mechanisms impact the clonal expansion and ultimately the disease course. Treatment decision making in MDS is largely based on prognostic scores. Research during the past decade provided strong evidence that specific genetic markers impact the disease course and prognosis. However, none of the prognostic scoring systems in use at the beginning of my doctoral studies included these genetic markers. Hence, in the three studies constituting this thesis we showed that:

In study I: A novel scoring system, the IPSS-M, refines prognostication in MDS

- Novel genetic markers such as *FLT3* and *MLL*-PTD impact prognosis in MDS and co-mutation patterns in *SF3B1*-mutated cases shape the course of the disease
- A novel prognostic score based on 22 clinical, cytogenetic and molecular variables is proposed
    - The IPSS-M score improves discrimination compared to the IPSS-R and is validated both in non-proliferative MDS/MPN and secondary or therapy-related MDS
    - Clinical implementation is facilitated by the web- or app-based IPSS-M calculator which delivers both a unique score and a risk category in individual patient
- We suggest using the IPSS-M scoring system in all newly diagnosed MDS patients

In study II: Combined genomic/transcriptomic characterization of MDS^{RS+} provides novel insights in the heterogeneity of the underlying disease biology and variety of outcome

- MDS^{RS+} are characterized by three distinct genetic subsets, *SF3B1*-, *SRSF2* or *TP53*^{multi}-mutated. These underlying genetic events shape the course of the disease independently of the RS fraction
- We propose that *SF3B1*-mutated MDS^{RS+} remains a separate entity in current classifications
- The three distinctive transcriptome profiles EMK-, INT- and IMP-like seem to be associated to the underlying MEP-content in the HSPC compartment The MEP fraction predicts OS independently of IPSS-M
- Prospective studies are needed to confirm the prognostic impact of flowcytometry-based MEP quantification which would facilitate implementation in clinical routine

In study III: Integration of dynamic clinical variables refines IPSS-M prognostication

- Most changes in transfusion states occur within a year after diagnosis
- A novel prognostic model combining IPSS-M + transfusion state at 8 months improves OS prediction compared to IPSS-M alone. It can be used to refine prognostication, guide treatment decision-making during the early disease course
- Early individual transfusion trajectories inform outcome and future transfusion requirement. This advocates for the development of prognostic model accounting for time-varying patient and disease characteristics

7 Points of perspective

Research and discoveries in the field of MDS and cancer in general have increased exponentially for the past two decades resulting in advances in the management of patients but also highlighting the heterogeneity of cancer biology. Paradoxically, the more we learn about MDS and underlying mechanisms, the more complex it appears. Hence, we can assume that there are as many types of MDS as there are patients with MDS and what works in one case will not necessarily work in another.

The evolution of both diagnostic and prognostic classifications reflects well the increasing complexity the MDS community has to deal with. The French American British classification in 1976 recognized only five types of MDS based on morphological criteria only and the first scoring system, IPSS, was constituted of three simple laboratory and cytogenetic parameters and could be rapidly mentally calculated by clinicians. The recently published classifications counted more than 20 MDS or MDS-related subsets. Moreover, due to the variety of novel predictive markers, prognostic scoring systems have reached a turning point where the number of variables to account for requires an algorithm computation such as in the IPSS-M.

Future research on the disease biology as well as discovery of new treatment targets will deepen our understanding of MDS. The number of variables to account for will increase exponentially, and even more considering changes of these variables during the disease course. To improve patient care and elaborate successful treatment strategy in each particular MDS case, novel machine learning-based technologies will be warranted to perform integrative multiparameter analyses and assist clinicians in tailoring the management of patients with MDS.

However, as the saying goes: prevention is better than cure. Prevention of MDS was not really discussed until a few years ago but this area of research will surely expand in the near future. Hence, advances in the field of germline predisposition syndromes and pre-MDS conditions such as CHIP or CCUS will hopefully give us the possibility to intervene before transformation to malignant disease.

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