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The mutational landscape and microenvironment in Myelodysplastic Syndromes with deletion of chromosome 5q

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The mutational landscape and microenvironment in Myelodysplastic Syndromes with deletion of chromosome 5q

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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“You can’t connect the dots looking forward, you can only connect them looking backwards. So you have to trust that the dots will somehow connect in your future. You have to trust in something - your gut, destiny, life, karma, whatever. Because believing that the dots will connect down the road, will you give the confidence to follow your heart even when it leads you off the well-worn path - and that will make all the difference.”

- *Steve Jobs*

Für Julia, Smilla und Yannick

ABSTRACT

Myelodysplastic Syndromes (MDS) with deletion of chromosome 5q (del(5q) are malignant bone marrow disorders characterized by macrocytic anemia, chronic transfusion dependency and an increased risk of progression to acute myeloid leukemia. A high proportion of patients with lower-risk del(5q) myelodysplastic syndromes will respond to lenalidomide treatment, but more than 40% of patients have progressed to acute leukemia five years after starting treatment. In this thesis, we characterized the rare MDS stem cells and bone marrow microenvironment of low-risk del(5q) MDS and studied whether certain somatic mutations are associated with disease progression.

We isolated stem and progenitor cells from lower-risk MDS patients by flow cytometric cell sorting and characterized these populations functionally and molecularly. By fluorescence in situ hybridisation we found the distinct stem and progenitor cells to be clonally involved. This allowed us to determine that the hierarchical organization in del(5q) MDS is similar to that of healthy bone marrow. Exploiting the fact that the vast majority of MDS cases harbour somatic mutations we employed a fate-mapping approach and observed that all somatic mutations found in the bulk bone marrow could be traced back to MDS stem cells. We observed that the del(5q) abnormality preceded the acquisition of additional mutations. Hypothesizing that the acquisition of certain somatic mutations might help explain progression in del(5q) MDS, we used a cohort of 35 longitudinally sampled patients to study whether certain mutations are associated with disease progression. Overall, the mutational landscape in a pure del(5q) cohort differed from low-risk MDS in general. Thirteen patients progressed to high-risk MDS or leukemia at a median of 85 months after diagnosis. We found progression to be associated with the detection of a limited subset of new mutations, i.e., *TP53, TET2,* and *RUNX1*. Of nine patients who progressed to AML, all were treated with lenalidomide and 7 were found to have mutations in *TP53*, which were present in the earliest sample in one case and acquired in the remaining six cases. Importantly, the new mutations were detected well before signs of clinical progression occurred.

Whether or not the microenvironment is perturbed or merely a bystander has been a heavily contentious issue in the literature - not only for MDS but for myeloid diseases in general. Comparing gene expression in cultured mesenchymal stromal cells from del(5q) and normal patients demonstrated no significant differences. Bone marrow biopsies in del(5q) MDS patients before and during lenalidomide treatment revealed significantly higher microvessel density (MVD) in del(5q) MDS compared to normal controls. In all patients analyzed, MVD decreased upon lenalidomide treatment, increasing again upon therapeutic failure. Analysis of staining patterns did not reveal quantitative differences in the expression of previously associated MSC markers between del(5q) MDS and normal bone marrow, suggesting that lenalidomide's main therapeutic effect is independent of reshaping the cellular composition of the microenvironment. Furthermore, as abnormal megakaryocytes with hypolobated nuclei constitute one of the hallmarks of del(5q) MDS and as megakaryocytes have recently been implicated as important niche cells in the regulation of HSC, we studied megakaryocytes as a component of the non-mesenchymal niche in MDS. We provide evidence that the pathognomonic hypolobation is directly associated with the clonal del(5q) aberration. Despite lenalidomide leading to complete clinical and cytogenetic responses, the pathognomonic megakaryocytes with hypolobated nuclei persisted in all patients. Our observation of high clonal involvement in MEP suggests that the entire megakaryocytic lineage from HSC to MEP to megakaryocytes might be resistant to treatment with lenalidomide.

In aggregate, our findings indicate no major perturbations in the mesenchymal niche. Instead, we find hematopoietic niche cells in the form of megakaryocytes to be treatment-resistant. As HSC continue to acquire somatic mutations, the risk of progression is associated with a limited set of mutations, warranting regular mutational profiling in patients treated with lenalidomide.

LIST OF SCIENTIFIC PAPERS

I. **MYELODYSPLASTIC SYNDROMES ARE PROPAGATED BY RARE AND DISTINCT HUMAN CANCER STEM CELLS IN VIVO.**
Woll, P. S., Kjällquist, U., Chowdhury, O., Doolittle, H., Wedge, D. C., Thongjuea, S., Erlandsson, R., Ngara, M., Anderson, K., Deng, Q., Mead, A. J., Stenson, L., Giustacchini, A., Duarte, S., Giannoulatou, E., Taylor, S., Karimi, M., Scharenberg, C., Mortera-Blanco, T., Macaulay, I. C., Clark, S.-A., Dybedal, I., Josefsen, D., Fenaux, P., Hokland, P., Holm, M. S., Cazzola, M., Malcovati, L., Tauro, S., Bowen, D., Boultwood, J., Pellagatti, A., Pimanda, J. E., Unnikrishnan, A., Vyas, P., Göhring, G., Schlegelberger, B., Tobiasson, M., Kvalheim, G., Constantinescu, S. N., Nerlov, C., Nilsson, L., Campbell, P. J., Sandberg, R., Papaemmanuil, E., Hellström-Lindberg, E., Linnarsson, S., and Jacobsen, S. E. W. (2014)
Cancer Cell, 25, 794–808.

II. **PROGRESSION IN PATIENTS WITH LOW- AND INTERMEDIATE-1-RISK DEL(5Q) MYELODYSPLASTIC SYNDROMES IS PREDICTED BY A LIMITED SUBSET OF MUTATIONS.**
Scharenberg, C., Giai, V., Pellagatti, A., Saft, L., Dimitriou, M., Jansson, M., Jädersten, M., Grandien, A., Douagi, I., Neuberg, D. S., LeBlanc, K., Boultwood, J., Karimi, M., Jacobsen, S. E. W., Woll, P. S., and Hellström-Lindberg, E. (2017)
Haematologica, 102, 498–508.

III. **MEGAKARYOCYTES HARBOUR THE DEL(5Q) ABNORMALITY DESPITE COMPLETE CLINICAL AND CYTOGENETIC REMISSION INDUCED BY LENALIDOMIDE TREATMENT.**
Scharenberg C., Jansson M., Saft L., Hellström-Lindberg E. (2017)
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RELATED PUBLICATIONS NOT INCLUDED IN THIS THESIS

I. **THE TRANSPORTER ABCB7 IS A MEDIATOR OF THE PHENOTYPE OF ACQUIRED REFRACTORY ANEMIA WITH RING SIDEROBLASTS.**
Nikpour, M.*, Scharenberg, C.*, Liu, A., Conte, S., Karimi, M., Mortera-Blanco, T., Giai, V., Fernandez-Mercado, M., Papaemmanuil, E., Högstrand, K., Jansson, M., Vedin, I., Wainscoat, J.S., Campbell, P., Cazzola, M., Boultwood, J., Grandien, A. & Hellström-Lindberg, E.
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II. **THE AGE AND GENOMIC INTEGRITY OF NEURONS AFTER CORTICAL STROKE IN HUMANS.**
Huttner, H.B., Bergmann, O., Salehpour, M., Rácz, A., Tatarishvili, J., Lindgren, E., Csonka, T., Csiba, L., Hortobágyi, T., Méhes, G., Englund, E., Solnestam, B.W., Zdunek, S., Scharenberg, C., Ström, L., Ståhl, P., Sigurgeirsson, B., Dahl, A., Schwab, S., Possnert, G., et al
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LIST OF ABBREVIATIONS

AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
BM	Bone marrow
CCyR	complete cytogenetic remission
CD	Cluster of Differentiation
CFU	Colony forming unit
CMML	Chronic myelomonocytic leukemia
CMP	Common myeloid progenitor
CR	Complete remission
G-CSF	Granulocyte colony-stimulating factor
GMP	granulocytic monocytic progenitor
Hb	Hemoglobin
HCT	Hematopoietic cell transplantation
HI	Hematological improvement
HSC	Hematopoietic stem cell
HSC	Hematopoietic Stem Cell
IPSS	International Prognostic Scoring System
IPSS-R	Revised IPSS
LMPP	Lympho-Myeloid primed Progenitor

LTC-IC	Long-term culture initiating cell
MDS	Myelodysplastic syndromes
MEP	megakaryo-erythroid progenitor
MPN	Myeloproliferative neoplasm
MPP	Multipotent Progenitor
NK	Natural killer cell
NOD/SCID	Non-obese diabetic/severe combined immune deficient
NSG	Non-obese diabetic/severe combined immune deficient, gamma-null (common gamma-chain deficient)
PB	Peripheral blood
PD	Progressive disease
PR	Partial remission
RA	Refractory anemia
RAEB	Refractory anemia with excess of blasts
RARS	Refractory anemia with ringsideroblasts
RCMD	Refractory cytopenia with multilineage dysplasia
RCMD-RS	Refractory cytopenia with multilineage dysplasia and ringsideroblasts
SD	Stable disease
WBC	White blood count
WHO	World Health Organization
WPSS	WHO-based Prognostic Scoring System

LIST OF GENE NAME ABBREVIATIONS

ASXL1	additional sex combs like 1 (Drosophila)
ATRX	alpha thalassemia/mental retardation syndrome X-linked
BCOR	BCL6 corepressor
CBL	Cbl proto-oncogene, E3 ubiquitin protein ligase
CDKN2A	cyclin-dependent kinase inhibitor 2A
CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha
CSF1R	colony stimulating factor 1 receptor
CSF3R	colony stimulating factor 3 receptor (granulocyte)
DDX39B	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39B
DDX46	DEAD (Asp-Glu-Ala-Asp) box polypeptide 46
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha
ELANE	elastase, neutrophil expressed
EPOR	erythropoietin receptor
ETV6	ets variant 6
EZH2	enhancer of zeste homolog 2 (Drosophila)
FLNB	filamin B, beta
FLT3	fms-related tyrosine kinase 3
GATA1	GATA binding protein 1 (globin transcription factor 1)
GATA2	GATA binding protein 2

GNAS	GNAS complex locus
IDH1	isocitrate dehydrogenase 1 (NADP+), soluble
IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial
JAK2	Janus kinase 2
JAK3	Janus kinase 3
KDM6A	lysine (K)-specific demethylase 6A
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
MLL	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)
MPL	myeloproliferative leukemia virus oncogene
NPM1	nucleophosmin (nucleolar phosphoprotein B23, numatrin)
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
PPP1R8	protein phosphatase 1, regulatory subunit 8
PRPF40B	PRP40 pre-mRNA processing factor 40 homolog B (S. cerevisiae)
PRPF8	PRP8 pre-mRNA processing factor 8 homolog (S. cerevisiae)
PTEN	phosphatase and tensin homolog
PTPN11	protein tyrosine phosphatase, non-receptor type 11
PUF60	poly-U binding splicing factor 60KDa
RBM17	RNA binding motif protein 17
RBM22	RNA binding motif protein 22

RBM25	RNA binding motif protein 25
RBM5	RNA binding motif protein 5
RUNX1	runt-related transcription factor 1
SF1	splicing factor 1
SF3A1	splicing factor 3a, subunit 1, 120kDa
SF3B1	splicing factor 3b, subunit 1, 155kDa
SMNDC1	survival motor neuron domain containing 1
SRSF1	serine/arginine-rich splicing factor 1
SRSF2	serine/arginine-rich splicing factor 2
TCERG1	transcription elongation regulator 1
TET2	tet methylcytosine dioxygenase 2
TP53	tumor protein p53
U2AF1	U2 small nuclear RNA auxiliary factor 1
U2AF2	U2 small nuclear RNA auxiliary factor 2
WT1	Wilms tumor 1
YBX1	Y box binding protein 1
ZRSR2	zinc finger (CCCH type), RNA-binding motif and serine/arginine rich 2

1 NORMAL HEMATOPOIESIS AND STEM CELLS

Healthy individuals depend on the adequate production of vast numbers of differentiated blood cells every day. In a carefully orchestrated process known as hematopoiesis, mature blood cells are produced from undifferentiated stem and progenitor cells via a complex series of steps that are still incompletely understood. With notable exception mainly in the lymphoid compartment, the lifespan of most hematopoietic cells is extremely short – from hours to days. This short life span necessitates the continuous production of vast numbers of cells to replace these lost cells. During each hour of each day over the lifetime of the individual 15 x 10^9 erythrocytes and 15 x 10^9 white blood cells are produced (Williams, 2000). When early anatomists examined the bone marrow they noted that the various hematopoietic lineages and differentiation stages exhibited a wide spectrum of different cell morphologies. More than 100 years ago, Alexander Maximov was the first to propose the existence of blood stem cells sitting at the apex of the hierarchical production of all blood cells (Maximov, 1909).

1.1 HEMATOPOIETIC STEM CELLS

The key to sustained hematopoiesis is the hematopoietic stem cell. Studies aimed at elucidating these enigmatic cells were prompted by the clinical need for cells capable of protecting humans exposed to minimum lethal doses of irradiation or chemotherapy (summarized in (Thomas, 1991)). Thus, the original path to the identification of hematopoietic stem cells started in the wake of the bombings of Hiroshima and Nagasaki at the end of the second World War. Those individuals who died from exposure to lower radiation doses either bled to death due to low platelet counts or had severely compromised immune function and succumbed to otherwise nonpathogenic infections. The first explanation for the aforementioned observations came from experiments with mice that were given varying doses of whole body-irradiation. At the minimal lethal dose, the mice died approximately two weeks after radiation exposure from hematopoietic failure. Interestingly, however, using lead to shield the spleen or a single bone saved the mice from otherwise lethal doses of radiation (Jacobsen et al., 1949). Infusion of syngeneic marrow after irradiation similarly rescued these irradiated mice from otherwise fatal failure of the hematopoietic system (Lorenz et al., 1951). That this protective effect was cell-mediated (via direct repopulation of the hematopoietic system) rather than due to released humoral factors that caused the recipients' cells to repair the irradiation damage was shown later by three independent groups in 1956 (Ford et al., 1956; Makinodan, 1956).

Despite that these repopulation experiments demonstrated the functional importance of stem cells, it were the seminal experiments performed by Till, McCulloch, Wu, Becker, and Siminovitch, who discovered that mice at their time of death, i.e., two weeks after irradiation, had developed colonies of myeloid, erythroid and megakaryocytic cells in their spleens. Interestingly, the number of formed spleen colonies correlated directly with the number of

bone marrow cells originally injected, with approximately 1 colony being generated per 7,000 bone marrow cells injected. Accordingly, the cells that gave rise to these colonies were dubbed colony-forming units-spleen (CFU-S) (Till and McCulloch, 1961). Using low doses of irradiation Becker et al. pre-irradiated bones and thereby induced unique chromosomal breaks in most hematopoietic cells, allowing to test whether these colonies of blood cells are clonal, i.e., derived from single precursor cells (Becker et al., 1963). The surviving cells exhibited chromosomal breaks that were radiation-induced and repaired, thus marking each clonogenic hematopoietic cell. Of note, all dividing cells within a single spleen colony, which contained different types of blood cells, contained the same unique chromosomal marker. In addition, each colony displayed its own unique chromosomal marker. Furthermore, when single spleen colonies were re-injected into a second set of lethally-irradiated mice, donor-derived spleen colonies contained the same unique chromosomal marker, indicating that these colonies had been regenerated from the same single cell that had generated the initial colony (Siminovitch et al., 1963). In rare cases, these colonies contained sufficient numbers of regenerative cells that were able to both prevent death from radiation-induced loss of blood cells in secondary recipients (radioprotection), and to spawn myeloerythroid and lymphoid cells exhibiting markers of the donor-injected cells (Wu et al., 1968).

Siminovitch et al. proposed the existence of a population of radioprotective cells within the bone marrow, termed hematopoietic stem cells, that are capable of both self-renewal as well as multi-lineage differentiation (Siminovitch et al., 1963). This remains the enduring definition of stem cells (Weissman, 2000).

In the decades that followed, substantial efforts were aimed at the purification and characterization of these cells. In the beginning, enrichment of the progenitor fraction was done by physical means (Visser and De Vries, 1990). However, with the invention of flow cytometric cell sorting by the Herzenberg group at Stanford, an ever-increasing panel of cell surface markers could be used to purify rare populations (Hardy et al., 1982; 1983; Hulett et al., 1969). The accompanying development of clonal assays of all major hematolymphoid precursors in mice (Ezine et al., 1987; Whitlock et al., 1987), facilitated the study of the isolated candidate progenitors' characteristics. In the mouse, this approach ultimately led to the isolation of clonogenic multipotent progenitors with a distinctive marker profile that proved to be hematopoietic stem cells (Spangrude et al., 1988; Uchida and Weissman, 1992).

Using *in vivo* limiting dilution analysis, two classes of multipotent cells - long-term (LT-HSCs) and short-term hematopoietic stem cells (ST-HSCs) - could be separated from one another suggesting heterogeneity within the most primitive compartment (Morrison and Weissman, 1994). While the long-term subset continuously self-renews for the life of the host, the short-term subset retains self-renewal capacity for approximately 8 weeks and then gives rise to multipotent progenitors (MPPs) (Morrison et al., 1997). Based on a difference in surface markers, these three different stem cell subsets can be prospectively isolated. The resultant downstream progeny are two kinds of oligolineage-restricted cells: common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). While CLPs at the

clonal level are restricted to give rise to B lymphocytes, T lymphocytes, and natural killer (NK) cells (Kondo et al., 1997), the common myeloid progenitors give rise to myelomonocytic (GMP) and megakaryocytic/erythroid progenitors (MEPs) (Akashi et al., 2000).

1.1.1 Human HSC

Attempts at isolating human Hematopoietic Stem and Progenitor cells were initially hampered by the fact that the cell surface markers differ significantly between mouse and man. Thus, inherent characteristics of stem cells, conserved across different species, such as the low uptake of certain fluorescent dyes like Rhodamine 123 and Hoechst 33342, were exploited for the isolation of these cells by FACS (Bertoncello et al., 1985; 1988; Chaudhary and Roninson, 1991; Pallavicini et al., 1985; Spangrude and Johnson, 1990).

CD34 was the first cell surface marker shown to enrich for primitive human progenitors (Civin et al., 1984). Within normal bone marrow, this marker is present on 1-2% of cells. After prospective isolation of cells bearing this marker, Civin et al. demonstrated that almost all progenitor cell activity, as assessed by in vitro colony-forming-unit (CFU) assays, was confined to CD34+ cells. Due to the lack of sensitive functional assays, long-term repopulation could not be assessed. However, by co-culturing with either human or murine stromal cells, candidate populations of candidate human stem cells could be interrogated for their ability to sustain hematopoietic colony growth after more mature progenitor cells had formed colonies (Simmons et al., 1987; Sutherland et al., 1989; Testa and Dexter, 1991). After an initial co-culture with stromal cells for 5-12 weeks, cells are plated in methylcellulose and assessed for their ability to form erythroid and myeloid colonies. The cells were termed Long-Term-Culture-Initiating Cells (LTC-ICs). However, the shortcomings of these assays were that they only assessed the myeloid potential of the candidate stem cells and not multi-lineage potential. Moreover, these assays fail to detect the capacity for self-renewal (Spooncer et al., 1985). With the advent of humanized mouse models, xenotransplantation assays became feasible (Kamel-Reid and Dick, 1988; McCune et al., 1988; Mosier et al., 1988). In these assays, human target cells are transplanted into immunocompromised mice. Successive improvements yielded a model, where Non-Obese Diabetic (NOD) were crossed with Severe Combined Immune Deficient (SCID) mice became the preferred host (Shultz et al., 1995). However, the high incidence of thymic lymphomas and the fact that murine NK cells persisted in these mice hampered the length with which these experiments could continue. Generating mice with the additional absence of the Interleukin-2-gamma chain (Shultz et al., 2005), led to a complete absence of murine B, T and NK cells. These mice, dubbed NSG mice (NOD/SCID/gamma), did not develop lymphomas and enabled long-term experiments with a 5-fold improvement in human engraftment.

In addition to murine xenotransplantation assays, functional evidence for human stem cells comes from patients that are being transplanted with autologous bone marrow or G-CSF-mobilized peripheral blood for several hematologic malignancies. Usually, unseparated hematopoietic cells from bone marrow - or nowadays collected from G-CSF-mobilized peripheral blood - are harvested, stored frozen and re-infused after conditioning the host bone marrow with radiation and/or chemotherapy and immunosuppressive treatment enabling the engraftment of the given cells. However, evidence for the ability of certain cell surface markers to enrich for stem cells comes from breast-cancer patients receiving mobilized peripheral blood cells selected for expression of CD34 and CD90 and resulting in long-term multilineage engraftment (Negrin et al., 2000). Similar studies in patients with lymphoma and myeloma confirmed that the long-term repopulating activity is confined to this population of cells (Michallet et al., 2000; Vose et al., 2001). However, as the transplanted cells are not permanently marked (e.g. genetically), these experiments do not exclude the possibility of endogenous reconstitution, and are therefore not an unequivocal proof of stem cell activity.

With progression of the field, the phenotype of human HSC has become increasingly refined. The subset of human bone marrow cells defined by CD34+CD38-CD90+ enriches for multipotent human stem cells (Baum et al., 1992). Majeti et al. demonstrated the existence of human multipotent progenitors capable of giving rise to lymphoid- as well as myeloid cells but lacking the ability to self-renew (Majeti et al., 2007). Using the immunophenotype of lineage-CD34+CD38-CD90+CD45RA- they were able to separate HSC from non-self-renewing MPP, defined by lineage-CD34+CD38-CD90-CD45RA+. Further characterization of these cells by a different group demonstrated that single cells could generate lymphoid colonies as well as myelomonocytic cells (Doulatov et al., 2010). Using a slight variation in markers, a similar population was identified as the murine equivalent to LMPP (Goardon et al., 2011).

The more mature downstream myeloid progenitors are found within the lineage-CD34+CD38+ subset. By prospectively isolating cells based on the expression of CD123 and CD45RA and using colony forming assays in methylcellulose, cell populations could be purified analogous to what was known from murine studies (Manz et al., 2002). Analysis of gene expression demonstrated the expression of lineage-restricted genes in these subsets. Furthermore, these populations neither have any significant LTC-IC activity nor do they possess the ability to differentiate to lymphoid cells in vitro.

Our current understanding of the hematopoietic hierarchy, with its various stem and progenitor cell compartments giving rise to all mature blood cell elements is depicted in Figure 1.

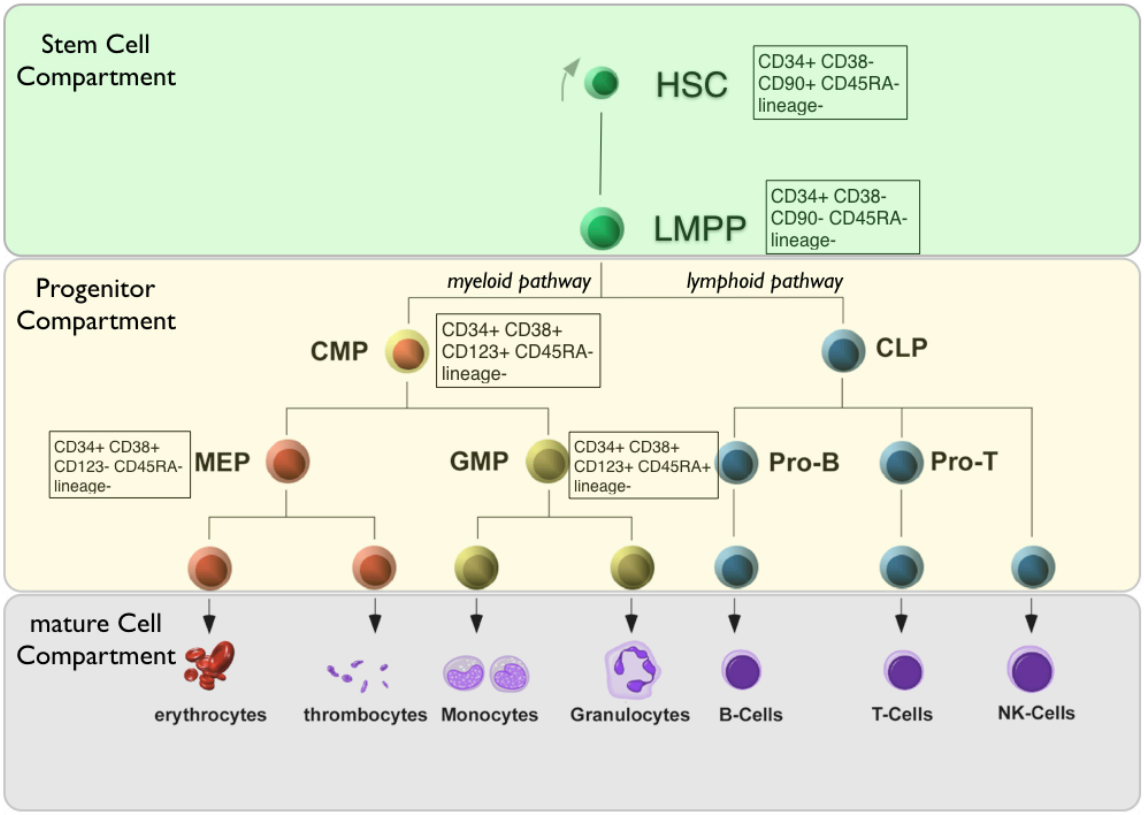

Figure 1. The Hematopoietic Hierarchy. The stem cell compartment is somewhat heterogenous and consists of different subpopulations that all can give rise to both all the various myeloid and lymphoid cells, but differ in their ability to self-renew. Hematopoietic stem cells (HSC) can self- renew for the entire lifetime of the organism, the Multipotent progenitor population / lympho-myeloid progenitors (LMPP) can still give rise to all mature blood cells but lack any self-renewal capacity. Among the progenitor cells, the common lymphoid progenitor (CLP) gives rise to cells of the B- cell, T-cell and NK-cell lineage, while the common myeloid progenitors (CMP) give rise to all myeloid cells. They themselves give rise to bipotent progenitors, i.e., the megakaryo-erythroid- progenitor (MEP) and granulocyte-monocyte-progenitor (GMP), which produce mature offspring, eg erythrocytes, thrombocytes, monocytes, and granulocytes. CD markers shown depict the immunophenotype used for isolating these subpopulations. Drawn from scratch with Keynote.

1.2 CANCER STEM CELLS

While normal hematopoiesis has clearly been shown to be hierarchically organized, it is still a matter of debate whether this feature remains true for malignant hematopoiesis. This debate extends to malignancies in general and is based on two principle models (Clevers, 2011; Magee et al., 2012; Reya et al., 2001; Visvader and Lindeman, 2008). In the stochastic

model, all cells within a tumor are thought to have equal potential for tumor propagation. The alternative (termed hierarchical) model posits that only a small and distinct subpopulation of cells possess the ability to spawn the malignancy. Central to this model is the existence of stem cells, having the unique potential for self-renewal as well as the ability to produce all other cells in the clonal malignant offspring. The existence of rare and distinct cancer stem cells has been demonstrated in leukemia (Bonnet and Dick, 1997; Lapidot et al., 1994) as well as solid organ malignancies (Ailles and Weissman, 2007; Al-Hajj et al., 2003; Dalerba et al., 2007; Singh et al., 2004). However, melanoma xeno-transplantation studies are experimental evidence for the stochastic model as 25% of all cells, without any selection, were able to reconstitute the tumor in mice (Quintana et al., 2008). Thus, it is likely that both models exist and that tumor development patterns differ between various malignancies.

Based on the seminal work by John Dick and colleagues, who demonstrated that only CD34+CD38- cells from AML patients were able to propagate the disease, it was assumed that cancer stem cells in Acute Myeloid Leukemia (AML) might arise from normal HSC (Bonnet and Dick, 1997; Lapidot et al., 1994). This concept was however challenged by a study demonstrating that CD38-antibodies impacted the engraftment of CD38+ cells (Taussig et al., 2008). In addition, subsequent studies demonstrated that CD34+CD38+ cells possess significant leukemia-initiating capacity. Jamieson and co-workers proposed transformed GMP as the candidate leukemic stem cells in blast-crisis CML (Jamieson et al., 2004). Similarly, a subgroup of AML-patients was found to have leukemia initiating cells with a GMP-like immunophenotype of CD34+CD38+CD45RA+ (Goardon et al., 2011). In addition, recent studies from our group in MDS patients with monosomy 7, demonstrated a significantly expanded GMP-population suggesting that perturbations in the stem and progenitor compartments of high-risk MDS are more similar to AML than low-risk MDS (Dimitriou et al., 2016). Hence, the term "cancer stem cell" does not necessarily imply that a normal stem cell has been transformed to be the cell of origin. The only prerequisite is the ability to self-renew. Normal stem cells already have this ability while downstream committed progenitors or mature cells need to acquire this feature aberrantly.

Despite their usefulness, one of the shortcomings of xenotransplantation models is related to taking the cells of interest out of their natural habitat. Despite the fact that AML is quite an aggressive malignancy, only about half of all samples engraft in immunocompromised mice. It has become clear that other supporting cells and the stromal microenvironment can play important roles in sustaining the leukemia. Moreover, growth factors and adhesion molecules may species-specific and not permit engraftment of human cells in mice. Applying genetic lineage tracing to murine models of cancerogenesis clearly identified examples of cancer stem cells (Chen et al., 2012; Schepers et al., 2012). Definitive evidence for human cancer stem cells would require proof of their existence in the patients themselves.

2 THE MYELODYSPLASTIC SYNDROMES

2.1 HISTORY

The concept of anemia anteceding acute leukemia was recognized by von Leube at the beginning of the 20th century (Leube, 1900). Subsequently, the condition for patients suffering from anemia that could not be cured by supplements was dubbed 'refractory anemia'. Chevalier is credited with establishing the concept of leukemia being linked to antecedent anemia, he proposed the term 'odo-leukemia' from the greek word 'odo' for 'threshold' (Chevallier, 1942). Eventually 'pre-leukemia' became the term that was accepted in the literature for a disease condition that encompassed cytopenia and dysplastic features in one or more of the hematopoietic lineages in the bone marrow, having an increased risk of evolution to leukemia (Block and Jacobson, 1953; Hamilton-Paterson, 1949). The geneticist Hans Grüneberg discovered iron-laden precursors of red blood cells in both mice and man and dubbed them as siderocytes and sideroblasts (Doniach et al., 1943; Grüneberg, 1939). Some 15 years later, Sven-Erik Björkman published four cases of acquired idiopathic sideroblastic anemia and described the curious finding of ringsideroblasts (Björkman, 1956).

In 1969 William Dameshek suggested to include these pre-leukemias under his umbrella of myeloproliferative diseases (Dameshek, 1951) but Harriet Gilbert recognized that some preleukemic cases were distinct from the classic myeloproliferative disorders. She coined the term "myelodysplastic disease" for pre-leukemic cases with lack of cell maturation (Gilbert, 1970). It soon became apparent that some patients with preleukemia never developed leukemia and Fisher and colleagues suggested to favour "myelodysplasia" over "preleukemia" (Fisher et al., 1973). In 1982, the French-American-British (FAB) cooperative group suggested criteria for the myelodysplastic syndromes (Bennett et al., 1982). This milestone greatly enabled further investigations into the prognosis and management of the disease.

2.2 EPIDEMIOLOGY

MDS commonly occurs in older individuals with a slight male predominance. The median age at diagnosis is above 70 years in most studies (Ma et al., 2007; Nordic MDS Group, 2017; Smith et al., 2011).

The exact incidence of de novo MDS has been difficult to determine. While MDS in Sweden has been included in cancer registries since the 1980s, the diagnosis of MDS is excluded from cancer registries in some countries. Moreover, many older individuals with cytopenias are incompletely evaluated in the case of significant comorbidities. The single most important risk factor for development of MDS is aging. MDS increases exponentially with age - while the yearly incidence in people below 30 years of age is around 0.1-0.4 per 100 000, people in their 80s have an incidence of 30 per 100 000.

The vast majority of primary (*de novo*) MDS cases are viewed as idiopathic in nature, although the causes are beginning to be elucidated and constitutes the topic of this thesis. About 10-15% of MDS are secondary or therapy-related. Environmental factors such radiation and chemical exposure or previous treatment with cytotoxic drugs are highly associated with MDS. In clinical practice it can be difficult to establish a causal link between exposure to a particular substance and the development of MDS, but findings of particular chromosomal aberrations (chromosomes 5 and 7), complex karyotype (>3 acquired chromosome abnormalities, and mutations in TP53 suggest secondary rather than *de novo* MDS.

2.3 CLINICAL PRESENTATION AND MORPHOLOGICAL DIAGNOSIS

The typical clinical presentation of a patient with MDS is one of unexplained anemia, usually macrocytic, with or without additional cytopenias. Symptoms of anemia include fatigue, pallor, weakness, dyspnea, cognitive impairment and headache (Foucar et al., 1985; Goldberg et al., 2010; Jansen et al., 2003; Meyers et al., 2005). The most pervasive symptom is fatigue which can be out of proportion to the degree of anemia (Abel et al., 2016; 2014). In a minority of patients, myelodysplasia manifests itself as sole thrombo- or leukocytopenia, with bleedings or infections as the initial presenting symptoms. Even more rarely, patients present initially with autoimmune manifestations, e.g., vasculitis, arthritis and pulmonary effusions (Billström et al., 1995; Giannouli et al., 2004).

Two hallmarks characterize MDS: firstly, abnormal cell morphology (dysplasia) and secondly, quantitative changes in one or more hematopoietic lineages (cytopenias). Performing complete blood counts almost invariably reveals a macrocytic or normocytic anemia while neutropenia and thrombocytopenia are more variable. Up to half of the patients present with pancytopenia at time of diagnosis.

The diagnostic evaluation of patients suspected of having MDS begins with family and past medical history and physical examination, followed by complete blood counts, examination of the peripheral blood smear and bone marrow aspirate and biopsy as well as cytogenetic studies. Additional laboratory test are also performed to rule out other disorders that can mimic MDS (Steensma, 2012). Medications, alcohol abuse, nutritional deficiencies (e.g., vitamin B12, folate, iron) can all lead to morphological dysplasia. Furthermore, HIV infection and immune-mediated cytopenia need to be ruled out.

Evidence of dysplasia in white and red blood cells can usually be demonstrated in the peripheral blood smear while the morphology of platelets is usually normal. Figure 2 illustrates and summarizes the morphologic abnormalities in MDS.

While a blood smear may suggest MDS, examination of the bone marrow aspirate is essential for the diagnosis. It can reveal dysplasia in one or more lineages, with significant dysplasia being defined by presence of dysplastic features in at least 10% of hematopoieitic precursor cells of a particular lineage (binucleate erythroid precursors, megaloblastoid erythroid maturation, ring sideroblasts, hypolobulation and hypogranulation of neutrophils, and small megakaryocytes with abnormal segmentation of their nuclei).

The absolute numbers of neutrophils and platelets are poorly correlated with the risk for infection and bleeding. However, the cellular dysfunction is often associated with the dysplasia itself, e.g., hypogranular neutrophils demonstrate impaired phagocytosis and microbicidal activity (Boogaerts et al., 1983) and platelet dysfunction is due to lack of expression of procoagulant molecules (Manoharan et al., 2002).

According to the criteria of the Word Health Organization (WHO) (Arber et al., 2016), at least 500 nucleated cells and 20 megakaryocytes have to be assessed. Based on this evaluation, the percentage of blasts in the marrow can be determined, and the morphology of blasts and other cells can be cytologically assessed. Impaired maturation of myeloid cells is usually apparent. While the percentage of granulocytic precursors is variable, it is decreased more often than increased. Sometimes, maturation arrest can occur, usually at the myelocyte stage. Cytoplasmic maturation may progress more rapidly than the nucleus.

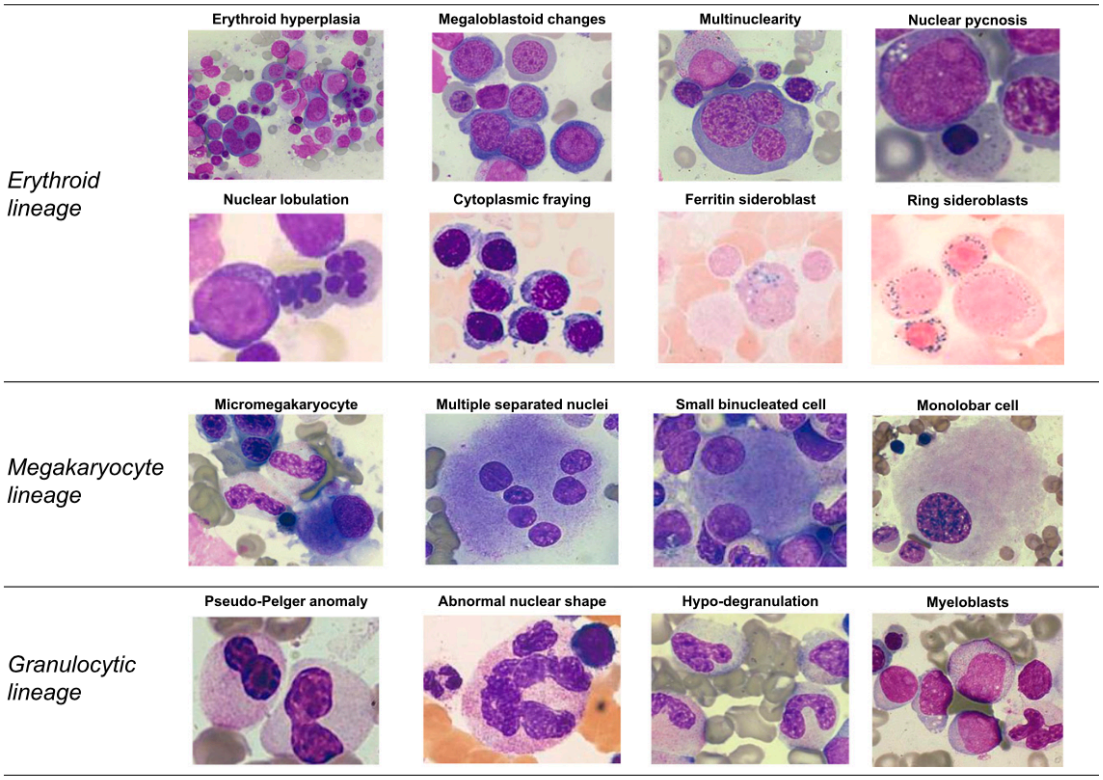

Figure 2. Representative examples of morphologic abnormalities of myelodysplasia. Reproduced from (Cazzola et al., 2013) with permission from Blood. © the American Society of Hematology.

Examination of the bone marrow core (so-called trephine) biopsy provides important additional information, namely, the degree of cellularity, morphology of megakaryocytes, presence of fibrosis and overall architecture.

Another method increasingly used in the evaluation of patients with suspected MDS is flow cytometry. While exceptionally suited for the detection of abnormal cell populations due to their aberrant expression of surface markers, it is currently viewed as an additional method and should be interpreted in the context of morphology of the bone marrow (Kern et al., 2013; 2010; van de Loosdrecht et al., 2009; 2008; Wells et al., 2003).

Cytogenetic studies on the other hand are currently essential in the evaluation of MDS. If morphologic assessment of the marrow proves to be equivocal, the finding of a cytogenetic abnormality can support the diagnosis (Arber et al., 2016). The importance of karyotypic abnormalities is discussed in more depth in the chapter on the molecular genetics of MDS.

2.4 CLASSIFICATION

The importance of the initial classification of MDS based on the FAB criteria put forward in 1982 cannot be overstated, as this classification paved the way for subsequent studies on prognosis and clinical management of the disease (Bennett et al., 1982). Five morphological subgroups were initially defined, based largely on percentages of bone marrow blasts and ringsideroblasts, i.e., refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess of blasts (RAEB), chronic myelomonocytic leukemia (CMML), and RAEB in transformation (RAEB-t). To distinguish MDS from AML, the level of bone marrow blasts was set at 30%.

Refining these initial criteria almost 20 years later, the WHO proposed their guidelines for the classification of MDS, taking into consideration the number of dysplastic cell lines, blast percentage, presence of ringed sideroblasts, myeloproliferative features, number of monocytes in blood and deletion of chromosome 5. Revisions of the WHO classification were published in 2008 and 2016 (Arber et al., 2016; Swerdlow, 2008). This most recent iteration incorporates further refinements of morphologic assessment, cytopenic evaluation as well as genetic information (Table 1).

Table 15. PB and BM findings and cytogenetics of MDS

Name	Dysplastic lineages	Cytopenias*	Ring sideroblasts as % of marrow erythroid elements	BM and PB blasts	Cytogenetics by conventional karyotype analysis
MDS with single lineage dysplasia (MDS-SLD)	1	1 or 2	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia (MDS-MLD)	2 or 3	1-3	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with ring sideroblasts (MDS-RS)					
MDS-RS with single lineage dysplasia (MDS-RS-SLD)	1	1 or 2	≥15%/≥5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS with multilineage dysplasia (MDS-RS-MLD)	2 or 3	1-3	≥15%/≥5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	1-3	1-2	None or any	BM <5%, PB <1%, no Auer rods	del(5q) alone or with 1 additional abnormality except −7 or del (7q)
MDS with excess blasts (MDS-EB)					
MDS-EB-1	0-3	1-3	None or any	BM 5%-9% or PB 2%-4%, no Auer rods	Any
MDS-EB-2	0-3	1-3	None or any	BM 10%-19% or PB 5%-19% or Auer rods	Any
MDS, unclassifiable (MDS-U)					
with 1% blood blasts	1-3	1-3	None or any	BM <5%, PB = 1%,‡ no Auer rods	Any
with single lineage dysplasia and pancytopenia	1	3	None or any	BM <5%, PB <1%, no Auer rods	Any
based on defining cytogenetic abnormality	0	1-3	<15%§	BM <5%, PB <1%, no Auer rods	MDS-defining abnormality
Refractory cytopenia of childhood	1-3	1-3	None	BM <5%, PB <2%	Any

*Cytopenias defined as: hemoglobin, <10 g/dL; platelet count, $<100 \times 10^9$/L; and absolute neutrophil count, $<1.8 \times 10^9$/L. Rarely, MDS may present with mild anemia or thrombocytopenia above these levels. PB monocytes must be $<1 \times 10^9$/L

†If *SF3B1* mutation is present.

‡One percent PB blasts must be recorded on at least 2 separate occasions.

§Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia, and are classified as MDS-RS-SLD.

Table 1. Peripheral blood and bone marrow findings and cytogenetics of MDS. Repodruced from (Arber et al., 2016) with permission from Blood. © the American Society of Hematology.

The natural course of MDS is quite variable amongst individual patients, reflecting in part different patient characteristics (e.g. age, previous exposure to chemotherapy) as well as known and unknown differences in pathogenesis of different MDS subtypes. While the classification systems primarily based on pathology such as the FAB and WHO have been invaluable for diagnosis they have their limitations in terms of prognosis. Thus, the existing classification schemes were further refined, including age and sex, morphologic features, blast percentages, presence of cytopenias, cytogenetic abberations, clinical characteristics, and transfusion requirements as additional variables.

2.5 PROGNOSIS

The old term “pre-leukemia” may be over-simplifying and in fact be a deceiving concept since the majority of patients with MDS do not transform into AML - rather, they succumb to the sequelae of bone marrow failure, e.g., infection, bleeding and symptomatic anemia.

The natural course of MDS is quite variable amongst individual patients, reflecting in part different patient characteristics (e.g. age, previous exposure to chemotherapy) as well as known and unknown differences in pathogenesis of different MDS subtypes. While the classification systems primarily based on pathology such as the FAB and WHO have been invaluable for diagnosis they have their limitations in terms of prognosis. Since the treatment recommendations rely on an accurate prognostic stratification, it is thus necessary to stratify patients according to their risk in order to be able to assess an individual patient’s prognosis and make therapeutic decisions. Several investigators have proposed prognostic scoring systems that divide patients into lower and higher risk subgroups, usually incorporating clinical as well as pathological information. Each system has its strengths and limitations but most importantly, all prognostic systems explain only a minor part of the observable variability in outcome. By initially observing an individual’s disease course - as opposed to abruptly starting therapy - one can glean important information about tempo and prognosis for the particular patient.

Based on number of cytopenias, percentage of bone marrow blasts and karyotype Greenberg et al. established the “International Prognostic Scoring System” (IPSS) for MDS in 1997 (Greenberg et al., 1997). Based on a multivariate analysis of a study cohort consisting of more than 800 MDS patients from the United States, Europe and Japan, four risk categories were defined which predicted risk of evolution to AML as well as overall survivial: Low, Intermediate-1 (Int-1), Intermediate-2 (Int-2), and High-risk. Upon stratification according to age, there was a significantly longer overall survival in patients below 70 years of age, but only in patients belonging to Low and Int-1 risk categories. In contrast, there was no association of age and survival in the higher risk categories, possibly because of high disease related mortality. The risk of AML progression was not associated with age.

To calculate the IPSS score, values from zero to two are determined for each of three variables according to the Table2. The IPSS score is calculated as the sum of each of these three values. It defines four risk groups for both overall survival as well as transformation into AML: low (0 points), intermediate-1 (0.5-1 points), intermediate-2 (1.5-2 points) and high (2.5-3). The IPSS also stratified survival according to patient age. While the median survival of low risk patients strongly depended on age (see table), the median survival of high risk patients was independent of age (between 4-6 months).

TABLE 2. The 1997 International Prognostic Scoring System (IPSS) for Myelodysplastic Syndromes[a]

	Category score[b]				
Prognostic factor	0 (best)	0.5	1.0	1.5	2.0 (worst)
Marrow blasts (%)	<5	5-10	—	11-20	21-30[c]
Karyotype	Good: normal, isolated −Y, isolated del(5q), or isolated del(20q)	Intermediate: all karyotypes not defined as good or poor	Poor: abnormal chromosome 7 or a complex karyotype (≥3 anomalies)	—	—
Peripheral blood cytopenias[d]	0 or 1	2 or 3	—	—	—

		Median survival (y)			
Risk category	Total score	All patients	Patients aged <60 y (n=205)	Patients aged ≥60 y (n=611)	Time until 25% of surviving patients developed leukemia (y)
Low	0	5.7	11.8	4.8	9.4
Intermediate-1	0.5 or 1.0	3.5	5.2	2.7	3.3
Intermediate-2	1.5 or 2.0	1.2	1.8	1.1	1.1
High	≥2.5	0.4	0.3	0.5	0.2

[a]Although replaced by the revised IPSS in 2012, numerous clinical trial protocols still use the original IPSS for determination of eligibility.
[b]Scoring system: A point value from 0 to 2.0 is determined for each of the 3 prognostic factors, and the 3 values are summed to obtain the total IPSS score.
[c]No longer considered myelodysplastic syndrome (redefined as acute myeloid leukemia by the World Health Organization in 2001).
[d]The IPSS definition of peripheral blood cytopenias: hemoglobin level less than 10 g/dL; absolute neutrophil count less than 1.8×10^9L; and platelet count less than 100×10^9L.
Adapted from *Blood*.[17]

Table 2. The 1997 International Prognostic Scoring System (IPSS) for Myelodysplastic Syndromes. Original publication by (Greenberg et al., 1997). Table reprinted from (Steensma, 2015), with permission from Elsevier.

The revised IPSS (IPSS-R) was published in 2012, it incorporated five rather than three subgroups based on cytogenetics, divided marrow blast percentage 5% into two sub-groups and expanded assessment of different cytopenias (Greenberg et al., 2012).

To calculate the revised IPSS-score, individual scores from zero to four are determined for each of five variables (Table 3):

1. Bone marrow blast percentage
2. Karyotype
3. Hemoglobin level
4. Platelet count
5. Absolute neutrophil count

The IPSS-R score is calculated from the sum of these five values. It defines five risk groups for both overall survival and AML progression. Patient age is an optional variable which can be incorporated to predict overall survival, but not evolution to AML.

TABLE 3. 2012 Revised International Prognostic Scoring System (IPSS-R)[a–c]

Updated cytogenetic classification for use in the IPSS-R (n=7012)

Risk group	Included karyotypes	Median survival (y)	Time until 25% of patients developed AML (y)	Patients in this group (%)
Very good	del(11q), -Y	5.4	Not reached	4
Good	Normal, del(20q), del(5q) alone or with 1 other anomaly, del(12p)	4.8	9.4	72
Intermediate	+8, del(7q), i17q, +19, any other single or double abnormality not listed, 2 or more independent clones	2.7	2.5	13
Poor	Abnormal 3q, -7, double abnormality include -7/del(7q), complex with 3 abnormalities	1.5	1.7	4
Very poor	Complex with >3 abnormalities	0.7	0.7	7

IPSS-R

Parameter	Categories and associated scores				
	Very good	Good	Intermediate	Poor	Very Poor
Cytogenetic risk group	0	1	2	3	4
Marrow blasts (%)	<2	2-<5	5-10	>10	
	0	1	2	3	
Hemoglobin (g/dL)	≥10	8-<10	<8		
	0	1	1.5		
Absolute neutrophil count ($\times 10^9$/L)	≥0.8	<0.8			
	0	0.5			
Platelet count ($\times 10^9$/L)	>100	50-100	<50		
	0	0.5	1		

IPSS-R (see: http://www.mds-foundation.org/ipss-r-calculator/)

Risk group	Points	% patients in this risk group (n=7012; AML data on 6485)	Median survival, years	Median survival for pts under 60 years	Time until 25% of patients develop AML, years
Very low	0-1.5	19%	8.8	Not reached	Not reached
Low	2.0-3.0	38%	5.3	8.8	10.8
Intermediate	3.5-4.5	20%	3.0	5.2	3.2
High	5.0-6.0	13%	1.5	2.1	1.4
Very high	>6.0	10%	0.8	0.9	0.7

[a]AML = acute myeloid leukemia.
[b]SI conversion factors: To convert hemoglobin values to g/L, multiply by 10.0.
[c]Possible range of summed scores: 0-10.
Adapted from *Blood*.[28]

Table 3. The 2012 Revised International Prognostic Scoring System (IPSS-R) for Myelodysplastic Syndromes. Original publication by (Greenberg et al., 2012). Table reprinted from (Steensma, 2015), with permission from Elsevier.

It is worth noting that the IPSS is based on data from MDS patients at diagnosis and can therefore not be used during an individual's disease course or at time of progression. Therefore, Malcovati et al proposed a time-dependent prognostic scoring system based on the WHO classification which allowed repeated assessment of an individual's prognosis at any time point of the disease including at time of progression. This WHO-classification based prognostic scoring system (WPSS) is based on the different WHO subgroups, karyotype and incorporates transfusion dependency (Malcovati et al., 2007). The latter is an independent negative prognostic factor. The WPSS defines five prognostic groups on a spectrum from very low to very high risk. Patients over 70 years and with very low risk MDS have a similar overall survival as the general population. Moreover, the presence of fibrosis and co-morbidity have also been identified as independent negative factors.

Taking into account the patient's age, comorbidities, and functional status, and based on the predicted risk of disease, clinicians are able to tailor therapeutic recommendations to each patient's situation.

2.6 TREATMENT

2.6.1 Hematopoietic Cell Transplantation

Currently, the only potentially curative treatment for MDS is allogeneic hematopoietic cell transplantation (HCT) (Parmar et al., 2011). In 1956, E Donnall Thomas performed the first successful transplantation of bone marrow from an identical twin into a patient with leukemia after prior radiotherapy. After other reports of failure (Rozman et al., 1978), Bhaduri et al. reported the first case of successful transplantation for a patient with MDS in 1979 (Bhaduri et al., 1979). By the early 1980s, this treatment modality had become established at several large cancer centers (Storb, 2003).

Significant advances in bone marrow / hematopoietic cell transplantation have been made during the last decades: selection of donors, cell source and post-transplant immuno-suppression have all undergone significant developments (Giralt, 2005). Collectively, this has led to a large decrease in transplantation related mortality (TRM) (Gyurkocza et al., 2010; Storb et al., 2001).

Unfortunately, given that the median age of patients at time of their MDS diagnosis is greater than 70 years, only a minority of patients are eligible for HCT, mainly due to comorbidities and reduced physical fitness (Deeg and de Lima, 2013). While this therapeutic modality has been used in less than 10% of the patients, its use has recently increased (de Witte et al., 2017; Gyurkocza and Deeg, 2012; Kröger, 2012). The majority of MDS patients >70 years nowadays receive reduced-intensity conditioning (RIC) before the infusion of allogeneic hematopoietic cells (Koreth et al., 2013), thus relying upon an immunological attack - the so-called graft-versus-leukemia (GVL) effect - of the infused cells to eradicate neoplastic clones of MDS cells. In general, the outcomes for MDS patients using myeloablative versus reduced-intensity conditiong are similar. However, reduced-intensity conditioning is inferior to myeloablative conditioning in terms of a higher relapse rate (Luger et al., 2012; Martino et al., 2006), while the risk for transplantation-related mortality is lower (Deeg et al., 2012; Koenecke et al., 2015). In the best clinical scenario, younger patients less than 40 years of age can experience long-term disease free survival in more than 50% of cases. In reality, only about one-third of MDS patients are considered cured - the 5-year overall as well as relapse-free survival rates after transplantation for MDS have been reported to be around 33% with strong inverse correlation to the IPSS-R score (Koenecke et al., 2015).

Thus, the current Nordic guidelines recommend that all MDS patients who are potential candidates for a transplant should be evaluated by a physician with expertise in hematopoietic cell transplantation early in the course of their disease (Nordic MDS Group, 2017). Therapeutic decisions are currently guided by overall clinical assessment and risk stratification systems (IPSS-R and/or others). The prognostic evaluation should determine the particular therapy for each individual patient. The goal for lower-risk patients are mainly focused on quality of life and symptom control. For higher-risk patients delay of disease progression and extension of survival have been achieved with azacitidine. Previous analyses based on mathematical modelling suggested that higher-risk patients derive a net gain of life from referral for HCT soon after their diagnosis while lower-risk patients benefit from waiting before being referred for HCT (Cutler et al., 2004; Koreth et al., 2013).

2.6.2 Supportive Care

About 40% of lower-risk patients and 60-80% of higher-risk patients are dependent on red blood cell transfusions (Cazzola et al., 2008; Hellström-Lindberg and Malcovati, 2008; (Nordic MDS Group, 2017). The requirement for regular transfusions have been associated with inferior survival compared to patients who do not require transfusions. Likely explanations are that this simply indicates more severe hematopoietic failure or that the increased transfusion frequency itself is harmful due to iron overload or additional mechanisms (Malcovati, 2007).

In patients with evidence of iron overload and a relatively good prognosis therapy with the iron chelators deferoxamine or deferasirox can be considered, although there are currently no randomized controlled studies that have demonstrated a benefit from iron chelation in MDS (Nordic MDS Group, 2017).

After infections, the second most common cause of morbidity and mortality is bleeding (Kantarjian et al., 2007). Supportive platelet transfusions can decrease bleeding risk, however, patients frequently develop alloimmunization with repeated platelet transfusion. While transfusion support can ameliorate symptoms and thus improve quality of life, there appears to be no direct impact on survival (Nachtkamp et al., 2009).

2.6.3 Hematopoietic Lineage Growth Factors

The erythropoesis-stimulating agents epoetin and darbepoetin (EPO) yield erythroid response rates in up to 50% of patients and are recommended as first-line treatment for the anemia of lower-risk MDS (Hellström-Lindberg and Malcovati, 2008; Malcovati et al., 2013; Nordic MDS Group, 2017). The response rate is inversely correlated with the individual's endogenous erythropoietin levels. While patients with high serum erythropoietin levels rarely

respond, an initial trial for 2-3 months is appropriate in patients with serum erythropoietin levels of 500 U/L or less (Buckstein et al., 2017; Hellström-Lindberg et al., 2003). Combining EPO with granulocyte colony-stimulating factor (G-CSF) has been shown to be superior to EPO alone in RARS patients (Jädersten et al., 2008; Park et al., 2008), likely due to synergistic effects via inbition of erythroid apoptosis (Tehranchi et al., 2003).

While G-CSF and GM-CSF have been shown to increase the number of neutrophils, they have failed to demonstrate an increase in survival (Vadhan-Raj et al., 1987). Rather, one study found G-CSF to be associated with shorter survival in patients with excess blasts, however, progression to AML was similar between the two groups. The current recommendation is to reserve G-CSF for patients with recurrent infections while being mindful of the fact that simply increasing the number of circulating neutrophils does not help if these cells are dysfunctional.

The past few years have witnessed an increased usage of thrombopoietin (TPO) receptor agonists in MDS (Brierley and Steensma, 2015). While formally approved solely for immune thrombocytopenia, eltrombopag and romiplostim have been used in MDS, owing to the fact that some MDS patients also have immune-mediated thrombocytopenia (Giagounidis et al., 2014). Analogous to EPO-treatment, patients who respond best have endogenous TPO levels less than 500 pg/L and an infrequent requirement for platelet transfusions {Sekeres:2014ck}.

Lastly, a main staple in supportive therapy is e-aminocaproic acid, an antifibrinolytic drug that has been shown to decrease bleeding in thrombocytopenic patients (Salacz et al., 2007).

2.6.4 Immunomodulatory drugs

Based on findings that autoreactive T cells can play a role in suppressing hematopoiesis (Epling-Burnette et al., 2007), treatments similar to those used for aplastic anemia, e.g., cyclosporine and antithymocyte globulin were evaluated in patients with MDS (Parikh et al., 2012). However, response to treatment was heterogenous (Sauntharajah et al., 2003; Sloand et al., 2008), with benefits mostly seen in those patients with the most benign disease expressions (Molldrem et al., 1997; 2002).

Thalidomide was another agent evaluated for its immunomodulatory effects together with its potential for inhibition of neoangiogenesis. While up to 25% of patients showed a favorable response, the adverse events of sedation, constipation and neuropathy were quite significant (Raza et al., 2001). Based on these findings, the second-generation immunomodulatory drug lenalidomide was evaluated in lower-risk patients (List et al., 2005). A subsequent study (List et al., 2006) estasblished that lenalidomide is most effective in low- to int-1 risk patients with deletion of chromosome 5q31. In this subgroup, two-thirds achieve transfusion independence, an increase in hemoglobin level and a median response duration of more than 2 years (Fenaux et al., 2011). Moreover, cytogenetic remission were seen in up to 50% of patients. In patients with normal karyotype or chromosomal abnormalities other than 5q, responses are

less frequent (Raza et al., 2008). Only 25% become transfusion independent and the median response duration is less than a year. Myelosuppression is lenalidomide's most common adverse effect and patients with thrombocytopenia, excess blasts or complex karyotypic abnormalities are unlikely to respond to lenalidomide.

2.6.5 Hypomethylating agents (DNMT inhibitors)

The transcription of genes is affected by epigenetic changes. An important epigenetic modification is the process of DNA methylation where cytidine residues are covalently modified by enzymes that add methyl-groups. These enzymes are called DNA methyltransferases. Azacitidine and decitabine are nucleoside analogues of cytosine and inhibit DNA methyltransferase I. Azacitidine decreases methylation of DNA and is thought to reverse gene silencing (Issa and Kantarjian, 2009). In addition, these analogues induce DNA damage similar to cytarabine and other nucleoside analogues (Issa, 2013). Azacitidine was evaluated in higher-risk patients (int-2 and high-risk, (Fenaux et al., 2009)). Compared to either best supportive care, low-dose cytarabine or AML-induction therapy, the azacitidine group exhibited a significant increase in median survival (24 months for azacitidine vs. 15 months for patients in other groups). While only a minority of patients (16%) achieved a complete response rate, this is not a requirement for achieving a survival benefit. The clinical response to hypomethylating agents might not be readily apparent as responses might be slow (Gore et al., 2013). Thus, an adequate therapeutic trial of azacitidine consists of 6 treatment cycles. The most common adverse effects, i.e., thrombocytopenia and neutropenia, are in clinical practice difficult to distinguish from the underlying disease (Nordic MDS Group, 2017).

3 THE GENETIC BASIS OF MDS

3.1 RECURRENT CYTOGENETIC ABNORMALITIES

At time of diagnosis, about 50% of patients with MDS have clonal chromosomal abnormalities (Billström et al., 1988; Haase et al., 2007; Mallo et al., 2011; Mauritzson et al., 2002; Sole et al., 2000; 1992). These chromosomal changes can manifest as structural abnormalities involving one chromosome (e.g., inversion, interstitial deletion), numerical changes (e.g., monosomy or trisomy) or be a balanced translocation between two chromosomes. About 15% of patients have multiple abnormalities and thus exhibit complex karyotypes. During the course of the disease, additional chromosomal aberrations may develop on top of previous ones or emerge in patients with no previous karyotypic abnormality. These changes are invariably associated with poor prognosis and progression to acute leukemia (Bochtler et al., 2015). However, none of the chromosomal abnormalities are specific for MDS and are also found in other myeloid diseases.

Good-prognosis cytogenetics

Anomaly	Frequency (%)	Median survival (mo)
No (normal karyotype)	49.5	53.4
del(5q), NC	10.7	77.2
del(5q), isolated	8.2	80
-Y, sole	3.5	36
-Y, NC	2.7	39
del(5q), +1	2.5	47
del(20q), NC	2.2	71
del(20q), isolated	1.9	71
+8,+1	1.2	44
+21, NC	1.1	100.8
del(12p), NC	0.8	108
+21, +1	0.8	80
t(1q), NC	0.6	34.7
t(7q), NC	0.6	34.7
-X, NC	0.5	56.4
t(11q),NC	0.5	32.1
-21, NC	0.5	32
del(9q), NC	0.4	NR
del(15q), NC	0.4	NR
t(15q),NC	0.4	NR
-Y,+1	0.4	84.6
+ 1/+1q, NC	0.4	34.7

Intermediate-prognosis cytogenetics

Anomaly	Frequency (%)	Median survival (mo)
+8, NC	5	23
+8, isolated	3.8	22
-7, NC	3.2	14
Any 3 abnormalities	2.8	17.1
-7, sole	2.3	14
del(11q), NC	0.9	26.1
-7,+1	0.9	14.4
del(7q), isolated and NC	0.6	19
del(11q), isolated	0.6	15.9
t(11q23), NC	0.5	20
Rea 3q, NC	0.5	19.9
+19, NC	0.4	19.8
-5,NC	0.4	14.6

Poor-prognosis cytogenetics

Anomaly	Frequency (%)	Median survival (mo)
Complex, all	13.4	8.7
Four to 6 abnormalities	5.3	9
More than 6 abnormalities	3.9	5
t(5q), NC	0.4	4.4

NC indicates noncomplex karyotype; and NR, not reached.

Table 4. Frequency and median survival of cytogenetic prognostic subgroups. Data is sorted by frequency of cytogenetic aberration. This table is based on original data found in table 4 from (Haase et al., 2007).

Importantly, irrespective of blast count, AML is diagnosed if any of the following cytogenetic abnormalities are present:

- t(8;21)(q22;q22): *RUNX1-RUNX1T1* (previously *AML1-ETO*)
- inv(16)(p13.1q22) or t(16;16)(p13.1;q22): *CBFB-MYH11*
- t(15;17)(q22;q21.1): *PML-RARA*

The karyotypic abnormalities that are most commonly found in MDS are del(5q), del(7q) or monosomy 7 (-7), trisomy 8 (+8), del(20q) as well as loss of the Y chromosome (Billström et al., 1988; Haase et al., 2007). Although the same cytogenetic lesions also occur in de novo AML, the balanced translocations typical for AML are uncommon in MDS (Mauritzson et al., 2002). Instead, the chromosomal abnormalities in MDS are usually unbalanced, indicating gain or loss of chromosomal material. In therapy-related MDS/AML cytogenetic abnormalities are even more prevalent, as up to 80% have chromosomal aberrations, in particular deletions of chromosomes 5 and 7. Deletion of the long arm of chromosome 5 with or without additional karyotypic abnormalities is not only the most frequent chromosomal aberration, it is the only one constituting its own entity in the WHO classification (Arber et al., 2016).

Despite the fact that other chromosomal abnormalities in MDS are not associated with specific clinical or morphological subsets using the WHO classification system, they do play a substantial role in the pathogenesis and prognosis and have a major impact in the clinical management of patients.

3.2 RECURRENTLY MUTATED GENES

The recent advances in inexpensive high-throughput genome sequencing technologies have enabled the discovery of genetic lesions that drive the pathogenesis of the majority of human cancers (Vogelstein et al., 2013), including the myelodysplastic syndromes.

Based on panels of genes that were previously found to be mutated in other myeloid diseases, a number of studies during the last years have described the mutational landscape of somatic point mutations in MDS (Bejar et al., 2011; Haferlach et al., 2014; Papaemmanuil et al., 2013; Yoshida et al., 2011). Based on current data, up to 90% of patients bear mutations in at least one recurrently mutated gene and it is believed that the remaining 10% of patients have mutations in genes yet to be identified.

MDS is the result of the sequential accrual of somatic mutations in hematopoietic stem cells. Based on calculations, these stem cells amass approximately 1.3 mutations in exons every decade (Welch et al., 2012) but the majority of these are innocent bystanders with no functional alteration (Mardis et al., 2009). However, this calculation presumes that the rate of mutation is constant - in reality, the process of mutation accrual could be dynamic as one

mutation might affect the acquisition of 2nd or 3rd hits and so forth (Papaemmanuil et al., 2013). The spectrum of recurrent somatic mutations in MDS and other myeloid diseases can be organized into several groups based on their biologic pathways.

3.2.1 Mutations in splicing factors

The alternative splicing of pre-mRNA is a hallmark of eukaryotes that enables the generation off different proteins from the same gene. Interestingly, aberrant alternative splicing is also one of the hallmarks of cancer (Lawrence et al., 2014). The seminal study by Yoshida et al. identified recurrent mutations in genes that are part of the spliceosome in up to 60% of cases of MDS (Yoshida et al., 2011).

Table 3. Most common driver genes in patients with MDS and MDS/MPN

Biological pathways and genes	Frequency, %*	Timing of mutation acquisition†	Relationship between mutant gene and clinical phenotype	Prognostic or predictive relevance of mutant gene
RNA splicing				
SF3B1	15-30%	More often a founding mutation	Strictly associated with ring sideroblasts phenotype (RARS, RARS-T)	Associated with good overall survival and low risk of leukemic evolution
SRSF2	10-20%	More often a founding mutation	Associated with RCMD or RAEB, co-mutated with *TET2* in CMML	Associated with poor overall survival and high risk of leukemic evolution
U2AF1	<10%	More often a founding mutation	Mainly associated with RCMD or RAEB	Associated with high risk of leukemic evolution
ZRSR2	<10%	More often a founding mutation	Not defined	Not defined
DNA methylation				
TET2	20-30%	More often a founding mutation	Found in all MDS subtypes, high mutation frequency (50-60%) in CMML	No impact on overall survival, may predict response to hypomethylating agents
DNMT3A	~10%	More often a founding mutation	Found in all MDS subtypes, co-mutated with *SF3B1* in RARS	Associated with unfavorable clinical outcome (negative prognostic relevance mitigated by *SF3B1* co-mutation in RARS)
IDH1/IDH2	~5%	More often a founding mutation	Associated with RCMD or RAEB	Associated with unfavorable clinical outcome
Chromatin modification				
ASXL1	15-20%	More often a subclonal mutation	Associated with RCMD or RAEB, high mutation frequency (40%) in CMML	Associated with unfavorable clinical outcome in all myeloid neoplasms (MDS, MDS/MPN, MPN)
EZH2	~5%	More often a subclonal mutation	Associated with RCMD or RAEB	Associated with unfavorable clinical outcome in all myeloid neoplasms
Transcription				
RUNX1	~10%	Typical subclonal mutation	Associated with RCMD or RAEB	Associated with unfavorable clinical outcome
BCOR	<5%	Typical subclonal mutation	Associated with RCMD or RAEB	Associated with unfavorable clinical outcome
DNA repair control				
TP53	~5%	Typical subclonal mutation	Associated with advanced disease and complex karyotype, mutated in 20% of patients with MDS with del(5q)	Associated with poor overall survival and high risk of leukemic evolution, predicts poor response to lenalidomide in MDS with del(5q)
Cohesin				
STAG2	<10%	More often a subclonal mutation	Associated with RCMD or RAEB. Mutated in about 10% of patients with AML	Associated with unfavorable clinical outcome
RAS pathway				
CBL	<5%	More often a subclonal mutation	Found in different MDS subtypes, associated with JMML in children	Not defined in MDS
NRAS/KRAS	<5%	More often a subclonal mutation	Found in different MDS subtypes, associated with JMML in children	Not defined in MDS
NF1	<5%	More often a subclonal mutation	Found in different MDS subtypes, associated with JMML in children	Not defined in MDS
DNA replication				
SETBP1	<5%	More often a subclonal mutation	Found in 25% of patients with aCML and in subsets of patients with advanced MDS or CMML	Associated with poor overall survival and high risk of leukemic evolution
Receptors				
CSF3R	<1%	Founding driver mutation in CNL	Strictly associated with CNL, found in a subset of patients with aCML	Mutation type may predict response to specific inhibitors

*Approximate proportion of patients with MDS carrying the mutant gene reported in studies published so far.
†Based on values for mutant allele burden or variant allele frequency.

Table 5. Most common driver genes in patients with MDS and MDS/MPN. Reproduced from (Cazzola et al., 2013) with permission from Blood. © the American Society of Hematology.

The mutations in *SF3B1*, *U2AF1* and *SRSF2* are invariably found within defined hotspots of these genes leading to loss of function, altering the operation of the splicing machinery (Graubert et al., 2011; Inoue et al., 2016; Papaemmanuil et al., 2011). The most common mutation within the splicing component affect SF3B1 and these mutations occur in up to 90% of MDS patients with ringssideroblasts (RARS) (Malcovati et al., 2015; 2011). In this context, *SF3B1* mutations are associated with a good prognosis. Current data suggest that the ringsideroblast phenotype is the result of aberrant splicing of genes central in iron homeostasis (Conte et al., 2015). The second most frequent splicing factor mutation in MDS is *SRSF2*. In the overlap syndromes of MDS/MPN this gene is also commonly mutated. It has been shown that mutations in *SRSF2* altered binding of the protein to splice enhancers and that this subsequently leads to mis-splicing of several important downstream genes one of which is EZH2 (Kim et al., 2015). In 10 to 15% of MDS cases mutations are found in *U2AF1* (Graubert et al., 2011). Loss-of-function is reported to lead to increased exon skipping (Ilagan et al., 2015; Przychodzen et al., 2013). *SRSF2* and *U2AF1* have not been associated with a similar favourable prognosis as *SF3B1*. Despite the aforementioned findings, no single mis-spliced isoform has hitherto been implicated in the pathogenesis of MDS. Moreover, studies using mouse models of splicing genes have not yielded similar patterns of altered splicing, neither between mice and men nor between individual mice (Kim et al., 2015; Kon et al., 2017; Mupo et al., 2017; Obeng et al., 2016). Perhaps the role that these mutations play in MDS pathobiology is unrelated to their known function within the splicing machinery.

3.2.2 Epigenetic regulators

Another set of genes found to be recurrently mutated in MDS affect genes that are involved in epigenetic regulation (Shih et al., 2012). Epigenetic regulation is the result of post-translational modification of either histones or the DNA itself.

3.2.2.1 Histone modification

The tails of histones can be covalently modified by enzymes, leading to changes in the structure of chromatin and affecting the binding of protein involved in gene regulation. Amongst the central regulators of transcriptional silencing in development are the PRCs composed of two separate protein complexes, PRC1 and PRC2. Their action on histones H2A and H3 results in the compaction of chromatin (Cao et al., 2002; Francis et al., 2004; Wang et al., 2004). Genes that are part of both protein complexes have been found to be mutated in MDS .

Mutation in two components of PRC1, i.e., *BCOR* and *BCORL1*, have been shown to be recurrently mutated in 5% of MDS cases and found to portend an unfavorable prognosis (Cao et al., 2016; Damm et al., 2013; Gao et al., 2012; Gearhart et al., 2006; Huynh et al., 2000). *EZH2* is mutated in about 5% of MDS patients, the resulting protein is part of the catalytic

subunit of PRC2. Studies in transgenic mice have confirmed that loss of *Ezh2* results in MDS (Sashida et al., 2014).

ASXL1 is in itself not part of the PRC but has been shown to indirectly affect PRC2 leading to deubiquitylation of histone H2A (Abdel-Wahab et al., 2012; Scheuermann et al., 2010). Approximately 20% of MDS patients have recurrent mutations in *ASXL1* (Haferlach et al., 2014), these mutations are confined to exons 11 and 12, leading to truncation of the protein and thus increasing its deubiquitylation (Abdel-Wahab et al., 2012; Balasubramani et al., 2015). The findings that mutations in *BCOR*, *EZH2* and *ASXL1* have been shown to disrupt normal hematopoieitc differentiation offers an explanation for their putative role in dysplasia and cytopenia (Abdel-Wahab et al., 2012; Cao et al., 2016; Sashida et al., 2014).

3.2.2.2 DNA methylation

Regulatory regions of the DNA can either be accessible or inaccessible. Which state is favoured is largely controlled by the methylation of cytosines in repetitive CpG elements. DNA methyltransferases (DNMTs) add methyl groups while the TET family of proteins are responsible for demethylation via a mutistep process (Ko et al., 2015; Tahiliani et al., 2009; Yang et al., 2015). Approximately 15% of MDS cases exhibit mutations in *DNMT3A* leading to loss-of-function of the protein (Haferlach et al., 2014; Ley et al., 2010; Papaemmanuil et al., 2013; Walter et al., 2011). *TET2* is one of the most frequently mutated genes (Haferlach et al., 2014), with 30% of mutations resulting in inactivation of the target protein (Okano et al., 1998). The state of hypermethylation of cytosines at enhancer sites is believed to repress several genes with important roles in myeloid differentiation (Kallin et al., 2012; Rasmussen et al., 2015; Yamazaki et al., 2015). *DNMT3A* and *TET2* are both expressed in hematopoietic stem cells - in a complementary, interconnected, yin-and-yang kind of fashion - having essential roles in self-renewal and myeloid differentiation (Challen et al., 2011; Mayle et al., 2015; Moran-Crusio et al., 2011; Zhang et al., 2016).

It was initially puzzling how mutations in enzymes involved in the citric acid cycle play a role in the pathogenesis of MDS and leukemia. However, it was found that cells with mutations in *IDH1* and *IDH2* accumulated a novel oncometabolite, 2-hydroxyglutarate (2-HG), instead of alpha-ketoglutarate (Dang et al., 2009; Ward et al., 2010; Xu et al., 2011). 2-HG was shown to diffuse to the nucleus leading to inhibition of - amongst others - TET2 and thus also affecting DNA methylation (Lu et al., 2012). Mutations in *IDH1* or *IDH2* occur in ~5% of patients with MDS (Haferlach et al., 2014). While the oncometabolite was shown to be sufficient to promote leukemogenesis, the effects were found to be reversible (Losman et al., 2013). This has led to the development of 2-HG inhibitors which are currently being evaluated in clinical trials.

3.2.3 Cohesin complex

An important part of DNA repair is the formation of a protein complex that holds together sister chromatids preventing the collapse of the replication fork thereby enabling DNA repair (Losada, 2014). This protein complex is made up of the co-called cohesins, *STAG2*, *SMC3*, *SMC1A* and *RAD21*. Taken together, inactivating mutations in cohesin genes are found in 15% of MDS cases (Haferlach et al., 2014; Papaemmanuil et al., 2013). Perhaps somewhat surprisingly given their function, cohesin mutations have not been associated with chromosomal abnormalities (Thota et al., 2014). Rather, it is now believed that cohesin mutations lead to altered gene expression due to a failure to stabilize loops of DNA that normally facilitate the interaction of promoters and distant enhancers (Horsfield et al., 2007; Leeke et al., 2014; Marsman et al., 2014; Mazumdar et al., 2015; Viny and Levine, 2017).

3.2.4 Transcription factors

A small group of hematopoietic transcription factors have been found to be recurrently mutated in MDS. Taking clues from inherited bone marrow failure disorders, where germ-line mutations in *RUNX1*, *GATA2* and *ETV6* had been linked to development of MDS and AML (Growney et al., 2005; Hahn et al., 2011; Zhang et al., 2015), these genes were also included in targeted sequencing panels for the study of MDS. Mutations in *RUNX1* are often associated with severe thrombocytopenia (Owen et al., 2008), they occur in approximately 10% of MDS cases (Haferlach et al., 2014). The protein forms the subunit of the core binding factor and is responsible for DNA-binding. Several target genes of *RUNX1* have important roles in hematopoiesis (Ito et al., 2015).

The zinc finger protein *GATA2* is highly expressed in HSCs and was shown to be essential for normal hematopoietic differentiation (Tsai et al., 1994). Somatic mutations in *GATA2* account for only 1% of MDS cases (Haferlach et al., 2014). The transcription factor Wilms tumor protein *WT1* has been shown to recruit *TET2* to specific loci and is mutated in fewer than 5% of cases of MDS (Rampal et al., 2014; Wang et al., 2015). There are other transcription factors with mutation frequencies below 1%.

3.2.5 Cell signaling genes

Genes for components of cell signaling pathways were shown to be mutated in a host of other myeloid malignancies and were thus obvious candidated to study in MDS (Furitsu et al., 1993; Kottaridis et al., 2001; Kralovics et al., 2005). Compared to AML, CMML or PV/ET, the frequencies with which these genes are mutated in MDS is quite low. Taken together, mutations in components of the MAPK pathway, e.g., *NRAS*, *KRAS*, *NF1* and *PTPN11*, are the most prevalent in MDS, accounting for approximately 10% of MDS cases (Haferlach et al., 2014; Papaemmanuil et al., 2013). In general, most mutations in signaling pathway components lead to the constitutive activation of the protein product. Of note, these mutations

often occur late in the course of the disease in more aggressive subclones that signify the transition to secondary AML (Murphy et al., 2013; Takahashi et al., 2013).

3.2.6 TP53

The single most frequently mutated tumor suppressor gene amongst all human cancers is *TP53* (Lawrence et al., 2014). In the clinical condition known as the Li-Fraumeni syndrome, humans are born with a single mutant allele of *TP53* (Li and Fraumeni, 1969). These people have a drastically increased risk for many types of cancer, including MDS and AML. In patients with MDS, somatic mutations of *TP53* are usually associated with low platelet counts, a high blast count, a complex karyotype and previous chemotherapy exposure. Initially thought of as a tumor promoting oncogene (hence the name, tumor protein 53), the groups of Vogelstein and Levine revealed its role as a tumor suppressor gene (Baker et al., 1989; Finlay et al., 1989).

p53 has been dubbed the "guardian of the genome" (Lane, 1992) given that it functions as a mediator of cellular stress. In the appropriate context, p53 increases pro-apoptotic genes and mediates cell cycle arrest (Lowe et al., 2004). Accumulation of additional genomic aberrations and chromosomal instability together with chemotherapy resistance observed in patients with inactivation of the p53 pathway are likely due to inadequate activation of the DNA damage response and loss of cell cycle arrest measures, respectively .

Several earlier reports had demonstrated the negative prognostic impact of *TP53* mutations in a number of hematological malignancies, including MDS (Horiike et al., 2003; Mori et al., 1995; Nakai et al., 1994; Preudhomme et al., 1994; Sugimoto et al., 1993). Mainly seen in high-risk and secondary (therapy-related) MDS (Adamson et al., 1995; Christiansen et al., 2001; Kaneko et al., 1995; Kita-Sasai et al., 2001; Kitagawa et al., 1994; Lai et al., 1995; Ludwig et al., 1992; Side et al., 2004), TP53 mutations occured frequently in the context of complex karyotypes, and have also been associated with disease progression and poor response to therapy (Padua et al., 1998; Wattel et al., 1994). In lower-risk MDS, on the other hand, TP53 mutations were rarely observed (Fidler et al., 2004; Iwasaki et al., 2008). However, these studies suffered from small sample size and the inherent low sensitivity associated with usage of conventional Sanger sequencing techniques. Using sensitive deep sequencing, our group previously reported *TP53* mutations in approximately 20% of low-risk del(5q) MDS patients (Jädersten et al., 2011). These mutations were detected years before disease progression and conferred an increased risk of evolution to overt leukemia.

3.3 CLONAL HEMATOPOIESIS

Cancer is now known to result from the stepwise accumulation of somatic mutations. However, it has been difficult to define the initial stages preceding the development of overt hematological malignancies.

Recently, three studies provided evidence for somatic mutations in blood DNA by using existing datasets of exome sequencing on peripheral blood samples from collectively more than 30,000 patients without known hematological cancers (Genovese et al., 2014; Jaiswal et al., 2014; Xie et al., 2014). While somatic mutations are rare (less than 1%) in people under 40, they increase with age, with frequencies up to 10% and 20% in patients over the age of 65 and 90, respectively. The acronym CHIP for Clonal Hematopoiesis of Indeterminate Potential has been adopted to describe this phenomenon (Steensma et al., 2015). Individuals with detectable mutations usually had only a single mutation. The majority of driver mutations sustaining clonal hematopoiesis occurred was explained by mutations in only three genes: *DNMT3A*, *TET2* and *ASXL1*. Additional recurrently mutated genes were: *JAK2*, *TP53*, *SF3B1*, *CBL*, *SRSF2*, *GNAS*, *PPM1D*, *BCOR*, and *BCORL1*. Driven by usually one of these somatic mutations, a single founding cell expands to a detectable clone.

After the initial hit occurring in a single stem cell there seems to be a substantial expansion of the formed clone as median clone size was approximately 18% of the cells in the peripheral blood. However, the large clone size observed in this study is related to the sensitivity of the method used for mutation detection. Studying clonal hematopoiesis by deep targeted sequencing (coverage >1000x) identified a clone size of 3% (McKerrell et al., 2015).

While previous studies had demonstrated that certain chromosomal translocations were transiently detected in healthy individuals (Müller et al., 1995), somatic mutations in clonal hematopoiesis seem to persist for years with no indication of spontaneous resolution. In a limited number of patients were follow-up analysis was possible, all somatic mutations persisted after 4-8 years (Jaiswal et al., 2014). Clonal hematopoiesis was a strong predictor for the development of hematological malignancy (Hazard ratio of ~12), confirming that the initial mutation forms a pre-malignant state. Direct evidence for the evolution from clonal hematopoiesis to hematologic malignancy was possible for two people who developed AML as the leukemic samples were found to contain the somatic mutations at high allele fractions (Genovese et al., 2014).

Clonal hematopoiesis is now viewed as part of the natural evolution from a pre-malignant state to overt leukemia (Ding et al., 2012b; Jan et al., 2012; Sperling et al., 2017), shown in Figure 2.

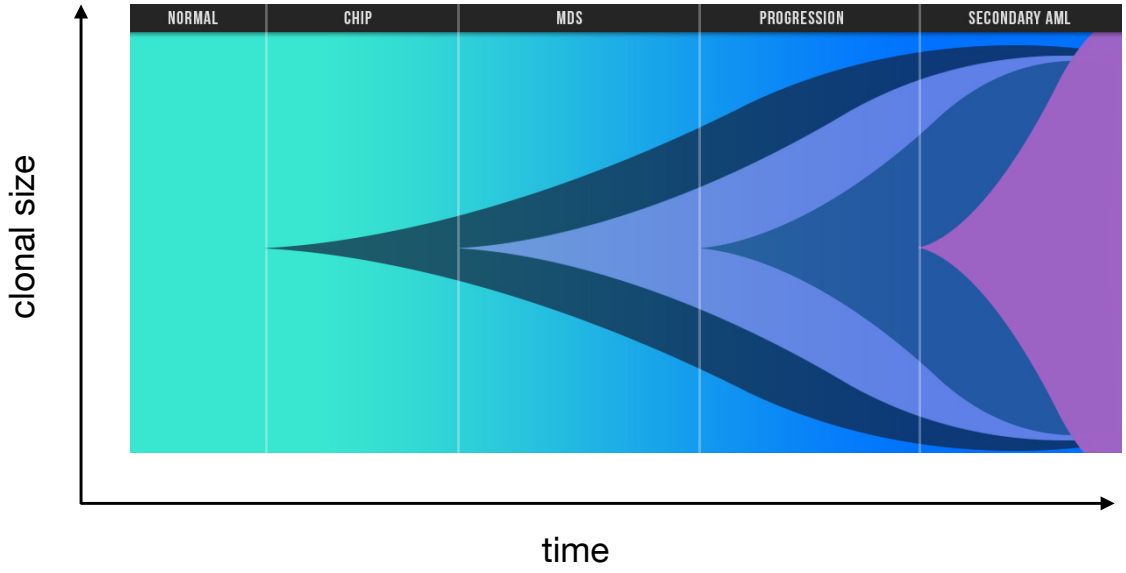

Figure 2. The natural evolution from normal hematopoiesis to the pre-malignant state of CHIP to MDS that progresses to overt secondary AML.

4 CLONAL EVOLUTION AND CLONAL ORIGIN

4.1 CLONAL EVOLUTION OF THE DISEASE

During cancer progression malignant cells are faced with genetic and epigenetic, as well as microenviromental selective pressure. The reiterative process of clones that expand, diversify genetically and subsequent selection of clones leads to the increased heterogeneity of the primary cancer. In a landmark article, Peter Nowell suggested that cancer followed the example of evolution by natural selection as originally proposed by Darwin (Darwin, 1859; Nowell, 1976). He hypothesized that cancers evolve over time by the stepwise acquisition of genetic variation. The term clonal evolution describes this increase in complexity during the progression of cancer. For a more in-depth discussion of these important concepts the reader is referred to the fascinating articles by Greaves and Maley (Greaves, 2010; Greaves and Maley, 2012).

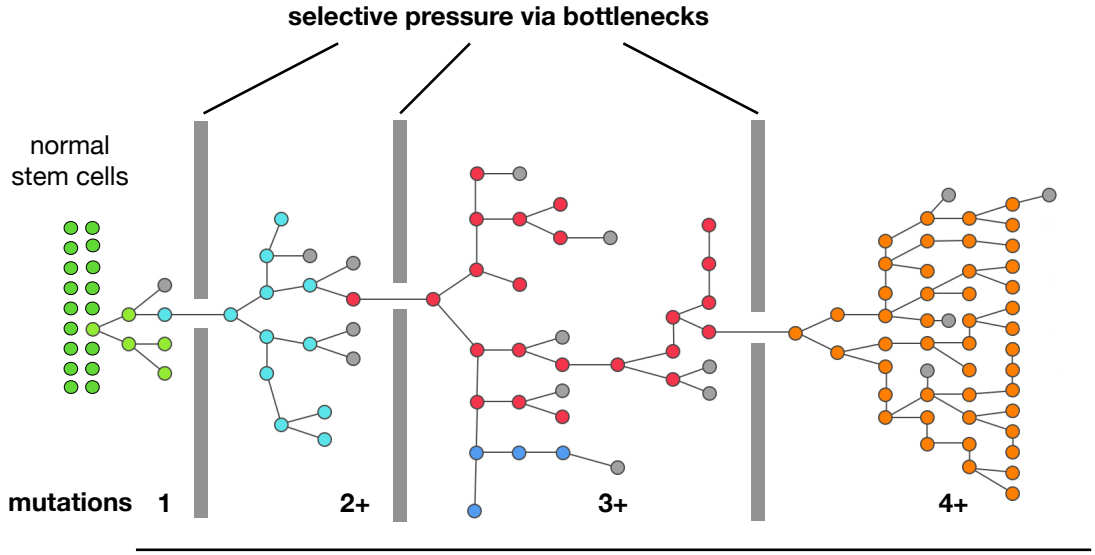

Figure 3. Clonal evolution is influenced by selective pressure from constraints ("bottlenecks") of intracellular events (genetic and epigenetic), the microenvironment or therapy. *Original concept from* (Nowell, 1976) *and adapted from* (Greaves, 2010).

4.1.1 The “good-old days” - the pre-sequencing era

The concept that evolution of MDS occurs by clonal expansion at the expense of normal progenitors was formulated by Jacobson et al. based on experiments involving G6PDH isoenzyme analysis and cytogenetic studies in cultured CFU-C. They found that the proportion of cells expressing type A G6PDH correlated with a proportional decline of karyotypically normal metaphases (Jacobson et al., 1978).

Studies using sequential cytogenetic analysis demonstrated that clonal evolution correlated with phenotypic evolution. The term clonal evolution is used in the sense of acquisition of additional karyotypic abnormalities. Several studies demonstrated that evolution of karyotypic abnormalities conferred an increased risk of transformation towards leukemia, with complex karyotypes, i.e., greater or equal to 3 karyotypic abnormalities within the same clone, being associated with the greatest risk (Anderson and Bagby, 1982; Ayraud et al., 1983; Gold et al., 1983; Nowell, 1982; Streuli et al., 1980; Yunis et al., 1988).

By comparison, secondary MDS usually progresses rapidly to AML and showed a predominance of hypodiploid clones with a complex karyotype (Pedersen-Bjergaard et al., 1984; Rowley et al., 1981). Interestingly, no fixed cytogenetic abnormality could be specifically associated with leukemic transformation. Hence, it appears that a common phenotypic endpoint can be reached via a broad variety of chromosomal rearrangements.

Importantly, evolution of karyotype was found to be neither necessary nor sufficient for leukemic progression, as evolution of karyotype was also observed in non-leukemic progression of phenotype with both hypoplastic and myeloproliferative states as possible outcomes in MDS (Mufti et al., 1982; Tomonaga et al., 1984). Conversely, progression to leukemia had also been demonstrated to occur in the absence of any chromosome rearrangement, indicating that karyotypic changes - while sometimes sufficient - are not necessary for development of leukemia (Tricot et al., 1985).

4.1.2 The genomic days

Modern day genomics have confirmed cancer to be a complex, adaptive system following Darwinian principles. It appears that most mutational processes act at the level of the DNA sequence. While in principle being purposeless genetic variation, mutations alter the cell’s fitness which underlies the selection of clones. By using a car-analogy, mutagenic lesions are classified as either ‘driver’ or ‘passenger’ depending on whether or not a relevant role in oncogenesis has been ascribed to the gene.

By performing whole-genome sequencing of paired samples from bone marrow and skin in secondary AML patients, Walter and coworkers identified somatic mutations specific to secondary AML (Walter et al., 2012). Examination of bone marrow from the antecedent MDS stage revealed that founding clones with 180-660 mutations persisted at the secondary AML stage. Acquisition of additional, new mutations led to the formation of subclones

containg an ever-increasing number of mutations. Similar to what has been observed for de novo AML (Welch et al., 2012), the majority appeared to be passenger mutations randomly acquired during aging with no apparent causal role in MDS pathogenesis.

In a follow-up study of 157 patients (Walter et al., 2013), 83% had at least one cytogenetic aberration or somatic mutation with 17 genes being significantly mutated. A founding clone and at least one subclone was observed in all cases. No single gene was found to be mutated exclusively in a founding clone or subclone. Thus, reiterating what had been observed by cytogenetic studies in the 1980s, these modern-day genomic approaches suggest that many genetic paths can lead to the initiation of MDS.

By using whole genome sequencing and transplantation into immunocompromised mice, Klco and colleagues demonstrated that genetic clonal architecture in AML correlated with the functional heterogeneity (Klco et al., 2014). In general, the patient-specific clonal architecture of the bone marrow was well represented in the patients' peripheral blood. Relapsing AML subclones could not be predicted by engraftment or recurring AML mutations. Moreover, the finding that the clonal architecture was not recapitulated in the xenografts suggested two important conclusions. Firstly, leukemic stem cells are likely also genetically heterogenous and secondly, the selective growth pressure that cells encounter in the microenvironment of the patient can not be approximated by xenotransplantation into murine microenvironments.

Previous data from our own group in del(5q) patients had shown clonal heterogeneity related to small TP53-mutated subclones which predicted for an unfavourable outcome and that these subclones expanded with progression of the disease (Jädersten et al., 2011; 2009).

5 PATHOGENESIS OF THE 5Q-SYNDROME

In 1974 van den Berghe et al described three patients with the clinical features of refractory anemia characterized by erythroid hypoplasia, megakaryocytes with hypolobated nuclei and normal to elevated platelet counts (Van den Berghe et al., 1974). All three patients had an interstitial deletion of the long arm of chromosome 5. After identification of the Philadelphia chromosome with the typical translocation of t(9;22), the sole deletion at 5q became the second example of a chromosomal aberration linked to a particular malignancy. The 5q-syndrome has been acknowledged as a distinct entity ever since the first iteration of the WHO classification of MDS.

The 2016 WHO classification of MDS uses the identification off del(5q) or del(5q) plus one other cytogenetic abnormality with the exception of -7/del(7q) in the definition of the 5q-syndrome (Arber et al., 2016). It is recognized as a distinctive type of MDS with the typical patient being an older woman. The female predominance of 70% is in contrast to the male predominance in other forms of MDS. Median age at diagnosis is between 65-70 years of age. Typically, patients present with a macrocytic anemia, normal or elevated platelet counts and an absence of significant neutropenia.

It has now been established that del(5q) leads to haploinsufficiency of a small number of genes (*RPS14, CSNK1A1, APC, HSPA9, DIAPH1, DDX41, EGR1, NPM1, TIFAB*) and two micro-RNAs (miR-145 and miR-146a). While point mutations in the remaining normal allele have been found in *CSNK1A1* or *DDX41*, no other genes has been found to be deleted or mutated.

In a seminal study using targeted short hairpin RNA (shRNA), *RPS14* haploinsufficiency was found to lead to abnormal erythroid differentiation via blocking ribosomal RNA processing (Ebert et al., 2008). Subsequently, mouse models with conditional heterozygous inactivation of Rps14 phenocopied the macrocytic anemia seen in the 5q- syndrome (Barlow et al., 2010). This effect was shown to be p53 dependent and involve upregulation of components of innate immune signaling.

An important gene central to the pathogenesis of the 5q-syndrome is the serine-threonine kinase *CSNK1A1* (Schneider et al., 2014). In *Csnk1a1*-heterozygously deleted cells, expression of the gene product, i.e., CK1a, is approximately 50% and this leads to increased stem cell self-renewal via upregulation of the WNT pathway. In contrast, homozygous deletion of *Csnk1a1*, leads to accumulation of p53 with loss of the competitive advantage observed in the heterozygous state. The gene-dosage effect of CSNK1A1 is central to the understanding of the treatment response to lenalidomide in MDS with deletion of 5q. CK1a can be targeted for degradation by the ubiquitin ligase CRL4 upon interaction with its co-factor CRBN. In the absence of lenalidomide, the affinity for CK1a is low. The binding of lenalidomide, on the other hand, results in a conformational change in CRBN increasing its affinity and thereby targeting CK1a for degradation via the ubiquitylation pathway (Krönke et al., 2015). The lower expression level of CK1a in del(5q) HSC lacking one copy of

CSK1A1 leads to a competitive advantage over normal HSC. Treatment with lenalidomide decreases CK1a levels in both normal and 5q-deleted HSC. While CK1a-levels drop below a critical threshold optimal for survival in *CSKN1A1*-heterozygous cells, wt-*CSNK1A1* cells prevail and gain the competive advantage.

The observation that p53 is somehow pathogenetically linked to deletion of chromosome 5q has recently been confirmed by a number of studies. The combined genetic approach of next-generation sequencing and mass spectrometry-based genotyping reported *TP53* as one of several predictors of poor survival in a large cohort of MDS patients (Bejar et al., 2011). Intriguingly, *TP53* mutations seem to be particularly frequent in MDS and AML with complex karyotypes including del(5q) and are often associated with a short survival (Kulasekararaj et al., 2013; Sebaa et al., 2012). In multivariate analyses of secondary AML, *TP53* was found to be an independent adverse prognostic factor for overall survival (Milosevic et al., 2012). Zemanova et al. reported del(5q) to be unstable and associated with different types of cryptic unbalanced chromosomal rearrangements (Zemanova et al., 2014). Recent mouse models suggest that loss of *Tp53* activity in the context of haploinsufficiency of the del(5q) genes Apc and Egr1 are sufficient for malignant transformation into AML (Stoddart et al., 2014). Several lines of evidence now suggest that the complete clinical phenotype of del(5q) MDS is the result of combinations of several genes (Graubert et al., 2009).

6 THE HEMATOPOIETIC MICROENVIRONMENT IN MDS

The ordered differentiation of distinct cellular entities of the complete hematopoieitic hierarchy are influenced by a variety of supportive microenvironmental niches. The cellular components of these niches are unique to the differentiation stage of the hematopoietic cells. An emerging model suggests that hematopoietic and mesenchymal cells are intertwined in a symbiotic relationship, each at particular stages in their respective differentiation (Morrison and Scadden, 2014). Distinct components of each niche support the maintenance, proliferation and differentiation of HSCs. Related to the finding that HSCs constitute the cell of origin in MDS, a central question has been which niche cells influence HSCs. Some niche cells are spawned descendents from HSCs themselves, e.g., macrophages and megakaryocytes (Bruns et al., 2014; Winkler et al., 2010; Zhao et al., 2014). In addition, various stromal cells such as vascular endothelial cells lining the sinusoids, osteoblast, adipocytes and mesenchymal stem cells have all been shown to function as niche cells in the microenvironment of HSC by altering their function and differentiation (Calvi et al., 2003; Ding et al., 2012a; Kfoury and Scadden, 2015; Méndez-Ferrer et al., 2010; Zhang et al., 2003).

In mouse models the selective disruption of certain genes only in certain stromal elements was sufficient to induce abnormal differentiation of HSC and dysplastic features (Raaijmakers et al., 2010; Walkley et al., 2007). On the other hand, myeloid neoplasms such as CML have been shown to alter the bone marrow microenvironment (Schepers et al., 2013). While it is possible to induce MDS-like disease in mouse models solely by stromal defects, it remains to be investigated whether or not human MDS is caused by defects confined to the microenvironment or whether the intrinsic genetic and epigenetic alterations of HSCs in MDS are necessary constituents of the human disease.

7 AIMS OF THIS THESIS

The overall aims of this thesis were to characterize hematopoietic stem cells and the microenvironment from MDS patients with deletions of chromosome 5q and to elucidate mechanisms underlying disease progression and response to treatment.

Specific aims were as follows:

I. To elucidate the nature of the disease-initiating cell

II. Determine which mutations are associated with disease progression of lower-risk patients with deletion of chromosome 5q

III. To study putative abnormalities in the microenvironment of patients with del(5q) MDS

For details about the protocols of the experiments, the reader is referred to the material and methods section of the individual papers.

8 SUMMARY OF RESULTS & DISCUSSION

8.1 CHARACTERIZATION OF STEM AND PROGENITOR CELLS IN DEL(5Q) MDS (PAPER I AND II)

One key information for devising new therapies is which cells to target. Whether cancer cells are stochastically or hierarchically organized is fundamental to therapeutic targeting. Previous studies had suggested that the dysplastic cells originate from transformed normal hematopoietic stem cells (Nilsson et al., 2000). The concept of human cancer stem cells rests on the existence of rare self-renewing stem cells capable of spawning descendents which are functionally and molecularly distinct and unable to form new tumors. If the hierarchical relationship between stem cells and progenitors is conserved in MDS, one could potentially use acquired somatic mutations as genetic tools by interrogating which candidate stem and progenitor populations harbour the somatic mutations found in bulk MDS bone marrow.

We used flow cytometric cell sorting to prospectively isolate stem and progenitor cells from low and intermediate-1 risk MDS patients, both with (paper II) and without (paper I and II) deletion of 5q. We observed phenotypically distinct stem and progenitor cell compartments similar to the normal hematopoietic hierarchical organization found in healthy bone marrow. Given that the flow cytometric profiles resembled those of normal controls, it was important to determine whether these stem and progenitor populations were clonally involved MDS cells or residual normal cells. Using flourescence in situ hybridisation we found that the prospectively isolated stem and progenitor cells were clonally involved. In order to rule out that aberrant expression of cell surface markers had compromised the fidelity with which these populations could be identified prospectively, we sought to characterize these populations functionally and molecularly (paper I). Colony-forming assays in methylcellulose confirmed that GMPs and MEPs exclusively gave rise to myeloid and erythroid colonies, respectively. Using long-term culture CFU-assays and xenotransplantation in NSG-mice we assessed whether the hierarchical organization is conserved in MDS. Only the lin-CD34+CD38-CD90+CD45RA- stem cells could give rise to CFUs after long-term culture and were able to engraft immunocompromised mice. We found no evidence of engraftment of any downstream progenitor population in the investigated patients, suggesting that self-renewal is confined to MDS stem cells. Consistent with the functional evaluation of the different subsets, the gene expression signatures of stem cells, GMPs and MEPs were similar to their respective counterpart in healthy bone marrow.

We also investigated the impact of lenalidomide therapy in del(5q) patients. Longitudinal samples from del(5q) patients treated with lenalidomide demonstrated that that the distribution of HSC and multipotent progenitors remained similar to healthy controls, irrespective of whether the sample was taken at diagnosis or during lenalidomide treatment (paper II). Notably, we observed that the frequency of GMP at diagnosis was significantly suppressed in del(5q) MDS with a concomitant increase in CMP (paper I and paper II). This finding in MDS patients with the deletion 5q is in line with findings reported from other studies of low-risk MDS (Pang et al., 2013; Will et al., 2012) and thus appears to be not

confined to del(5q) MDS. Importantly, upon treatment with lenalidomide, the frequencies reverted to their normal distribution (paper II). In a small number of patients (n=5) who progressed to leukemia, we had access to longitudinal samples. This allowed us study the kinetic changes in stem and progenitor cell subsets within the same patient over time, both during treatment and progression of the disease. The only common finding was that one predominant subset expanded prior to progression, the type of subset, however, varied from patient to patient (paper II). Assessment of del(5q) clonal size in sorted stem and progenitor cells as well as bulk bone marrow demonstrated persistence of del(5q) during lenalidomide treatment and despite the fact that several of the patients exhibited a complete clinical response to lenalidomide. The only patient who initially showed complete clearance of del(5q) from the mononuclear cell compartment of bulk bone marrow, i.e., the compartment that is analyzed in clinical routine, had minimal involvement in all myeloid progenitors. In contrast, more than half of the lin-CD34+CD38-CD90+CD45RA- stem cell compartment remained part of the del(5q) clone. These findings are in line with a previous report suggesting a selective resistance of del(5q) HSC to lenalidomide treatment (Tehranchi et al., 2010).

8.2 MINING THE MUTATIONAL LANDSCAPE OF MDS FOR MARKERS OF CANCER STEM CELLS AND DISEASE PROGRESSION (PAPER I AND II)

One inherent shortcoming of previous studies of cancer stem cell function is the assessment outside of the cells' natural habitat. Prior to this study, the existence of human cancer stem cells has not been demonstrated in the patients themselves. The concept had however been demonstrated in murine models by fate-mapping using genetic lineage-tracing (Chen et al., 2012; Schepers et al., 2012).

We sought to exploit the fact that the vast majority of MDS cases harbour somatic mutations and used these mutations in a fate-mapping approach. We employed both targeted sequencing of 84 genes previously implicated in myeloid malignancies as well as exome sequencing of unfractionated bone marrow. We then performed targeted sequencing of individually picked colonies derived from long-term clones of single cells and flow-sorted MDS stem and progenitor populations. Our finding of all detected mutations being acquired in the MDS stem cells demonstrated that only these cells self-renew in the patients provided that only self renewing cells can give rise to stable mutations. If mutations were only detectable in downstream progenitors, this would indicate that these cells had re-acquired the ability to self-renew - a feature that is normally lost during lineage commitment. Overall, the mutational landscape in del(5q) MDS patients appeared to be relatively stable, while additional mutations were identified in two other patients who later progressed to AML. Importantly, the acquisition of new mutations pre-dated clinical signs of progression. We were able to elucidate the chronological order in which these mutations were acquired by performing targeted sequencing of whole-genome-amplified single-cell colonies derived from long-term cultures of single MDS stem cells. Both patients had acquired recurrent mutations

in *TP53* prior to clinical signs of disease progression, e.g., an increase in blasts. One patient was found to have an additional putative passenger-mutation in *PIFO* at a high frequency which was confined to MEP-progenitors and absent from the stem cell compartment. Strikingly, the MEP-compartment was significantly expanded, suggesting that these MEPs had reacquired the ability to self-renew. This observation is in line with current models of leukemic evolution in AML where increased clonal off-branching is observed (Ding et al., 2012b; Walter et al., 2012; Welch et al., 2012).

An additional curious finding from this study was that somatic mutations were acquired in cells that already had the del(5q) abnormality present. A notable exception to this were patients with ringsideroblasts; in these patients, *SF3B1* mutations preceded the acquisition of del(5q). Taken together, the experiments in paper I provide definitive evidence that rare human cancer stem cells exist in vivo. This finding has broad biological implications for our understanding of cancer biology and clinical implications by specifically targeting the MDS stem cell population.

The molecular mechanisms why certain del(5q) MDS patients progress have not been elucidated. Therefore, we are currently unable to predict which patients are likely to progress. Jädersten et al. previously found that the presence of small *TP53*-mutated subclones predict adverse outcome and demonstrated that these subclones expand with progression of disease (Jädersten et al., 2011). In addition, the advent of next-generation sequencing methodologies has demonstrated that the majority of MDS patients harbour recurrent somatic mutations in a number of myeloid candidate genes (Bejar et al., 2011; Haferlach et al., 2014; Papaemmanuil et al., 2013; Yoshida et al., 2011). As several of these genes are associated with clinical outcome, we hypothesized that the acquisition of certain somatic mutations might help explain disease progression in del(5q) MDS. We used our cohort of longitudinal patient samples to study whether certain mutations are associated with disease progression. The vast majority of patients (84%) were found to harbour at least one recurrent mutation. The presence of mutations did not correlate with other clinical parameters, e.g., blood counts, age, other cytogenetic aberrations. Of note, the mutational landscape in a pure del(5q) cohort differed from low-risk MDS in general; splicing gene mutations occured less frequently and overall the mutational spectrum resembled that of higher-risk MDS patients. While 22 patients in our cohort were treated with lenalidomide, 13 were not. We did not detect any difference between these two groups in terms of number or type of mutations. At diagnosis, about one-third had no mutation and the presence of one or more mutation did not predict progression. However, absence of mutations was suggestive of freedom from progression.

Thirteen patients progressed to high-risk MDS or leukemia at a median of 85 months after diagnosis. Conspicuously, progression was associated with the detection of a finite subset of new mutations, namely *TP53, TET2*, and *RUNX1*. Two single cases harboured mutations in *SF3B1* and *PTPN11* but due to the low number no significant conclusion could be drawn from this. Of 9 patients who progressed to AML, 7 had mutations in *TP53*, which were present in the earliest sample in one case and acquired in the remaining six cases. Of note,

five of the six patients had been analyzed previously by deep sequencing and found to have no mutation at diagnosis, further arguing that these mutations had been acquired along the way. Immunohistochemical detection of p53 was strongly correlated with detection of mutation of *TP53* by targeted sequencing, confirming our earlier findings (Saft et al., 2014). We detected mutations in *RUNX1* and *TET2* in the remaining two patients with leukemic progression; these mutations were present at both time-points at which these patients were sampled. Patients who progressed to higher-risk MDS were all found to carry mutations in *TET2*. Remarkably, testing positive for any of three mutations (*TP53*, *TET2*, *RUNX1*) conveyed a high probability of progression (13/16, 81%), regardless of whether or not the mutations were present at diagnosis or acquired subsequently. An important finding was that the new mutations were detected well before signs of clinical progression occured; the median time was 42 months from mutation detection to clinical evidence of progressive disease.

Our finding that the frequency of mutation is higher in later samples than at diagnosis is in line with a model where initial mutations occur in HSC leading to clonal hematopoiesis which might develop into MDS. Acquisition of additional mutations then leads to disease progression. The fact that several patients showed increased allele burdens and acquisition of new mutation during treatment and natural course of the disease suggest that clonal evolution is frequent event in patients with lower-risk del(5q) MDS. Of 13 patients with progressive disease, 12 were treated with lenalidomide. *TP53* was the major culprit and identified in 7 of 9 patients who developed leukemia. Since normal function of p53 is required for apoptosis of erythroid cells due to haploinsufficiency of *RPS14*, this might help to explain the apparent propensity for del(5q) cells developing *TP53* mutations. In theory, treatment with lenalidomide might select for *TP53* mutations. In that regard it is interesting to note that some patients exhibited more than one *TP53* mutation. Furthermore, our study highlights the marked clonal heterogeneity and instability, likely explaining disease progression of lower-risk del(5q) MDS treated with lenalidomide. Curiously, compared to other MDS subtypes, isolated del(5q) in lower-risk MDS has generally been associated with low risk for leukemic transformation, while presence of del(5q) is associated with an adverse prognosis in either de novo AML or in MDS with complex karyotypes (Milosevic et al., 2012; Sebaa et al., 2012; Zemanova et al., 2014). *TP53* mutations migh help explain this observation.

While we observed *TP53* mutations in the majority of patients which progressed to leukemia or higher-risk disease, additional mutations were associated with progression or loss of treatment response. Mutations in RUNX1 were univariably associated with disease progression and observed in 3 of 13 patients. Due to allele burden we were able to exclude the possibility of *RUNX1* germline mutations. *RUNX1* has a well-documented association with poor prognosis in MDS as well as AML (Chen et al., 2007; Steensma et al., 2005; Tang et al., 2009). We observed mutations in *TET2* in 6 of 13 patients who showed signs of disease progression. Three patients were found to harbour mutations in both *TET2* as well as *TP53*; however, our data cannot provide evidence that this occured by independent mutational processes. *TET2* mutations are quite common in myeloid neoplasms in general. Their impact

in MDS is a contentious issue (Abdel-Wahab et al., 2009; Smith et al., 2010), while one study found *TET2* mutations to be associated with shorter survival in MDS patients undergoing hematopoietic cell transplantation (Bejar et al., 2014).

8.3 THE ROLE OF THE MICROENVIROMENT IN MDS WITH DELETION OF 5Q (PAPER II AND III)

The microenvironment provides input to all cells in the bone marrow, thereby regulating their maintenance, proliferation and differentiation. In the setting of hematological disease, changes in the bone marrow microenvironment can be the consequence of chronic pertubation of bone marrow homeostasis or the neoplastic clone actively instigating a remodelling towards a cancer-supportive microenvironment (Li and Neaves, 2006; Raaijmakers, 2011; Schepers et al., 2013; Vener et al., 2008; Walkley et al., 2007). While it is generally believed that the inability to produce mature progeny has mainly intrinsic reasons, i.e., due to defects in the hematopoietic stem and progenitor cells themselves, we sought to determine to what extent the microenvironment might contribute. To this end, we cultured mesenchymal stromal cells from untreated del(5q) patients as well as healthy volunteers. Comparing their gene expression profiles, we confirmed the absence of expression of hematopoietic genes, suggesting that our cultures were not contaminated by remaining hematopoietic cells, i.e., macrophages, a finding that can lead to erroneous conclusions and has led to significant debate in the past (Ramakrishnan et al., 2006; Simmons et al., 1987). Fluorescence in situ hybridisation analysis on del(5q) stromal cells failed to detect the clonal del(5q) abnormality above background, suggesting negligible contamination by macrophages. Our studies of MSC from a pure cohort of low to intermediate-1 risk patients with deletion of 5q are in line with previous findings that the marrow stroma is not derived from the malignant clone in MDS (Ramakrishnan et al., 2006).

MSC cultures from untreated del(5q) MDS patients as well as healthy volunteers expressed gene signatures typical for MSC, albeit no genes were expressed in a statistically significant manner (paper II), not even when specifically examining genes that had previously been implicated in the interaction of niche cells with hematopoietic stem cells (Méndez-Ferrer et al., 2010). While seemingly at odds with recent reports of stromal abnormalities in multiple subtypes of MDS (Geyh et al., 2013; Medyouf et al., 2014), our studies confirm earlier studies by other groups who found the stromal abnormalities to be reversible and MDS stroma capable of supporting normal hematopoiesis in vitro (Deeg et al., 2000; Soenen-Cornu et al., 2005).

As *in vitro* cultures are by nature artificial approximations and hence might fail to detect abnormalities, we examined bone marrow biopsies in del(5q) MDS patients before and during lenalidomide treatment and compared them to healthy controls. We observed a significantly higher microvessel density (MVD) in del(5q) MDS compared to normal controls. Lenalidomide treatment, at least during the initial phase, decreased microvessel

density in all patients analyzed. Upon therapeutic failure, microvessel density increased in 4 of 5 patients and was paralleled by a concomitant increase in bone marrow cellularity. We also observed that lenalidomide decreased the number of CD68+ macrophages which mirrored a decrease in cellularity. However, we failed to detect an increase in macrophages in del(5q) patients prior to treatment compared to controls. Based mainly on murine but also some human studies, several surrogate markers for mesenchymal stem cells have been put forward, e.g. nestin, CD271, CD146. Analysis of staining patterns for these markers demonstrated labeling confined to rare perivascular mesenchymal cells, e.g., endothelial and advential sinusoidal cells. The staining pattern we observed is consistent to what has been reported from murine and human studies. Importantly, we were unable to detect quantitative differences in expression patterns for any of these markers between del(5q) MDS and normal bone marrow. While these findings do not formally rule out any microenvironmental irregularities, they demonstrate that - based on the MSC markers tested - lenalidomide does not exert its main therapeutic effect by reshaping the cellular composition of the microenvironment. Our data in patients with del(5q) MDS neither suggests that the microenvironment constrains healthy hematopoiesis. However, the issue whether the microenviroment is perturbed or merely a bystander has been a heavily contentious issue in the literature, not only for MDS but for myeloid diseases in general (Deeg, 2002; Ferrer et al., 2013; Medyouf et al., 2014; Raaijmakers, 2011; Raaijmakers and Scadden, 2008; Schepers et al., 2015). Some of the conflicting findings are probably explained by the heterogeneity of the diseases studied, particularly in MDS. In order to draw relevant conclusions, we propose to study carefully selected subgroups of patients with closely matched clinical characteristics, similar to what we have done in low to intermediate-1 del(5q) MDS.

Lenalidomide produces complete cytogenetic remission, eradicating most mature and progenitor del(5q) cells. However, the rare disease-sustaining MDS stem cells fail to be eradicated during lenalidomide therapy. These resistant cancer stem cells are the probable reason for why the majority of patients relapse (Fenaux et al., 2011; Giagounidis et al., 2012; Saft et al., 2014; Tehranchi et al., 2010). We hypothesized this resistance to be the cause for continued accrual of somatic mutations in the stem cell compartment. Whether the resistance is confined to stem cells only or extends to other populations is unknown.

Abnormal megakaryocytes with hypolobated nuclei are one of the hallmarks of del(5q) MDS. As megakaryocytes have recently been implicated as important niche cells in the regulation of HSC (Bruns et al., 2014; Zhao et al., 2014), we hypothesized that these cells might have potential implications for HSC responding to lenalidomide treatment. Therefore, we sought to study megakaryocytes as a component of the non-mesenchymal niche in MDS and to determine the response of abnormal megakaryocytes with hypolobated nuclei to lenalidomide therapy.

Examining the bone marrows of 22 del(5q) patients we observed the persistence of the pathognomonic megakaryocytes with hypolobated nuclei in all patients, despite lenalidomide leading to complete clinical responses. As clinical response might not correlate with complete

cytogenetic remission, we investigated a subset of 21 patients from the MDS-004 study. Four patients had available biopsies at time of complete cytogenetic remission. The del(5q) abnormality was completely absent in 3 patients at 12 weeks post-randomization, and at 48 weeks in the remaining patient. In all 4 cases, megakaryocytes with hypolobated nuclei persisted. We were able to validate these findings in our own cohort of 22 consecutive lenalidomide-treated del(5q) MDS patients seen at Karolinska University Hospital. In all patients that had biopsy material available at time of CCyR, we were able to clearly identify megakaryocytes with hypolobated nuclei. Furthermore, we provide evidence that the pathognomonic nuclear hypolobation is directly associated with the clonal del(5q) aberration. Irrespective of nuclear content, megakaryocytes of 2N, 4N, 8N or 16N harboured del(5q), suggesting that the feature of hypolobation is not correlated to nuclear content. This is in line with findings from murine models of del(5q) MDS (Barlow et al., 2010).

Given that megakaryocytes are rare and readily identifiable on routine morphology, we propose that focussing on megakaryocytes is a viable strategy for evaluating patients for long-term remission and where discontinuation of lenalidomide is considered. Remarkably, one of our patients who exhibited complete hematological remission 2 years after discontinuation of lenalidomide, demonstrated clonal involvement in 2 of 23 megakaryocytes.

Our findings suggest that the entire megakaryocyte lineage from HSC to MEP to megakaryocytes might be resistant to treatment with lenalidomide. Whether or not this resistance might help explain the high relapse-rate of lenalidomide-treated patients remains an open question. Of note, HSC primed for differentiation towards the megakaryocytic lineage have been shown to reside at the apex of the HSC hierarchy (Sanjuan-Pla et al., 2013) and there is evidence for fast-track differentiation from HSC via MEP to megakaryocytes (Haas et al., 2015; Yamamoto et al., 2013). We believe this to underlie the high-degree of lenalidomide resistance in the megakaryocytic lineage as a whole and entertain the notion that HSC and megakaryocytes can favour each other's existence in a symbiotic manner.

9 CONCLUDING REMARKS & FUTURE DIRECTIONS

We have demonstrated that a fate-mapping approach based on tracing somatic mutations back to prospectively isolated cell populations is a viable strategy for identifying cancer-initiating cells. Similar approaches should prove informative in a wide variety of malignancies. In the case of del(5q) MDS, we have shown that the disease-driving cell is the normal hematopoietic stem cell where somatic mutations are accrued. An obvious explanation for this is that HSC are the only cells where the transcriptional machinery is rigged for self-renewal; all downstream progenitors would need to reactivate the self-renewal machinery first - otherwise, mutations in these populations remain transitory events. Of note, specifically in the case for MDS with deletion of 5q, we observed the acquisition of somatic mutations after the deletion of 5q had occurred, arguing that progression of the disease results from the accumulation of additional mutations in the stem cell compartment.

Our findings in paper II extended these observations and demonstrated that patients with lower-risk del(5q) have a high tendency towards clonal evolution and that this is clearly linked to progression, particularly in patients treated with lenalidomide. This is in contrast to lenalidomide-treated MDS patients without deletion of 5q, where the rate of clonal evolution is much lower (Chesnais et al., 2016).

Our longitudinal data clearly indicate that progression results from an increase in the mutational burden over time and is thus unlikely to be predicted by mutational profiling solely at diagnosis. While mutations in TP53 detected at diagnosis have been shown to predict disease progression even in low-risk MDS (Jädersten et al., 2009; 2011), our longitudinal follow-up of these patients in paper II demonstrated the emergence of TP53 mutations shortly before time of progression, arguing that these are acquired rather than present at undetectable levels at diagnosis. Intriguingly, the patients who progressed in our study showed a strong bias towards having been treated with lenalidomide. While this might be the result of a higher treatment incentive in patients with a less benign disease course, it is possible that lenalidomide exerts selective pressure on resistant driver subclones within the HSC compartment. Lenalidomide exerts its main therapeutic effects via decreasing the need for transfusion (List et al., 2006) and by a HSC-specific mechanism involving targeted destruction of CK1a via the ubiquitylation pathway (Krönke et al., 2015). Given that both the successful reversal of anemia and abrogation of the clonal advantage of del(5q) depend on intact TP53 (Barlow et al., 2010; Schneider et al., 2014), it seems likely that lenalidomide's therapeutic effect is dependent upon unmutated, wild-type TP53. The recent findings by Lode et al. confirm our observations, as these investigators reported that a statistically significant higher exposure to lenalidomide was observed in patients with progressive disease and specifically in patients with TP53 clonal evolution (Lodé et al., 2017).

Several centers are already using targeted sequencing panels to aid in the diagnosis of MDS - this will likely become part of the standard work-up of patients with suspected MDS over the next few years, both for aiding in and confirming the diagnosis and for determining prognosis. Similar to what has recently been published for AML (Papaemmanuil et al., 2016),

a large international effort is underway by the International Working Group for MDS Molecular Prognosis (MDS IWG-PM) in order to assess the value of mutation testing in risk stratification. Preliminary results (ASH abstract 2015) on approximately 2000 patients suggest that a mutation in any of the following genes, *TP53, RUNX1, NRAS, EZH2*, conferred high-risk for progression. This prognostic information was independent from the IPSS-R score. These findings suggest that mutational profiling can contribute critical information that allows the forecasting of the natural history of MDS in a particular patient. However, as shown in this body of work, patients are continually acquiring mutations during their disease course. We therefore suggest that assessment of mutational status be performed at regular follow-up visits, particularly in patients with *TP53* mutations.

Our flow cytometric analysis in papers I and II demonstrated that unless the patient showed clinical signs of progression, neither lenalidomide treatment nor the presence of mutations led to profound changes in the relative organization of stem and progenitor cell populations. Although the absolute size of these subsets are impossible to quantitate due to the inherent technical variability of the bone marrow draw (Pang et al., 2011), the lack of apparent relative expansions suggests that available niche space might constrain expansion to a certain extent. Thus, it seems likely, that certain particular driver mutations must be acquired leading to transformative events, e.g., remodeling of the microenvironment, to allow for expansion of transformed clones (Schepers et al., 2013; 2015). Our finding of the whole megakaryocytic branch of hematopoiesis being lenalidomide-resistant is important in this context (paper III). If human megakaryocytes regulate HSC pool size similarly to what has been reported in mouse models (Bruns et al., 2014; Zhao et al., 2014), it is conceivable that del(5q) megakaryocytes might favour del(5q) stem cells over normal ones. This hypothesis is easily testible in mouse models by specifically deleting the syntenic regions equivalent to human del(5q) (Barlow et al., 2010) in murine megakaryocytes. Perhaps, specifically targeting del(5q) megakaryocytes might offer a viable therapeutic modality for patients.

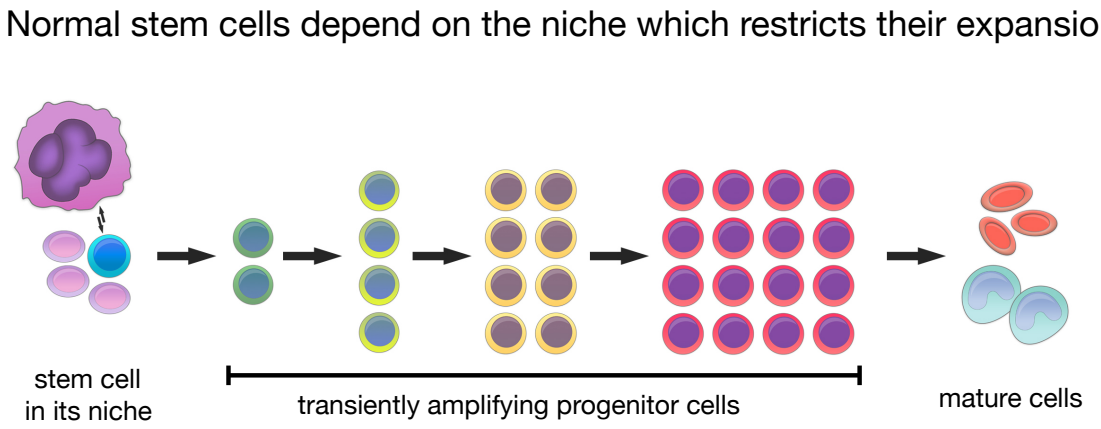

Figure 4. Model of the hematopoietic niche (mesenchymal and non-mesenchymal/hematopoietic) regulating and restricting the expansion of stem cells. Modified after (Lobo et al., 2007).

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11 REFERENCES

Abdel-Wahab, O., Adli, M., LaFave, L.M., Gao, J., Hricik, T., Shih, A.H., Pandey, S., Patel, J.P., Chung, Y.R., Koche, R., et al. (2012). ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. Cancer Cell *22*, 180–193.

Abdel-Wahab, O., Mullally, A., Hedvat, C., Garcia-Manero, G., Patel, J., Wadleigh, M., Malinge, S., Yao, J., Kilpivaara, O., Bhat, R., et al. (2009). Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood *114*, 144–147.

Abel, G.A., Efficace, F., Buckstein, R.J., Tinsley, S., Jurcic, J.G., Martins, Y., Steensma, D.P., Watts, C.D., Raza, A., Lee, S.J., et al. (2016). Prospective international validation of the Quality of Life in Myelodysplasia Scale (QUALMS). Haematologica *101*, 781–788.

Abel, G.A., Klaassen, R., Lee, S.J., Young, N.L., Cannella, L., Steensma, D.P., and Efficace, F. (2014). Patient-reported outcomes for the myelodysplastic syndromes: a new MDS-specific measure of quality of life. Blood *123*, 451–452.

Adamson, D.J., Dawson, A.A., Bennett, B., King, D.J., and Haites, N.E. (1995). p53 mutation in the myelodysplastic syndromes. Br J Haematol *89*, 61–66.

Ailles, L.E., and Weissman, I.L. (2007). Cancer stem cells in solid tumors. Curr. Opin. Biotechnol. *18*, 460–466.

Akashi, K., Traver, D., Miyamoto, T., and Weissman, I.L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature *404*, 193–197.

Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J., and Clarke, M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proceedings of the National Academy of Sciences *100*, 3983–3988.

Anderson, R.L., and Bagby, G.C. (1982). The prognostic value of chromosome studies in patients with the preleukemic syndrome (hemopoietic dysplasia). Leukemia Research *6*, 175–181.

Arber, D.A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M.J., Le Beau, M.M., Bloomfield, C.D., Cazzola, M., and Vardiman, J.W. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood *127*, 2391–2405.

Ayraud, N., Donzeau, M., Raynaud, S., and Lambert, J.C. (1983). Cytogenetic study of 88 cases of refractory anemia. Cancer Genet. Cytogenet. *8*, 243–248.

Baker, S.J., Fearon, E.R., Nigro, J.M., Hamilton, S.R., Preisinger, A.C., Jessup, J.M., vanTuinen, P., Ledbetter, D.H., Barker, D.F., Nakamura, Y., et al. (1989). Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Science *244*, 217–221.

Balasubramani, A., Larjo, A., Bassein, J.A., Chang, X., Hastie, R.B., Togher, S.M., Lähdesmäki, H., and Rao, A. (2015). Cancer-associated ASXL1 mutations may act as gain-of-function mutations of the ASXL1-BAP1 complex. Nat Commun *6*, 7307.

Barlow, J.L., Drynan, L.F., Hewett, D.R., Holmes, L.R., Lorenzo-Abalde, S., Lane, A.L., Jolin, H.E., Pannell, R., Middleton, A.J., Wong, S.H., et al. (2010). A p53-dependent mechanism underlies macrocytic anemia in a mouse model of human 5q- syndrome. Nat Med *16*, 59–66.

Baum, C.M., Weissman, I.L., Tsukamoto, A.S., Buckle, A.M., and Peault, B. (1992). Isolation of a candidate human hematopoietic stem-cell population. Proceedings of the National Academy of Sciences *89*, 2804–2808.

Becker, A.J., McCulloch, E.A., and Till, J.E. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature *197*, 452–454.

Bejar, R., Lord, A., Stevenson, K., Bar-Natan, M., Pérez-Ladaga, A., Zaneveld, J., Wang, H., Caughey, B., Stojanov, P., Getz, G., et al. (2014). TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood *124*, 2705–2712.

Bejar, R., Stevenson, K., Abdel-Wahab, O., Galili, N., Nilsson, B., Garcia-Manero, G., Kantarjian, H., Raza, A., Levine, R.L., Neuberg, D., et al. (2011). Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med *364*, 2496–2506.

Bennett, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A., Gralnick, H.R., and Sultan, C. (1982). Proposals for the classification of the myelodysplastic syndromes. Br J Haematol *51*, 189–199.

Bertoncello, I., Hodgson, G.S., and Bradley, T.R. (1985). Multiparameter analysis of transplantable hemopoietic stem cells: I. The separation and enrichment of stem cells homing to marrow and spleen on the basis of rhodamine-123 fluorescence. Exp Hematol *13*, 999–1006.

Bertoncello, I., Hodgson, G.S., and Bradley, T.R. (1988). Multiparameter analysis of transplantable hemopoietic stem cells. II. Stem cells of long-term bone marrow-reconstituted recipients. Exp Hematol *16*, 245–249.

Bhaduri, S., Kubanek, B., Heit, W., Pflieger, H., Kurrle, E., Fliedner, T.M., and Heimpel, H. (1979). A case of preleukemia--reconstitution of normal marrow function after bone marrow transplantation (BMT) from identical twin. Blut *38*, 145–149.

Billström, R., Johansson, H., Johansson, B., and Mitelman, F. (1995). Immune-mediated complications in patients with myelodysplastic syndromes--clinical and cytogenetic features. Eur. J. Haematol. *55*, 42–48.

Billström, R., Thiede, T., Hansen, S., Heim, S., Kristoffersson, U., Mandahl, N., and Mitelman, F. (1988). Bone marrow karyotype and prognosis in primary myelodysplastic syndromes. Eur. J. Haematol. *41*, 341–346.

Björkman, S.E. (1956). Chronic refractory anemia with sideroblastic bone marrow; a study of four cases. Blood *11*, 250–259.

Block, M., and Jacobson, L.O. (1953). Preleukemic acute human leukemia. J Am Med Assoc *152*, 1018–1028.

Bochtler, T., hling, S.F.O., and mer, A.K.A. (2015). Role of chromosomal aberrations in clonal diversity and progression of acute myeloid leukemia. *29*, 1243–1252.

Bonnet, D., and Dick, J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med *3*, 730–737.

Boogaerts, M.A., Nelissen, V., Roelant, C., and Goossens, W. (1983). Blood neutrophil function in primary myelodysplastic syndromes. Br J Haematol *55*, 217–227.

Brierley, C.K., and Steensma, D.P. (2015). Thrombopoiesis-stimulating agents and myelodysplastic syndromes. Br J Haematol *169*, 309–323.

Bruns, I., Lucas, D., Pinho, S., Ahmed, J., Lambert, M.P., Kunisaki, Y., Scheiermann, C., Schiff, L., Poncz, M., Bergman, A., et al. (2014). Megakaryocytes regulate hematopoietic stem cell quiescence through CXCL4 secretion. Nature Publishing Group *20*, 1315–1320.

Buckstein, R., Balleari, E., Wells, R., Santini, V., Sanna, A., Salvetti, C., Crisà, E., Allione, B.,

Danise, P., Finelli, C., et al. (2017). ITACA: A new validated international erythropoietic stimulating agent-response score that further refines the predictive power of previous scoring systems. Am. J. Hematol. *92*, 1037–1046.

Calvi, L.M., Adams, G.B., Weibrecht, K.W., Weber, J.M., Olson, D.P., Knight, M.C., Martin, R.P., Schipani, E., Divieti, P., Bringhurst, F.R., et al. (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. Nature *425*, 841–846.

Cao, Q., Gearhart, M.D., Gery, S., Shojaee, S., Yang, H., Sun, H., Lin, D.-C., Bai, J.-W., Mead, M., Zhao, Z., et al. (2016). BCOR regulates myeloid cell proliferation and differentiation. Leukemia *30*, 1155–1165.

Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R.S., and Zhang, Y. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science *298*, 1039–1043.

Cazzola, M., Cazzola, M., Porta, Della, M.G., Porta, Della, M.G., Malcovati, L., and Malcovati, L. (2013). The genetic basis of myelodysplasia and its clinical relevance. Blood *122*, 4021–4034.

Cazzola, M., Porta, Della, M.G., and Malcovati, L. (2008). Clinical relevance of anemia and transfusion iron overload in myelodysplastic syndromes. Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program *2008*, 166–175.

Challen, G.A., Sun, D., Jeong, M., Luo, M., Jelinek, J., Berg, J.S., Bock, C., Vasanthakumar, A., Gu, H., Xi, Y., et al. (2011). Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet *44*, 23–31.

Chaudhary, P.M., and Roninson, I.B. (1991). Expression and activity of P-glycoprotein, a multidrug efflux pump, in human hematopoietic stem cells. Cell *66*, 85–94.

Chen, C.-Y., Lin, L.-I., Tang, J.-L., Ko, B.-S., Tsay, W., Chou, W.-C., Yao, M., Wu, S.-J., Tseng, M.-H., and Tien, H.-F. (2007). RUNX1 gene mutation in primary myelodysplastic syndrome--the mutation can be detected early at diagnosis or acquired during disease progression and is associated with poor outcome. Br J Haematol *139*, 405–414.

Chen, J., Li, Y., Yu, T.-S., McKay, R.M., Burns, D.K., Kernie, S.G., and Parada, L.F. (2012). A restricted cell population propagates glioblastoma growth after chemotherapy. Nature *488*, 522–526.

Chesnais, V., Renneville, A., Toma, A., Lambert, J., Passet, M., Dumont, F., Chevret, S., Lejeune, J., Raimbault, A., Stamatoullas, A., et al. (2016). Effect of lenalidomide treatment on clonal architecture of myelodysplastic syndromes without 5q deletion. Blood *127*, 749–760.

Chevallier, P. (1942). Sur la terminologie des leucoses et les affections-frontieres: les odoleucoses. Sangre Barc *1942–1943*, 587–593.

Christiansen, D.H., Andersen, M.K., and Pedersen-Bjergaard, J. (2001). Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. Journal of Clinical Oncology *19*, 1405–1413.

Civin, C.I., Strauss, L.C., Brovall, C., Fackler, M.J., Schwartz, J.F., and Shaper, J.H. (1984). Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol *133*, 157–165.

Clevers, H. (2011). The cancer stem cell: premises, promises and challenges. Nature Medicine *17*, 313–319.

Conte, S., Katayama, S., Vesterlund, L., Karimi, M., Dimitriou, M., Jansson, M., Mortera-Blanco, T., Unneberg, P., Papaemmanuil, E., Sander, B., et al. (2015). Aberrant splicing of genes involved in haemoglobin synthesis and impaired terminal erythroid maturation in SF3B1 mutated refractory anaemia with ring sideroblasts. Br J Haematol *171*, 478–490.

Cutler, C.S., Lee, S.J., Greenberg, P., Deeg, H.J., Perez, W.S., Anasetti, C., Bolwell, B.J., Cairo, M.S., Gale, R.P., Klein, J.P., et al. (2004). A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. Blood *104*, 579–585.

Dalerba, P., Dylla, S.J., Park, I.-K., Liu, R., Wang, X., Cho, R.W., Hoey, T., Gurney, A., Huang, E.H., Simeone, D.M., et al. (2007). Phenotypic characterization of human colorectal cancer stem cells. Proceedings of the National Academy of Sciences *104*, 10158–10163.

Dameshek, W. (1951). Some speculations on the myeloproliferative syndromes. Blood *6*, 372–375.

Damm, F., Chesnais, V., Nagata, Y., Yoshida, K., Scourzic, L., Okuno, Y., Itzykson, R., Sanada, M., Shiraishi, Y., Gelsi-Boyer, V., et al. (2013). BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. Blood *122*, 3169–3177.

Dang, L., White, D.W., Gross, S., Bennett, B.D., Bittinger, M.A., Driggers, E.M., Fantin, V.R., Jang, H.G., Jin, S., Keenan, M.C., et al. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature *462*, 739–744.

Darwin, C. (1859). On the Origin of Species (Oxford University Press).

de Witte, T., Bowen, D., Robin, M., Malcovati, L., Niederwieser, D., Yakoub-Agha, I., Mufti, G.J., Fenaux, P., Sanz, G., Martino, R., et al. (2017). Allogeneic hematopoietic stem cell transplantation for MDS and CMML: recommendations from an international expert panel. Blood *129*, 1753–1762.

Deeg, H.J., Beckham, C., Loken, M.R., Bryant, E., Lesnikova, M., Shulman, H.M., and Gooley, T. (2000). Negative regulators of hemopoiesis and stroma function in patients with myelodysplastic syndrome. Leuk. Lymphoma *37*, 405–414.

Deeg, H.J. (2002). Marrow stroma in MDS: culprit or bystander? Leukemia Research *26*, 687–688.

Deeg, H.J., and de Lima, M. (2013). Hematopoietic stem cell transplantation for older patients with myelodysplastic syndromes. J Natl Compr Canc Netw *11*, 1227–1233.

Deeg, H.J., Scott, B.L., Fang, M., Shulman, H.M., Gyurkocza, B., Myerson, D., Pagel, J.M., Platzbecker, U., Ramakrishnan, A., Radich, J.P., et al. (2012). Five-group cytogenetic risk classification, monosomal karyotype, and outcome after hematopoietic cell transplantation for MDS or acute leukemia evolving from MDS. Blood *120*, 1398–1408.

Dimitriou, M., Woll, P.S., Mortera-Blanco, T., Karimi, M., Wedge, D.C., Doolittle, H., Douagi, I., Papaemmanuil, E., Jacobsen, S.E.W., and Hellström-Lindberg, E. (2016). Perturbed hematopoietic stem and progenitor cell hierarchy in myelodysplastic syndromes patients with monosomy 7 as the sole cytogenetic abnormality. Oncotarget *7*, 72685–72698.

Ding, L., Saunders, T.L., Enikolopov, G., and Morrison, S.J. (2012a). Endothelial and perivascular cells maintain haematopoietic stem cells. Nature *481*, 457–462.

Ding, L., Ley, T.J., Larson, D.E., Miller, C.A., Koboldt, D.C., Welch, J.S., Ritchey, J.K., Young, M.A., Lamprecht, T., McLellan, M.D., et al. (2012b). Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature *481*, 506–510.

Doniach, I., Grüneberg, H., and Pearson, J.E.G. (1943). The occurrence of siderocytes in adult human blood. J. Pathol. 23–31.

Doulatov, S., Notta, F., Eppert, K., Nguyen, L.T., Ohashi, P.S., and Dick, J.E. (2010). Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. Nat Immunol *11*, 585–593.

Ebert, B.L., Pretz, J., Bosco, J., Chang, C.Y., Tamayo, P., Galili, N., Raza, A., Root, D.E., Attar, E., Ellis, S.R., et al. (2008). Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature *451*, 335–339.

Epling-Burnette, P.K., Painter, J.S., Rollison, D.E., Ku, E., Vendron, D., Widen, R., Boulware, D., Zou, J.X., Bai, F., and List, A.F. (2007). Prevalence and clinical association of clonal T-cell expansions in Myelodysplastic Syndrome. Leukemia *21*, 659–667.

Ezine, S., Jerabek, L., and Weissman, I. (1987). The phenotype of thymocytes derived from a single clonogenic precursor. J Immunol *139*, 2195–2199.

Fenaux, P., Giagounidis, A., Selleslag, D., Beyne-Rauzy, O., Mufti, G., Mittelman, M., Muus, P., Boekhorst, Te, P., Sanz, G., Del Cañizo, C., et al. (2011). A randomized phase 3 study of lenalidomide versus placebo in RBC transfusion-dependent patients with Low-/Intermediate-1-risk myelodysplastic syndromes with del5q. Blood *118*, 3765–3776.

Fenaux, P., Mufti, G.J., Hellström-Lindberg, E., Santini, V., Finelli, C., Giagounidis, A., Schoch, R., Gattermann, N., Sanz, G., List, A., et al. (2009). Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. *10*, 223–232.

Ferrer, R.A., Wobus, M., List, C., Wehner, R., Schönefeldt, C., Brocard, B., Mohr, B., Rauner, M., Schmitz, M., Stiehler, M., et al. (2013). Mesenchymal stromal cells from patients with myelodyplastic syndrome display distinct functional alterations that are modulated by lenalidomide. Haematologica *98*, 1677–1685.

Fidler, C., Watkins, F., Bowen, D.T., Littlewood, T.J., Wainscoat, J.S., and Boultwood, J. (2004). NRAS, FLT3 and TP53 mutations in patients with myelodysplastic syndrome and a del(5q). Haematologica *89*, 865–866.

Finlay, C.A., Hinds, P.W., and Levine, A.J. (1989). The p53 proto-oncogene can act as a suppressor of transformation. Cell *57*, 1083–1093.

Fisher, W.B., Armentrout, S.A., Weisman, R., and Graham, R.C. (1973). "Preleukemia." A myelodysplastic syndrome often terminating in acute leukemia. Arch. Intern. Med. *132*, 226–232.

Ford, C.E., Hamerton, J.L., Barnes, D.W., and Loutit, J.F. (1956). Cytological identification of radiation-chimaeras. Nature *177*, 452–454.

Foucar, K., Langdon, R.M., Armitage, J.O., Olson, D.B., and Carroll, T.J. (1985). Myelodysplastic syndromes. A clinical and pathologic analysis of 109 cases. Cancer *56*, 553–561.

Francis, N.J., Kingston, R.E., and Woodcock, C.L. (2004). Chromatin compaction by a polycomb group protein complex. Science *306*, 1574–1577.

Furitsu, T., Tsujimura, T., Tono, T., Ikeda, H., Kitayama, H., Koshimizu, U., Sugahara, H., Butterfield, J.H., Ashman, L.K., and Kanayama, Y. (1993). Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. J. Clin. Invest. *92*, 1736–1744.

Gao, Z., Zhang, J., Bonasio, R., Strino, F., Sawai, A., Parisi, F., Kluger, Y., and Reinberg, D. (2012). PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. Mol Cell *45*, 344–356.

Gearhart, M.D., Corcoran, C.M., Wamstad, J.A., and Bardwell, V.J. (2006). Polycomb group and SCF ubiquitin ligases are found in a novel BCOR complex that is recruited to BCL6 targets. Mol Cell Biol *26*, 6880–6889.

Genovese, G., Kähler, A.K., Handsaker, R.E., Lindberg, J., Rose, S.A., Bakhoum, S.F., Chambert, K., Mick, E., Neale, B.M., Fromer, M., et al. (2014). Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. N Engl J Med *371*, 141126135947008–2487.

Geyh, S., Oz, S., Cadeddu, R.P., Frobel, J., Brückner, B., Kündgen, A., Fenk, R., Bruns, I., Zilkens, C., Hermsen, D., et al. (2013). Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. Leukemia *27*, 1841–1851.

Giagounidis, A.A.N., Kulasekararaj, A., Germing, U., Radkowski, R., Haase, S., Petersen, P., Göhring, G., Busche, G., Aul, C., Mufti, G.J., et al. (2012). Long-term transfusion independence in del(5q) MDS patients who discontinue lenalidomide. Leukemia *26*, 855–858.

Giagounidis, A., Mufti, G.J., Fenaux, P., Sekeres, M.A., Szer, J., Platzbecker, U., Kuendgen, A., Gaidano, G., Wiktor-Jedrzejczak, W., Hu, K., et al. (2014). Results of a randomized, double-blind study of romiplostim versus placebo in patients with low/intermediate-1-risk myelodysplastic syndrome and thrombocytopenia. Cancer *120*, 1838–1846.

Giannouli, S., Voulgarelis, M., Zintzaras, E., Tzioufas, A.G., and Moutsopoulos, H.M. (2004). Autoimmune phenomena in myelodysplastic syndromes: a 4-yr prospective study. Rheumatology (Oxford) *43*, 626–632.

Gilbert, H.S. (1970). A reappraisal of the "myeloproliferative disease" concept. Mt. Sinai J. Med. *37*, 426–435.

Giralt, S. (2005). Reduced-intensity conditioning regimens for hematologic malignancies: what have we learned over the last 10 years? Hematology Am Soc Hematol Educ Program *2005*, 384–389.

Goardon, N., Marchi, E., Atzberger, A., Quek, L., Schuh, A., Soneji, S., Woll, P., Mead, A., Alford, K.A., Rout, R., et al. (2011). Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell *19*, 138–152.

Gold, E.J., Conjalka, M., Pelus, L.M., Jhanwar, S.C., Broxmeyer, H., Middleton, A.B., Clarkson, B.D., and Moore, M.A. (1983). Marrow cytogenetic and cell-culture analyses of the myelodysplastic syndromes: insights to pathophysiology and prognosis. Journal of Clinical Oncology *1*, 627–634.

Goldberg, S.L., Chen, E., Corral, M., Guo, A., Mody-Patel, N., Pecora, A.L., and Laouri, M. (2010). Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. J Clin Oncol *28*, 2847–2852.

Gore, S.D., Fenaux, P., Santini, V., Bennett, J.M., Silverman, L.R., Seymour, J.F., Hellström-Lindberg, E., Swern, A.S., Beach, C.L., and List, A.F. (2013). A multivariate analysis of the relationship between response and survival among patients with higher-risk myelodysplastic syndromes treated within azacitidine or conventional care regimens in the randomized AZA-001 trial. Haematologica *98*, 1067–1072.

Graubert, T.A., Payton, M.A., Shao, J., Walgren, R.A., Monahan, R.S., Frater, J.L., Walshauser, M.A., Martin, M.G., Kasai, Y., and Walter, M.J. (2009). Integrated genomic analysis implicates haploinsufficiency of multiple chromosome 5q31.2 genes in de novo myelodysplastic syndromes pathogenesis. PLoS ONE *4*, e4583.

Graubert, T.A., Shen, D., Ding, L., Okeyo-Owuor, T., Lunn, C.L., Shao, J., Krysiak, K., Harris, C.C., Koboldt, D.C., Larson, D.E., et al. (2011). Recurrent mutations in the U2AF1 splicing factor

in myelodysplastic syndromes. Nat Genet *44*, 53–57.

Greaves, M. (2010). Cancer stem cells: back to Darwin? Semin. Cancer Biol. *20*, 65–70.

Greaves, M., and Maley, C.C. (2012). Clonal evolution in cancer. Nature *481*, 306–313.

Greenberg, P., Cox, C., LeBeau, M.M., Fenaux, P., Morel, P., Sanz, G., Sanz, M., Vallespi, T., Hamblin, T., Oscier, D., et al. (1997). International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood *89*, 2079–2088.

Greenberg, P.L., Tuechler, H., Schanz, J., Sanz, G., Garcia-Manero, G., Solé, F., Bennett, J.M., Bowen, D., Fenaux, P., Dreyfus, F., et al. (2012). Revised international prognostic scoring system for myelodysplastic syndromes. Blood *120*, 2454–2465.

Growney, J.D., Shigematsu, H., Li, Z., Lee, B.H., Adelsperger, J., Rowan, R., Curley, D.P., Kutok, J.L., Akashi, K., Williams, I.R., et al. (2005). Loss of Runx1 perturbs adult hematopoiesis and is associated with a myeloproliferative phenotype. Blood *106*, 494–504.

Grüneberg, H. (1939). Inherited Macrocytic Anemias in the House Mouse. Genetics *24*, 777–810.

Gyurkocza, B., and Deeg, H.J. (2012). Allogeneic hematopoietic cell transplantation for MDS: for whom, when and how? Blood Rev. *26*, 247–254.

Gyurkocza, B., Rezvani, A., and Storb, R.F. (2010). Allogeneic hematopoietic cell transplantation: the state of the art. Expert Rev Hematol *3*, 285–299.

Haas, S., Hansson, J., Klimmeck, D., Loeffler, D., Velten, L., Uckelmann, H., Wurzer, S., Prendergast, Á.M., Schnell, A., Hexel, K., et al. (2015). Inflammation-Induced Emergency Megakaryopoiesis Driven by Hematopoietic Stem Cell-like Megakaryocyte Progenitors. Cell Stem Cell *17*, 422–434.

Haase, D., Germing, U., Schanz, J., Pfeilstöcker, M., Nösslinger, T., Hildebrandt, B., Kündgen, A., Lübbert, M., Kunzmann, R., Giagounidis, A.A.N., et al. (2007). New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood *110*, 4385–4395.

Haferlach, T., Nagata, Y., Grossmann, V., Okuno, Y., Bacher, U., Nagae, G., Schnittger, S., Sanada, M., Kon, A., Alpermann, T., et al. (2014). Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia *28*, 241–247.

Hahn, C.N., Chong, C.-E., Carmichael, C.L., Wilkins, E.J., Brautigan, P.J., Li, X.-C., Babic, M., Lin, M., Carmagnac, A., Lee, Y.K., et al. (2011). Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. Nat Genet *43*, 1012–1017.

Hamilton-Paterson, J. (1949). Pre-leukaemic anaemia. Acta Haematol. *2*, 309–316.

Hardy, R.R., Hayakawa, K., Haaijman, J., and Herzenberg, L.A. (1982). B-cell subpopulations identified by two-colour fluorescence analysis. Nature *297*, 589–591.

Hardy, R.R., Hayakawa, K., Parks, D.R., and Herzenberg, L.A. (1983). Demonstration of B-cell maturation in X-linked immunodeficient mice by simultaneous three-colour immunofluorescence. Nature *306*, 270–272.

Hellström-Lindberg, E., and Malcovati, L. (2008). Supportive care, growth factors, and new therapies in myelodysplastic syndromes. Blood Rev. *22*, 75–91.

Hellström-Lindberg, E., Gulbrandsen, N., Lindberg, G., Ahlgren, T., Dahl, I.M.S., Dybedal, I., Grimfors, G., Hesse-Sundin, E., Hjorth, M., Kanter-Lewensohn, L., et al. (2003). A validated

decision model for treating the anaemia of myelodysplastic syndromes with erythropoietin + granulocyte colony-stimulating factor: significant effects on quality of life. Br J Haematol *120*, 1037–1046.

Horiike, S., Kita-Sasai, Y., Nakao, M., and Taniwaki, M. (2003). Configuration of the TP53 gene as an independent prognostic parameter of myelodysplastic syndrome. Leuk. Lymphoma *44*, 915–922.

Horsfield, J.A., Anagnostou, S.H., Hu, J.K.-H., Cho, K.H.Y., Geisler, R., Lieschke, G., Crosier, K.E., and Crosier, P.S. (2007). Cohesin-dependent regulation of Runx genes. Development *134*, 2639–2649.

Hulett, H.R., Bonner, W.A., Barrett, J., and Herzenberg, L.A. (1969). Cell sorting: automated separation of mammalian cells as a function of intracellular fluorescence. Science *166*, 747–749.

Huynh, K.D., Fischle, W., Verdin, E., and Bardwell, V.J. (2000). BCoR, a novel corepressor involved in BCL-6 repression. Genes Dev *14*, 1810–1823.

Ilagan, J.O., Ramakrishnan, A., Hayes, B., Murphy, M.E., Zebari, A.S., Bradley, P., and Bradley, R.K. (2015). U2AF1 mutations alter splice site recognition in hematological malignancies. Genome Research *25*, 14–26.

Inoue, D., Bradley, R.K., and Abdel-Wahab, O. (2016). Spliceosomal gene mutations in myelodysplasia: molecular links to clonal abnormalities of hematopoiesis. Genes Dev *30*, 989–1001.

Issa, J.-P.J. (2013). The myelodysplastic syndrome as a prototypical epigenetic disease. Blood *121*, 3811–3817.

Issa, J.-P.J., and Kantarjian, H.M. (2009). Targeting DNA methylation. Clinical Cancer Research *15*, 3938–3946.

Ito, Y., Bae, S.-C., and Chuang, L.S.H. (2015). The RUNX family: developmental regulators in cancer. Nat Rev Cancer *15*, 81–95.

Iwasaki, T., Murakami, M., Sugisaki, C., Sobue, S., Ohashi, H., Asano, H., Suzuki, M., Nakamura, S., Ito, M., and Murate, T. (2008). Characterization of myelodysplastic syndrome and aplastic anemia by immunostaining of p53 and hemoglobin F and karyotype analysis: differential diagnosis between refractory anemia and aplastic anemia. Pathol. Int. *58*, 353–360.

Jacobsen, L.O., Marks, E.K., Robson, M.J., Gaston, E.O., and Zirkle, L.E. (1949). Effect of spleen protection on mortality following X-irradiation. J Lab Clin Med *34*, 1538–1543.

Jacobson, R.J., Salo, A., and Fialkow, P.J. (1978). Agnogenic myeloid metaplasia: a clonal proliferation of hematopoietic stem cells with secondary myelofibrosis. Blood *51*, 189–194.

Jaiswal, S., Fontanillas, P., Flannick, J., Manning, A., Grauman, P.V., Mar, B.G., Lindsley, R.C., Mermel, C.H., Burtt, N., Chavez, A., et al. (2014). Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. N Engl J Med *371*, 141126135947008–2498.

Jamieson, C.H.M., Ailles, L.E., Dylla, S.J., Muijtjens, M., Jones, C., Zehnder, J.L., Gotlib, J., Li, K., Manz, M.G., Keating, A., et al. (2004). Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med *351*, 657–667.

Jan, M., Snyder, T.M., Corces-Zimmerman, M.R., Vyas, P., Weissman, I.L., Quake, S.R., and Majeti, R. (2012). Clonal Evolution of Preleukemic Hematopoietic Stem Cells Precedes Human Acute Myeloid Leukemia. Sci Transl Med *4*, 149ra118–149ra118.

Jansen, A.J.G., Essink-Bot, M.-L., Beckers, E.A.M., Hop, W.C.J., Schipperus, M.R., and Van

Rhenen, D.J. (2003). Quality of life measurement in patients with transfusion-dependent myelodysplastic syndromes. Br J Haematol *121*, 270–274.

Jädersten, M., Malcovati, L., Dybedal, I., Porta, Della, M.G., Invernizzi, R., Montgomery, S.M., Pascutto, C., Porwit, A., Cazzola, M., and Hellström-Lindberg, E. (2008). Erythropoietin and granulocyte-colony stimulating factor treatment associated with improved survival in myelodysplastic syndrome. Journal of Clinical Oncology *26*, 3607–3613.

Jädersten, M., Saft, L., Pellagatti, A., Göhring, G., Wainscoat, J.S., Boultwood, J., Porwit, A., Schlegelberger, B., and Hellström-Lindberg, E. (2009). Clonal heterogeneity in the 5q- syndrome: p53 expressing progenitors prevail during lenalidomide treatment and expand at disease progression. Haematologica *94*, 1762–1766.

Jädersten, M., Saft, L., Smith, A., Kulasekararaj, A., Pomplun, S., Göhring, G., Hedlund, A., Hast, R., Schlegelberger, B., Porwit, A., et al. (2011). TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. Journal of Clinical Oncology *29*, 1971–1979.

Kallin, E.M., Rodríguez-Ubreva, J., Christensen, J., Cimmino, L., Aifantis, I., Helin, K., Ballestar, E., and Graf, T. (2012). Tet2 facilitates the derepression of myeloid target genes during CEBPα-induced transdifferentiation of pre-B cells. Mol Cell *48*, 266–276.

Kamel-Reid, S., and Dick, J.E. (1988). Engraftment of immune-deficient mice with human hematopoietic stem cells. Science *242*, 1706–1709.

Kaneko, H., Misawa, S., Horiike, S., Nakai, H., and Kashima, K. (1995). TP53 mutations emerge at early phase of myelodysplastic syndrome and are associated with complex chromosomal abnormalities. Blood *85*, 2189–2193.

Kantarjian, H., Giles, F., List, A., Lyons, R., Sekeres, M.A., Pierce, S., Deuson, R., and Leveque, J. (2007). The incidence and impact of thrombocytopenia in myelodysplastic syndromes. Cancer *109*, 1705–1714.

Kern, W., Haferlach, C., Schnittger, S., Alpermann, T., and Haferlach, T. (2013). Serial assessment of suspected myelodysplastic syndromes: significance of flow cytometric findings validated by cytomorphology, cytogenetics, and molecular genetics. Haematologica *98*, 201–207.

Kern, W., Haferlach, C., Schnittger, S., and Haferlach, T. (2010). Clinical utility of multiparameter flow cytometry in the diagnosis of 1013 patients with suspected myelodysplastic syndrome: correlation to cytomorphology, cytogenetics, and clinical data. Cancer *116*, 4549–4563.

Kfoury, Y., and Scadden, D.T. (2015). Mesenchymal cell contributions to the stem cell niche. Cell Stem Cell *16*, 239–253.

Kim, E., Ilagan, J.O., Liang, Y., Daubner, G.M., Lee, S.C.-W., Ramakrishnan, A., Li, Y., Chung, Y.R., Micol, J.-B., Murphy, M.E., et al. (2015). SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition. Cancer Cell *27*, 617–630.

Kita-Sasai, Y., Horiike, S., Misawa, S., Kaneko, H., Kobayashi, M., Nakao, M., Nakagawa, H., Fujii, H., and Taniwaki, M. (2001). International prognostic scoring system and TP53 mutations are independent prognostic indicators for patients with myelodysplastic syndrome. Br J Haematol *115*, 309–312.

Kitagawa, M., Yoshida, S., Kuwata, T., Tanizawa, T., and Kamiyama, R. (1994). p53 expression in myeloid cells of myelodysplastic syndromes. Association with evolution of overt leukemia. Am. J. Pathol. *145*, 338–344.

Klco, J.M., Spencer, D.H., Miller, C.A., Griffith, M., Lamprecht, T.L., O'Laughlin, M., Fronick, C., Magrini, V., Demeter, R.T., Fulton, R.S., et al. (2014). Functional heterogeneity of genetically

defined subclones in acute myeloid leukemia. Cancer Cell *25*, 379–392.

Ko, M., An, J., Pastor, W.A., Koralov, S.B., Rajewsky, K., and Rao, A. (2015). TET proteins and 5-methylcytosine oxidation in hematological cancers. Immunol. Rev. *263*, 6–21.

Koenecke, C., Göhring, G., de Wreede, L.C., van Biezen, A., Scheid, C., Volin, L., Maertens, J., Finke, J., Schaap, N., Robin, M., et al. (2015). Impact of the revised International Prognostic Scoring System, cytogenetics and monosomal karyotype on outcome after allogeneic stem cell transplantation for myelodysplastic syndromes and secondary acute myeloid leukemia evolving from myelodysplastic syndromes: a retrospective multicenter study of the European Society of Blood and Marrow Transplantation. Haematologica *100*, 400–408.

Kon, A., Yamazaki, S., Nannya, Y., Kataoka, K., Ota, Y., Nakagawa, M.M., Yoshida, K., Shiozawa, Y., Morita, M., Yoshizato, T., et al. (2017). Physiological Srsf2 P95H expression causes impaired hematopoietic stem cell functions and aberrant RNA splicing in mice. Blood blood–2017–01–762393.

Kondo, M., Weissman, I.L., and Akashi, K. (1997). Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell *91*, 661–672.

Koreth, J., Pidala, J., Perez, W.S., Deeg, H.J., Garcia-Manero, G., Malcovati, L., Cazzola, M., Park, S., Itzykson, R., Ades, L., et al. (2013). Role of reduced-intensity conditioning allogeneic hematopoietic stem-cell transplantation in older patients with de novo myelodysplastic syndromes: an international collaborative decision analysis. J Clin Oncol *31*, 2662–2670.

Kottaridis, P.D., Gale, R.E., Frew, M.E., Harrison, G., Langabeer, S.E., Belton, A.A., Walker, H., Wheatley, K., Bowen, D.T., Burnett, A.K., et al. (2001). The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood *98*, 1752–1759.

Kralovics, R., Passamonti, F., Buser, A.S., Teo, S.-S., Tiedt, R., Passweg, J.R., Tichelli, A., Cazzola, M., and Skoda, R.C. (2005). A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med *352*, 1779–1790.

Kröger, N. (2012). Allogeneic stem cell transplantation for elderly patients with myelodysplastic syndrome. Blood *119*, 5632–5639.

Krönke, J., Fink, E.C., Hollenbach, P.W., MacBeth, K.J., Hurst, S.N., Udeshi, N.D., Chamberlain, P.P., Mani, D.R., Man, H.W., Gandhi, A.K., et al. (2015). Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. Nature *523*, 183–188.

Kulasekararaj, A.G., Mohamedali, A.M., and Mufti, G.J. (2013). Recent advances in understanding the molecular pathogenesis of myelodysplastic syndromes. Br J Haematol *162*, 587–605.

Lai, J.L., Preudhomme, C., Zandecki, M., Flactif, M., Vanrumbeke, M., Lepelley, P., Wattel, E., and Fenaux, P. (1995). Myelodysplastic syndromes and acute myeloid leukemia with 17p deletion. An entity characterized by specific dysgranulopoïesis and a high incidence of P53 mutations. Leukemia *9*, 370–381.

Lane, D.P. (1992). Cancer. p53, guardian of the genome. Nature *358*, 15–16.

Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M.A., and Dick, J.E. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature *367*, 645–648.

Lawrence, M.S., Stojanov, P., Mermel, C.H., Robinson, J.T., Garraway, L.A., Golub, T.R., Meyerson, M., Gabriel, S.B., Lander, E.S., and Getz, G. (2014). Discovery and saturation analysis

of cancer genes across 21 tumour types. Nature *505*, 495–501.

Leeke, B., Marsman, J., O'Sullivan, J.M., and Horsfield, J.A. (2014). Cohesin mutations in myeloid malignancies: underlying mechanisms. Exp Hematol Oncol *3*, 13.

Leube, von, W. (1900). Rapid verlaufende schwere Anämie mit gleichzeitiger leukämischer Veränderung des Blutbildes. Klin Wochensch Berlin.

Ley, T.J., Ding, L., Walter, M.J., McLellan, M.D., Lamprecht, T., Larson, D.E., Kandoth, C., Payton, J.E., Baty, J., Welch, J., et al. (2010). DNMT3A mutations in acute myeloid leukemia. N Engl J Med *363*, 2424–2433.

Li, F.P., and Fraumeni, J.F. (1969). Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann. Intern. Med. *71*, 747–752.

Li, L., and Neaves, W.B. (2006). Normal stem cells and cancer stem cells: the niche matters. Cancer Research *66*, 4553–4557.

List, A., Dewald, G., Bennett, J., Giagounidis, A., Raza, A., Feldman, E., Powell, B., Greenberg, P., Thomas, D., Stone, R., et al. (2006). Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med *355*, 1456–1465.

List, A., Kurtin, S., Roe, D.J., Buresh, A., Mahadevan, D., Fuchs, D., Rimsza, L., Heaton, R., Knight, R., and Zeldis, J.B. (2005). Efficacy of lenalidomide in myelodysplastic syndromes. N Engl J Med *352*, 549–557.

Lobo, N.A., Shimono, Y., Qian, D., and Clarke, M.F. (2007). The biology of cancer stem cells. Annu. Rev. Cell Dev. Biol. *23*, 675–699.

Lodé, L., Ménard, A., Flet, L., Richebourg, S., Loirat, M., Eveillard, M., Le Bris, Y., Godon, C., Theisen, O., Gagez, A.-L., et al. (2017). Emergence and evolution of TP53 mutations are a key feature of disease progression in myelodysplastic patients with lower-risk del(5q) treated with lenalidomide. Haematologica haematol.2017.181404.

Lorenz, E., Uphoff, D., Reid, T.R., and Shelton, E. (1951). Modification of irradiation injury in mice and guinea pigs by bone marrow injections. J. Natl. Cancer Inst. *12*, 197–201.

Losada, A. (2014). Cohesin in cancer: chromosome segregation and beyond. Nat Rev Cancer *14*, 389–393.

Losman, J.-A., Looper, R.E., Koivunen, P., Lee, S., Schneider, R.K., McMahon, C., Cowley, G.S., Root, D.E., Ebert, B.L., and Kaelin, W.G. (2013). (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. Science *339*, 1621–1625.

Lowe, S.W., Cepero, E., and Evan, G. (2004). Intrinsic tumour suppression. Nature *432*, 307–315.

Lu, C., Ward, P.S., Kapoor, G.S., Rohle, D., Turcan, S., Abdel-Wahab, O., Edwards, C.R., Khanin, R., Figueroa, M.E., Melnick, A., et al. (2012). IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature *483*, 474–478.

Ludwig, L., Schulz, A.S., Janssen, J.W., Grünewald, K., and Bartram, C.R. (1992). P53 mutations in myelodysplastic syndromes. Leukemia *6*, 1302–1304.

Luger, S.M., Ringdén, O., Zhang, M.-J., Pérez, W.S., Bishop, M.R., Bornhauser, M., Bredeson, C.N., Cairo, M.S., Copelan, E.A., Gale, R.P., et al. (2012). Similar outcomes using myeloablative vs reduced-intensity allogeneic transplant preparative regimens for AML or MDS. Bone Marrow Transplant *47*, 203–211.

Ma, X., Does, M., Raza, A., and Mayne, S.T. (2007). Myelodysplastic syndromes: incidence and survival in the United States. Cancer *109*, 1536–1542.

Magee, J.A., Piskounova, E., and Morrison, S.J. (2012). Cancer stem cells: impact, heterogeneity, and uncertainty. Cancer Cell *21*, 283–296.

Majeti, R., Park, C.Y., and Weissman, I.L. (2007). Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. Cell Stem Cell *1*, 635–645.

Makinodan, T. (1956). Circulating rat cells in lethally irradiated mice protected with rat bone marrow. Proc. Soc. Exp. Biol. Med. *92*, 174–179.

Malcovati, L. (2007). Impact of transfusion dependency and secondary iron overload on the survival of patients with myelodysplastic syndromes. Leukemia Research *31 Suppl 3*, S2–S6.

Malcovati, L., Germing, U., Kuendgen, A., Porta, Della, M.G., Pascutto, C., Invernizzi, R., Giagounidis, A., Hildebrandt, B., Bernasconi, P., Knipp, S., et al. (2007). Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol *25*, 3503–3510.

Malcovati, L., Hellström-Lindberg, E., Bowen, D., Ades, L., Cermak, J., Del Cañizo, C., Porta, Della, M.G., Fenaux, P., Gattermann, N., Germing, U., et al. (2013). Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood *122*, 2943–2964.

Malcovati, L., Karimi, M., Papaemmanuil, E., Ambaglio, I., Jädersten, M., Jansson, M., Elena, C., Gallì, A., Walldin, G., Porta, Della, M.G., et al. (2015). SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood *126*, 233–241.

Malcovati, L., Papaemmanuil, E., Bowen, D.T., Boultwood, J., Porta, Della, M.G., Pascutto, C., Travaglino, E., Groves, M.J., Godfrey, A.L., Ambaglio, I., et al. (2011). Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood *118*, 6239–6246.

Mallo, M., Cervera, J., Schanz, J., Such, E., Garcia-Manero, G., Luño, E., Steidl, C., Espinet, B., Vallespi, T., Germing, U., et al. (2011). Impact of adjunct cytogenetic abnormalities for prognostic stratification in patients with myelodysplastic syndrome and deletion 5q. Leukemia *25*, 110–120.

Manoharan, A., Brighton, T., Gemmell, R., Lopez, K., Moran, S., and Kyle, P. (2002). Platelet dysfunction in myelodysplastic syndromes: a clinicopathological study. Int. J. Hematol. *76*, 272–278.

Manz, M.G., Miyamoto, T., Akashi, K., and Weissman, I.L. (2002). Prospective isolation of human clonogenic common myeloid progenitors. Proc Natl Acad Sci USA *99*, 11872–11877.

Mardis, E.R., Ding, L., Dooling, D.J., Larson, D.E., McLellan, M.D., Chen, K., Koboldt, D.C., Fulton, R.S., Delehaunty, K.D., McGrath, S.D., et al. (2009). Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med *361*, 1058–1066.

Marsman, J., O'Neill, A.C., Kao, B.R.-Y., Rhodes, J.M., Meier, M., Antony, J., Mönnich, M., and Horsfield, J.A. (2014). Cohesin and CTCF differentially regulate spatiotemporal runx1 expression during zebrafish development. Biochim. Biophys. Acta *1839*, 50–61.

Martino, R., Iacobelli, S., Brand, R., Jansen, T., van Biezen, A., Finke, J., Bacigalupo, A., Beelen, D., Reiffers, J., Devergie, A., et al. (2006). Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. Blood *108*, 836–846.

Mauritzson, N., Albin, M., Rylander, L., Billström, R., Ahlgren, T., Mikoczy, Z., Björk, J., Strömberg, U., Nilsson, P.G., Mitelman, F., et al. (2002). Pooled analysis of clinical and cytogenetic features in treatment-related and de novo adult acute myeloid leukemia and myelodysplastic syndromes based on a consecutive series of 761 patients analyzed 1976-1993 and on 5098 unselected cases reported in the literature 1974-2001. Leukemia *16*, 2366–2378.

Maximov, A. (1909). Der Lymphozyt als gemeinsame Stammzelle der verschiedenen Blutelemente in der embryonalen Entwicklung und im postfetalen Leben der Säugetiere. Folia Haematologica *8*, 125–134.

Mayle, A., Yang, L., Rodriguez, B., Zhou, T., Chang, E., Curry, C.V., Challen, G.A., Li, W., Wheeler, D., Rebel, V.I., et al. (2015). Dnmt3a loss predisposes murine hematopoietic stem cells to malignant transformation. Blood *125*, 629–638.

Mazumdar, C., Shen, Y., Xavy, S., Zhao, F., Reinisch, A., Li, R., Corces, M.R., Flynn, R.A., Buenrostro, J.D., Chan, S.M., et al. (2015). Leukemia-Associated Cohesin Mutants Dominantly Enforce Stem Cell Programs and Impair Human Hematopoietic Progenitor Differentiation. Cell Stem Cell *17*, 675–688.

McCune, J.M., Namikawa, R., Kaneshima, H., Shultz, L.D., Lieberman, M., and Weissman, I.L. (1988). The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science *241*, 1632–1639.

McKerrell, T., Park, N., Moreno, T., Grove, C.S., Ponstingl, H., Stephens, J., Understanding Society Scientific Group, Crawley, C., Craig, J., Scott, M.A., et al. (2015). Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. CellReports *10*, 1239–1245.

Medyouf, H., Mossner, M., Jann, J.-C., Nolte, F., Raffel, S., Herrmann, C., Lier, A., Eisen, C., Nowak, V., Zens, B., et al. (2014). Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. Cell Stem Cell *14*, 824–837.

Meyers, C.A., Albitar, M., and Estey, E. (2005). Cognitive impairment, fatigue, and cytokine levels in patients with acute myelogenous leukemia or myelodysplastic syndrome. Cancer *104*, 788–793.

Méndez-Ferrer, S., Michurina, T.V., Ferraro, F., Mazloom, A.R., Macarthur, B.D., Lira, S.A., Scadden, D.T., Ma'ayan, A., Enikolopov, G.N., and Frenette, P.S. (2010). Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature *466*, 829–834.

Michallet, M., Philip, T., Philip, I., Godinot, H., Sebban, C., Salles, G., Thiebaut, A., Biron, P., Lopez, F., Mazars, P., et al. (2000). Transplantation with selected autologous peripheral blood CD34+Thy1+ hematopoietic stem cells (HSCs) in multiple myeloma: impact of HSC dose on engraftment, safety, and immune reconstitution. Exp Hematol *28*, 858–870.

Milosevic, J.D., Puda, A., Malcovati, L., Berg, T., Hofbauer, M., Stukalov, A., Klampfl, T., Harutyunyan, A.S., Gisslinger, H., Gisslinger, B., et al. (2012). Clinical significance of genetic aberrations in secondary acute myeloid leukemia. Am. J. Hematol. *87*, 1010–1016.

Molldrem, J.J., Caples, M., Mavroudis, D., Plante, M., Young, N.S., and Barrett, A.J. (1997). Antithymocyte globulin for patients with myelodysplastic syndrome. Br J Haematol *99*, 699–705.

Molldrem, J.J., Leifer, E., Bahceci, E., Saunthararajah, Y., Rivera, M., Dunbar, C., Liu, J., Nakamura, R., Young, N.S., and Barrett, A.J. (2002). Antithymocyte globulin for treatment of the bone marrow failure associated with myelodysplastic syndromes. Ann. Intern. Med. *137*, 156–163.

Moran-Crusio, K., Reavie, L., Shih, A., Abdel-Wahab, O., Ndiaye-Lobry, D., Lobry, C., Figueroa, M.E., Vasanthakumar, A., Patel, J., Zhao, X., et al. (2011). Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell *20*, 11–24.

Mori, N., Hidai, H., Yokota, J., Okada, M., Motoji, T., Oshimi, K., and Mizoguchi, H. (1995). Mutations of the p53 gene in myelodysplastic syndrome and overt leukemia. Leukemia Research *19*, 869–875.

Morrison, S.J., and Weissman, I.L. (1994). The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. Immunity *1*, 661–673.

Morrison, S.J., Wandycz, A.M., Hemmati, H.D., Wright, D.E., and Weissman, I.L. (1997). Identification of a lineage of multipotent hematopoietic progenitors. Development *124*, 1929–1939.

Morrison, S.J., and Scadden, D.T. (2014). The bone marrow niche for haematopoietic stem cells. Nature *505*, 327–334.

Mosier, D.E., Gulizia, R.J., Baird, S.M., and Wilson, D.B. (1988). Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature *335*, 256–259.

Mufti, G.J., Hamblin, T.J., and Seabright, M. (1982). Acute transformation of a myeloproliferative state in sideroblastic anaemia with abnormal karyotype. J. Med. Genet. *19*, 478.

Mupo, A., Seiler, M., Sathiaseelan, V., Pance, A., Yang, Y., Agrawal, A.A., Iorio, F., Bautista, R., Pacharne, S., Tzelepis, K., et al. (2017). Hemopoietic-specific Sf3b1-K700E knock-in mice display the splicing defect seen in human MDS but develop anemia without ring sideroblasts. Leukemia *31*, 720–727.

Murphy, D.M., Bejar, R., Stevenson, K., Neuberg, D., Shi, Y., Cubrich, C., Richardson, K., Eastlake, P., Garcia-Manero, G., Kantarjian, H., et al. (2013). NRAS mutations with low allele burden have independent prognostic significance for patients with lower risk myelodysplastic syndromes. Leukemia *27*, 2077–2081.

Müller, J.R., Janz, S., Goedert, J.J., Potter, M., and Rabkin, C.S. (1995). Persistence of immunoglobulin heavy chain/c-myc recombination-positive lymphocyte clones in the blood of human immunodeficiency virus-infected homosexual men. Proceedings of the National Academy of Sciences *92*, 6577–6581.

Nachtkamp, K., Kündgen, A., Strupp, C., Giagounidis, A., Kobbe, G., Gattermann, N., Haas, R., and Germing, U. (2009). Impact on survival of different treatments for myelodysplastic syndromes (MDS). Leukemia Research *33*, 1024–1028.

Nakai, H., Kaneko, H., Nakao, M., Horiike, S., and Misawa, S. (1994). Is inactivation of the p53 gene a common event in leukemias and myelodysplastic syndrome with monosomy 17p? Leukemia *8*, 1247–1248.

Negrin, R.S., Atkinson, K., Leemhuis, T., Hanania, E., Juttner, C., Tierney, K., Hu, W.W., Johnston, L.J., Shizurn, J.A., Stockerl-Goldstein, K.E., et al. (2000). Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with metastatic breast cancer. Biol Blood Marrow Transplant *6*, 262–271.

Nilsson, L., Astrand-Grundström, I., Arvidsson, I., Jacobsson, B., Hellström-Lindberg, E., Hast, R., and Jacobsen, S.E. (2000). Isolation and characterization of hematopoietic progenitor/stem cells in 5q-deleted myelodysplastic syndromes: evidence for involvement at the hematopoietic stem cell level. Blood *96*, 2012–2021.

Nordic MDS Group (2017). Guidelines for the diagnosis and treatment of Myelodysplastic Syndrome and Chronic Myelomonocytic Leukemia. 1–47. Available at: https://nmds.org/index.php/guidelines (accessed 26/01/18).

Nordic MDS Group Myelodysplastiskt syndrom (MDS) - Rapport från nationella kvalitetsregistret för diagnosår 2009-2014. Available at: https://www.cancercentrum.se/syd/cancerdiagnoser/blod-lymfom-myelom/myelodysplastiskt-syndrom-mds/kvalitetsregister/ (accessed 26/01/18).

Nowell, P.C. (1976). The clonal evolution of tumor cell populations. Science *194*, 23–28.

Nowell, P.C. (1982). Cytogenetics of preleukemia. Cancer Genet. Cytogenet. *5*, 265–278.

Obeng, E.A., Chappell, R.J., Seiler, M., Chen, M.C., Campagna, D.R., Schmidt, P.J., Schneider, R.K., Lord, A.M., Wang, L., Gambe, R.G., et al. (2016). Physiologic Expression of Sf3b1(K700E) Causes Impaired Erythropoiesis, Aberrant Splicing, and Sensitivity to Therapeutic Spliceosome Modulation. Cancer Cell *30*, 404–417.

Okano, M., Xie, S., and Li, E. (1998). Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet *19*, 219–220.

Owen, C.J., Toze, C.L., Koochin, A., Forrest, D.L., Smith, C.A., Stevens, J.M., Jackson, S.C., Poon, M.-C., Sinclair, G.D., Leber, B., et al. (2008). Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. Blood *112*, 4639–4645.

Padua, R.A., Guinn, B.A., Al-Sabah, A.I., Smith, M., Taylor, C., Pettersson, T., Ridge, S., Carter, G., White, D., Oscier, D., et al. (1998). RAS, FMS and p53 mutations and poor clinical outcome in myelodysplasias: a 10-year follow-up. Leukemia *12*, 887–892.

Pallavicini, M.G., Summers, L.J., Dean, P.N., and Gray, J.W. (1985). Enrichment of murine hemopoietic clonogenic cells by multivariate analyses and sorting. Exp Hematol *13*, 1173–1181.

Pang, W.W., Pluvinage, J.V., Price, E.A., Sridhar, K., Arber, D.A., Greenberg, P.L., Schrier, S.L., Park, C.Y., and Weissman, I.L. (2013). Hematopoietic stem cell and progenitor cell mechanisms in myelodysplastic syndromes. Proc Natl Acad Sci USA *110*, 3011–3016.

Pang, W.W., Price, E.A., Sahoo, D., Beerman, I., Maloney, W.J., Rossi, D.J., Schrier, S.L., and Weissman, I.L. (2011). Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc Natl Acad Sci USA *108*, 20012–20017.

Papaemmanuil, E., Cazzola, M., Boultwood, J., Malcovati, L., Vyas, P., Bowen, D., Pellagatti, A., Wainscoat, J.S., Hellström-Lindberg, E., Gambacorti-Passerini, C., et al. (2011). Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med *365*, 1384–1395.

Papaemmanuil, E., Gerstung, M., Bullinger, L., Gaidzik, V.I., Paschka, P., Roberts, N.D., Potter, N.E., Heuser, M., Thol, F., Bolli, N., et al. (2016). Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med *374*, 2209–2221.

Papaemmanuil, E., Gerstung, M., Malcovati, L., Tauro, S., Gundem, G., Van Loo, P., Yoon, C.J., Ellis, P., Wedge, D.C., Pellagatti, A., et al. (2013). Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood *122*, 3616–27–quiz3699.

Parikh, A.R., Olnes, M.J., and Barrett, A.J. (2012). Immunomodulatory treatment of myelodysplastic syndromes: antithymocyte globulin, cyclosporine, and alemtuzumab. Semin. Hematol. *49*, 304–311.

Park, S., Grabar, S., Kelaidi, C., Beyne-Rauzy, O., Picard, F., Bardet, V., Coiteux, V., Leroux, G., Lepelley, P., Daniel, M.-T., et al. (2008). Predictive factors of response and survival in myelodysplastic syndrome treated with erythropoietin and G-CSF: the GFM experience. Blood *111*, 574–582.

Parmar, S., de Lima, M., Deeg, H.J., and Champlin, R. (2011). Hematopoietic stem cell transplantation for myelodysplastic syndrome: a review. Semin Oncol *38*, 693–704.

Pedersen-Bjergaard, J., Philip, P., Pedersen, N.T., Hou-Jensen, K., Svejgaard, A., Jensen, G., and Nissen, N.I. (1984). Acute nonlymphocytic leukemia, preleukemia, and acute myeloproliferative syndrome secondary to treatment of other malignant diseases. II. Bone marrow cytology, cytogenetics, results of HLA typing, response to antileukemic chemotherapy, and survival in a total series of 55 patients. Cancer *54*, 452–462.

Preudhomme, C., Vanrumbeke, M., Lai, J.L., Lepelley, P., Wattel, E., and Fenaux, P. (1994). Inactivation of the p53 gene in leukemias and myelodysplastic syndrome (MDS) with 17p monosomy. Leukemia *8*, 2241–2242.

Przychodzen, B., Jerez, A., Guinta, K., Sekeres, M.A., Padgett, R., Maciejewski, J.P., and Makishima, H. (2013). Patterns of missplicing due to somatic U2AF1 mutations in myeloid neoplasms. Blood *122*, 999–1006.

Quintana, E., Shackleton, M., Sabel, M.S., Fullen, D.R., Johnson, T.M., and Morrison, S.J. (2008). Efficient tumour formation by single human melanoma cells. Nature *456*, 593–598.

Raaijmakers, M.H.G.P. (2011). Niche contributions to oncogenesis: emerging concepts and implications for the hematopoietic system. Haematologica *96*, 1041–1048.

Raaijmakers, M.H.G.P., and Scadden, D.T. (2008). Evolving concepts on the microenvironmental niche for hematopoietic stem cells. Curr. Opin. Hematol. *15*, 301–306.

Raaijmakers, M.H.G.P., Mukherjee, S., Guo, S., Zhang, S., Kobayashi, T., Schoonmaker, J.A., Ebert, B.L., Al-Shahrour, F., Hasserjian, R.P., Scadden, E.O., et al. (2010). Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature *464*, 852–857.

Ramakrishnan, A., Awaya, N., Bryant, E., and Torok-Storb, B. (2006). The stromal component of the marrow microenvironment is not derived from the malignant clone in MDS. Blood *108*, 772–773.

Rampal, R., Alkalin, A., Madzo, J., Vasanthakumar, A., Pronier, E., Patel, J., Li, Y., Ahn, J., Abdel-Wahab, O., Shih, A., et al. (2014). DNA hydroxymethylation profiling reveals that WT1 mutations result in loss of TET2 function in acute myeloid leukemia. CellReports *9*, 1841–1855.

Rasmussen, K.D., Jia, G., Johansen, J.V., Pedersen, M.T., Rapin, N., Bagger, F.O., Porse, B.T., Bernard, O.A., Christensen, J., and Helin, K. (2015). Loss of TET2 in hematopoietic cells leads to DNA hypermethylation of active enhancers and induction of leukemogenesis. Genes Dev *29*, 910–922.

Raza, A., Meyer, P., Dutt, D., Zorat, F., Lisak, L., Nascimben, F., Randt, du, M., Kaspar, C., Goldberg, C., Loew, J., et al. (2001). Thalidomide produces transfusion independence in long-standing refractory anemias of patients with myelodysplastic syndromes. Blood *98*, 958–965.

Raza, A., Reeves, J.A., Feldman, E.J., Dewald, G.W., Bennett, J.M., Deeg, H.J., Dreisbach, L., Schiffer, C.A., Stone, R.M., Greenberg, P.L., et al. (2008). Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-1 risk myelodysplastic syndromes with karyotypes other than deletion 5q. Blood *111*, 86–93.

Reya, T., Morrison, S.J., Clarke, M.F., and Weissman, I.L. (2001). Stem cells, cancer, and cancer stem cells. Nature *414*, 105–111.

Rowley, J.D., Golomb, H.M., and Vardiman, J.W. (1981). Nonrandom chromosome abnormalities in acute leukemia and dysmyelopoietic syndromes in patients with previously treated malignant disease. Blood *58*, 759–767.

Rozman, C., Grañena, A., Nomdedeu, B., Vives-Corrons, J.L., Montserrat, E., Hernandez-Nieto, L., and Feliu, E. (1978). Attempt of isogeneic bone marrow transplantation without conditioning in a

preleukaemic syndrome, negative result [proceedings]. Pathol. Biol. *26*, 47.

Saft, L., Karimi, M., Ghaderi, M., Matolcsy, A., Mufti, G.J., Kulasekararaj, A., Göhring, G., Giagounidis, A., Selleslag, D., Muus, P., et al. (2014). p53 protein expression independently predicts outcome in patients with lower-risk myelodysplastic syndromes with del(5q). Haematologica *99*, 1041–1049.

Salacz, M.E., Lankiewicz, M.W., and Weissman, D.E. (2007). Management of thrombocytopenia in bone marrow failure: a review. J Palliat Med *10*, 236–244.

Sanjuan-Pla, A., Macaulay, I.C., Jensen, C.T., Woll, P.S., Luis, T.C., Mead, A., Moore, S., Carella, C., Matsuoka, S., Bouriez Jones, T., et al. (2013). Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. Nature *502*, 232–236.

Sashida, G., Harada, H., Matsui, H., Oshima, M., Yui, M., Harada, Y., Tanaka, S., Mochizuki-Kashio, M., Wang, C., Saraya, A., et al. (2014). Ezh2 loss promotes development of myelodysplastic syndrome but attenuates its predisposition to leukaemic transformation. Nat Commun *5*, 4177.

Sauntharajah, Y., Nakamura, R., Wesley, R., Wang, Q.J., and Barrett, A.J. (2003). A simple method to predict response to immunosuppressive therapy in patients with myelodysplastic syndrome. Blood *102*, 3025–3027.

Schepers, A.G., Snippert, H.J., Stange, D.E., van den Born, M., van Es, J.H., van de Wetering, M., and Clevers, H. (2012). Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. Science *337*, 730–735.

Schepers, K., Campbell, T.B., and Passegué, E. (2015). Normal and leukemic stem cell niches: insights and therapeutic opportunities. Cell Stem Cell *16*, 254–267.

Schepers, K., Pietras, E.M., Reynaud, D., Flach, J., Binnewies, M., Garg, T., Wagers, A.J., Hsiao, E.C., and Passegué, E. (2013). Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. Cell Stem Cell *13*, 285–299.

Scheuermann, J.C., de Ayala Alonso, A.G., Oktaba, K., Ly-Hartig, N., McGinty, R.K., Fraterman, S., Wilm, M., Muir, T.W., and Müller, J. (2010). Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. Nature *465*, 243–247.

Schneider, R.K., Ademà, V., Heckl, D., Järås, M., Mallo, M., Lord, A.M., Chu, L.P., McConkey, M.E., Kramann, R., Mullally, A., et al. (2014). Role of Casein Kinase 1A1 in the Biology and Targeted Therapy of del(5q) MDS. Cancer Cell *26*, 509–520.

Sebaa, A., Ades, L., Baran-Marzack, F., Mozziconacci, M.-J., Penther, D., Dobbelstein, S., Stamatoullas, A., Récher, C., Prebet, T., Moulessehoul, S., et al. (2012). Incidence of 17p deletions and TP53 mutation in myelodysplastic syndrome and acute myeloid leukemia with 5q deletion. Genes Chromosomes Cancer *51*, 1086–1092.

Shih, A.H., Abdel-Wahab, O., Patel, J.P., and Levine, R.L. (2012). The role of mutations in epigenetic regulators in myeloid malignancies. Nat Rev Cancer *12*, 599–612.

Shultz, L.D., Schweitzer, P.A., Christianson, S.W., Gott, B., Schweitzer, I.B., Tennent, B., McKenna, S., Mobraaten, L., Rajan, T.V., and Greiner, D.L. (1995). Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol *154*, 180–191.

Shultz, L.D., Lyons, B.L., Burzenski, L.M., Gott, B., Chen, X., Chaleff, S., Kotb, M., Gillies, S.D., King, M., Mangada, J., et al. (2005). Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol *174*, 6477–6489.

Side, L.E., Curtiss, N.P., Teel, K., Kratz, C., Wang, P.W., Larson, R.A., Le Beau, M.M., and Shannon, K.M. (2004). RAS, FLT3, and TP53 mutations in therapy-related myeloid malignancies with abnormalities of chromosomes 5 and 7. Genes Chromosomes Cancer *39*, 217–223.

Siminovitch, L., McCulloch, E.A., and Till, J.E. (1963). The distribution of colony-forming cells among spleen colonies. J Cell Comp Physiol *62*, 327–336.

Simmons, P.J., Przepiorka, D., Thomas, E.D., and Torok-Storb, B. (1987). Host origin of marrow stromal cells following allogeneic bone marrow transplantation. Nature *328*, 429–432.

Singh, S.K., Hawkins, C., Clarke, I.D., Squire, J.A., Bayani, J., Hide, T., Henkelman, R.M., Cusimano, M.D., and Dirks, P.B. (2004). Identification of human brain tumour initiating cells. Nature *432*, 396–401.

Sloand, E.M., Wu, C.O., Greenberg, P., Young, N., and Barrett, J. (2008). Factors affecting response and survival in patients with myelodysplasia treated with immunosuppressive therapy. J Clin Oncol *26*, 2505–2511.

Smith, A., Howell, D., Patmore, R., Jack, A., and Roman, E. (2011). Incidence of haematological malignancy by sub-type: a report from the Haematological Malignancy Research Network. Br J Cancer *105*, 1684–1692.

Smith, A.E., Mohamedali, A.M., Kulasekararaj, A., Lim, Z., Gäken, J., Lea, N.C., Przychodzen, B., Mian, S.A., Nasser, E.E., Shooter, C., et al. (2010). Next-generation sequencing of the TET2 gene in 355 MDS and CMML patients reveals low-abundance mutant clones with early origins, but indicates no definite prognostic value. Blood *116*, 3923–3932.

Soenen-Cornu, V., Tourino, C., Bonnet, M.-L., Guillier, M., Flamant, S., Kotb, R., Bernheim, A., Bourhis, J.-H., Preudhomme, C., Fenaux, P., et al. (2005). Mesenchymal cells generated from patients with myelodysplastic syndromes are devoid of chromosomal clonal markers and support short- and long-term hematopoiesis in vitro. Oncogene *24*, 2441–2448.

Sole, F., Espinet, B., Sanz, G.F., Cervera, J., Calasanz, M.J., Luño, E., Prieto, F., Granada, I., Hernández, J.M., Cigudosa, J.C., et al. (2000). Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary myelodysplastic syndromes. Grupo Cooperativo Español de Citogenética Hematológica. Br J Haematol *108*, 346–356.

Sole, F., Prieto, F., Badia, L., Woessner, S., Florensa, L., Caballin, M.R., Coll, M.D., Besses, C., and Sans-Sabrafen, J. (1992). Cytogenetic studies in 112 cases of untreated myelodysplastic syndromes. Cancer Genet. Cytogenet. *64*, 12–20.

Spangrude, G.J., and Johnson, G.R. (1990). Resting and activated subsets of mouse multipotent hematopoietic stem cells. Proceedings of the National Academy of Sciences *87*, 7433–7437.

Spangrude, G.J., Heimfeld, S., and Weissman, I.L. (1988). Purification and characterization of mouse hematopoietic stem cells. Science *241*, 58–62.

Sperling, A.S., Gibson, C.J., and Ebert, B.L. (2017). The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. Nat Rev Cancer *17*, 5–19.

Spooncer, E., Lord, B.I., and Dexter, T.M. (1985). Defective ability to self-renew in vitro of highly purified primitive haematopoietic cells. Nature *316*, 62–64.

Steensma, D.P. (2012). Dysplasia has A differential diagnosis: distinguishing genuine myelodysplastic syndromes (MDS) from mimics, imitators, copycats and impostors. Curr Hematol Malig Rep *7*, 310–320.

Steensma, D.P. (2015). Myelodysplastic Syndromes: Diagnosis and Treatment. Mayo Clin. Proc. *90*, 969–983.

Steensma, D.P., Bejar, R., Jaiswal, S., Lindsley, R.C., Sekeres, M.A., Hasserjian, R.P., and Ebert, B.L. (2015). Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood *126*, 9–16.

Steensma, D.P., Gibbons, R.J., Mesa, R.A., Tefferi, A., and Higgs, D.R. (2005). Somatic point mutations in RUNX1/CBFA2/AML1 are common in high-risk myelodysplastic syndrome, but not in myelofibrosis with myeloid metaplasia. Eur. J. Haematol. *74*, 47–53.

Stoddart, A., Fernald, A.A., Wang, J., Davis, E.M., Karrison, T., Anastasi, J., and Le Beau, M.M. (2014). Haploinsufficiency of del(5q) genes, Egr1 and Apc, cooperate with Tp53 loss to induce acute myeloid leukemia in mice. Blood *123*, 1069–1078.

Storb, R.F., Champlin, R., Riddell, S.R., Murata, M., Bryant, S., and Warren, E.H. (2001). Non-myeloablative transplants for malignant disease. Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program 375–391.

Storb, R. (2003). Allogeneic hematopoietic stem cell transplantation--yesterday, today, and tomorrow. Exp Hematol *31*, 1–10.

Streuli, R.A., Testa, J.R., Vardiman, J.W., Mintz, U., Golomb, H.M., and Rowley, J.D. (1980). Dysmyelopoietic syndrome: sequential clinical and cytogenetic studies. Blood *55*, 636–644.

Sugimoto, K., Hirano, N., Toyoshima, H., Chiba, S., Mano, H., Takaku, F., Yazaki, Y., and Hirai, H. (1993). Mutations of the p53 gene in myelodysplastic syndrome (MDS) and MDS-derived leukemia. Blood *81*, 3022–3026.

Sutherland, H.J., Eaves, C.J., Eaves, A.C., Dragowska, W., and Lansdorp, P.M. (1989). Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis in vitro. Blood *74*, 1563–1570.

Swerdlow, S.H. (2008). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (World Health Organization).

Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L., et al. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science *324*, 930–935.

Takahashi, K., Jabbour, E., Wang, X., Luthra, R., Bueso-Ramos, C., Patel, K., Pierce, S., Yang, H., Wei, Y., Daver, N., et al. (2013). Dynamic acquisition of FLT3 or RAS alterations drive a subset of patients with lower risk MDS to secondary AML. Leukemia *27*, 2081–2083.

Tang, J.-L., Hou, H.-A., Chen, C.-Y., Liu, C.-Y., Chou, W.-C., Tseng, M.-H., Huang, C.-F., Lee, F.-Y., Liu, M.-C., Yao, M., et al. (2009). AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. Blood *114*, 5352–5361.

Taussig, D.C., Miraki-Moud, F., Anjos-Afonso, F., Pearce, D.J., Allen, K., Ridler, C., Lillington, D., Oakervee, H., Cavenagh, J., Agrawal, S.G., et al. (2008). Anti-CD38 antibody-mediated clearance of human repopulating cells masks the heterogeneity of leukemia-initiating cells. Blood *112*, 568–575.

Tehranchi, R., Fadeel, B., Forsblom, A.-M., Christensson, B., Samuelsson, J., Zhivotovsky, B., and Hellström-Lindberg, E. (2003). Granulocyte colony-stimulating factor inhibits spontaneous cytochrome c release and mitochondria-dependent apoptosis of myelodysplastic syndrome hematopoietic progenitors. Blood *101*, 1080–1086.

Tehranchi, R., Woll, P.S., Anderson, K., Buza-Vidas, N., Mizukami, T., Mead, A.J., Astrand-Grundström, I., Strömbeck, B., Horvat, A., Ferry, H., et al. (2010). Persistent malignant stem cells in del(5q) myelodysplasia in remission. N Engl J Med *363*, 1025–1037.

Testa, N.G., and Dexter, T.M. (1991). The biology of long-term bone marrow cultures and its application to bone marrow transplantation. Current Opinion in Oncology *3*, 272–278.

Thomas, E.D. (1991). Frontiers in bone marrow transplantation. Blood Cells *17*, 259–267.

Thota, S., Viny, A.D., Makishima, H., Spitzer, B., Radivoyevitch, T., Przychodzen, B., Sekeres, M.A., Levine, R.L., and Maciejewski, J.P. (2014). Genetic alterations of the cohesin complex genes in myeloid malignancies. Blood *124*, 1790–1798.

Till, J.E., and McCulloch, E.A. (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat. Res. *14*, 213–222.

Tomonaga, M., Tomonaga, Y., Kusano, M., and Ichimaru, M. (1984). Sequential karyotypic evolutions and bone marrow aplasia preceding acute myelomonocytic transformation from myelodysplastic syndrome. Br J Haematol *58*, 53–60.

Tricot, G., Boogaerts, M.A., De Wolf-Peeters, C., Van den Berghe, H., and Verwilghen, R.L. (1985). The myelodysplastic syndromes: different evolution patterns based on sequential morphological and cytogenetic investigations. Br J Haematol *59*, 659–670.

Tsai, F.Y., Keller, G., Kuo, F.C., Weiss, M., Chen, J., Rosenblatt, M., Alt, F.W., and Orkin, S.H. (1994). An early haematopoietic defect in mice lacking the transcription factor GATA-2. Nature *371*, 221–226.

Uchida, N., and Weissman, I.L. (1992). Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin- Sca-1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow. J. Exp. Med. *175*, 175–184.

Vadhan-Raj, S., Keating, M., LeMaistre, A., Hittelman, W.N., McCredie, K., Trujillo, J.M., Broxmeyer, H.E., Henney, C., and Gutterman, J.U. (1987). Effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. N Engl J Med *317*, 1545–1552.

van de Loosdrecht, A.A., Alhan, C., Béné, M.C., Porta, Della, M.G., Dräger, A.M., Feuillard, J., Font, P., Germing, U., Haase, D., Homburg, C.H., et al. (2009). Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. Haematologica 2009 94: 1124–1134.

van de Loosdrecht, A.A., Westers, T.M., Westra, A.H., Dräger, A.M., van der Velden, V.H.J., and Ossenkoppele, G.J. (2008). Identification of distinct prognostic subgroups in low- and intermediate-1-risk myelodysplastic syndromes by flow cytometry. Blood *111*, 1067–1077.

Van den Berghe, H., Cassiman, J.J., David, G., Fryns, J.P., Michaux, J.L., and Sokal, G. (1974). Distinct haematological disorder with deletion of long arm of no. 5 chromosome. Nature *251*, 437–438.

Vener, C., Fracchiolla, N.S., Gianelli, U., Calori, R., Radaelli, F., Iurlo, A., Caberlon, S., Gerli, G., Boiocchi, L., and Deliliers, G.L. (2008). Prognostic implications of the European consensus for grading of bone marrow fibrosis in chronic idiopathic myelofibrosis. Blood *111*, 1862–1865.

Viny, A.D., and Levine, R.L. (2017). Cohesin mutations in myeloid malignancies made simple. Curr. Opin. Hematol. 1.

Visser, J.W., and De Vries, P. (1990). Identification and purification of murine hematopoietic stem

cells by flow cytometry. Methods Cell Biol *33*, 451–468.

Visvader, J.E., and Lindeman, G.J. (2008). Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer *8*, 755–768.

Vogelstein, B., Papadopoulos, N., Velculescu, V.E., Zhou, S., Diaz, L.A., and Kinzler, K.W. (2013). Cancer genome landscapes. Science *339*, 1546–1558.

Vose, J.M., Bierman, P.J., Lynch, J.C., Atkinson, K., Juttner, C., Hanania, C.E., Bociek, G., and Armitage, J.O. (2001). Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with recurrent indolent non-Hodgkin's lymphoma. Biol Blood Marrow Transplant *7*, 680–687.

Walkley, C.R., Olsen, G.H., Dworkin, S., Fabb, S.A., Swann, J., McArthur, G.A., Westmoreland, S.V., Chambon, P., Scadden, D.T., and Purton, L.E. (2007). A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. Cell *129*, 1097–1110.

Walter, M.J., Ding, L., Shen, D., Shao, J., Grillot, M., McLellan, M., Fulton, R., Schmidt, H., Kalicki-Veizer, J., O'Laughlin, M., et al. (2011). Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia *25*, 1153–1158.

Walter, M.J., Shen, D., Shao, J., Ding, L., White, B.S., Kandoth, C., Miller, C.A., Niu, B., McLellan, M.D., Dees, N.D., et al. (2013). Clonal diversity of recurrently mutated genes in myelodysplastic syndromes. Leukemia *27*, 1275–1282.

Walter, M.J., Shen, D., Ding, L., Shao, J., Koboldt, D.C., Chen, K., Larson, D.E., McLellan, M.D., Dooling, D., Abbott, R., et al. (2012). Clonal architecture of secondary acute myeloid leukemia. N Engl J Med *366*, 1090–1098.

Wang, H., Wang, L., Erdjument-Bromage, H., Vidal, M., Tempst, P., Jones, R.S., and Zhang, Y. (2004). Role of histone H2A ubiquitination in Polycomb silencing. Nature *431*, 873–878.

Wang, Y., Xiao, M., Chen, X., Chen, L., Xu, Y., Lv, L., Wang, P., Yang, H., Ma, S., Lin, H., et al. (2015). WT1 recruits TET2 to regulate its target gene expression and suppress leukemia cell proliferation. Mol Cell *57*, 662–673.

Ward, P.S., Patel, J., Wise, D.R., Abdel-Wahab, O., Bennett, B.D., Coller, H.A., Cross, J.R., Fantin, V.R., Hedvat, C.V., Perl, A.E., et al. (2010). The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. Cancer Cell *17*, 225–234.

Wattel, E., Preudhomme, C., Hecquet, B., Vanrumbeke, M., Quesnel, B., Dervite, I., Morel, P., and Fenaux, P. (1994). p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood *84*, 3148–3157.

Weissman, I.L. (2000). Stem cells: units of development, units of regeneration, and units in evolution. Cell *100*, 157–168.

Welch, J.S., Ley, T.J., Link, D.C., Miller, C.A., Larson, D.E., Koboldt, D.C., Wartman, L.D., Lamprecht, T.L., Liu, F., Xia, J., et al. (2012). The origin and evolution of mutations in acute myeloid leukemia. Cell *150*, 264–278.

Wells, D.A., Benesch, M., Loken, M.R., Vallejo, C., Myerson, D., Leisenring, W.M., and Deeg, H.J. (2003). Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. Blood *102*, 394–403.

Whitlock, C.A., Tidmarsh, G.F., Muller-Sieburg, C., and Weissman, I.L. (1987). Bone marrow stromal cell lines with lymphopoietic activity express high levels of a pre-B neoplasia-associated molecule. Cell *48*, 1009–1021.

Will, B., Zhou, L., Vogler, T.O., Ben-Neriah, S., Schinke, C., Tamari, R., Yu, Y., Bhagat, T.D., Bhattacharyya, S., Barreyro, L., et al. (2012). Stem and progenitor cells in myelodysplastic syndromes show aberrant stage-specific expansion and harbor genetic and epigenetic alterations. Blood *120*, 2076–2086.

Williams, D.A. (2000). Stem Cell Model of Hematopoiesis. In Hematology, Basic Principles and Practice (3rd edition).

Winkler, I.G., Sims, N.A., Pettit, A.R., Barbier, V., Nowlan, B., Helwani, F., Poulton, I.J., van Rooijen, N., Alexander, K.A., Raggatt, L.J., et al. (2010). Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood *116*, 4815–4828.

Wu, A.M., Till, J.E., Siminovitch, L., and McCulloch, E.A. (1968). Cytological evidence for a relationship between normal hemotopoietic colony-forming cells and cells of the lymphoid system. J. Exp. Med. *127*, 455–464.

Xie, M., Lu, C., Wang, J., McLellan, M.D., Johnson, K.J., Wendl, M.C., McMichael, J.F., Schmidt, H.K., Yellapantula, V., Miller, C.A., et al. (2014). Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nature Medicine *20*, 1472–1478.

Xu, W., Yang, H., Liu, Y., Yang, Y., Wang, P., Kim, S.-H., Ito, S., Yang, C., Wang, P., Xiao, M.-T., et al. (2011). Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. Cancer Cell *19*, 17–30.

Yamamoto, R., Morita, Y., Ooehara, J., Hamanaka, S., Onodera, M., Rudolph, K.L., Ema, H., and Nakauchi, H. (2013). Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. Cell *154*, 1112–1126.

Yamazaki, J., Jelinek, J., Lu, Y., Cesaroni, M., Madzo, J., Neumann, F., He, R., Taby, R., Vasanthakumar, A., Macrae, T., et al. (2015). TET2 Mutations Affect Non-CpG Island DNA Methylation at Enhancers and Transcription Factor-Binding Sites in Chronic Myelomonocytic Leukemia. Cancer Research *75*, 2833–2843.

Yang, L., Rau, R., and Goodell, M.A. (2015). DNMT3A in haematological malignancies. Nat Rev Cancer *15*, 152–165.

Yoshida, K., Sanada, M., Shiraishi, Y., Nowak, D., Nagata, Y., Yamamoto, R., Sato, Y., Sato-Otsubo, A., Kon, A., Nagasaki, M., et al. (2011). Frequent pathway mutations of splicing machinery in myelodysplasia. Nature *478*, 64–69.

Yunis, J.J., Lobell, M., Arnesen, M.A., Oken, M.M., Mayer, M.G., Rydell, R.E., and Brunning, R.D. (1988). Refined chromosome study helps define prognostic subgroups in most patients with primary myelodysplastic syndrome and acute myelogenous leukaemia. Br J Haematol *68*, 189–194.

Zemanova, Z., Michalova, K., Buryova, H., Brezinova, J., Kostylkova, K., Bystricka, D., Novakova, M., Sarova, I., Izakova, S., Lizcova, L., et al. (2014). Involvement of deleted chromosome 5 in complex chromosomal aberrations in newly diagnosed myelodysplastic syndromes (MDS) is correlated with extremely adverse prognosis. Leukemia Research *38*, 537–544.

Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W.-G., Ross, J., Haug, J., Johnson, T., Feng, J.Q., et al. (2003). Identification of the haematopoietic stem cell niche and control of the niche size. Nature *425*, 836–841.

Zhang, M.Y., Churpek, J.E., Keel, S.B., Walsh, T., Lee, M.K., Loeb, K.R., Gulsuner, S., Pritchard, C.C., Sanchez-Bonilla, M., Delrow, J.J., et al. (2015). Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. Nat Genet *47*, 180–185.

Zhang, X., Su, J., Jeong, M., Ko, M., Huang, Y., Park, H.J., Guzman, A., Lei, Y., Huang, Y.-H., Rao, A., et al. (2016). DNMT3A and TET2 compete and cooperate to repress lineage-specific transcription factors in hematopoietic stem cells. Nat Genet *48*, 1014–1023.

Zhao, M., Perry, J.M., Marshall, H., Venkatraman, A., Qian, P., He, X.C., Ahamed, J., and Li, L. (2014). Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. Nature Publishing Group *20*, 1321–1326.