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Molecular Pathogenesis of Leukemia Progression in MLL-ENL Mediated Leukemogenesis

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DOCTORAL DISSERTATION

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Abstract

Development of acute myeloid leukemia (AML) involves stepwise processes. Genetic and/or epigenetic events emerging in normal immature hematopoietic cells give rise to leukemia initiating cells (LICs), which have a competitive advantage over normal hematopoietic progenitors. This is followed by acquisition of additional genetic perturbations that further promote proliferation and/or inhibit differentiation of LICs, ultimately leading to transformation. Balanced chromosomal translocations that involve the mixed lineage leukemia-1 (MLL1/KMT2A) gene can generate chimeric MLL fusion proteins. MLL-rearrangements (MLLr) account for 10% of human acute leukemia and represent a group of leukemia with distinct molecular features and associate with poor prognosis in AML. The powerful oncogenic activity of MLL fusion proteins has been frequently exploited in mouse models to explore the pathogenesis of leukemic initiation, evolution, therapy resistance, and relapse. However, the molecular pathogenesis of leukemia transformation remains to a large extent unknown. In this thesis, we explored an inducible transgenic mouse strain (iMLL-ENL mice) previously generated in our laboratory, in which a human *MLL-ENL* fusion gene can be conditionally induced by Doxycycline.

In the first study (article I), we identified a highly recurrent secondary driver mutation, MSN R295C, and explored its mechanistic consequences in AML development. By demonstrating the indispensable role of MSN R295C in AML progression, this study provided new insight to the molecular pathogenesis in *MLL*r AML. Further studies addressing the function of MSN R295C revealed a potential mechanism that involved the highly conserved structure and function of ERM proteins. These studies demonstrated a critical role of ERM proteins in AML, with implications also for human cancer.

In the second study (article II), we explored the influence of the fetal specific gene Lin28b on AML development by using the same AML model described in Article I. The study proposes that fetal-specific genes such as Lin28b might constrain AML development during fetal development, which in an extension also might influence on the relatively low frequency of AML in children. In addition, we proposed a LIN28B/ MYBBP1A /MYB tumor suppressor axis, which might explain the tumor suppressor activity of LIN28B in MLLr AML. In this view, this study shed new light on the prospects of Lin28b and/or its downstream effector as a novel therapeutic target in MYB-dependent cancers.

In the third study (article III), we detailed attempts to ex vivo expand hematopoietic stem cells (HSCs). Although HSCs were successfully expanded, we found that the output cells following ex vivo expansion of candidate HSCs are heterogeneous, with only 0.1% of cultured cells retaining the original input HSC phenotype. By applying barcoding methodologies to assess the clonality associated with the ex vivo expansion, we found that even within the strictly defined LT-HSC, there still exist significant functional heterogeneity among individual candidate HSCs. This emphasizes the need to evaluate function rather than merely phenotype.

Taken together, the thesis' main content revolves around the use of mouse models to approach acute myeloid leukemia (AML) (Articles I and II) and hematopoietic stem cell function (Article III).

Key words AML; MLL fusions; murine model; secondary mutation; ERM; clonal evolution; Lin28b; HSCs.

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Molecular Pathogenesis of Leukemia Progression in MLL-ENL Mediated Leukemogenesis

Ouyang Yuan

*Do all things with love,
passion and dedication!
– Patrick Driessen*

要意志坚强，要勤奋，要探索，要发现，而且永不屈服。
——赫胥黎

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II. A fetal tumor suppressor axis abrogates mll-fusion driven acute myeloid leukemia.

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III. Ex vivo expansion potential of murine hematopoietic stem cells: a rare property only partially predicted by phenotype.

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Abbreviations

AEP AF4 family/ENL family/P-TEFb complex

AGM aorta-gonad-mesonephros

ALL acute lymphocytic leukemia

AML and acute myeloid leukemia

ASH2L ASH2 Like, Histone Lysine Methyltransferase Complex Subunit

BAL biphenotypic acute leukemia

BCR breakpoint cluster region

BLPs B-cell-biased lymphoid progenitors

BM bone marrow

BMT bone marrow transplantation

CBP CREB-binding protein

CFU-S colony forming unit-spleen

CH Clonal hematopoiesis

CLL chronic lymphocytic leukemia

CLPs common lymphoid progenitors

CML chronic myeloid leukemia

dHSCs definitive HSCs

ERM Ezrin-Radixin-Moesin

FACS Fluorescence-Activated Cell Sorting

FL fetal liver

GMLPs granulocyte monocyte lymphoid progenitors

GMP granulocyte-macrophage progenitors

GOF gain-of-function

HCT Hematopoietic stem cell transplantation

H3K4 histone H3 lysine residue 4

H4K16 histone H4 lysine 16

H3K27 histone H3 lysine residue 27

H3K79 lysine 79 residues in histone H3

HPCs hematopoietic progenitor cells

HSCs Hematopoietic stem cells

HSPCs hematopoietic stem and progenitor cells

LEDGF lens epithelium-derived growth factor

LICs leukemia initiating cells

LMPP lymphoid-primed multipotent progenitors

LOF loss-of-function

LSCs leukemic stem cells

LSK Lin-Sca1+cKit+

LT-HSCs long-term repopulating HSCs

MEN1 menin

miR microRNA

MLL Mixed lineage leukemia

MLL-r MLL-rearranged

mRNP messenger ribonucleoprotein

MSN Moesin

MkP megakaryocyte progenitors

MDS myelodysplastic syndrome

MOF males absent on first

MPN myeloproliferative neoplasm

MPPs multipotent progenitors

NK natural killer

PHD plant homology domains

pMeg/E pre-megakaryocyte-erythroid progenitor

preCFU-E pre-colony-forming-unit-erythroid

preGMs pre-granulocytes-monocytes

PVA polyvinyl alcohol

RBPB5 retinoblastoma binding protein 5

sAML Secondary AML

Sca1 stem cell antigen 1

SNL speckled nuclear localization

TrxG trithorax group

VAF variant allele frequency

WDR5 WD Repeat Domain 5

WHO World Health Organization

VAF variant allele frequency

Introduction

Hematopoiesis

Except for some long-lived hematopoietic cell types, such as memory T cells, most hematopoietic cells are short-lived. The latter includes erythrocytes, platelets, myeloid cells and most lymphocytes. These various blood cell lineages must be continuously regenerated to maintain homeostasis of the blood system in order to maintain crucial downstream functions such as oxygen transport, hemostasis and immunological defense mechanisms. Recent estimates suggest that the adult bone marrow (BM) produces 2–6×10^{10} cells/kg/day in mice and 5–7×10^{10} cells/kg/day in humans under homeostatic conditions/steady-state hematopoiesis. Moreover, upon situations of stress, such as for instance an infection, these numbers can be increased several-fold (*1*).

Blood lineages and the hematopoietic hierarchy

The blood system is typically divided into two branches, the lymphoid and myeloid lineages. The lymphoid lineage includes B cells, T cells and natural killer (NK) cells, whereas the myeloid lineage includes monocytes and granulocytes, thrombocytes and erythrocytes. Hematopoietic stem cells (HSCs) give rise to these mature cells through a sequential multistep differentiation cascade, which is referred to as the hematopoietic hierarchy (Figure 1). The hematopoietic hierarchy involves several intermediate progenitors that lose self-renewal capacity but not proliferation potential and become progressively restricted in their differentiation potential. This organization leads to a massive amplification of hematopoietic cell generation (*2*).

Initial steps of HSCs differentiation involve multipotent progenitors (MPPs) (*3*), and how MPPs commit to specific lineages has been debated. In the latest model, granulocyte monocyte lymphoid progenitors (GMLPs), highly overlapping with the previously identified lymphoid-primed multipotent progenitors (LMPP), were identified to be located downstream of MPPs. GMLPs harbor lymphoid and myeloid potential, while lacking megakaryocyte and erythroid (MegE) potential (*4, 5*). This suggests that the separation of MegE lineage from granulocytes, monocytes and lymphoid lineage occur downstream of MPP. MegE cells are proposed to be generated from HSCs and MPPs via pre-megakaryocyte-erythroid progenitor (pMeg/E) that can generate megakaryocyte progenitors (MkP) and erythroid

progenitors (pre-colony-forming-unit-erythroid; preCFU-E, CFU-E) (*6, 7*). GMLPs are committed to myeloid differentiation via pre-granulocytes-monocytes (preGMs), or to lymphoid differentiation via common lymphoid progenitors (CLPs) (*8*). preGM can be further subdivided into GATA1+ and GATA1- fraction, in which GATA1+ preGMs generate mast cells and eosinophils, while GATA1- preGMs generate monocytes and neutrophils (*9*).
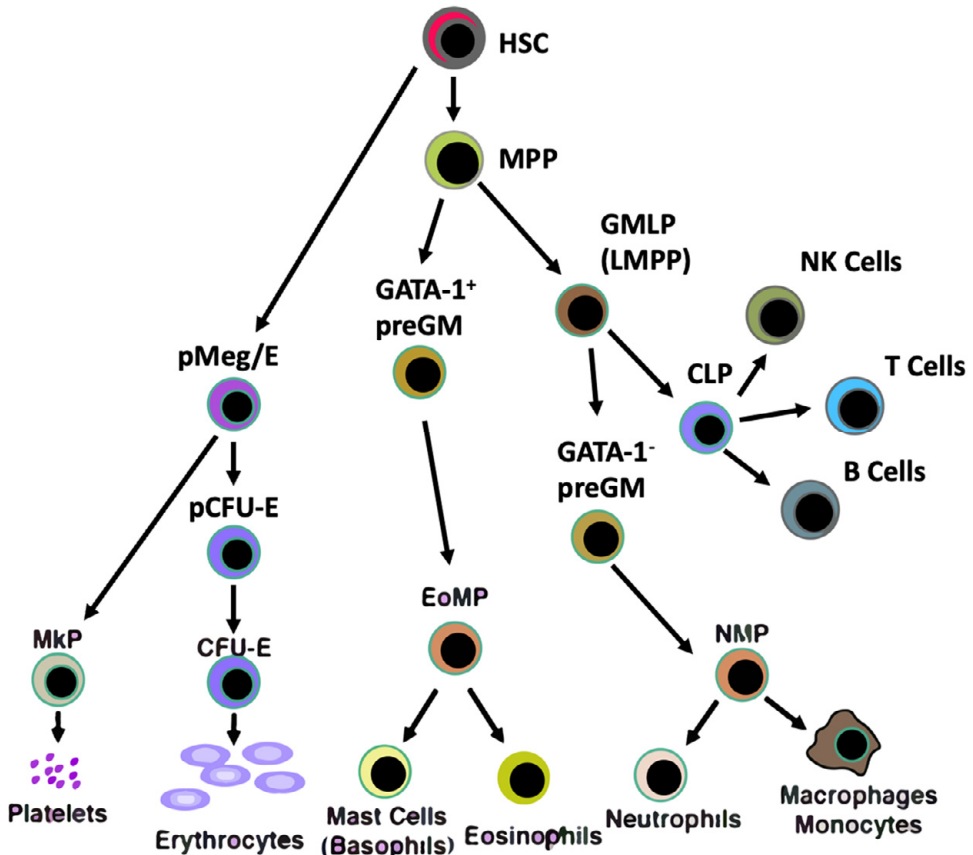

Figure 1. Schematic depiction of the proposed hematopoietic hierarchy and hematopoietic cells produced by HSCs. EoMP: Eosinophil Mast Cell progenitor, NMP: Neutrophil Monocyte progenitor.

Hematopoietic stem cells

The Discovery and definition of hematopoietic stem cells

In 1945, civilian populations in Hiroshima and Nagasaki were exposed to atomic bomb explosions. Many who survived the initial atomic bomb explosion were found to die of hematopoietic failure caused by exposure to gamma irradiation (*10*). Since then, hematopoietic researchers started to explain how blood cells were formed. With the findings that the hematopoietic failure could be rescued by shielding the spleen with lead from irradiation (*11*) or by injecting spleen or marrow cells (*12, 13*), researchers suggested the existence of proliferating hematopoietic progenitor cells in the bone marrow and spleen. In the 1960s, Till and McCulloch found a linear relationship between the number of transplanted bone marrow cells and the survival of lethally irradiated mice (*14*). Additionally, they identified gross nodules in the spleens of the recipients after irradiation and bone marrow cells transplantation. Those nodules were found to consist of colonies of rapidly proliferating hematopoietic tissue. Clusters of undifferentiated and differentiated cells, including erythroblasts, myelocytes, metamyelocytes and megakaryocytes were present (*15*). These results suggested that bone marrow cells were able to give rise to colonies in the spleen, referred to as colony forming unit-spleen (CFU-S), whose number was linearly correlated to the number of transplanted bone marrow cells. Next, the same group studied the clonality in the CFU-S by tracing the abnormal karyotype induced by sub-lethal irradiation. In this study, they showed that the vast majority of the mixed myeloerythroid progeny in the spleen colonies were clonal and originated from one individual cell (*16*). However, it was not until the 1980s that CFU-S colonies were found to fail to maintain hematopoiesis in the long-term and that they were derived from hematopoietic progenitor cells (HPCs) rather than from hematopoietic stem cells (HSCs), although colony forming cells in CFU-S could give rise to new CFU-S that contain both myeloid and lymphoid lineages following secondary transplantation (*17-19*).

In later work, donor cells were individually labelled by taking advantage of random retroviral genetic integration prior to transplantation. This approach allowed for long-term multilineage reconstitution analysis and estimation of the longevity (self-renewal capacity) of the individual donor cells through transplantations. By applying this strategy, several studies indicated the existence of cells with long-term multilineage differentiation potential and the ability to self-renew, which are today regarded as the two key defining properties of HSCs (*20-22*).

The next development in the identification, and also isolation, of HSCs are taking advantage of specific cell surface markers. Currently, the most common ones include mature lineage markers, stem cell antigen 1 (Sca1), tyrosine kinase receptor cKit, CD34, Flt3, CD48 and CD150. In mouse, the most primitive hematopoietic

stem and progenitor cells (HSPCs) are negative for mature lineage marker, but express Sca1 and cKit, referred to as Lin-Sca1+cKit+ (LSK) cells (*23-26*). Within the LSK fraction, HSCs can be further enriched as positive for CD150 and negative for CD34, Flt3, and CD48 (*27-29*).

With Fluorescence-Activated Cell Sorting (FACS)-based protocols, HSCs have been identified as very rare cells within the bone marrow that are capable of multilineage differentiation and long-term self-renewal in the bone marrow transplantation (BMT) assay (*30*). These properties also position HSCs as the preferred targets for gene therapy/gene editing approaches aimed to cure inherited blood cell disorders.

Hematopoietic stem cells during ontogeny

During embryonic development, hematopoiesis is characterized by successive waves of development, with the initial "primitive hematopoiesis" followed by "definitive hematopoiesis" (*31*). Primitive hematopoiesis occurs as early as day E7.5 in the yolk sac blood islands and is followed by the definitive hematopoiesis emerging at approximately E10.5. Primitive hematopoiesis is transient and produces mainly erythrocytes for oxygen transport but has little HSC activity. Definitive hematopoiesis, however, persist for life and involve definitive HSCs (dHSCs). dHSCs have been found to originate in the aorta-gonad-mesonephros (AGM) region (32, 33) and concomitantly migrate to other tissues, including the placenta (34, 35), vitelline/umbilical arteries (36, 37), the embryonic head (38) and the yolk sac (33, 39). Subsequently, dHSCs migrate to the fetal liver (FL) where they expand massively during E12-E16, coinciding with the extinction of their production by the AGM and placenta (*31, 40*). From E17.5 until week 2 after birth, dHSCs leave the liver and migrate to the BM and remain anchored in the BM thereafter throughout life (*41*). FL HSCs features are retained after seeding in the BM until week 4 after birth, when FL dHSCs characteristics switch to HSCs with adult characteristics (*41-43*).

FL HSCs are phenotypically and functionally distinct from adult HSCs with regards to their surface marker phenotype, cell cycle activities and differentiation potential (*42, 44-46*). The vast majority (95-100%) of FL HSCs are actively cycling, which is important to rapidly develop the blood system, while most adult HSCs are dormant, with estimations of only ~5% actively cycling to maintain homeostasis. FL HSCs repopulate mice more efficiently than adult HSCs in transplantation (*47*). FL HSCs produce a higher output of myeloid and erythroid cells, while adult HSCs generate a more balanced output consisting of all lineages (*46*). Additionally, some blood cell subsets are in fact only generated during FL hematopoiesis and not during adult hematopoiesis, such as tissue resident macrophages (*48*), lymphoid peritoneal

B1a B cells (*49*) and epidermal Vγ3δ+ T cells (*50*). Several studies have additionally revealed distinct molecular landscapes of FL and adult HSCs (*51, 52*).

Upon organismal aging, hallmarks of aged HSCs include impaired self-renewal capacity and a skewed differentiation potential towards myeloid cells after transplantation, which is accompanied by loss of lymphoid and erythroid potential (*53-56*). Age-associated features of HSCs are believed to be primarily driven by cell intrinsic alterations, which is suggested by experiments in which young HSCs were transplanted into aged recipients (*54*). The aged hematopoietic system is also characterized with distinct composition of HSPCs, in which the frequency of myeloid progenitors is increased while the frequency of lymphoid progenitors is decreased. The transcriptional profile of aged HSCs additionally support their observed myeloid biased differentiation potential (*57, 58*). Collectively, such findings support the interpretation that the phenotypes of the aged hematopoietic system are at least in part a consequence of cell-intrinsic age-associated alterations in HSCs.

Hematopoietic stem cell transplantation (HCT)

HCT is tremendously important both clinically and in experimental hematology. The mouse as a model system has been an indispensable tool for studying hematopoiesis since most fundamental hematological aspects are conserved between mice and humans. Because of this, HCT experiments into mice has been the mainstay of research into in vivo HSC biology.

When studied in isolation, the most primitive HSCs are not able to generate mature blood cells rapidly enough to provide a rescue from the immediate consequences of lethal irradiation (*59*). Therefore, co-transplantation of HPCs to initially and rapidly support the host is needed (*60, 61*).

Transplantation of HSCs and subsequent quantitative and qualitative monitoring has been used to determine the fate and activity of candidate HSCs (*62*). Typically, to distinguish donor HSCs and their progeny from host cells after transplantation, congenic mice that express cell surface makers CD45.1 and/or CD45.2 are used. Of note, only leukocytes express the CD45 antigen and thus donor cell reconstitution is assessed by investigation of Myeloid, B and T cell reconstitution, since erythrocytes and platelets are devoid of CD45 expression. Long-term and multilineage reconstitution potential of transplanted HSCs is typically assessed by the analysis of peripheral blood and bone marrow 16 weeks or longer post transplantation.

A common experimental setup is known as competitive transplantation, which assess the function of candidate HSCs against a known amount of competitor cells

(*63*). By comparing the lineage reconstitution derived from the donor HSCs to the portion of competitor-derived cells, with a known number of repopulating units, the repopulating activity of the candidate HSCs can be analyzed.

The self-renewal capacity of HSCs can be estimated by serial transplantations, in which candidate HSCs from primary recipient mice are assessed in secondary transplantation by repopulating analysis. Only the cells that can repopulate the secondary recipients are defined to have self-renewal capacity.

Acute leukemia

While historical evidence has indicated the existence of leukemia for thousands of years, it was only until the middle of the 19th century that such diseases were more directly studied. Reports included cases of acute hyperplasia of the spleen, with unexplainable milky blood accompanied by pus (*64*). In 1844, Alfred Donné performed microscopic examinations of the blood of a leukemic patient and detected an accumulation of leukocytes. He was the first to propose a maturation arrest as a cause of the leukocyte accumulation in the leukemic patient, which still is seen as a main feature of leukemia (*65, 66*). In the following year, John Bennett described the disease as a primary systemic blood disorder, which was considered a secondary manifestation caused by infection, that was named leucocythemia (*67, 68*).

In 1846, Henry Fuller observed that an abnormal proportion of the leukemic patient's blood was spherical, finely granular, and larger in size than ordinary leukocytes. With this abnormal morphology, Fuller became the first one to diagnose a patient with leukemia (*69*). In 1847, Rudolf Virchow coined the term leukemia 'leukämie', which is a combination of the Greek words "leukos/white" and "heima/blood" (*70*).

Later on, leukemia was found to be a very heterogeneous diseases, and several attempts were made to further categorize it. However, it was not until 1913 that leukemia was classified into four types: chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), and acute myeloid leukemia (AML) (*71*). Leukemias are considered acute or chronic based on the percentage of blast cells in the bone marrow or blood, while their lineage/phenotype governs their further subdivision into myeloid or lymphoid subtypes.

Acute myeloid leukemia

AML affects both children and adults. In fact, it is considered the most common type of acute leukemia in adults, with an incidence progressively increasing with age. The annual incidence of AML for those aged > 65 years is 20.1 per 100,000 in comparison to 2.0 per 100,000 for those < 65 years old (*72*).

In 2019, it was estimated in United States (US) that >10,900 patients were expected to die from AML, which represents 62% of all leukemia-related deaths and the 5th worst 5-year overall survival (OS) (24%) of all cancers in US (*73*). As of 2018, the estimated median OS of AML was 8.5 months, with a 2-year and a 5-year OS of 32.0% and 24.0%, respectively (*74*). A diagnosis of AML at age ≥ 75 years predicts the worst median OS (2.3 months) of all cancer types (*75*).

Secondary and de novo AML

AML can be the end result of various hematological diseases and/or malignancies, including myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN) and chronic leukemia, as a secondary disease/malignancy. Secondary AML can also develop as a consequence of chemotherapy and/or radiation therapy to pre-leukemic neoplasm (*76*). In contrast, AML that develops without any prior disease or exposure to therapies is referred to as de novo AML.

Infant, childhood and adult AML

Infant acute leukemia is historically defined by its occurrence in children under 1 year of age. This definition has been extended to include children up to 2 years of age, as patients aged between 1 to 2 years have been suggested to display similar clinical and biological patterns of disease (*77*). Childhood acute leukemia affects patients under 18 years of age. Infant AML account for ~25% of all infant leukemia cases. In childhood leukemia, the frequency of AML is ~33%, which rise to ~47% in adult AML (*78*).

The 5-year event-free survival rates of childhood ALL range between 76% - 86% in children receiving standard therapy in developed countries, while that of childhood AML range between 49% - 63% in some of the more successful clinical trials (*79*).

Clinical signs

AML is characterized by blocked differentiation and clonal proliferation of blast cells, leading to features of BM failure. If not treated, AML results in death within

months (*80*). The clinical symptoms of AML are diverse and nonspecific and usually correlated with the leukemic infiltration of the bone marrow. Leukemic infiltration of the bone marrow can repress the normal hematopoiesis and cause cytopenia. Typically, patients present with signs and symptoms of fatigue, bleeding, or infections and fever due to reductions in red cells, platelets, or white cells, respectively. In addition, leukemic infiltration of other tissues, including the liver, spleen, skin, lymph nodes, bone, gingiva, and the central nervous system, can produce a variety of other symptoms, including hepatomegaly, splenomegaly, leukemia cutis, lymphadenopathy and bone pain.

Diagnosis and Classification

Until the 1970s, the diagnosis was based solely on the pathological and cytologic examination of bone marrow and blood. In 1976, a group of French, American, and British leukemia experts divided AML into subtypes, M1 - M6 initially then M7 and M0 were added by 1987. This categorization is based on the cell type and the stage of maturity in which leukemia initially develops and was termed the FAB classification (*81*). This classification is based largely on the microscopic morphology following defined staining, but without taking into account many other factors that are now known to affect prognosis.

In 2001, and with the wider availability of chromosome analysis, a new classification for hematopoietic and lymphoid neoplasms based on recurrent cytogenetic abnormalities was proposed by the World Health Organization (WHO) together with the Society for Hematopathology and the European Association of Hematopathology (*82, 83*). The threshold for the diagnosis of AML was reduced from 30% to 20% blasts in the blood or marrow. In addition, patients with the clonal, recurring cytogenetic abnormalities t(8;21)(q22;q22), inv(16)(p13q22) or t(16;16)(p13;q22), and t(15;17)(q22;q12) should be considered to have AML regardless of the blast percentage (*83*). This classification was revised in 2008 (*84*) and was further refined in 2016 based on molecular alterations (*85*).

Risk factor of AML

Exactly why leukemia develops is still not fully understood. However, certain risk factors that increase the risk of AML development have been identified including age, male gender, irradiation, smoking, exposure to certain solvent chemicals (benzene, formaldehyde), chemotherapy history, genetic syndrome (Fanconi anemia, Bloom syndrome, Ataxia-telangiectasia, Diamond-Blackfan anemia, Shwachman-Diamond syndrome, Li-Fraumeni syndrome, Neurofibromatosis type 1, Kostmann syndrome, Down syndrome, Trisomy 8) and a family history with AML (*86, 87*).

Treatment of AML

During the 1970s a chemotherapy combination consisting of a daunorubicin and cytarabine was introduced, which remained the standard therapy for most AML patients until very recently (*88*). The current cure rate of AML is 35 to 40% of adult patients who are 60 years of age or younger and 5 to 15% of patients who are older than 60 years of age (*89*). Other drugs, including FLT3 inhibitors (*90*), Gemtuzumab ozogamicin (*91-94*), CPX-351 (*95*), cladribine (*96*) and clofarabine (*97*) have been proposed to improve induction therapy, which is used to eliminate leukemia cells and induce a remission, in several trials with or without combination of daunorubicin and cytarabine. Allogeneic stem cell transplantation is recommended for patients with high-risk cytogenetic characteristics, which predisposes for an adverse prognosis and a high risk of relapse following chemotherapy treatment. In addition, allogeneic stem cell transplantation is the only curative option for patients with poor induction therapy response and therapy related/secondary AML (*98*).

Stepwise development of AML

Emergence of subclones and clonal evolution in leukemia

AML develops as a consequence of the accumulation of mutations (*99*). Findings throughout the past two decades have provided evidence that AML arises from primitive hematopoietic cells that harbor mutations and epigenetic aberrations, initially leading to formation and expansion of leukemia initiating cells (LICs), and ultimately progressing to fully transformed leukemic stem cells (LSCs) (*100*). Thus, AML development is thought to be a multistep process. LICs, implied as pre-LSCs, are characterized by increased self-renewal, blocked differentiation, and reduced programmed cell death. They are able to outcompete normal HSCs and give rise to a population carrying a distinct genetic profile, which is known as a subclone.

Clonal hematopoiesis (CH) is identified based on the detection of mutations relevant to myeloid malignancies, with a variant allele frequency (VAF) above 2% in the peripheral blood (*101-105*). It has been estimated that at least 5%–10% of individuals over age 65 have such a hematopoietic clonal expansion, with the percentage further increasing with age (*103, 106, 107*). The physiological consequences of CH have been investigated intensively. Overall, detection of any CH has been shown to be associated with an 11-14-fold increase in the risk of developing hematologic malignancies. This increases to ~50-fold for clones with VAF > 10% (*102, 105, 108, 109*). However, many instances of CH show little or no overt effects, and only 4% of individuals with CH have been estimated to ever develop a hematologic malignancy. In such cases, CH represents an antecedent permissive state for leukemia development, and the subsequent acquisition of secondary mutations in the subclones result in a fulminant leukemia (*102, 110-114*).

Classical model of clonal progression represents a linear path. Thus, subclone undergoes clonal evolution through the acquisition of multiple additional mutations that ultimately lead to disease development (*113, 115*). Myelodysplastic syndrome (MDS), a closely related pre-leukemic disease in the clinic that frequently progresses to AML, provides a biological system to determine the clonal evolution underlying AML transformation. Single-cell targeted sequencing of highly fractionated MDS stem cells has revealed a pattern of nonlinear, parallel clonal evolution during MDS progression to AML, with subclones not detected in MDS that were found to progress to AML (*116*). In line with this, several high-throughput sequencing studies have suggested a branching pattern for clonal evolution, which represents a greater complexity in the clonal architecture of leukemia. This leads to a mixture of different subclones in leukemic cells that have different potential to contribute to disease relapse.

Initiating mutations in most AML cases are largely unknown because preleukemic cells are clinically silent and AML is typically diagnosed without observation of a pre-leukemic phase. Two independent studies that traced AML patients back to their healthy status detected the mutations years before AML diagnosis (median time: 6.3 and 9.6 years). This indicates a rather long period of latency that precedes AML transformation (*117, 118*). Studies focusing on the critical window between clonal expansion and leukemic transformation can provide insights into the cellular origins and mechanisms of leukemia onset.

Leukemic stem cells

LSCs, as fully transformed leukemic cells, can initiate disease when transplanted into irradiated animals. They possess high self-renewal capacity that allows for serial propagation. LSCs reside at the apex of the AML development hierarchy, giving rise to non-LSC blasts that are unable to self-renew (*110*). In addition, LSCs have been shown to be mostly quiescent (*119*) and resistant to chemotherapy (*120*). These properties allow them to be the main cause of relapse. Therefore, further characterization of LSCs is critical for development of targeted therapies that can help improve treatment efficacy and patients' survival.

Nearly 30 years ago, John Dick and colleagues showed that LSCs are very rare cells in AML (1 in 250,000 in peripheral blood of AML patient). They suggested that LSCs activity were contained within the CD34+CD38− fraction, given that it was the only population of human AML cells that could engraft SCID mice and produce AML (*121*).

Later studies have proposed the existence of LSCs in the CD34+CD38+ cell fraction, known as GMP (granulocyte-macrophage progenitors)-like LSCs (*122*). In addition, LSC activity was also detected in the CD34- compartments in a few AML cases, in which the frequency of CD34+ cells was <20%, (*123, 124*). However, the

Lin−CD34+CD38− immunophenotype is the one encompassing the vast majority of LSC activity in more than 90% of AML cases (*125*). In comparison to normal HSCs, which also reside within the CD34+CD38− compartment, LSCs have been reported to lack the expression of CD90, suggesting it as a marker to distinguish normal stem cells from their malignant counterparts in at least some instances (*122, 125*).

In murine leukemic models, the immunophenotype varies between studies (*126*). In an MLL-AF9 retrovirus mediated AML model, the LSCs were found in the GMP-like leukemic cells (IL-7R-Lin-Sca-1-c-Kit+CD34+ FcγRII/III+) (*127*). Other studies have suggested that LSCs presented a mature myeloid immunophenotype (CD11b+Gr1+) rather than the immature HSPC immunophenotype (*128*). Advances in the characterization and understanding of LSCs provide insight to AML pathogenesis and therapies based on elimination of LICs.

Progression from pre-leukemic to fully transformed leukemia

The laboratory of John E. Dick et al proved the existence of pre leukemic HSCs in an AML patient in remission which harbored the presumed first event, a DNMT3A mutation, that were still capable of undergoing multilineage differentiation in spite of clonal expansion (*99*). In line with this, pre leukemic HSCs with AML1/ETO (8;21chromosomal translocation) were detected during remission and were able to differentiate to myeloid and B cells, but not to T cells (*111, 129*). Modelling of pre-leukemic events using murine models has provided important conceptual insights into the pre-leukemic stages at the HSPC level and their functional relevance for leukemia development. In these mouse models, MLL fusions (*130, 131*), CBFβ–SMMHC fusion (*132*), PU.1 deficiency (*133*) and CEBPα mutations (*134*) induced distinct pre-leukemic phases, which were characterized by myeloid-biased HSC, blocked differentiation and accumulation of immature myeloid cells in the bone marrow and blood. In these models, while all the mice developed leukemia with a latency of 2–6 months, not all pre leukemic cells were able to fully transform into AML. Some pre-leukemic events were found to be insufficient to induce full transformation, and if AML occurred, it was of a long latency (*135-143*). This indicated the requirement of secondary cooperating oncogenic insults for leukemia development in these models (*136-140, 144*).

The molecular events leading to transformation from pre-leukemia to AML are still largely unclear. Attempts have been made to identify the pathogenesis of the evolutionary trajectory that leads to the eventual progression into AML. It has been widely accepted that the acquisition of additional genetic lesions, as well as cell extrinsic regulations, including by the BM microenvironment (*145*) and immunoediting (*146*) are all relevant mechanisms in the progression to AML (*100*).

Genetic lesions acquired by pre-leukemic cells lead to dysregulations of their transcriptional profile and activation of distinct signaling pathways, which are indispensable for leukemic transformation. For instance, PU.1 reduction lead to downregulation of the Jun transcription factor family (*133*) and MLL fusions lead to the upregulation of members of the *HOX* transcription factor family (*147*). Several signaling pathway activated by genetic lesions have been shown to act as pervasive pre-leukemic to AML transformation mechanism, including RAS signaling activated by *RAS* mutations (*148*), RTK signaling activated by *CKIT* and *FLT3* mutations (*149, 150*) and Wnt/β-Catenin signaling activated by *Mdmx* overexpression (*140*). Notably, AML LSCs have been shown to express higher levels of activated RAC and CDC42 compared to pre leukemic cells (*128*).

To delineate the mutational profile in leukemia, deep sequencing of paired pre leukemic and leukemic patient samples have been conducted. Findings from such endeavours suggest that mutations in genes involving RAS and RTK signaling pathways, including *FLT3*, *PTPN11*, *WT1*, *IDH1*, *NPM1*, *IDH2* and *NRAS* were acquired and associated with faster AML progression and worse prognosis (*151*). Such accumulated somatic mutations confer selective advantage that drive successive waves of clonal expansion, with the fittest clone becoming dominant (*151*). Several other secondary mutations have been found and have been shown to be able to trigger leukemic transformation in murine models (*139, 152-155*).

Those observations also suggest that the altered molecular features resulting from the initial event could promote the acquisition of subsequent genetic alterations, which leads to complete leukemic transformation. This phenomenon is supported by studies that reported the capacity of some first events to impair genome stability and normal mitotic checkpoint, leading to a dysregulation of the DNA repair mechanism (*156-158*).

Genetic alterations in AML

Genetic characterization compared to solid tumors

Similar to other hematological malignances, AML is a complex, dynamic disease, characterized by multiple somatically acquired driver mutations that are instrumental in the classification of different AML entities (*159*). Around 53-59% of adult AML patients harbor chromosomal abnormalities of which about a third includes recurrent cytogenetic changes that define specific AML entities. These include t(15;17)(q24;q21) encoding PML-RARA, t(8;21)(q22;q22) encoding RUNX1-RUNXT1 (also known as AML1-ETO), inv(16)(p13q22) /t(16;16)(p13;q22) encoding CBFB-MYH11, t(6;9)(p23;q34) encoding DEK-NUP214, t(9;11)(p22;q23) encoding MLL-AF9, inv(3)(q21q26) /t(3;3)(q21;q26) which affect GATA2 and MECOM regulation, and t(1;22)(p13;q13) encoding

RBM15-MKL1 (*159, 160*). Studies in large population-based cohorts have revealed the genomic landscape and the genetic heterogeneity in de novo AML (*161, 162*) (Figure 2). Overall, AML genomes have fewer mutations than most other adult cancers, with an average of only 13 mutations found per patient. This is in contrast to for instance in lung cancer, where more than 200 mutations have been identified per patient on average (*161*). Genes that were found to be recurrently mutated in AML included *CEBPA, FLT3, DNMT3A, IDH1, IDH2, NPM1, NRAS, RUNX1, TET2, TP53, WT1, KRAS, U2AF1, KIT, PTPN11, PHF6, SMC3, FAM5C, SMC1A, RAD21, STAG2, HNRNPK* and *EZH2* (*161, 162*). In addition, these mutated genes were classified into different functional categories: transcription factor fusions, the gene encoding nucleophosmin (*NPM1*), tumor suppressor genes, DNA methylation-related genes, signaling genes, chromatin-modifying genes, myeloid transcription factor genes, cohesin complex genes, and spliceosome complex genes (*161*). A study profiling the landscape of mutations in 111 cancer relevant genes in a large cohort of AML patients proposed classifying AML into 11 groups with different phenotypes and outcomes (*163*). Another study identified a distinct genetic subtype, the presence of mutations in *SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR,* or *STAG2*, of secondary AML (sAML) and elderly de novo AML populations, which is related to worse clinical outcomes (*164*). These findings have provided important insights into the molecular abnormalities underlying AML with normal cytogenetics and those with chromosomal abnormalities that were previously poorly understood.
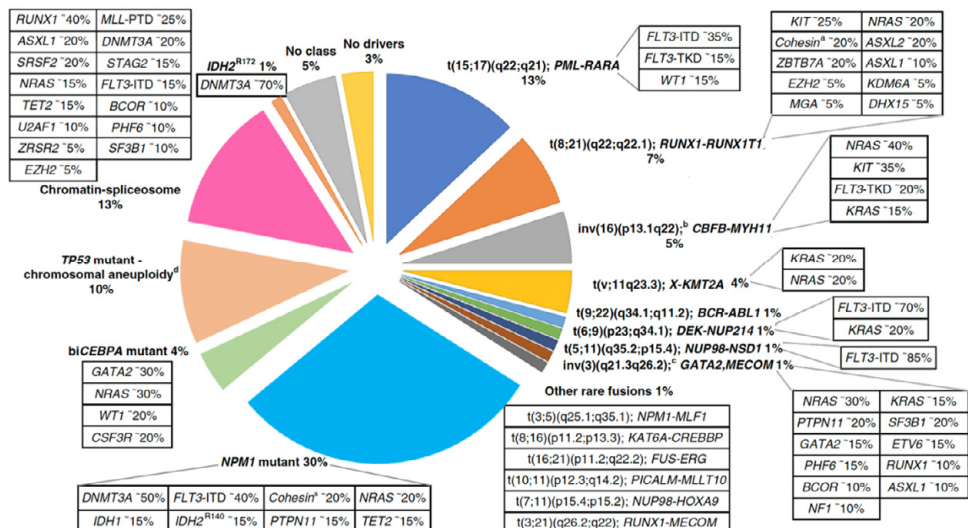

Figure 2. Genomic landscape and the genetic heterogeneity in de novo AML (adapted from D. Grimwade et al, 2016).

Class I, II and III mutations in AML

Genetic alterations in AML can be divided into different classes. Class I mutations lead to consistently activated tyrosine kinases or their downstream effectors, and include BCR-ABL, *CKIT, FLT3, KRAS, NRAS* and *PTPN11* mutants. These mutations confer the hematopoietic progenitors with survival/proliferation advantage. Class II mutations result in loss of function of transcriptional factors that are important for normal hematopoietic differentiation, and include AML1-ETO, PML-RARα, CBFβ-MYH11, *CEBPA* and *RUNX1* mutants (*165, 166*). In the classical "two-hit" model, it was proposed that leukemogenesis is induced upon the acquisition of both class I and class II mutations (*167*).

Class III mutations involve genes that associate with DNA methylation, such as *DNMT3A, DNMT3b, TET1, TET2, IDH1* and *IDH2,* and chromatin modifications, such as MLL fusions, MLL PTD, NUP98-NSD1, *ASXL1, EZH2*, leading to epigenetic dysregulation of their target genes (*165, 168*).

Other groups of genes, such as DNA repair regulators, including TP53 and NPM1, and spliceosome machinery regulators, including SRSF2, SF3B1 and UA2AF1, are also frequently mutated and are considered key for AML pathogenesis (*166, 168*).

Genetic alterations play a significant role for determining prognosis and treatment decision-making. With this in mind, the European Leukemia Net (ELN) published recommendations for AML diagnosis and management, and categorized AML into 3 prognostic groups; favourable, intermediate and adverse, based on genetic abnormalities and their correlation with prognosis (*98, 160, 169*).

Temporal order of mutations

The co-occurrence and exclusivity of the most frequent variants suggests biological cooperation between certain mutational events. For instance, 73% of the NPM1-mutated AML carry mutations in DNA-methylation genes (*DNMT3A, IDH1, IDH2,* and *TET2*) (*170*). In addition, *FLT3, DNMT3A,* and *NPM1* mutations co-occur significantly in AML patients, with many patients harboring mutations in both *NPM1* and *DNMT3A* or in *NPM1* and *FLT3* (*161*). In contrast, *NPM1* and *DNMT3A* mutations were found to be mutually exclusive of PML-RARA, MYH11-CBFB, and MLL-containing fusion genes, and *RUNX1* and *TP53* mutations were mutually exclusive of *FLT3* and *NPM1* mutations (*161*).

During the last 10 years, single-cell–based analysis has allowed for the identification of early and initiating/founder mutations in AML during the process of leukemogenesis and have been found to be enriched for epigenetic modifiers such as DNMT3A, TET2, ASXL1 and MLL fusions (*99, 113, 115, 116*). Other mutations, including in FLT3, PTPN11, WT1, IDH1, NPM1, IDH2 and NRAS, tend

to be newly acquired during the pre-leukemia stage and associate with fully transformed leukemia (*151*).

Furthermore, by manipulating co-mutations in murine models, studies have shown that the cooperation of mutations could influence not only on disease latency, but also the lineage assignment of developing leukemia, depending on the order of those mutations (*152, 171*).

Function of gene mutation

Genomic variants can be categorized into loss-of-function (LOF) mutations and gain-of-function (GOF) mutations based on their effect on function of the gene. LOF mutations, also known as inactivating mutations, result in the lack of production or the product having less or no function. Moreover, according to the degree of LOF mutations, these mutations are further classified as null mutations, which lead to the entire loss of the function, and leaky mutations, which lead to partial loss of the function. Key LOF mutations in AML include for *TP53, DNMT3A, ASXL1* and *TET2*, but it should also be noted that wild type copies are often retained on the second allele, highlighting the effects of gene dosage.

GOF mutations, on the other hand, also known as activating mutations, produce proteins with enhanced activity or novel functions. Many GOF mutations have been identified and studied in human disease. In AML, there have been many frequent GOF mutations identified, and whose functions have been intensively explored. For example, mutations in *RAS, BRAF* and *KIT*, located to their kinase domains, lead to constitutive activation of these oncogenes.

However, illuminating the functions of GOF mutations remains challenging (*172*). First, as opposed to the known function of the gene product, it can be difficult to find out novel functions resulting from GOF mutations. Second, the computational and experimental methodology for precisely predicting and exploring the function of GOF mutations is very much still lacking. Third, many GOF mutations are located in the regions that does not associate with known structural/functional domains.

Mixed lineage leukemia (*MLL*) rearranged leukemia

Structure and interactome of MLL

Modification of chromatin structure plays crucial role in regulation of cellular gene expression, and a major type of epigenetic regulation of gene expression. Several groups of proteins have been shown to regulate gene expression through modification of chromatin structure. The trithorax group (TrxG) promotes gene

expression through methylation of histone H3 lysine residue 4 (H3K4). In contrast, the polycomb group (PcG) represses gene expression through methylation of histone H3 lysine residue 27 (H3K27) (*173*). Aberrant transcription caused by dysfunction of TrxG or PcG can lead to cancer development in model systems, suggesting the involvement of these chromatin modifiers in cancer pathogenesis (*174, 175*). The Mixed lineage leukemia gene-1 (*MLL*; also known as *KMT2A* or *MLL1*), located on chromosome 11q23, is a member of the TrxG group genes. MLL1 is a large (3969 amino acids) DNA binding multidomain protein. A key transcriptional target of MLL includes the HOX gene cluster, which is positively regulated by MLL.

MLL is cleaved by the enzyme Taspase 1 into two fragments, one N-terminal (MLL-N) and one C-terminal (MLL-C). These two fragments reassociate and further assemble into multiprotein complexes through intramolecular interactions mediated between the two interaction motifs FYRN on MLL-N and FYRC on MLL-C (Figure 3) that regulate the transcription of target genes.

The MLL-N encompasses several domains involved in DNA binding, including three short AT hooks (ATH1-3), two speckled nuclear localization motifs (SNL1 and SNL2), a Cysteine-n-n-Cysteine zinc-finger (CxxC) domain, and four plant homology domains (PHD) which also includes a bromodomain (*176-179*).

MLL-C contains domains involved in transcriptional regulation including a domain that recruits the histone acetyltransferase CREB-binding protein (CBP) that is a positive regulator of transcription and the SET (Su(var)3–9, enhancer of zeste, trithorax) domain, located at the extreme C terminus, which is responsible for its H3K4 methyltransferase activity and mediates chromatin modifications associated with transcriptional activation (*180-182*).

Of note, a number of other proteins such as WD Repeat Domain 5 (WDR5), are required for the assembly and targeting of the MLL complex, and therefore contribute to MLL1 activity (*183, 184*). MLL-C interacts with WDR5, males absent on first (MOF), retinoblastoma binding protein 5 (RBPB5) and ASH2 Like, Histone Lysine Methyltransferase Complex Subunit (ASH2L). WDR5 is essential for binding of the MLL core complex to the K4-dimethylated H3 tail as well as for global H3 K4 trimethylation and HOX gene activation (*185*). In addition, MOF-mediated histone acetyltransferase activity specific for histone H4 lysine 16 (H4K16) is also required for optimal transcription activation on MLL1 target genes, suggesting that both methylation and acetylation are involved in the activator-based mechanism for the MLL1 core complex (*186*). RBPB5, mediating the interaction of ASH2L with the MLL-C, has a crucial role in the structural organization of the complex, being the only component that makes direct contact with each of the other proteins (*187*). Omission of either RBBP5 or ASH2L resulted in substantial loss of H3K4 histone methyltransferases activity, which was completely lost upon the absence of WDR5 (*187*).

30

MLL-N interacts with menin (MEN1) and lens epithelium-derived growth factor (LEDGF), that are involved in the positioning of the MLL complex to specific loci. Menin is a tumor suppressor whose loss of function causes the human cancer syndrome known as multiple endocrine neoplasia type 1. MEN1 has been shown to link the MLL1-N with LEDGF, a chromatin-associated protein previously implicated in leukemia, autoimmunity, and HIV-1 pathogenesis (*188*). LEDGF is required for both MLL-dependent transcription and leukemic transformation (*188*). Menin and MLL both associate with the *Hoxa9* promoter, and in the absence of Menin, MLL and MLL fusions fail to regulate *Hoxa9* expression, which is believed to be an essential oncogenic cofactor for MLL fusion-mediated transformation (*189*).
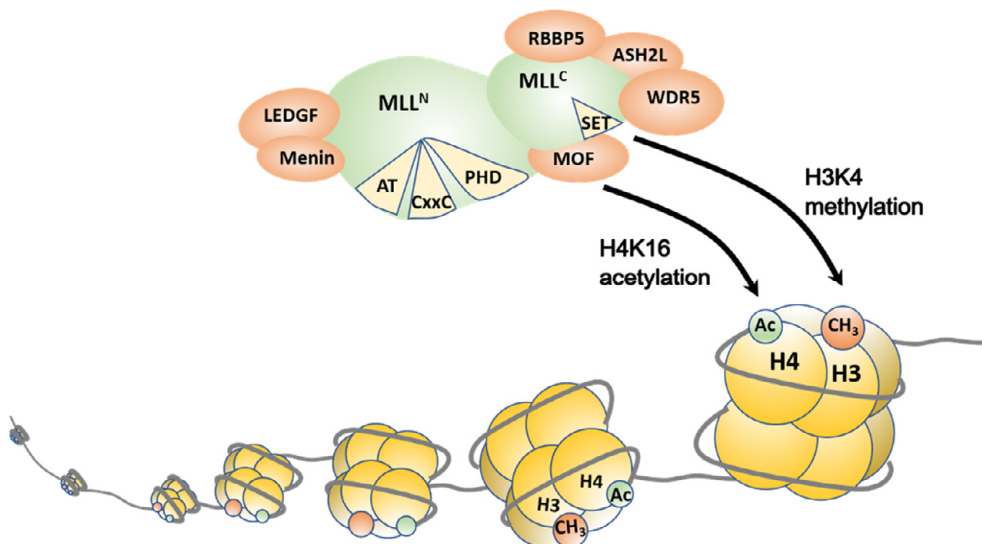
Figure 3. Composition of MLL complex and its function.

The role of MLL in normal development/hematopoiesis

MLL is broadly expressed in hematopoietic cells including stem and progenitor cells. *Mll-/–* mice are embryonic lethal, whereas *Mll+/–* mice are anemic, with pronounced growth retardation, specific axial skeleton defects and defective hematopoietic precursors (*190, 191*). To define the role of MLL in the development of the hematopoietic system, P. Ernst and colleagues examined the potential of cells lacking MLL and found that *Mll*-deficient ES cells failed to contribute to fetal liver hematopoietic stem cell/progenitor populations in chimeric embryos. These results demonstrate an intrinsic requirement of *Mll* for the generation of HSCs in the embryo (*192*). Knockout mouse models have demonstrated the crucial role of MLL in the control of *Hox* gene expression

during the development of the axial skeleton and hematopoietic systems in mammals (*193*). The expression of stage-specific *Hox* genes is initiated appropriately but not maintained during development in the absence of MLL *(190)*. The *Hox* genes, in which loss of *Hoxa9* impair HSPC function, have been shown to play an essential role during embryonic development (*194, 195*).

Fusion partners in MLL-rearranged leukemia

Balanced chromosomal 11q23 translocations that involve the *MLL1/KMT2A* gene result in the formation of chimeric fusion proteins with novel gene regulatory properties and can give rise to a genetically distinct subset of leukemia, namely *MLL*-rearranged (*MLL*-r) AML and ALL. Overall, leukemias that bear *MLL* rearrangements are found in approximately 10% of human leukemias (*196*). *MLL* rearrangements are found in >70% of infant leukemias. In older children and adults, they account for ∼10% of all acute leukemias (*197*). *MLL* rearrangements are also found in approximately 10% of AMLs in adults and comprise 35–50% of AML cases in infants.

MLL translocations, as many other translocations found in leukemia, are caused by impaired DNA double strand break repair. The 8.3 kb breakpoint cluster region (BCR) between exons 8 and 13 is the target for most MLL rearrangements and a chimeric fusion protein is created. Consequently, all identified MLL fusions contain the first 8–13 exons of MLL, containing the MLL1-N fragment with its AT hooks, two SNL, as well as CxxC domain, and a variable number of exons from a fusion partner gene (*198*). The Taspase 1 cleavage site along with MLL-C contains domains with histone H3 lysine 4 (H3K4) methyltransferase activity are lost in MLL fusions. Another type of MLL rearrangement, MLL–PTD (partial tandem duplication), is a result of internal tandem duplication of select exons and also identified in AML (*199, 200*).

So far, a total of 135 different *MLL* rearrangements have been identified, of which 94 translocation partner genes have been characterized at the molecular level. Partially, 35 out of them are recurrent and only 9 specific partner genes, AF4, AF9, ENL, AF10, AF6, ELL, AF1p, AF17 and SEPT6, account for more than 90% of all illegitimate recombination of the *MLL* gene (*78*). MLL-AF4, MLL-AF9, MLL-ENL and MLL-AF10 are the most common MLL fusions identified in AML, which account for 36%, 19%, 13% and 8% of *MLL*-r leukemia, respectively (*78*). These MLL fusions can be found in both lymphoid and myeloid leukemia with a clear lineage preference for MLL-AF4 in ALL, while MLL-ENL and MLL-AF10 associate with AML in both adult and pediatric cases. Besides, the distribution of different MLL fusions varies with age, for example with increased frequency of MLL-PTD in older AML patients and increased frequency of MLL-AF4 in adult ALL (Figure 4).

In general, *MLL*-r AML tends to associate with a poor prognosis (*78, 160, 201*). Studies suggest that patients with MLL-AF9 have a more favourable prognosis compared to the other MLL fusions in adult AML (*201*). In paediatric *MLL*-r AML however, larger differences in outcome were observed between subgroups of different translocation partners, suggesting that individual MLL fusions might have distinct impact on prognosis (*202*).
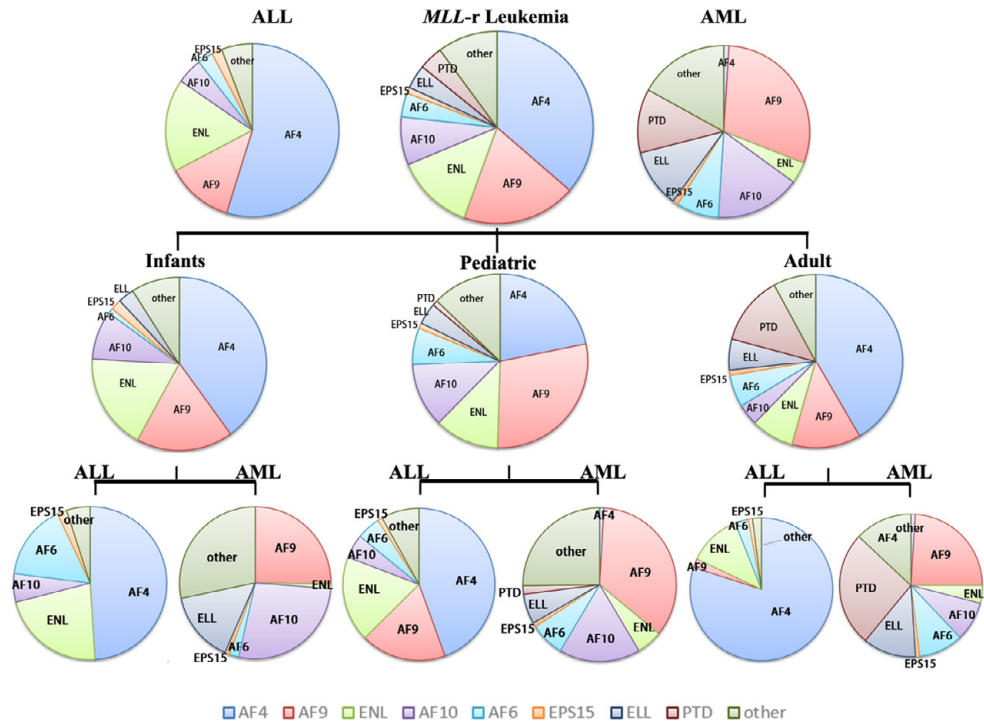
Figure 4. Frequency of different MLL fusions according to age classes and disease type.

The molecular impact of MLL fusion proteins

It has been shown that most, if not all, MLL fusion protein complexes are associated with AF4 family/ENL family/P-TEFb complex (AEP) and DOT1L. AF4 family is composed of AF4, AF5q31, and LAF4. ENL family is consists of ENL and AF9. P-TEFb elongation factor is composed of cyclinT1/2 and CDK9. AEP is involved in the regulation of MLLr targeting genes by promoting transcriptional elongation and ablation of AEP from MLL fusion complex eradicates the leukemic transformation (*203*) (Figure 5).

DOT1L, a histone methyltransferase, methylates lysine 79 residues in histone H3 (H3K79). DOT1L is commonly involved in MLL fusion complex and the deletion

of DOT1L rescues MLL fusion-mediated leukemogenesis (*204-206*). In line with this, H3K79 methylation levels have been found to be increased on the *Hoxa9* and *Meis1* promoters in MLL-ENL mediated leukemia (*207*). Thus, these results suggest that the loss of H3K4 methyltransferase activity in wild-type MLL might be compensated by the acquisition of H3K79 methyltransferase activity in MLL fusions, which further perturb transcriptional control.

MLL-r leukemia has been proposed to show a highly uniform and distinct pattern and often share common transcriptional signatures within this entity (*208*). This is supported by accumulating evidence suggesting that many MLL fusion partners belong to a network involved in transcriptional regulation through remodelling target chromatin (*209*). *Hoxa* cluster genes and their interacting partners *Meis1* and *Pbx3* have been proposed as key transcriptional targets of MLL fusions (*127, 207, 210-212*). In addition, combined overexpression of *Hoxa9* and *Meis1* leads to leukemia similar to that seen in *MLL*-r models (*212, 213*). In addition, *c-Myb* has been shown to be an essential downstream target for *MLL*-r AML (*214*).

MLL fusion proteins do not act by direct activation of a specific enzyme, making it difficult to target the fusion proteins with small molecules. However, attempts have been made to target these known partners of MLL fusion for the treatment of AML. For instance, VTP50469, a small molecule that breaks up the interaction between Menin and the MLL fusion complex, leads to suppression of a subset of MLL fusion target genes by releasing MLL from these gene loci (*215*). DOT1L inhibitors, EPZ004777 and EPZ-5676 also show tumor regression activity in *MLL*-r leukemia (*216, 217*). In addition, combination therapy of Menin and DOT1L inhibitors have shown improved therapeutic outcomes in patients with *MLL*-r leukemia (*218*).
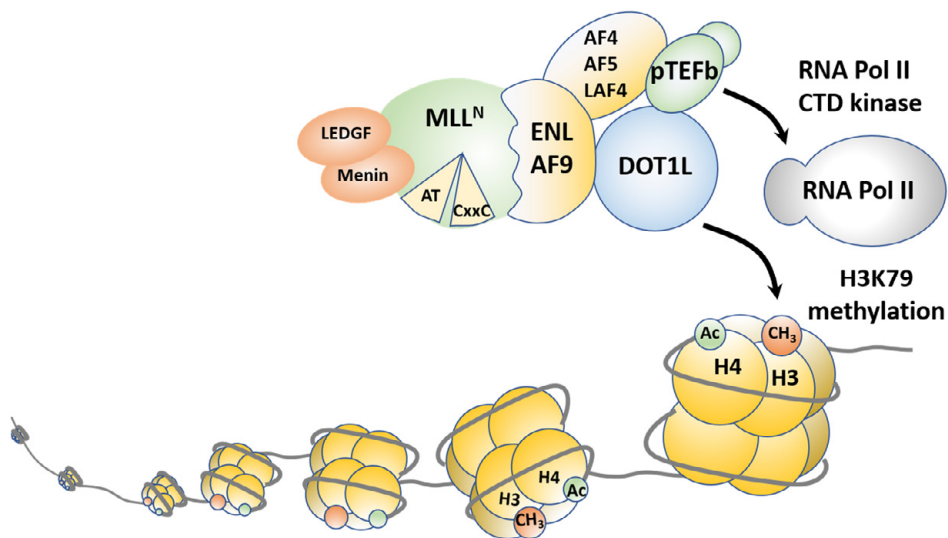

Figure 5. Protein complexes of major MLL fusions.

Murine models of MLL-R leukemia

The powerful oncogenic activity of MLL-r proteins have frequently been exploited in mouse models to explore the pathogenesis of leukemia initiation, evolution, therapy resistance and relapse (*219*). MLL-ENL, MLL-AF9 and MLL-AF4, the most common MLL translocations found in human acute lymphoid and myeloid acute leukemia, are often used to mediate leukemia in these models. In many such studies, MLL-r have been introduced by viral delivery to immature murine hematopoietic cells, followed by their transplantation into recipient mice (*220*). Using such approaches, MLL-ENL has been shown to be able to induce AML and biphenotypic acute leukemia (BAL) (*221-223*). MLL-AF9 on the other hand only led to the development of AML in murine BM progenitors (*127, 224-226*) and MLL-AF4 led to B cell ALL (*227*).

Similar experimental strategies have been used to address the leukemogenic effect of MLL-r in human cells. Barabé et al showed that MLL-ENL and MLL-AF9 both were able to induce AML and B cell ALL when introduced virally into human CD34+ cord blood cells (*228*). Later, Wei et al showed that the phenotype of MLL-AF9 mediated leukemia was dependent on the strain of immunodeficient mice used for the transplantation, indicating the importance of the microenvironment in determining the lineage fate in MLL-AF9 mediated leukemia (*229*).

As an alternative to viral delivery, a wide range of transgenic mouse models have been generated for studying the pathogenesis of leukemia initiation and progression (*131, 152, 230-244*) (Table 1). The rationale for this approach is to circumvent several of the experimental caveats associated with viral delivery of MLL fusion genes, including poor delivery of the actual fusion genes, unphysiological expression levels caused by the strong promoters typically associated with viral vectors, and insertional mutagenesis. Because of this, our lab previously generated an inducible MLL-ENL mouse model (iMLL-ENL) model (*131*), which allowed us to firmly establish the precise identity of the hematopoietic progenitor cells capable of initiating AML, without having to subject candidate cells to any form of in vitro culture prior to assessment of their leukemic capacity. With our model, we showed that GMLPs, pGMs, GMPs, CLPs, B-cell-biased lymphoid progenitors (BLPs), DN1 T cells, were LICs that could give rise to AML (*131, 152*). By contrast, very primitive populations such as HSCs and MPPs, were protected against MLL fusion mediated transformation, indicating that HSCs are not LICs in this system (*131*). These findings are in line with later studies which showed that haematopoietic differentiation is indispensable for AML formation (*245, 246*).

Table 1 *MLL*r mouse models that have been reported in studies.

MLL fusion	Driver Promoter	Disease	Disease Latency	Reference
MLL-ENL translocator	Lmo2-Cre (HSC)	AML	Up to 120 d	Forster et al 2003
	Lck-Cre (T cells)	Myeloproliferative-disease-like myeloid leukemia or small T-cell lymphoma	Up to 550d	Drynan et al 2005
	Rag1-Cre (lymphoid progenitors)	AML	Up to 170 d	Cano et al 2008
MLL-ENL constitutive knock-in	endogenous Mll locus	Long latency MPD with progression to AML upon DNA Damage Response inhibition	Mean of 592 d for MPD Mean of 62d with DDR	Takacova et al 2012
DOX-inducible MLL-ENL	Col1a1	AML	10–15 w depending on targeted cell transplanted (GMLP, pGM<CLP<GMP)	Ugale et al 2014
	Hprt	Biphenotypic mixed lineage leukemia	72d	Stavropoulou et al 2018
Mll-AF9 translocator	Lmo2-Cre (HSC)	Myeloproliferative-disease-like myeloid leukemia	Up to 350 d	Drynan et al 2005
DOX-inducible MLL-AF9	Hprt	AML	median 73d	Stavropoulou et al 2016
Mll-AF9 constitutive knock-in	Endogenous MLL	MPD-like myeloid leukemias	median of 220 d	Chen et al 2006
Mll-AF4 constitutive knock-in	Endogenous MLL	Lymphoid and myeloid hyperplasia, B-cell lymphoma, and myeloid malignancies	median of 520 d	Chen et al 2006
Mll-AF4 conditional invertor	Lmo2-Cre (HSC)	Embryonic lethal	NA	Metzler et al 2006
	Rag1-Cre (B, T cells)	Diffuse large B-cell lymphomas	Mean 317–466 d	
	Lck-Cre (T cells)		Mean 416–472 d	
	CD19-Cre (B cells)		Mean 460–475 d	
Mll-AF4 conditional invertor	Vav-Cre (all definitive hematopoietic cells)	B cell lymphoma (most common), T cell lymphoma	Median 556 d	Barrett et al 2016
	VE-Cadherin-Cre (hemogenic endothelium+all definitive hematopoietic cells)	B cell lymphoma (most common), T cell lymphoma, lymphoproliferative disorder	Median 437 d	
Mll-AF4 conditional knock-in	Mx1-Cre	Pre-B ALL	152 d	Krivtsov et al 2008

Cooperating lesions in MLL-R leukemia

While MLL-r leukemias appear to associate with rather few secondary mutations (*220*), a substantial fraction of MLL-r AML harbour activating mutations in *RAS*, *FLT3*, *PTPN11* and *BRAF* (*226, 247*). This has lent support for a two-step model of leukemogenesis in which the initial MLL-r act to mainly inhibit differentiation, while the secondary mutation provides a strong proliferative signal.

Consistent with data from human patients, secondary genetic/activating lesions in *Kras*, *Braf, Cbl, Ptpn11*, *Gnb2* and *Flt3* have been reported upon transformation in

MLL fusions driven murine AML models (*171, 226, 248*). In support of the relevance of these mutations for AML development, their co-expression with different MLL fusion genes can accelerate leukemogenesis and produce more aggressive and rapid AML in MLL-fusion mouse models (*154, 171, 249, 250*).

Moesin (MSN)

Structure, function, and regulation of the moesin

Moesin is a member of Ezrin-Radixin-Moesin (ERM) protein family, which are best known for their activity to crosslink the plasma membrane and the actin cytoskeleton (*251*). Processes facilitated by MSN include the control of cell membrane dynamics, adhesion, cell survival, cell motility, cell polarity and morphogenesis (*252, 253*).

The structure of the Moesin protein consists of three functional regions: 1) a ∼300 amino acid N-terminal compound FERM domain; 2) a ∼200 amino acid linker region that is mostly α-helical; and 3) a ∼70 amino acid C-terminal tail (*254*). The MSN FERM domain contains F1 (residues 4–82), F2 (residues 96–195) and F3 (residues 204–297) subdomains. The F1 subdomain contains a 5-stranded mixed β sheet packed against an α helix with a short 3_{10} helix. F2 is composed of five α helices, with an excursion of 36 residues between helices B and C that contains a long loop and a short a helix. F3 consists of a seven-stranded β sandwich followed by a long capping α helix (*254*).

The FERM-folded conformation of these 3 subdomains is mainly attributed to the conformational rearrangement of the F1-F2 linker, resulting in a three-leaf clover architecture, with the F3 subdomain residing on the top of the F1 and F2 subdomains (*255*). Notably, interactions mediated by the R295 residue in F3 and E87 residue in the F1 domain has been proposed to maintain the FERM cloverleaf conformation stability (*255*). Disruption of interactions between F3 and F1, on the other hand, leads to F3 subdomain movements and destabilize the FERM domain conformation (*255*).

The FERM domain is highly conserved in all ERM-protein family members, which contains a crucial binding site to protein ligands (*256*). A "hydrophobic shallow groove" formed by strand β5C (residues 245-251) and α1C-helix (residues 274–297) which binds to tails of transmembrane proteins, such as CD44, ICAM-2 and NEP has been reported (*257, 258*). The interconversion between the active and inactive MSN conformation is important to avoid unnecessary or inappropriate interactions with adhesion molecules and actin filaments (*256, 259*). When inactive, the MSN protein is present in the cytosol and the FERM domains are masked by residues 494–500 in the C-terminal tail domain through head–tail interaction (*254,*

257). Alternatively, MSN can be kept in an inactivate conformation by homodimerization in which the FERM domain is masked by the residues 295–304 in the FERM C-terminal domain from another molecule (*260*). The interaction between strand β5C and the FERM C-terminal residues (residues 295–304) was suggested to be crucial to maintain the ERM homodimerization, an important mechanism for the stabilization and inactivation of MSN (*257*).

MSN gets activated when the FERM domain binds to phosphatidylinositol (4,5)-bisphosphate (PIP2) and the C terminal tail gets released from the FERM domain in which the T558 residue become phosphorylated (*261, 262*). The activated MSN is located at the plasma membrane, where the FERM domain binds to adhesion molecule ligands, including for CD44, CD43, ICAM1 and ICAM2, while the C-terminal tail binds to the actin cytoskeleton (*256, 259*).

The role of MSN in normal hematopoiesis and malignancy

In hematopoiesis, both MSN and Ezrin are expressed, whereas Radixin is mostly absent. MSN is the quantitatively dominant ERM protein in human blood lymphocytes, monocytes and neutrophils and the only ERM family member expressed in platelets (*263*). A germline R171W mutation leads to loss of MSN expression and associates with an X-linked immunodeficiency syndrome (*264, 265*).

Of note, ERM proteins have been proposed to be responsible for a crucial step in Ras activation in that they are recruited to adhesion molecules at the plasma membrane and bind to F-actin, where they can form a complex that acts as a scaffold and activate Ras (*266, 267*). Thus, ERMs are vital in the oncogenic mechanism in the Ras signaling driven tumorigenesis (*268*). Increasing evidence suggests that the dysregulation of cell signaling resulting from both altered MSN expression and its subcellular localization play crucial roles during cancer progression (*269-273*). For instance, altered subcellular distribution of MSN together with increased expression levels influence the invasive and metastatic ability of tumor cells in oral squamous cell carcinoma (*270*). MSN has also been proven to be indispensable for the invasiveness and colonization capacity of melanoma cells (*271*). Intriguingly, the specific RRRKPDT motif at FERM domain C-terminal residues 293–299 have been implicated in propagation of lung cancer via direct regulation by a small RNA, piR-L-163, which was found expressed in normal but not transformed lung fibroblasts and acted to limit cell proliferation (*274*). Besides, a MSN fusion, MSN-ALK, resulting from a (X;2)(q11–12;p23) translocation, was reported to underlie the pathogenesis of anaplastic large cell lymphoma (*273*). A role of MSN in human cancers is also supported by the spectrum of somatic mutations in MSN found across human cancers. However, limited information is available on the role of MSN in leukemia and, despite its high prevalence, in murine *MLL*-r AML (Study I) (*226*).

Lin28B

Pediatric and adult AML

AML accounts only for ~20% of all pediatric acute leukemia cases. Despite the higher prevalence of pediatric ALL, AML is the leading cause of childhood leukemic mortality (*275*). The incidence of AML in adults increases to ~50% and steadily increases with age (*78*).

The landscape of somatic variants in pediatric AML has been reported to be markedly different from that reported in adults. Cytogenetic alterations are present in 80% of infants, but only in 50% of adult AML patients (*79*). In addition, the mutations that are common in adult AML, including *DNMT3A* and *TP53* mutations, have been found to be absent from almost all pediatric cases (*275*).

As mentioned before, AML leukemogenesis is driven by the accumulation of genetic aberrations that lead to pre leukemia and ultimately fully transformed leukemia. Pediatric AML, however, has one of the lowest rates of mutation among all cancers, including adult AML, and often lack the history of MPN or MDS underlying the pre leukemic stage (*276*). This has challenged the stepwise model of AML development. Therefore, I believe based on different lineage preferences, biological processes, and molecular landscape, that AML in children and adults should be viewed as two distinct diseases. This concept is also supported by murine models that showed that the age of the cell of origin significantly impacts NUP98-HOXA9 driven leukemia latency, phenotype, molecular profile, as well as the leukemic niche (*277*). Specifically, young cells could drive myeloid, lymphoid or mixed phenotype acute leukaemia, whereas adult cells only give rise to AML, with a shorter disease latency. In addition, adult leukemic cells, but not young leukemic cells, lead to the remodeling of the BM niche. Conversely, young cells were observed to be more prone to MLL fusions than adult cells in terms of leukemia latency and lineage dictation (*278, 279*). This suggest that young hematopoietic progenitors may be inherently more susceptible to transformation by MLL fusion than adult progenitors, which may explain the relatively fewer identified cooperating mutations in infant *MLL*-r leukemias (*280*).

However, when compared to neonatal cells, the fetal cells were found to give rise to leukemia with longer disease latency in a mouse model (*279*). This is in line with the observations that overt leukemic transformation usually occurs postnatally, even though the driver lesions were acquired prenatally (*281-284*). In further support of this, *Lin28b*, a fetal master regulator, was found to play a role in the suppression of MLL fusion driven AML initiation during the fetal stage (*279*).

The role of LIN28B in normal hematopoiesis

LIN28B is a highly conserved RNA-binding protein with specific expression in the mouse and human fetal liver and thymus. In addition, forced expression of *Lin28* reprograms HSPCs from adult into their fetal counterparts, with multilineage reconstitution potential that includes generation of fetal-like lymphopoiesis (*49, 285*). The best studied mechanism of LIN28B in hematopoiesis is its ability to negatively regulate the *let-7* microRNA (miR) biogenesis, leading to depression of transcripts under *let-7* miR control (*49, 286-288*). Apart from this, several studies have shown that LIN28B can regulate translation of targeted mRNAs by direct binding (*289-292*). For instance, LIN28B could enhance mRNA translation efficiency by facilitating the recruitment of RNA helicase A to the translational machinery (*293*). A more recent structural study has proposed that LIN28 might activate translation by gaining access to dormant messenger ribonucleoprotein (mRNPs) via co-association to the Y-box binding protein 1 (YB-1) (*294*).

The role of LIN28B in malignancy

The role of LIN28B in leukemogenesis is a subject of debate. One study suggested LIN28 to be indispensable in *MLL*-r leukemia by inhibiting miR-150 maturation via MLL-Fusion/MYC/LIN-28 axis (*295*). In addition, LIN28 has been shown to promote proliferation and repress differentiation in *MLL*-r leukemic cells by negatively regulating *Let-7* (*296, 297*). These studies collectively suggest an oncogenic role of LIN28B in MLL-r AML. In contrast, other studies suggested the tumor suppressor activities of LIN28 (*279, 298*) in *MLL*-r leukemia. Downregulation of *miR-128a* induces Lin28a expression and rescue cell differentiation in AML (*298*). The diverse function of LIN28 in leukemia might result from the complexity of its targets.

Summary of articles

Article I

A somatic mutation in moesin drives progression into acute myeloid leukemia

Background to Article I

It has been widely accepted that development of acute myeloid leukemia (AML) involves stepwise processes. Genetic and/or epigenetic events appeared in normal immature hematopoietic cells give rise to leukemia initiating cells (LICs), harboring a competitive advantage over normal hematopoietic progenitors. This is followed by acquisition of additional genetic perturbations that further promote proliferation and/or inhibit differentiation of LICs thereafter lead to the formation of LSCs and AML (*100, 113, 299*). One approach to study this stepwise process is to apply the genetic information available from human AML studies to relevant animal models.

Balanced chromosomal translocations that involve the mixed lineage leukemia-1 (*MLL1/KMT2A*) gene can generate chimeric MLL fusion proteins. *MLL*-rearrangements (*MLL*-r) can be found in 35–50% of infant AML cases and ~3% of de novo AML cases in older children and adults (*280, 300*). In general, *MLL*-r are correlated to poor prognosis in AML (*163, 280, 300*). Notably, a substantial group of *MLL*-r AML are found to harbor somatic mutations, with activating mutations in RAS, FLT3, PTPN11, and BRAF being the most common. This has lent support to a two-step model of leukemogenesis in which the initial *MLL-r* and secondary mutations cooperate to form AML. However, this concept is challenged by the fact that in pediatric *MLL*-r acute lymphoblastic leukemia (ALL), few secondary mutations are found, which are often subclonal and frequently lost during relapse (*280*). This indicate that MLL fusion products can drive leukemia by itself in certain cell types which are more prone to *MLL*-r induced transformation and/or that genetic predisposition can act as a cooperative event in *MLL*-r instead of somatically acquired secondary mutations.

MLL fusion proteins has been frequently applied in mouse models to study the pathogenesis of leukemic initiation, evolution, relapse and therapy resistance. Secondary mutations have been identified in mouse models based on virally

expression of MLL fusion (*171, 226*). Although these studied identified some secondary mutations consistent with human AML, substantial differences of mutation patterns exist between these few murine studies and human leukemic samples. To be noted, the observation of extensive sub clonality in these viral delivery murine models does not accurately reflect the monoclonality of human AML. In this paper, we explored an inducible transgenic mouse strain (iMLL-ENL mice) previously generated in our laboratory, in which a human *MLL-ENL* fusion gene can be induced by Doxycycline, with the aim of identifying secondary events that underlie progression into overt AML (*131*).

Summary of Article I

In the initial stages of our work, we observed a three-phase patten of AML development, which is consist of initial pre-leukemic cell expansion phase, an intermediate contraction and later AML transformation phases. In addition, we demonstrated the clonality of the AML arising in the model used, indicating the relevance of the used model for mimicking human AML progression. Next, we set out to identify potential secondary mutations that could lead to the disease pattern of MLL-ENL induced AML. By subjecting multiple independent AMLs to exome sequencing, we identified activating mutations in *Hras*, *Ptpn11* and *Braf*, which involve the RAS pathway and have been identified in human *MLL*r AML.

Most strikingly, we found a highly recurrent de novo mutation in Moesin, that leads to substitution of the Arginine 295 residue to Cysteine. We further validated that R295C mutant MSN dramatically accelerated *MLL*-r AML transformation by inhibiting the differentiation of LICs. The ablation of R295C mutant MSN in the transformed leukemic cells led to impaired self-renewal capacity of leukemic cells.

While the MSN R295C mutation has been reported previously in one case of a CALM/AF10 mouse model (*301*) and as a recurrent somatic mutation in a retroviral MLL-AF9 AML model (*226*). However, none of these studies investigated the function of the MSN R295C mutation.

The most well established functions of MSN involve regulation of cell polarity, adhesion and migration (*251*). In hematopoiesis, MSN is the most dominantly expressed ERM protein and in several types of human cancer, MSN has been suggested to have oncogenic functions (*269-272*). MSN-ALK fusion, formed by a (X;2)(q11–12;p23) translocation, has been reported to underly the pathogenesis of anaplastic large cell lymphoma (*273*). Overall, no studies have previously shown a direct association of MSN with AML. In addition, neither expression levels nor mutations have to date been associated with human AML (data from TCGA).

A loss of function mutation on MSN, a germline R171W mutation, has been shown to associate with an X-linked immunodeficiency syndrome (*265*). Our results

demonstrate that the R295C mutation is gain of function mutation rather than loss of function mutation and its role in AML is dependent on MLL-ENL expression.

Phosphorylation on the T558 residue facilitate MSN activation (*262*). However, we observed that the phosphorylation on T558 interfered the function of the C295 mutant MSN. To be noted, one study observed that T558 phosphorylation of MSN led to inactivation of the Rho GTPase CDC42 in human myeloid cells (*302*). This is likely relevant to *MLL*-r AML, that relies on CDC42 activity to maintain self-renewal capacity of leukemic cells (*128, 303*).

Studies have shown that MSN protein structure is highly conserved and associated with its function. MSN contains a FERM domain, which consist of F1, F2 and F3 subdomains and which form a cloverleaf conformation (*255*). Notably, this FERM cloverleaf structure is maintained by interactions between R295 residue in F3 and E87 in the F1 domain (*255*). Breakdown of interactions between F3 and F1 would destabilize the FERM domain conformation and lead to F3 subdomain movements (*255*). The F3 subdomain contains several conserved secondary structures that are crucial for the function of MSN (*257*). The interaction between the F3 subdomain and the FERM C-terminal residues leads to homodimerization which is an inactive form of MSN (*257*). Inspired by these findings, we analysed the protein structure and found that the C295 mutation disturb the secondary structures formation of MSN. In addition, the C295 mutation interferes with the formation of homodimers and lead to increased number of monomers, which provided functional evidence for a disturbed FERM structure in C295 mutant MSN.

Notably, when exploring MSN mutations in human cancer, we observed two mutations on the Arginine 295 residue, S295 and H295, that also led to accelerated AML progression in the murine MLL-ENL model. Interestingly, we observed a corelation between the positive charge on the substituted residue and pathogenicity of mutations on R295. However, other substitutions on residues R293 and R294 did not show any effect on AML transformation. These findings indicate that R295 mutations might function by perturbing the salt bridge interaction specifically associated with R295.

Next, we profiled the interactome of C295 mutant MSN but did not observe any apparent alterations between WT and C295 mutant MSN. This result indicates that the oncogenic activities of C295 mutant MSN is not mediated by a pronounced disruption of the protein interactome.

Notably, we observed that C295 mutant MSN led to the activation of ERK signalling and upregulation of its downstream targets, *Myc* and *Ccnd1*. This finding is in line with previous studies show that mutations involving the RAS pathway are the most prominent secondary mutations in both human and murine *MLL*-r AML (*171, 226*).

Further studies are needed to illuminate how C295 mutant MSN activates the ERK signaling to trigger AML progression. Two studies have reported that the ERM

family protein Ezrin is involved in the spatiotemporal regulation of RAS activity (*266, 304*). Our interactome analysis also showed direct interaction between MSN and ERK. Thus, C295 mutant MSN might affect ERK activity by regulating the encounter with ERK.

Surveying the literature, we also noticed that MSN could bind to RNA with the RRRKPDT motif (293–299), which has been implicated in human lung cancer via its direct binding with a small RNA, piR-L-163 (*274*). Alternatively, C295 mutant MSN might function by dysregulating the RNA binding. If and how this might be connected to increased ERK activity remains unknown.

In conclusion, we in this paper demonstrated the stepwise development of AML in a model of *MLL*-r AML. With the identification of a recurrent MSN R295C mutation and validation of its role in leukemogenesis, we provide new insight to the molecular pathogenesis in *MLL*-r AML. Besides, our study proposes a critical role of ERM proteins in AML, with implications also for human cancer. In this view, uncovering of the biological roles and specific function of ERM proteins could lead to therapeutic benefit in human cancer, including for AML.

Article II

A fetal tumor suppressor axis abrogates mll-fusion driven acute myeloid leukemia

Background to Article II

The lineage preference, biological processes and molecular landscape in pediatric AML vary from adult AML. Thus, AML in children and adults should be viewed as two distinct diseases. Chromosomal translocations that involve the Mixed-lineage leukemia 1 (*MLL1/KMT2A*) gene generate some of the most aggressive forms of AML, with an incidence of MLL-rearrangements (*MLL*-r) in ~35-50% of infant, ~15-20% of pediatric and ~10% of adult AML cases (*305, 306*). The molecular intricacies of pediatric *MLL*-leukemogenesis remain largely elusive. In contrast to the situation in adults, pediatric MLL-fusion leukemias have been proposed to be sufficient to drive transformation, without the need for additional molecular lesions (*275, 281, 283, 307*). In line with this, several studies showed that young cells were more prone to MLL fusion than adult cells in terms of leukemia latency and lineage dictation (*278, 279*).

Despite that MLL fusions appear for the most part to arise in utero, the incidence of congenital/neonatal AML leukemia is very low (*308*), suggesting fetal hematopoietic stem/progenitor cells are protected against the *MLL*-r driven

leukemogenesis. Although it is well established that there exist distinct prenatal signals that promote organismal growth and cellular proliferation (*309*), little is known about their relationship to oncogenesis (*310*).

It has previously been established that enforced expression of LIN28B in adult hematopoietic stem/progenitor cells is sufficient to reestablish several aspects of fetal hematopoiesis (*49*). Here, we attempted to approach this issue by enforcing overexpression of LIN28B, a master regulator of fetal hematopoiesis, in adult *MLL*-r driven leukemogenesis (*49, 285*).

Summary of Article II

Using our dual-transgenic mouse model, LIN28B potently impeded the development of MLL-ENL induced AML, with more than 50% of the mice in the L28BME group failing to succumb to disease. Interestingly, when interrogating AML developing in this group, we failed to observe expression of LIN28B, whose expression became silenced by unclear mechanism. This finding further confirmed the tumor suppressor function of LIN28B in ME driven leukemogenesis. Likewise, the enforced expression of LIN28B also interferes with AML progression induced by a combination of activating RAS mutations with MLL-fusions, which lead to very aggressive AML (*250*). Thus, LIN28B can also disrupt AML in settings involving relevant secondary driver mutations.

Some studies have suggested an oncogenic role of LIN28 (*295-297*). However, recent studies have suggested that expression of LIN28 has a tumor suppressive function in AML (*279, 298*). In accordance with the latter view, we found that a vast majority of both pediatric and adult AML patient samples lack expression of *LIN28B*, which is also not expressed in our inducible ME mouse model. The role of Lin28 as a negative regulator of the *let-7* class of miRs is well established (*286*). In our model, we have observed that MLL-ENL elevated the expression of some *let-7* miR family members, whereas whose expression is disrupted in the presence of Lin28b. However, we failed to observe any pronounced delays in leukemic development upon repressing *let-7* miRs, indicating the tumor suppressor activity of Lin28b is largely let-7 independent.

Transcriptional profiling revealed a striking depletion of c-MYB target genes upon enforced expression of LIN28B, which was accompanied by significant decreases in *MLL*-leukemogenesis and LSC signatures and an enrichment for expression signatures associated to differentiation and apoptosis. MYB is an essential target of *MLL*-rearrangements and plays critical roles in *MLL*r leukemogenesis, with partial or transient suppression on MYB being sufficient to impede leukemia (*311, 312*). Intriguingly, all of these direct and indirect regulators of MYB, including for *Myb* itself, were found to be unaltered upon LIN28B expression, suggesting that MYB itself is a direct downstream target of LIN28B or its effectors.

Several studies showed that LIN28B could modulate translation by direct binding to mRNAs (*289-292*). Therefore, we mapped the LIN28B-bound RNA profile, in which Mybbp1a mRNA was identified and validated as a LIN28B binding target. Mybbp1a is a negative regulator of MYB activity (*313*). Similar to LIN28B, MYBBP1A protein expression levels are also higher in fetal as opposed to adult hematopoietic progenitors and critical for early embryonic development (*314, 315*). While MYBBP1A has not been extensively studied in the context of AML, its tumor suppressive activities have been suggested in several other human cancers, in which its decreased expression levels associate with worse disease prognosis (*316*). Mechanistically, loss of MYBBP1A is correlated with increased MYB activity and induce a metabolic shift towards oxidate phosphorylation, both of which have been proposed to be indispensable for LIC maintenance (*316, 317*).

We observed that MYBBP1A protein expression level was increased upon LIN28B expression. Enforced overexpression of MYBBP1A recapitulated several of the tumor suppressor phenotypes of LIN28B in both murine and human *MLL*r leukemia. These findings suggested a close physical and functional connection between Mybbp1a and LIN28B. LIN28B has been shown to enhance mRNA translation by different mechanisms, including recruitment of RNA helicase A to the translational machinery and gain of access to dormant mRNPs via co-association to YB-1 (*293, 294*). Here, we demonstrated that higher MYBBP1A levels might be caused by repressed proteasomal degradation resulting from LIN28B expression. Intriguingly, LIN28B was previously shown to coimmunoprecipitate with MYBBP1A in embryonic fibroblasts (*318*), suggesting that these two proteins can also interact physically. Finally, since LIN28B is regarded as a master regulator of fetal hematopoiesis with diverse functions and interactomes, our results do not exclude the existence of other LIN28B-mediated interactions that synergistically mediates its tumor suppressor activities.

Taken together, this work provides insight to the nature of fetal HSPCs that are protected against to leukemogenesis. Out data show that forced LIN28B expression in adult HSPCs could induce several, if not all, features of fetal hematopoiesis, and which serve to repress *MLL*-r driven leukemia. Moreover, we propose LIN28B/ MYBBP1A /MYB as a novel tumor suppressor axis that restricts *MLL*-r AML and perhaps also other AML subtypes with a MYB involvement. In this view, the developmentally restricted expression of LIN28B provides a natural protection against MYB-dependent tumors, while its abrupt decline a few weeks after birth presents an opportune for oncogenesis.

Article III

Ex vivo expansion potential of murine hematopoietic stem cells: a rare property only partially predicted by phenotype

Background to Article III

Hematopoietic stem cells (HSCs) maintain homeostasis and hematopoietic cells generation for life (*30*). This is achieved by their combined capacity of *multilineage differentiate* and *self-renewal*. Thus, HSCs represent the functional unit for hematopoietic regeneration in the clinical bone marrow transplantation (BMT) and are the preferred targets for gene therapy/gene editing approaches for the treatment of inherited blood disorders. However, the scarcity of HSCs in vivo (the frequency of about 0.005% of bone marrow cells) has hindered their broad clinical application as well as for experimental research. To be able to maintain or even expand human HSCs ex vivo is a pre-requisite for applications of gene therapy or to facilitate BMT outcome from limited donor sources (*319*).

While intensive efforts have been put towards the development of *in vitro* culture conditions that would allow for ex vivo HSC maintenance, most results have to date been disappointing (*30*). Meanwhile, studies using relevant animal models such as the mouse, could provide insight to understand and manipulate the fate processes of HSCs. More recently, an alternative polyvinyl alcohol (PVA)-based culture system has been proposed to support massive expansion (236-899-fold) of murine HSCs over a month (*320*). However, despite the remarkable magnitude of HSC expansion reported in this study, many details of this culture condition remain unknown. Therefore, in this study we set out to characterize the PVA-based culture system in more details.

Summary of Article III

First, we focused on the requirements for input cells for an in vitro culture. It was reported that PVA-based system can support selective HSC expansion when culturing less pure or even unfractionated BM cell populations (*321*). In line with this, we observed an expansion of long-term repopulating HSCs (LT-HSCs) when starting the cultures with primitive population of cKit+ cells. At the same time, we noticed some unfavourable aspects relevant to the less defined HSC-containing cells as input. For instance, the very low frequency of HSCs in the culture made it difficult to track the fates of candidate HSCs. Massive cell death of unresponsive non-HSCs might also cause indirect effects on HSCs. Apart from that, maintaining the non-HSCs requires larger culture volumes, which is laborious, time-consuming

and associates with higher costs. Therefore, we next evaluated more stringently purified HSCs as input cells for culture.

HSCs are phenotypically defined as Lin-Sca+cKit+ (LSK) CD48-CD150+ (*322*). Additional markers, including CD201 have been proposed to further enrich for LT-HSCs activity (*323*). In our study, we demonstrated that the inclusion of CD201 in candidate HSC isolation for *in vitro* culture is critical for robust expansion of *bona fide* LT-HSCs. By contrast, the addition of CD41, another marker suggested to discriminate differential HSC potentials (*324*), offered little further enrichment for functional LT-HSCs.

The average cell output from 50 initial HSCs (LSK CD48-CD150+CD201+/high) following 3-week culture was about 13.6 x 10^6 cells. These cells comprised of a highly heterogenous pool of stem and multipotent progenitor cells (HSPCs), with candidate phenotypic HSCs representing only about 0.1% of total culture. We further demonstrated that while the total pool of cultured cells could support robust hematopoietic regeneration *in vivo*, the actual long-term repopulating activity was restricted to a subfraction of LSK CD48-CD150+CD201+ cultured cells. Hence, our data suggest that additional markers such as EPCR and CD48 are needed to better predict the LT-HSC activity both before and after culture.

When starting the cultures with lower number of input HSCs (10-cell cultures), we observed heterogeneous reconstitution patterns in competitive transplantation assay. This indicates that even cells with a strictly defined LT-HSC phenotype, (phenotypically "homogeneous" cell fraction) can result in highly heterogeneous outputs following transplantation. Our clonal barcoding experiments further supported this view. This leads to the question whether the PVA-based culture system supports the expansion of only certain HSC clones/subsets. Connected to this, one relevant observation was the similar expansion of HSCs isolated from fetal liver and adult BM, despite the fact that fetal liver HSCs show higher repopulating activity than BM HSCs when unmanipulated (*47*). Further studies are needed to address this question.

Successful HSPCs transplantation requires conditioning, which typically involves irradiation and/or chemotherapy. The treatment allows for the transplanted cells to be accepted by the host, by providing the space in the BM niche and reducing responses from the host immune system. However, it is now well known that conditioning leads to many undesired short- and long-term effects that impacts on the recipient's health, but also on the fate transplanted cells. Unconditioned HSPCs transplantation therefore represents a desirable approach for some situations. While to date the requirement for a large number of HSPCs has been a major limitation for unconditioned transplantation (*325, 326*), the newer HSC ex vivo expansion system provides prospects for such approaches. In the final part of this study, we explored the potential of ex vivo expanded HSPCs to engraft unconditioned recipients.

By matching hosts for CD45-isoforms, we could successfully achieve stable, long-term multilineage engraftment in adult unconditional hosts from the cultured progeny of only 100 candidate HSCs. Intriguingly, despite being activated to proliferate *in vitro*, many candidate HSCs rapidly returned to a quiescent state *in vivo*, with the most dormant HSCs exhibiting the most stringent HSC phenotype (SLAM HSCs bearing high levels of EPCR).

Although many aspects of the PVA-based culture system remain to be explored, it represents a powerful approach to maintain and expand murine HSCs. We believe that our detailed characterization of the cells following expansion in PVA-based culture conditions is valuable not only for future applications of this system, but also for HSC biology in general.

Populärvetenskaplig Sammanfattning

Akut myeloid leukemi (AML) är en typ av blodcancer. Sjukdomen kännetecknas av att utmognaden av blodceller hindras och att produktionen av normala blodceller minskar. AML orsakas av genförändringar i vårt DNA. En mängd olika genetiska förändringar har hittats i AML, men vissa är vanligare och skadligare än andra. Normalt krävs det fler än en genetisk förändring för att AML ska utvecklas. De förändringar som uppstår först, de primära förändringarna, leder till ett s.k. pre-leukemiskt tillstånd (pre-AML). Ytterligare förändringar, sekundära förändringar, leder sedan till fullt utvecklad AML.

Forskning har visat att de första förändringarna inträffar flera år innan AML-diagnos. Det går därför inte att studera det tidiga skeendet i människor, men med hjälp av djurmodeller är det möjligt studera vilken kombination av genförändringar (mutationer) som föranleder AML, och huruvida den ordning i vilken de inträffar är av betydelse för sjukdomsutvecklingen. Ofta används möss med liknande genetiska förändringar som hittats hos AML-patienter. Förståelse för utvecklingen av AML är viktigt för att kunna framställa behandlingar som förhindrar, fördröjer eller botar sjukdomen.

De flesta celler i våra kroppar har normalt 23 kromosompar som innehåller vårt DNA. Nästan hälften av alla AML-patienter har förändringar av kromosomerna i cellerna som utvecklar leukemin. Vissa kromosomförändring är vanligare än andra, t.ex. den att en bit av kromosom nummer 11 har bytt plats med en del av någon annan kromosom. Denna typ av förändring kallas för translokation eller rearrangemang och kan leda att delar av två gener som normalt sitter på olika kromosomer fogas samman och bildar en s.k. fusionsgen. Genen *MLL1* (även kallad *KMT2A*) finns på kromosom 11 just på den plats där det är vanligare att kromosomen delat sig i AML. MLL-fusionsgener är särskilt farliga och hittas hos ca 10 % av vuxna AML-patienter och förknippas med en dålig prognos.

På grund av deras starka cancerframkallande effekt har MLL-fusionsgener använts frekvent i musstudier inom cancerforskning. Vi har framställt en experimentell musmodell för en MLL-fusionsgen. I vårt fall har MLL satts ihop med ENL-genen (MLL-ENL). Denna musmodell utvecklar en blodcancer som liknar human AML, och vi har använt denna i vår forskning för att studera AML.

I artikel 1 ville vi studera vad som händer under skedet från det att den initiala genförändringen uppstår (i vårt fall införandet av MLL-ENL genen) till det att AML

uppstår. Vi upptäckte att i 65.6 % av fallen hade *moesin* (*Msn*)-genen muterats. Mutationer av denna gen har ännu inte påträffats hos AML-patienter men däremot hos patienter med andra cancertyper, och tyder på att det är en gen som är inblandad i flera olika cancrar.

För att förstå Msn:s roll i AML, införde vi en *Msn*-mutation i vår AML-ENL-modell. När dessa två genförändringar kombinerades försvann den vänteperiod som vanligen föreligger från det att AML-ENL-fusiongenen introduceras till att AML utvecklas. *Msn*-mutationen kunde dock inte ensam utveckla AML. Vi upptäckte också att vi kunde hämma sjukdomen i redan utvecklad AML genom att minska nivåerna av Msn. Detta var en spännande upptäckt då en liknande strategi skulle kunna användas som en framtida behandlingsform även för AML-patienter. Vår upptäckt gav också en potentiell förklaring till skillnader i tiden från de initiala genförändringarna till AML-utveckling, nämligen skillnader i uppkomsthastighet av de sekundära förändringarna, såsom mutation av *Msn*. Ytterligare forskning krävs dock för ökad förståelse för hur dessa genetiska förändringar uppstår och leder till sjukdom.

Hos barn med AML är det ännu vanligare med MLL-fusioner (15-20 %). Dessa patienter har MLL-fusioner redan under tiden i livmodern, men utvecklar inte leukemi förrän efter födseln. Detta tyder på att blodceller av någon anledning är skyddade mot MLL-fusionsgensdriven leukemi under fostertiden. Blodsystemet under fostertiden, då blodcellsutveckling framför allt sker i levern, skiljer sig markant från efter födseln, då blodcellsutvecklingen sker i benmärgen. Forskning har visat att *Lin28*-genen endast är aktiv endast under fostertiden och om man aktiverar den hos vuxna benmärgsblodceller förändras dessa till att likna fetala leverblodceller.

I artikel 2 undersökte vi hur fetala leverblodceller förhindrar utvecklingen av MLL-fusionsgensdriven leukemi. Vi upptäckte att om *Lin28b* aktiveras i vuxna benmärgsblodceller med MLL-ENL uppkommer ingen AML. Vi såg at Lin28b kunde binda till olika typer av RNA, bl.a. *Mybbp1a*-mRNA (en typ av kodningsmall som kopieras från DNA och innehåller instruktioner för hur genens protein ska se ut). När Lin28 band till *Mybbp1a*-mRNA ledde det till att mer av Mybbp1a-protein bildades, som i sin tur hämmade MYB (ett viktigt protein i MLL-fusionsgensorsakad leukemi). Våra studier visade därmed att Lin28/Mybbp1a/MYB-serien kan hämma cancerutveckling och att Lin28 utgör ett nytt möjligt terapeutiskt mål för behandling av MYB-beroende cancerformer.

Blodstamceller är långlivade och kan producera alla typer av blodceller. Blodstamceller är därför optimala mål för genterapi/genredigeringsmetoder som syftar till att bota blodcellssjukdomar. Transplantation av blodstamceller används både kliniskt och i experimentella studier. Blodstamceller är relativt ovanliga och det finns därför väldigt få av dem i benmärgen. Denna begränsning har varit en utmaning för användningen av blodcellstransplantation/genterapi i både klinik och

för forskning. Forskare nyligen lyckats etablera ett tillvägagångsätt för att öka antalet blodstamceller genom att odla dem i inkubatorer utanför kroppen. I artikel 3 studerade vi denna metod och lyckades öka antalet blodstamceller ~300 gånger under en treveckorsperiod. Dessa stamceller hade vid slutskedet samma markörer på cellytan som används för att identifiera blodstamceller som kommer direkt ifrån kroppen, samt samma funktion. Det är viktigt att poängtera att även om antalet stamceller ökade så var 99,9 % av de celler som producerades i odlingen inte stamceller utan mer mogna blodceller. Expansionen som detta odlingssystem medger underlättar grund- och klinisk blodcellsforskning.

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References

1. R. Sender, R. Milo, The distribution of cellular turnover in the human body. *Nature medicine* **27**, 45-48 (2021).

2. D. Bryder, D. J. Rossi, I. L. Weissman, Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *The American journal of pathology* **169**, 338-346 (2006).

3. R. Yamamoto *et al.*, Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. *Cell* **154**, 1112-1126 (2013).

4. J. Adolfsson *et al.*, Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential: a revised road map for adult blood lineage commitment. *Cell* **121**, 295-306 (2005).

5. Y. Arinobu *et al.*, Reciprocal activation of GATA-1 and PU. 1 marks initial specification of hematopoietic stem cells into myeloerythroid and myelolymphoid lineages. *Cell Stem Cell* **1**, 416-427 (2007).

6. S. Haas *et al.*, Inflammation-Induced Emergency Megakaryopoiesis Driven by Hematopoietic Stem Cell-like Megakaryocyte Progenitors. *Cell Stem Cell* **17**, 422-434 (2015).

7. B. K. Tusi *et al.*, Population snapshots predict early haematopoietic and erythroid hierarchies. *Nature* **555**, 54-60 (2018).

8. C. J. Pronk *et al.*, Elucidation of the phenotypic, functional, and molecular topography of a myeloerythroid progenitor cell hierarchy. *Cell Stem Cell* **1**, 428-442 (2007).

9. R. Drissen *et al.*, Distinct myeloid progenitor–differentiation pathways identified through single-cell RNA sequencing. *Nature Immunology* **17**, 666-676 (2016).

10. I. L. Weissman, J. A. Shizuru, The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. *Blood, The Journal of the American Society of Hematology* **112**, 3543-3553 (2008).

11. L. Jacobson *et al.*, The role of the spleen in radiation injury and recovery. *The Journal of laboratory and clinical medicine* **35**, 746-770 (1950).

12. E. Lorenz, D. Uphoff, T. Reid, E. Shelton, Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *Journal of the National Cancer Institute* **12**, 197-201 (1951).

13. L. Jacobson, E. Simmons, E. Marks, J. Eldredge, Recovery from radiation injury. *Science (New York, N.Y.)* **113**, 510-511 (1951).

14. E. A. McCulloch, J. E. Till, The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. *Radiation research* **13**, 115-125 (1960).

15. J. E. Till, E. A. McCulloch, A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiation research* **14**, 213-222 (1961).

16. A. J. Becker, E. A. McCulloch, J. E. Till, Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. (1963).

17. L. Siminovitch, E. A. McCulloch, J. E. Till, THE DISTRIBUTION OF COLONY-FORMING CELLS AMONG SPLEEN COLONIES. *Journal of cellular and comparative physiology* **62**, 327-336 (1963).

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

19. R. Schofield, The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* **4**, 7-25 (1978).

20. G. Keller, C. Paige, E. Gilboa, E. F. Wagner, Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent haematopoietic precursors. *Nature* **318**, 149-154 (1985).

21. J. E. Dick, M. C. Magli, D. Huszar, R. A. Phillips, A. Bernstein, Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic system of W/Wv mice. *Cell* **42**, 71-79 (1985).

22. I. R. Lemischka, D. H. Raulet, R. C. Mulligan, Developmental potential and dynamic behavior of hematopoietic stem cells. *Cell* **45**, 917-927 (1986).

23. S. J. Morrison, I. L. Weissman, The Long-Term Repopulating Subset of Hematopoietic Stem-Cells Is Deterministic and Isolatable by Phenotype. *Immunity* **1**, 661-673 (1994).

24. G. J. Spangrude, S. Heimfeld, I. L. Weissman, Purification and characterization of mouse hematopoietic stem cells. *Science (New York, N.Y.)* **241**, 58-62 (1988).

25. C. L. Li, G. R. Johnson, Murine hematopoietic stem and progenitor cells: I. Enrichment and biologic characterization. *Blood* **85**, 1472-1479 (1995).

26. K. Ikuta, I. L. Weissman, Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. *Proceedings of the National Academy of Sciences* **89**, 1502-1506 (1992).

27. M. J. Kiel *et al.*, SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* **121**, 1109-1121 (2005).

28. J. Adolfsson *et al.*, Upregulation of flt3 expression within the bone marrow Lin(-)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity* **15**, 659-669 (2001).

29. M. Osawa, K. Hanada, H. Hamada, H. Nakauchi, Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science (New York, N.Y.)* **273**, 242-245 (1996).

30. A. Konturek-Ciesla, D. Bryder, Stem Cells, Hematopoiesis and Lineage Tracing: Transplantation-Centric Views and Beyond. *Frontiers in Cell and Developmental Biology* **10**, (2022).

31. A. Medvinsky, S. Rybtsov, S. Taoudi, Embryonic origin of the adult hematopoietic system: advances and questions. *Development (Cambridge, England)* **138**, 1017-1031 (2011).

32. A. Medvinsky, E. Dzierzak, Definitive hematopoiesis is autonomously initiated by the AGM region. *Cell* **86**, 897-906 (1996).

33. A. M. Müller, A. Medvinsky, J. Strouboulis, F. Grosveld, E. Dzierzakt, Development of hematopoietic stem cell activity in the mouse embryo. *Immunity* **1**, 291-301 (1994).

34. K. Ottersbach, E. Dzierzak, The murine placenta contains hematopoietic stem cells within the vascular labyrinth region. *Developmental cell* **8**, 377-387 (2005).

35. C. Gekas, F. Dieterlen-Lièvre, S. H. Orkin, H. K. Mikkola, The placenta is a niche for hematopoietic stem cells. *Developmental cell* **8**, 365-375 (2005).

36. S. Gordon-Keylock, M. Sobiesiak, S. Rybtsov, K. Moore, A. Medvinsky, Mouse extraembryonic arterial vessels harbor precursors capable of maturing into definitive HSCs. *Blood, The Journal of the American Society of Hematology* **122**, 2338-2345 (2013).

37. M. F. de Bruijn, N. A. Speck, M. C. Peeters, E. Dzierzak, Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. *The EMBO journal* **19**, 2465-2474 (2000).

38. Z. Li *et al.*, Mouse embryonic head as a site for hematopoietic stem cell development. *Cell Stem Cell* **11**, 663-675 (2012).

39. P. Kumaravelu *et al.*, Quantitative developmental anatomy of definitive haematopoietic stem cells/long-term repopulating units (HSC/RUs): role of the aorta-gonad-mesonephros (AGM) region and the yolk sac in colonisation of the mouse embryonic liver. (2002).

40. H. Ema, H. Nakauchi, Expansion of hematopoietic stem cells in the developing liver of a mouse embryo. *Blood, The Journal of the American Society of Hematology* **95**, 2284-2288 (2000).

41. A. Sanchez-Aguilera *et al.*, Guanine nucleotide exchange factor Vav1 regulates perivascular homing and bone marrow retention of hematopoietic stem and progenitor cells. *Proceedings of the National Academy of Sciences* **108**, 9607-9612 (2011).

42. M. B. Bowie *et al.*, Identification of a new intrinsically timed developmental checkpoint that reprograms key hematopoietic stem cell properties. *Proceedings of the National Academy of Sciences* **104**, 5878-5882 (2007).

43. M. B. Bowie *et al.*, Hematopoietic stem cells proliferate until after birth and show a reversible phase-specific engraftment defect. *The Journal of clinical investigation* **116**, 2808-2816 (2006).

44. S. J. Morrison, H. D. Hemmati, A. M. Wandycz, I. L. Weissman, The purification and characterization of fetal liver hematopoietic stem cells. *Proceedings of the National Academy of Sciences* **92**, 10302-10306 (1995).

45. K. Kikuchi, M. Kondo, Developmental switch of mouse hematopoietic stem cells from fetal to adult type occurs in bone marrow after birth. *Proceedings of the National Academy of Sciences* **103**, 17852-17857 (2006).

46. E. M. Pietras, M. R. Warr, E. Passegué, Cell cycle regulation in hematopoietic stem cells. *The Journal of cell biology* **195**, 709-720 (2011).

47. V. I. Rebel, C. L. Miller, C. J. Eaves, P. M. Lansdorp, The repopulation potential of fetal liver hematopoietic stem cells in mice exceeds that of their liver adult bone marrow counterparts. *Blood* **87**, 3500-3507 (1996).

48. F. Ginhoux *et al.*, Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science (New York, N.Y.)* **330**, 841-845 (2010).

49. J. Yuan, C. K. Nguyen, X. Liu, C. Kanellopoulou, S. A. Muljo, Lin28b reprograms adult bone marrow hematopoietic progenitors to mediate fetal-like lymphopoiesis. *Science (New York, N.Y.)* **335**, 1195-1200 (2012).

50. W. L. Havran, J. P. Allison, Origin of Thy-1+ dendritic epidermal cells of adult mice from fetal thymic precursors. *Nature* **344**, 68-70 (1990).

51. J. K. Manesia *et al.*, Distinct molecular signature of murine fetal liver and adult hematopoietic stem cells identify novel regulators of hematopoietic stem cell function. *Stem cells and development* **26**, 573-584 (2017).

52. M. Jassinskaja *et al.*, Comprehensive proteomic characterization of ontogenic changes in hematopoietic stem and progenitor cells. *Cell reports* **21**, 3285-3297 (2017).

53. K. Sudo, H. Ema, Y. Morita, H. Nakauchi, Age-associated characteristics of murine hematopoietic stem cells. *The Journal of experimental medicine* **192**, 1273-1280 (2000).

54. D. J. Rossi *et al.*, Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proceedings of the National Academy of Sciences* **102**, 9194-9199 (2005).

55. S. J. Morrison, A. M. Wandycz, K. Akashi, A. Globerson, I. L. Weissman, The aging of hematopoietic stem cells. *Nature medicine* **2**, 1011-1016 (1996).

56. B. Dykstra, S. Olthof, J. Schreuder, M. Ritsema, G. De Haan, Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. *Journal of Experimental Medicine* **208**, 2691-2703 (2011).

57. A. Grover *et al.*, Single-cell RNA sequencing reveals molecular and functional platelet bias of aged haematopoietic stem cells. *Nat Commun* **7**, 1-12 (2016).

58. A. Rundberg Nilsson, S. Soneji, S. Adolfsson, D. Bryder, C. J. Pronk, Human and murine hematopoietic stem cell aging is associated with functional impairments and intrinsic megakaryocytic/erythroid bias. *PloS one* **11**, e0158369 (2016).

59. H. Nakauchi, H. Takano, H. Ema, M. Osawa, Further characterization of CD34-low/negative mouse hematopoietic stem cells. *Ann N Y Acad Sci* **872**, 57-66; discussion 66-70 (1999).

60. L. Yang *et al.*, Identification of Lin(-)Sca1(+)kit(+)CD34(+)Flt3- short-term hematopoietic stem cells capable of rapidly reconstituting and rescuing myeloablated transplant recipients. *Blood* **105**, 2717-2723 (2005).

62

61. T. Na Nakorn, D. Traver, I. L. Weissman, K. Akashi, Myeloerythroid-restricted progenitors are sufficient to confer radioprotection and provide the majority of day 8 CFU-S. *J Clin Invest* **109**, 1579-1585 (2002).

62. L. E. Purton, D. T. Scadden, Limiting factors in murine hematopoietic stem cell assays. *Cell Stem Cell* **1**, 263-270 (2007).

63. D. E. Harrison, C. T. Jordan, R. K. Zhong, C. M. Astle, Primitive hemopoietic stem cells: direct assay of most productive populations by competitive repopulation with simple binomial, correlation and covariance calculations. *Exp Hematol* **21**, 206-219 (1993).

64. K. R. Kampen, The discovery and early understanding of leukemia. *Leukemia research* **36**, 6-13 (2012).

65. A. Donné, *Cours de microscopie complémentaire des études médicales: anatomie microscopique et physiologie des fluides de l'économie*. (Baillière, 1844).

66. K. R. Kampen, The discovery and early understanding of leukemia. *Leukemia research* **36**, 6-13 (2012).

67. J. Bennett. (Last Edinburgh edn.,(S. Samuel & W. Wood, New York, 1860), 1860).

68. J. H. Bennett, Case of hypertrophy of the spleen and liver, in which death took place from suppuration of the blood. *Edinburgh Med Sug J* **64**, 413-423 (1845).

69. H. Fuller, Particulars of a case in which enormous enlargement of the spleen and liver, together with dilation of all the blood vessels of the body were found co-incident with a peculiarly altered condition of the blood. *Lancet* **2**, 43-44 (1846).

70. R. Virchow, *Gesammelte abhandlungen zur wissenschaftlichen medicin*. (BoD–Books on Demand, 2022).

71. H. Reschad, V. Schilinng-Torgau, Ueber eine neue leukamie dursh echte uebergangsformen und ihre bedeutung fur dies selbstãndigkeit diezer zellen. *Munch. Med. Wochenschr* **60**, 1981-1984 (1913).

72. N. Howlader *et al.*, SEER cancer statistics review, 1975–2016. *National Cancer Institute* **1**, (2019).

73. N. C. Institute, Surveillance, epidemiology, and end results program. *Cancer stat facts: Non-Hodgkin lymphoma*, (2017).

74. R. M. Shallis, R. Wang, A. Davidoff, X. Ma, A. M. Zeidan, Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. *Blood reviews* **36**, 70-87 (2019).

75. S. F. Huntington *et al.*, Treatment patterns, economic burden, and overall survival in US Medicare Advantage beneficiaries newly diagnosed with acute myeloid leukemia (AML) in 2015–2020. *Leukemia & lymphoma* **63**, 1180-1190 (2022).

76. E. Estey, H. Dohner, Acute myeloid leukaemia. *Lancet* **368**, 1894-1907 (2006).

77. S. Blais *et al.*, Is acute myeloblastic leukemia in children under 2 years of age a specific entity? A report from the FRENCH ELAM02 Study Group. *HemaSphere* **3**, (2019).

78. C. Meyer *et al.*, The MLL recombinome of acute leukemias in 2017. *Leukemia* **32**, 273-284 (2018).

79.   C. H. Pui, W. L. Carroll, S. Meshinchi, R. J. Arceci, Biology, risk stratification, and therapy of pediatric acute leukemias: an update. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **29**, 551-565 (2011).

80.   B. Lowenberg, J. R. Downing, A. Burnett, Acute myeloid leukemia. *New England Journal of Medicine* **341**, 1051-1062 (1999).

81.   J. M. Bennett *et al.*, Proposals for the classification of the acute leukaemias French-American-British (FAB) co-operative group. *British journal of haematology* **33**, 451-458 (1976).

82.   J. W. Vardiman, N. L. Harris, R. D. Brunning, The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood, The Journal of the American Society of Hematology* **100**, 2292-2302 (2002).

83.   E. S. Jaffe, *Pathology and genetics of tumours of haematopoietic and lymphoid tissues*. (Iarc, 2001), vol. 3.

84.   J. W. Vardiman *et al.*, The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood, The Journal of the American Society of Hematology* **114**, 937-951 (2009).

85.   S. H. Swerdlow *et al.*, *WHO classification of tumours of haematopoietic and lymphoid tissues*. (International agency for research on cancer Lyon, 2008), vol. 2.

86.   F. M. Ghazawi *et al.*, Analysis of acute myeloid leukemia incidence and geographic distribution in Canada from 1992 to 2010 reveals disease clusters in Sarnia and other industrial US border cities in Ontario. *Cancer* **125**, 1886-1897 (2019).

87.   S. S. Strom, R. Oum, K. Y. Elhor Gbito, G. Garcia-Manero, Y. Yamamura, De novo acute myeloid leukemia risk factors: a Texas case-control study. *Cancer* **118**, 4589-4596 (2012).

88.   J. Yates, H. Wallace Jr, R. Ellison, J. Holland, Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. *Cancer chemotherapy reports* **57**, 485-488 (1973).

89.   H. Döhner *et al.*, Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood, The Journal of the American Society of Hematology* **115**, 453-474 (2010).

90.   R. M. Stone *et al.*, Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. *N Engl J Med* **377**, 454-464 (2017).

91.   S. H. Petersdorf *et al.*, A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. *Blood* **121**, 4854-4860 (2013).

92.   A. K. Burnett *et al.*, Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **29**, 369-377 (2011).

93. A. K. Burnett *et al.*, Addition of gemtuzumab ozogamicin to induction chemotherapy improves survival in older patients with acute myeloid leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **30**, 3924-3931 (2012).

94. S. Castaigne *et al.*, Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet* **379**, 1508-1516 (2012).

95. J. E. Lancet *et al.*, CPX-351 (cytarabine and daunorubicin) Liposome for Injection Versus Conventional Cytarabine Plus Daunorubicin in Older Patients With Newly Diagnosed Secondary Acute Myeloid Leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **36**, 2684-2692 (2018).

96. J. Holowiecki *et al.*, Cladribine, but not fludarabine, added to daunorubicin and cytarabine during induction prolongs survival of patients with acute myeloid leukemia: a multicenter, randomized phase III study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **30**, 2441-2448 (2012).

97. A. K. Burnett *et al.*, A comparison of clofarabine with ara-C, each in combination with daunorubicin as induction treatment in older patients with acute myeloid leukaemia. *Leukemia* **31**, 310-317 (2017).

98. H. Döhner *et al.*, Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* **129**, 424-447 (2017).

99. L. I. Shlush *et al.*, Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* **506**, 328-333 (2014).

100. K. Mitchell, U. Steidl, Targeting Immunophenotypic Markers on Leukemic Stem Cells: How Lessons from Current Approaches and Advances in the Leukemia Stem Cell (LSC) Model Can Inform Better Strategies for Treating Acute Myeloid Leukemia (AML). *Cold Spring Harbor perspectives in medicine* **10**, (2020).

101. G. Genovese *et al.*, Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* **371**, 2477-2487 (2014).

102. S. Jaiswal *et al.*, Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* **371**, 2488-2498 (2014).

103. M. Xie *et al.*, Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nature medicine* **20**, 1472-1478 (2014).

104. L. I. Shlush, Age-related clonal hematopoiesis. *Blood* **131**, 496-504 (2018).

105. D. P. Steensma *et al.*, Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* **126**, 9-16 (2015).

106. L. Busque *et al.*, Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nature genetics* **44**, 1179-1181 (2012).

107. N. K. Gillis *et al.*, Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. *Lancet Oncol* **18**, 112-121 (2017).

108. M. R. Corces, H. Y. Chang, R. Majeti, Preleukemic hematopoietic stem cells in human acute myeloid leukemia. *Front Oncol* **7**, 263 (2017).

109. T. Gao *et al.*, Interplay between chromosomal alterations and gene mutations shapes the evolutionary trajectory of clonal hematopoiesis. *Nat Commun* **12**, 338 (2021).

110. D. Bonnet, J. E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature medicine* **3**, 730-737 (1997).

111. M. R. Corces-Zimmerman, W. J. Hong, I. L. Weissman, B. C. Medeiros, R. Majeti, Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. *Proc Natl Acad Sci U S A* **111**, 2548-2553 (2014).

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

113. R. L. Bowman, L. Busque, R. L. Levine, Clonal hematopoiesis and evolution to hematopoietic malignancies. *Cell Stem Cell* **22**, 157-170 (2018).

114. J. S. Welch *et al.*, The origin and evolution of mutations in acute myeloid leukemia. *Cell* **150**, 264-278 (2012).

115. M. Jan *et al.*, Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Sci Transl Med* **4**, 149ra118 (2012).

116. J. Chen *et al.*, Myelodysplastic syndrome progression to acute myeloid leukemia at the stem cell level. *Nature medicine* **25**, 103-110 (2019).

117. P. Desai *et al.*, Somatic mutations precede acute myeloid leukemia years before diagnosis. *Nature medicine* **24**, 1015-1023 (2018).

118. S. Abelson *et al.*, Prediction of acute myeloid leukaemia risk in healthy individuals. *Nature* **559**, 400-404 (2018).

119. Y. Guan, B. Gerhard, D. E. Hogge, Detection, isolation, and stimulation of quiescent primitive leukemic progenitor cells from patients with acute myeloid leukemia (AML). *Blood* **101**, 3142-3149 (2003).

120. F. Ishikawa *et al.*, Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat Biotechnol* **25**, 1315-1321 (2007).

121. T. Lapidot *et al.*, A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* **367**, 645-648 (1994).

122. N. Goardon *et al.*, Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. *Cancer cell* **19**, 138-152 (2011).

123. M. P. Martelli *et al.*, CD34+ cells from AML with mutated NPM1 harbor cytoplasmic mutated nucleophosmin and generate leukemia in immunocompromised mice. *Blood* **116**, 3907-3922 (2010).

124. D. C. Taussig *et al.*, Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34(-) fraction. *Blood* **115**, 1976-1984 (2010).

125. D. Thomas, R. Majeti, Biology and relevance of human acute myeloid leukemia stem cells. *Blood* **129**, 1577-1585 (2017).

126. P. N. Kelly, A. Dakic, J. M. Adams, S. L. Nutt, A. Strasser, Tumor growth need not be driven by rare cancer stem cells. *Science (New York, N.Y.)* **317**, 337-337 (2007).

127. A. V. Krivtsov *et al.*, Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature* **442**, 818-822 (2006).

128. T. C. Somervaille, M. L. Cleary, Identification and characterization of leukemia stem cells in murine MLL-AF9 acute myeloid leukemia. *Cancer cell* **10**, 257-268 (2006).

129. T. Miyamoto, I. L. Weissman, K. Akashi, AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. *Proc Natl Acad Sci U S A* **97**, 7521-7526 (2000).

130. F. Barabe, J. A. Kennedy, K. J. Hope, J. E. Dick, Modeling the initiation and progression of human acute leukemia in mice. *Science (New York, N.Y.)* **316**, 600-604 (2007).

131. A. Ugale *et al.*, Hematopoietic stem cells are intrinsically protected against MLL-ENL-mediated transformation. *Cell reports* **9**, 1246-1255 (2014).

132. Y.-H. Kuo *et al.*, Cbfβ-SMMHC induces distinct abnormal myeloid progenitors able to develop acute myeloid leukemia. *Cancer cell* **9**, 57-68 (2006).

133. U. Steidl *et al.*, Essential role of Jun family transcription factors in PU. 1 knockdown–induced leukemic stem cells. *Nature genetics* **38**, 1269-1277 (2006).

134. O. Bereshchenko *et al.*, Hematopoietic stem cell expansion precedes the generation of committed myeloid leukemia-initiating cells in C/EBPα mutant AML. *Cancer cell* **16**, 390-400 (2009).

135. W. Chen *et al.*, A murine Mll-AF4 knock-in model results in lymphoid and myeloid deregulation and hematologic malignancy. *Blood* **108**, 669-677 (2006).

136. F. Bernardin *et al.*, TEL-AML1, expressed from t(12;21) in human acute lymphocytic leukemia, induces acute leukemia in mice. *Cancer research* **62**, 3904-3908 (2002).

137. C. Schessl *et al.*, The AML1-ETO fusion gene and the FLT3 length mutation collaborate in inducing acute leukemia in mice. *The Journal of clinical investigation* **115**, 2159-2168 (2005).

138. L. Scourzic *et al.*, DNMT3A R882H mutant and Tet2 inactivation cooperate in the deregulation of DNA methylation control to induce lymphoid malignancies in mice. *Leukemia* **30**, 1388 (2016).

139. S. Nishida *et al.*, AML1-ETO rapidly induces acute myeloblastic leukemia in cooperation with the Wilms tumor gene, WT1. *Blood* **107**, 3303-3312 (2006).

140. K. Ueda *et al.*, MDMX acts as a pervasive preleukemic-to-acute myeloid leukemia transition mechanism. *Cancer cell* **39**, 529-+ (2021).

141. M. Ko *et al.*, Ten-Eleven-Translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. *Proc Natl Acad Sci U S A* **108**, 14566-14571 (2011).

142. K. Moran-Crusio *et al.*, Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer cell* **20**, 11-24 (2011).

143. O. A. Guryanova *et al.*, Dnmt3a regulates myeloproliferation and liver-specific expansion of hematopoietic stem and progenitor cells. *Leukemia* **30**, 1133-1142 (2016).

144. H. Tamai *et al.*, Activated K-Ras protein accelerates human MLL/AF4-induced leukemo-lymphomogenicity in a transgenic mouse model. *Leukemia* **25**, 888-891 (2011).

145. D. S. Krause *et al.*, Differential regulation of myeloid leukemias by the bone marrow microenvironment. *Nature medicine* **19**, 1513-1517 (2013).

146. D. Mittal, M. M. Gubin, R. D. Schreiber, M. J. Smyth, New insights into cancer immunoediting and its three component phases—elimination, equilibrium and escape. *Current opinion in immunology* **27**, 16-25 (2014).

147. P. M. Ayton, M. L. Cleary, Transformation of myeloid progenitors by MLL oncoproteins is dependent on Hoxa7 and Hoxa9. *Genes & development* **17**, 2298-2307 (2003).

148. V. Grossmann *et al.*, High incidence of RAS signalling pathway mutations in MLL-rearranged acute myeloid leukemia. *Leukemia* **27**, 1933-1936 (2013).

149. L. Y. Shih *et al.*, Cooperating mutations of receptor tyrosine kinases and Ras genes in childhood core-binding factor acute myeloid leukemia and a comparative analysis on paired diagnosis and relapse samples. *Leukemia* **22**, 303-307 (2008).

150. N. A. Zorko *et al.*, Mll partial tandem duplication and Flt3 internal tandem duplication in a double knock-in mouse recapitulates features of counterpart human acute myeloid leukemias. *Blood* **120**, 1130-1136 (2012).

151. H. Makishima *et al.*, Dynamics of clonal evolution in myelodysplastic syndromes. *Nature genetics* **49**, 204 (2017).

152. A. Ugale *et al.*, MLL-ENL-mediated leukemia initiation at the interface of lymphoid commitment. *Oncogene* **36**, 3207-3212 (2017).

153. L. M. Kelly *et al.*, PML/RARα and FLT3-ITD induce an APL-like disease in a mouse model. *Proceedings of the National Academy of Sciences* **99**, 8283-8288 (2002).

154. L. Chen *et al.*, Mutated Ptpn11 alters leukemic stem cell frequency and reduces the sensitivity of acute myeloid leukemia cells to Mcl1 inhibition. *Leukemia* **29**, 1290-1300 (2015).

155. J. S. Jutzi *et al.*, Altered NFE2 activity predisposes to leukemic transformation and myelosarcoma with AML-specific aberrations. *Blood* **133**, 1766-1777 (2019).

156. A. Boyapati *et al.*, A leukemia fusion protein attenuates the spindle checkpoint and promotes aneuploidy. *Blood* **109**, 3963-3971 (2007).

157. M. Alcalay *et al.*, Acute myeloid leukemia fusion proteins deregulate genes involved in stem cell maintenance and DNA repair. *The Journal of clinical investigation* **112**, 1751-1761 (2003).

158. L. F. Peterson *et al.*, Acute myeloid leukemia with the 8q22; 21q22 translocation: secondary mutational events and alternative t (8; 21) transcripts. *Blood, The Journal of the American Society of Hematology* **110**, 799-805 (2007).

159. D. A. Arber *et al.*, The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood, The Journal of the American Society of Hematology* **127**, 2391-2405 (2016).

160. H. Döhner *et al.*, Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood, The Journal of the American Society of Hematology* **129**, 424-447 (2017).

161. N. Cancer Genome Atlas Research *et al.*, Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med* **368**, 2059-2074 (2013).

162. J. W. Tyner *et al.*, Functional genomic landscape of acute myeloid leukaemia. *Nature* **562**, 526-531 (2018).

163. E. Papaemmanuil *et al.*, Genomic classification and prognosis in acute myeloid leukemia. *New England Journal of Medicine* **374**, 2209-2221 (2016).

164. R. C. Lindsley *et al.*, Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood, The Journal of the American Society of Hematology* **125**, 1367-1376 (2015).

165. C. Thiede, Mutant DNMT3A: teaming up to transform. *Blood, The Journal of the American Society of Hematology* **119**, 5615-5617 (2012).

166. T. Kitamura *et al.*, The molecular basis of myeloid malignancies. *Proc Jpn Acad Ser B Phys Biol Sci* **90**, 389-404 (2014).

167. L. M. Kelly, D. G. Gilliland, Genetics of myeloid leukemias. *Annu Rev Genomics Hum Genet* **3**, 179-198 (2002).

168. F. Pourrajab, M. R. Zare-Khormizi, A. S. Hashemi, S. Hekmatimoghaddam, Genetic Characterization and Risk Stratification of Acute Myeloid Leukemia. *Cancer Manag Res* **12**, 2231-2253 (2020).

169. K. Mrózek *et al.*, Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. *Journal of clinical oncology* **30**, 4515 (2012).

170. F. Pourrajab, M. R. Zare-Khormizi, A. S. Hashemi, S. Hekmatimoghaddam, Genetic characterization and risk stratification of acute myeloid leukemia. *Cancer management and research* **12**, 2231 (2020).

171. A. Hyrenius-Wittsten *et al.*, De novo activating mutations drive clonal evolution and enhance clonal fitness in KMT2A-rearranged leukemia. *Nat Commun* **9**, 1770 (2018).

172. X. Xu, B. Schneider, Therapeutic targeting potential of chromatin-associated proteins in MLL-rearranged acute leukemia. *Cellular oncology (Dordrecht)* **42**, 117-130 (2019).

173. A. Shilatifard, Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. *Annu. Rev. Biochem.* **75**, 243-269 (2006).

174. A. Sparmann, M. van Lohuizen, Polycomb silencers control cell fate, development and cancer. *Nature Reviews Cancer* **6**, 846-856 (2006).

175. R. C. Rao, Y. Dou, Hijacked in cancer: the KMT2 (MLL) family of methyltransferases. *Nature Reviews Cancer* **15**, 334-346 (2015).

176. N. J. Zeleznik-Le, A. M. Harden, J. D. Rowley, 11q23 translocations split the "AT-hook" cruciform DNA-binding region and the transcriptional repression domain from the activation domain of the mixed-lineage leukemia (MLL) gene. *Proc Natl Acad Sci U S A* **91**, 10610-10614 (1994).

177. M. Birke *et al.*, The MT domain of the proto-oncoprotein MLL binds to CpG-containing DNA and discriminates against methylation. *Nucleic Acids Res* **30**, 958-965 (2002).

178. Z. Wang *et al.*, Pro isomerization in MLL1 PHD3-bromo cassette connects H3K4me readout to CyP33 and HDAC-mediated repression. *Cell* **141**, 1183-1194 (2010).

179. P. Y. Chang *et al.*, Binding of the MLL PHD3 finger to histone H3K4me3 is required for MLL-dependent gene transcription. *Journal of molecular biology* **400**, 137-144 (2010).

180. T. A. Milne *et al.*, MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol Cell* **10**, 1107-1117 (2002).

181. T. Nakamura *et al.*, ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Molecular Cell* **10**, 1119-1128 (2002).

182. P. Ernst, J. Wang, M. Huang, R. H. Goodman, S. J. Korsmeyer, MLL and CREB bind cooperatively to the nuclear coactivator CREB-binding protein. *Molecular and cellular biology* **21**, 2249-2258 (2001).

183. B. D. Crawford, J. L. Hess, MLL core components give the green light to histone methylation. *ACS chemical biology* **1**, 495-498 (2006).

184. R. J. Sims, 3rd, D. Reinberg, Histone H3 Lys 4 methylation: caught in a bind? *Genes & development* **20**, 2779-2786 (2006).

185. J. Wysocka *et al.*, WDR5 associates with histone H3 methylated at K4 and is essential for H3K4 methylation and vertebrate development. *Cell* **121**, 859-872 (2005).

186. Y. Dou *et al.*, Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. *Cell* **121**, 873-885 (2005).

187. Y. Dou *et al.*, Regulation of MLL1 H3K4 methyltransferase activity by its core components. *Nature structural & molecular biology* **13**, 713-719 (2006).

188. A. Yokoyama, M. L. Cleary, Menin critically links MLL proteins with LEDGF on cancer-associated target genes. *Cancer cell* **14**, 36-46 (2008).

189. A. Yokoyama *et al.*, The menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. *Cell* **123**, 207-218 (2005).

190. B. D. Yu, J. L. Hess, S. E. Horning, G. A. J. Brown, S. J. Korsmeyer, Altered Hox expression and segmental identity in Mll-mutant mice. *Nature* **378**, 505-508 (1995).

191. H. Yagi *et al.*, Growth disturbance in fetal liver hematopoiesis of Mll-mutant mice. *Blood* **92**, 108-117 (1998).

192. P. Ernst *et al.*, Definitive hematopoiesis requires the mixed-lineage leukemia gene. *Developmental cell* **6**, 437-443 (2004).

193. C. Abramovich, R. K. Humphries, Hox regulation of normal and leukemic hematopoietic stem cells. *Current opinion in hematology* **12**, 210-216 (2005).

194. H. J. Lawrence *et al.*, Loss of expression of the Hoxa-9 homeobox gene impairs the proliferation and repopulating ability of hematopoietic stem cells. *Blood* **106**, 3988-3994 (2005).

195. H. J. Lawrence *et al.*, Mice bearing a targeted interruption of the homeobox gene HOXA9 have defects in myeloid, erythroid, and lymphoid hematopoiesis. *Blood* **89**, 1922-1930 (1997).

196. J. Huret, P. Dessen, A. Bernheim, An atlas on chromosomes in hematological malignancies. Example: 11q23 and MLL partners. *Leukemia* **15**, 987-989 (2001).

197. A. C. Winters, K. M. Bernt, MLL-Rearranged Leukemias-An Update on Science and Clinical Approaches. *Frontiers in pediatrics* **5**, 4 (2017).

198. P. M. Ayton, M. L. Cleary, Molecular mechanisms of leukemogenesis mediated by MLL fusion proteins. *Oncogene* **20**, 5695-5707 (2001).

199. M. A. Caligiuri *et al.*, Partial tandem duplication of ALL1 as a recurrent molecular defect in acute myeloid leukemia with trisomy 11. *Cancer research* **56**, 1418-1425 (1996).

200. S. A. Schichman *et al.*, ALL-1 partial duplication in acute leukemia. *Proceedings of the National Academy of Sciences* **91**, 6236-6239 (1994).

201. D. Grimwade *et al.*, Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood* **116**, 354-365 (2010).

202. B. V. Balgobind *et al.*, Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. *Blood* **114**, 2489-2496 (2009).

203. D. Biswas *et al.*, Function of leukemogenic mixed lineage leukemia 1 (MLL) fusion proteins through distinct partner protein complexes. *Proc Natl Acad Sci U S A* **108**, 15751-15756 (2011).

204. Y. Okada *et al.*, hDOT1L links histone methylation to leukemogenesis. *Cell* **121**, 167-178 (2005).

205. H. Okuda *et al.*, Cooperative gene activation by AF4 and DOT1L drives MLL-rearranged leukemia. *The Journal of clinical investigation* **127**, 1918-1931 (2017).

206. K. M. Bernt *et al.*, MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. *Cancer cell* **20**, 66-78 (2011).

207. T. A. Milne, M. E. Martin, H. W. Brock, R. K. Slany, J. L. Hess, Leukemogenic MLL fusion proteins bind across a broad region of the Hox a9 locus, promoting transcription and multiple histone modifications. *Cancer research* **65**, 11367-11374 (2005).

208. S. A. Armstrong *et al.*, MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nature genetics* **30**, 41-47 (2002).

209. F. Erfurth, C. S. Hemenway, A. C. de Erkenez, P. H. Domer, MLL fusion partners AF4 and AF9 interact at subnuclear foci. *Leukemia* **18**, 92-102 (2004).

210. M. P. Garcia-Cuellar, J. Steger, E. Füller, K. Hetzner, R. K. Slany, Pbx3 and Meis1 cooperate through multiple mechanisms to support Hox-induced murine leukemia. *Haematologica* **100**, 905-913 (2015).

211. Z. Li *et al.*, PBX3 is an important cofactor of HOXA9 in leukemogenesis. *Blood* **121**, 1422-1431 (2013).

212. B. B. Zeisig *et al.*, Hoxa9 and Meis1 are key targets for MLL-ENL-mediated cellular immortalization. *Molecular and cellular biology* **24**, 617-628 (2004).

213. E. Kroon *et al.*, Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. *The EMBO journal* **17**, 3714-3725 (1998).

214. J. L. Hess *et al.*, c-Myb is an essential downstream target for homeobox-mediated transformation of hematopoietic cells. *Blood* **108**, 297-304 (2006).

215. A. V. Krivtsov *et al.*, A Menin-MLL Inhibitor Induces Specific Chromatin Changes and Eradicates Disease in Models of MLL-Rearranged Leukemia. *Cancer cell* **36**, 660-673.e611 (2019).

216. S. R. Daigle *et al.*, Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. *Blood, The Journal of the American Society of Hematology* **122**, 1017-1025 (2013).

217. S. R. Daigle *et al.*, Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer cell* **20**, 53-65 (2011).

218. C. Dafflon *et al.*, Complementary activities of DOT1L and Menin inhibitors in MLL-rearranged leukemia. *Leukemia* **31**, 1269-1277 (2017).

219. K. Ottersbach *et al.*, The "Never-Ending" mouse models for MLL-rearranged acute leukemia are still teaching Us. *HemaSphere* **2**, (2018).

220. T. A. Milne, Mouse models of MLL leukemia: recapitulating the human disease. *Blood* **129**, 2217-2223 (2017).

221. C. Lavau, S. J. Szilvassy, R. Slany, M. L. Cleary, Immortalization and leukemic transformation of a myelomonocytic precursor by retrovirally transduced HRX–ENL. *The EMBO journal* **16**, 4226-4237 (1997).

222. A. Cozzio *et al.*, Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. *Genes & development* **17**, 3029-3035 (2003).

223. B. B. Zeisig, M. P. García-Cuéllar, T. H. Winkler, R. K. Slany, The Oncoprotein MLL–ENL disturbs hematopoietic lineage determination and transforms a biphenotypic lymphoid/myeloid cell. *Oncogene* **22**, 1629-1637 (2003).

224. T. C. Somervaille, M. L. Cleary, Identification and characterization of leukemia stem cells in murine MLL-AF9 acute myeloid leukemia. *Cancer cell* **10**, 257-268 (2006).

225. A. V. Krivtsov *et al.*, Cell of origin determines clinically relevant subtypes of MLL-rearranged AML. *Leukemia* **27**, 852-860 (2013).

226. S. Kotani *et al.*, Molecular pathogenesis of disease progression in MLL-rearranged AML. *Leukemia* **33**, 612-624 (2019).

227. A. Bursen *et al.*, The AF4· MLL fusion protein is capable of inducing ALL in mice without requirement of MLL· AF4. *Blood, The Journal of the American Society of Hematology* **115**, 3570-3579 (2010).

228. F. Barabé, J. A. Kennedy, K. J. Hope, J. E. Dick, Modeling the initiation and progression of human acute leukemia in mice. *Science (New York, N.Y.)* **316**, 600-604 (2007).

229. J. Wei *et al.*, Microenvironment determines lineage fate in a human model of MLL-AF9 leukemia. *Cancer cell* **13**, 483-495 (2008).

230. H. Tamai, K. Inokuchi, Establishment of MLL/AF4 transgenic mice with the phenotype of lymphoblastic leukemia or lymphoma. *J Nippon Med Sch* **80**, 326-327 (2013).

231. S. Takacova *et al.*, DNA damage response and inflammatory signaling limit the MLL-ENL-induced leukemogenesis in vivo. *Cancer cell* **21**, 517-531 (2012).

232. V. Stavropoulou *et al.*, MLL-AF9 Expression in Hematopoietic Stem Cells Drives a Highly Invasive AML Expressing EMT-Related Genes Linked to Poor Outcome. *Cancer cell* **30**, 43-58 (2016).

233. V. Stavropoulou *et al.*, A Novel Inducible Mouse Model of MLL-ENL-driven Mixed-lineage Acute Leukemia. *HemaSphere* **2**, e51 (2018).

234. M. Metzler *et al.*, A conditional model of MLL-AF4 B-cell tumourigenesis using invertor technology. *Oncogene* **25**, 3093-3103 (2006).

235. A. V. Krivtsov *et al.*, H3K79 methylation profiles define murine and human MLL-AF4 leukemias. *Cancer cell* **14**, 355-368 (2008).

236. A. Forster *et al.*, Engineering de novo reciprocal chromosomal translocations associated with Mll to replicate primary events of human cancer. *Cancer cell* **3**, 449-458 (2003).

237. L. F. Drynan *et al.*, Mll fusions generated by Cre-loxP-mediated de novo translocations can induce lineage reassignment in tumorigenesis. *Embo Journal* **24**, 3136-3146 (2005).

238. C. L. Dobson *et al.*, The mll-AF9 gene fusion in mice controls myeloproliferation and specifies acute myeloid leukaemogenesis. *The EMBO journal* **18**, 3564-3574 (1999).

239. J. Corral *et al.*, An Mll-AF9 fusion gene made by homologous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. *Cell* **85**, 853-861 (1996).

240. E. C. Collins, R. Pannell, E. M. Simpson, A. Forster, T. H. Rabbitts, Inter-chromosomal recombination of Mll and Af9 genes mediated by cre-loxP in mouse development. *EMBO Rep* **1**, 127-132 (2000).

241. W. Chen *et al.*, A murine Mll-AF4 knock-in model results in lymphoid and myeloid deregulation and hematologic malignancy. *Blood* **108**, 669-677 (2006).

242. W. Chen *et al.*, Malignant transformation initiated by Mll-AF9: gene dosage and critical target cells. *Cancer cell* **13**, 432-440 (2008).

243. F. Cano, L. F. Drynan, R. Pannell, T. H. Rabbitts, Leukaemia lineage specification caused by cell-specific Mll-Enl translocations. *Oncogene* **27**, 1945-1950 (2008).

244. N. A. Barrett *et al.*, Mll-AF4 Confers Enhanced Self-Renewal and Lymphoid Potential during a Restricted Window in Development. *Cell reports* **16**, 1039-1054 (2016).

245. E. Ohlsson *et al.*, Initiation of MLL-rearranged AML is dependent on C/EBPalpha. *The Journal of experimental medicine* **211**, 5-13 (2014).

246. M. Ye *et al.*, Hematopoietic Differentiation Is Required for Initiation of Acute Myeloid Leukemia. *Cell Stem Cell* **17**, 611-623 (2015).

247. V. P. Lavallee *et al.*, The transcriptomic landscape and directed chemical interrogation of MLL-rearranged acute myeloid leukemias. *Nature genetics* **47**, 1030-1037 (2015).

248. T. Verbiest *et al.*, Tracking preleukemic cells in vivo to reveal the sequence of molecular events in radiation leukemogenesis. *Leukemia* **32**, 1435-1444 (2018).

249. D. H. Christiansen, M. K. Andersen, F. Desta, J. Pedersen-Bjergaard, Mutations of genes in the receptor tyrosine kinase (RTK)/RAS-BRAF signal transduction pathway in therapy-related myelodysplasia and acute myeloid leukemia. *Leukemia* **19**, 2232-2240 (2005).

250. J. Zuber *et al.*, Mouse models of human AML accurately predict chemotherapy response. *Genes & development* **23**, 877-889 (2009).

251. W. T. Lankes, H. Furthmayr, Moesin: a member of the protein 4.1-talin-ezrin family of proteins. *Proceedings of the National Academy of Sciences* **88**, 8297-8301 (1991).

252. A. L. Neisch, R. G. Fehon, Ezrin, Radixin and Moesin: key regulators of membrane–cortex interactions and signaling. *Curr Opin Cell Biol* **23**, 377-382 (2011).

253. A. García-Ortiz, J. M. Serrador, ERM Proteins at the Crossroad of Leukocyte Polarization, Migration and Intercellular Adhesion. *International journal of molecular sciences* **21**, 1502 (2020).

254. M. A. Pearson, D. Reczek, A. Bretscher, P. A. Karplus, Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain. *Cell* **101**, 259-270 (2000).

255. P. Zhang *et al.*, Crystal structure of the FERM-folded talin head reveals the determinants for integrin binding. *Proceedings of the National Academy of Sciences* **117**, 32402-32412 (2020).

256. S. Tsukita, S. Yonemura, S. Tsukita, ERM proteins: head-to-tail regulation of actin-plasma membrane interaction. *Trends in biochemical sciences* **22**, 53-58 (1997).

257. K. Kitano, F. Yusa, T. Hakoshima, Structure of dimerized radixin FERM domain suggests a novel masking motif in C-terminal residues 295-304. *Acta Crystallogr Sect F Struct Biol Cryst Commun* **62**, 340-345 (2006).

258. T. Mori *et al.*, Structural basis for CD44 recognition by ERM proteins. *The Journal of biological chemistry* **283**, 29602-29612 (2008).

259. N. Oshiro, Y. Fukata, K. Kaibuchi, Phosphorylation of moesin by rho-associated kinase (Rho-kinase) plays a crucial role in the formation of microvilli-like structures. *The Journal of biological chemistry* **273**, 34663-34666 (1998).

260. R. Gary, A. Bretscher, Heterotypic and homotypic associations between ezrin and moesin, two putative membrane-cytoskeletal linking proteins. *Proceedings of the National Academy of Sciences* **90**, 10846-10850 (1993).

261. F. Roch *et al.*, Differential roles of PtdIns(4,5)P2 and phosphorylation in moesin activation during Drosophila development. *Journal of cell science* **123**, 2058-2067 (2010).

262. L. Huang, T. Y. Wong, R. C. Lin, H. Furthmayr, Replacement of threonine 558, a critical site of phosphorylation of moesin in vivo, with aspartate activates F-actin binding of moesin. Regulation by conformational change. *The Journal of biological chemistry* **274**, 12803-12810 (1999).

263. A. Shcherbina, A. Bretscher, D. M. Kenney, E. Remold-O'Donnell, Moesin, the major ERM protein of lymphocytes and platelets, differs from ezrin in its insensitivity to calpain. *FEBS letters* **443**, 31-36 (1999).

264. G. Bradshaw *et al.*, Exome Sequencing Diagnoses X-Linked Moesin-Associated Immunodeficiency in a Primary Immunodeficiency Case. *Front Immunol* **9**, 420 (2018).

265. C. Lagresle-Peyrou *et al.*, X-linked primary immunodeficiency associated with hemizygous mutations in the moesin (MSN) gene. *J Allergy Clin Immunol* **138**, 1681-1689 e1688 (2016).

266. K. J. Geißler *et al.*, Regulation of Son of sevenless by the membrane-actin linker protein ezrin. *Proceedings of the National Academy of Sciences* **110**, 20587-20592 (2013).

267. T. Sperka *et al.*, Activation of Ras requires the ERM-dependent link of actin to the plasma membrane. *PloS one* **6**, (2011).

268. L. B. Riecken *et al.*, CPI-17 drives oncogenic Ras signaling in human melanomas via Ezrin-Radixin-Moesin family proteins. *Oncotarget* **7**, 78242 (2016).

269. J. Clucas, F. Valderrama, ERM proteins in cancer progression. *Journal of cell science* **127**, 267-275 (2014).

270. H. Kobayashi *et al.*, Clinical significance of cellular distribution of moesin in patients with oral squamous cell carcinoma. *Clinical cancer research* **10**, 572-580 (2004).

271. A. Estecha *et al.*, Moesin orchestrates cortical polarity of melanoma tumour cells to initiate 3D invasion. *Journal of cell science* **122**, 3492-3501 (2009).

272. Y. Qin *et al.*, Interfering MSN-NONO complex–activated CREB signaling serves as a therapeutic strategy for triple-negative breast cancer. *Science advances* **6**, eaaw9960 (2020).

273. F. Tort *et al.*, Molecular characterization of a new ALK translocation involving moesin (MSN-ALK) in anaplastic large cell lymphoma. *Laboratory Investigation* **81**, 419-426 (2001).

274. Y. Mei *et al.*, A piRNA-like small RNA interacts with and modulates p-ERM proteins in human somatic cells. *Nat Commun* **6**, 7316 (2015).

275. H. Bolouri *et al.*, The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. *Nature medicine* **24**, 103-112 (2018).

276. X. Ma *et al.*, Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours. *Nature* **555**, 371-376 (2018).

277. S. Chaudhury *et al.*, Age-specific biological and molecular profiling distinguishes paediatric from adult acute myeloid leukaemias. *Nat Commun* **9**, 5280 (2018).

278. S. J. Horton *et al.*, MLL-AF9-mediated immortalization of human hematopoietic cells along different lineages changes during ontogeny. *Leukemia* **27**, 1116-1126 (2013).

279. T. Okeyo-Owuor *et al.*, The efficiency of murine MLL-ENL-driven leukemia initiation changes with age and peaks during neonatal development. *Blood advances* **3**, 2388-2399 (2019).

280. A. K. Andersson *et al.*, The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. *Nature genetics* **47**, 330-337 (2015).

281. A. Cazzola *et al.*, Prenatal Origin of Pediatric Leukemia: Lessons From Hematopoietic Development. *Front Cell Dev Biol* **8**, 618164 (2020).

282. K. B. Gale *et al.*, Backtracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. *Proc Natl Acad Sci U S A* **94**, 13950-13954 (1997).

283. L. K. Jones *et al.*, Cryptic rearrangement involving MLL and AF10 occurring in utero. *Leukemia* **17**, 1667-1669 (2003).

284. J. P. Shrivastava, K. K. Magnani, L. Tripathi, K. S. Mangal, Congenital acute myeloid leukemia: A rare diagnostic enigma case report with review of literature. *South Asian journal of cancer* **5**, 212-213 (2016).

285. M. R. Copley *et al.*, The Lin28b–let-7–Hmga2 axis determines the higher self-renewal potential of fetal haematopoietic stem cells. *Nature cell biology* **15**, 916-925 (2013).

286. S. R. Viswanathan, G. Q. Daley, R. I. Gregory, Selective blockade of microRNA processing by Lin28. *Science (New York, N.Y.)* **320**, 97-100 (2008).

287. Y. Zhou *et al.*, Lin28b promotes fetal B lymphopoiesis through the transcription factor Arid3a. *The Journal of experimental medicine* **212**, 569-580 (2015).

288. S. Manier *et al.*, The LIN28B/let-7 axis is a novel therapeutic pathway in multiple myeloma. *Leukemia* **31**, 853-860 (2017).

289. M. L. Wilbert *et al.*, LIN28 binds messenger RNAs at GGAGA motifs and regulates splicing factor abundance. *Mol Cell* **48**, 195-206 (2012).

290. S. Wang *et al.*, Enhancement of LIN28B-induced hematopoietic reprogramming by IGF2BP3. *Genes & development* **33**, 1048-1068 (2019).

291. M. Hafner *et al.*, Identification of mRNAs bound and regulated by human LIN28 proteins and molecular requirements for RNA recognition. *Rna* **19**, 613-626 (2013).

292. A. Basak *et al.*, Control of human hemoglobin switching by LIN28B-mediated regulation of BCL11A translation. *Nature genetics* **52**, 138-145 (2020).

293. J. Jin *et al.*, Evidence that Lin28 stimulates translation by recruiting RNA helicase A to polysomes. *Nucleic Acids Res* **39**, 3724-3734 (2011).

294. A. Samsonova *et al.*, Lin28, a major translation reprogramming factor, gains access to YB-1-packaged mRNA through its cold-shock domain. *Commun Biol* **4**, 359 (2021).

295. X. Jiang *et al.*, Blockade of miR-150 maturation by MLL-fusion/MYC/LIN-28 is required for MLL-associated leukemia. *Cancer cell* **22**, 524-535 (2012).

296. L. Chen, Y. Sun, J. Wang, H. Jiang, A. G. Muntean, Differential regulation of the c-Myc/Lin28 axis discriminates subclasses of rearranged MLL leukemia. *Oncotarget* **7**, 25208-25223 (2016).

297. J. Zhou *et al.*, Inhibition of LIN28B impairs leukemia cell growth and metabolism in acute myeloid leukemia. *Journal of Hematology & Oncology* **10**, 138 (2017).

298. L. De Luca *et al.*, Knockdown of miR-128a induces Lin28a expression and reverts myeloid differentiation blockage in acute myeloid leukemia. *Cell death & disease* **8**, e2849 (2017).

299. J. C. Wang, J. E. Dick, Cancer stem cells: lessons from leukemia. *Trends in cell biology* **15**, 494-501 (2005).

300. A. V. Krivtsov, S. A. Armstrong, MLL translocations, histone modifications and leukaemia stem-cell development. *Nature Reviews Cancer* **7**, 823-833 (2007).

301. S. Dutta *et al.*, The target cell of transformation is distinct from the leukemia stem cell in murine CALM/AF10 leukemia models. *Leukemia* **30**, 1166-1176 (2016).

302. X. Liu *et al.*, Moesin and myosin phosphatase confine neutrophil orientation in a chemotactic gradient. *Journal of Experimental Medicine* **212**, 267-280 (2015).

303. B. Mizukawa *et al.*, The cell polarity determinant CDC42 controls division symmetry to block leukemia cell differentiation. *Blood, The Journal of the American Society of Hematology* **130**, 1336-1346 (2017).

304. T. Sperka *et al.*, Activation of Ras requires the ERM-dependent link of actin to the plasma membrane. *PloS one* **6**, e27511 (2011).

305. A. V. Krivtsov, S. A. Armstrong, MLL translocations, histone modifications and leukaemia stem-cell development. *Nature reviews. Cancer* **7**, 823-833 (2007).

306. P. Brown, Treatment of infant leukemias: challenge and promise. *Hematology. American Society of Hematology. Education Program* **2013**, 596-600 (2013).

307. S. S. Chaudhury, J. K. Morison, B. E. Gibson, K. Keeshan, Insights into cell ontogeny, age, and acute myeloid leukemia. *Experimental hematology* **43**, 745-755 (2015).

308. V. V. Tewari, R. Mehta, K. Tewari, Congenital Acute Leukemia: A Rare Hematological Malignancy. *Journal of Neonatal Biology* **6**, 1-4 (2017).

309. M. A. Basson, Signaling in cell differentiation and morphogenesis. *Cold Spring Harb Perspect Biol* **4**, (2012).

310. M. Filbin, M. Monje, Developmental origins and emerging therapeutic opportunities for childhood cancer. *Nature medicine* **25**, 367-376 (2019).

311. J. Zuber *et al.*, An integrated approach to dissecting oncogene addiction implicates a Myb-coordinated self-renewal program as essential for leukemia maintenance. *Genes & development* **25**, 1628-1640 (2011).

312. K. Ramaswamy *et al.*, Peptidomimetic blockade of MYB in acute myeloid leukemia. *Nat Commun* **9**, 110 (2018).

313. F. J. Tavner *et al.*, Molecular cloning reveals that the p160 Myb-binding protein is a novel, predominantly nucleolar protein which may play a role in transactivation by Myb. *Molecular and cellular biology* **18**, 989-1002 (1998).

314. S. Mori *et al.*, Myb-binding protein 1A (MYBBP1A) is essential for early embryonic development, controls cell cycle and mitosis, and acts as a tumor suppressor. *PloS one* **7**, e39723 (2012).

315. M. Jassinskaja *et al.*, Ontogenic shifts in cellular fate are linked to proteotype changes in lineage-biased hematopoietic progenitor cells. *Cell reports* **34**, 108894 (2021).

316. B. Felipe-Abrio, E. M. Verdugo-Sivianes, C. Sáez, A. Carnero, Loss of MYBBP1A Induces Cancer Stem Cell Activity in Renal Cancer. *Cancers* **11**, (2019).

317. L. de Beauchamp, E. Himonas, G. V. Helgason, Mitochondrial metabolism as a potential therapeutic target in myeloid leukaemia. *Leukemia* **36**, 1-12 (2022).

318. Y. Lai, M. Qiao, M. Song, S. T. Weintraub, Y. Shiio, Quantitative proteomics identifies the Myb-binding protein p160 as a novel target of the von Hippel-Lindau tumor suppressor. *PloS one* **6**, e16975 (2011).

319. A. C. Wilkinson, K. J. Igarashi, H. Nakauchi, Haematopoietic stem cell self-renewal in vivo and ex vivo. *Nature reviews. Genetics* **21**, 541-554 (2020).

320. A. C. Wilkinson *et al.*, Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation. *Nature*, (2019).

321. K. Ochi, M. Morita, A. C. Wilkinson, A. Iwama, S. Yamazaki, Non-conditioned bone marrow chimeric mouse generation using culture-based enrichment of hematopoietic stem and progenitor cells. *Nat Commun* **12**, 3568 (2021).

322. G. A. Challen, E. M. Pietras, N. C. Wallscheid, R. A. J. Signer, Simplified murine multipotent progenitor isolation scheme: Establishing a consensus approach for multipotent progenitor identification. *Exp Hematol* **104**, 55-63 (2021).

323. D. G. Kent *et al.*, Prospective isolation and molecular characterization of hematopoietic stem cells with durable self-renewal potential. *Blood* **113**, 6342-6350 (2009).

324. C. Gekas, T. Graf, CD41 expression marks myeloid-biased adult hematopoietic stem cells and increases with age. *Blood* **121**, 4463-4472 (2013).

325. D. Bhattacharya, D. J. Rossi, D. Bryder, I. L. Weissman, Purified hematopoietic stem cell engraftment of rare niches corrects severe lymphoid deficiencies without host conditioning. *J Exp Med* **203**, 73-85 (2006).

326. A. Czechowicz, D. Kraft, I. L. Weissman, D. Bhattacharya, Efficient transplantation via antibody-based clearance of hematopoietic stem cell niches. *Science* **318**, 1296-1299 (2007).