HHS Public Access

Author manuscript

Stem Cell Res. Author manuscript; available in PMC 2023 April 19.

Published in final edited form as:

Stem Cell Res. 2021 April ; 52: 102195. doi:10.1016/j.scr.2021.102195.

Induced pluripotent stem cell models of myeloid malignancies and clonal evolution

Andreea Reilly,

Sergei Doulatov*

Division of Hematology, Department of Medicine, Department of Genome Sciences, Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA 98195, United States

Abstract

Reprogramming of cells from patients with genetic disorders to pluripotency is a promising avenue to understanding disease biology. A number of induced pluripotent stem cell (iPSC) models of inherited monogenic blood disorders have been reported over the past decade. However, the application of iPSCs for modeling of hematological malignancies has only recently been explored. Blood malignancies comprise a spectrum of genetically heterogeneous disorders marked by the acquisition of somatic mutations and chromosomal aberrations. This genetic heterogeneity presents unique challenges for iPSC modeling, but also opportunities to capture genetically distinct states and generate models of stepwise progression from normal to malignant hematopoiesis. Here we briefly review the current state of this field, highlighting current models of acquired pre-malignant and malignant blood disorders and clonal evolution, and challenges including barriers to reprogramming and differentiation of iPSCs into bona fide hematopoietic stem cells.

Keywords

iPSC; Myeloid malignancies; HSC

1. iPSC models of human disease

Disease models are necessary in order to dissect the biological steps that go awry and to find ways to ameliorate them. Mouse models remain the gold standard in cancer biology. However there is an increasing appreciation for the need for human cell models. Human and mouse hematopoietic stem cells (HSCs) and leukemia-initiating cells are biologically

*Corresponding author at: Division of Hematology, Department of Medicine, University of Washington, 1795 N.E. Pacific Street, Box 357710, Seattle, WA 98195, United States, doulatov@uw.edu (S. Doulatov).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Andreea Reilly: Conceptualization, Writing - review & editing. **Sergei Doulatov:** Conceptualization, Writing - review & editing.

distinct (Doulatov et al., 2012; Eppert et al., 2011). Malignant transformation of normal HSCs typically occurs over many years and involves a temporally ordered acquisition of genetic lesions, which is difficult to recapitulate in model organisms. Cancer-derived cell lines have been extensively used; however these lines are adapted for in vitro growth and harbor numerous genetic and cytogenetic abnormalities. Primary human cells are an ideal source for in vitro and in vivo xenograft studies. However, the availability of primary cells is limiting, especially in rare and pre-neoplastic disorders. Most pre-malignant (e.g. myelodysplastic syndrome, MDS) and leukemic cells cannot be readily cultured in vitro, have a high degree of genetic heterogeneity, and limited capacity for clonal expansion, which presents a barrier for genetic manipulation, gene editing, and functional studies. Thus, there is a significant need for scalable genetically accurate models of benign and malignant hematological disorders. A growing number of iPSC models of hematological disease have been reported, including inherited bone marrow failure (BMF) and acquired malignancies.Fig. 1.

iPSCs can be generated from patient blood samples or skin biopsies by reprogramming with the Yamanaka factors OCT4, SOX2, KLF4, and MYC (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). iPSCs retain the genome of the somatic cell and all of its genetic lesions, but not its epigenome, which is extensively reorganized during reprogramming. Current methods based on episomal vectors, Sendai virus, or RNA reprogramming are non-integrating and achieve high efficiencies (Schlaeger et al., 2015), except when disease genes interfere with the reprogramming process, for instance in Fanconi anemia (Müller et al., 2012). Comprehensive genomic analysis suggests that iPSCs are completely reprogrammed and, at least for disease modeling purposes, they can be considered essentially equivalent to embryonic stem cells (ESCs) (Choi et al., 2015). iPSCs can be stably maintained in culture over time, and protocols have been established to differentiate them into all major somatic cell types. These protocols strive to reproduce the developmental cues during embryogenesis following the normal developmental sequence from gastrulation to the desired cell type. Protocols for blood differentiation of iPSCs generate hematopoietic stem and progenitor cells (HSPCs) marked by $CD34^+$ and $CD45^+$, and most blood cell lineages, including myeloid, erythroid, and lymphoid (Ditadi et al., 2017). iPSCs can be clonally expanded and gene edited to generate isogenic control lines, which enables functional studies in a defined genetic background. These features make iPSCs an attractive alternative for cell therapy. However, technical challenges have prevented a wider adoption. Differentiation of patient iPSCs to HSPCs is laborious, costly, and the efficiency is variable depending on patient genotype. Additionally, iPSC-derived HSPCs have a limited expansion capacity, recapitulate fetal instead of adult hematopoiesis, and robust generation of bona fide HSCs for autologous bone marrow transplantation has remained a long-standing challenge (Vo and Daley, 2015). Recent advances have improved our understanding of blood development and used transcription factors to induce HSC properties for disease modeling and drug screening (Fig. 1) (Doulatov et al., 2013; Sugimura et al., 2017). This review will examine the considerations and biological insights from iPSC models, in particular for clonal evolution in complex acquired disorders, such as myeloproliferative neoplasms (MPN), MDS and acute myeloid leukemia (AML).

2. Hematopoietic differentiation of iPSCs

Blood development involves spatially and temporally distinct waves, which must be recapitulated during differentiation of pluripotent cells. In the mouse embryo, the first waves of blood cells arise at 7.5 days post-conception (dpc) in the extra-embryonic yolk sac vasculature. These early waves produce primitive and erythro-myeloid progenitors (EMPs) with lineage-restricted potential (Palis and Yoder, 2001). A distinct wave of "definitive" progenitors and pre-HSCs arises in the dorsal aorta and arteries of the developing conceptus around 9.5 dpc, with the first transplantable HSCs arising at 10.5 dpc (Ivanovs et al., 2011; Medvinsky and Dzierzak, 1996). Broadly speaking, the goal of iPSC differentiation is to recapitulate the later definitive wave of hematopoiesis that specifies HSCs. Although existing differentiation protocols recapitulate both primitive and definitive waves of specification, they give rise to progenitors with fetal properties (for instance, HbF-expressing erythroid cells) and no transplantable HSCs (e.g. Vo and Daley, 2015).

The most salient reason is that our understanding of early hematopoietic development remains incomplete (Dzierzak and Bigas, 2018; Dzierzak and Speck, 2008). HSPCs arise from specialized hemogenic endothelial (HE) precursors. This endothelial-to-hematopoietic transition (EHT) is critically dependent on Notch signaling (Robert-Moreno et al., 2008) and requires induction of blood-specific transcription factors RUNX1, GATA2, GFI1, ETV6, among others. Detailed protocols established by the Keller group and others specify definitive HE precursors by BMP4 and Wnt, followed by Notch-dependent EHT (Ditadi et al., 2015; Kennedy et al., 2012; Sturgeon et al., 2014). During EHT, specification of HSCs, as opposed to lineage-restricted progenitors, is closely dependent on activation of the medial HOXA genes: HOXA5, HOXA7, and HOXA9 (Dou et al., 2016), which in turn is regulated by the Trithorax complex (MLL) and Polycomb repressive complex (PRC1 and PRC2) (Jude et al., 2007; Lee et al., 2006). HOXA dependence appears to be a unique property of human HSCs; by contrast, mouse HSCs are HOXB-dependent (HOXB4 and HOXB5) and can be efficiency specified from ESCs by ectopic HOXB4 expression (Kyba et al., 2002; Wang et al., 2005). Medial HOXA genes are inactive in human iPSC-HSPCs and conditions to activate them remain a focus of intense study (Dou et al., 2016; Ng et al., 2016; Wang et al., 2005).

Several groups have previously explored activation of the HOXA genes to specify multipotential HSPCs from iPSCs by: 1) expression of HOXA9 and other factors in already specified iPSC-HSPCs (Doulatov et al., 2013), 2) induction of HOXA5 and HOXA9 during EHT (Sugimura et al., 2017), and 3) suppression of EZH1 (Vo et al., 2018). The first approach does not enable complete re-activation of HSC potential, but generates expandable multipotential HSPCs (CD34-5F), a useful system for disease modeling and drug screens. HOXA5 and HOXA9 induction during EHT gives rise to HSPCs with increased repopulation potential, although this approach is technically more challenging since the timing of expression during EHT is important. Lastly, inactivation of PRC2 component EZH1 reactivates lymphoid potential from iPSCs, and EZH1 deletion drives precocious HSC formation in mice (Vo et al., 2018). Heterozygous loss of EZH1 confers increased HSC potential compared with loss of both alleles suggesting that fine-tuning of PRC2 activity may be required to reactivate HSC gene expression.

Taken together, these studies highlight the importance of HOXA expression for specifying HSCs from iPSCs and present novel strategies for disease modeling studies. Current protocols use embryoid body or stromal co-culture differentiation of iPSCs to $CD34^+CD45^+$ HSPCs, followed by conditional expression of HSC transcription factors. Further refinements would enable activation of HOXA in $CD34^+CD43^-$ HE, or replacing ectopic HOXA delivery with selective inactivation of EZH1. These would create a technically simplified and robust protocol to use iPSCs for disease modeling studies in the near term. The ultimate goal is to replace transgenes with morphogens to instruct definitive HSC potential during iPSC differentiation paving the way for their use in bone marrow transplantation and cell replacement therapies.

3. The spectrum of myeloid malignancies

To date, iPSCs have been most extensively used to model inherited bone marrow failure (BMF) disorders. BMF are monogenic disorders in which the differentiation of one or more blood lineages is severely curtailed (Dokal and Vulliamy, 2010). The advantages of using iPSCs for modeling BMF is that these disorders are rare and primary patient cells are limiting; the monogenic origin simplifies generation of iPSCs from available patient cells (such as skin biopsy) as well as gene editing for isogenic controls; and in many cases the mouse models, if available, only partially capture the relevant molecular phenotypes. iPSC models of BMF that have been created include: Fanconi Anemia, Congenital Neutropenia, Familial Platelet Disorder, and Diamond-Blackfan Anemia, among others (Connelly et al., 2014; Dannenmann et al., 2019; Garçon et al., 2013; Jung et al., 2018; Liu et al., 2014; Sakurai et al., 2014). By contrast, the use of iPSCs for modeling myeloid malignancies has only been reported more recently and presents additional challenges, notably genetic and epigenetic heterogeneity, barriers to reprogramming, context of the aging adult HSCs and pro-inflammatory microenvironment, and re-establishment of premalignant or malignant state upon differentiation (Papapetrou, 2016).

Myeloid malignancies including myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS), and acute myeloid leukemias (AML) comprise a spectrum of clonal disorders marked by accumulation of genetic alterations that confer aberrant HSC self-renewal and differentiation (Cai and Levine, 2019; Sperling et al., 2017). Myeloid neoplasms are driven by recurrent somatic mutations and cytogenetic alterations involving splicing, epigenetic regulation, signaling, transcription factors, and DNA damage response genes (Papaemmanuil et al., 2013; Sperling et al., 2017; Welch et al., 2012). Individual patients typically harbor multiple driver mutations which are acquired in a specific order during disease evolution. Several studies have highlighted the importance of the order of mutation acquisition and the temporal sequence of events (Ortmann et al., 2015) but this remains poorly understood. Development of malignancy is preceded by an extended period of premalignant clonal evolution termed age-related clonal hematopoiesis (CH), in which a driver mutation in an HSC confers a fitness advantage leading to clonal expansion (Sperling et al., 2017; Steensma et al., 2015). Sequencing of AML patient and control blood samples has shown the presence of CH years before diagnosis (Desai et al., 2018; Shlush et al., 2014). While CH is associated with an increased risk of malignancy, most individuals with CH do not develop overt malignancy. It is thus critical to develop genetically accurate

models of disease that inform the mechanisms of progression from CH or preleukemia to MDS/MPN/AML.

Genetically engineered mouse models and, more recently, human xenotransplant models have been the gold standard in studying myeloid malignancies (Morotti et al., 2017; Mullally et al., 2012; Thomas and Majeti, 2017). For human cell models, primary patient HSPCs generally expand poorly in vitro, and at least for MDS and preleukemia, poorly engraft immunodeficient mice, despite advancements in the development of humanized mouse strains (Song et al., 2019). iPSC models provide a genetically relevant context, as compared to artificial gene overexpression, in which to study how combinations of mutations and chromosomal aberrations promote leukemic transformation. Premalignant subclones can be captured by reprogramming to create models of disease progression. In addition, gene editing can be used to introduce combinations of mutations into iPSCs followed by HSPC differentiation. Despite the challenges outlined above, the reprogramming approach enables functional studies of leukemia-associated genetic abnormalities in a genetically accurate context.

4. Malignant cell epigenetic state

The epigenome of the somatic cell is largely erased during reprogramming. Thus, one important question is whether the malignant cell epigenome is re-established during differentiation. The classical experiments with somatic cell nuclear transfer (SCNT) by John Gurdon in the early 1960s were the first to demonstrate that the oocyte can reprogram a mature differentiated cell into a pluripotent state. However, it remained unclear if the epigenome of a cancer cell could also be reprogrammed to pluripotency and re-established during differentiation. The classical study demonstrating this was carried out by Hochedlinger and Jaenisch who performed SCNT with murine cancer cell lines and primary tumors, including p53-null lymphoma, moloney murine leukemia virus-induced leukemia, PML-RAR leukemia, and a hypomethylated Chip/c lymphoma (Hochedlinger et al., 2004). All of the reprogrammed cancer cell lines, but none of the primary tumor cells, were able to form blastocysts. From a total of 57 blastocysts derived from multiple cancer types, only two ESC lines were established from RAS-inducible melanoma nuclei. Importantly, RAS-mutant ESCs gave rise to live chimeras which developed RAS-mutant tumors at a much higher frequency. These seminal findings suggest that: 1) the oocyte environment can suppress a malignant epigenetic state, but specific genetic alterations are incompatible with the establishment or maintenance of an ES cell state, and 2) that the malignant epigenetic state can be in some cases re-established during differentiation and give rise to tumors.

5. iPSC models of myeloproliferative neoplasms

The first successful attempts at reprogramming acquired hematological disorders were from patients with chronic myeloid leukemia (CML) driven by the Philadelphia chromosome. Primary $CD34^+$ HSPCs from CML patients, as well as cells from the human CML KMB7 cell line, were reprogrammed, and the CML-iPSCs were shown to harbor the BCR-ABL translocation (Carette et al., 2010; Hosoi et al., 2014; Hu et al., 2011). The CML-iPSC model recapitulated the features of the disease and provided insights into the sensitivity

of CML to imatitnib, a classical BCR-ABL tyrosine kinase inhibitor (Druker et al., 2001). Since pluripotent cells do not require tyrosine kinase signaling for proliferation, CML-derived iPSCs were resistant to imatinib. By contrast, CML iPSC-derived HSPCs were sensitive to imatinib (Carette et al., 2010; Hosoi et al., 2014). These results suggest that the iPSC model recapitulates the BCR-ABL-dependent oncogenic state of CML cells.

BCR-ABL-negative myeloproliferative neoplasms (MPN), including polycythemia vera, essential thrombocytosis, and myelofibrosis, are characterized by myeloproliferation and risk of transformation to AML (Spivak, 2017). The JAK2-V617F mutation is the most prevalent mutation in MPN (Kilpivaara and Levine, 2008). Multiple groups have isolated iPSC lines harboring homozygous and heterozygous JAK2-V617F mutations from MPN patients and showed that the JAK2-V617F lines had an enhanced myeloid potential compared to healthy donor iPSCs, consistent with the disease phenotype (Saliba et al., 2013; Ye et al., 2009). These studies provided evidence that clones from malignant hematological cancers can be captured through iPSC reprogramming and used to investigate aberrant JAK/STAT signaling and its contribution to MPN pathogenesis.

In juvenile myelomonocytic leukemia (JMML) hyperactive RAS/MAPK (Niemeyer, 2018) and JAK/STAT (Kotecha et al., 2008) signaling leads to extensive proliferation of the granulocytic and monocyte lineages, often in young children (Niemeyer and Kratz, 2008). Reprogramming bone marrow samples from two patients with different JMML-associated mutations, as well as a healthy patient, yielded control and JMML-derived iPSC lines (Tasian et al., 2019). Characterizing the iPSC-derived myeloid cells revealed differences in the hyperactive signaling pathways: *PTPN11* mutants exhibited increased RAS/MAPK signaling, while *CBL* mutants had elevated JAK/STAT. Importantly, treatment with pathway-specific kinase inhibitors diminished the constitutive signaling (Tasian et al., 2019). Childhood leukemias, such as JMML, can arise form somatic mutations in fetal progenitors (Böiers et al., 2018; Tarnawsky et al., 2018), making iPSCs particularly relevant as an alternative to more controversial primary fetal human cells. This study demonstrates how iPSC models facilitate drug testing in a rare MPN, and that iPSC models are suitable for both adult and pediatric malignancies.

6. iPSC models of myelodysplastic syndromes

Myelodysplastic syndromes (MDS) are clonal disorders characterized by cytopenias and morphologic dysplasia affecting one or more blood lineages (Bejar, 2014; Ogawa, 2019). The most common driver mutations in MDS affect splicing (*SF3B1*, *SRSF2*), epigenetic regulation (*TET2*, *ASXL1*), and deletions on long arm of chromosomes 5 and 7 (Papaemmanuil et al., 2013). These mutations impart clonal advantage to HSCs and perturb differentiation giving rise to aberrant dysplastic cells. Over time, normal HSCs are outcompeted leading to cytopenias, while continued accumulation of oncogenic hits predisposes to AML progression.

Kotini et al. established iPSCs from MDS patients with different oncogenic lesions, including deletion of 7q [del(7q)], 20q, and *GATA2* mutations (Kotini et al., 2017), and demonstrated that the MDS stage correlates with hematopoietic differentiation efficiency

(Hsu et al., 2019; Kotini et al., 2017). iPSC lines from advanced MDS showed decreased HSPC differentiation, and correction of del(7q) restored hematopoietic differentiation (Kotini et al., 2017). By contrast, introduction of del(7q) into premalignant iPSCs abrogated hematopoietic differentiation potential resembling high-risk MDS. Even though in this study the clones representing the different disease stages were derived from distinct patients, the experiments were able to demonstrate how the acquisition of particular genomic lesions can exacerbate the disease phenotype. It is unclear why MDS iPSCs display reduced hematopoietic differentiation. One possibility is that common driver mutations affect genes that are required for hematopoietic specification. Alternatively, driver mutations may block MDS HSPCs differentiation into mature cells leading to reduced hematopoietic output. Since current differentiation protocols do not support robust HSC specification, modeling of HSC-specific phenotypes in MDS such as clonal dominance remains difficult using iPSC models.

Mutations in splicing factor *SF3B1* are found in ~ 25% of MDS and ~ 80% of MDS with ring sideroblasts (MDS-RS) suggesting a causal connection between mutant SF3B1 and ring sideroblasts (RS), erythroid precursors with iron-laden mitochondria (Malcovati et al., 2015, 2011; Papaemmanuil et al., 2011). However, the mechanisms by which *SF3B1* mutations cause RS formation remains poorly understood since *SF3B1*-mutant mouse models and human cell lines do not recapitulate RS formation in vitro (Mupo et al., 2017; Obeng et al., 2016). Hsu et al. showed that *SF3B1*-mutant iPSCs give rise to erythroid precursors with damaged mitochondria and RS (Hsu et al., 2019). Mutant SF3B1 was sufficient for RS formation, while *EZH2* co-mutations modified mitochondrial activity during erythroid differentiation (Hsu et al., 2019). The authors used the 5F transcription factors system to conditionally expand $CD34^+$ HSPCs, to enable genetic, proteomic, biochemical and drug screening studies (Doulatov et al., 2017). This study shows that iPSC models can recapitulate key dysplastic features of MDS differentiation that may be difficult to capture in other models.

Cytogenetic aberrations, especially deletions on chromosomes 5 and 7, encompassing hundreds of genes, are common in MDS (Jerez et al., 2012a, 2012b). Modeling these disorders in mouse models has been problematic because the corresponding syntenic regions are present on multiple chromosomes. Recent experiments have demonstrated that reprogramming can be utilized to generate models of chromosomal deletions. Del(7q) iPSCs were derived from the bone marrow of MDS patients, or were engineered using a Cre-Lox strategy (Kotini et al., 2015). More recently, Kotini et al. showed that CRISPR-Cas9 could be used to generate large deletions more precisely (Kotini and Papapetrou, 2020). Using this model in conjunction with a spontaneous complementation strategy, Kotini et al. identified the critical 7q region responsible for hematopoietic differentiation (Kotini et al., 2015). In Shwachman-Diamond Syndrome (SDS), a BMF disorder with predisposition to MDS/AML, Ruiz-Gutierrez et al. developed a model where del (7q) was engineered into SDS iPSCs, a model of progression from BMF to MDS (Ruiz-Gutierrez et al., 2019). Del(5q) is a hallmark of an MDS subtype marked by macrocytic anemia and good prognosis, however 5q loss is also associated with high risk MDS and AML with *TP53* mutations. Hsu et al. derived *TP53*-mutant iPSCs with del(5q) and showed that 5q loss contributes to impaired DNA damage response (Hsu et al., 2019). These studies show that iPSCs are a useful model

of MDS with chromosomal deletions identifying key genes involved in pathogenesis using forward and reverse genetic screening strategies.

7. iPSC models of AML and generation of transplantable leukemias

A consistent feature of normal pluripotent cell differentiation is the lack of transplantable HSCs. In contrast, recent studies have shown that differentiation of iPSCs derived from AML patients generates transplantable leukemia-initiating stem cells (LSCs). Kotini et al. and Chao et al. showed that iPSCs can be reprogrammed from fully transformed AML samples and give rise to LSCs that robustly engraft and cause acute disease in mice (Chao et al., 2017; Kotini et al., 2017). This indicates that oncogenic mutations can specify the LSC epigenetic state during differentiation. Measuring the engraftment potential of HSPCs derived from low-risk MDS, high-risk MDS, and AML revealed that only the AML iPSCs were able to give rise to leukemia in vivo, and could be maintained long term in vitro (Kotini et al., 2017). Chao et al. further compared the engraftment potential of KRAS G13D-mutant and wild-type AML iPSC subclones derived from the same patient. AML cells derived from *KRAS*-mutant iPSCs engrafted, while the *KRAS* wild type cells did not (Chao et al., 2017). Both lines were equally sensitive to MLL inhibitor EPZ-5676, but only *KRAS* mutant cells were responsive to MEK inhibitor PD98059 and Trametinib (Chao et al., 2017). The relapse sample from the same patient showed that the *KRAS* wild-type clone became dominant, and accordingly *KRAS* wild-type cells were more resistant to the chemotherapy drug cytarabine. These findings suggest that AML mutations can instruct a malignant epigenetic state that is compatible with in vivo engraftment. Moreover, AML-derived LSCs can identify drug sensitivity of diagnostic and relapse subclones.

LSCs are a rare and epigenetically distinct cell population that can initiate leukemia in vivo (Dick and Bonnet, 1997; Lapidot et al., 1994). Interestingly, AML iPSCs generate heterogeneous leukemic populations with LSC-like properties. Wesely et. al. recently isolated two different AML iPSC-derived hematopoietic populations termed iLSC and iBlast and demonstrated that only the iLSC could initiate leukemia in vivo. Further characterization of the iLSCs revealed RUNX1 as a critical player in the maintenance of the iLSC population. Attenuating RUNX1 protein by 50% in the iLSC population abrogated their ability to engraft or to survive long term in vitro (Wesely et al., 2020). Moreover, RUNX1 depletion in primary AML samples of multiple subtypes impaired their proliferative potential. In addition, the group was able to identify a RUNX1 target gene, TSPAN18, that upon overexpression was able to partially rescue the RUNX1 depletion effect (Wesely et al., 2020). These studies collectively show how patient-derived iPSC models inform leukemogenesis in multiple ways: first, to gain insight into the genetic lesions that contribute to leukemic transformation. Second, to provide evidence for the LSC model, better characterize LSC properties, and uncover therapeutic vulnerabilities.

8. Barriers to reprogramming

Despite these and other reports of AML iPSCs, malignant transformation imposes significant barriers to reprogramming. Some of the malignant clones that reprogrammed harbored MLL rearrangements (Chao et al., 2017; Lee et al., 2017), suggesting that MLL

rearrangements might enhance reprogramming potential. In another study, bone marrow from an AML patient with the high-risk der(7)t(7;13) translocation was reprogrammed. The iPSC lines derived did not harbor the translocation and were cytogenetically normal (Yamasaki et al., 2019). Similarly, reprogramming an AML patient sample with a t(8;21) translocation resulted in only the successful reprogramming of cytogenetically normal iPSC lines (Hoffmann et al., 2016). These studies provide further evidence that particular chromosomal abnormalities and point mutations are selected against during reprogramming. One possibility is that genes mutated in myeloid neoplasms often involve epigenetic regulators which are required for successful reprogramming. Another possibility is that high mutation burden and aneuploidy can trigger cell stress responses such as p53 activation that impede reprogramming (Marión et al., 2009). Thus, likely not all genetic abnormalities found in leukemias can be directly reprogrammed to a pluripotent state.

One strategy to circumvent reprogramming barriers is engineering mutations directly into iPSCs by using CRISPR/Cas9. For example, a high-risk MDS patient sample reprogrammed by Kotini et al., yielded iPSCs derived from normal cells or from a high-risk MDS clone (Kotini et al., 2017). The normal iPSC line was genetically edited to introduce a truncating mutation in ASXL1, and a further deletion of 7q in the ASXL1-mutant clone. This yielded a panel of patient-derived iPSC lines representative of each stage of disease progression, from normal to preleukemic to MDS. Kotini et al. also performed this manipulation starting with a preleukemic clone that harbored a *GATA2* mutation. Inactivation of the second *GATA2* allele generated a low-risk MDS clone, while deletion of 7q using Cre/Lox-based recombination strategy generated a high-risk MDS clone (Kotini et al., 2017). More recently, the same authors developed a CRISPR/Cas9-based system to more simply introduce precise chromosomal deletions in iPSCs (Kotini and Papapetrou, 2020). Thus even though barriers to reprogramming impede the capture of all disease clones in a patient, normal or preleukemic subclones can be genetically edited to produce a set of clones on the same genetic background that simulate the disease development.

While reprogramming barriers often impede reprogramming of leukemic or fully malignant cells, this favors reprogramming of rare preleukemic cells that remain in the marrow of MDS and AML patients, which can inform the history of clonal evolution in myeloid neoplasms.

9. Clonal evolution and preleukemia

Myeloid malignancies are the end product of age-related molecular evolution of HSCs (Bowman et al., 2018). Most mutations are benign while rare driver mutations confer a competitive advantage. This dynamic clonal expansion occurs over many years subject to Darwinian forces of mutation, selection, and drift, and in many cases is only discovered once the patient presents with overt malignancy (Greaves, 2015; Nowell, 1976). Pioneering studies have shown that clonal expansions in the blood are surprisingly common in aging, and are driven by oncogenic mutations most commonly in epigenetic modifiers *DNMT3A*, *TET2*, and *ASXL1* (Buscarlet et al., 2017; Genovese et al., 2014; Jaiswal et al., 2014; Shlush et al., 2014). Similar clonal expansions have since been reported in solid tissues maintained by stem cells (Blokzijl et al., 2016; Yokoyama et al., 2019) suggesting that

clonal evolution is a common physiological feature of normal aging. Although CH is widely considered to be a common preleukemic state, dissecting the order of mutations and how they cooperate towards malignant transformation remains a challenge. Preleukemic clones can be detected in the bone marrow long after disease initiation and can serve as a reservoir for further clonal evolution and relapse (Corces-Zimmerman et al., 2014; Jan et al., 2012; Shlush et al., 2017). However, preleukemic clones are typically very rare since they are outcompeted by their more malignant descendants and their direct detection has been a challenge.

Whole genome and exome sequencing has been the main tool to track clonal histories. Driver mutations in AML can be found at different variant allele frequencies (VAF) (Ding et al., 2012; Ley et al., 2010; Mardis et al., 2009), which can be used to reconstruct the chronological order of driver mutations (Papaemmanuil et al., 2013; Welch et al., 2012). However, many mutations have similar allele frequencies making it difficult to reconstruct the order (Makishima et al., 2017; Nagata et al., 2019). Access to serially collected samples can greatly improve the resolution (Da Silva-Coelho et al., 2017; Makishima et al., 2017). However, serially-collected samples are only available for a small number of patients. Additionally, bulk sequencing does not offer definitive evidence of whether mutations are present in the same subclone. Single-cell DNA or RNA sequencing of patient samples can accurately inform the clonal hierarchy (Miles et al., 2020; Nagata et al., 2019; Paguirigan et al., 2015; Petti et al., 2019; van Galen et al., 2019). These approaches use single cell targeted DNA sequencing, RNAseq, or combination of RNAseq and DNA sequencing (e.g. Genotyping of Transcriptomes). These methods enable the sampling of transcriptomes of genetically distinct subclones, but technical limitations remain (Baslan and Hicks, 2017; Luquette et al., 2019). Fundamentally, preleukemic clones can be rare and difficult to detect. More importantly, these methods do not permit functional interrogation of the observed subclones to inform how individual mutations affect HSPC self-renewal and differentiation (Fig. 2).

Reprogramming is essentially a single cell technology in which single somatic cells reprogrammed to pluripotency retain their genome and any somatic mutations (Fig. 2). By contrast with sequencing-only approaches, iPSCs can be differentiated to HSPCs, lending themselves to both genetic and functional interrogation of clonal progression. Recently, iPSC reprogramming has been used to capture genetically distinct preleukemic subclones from individual MDS patients (Hsu et al., 2019). Consistent with prior findings, malignant clones displayed a strong barrier to reprogramming, especially in patients with complex karyotype. By contrast, rare premalignant and normal HSCs were preferentially reprogrammed. We can thus take advantage of this ‘barrier’ to capture preleukemic intermediates which may be too rare to be directly detected by other approaches. This provides an opportunity to sample whole genomes of preleukemic cells. In addition, differentiation of clonal and subclonal iPSC to HSPCs can be performed in parallel. Echoing previous results, progressive acquisition of mutations resulted in reduced HSPC differentiation potential, providing an opportunity to investigate the functional impact of cooperating mutations and mutation order on gene expression, proliferation, and differentiation.

Isogenic iPSC lines can capture inter-clonal genetic heterogeneity as well as intra-clonal epigenetic heterogeneity (Beke et al., 2020). However, the delivery method of the OSKM factors can affect the reprogramming efficiency (Schlaeger et al., 2015), with the episomal method generally capturing more subclones. It is important to note that not all oncogenic mutations are compatible with reprogramming or cell culture, skewing which clones can be positively identified by reprogramming and the ratios of the clones captured (Shakiba et al., 2019). Despite these caveats of iPSC technology, it remains one of the few model systems to study the functional consequences of sequential acquisition of mutations in a genetically accurate context.

10. Summary and perspectives

There is a need to define the evolutionary steps that lead to tumorigenesis in order to create more effective treatments and preventative care that allows for early intervention. This means understanding the interplay between mutations in early clones, identifying which mutation combinations pose increased risk, and determining the mechanisms that generate clonal complexity. Employing reprogramming technology to develop models of hematological disease has proven useful for providing insight into these topics. There are several advantages of this system: first, it bears a high degree of disease relevance since the model is patient-derived. Second, it offers an expandable supply of material for downstream assays. Third, it can model complex malignancies with increased clonal heterogeneity. And fourth, reprogramming enables capture of rare preleukemic clones that can otherwise be difficult to detect (Hsu et al., 2019). Studies so far have demonstrated the viability of patient-derived iPSCs as a drug screening platform, and as a way to phenotypically characterize cells from different disease stages. iPSC models have provided insight into how genetic aberrations can alter biological pathways that contribute to aberrant hematopoiesis in MPN, MDS, and AML. Additionally, the discovery that some iPSC lines produce serially transplantable leukemias lead to a deeper understanding of LSC biology and the exposure of new therapeutic targets in AML. Still, it is important to understand the limitations of iPSC models. iPSCs and reprogramming are time and labor intensive which is a challenge for large patient cohorts. Some genetic lesions may not be compatible with reprogramming. Most importantly, the methods for generation of HSCs from iPSC and developmental maturation into adult-like HSCs require further inspiration. We envision that iPSC models can complement other methods and model systems, including single-cell RNA and DNA sequencing, mouse models, or primary normal and patient $CD34^+$ cells. iPSC reprogramming provides a valuable prototype of the human disease context, and future breakthroughs will usher in more efficacious therapeutics for hematologic malignancies.

Acknowledgements

S.D. is supported by the US National Institutes of Health (NIH) New Innovator Award DP2-HL147126, R01-HL151651, R21-HL139864 and American Society of Hematology Scholar Award.

References

Baslan T, Hicks J, 2017. Unravelling biology and shifting paradigms in cancer with single-cell sequencing. Nat. Rev. Cancer 17 (9), 557–569. 10.1038/nrc.2017.58. [PubMed: 28835719]

Bejar R, 2014. Clinical and genetic predictors of prognosis in myelodysplastic syndromes. Haematologica 99 (6), 956–964. 10.3324/haematol.2013.085217. [PubMed: 24881041]

Beke A, Laplane L, Riviere J, Yang Q, Torres-Martin M, Dayris T, Rameau P, Saada V, Bilhou-Nabera C, Hurtado A, Lordier L, Vainchenker W, Figueroa ME, Droin N, Solary E, 2020. Multilayer intraclonal heterogeneity in chronic myelomonocytic leukemia. Haematologica haematol. 2018, 208488. 10.3324/haematol.2018.208488.

Blokzijl F, de Ligt J, Jager M, Sasselli V, Roerink S, Sasaki N, Huch M, Boymans S, Kuijk E, Prins P, Nijman IJ, Martincorena I, Mokry M, Wiegerinck CL, Middendorp S, Sato T, Schwank G, Nieuwenhuis EES, Verstegen MMA, van der Laan LJW, de Jonge J, IJzermans JNM, Vries RG, van de Wetering M, Stratton MR, Clevers H, Cuppen E, van Boxtel R, 2016. Tissue-specific mutation accumulation in human adult stem cells during life. Nature 538 (7624), 260–264. 10.1038/nature19768. [PubMed: 27698416]

Böiers C, Richardson SE, Laycock E, Zriwil A, Turati VA, Brown J, Wray JP, Wang D, James C, Herrero J, Sitnicka E, Karlsson S, Smith AJH, Jacobsen SEW, Enver T, 2018. A Human IPS Model Implicates Embryonic B-Myeloid Fate Restriction as Developmental Susceptibility to B Acute Lymphoblastic Leukemia-Associated ETV6-RUNX1. Dev. Cell 44, 362–377.e7. 10.1016/j.devcel.2017.12.005. [PubMed: 29290585]

Bowman RL, Busque L, Levine RL, 2018. Clonal Hematopoiesis and Evolution to Hematopoietic Malignancies. Cell Stem Cell 22 (2), 157–170. 10.1016/j.stem.2018.01.011. [PubMed: 29395053]

Buscarlet M, Provost S, Zada YF, Barhdadi A, Bourgoin V, Lépine G, Mollica L, Szuber N, Dubé MP, Busque L, 2017. DNMT3A and TET2 dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. Blood 130, 753–762. 10.1182/blood-2017-04-777029. [PubMed: 28655780]

Cai SF, Levine RL, 2019. Genetic and epigenetic determinants of AML pathogenesis. Semin. Hematol. 56 (2), 84–89. 10.1053/j.seminhematol.2018.08.001. [PubMed: 30926095]

Carette JE, Pruszak J, Varadarajan M, Blomen VA, Gokhale S, Camargo FD, Wernig M, Jaenisch R, Brummelkamp TR, 2010. Generation of iPSCs from cultured human malignant cells. Blood 115, 4039–4042. 10.1182/blood-2009-07-231845. [PubMed: 20233975]

Chao MP, Gentles AJ, Chatterjee S, Lan F, Reinisch A, Corces MR, Xavy S, Shen J, Haag D, Chanda S, Sinha R, Morganti RM, Nishimura T, Ameen M, Wu H, Wernig M, Wu JC, Majeti R, 2017. Human AML-iPSCs Reacquire Leukemic Properties after Differentiation and Model Clonal Variation of Disease. Cell Stem Cell 20 (3), 329–344.e7. 10.1016/j.stem.2016.11.018. [PubMed: 28089908]

Choi J, Lee S, Mallard W, Clement K, Tagliazucchi GM, Lim H, Choi IY, Ferrari F, Tsankov AM, Pop R, Lee G, Rinn JL, Meissner A, Park PJ, Hochedlinger K, 2015. A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs. Nat. Biotechnol. 33 (11), 1173–1181. 10.1038/nbt.3388. [PubMed: 26501951]

Connelly JP, Kwon EM, Gao Y, Trivedi NS, Elkahloun AG, Horwitz MS, Cheng L, Liu PP, 2014. Targeted correction of RUNX1 mutation in FPD patient-specific induced pluripotent stem cells rescues megakaryopoietic defects. Blood 124, 1926–1930. 10.1182/blood-2014-01-550525. [PubMed: 25114263]

Corces-Zimmerman MR, Hong W-J, Weissman IL, Medeiros BC, Majeti R, 2014. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc. Natl. Acad. Sci. U. S. A. 111 (7), 2548–2553. 10.1073/pnas.1324297111. [PubMed: 24550281]

da Silva-Coelho P, Kroeze LI, Yoshida K, Koorenhof-Scheele TN, Knops R, van de Locht LT, de Graaf AO, Massop M, Sandmann S, Dugas M, Stevens-Kroef MJ, Cermak J, Shiraishi Y, Chiba K, Tanaka H, Miyano S, de Witte T, Blijlevens NMA, Muus P, Huls G, van der Reijden BA, Ogawa S, Jansen JH, 2017. Clonal evolution in myelodysplastic syndromes. Nat. Commun. 8 (1) 10.1038/ncomms15099.

Dannenmann B, Zahabi A, Mir P, Oswald B, Bernhard R, Klimiankou M, Morishima T, Schulze-Osthoff K, Zeidler C, Kanz L, Lachmann N, Moritz T, Welte K, Skokowa J, 2019. Human iPSC-based model of severe congenital neutropenia reveals elevated UPR and DNA damage in CD34 + cells preceding leukemic transformation. Exp. Hematol. 71, 51–60. 10.1016/j.exphem.2018.12.006. [PubMed: 30615903]

Desai P, Mencia-trinchant N, Savenkov O, Simon MS, Cheang G, Lee S, Samuel M, Ritchie EK, Guzman ML, Ballman KV, Roboz GJ, Hassane DC, 2018. leukemia years before diagnosis. Nat. Med. 24 10.1038/s41591-018-0081-z.

Dick JE, Bonnet D, 1997. Human Acute Myeloid Leukaemia is organised as a heirarchy that originates from a primitive haematopoetic cell. Nat. Med. 3, 730–737. [PubMed: 9212098]

Ding L.i., Ley TJ, Larson DE, Miller CA, Koboldt DC, Welch JS, Ritchey JK, Young MA, Lamprecht T, McLellan MD, McMichael JF, Wallis JW, Lu C, Shen D, Harris CC, Dooling DJ, Fulton RS, Fulton LL, Chen K, Schmidt H, Kalicki-Veizer J, Magrini VJ, Cook L, McGrath SD, Vickery TL, Wendl MC, Heath S, Watson MA, Link DC, Tomasson MH, Shannon WD, Payton JE, Kulkarni S, Westervelt P, Walter MJ, Graubert TA, Mardis ER, Wilson RK, DiPersio JF, 2012. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature 481 (7382), 506–510. 10.1038/nature10738. [PubMed: 22237025]

Ditadi A, Sturgeon CM, Keller G, 2017. A view of human haematopoietic development from the Petri dish. Nat. Rev. Mol. Cell Biol. 18 (1), 56–67. 10.1038/nrm.2016.127. [PubMed: 27876786]

Ditadi A, Sturgeon CM, Tober J, Awong G, Kennedy M, Yzaguirre AD, Azzola L, Ng ES, Stanley EG, French DL, Cheng X, Gadue P, Speck NA, Elefanty AG, Keller G, 2015. Human definitive haemogenic endothelium and arterial vascular endothelium represent distinct lineages. Nat. Cell Biol. 17 (5), 580–591. 10.1038/ncb3161. [PubMed: 25915127]

Dokal I, Vulliamy T, 2010. Inherited bone marrow failure syndromes. Haematologica 95 (8), 1236–1240. 10.3324/haematol.2010.025619. [PubMed: 20675743]

Dou DR, Calvanese V, Sierra MI, Nguyen AT, Minasian A, Saarikoski P, Sasidharan R, Ramirez CM, Zack JA, Crooks GM, Galic Z, Mikkola HKA, 2016. Medial HOXA genes demarcate haematopoietic stem cell fate during human development. Nat. Cell Biol. 18 (6), 595–606. 10.1038/ncb3354. [PubMed: 27183470]

Doulatov S, Notta F, Laurenti E, Dick J, 2012. Hematopoiesis: A human perspective. Cell Stem Cell 10 (2), 120–136. 10.1016/j.stem.2012.01.006. [PubMed: 22305562]

Doulatov S, Vo L, Chou S, Kim P, Arora N, Li H.u., Hadland B, Bernstein I, Collins J, Zon L, Daley G, 2013. Induction of multipotential hematopoietic progenitors from human pluripotent stem cells via respecification of lineage-restricted precursors. Cell Stem Cell 13 (4), 459–470. 10.1016/j.stem.2013.09.002. [PubMed: 24094326]

Doulatov S, Vo LT, Macari ER, Wahlster L, Kinney MA, Taylor AM, Barragan J, Gupta M, McGrath K, Lee H-Y, Humphries JM, DeVine A, Narla A, Alter BP, Beggs AH, Agarwal S, Ebert BL, Gazda HT, Lodish HF, Sieff CA, Schlaeger TM, Zon LI, Daley GQ, 2017. Drug discovery for Diamond-Blackfan anemia using reprogrammed hematopoietic progenitors. Sci. Transl. Med. 9 (376), eaah5645. 10.1126/scitranslmed.aah5645. [PubMed: 28179501]

Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL, 2001. Efficacy and Safety of a Specific Inhibitor of the Bcr-Abl Tyrosine. English J. 344, 1031–1037. 10.1056/NEJM200104053441401.

Dzierzak E, Bigas A, 2018. Blood Development: Hematopoietic Stem Cell Dependence and Independence. Cell Stem Cell 22 (5), 639–651. 10.1016/j.stem.2018.04.015. [PubMed: 29727679]

Dzierzak E, Speck NA, 2008. Of lineage and legacy: The development of mammalian hematopoietic stem cells. Nat. Immunol. 9 (2), 129–136. 10.1038/ni1560. [PubMed: 18204427]

Eppert K, Takenaka K, Lechman ER, Waldron L, Nilsson B, van Galen P, Metzeler KH, Poeppl A, Ling V, Beyene J, Canty AJ, Danska JS, Bohlander SK, Buske C, Minden MD, Golub TR, Jurisica I, Ebert BL, Dick JE, 2011. Stem cell gene expression programs influence clinical outcome in human leukemia. Nat. Med. 17 (9), 1086–1093. 10.1038/nm.2415. [PubMed: 21873988]

Garçon L, Ge J, Manjunath SH, Mills JA, Apicella M, Parikh S, Sullivan LM, Podsakoff GM, Gadue P, French DL, Mason PJ, Bessler M, Weiss MJ, 2013. Ribosomal and hematopoietic defects in induced pluripotent stem cells derived from Diamond Blackfan anemia patients. Blood 122, 912–921. 10.1182/blood-2013-01-478321. [PubMed: 23744582]

Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, Chambert K, Mick E, Neale BM, Fromer M, Purcell SM, Svantesson O,

Landén M, Höglund M, Lehmann S, Gabriel SB, Moran JL, Lander ES, Sullivan PF, Sklar P, Grönberg H, Hultman CM, McCarroll SA, 2014. Clonal hematopoiesis and blood-cancer risk inferred

from blood DNA sequence. N. Engl. J. Med. 371 (26), 2477–2487. 10.1056/NEJMoa1409405. [PubMed: 25426838]

Greaves M, 2015. Evolutionary determinants of cancer. Cancer Discov. 5 (8), 806–820. 10.1158/2159-8290.CD-15-0439. [PubMed: 26193902]

Hochedlinger K, Blelloch R, Brennan C, Yamada Y, Kim M, Chin L, Jaenisch R, 2004. Reprogramming of a melanoma genome by nuclear transplantation. Genes Dev. 18, 1875–1885. 10.1101/gad.1213504. [PubMed: 15289459]

Hoffmann D, Göhring G, Heuser M, Ganser A, Schambach A, Morgan MA, 2016. Letter to the Editor: Production of mature healthy hematopoietic cells from induced pluripotent stem cells derived from an AML diagnostic sample containing the t(8;21) translocation. Stem Cells 34 (3), 797–799. 10.1002/stem.2207. [PubMed: 26388310]

Hosoi M, Kumano K, Taoka K, Arai S, Kataoka K, Ueda K, Kamikubo Y, Takayama N, Otsu M, Eto K, Nakauchi H, Kurokawa M, 2014. Generation of induced pluripotent stem cells derived from primary and secondary. 42 (9), 816–825. 10.1016/j.exphem.2014.03.010.

Hsu J, Reilly A, Hayes BJ, Clough CA, Konnick EQ, Torok-Storb B, Gulsuner S, Wu D, Becker PS, Keel SB, Abkowitz JL, Doulatov S, 2019. Reprogramming identifies functionally distinct stages of clonal evolution in myelodysplastic syndromes. Blood 134, 186–198. 10.1182/blood.2018884338. [PubMed: 31010849]

Hu K, Yu J, Suknuntha K, Tian S, Montgomery K, Choi KD, Stewart R, Thomson JA, Slukvin II, 2011. Efficient generation of transgene-free induced pluripotent stem cells from normal and neoplastic bone marrow and cord blood mononuclear cells. Blood 117, 109–119. 10.1182/blood-2010-07-298331.

Ivanovs A, Rybtsov S, Welch L, Anderson RA, Turner ML, Medvinsky A, 2011. Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. J. Exp. Med. 208, 2417–2427. 10.1084/jem.20111688. [PubMed: 22042975]

Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, Lindsley RC, Mermel CH, Burtt N, Chavez A, Higgins JM, Moltchanov V, Kuo FC, Kluk MJ, Henderson B, Kinnunen L, Koistinen HA, Ladenvall C, Getz G, Correa A, Banahan BF, Gabriel S, Kathiresan S, Stringham HM, McCarthy MI, Boehnke M, Tuomilehto J, Haiman C, Groop L, Atzmon G, Wilson JG, Neuberg D, Altshuler D, Ebert BL, 2014. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. N. Engl. J. Med. 371 (26), 2488–2498. 10.1056/NEJMoa1408617. [PubMed: 25426837]

Jan M, Snyder TM, Corces-Zimmerman MR, Vyas P, Weissman IL, Quake SR, Majeti R, 2012. Clonal Evolution of Preleukemic Hematopoietic Stem Cells Precedes Human Acute Myeloid Leukemia. Sci. Transl. Med. 4 10.1126/scitranslmed.3004315, 149ra118–149ra118.

Jerez A, Gondek LP, Jankowska AM, Makishima H, Przychodzen B, Tiu RV, O'Keefe CL, Mohamedali AM, Batista D, Sekeres MA, McDevitt MA, Mufti GJ, Maciejewski JP, 2012a. Topography, clinical, and genomic correlates of 5q myeloid malignancies revisited. J. Clin. Oncol. 30 (12), 1343–1349. 10.1200/JCO.2011.36.1824. [PubMed: 22370328]

Jerez A, Sugimoto Y, Makishima H, Verma A, Jankowska AM, Przychodzen B, Visconte V, Tiu RV, O'Keefe CL, Mohamedali AM, Kulasekararaj AG, Pellagatti A, McGraw K, Muramatsu H, Moliterno AR, Sekeres MA, McDevitt MA, Kojima S, List A, Boultwood J, Mufti GJ, Maciejewski JP, 2012b. Loss of heterozygosity in 7q myeloid disorders: Clinical associations and genomic pathogenesis. Blood 119, 6109–6117. 10.1182/blood-2011-12-397620. [PubMed: 22553315]

Jude CD, Climer L, Xu D, Artinger E, Fisher JK, Ernst P, 2007. Unique and Independent Roles for MLL in Adult Hematopoietic Stem Cells and Progenitors. Cell Stem Cell 1 (3), 324–337. 10.1016/j.stem.2007.05.019. [PubMed: 18371366]

Jung M, Cordes S, Zou J, Yu SJ, Guitart X, Hong SG, Dang V, Kang E, Donaires FS, Hassan SA, Albitar M, Hsu AP, Holland SM, Hickstein DD, Townsley D, Dunbar CE, Winkler T, 2018. GATA2 deficiency and human hematopoietic development modeled using induced pluripotent stem cells. Blood Adv. 2, 3553–3565. 10.1182/bloodadvances.2018017137. [PubMed: 30538114]

Kennedy M, Awong G, Sturgeon C, Ditadi A, LaMotte-Mohs R, Zúñiga-Pflücker J, Keller G, 2012. T Lymphocyte Potential Marks the Emergence of Definitive Hematopoietic Progenitors

in Human Pluripotent Stem Cell Differentiation Cultures. Cell Rep. 2 (6), 1722–1735. 10.1016/j.celrep.2012.11.003. [PubMed: 23219550]

Kilpivaara O, Levine RL, 2008. JAK2 and MPL mutations in myeloproliferative neoplasms: Discovery and science. Leukemia 22 (10), 1813–1817. 10.1038/leu.2008.229. [PubMed: 18754026]

Kotecha N, Flores NJ, Irish JM, Simonds EF, Sakai DS, Archambeault S, Diaz-Flores E, Coram M, Shannon KM, Nolan GP, Loh ML, 2008. Single-Cell Profiling Identifies Aberrant STAT5 Activation in Myeloid Malignancies with Specific Clinical and Biologic Correlates. Cancer Cell 14 (4), 335–343. 10.1016/j.ccr.2008.08.014. [PubMed: 18835035]

Kotini AG, Chang C-J, Boussaad I, Delrow JJ, Dolezal EK, Nagulapally AB, Perna F, Fishbein GA, Klimek VM, Hawkins RD, Huangfu D, Murry CE, Graubert T, Nimer SD, Papapetrou EP, 2015. Functional analysis of a chromosomal deletion associated with myelodysplastic syndromes using isogenic human induced pluripotent stem cells. Nat. Biotechnol. 33 (6), 646–655. 10.1038/nbt.3178. [PubMed: 25798938]

Kotini AG, Chang C-J, Chow A, Yuan H, Ho T-C, Wang T, Vora S, Solovyov A, Husser C, Olszewska M, Teruya-Feldstein J, Perumal D, Klimek VM, Spyridonidis A, Rampal RK, Silverman L, Reddy EP, Papaemmanuil E, Parekh S, Greenbaum BD, Leslie CS, Kharas MG, Papapetrou EP, 2017. Stage-Specific Human Induced Pluripotent Stem Cells Map the Progression of Myeloid Transformation to Transplantable Leukemia. Cell Stem Cell 20 (3), 315–328.e7. 10.1016/j.stem.2017.01.009. [PubMed: 28215825]

Kotini AG, Papapetrou EP, 2020. Engineering of targeted megabase-scale deletions in human induced pluripotent stem cells. Exp. Hematol. 87, 25–32. 10.1016/j.exphem.2020.06.001. [PubMed: 32544417]

Kyba M, Perlingeiro RCR, Daley GQ, 2002. HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell 109 (1), 29–37. 10.1016/S0092-8674(02)00680-3. [PubMed: 11955444]

Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE, 1994. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 367 (6464), 645–648. 10.1038/367645a0. [PubMed: 7509044]

Lee J-H, Salci KR, Reid JC, Orlando L, Tanasijevic B, Shapovalova Z, Bhatia M, 2017. Brief Report: Human Acute Myeloid Leukemia Reprogramming to Pluripotency Is a Rare Event and Selects for Patient Hematopoietic Cells Devoid of Leukemic Mutations. Stem Cells 35 (9), 2095–2102. 10.1002/stem.v35.910.1002/stem.2655. [PubMed: 28758276]

Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K-I, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, Young RA, 2006. Control of Developmental Regulators by Polycomb in Human Embryonic Stem Cells. Cell 125 (2), 301–313. 10.1016/j.cell.2006.02.043. [PubMed: 16630818]

Ley TJ, Ding L.i., Walter MJ, McLellan MD, Lamprecht T, Larson DE, Kandoth C, Payton JE, Baty J, Welch J, Harris CC, Lichti CF, Townsend RR, Fulton RS, Dooling DJ, Koboldt DC, Schmidt H, Zhang Q, Osborne JR, Lin L, O'Laughlin M, McMichael JF, Delehaunty KD, McGrath SD, Fulton LA, Magrini VJ, Vickery TL, Hundal J, Cook LL, Conyers JJ, Swift GW, Reed JP, Alldredge PA, Wylie T, Walker J, Kalicki J, Watson MA, Heath S, Shannon WD, Varghese N, Nagarajan R, Westervelt P, Tomasson MH, Link DC, Graubert TA, DiPersio JF, Mardis ER, Wilson RK, 2010. DNMT3A mutations in acute myeloid leukemia. N. Engl. J. Med. 363 (25), 2424–2433. 10.1056/NEJMoa1005143. [PubMed: 21067377]

Liu G-H, Suzuki K, Li M.o., Qu J, Montserrat N, Tarantino C, Gu Y, Yi F, Xu X, Zhang W, Ruiz S, Plongthongkum N, Zhang K, Masuda S, Nivet E, Tsunekawa Y, Soligalla RD, Goebl A, Aizawa E, Kim NY, Kim J, Dubova I, Li Y, Ren R, Benner C, del Sol A, Bueren J, Trujillo JP, Surralles J, Cappelli E, Dufour C, Esteban CR, Belmonte JCI, 2014. Modelling Fanconi anemia pathogenesis and therapeutics using integration-free patient-derived iPSCs. Nat. Commun. 5 (1) 10.1038/ncomms5330.

Luquette LJ, Bohrson CL, Sherman MA, Park PJ, 2019. Identification of somatic mutations in single cell DNA-seq using a spatial model of allelic imbalance. Nat. Commun. 10 (1) 10.1038/s41467-019-11857-8.

Makishima H, Yoshizato T, Yoshida K, Sekeres MA, Radivoyevitch T, Suzuki H, rzychodzen B, Nagata Y, Meggendorfer M, Sanada M, Okuno Y, Hirsch C, Kuzmanovic T, Sato Y, Sato-Otsubo A, LaFramboise T, Hosono N, Shiraishi Y, Chiba K, Haferlach C, Kern W, Tanaka H, Shiozawa Y, Gómez-Seguí I, Husseinzadeh HD, Thota S, Guinta KM, Dienes B, Nakamaki T, Miyawaki S, Sauntharajah Y, Chiba S, Miyano S, Shih L-Y, Haferlach T, Ogawa S, Maciejewski JP, 2017. Dynamics of clonal evolution in myelodysplastic syndromes. Nat. Genet. 49 (2), 204–212. 10.1038/ng.3742. [PubMed: 27992414]

Malcovati L, Karimi M, Papaemmanuil E, Ambaglio I, Jädersten M, Jansson M, Elena C, Gallì A, Walldin G, Porta MGD, Raaschou-Jensen K, Travaglino E, Kallenbach K, Pietra D, Ljungström V, Conte S, Boveri E, Invernizzi R, Rosenquist R, Campbell PJ, Cazzola M, Lindberg EH, 2015. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood 126, 233–241. 10.1182/blood-2015-03-633537. [PubMed: 25957392]

Malcovati L, Papaemmanuil E, Bowen DT, Boultwood J, Della Porta MG, Pascutto C, Travaglino E, Groves MJ, Godfrey AL, Ambaglio I, Gallì A, Da Vià MC, Conte S, Tauro S, Keenan N, Hyslop A, Hinton J, Mudie LJ, Wainscoat JS, Futreal PA, Stratton MR, Campbell PJ, Hellström-Lindberg E, Cazzola M, 2011. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood 118, 6239–6246. 10.1182/blood-2011-09-377275. [PubMed: 21998214]

Mardis ER, Ding L.i., Dooling DJ, Larson DE, McLellan MD, Chen K, Koboldt DC, Fulton RS, Delehaunty KD, McGrath SD, Fulton LA, Locke DP, Magrini VJ, Abbott RM, Vickery TL, Reed JS, Robinson JS, Wylie T, Smith SM, Carmichael L, Eldred JM, Harris CC, Walker J, Peck JB, Du F, Dukes AF, Sanderson GE, Brummett AM, Clark E, McMichael JF, Meyer RJ, Schindler JK, Pohl CS, Wallis JW, Shi X, Lin L, Schmidt H, Tang Y, Haipek C, Wiechert ME, Ivy JV, Kalicki J, Elliott G, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson MA, Baty J, Heath S, Shannon WD, Nagarajan R, Link DC, Walter MJ, Graubert TA, DiPersio JF, Wilson RK, Ley TJ, 2009. Recurring mutations found by sequencing an acute myeloid leukemia genome. N. Engl. J. Med. 361 (11), 1058–1066. 10.1056/NEJMoa0903840. [PubMed: 19657110]

Marión RM, Strati K, Li H, Murga M, Blanco R, Ortega S, Fernandez-Capetillo O, Serrano M, Blasco MA, 2009. A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. Nature 460 (7259), 1149–1153. 10.1038/nature08287. [PubMed: 19668189]

Medvinsky A, Dzierzak E, 1996. Definitive hematopoiesis is autonomously initiated by the AGM region. Cell 86 (6), 897–906. 10.1016/S0092-8674(00)80165-8. [PubMed: 8808625]

Miles LA, Bowman RL, Merlinsky TR, Csete IS, Ooi A, Durruthy-Durruthy R, Bowman M, Famulare C, Patel MA, Mendez P, Ainali C, Manivannan M, Sahu S, Goldberg AD, Bolton K, Zehir A, Rampal R, Carroll MP, Meyer SE, Viny AD, Levine RL, 2020. Single cell mutational profiling delineates clonal trajectories in myeloid malignancies. bioRxiv. 10.1101/2020.02.07.938860.

Morotti A, Rocca S, Carrà G, Saglio G, Brancaccio M, 2017. Modeling myeloproliferative neoplasms: From mutations to mouse models and back again. Blood Rev. 31 (3), 139–150. 10.1016/j.blre.2016.11.004. [PubMed: 27899218]

Mullally A, Lane SW, Brumme K, Ebert BL, 2012. Myeloproliferative Neoplasm Animal Models. Hematol. Oncol. Clin. North Am. 26 (5), 1065–1081. 10.1016/j.hoc.2012.07.007. [PubMed: 23009938]

Müller LUW, Milsom MD, Harris CE, Vyas R, Brumme KM, Parmar K, Moreau LA, Schambach A, Park IH, London WB, Strait K, Schlaeger T, DeVine AL, Grassman E, D'Andrea A, Daley GQ, Williams DA, 2012. Overcoming reprogramming resistance of Fanconi anemia cells. Blood 119, 5449–5457. 10.1182/blood-2012-02-408674. [PubMed: 22371882]

Mupo A, Seiler M, Sathiaseelan V, Pance A, Yang Y, Agrawal AA, Iorio F, Bautista R, Pacharne S, Tzelepis K, Manes N, Wright P, Papaemmanuil E, Kent DG, Campbell PC, Buonamici S, Bolli N, Vassiliou GS, 2017. Hemopoietic-specific Sf3b1-K700E knock-in mice display the splicing defect seen in human MDS but develop anemia without ring sideroblasts. Leukemia 31 (3), 720–727. 10.1038/leu.2016.251. [PubMed: 27604819]

Nagata Y, Makishima H, Kerr CM, Przychodzen BP, Aly M, Goyal A, Awada H, Asad MF, Kuzmanovic T, Suzuki H, Yoshizato T, Yoshida K, Chiba K, Tanaka H, Shiraishi Y, Miyano S, Mukherjee S, LaFramboise T, Nazha A, Sekeres MA, Radivoyevitch T, Haferlach T, Ogawa S, Maciejewski JP, 2019. Invariant patterns of clonal succession determine specific clinical features of myelodysplastic syndromes. Nat. Commun. 10, 1–14. 10.1038/s41467-019-13001-y. [PubMed: 30602773]

Ng ES, Azzola L, Bruveris FF, Calvanese V, Phipson B, Vlahos K, Hirst C, Jokubaitis VJ, Yu QC, Maksimovic J, Liebscher S, Januar V, Zhang Z, Williams B, Conscience A, Durnall J, Jackson S, Costa M, Elliott D, Haylock DN, Nilsson SK, Saffery R, Schenke-Layland K, Oshlack A, Mikkola HKA, Stanley EG, Elefanty AG, 2016. Differentiation of human embryonic stem cells to HOXA + hemogenic vasculature that resembles the aorta-gonad-mesonephros. Nat. Biotechnol. 34 (11), 1168–1179. 10.1038/nbt.3702. [PubMed: 27748754]

Niemeyer CM, 2018. JMML genomics and decisions. Hematology 2018, 307–312. 10.1182/asheducation-2018.1.307. [PubMed: 30504325]

Niemeyer CM, Kratz CP, 2008. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia: Molecular classification and treatment options. Br. J. Haematol. 140 (6), 610–624. 10.1111/bjh.2008.140.issue-610.1111/j.1365-2141.2007.06958.x. [PubMed: 18302710]

Nowell P, 1976. The clonal evolution of tumor cell populations. Science (80-. 194 (4260), 23–28. 10.1126/science:959840.

Obeng E, Chappell R, Seiler M, Chen M, Campagna D, Schmidt P, Schneider R, Lord A, Wang L, Gambe R, McConkey M, Ali A, Raza A, Yu L, Buonamici S, Smith P, Mullally A, Wu C, Fleming M, Ebert B, 2016. Physiologic Expression of Sf3b1K700E Causes Impaired Erythropoiesis, Aberrant Splicing, and Sensitivity to Therapeutic Spliceosome Modulation. Cancer Cell 30 (3), 404–417. 10.1016/j.ccell.2016.08.006. [PubMed: 27622333]

Ogawa S, 2019. Genetics of MDS. Blood 133, 1049–1059. 10.1182/blood-2018-10-844621. [PubMed: 30670442]

Ortmann CA, Kent DG, Nangalia J, Silber Y, Wedge DC, Grinfeld J, Baxter EJ, Massie CE, Papaemmanuil E, Menon S, Godfrey AL, Dimitropoulou D, Guglielmelli P, Bellosillo B, Besses C, Döhner K, Harrison CN, Vassiliou GS, Vannucchi A, Campbell PJ, Green AR, 2015. Effect of mutation order on myeloproliferative neoplasms. N. Engl. J. Med. 372 (7), 601–612. 10.1056/NEJMoa1412098. [PubMed: 25671252]

Paguirigan AL, Smith J, Meshinchi S, Carroll M, Maley C, Radich JP, 2015. Single-cell genotyping demonstrates complex clonal diversity in acute myeloid leukemia. Sci. Transl. Med. 7, 1–10. 10.1126/scitranslmed.aaa0763.

Palis J, Yoder MC, 2001. Yolk-sac hematopoiesis: The first blood cells of mouse and man. Exp. Hematol. 29, 927–936. 10.1016/S0301-472X(01)00669-5. [PubMed: 11495698]

Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D, Pellagatti A, Wainscoat JS, Hellstrom-Lindberg E, Gambacorti-Passerini C, Godfrey AL, Rapado I, Cvejic A, Rance R, McGee C, Ellis P, Mudie LJ, Stephens PJ, McLaren S, Massie CE, Tarpey PS, Varela I, Nik-Zainal S, Davies HR, Shlien A, Jones D, Raine K, Hinton J, Butler AP, Teague JW, Baxter EJ, Score J, Galli A, Della Porta MG, Travaglino E, Groves M, Tauro S, Munshi NC, Anderson KC, El-Naggar A, Fischer A, Mustonen V, Warren AJ, Cross NCP, Green AR, Futreal PA, Stratton MR, Campbell PJ, 2011. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N. Engl. J. Med. 365 (15), 1384–1395. 10.1056/NEJMoa1103283. [PubMed: 21995386]

Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, Yoon CJ, Ellis P, Wedge DC, Pellagatti A, Shlien A, Groves MJ, Forbes SA, Raine K, Hinton J, Mudie LJ, McLaren S, Hardy C, Latimer C, Della Porta MG, O'Meara S, Ambaglio I, Galli A, Butler AP, Walldin G, Teague JW, Quek L, Sternberg A, Gambacorti-Passerini C, Cross NCP, Green AR, Boultwood J, Vyas P, Hellstrom-Lindberg E, Bowen D, Cazzola M, Stratton MR, Campbell PJ, 2013. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 122, 3616–3627. 10.1182/blood-2013-08-518886. [PubMed: 24030381]

Papapetrou EP, 2016. Patient-derived induced pluripotent stem cells in cancer research and precision oncology. Nat. Med. 22 (12), 1392–1401. 10.1038/nm.4238. [PubMed: 27923030]

Petti AA, Williams SR, Miller CA, Fiddes IT, Srivatsan SN, Chen DY, Fronick CC, Fulton RS, Church DM, Ley TJ, 2019. A general approach for detecting expressed mutations in AML cells using single cell RNA-sequencing. Nat. Commun. 10 (1) 10.1038/s41467-019-11591-1.

Robert-Moreno À, Guiu J, Ruiz-Herguido C, López ME, Inglés-Esteve J, Riera L, Tipping A, Enver T, Dzierzak E, Gridley T, Espinosa L, Bigas A, 2008. Impaired embryonic haematopoiesis yet normal arterial development in the absence of the Notch ligand Jagged1. EMBO J. 27 (13), 1886–1895. 10.1038/emboj.2008.113. [PubMed: 18528438]

Ruiz-Gutierrez M, Bölükbaşi ÖV, Alexe G, Kotini AG, Ballotti K, Joyce CE, Russell DW, Stegmaier K, Myers K, Novina CD, Papapetrou EP, Shimamura A, 2019. Therapeutic discovery for marrow failure with MDS predisposition using pluripotent stem cells. JCI Insight 4. 10.1172/jci.insight.125157.

Sakurai M, Kunimoto H, Watanabe N, Fukuchi Y, Yuasa S, Yamazaki S, Nishimura T, Sadahira K, Fukuda K, Okano H, Nakauchi H, Morita Y, Matsumura I, Kudo K, Ito E, Ebihara Y, Tsuji K, Harada Y, Harada H, Okamoto S, Nakajima H, 2014. Impaired hematopoietic differentiation of RUNX1-mutated induced pluripotent stem cells derived from FPD/AML patients. Leukemia 28 (12), 2344–2354. 10.1038/leu.2014.136. [PubMed: 24732596]

Saliba J, Hamidi S, Lenglet G, Langlois T, Yin J, Cabagnols X, Secardin L, Legrand C, Galy A, Opolon P, Benyahia B, Solary E, Bernard OA, Chen L, Debili N, Raslova H, Norol F, Vainchenker W, Plo I, Di Stefano A, Dello Sbarba P, 2013. Heterozygous and Homozygous JAK2V617F States Modeled by Induced Pluripotent Stem Cells from Myeloproliferative Neoplasm Patients. PLoS One 8 (9), e74257. 10.1371/journal.pone.0074257. [PubMed: 24066127]

Schlaeger TM, Daheron L, Brickler TR, Entwisle S, Chan K, Cianci A, DeVine A, Ettenger A, Fitzgerald K, Godfrey M, Gupta D, McPherson J, Malwadkar P, Gupta M, Bell B, Doi A, Jung N, Li X, Lynes MS, Brookes E, Cherry ABC, Demirbas D, Tsankov AM, Zon LI, Rubin LL, Feinberg AP, Meissner A, Cowan CA, Daley GQ, 2015. A comparison of non-integrating reprogramming methods. Nat. Biotechnol. 33 (1), 58–63. 10.1038/nbt.3070. [PubMed: 25437882]

Shakiba N, Fahmy A, Jayakumaran G, McGibbon S, David L, Trcka D, Elbaz J, Puri MC, Nagy A, van der Kooy D, Goyal S, Wrana JL, Zandstra PW, 2019. Cell competition during reprogramming gives rise to dominant clones. Science 364 (6438), eaan0925. 10.1126/science:aan0925. [PubMed: 30898844]

Shlush LI, Mitchell A, Heisler L, Abelson S, Ng SWK, Trotman-Grant A, Medeiros JJF, Rao-Bhatia A, Jaciw-Zurakowsky I, Marke R, McLeod JL, Doedens M, Bader G, Voisin V, Xu ChangJiang, McPherson JD, Hudson TJ, Wang JCY, Minden MD, Dick JE, 2017. Tracing the origins of relapse in acute myeloid leukaemia to stem cells. Nature 547 (7661), 104–108. 10.1038/nature22993. [PubMed: 28658204]

Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW, McLeod JL, Doedens M, Medeiros JJF, Marke R, Kim HJ, Lee K, McPherson JD, Hudson TJ, Pan-Leukemia Gene Panel Consortium, HALT T., Brown AMK, Yousif F, Trinh QM, Stein LD, Minden MD, Wang JCY, Dick JE, 2014. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature 506 (7488), 328–333. 10.1038/nature13038. [PubMed: 24522528]

Song Y, Rongvaux A, Taylor A, Jiang T, Tebaldi T, Balasubramanian K, Bagale A, Terzi YK, Gbyli R, Wang X, Fu X, Gao Y, Zhao J, Podoltsev N, Xu M, Neparidze N, Wong E, Torres R, Bruscia EM, Kluger Y, Manz MG, Flavell RA, Halene S, 2019. A highly efficient and faithful MDS patient-derived xenotransplantation model for pre-clinical studies. Nat. Commun. 10, 1–14. 10.1038/s41467-018-08166-x. [PubMed: 30602773]

Sperling AS, Gibson CJ, Ebert BL, 2017. The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. Nat. Rev. Cancer 17 (1), 5–19. 10.1038/nrc.2016.112. [PubMed: 27834397]

Spivak JL, 2017. Myeloproliferative Neoplasms. N. Engl. J. Med. 376 (22), 2168–2181. 10.1056/NEJMra1406186. [PubMed: 28564565]

Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, Ebert BL, 2015. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood 126, 9–16. 10.1182/blood-2015-03-631747. [PubMed: 25931582]

Sturgeon CM, Ditadi A, Awong G, Kennedy M, Keller G, 2014. Wnt signaling controls the specification of definitive and primitive hematopoiesis from human pluripotent stem cells. Nat. Biotechnol. 32 (6), 554–561. 10.1038/nbt.2915. [PubMed: 24837661]

Sugimura R, Jha DK, Han A, Soria-Valles C, da Rocha EL, Lu Y-F, Goettel JA, Serrao E, Rowe RG, Malleshaiah M, Wong I, Sousa P, Zhu TN, Ditadi A, Keller G, Engelman AN, Snapper SB, Doulatov S, Daley GQ, 2017. Haematopoietic stem and progenitor cells from human pluripotent stem cells. Nature 545 (7655), 432–438. 10.1038/nature22370. [PubMed: 28514439]

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S, 2007. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell 131 (5), 861–872. 10.1016/j.cell.2007.11.019. [PubMed: 18035408]

Takahashi K, Yamanaka S, 2006. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell 126 (4), 663–676. 10.1016/j.cell.2006.07.024. [PubMed: 16904174]

Tarnawsky SP, Yu WM, Qu CK, Chan RJ, Yoder MC, 2018. Hematopoietic-restricted Ptpn11E76K reveals indolent MPN progression in mice. Oncotarget 9, 21831–21843. 10.18632/oncotarget.25073. [PubMed: 29774106]

Tasian SK, Casas JA, Posocco D, Gandre-Babbe S, Gagne AL, Liang G.e., Loh ML, Weiss MJ, French DL, Chou ST, 2019. Mutation-specific signaling profiles and kinase inhibitor sensitivities of juvenile myelomonocytic leukemia revealed by induced pluripotent stem cells. Leukemia 33 (1), 181–190. 10.1038/s41375-018-0169-y. [PubMed: 29884903]

Thomas D, Majeti R, 2017. Biology and relevance of human acute myeloid leukemia stem cells. Blood 129, 1577–1585. 10.1182/blood-2016-10-696054. [PubMed: 28159741]

van Galen P, Hovestadt V, Wadsworth II MH, Hughes TK, Griffin GK, Battaglia S, Verga JA, Stephansky J, Pastika TJ, Lombardi Story J, Pinkus GS, Pozdnyakova O, Galinsky I, Stone RM, Graubert TA, Shalek AK, Aster JC, Lane AA, Bernstein BE, 2019. Single-Cell RNA-Seq Reveals AML Hierarchies Relevant to Disease Progression and Immunity. Cell 176 (6), 1265–1281.e24. 10.1016/j.cell.2019.01.031. [PubMed: 30827681]

Vo LT, Daley GQ, 2015. De novo generation of HSCs from somatic and pluripotent stem cell sources. Blood 125, 2641–2648. 10.1182/blood-2014-10-570234. [PubMed: 25762177]

Vo LT, Kinney MA, Liu X, Zhang Y, Barragan J, Sousa PM, Jha DK, Han A, Cesana M, Shao Z, North TE, Orkin SH, Doulatov S, Xu J, Daley GQ, 2018. Regulation of embryonic haematopoietic multipotency by EZH1. Nature 553 (7689), 506–510. 10.1038/nature25435. [PubMed: 29342143]

Wang L, Menendez P, Shojaei F, Li L, Mazurier F, Dick JE, Cerdan C, Levac K, Bhatia M, 2005. Generation of hematopoietic repopulating cells from human embryonic stem cells independent of ectopic HOXB4 expression. J. Exp. Med. 201, 1603–1614. 10.1084/jem.20041888. [PubMed: 15883170]

Welch J, Ley T, Link D, Miller C, Larson D, Koboldt D, Wartman L, Lamprecht T, Liu F, Xia J, Kandoth C, Fulton R, McLellan M, Dooling D, Wallis J, Chen K, Harris C, Schmidt H, Kalicki-Veizer J, Lu C, Zhang Q, Lin L, O'Laughlin M, McMichael J, Delehaunty K, Fulton L, Magrini V, McGrath S, Demeter R, Vickery T, Hundal J, Cook L, Swift G, Reed J, Alldredge P, Wylie T, Walker J, Watson M, Heath S, Shannon W, Varghese N, Nagarajan R, Payton J, Baty J, Kulkarni S, Klco J, Tomasson M, Westervelt P, Walter M, Graubert T, DiPersio J, Ding L.i., Mardis E, Wilson R, 2012. The origin and evolution of mutations in acute myeloid leukemia. Cell 150 (2), 264–278. 10.1016/j.cell.2012.06.023. [PubMed: 22817890]

Wesely J, Kotini AG, Izzo F, Luo H, Yuan H, Sun J, Georgomanoli M, Zviran A, Deslauriers AG, Dusaj N, Nimer SD, Leslie C, Landau DA, Kharas MG, Papapetrou EP, 2020. Acute Myeloid Leukemia iPSCs Reveal a Role for RUNX1 in the Maintenance of Human Leukemia Stem Cells. Cell Rep. 31 (9), 107688. 10.1016/j.celrep.2020.107688. [PubMed: 32492433]

Yamasaki AE, King NE, Matsui H, Jepsen K, Panopoulos AD, 2019. Two iPSC lines generated from the bone marrow of a relapsed/refractory AML patient display normal karyotypes and myeloid differentiation potential. Stem Cell Res. 41, 101587. 10.1016/j.scr.2019.101587. [PubMed: 31739201]

Ye Z, Zhan H, Mali P, Dowey S, Williams DM, Jang YY, Dang CV, Spivak JL, Moliterno AR, Cheng L, 2009. Human-induced pluripotent stem cells from blood cells of healthy donors and

patients with acquired blood disorders. Blood 114, 5473–5480. 10.1182/blood-2009-04-217406. [PubMed: 19797525]

Yokoyama A, Kakiuchi N, Yoshizato T, Nannya Y, Suzuki H, Takeuchi Y, Shiozawa Y, Sato Y, Aoki K, Kim SK, Fujii Y, Yoshida K, Kataoka K, Nakagawa MM, Inoue Y, Hirano T, Shiraishi Y, Chiba K, Tanaka H, Sanada M, Nishikawa Y, Amanuma Y, Ohashi S, Aoyama I, Horimatsu T, Miyamoto S, Tsunoda S, Sakai Y, Narahara M, Brown JB, Sato Y, Sawada G, Mimori K, Minamiguchi S, Haga H, Seno H, Miyano S, Makishima H, Muto M, Ogawa S, 2019. Age-related remodelling of oesophageal epithelia by mutated cancer drivers. Nature 565 (7739), 312–317. 10.1038/s41586-018-0811-x. [PubMed: 30602793]

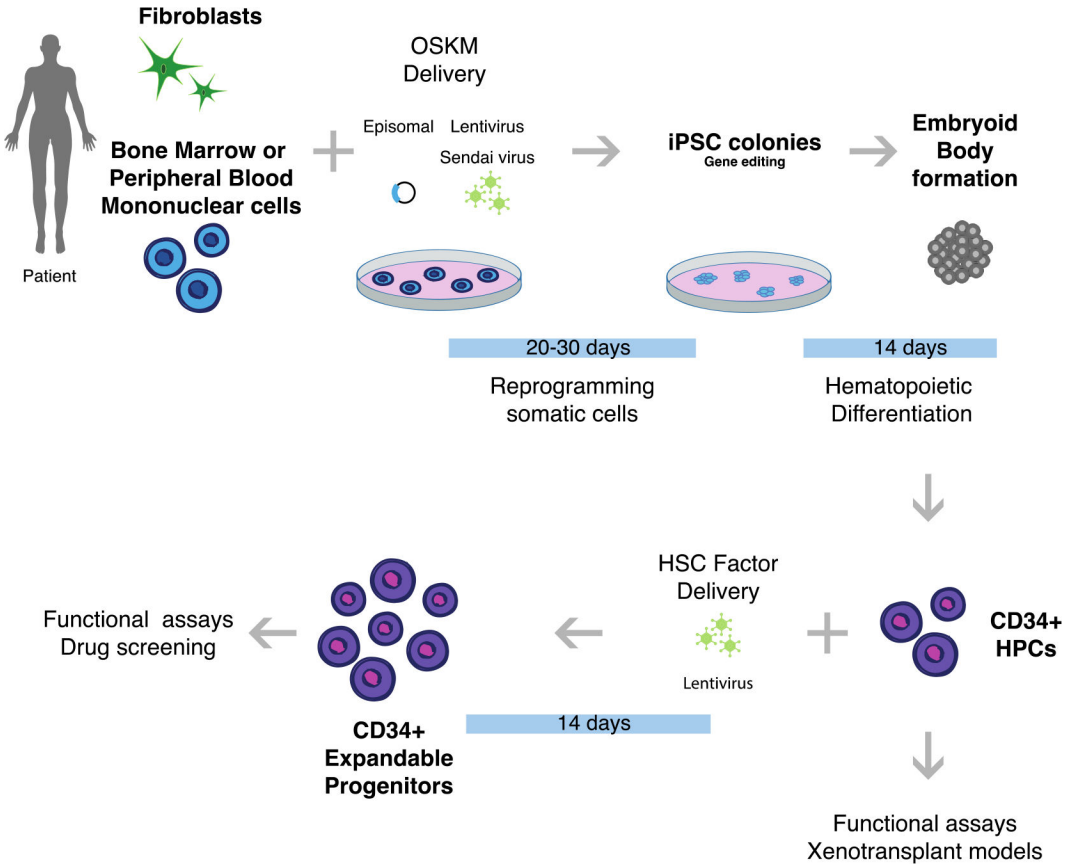
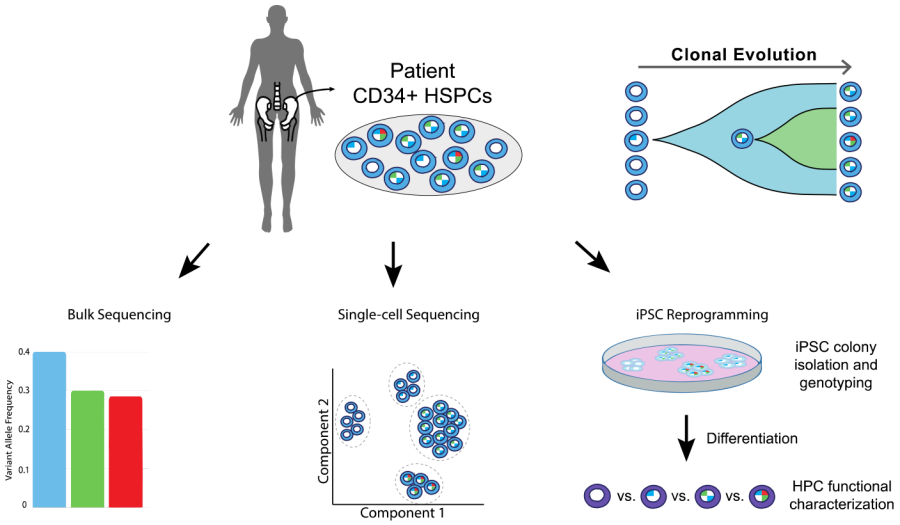


Fig. 1.

Overview of the iPSC reprogramming process for hematological diseases. Workflow diagram for iPSC model generation. Starting with a sample of somatic cells from the patient (e.g. fibroblasts or bone marrow), the OSKM Yamanaka factors are introduced by one of several methods, such as episomal vectors or lentivirus. After several weeks, iPSC colonies are isolated, expanded, phenotyped for expression of pluripotency markers, and genotyped for the patient-specific driver mutations. At this point, gene editing of iPSCs is also an option. The iPSC lines then undergo in vitro differentiation (10–14 days) to $CD34^{+}CD45^{+}$ HSPCs. Lentiviral delivery of HSC factors (e.g. 5 factors: HOXA9, MYB, ERG, SOX4, RORA) to $CD34^{+}$ HSPCs can create conditionally expandable progenitor lines for downstream hematopoietic differentiation or molecular assays.



Technology:	Bulk Sequencing	Single-cell Sequencing	iPSC Reprogramming
Detection of preleukemic clones	+/−	+	+
Detection of leukemic clones	+	+	+/−
Detection of rare clones	+/−	+	+/−
Suitable for tracking mutation order	+/−	+	+
Technical ease	+	+/−	−
Functional readout	−	−	+
Suitable for use in clinic	+	−	−

Fig. 2.

Comparison of single-cell technologies for detection of disease clones. Diagram of a patient with acquired hematologic malignancy and the readout of each technology for the patient CD34 + HSPC sample. Table compares criteria for each single-cell method. “+” indicates the method is useful for the application, “+/−” indicates useful in some cases, and “−” indicates not usually useful.