Induced Pluripotent Stem Cells: an Appropriate Model for Acute Myeloid Leukemia?

A literature review on the prospects of induced pluripotent stem cells to serve as a model for monitoring disease mechanisms and drug sensitivity in acute myeloid leukemia.

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Abstract

Acute myeloid leukemia is a highly heterogeneous disease with poor treatment outcomes. As such, it begs for a better understanding of molecular mechanisms underlying specific incidences. Induced pluripotent stem cells are able to provide models that in theory should contribute to this increased understanding. Due to various complications, induced pluripotent stem cells have not been a major focus in AML research. Gradually now, insights are being offered that these complications can be eliminated in some cases and AML research can also profit from this development.

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Introduction

Acute myeloid leukemia (AML) is comprised of a large family of severe diseases characterized by hematopoietic dysfunction and being ultimately fatal within months of diagnosis if left untreated. In recent years, identification of molecular abnormalities in AML have provided a better comprehension of the disease pathogenesis. This has led to improved classification, but outcomes after diagnosis remain poor (Meyers, Yu, Kaye, & Davis, 2013).

Two main complications impede improving these poor outcomes. The first is that AML is driven by leukemic stem cells, which are refractory to chemotherapy and thus often lead to relapse (Shlush et al., 2017). The other is the heterogeneity of the disease. Many genetic abnormalities have been identified in AML, of which each patient carries 13 on average, leading to many unique combinations. Besides this, even within patients multiple genetically distinct clones can exist with differential chemotherapy response rates (The Cancer Genome Atlas Research Network, 2013). Due to this, personalized targeted therapy could proof to be advantageous especially for AML. Yet, proper research models that can contribute to its development are rare. *In vitro* studies are difficult to conduct since AML sample numbers are limited and leukemic blasts respond to culturing by rapidly undergoing apoptosis.

Previously, embryonic stem cells (ESCs) have generally been used as a model in response to such problems. These however, carry the weight of extensive ethical discussions. The use of induced pluripotent stem cells (iPSCs) as a model could be an opportunity to overcome both these difficulties (Lim, Inoue-Yokoo, Tan, Lai, & Sugiyama, 2013).

For other diseases, these iPSCs have been relatively easily generated, thereby presenting opportunities to study more of the specific subtypes, even patient or stage specific (Park et al., 2008). Since AML is caused by the accumulation of multiple genetic, often including chromosomal, abnormalities, it is difficult to mimic this complex genetic background in normal healthy hematopoietic stem/progenitor cells (HSPCs). By generating iPSC lines from patient samples, this complex genetic background is maintained while the cells are also easily expandable. As iPSC lines are typically clonal, they might even generate models for distinct subclones within individual patients. Nonetheless, since reprogramming to iPSCs resets epigenetics, it could prove challenging to model the epigenetic contributions to AML.

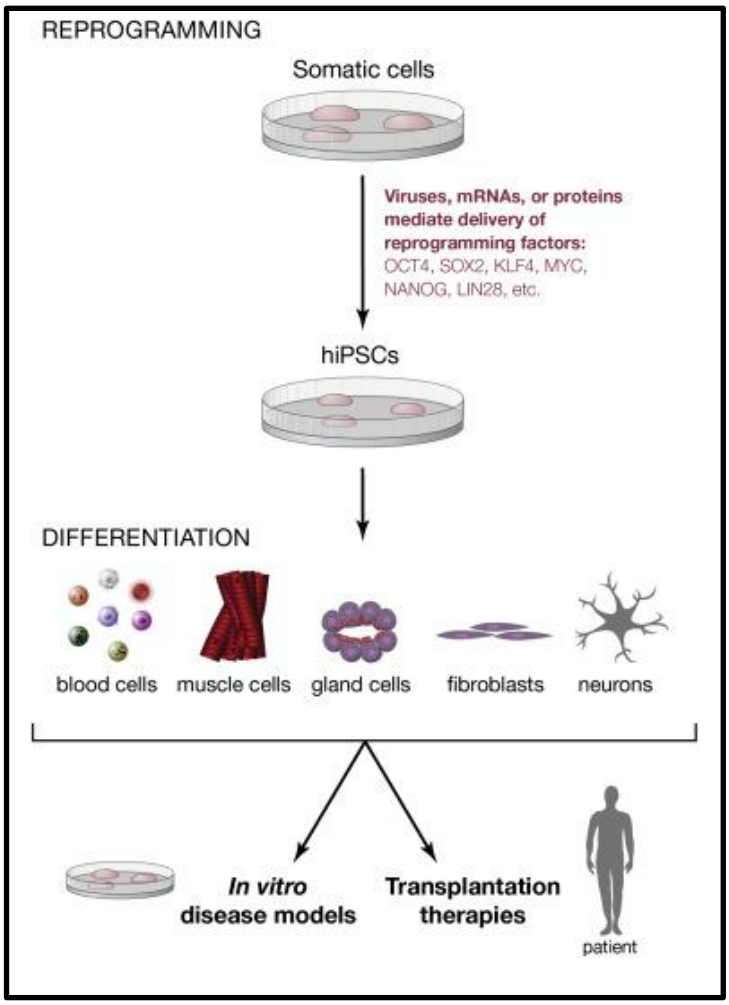
Here, I will first review recent progress in iPSC research and AML. Then the genetic and epigenetic landscape of AML will be considered before reviewing recent studies in AML involving iPSCs. With this in hand I will try to provide some preliminary conclusion to whether iPSCs can serve as a proper model, both for pathogenesis comprehension and drug screening.

Induced Pluripotent Stem Cells

Historical background

Since the 1980s, ESCs are known to have the property of indefinite growth combined with a maintained pluripotency (Evans & Kaufman, 1981; Martin, 1981). As such, they were thought to be of great use in studying development and pathogenesis of certain diseases, drug screening, regenerative medicine and for transplantation therapies. However, there were major downsides to this approach. First of all, there were ethical reasons to hold back from this, since human embryos were required to cultivate ESCs. Additionally, it was difficult to match the biological characteristics of the ESCs with the cells of the patient or disease of interest.

Only little more than a decade ago, it was discovered by the lab of Shinya Yamanaka that it was possible to induce pluripotent stem cells from mice by simply introducing four transcription factors to somatic cells. Under the influence of *Oct3/4*, *Sox2*, *c-Myc* and *Klf4*, adult fibroblasts were able to reprogram back to a state that matched ESCs in terms of morphology, growth properties and ESC marker genes (Takahashi & Yamanaka, 2006). The name that was given to these reprogrammed cells is induced pluripotent stem cells. These mouse iPSCs were also transplanted into blastocysts, where they could give rise to germline competent adult chimeras, proving their pluripotency (Okita, Ichisaka, & Yamanaka, 2007).



One year later, this process was also successfully carried out with differentiated human somatic cells (fibroblasts). Treatment of these cells with the same factors as described above resulted in human iPSCs that were equal to their ESC counterparts in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes and telomerase activity. Also, these iPSCs were proven to differentiate into the different types of cells that account for the three germ layers, both in vitro and in teratomas (Takahashi et al., 2007). This discovery has led to the use of iPSCs as a disease- and patient specific model, tackling the major downsides of ESCs.

Figure 1. *Schematic overview of iPSC technology* (Hockemeyer & Jaenisch, 2016).

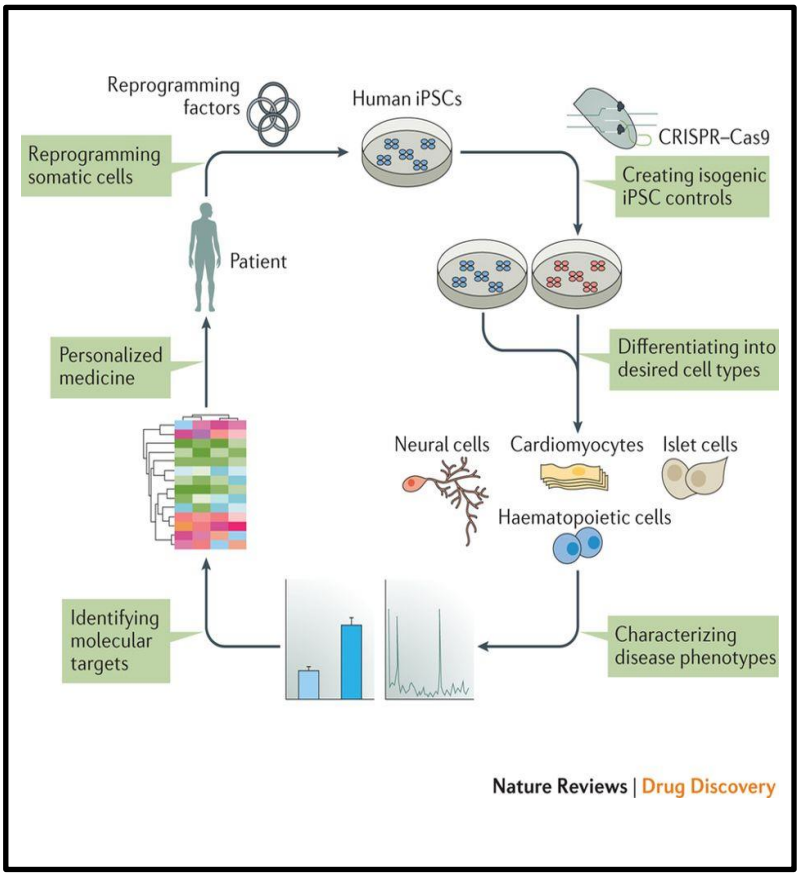
Recent progress

When initially discovered, the efficiency of reprogramming somatic cells back to iPSCs was very low. Only a very small portion of the somatic cells (~0.001%) formed actual iPSCs (Okita et al., 2007; Takahashi & Yamanaka, 2006; Takahashi et al., 2007). Furthermore, their overexpression and the fact that *c-Myc* and *Klf4* are oncogenes raised concerns regarding safety. The legitimacy of these concerns was proven when the first germline competent iPSCs were reported, of which ~20% formed tumors caused by the reactivation of *c-Myc* (Okita et al., 2007). Finally, the method of delivery of the factors – a retrovirus – brought along risks of insertional mutagenesis. Next to the factors discovered by Yamanaka, the so-called Yamanaka factors, Thomson proved that the use of *Nanog* and *Lin28* instead of *c-Myc* and *Klf4* also generated iPSCs, albeit at equally low rate (Yu et al., 2007).

In recent years, much progress has been made concerning reprogramming techniques. Firstly, researchers have been looking for a way to overcome the low efficiency. Other transcription factors, microRNAs and small molecules have been found to possibly improve reprogramming efficiency (Deng et al., 2015). Also introducing factors in a sequential manner (Oct4-Klf4; c-Myc; Sox2) has been shown to improve reprogramming (Liu et al., 2013). In addition, the choice of somatic cell can also affect the process, since they exhibit different epigenetics and expression profiles.

The delivery method can range from virus-based integrative methods such as a retro- or lentivirus, to non-integrative protein introduction. Each of these has its own advantages and downsides (Brouwer, Zhou, & Nadif Kasri, 2015). The most commonly used method is the use of a retrovirus, being very efficient. The disadvantage of this method however, is that it involves genomic integration, thereby altering the hosts genome and leading to the possibility of transgene expression. Also, the use of a retrovirus is dependent on the type of somatic cell, since it requires cell division in order to integrate. At first several of these retroviruses, each containing a single factor, needed to be used for the integration of all factors. Later, this was solved by a lentivirus that contained all reprogramming factors, reducing the number of integration sites and thus increasing safety (Carey et al., 2008).

By now, ways to circumvent these safety issues have been established. These are the non-integrative delivery systems such as adenovirus, Sendai virus, episomal vectors, and direct protein delivery. The main disadvantage of these methods however, is that they are all highly inefficient, and require multiple transfections (Brouwer et al., 2015).



One of the main recent breakthroughs in molecular biology, site-specific gene editing by *CRISPR*-Cas9, also has its influence on iPSC disease modeling. As described by Shi, Inoue, Wu and Yamanaka in a 2016 review on iPSC technology: "This gene editing technology enables researchers to introduce disease-causing mutations into wild-type iPSCs and to eliminate such mutations in patient iPSCs to create isogenic controls for iPSC-based disease modelling" (Shi, Inoue, Wu, & Yamanaka, 2016). For AML this means that it would be easier to model the relative contribution of specific gene mutations to disease pathogenesis.

Figure 2. *Overview of iPSC-based disease modeling, including gene-editing* (Shi et al., 2016).

In summary, iPSCs technologies have made rapid improvements in the twelve years since discovery. These include enhanced efficiency, better insights in the effects of different factors and modes of introduction. Next to that, advancements in gene-editing have simplified the means to compare iPSC models with controls, thereby strengthening their scientific value as a research model.

iPSCs as a research model

So far, iPSCs have been used to study many different diseases of which a cell type relevant to that disease was derived. Most research has been done with modeling neurodegenerative diseases such as Parkinson disease and Alzheimer disease, as well as with cardiovascular diseases (Ebert, Liang, & Wu, 2012). These have in common that they are mainly composed of a single, matured cell type, making the model easy to culture. For more sophisticated models, containing multiple cell types, organoids have been created with an iPSC origin (Takebe et al., 2013).

Hematological disorders have also been studied with the use of iPSC models. Cells were derived from human chronic myeloid leukemia (CML) cells, and these were found to be pluripotent despite their oncogenic mutations (Carette et al., 2010). However, although the parental cell line had an oncogene addiction – a dependence of continuous signaling of BCR-ABL – the iPSCs did not have this dependency and were thus resistant to BCR-ABL inhibitor imatinib. This indicated that epigenetics also plays a role, something that can be a major downside to the use of iPSCs as a reliable research model. On the other hand, Amabile et al. have shown that in CML, by using CML cell lines and leukemic iPSCs, genetic and epigenetic alterations are both required to maintain leukemic potential (Amabile et al., 2015).

In addition to the above-mentioned use of iPSC technology, it can also be used to screen drugs for both efficacy and toxicity, thereby contributing to the ideal of personalized medicine (Ebert et al., 2012). For both, the main advantage is that it enables researchers to copy patient specific phenotypes to a culture, also for cells that are hard to access. Drugs can also be screened on multiple, disease-relevant cell types.

Hematopoiesis and Acute Myeloid Leukemia

Normal hematopoiesis

Hematopoiesis is the formation of differentiated blood cells out of hematopoietic stem cells (HSCs). These HSCs reside in the bone marrow and have the ability to give rise to the full spectrum of blood cell types as well as self-renewal (Alexander & Frenette, 2016). The main purpose of blood, is transportation. Both cells, that are required for immune responses, and oxygen molecules are carried through the body and delivered at their destination.

Blood can be divided into two main cell lineages, both derived from the HSC. First there is a lymphoid branch, that gives rise to cells of the adaptive immune system, B and T lymphocytes, and natural killer cells. Next to that is a more diverse branch of myeloid cells. From a common myeloid progenitor, erythrocytes and thrombocytes arise. The former of which is responsible for the transportation of oxygen and the latter for initiating a blood clot in case of bleeding. The myeloid progenitor can also differentiate into a myeloblast, which is in turn a common progenitor for cells of the innate immune system (Elisa & Dick, 2012).

The production of different hematopoietic cell types is very precisely regulated. This happens under the influence of different growth factors and transcription factors in the bone marrow. Cells

generally only move to the peripheral blood afterwards, upon their maturation (Lapidot & Petit, 2002).

Acute myeloid leukemia

AML is a cluster of diseases which is characterized by a clonal expansion of malignant, poorly differentiated myeloid cells in bone marrow and peripheral blood. This results in a loss of blood function typically leading to fatigue and weight loss, and, if left untreated, is fatal within months of diagnosis by infection or bleeding (De Kouchkovsky & Abdul-Hay, 2016). AML manifests itself primarily in patients aged over 60, although a small subset concerns children and young adults. Due to the former group's inability to undergo intensive chemotherapy, their cure rate is only 5-15%, with that of the latter group being 35-40% (Döhner, Weisdorf, & Bloomfield, 2015). This is partly due to a high relapse frequency.

One of the difficulties in managing AML is its profound heterogeneity, both inter- and intrapatient. Current classification is still primarily based on cytogenetic differences (Arber et al., 2016), and since the recognition of AML's great molecular heterogeneity has only gained momentum since the start of the millennium, translation to the clinic still proves to be a challenge (Döhner et al., 2015). Recent progress in understanding the molecular pathogenesis of AML has given rise to the possibility of targeted treatment, and this approach is gradually incorporated in prognostics (Grossmann et al., 2012). However the prospected clinical value this may have, initial treatment approach has remained largely unchanged with chemotherapy and possibly stem cell transplantation. Moreover, advancements in genetic and epigenetic analysis have shown even more diversity in AML, and therefore show the importance of taking even larger steps towards individualized therapy (Kadia, Ravandi, Cortes, & Kantarjian, 2015).

The origin of AML can be found in several different causes. The first possibility is that it occurs as a consequence of prior therapy. DNA damage is induced by chemotherapies such as alkylating agents, topoisomerase-II-inhibitors and antimetabolites, or by ionizing radiation, both leading to the disturbed hematopoiesis that characterizes AML (Sill, Olipitz, Zebisch, Schulz, & Wölfler, 2010). Next to prior therapy it can also be induced by environmental toxins. Thirdly, it can be a result of underlying hematological disease, such as myelodysplasia (MDS), where it progresses from low risk MDS to high risk MDS before manifesting itself as secondary AML. Finally AML can occur *de novo* in healthy individuals as a result of the accumulation of mutations. This accounts for the commonly late onset.

Genetics and epigenetics

In order to question the viability of iPSCs as a model in AML, first the genetics and epigenetics that play a role in the disease must be identified. Since the reprogramming of cells might involve the destruction of parts of these landscapes, it is good to know the respective genomics and epigenomics that are connected to the disease and their biological role, to conclude which functions might be properly tested with such a model, and which might be suspect to disturbance. This mapping in adult *de novo* AML was done in 2013 by The Cancer Genome Atlas Research Network (TCGA). Genomes of 200 samples that were clinically diagnosed with adult *de novo* AML were sequenced, as were RNA and microRNA, and DNA-methylation analysis was performed (The Cancer Genome Atlas Research Network, 2013).

First of all, it is suggested that somatic mutations play a role in AML pathogenesis. This conclusion is drawn from the fact that over the past few decades structural chromosome variations have been found as trustworthy diagnostic and prognostic markers. On the other hand, there is a big group

of nearly 50% that has a normal karyotype of which many lack structural abnormalities. New sequencing techniques have marked mutations in *FLT3*, *NPM1, KIT, CEBPA, TET2, DNMT3A* and *IDH1* as recurrent, but there are also patients that do not carry mutations in any of these recognized driver genes (Shen et al., 2011).

Previously, patients have been classified based on a cytogenetic risk profile. This included a favorable risk profile (*PML-RARA, RUNX1-RUNX1T1,* or *MYH11*-CBFB fusions), an intermediate risk profile (mostly normal karyotype), and an unfavorable risk profile (monosomy or complex alterations). Because therapy outcomes between intermediate risk varies greatly, studies have focused on specifying this category by means of establishing new biomarkers. However, this had not led to completely accurate classification schemes. Improving these schemes was listed as the aim of the authors of the TCGA paper.

The 200 samples were selected in a manner that they represented a realistic subtype distribution. Copy number alterations were, as expected, primarily found in samples that were classified as unfavorable risk. Furthermore, they sought for mutations in coding sequences. Somatic variants were divided into four tiers, with tier 1 mutations being the most likely to have influence on biological function. These were the mutations that were found in coding regions of the genome, and in those an average of 13 mutations per sample was observed. A small number (3) of samples contained no variants in the coding regions, but these all contained fusions known to initiate AML.

Next, the authors looked at recurrent tier 1 mutations of samples grouped according to known risk profile, fusion events or *TP53* mutations. The mean of all samples for these recurrent tier 1 mutations was 5.24, while that of the groups carrying *MLL* and *PML-RARA* was significantly lower (2.09 and 3.25 respectively). This lead to the suggestion that these subgroups might be less dependent on other mutations for AML initiation (figure 3). However, sample sets were not sufficiently big enough to draw strong conclusions from this. With a test were then 23 genes identified that had a significantly elevated mutation prevalence, including genes known and unknown to play a role in AML pathogenesis, and many involved in the epigenetic machinery.

After sequencing the whole genome of 50 samples, there turned out to be a strong correlation between the number of mutations in coding and non-coding regions, suggesting a random distribution of mutations. This is in line with earlier studies, that reported that most mutations in AML occur randomly in HSPCs, before the initiating event, being the reason that AML mutations are present in almost all cells in AML samples (Welch et al., 2012). More than halve of the samples however, did have a subclone next to its founding clone, and one sample had two (The Cancer Genome Atlas Research Network, 2013). This heterogeneity within has important consequences since they might carry different optimal therapeutic targets.

For further analysis mutations were grouped into groups according to biological function. This showed that recurrent mutations in some genes were mutually exclusive with fusion events, showing they might have an equal role with regard to AML initiation as transcription factor fusions. Besides this mutual exclusivity there were also genes that significantly co-occurred (*NMP1* and *DNMT3A, NMP1* and *FLT3)*. Mutations in different biological classes were found to be mutually exclusive as well, suggesting alteration of one of these pathways suffices for the pathogenesis of AML.

Lastly, microRNA, mRNA and DNA methylation analysis showed significant differences between subgroups. A combination of *NPM1, DNMT3A* and *FLT3* mutations showed a unique expression pattern. This confirms the hypothesis that not only genetic but also epigenetic features can specify

AML, leading the authors to suggest that based on these epigenetic features the above combination could be identified as a new subtype.

These results together show the heterogeneity of the disease, in terms of both genetic and epigenetic features, as well as the existence of subclones. As this decreases the possibility of a proper, representative, research model, iPSCs might be relevant for future AML research.

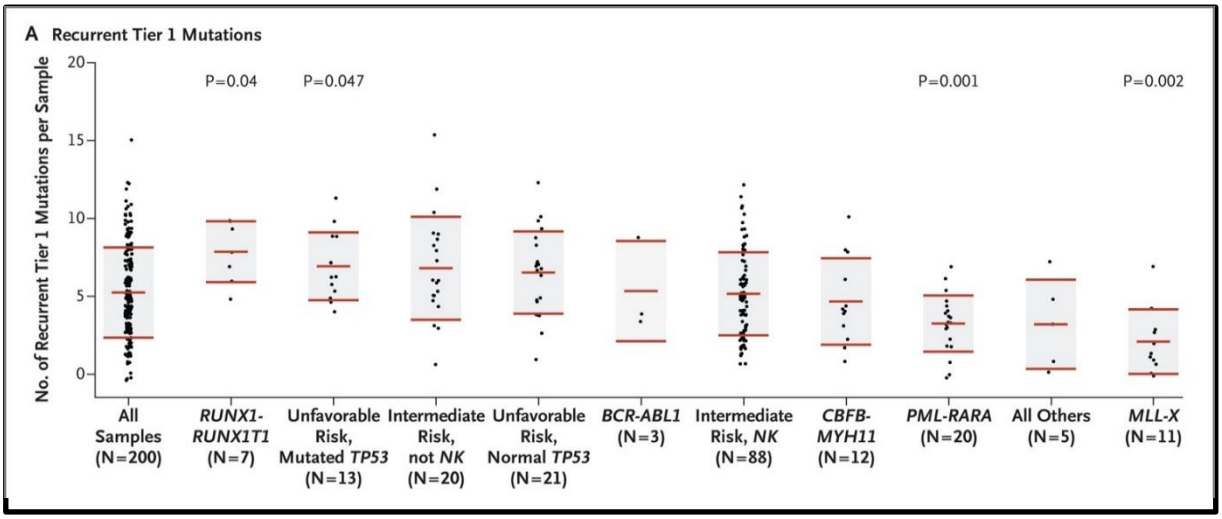


Figure 3. *Recurrent tier 1 mutations per group* (The Cancer Genome Atlas Research Network, 2013).

Induced Pluripotent Stem Cells as a Model for Acute Myeloid Leukemia

Reprogramming

Research encompassing iPSCs derived from AML samples has so far been rather limited. Recently however, several studies have been conducted in which iPSCs have been constructed from somatic AML patient cells. Firstly, researchers lately established an iPSC line derived from leukemia-infiltrated skin cells (Zhu et al., 2017). These were taken from an adolescent M6 AML patient, having a normal karyotype and no leukemia fusion genes. With ESCs as a positive control is was found that the skin-derived iPSCs showed pluripotency by analyzing pluripotency marker expression. Furthermore, iPSCs matched their somatic paternal cells in terms of karyotype after ten generations of cell culturing (figure 4).

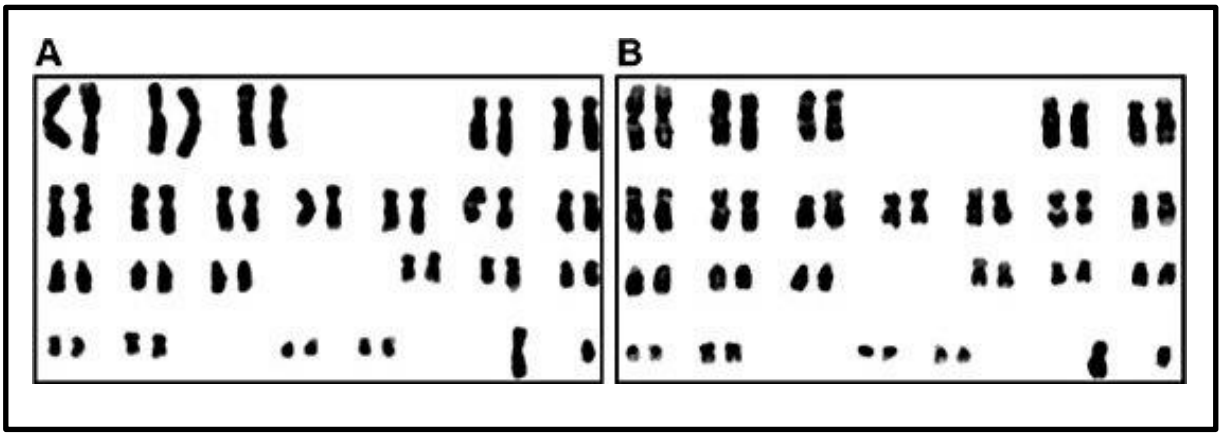


Figure 4. *Showing equal karyotype of somatic skin cells (A) and iPSCs (B)* (Zhu et al., 2017).

Injection of the iPSCs in mice led to teratoma formation carrying all three germ layers, hence proving their differentiation potential. Unfortunately, iPSCs were not differentiated back to

somatic cells in this study. As such, it can prove to be of use for the development of iPSC-based stem cell transplantation and this is further suggested by the fact that known leukocyte antigens showed negative or decreased expression in iPSCs. For disease modelling and drug discovery however, these results carry little direct scientific weight.

Despite this small success, difficulties with reprogramming AML cells are reported (Lee Jong-Hee et al., 2017). In this study, multiple subtypes (13) of AML were used to record their reprogramming viability. These subtypes were scattered across risk stratification representing favorable and unfavorable as well as intermediate risk profile subtypes. Out of 16 patient samples, only seven samples were able to produce iPSCs. From these seven again, only one sample was found to give rise to iPSC colonies that originated from the transformed AML cells. The remainder did produce iPSCs, but these did not derive from the AML cells (figure 5).

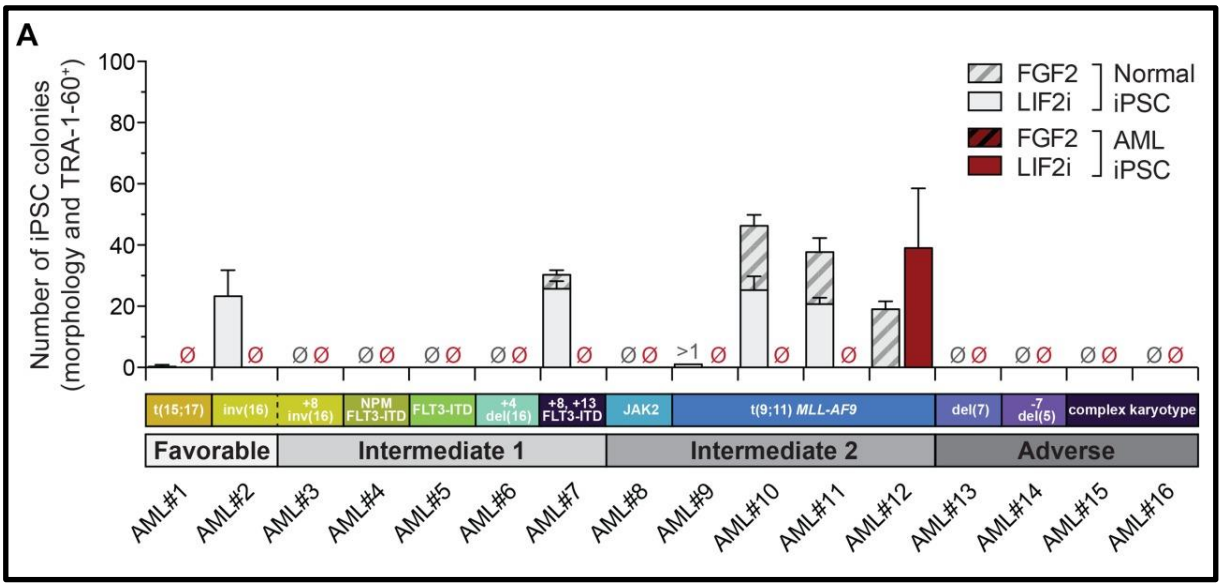


Figure 5. *Only one from sixteen samples gave rise to AML-iPSCs* (Lee Jong-Hee et al., 2017).

The single sample that was successfully reprogrammed carried the MLL-AF9 rearrangement, and this fusion was seen in all analyzed clones. Interestingly, the MLL rearrangement is not solely responsible for successful reprogramming, since other samples (#9, #10 and #11, figure 5) with the same karyotype did not induce pluripotent AML clones. Further investigation of these AML iPSCs showed a potential for differentiation to the hematopoietic lineage, but terminal differentiation was strongly blocked, similar to the primary AML sample cells.

Given the rarity of reprogramming and its limitation to MLL mutations, the authors also investigated the influence of the MLL-AF9 fusion product on this reprogramming. Overexpression of both MLL-AF9 and Yamanaka factors in regular AML patient samples showed no improvement on successfully inducing pluripotency. Only normal iPSCs were generated and no AML iPSCs. This suggests that reprogramming preference for cells devoid of AML aberrations is due to other factors, potentially correlated with MLL rearrangements.

Resemblance of primary samples

Another recent study by Chao *et al.* involving iPSCs and AML focused primarily on the epigenetic contribution to AML pathogenesis. Due to the epigenetic reprogramming that is entailed by iPSC generation, the authors were provided a platform to investigate relative contributions of both genetics and epigenetics to AML (Chao et al., 2017). This study was performed using two samples of AML, both harboring MLL rearrangements again. These rearrangements were also present in

multiple iPSC clones per sample and no additional chromosomal aberrations, demonstrating the ability to reprogram these MLL rearranged AML cells without altering their genetic properties.

Next, these AML iPSCs were differentiated into hematopoietic progenitors. Progenitor cells from control iPSCs turned out to form both myeloid and erythroid colonies, whereas those from AML iPSCs gave rise to mostly myeloid colonies. Transplanting these AML iPSCs *in vivo* led to an aggressive myeloid leukemia in mice, of which the engrafted cells matched both the mutations and immunophenotype of the patients' original leukemic blasts.

With the reacquisition of these leukemic properties given the authors turned toward the effect this has on DNA methylation and gene expression. DNA methylation arrays showed a resemblance between hematopoietic cells that were derived from AML-iPSCs, primary AML cells and engrafted patient AML cells. Furthermore, it was shown that undifferentiated AML iPSCs had significantly different methylation patters when compared to primary AML samples. Notably, primary AML samples showed similar DNA methylation patters relative to differentiated AML iPSCs, both demonstrating hypermethylation of pluripotency gene sets and hypomethylation of the hematopoietic and leukemic gene sets. Similar results were obtained from gene expression analysis. In sum these results show that DNA methylation and gene expression patters were reset after reprogramming, but later reacquired upon differentiation.

Drug screenings were also incorporated in the studies. The main outcome of this was that targeting the rearranged *MLL* by a *DOT1L* inhibitor provided different results, based on differentiation state of the AML iPSCs. Besides this, subclones of the samples were compared after reprogramming and differentiation. This led to the observation that *KRAS* wild-type subclones were more resistant to chemotherapy than the *KRAS* mutant subclone, something indeed witnessed in the clinic. The *KRAS* mutant, which had been dominant at diagnosis of this patient, was absent in the sample taken at relapse.

A fourth paper recently published concerning AML and iPSCs specified stages of MDS/AML progression (Kotini et al., 2017). Since progression of AML from MDS is, in contrast to *de novo* AML, has been no major subject of study. Reasons for this reside in the limited availability and poor growth of primary MDS samples.

IPSC lines were generated from four patients (one low risk MDS, two high risk MDS, one secondary AML). Here also, the AML patient harbored an *MLL* rearrangement. A representative panel of iPSC lines was then selected to characterize the different disease stages. Based on cell surface markers evidence was found of a delay in differentiation of low risk MDS iPSCs that further increased in high risk MDS, while MDS/AML iPSCs differentiation was blocked after the HPC stage. This disease-stage specific phenotypic difference was also observable after transplantation in mice. Transplantation of HPC of normal iPSCs and those of both low and high risk MDS showed no engraftment potential. MDS/AML HPCs however, exhibited high levels of engraftment in multiple animals and ultimately gave rise to an AML phenotype. This shows that the iPSCs of different disease stages produce a phenotypic resemblance with transplantable leukemia at the end of the spectrum.

Finally, the authors tried to model the stage-specific drug response of 5-Azacytidine (5-AzaC), a current first-line therapy in MDS with a 30-50% response rate (Kotini et al., 2017). Biomarkers to predict responders are, however, limited (Bejar & Steensma, 2014) illustrating the possible significance of iPSC-based models. HPCs from different stages were cultured in presence of absence of 5-AzaC to test for the effect of the therapeutic on differentiation. This led to the

observation that 5-AzaC rescued certain myeloid colonies in low risk MDS, while having no effect in normal or other stage iPSC lines. Oppositely, in high risk MDS, 5-AzaC selectively inhibited growth of the MDS clone, without having that effect in the other iPSC derived lineages. Together these results show a differentiation in drug effectiveness on the basis of disease stage, thereby confirming the potential of iPSCs as model for drug screening.

Discussion

Progress in iPSC-based research has so far been limited to few diseases. This is not surprising given the fact that they were first reported only little more than a decade ago. Despite this, their potential has been globally recognized giving researchers a platform to model diseases with effective genetic aberrations. They provide a solution to multiple difficulties previously experienced, including ethical considerations and sample scarcity.

One of the reasons iPSCs could be of interest for AML research is the disease's heterogeneity, both inter- and intrapatient, as demonstrated earlier. (The Cancer Genome Atlas Research Network, 2013). IPSCs could be of use in modeling the pathogenesis of specified subtypes based on both genetic and epigenetic characteristics. It should be noted however, that iPSCs as a model for AML has only recently celebrated its first minor successes, and large-scale research has not been conducted yet. This is visible in the limited number of papers reporting on iPSC cell lines derived from AML samples.

The initial step, induction itself, already proved problematic (Lee Jong-Hee et al., 2017). Although it is possible to induce some AML cells to iPSCs, the means to achieve this should be carefully considered. While the first generation iPSCs were still generated using invasive retro- or lentivirus methods, new technologies such as the use of a non-invasive Sendai virus should prevent iPSC cell lines to have genomic alterations relative to their primary cell samples (Fusaki, Ban, Nishiyama, Saeki, & Hasegawa, 2009).

As mentioned, reprogramming AML samples is difficult and success only concerned samples harboring *MLL* rearrangements, which in total make up about 10% of AML incidences (Winters & Bernt, 2017). The underlying biology of this refractoriness to reprogramming is something that requires attention for the coming decade, if the potential of iPSCs in AML research are to be fully exploited. One could argue that epigenetics are most likely to play a big role here, since many mutations in AML are found to be in epigenetic machinery. Also, the common abnormality in the AML samples that were reprogrammed is *MLL,* being a transcription regulator itself (Rolf, 2011).

Regardless the practical complications of reprogramming, iPSC lines that were successfully generated did seem to resemble the samples they were derived from, genetically and phenotypically, and both *in vitro* and *in vivo* (Chao et al., 2017; Kotini et al., 2017; Lee Jong-Hee et al., 2017). This does not automatically mean however, that they are able to mimic patient, stage, or subclone specific AML cells. As discussed, AML is a highly heterogeneous disease with, next to known driver mutations, epigenetics playing a complex role in pathogenesis too (The Cancer Genome Atlas Research Network, 2013). Yet, only one study investigated the subclone-specific resemblance of iPSC lines and results were positive (Chao et al., 2017), but it is too early to draw any conclusion from this.

Since reprogramming involves the destruction of epigenetic DNA methylation patters, the resemblance of these patterns should be of increased interest when comparing cells derived from AML iPSCs and primary leukemic blasts. Interestingly, these DNA methylation patterns and gene expression, though reset when generating the iPSCs, were reacquired upon maturation (Chao et

al., 2017). Although the experiments were only performed with a limited number of patient samples this could hold promising implications for the use of iPSCs as a research model in AML. These implications are strengthened by the strong upcoming of gene editing techniques. These could simplify the research by enabling researchers to verify their findings using control groups that are modified by *CRISPR/*Cas9.

Taken together, it must be concluded that AML pathogenesis and drug sensitivity research can benefit greatly from the presence of a model that mimics its characteristics, is easily edited and widely available. iPSCs can be this model, but at this point this is too early to say. For *MLL*-harboring AML prospects are positive, though further research is required, starting with the biological mechanisms underlying variation in reprogramming potential.

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