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Human cell models for inherited platelet disorders: strengths, limitations and emerging technologies

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Abstract

Inherited platelet disorders (IPD) comprise a clinically and genetically heterogeneous group of conditions affecting platelet number, function, or both. Although next-generation sequencing has accelerated gene discovery, it has also produced a growing number of variants of uncertain significance, creating a pressing need for experimental systems that can establish causal genotype–phenotype relationships. Because megakaryocytes are scarce in the bone marrow and murine models incompletely recapitulate human platelet biology, human cell-based models are indispensable for studying megakaryopoiesis and platelet formation. Here we review the evolution of such models and their application to IPD research. Leukemia-derived megakaryocytic cell lines provided the first genetically tractable models but incompletely reproduce physiological thrombopoiesis. Megakaryocytes derived from primary hematopoietic stem cells remain the gold standard for patient-specific disease modeling, offering high biological relevance but requiring donor material and being subject to significant inter-donor variability. Induced pluripotent stem cells provide a renewable source of megakaryocytes for disease modeling, although efficient platelet production and functional characterization remain challenging. In contrast, the immortalized megakaryocyte cell line offers a scalable and genetically tractable platform that faithfully recapitulates megakaryocyte maturation, supports platelet production, and enables selected platelet function assays. We further discuss emerging three-dimensional and organoid systems that begin to capture the complexity of the bone-marrow niche. Across these platforms we discuss applications in disease mechanism discovery, functional validation of novel variants, and preclinical evaluation of therapies. No single model is universally optimal; rather, model choice should be guided by the biological question, with validation against primary patient material remaining essential.

Introduction to megakaryopoiesis, platelet function and inherited platelet disorders

Humans generate around 10^{11} platelets per day, making platelets one of the most abundant blood cell types.¹ In the bone marrow, hematopoietic stem cells (HSCs) undergo three important developmental stages to produce platelets (Figure 1). The first phase determines the lineage fate and is driven by the interaction of thrombopoietin (TPO) with its receptor cMPL and the upregulation of transcription factors RUNX1, GATA-1 and FLI1.² In the classical hematopoietic hierarchy, HSCs differentiate into common myeloid progenitor cells and subsequently to bipotent megakaryocyte-erythroid progenitors, from which megakaryocytes arise. This stepwise route is, however, not obligatory. Lineage-tracing studies have shown that a subset of HSCs is megakaryocyte-primed and can generate megakaryocytes directly, effectively bypassing the intermediate progenitor stages and unipotent megakaryocyte progenitor have been identified.³ These two parallel routes converge into a common downstream pathway. Megakaryocyte commitment is accompanied by the expression of lineage-specific markers, beginning with integrin α_{IIb} (CD41) and integrin β_3 (CD61), and followed at later stages by GPIX (CD42a) and GPIb α (CD42b). Next, megakaryocytes undergo a cytoplasmic maturation and polyploidization phase. In this phase, megakaryocyte markers CD61 and CD41 increase and megakaryocytes undergo endomitosis, during which the cells replicate DNA without cytokinesis and become polyploid (up to 128N).² Lastly, mature megakaryocytes undergo membrane reorganization and form proplatelets.³ These proplatelets have filamentous structures with bulbous ends from which they split of platelets into the blood stream. Here the platelets remain in their resting state until a vessel wall damage is detected. As a result of the vessel damage, von Willebrand factor (VWF) becomes exposed, to which platelets will start to adhere. Platelet adhesion triggers intracellular signaling cascades, leading to platelet activation. Activation is characterized by a shape change from discoid to spherical with the extension of pseudopodia, degranulation of alpha (α)-granules and dense granules, and the release of soluble mediators such as ADP, thromboxane A2 and serotonin. These mediators act in an autocrine and paracrine fashion to recruit and activate additional platelets, thereby amplifying the response.^{2,3}

Germline variants in platelet related genes can disturb the delicate process of hemostasis and result in inherited platelet disorders (IPD).⁴ Broadly, they can be subdivided into two main groups. The first group, platelet function disorders, is, as the name suggests, defined by impaired platelet functioning. The second group, inherited thrombocytopenia, is caused by an impaired platelet formation and is characterized by a platelet count below 150 000 platelets per μ L blood. However, these two groups are not mutually exclusive, as patients with thrombocytopenia often also exhibit functional defects. Patients with an IPD commonly suffer from variable degrees of mucocutaneous bleeding symptoms and excessive postoperative or trauma-related bleeding, among other clinical symptoms outside the blood system.⁵ To date, more than 70 genes have been identified as being involved in platelet production and function. However, the precise roles of many of these genes remain only partially understood.⁶ Due to this genetic and clinical heterogeneity, IPD remain difficult to diagnose and can be diagnosed incidentally, as not all patients present obvious bleeding symptoms.^{6,7} Thus, studying IPD in the laboratory is important for improving our understanding of the genes that regulate megakaryopoiesis and platelet formation.

Why are cell-based disease models needed for IPD research?

There is a growing need for reliable cell-based disease models for IPD to improve our understanding of disease mechanisms and facilitate the interpretation of variants of uncertain significance (VUS) identified through next-generation sequencing approaches.^{4,6} Such models can enable the functional validation of novel variants by allowing direct assessment of their impact on megakaryocyte differentiation, platelet production, and platelet function. Although multigene panels used to screen IPD patients have substantially increased diagnostic yield, they have also led to the identification of numerous VUS, which account for approximately 50% of all detected

variants.⁸ While *in silico* predictions can provide indications of pathogenicity, experimental models are required to establish causal relationships between genotype and phenotype. Cell-based systems enable the investigation of how specific variants affect processes such as protein expression, intracellular signaling, cytoskeletal dynamics, and proplatelet formation, thereby distinguishing pathogenic (functional) variants from benign (nonfunctional) variants. In addition, disease models provide a valuable platform for investigating newly implicated genes and elucidating the molecular pathways that govern megakaryopoiesis and platelet biology. Many genes associated with IPDs remain poorly characterized, and their contributions to platelet formation and function cannot be fully understood without experimental interrogation.⁶ By introducing gene knockouts or patient-specific DNA variants into relevant cellular systems, researchers can identify disrupted pathways, characterize interacting partners, and uncover broader regulatory networks. This not only enhances mechanistic insight into disease pathogenesis but also contributes to a more comprehensive understanding of normal megakaryopoiesis and platelet biology. Finally, disease models constitute an important platform for the development and preclinical evaluation of novel therapeutic strategies. They enable the testing of pharmacological compounds as well as gene-correction approaches, including CRISPR/Cas9-mediated genome editing aimed at restoring platelet production or function.⁹ Importantly, patient-specific models enable personalized approaches, where therapeutic efficacy and potential off-target effects can be assessed within a genetically relevant context. Consequently, these models have the potential to facilitate the translation of basic discoveries into clinically relevant therapies and to advance precision medicine in the management of IPD.

Mouse models were developed as one of the first models to study IPD and have provided important insights into understanding disease pathogenesis.¹⁰ However, creating a mouse model to study multiple individual variants is often time-consuming and not cost-effective. Additionally, their ability to recapitulate the human disease phenotype varies, with some models closely resembling patient manifestations while others only partially reproduce the clinical or molecular features of the disorder.¹⁰ These discrepancies may reflect species-specific differences in megakaryocytes and platelet physiology, as well as differences in gene function or regulation between mice and humans.^{10,11} In addition, many mouse models are often based on complete knockouts, which may not accurately mimic the effects of specific patient-associated variants, such as specific missense variants or gain-of-function defects.^{10,12} These aspects are next illustrated by some examples. Megakaryopoiesis differs between mice and humans: mice have approximately threefold higher platelet counts ($\sim 800\text{--}1200 \times 10^9/\text{L}$ vs. $\sim 150\text{--}400 \times 10^9/\text{L}$ in humans), smaller platelets (mean platelet volume $\sim 5\text{--}7$ fL vs. $\sim 7\text{--}12$ fL in humans), and a shorter platelet lifespan ($\sim 4\text{--}5$ days vs. $\sim 7\text{--}10$ days in humans).¹³ Murine platelet function differs from that of human platelets in several important respects. For example, mice platelets need higher concentration of ADP to achieve aggregation comparable to human platelets. In addition, disease-specific mouse models also often fail to fully recapitulate the corresponding human phenotypes. For instance, *Itgb3*^{-/-} mice exhibit mild microcytic hypochromic anemia and splenomegaly due to extramedullary hematopoiesis, whereas these non-platelet manifestations are not present in patients with Glanzmann thrombasthenia.¹⁴ Likewise, *Sln14* K208N knock in mice, carrying the murine homolog of the pathogenic human K192N variant associated with thrombocytopenia, exhibit severe anemia, splenomegaly, and abnormal thrombus formation, but show no major platelet defect or bleeding phenotype.¹² Similarly, *Src* knock out mice show impaired osteoclast function but no bleeding or platelet phenotype while in humans, *SRC* gain-of-function variants cause syndromic thrombocytopenia.¹⁵ Heterozygous *Myh9* knockout mice develop hearing deficits but do not exhibit the macrothrombocytopenia characteristics of patients with MYH9-related disease.¹⁶ Lastly, mice haploinsufficient for *Runx1* display at most mild platelet abnormalities and do not spontaneously develop myeloid malignancy, meaning a cardinal feature of *RUNX1* deficiencies in humans is absent in this model.¹⁷

Therefore, human cell-based models are often preferred for studying human platelet disorders and variant-specific mechanisms. In addition, generating and characterizing mouse models is time-consuming, costly, and difficult to scale, particularly because many VUS identified in patients are often case-specific, making the generation of an individual mouse model for each variant impossible. These limitations have increased the need for accessible and scalable human *in vitro* models to study IPDs. However, studying primary human megakaryocytes also presents significant challenges. Less than 1% of all cells in the bone marrow are megakaryocytes, making their isolation for further laboratory analysis not only highly invasive, but also technically difficult.¹⁸ Although advances in thrombopoiesis and platelet-generation technologies have recently been reviewed in detail¹⁹, including *in vivo* platelet biogenesis, *ex vivo* platelet production systems, and transfusion applications, comparatively less attention has been devoted to systematically comparing the strengths and limitations of the human cellular platforms currently used to study IPDs. This review addresses this gap by providing an in-depth overview of the evolution and use of human cell-based models for IPD research, including established leukemia cell lines, HSCs, induced pluripotent stem cells (iPSC) and the immortalized megakaryocyte cell line (imMKCL) (Figure 2). We discuss how these models have been applied to investigate platelet-related genes and disease mechanisms, as well as their emerging roles in VUS interpretation, functional validation, and therapeutic compound screening.

The first-generation cell models: leukemia-derived megakaryocytic cell lines

Human hematopoietic leukemia-derived cell lines with megakaryocytic characteristics, including K562 (chronic myeloid leukemia), HEL (human erythroleukemia), MEG-01 (megakaryoblastic leukemia), DAMI (megakaryoblastic leukemia-derived) and UT-7 (megakaryoblastic leukemia) cells were among the first *in vitro* models used to study megakaryopoiesis without reliance on primary donor material.²⁰ In this section, we summarize their characteristics and applications for IPD research.

K562 is a human chronic myeloid leukemia-derived multipotent hematopoietic cell line established in 1975 by Lozzio and Lozzio from the pleural effusion of a patient with chronic myeloid leukemia in blast crisis.²¹ The cells exhibit rapid and sustained proliferation in culture. K562 cells express low levels of key megakaryocyte markers, such as integrin $\alpha_{IIb}\beta_3$ and GPIb α , and α -granules proteins including PF4 and VWF. However, they remain arrested in a promegakaryoblast stage and lack dense granules. The development of K562 cells paved the way for the generation of additional leukemia-derived cell lines with megakaryocytic characteristics.

The human erythroleukemia (HEL) cell line, which displays similar properties, was derived from a patient with acute myeloid leukemia post-Hodgkin lymphoma.²² Both K562 and HEL cells exhibit features of megakaryocytic, erythroid and macrophage lineages, rendering them useful in chronic myeloid leukemia research. Although stimulation with phorbol 12-myristate 13-acetate (PMA) can induce an increase in cell size and polyploidy, particularly in HEL cells, these cell lines lack mature megakaryocytic characteristics and are therefore suboptimal models for studying later stages of megakaryopoiesis, such as proplatelet formation.

The UT-7 cell line was established by Komatsu *et al.* from the bone marrow of a patient with acute megakaryoblastic leukemia.²³ UT-7 cells are dependent on cytokines, such as GM-CSF, IL-3 or erythropoietin, which makes them suited for studying inherited cytopenia defects caused by cytokine receptor signaling. Basso-Valentina *et al.* used UT-7 cells overexpressing WT or mutant MPL to characterize the p.Arg464Gly variant identified in a patient with congenital amegakaryocytic thrombocytopenia, showing that the variant impairs receptor maturation and cell-surface localization, with reduced TPO-induced STAT3, STAT5, AKT and ERK phosphorylation and loss of TPO-dependent proliferation.²⁴ Co-expression of the mutant chaperone CALRdel52 restored MPL trafficking and rescued the response to eltrombopag. The

same group subsequently used UT-7 sublines expressing MPL, G-CSFR or EPOR to show that *ANKRD26* regulates type I cytokine receptor signaling by interfering with receptor internalization, so that the sustained *ANKRD26* expression found in patients prolongs downstream signaling.²⁵ Together these studies illustrate the value of UT-7 in researching receptor trafficking, signaling and drug responsiveness, although, like the other leukemia-derived lines, UT-7 does not undergo terminal maturation or physiological proplatelet formation.

The MEG-01 cell line was the first pure megakaryoblastic cell line, lacking B-, T-lymphoid and myeloid markers, and was established from the bone marrow of a patient with chronic myeloid leukemia in blast crisis.²⁶ MEG-01 cells are hyperdiploid and express megakaryocyte markers including $\alpha_{IIb}\beta_3$, VWF and fibrinogen, the expression of which is further increased following PMA stimulation. They were also the first cell line shown to produce some platelet-like particles (PLP) which display platelet-associated structures and markers, including integrin $\alpha_{IIb}\beta_3$.²⁷ However, PLP production remains highly inefficient, with only approximately 0.3 PLPs generated per megakaryocyte, compared with thousands of platelets normally released by mature bone marrow megakaryocyte *in vivo*. Consequently, MEG-01 cells have limited utility for accurately modeling physiological thrombopoiesis and platelet biogenesis. Štika *et al.* employed CRISPR/Cas9-mediated gene editing in MEG-01 cells to introduce a patient-specific variant (p.Thr20Ile) in *CYCS* and demonstrated that alterations in caspase activity and increased mitochondrial respiration were associated with *CYCS*-related thrombocytopenia.²⁸ In another study by Ferrari *et al.*, MEG-01 cells were transfected with p.Pro103Arg mutant *RUNX1* construct and functional assays revealed dysregulation of *ANKRD26* and impaired PLP production, thereby explaining the thrombocytopenic phenotype observed in patients carrying this variant.²⁹ Daimon *et al.* used lentiviral transduction to express *CDC42* p.Tyr64Cys mutation in MEG-01 cells to study PLP formation.³⁰ They observed shorter cytoplasmic protrusions, supporting their hypothesis that *CDC42*-associated aberrant actin modulation underlies the macrothrombocytopenia observed in affected individuals. Overall, these examples highlight MEG-01 cells as a useful cell model for studying thrombocytopenia mechanisms using diverse gene-editing approaches, although platelet formation assessment is largely restricted to PLP assays. Finally, DAMI cells were isolated from peripheral blood of a patient with megakaryoblastic leukemia.³¹ DAMI is the most extensively studied megakaryocyte cell line, with nearly all cells (>89%) uniformly expressing megakaryocyte markers integrin $\alpha_{IIb}\beta_3$. They are polyploid (13% >4N) and this increases upon PMA stimulation, which has contributed to DAMI cells becoming a widely used model for studying megakaryopoiesis. Fletcher *et al.* used transiently transfected DAMI cells with mutant *SLFN14* (p.Lys218Glu, p.Lys219Asn and p.Val220Asp) to study *SLFN14*-related thrombocytopenia, demonstrating that mutated *SLFN14* colocalizes with ribosomes and mediates rRNA endonucleolytic degradation.³² More recently, Song *et al.* employed DAMI cells to elucidate the role of CREG1 protein in megakaryocytes and confirmed that siRNA-mediated CREG1 knockdown inhibits PMA-induced megakaryocyte differentiation.³³ Overall, MEG-01 and DAMI cells are more megakaryocyte-specific than K562 and HEL cells and, although PMA stimulation can induce proplatelet-like protrusions and limited number of PLPs. An important limitation here is that PMA directly activates protein kinase C, resulting in cell cycle arrest, increase in cell size and upregulation of megakaryocytic markers.³⁴ This resembles megakaryocyte differentiation, but differs from TPO-induced differentiation and therefore does not fully reflect physiological megakaryopoiesis, proplatelet formation or platelet production. Notably, CRISPR/Cas9-mediated genome editing is considered more physiologically relevant than overexpression approaches, as it avoids non-physiological protein overexpression. Additionally, as no physiological platelets can be formed, functional testing is not possible with this model.

Megakaryocytes derived from primary hematopoietic stem cells for disease modeling

HSCs can be isolated from bone marrow, umbilical cord, fetal liver cells or peripheral blood, with slight differences across sources but broadly comparable overall megakaryocyte differentiation potential.³⁵ The easiest and least invasive approach is to isolate CD34⁺ HSCs from peripheral blood via magnetic bead isolation. Some studies have used CD45⁺ peripheral blood mononuclear cells for further differentiation, however, this approach is less commonly used, as it results in cultures with lower megakaryocyte purity and greater variability.^{36–38} Under the influence of TPO, these stem cells will differentiate into mature megakaryocytes, proplatelet forming megakaryocytes and eventually platelets within 10 to 14 days. Table 1 provides an overview of studies using patient-derived or genetically modified CD34⁺ HSCs to investigate IPD, highlighting their applications in elucidating disease mechanisms, functionally validating novel variants, and evaluating emerging therapeutic strategies.

HSC-derived megakaryocytes have primarily been used to elucidate disease mechanisms in IPD and are often among the first models employed to investigate newly discovered platelet-related genes. This approach has proven particularly valuable for genes such as *SLFN14*, *IKZF5*, *KDSR* and *DIAPH1*, which were initially identified through WES/WGS studies but whose roles in megakaryopoiesis and platelet biology remained incompletely understood.^{39–42} Similarly, HSC models have provided important mechanistic insights into established platelet genes, including *ETV6*, *MYH9*, *PLAU*, *RUNX1*, *RNU4ATAC*, *FLI1*, *GATA1*, *ANKRD26*, and *NBEAL2*, helping to unravel the molecular pathways underlying disease pathogenesis and megakaryocyte differentiation.^{43–53} Beyond mechanistic studies, patient-derived HSCs have proven valuable for the functional characterization of novel very specific variants in known platelet genes. Patient-derived HSCs have been used to characterize variants in *FLI1*, *GP1BA*, *ITGB3* and *SRC*, enabling direct assessment of variant-associated defects in megakaryocyte differentiation and platelet formation.^{15,36,54,55} In addition to platelet formation, this model allows platelet function to be assessed. Readouts such as PAC-1 binding, reporting $\alpha_{IIb}\beta_3$ activation, and spreading assays that quantify filopodia formation can clarify the defects underlying established function disorders such as Glanzmann thrombasthenia, as well as the functional defects seen alongside thrombocytopenia, for example in patients with pathogenic *ETV6* variants.^{43,55} A major advantage of this approach is that patient-derived cells recapitulate disease phenotypes without requiring genetic manipulation, facilitating the study of patient-specific disease mechanisms. More recently, genetic modification of HSCs using CRISPR/Cas9 has emerged as a powerful complementary strategy.^{50,56} Montenont *et al.* developed the CRIMSON platform, which utilizes CD34⁺-derived, CRISPR-edited megakaryocytes to investigate loss-of-function effects in platelet-related genes as well as VUS.^{50,56} By relying on healthy donor-derived HSCs, this approach reduces the need for patient samples while enabling stable genetic modification. However, because analyses are performed on unsorted pools of edited cells comprising mixtures of clones carrying different gene edits, phenotypic interpretation may be complicated by clonal heterogeneity.

In addition to disease modeling, HSC-derived megakaryocytes provide a physiologically relevant platform for the preclinical evaluation of therapeutic strategies, including pharmacological and gene-based approaches. CD34⁺-derived megakaryocytes have been widely used to investigate TPO receptor agonists, with eltrombopag representing the most extensively studied example. TPO is a key cytokine regulating both early and late stages of HSC differentiation and promotes megakaryocyte expansion, maturation and platelet production, making HSC-derived megakaryocytes an ideal system for evaluating novel TPO analogues.⁵⁷ HSCs have also played an important role in the development of gene therapies for IPD. Wilcox *et al.* demonstrated phenotypic correction of Glanzmann thrombasthenia using retroviral-mediated gene transfer in patient-derived cells.⁵⁸ Although retroviral vectors can restore gene function, their propensity for random genomic integration increases the risk of insertional mutagenesis. Lentiviral vectors

exhibit a more favorable integration profile and have subsequently been explored as a therapeutic strategy for disorders such as *GATA1* deficiency or Bernard-Soulier syndrome.^{59,60} A recent study by Lee *et al.*⁶¹ created a model for and *RUNX1*-related familial platelet disorder with associated myeloid malignancies, using shRNA on healthy donor HSCs, for preclinical testing for novel compounds. This model was used in testing RepSox, a small-molecule inhibitor of the TGF- β 1 receptor pathway, as a novel treatment option. This was the first demonstration that a drug reverses *RUNX1* deficiency-induced hemostatic defects, and it shows that HSC-derived megakaryocytes can support a full preclinical pipeline rather than mechanistic study alone. In addition to evaluating phenotypic rescue *in vitro*, genetically modified HSCs may ultimately serve as a source for transplantation-based therapeutic approaches.

Despite their biological relevance and broad applicability, HSC-based models present several practical and experimental limitations. Obtaining enough HSCs typically requires relatively large blood volumes, often ranging from 30 to 40 mL. This is a limiting factor when studying young children. Furthermore, patient-derived HSCs are generally compared with cells from healthy donors, who can exhibit considerable variability in differentiation capacity and proplatelet formation, potentially confounding experimental results. The interpretation of VUS may also be challenging, as patient-derived cells often contain additional genetic factors that can contribute to the observed phenotype. Raslova *et al.* addressed this limitation in Paris-Trousseau syndrome, which is characterized by *FLI1* haploinsufficiency, by using lentiviral-mediated *FLI1* overexpression to rescue the phenotype in patient-derived megakaryocytes and thereby strengthen causal inference.⁴⁸ Similarly, other groups have compared patient-derived megakaryocytes with healthy donor-derived cells engineered to express the variant of interest.^{15,43} However, such overexpression approaches carry the risk of non-physiological gene expression levels that may introduce artefacts and complicate interpretation of the resulting phenotypes.⁶²

Megakaryocytes derived from induced Pluripotent Stem Cells for disease modeling

Induced pluripotent stem cells (iPSCs) were first generated by Yamanaka and colleagues, who reprogrammed mouse fibroblasts in 2006 and human fibroblasts in 2007 using the four reprogramming factors Oct4, Sox2, Klf4 and c-Myc (Yamanaka factors).^{63,64} These factors reset somatic cells to an embryonic-stem-cell-like state, conferring pluripotency and unlimited *ex vivo* expansion. In 2010, Takayama *et al.* demonstrated that iPSCs can be differentiated into megakaryocytes and PLPs with *in vivo* functionality, establishing iPSCs as a powerful platform for modeling IPD.⁶⁵ Table 2 provides an overview of patient-derived and genome-edited iPSC models that have been used to study IPD, highlighting their applications in disease-mechanism discovery, variant interpretation, and therapeutic development.

iPSCs are now widely used to study the mechanisms of platelet related genes. Multiple groups have generated patient-specific iPSC lines to study established disorders, including Glanzmann thrombasthenia, Bernard-Soulier syndrome, Hermansky-Pudlak syndrome, congenital amegakaryocytic thrombocytopenia, platelet-type bleeding disorder 17, *FLNA*- and *MYH9*-related disease and familial platelet disorder with associated myeloid malignancies (Table 2).^{66–82} Beyond known conditions, iPSC models have also elucidated the function of newly implicated genes; for example, Bariana *et al.* used patient-derived iPSCs to uncover the role of *KDSR* in sphingolipid metabolism during platelet biogenesis.⁴¹ In parallel, CRISPR/Cas9 editing of healthy-donor iPSCs enables controlled introduction of specific variants in genes such as *GP1BA* and *RUNX1*, supporting systematic genotype–phenotype studies.^{83,84} Functional characterization is also possible in this model. Orban *et al.* observed similar defects in agonist-induced activation, spreading and adhesion and cytoskeletal rearrangement in blood-derived platelets and platelets formed by iPSC from patients with Glanzmann Thrombasthenia.⁶⁶ The limitations of this approach are illustrated by Vo *et al.*, who found that *in vitro*-generated platelet-like particles from iPSC-

derived megakaryocytes were only approximately 50% platelets and mostly injured, and therefore assessed platelet function on platelets released *in vivo* after infusing the megakaryocytes into mice.⁸⁵ The main constraint is the low platelet yield, which limits assays requiring large platelet numbers. Patient-derived iPSC also provide a robust platform for evaluating therapeutic strategies, including gene correction to personalized pharmacological screening. Sullivan *et al.* restored integrin $\alpha_{IIb}\beta_3$ expression and function in Glanzmann thrombasthenia by inserting an α_{IIb} transgene under the *GP1BA* promoter at the AAVS1 safe-harbor locus.⁸⁶ Similarly, Iizuka *et al.* corrected germline *RUNX1* mutations using TALENs, normalizing megakaryocyte differentiation and maturation.⁸⁷ Pharmacological approaches have also been explored: Krutein *et al.* showed that stabilizing *RUNX1* protein levels through CDK or proteasome inhibition improves megakaryocyte output in patient-derived models.⁸⁸ This system was also used on iPSC derived megakaryocytes from patients carrying heterozygous *GP1BA* variants (p.Asn103Asp, p.Leu160Pro, p.Asn150Ser), to prove ROCK1/2 inhibition with Y27632 increases platelet number and restored platelet size in megakaryocytes, but had no effect in biallelic BSS, indicating a distinct underlying mechanism.⁸⁹ Moreover, integrating iPSC-derived megakaryocytes with a 3D silk bone-marrow bioreactor allowed Di Buduo *et al.* to predict patient-specific responses to eltrombopag in *MYH9*-related disease, illustrating the potential of iPSC systems for precision medicine.³⁸

Directed differentiation of iPSCs closely mirrors physiological megakaryopoiesis and offers flexibility to generate additional hematopoietic lineages from the same starting material. However, conventional protocols typically require 3-5 weeks and often yield modest numbers of megakaryocytes with substantial batch-to-batch variability. To overcome these limitations Moreau *et al.* developed a forward-programming strategy in which doxycycline-inducible overexpression of *GATA1*, *FLI1* and *TAL1* directly reprograms iPSCs into megakaryocytes.⁹⁰ This approach produces mature megakaryocytes within ~7 days, increases yield up to 2×10^5 megakaryocytes per input iPSC, and enables long-term expansion of proliferative megakaryocyte progenitors. However, megakaryocytes derived from this model present with an embryonic signature.

The immortalized megakaryocyte progenitor cell line as a platform for platelet disease modeling

As previously mentioned, the leukemia-derived megakaryocyte-like cell lines are unable to form physiological (pro)platelets. Therefore, there is a need for a genetically modifiable, human megakaryocyte system that can proliferate robustly and undergo terminal maturation to form physiological proplatelets and platelets. With rising demand for platelet transfusions, driven largely by chemotherapy-induced thrombocytopenia, the imMKCL was developed to generate transfusion-grade *in vitro* platelets.⁹¹ This iPSC-derived cell line employs a doxycycline (Dox) inducible overexpression of *c-MYC*, *BCL-XL* and *BMI1* via TET-coupled upregulation, enabling continuous proliferation and apoptosis inhibition under Dox-ON conditions. Removing doxycycline from the culture medium (Dox-OFF) halts this overexpression and initiates imMKCL differentiation into more mature polyploid megakaryocytes in just four days of differentiation. These megakaryocytes are polyploid (20% of cells are $\geq 8N$) and almost all (>90%) day four differentiated imMKCL are CD41⁺ and CD42⁺.⁹¹ After five days, proplatelets can be observed and at day six platelets can be isolated. These platelets are able to induce clot retraction and show increased PAC-1 binding upon stimulation with agonists but they are larger than blood-derived platelets.⁹¹ In static culture, Ito *et al.* reported yields of ~18 platelets per megakaryocyte, increasing to ~24 platelets per megakaryocyte under turbulent culture conditions.⁹² More recently, Sugimoto *et al.* reported the first in-human transfusion of iPSC derived platelets in a patient with severe aplastic anemia and alloimmune platelet transfusion refractoriness.⁹³ No significant adverse events were observed and flow cytometry detected transfused iPSC-derived platelets in the circulation, but a rapid gradual decrease was observed 24 hours, indicating transient platelet persistence. This

study marked a groundbreaking advancement in regenerative medicine for thrombocytopenia and platelet disorders, establishing the first autologous iPSC-derived platelets in a patient with alloimmune platelet transfusion refractoriness and no compatible donors.

Kahr *et al.* were the first to use the imMKCL to study IPD.⁹⁴ Using lentiviral CRISPR/Cas9, they generated a *ARPC1B* knockout, revealing Arp2/3-dependent actin-branching defects, impaired proplatelet formation, and *compensatory* *ARPC1A* upregulation, mimicking patient phenotypes. The same group later produced knockouts of *SEC22B* and *NBEAL2*, to make a model for gray platelet syndrome and study α -granule biogenesis.⁹⁵ To our knowledge, our group was the first to use non-viral CRISPR/Cas9 with single cell sorting in imMKCL to study platelet formation disorders. In Ver Donck *et al.* we used Homology Directed Repair (HDR)-mediated CRISPR/Cas9, to generate two distinct clones containing a p.Lys219Asn variant in *SLFN14*, that served as a model to study *SLFN14*-related thrombocytopenia.³⁹ Deep phenotyping revealed a ribosomal defect with increased RNA degradation, providing a mechanistic explanation for the thrombocytopenia observed in patients. More recently, we established two additional thrombocytopenia models using imMKCLs to investigate megakaryocyte transcriptome dynamics.⁹⁶ *GP9* knockouts recapitulate Bernard Soulier syndrome, while the *IKZF5* p.Tyr121Phe heterozygous variant causes mild thrombocytopenia. RNA-seq across differentiation stages showed that patient platelet transcriptomes cluster with immature imMKCLs (day 0–1), whereas healthy controls cluster with mature imMKCLs (day 4–5). This suggests that platelet RNA profiles in thrombocytopenia patients reflect immature megakaryocyte origin, limiting their utility for mechanistic interpretation. However, iPSC-derived megakaryocytes and platelets display a more primitive transcriptomic profile compared with HSC-derived megakaryocytes and blood-derived platelets, and transcriptomic differences may arise from culture conditions rather than solely reflecting disease-associated phenotypes.⁹⁶ This underscores the importance of including appropriate, more physiologically relevant controls. Overall, imMKCLs are highly genetically tractable, recapitulate megakaryocyte transcriptomic signatures, and provide a scalable, reproducible platform for screening IPD-associated variants without requiring fresh patient samples.^{39,96} Nonetheless, these cells remain artificially produced and thus comparison with patient-derived material remains essential to validate findings and exclude off-target CRISPR effects.

Emerging technologies for modeling megakaryopoiesis and platelet disorders

Despite extensive efforts to elucidate megakaryopoiesis and thrombopoiesis using cell models, robust experimental models that fully recapitulate the complexity of the bone marrow niche remain scarce. A silk fibroin-based 3D bone marrow system was developed in which silk from silkworm cocoons is molded into porous sponge-like scaffolds that mimic the bone-marrow 3D architecture and support HSC differentiation into megakaryocytes and (pro)platelets.^{97,98} These scaffolds were the first to replicate bone marrow structure and could be functionalized using extracellular matrix components, such as fibronectin and collagen. This model enabled the assessment of treatment response of patients with MYH9- and ANKRD26-related thrombocytopenia to elthrombopag.³⁷ Beyond extracellular matrix interactions and structural cues, physiologically accurate megakaryopoiesis also requires cell–cell interactions with diverse bone-marrow populations. Recently, Khan *et al.* developed the first ever vascularized, multilineage human bone marrow-like organoids.⁹⁹ This system exists of iPSCs committed to mesenchymal, endothelial, and hematopoietic (erythroid, megakaryocyte, myelomonocytic and HSC) lineages, creating a unique environment to study different interactions in a bone marrow-like milieu. These organoids have already been used to evaluate anti-fibrotic and chemotherapeutic responses and hold significant promise for future modeling of IPD.⁹⁹ However, their complexity, long differentiation timelines, and technical demands make them poorly suited for rapid functional validation of VUS, where scalable and genetically tractable models are required.

Discussion

Over the past decades, human cell-based models have become indispensable tools for unraveling the molecular mechanisms underlying IPDs. Leukemia-derived cell lines such as K562, HEL, MEG-01, and DAMI provided the earliest tractable systems for studying megakaryopoiesis and, despite their well-recognized limitations in recapitulating physiological (pro)platelet formation, continue to serve as accessible platforms for initial mechanistic studies and gene-editing experiments (Table 3). Primary HSCs remain the gold standard for patient-specific disease modeling, offering unmatched biological relevance, although their dependence on fresh donor material and substantial inter-donor variability can complicate experimental interpretation. The advent of iPSC-based models has broadened the field considerably, enabling the generation of patient-specific megakaryocytes from individuals carrying rare or unique variants and providing a renewable source of cells for both mechanistic and preclinical studies. More recently, the imMKCL has emerged as a practical and genetically tractable alternative that circumvents the logistical challenges of primary cell isolation while faithfully reproducing key features of megakaryocyte biology at the transcriptomic and proteomic levels. Despite this progress, current models still fall short of fully capturing the complexity of the bone-marrow niche and emerging three-dimensional organoid systems incorporating multiple cell lineages and vascular components may help address this gap. Taken together, the field has shifted away from reliance on any single model toward a more integrative strategy in which the choice of system is guided by the specific biological question at hand. Continued refinement of these platforms, alongside rigorous validation against primary patient material, will be essential for translating mechanistic discoveries into meaningful advances in the diagnosis and treatment of IPDs.

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Table 1 HSC as a model to study IPD. MK: megakaryocyte. References are ordered based on publication date.

Reference	Disorder	Gene involved	Patient derived or genetic modification	OUTCOME
Identification of molecular mechanisms of platelet related genes				
Raslova <i>et al.</i> 2004 ⁴⁸	Paris-Trousseau syndrome	<i>FLI1</i>	patients derived and lentiviral transduction in patient HSC	Hemizygous loss of <i>FLI1</i> combined with its transient monoallelic expression in MK progenitors creates a subpopulation of <i>FLI1</i> -null cells that fail to differentiate and undergo massive lysis.
Veljkovic <i>et al.</i> 2009 ⁴⁴	Quebec platelet disorder	<i>PLAU</i>	patients derived	Abnormal overexpression of urokinase plasminogen activator emerges specifically during MK differentiation and the protein is co-stored with plasminogen in α -granules, where it drives the characteristic α -granule protein degradation.
Bluteau <i>et al.</i> 2012 ⁵¹	Familial platelet defect with predisposition to myeloid malignancies	<i>RUNX1</i> , <i>MYL9</i> , <i>MYH9</i> and <i>MYH10</i>	patients derived	MKs differentiated from patients showed a 60-80% reduction in MK output, low ploidy and a major defect in proplatelet formation. <i>RUNX1</i> directly regulates <i>MYL9</i> and <i>MYH9</i> and represses <i>MYH10</i> , so haploinsufficiency decreases <i>MYL9</i> and <i>MYH9</i> , while <i>MYH10</i> persists in mature MKs.
Chen <i>et al.</i> 2013 ⁵³	MYH9-related thrombocytopenia	<i>MYH9</i>	patients derived	MKs from patients with MYH9-related thrombocytopenia show reduced proplatelet formation, altered myosin IIA and actin distribution, and elevated traction forces during spreading, indicating excess actomyosin contractility.
Bluteau <i>et al.</i> 2014 ⁵²	Thrombocytopenia 2	<i>ANKRD26</i> , <i>RUNX1</i> and <i>FLI1</i>	patient derived with shRNA knockdown of <i>RUNX1</i> and <i>FLI1</i>	Variants in the 5' UTR of <i>ANKRD26</i> abolish <i>RUNX1</i> and <i>FLI1</i> binding, prevent the physiological silencing of <i>ANKRD26</i> during late megakaryopoiesis, resulting in persistent expression and an increase in TPO/MPL signaling and MAPK/ERK hyperactivation, impairing proplatelet formation, a defect fully rescued by ERK inhibition
Stritt <i>et al.</i> 2016 ⁴²	Macrothrombocytopenia	<i>DIAPH1</i>	patients derived	A gain-of-function truncating variant (Arg1213Ter) in <i>DIAPH1</i> causes autosomal dominant macrothrombocytopenia and sensorineural hearing loss by constitutively activating <i>DIAPH1</i> , leading to dysregulated actin and microtubule cytoskeletal organization that impairs proplatelet formation.
Poggi <i>et al.</i> 2017 ⁴³	Thrombocytopenia and platelet function	<i>ETV6</i>	patients derived and lentiviral transduction of healthy control HSC with mutant	Germline variants in the transcriptional repressor <i>ETV6</i> cause autosomal dominant thrombocytopenia by impairing proplatelet formation through downregulation of actin cytoskeletal regulators <i>CDC42</i> and <i>RHOA</i> , while also elevating circulating CD34 ⁺ progenitors and predisposing carriers to hematologic malignancy.
Wijgaerts <i>et al.</i> 2017 ⁴⁹	Gray platelet syndrome	<i>NBEAL2</i> and <i>GATA1</i>	patients derived	<i>GATA1</i> regulates <i>NBEAL2</i> expression via a long-distance enhancer, providing a molecular link between the alpha-granule defects seen in <i>GATA1</i> -deficient macrothrombocytopenia and Gray Platelet Syndrome.
Bariana <i>et al.</i> 2018 ⁴¹	Thrombocytopenia	<i>KDSR</i>	patients derived	Compound heterozygous loss-of-function variants in <i>KDSR</i> , cause thrombocytopenia by impairing proplatelet formation through cytoskeletal disorganization.
Heremans <i>et al.</i> 2018 ⁴⁶	Roifman syndrome	<i>RNU4ATAC</i>	patients derived	<i>RNU4ATAC</i> variants that disrupt minor intron splicing, leading to a blockade in B-cell maturation and defective megakaryopoiesis due to significant intron retention in critical genes such as <i>MAPK1</i> , <i>DIAPH1</i> , and <i>HPS1</i> .
Lentaigne <i>et al.</i> 2019 ⁴⁰	Thrombocytopenia	<i>IKZF5</i>	patients derived	Pathogenic variants in the transcription factor <i>IKZF5</i> disrupt chromatin binding, disrupting lineage specific differentiation defect in MKs.
Montenont <i>et al.</i> 2021 ⁵⁰	Platelet function disorders	<i>GP6</i> , <i>RASGRP2</i> , <i>ITGA2B</i> , <i>B2M</i> and <i>COMMD7</i>	healthy control HSC with NHEJ mediated CRISPR Cas9	An efficient CRISPR/Cas9-based platform for screening platelet gene functions in CD34 ⁺ -derived MKs.
Basso-Valentina and	Thrombocytopenia 2	<i>ANKRD26</i>	patient derived with lentiviral	<i>ANKRD26</i> regulates blood cell proliferation via MPL, EPOR and G-CSFR and thus is required for early megakaryopoiesis.

Donada <i>et al.</i> 2023 ²⁴			transduction of WT <i>ANKRD26</i>	
Ver Donck <i>et al.</i> 2023 ³⁹	Thrombocytopenia	<i>SLFN14</i>	patients derived	p.Lys219Asn mutant <i>SLFN14</i> results in ribosome dysfunction and increased rRNA degradation
Bigot <i>et al.</i> 2023 ⁴⁷	Thrombocytopenia and leukemia predisposition syndrome	<i>ETV6</i>	patients derived	Single-cell RNA sequencing of CD34 ⁺ -derived megakaryopoiesis revealed that <i>ETV6</i> deficiency causes aberrant differentiation beginning at the MK-erythroid progenitor stage, driven by upregulation of ribosome biogenesis and RPS6 overexpression, and that inhibiting translation with CX-5461 partially rescues platelet-like particle production.
Del Carpio-Cano <i>et al.</i> 2024 ⁴⁵	Familial platelet defect with predisposition to myeloid malignancies	<i>RUNX1</i>	short hairpin RNA <i>RUNX1</i> knock down	<i>RUNX1</i> haploinsufficiency causes defective platelet endocytosis of albumin, fibrinogen, and IgG, while paradoxically enhancing endocytosis in MKs with defective intracellular trafficking via distinct caveolin-1 and RAB11 pathways, leading to net depletion of these proteins from α -granules.
Functional validation of novel variants				
Balduini <i>et al.</i> 2008 ³⁶	Bernard-Soulier Syndrome type Bolzano	<i>GP1BA</i>	patients derived	Macrothrombocytopenia in Bernard-Soulier Syndrome type Bolzano is caused by an intrinsic defect in proplatelet formation characterized by reduced output, enlarged proplatelet tips, and deranged α -tubulin distribution despite normal MK differentiation and maturation.
Ghevaert <i>et al.</i> 2008 ⁵⁵	Glanzmann Thrombasthenia-like syndrome	<i>ITGB3</i>	patients derived	Glanzmann thrombasthenia-like syndrome is caused by a dominant <i>ITGB3</i> mutation (p.Asp749His) that disrupts a conserved cytoplasmic salt bridge, resulting in constitutive $\alpha_{IIb}\beta_3$ activation and abnormal proplatelet formation leading to macrothrombocytopenia.
Turro <i>et al.</i> 2016 ¹⁵	Thrombocytopenia	<i>SRC</i>	patients derived and lentiviral transduction of healthy control HSC with mutant <i>SRC</i>	A dominant gain-of-function <i>SRC</i> mutation (p.Glu527Lys) causes a novel syndrome of thrombocytopenia, myelofibrosis, and bone pathologies by inducing constitutive kinase activity that triggers abnormal actin organization and a severe proplatelet formation defect.
Saultier <i>et al.</i> 2017 ⁵⁴	Macrothrombocytopenia	<i>FLI1</i>	patients derived	Two novel dominant <i>FLI1</i> variants (p.Arg337Gln and p.Lys345Glu) cause macrothrombocytopenia through defective MK maturation and a profound dense granule biogenesis deficiency. <i>MYH10</i> is a reliable biomarker for the disorder.
Kosaka <i>et al.</i> 2025 ⁵⁶	Glanzmann Thrombasthenia	<i>ITGA2B</i> and <i>ITGB3</i>	healthy donor and HDR mediated CRISPR Cas9	An efficient CRISPR/Cas9-based platform for screening variants of unknown significance in CD34 ⁺ -derived MK.
(Pre)clinical testing of therapeutic strategies				
Wilcox <i>et al.</i> 2000 ⁵⁸	Glanzmann Thrombasthenia	<i>ITGB3</i>	patients derived with retroviral transduction	<i>Ex vivo</i> phenotypic correction of Glanzmann thrombasthenia using an α_{IIb} promoter-driven retroviral vector to restore integrin β_3 expression and functional fibrinogen binding in MK derived from transduced patient CD34 ⁺ cells.
Basso-Valentina <i>et al.</i> 2021 ²⁴	Congenital amegakaryocytic thrombocytopenia (CAMT)	<i>MPL</i> and <i>CALR</i>	patient derived with retroviral transduction for <i>CALR</i> ^{del52}	The homozygous <i>MPL</i> p.Arg464Gly variant causes CAMT through defective receptor trafficking to the cell surface; retroviral expression of <i>CALR</i> ^{del52} mutant did not restore trafficking but rendered <i>MPL</i> responsive to eltrombopag, restoring MK differentiation of patient MK.
Martinez-Navajas <i>et al.</i> 2023 ⁶⁰	Bernard-Soulier syndrome	<i>GP9</i>	patient derived with lentiviral transduction	Lentiviral delivery of GPIX to HSCs isolated from the peripheral blood of two unrelated patients with Bernard-Soulier syndrome restored GPIX expression in the derived MKs and platelets and reduced platelet size, correcting the macrothrombocytopenic phenotype and demonstrating the potential of lentiviral gene therapy for this disorder.
Lee <i>et al.</i> 2023 ⁶¹	Familial platelet defect with predisposition to myeloid malignancies	<i>RUNX1</i>	lentiviral shRNA knockdown in control HSC	shRNA-mediated knockdown of <i>RUNX1</i> in CD34 ⁺ -derived MKs reduced agonist responsiveness, and after infusion into immunodeficient mice released fewer platelets and failed to restore thrombus formation. The TGF β 1 pathway inhibitor RepSox corrected the thrombopoietic defect, platelet half-life and agonist response, representing the first pharmacological reversal of <i>RUNX1</i> deficiency-induced hemostatic defects.

Table 2 iPSC as a model to study IPD. MK: megakaryocyte. References are ordered based on publication date.

Reference	Disorder	Gene involved	Genetic modification	OUTCOME
Identification of molecular mechanisms of platelet related genes				
Hirata <i>et al.</i> 2013 ⁷¹	Congenital amegakaryocytic thrombocytopenia	<i>MPL</i>	patient derived	MPL signaling is indispensable for early human hematopoiesis and its loss causes congenital amegakaryocytic thrombocytopenia through the dysregulation of the FLI1-mediated program that normally governs the balance between megakaryopoiesis and erythropoiesis.
Sakurai <i>et al.</i> 2014 ⁷²	Familial platelet disorder with associated myeloid malignancies	<i>RUNX1</i>	patient derived	RUNX1 mutations in familial platelet disorder cause impaired hematopoietic and MK differentiation. Overexpression of mutant-RUNX1 in WT-iPSC does not recapitulate the disease phenotype.
Orban <i>et al.</i> 2015 ⁶⁶	Glanzmann Thrombasthenia	<i>ITGA2B</i> and <i>ITGB3</i>	patient derived	Generated and characterized a patient-derived iPSC line with a compound heterozygous variant in <i>ITGA2B</i> (p.Pro614Leu and p.Asn826_Trp827) that recapitulates Glanzmann thrombasthenia disease phenotype.
Antony-Debré <i>et al.</i> 2015 ⁷³	Familial platelet disorder with associated myeloid malignancies	<i>RUNX1</i>	patient derived	RUNX1 dosage determines the disease phenotype. Haploinsufficiency disrupts megakaryopoiesis and proplatelet formation with thrombocytopenia alone, whereas the dominant-negative p.Arg174Gln variant additionally drives granulomonocytic amplification and genomic instability, explaining leukemic predisposition
Maguire <i>et al.</i> 2016 ⁶⁷	Hermansky–Pudlak syndrome type 2	<i>AP3B1</i>	patient derived	Generated and characterized a patient-derived iPSC line for Hermansky–Pudlak syndrome type 2 carrying mutations in the <i>AP3B1</i> gene, providing a critical resource for modeling the disease's deficiencies in organelle biogenesis.
Lopez-Onieva <i>et al.</i> 2016 ⁸⁰	Bernard-Soulier Syndrome	<i>GP9</i>	patient derived	Generated and characterized a patient-derived iPSC line with a p.Asn45Ser variant in <i>GPIX</i> to model Bernard-Soulier syndrome
Lopez-Onieva <i>et al.</i> 2016 ⁸¹	Bernard-Soulier Syndrome	<i>GP9</i>	patient derived	Generated and characterized a patient-derived iPSC line with a p.Trp71Arg variant in <i>GPIX</i> to model Bernard-Soulier syndrome
Lopez-Onieva <i>et al.</i> 2017 ⁸²	Bernard-Soulier Syndrome	<i>GP9</i>	patient derived	Generated and characterized a patient-derived iPSC line with a p.Phe55Ser variant in <i>GPIX</i> to model Bernard-Soulier syndrome
Rabbolini <i>et al.</i> 2017 ⁷⁴	platelet-type bleeding disorder 17	<i>GFI1B</i>	patient derived	GFI1B mutations in non-DNA-binding (p.Cys168Phe) domains result in milder, heterogeneous platelet phenotypes compared to mutations that result in deletion of DNA-binding domains (c.2520 + 1_2520 + 8delGTGGGCAC). Increased platelet CD34 expression as a universal marker of perturbed GFI1B function.
Vo <i>et al.</i> 2017 ⁸⁵	Paris-Trousseau syndrome	<i>FLI1</i>	patient derived with viral over-expression	Hemizygous deletion of <i>FLI1</i> causes Paris-Trousseau syndrome and leads to defective megakaryopoiesis and impaired platelet function—defects can be rescued and even enhanced by <i>FLI1</i> overexpression.
Hu <i>et al.</i> 2017 ⁷⁸	Glanzmann Thrombasthenia	<i>ITGA2B</i>	patient derived and lentiviral over-expression	Generated and characterized a patient-derived iPSC line with compound heterozygous c.480CNG and c.574+5GNC variants in <i>ITGA2B</i> to model Glanzmann Thrombasthenia. Overexpression of WT <i>ITGA2B</i> restores phenotype.
Lamolda <i>et al.</i> 2019 ⁶⁹	Familial platelet disorder with associated myeloid malignancies	<i>RUNX1</i>	patient derived	Generated and characterized a patient-derived iPSC line with a novel p.Thr196Ala variant in <i>RUNX1</i> to model FPDMM
Mekchay <i>et al.</i> 2019 ⁷⁵	Bernard-Soulier Syndrome	<i>GP1BA</i> and <i>GP1BB</i>	patient derived	Generated and characterized a patient-derived iPSC line with variants in <i>GP1BA</i> (p.Asn221Lys) and <i>GP1BB</i> (Glu136Ter) to model Bernard Soullier Syndrome. Overexpression of WT <i>GP1BA</i> or <i>GP1BB</i> restores phenotype.
Tangprasittipap <i>et al.</i> 2019 ⁷⁰	Thrombocytopenia	<i>MYH9</i>	patient derived	Generated and characterized a patient-derived iPSC line with a p.Arg702Cys variant in <i>MYH9</i> to model MYH9 related thrombocytopenia

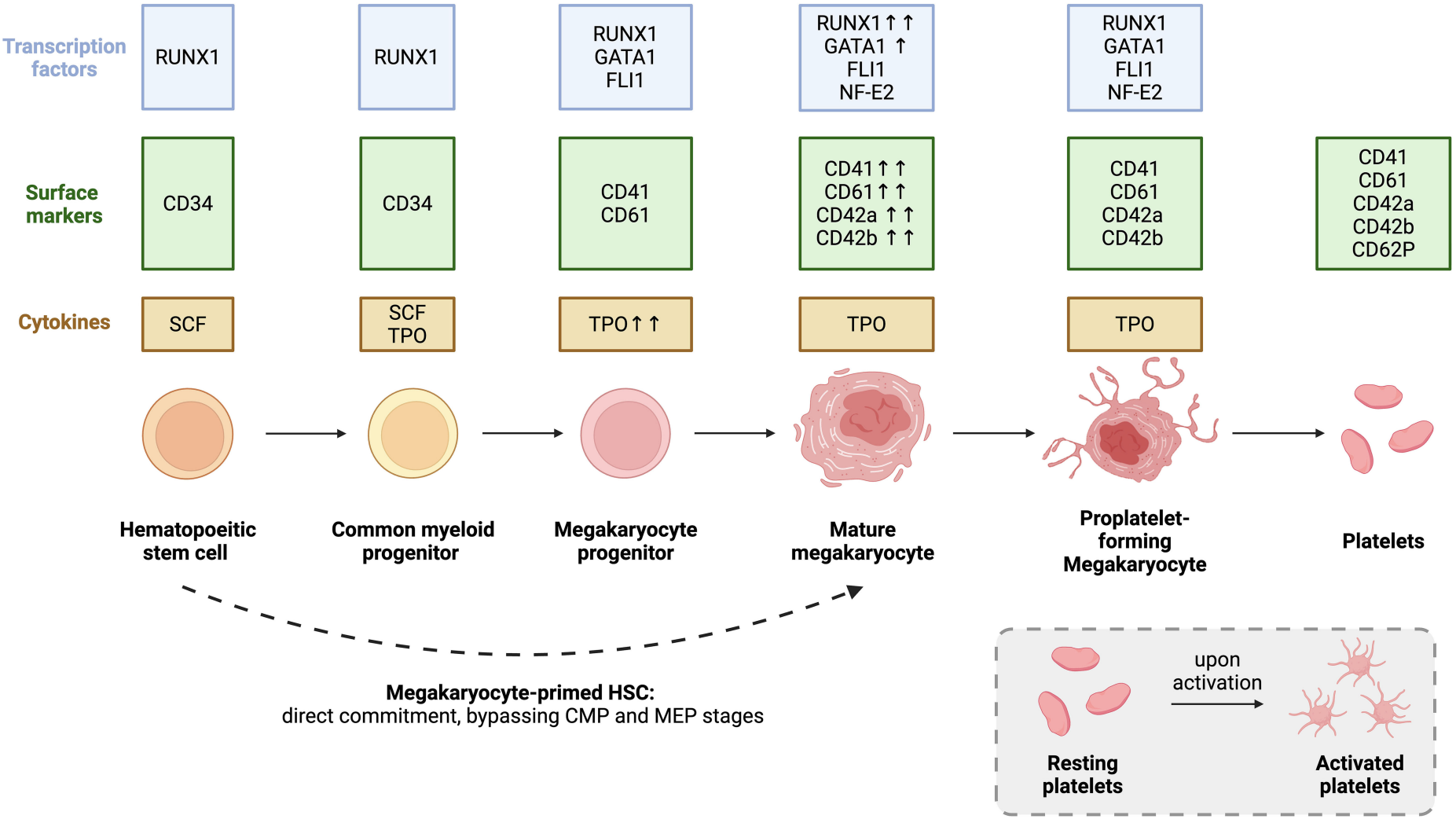
Van Oorschot <i>et al.</i> 2019 ⁷⁶	Platelet function disorder	<i>GFI1B</i>	patient derived	The dominant-negative p.Gln287Ter variant in <i>GFI1B</i> cause bleeding disorders by sequestering the LSD1-RCOR-HDAC complex, thereby deregulating the broad developmental program required for normal megakaryopoiesis.
Donada <i>et al.</i> 2019 ⁷⁷	Macrothrombocytopenia	<i>FLNA</i>	patient derived	Mutations in <i>FLNA</i> cause macrothrombocytopenia by disrupting the FLNa/ $\alpha_{IIb}\beta_3$ interaction, which leads to RhoA overactivation and impaired proplatelet formation in MKs.
Bariana <i>et al.</i> 2019 ⁴⁰	Thrombocytopenia	<i>KDSR</i>	patient derived with transduction	A compound heterozygous loss of <i>KDSR</i> causes thrombocytopenia by disrupting <i>de novo</i> sphingolipid synthesis, which leads to impaired MK cytoskeletal organization and proplatelet formation.
Borst <i>et al.</i> 2021 ⁷⁹	Thrombocytopenia	<i>ETV6</i> and <i>RUNX1</i>	patient derived and CRISPR	Variants in <i>ETV6</i> and <i>RUNX1</i> result in defective proplatelet formation through different mechanisms: mutant <i>ETV6</i> results in an excess of immature, less-responsive MKs, whereas <i>RUNX1</i> mutants yield a deficiency of MKs that are more responsive to agonists
Pawinwongchai <i>et al.</i> 2021 ⁸³	platelet-type von Willebrand disease	<i>GP1BA</i>	CRISPR Cas9	Both loss- and gain-of-function variants in <i>GP1BA</i> cause macrothrombocytopenia by reducing MAPK/ERK activation and enhancing ROCK/MLC2 phosphorylation, a defect successfully rescued <i>in vitro</i> by ROCK inhibition
Tanaka <i>et al.</i> 2024 ⁸⁴	Familial platelet disorder with associated myeloid malignancies	<i>RUNX1</i>	CRISPR Cas9	<i>RUNX1</i> transactivation domain mutations cause defective megakaryopoiesis and aberrant DNA hypermethylation by downregulating <i>FLI1</i> , a phenotype that can be functionally rescued through <i>FLI1</i> overexpression
Lordier <i>et al.</i> 2025 ⁸⁹	Bernard Soulier Syndrome	<i>GP1BA</i>	patient derived	The heterozygous p.Asn103Asp variant in <i>GP1BA</i> does not affect MK differentiation but reduces VWF affinity and increases SRC activation downstream of GPIb α , overactivating the RhoA pathway; resulting in a pre-activation of integrin $\alpha_{IIb}\beta_3$ and excessive stress fiber formation, disrupting proplatelet formation after adhesion on fibrinogen.
(Pre)clinical testing of therapeutic strategies				
Sullivan <i>et al.</i> 2014 ⁸⁶	Glanzmann Thrombasthenia	<i>ITGA2B</i>	patient derived	A MK-specific transgene expression strategy using the <i>Gp1ba</i> promoter targeted to the AAVS1 locus to successfully restore surface expression and biological function of the $\alpha_{IIb}\beta_3$ integrin in Glanzmann thrombasthenia derived iPSC.
Iizuka <i>et al.</i> 2015 ⁸⁷	Familial platelet disorder with associated myeloid malignancies	<i>RUNX1</i>	patient derived with TALEN plasmid transfection	A TALEN-mediated gene correction of the <i>RUNX1</i> p.Arg174Ter nonsense mutation in patient-derived iPSC successfully restores normal MK differentiation and maturation
Krutein <i>et al.</i> 2020 ⁸⁸	Familial platelet disorder with associated myeloid malignancies	<i>RUNX1</i>	patient derived	Pharmacological inhibition of <i>RUNX1</i> proteolytic degradation—specifically using proteasome or CDK inhibitors—successfully restores steady-state <i>RUNX1</i> protein levels and improves MK differentiation and function in patient-derived iPSC
Di Buduo <i>et al.</i> 2021 ³⁷	Thrombocytopenia	<i>MYH9</i>	patient derived	The 3D silk bone marrow model can be used to predict clinical response to Eltrombopag by assessing <i>ex vivo</i> platelet production of patient derived iPSC.
Lordier <i>et al.</i> 2025 ⁸⁹	Bernard Soulier Syndrome	<i>GP1BA</i>	patient derived	The SRC inhibitor dasatinib restored stress fiber formation in p.Asn103Asp iPSC MK. ROCK1/2 inhibition with Y27632 on MK in the 3D silk bone marrow model increased platelet number and restored platelet size in MK carrying heterozygous <i>GP1BA</i> variants (p.Asn103Asp, p.Leu160Pro, p.Asn150Ser), but had no effect in biallelic BSS, indicating a distinct underlying mechanism

Table 3 Overview of the advantages and disadvantages of using cell lines, primary megakaryocytes (MK), iPSC-derived MK or immortalized megakaryocyte cell line (imMKCL) to study inherited platelet disorders.

Group	Advantages	Disadvantages
Cell lines	• Easy to culture. • Low cost and scalable • Useful for rapid mechanistic studies • MEG-01 and DAMI are more MK-relevant than K562/HEL • No need for fresh patient material	• Not physiologically accurate • Often immature and lineage-mixed • Limited ability to model late megakaryopoiesis and platelet production. • Only platelet-like particles
CD34 ⁺ -derived megakaryocytes	• Physiological model • Recapitulates patient-specific phenotypes • Useful for variant validation, disease mechanism studies, personalized therapeutic testing and rescue experiments	• Limited MK numbers • Sample collection difficult in young children • Donor variability • Genetic background may complicate variant interpretation • Difficult to genetically modify • Limited platelet numbers that are difficult to purify
iPSC-derived megakaryocytes	• Patient-specific and renewable • Scalable once established • Useful for disease modeling and gene correction • Avoids repeated primary sampling • Genetically modifiable	• Time-consuming • High technical variability • Lower differentiation capacity • Retention of embryonic-like signature • Limited platelet numbers that are difficult to purify
imMKCL-derived megakaryocytes	• Highly scalable • Reproducible • Genetically modifiable and tractable • No need for fresh patient material • Generation of functional platelets	• Needs validation against patient-derived cells • Retention of embryonic-like signature • Platelets are different from blood-derived platelets

Figure 1. Overview of megakaryopoiesis and thrombopoiesis. At each stage, important transcription factors (blue), surface markers (green) and cytokines (yellow) are indicated above the cell type. Figure based on Bianchi *et al.*² and Noetzli *et al.*³ and created with Biorender.

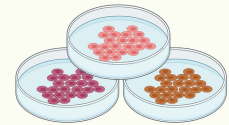
Figure 2. Human cell models commonly used to study inherited platelet disorders. DOX: doxycycline, HSC: hematopoietic stem cell, imMKCL: immortalized megakaryocyte cell line, iPSC: induced pluripotent stem cell, OSKM: Oct4, Sox2, Klf4 and c-Myc (Yamanaka factors), PLP: platelet-like particle, PMA: phorbol 12-myristate 13-acetate, SCF: stem cell factor TPO: thrombopoietin.



**Time to mature
megakaryocytes**

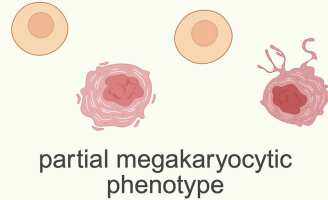
**Platelet output per
megakaryocyte**

cell lines



K562, HEL, MEG-01,
DAMI, UT-7

PMA



partial megakaryocytic
phenotype



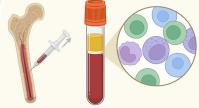
platelet-like particles
(MEG-01)

-

-

cell lines with megakaryocytic characteristics

HSC



30-40 ml blood

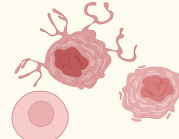


CD34⁺ HSC

+ TPO



megakaryocyte
progenitor



polyloid MK and
proplatelet formation



platelets

10 - 14 days

<10 platelets

hematopoietic stem cells

ipsc



fibroblast,
PBMC, T-cell or
CD34⁺ HSC

OSKM



iPSC

mesoderm
induction



CD34⁺
progenitor

+ TPO
+ SCF



megakaryocyte



proplatelet
formation



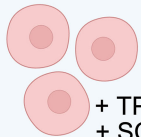
platelets

3 - 5 weeks
~7 days by **forward
programming**

<10 platelets

induced pluripotent stem cells

imMKCL

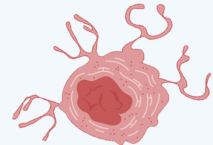


+ TPO
+ SCF

-DOX



day 4:
megakaryocyte



day 5:
proplatelets



day 6:
platelets

4 days for mature MK

~ 18 platelets in static
~24 with turbulence

immortalized megakaryocyte cell line