

Pediatric Tumors as Disorders of Development: The Case for In Vitro Modeling Based on Human Stem Cells

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Cullen D. Clairmont^{1,2} , Joanna J. Gell^{1,2,3,4}, and Ching C. Lau^{1,2,3,4}

Abstract

Despite improvements in patient outcomes, pediatric cancer remains a leading cause of non-accidental death in children. Recent genetic analysis of patients with pediatric cancers indicates an important role for both germline genetic predisposition and cancer-specific somatic driver mutations. Increasingly, evidence demonstrates that the developmental timepoint at which the cancer cell-of-origin transforms is critical to tumor identity and therapeutic response. Therefore, future therapeutic development would be bolstered by the use of disease models that faithfully recapitulate the genetic context, cell-of-origin, and developmental window of vulnerability in pediatric cancers. Human stem cells have the potential to incorporate all of these characteristics into a pediatric cancer model, while serving as a platform for rapid genetic and pharmacological testing. In this review, we describe how human stem cells have been used to model pediatric cancers and how these models compare to other pediatric cancer model modalities.

Plain Language Summary

Today, pediatric cancer is a leading cause of non-accidental death in children. In order to further improve outcomes, it is important for researchers and clinicians alike to recognize how pediatric cancers are distinct from adult cancers. Inherited risk of cancer may play a greater role in pediatric cancer risk, and subsequent tumor-specific acquired driver mutations initiate tumor formation. However, there is substantial interaction between inherited and acquired mutations, which supports consideration of both simultaneously. Recent advancements in biotechnology, have improved matching between early cells of development and pediatric cancer cells, although cell-of-origin for certain pediatric central nervous system tumors remain elusive. Increasingly, evidence, particularly in pediatric medulloblastoma, demonstrates that the developmental timepoint at which the cancer cell-of-origin transforms is critical to tumor identity and therapeutic response. Therefore, future therapeutic development would be bolstered by the use of disease models that faithfully recapitulate the genetic context, cell-of-origin, and developmental window of pediatric cancers. Human stem cells have the potential to incorporate all of these characteristics into a pediatric cancer model, while serving as a platform for rapid genetic and pharmacological testing. In this review, we describe how human stem cells have been used to model pediatric cancers, how human these models compare to other pediatric cancer model modalities, and how these models can be improved in the future.

¹University of Connecticut School of Medicine, Farmington, CT, USA

²The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA

³Connecticut Children's Medical Center, Center for Cancer and Blood Disorders, Hartford, CT, USA

⁴Division of Pediatric Hematology-Oncology, Department of Pediatrics, UConn Health, Farmington, CT, USA

Corresponding Author:

Ching C. Lau, The Jackson Laboratory for Genomic Medicine, 10 Discovery Drive, Farmington, CT 06032, USA.

Email: Ching.lau@jax.org



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Introduction

Children are not small adults, and pediatric cancers are not adult cancers that happen to occur in young patients. Pediatric cancers differ from adult cancers in numerous ways. Approximately 50% of adult cancers are caused by years of accumulated cellular and DNA damage from lifestyle risk factors, such as smoking, alcohol consumption, and obesity, that could not have accumulated within childhood.¹ Pediatric cancers consist of different etiologies. While acute lymphoblastic leukemia (ALL), neuroblastoma, and medulloblastoma (MB) are relatively common in children, these are rarely found in adults.² Pediatric cancers often behave differently from their adult counterparts. For example, pediatric thyroid cancer is associated with larger tumor size and more aggressive pathological features compared to that of adults.³

Although pediatric cancer is relatively rare and the 5-year overall survival rate has improved to nearly 85%, it is still the second leading cause of death for children 5-9 years old and the third leading cause of death for children 10-14 years old in the United States.⁴⁻⁷ Further, survivors of childhood cancer have reduced lifespan and increased risk of late effects of treatment.^{8,9} An estimated two-thirds of childhood cancer survivors will develop chronic health conditions, including neurocognitive impairment, pain, and compromised mental health.¹⁰ Adult survivors of pediatric cancer also have five-fold increased risk of serious cardiovascular disease and death.^{11,12} Therefore, pediatric cancers continue to place a substantial burden on society.

In order to develop safer and more effective therapies, the unique biologic underpinnings of pediatric cancers must be investigated. Disease modeling is a powerful technique that leverages our understanding of pathogenesis in human patients to develop a biological system that mimics the human disease. Although pharmacological testing with a disease model is often a prerequisite to clinical trials, many disease models have limited translatability due to incomplete disease validity. In the case of pediatric oncology, a faithful model would precisely recapitulate the genetic context, cancer cell-of-origin, and the narrow developmental window during which the disease mutation will have its impact. We propose that modern techniques for human stem cell genetic engineering and in vitro differentiation can be utilized to better mirror the abnormal development observed in pediatric cancers.

Main Body

Genetic Context

The genetic context that contributes to pediatric cancer pathogenesis can be largely divided into germline and somatic driver mutations. In general, germline mutations, either inherited from parents or occurring *de novo*, predispose patients to developing certain cancers, while somatic driver mutations can accumulate in a subpopulation of cells and lead to tumor development.

Trisomy 21, or Down Syndrome (DS), is a germline aneuploidy that is well known to increase risk for childhood leukemia.¹³ A large cohort study found that the extent of increased risk in DS children under 5 years old is highly variable between subtypes: 28-fold for ALL, 399-fold for acute myeloid leukemia (AML), and 1500-fold for acute megakaryoblastic leukemia.¹⁴ Interestingly, DS is also protective against many adult solid tumors.¹⁵ There are also heritable, monogenic syndromes that result in cancer predisposition, including retinoblastoma, familial adenomatous polyposis, neurofibromatosis, von Hippel-Lindau syndrome, and Li-Fraumeni syndrome (LFS).¹⁶ A study of 1120 pediatric cancer cases, including leukemia and solid tumors, found that 8.5% of participants had a pathogenic or likely pathogenic germline mutation in one of 60 such genes, while another study of 914 cases investigating 162 genes found a rate of 6%.^{17,18} In a study of 751 patients with pediatric solid tumors, pathogenic or likely pathogenic variants were found in 13% of patients.¹⁹ Interestingly, another study found likely harmful germline variants in 11% of pediatric ALL cases, compared to 8% of adult ALL cases.²⁰

However, these findings may underestimate germline predisposition in pediatric cancers partly because they do not appreciate the effect of common gene variants with low penetrance, which are detected through genome-wide association studies (GWAS). GWAS of pediatric cancers, although largely limited to the relatively common ALL, have also identified several cancer-associated loci in pediatric solid tumors, including in neuroblastoma, Wilms tumor, Ewing sarcoma (EWS), osteosarcoma, Langerhans cell histiocytosis, hepatoblastoma, retinoblastoma, and germ cell tumors (GCTs).²¹⁻²³ Notably, analysis of GWAS data found that germline allelic variants in pediatric and young adult cancers had significantly greater odds ratios compared to adult cancers, which indicates that germline variants may explain a greater proportion of the disease in young onset cancer cases.²⁴

Conversely, pediatric cancers have low somatic mutational burden.²⁵ Frequencies of somatic mutations involving coding sequences in pediatric cancers were found to be 14 times lower than in adult cancers.¹⁸ Analysis of 77 significantly mutated genes found that many mutations were mutually exclusive across cancer types and that pediatric cancers often developed from a single putative driver gene, which is in stark contrast to the frequent mutation accumulation in adult cancers.¹⁸ Approximately 5.5% cases of pediatric cancer cases do display hypermutation, often due to mismatch repair deficiency and DNA polymerase mutations, comparatively lower than the 1 in 6 adult cancers.²⁶ In addition, a study of 1699 cases of pediatric leukemia and solid tumors found that of the 142 driver mutations identified, only 45% matched previously identified adult somatic driver mutations.²⁷ It appears that mutations in genes involved in epigenetic and transcriptional regulation are relatively more common in pediatric than adult cancers.^{18,28–30} As one example, histone 3 variant 3 is present in about 50% of pediatric diffuse intrinsic pontine gliomas (DIPGs) and in only 0.2% of adult DIPGs.^{31–33} Furthermore, single nucleotide variations and indels are relatively rare in pediatric cancers, while specific structural variations, such as those resulting in gene fusions or chromosomal abnormalities, are more common.³⁴ A recently published review discusses genetic and epigenetic signatures of pediatric cancer in greater detail.³⁰

There is emerging evidence of how germline and somatic mutation interact and result in tumor progression. First, genes commonly mutated in both the germline and somatically indicate a direct role for germline mutations in tumor evolution.³⁵ Second, germline mutations may increase the risk for development of certain somatic mutations.³⁶ For example, there is strong concordance between germline mutations in *GATA2* and somatic mutational profile, including monosomy 7 and mutations in *SETBP1*, *RUNX1*, and *ASXL1*, in pediatric myelodysplastic syndrome.³⁷ Finally, there are functional relationships between germline and somatic mutations. One study found that germline variants in an enhancer-like DNA element that binds to the EWSR1-FLI1 fusion protein in EWS resulted in variable responsiveness to CDK2 therapy via changes in MYBL2 expression levels.³⁸ A recent GWAS validation study also identified how variants in a super-enhancer region of the *LMO1* oncogene altered binding with GATA3 and neuroblastoma dependency.³⁹ This evidence supports the consideration of evaluating germline and somatic mutations simultaneously in modeling pediatric cancer.

Cell-of-origin and Developmental Window

Increasingly, pediatric cancers, particularly pediatric brain tumors, are viewed as a form of abnormal development, in which cancers arise from stem-like progenitor populations.^{31,40} The histopathological similarities between pediatric tumors and precursor cells of development served as the first line of evidence in support of this hypothesis. More

recently, lineage tracing in genetically engineered mouse models of cancer provides strong evidence for the impact of the cell-of-origin on cancer development. By causing a somatic loss of *Nfl* in different neural progenitor populations and observing which models formed tumors, researchers found that neuroglial progenitors and pre-oligodendrocyte precursor cells gave rise to low-grade gliomas with different latencies.⁴¹ Another approach that is possible with human samples is done by analyzing somatic mutations in different healthy tissues in one patient to infer the phylogenetic relationship of that patient's cancer with development. Simply, somatic mutations that are present in the tumor and one healthy tissue source, but not in other healthy tissues, indicate a more recent common progenitor. This technique has shed light on the origins of neuroblastoma,⁴² Wilms tumor,⁴³ malignant rhabdoid tumors,⁴⁴ and pediatric AML.⁴⁵

Advances in single cell sequencing and multi-omics have empowered researchers with a method to correlate precursor cells and cancer cells,⁴⁶ leading to the identification of many putative cells-of-origin, such as the oligodendrocyte precursor for diffuse midline H3K27 M gliomas,⁴⁷ cerebellar granule neuron progenitor (CGNP) for Sonic hedgehog MB (SHH-MB),⁴⁸ and fetal adrenal neuroblast for neuroblastoma.⁴⁹ A study that generated a single cell transcriptome atlas of more than 65,000 human and mouse cells located in the embryonal pons and forebrain found that specific neuroectoderm and neuronal lineages matched different CNS tumor subtypes.⁵⁰ These researchers identified the narrow window of E16-E18 gliogenic progenitors in mice, as being highly similar to pediatric ependymoma and pilocytic astrocytoma. There is also increasing evidence showing that different MB subtypes correlate with different developmental stages of CGNPs.^{51–54} In particular, researchers found that CGNPs at weeks 10–15 into embryogenesis were most similar to the Group 3 subtype, while 20–30 week CGNPs were most similar to Group 4.⁵⁵ Notably, several pediatric CNS tumors, namely intracranial GCTs, still have uncertain cells-of-origin, which may be resolved through improvements in sequencing technology and lineage tracing.^{40,46}

Slight differences in the developmental timepoint of transformation can have major consequences for tumor formation, identity, and therapeutic response. For example, intracranial atypical teratoid/rhabdoid tumors only formed in a murine model when SMARCB1 was inactivated between E6-E10 and not at later developmental timepoints.^{56,57} Similarly, researchers seeking to model pediatric high grade gliomas in mice found that mutations in H3.3K27 M and loss of *Trp53* induced in utero, not postnatally, resulted in tumorigenesis.⁵⁸ Further, the different developmental origins of MB may have major therapeutic implications. Notably, early embryonic CGNPs, which correspond to infant onset SHH-MB, have been shown to exhibit reduced response to Smoothed inhibition, unlike later stage CGNPs.⁵³ Similarly, MBs transformed from *Gfap* + neural stem cells were found to be more aggressive and radioresistant compared to MBs generated

from *Atoh1*+ committed neural progenitors.⁵⁹ The origin of neuroblastoma from neural crest cells is critical to tumor initiation. While overexpression of *MYCN* in multipotent murine sympathoadrenal progenitors increased proliferation and neural lineage commitment, it failed to produce tumors, unlike neural crest-derived *MYCN* models.^{60,61} Finally, research with mouse models of neurofibromatosis type 1 have revealed that developmentally transient, migrating glial progenitors during gliogenesis were specifically responsible for development of low-grade gliomas sensitive to MEK/ERK/MAPK inhibition.⁶² This evidence emphasizes the profound effect of the cell-of-origin and transformation developmental timepoint on treatment targets and efficacy.

Genetic context, cell-of-origin, and developmental origin must be considered when generating a model of pediatric cancer. Basic germline or somatic mutations can be easily engineered using modern CRISPR/Cas9 techniques. Reprogramming of somatic cells from patients with germline predisposition into pluripotent stem cells is an alternative approach to maintain genetic context of pediatric cancer in a model. Information about cell-of-origin can be directly translated into the differentiation of the stem cell model. As in vitro methods for stem cell differentiation improve and become highly tunable to developmental stage, stem cell

models can more accurately match developmental origin of transformation (Figure 1).

The Landscape of Pediatric Cancer Modeling

Primary Culture and Immortalized Cell Lines. Primary cancer cell culture involves the extraction of tumor cells from patients' clinical specimens and the expansion of those cells in vitro. Primary cultures can be established as a monolayer or in more complex 3D structures. Patient-derived organoids have been described for pediatric sarcomas and multiple pediatric brain tumors.^{63,64} By introducing primary cultures of pediatric brain tumors to cerebral organoids or 3D extracellular matrix-containing scaffolds, important features, including tumor heterogeneity and cell invasion, can be studied.⁶⁵⁻⁶⁷ Primary cultures are typically limited by slow growth and limited sample supply, unlike cancer cell lines, which are immortal. Cancer cell lines often have significant alterations in their genome, allowing for rapid and continuous proliferation but causing deviation from the original sample. The establishment of pediatric cancer cell lines has lagged behind those of adult cancers, due to the scarcity of clinical samples and low rates of successful cell line establishment; as a result, there are still many pediatric cancers without a reliable model.^{68,69}

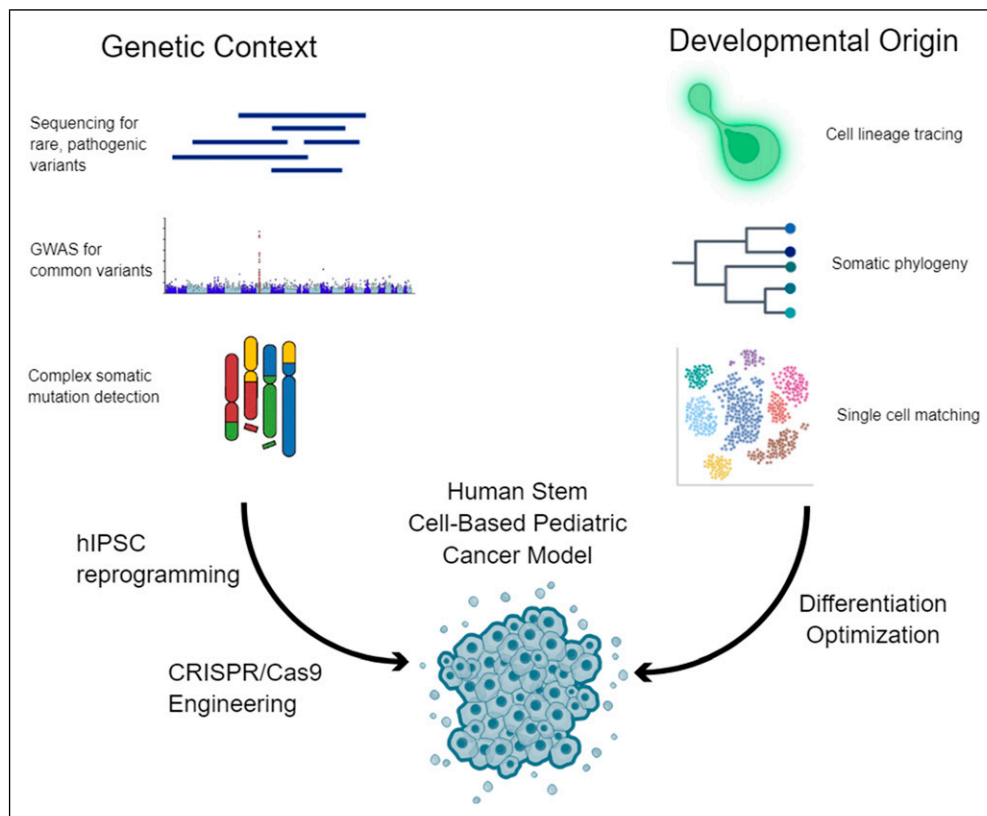


Figure 1. Techniques to investigate the genetic context and developmental origin of pediatric cancer, which will inform human stem cell-based models. Created with the Mind the Graph platform (www.mindthegraph.com) and Biorender.com. Includes image adapted from Balachandran & Beck (2020).¹⁷⁰

The challenges experienced with cell lines are exemplified with cell lines derived from seminomas, a pediatric/adolescent GCT subtype that resembles primordial germ cells (PGCs). Seminomas have been difficult to culture, but three seminoma cell lines have been established. However, each of these have significant drawbacks: JKT-1's expression profile more closely resembles a different GCT subtype, SEM-1 exhibits histology that is a mixture of multiple GCT subtypes, and TCam-2 has a highly unusual mutation in BRAF.^{70,71} For seminomas, a stem cell-derived in vitro model may be a good alternative. To this end, researchers demonstrated that overexpressing *PRDM14* in stem cell-derived PGCs delayed differentiation and increased proliferation, important mechanisms of seminoma pathogenesis.⁷²

Patient-derived xenograft (PDX) models. Animal PDX models are established through transplantation of human samples into animals. Upon transplantation of human tumor samples, the tumor can grow and progress within the in vivo environment at orthotopic or heterotopic locations. A recent publication describing the establishment of 131 pediatric PDX models reported high concordance of key features with the original tumor, including exome, expression profile, and histology.⁷³ However, PDX models have several limitations. After multiple passages, PDX models exhibit genomic instability caused by selection pressure for oncogenic phenotypes.^{74,75} In this way, the genetic mutations in PDX models quickly begin to “drift” away from the patient's driver mutations and become progressively less representative of the phenotype of the human tumor.⁷⁶ Human induced pluripotent stem cells (hiPSCs), on the other hand, have a comparatively low mutational rate even after many passages; in this way, the phenotype of harbored mutations could be reliably assayed.^{77,78} PDX models also typically involve the transplantation into immunodeficient adult mice, which substantially limits insights into tumor-immune microenvironment and developmental window. In addition to immune humanized models, chimera models, established using human stem cells, can be used to improve in vivo modeling of human pediatric cancers.⁷⁹ In one study, hiPSCs were genetically engineered to harbor Dox-inducible oncogenic mutations, differentiated into neural crest cells, and transplanted into E8.5 mice to create a mouse-human chimera harboring neuroblastoma.

Genetically engineered mouse models (GEMMs). GEMMs are created by leveraging our knowledge of human cancer genetics to engineer oncogenic mutations into the mouse. With GEMMs, researchers can take advantage of conditional and cell-specific oncogenic activation to tightly control developmental programs and faithfully model pediatric tumorigenesis in vivo.⁸⁰ The most well established GEMM for pediatric cancer is the tyrosine hydroxylase-*MYCN* transgenic mouse, which, through *MYCN* overexpression in neuroectodermal cells, causes human-like neuroblastoma formation.⁸¹ GEMMs for pediatric high-grade glioma and MB have also been

validated.^{82,83} One interesting approach for modeling MB in mice includes isolating postnatal cerebellar cells, infecting them with *Myc* and dominant-negative *p53*, and transplanting them into the cerebellum of an immunocompromised recipient.⁸⁴ Distinct from the *Ptch1* knockout, SHH-associated MB model, this transplant model resembled large cell/anaplastic MB in humans. However, the differences between humans and mice are an unavoidable limitation for GEMMs. Slight differences in interspecies development can be critical: researchers have identified that unique attributes of the human rhombic lip development cause an expansion of one MB cell-of-origin, thereby increasing risk for certain subtypes.²⁹ The distinct genetic background of animal models from human patients also limits construct validity. Nevertheless, through rigorous cross-species genetics, ependymoma has been studied by engineering mouse neural stem cells with alterations found in human cancers, including overexpression of *Ephb2*,⁸⁵ *RELA* fusion,⁸⁶ and ZFTA translocations.⁸⁷ However, differences between the human and model organism genomes may be more challenging to overcome for certain pediatric cancers.

Human Stem Cell Modeling

Primed hiPSCs have potency analogous to the inner cell mass that gives rise to the entire embryo. The cancer cell-of-origin for pediatric cancers can be described as a daughter of the inner cell mass that has differentiated throughout early development and gained cancer-initiating somatic mutations. In this way, hiPSCs can be reprogrammed from patients with germline predisposition and/or engineered to harbor disease-relevant mutations and be differentiated in vitro to the correct cell-of-origin, in order to accurately recapitulate core features of pathogenesis. Such stem cell-derived pediatric cancer models are easily adapted for multiple purposes. As a simple 2D in vitro platform, stem cell derived models can be used for high throughput drug screening and rapid GWAS validation, a highly desired resource to meet the recent explosion in GWAS data.^{88,89} While in vitro culture systems lack certain complexity found in *in vivo* models, advances in 3D, stem cell-based in vitro systems can mitigate this problem.⁹⁰ Increasingly, organoid models are being developed for pediatric tumors to better recapitulate tumor progression (Figure 2).

Medulloblastoma. Using human neural stem cells and progenitors derived from the cerebellar anlage of human embryos, researchers created a human-based model of Group 3 MB.⁹¹ Isolated cells were transduced with c-MYC and dominant negative *p53*, as well as constitutively active AKT and hTERT, before being transplanted into the mouse cerebellum, wherein Group 3 MB-like tumors developed. Further, authors leveraged this model and a novel in silico approach to predict the efficacy of CDK 4/6 inhibitors in Group 3 MBs, a finding that has since been validated.^{92–94} Based on this MB model, researchers subsequently found that Fronzoside A enhanced

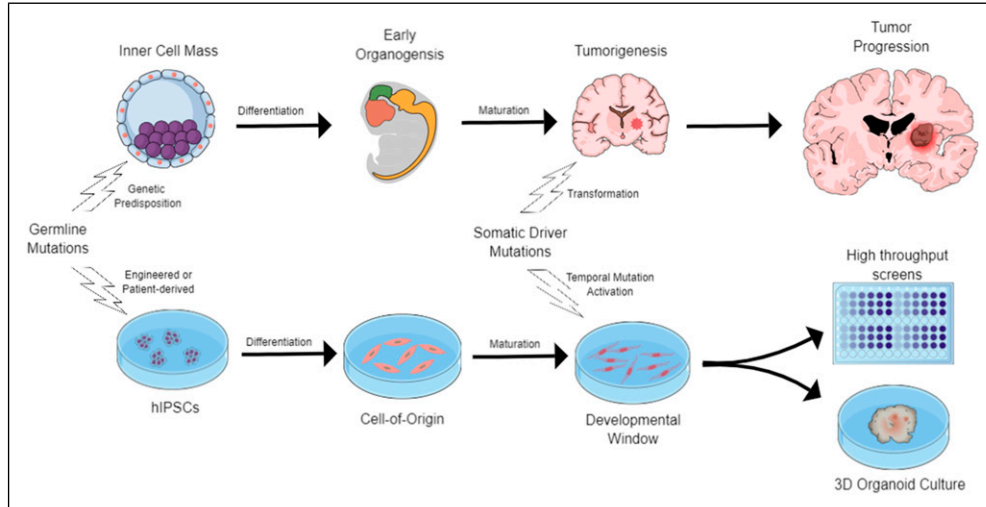


Figure 2. Human stem cells as a multi-faceted, in vitro model of aberrant development in pediatric cancers. Created with the Mind the Graph platform (www.mindthegraph.com).

antitumor effects of immune cells at the site of the tumor, resulting in cytotoxicity and increased survival of xenograft-receipt mice.⁹⁵ Group 3 MB has also been modeled using human cerebellar organoids that were transfected in vivo in mice to overexpress *Gfi1*, *c-MYC*, and *Otx2*.⁹⁶ This model has a DNA methylation signature that clustered closely with the human disease and exhibited over-proliferation, which was inhibited by tazemetostat, a *EZH2*-specific inhibitor.

Human iPSCs from patients with Gorlin syndrome, who harbor a germline *PTCH1* mutation, have been differentiated into neuroepithelial cells and transduced to overexpress *MYCN* as a model for SHH-MB.^{97–100} This hiPSC model, based on transcriptome profile, was found to be more similar to pediatric SHH-MB than the murine model, which was more similar to adult SHH-MB.⁹⁷ A stem-cell derived model of infant SHH-MB was also found to be more aggressive than a model derived from embryonic neuroepithelial cells, due to increased OCT4 and mTOR activity, consistent with mouse findings.^{59,101} This study demonstrates how human stem cells can recapitulate tight developmental windows that may be otherwise inaccessible.¹⁰¹

Pediatric Glioma. Many human stem cell models for glioblastoma have been described,⁹⁹ including genetically engineered, hiPSC-derived neural progenitor¹⁰² and cerebral organoid models,¹⁰³ as well as a new technique called Glioma Cerebral Organoids (GLICO), which combines patient-derived glioma stem cells with hiPSC-derived cerebral organoids.¹⁰⁴ However, relatively few human stem cell models specific for pediatric glioma have been generated. In modeling diffuse intrinsic pontine glioma, authors differentiated embryonic stem cells into neural progenitor cells and transduced the following genetic manipulations: *PDGFRA* overexpression, *TP53* knockdown, and introduction of mutant H3.3K27 M.¹⁰⁵ The authors demonstrated that this

combination of manipulations synergistically resulted in growth advantage and tumorigenesis of the engineered cells. A small drug screen based on this model revealed that menin inhibition decreased survival of this model in vitro and in vivo. Notably, in a later study, H3.3-K27 M and TP53 inactivation alone in neural stem cells, not oligodendrocyte progenitors, was found to be tumorigenic.¹⁰⁶ In order to model pediatric high grade glioma, researchers engineered human embryonic stem cells with mutations in *H3.3G34 R*, *ATRX*, and *TP53*.¹⁰⁷ Interestingly, tumors only formed from forebrain neural progenitors, not hindbrain progenitors, when *N-MYC* was also overexpressed, corresponding to the location found in pediatric patients.

Another research group also used a stem cell approach to model pediatric low-grade gliomas. First of all, the authors found that *NF1* loss and *KIAA1549:BRAF* fusion in hiPSC-derived neural and glial progenitors, but not differentiated astrocytes, resulted in glioma-like tumors in *Rag1*^{-/-} mice. Interestingly, immunocompetent mice with a *Cxcl10* knockout were permissive to tumor formation. Finally, inhibition of MEK was found to promote apoptosis and reduce proliferation in this glioma model. In a substantially different human model, neural progenitors engineered with R132H mutant IDH1, as well as *TP53* and *ATRX* knockdown, synergistically caused in vivo invasion and clustered with IDH1 mutant human low-grade glioma based on DNA methylation and transcriptome.

Neuroblastoma. An early human neural crest stem cell model of neuroblastoma was described by Newman et al (2017).¹⁰⁸ The authors found that overexpression of *MYCN* in neural crest stem cells promoted proliferation and migration and resulted in tumorigenesis. The authors subsequently found that gene silencing of non-canonical alternative nonhomologous end-joining components, namely Lig1, Lig3, and PARP1, diminished tumor characteristics in this model.

Building on this model, researchers found that CD55 via the JNK pathway promoted tumorigenesis and progression.¹⁰⁹ Recently, a neuroblastoma model was generated that integrated *MYCN* overexpression with copy number gain in chromosomal arms 17q and 1q.¹¹⁰ These copy number alterations resulted in impaired trunk neural crest differentiation and enhanced tumorigenic hallmarks. Because the copy number alterations alone did not result in tumorigenesis, the authors hypothesized that these alterations provide an early selection advantage and mark a tumor-initiating event that requires a second hit, like in *MYCN*, for tumor development. Integration of tumor-initiating structural variants and chromosomal aberrations may be valuable for multiple pediatric cancer models.

Neurofibroma. Neurofibromatosis type 1 (NF1) is a multi-cancer predisposition syndrome caused by the germline mutation in *NF1*. While GEMMs have provided some insight, tumors derived from some of these models can be substantially different from the human tumor. For example, the human NF1-low grade glioma has significantly more microglia than the mouse model, a problem potentially addressed with hiPSC-derived microglia.¹¹¹ Investigating neurofibromas in NF1, researchers found that *NF1-null* hiPSC-derived Schwann cells exhibited differentiation defects and were able to form humanized neurofibromas when injected into the sciatic nerve of immunodeficient mice.^{112,113} Further, engineered loss of *TP53* in *NF1-null* Schwann cells resulted in the development of malignant tumors. A rare subpopulation of NF1 patients will have a microdeletion of 17q11.2 that contains *NF1* and flanking regions, which not only increases risk for neurofibromas, but also results in severe developmental delays. A forebrain cerebral organoid was generated from hiPSCs harboring this deletion; the model exhibited deficits in differentiation, as well as hyperproliferation.¹¹⁴

Pediatric cancer predisposition syndromes, leukemia, and other tumors. Cancer predisposition syndromes are particularly amenable to stem cell modeling because of the presence of germline mutations that will be present in derived hiPSCs.¹¹⁵ Retinoblastoma is a childhood malignancy that occurs due to biallelic inactivation of *RBI*. Recently, retinal organoids have been engineered from hiPSCs with *RBI* mutations and were found to closely cluster with patient samples based on copy number variations and DNA methylation profile.^{116,117} Familial adenomatous polyposis (FAP) is caused by a loss of function in the *APC* gene, and colonic organoids derived from FAP patients exhibit epithelial hyperproliferation.¹¹⁸ Using these models, potential drug candidates, including retinoic acid, curcumin, and geneticin, and modes of resistance have been tested.^{118,119} *APC* heterozygosity in hiPSC-derived intestinal organoids was found to be responsible for metabolic and cell adherence changes associated with malignancy.¹²⁰ Although hiPSC lines have been derived for Beckwith-Wiedemann syndrome,¹²¹ von Hippel-Lindau

syndrome,¹²² xeroderma pigmentosum,¹²³ and multiple endocrine neoplasia 2A,¹²⁴ no malignancy phenotypes have been described.¹²⁵ However, a model for insulinoma, a pancreatic neuroendocrine tumors found in multiple endocrine neoplasia type 1, was recently generated from patient-derived hiPSCs differentiated into β -cells in vitro and transplanted into immunodeficient mice.¹²⁶

Fanconi anemia (FA) with bone marrow failure is caused by mutations in several genes that result in genomic instability and increased risk for multiple cancers, including AML, at a young age. Multiple studies have shown that FA patient-derived iPSCs exhibit abnormal hematopoietic differentiation.¹²⁷⁻¹³⁰ These models demonstrated that anti-inflammatory compounds, and inhibition of MYC with the BET bromodomain inhibitor JQ1 can rescue cellular dysfunction in FA.^{127,131} Organ-on-a-chip is an in vitro culture system that uses microfluidics to model organ physiology. In one study, hematopoietic stem cells isolated from patients with Shwachman–Diamond syndrome, another genetic bone marrow failure syndrome with increased risk for leukemia, were adapted to a bone marrow-on-a-chip and exhibited widespread deficits in hematopoiesis.¹³² More recently, researchers used a leukemia-on-a-chip model to demonstrate how B-cell ALL blasts promote a chemoprotective niche.¹³³ Although hiPSCs derived from various pediatric leukemia cells exhibited abnormal leukemogenesis that favored certain lineages, they were unable to recapitulate leukemia in vivo.¹³⁴ Similarly, hemopoietic differentiation of hiPSCs with the ETV6-RUNX1 fusion, the most common fusion associated with childhood ALL, resulted in expansion of CD19⁻IL-7R⁺ progenitors, reduced B-cell commitment, and molecular signatures resembling a pre-leukemic state.¹³⁵

In human stem cells, modeling of EWS was initially attempted by dox-inducible expression of *EWS-FLI1* and knockdown of *TP53* in embryoid bodies; although this model exhibited characteristics of transformation, it failed to form tumors via xenografting.¹³⁶ However, by introducing the t(11;22)(q24;q12) translocation and oncogenic alterations in *STAG2*, *TP53*, and *CDKN2A* in mesenchymal stem cells specifically from European patient resulted in formation of EWS-like tumor.¹³⁷ LFS hiPSC-derived osteoblasts exhibit reduced differentiation capacity and expression enrichment associated with poor outcomes in LFS patients with osteosarcoma.¹³⁸ Interestingly, *MYCN* overexpression in a LFS hiPSC-derived bone and cartilage progenitor resulted in chondroblastic osteosarcoma-like tumor.¹³⁹ Knockout of *TP53* has been used to model rhabdomyosarcoma and atypical teratoid/rhabdoid tumor in combination with the PAX3-FOXO1 fusion or SMARCB1 deficiency, respectively.^{140,141} A recently published article used hiPSCs derived from a patient with germline isochromosome 12p to model testicular seminoma.¹⁴² This model did appear to upregulate signaling related to transformation but failed to result in tumor formation in vivo; the authors hypothesize that additional somatic driver mutations are required.

Germline Aneuploidy. Genomic instability is known to cause aneuploidy, but recent evidence also demonstrates how aneuploidy can promote instability.^{143–145} Growing literature have illuminated the diverse role of aneuploidy in tumor initiation, evolution, drug resistance, and immunogenicity.^{146–148} Studies using mouse embryonic stem cells have shown that multiple chromosomal gains independently promote proliferation and metastasis.^{149,150} Further, studies have shown that chromosomal aberrations that accumulate in hPSCs in vitro not only recapitulate aberrations found in human cancer, but also promote tumorigenicity.^{151,152} Therefore, there would be substantial value in using human pluripotent stem cells with aneuploidy to model tumorigenesis. However, engineering aneuploidy or other complex variants can be difficult. Alternatively, hPSCs can be derived from patients with germline aneuploidy. When such resources are generated, many experiments are possible, including investigation of diverse cell lineages.

Trisomy 21 hPSCs, engineered with a truncated *GATA1* and *STAG2* loss-of-function, exhibit downregulation of myeloid differentiation and expansion of the immature megakaryocytic population.¹⁵³ Expression of truncated *GATA1* in trisomy 21 in early hematopoietic progenitor cells was found to be sufficient to cause transient abnormal myelopoiesis, namely reduced megakaryocytic commitment.^{154,155} As an alternative approach, investigators studying the role of endothelial cell dysfunction in DS hypothesized that decreased proliferation, migration, and inflammatory response, as well as impaired mesodermal differentiation, may contribute to both to leukemia predisposition and solid tumor protection.^{156,157}

Klinefelter syndrome, characterized by 47,XXY, is associated with extragonadal germ cell tumors, and Turner syndrome, characterized by 45,XO, with gonadoblastoma, particularly in patients with cryptic Y chromosome material.^{158,159} Both 47,XXY, and 45,XO, hPSCs have been generated and preliminarily differentiated to study pluripotency and neurogenesis, but little investigation into the cancer cell-of-origin, or germ cell progenitors, have been pursued.^{160–162} Perhaps not surprisingly, in vitro differentiation of 47,XXY, hPSCs into the germ cell lineage is significantly less efficient due to increased germ cell death.¹⁶³ Therefore, a Klinefelter hPSC-derived model of germ cell tumors may be technically challenging to generate. Interestingly, primordial germ cell-like cells generated from 45,XO, hPSCs exhibited typical epigenetic and transcriptomic features, although further germ cell differentiation was not investigated.¹⁶⁴

Conclusion

In this review, we examined the critical genetic and developmental information to consider when modeling pediatric cancers and described current human stem cell models being developed for diverse pediatric cancer types. Understanding that pediatric cancers arise from a deviation in normal

development and differentiation, we posit that human stem cell models are well suited to model tumorigenesis in childhood tumors. While there has been substantial progress in human stem cell modeling, as evident by the numerous examples described in this review, there is substantial room for improvement. Greater precision, regarding genetic context and cell-of-origin, is needed. The timing of driver mutations during development is critical to tumor identity and clinical manifestation. As genomic analyses, such as phylogenetic tracing and single cell sequencing, of patient samples pinpoint the developmental ancestor of transformation, stem cell models must be tuned to better mimic the cell-of-origin. Using modern cellular and genetic engineering techniques, researchers are empowered to activate driver mutations at precise developmental windows, while appreciating germline mutations that may have contributed to a predisposing environment. Future validation and characterization of pediatric cancer stem cell models need to have a keen focus on the dynamics of development. Gene expression analysis and functional assays demonstrating increased proliferation or tumorigenicity are insufficient. Models that only demonstrate features common to most cancers are unlikely to produce translatable insights; instead, models should be generated and validated based on tumor-specific properties. For example, changes in differentiation trajectory, such as loss of differentiation potential or deviations from typical differentiation programs, are key to elements of pediatric tumorigenesis. Other factors to consider include tumor heterogeneity, interactions with other cells of development, and changes in functions particular to the cell-of-origin. Without these considerations, models have limited translatability and risk scientific discoveries reflective of only the model itself, not the human disease.

Pediatric oncology research is often viewed and treated as a subcategory of adult oncology. Receiving only 4–5% of federal United States cancer research funding, pediatric cancer research is lagging behind its adult counterpart.^{165,166} Targeted therapeutics tested in pediatric cancer are often repurposed drugs initially tested in adult populations, which may explain why Phase I clinical trials of targeted therapies for pediatric solid tumors demonstrate objective response in only 3.1% of cases, compared to 19.8% in adult trials.^{167–169} Given the vast differences in the pathogenesis between adult and pediatric cancers, there would be substantial benefit in closer collaboration between pediatric oncology and developmental biology. Expertise about cell lineage, cell-cell interactions, and cellular differentiation would be highly valuable to designing pediatric cancer models.

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ORCID iD

Cullen D. Clairmont  <https://orcid.org/0000-0002-9672-2205>

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