1-1-2024

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Cancer Control
Volume 31: 1–15

DOI: 10.1177/10732748241270564
journals.sagepub.com/home/ccx
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Pediatric Tumors as Disorders of Development: The Case for In Vitro Modeling Based on Human Stem Cells

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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.

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Data Availability Statement included at the end of the article

Keywords
cell of origin, developmental window, disease modeling, pediatric cancers, pluripotent stem cells

Received November 30, 2023. Received revised May 31, 2024. Accepted for publication June 13, 2024.

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 *Nf1* 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 simar 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 Smoothened 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]

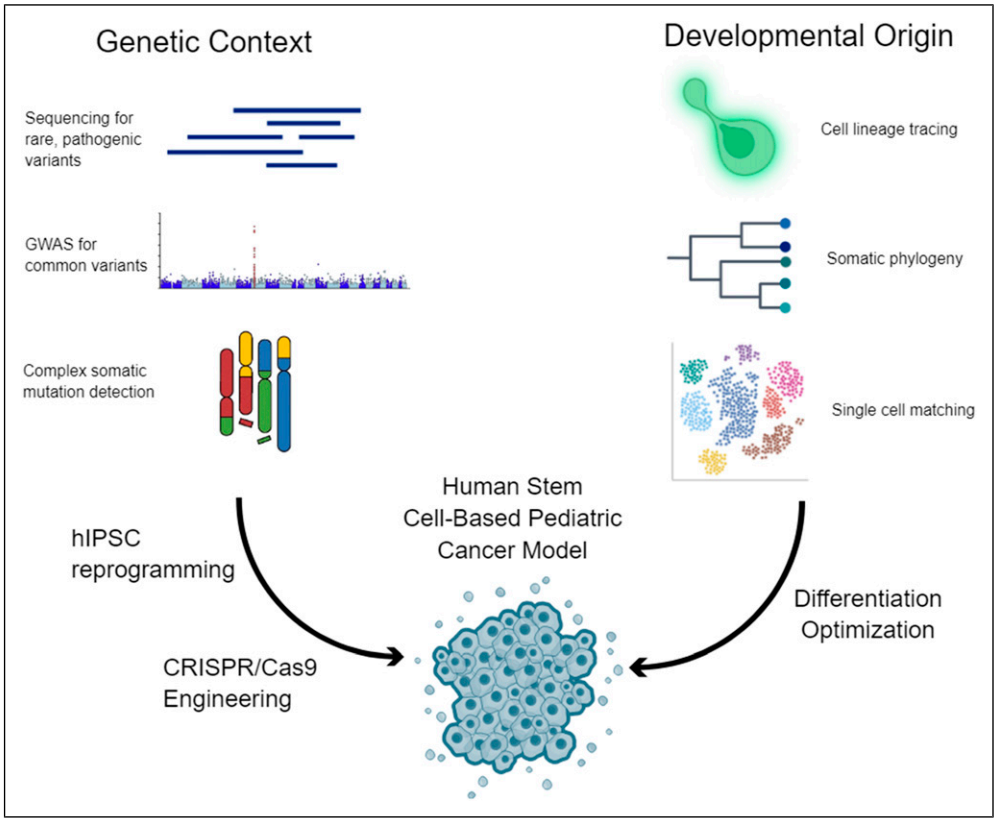

Figure 1. Techniques to investigate the genetic context and developmental origin of pediatric cancer, which will inform human stem cell-based models. Created with the Mind the Graph platform (www.mindthegraph.com) and Biorender.com. Includes image adapted from Balachandran & Beck (2020).[170]

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 Frondoside A enhanced

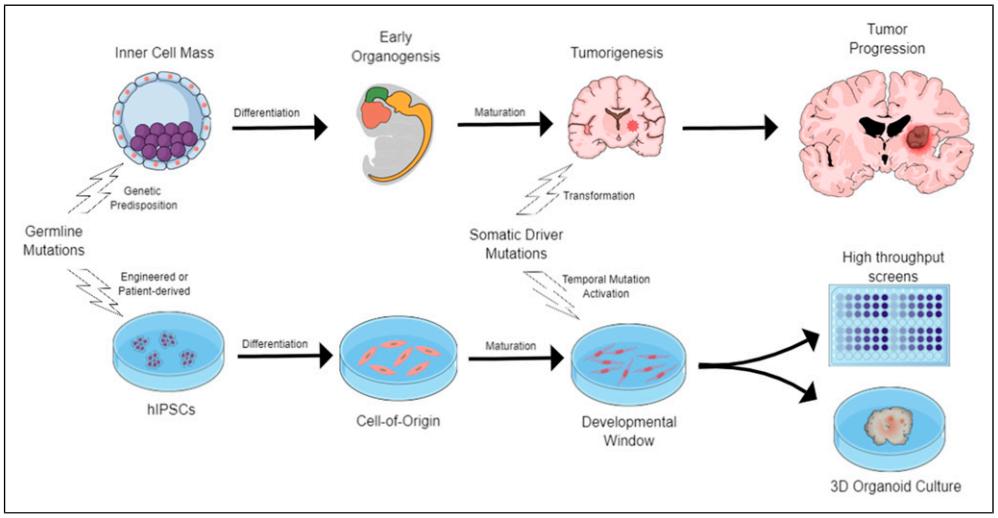

Figure 2. Human stem cells as a multi-faceted, in vitro model of aberrant development in pediatric cancers. Created with the Mind the Graph platform (www.mindthegraph.com).

antitumor effects of immune cells at the site of the tumor, resulting in cytotoxicity and increased survival of xenograft-recipient 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 progenitor[102] 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 multicancer 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 *RB1*. Recently, retinal organoids have been engineered from hIPSCs with *RB1* 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 $CD19^{-}IL\text{-}7R^{+}$ 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 hIPSCs in vitro not only recapitulate aberrations found in human cancer, but also promote tumorgenicity.[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, hIPSCs 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 hIPSCs, 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, hIPSCs 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, hIPSCs into the germ cell lineage is significantly less efficient due to increased germ cell death.[163] Therefore, a Klinefelter hIPSC-derived model of germ cell tumors may be technically challenging to generate. Interestingly, primordial germ cell-like cells generated from 45,XO, hIPSCs 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.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work

was supported by the CCMC Martin J Gavin Endowment, West Family Trust, JAX Director Innovation Fund, National Human Genome Research Institute (NHGRI) (5T32HG010463-05), CureSearch for Childrens Cancer (685676) and Congressionally Directed Medical Research Program (W81XWH-22-1-0177).

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Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study." Retain the statement provided in quotes.

References

1. Colditz GA, Wolin KY, Gehlert S. Applying what we know to accelerate cancer prevention. *Sci Transl Med*. 2012;4(127):127rv4. doi:10.1126/scitranslmed.3003218
2. Kattner P, Strobel H, Khoshnevis N, et al. Compare and contrast: pediatric cancer versus adult malignancies. *Cancer Metastasis Rev*. 2019;38(4):673-682. doi:10.1007/s10555-019-09836-y
3. Kim SY, Yun HJ, Chang H, et al. Aggressiveness of differentiated thyroid carcinoma in pediatric patients younger than 16 years: a propensity score-matched analysis. *Front Oncol*. 2022;12. https://www.frontiersin.org/articles/10.3389/fonc.2022.872130. Accessed November 15, 2023.
4. Survival cancer trends progress report. Accessed November 15, 2023.https://progressreport.cancer.gov/after/survival
5. Cancer facts & figures 2020. Accessed November 15, 2023.https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2020.html
6. Cancer in children and adolescents - NCI. Published September 29, 2023. Accessed November 24, 2023.https://www.cancer.gov/types/childhood-cancers/child-adolescent-cancers-fact-sheet
7. FastStats. Published July 25, 2023. Accessed November 15, 2023.https://www.cdc.gov/nchs/fastats/child-health.htm
8. Yeh JM, Ward ZJ, Chaudhry A, et al. Life expectancy of adult survivors of childhood cancer over 3 decades. *JAMA Oncol*. 2020;6(3):350-357. doi:10.1001/jamaoncol.2019.5582
9. Suh E, Stratton KL, Leisenring WM, et al. Late mortality and chronic health conditions in long-term survivors of early-adolescent and young adult cancers: a retrospective cohort analysis from the Childhood Cancer Survivor Study. *Lancet Oncol*. 2020;21(3):421-435. doi:10.1016/S1470-2045(19)30800-9
10. Phillips SM, Padgett LS, Leisenring WM, et al. Survivors of childhood cancer in the United States: prevalence and burden of morbidity. *Cancer Epidemiol Biomarkers Prev*. 2015;24(4):653-663. doi:10.1158/1055-9965.EPI-14-1418
11. Armstrong GT, Oeffinger KC, Chen Y, et al. Modifiable risk factors and major cardiac events among adult survivors of childhood cancer. *J Clin Oncol*. 2013;31(29):3673-3680. doi:10.1200/JCO.2013.49.3205
12. Chow EJ, Chen Y, Armstrong GT, et al. Underdiagnosis and undertreatment of modifiable cardiovascular risk factors among survivors of childhood cancer. *J Am Heart Assoc*. 2022;11(12):e024735. doi:10.1161/JAHA.121.024735
13. Taub JW. Relationship of chromosome 21 and acute leukemia in children with Down syndrome. *J Pediatr Hematol Oncol*. 2001;23(3):175-178. doi:10.1097/00043426-200103000-00012
14. Marlow EC, Ducore J, Kwan ML, et al. Leukemia risk in a cohort of 3.9 million children with and without down syndrome. *J Pediatr*. 2021;234:172-180. doi:10.1016/j.jpeds.2021.03.001
15. Hasle H, Friedman JM, Olsen JH, Rasmussen SA. Low risk of solid tumors in persons with Down syndrome. *Genet Med*. 2016;18(11):1151-1157. doi:10.1038/gim.2016.23
16. Rahner N, Steinke V. Hereditary cancer syndromes. *Dtsch Ärztebl Int*. 2008;105(41):706-714. doi:10.3238/arztebl.2008.0706
17. Zhang J, Walsh MF, Wu G, et al. Germline mutations in predisposition genes in pediatric cancer. *N Engl J Med*. 2015;373(24):2336-2346. doi:10.1056/NEJMoa1508054
18. Gröbner SN, Worst BC, Weischenfeldt J, et al. The landscape of genomic alterations across childhood cancers. *Nature*. 2018;555(7696):321-327. doi:10.1038/nature25480
19. Fiala EM, Jayakumaran G, Mauguen A, et al. Prospective pan-cancer germline testing using MSK-IMPACT informs clinical translation in 751 patients with pediatric solid tumors. *Nat Can (Ott)*. 2021;2:357-365. doi:10.1038/s43018-021-00172-1
20. Douglas SPM, Lahtinen AK, Koski JR, et al. Enrichment of cancer-predisposing germline variants in adult and pediatric patients with acute lymphoblastic leukemia. *Sci Rep*. 2022;12(1):10670. doi:10.1038/s41598-022-14364-x
21. Alonso-Luna O, Mercado-Celis GE, Melendez-Zajgla J, Zapata-Tarres M, Mendoza-Caamal E. The genetic era of childhood cancer: identification of high-risk patients and germline sequencing approaches. *Ann Hum Genet*. 2023;87(3):81-90. doi:10.1111/ahg.12502
22. Plon SE, Lupo PJ. Genetic predisposition to childhood cancer in the genomic era. *Annu Rev Genom Hum Genet*. 2019;20:241-263. doi:10.1146/annurev-genom-083118-015415
23. Capasso M, Montella A, Tirelli M, Maiorino T, Cantalupo S, Iolascon A. Genetic predisposition to solid pediatric cancers. *Front Oncol*. 2020;10:590033. doi:10.3389/fonc.2020.590033
24. Raynor La., Pankratz N, Spector LG. An analysis of measures of effect size by age of onset in cancer genomewide association studies. *Genes Chromosomes Cancer*. 2013;52(9):855-859. doi:10.1002/gcc.22081
25. Sweet-Cordero EA, Biegel JA. The genomic landscape of pediatric cancers: implications for diagnosis and treatment. *Science*. 2019;363(6432):1170-1175. doi:10.1126/science.aaw3535
26. Campbell BB, Light N, Fabrizio D, et al. Comprehensive analysis of hypermutation in human cancer. *Cell*. 2017;171(5):1042-1056.e10. doi:10.1016/j.cell.2017.09.048

27. Ma X, Liu Y, Liu Y, et al. Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours. *Nature*. 2018;555(7696):371-376. doi:10.1038/nature25795
28. Huether R, Dong L, Chen X, et al. The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nat Commun*. 2014;5:3630. doi:10.1038/ncomms4630
29. Hendrikse LD, Haldipur P, Saulnier O, et al. Failure of human rhombic lip differentiation underlies medulloblastoma formation. *Nature*. 2022;609(7929):1021-1028. doi:10.1038/s41586-022-05215-w
30. Chen X, Yang W, Roberts CWM, Zhang J. Developmental origins shape the paediatric cancer genome. *Nat Rev Cancer*. 2024;24:382-398. doi:10.1038/s41568-024-00684-9. Published online May 2.
31. Azzarelli R, Simons BD, Philpott A. The developmental origin of brain tumours: a cellular and molecular framework. *Development*. 2018;145(10):dev162693. doi:10.1242/dev.162693
32. Schwartzentruber J, Korshunov A, Liu XY, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*. 2012;482(7384):226-231. doi:10.1038/nature10833
33. Wu G, Broniscer A, McEachron TA, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet*. 2012;44(3):251-253. doi:10.1038/ng.1102
34. Rahal Z, Abdulhai F, Kadara H, Saab R. Genomics of adult and pediatric solid tumors. *Am J Cancer Res*. 2018;8(8):1356-1386.
35. Rahman N. Realizing the promise of cancer predisposition genes. *Nature*. 2014;505(7483):302-308. doi:10.1038/nature12981
36. Shahani SA, Marcotte EL. Landscape of germline cancer predisposition mutations testing and management in pediatrics: implications for research and clinical care. *Front Pediatr*. 2022;10:1011873. https://www.frontiersin.org/articles/10.3389/fped.2022.1011873. Accessed November 16, 2023.
37. Pastor V, Hirabayashi S, Karow A, et al. Mutational landscape in children with myelodysplastic syndromes is distinct from adults: specific somatic drivers and novel germline variants. *Leukemia*. 2017;31(3):759-762. doi:10.1038/leu.2016.342
38. Musa J, Cidre-Aranaz F, Aynaud MM, et al. Cooperation of cancer drivers with regulatory germline variants shapes clinical outcomes. *Nat Commun*. 2019;10:4128. doi:10.1038/s41467-019-12071-2
39. Oldridge DA, Wood AC, Weichert-Leahey N, et al. Genetic predisposition to neuroblastoma mediated by a LMO1 super-enhancer polymorphism. *Nature*. 2015;528(7582):418-421. doi:10.1038/nature15540
40. Marino S, Gilbertson RJ. Harnessing brain development to understand brain tumours. *Dev Camb Engl*. 2021;148(2):dev193342. doi:10.1242/dev.193342
41. Solga AC, Toonen JA, Pan Y, et al. The cell of origin dictates the temporal course of neurofibromatosis-1 (Nf1) low-grade glioma formation. *Oncotarget*. 2017;8(29):47206-47215. doi:10.18632/oncotarget.17589
42. Coorens THH, Farndon SJ, Mitchell TJ, et al. Lineage-independent tumors in bilateral neuroblastoma. *N Engl J Med*. 2020;383(19):1860-1865. doi:10.1056/NEJMoa2000962
43. Coorens THH, Treger TD, Al-Saadi R, et al. Embryonal precursors of Wilms tumor. *Science*. 2019;366(6470):1247-1251. doi:10.1126/science.aax1323
44. Custers L, Khabirova E, Coorens THH, et al. Somatic mutations and single-cell transcriptomes reveal the root of malignant rhabdoid tumours. *Nat Commun*. 2021;12(1):1407. doi:10.1038/s41467-021-21675-6
45. Brandsma AM, Bertrums EJM, van Roosmalen MJ, et al. Mutation signatures of pediatric acute myeloid leukemia and normal blood progenitors associated with differential patient outcomes. *Blood Cancer Discov*. 2021;2(5):484-499. doi:10.1158/2643-3230.BCD-21-0010
46. Coorens THH, Behjati S. Tracing and targeting the origins of childhood cancer. *Annu Rev Cell Biol*. 2022;6(1):35-47. doi:10.1146/annurev-cancerbio-070620-091632
47. Filbin MG, Tirosh I, Hovestadt V, et al. Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. *Science*. 2018;360(6386):331-335. doi:10.1126/science.aao4750
48. Hovestadt V, Smith KS, Bihannic L, et al. Resolving medulloblastoma cellular architecture by single-cell genomics. *Nature*. 2019;572(7767):74-79. doi:10.1038/s41586-019-1434-6
49. Jansky S, Sharma AK, Körber V, et al. Single-cell transcriptomic analyses provide insights into the developmental origins of neuroblastoma. *Nat Genet*. 2021;53(5):683-693. doi:10.1038/s41588-021-00806-1
50. Jessa S, Blanchet-Cohen A, Krug B, et al. Stalled developmental programs at the root of pediatric brain tumors. *Nat Genet*. 2019;51(12):1702-1713. doi:10.1038/s41588-019-0531-7
51. Rausch T, Jones DTW, Zapatka M, et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. *Cell*. 2012;148(1):59-71. doi:10.1016/j.cell.2011.12.013
52. Northcott PA, Hielscher T, Dubuc A, et al. Pediatric and adult sonic hedgehog medulloblastomas are clinically and molecularly distinct. *Acta Neuropathol*. 2011;122(2):231-240. doi:10.1007/s00401-011-0846-7
53. Smit MJ, Martini TEI, Armandari I, et al. The developmental stage of the medulloblastoma cell-of-origin restricts Sonic hedgehog pathway usage and drug sensitivity. *J Cell Sci*. 2022;135(11):jeb258608. doi:10.1242/jcs.258608
54. Gold MP, Ong W, Masteller AM, et al. Developmental basis of SHH medulloblastoma heterogeneity. *Nat Commun*. 2024;15(1):270. doi:10.1038/s41467-023-44300-0
55. Hooper CM, Hawes SM, Kees UR, Gottardo NG, Dallas PB. Gene expression analyses of the spatio-temporal relationships of human medulloblastoma subgroups during early human

neurogenesis. *PLoS One*. 2014;9(11):e112909. doi:10.1371/journal.pone.0112909
56. Barbet V, Broutier L. Future match making: when pediatric oncology meets organoid technology. *Front Cell Dev Biol*. 2021;9:674219. https://www.frontiersin.org/articles/10.3389/fcell.2021.674219. Accessed November 28, 2023.
57. Han ZY, Richer W, Fréneaux P, et al. The occurrence of intracranial rhabdoid tumours in mice depends on temporal control of Smarcb1 inactivation. *Nat Commun*. 2016;7(1):10421. doi:10.1038/ncomms10421
58. Pathania M, De Jay N, Maestro N, et al. H3.3K27M cooperates with Trp53 loss and PDGFRA gain in mouse embryonic neural progenitor cells to induce invasive high-grade gliomas. *Cancer Cell*. 2017;32(5):684-700. doi:10.1016/j.ccell.2017.09.014
59. Malawsky DS, Weir SJ, Ocasio JK, et al. Cryptic developmental events determine medulloblastoma radiosensitivity and cellular heterogeneity without altering transcriptomic profile. *Commun Biol*. 2021;4(1):616. doi:10.1038/s42003-021-02099-w
60. Mobley BC, Kwon M, Kraemer BR, et al. Expression of MYCN in multipotent sympathoadrenal progenitors induces proliferation and neural differentiation, but is not sufficient for tumorigenesis. *PLoS One*. 2015;10(7):e0133897. doi:10.1371/journal.pone.0133897
61. Olsen RR, Otero JH, García-López J, et al. MYCN induces neuroblastoma in primary neural crest cells. *Oncogene*. 2017;36(35):5075-5082. doi:10.1038/onc.2017.128
62. Jecrois ES, Zheng W, Bornhorst M, et al. Treatment during a developmental window prevents NF1-associated optic pathway gliomas by targeting Erk-dependent migrating glial progenitors. *Dev Cell*. 2021;56(20):2871-2885. doi:10.1016/j.devcel.2021.08.004
63. Lago C, Federico A, Leva G, et al. Patient-and xenograft-derived organoids recapitulate pediatric brain tumor features and patient treatments. *EMBO Mol Med*. 2023;15(12):e18199. doi:10.15252/emmm.202318199
64. Xu S, Tan S, Guo L. Patient-derived organoids as a promising tool for multimodal management of sarcomas. *Cancers*. 2023;15(17):4339. doi:10.3390/cancers15174339
65. Sood D, Tang-Schomer M, Pouli D, et al. 3D extracellular matrix microenvironment in bioengineered tissue models of primary pediatric and adult brain tumors. *Nat Commun*. 2019;10(1):4529. doi:10.1038/s41467-019-12420-1
66. Schwark K, Messinger D, Cummings JR, et al. Receptor tyrosine kinase (RTK) targeting in pediatric high-grade glioma and diffuse midline glioma: pre-clinical models and precision medicine. *Front Oncol*. 2022;12:922928. doi:10.3389/fonc.2022.922928
67. da Silva B, Mathew RK, Polson ES, Williams J, Wurdak H. Spontaneous glioblastoma spheroid infiltration of early-stage cerebral organoids models brain tumor invasion. *SLAS Discov*. 2018;23(8):862-868. doi:10.1177/2472555218764623
68. Chew N, Habarakada D, Firestein R, Daniel P. A protocol to establish cell line models from rare pediatric solid tumors. *STAR Protoc*. 2023;4(3):102537. doi:10.1016/j.xpro.2023.102537
69. Sun CX, Daniel P, Bradshaw G, et al. Generation and multidimensional profiling of a childhood cancer cell line atlas defines new therapeutic opportunities. *Cancer Cell*. 2023;41(4):660-677.e7. doi:10.1016/j.ccell.2023.03.007
70. Russell SM, Lechner MG, Mokashi A, et al. Establishment and characterization of a new human extragonadal germ cell line, SEM-1, and its comparison with TCam-2 and JKT-1. *Urology*. 2013;81(2):464.e1-464.e4649. doi:10.1016/j.urology.2012.09.029
71. Eckert D, Nettersheim D, Heukamp LC, Kitazawa S, Biermann K, Schorle H. TCam-2 but not JKT-1 cells resemble seminoma in cell culture. *Cell Tissue Res*. 2008;331(2):529-538. doi:10.1007/s00441-007-0527-y
72. Gell JJ, Zhao J, Chen D, Hunt TJ, Clark AT. PRDM14 is expressed in germ cell tumors with constitutive overexpression altering human germline differentiation and proliferation. *Stem Cell Res*. 2018;27:46-56. doi:10.1016/j.scr.2017.12.016
73. Marques Da Costa ME, Zaidi S, Scoazec JY, et al. A biobank of pediatric patient-derived-xenograft models in cancer precision medicine trial MAPPYACTS for relapsed and refractory tumors. *Commun Biol*. 2023;6(1):949. doi:10.1038/s42003-023-05320-0
74. Ben-David U, Beroukhim R, Golub TR. Genomic evolution of cancer models: perils and opportunities. *Nat Rev Cancer*. 2019;19(2):97-109. doi:10.1038/s41568-018-0095-3
75. Zhuo J, Su R, Tan W, Lian Z, Lu D, Xu X. The ongoing trends of patient-derived xenograft models in oncology. *Cancer Commun*. 2020;40(11):559-563. doi:10.1002/cac2.12096
76. Ben-David U, Ha G, Tseng YY, et al. Patient-derived xenografts undergo mouse-specific tumor evolution. *Nat Genet*. 2017;49(11):1567-1575. doi:10.1038/ng.3967
77. Halliwell J, Barbaric I, Andrews PW. Acquired genetic changes in human pluripotent stem cells: origins and consequences. *Nat Rev Mol Cell Biol*. 2020;21(12):715-728. doi:10.1038/s41580-020-00292-z
78. Thompson O, von Meyenn F, Hewitt Z, et al. Low rates of mutation in clinical grade human pluripotent stem cells under different culture conditions. *Nat Commun*. 2020;11(1):1528. doi:10.1038/s41467-020-15271-3
79. Cohen MA, Zhang S, Sengupta S, et al. Formation of human neuroblastoma in mouse-human neural crest chimeras. *Cell Stem Cell*. 2020;26(4):579-592. doi:10.1016/j.stem.2020.02.001
80. Chesler L, Weiss WA. Genetically engineered murine models – contribution to our understanding of the genetics, molecular pathology and therapeutic targeting of neuroblastoma. *Semin Cancer Biol*. 2011;21(4):245-255. doi:10.1016/j.semcancer.2011.09.011
81. Weiss WA, Aldape K, Mohapatra G, Feuerstein BG, Bishop JM. Targeted expression of MYCN causes neuroblastoma in transgenic mice. *EMBO J*. 1997;16(11):2985-2995. doi:10.1093/emboj/16.11.2985
82. Schoof M, Godbole S, Albert TK, et al. Mouse models of pediatric high-grade gliomas with MYCN amplification reveal intratumoral heterogeneity and lineage signatures. *Nat Commun*. 2023;14(1):7717. doi:10.1038/s41467-023-43564-w

83. Roussel MF, Stripay JL. Modeling pediatric medulloblastoma. *Brain Pathol*. 2020;30(3):703-712. doi:10.1111/bpa.12803
84. Pei Y, Moore CE, Wang J, et al. An animal model of MYC-driven medulloblastoma. *Cancer Cell*. 2012;21(2):155-167. doi:10.1016/j.ccr.2011.12.021
85. Johnson RA, Wright KD, Poppleton H, et al. Cross-species genomics matches driver mutations and cell compartments to model ependymoma. *Nature*. 2010;466(7306):632-636. doi:10.1038/nature09173
86. Ozawa T, Arora S, Szulzewsky F, et al. A *de novo* mouse model of C11orf95-RELA fusion-driven ependymoma identifies driver functions in addition to NF-κB. *Cell Rep*. 2018;23(13):3787-3797. doi:10.1016/j.celrep.2018.04.099
87. Kupp R, Ruff L, Terranova S, et al. ZFTA translocations constitute ependymoma chromatin remodeling and transcription factors. *Cancer Discov*. 2021;11(9):2216-2229. doi:10.1158/2159-8290.CD-20-1052
88. Mills MC, Rahal C. A scientometric review of genome-wide association studies. *Commun Biol*. 2019;2(1):9-11. doi:10.1038/s42003-018-0261-x
89. Lichou F, Trynka G. Functional studies of GWAS variants are gaining momentum. *Nat Commun*. 2020;11(1):6283. doi:10.1038/s41467-020-20188-y
90. Lago C, Gianesello M, Santomaso L, et al. Medulloblastoma and high-grade glioma organoids for drug screening, lineage tracing, co-culture and in vivo assay. *Nat Protoc*. 2023;18(7):2143-2180. doi:10.1038/s41596-023-00839-2
91. Hanaford AR, Archer TC, Price A, et al. DiSCoVERing innovative therapies for rare tumors: combining genetically accurate disease models with in silico analysis to identify novel therapeutic targets. *Clin Cancer Res*. 2016;22(15):3903-3914. doi:10.1158/1078-0432.CCR-15-3011
92. Buzzetti M, Morlando S, Solomos D, et al. Pre-therapeutic efficacy of the CDK inhibitor dinaciclib in medulloblastoma cells. *Sci Rep*. 2021;11(1):5374. doi:10.1038/s41598-021-84082-3
93. Cook Sangar ML, Genovesi LA, Nakamoto MW, et al. Inhibition of CDK4/6 by palbociclib significantly extends survival in medulloblastoma patient-derived xenograft mouse models. *Clin Cancer Res*. 2017;23(19):5802-5813. doi:10.1158/1078-0432.CCR-16-2943
94. Pribnow A, Jonchere B, Liu J, et al. Combination of ribociclib and gemcitabine for the treatment of medulloblastoma. *Mol Cancer Therapeut*. 2022;21(8):1306-1317. doi:10.1158/1535-7163.MCT-21-0598
95. Xue Y, Fu Y, Zhao F, et al. Frondoside A inhibits an MYC-driven medulloblastoma model derived from human-induced pluripotent stem cells. *Mol Cancer Therapeut*. 2021;20(6):1199-1209. doi:10.1158/1535-7163.MCT-20-0603
96. Ballabio C, Anderle M, Gianesello M, et al. Modeling medulloblastoma in vivo and with human cerebellar organoids. *Nat Commun*. 2020;11(1):583. doi:10.1038/s41467-019-13989-3
97. Huang M, Tailor J, Zhen Q, et al. Engineering genetic predisposition in human neuroepithelial stem cells recapitulates medulloblastoma tumorigenesis. *Cell Stem Cell*. 2019;25(3):433-446.e7. doi:10.1016/j.stem.2019.05.013
98. Susanto E, Marin Navarro A, Zhou L, et al. Modeling SHH-driven medulloblastoma with patient iPS cell-derived neural stem cells. *Proc Natl Acad Sci U S A*. 2020;117(33):20127-20138. doi:10.1073/pnas.1920521117
99. Khamis ZI, Sarker DB, Xue Y, et al. Modeling human brain tumors and the microenvironment using induced pluripotent stem cells. *Cancers*. 2023;15(4):1253. doi:10.3390/cancers15041253
100. Ikemoto Y, Miyashita T, Nasu M, et al. Gorlin syndrome-induced pluripotent stem cells form medulloblastoma with loss of heterozygosity in PTCH1. *Aging*. 2020;12(10):9935-9947. doi:10.18632/aging.103258
101. Čančer M, Hutter S, Holmberg KO, et al. Humanized stem cell models of pediatric medulloblastoma reveal an oct4/mTOR Axis that promotes malignancy. *Cell Stem Cell*. 2019;25(6):855-870.e11. doi:10.1016/j.stem.2019.10.005
102. Sancho-Martinez I, Nivet E, Xia Y, et al. Establishment of human iPSC-based models for the study and targeting of glioma initiating cells. *Nat Commun*. 2016;7(1):10743. doi:10.1038/ncomms10743
103. Ogawa J, Pao GM, Shokhirev MN, Verma IM. Glioblastoma model using human cerebral organoids. *Cell Rep*. 2018;23(4):1220-1229. doi:10.1016/j.celrep.2018.03.105
104. Linkous A, Balamatsias D, Snuderl M, et al. Modeling patient-derived glioblastoma with cerebral organoids. *Cell Rep*. 2019;26(12):3203-3211.e5. doi:10.1016/j.celrep.2019.02.063
105. Funato K, Major T, Lewis PW, Allis CD, Tabar V. Use of human embryonic stem cells to model pediatric gliomas with H3.3K27M histone mutation. *Science*. 2014;346(6216):1529-1533. doi:10.1126/science.1253799
106. Haag D, Mack N, Benites Goncalves da Silva P, et al. H3.3-K27M drives neural stem cell-specific gliomagenesis in a human iPSC-derived model. *Cancer Cell*. 2021;39(3):407-422.e13. doi:10.1016/j.ccell.2021.01.005
107. Funato K, Smith RC, Saito Y, Tabar V. Dissecting the impact of regional identity and the oncogenic role of human-specific *NOTCH2NL* in an hESC model of H3.3G34R-mutant glioma. *Cell Stem Cell*. 2021;28(5):894-905.e7. doi:10.1016/j.stem.2021.02.003
108. Newman EA, Chukkapalli S, Bashllari D, et al. Alternative NHEJ pathway proteins as components of MYCN oncogenic activity in human neural crest stem cell differentiation: implications for neuroblastoma initiation. *Cell Death Dis*. 2017;8(12):3208. doi:10.1038/s41419-017-0004-9
109. Weng Z, Lin J, He J, et al. Human embryonic stem cell-derived neural crest model unveils CD55 as a cancer stem cell regulator for therapeutic targeting in *MYCN* -amplified neuroblastoma. *Neuro Oncol*. 2022;24(6):872-885. doi:10.1093/neuonc/noab241
110. Saldana-Guerrero IM, Montano-Gutierrez LF, Boswell K, et al. A human neural crest model reveals the developmental impact of neuroblastoma-associated chromosomal

aberrations. *Nat Commun*. 2024;15(1):3745. doi:10.1038/s41467-024-47945-7

111. Wegscheid ML, Anastasaki C, Gutmann DH. Human stem cell modeling in neurofibromatosis type 1 (NF1). *Exp Neurol*. 2018;299:270-280. doi:10.1016/j.expneurol.2017.04.001
112. Mo J, Anastasaki C, Chen Z, et al. Humanized neurofibroma model from induced pluripotent stem cells delineates tumor pathogenesis and developmental origins. *J Clin Invest*. 2021;131(1):e139807. doi:10.1172/JCI139807
113. Mazuelas H, Magallón-Lorenz M, Fernández-Rodríguez J, et al. Modeling iPSC-derived human neurofibroma-like tumors in mice uncovers the heterogeneity of Schwann cells within plexiform neurofibromas. *Cell Rep*. 2022;38(7):110385. doi:10.1016/j.celrep.2022.110385
114. Wegscheid ML, Anastasaki C, Hartigan KA, et al. Patient-derived iPSC-cerebral organoid modeling of the 17q11.2 microdeletion syndrome establishes *CRLF3* as a critical regulator of neurogenesis. *Cell Rep*. 2021;36(1):109315. doi:10.1016/j.celrep.2021.109315
115. Draper GM, Panken DJ, Largaespada DA. Modeling human cancer predisposition syndromes using CRISPR/Cas9 in human cell line models. *Genes Chromosomes Cancer*. 2023;62(9):493-500. doi:10.1002/gcc.23140
116. Li YP, Wang YT, Wang W, et al. Second hit impels oncogenesis of retinoblastoma in patient-induced pluripotent stem cell-derived retinal organoids: direct evidence for Knudson's theory. *PNAS Nexus*. 2022;1(4):pgac. 162. doi:10.1093/pnasnexus/pgac162
117. Norrie JL, Nityanandam A, Lai K, et al. Retinoblastoma from human stem cell-derived retinal organoids. *Nat Commun*. 2021;12(1):4535. doi:10.1038/s41467-021-24781-7
118. Crespo M, Vilar E, Tsai SY, et al. Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. *Nat Med*. 2017;23(7):878-884. doi:10.1038/nm.4355
119. Telang N. Stem cell models for genetically predisposed colon cancer. *Oncol Lett*. 2020;20(5):138. doi:10.3892/ol.2020.11998.
120. Sommer CA, Capilla A, Molina-Estevez FJ, et al. Modeling APC mutagenesis and familial adenomatous polyposis using human iPS cells. *PLoS One*. 2018;13(7):e0200657. doi:10.1371/journal.pone.0200657
121. Chang S, Hur SK, Naveh NSS, et al. Derivation and investigation of the first human cell-based model of Beckwith-Wiedemann syndrome. *Epigenetics*. 2021;16(12):1295-1305. doi:10.1080/15592294.2020.1861172
122. Schuster J, Fatima A, Schwarz F, Klar J, Laan L, Dahl N. Generation of human induced pluripotent stem cell (iPSC) lines from three patients with von Hippel-Lindau syndrome carrying distinct VHL gene mutations. *Stem Cell Res*. 2019;38:101474. doi:10.1016/j.scr.2019.101474
123. Ohnishi H, Kawasaki T, Deguchi T, Yuba S. Generation of xeroderma pigmentosum-A patient-derived induced pluripotent stem cell line for use as future disease model. *Cell Reprogr*. 2015;17(4):268-274. doi:10.1089/cell.2014.0104
124. Hadoux J, Féraud O, Griscelli F, et al. Generation of an induced pluripotent stem cell line from a patient with hereditary multiple endocrine neoplasia 2A (MEN2A) syndrome with RET mutation. *Stem Cell Res*. 2016;17(1):154-157. doi:10.1016/j.scr.2016.06.008
125. Turhan A, Foudi A, Hwang JW, Desterke C, Griscelli F, Bennaceur-Griscelli A. Modeling malignancies using induced pluripotent stem cells: from chronic myeloid leukemia to hereditary cancers. *Exp Hematol*. 2019;71:61-67. doi:10.1016/j.exphem.2019.01.003
126. Cheng Z, Guo D, Ruzi A, et al. Modeling MEN1 with patient-origin iPSCs reveals GLP-1R mediated hypersecretion of insulin. *Cells*. 2022;11(15):2387. doi:10.3390/cells11152387
127. Liu GH, Suzuki K, Li M, et al. Modelling Fanconi anemia pathogenesis and therapeutics using integration-free patient-derived iPSCs. *Nat Commun*. 2014;5(1):4330. doi:10.1038/ncomms5330
128. Suzuki NM, Niwa A, Yabe M, et al. Pluripotent cell models of fanconi anemia identify the early pathological defect in human hemoangiogenic progenitors. *Stem Cells Transl Med*. 2015;4(4):333-338. doi:10.5966/sctm.2013-0172
129. Marion W, Boettcher S, Ruiz-Torres S, et al. An induced pluripotent stem cell model of Fanconi anemia reveals mechanisms of p53-driven progenitor cell differentiation. *Blood Adv*. 2020;4(19):4679-4692. doi:10.1182/bloodadvances.2020001593
130. Rowe RG, Marion W, Ruiz-Torres S, et al. Modeling fanconi anemia using human induced pluripotent stem cells by reversible complementation. *Blood*. 2018;132(Supplement 1):3856. doi:10.1182/blood-2018-99-111701
131. Rodríguez A, Zhang K, Färkkilä A, et al. MYC promotes bone marrow stem cell dysfunction in fanconi anemia. *Cell Stem Cell*. 2021;28(1):33-47. doi:10.1016/j.stem.2020.09.004
132. Chou DB, Frismantas V, Milton Y, et al. On-chip recapitulation of clinical bone marrow toxicities and patient-specific pathophysiology. *Nat Biomed Eng*. 2020;4(4):394-406. doi:10.1038/s41551-019-0495-z
133. Ma C, Witkowski MT, Harris J, et al. Leukemia-on-a-chip: dissecting the chemoresistance mechanisms in B cell acute lymphoblastic leukemia bone marrow niche. *Sci Adv*. 2020;6(44):eaba5536. doi:10.1126/sciadv.aba5536
134. Li T, Zhang Y, Li Y, et al. Modeling leukemia with pediatric acute leukemia patient-derived iPSCs. *Stem Cell Res*. 2021;54:102404. doi:10.1016/j.scr.2021.102404
135. Böiers C, Richardson SE, Laycock E, et al. A human IPS model implicates embryonic B-myeloid fate restriction as developmental susceptibility to B acute lymphoblastic leukemia-associated ETV6-RUNX1. *Dev Cell*. 2018;44(3):362-377. doi:10.1016/j.devcel.2017.12.005
136. Gordon DJ, Motwani M, Pellman D. Modeling the initiation of Ewing sarcoma tumorigenesis in differentiating human embryonic stem cells. *Oncogene*. 2016;35(24):3092-3102. doi:10.1038/onc.2015.368
137. Sole A, Grossetête S, Heintzé M, et al. Unraveling ewing sarcoma tumorigenesis originating from patient-derived mesenchymal

stem cells. *Cancer Res*. 2021;81(19):4994-5006. doi:10.1158/0008-5472.CAN-20-3837

138. Lee DF, Su J, Kim HS, et al. Modeling familial cancer with induced pluripotent stem cells. *Cell*. 2015;161(2):240-254. doi:10.1016/j.cell.2015.02.045
139. Mukae K, Takenobu H, Endo Y, et al. Development of an osteosarcoma model with MYCN amplification and TP53 mutation in hiPS cell-derived neural crest cells. *Cancer Sci*. 2023;114(5):1898-1911. doi:10.1111/cas.15730
140. Searcy MB, Larsen RK, Stevens BT, et al. PAX3-FOXO1 dictates myogenic reprogramming and rhabdomyosarcoma identity in endothelial progenitors. *Nat Commun*. 2023;14(1):7291. doi:10.1038/s41467-023-43044-1
141. Terada Y, Jo N, Arakawa Y, et al. Human pluripotent stem cell-derived tumor model uncovers the embryonic stem cell signature as a key driver in atypical teratoid/rhabdoid tumor. *Cell Rep*. 2019;26(10):2608-2621. doi:10.1016/j.celrep.2019.02.009
142. Cheng K, Seita Y, Whelan EC, et al. Defining the cellular origin of seminoma by transcriptional and epigenetic mapping to the normal human germline. *Cell Reports*. 2024;43(6). doi:10.1016/j.celrep.2024.114323
143. Garribba L, Santaguida S. The dynamic instability of the aneuploid genome. *Front Cell Dev Biol*. 2022;10:838928. doi:10.3389/fcell.2022.838928
144. Ganmore I, Smooha G, Izraeli S. Constitutional aneuploidy and cancer predisposition. *Hum Mol Genet*. 2009;18(R1):R84-R93. doi:10.1093/hmg/ddp084
145. Garribba L, De Feudis G, Martis V, et al. Short-term molecular consequences of chromosome mis-segregation for genome stability. *Nat Commun*. 2023;14(1):1353. doi:10.1038/s41467-023-37095-7
146. Vasudevan A, Schukken KM, Sausville EL, Girish V, Adebambo OA, Sheltzer JM. Aneuploidy as a promoter and suppressor of malignant growth. *Nat Rev Cancer*. 2021;21(2):89-103. doi:10.1038/s41568-020-00321-1
147. Bach DH, Zhang W, Sood AK. Chromosomal instability in tumor initiation and development. *Cancer Res*. 2019;79(16):3995-4002. doi:10.1158/0008-5472.CAN-18-3235
148. Sansregret L, Swanton C. The role of aneuploidy in cancer evolution. *Cold Spring Harb Perspect Med*. 2017;7(1):a028373. doi:10.1101/cshperspect.a028373
149. Xiao R, Xu D, Zhang M, et al. Aneuploid embryonic stem cells drive teratoma metastasis. *Nat Commun*. 2024;15(1):1087. doi:10.1038/s41467-024-45265-4
150. Zhang M, Cheng L, Jia Y, et al. Aneuploid embryonic stem cells exhibit impaired differentiation and increased neoplastic potential. *EMBO J*. 2016;35(21):2285-2300. doi:10.15252/embj.201593103
151. Ben-David U, Arad G, Weissbein U, et al. Aneuploidy induces profound changes in gene expression, proliferation and tumorigenicity of human pluripotent stem cells. *Nat Commun*. 2014;5(1):4825. doi:10.1038/ncomms5825
152. Andrews PW, Barbaric I, Benvenisty N, et al. The consequences of recurrent genetic and epigenetic variants in human pluripotent stem cells. *Cell Stem Cell*. 2022;29(12):1624-1636. doi:10.1016/j.stem.2022.11.006
153. Barwe SP, Sebastian A, Sidhu I, Kolb EA, Gopalakrishnapillai A. Modeling down syndrome myeloid leukemia by sequential introduction of GATA1 and STAG2 mutations in induced pluripotent stem cells with trisomy 21. *Cells*. 2022;11(4):628. doi:10.3390/cells11040628
154. Matsuo S, Nishinaka-Arai Y, Kazuki Y, et al. Pluripotent stem cell model of early hematopoiesis in Down syndrome reveals quantitative effects of short-form GATA1 protein on lineage specification. *PLoS One*. 2021;16(3):e0247595. doi:10.1371/journal.pone.0247595
155. Barwe SP, Sidhu I, Kolb EA, Gopalakrishnapillai A. Modeling transient abnormal myelopoiesis using induced pluripotent stem cells and CRISPR/Cas9 technology. *Mol Ther Methods Clin Dev*. 2020;19:201-209. doi:10.1016/j.omtm.2020.09.007
156. Perepitchka M, Galat Y, Beletsky IP, Iannaccone PM, Galat V. Down syndrome iPSC model: endothelial perspective on tumor development. *Oncotarget*. 2020;11(36):3387-3404. doi:10.18632/oncotarget.27712
157. Galat Y, Perepitchka M, Elcheva I, Iannaccone S, Iannaccone PM, Galat V. iPSC-derived progenitor stromal cells provide new insights into aberrant musculoskeletal development and resistance to cancer in down syndrome. *Sci Rep*. 2020;10(1):13252. doi:10.1038/s41598-020-69418-9
158. Bonouvrie K, van der Werff Ten Bosch J, van den Akker M. Klinefelter syndrome and germ cell tumors: review of the literature. *Int J Pediatr Endocrinol*. 2020;2020:18. doi:10.1186/s13633-020-00088-0
159. Kwon A, Hyun SE, Jung MK, et al. Risk of gonadoblastoma development in patients with turner syndrome with cryptic Y chromosome material. *Horm Cancer*. 2017;8(3):166-173. doi:10.1007/s12672-017-0291-8
160. Waldhorn I, Turetsky T, Steiner D, et al. Modeling sex differences in humans using isogenic induced pluripotent stem cells. *Stem Cell Rep*. 2022;17(12):2732-2744. doi:10.1016/j.stemcr.2022.10.017
161. Panula S, Kurek M, Kumar P, et al. Human induced pluripotent stem cells from two azoospermic patients with Klinefelter syndrome show similar X chromosome inactivation behavior to female pluripotent stem cells. *Hum Reprod*. 2019;34(11):2297-2310. doi:10.1093/humrep/dez134
162. Veerasubramanian N, Karthikeyan V, Hegde S, Dhanushkodi A, Parveen S. Generation of induced pluripotent stem cells from turner syndrome (45XO) fetal cells for downstream modelling of neurological deficits associated with the syndrome. *J Vis Exp*. 2021;178. doi:10.3791/62240
163. Botman O, Hibaoui Y, Giudice MG, et al. Modeling klinefelter syndrome using induced pluripotent stem cells reveals impaired germ cell differentiation. *Front Cell Dev Biol*. 2020;8:567454. doi:10.3389/fcell.2020.567454
164. de Souza AF, Bressan FF, Pieri NCG, et al. Generation of primordial germ cell-like cells from iPSCs derived from turner syndrome patients. *Cells*. 2021;10(11):3099. doi:10.3390/cells10113099

165. NCI budget fact book - NCI. Published May 10, 2022. Accessed November 16, 2023.https://www.cancer.gov/about-nci/budget/fact-book
166. *NCI Funded Research Portfolio Home*. Accessed November 24, 2023.https://fundedresearch.cancer.gov/nciportfolio/
167. Camille C, Thomas G, Carine B, Antoine I. Encouraging trends in modern phase 1 oncology trials. *N Engl J Med*. 2018; 378(23):2242-2243. doi:10.1056/NEJMc1803837
168. Laetsch TW, DuBois SG, Bender JG, Macy ME, Moreno L. Opportunities and challenges in drug development for pediatric cancers. *Cancer Discov*. 2021;11(3):545-559. doi:10.1158/2159-8290.CD-20-0779
169. Dorris K, Liu C, Li D, et al. A comparison of safety and efficacy of cytotoxic versus molecularly targeted drugs in pediatric phase I solid tumor oncology trials. *Pediatr Blood Cancer*. 2017;64(3):e26258. doi:10.1002/pbc.26258
170. Balachandran P, Beck CR. Structural variant identification and characterization. *Chromosome Res*. 2020;28(1):31-47. doi:10.1007/s10577-019-09623-z