Precision medicine in pediatric oncology: lessons learned and next steps

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Abstract

The maturation of genomic technologies has enabled new discoveries in disease pathogenesis as well as new approaches to patient care. In pediatric oncology, patients may now receive individualized genomic analysis to identify molecular aberrations of relevance for diagnosis and/or treatment. In this context, several recent clinical studies have begun to explore the feasibility and utility of genomics-driven precision medicine. Here, we review the major developments in this field, discuss current

limitations, and explore aspects of the clinical implementation of precision medicine which lack consensus. Lastly, we discuss ongoing scientific efforts in this arena, which may yield future clinical applications.

Introduction

Medicine and society: the precision medicine era

Precision medicine is broadly defined by the National Institutes of Health as "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person." The Obama administration's January 2015 announcement of the Precision Medicine Initiative (PMI) takes a step forward in efforts to move precision medicine into clinical practice[1]. With $215 million in planned funding for fiscal year

2016, the PMI aims to leverage next generation sequencing capabilities, improved biospecimen analytics, and tools for management of large data sets, to generate outcome data that will facilitate movement from the research realm into clinical care. Recently, the Obama campaign's National Cancer Moonshot Initiative, announced during the 2016 State of the Union address and motivated by the death of Vice President Joseph Biden's son to brain cancer, is now expanding governmental involvement and financial support upwards of $4 billion[2].

Indeed, across multiple disciplines, the widespread utilization of high-throughput genomic technologies has enabled more detailed clinical characterization and management according to genomic knowledge. In pulmonology, cystic fibrosis patients with the pathogenic *CFTR* G551D mutation preferentially respond to the drug ivacaftor[3]. Cardiovascular medicine has 12 drugs with pharmacogenetic labeling from the FDA, and genotype data is helping to better predict risk for cardiovascular disease and characterize disease subtypes. Identification of patients with mutations linked to familial hypercholesterolemia, arrhythmias, and cardiomyopathies creates opportunities for prevention of myocardial infarction and sudden cardiac death[4]. Researchers in gastroenterology are using precision medicine tools to improve biomarkers for numerous diseases and are interrogating the microbiome environment in GI disease. In the intensive care unit, researchers have begun to define clinically-feasible assays to rapidly detect sepsis through accumulation of specific metabolites in blood[5].

Precision medicine and cancer

While the tools of precision medicine are being applied broadly, cancer has been at the vanguard of these efforts (**Figure 1**), and near-term goals of the PMI are most accessible in oncology. The emergence of biomarker-driven targeted therapies is already a reality for some oncology patients.

Thus, lung cancer patients with *EGFR* alterations receive EGFR-targeting therapies[6], whereas those with *ALK* alterations receive ALK-targeting therapies[7]. Furthermore, as molecular subclasses of cancer are established, clinical study design has adapted accordingly, moving towards umbrella-designs or biomarker-driven study where patients are enrolled based upon molecular features. The National Cancer Institute (NCI), which is leading the Moonshot Initiative efforts, has outlined several areas of focus for ongoing oncology PMI research and implementation: expanding clinical study, enhancing drug discovery and development, developing new cell line models, furthering the promise of immunotherapy, and improving early detection and prevention through vaccines, chemoprevention, and biomarker discovery[2]. Moreover, pediatric cancer has been emphasized as a specific target area for advancing precision medicine into clinical care.

Early Clinical Studies in pediatric oncology

At diagnosis, pediatric cancer patients tend to have lower rates of mutation across their genomes when compared against all adult cancers[8-10]. By contrast, pediatric tumors that are treatment-refractory and recurrent generally have higher mutation rates, more comparable to adult tumors[11-13]. These data can be used to support claims that, at diagnosis, there may be less molecular complexity per individual cancer, which may enable efficacy for targeted agents by decreasing the number of altered cellular pathways, as well as the claim that there are generally few recurrently mutated targetable genes in pediatric cancers, which may limit the availability and use of some targeted agents. The relative paucity of targetable mutations in pediatrics is compounded by limited access to newer targeted therapeutic agents due to availability of fewer pediatric clinical studies and smaller number of eligible patients for each study.

Despite these challenges, initial pilot studies of genomic medicine in pediatric oncology have been both fruitful and encouraging (**Figure 2**), with several major conclusions. First, although pediatric tumors typically lack frequent targetable kinase alterations such as those in common adult cancers such as lung (*EGFR*) or breast cancer (*HER2*), pediatric tumors appear to be enriched for targetable gene fusions. Second, there has been a surprising frequency of rare mutations in actionable genes in unexpected tumor types[14]. Third, the studies have re-emphasized importance of pathogenic germline mutations in pediatric cancers, even among patients lacking a notable family history of cancer. Finally, there have been notable cases of patients with a change in diagnosis or risk stratification due to genomic aberrations discovered on molecular testing.

Below, we summarize the early findings from three key pediatric precision oncology studies, including two from the NHGRI and NCI-funded Clinical Sequencing Exploratory Research (CSER) program[15-17] (**Table 1**). All of these studies are still ongoing and we will await the results of a larger, more definitive cohort in the future. For readers less familiar with genome sequencing technologies, we have included an **Appendix S1** which details the basic modalities, their pros and cons, and their compatibility with different biospecimen types.

PEDS-MIONCOSEQ

The University of Michigan Pediatric Michigan Oncology Sequencing (PEDS-MIONCOSEQ) study[15] is based upon their earlier adult sequencing efforts[18]. The results from the first 102 patients enrolled on PEDS-MIONCOSEQ have now been reported[15]. Primary study population included pediatric and young adult cancer patients with refractory, relapsed disease while 20% cases included had newly diagnosed high-risk or rare disease, all of whom had undergone extensive testing by available standard of care testing. Majority of these patients had either failed or had no proven

143 therapeutic options available to them and were looking for novel therapies. This was the only
144 pediatric study, which included all subtypes of pediatric malignancies including hematopoietic, brain
145 and solid tumors. Ninety-one patients underwent genomic analyses with whole exome sequencing
146 (WES) of tumor and germline DNA as well as RNA sequencing of tumor RNA. Clinical decision-
147 making was made through a multidisciplinary tumor board and patient follow up was updated
148 quarterly. Typical turn-around time and cost estimates were 54 days and $6000, respectively. Overall,
149 42 patients (46%) had a potentially actionable findings, most of which were not detected by standard
150 diagnostic tests that did not include sequencing. The actionable findings included 9 patients with
151 germline findings, 10 patients with an actionable gene fusion found via RNA-seq, and two patients
152 who had their diagnosis changed. Twenty three patients had an individualized care decision made
153 based upon sequencing results, which included 14 patients receiving different therapies, 9 patients
154 with genetic counseling, and 1 patient with both. Nine of 14 patients with a change in management
155 had a clinical response lasting more than 6 months in duration.

156

157 BASIC3

158 Data have been reported for the first 150 children with solid and brain tumors enrolled on the Baylor
159 College of Medicine Advancing Sequencing in Childhood Cancer Care (BASIC3) study[16]. All
160 patients underwent germline WES and those with available tumor (121/150; 81%) also underwent
161 tumor WES. Unique among pediatric studies to date, the BASIC3 study included only newly-
162 diagnosed, untreated patients. The clinical relevance of sequencing findings was described using a
163 standardized scale defined by the study investigators. In total 47/121 (39%) patients who underwent
164 both tumor and germline sequencing were considered to have a potentially clinically-relevant finding.
165 4/121 (3%) of patients harbored a category I somatic mutation (i.e. known pathogenic in that disease),
166 and 29/121 (24%) had a category II somatic mutation (i.e. a gene of potential clinical relevance,
167 including known targetable genes). 15/150 patients (10%) undergoing germline sequencing had a

diagnostic germline finding related to their phenotype (cancer and/or other diseases), including 13 (8.6%) with pathogenic or likely pathogenic mutations in known cancer susceptibility genes. No patients were treated with molecularly targeted agents based on study results.

iCat

The Individualized Cancer Therapy (iCat) study is a multi-institutional effort coordinated through Dana-Farber/Boston Children's Hospital[19], with results of sequencing of 101 extra-cranial solid tumor patients reported, including 80% with recurrent or refractory disease looking for novel therapeutic options. Molecular profiling was completed on tumor tissue DNA for 89 patients. Molecular profiling was performed with a heterogeneous variety of techniques: 13 patients via OncoMap alone (a Sequenom assay for 41 genes), 27 patients by OncoMap and array comparative genomic hybridization (aCGH), 25 patients by OncoPanel (targeted Illumina sequencing for 275 genes and 91 introns for rearrangements) and aCGH, and 24 patients by OncoPanel alone. Clinical recommendations were based on consensus opinion with members of the multidisciplinary panel ranking potential findings on a 1 (strongest) to 5 (weakest) scale. In total, 31% of patients received iCat recommendations and 43% patients were judged to have findings of clinical significance, including frequent focal copy number alterations (20), the majority of which were *MYC*/*MYCN* amplifications detectable by conventional methods. Three patients (3%) were treated with targeted therapies based upon study findings, but there were no objective responses. Three patients had a change in disease diagnosis based upon tumor profiling.

INFORM

The Individualized Therapy for Relapsed Malignancies in Childhood (INFORM) study is a multi-institutional German effort coordinated through the German Cancer Research Center (DKFZ)[20]. Fifty seven patients were enrolled (50 relapsed/refractory and 7 primary patients), of whom 52 received molecular profiling. Molecular profiling was performed with WES and RNA-seq. Low-coverage whole genome sequencing (WGS) was used for copy number events; DNA methylation and gene expression microarrays were also performed. Typical turn-around time and cost estimates were 28 days and □7000 (~$8000), respectively. Clinical recommendations were based on a standardized, seven-step scoring algorithm to prioritize molecular targets. In total, 26 patients (50%) had a clinically-relevant finding (limited to fusions, gene expression, copy number and mutations/indels; DNA methylation was not directly used). Two (4%) patients had a germline finding which supported a cancer predisposition syndrome. Ten (19%) patients had treatments altered based upon molecular findings, including two (4%) patients who had prolonged tumor response >6 months. Five (10%) patients had a change in diagnosis based upon tumor profiling.

Lessons from the early studies

There are several important issues highlighted by these studies. First, clinical genomic analysis has the potential to identify potentially clinically relevant alterations in a substantial fraction of pediatric cancer patients as demonstrated by all three studies. Second, both the tumor and germline alterations identified in these studies target a diverse set of genes, including many which were not previously known to be associated with the patient's cancer type or in pediatric cancer, emphasizing the potential yield of genome-scale testing for these patients.

Third, the PEDS-MIONCOSEQ and INFORM studies demonstrates the utility of RNA-seq to identify actionable gene fusions. In the PEDS-MIONCOSEQ study, 33 of 91 patients had a driver gene

fusion, 10 of which were actionable. In the INFORM study, 5 of 52 patients had an actionable gene fusion. While the iCat study attempted to identify translocations via DNA sequencing of targeted introns, this method was not particularly effective. Only 1 targetable translocation was found, which is surprising given that the iCat study had very high proportion of sarcomas patients (n = 61). By contrast, the PEDS-MIONCOSEQ and INFORM studies had directly targetable fusions in 5 of 44 sarcoma patients. Fourth, there were 10 patients collectively in the iCat, PEDS-MIONCOSEQ, and INFORM studies whose diagnosis was changed by tumor profiling, which is significant given the detailed pathologic review each patient had as part of clinical evaluation, including many of the refractory patients being reviewed by more than one treating center before enrollment on these studies.

Fifth, the PEDS-MIONCOSEQ, INFORM and iCat studies demonstrated the potential utility of genomics to guide selection of targeted therapies. While the PEDS-MIONCOSEQ and INFORM studies demonstrated that a small set of patients (n=9 (10%) and 2 (4%), respectively) had a clinical response following initiation of a targeted therapy, iCat study failed to show objective responses in their patient population (n=3). The difference most likely is due to biological nature of malignancies and genomic lesion being targeted. PEDS-MIONCOSEQ and INFORM responders included patients with SNV or actionable fusion in hematological malignancies and actionable fusions in solid tumors, which historically have shown to be more responsive to single agent targeted therapy. In comparison, all three iCat patients who were treated based on study recommendations were refractory solid tumor patients with mutations in FGF, PI3K and ALK pathway and were treated with a single agent targeted therapy. These differential responses to single agent targeted therapy highlights the importance of optimal patient selection, role of RNA-Seq in genomic analysis of pediatric patients and role of multi-agent targeted therapy for the hardest to treat refractory solid tumors. In contrast, the BASIC3 study highlights spectrum of genomic changes in newly diagnosed and untreated patients but did not require

change in management based on study results, as it would ethically and logistically very challenging to integrate targeted therapy in combination with or instead of standard frontline therapy.

Lastly, these studies highlight the prevalence of pathogenic germline mutations: roughly 10% in both PEDS-MIONCOSEQ and BASIC3, and 4% in INFORM, while iCat study did not specifically address germline mutations. These data are consistent with recent data from the Pediatric Cancer Genome Project (PCGP), a collaboration between St Jude and Washington University with a goal to characterize pediatric cancer genomes[21]. By analyzing germline sequencing data of 1120 patients for 60 known cancer predisposition genes, the PCGP found that there was an overall 8.5% prevalence of likely pathogenic variants in the germline of pediatric cancer patients[14]. In addition, almost half of these patients with pathogenic variants in both PEDS-MIONCOSEQ and PCGP studies had no significant family history. This information is of great significance to providers caring for patients as well as for their families, as most of these parents and siblings are in relatively younger age group and would benefit from early screening.

Molecular targets in pediatric cancers:

While molecular targets in adult tumors have been the focus of most pharmaceutical efforts[22], pediatric patients have largely not yet benefited from these due to limited overlap with molecular events driving adult tumors, small number of patients and safety concerns in young children. However, this is beginning to change as we start to catalogue actionable events driving pediatric tumors through precision oncology studies discussed earlier and other efforts[9,11,21,23]. A selection of most common molecular events and targeted agents are detailed in **Table 2**[24-46].

Extending the utility of drugs initially developed for adult cancers and repurposing them for pediatric tumors sharing the same target have become a major source of new clinical studies for pediatrics, and there are several particularly notable examples. First, crizotinib, initially promoted in *ALK* fusion-positive lung cancers[47], has demonstrated impressive responses in patients with a variety of molecular aberrations (*ALK*, *NTRK1/2/3*, and *ROS1* translocations) as well as in different tumor types e.g. anaplastic large cell lymphoma, inflammatory myofibroblastic tumors, neuroblastoma and sarcomas[15,33]. Second, for brain tumors, *SMO* inhibitors such as vismodegib, first developed for basal cell carcinoma[48], have demonstrated promise for medulloblastoma patients with *PTCH1* mutations[36,49]. Third, *PARP1* inhibitors, which were initially applied to *BRCA1/2* mutant breast and ovarian cancers[50], are being explored as a therapeutic strategy for Ewing's sarcoma patients with *EWSR1-FLI1* fusions[37,38], although initial studies of olaparib monotherapy suggest that its activity as a single agent is limited[51,52]. Lastly, a number of exciting molecular strategies for treating neuroblastoma are being investigated, such as CDK4/6 inhibitors and aurora kinase inhibitors, both of which have shown selectivity for MYCN-amplified cell lines in vitro[39].

Drug availability in pediatric oncology

Access to pediatric oncology drugs is unfortunately not a new problem. There have been prior issues with shortages in anticancer agents[53], which has prompted discussion by many institutions including the Food and Drug Administration (FDA)[54]. For new discoveries, methods to incentivize pharmaceutical companies have been extensively discussed[55], and there are two existing laws which promote pediatric drug development—the Best Pharmaceuticals for Children Act (BPCA) and the Pediatric Research Equity Act (PREA). The BPCA offers additional patent exclusivity for on-patent drugs tested for pediatric use. The PREA enables the FDA to mandate pediatric drug studyies as a last resort if other incentives do not succeed.

Recently, accelerated FDA approval of "breakthrough" drugs such as crizotinib[56] has generated much interest and discussion[57,58]. Because of such extraordinary examples of targeted agents, "seamless" or "first-in-human" studies, which are streamlined and do not employ traditional phase 1/phase 2/phase 3 paradigms, have been used on more than 40 oncologic therapies[59]. These studies may provide a basis to test novel compounds in pediatric patients more quickly. However, accelerated study designs also have significant limitations when applied for pediatrics, including lack of control group and poor ability to identify toxicities, particularly in an age-dependent fashion. Ultimately, while modified study design may help, increased access to targeted therapies will also require greater collaboration with industry to move experimental therapeutics into the clinic for childhood cancers via traditional clinical studies as well.

Logistical challenges in precision oncology

Cost

Genomic profiling of pediatric cancer patients presents numerous challenges (**Table 3**). First is cost. The PEDS-MIONCOSEQ study had a cost of $6000 for WES and RNA-Seq with about half going towards biochemical reagents and the other half for computational analyses, laboratory personnel and capital depreciation[15,60]. The INFORM study had a cost of €7000 (~$8000), which included WES, RNA-seq, low-coverage WGS, a gene expression array, and a DNA methylation array[20]. However, these cost estimates are probably lower than actual costs as it does not include the time spent in clinical analysis, annotation, discussion and deliberation on the results. On the other hand, traditional sequencing assays such as BRCA gene sequencing can cost up to $5000 for a single gene or small panel of genes and thus making a genome wide approach more cost effective[61].

309

310 The cost of reagents is going down however the future cost of sequencing may not come down
311 significantly due to rising bioinformatics costs deriving mainly from (1) data storage, (2)
312 computational pipeline generation, and (3) data processing time[60,62,63]. Indeed, data storage and
313 processing time are increasingly facilitated through cloud computing, which is a pay-for-service
314 paradigm.

315

316 In addition to the cost of reagents and computational resources, there are also considerable costs for a
317 clinical genomic infrastructure, including increased personnel such as technologists, bioinformaticians
318 and genetic counselors. Building a genomics team to generate and analyze sequencing data therefore
319 requires institutional support from the hospital or health care system. Likewise, there may be costs
320 associated with training physicians to understand genomic data and reports through ongoing medical
321 education.

322

323 Turn-around time

324 The median reported turnaround time for PEDS-MIONCOSEQ and INFORM studies were 54 and 28
325 days, respectively, while other studies did not report the time[15,20]. Reductions in turn-around time
326 will likely result through streamlined computational analyses, which at present can take up to 4
327 weeks. This may be lessened through targeted analyses, which focus only on a limited set of genes.
328 Ultimately, the most promising way to reduce turn-around-time will likely stem from optimized
329 computational pipelines that process data more quickly and in a parallelized fashion[62,63].

330

331 Obtaining adequate tumor material

Genomic profiling requires sufficient tumor material from biopsy or resection. The tumor material also needs to be of sufficient quality (e.g. not fully necrotic tissue). Given these considerations, some children have undergone invasive procedures (e.g. biopsy) for the sole purpose of obtaining material for genomic testing. While there have been no major patient complications reported to date, there is a possibility of complications for any procedure. As sequencing methods improve, we anticipate that the need for additional biopsies will be low, due to improved ability to molecularly profile FFPE archived tissue or by further optimization of liquid biopsies techniques.

Rational combination of targeted therapies

Even when a targeted therapy is potentially available for a particular patient, the optimal way to implement this treatment is unclear. For example, early lessons with use of cytotoxic chemotherapy showed us the benefits of rationale combination in treatment of cancer and many in the scientific community assume the same with targeted agents. However, we need more rigorous pre-clinical and clinical testing to understand better, which are the optimal agents to combine for each molecular aberration and with least toxicity. The combination therapy is likely to include multiple targeted agents or targeted agents in combination with chemotherapy, radiation or immunotherapy, and it will most likely depend on the molecular aberration, tumor type being treated and host immune response.

Recently, the SHIVA, a phase-II randomized study in adults with refractory solid tumors, offered a cautionary tale[64]. All included patients harbored a molecular alteration within one of three pathways (hormone receptor, PI3K/AKT/mTOR, and RAF/MEK). Eleven molecularly targeted agents for these pathways were available. Patients were randomized to receive a targeted agent as monotherapy or standard therapy via physician's choice. With a median follow-up of 11 months, progression free survival was not different between the two groups.

The SHIVA study has been cited by skeptics to argue that the efficacy of precision medicine may be low[65]. However, the SHIVA study should be interpreted with caution due to multiple serious limitations. Perhaps most importantly, it is probably unrealistic to expect that multiply refractory metastatic cancers will respond to targeted agent monotherapy; these tumors have many different pathways dysregulated. In addition, their next generation sequencing panel was very limited making it likely that a true driver molecular event was missed. Nonetheless, the SHIVA study does suggest that the patient selection, choice of sequencing panel and available targeted agents, all will play an important role in practice of precision oncology. In addition, it is certainly possible that populations most likely to benefit from targeted agents might be treatment-naïve tumors where pathway addiction is likely stronger and we will need similar studies in newly diagnosed patients to test its clinical utility.

Defining pathogenic variants in pediatrics

Relatively few variants have been specifically characterized to validate their pathogenicity. This leads to a challenge when tumor profiling produces variants that have not been specifically tested experimentally. To address this, the American College of Medical Genetics and Genomics (ACMG) updated its terminology for sequence variants in 2015[66]. The Human Genome Variation Society (HGVS) similarly has guidelines for terminology[67]. These guidelines distinguish criteria that are "pathogenic" compared to those that are "likely pathogenic", "likely benign", "benign" or "uncertain significance". Numerous efforts, including the Somatic Cancer working group of the Clinical Genome Resource (ClinGen), are currently focused on the challenge of defining standards for interpretation of somatic changes and their clinical actionability[68].

In practice, most clinical sequencing groups (BASIC3, PEDS-MIONCOSEQ) employ centralized sequence variant databases, generally ClinVar[69], bioinformatics algorithms for prediction of pathogenic variants, such as PolyPhen-2[70], as well as expert opinion[15,16]. One major challenge both clinically and scientifically is presented by variants of uncertain significance both for somatic and germline variants. For germline variants, there is no efficient way currently to interpret these variants, and they are generally discarded from clinical considerations unless so-called "trio" testing (mother, father, affected child) is available, which may provide useful information for interpretation of a given variant in a pediatric patient. Recent challenges and scrutiny in cardiology, where there are now doubts regarding the pathogenicity of germline variants in some inherited arrhythmia syndromes[4,71], highlights the unclear nature of many genomic variants.

Ethical challenges of germline findings

There have been many discussions of the ethical implications of germline genome profiling for pediatric cancers[72-75], as well as discussion of how best to share genomic information with patients[76,77]. The chance of finding incidental germline pathogenic variants, defined as variant that was unrelated to cancer or other known patient phenotype creates an ethical challenge for these patients. Indeed, in the BASIC3 study, 8 patients (5%) were found to have such a pathogenic germline variant. Similarly, a recent analysis of the 1,000 genomes project, which sequenced 1,000 adult genomes, found a 2.3% prevalence for incidental findings[67]. In response to this, some groups (e.g. PEDS-MIONCOSEQ) employ a flexible-default consent model in which parents can decide whether they wish to receive results pertaining to pathogenic germline variants. In the case of PEDS-MIONCOSEQ, a majority of parents (>80%) did wish to receive these results.

Even so, there is a risk that germline discoveries in a child may enable a potential for genetic discrimination in the future, particularly for germline variants not related to cancer or childhood disease generally. While genetic counselors are routinely involved with families and patients for whom a heritable cancer syndrome is suspected, it is not clear that genetic counselors should be involved in cases of incidental germline findings which do not pertain to cancer. At the same time, for a child with cancer who also has a complex medical condition without a known underlying genetic diagnosis, it is possible that an incidental germline finding may elucidate a unifying genetic diagnosis for an underlying medical syndrome. Ultimately, it may be most prudent to leave the decision of disclosure of incidental germline findings to parents and patients, though explicit counseling on the risks of this decision must be addressed prospectively.

Universalization of practice

The implementation of precision medicine is currently uneven and lacks standardization. There are numerous aspects of healthcare infrastructure which will ultimately impact the dissemination of precision medicine practices, including access to biomarker tests and therapies, integration with electronic health care records, establishment of national databases, and standardized regulatory and reimbursement processes, among others[78]. While such topics are beyond the purview of this review, the National Academy of Sciences has been active in discussing mechanisms to expand and standardize precision medicine through a rational, best-practices perspective[78]. Recently, the Institute of Medicine (IOM) assembled a Committee on Policy Issues in the Clinical Development and Use of Biomarkers for Molecularly Targeted Therapies[79]. In their report, the Committee has advocated for increased involvement and regulation by the secretary of Health and Human Services (HHS), in conjunction with the FDA, to standardize biomarker testing nationally[80].

Debated topics

Design and role of the precision tumor board

Though incorporated into all clinical sequencing efforts to date, the design of precision medicine tumor boards varies significantly. While all tumor boards have included clinical faculty in hematology/oncology and scientific experts in sequencing, the PEDS-MIONCOSEQ and BASIC3 studies also incorporated clinical cancer geneticists up-front as core members of the tumor board[15,16]. The PEDS-MIONCOSEQ study also has clinical ethicists as core members[15]. Methods to interpret the data also vary. For example, in the iCat study, members of the expert panel rank each actionable alteration in each patient, using a formal system[19]. By contrast, other groups (PEDS-MIONCOSEQ) discuss clinical sequencing findings, but do not have formal ranking systems.

Implementation of DNA sequencing

A version of DNA sequencing (e.g. WES or mutation panels) is an important component for any precision medicine sequencing panel. However, the precise implementation of DNA sequencing varies between groups and which is the most optimal approach is still not clear. The BASIC3 study analyzed the entire exome for somatic and germline mutations. Other groups performed WES but focus computational analyses to a list of known cancer genes (PEDS-MIONCOSEQ, PCGP, INFORM). Lastly, some advocate for targeted sequencing of only cancer-relevant genes and not sequence the whole exome (the OncoMap and OncoPanel approaches in the iCat study).

RNAseq or no RNAseq?

The role of RNA sequencing is even less clear. The use of RNA is associated with additional challenges, including (1) technical difficulties in extracting high-quality RNA from tissue samples, (2)

analytical complexities of tumor-stroma mixtures where the fraction of gene expression from each cell type is difficulty to ascertain, and (3) increased cost and time of the sequencing and computational analysis. Nevertheless, RNA sequencing also enables invaluable analyses. These include comprehensive gene fusion discovery, tumor expression subgroup analysis (e.g. medulloblastoma subgroups, Ph-like acute lymphoblastic leukemia), and cell-of-origin gene expression analyses for tumors of unknown primary. Given the clinical benefit of the discovery of actionable gene fusions, especially in pediatric leukemias and sarcomas[15,20], we advocate for the inclusion of RNA sequencing in precision oncology for pediatric cases.

Standardizing the term "Actionable Findings or Clinically Relevant":

All the pediatric precision oncology studies reviewed here used the term "actionable findings" or findings of "clinical relevance" to measure the impact of the study. However, the definition of these terms was variable between studies. While all studies included "druggable" genomic alterations in these categories, only PEDS-MIONCOSEQ, iCat and BASIC3 included alterations that are not druggable, but impacted diagnosis, prognosis or risk stratification as actionable or clinically relevant. In addition, only PEDS-MIONCOSEQ and BASIC3 considered pathogenic germline variants as actionable findings, with only BASIC3 considering non-cancer related germline findings as actionable.

There is a definite need for standardizing the reporting on what are considered actionable or clinically relevant findings, both in somatic and germline sequencing. In addition, the somatic findings need further prioritization based on strength of clinical evidence and germline findings needs sub-classification into actionable: a) cancer related, b) non-cancer related and c) pharmacogenomics

findings. Finally, we must recognize that as we identify new targets and develop new agents, the fraction of patients which are considered actionable is likely to change.

Subclone detection

Cancer is a multiclonal disease. Pediatric leukemias and sarcomas typically harbor at least 2 distinct genetic clones at diagnosis, with the dominant clone representing ~70 – 95% of tumor cells[81-83]. Brain tumors such as medulloblastoma generally present with one overwhelming dominant clone (>95% prevalence) while post-treatment recurrence originate from distant minor subclones[13,84]. The issue of multiple cancer clones raises several clinical and technical questions: How deep should sequencing be? What cut-offs should be used to detect clonal abundance? How prevalent should a clone be to impact patient care?

There are no established guidelines to answer these questions in the clinical context. Generally, WES aims for at least 100x coverage. To conceptualize what this means clinically, consider the following example: 100x coverage entails 100 reads at a given locus. If the tumor is 70% pure, then 70 of those reads represent tumor cells, and 30 reads would be stromal. Assuming one tumor clone, a homozygous mutation would therefore have 70 supporting reads and a heterozygous mutation would have 35 reads. If there are two clones, one that represents 80% of cancer cells and a second that represents 20%, then major clone would have 56 reads and the minor clone would have 14 reads. A heterozygous variant in the minor clone would therefore have 7 supporting reads.

Although the importance of subclones is well-established, it is not clear at what point subclones should be treated therapeutically. A targetable *ALK* mutation in a major clone will surely be a good

candidate for an ALK inhibitor, but what about an *ALK* mutation that is at 1% prevalence? Indeed, new evidence of subclonal *ALK* mutations suggests that this question has growing importance for neuroblastoma[85]. Furthermore, at 100x coverage, a heterozygous *ALK* mutation in 1% of neuroblastoma cells will likely be missed due to insufficient read coverage; but at 500x coverage this same mutation may be detected. Ultimately, additional research in this area is needed to help guide precision medicine efforts.

Patient enrollment

Patient selection is critical for precision medicine. Patients for whom cure rates are extremely high (e.g. standard risk acute lymphoblastic leukemia) may benefit less from tumor sequencing. Initial efforts emphasized genomic profiling of multiply relapsed and refractory patients. However, highly refractory tumors are unlikely to exhibit single pathway addiction due to the development of multiple resistance pathways during the course of therapy. Thus, many advocate for genomic profiling early in disease course, ideally at diagnosis for cases with higher probability of relapse, and to incorporate targeted therapy (if appropriate) into the treatment regimen earlier as well, as tumors that are more naïve may respond better to pathway inhibition. Many groups are also repeating genomic analysis at the time of relapse to assess for clonal evolution and newly acquired molecular features.

Future directions

NCI Pediatric MATCH Study

The NCI Pediatric Molecular Analysis for Therapeutic Choice (MATCH) study, a collaborative effort between the Children's Oncology Group and the National Cancer Institute, is an ongoing effort that aims to build on adult oncology study[86,87] to develop a protocol for targeted therapy using an

519 umbrella design. NCI Pediatric MATCH will use standardized DNA and RNA-based biomarker
520 profiling of patient tumor and blood samples to assign patients to phase II studies of targeted therapies
521 if one of a predefined set of actionable mutations is detected. A number of drug-biomarker pairs have
522 been prioritized for inclusion on the study based on factors including (1) prevalence of the genomic
523 alteration in pediatric cancer, (2) ability to detect the target using the study platform, (3) evidence
524 linking the target to activity of the agent, (4) clinical and preclinical data for specific agents, and (5)
525 other ongoing or planned biomarker-defined clinical studies. The study is anticipated to open with 5-8
526 arms (molecularly-targeted agents). Given the size of the NCI Pediatric MATCH study, the methods
527 employed for genomic profiling are likely to inform precision oncology approaches for pediatric
528 patients moving forward.

529

530 Liquid tumor biopsies

531 Currently, the clinical standard is to monitor genomic alterations via direct tumor biopsy or resection.
532 However, there is abundant evidence that circulating tumor cells (CTCs) and/or cell free DNA
533 (cfDNA) present in blood offer an opportunity to evaluate tumor biology non-invasively, even for
534 brain tumors[88-93]. In pediatric cancers, most evidence for CTCs and cfDNA has been in
535 neuroblastoma and other solid tumors[94-96].

536

537 In addition to being non-invasive, CTCs and cfDNA enable frequent monitoring of tumor course
538 during and after treatment. Technically, methods to isolate this genomic material are challenging,
539 costly, and labor-intensive. However, they are increasingly clinically feasible[89]. CTCs also entail
540 single-cell sequencing, which if done for populations of tumor cells, may enable more direct
541 quantification of tumor heterogeneity and clonal abundance. In the future, methodological advances
542 and decreasing sequencing costs may help advance clinical prospects for single-cell sequencing.

543

544 Tumor profiling at multiple time points

545 In addition to tumor profiling at diagnosis and relapse, some groups now advocate for molecular
546 analyses at more regular intervals during treatment. Molecular assays for minimal residual disease
547 (MRD) in leukemias, for example, now include both flow cytometry and polymerase chain reaction
548 (PCR). Sequencing may ultimately fulfill this role too, and multiple groups are exploring the clinical
549 feasibility and utility of sequencing for MRD[97-100].

550

551 Expanding the landscape of sequencing

552 As knowledge of tumor biology advances and sequencing becomes more easily implemented, the
553 range of clinically-relevant genomic tools may expand (**Figure 3**)[101]. DNA methylation
554 sequencing, or other forms of epigenomics, may be appropriate for some tumors such as brain tumors.
555 Here, recent elucidation of a CpG island methylator phenotype (CIMP) has advanced our
556 understanding of tumor subgroups and may be relevant to understanding driver genomic
557 alterations[102,103] and patient disease course[104]. Methylation sequencing may ultimately be
558 possible from non-invasive sources as well[105].

559

560 Moreover, as immunotherapy and cancer immunology advance, clinical sequencing may incorporate
561 efforts to decode tumor neoantigens and T-cell repertoires in patients. Such initiatives are already
562 being explored in patient samples and in actively treated patients[106-108]. Further efforts in patient
563 care may expand into small RNA and microRNA sequencing[109].

564

565 Rationally understanding drug metabolism

566 One of the biggest black boxes in medicine is how different patients metabolize medications, which
567 can significantly impact effect dose, therapeutic levels, and side effects. This is particularly critical
568 for cytotoxic chemotherapy (e.g. 6-mercaptopurine, methotrexate, cisplatin) as well as specific
569 toxicities associated with individual therapies (e.g. cardiomyopathy with anthracyclines, hearing loss
570 with vincristine). The application of genomic technologies, especially metabolomics, may provide
571 key insights as well as clinical tools to understand and rationally predict drug behavior and toxicity
572 profiles in patients *in vivo*[110]. Ultimately, patients may have individually tailored dosing regimens
573 based on their specific physiology. Such prospects have the possibility of dramatically changing the
574 way medicine is practiced.

575

576 **Concluding remarks**

577 Precision medicine has rapidly become one of the most pursued research and clinical objectives over
578 the past decade. The political landscape, including the Precision Medicine Initiative and the
579 Moonshot for cancer, indicate that funding and support for precision medicine initiatives will continue
580 to be robust. Early clinical evidence for pediatric precision medicine through the PEDS-
581 MIONCOSEQ, BASIC3, INFORM and iCat studies has been encouraging, with meaningful results
582 for some patients. Yet, precision medicine still faces numerous challenges in its implementation,
583 standardization and feasibility across multiple institutions. In the near future, large-scale prospective
584 consortia studies such as the NCI Pediatric MATCH study will further refine the implementation of
585 precision medicine in pediatric oncology.

586

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596

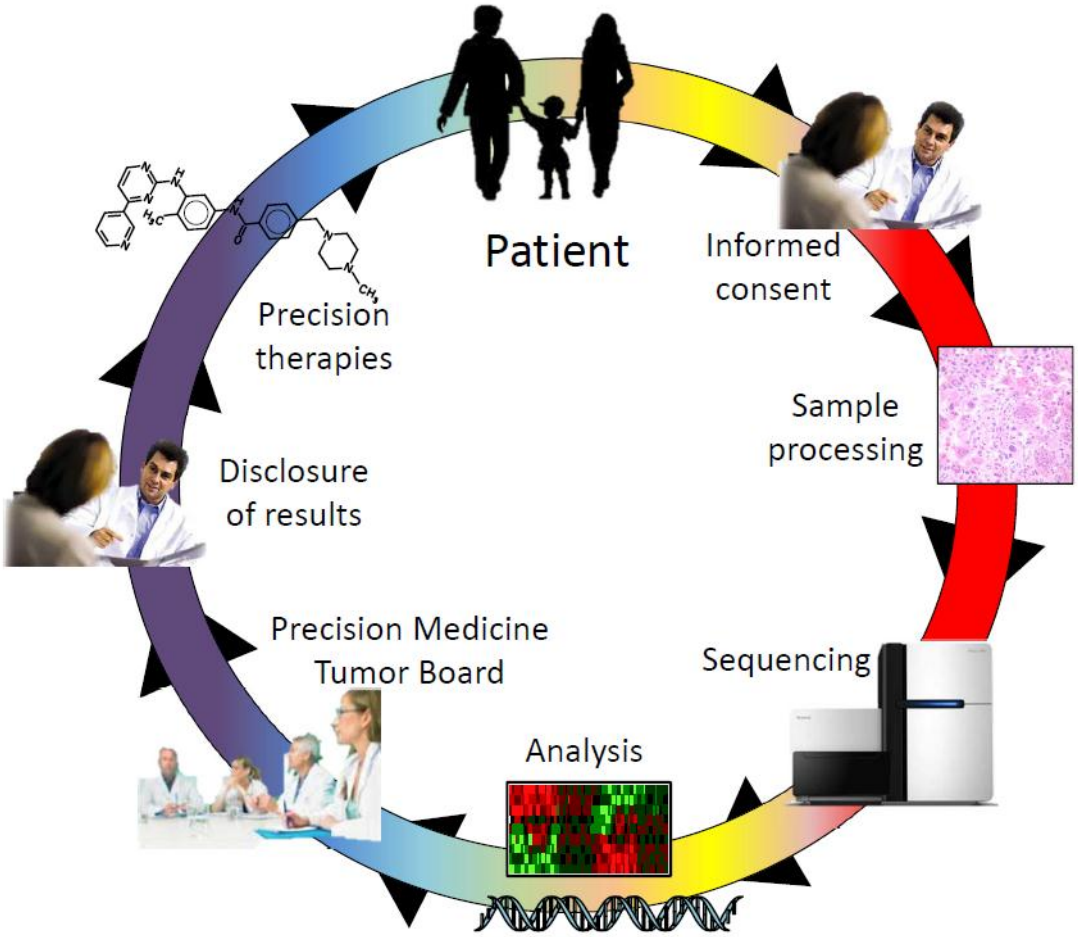
597 **Conflicts of Interest**

598 The authors declare no relevant conflicts of interest.

599

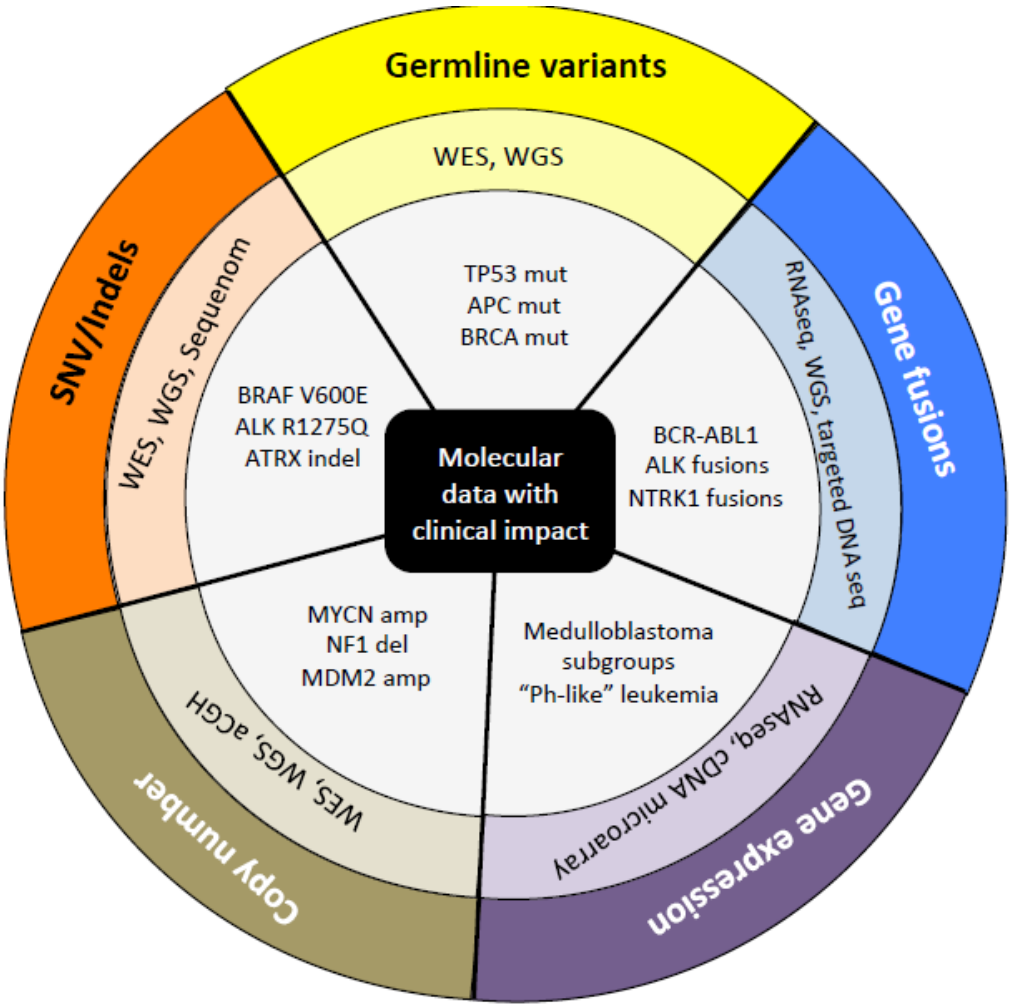
600 **Figure Legends**

601 **Figure 1: Overview of precision medicine in oncology.** Patients are enrolled for genomic profiling
602 following informed consent. Tumor samples are then acquired, processed, molecularly profiled
603 (typically through sequencing), and analyzed computationally. Molecular results are reviewed in a
604 precision medicine tumor board prior to disclosure of selected, relevant results to the patient. Where
605 available, targeted therapies may be initiated based upon molecular findings.



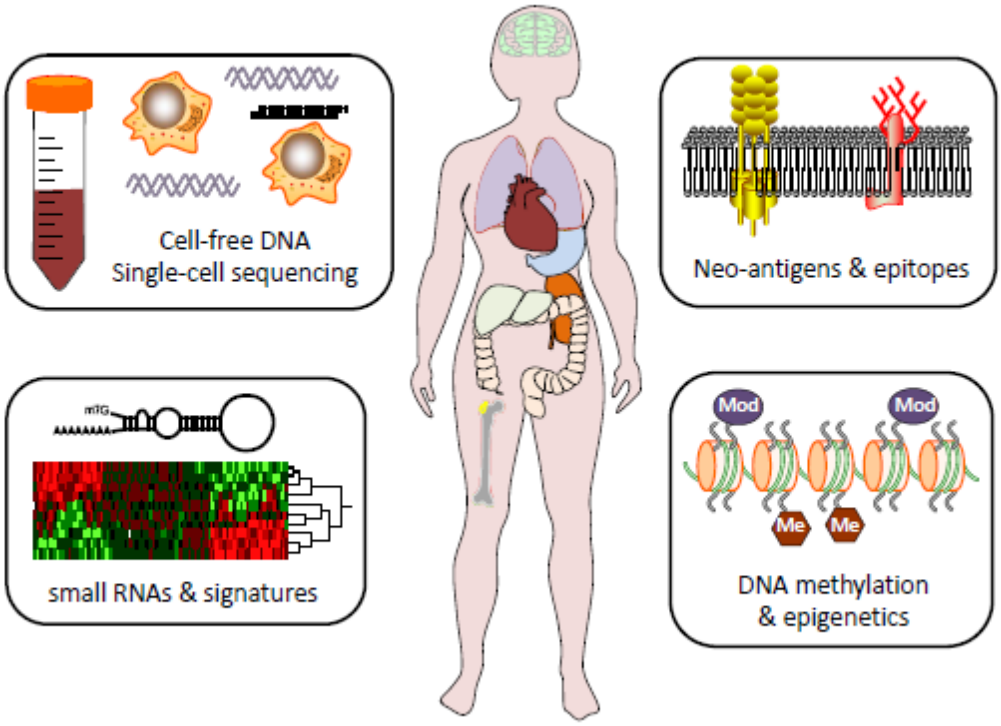
606

607 **Figure 2: Molecular data in precision oncology.** Pediatric cancers may harbor clinically relevant
608 germline and somatic variants, copy number aberrations, gene fusions, and gene expression patterns.
609 Here, the outer circle indicates the type of molecular event. The middle circle indicates the various
610 molecular assays used to profile a given molecular event. The inner circle provides several examples
611 of clinically-relevant findings enabled by molecular profiling. WES: whole exome sequencing.
612 WGS: whole genome sequencing. cDNA: complementary DNA. Mut: mutation. Amp:
613 amplification. Del: deletion. Indel: Insertion/deletion. SNV: single nucleotide variant. aCGH: array
614 comparative genome hybridization.



615

616 **Figure 3: Future directions in precision medicine.** In upcoming years, further research may define
617 clinical roles for multiple new areas of precision medicine. Four potential new areas include
618 epigenomic profiling, small RNA profiling, neo-antigens and epitope profiling, and single cell
619 sequencing and cell-free DNA (cfDNA).



620

621 **Tables (see attached files):**

622 Table I: Pilot studies of genomic medicine in pediatric oncology

623 Table II: Targeted agents in pediatric cancers

624 Table III: Challenges in precision medicine

625

626 **Appendix S1:** The basic modalities of genome sequencing technologies, their advantages and
627 disadvantages, and their compatibility with different biospecimen types

628

629 **References**

630 1. Collins FS, Varmus H. A new initiative on precision medicine. The New England journal of
631 medicine 2015:372(9):793-795.

2. McCarthy M. US president endorses "moonshot" effort to cure cancer. Bmj 2016:352:i213.

3. Ramsey BW, Davies J, McElvaney NG, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. The New England journal of medicine 2011:365(18):1663-1672.

4. Joyner MJ. Precision Medicine, Cardiovascular Disease and Hunting Elephants. Progress in cardiovascular diseases 2016.

5. Antcliffe D, Gordon AC. Metabonomics and intensive care. Critical care 2016:20(1):68.

6. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. The New England journal of medicine 2004:350(21):2129-2139.

7. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. The New England journal of medicine 2013:368(25):2385-2394.

8. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013:499(7457):214-218.

9. Pugh TJ, Morozova O, Attiyeh EF, et al. The genetic landscape of high-risk neuroblastoma. Nature genetics 2013:45(3):279-284.

10. Pugh TJ, Weeraratne SD, Archer TC, et al. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. Nature 2012:488(7409):106-110.

11. Eleveld TF, Oldridge DA, Bernard V, et al. Relapsed neuroblastomas show frequent RAS-MAPK pathway mutations. Nature genetics 2015:47(8):864-871.

12. Schramm A, Koster J, Assenov Y, et al. Mutational dynamics between primary and relapse neuroblastomas. Nature genetics 2015:47(8):872-877.

13. Morrissy AS, Garzia L, Shih DJ, et al. Divergent clonal selection dominates medulloblastoma at recurrence. Nature 2016:529(7586):351-357.

14. Zhang J, Walsh MF, Wu G, et al. Germline Mutations in Predisposition Genes in Pediatric Cancer. The New England journal of medicine 2015:373(24):2336-2346.

15. Mody RJ, Wu YM, Lonigro RJ, et al. Integrative Clinical Sequencing in the Management of Refractory or Relapsed Cancer in Youth. JAMA 2015:314(9):913-925.

16. Parsons DW, Roy A, Yang Y, et al. Diagnostic Yield of Clinical Tumor and Germline Whole-Exome Sequencing for Children With Solid Tumors. JAMA oncology 2016.

17. Green RC, Goddard KA, Jarvik GP, et al. Clinical Sequencing Exploratory Research Consortium: Accelerating Evidence-Based Practice of Genomic Medicine. American journal of human genetics 2016:98(6):1051-1066.

18. Roychowdhury S, Iyer MK, Robinson DR, et al. Personalized oncology through integrative high-throughput sequencing: a pilot study. Science translational medicine 2011:3(111):111ra121.

19. Harris MH, DuBois SG, Glade Bender JL, et al. Multicenter Feasibility Study of Tumor Molecular Profiling to Inform Therapeutic Decisions in Advanced Pediatric Solid Tumors: The Individualized Cancer Therapy (iCat) Study. JAMA oncology 2016.

20. Worst BC, van Tilburg CM, Balasubramanian GP, et al. Next-generation personalised medicine for high-risk paediatric cancer patients - The INFORM pilot study. European journal of cancer 2016:65:91-101.

21. Downing JR, Wilson RK, Zhang J, et al. The Pediatric Cancer Genome Project. Nature genetics 2012:44(6):619-622.

22. Roychowdhury S, Chinnaiyan AM. Translating genomics for precision cancer medicine. Annual review of genomics and human genetics 2014:15:395-415.

23. Janeway KA, Place AE, Kieran MW, et al. Future of clinical genomics in pediatric oncology. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013:31(15):1893-1903.

24. Perry JA, Kiezun A, Tonzi P, et al. Complementary genomic approaches highlight the PI3K/mTOR pathway as a common vulnerability in osteosarcoma. Proceedings of the National Academy of Sciences of the United States of America 2014:111(51):E5564-5573.

25. Franz DN, Belousova E, Sparagana S, et al. Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2013:381(9861):125-132.

26. Zhao Y, Adjei AA. The clinical development of MEK inhibitors. Nature reviews Clinical oncology 2014:11(7):385-400.

27. Chang TY, Dvorak CC, Loh ML. Bedside to bench in juvenile myelomonocytic leukemia: insights into leukemogenesis from a rare pediatric leukemia. Blood 2014:124(16):2487-2497.

28. Lu C, Zhang J, Nagahawatte P, et al. The genomic landscape of childhood and adolescent melanoma. The Journal of investigative dermatology 2015:135(3):816-823.

29. Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations. The New England journal of medicine 2015:373(8):726-736.

30. Bautista F, Paci A, Minard-Colin V, et al. Vemurafenib in pediatric patients with BRAFV600E mutated high-grade gliomas. Pediatric blood & cancer 2014:61(6):1101-1103.

31. Sievert AJ, Lang SS, Boucher KL, et al. Paradoxical activation and RAF inhibitor resistance of BRAF protein kinase fusions characterizing pediatric astrocytomas. Proceedings of the National Academy of Sciences of the United States of America 2013:110(15):5957-5962.

700 32. Mosse YP, Laudenslager M, Longo L, et al. Identification of ALK as a major familial
701 neuroblastoma predisposition gene. Nature 2008:455(7215):930-935.

702 33. Mosse YP, Lim MS, Voss SD, et al. Safety and activity of crizotinib for paediatric patients
703 with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology
704 Group phase 1 consortium study. The Lancet Oncology 2013:14(6):472-480.

705 34. Vaishnavi A, Capelletti M, Le AT, et al. Oncogenic and drug-sensitive NTRK1
706 rearrangements in lung cancer. Nature medicine 2013:19(11):1469-1472.

707 35. Doebele RC, Davis LE, Vaishnavi A, et al. An Oncogenic NTRK Fusion in a Patient with
708 Soft-Tissue Sarcoma with Response to the Tropomyosin-Related Kinase Inhibitor LOXO-
709 101. Cancer discovery 2015:5(10):1049-1057.

710 36. Gajjar A, Stewart CF, Ellison DW, et al. Phase I study of vismodegib in children with
711 recurrent or refractory medulloblastoma: a pediatric brain tumor consortium study. Clinical
712 cancer research : an official journal of the American Association for Cancer Research
713 2013:19(22):6305-6312.

714 37. Garnett MJ, Edelman EJ, Heidorn SJ, et al. Systematic identification of genomic markers of
715 drug sensitivity in cancer cells. Nature 2012:483(7391):570-575.

716 38. Brenner JC, Feng FY, Han S, et al. PARP-1 inhibition as a targeted strategy to treat Ewing's
717 sarcoma. Cancer research 2012:72(7):1608-1613.

718 39. Rader J, Russell MR, Hart LS, et al. Dual CDK4/CDK6 inhibition induces cell-cycle arrest
719 and senescence in neuroblastoma. Clinical cancer research : an official journal of the
720 American Association for Cancer Research 2013:19(22):6173-6182.

721 40. Puissant A, Frumm SM, Alexe G, et al. Targeting MYCN in neuroblastoma by BET
722 bromodomain inhibition. Cancer discovery 2013:3(3):308-323.

723 41. Bandopadhayay P, Bergthold G, Nguyen B, et al. BET bromodomain inhibition of MYC-
724 amplified medulloblastoma. Clinical cancer research : an official journal of the American
725 Association for Cancer Research 2014:20(4):912-925.

726 42. Li SQ, Cheuk AT, Shern JF, et al. Targeting wild-type and mutationally activated FGFR4 in
727 rhabdomyosarcoma with the inhibitor ponatinib (AP24534). PloS one 2013:8(10):e76551.

728 43. Watt TC, Cooper T. Sorafenib as treatment for relapsed or refractory pediatric acute
729 myelogenous leukemia. Pediatric blood & cancer 2012:59(4):756-757.

730 44. Tarlock K, Chang B, Cooper T, et al. Sorafenib treatment following hematopoietic stem cell
731 transplant in pediatric FLT3/ITD acute myeloid leukemia. Pediatric blood & cancer
732 2015:62(6):1048-1054.

733 45. Glade Bender JL, Lee A, Reid JM, et al. Phase I pharmacokinetic and pharmacodynamic
734 study of pazopanib in children with soft tissue sarcoma and other refractory solid tumors: a

children's oncology group phase I consortium report. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013:31(24):3034-3043.

46. DuBois SG, Marachelian A, Fox E, et al. Phase I Study of the Aurora A Kinase Inhibitor Alisertib in Combination With Irinotecan and Temozolomide for Patients With Relapsed or Refractory Neuroblastoma: A NANT (New Approaches to Neuroblastoma Therapy) Trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2016:34(12):1368-1375.

47. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. The New England journal of medicine 2010:363(18):1693-1703.

48. Von Hoff DD, LoRusso PM, Rudin CM, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. The New England journal of medicine 2009:361(12):1164-1172.

49. Rudin CM, Hann CL, Laterra J, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. The New England journal of medicine 2009:361(12):1173-1178.

50. Fong PC, Boss DS, Yap TA, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. The New England journal of medicine 2009:361(2):123-134.

51. Smith MA, Hampton OA, Reynolds CP, et al. Initial testing (stage 1) of the PARP inhibitor BMN 673 by the pediatric preclinical testing program: PALB2 mutation predicts exceptional in vivo response to BMN 673. Pediatric blood & cancer 2015:62(1):91-98.

52. Choy E, Butrynski JE, Harmon DC, et al. Phase II study of olaparib in patients with refractory Ewing sarcoma following failure of standard chemotherapy. BMC cancer 2014:14:813.

53. Decamp M, Joffe S, Fernandez CV, et al. Chemotherapy drug shortages in pediatric oncology: a consensus statement. Pediatrics 2014:133(3):e716-724.

54. Murphy D, Reaman G, Jensen CV. Pediatric oncology drug shortages: a multifaceted problem. Pediatrics 2014:133(3):e728-729.

55. Institute of Medicine. Addressing the Barriers to Pediatric Drug Development: Workshop Summary. Washington (DC): The National Academies Press; 2008.

56. Malik SM, Maher VE, Bijwaard KE, et al. U.S. Food and Drug Administration approval: crizotinib for treatment of advanced or metastatic non-small cell lung cancer that is anaplastic lymphoma kinase positive. Clinical cancer research : an official journal of the American Association for Cancer Research 2014:20(8):2029-2034.

57. Johnson JR, Ning YM, Farrell A, et al. Accelerated approval of oncology products: the food and drug administration experience. Journal of the National Cancer Institute 2011:103(8):636-644.

769 58. Kesselheim AS, Wang B, Franklin JM, et al. Trends in utilization of FDA expedited drug
770 development and approval programs, 1987-2014: cohort study. Bmj 2015:351:h4633.

771 59. Prowell TM, Theoret MR, Pazdur R. Seamless Oncology-Drug Development. The New
772 England journal of medicine 2016.

773 60. Muir P, Li S, Lou S, et al. The real cost of sequencing: scaling computation to keep pace with
774 data generation. Genome biology 2016:17(1):53.

775 61. Porter PL, Malone KE, Heagerty PJ, et al. Expression of cell-cycle regulators p27Kip1 and
776 cyclin E, alone and in combination, correlate with survival in young breast cancer patients.
777 Nat Med 1997:3(2):222-225.

778 62. Schatz MC, Langmead B, Salzberg SL. Cloud computing and the DNA data race. Nature
779 biotechnology 2010:28(7):691-693.

780 63. Stein LD. The case for cloud computing in genome informatics. Genome biology
781 2010:11(5):207.

782 64. Le Tourneau C, Delord JP, Goncalves A, et al. Molecularly targeted therapy based on tumour
783 molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre,
784 open-label, proof-of-concept, randomised, controlled phase 2 trial. The Lancet Oncology
785 2015:16(13):1324-1334.

786 65. Prasad V, Fojo T, Brada M. Precision oncology: origins, optimism, and potential. The Lancet
787 Oncology 2016:17(2):e81-86.

788 66. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence
789 variants: a joint consensus recommendation of the American College of Medical Genetics and
790 Genomics and the Association for Molecular Pathology. Genetics in medicine : official
791 journal of the American College of Medical Genetics 2015:17(5):405-424.

792 67. Dorschner MO, Amendola LM, Turner EH, et al. Actionable, pathogenic incidental findings
793 in 1,000 participants' exomes. American journal of human genetics 2013:93(4):631-640.

794 68. Rehm HL, Berg JS, Brooks LD, et al. ClinGen--the Clinical Genome Resource. The New
795 England journal of medicine 2015:372(23):2235-2242.

796 69. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among
797 sequence variation and human phenotype. Nucleic acids research 2014:42(Database
798 issue):D980-985.

799 70. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging
800 missense mutations. Nature methods 2010:7(4):248-249.

801 71. Van Driest SL, Wells QS, Stallings S, et al. Association of Arrhythmia-Related Genetic
802 Variants With Phenotypes Documented in Electronic Medical Records. Jama 2016:315(1):47-
803 57.

804 72. Scollon S, Bergstrom K, Kerstein RA, et al. Obtaining informed consent for clinical tumor
805 and germline exome sequencing of newly diagnosed childhood cancer patients. Genome
806 medicine 2014:6(9):69.

807 73. Everett JN, Mody RJ, Stoffel EM, et al. Incorporating genetic counseling into clinical care for
808 children and adolescents with cancer. Future oncology 2016:12(7):883-886.

809 74. McCullough LB, Slashinski MJ, McGuire AL, et al. Is Whole-Exome Sequencing an
810 Ethically Disruptive Technology? Perspectives of Pediatric Oncologists and Parents of
811 Pediatric Patients With Solid Tumors. Pediatric blood & cancer 2016:63(3):511-515.

812 75. Parsons DW, Roy A, Plon SE, et al. Clinical tumor sequencing: an incidental casualty of the
813 American College of Medical Genetics and Genomics recommendations for reporting of
814 incidental findings. Journal of clinical oncology : official journal of the American Society of
815 Clinical Oncology 2014:32(21):2203-2205.

816 76. Roberts JS, Shalowitz DI, Christensen KD, et al. Returning individual research results:
817 development of a cancer genetics education and risk communication protocol. Journal of
818 empirical research on human research ethics : JERHRE 2010:5(3):17-30.

819 77. Henderson GE, Wolf SM, Kuczynski KJ, et al. The challenge of informed consent and return
820 of results in translational genomics: empirical analysis and recommendations. The Journal of
821 law, medicine & ethics : a journal of the American Society of Law, Medicine & Ethics
822 2014:42(3):344-355.

823 78. National Academies of Sciences. Biomarker Tests for Molecularly Targeted Therapies: Key
824 to Unlocking Precision Medicine. Washington, DC: The National Academies Press; 2016.

825 79. Lyman GH, Moses HL. Biomarker Tests for Molecularly Targeted Therapies--The Key to
826 Unlocking Precision Medicine. The New England journal of medicine 2016:375(1):4-6.

827 80. In: Graig LA, Phillips JK, Moses HL, editors. Biomarker Tests for Molecularly Targeted
828 Therapies: Key to Unlocking Precision Medicine. Washington (DC)2016.

829 81. Ma X, Edmonson M, Yergeau D, et al. Rise and fall of subclones from diagnosis to relapse in
830 pediatric B-acute lymphoblastic leukaemia. Nature communications 2015:6:6604.

831 82. Farrar JE, Schuback HL, Ries RE, et al. Genomic profiling of pediatric acute myeloid
832 leukemia reveals a changing mutational landscape from disease diagnosis to relapse. Cancer
833 research 2016.

834 83. Chen X, Stewart E, Shelat AA, et al. Targeting oxidative stress in embryonal
835 rhabdomyosarcoma. Cancer cell 2013:24(6):710-724.

836 84. Wu X, Northcott PA, Dubuc A, et al. Clonal selection drives genetic divergence of metastatic
837 medulloblastoma. Nature 2012:482(7386):529-533.

838 85. Bellini A, Bernard V, Leroy Q, et al. Deep Sequencing Reveals Occurrence of Subclonal
839 ALK Mutations in Neuroblastoma at Diagnosis. Clinical cancer research : an official journal
840 of the American Association for Cancer Research 2015:21(21):4913-4921.

841 86. McNeil C. NCI-MATCH launch highlights new trial design in precision-medicine era.
842 Journal of the National Cancer Institute 2015:107(7).

843 87. Abrams J, Conley B, Mooney M, et al. National Cancer Institute's Precision Medicine
844 Initiatives for the new National Clinical Trials Network. American Society of Clinical
845 Oncology educational book / ASCO American Society of Clinical Oncology Meeting
846 2014:71-76.

847 88. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and
848 late-stage human malignancies. Science translational medicine 2014:6(224):224ra224.

849 89. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor
850 metastatic breast cancer. The New England journal of medicine 2013:368(13):1199-1209.

851 90. Forshew T, Murtaza M, Parkinson C, et al. Noninvasive identification and monitoring of
852 cancer mutations by targeted deep sequencing of plasma DNA. Science translational medicine
853 2012:4(136):136ra168.

854 91. Leary RJ, Sausen M, Kinde I, et al. Detection of chromosomal alterations in the circulation of
855 cancer patients with whole-genome sequencing. Science translational medicine
856 2012:4(162):162ra154.

857 92. Maheswaran S, Sequist LV, Nagrath S, et al. Detection of mutations in EGFR in circulating
858 lung-cancer cells. The New England journal of medicine 2008:359(4):366-377.

859 93. Muller C, Holtschmidt J, Auer M, et al. Hematogenous dissemination of glioblastoma
860 multiforme. Science translational medicine 2014:6(247):247ra101.

861 94. Combaret V, Iacono I, Bellini A, et al. Detection of tumor ALK status in neuroblastoma
862 patients using peripheral blood. Cancer medicine 2015:4(4):540-550.

863 95. Kurihara S, Ueda Y, Onitake Y, et al. Circulating free DNA as non-invasive diagnostic
864 biomarker for childhood solid tumors. Journal of pediatric surgery 2015:50(12):2094-2097.

865 96. Kuroda T, Morikawa N, Matsuoka K, et al. Prognostic significance of circulating tumor cells
866 and bone marrow micrometastasis in advanced neuroblastoma. Journal of pediatric surgery
867 2008:43(12):2182-2185.

868 97. Klco JM, Miller CA, Griffith M, et al. Association Between Mutation Clearance After
869 Induction Therapy and Outcomes in Acute Myeloid Leukemia. Jama 2015:314(8):811-822.

870 98. Kotrova M, Muzikova K, Mejstrikova E, et al. The predictive strength of next-generation
871 sequencing MRD detection for relapse compared with current methods in childhood ALL.
872 Blood 2015:126(8):1045-1047.

873 99. Pan X, Nariai N, Fukuhara N, et al. Monitoring of minimal residual disease in early T-cell
874 precursor acute lymphoblastic leukaemia by next-generation sequencing. British journal of
875 haematology 2016.

876 100. Stadt UZ, Escherich G, Indenbirken D, et al. Rapid Capture Next-Generation Sequencing in
877 Clinical Diagnostics of Kinase Pathway Aberrations in B-Cell Precursor ALL. Pediatric blood
878 & cancer 2016.

879 101. Soon WW, Hariharan M, Snyder MP. High-throughput sequencing for biology and medicine.
880 Molecular systems biology 2013:9:640.

881 102. Jha P, Pia Patric IR, Shukla S, et al. Genome-wide methylation profiling identifies an
882 essential role of reactive oxygen species in pediatric glioblastoma multiforme and validates a
883 methylome specific for H3 histone family 3A with absence of G-CIMP/isocitrate
884 dehydrogenase 1 mutation. Neuro-oncology 2014:16(12):1607-1617.

885 103. Turcan S, Rohle D, Goenka A, et al. IDH1 mutation is sufficient to establish the glioma
886 hypermethylator phenotype. Nature 2012:483(7390):479-483.

887 104. Mack SC, Witt H, Piro RM, et al. Epigenomic alterations define lethal CIMP-positive
888 ependymomas of infancy. Nature 2014:506(7489):445-450.

889 105. Legendre C, Gooden GC, Johnson K, et al. Whole-genome bisulfite sequencing of cell-free
890 DNA identifies signature associated with metastatic breast cancer. Clinical epigenetics
891 2015:7(1):100.

892 106. Gerlinger M, Quezada SA, Peggs KS, et al. Ultra-deep T cell receptor sequencing reveals the
893 complexity and intratumour heterogeneity of T cell clones in renal cell carcinomas. The
894 Journal of pathology 2013:231(4):424-432.

895 107. Gubin MM, Artyomov MN, Mardis ER, et al. Tumor neoantigens: building a framework for
896 personalized cancer immunotherapy. The Journal of clinical investigation 2015:125(9):3413-
897 3421.

898 108. Tran E, Ahmadzadeh M, Lu YC, et al. Immunogenicity of somatic mutations in human
899 gastrointestinal cancers. Science 2015:350(6266):1387-1390.

900 109. Nair VS, Pritchard CC, Tewari M, et al. Design and Analysis for Studying microRNAs in
901 Human Disease: A Primer on -Omic Technologies. American journal of epidemiology
902 2014:180(2):140-152.

903 110. Elie V, de Beaumais T, Fakhoury M, et al. Pharmacogenetics and individualized therapy in
904 children: immunosuppressants, antidepressants, anticancer and anti-inflammatory drugs.
905 Pharmacogenomics 2011:12(6):827-843.

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908 **TABLE 1: Pilot studies of genomic medicine in pediatric oncology**

	Institution	# patients enrolled	# patients analyzed	Tumor types included	Types of patients enrolled	Molecular profiling for somatic events	Molecular profiling for trans-criptional events	Molecular profiling for germline events	Profiling platform	CLIA lab?	M c -
BASIC3	Baylor College of Medicine	150	150 (GL), 121 (Tumor)	Solid	Newly diagnosed	WES	None	WES	Illumina HiSeq	Yes	W 2
PEDS-MI-ONCOSEQ	University of Michigan	102	91	Solid, brain and liquid	Relapsed, high risk newly diagnosed	WES, RNA-Seq	RNA-Seq	WES	Illumina HiSeq	Yes	W 1
iCat	Dana-Farber Cancer Institute and others	101	89	Solid	Relapsed, high risk newly diagnosed	Sequenom, aCGH, WES	None	Not done	Illumina HiSeq, Agilent	Yes	
INFORM	German Cancer Research Center (DKFZ) and others	57	52	Solid, brain and liquid	Relapsed, high risk newly diagnosed	WES, WGS, RNA-Seq, Methyl-ation array, RNA GeneChip array	RNA-Seq, GeneChip Array	WES, WGS	Illumina HiSeq, Affy-metrix Gene-Chip, Illumina methyl-array	NA	W 1 W 3

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910 **Abbreviations:** GL, germline; WES, whole exome sequencing; WGS, whole genome sequencing;
911 Methyl-array, methylation array; aCGH, array comparative genomic hybridization; SNV, single
912 nucleotide variant; CLIA, clinical laboratory improvements amendments; NR, not reported.
913 Patient enrollment numbers refer to data reported in [References 15, 16, 19, and 20].

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915 **TABLE 2: Targeted agents in pediatric cancers**

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Inhibitor target	Example molecular biomarkers*	Example therapeutics	Example pediatric tumors	References
PI3K/ mTOR	PIK3CA mutations PTEN loss TSC1/2 loss	Everolimus Temsirolimus Rapamycin	Sarcomas Subependymal giant cell astrocytomas	[24, 25]
MEK	BRAF mutation BRAF tandem duplication N/KRAS mutation PTPN11 mutation NF1 loss	Trametinib Selumetinib	Melanoma Plexiform neurofibroma Glioblastoma Juvenile myelomonocytic leukemia	[26, 27]
BRAF	BRAF V600E/K BRAF fusions	Vemurafenib Dabrafenib	Melanoma LCH Glioma Pilocytic astrocytomas (2nd generation inhibitors only)	[28 – 31]
ALK	ALK mutation/fusion NTRK1/2/3 fusion ROS1 fusion	Crizotinib	Neuroblastoma Embryonal sarcomas	[32, 33]
NTRK 1/2/3	NTRK1/2/3 fusion	Crizotinib LOXO-101	Infantile fibrosarcomas Mesonephric blastoma	[34, 35]
SMO	PTCH1 mutation SUFU mutations GLI1 amplification	Vismodegib	Medulloblastoma	[36]
PARP1	BRCA1/2 mutation	Olaparib	Ewing's Sarcoma	[37, 38]

	EWSR1-FLI fusion ATM mutation	Rucaparib		
CDK4/6	CDK4/6 amplification CyclinD1 amplification	Palbociclib	Neuroblastoma Rhabdomyosarcoma ATRT	[39]
BET bromodomain	BRD-NUT fusions MYCN amplification MYC translocations	JQ1, IBET726, OTX015	NUT midline carcinomas Neuroblastoma Medulloblastoma Burkitt Lymphoma	[40, 41]
AURKA	MYCN amplification	Alisertib	Neuroblastoma	[46]
FGFR	FGFR1/2/3 fusion, amplification, mutation	Ponatinib Dovitinib	Rhabdomyosarcoma	[42]
Multi-kinase inhibitors	FLT3 mutation or internal tandem duplication	Sorafenib	Acute myeloid leukemia	[43, 44]
	VEGFR, cKit, PDGFR expression	Pazopanib	Sarcomas	[45]

* Loss refers to genomic loss through either deletion or inactivating mutation

TABLE 3: Challenges in precision medicine

		Current Status	Considerations	Future possibilities

Challenges	**Cost***	$6000	• $3000 in direct sequencing costs • $1000 for library preparation • $2000 Lab personnel, capital cost	• Reductions in sequencing reagents • Reduced reliance on fee-for-service computational services
	Turn around time**	4-6 weeks	• 1-2 weeks for sequencing • 2-4 weeks for bioinformatics	Optimizing computational pipelines with targeted analyses for time reductions
	Lack of clinical trial availability	~20-40% of patients with actionable targets lack access to drugs	Limited pediatric safety/efficacy data available for many experimental therapies	Multi-institutional umbrella trial protocols such as the MATCH
	Rational combination of therapies	Targeted agents typically initiated in the relapse setting mostly as a single agent after standard-of-care	Relapsed/refractory patients likely have multiple intrinsic resistance mechanisms	• Introduction of targeted agents early in disease course • Combining targeted agents with other targeted agents, standard-of-care regimens or immunotherapy
	Incidental germline findings	~8 - 10% of patients harbor likely pathogenic variants	Flexible-default model of optional disclosure of germline findings to families	• Increased access for "trio" testing of families to define variants • Longitudinal studies on the impact of findings on families (e.g. psychological, access to care and adherence to cancer screening)

*Estimate for supplies and capital depreciation for the Peds-Mioncoseq study by Michigan group (Ref 15) only and does not include cost of analysis.

** Turnaround time estimates refer to the Peds-Mioncoseq study by Michigan group (Ref 15) only