Precision Medicine in Pediatric Oncology: Translating Genomic Discoveries into Optimized Therapies

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Abstract

Survival of children with cancers has dramatically improved over the past several decades. This success has been achieved through improvement of combined modalities in treatment approaches, intensification of cytotoxic chemotherapy for those with high-risk disease and refinement of risk stratification incorporating novel biologic markers in addition to traditional clinical and histologic features. Advances in cancer genomics have shed important mechanistic insights on disease biology and have identified “driver” genomic alterations, aberrant activation of signaling pathways, and epigenetic modifiers that can be targeted by novel agents. Thus, the recently described genomic and epigenetic landscapes of many childhood cancers have expanded the paradigm of precision medicine in the hopes of improving outcomes while minimizing toxicities. In this review, we will discuss the biologic rationale for molecularly targeted therapies in genomically-defined subsets of pediatric leukemias, solid tumors, and brain tumors.
Introduction

Survival rates for children diagnosed with cancer have improved substantially over the last five decades. Today, long-term survival is expected for approximately 80% of children treated with contemporary therapies (1). These successes have occurred as a result of multi-centered, randomized clinical trials that have largely tested the efficacy of dose intensification of conventional cytotoxic chemotherapy and the implementation of enhanced supportive care. Nevertheless, childhood cancer remains the leading cause of non-accident related death in children and for many cancers, further intensification of cytotoxic chemotherapy has failed to provide additional therapeutic benefit and has instead resulted in increased treatment-related mortality and undesirable long-term toxicity (2, 3). Therefore, innovative therapeutic approaches are urgently needed for patients with high-risk disease. Meanwhile, advances in cancer genomics have revolutionized the field of oncology and have become a crucial component in modern risk stratification algorithms and novel therapeutic avenues. The genomic and epigenetic landscapes of many childhood cancers have been recently elucidated and provide important insights into genetic alterations, signal transduction abnormalities and epigenetic dysregulation, all of which may represent actionable therapeutic targets. In this review, we will discuss how genomic discoveries are being translated from the bench into the clinic, resulting in the development of precision medicine trials for specific
subtypes of pediatric hematologic malignancies, solid tumors and brain tumors. Immunotherapeutic approaches will be discussed separately.

**Precision medicine therapeutics in pediatric hematologic malignancies**

**Acute lymphoblastic leukemia**

The treatment of Philadelphia-chromosome positive acute lymphoblastic leukemia (Ph+ALL) represents the first paradigm of precision medicine principals in pediatric oncology. Comprising 3% of pediatric ALL, Ph+ALL, harbors the canonical t(9;22)(q34;q11) translocation, resulting in the BCR-ABL1 oncoprotein. Ph+ALL was previously associated with a dismal outcome despite intensive multi-agent chemotherapy and required hematopoietic stem cell transplantation (HSCT) in first remission (4). However, targeted inhibition of the ABL1 kinase domain with imatinib in combination with a high-risk (HR) ALL cytotoxic chemotherapy backbone significantly improved survival among children with Ph+ALL, such that HSCT is no longer universally recommended in first remission (4). Recent large-scale genomic profiling studies have identified a new subtype of HR B-lineage ALL in which this paradigm of molecularly targeted therapy may be similarly efficacious. Philadelphia chromosome-like ALL (Ph-like ALL) is characterized by a gene expression profile (GEP) similar to that of Ph+ALL but lacks the hallmark BCR-ABL1 oncoprotein. Ph-like ALL patients who exhibit adverse clinical features face an exceptionally poor prognosis compared to patients with HR B-lineage ALL without the Ph-like GEP when treated with modern chemotherapy regimens (5). The frequency of Ph-like ALL increases with age, ranging from 15% in children and adolescents to over 20% in adults, with a peak among young adults between
21 to 39 years of age (6). A recent comprehensive genomic analysis of Ph-like ALL unraveled its heterogeneous genomic landscape characterized by a diverse range of mutations in genes that are crucial in B-cell development as well as genetic alterations activating kinases and cytokine receptor genes, rendering Ph-like ALL amenable to treatment with tyrosine kinase inhibitor (TKI) therapy (7). Approximately half of Ph-like ALL patients harbor rearrangements of the cytokine receptor like factor 2 (CRLF2) gene, and concomitant activating JAK mutations are present in half of the CRLF2-rearranged cases. The remainder of Ph-like ALL is characterized by kinase-activating alterations in the ABL-class genes ABL1, ABL2, PDGFRB, and CS1FR (13%), JAK2 and EPOR rearrangements (11%), other JAK/STAT pathway lesions such as mutations in IL7R or focal deletion of SH2B3 (13%), Ras-pathway mutations (4%), and other rare kinase fusions such as NTRK3 and DGKH (<1%) (6). A rapidly growing body of work from in vitro assays and patient-derived xenografts has demonstrated that the myriad of kinase alterations in Ph-like ALL converges to activate three common downstream pathways: the JAK-STAT, ABL, and PI3K-Akt-mTOR signaling pathways, each of which is targetable with the relevant TKIs, providing compelling rationale for testing the upfront efficacy of combinatorial TKI and conventional chemotherapy regimen (6, 8, 9).

Specifically, the Children’s Oncology Group (COG) is currently testing the incorporation of dasatinib or ruxolitinib into a conventional chemotherapy backbone to establish their efficacy in Ph-like ALL with ABL-class fusions or JAK/STAT pathway alterations, respectively (Figure 1) (Table 1).

Infant ALL represents another rare high-risk subset of B-ALL in which innovative approaches are needed to address the high rates of treatment failure and
improve outcomes. Rearrangement of the *MLL* gene at 11q23, now known as *KMT2A*, is a molecular hallmark of infant ALL that occurs in up to 75% of cases (10). *KMT2A*-rearrangements (*KMT2A*-R) represent the most important negative prognostic factor in infant ALL, particularly for those less than 90 days old. Efforts to decipher the biology of infant ALL have identified potential therapeutic candidates to be investigated in clinical trials, although the genomic landscape of infant ALL is characterized by the paucity of somatic mutations outside of *KMT2A*-R (11). AALL0631 was the first precision medicine trial in *KMT2A*-R infant ALL in which lestaurtinib, a FLT3 inhibitor, was added to chemotherapy given the frequent FLT3 overexpression within this subtype (12). Unfortunately, the addition of lestaurtinib failed to improve survival outcomes relative to standard treatment in *de novo* cases. Similarly, chemotherapy combined with quizartinib, a second-generation FLT3 inhibitor, did not have significant clinical activity in the relapsed setting (13). Alternatively, there is increasing evidence demonstrating widespread epigenetic dysregulation in *KMT2A*-R ALL. Therefore, the next generation of trials will test whether demethylating/hypomethylating agents (eg. decitabine, azacitidine, DOT1L inhibitors) or histone deacetylation inhibitors (eg. vorinostat, panobinostat, bromodomain inhibitors) in combination with an ALL chemotherapy backbone will improve outcomes (14).

Unlike B-ALL, the heterogeneity of genetic alterations in T-lineage ALL (T-ALL) has precluded the identification of prognostic factors for risk stratification and the development of targeted therapeutic regimens. Nevertheless, genome-wide analyses have identified lesions that dysregulate key signaling pathways, some of which may represent novel therapeutic targets. For instance, the Notch signaling pathway is the most
commonly deregulated pathway in T-ALL (15). Notch signaling inhibition by gamma-secretase inhibitors (GSIs) that block the second cleavage of Notch1 preventing its activation, have been investigated in preclinical and early phase studies. Unfortunately, the gastrointestinal toxicity associated with first-generation GSIs and their lack of efficacy in more common adult solid tumors have precluded further development in T-ALL trials. Alternative Notch-inhibiting strategies utilizing newer GSIs and anti-Notch1 immunotherapies are currently being investigated in adult trials (15). Furthermore, T-ALL is characterized by frequent activation of the PI3K-Akt-mTOR pathway, mostly as a result of inactivating mutations in PTEN. Inhibitors of this pathway have demonstrated preclinical efficacy, both alone and in combination with cytotoxic chemotherapy (16).

Bortezomib, a proteasome inhibitor, is the only targeted therapy that has reached the threshold of testing in a front line phase III trial for de novo pediatric T-ALL. The inclusion of bortezomib is based on the observation that constitutive activation of NF-κB often occurs in T-ALL blasts as a result of Notch or Akt activation. Furthermore, preclinical studies demonstrated that inhibition of NF-κB by bortezomib can enhance leukemia cell sensitivity to other traditional chemotherapeutic agents and may reverse steroid resistance, rendering it a promising target to incorporate into upfront clinical trials (15).

Despite survival rates approaching 90% in children with ALL who are treated with modern chemotherapy regimens, approximately 15-20% of patients still relapse and outcomes following relapse are dismal (17). Genomic characterization of matched patient samples at diagnosis, remission, and relapse has deepened our understanding of the intertwined and complex interactions between clonal architecture, outgrowth and
emergence of mutations, and mechanisms governing therapy resistance. Substantial genomic changes exist between diagnostic and relapsed ALL samples, illustrating the dynamic clonal evolution of leukemogenesis at recurrence (18). However, relapse-acquired mutations can often be detected at low levels at diagnosis, implying that ALL relapse can arise from the enrichment of rare, pre-existing subclones (18). This finding further suggests a paradigm shift in which subclonal mutations may not only play a “passenger” role, but rather may “drive” disease relapse. Recurrent genetic alterations associated with relapsed ALL include: 1) activating somatic mutations in Ras pathway-related genes; 2) somatic mutations in genes that confer chemo-resistance such as \textit{NT5C2}, \textit{CREBBP} and \textit{PRPS1}; and 3) epigenetic dysregulation (19). Collectively, these data provide compelling rationale for the incorporation of MEK inhibitors and epigenetic modifiers in future precision medicine trials for relapsed ALL.

\textbf{Acute myeloid leukemia}

Pediatric acute myeloid leukemia (AML) is a genetically heterogeneous disease with historically dismal outcomes, though the introduction of cytarabine and anthracycline-based intensive chemotherapy regimens now result in cure rates between 50 to 65\% (20). Intensification of conventional chemotherapy has been maximized given the high frequency of treatment-related mortality in this patient population. Therefore, the development of novel therapeutic strategies relies on the knowledge derived from recent gene discovery studies. Approximately 75\% of pediatric AML cases harbor genetic aberrations that may represent critical molecular targets. The most common cytogenetic abnormalities in childhood AML are the t(8;21)(q22;q22) and inv(16)(p13.1q22)
translocations involving core-binding factor (CBF-AML), KMT2A-rearranged AML, and t(15;17)(q22;q21). Another major subgroup is defined by activating mutations in genes such as FLT3, KIT, and RAS, resulting in dysregulated signal transduction (21). The first precision medicine paradigm in AML incorporated all-trans retinoic acid (ATRA) to target the pathognomonic PML-RARα fusion of acute promyelocytic leukemia (APL). The next-generation of AML targeted therapy trials focused on the addition of FLT3 inhibitors to therapy regimens for patients with FLT3 mutations, which occur in 10-15% of pediatric AML and confer a poor prognosis (21). The current COG phase III trial for de novo AML, AAML1031, assesses the combinatorial effect of sorafenib, a multi-TKI with activity against FLT3, and conventional chemotherapy for FLT3-mutant AML. Other potentially targetable mutations that are now being investigated in early phase clinical trials include those involving KIT and components of the Ras pathway (22). A phase I study of dasatinib has been completed in children with imatinib-resistant KIT mutations, while small molecule Ras-pathway inhibitors have not yet reached clinical trials despite promising results in preclinical models (23). The distinct epigenetic profile of pediatric AML may further identify a subset of patients who may benefit from the addition of epigenetic modifiers. Recent studies testing the efficacy of hypomethylating agents such as decitabine or azacitidine in very high risk or relapsed AML patients have showed early efficacy and may warrant further investigation (24).
Precision medicine therapeutics in pediatric solid and brain tumors

**Neuroblastoma**

The clinical presentation and survival rates of neuroblastoma patients vary greatly. Infants can present with tumors that regress spontaneously. Children with localized disease have >90% survival with minimal chemotherapy, while those with widely metastatic disease only have 40-50% survival, despite receiving intensive multimodal regimens. Tumors in older children and adolescents are often chemoresistant with a chronic, indolent course (25). The association between MYCN amplification and aggressive disease was first identified in the 1980s (26). Subsequently, DNA ploidy, gains of 17q, and deletions of 1p or 11q were identified as prognostic biomarkers (27, 28). Although some of these alterations have been incorporated into the INRG classification for risk group assignment (29), none of these findings have been translated into tailored therapies, due to insufficient understanding of the oncogenes or tumor-suppressors at these loci and their contribution to disease pathogenesis. Even for MYCN, whose role in tumorigenesis is well-understood (30), there are yet to be targeted approaches. This is attributable to difficulties in designing a small molecule that can bind its helix-loop-helix structure (31). One approach is to target MAX, the heterodimerization partner of MYC, which is required for DNA binding. Drugs targeting the MYC:MAX interaction have demonstrated potential in vitro and in vivo (32). Another method is to target synthetic lethal interactions through CDK1, CDK2, or CHK1 inhibition (33-35). There are no active pediatric trials with CDK1 or CDK2 inhibitors,
but the COG will be opening a Phase I study with a CHK1 inhibitor. Drugs that target mTOR, PI3K, Aurora kinase, and Akt decrease MYCN stabilization, providing an alternative therapeutic strategy (32, 36, 37). Pediatric dosing for mTOR and PI3K/mTOR inhibitors is available, although their utility in neuroblastoma has not been fully investigated. Aurora kinase and Akt inhibitors are currently in pediatric clinical phase testing. A final approach to targeting MYCN is through bromodomain and extra terminal (BET) inhibition, since MYC family genes are targets of these chromatin readers. Studies of BET inhibitors in neuroblastoma cell lines and mouse models have demonstrated cytotoxicity (38), however, these drugs have yet to make it into clinical trials due to concerns for off-target toxicities.

Next-generation sequencing technologies have shed light on neuroblastoma heterogeneity at diagnosis and relapse (Figure 2). Alterations most commonly occur in ALK at rates of 8-14% at diagnosis and 26-43% at relapse (39). Clinical trials with Crizotinib, which is FDA-approved for the treatment of ALK-rearranged non-small cell lung cancer, and early phase clinical trials with next-generation ALK inhibitors have generated excitement amongst clinicians. Although RAS-MAPK alterations are rare at diagnosis, relapsed/refractory neuroblastoma specimens demonstrate enrichment for activating mutations in this pathway, and susceptibility to MEK inhibition (40), offering another targeted approach which is in clinical trial. Interestingly, the CDK4/CDK6 cell cycle regulatory pathway is implicated in neuroblastoma pathogenesis through CCND1 and CDK4 amplification or CDKN2A deletion and the frequency of these alterations may be increased in the relapsed setting (39). In xenograft models, dual CDK4/CDK6
inhibition induced cell cycle arrest and senescence (41). Furthermore, CDK4 inhibition sensitized MYCN amplified neuroblastoma cells to doxorubicin-mediated death (42). Collectively, these discoveries underscore the potential for precision medicine to augment conventional therapies for neuroblastoma and highlight the need for biopsy at the time of relapse.

**Sarcomas**

Sarcomas comprise <15% of pediatric malignancies (43), but contribute largely to cancer-related morbidity and mortality. These patients desperately need new therapeutic strategies, yet have seen few advances over the decades. Recent studies have described the genomic landscape of Ewing sarcoma, rhabdomyosarcoma, and osteosarcoma (44-48). They have shed light on sarcoma biology, but have not yielded new treatments. This is due, in part, to the genomic profile of pediatric sarcomas, which is often characterized by gene rearrangements with a paucity of other alterations. Canonical translocations are ideal candidates for targeting, as they are drivers of oncogenesis only present in malignant cells. However, identifying tailored therapies has been challenging for at least two reasons. First, the chimeric protein is often not easily druggable. For example, the Ewing sarcoma translocation, EWS-FLI, does not form a fixed three-dimensional structure under physiologic conditions, impeding inhibitor design (49). Only recently, a small molecule, YK-4-279, was identified; it is able to block EWS-FLI protein interactions and result in cell cytotoxicity (50). It has transitioned to phase I testing in relapsed/refractory patients (51). Secondly, targeting EWS-FLI, as well as the alveolar
rhabdomyosarcoma PAX-FOXO fusion, is difficult due to an absence of inherent enzymatic activity. Unlike most oncogenic rearrangements that result in kinase activation, these chimeric proteins activate and repress transcriptional activity of a wide array of genes and reversal of these gene signatures is quite complicated. Trabectedin, a synthetic alkaloid, reversed the EWS-FLI signature in preclinical models, however, phase II testing did not demonstrate sufficient single-agent activity (51). Phase Ib combination testing is currently recruiting.

Incorporating precision medicine in the management of translocation-negative sarcomas has posed a challenge, as well. Fusion-negative rhabdomyosarcoma patients harbor alterations in multiple targetable pathways (Table 2). Unfortunately, these mutations occur at such a low rate that designing well-powered clinical trials can be problematic. Even in the complex and unstable osteosarcoma genome, which harbors multiple structural rearrangements and copy number changes, few targetable alterations are recurrent across multiple patients. Though we have gained new insights into sarcoma oncogenesis through genomic profiling, there are still many obstacles to leverage them into therapeutic strategies.

Despite these challenges, several candidates for targeted therapies have been identified. IGF-1R, PDGFR, VEGFR, and HER-2 have been observed to be overexpressed in osteosarcoma (52-55) and given their role in activating multiple downstream signaling pathways that affect growth, cell proliferation, and survival, they pose an attractive target. However, clinical trials with the FDA-approved agents Imatinib, Sorafenib, and Sunitib did not induce significant responses in osteosarcoma (56-59). Additionally, the anti-IGF-1R antibody Cixutumumab, the anti-VEGF antibody
Bevacizumab, and the anti-HER2 antibody Trastuzumab failed to demonstrate efficacy (60-62). Targeting the non-receptor tyrosine kinase Src sheds light on another therapeutic avenue for osteosarcoma patients, as it is implicated in the development of lung metastases through its activation of pathways necessary for cell survival, migration, adhesion, and invasion. *In vitro*, inhibition of Src resulted in decreased metastatic potential, but this was not replicated *in vivo* (63), highlighting that multiple pathways may be involved in the development of osteosarcoma metastases. A clinical trial for patients with recurrent osteosarcoma localized to the lung is currently underway. It is investigating the utility of Sarcatanib in patients who have had a complete surgical resection of their tumor. Ewing sarcomas have recurrent abnormalities in cohesin complex genes, including *TP53* alterations, *CDKN2A* deletions, and *STAG2* mutations (44-46). Synthetic lethality between cohesin complex mutations and poly (ADP-ribose) polymerase (PARP) has been observed (64), thus prompting several trials investigating PARP inhibitors in combination with DNA-damaging agents in Ewing sarcoma.

**CNS Tumors**

Recent advances in CNS tumor sequencing are shifting the field from a focus on histopathologic features to genetic identifiers. Rapid translation of molecular discoveries into informed therapies has gone hand in hand. Recently, genomic profiling elucidated four subgroups, based on molecular drivers of oncogenesis: WNT, SHH (Sonic Hedgehog), group 3, and group 4. These subgroups have been incorporated into the most recent 2016 WHO classification, which, takes into account molecular markers in addition to pathologic features (65), redefining the framework in which CNS tumors were
previously diagnosed. The WNT group harbors somatic mutations in the \textit{CTNNB1} gene or, in more rare instances, germline mutations in the \textit{APC} tumor suppressor gene (66). These alterations upregulate the WNT pathway, resulting in tumorigenesis. No targeted therapies exist for these patients, but because their overall survival approaches 90% (67), future studies may examine therapy de-intensification. The SHH group contain aberrations in the SHH pathway, most commonly in \textit{PTCH1}, but also in \textit{SMO}, \textit{SUFU} and \textit{GLI2} (68). A phase I study of the FDA-approved SMO inhibitor Vismodegib demonstrated activity in SHH medulloblastoma (69) and may offer a molecular-guided therapy for this subset of patients. It is important to note that tumors with abnormalities in \textit{SUFU} or \textit{GLI2} are downstream of SMO and thus, resistant to Vismodegib (70). Unfortunately, less is known about the specific genetic drivers in group 3 and group 4. Group 3 tumors have the worst prognosis and a high frequency of copy number abnormalities. They often have high MYC expression and a subset is \textit{MYCN} amplified. Group 4 tumors also harbor copy number alterations, and often have amplifications in \textit{CDK6} and \textit{MYCN}. As described above, there are challenges to targeting MYC, but bromodomain inhibition has shown promise in preclinical models of medulloblastoma with \textit{MYCN} amplification (71). Palbociclib, a dual CDK4/CDK6 inhibitor is currently in Phase I testing.

Molecular features have also been integrated in the most recent WHO classification of posterior fossa and supratentorial ependymomas, given the wide histopathologic variation across tumors, which results in lack of concordance even amongst experts (65, 72). Posterior fossa ependymomas have been subdivided into two groups. Group A tumors lack recurrent genetic alterations, but have increased
methylation of CpG islands, resulting in transcriptional silencing of targets of polycomb repressive complex 2. *In vitro*, drugs targeting the polycomb repressive complex 2 and DNA demethylating agents impair proliferation of ependymoma cells (73). Group B tumors have multiple chromosomal aberrations, but none that are amenable to targeting (74). Supratentorial ependymomas generally harbor 2 classes of gene rearrangements. The majority contains a fusion of c11orf95 and RELA, while the remainder has fusions involving the transcriptional co-activator YAP1 (75). Unfortunately, no targeted therapies exist for these chimeric proteins. Because 75% percent of ependymomas express aberrant ERBB2 and ERBB4 signaling (76), lapatanib, an ERBB inhibitor, has been the subject of active clinical investigation (77). It has failed to demonstrate efficacy, but this may reflect the need for higher levels of lapatanib *in vivo* and thus, still holds promise.

Pediatric low grade gliomas are characterized by numerous mutations and copy number alterations, including somatic alterations in *BRAF*. The most common alterations are translocations in *BRAF*, which generate fusion proteins that results in loss of BRAF regulation and activation of the MAP kinase pathway. The *BRAFV600E* mutation, which leads to constitutive activation of BRAF, is also found in low grade gliomas. These findings have led to several clinical trials examining BRAF inhibitors in the V600E mutated patients. BRAF inhibitors are contraindicated in patients with BRAF fusions, as it results in feedback loop-mediated upregulation of the pathway and accelerated tumor growth. Accordingly, MAP kinase inhibitors are under investigation in this cohort of patients. As our understanding of the molecular oncogenesis of CNS
tumors continues to grow, we are likely to identify new therapeutic strategies to enhance our current management of these patients.

In light of these novel therapeutic avenues, the COG is developing the pediatric counterpart of the adult NCI-MATCH trial to enroll children and adolescents with relapsed/refractory solid tumors, CNS tumors and lymphomas (Figure 3). In addition to offering molecular-targeted therapy for these patients, the trial will also provide a rich source of genomic data for future discovery.

**Precision medicine in pediatric oncology: new horizons and future challenges**

In addition to suggesting novel therapeutic targets, gene discovery studies enhance other aspects of the precision medicine algorithm including cancer surveillance, assessment of inherited susceptibility to therapy-related toxicity, and treatment response monitoring. The Pediatric Cancer Genome Project identified 8.5% of children and adolescents with cancer had germline mutations in cancer-predisposition genes, in which family history failed to predict the presence of an underlying predisposition syndrome in most patients (78). The most commonly affected genes were TP53, APC, BRCA2, NF1, PMS2, RB1 and RUNXI. In addition to several previously well-described predisposition syndromes such as Li-Fraumeni syndrome, new associations have been observed between germline mutations of TP53, PMS2 and RET mutations with Ewing’s sarcoma, APC and SDHB mutations with neuroblastoma and a diverse range of mutations in APC, VHL, CDH1, PTCH1, and SDHA with leukemia (78). Knowledge of these inherited predispositions has major implications on direct patient care and on disease surveillance.
as well as genetic counseling for patients and their families. Furthermore, several genome-wide association studies have identified polymorphisms in genes that predict susceptibility to common chemotherapy-related complications, such as polymorphisms in TPMT (79) and NUDT15, which are associated with abnormal thiopurine metabolism resulting in severe myelosuppression (80), polymorphisms in GRIA1 associated with asparaginase allergy (81) while those in CPA2 with asparaginase-induced pancreatitis (82), variants in ACP1, BMP7 and PROX1-ASI that predispose to glucocorticoid-induced osteonecrosis (83, 84), polymorphisms in HAS3 predisposing to anthracycline cardiomyopathy (85), polymorphisms in CEP72 associated with vincristine neuropathy (86), and variants in SLCO1B1 involved in methotrexate clearance (87). Given that genetic testing for TMPT status to inform adjustments in mercaptopurine dosage has already become standard practice in ALL, one could envision the implementation of a tailored pharmacogenomic panel relevant to a specific chemotherapy regimen to individualize therapy based on genetic risk factors that predict host toxicities. Lastly, advances in the technologies to detect cancer cells have paved innovative ways to measure treatment response and ultimately predict risk of relapse. Childhood leukemia treatment response measured by minimal residual disease (MRD) has been identified as a powerful independent prognostic factor (88). It has traditionally been measured by flow cytometry or other molecular-based techniques. However, sensitive next-generation sequencing-based MRD monitoring are being tested prospectively in several pediatric oncology consortia for clinical relevance (89). While MRD measurement has revolutionized the management of leukemia patients, similar, ultra-sensitive biomarkers are essentially non-existent for our solid tumor patients. Recently, several adult studies
demonstrated that detection of circulating tumor DNA (ctDNA) in the plasma can predict relapse earlier than can be seen on standard imaging studies. Very few investigations of the utility of ctDNA in pediatric solid tumors exist, however preliminary results using droplet digital PCR and next-generation sequencing are encouraging (90-92). In parallel, several challenges emerge from the new era of precision medicine including intratumoral genomic heterogeneity that makes it difficult to distinguish driver from passager mutations, lack of sustained response resulting from the emergence of acquired drug-resistant mutations and escape pathways, and incorporation of genomic sequencing technologies into pragmatic, real-time, cost-effective clinical workflows for treatment decision making. In addition, new ethical dilemmas have arisen due to the incidental detection of inherited susceptibility to cancer predisposition syndromes. Genomics-driven targeted therapies will require a strong collaboration between basic scientists, molecular pathologists, bioinformaticians, and oncologists to develop robust clinical trials to address these issues in an effort to further improve patient survival.

**Conclusion**

Advances in cancer genomics have deepened our understanding of the disease biology, uncovered actionable genomic alterations and offered unique opportunities for precision medicine approaches. Several molecular targeted therapy trials are currently underway in the hopes of balancing survival and toxicity for these high-risk patient populations. However, this exciting era of tailored therapy also opens up new challenges. The next generation of translational trials will aim: 1) to determine the optimal combination between novel therapeutic agents and conventional cytotoxic drugs; 2) to
investigate the underlying mechanisms governing therapy resistance as the emergence of drug-resistant mutations and compensatory feedback pathways can arise; and 3) to harness international collaboration to design statistically powered trials for rare, high-risk, genomically-defined entities. Hence, precision medicine illustrates a novel treatment paradigm to improve outcomes for children with cancer in this modern era of multi-omics.


**Figure 1:** Recurrent cytogenetic and molecular abnormalities in pediatric hematologic malignancies. The frequency of different genomic alterations of pediatric B-lineage, T-lineage ALL and AML is depicted. Of note, the cumulative incidence does not add up to 100% since more than one alteration can occur concomitantly.

**Figure 2:** Relative frequency of genomic alterations in neuroblastoma at diagnosis compared to relapse

**Figure 3:** Pediatric MATCH trial schema
<table>
<thead>
<tr>
<th>Leukemia subtype</th>
<th>Common alterations</th>
<th>Precision medicine approach</th>
<th>Clinical trials</th>
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<tbody>
<tr>
<td>Ph+ALL</td>
<td>BCR-ABL1</td>
<td>Chemotherapy + Imatinib</td>
<td>NCT03007147 – not yet recruiting</td>
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<td>Ph-like ALL</td>
<td>ABL-class fusions (ABL1, ABL2, CSF1R, PDGFRB)</td>
<td>Chemotherapy + Dasatinib</td>
<td>NCT02883049 – recruiting</td>
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<td>CRLF2 rearrangements and other JAK-STAT pathway lesions (JAK2, EPOR rearrangements and/or IL7R, SH2B3 mutations)</td>
<td>Chemotherapy + Ruxolitinib</td>
<td>NCT02723994 – recruiting</td>
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<td>Infant ALL</td>
<td>KMT2A rearrangements associated with FLT3 overexpression and epigenetic dysregulation</td>
<td>Chemotherapy + Azacitidine</td>
<td>NCT02828358 – recruiting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemotherapy + Bortezomib + Vorinostat</td>
<td>NCT02553460 – recruiting</td>
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<tr>
<td>T-ALL</td>
<td>Activated NF-κB pathway</td>
<td>Chemotherapy + Bortezomib</td>
<td>NCT02112916 – recruiting</td>
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<tr>
<td>Relapsed ALL</td>
<td>PI3K/AKT/mTOR pathway alterations</td>
<td>Chemotherapy + Everolimus</td>
<td>NCT01523977 – recruiting</td>
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<td>Epigenetic dysregulation</td>
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<td>NCT01614197 – recruiting</td>
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<td></td>
<td>Relapsed AML with core-binding factor mutations</td>
<td>Chemotherapy + Dasatinib</td>
<td>NCT02680951 – recruiting</td>
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Table 2: Recurrent alterations in fusion-negative rhabdomyosarcomas are listed as copy number gains/losses or mutations with associated amino acid change when published. Of note, frequency reported is across fusion-negative and fusion-positive rhabdomyosarcomas. Data from Shukla et al. (93), Chen et al. (48), Shern et al. (47), and Kashi et al. (94).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>Frequency</th>
</tr>
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<td>FGFR4</td>
<td>Mutation V550L, N535K, G528C</td>
<td>6-9%</td>
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<tr>
<td>IGF1R</td>
<td>Copy number gain</td>
<td>&lt;1-2%</td>
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<td>IGF2</td>
<td>Mutation</td>
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</tr>
<tr>
<td>PDGFRA</td>
<td>Mutation</td>
<td>1.4%</td>
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<td>ERBB2</td>
<td>Mutation</td>
<td>1.4%</td>
</tr>
<tr>
<td>MET</td>
<td>Copy number gain</td>
<td>1%</td>
</tr>
<tr>
<td>BRAF</td>
<td>Mutation V600E</td>
<td>&lt;1-1%</td>
</tr>
<tr>
<td>NRAS</td>
<td>Mutation G12A, Q61K, Q61H</td>
<td>8-12%</td>
</tr>
<tr>
<td>KRAS</td>
<td>Mutation G12A, G12C, G12D, G13D</td>
<td>4-6%</td>
</tr>
<tr>
<td>HRAS</td>
<td>Mutation G13R, Q61K</td>
<td>3-4%</td>
</tr>
<tr>
<td>NF1</td>
<td>Mutation C1939_N1942FS, L18551, W784C</td>
<td>3.4%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>Mutation Q546, H1047R, E542K</td>
<td>5-6%</td>
</tr>
<tr>
<td>CCND1</td>
<td>Mutation</td>
<td>1%</td>
</tr>
<tr>
<td>CCND2</td>
<td>Mutation</td>
<td>1%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Copy number loss</td>
<td>2%</td>
</tr>
<tr>
<td>MDM2</td>
<td>Copy number gain</td>
<td>8%</td>
</tr>
<tr>
<td>TP53</td>
<td>Mutation A276D, C176F, P250L, L308V</td>
<td>3.5%</td>
</tr>
<tr>
<td>Gene</td>
<td>Mutation</td>
<td>Frequency</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>Mutation G34E, T41A, S45Y</td>
<td>2-3.3%</td>
</tr>
<tr>
<td>MYOD1</td>
<td>Mutation L122R</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>BCOR</td>
<td>Mutation Copy number loss</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>FBXW7</td>
<td>Mutation R387P, R441G, R367P</td>
<td>4.8%</td>
</tr>
</tbody>
</table>
Figure 1:

- **B-ALL**
  - Hyperdiploidy (25%)
  - ETV6-RUNX1 (25%)
  - IKZF1 deletions (15%)
  - CRLF2-rearranged (8%)
  - Ph-like kinase fusions (7%)
  - KMT2A-rearranged (6%)
  - TCF3-rearranged (4%)
  - DUX4-rearranged (4%)
  - ZN384-rearranged (4%)
  - BCR-ABL1 (3%)
  - MEF2D-rearranged (3%)
  - IAMP21 (2%)
  - Hypodiploidy (1%)

- **T-ALL**
  - NOTCH mutations (50%)
  - TAL1/LMO2 fusions (50%)
  - TLX3 fusions (20%)
  - TLX1 fusions (10%)
  - LYL1 fusions (10%)
  - ETP (10%)
  - KMT2A-rearranged (5%)
  - NUP214-ABL1 (5%)
  - PICALM-MLLT10 (5%)

- **AML**
  - KMT2A-rearranged (20%)
  - RAS mutations (20%)
  - RUNX1-RUNXIT1 (12%)
  - FLT3-ITD mutations (10%)
  - WT1 mutations (10%)
  - CBFβ-MYH11 (8%)
  - NPM1 mutations (8%)
  - PML-RARα (7%)
  - Monosomy 5 and 7 (6%)
  - KIT mutations (5%)
  - CEBPA mutations (5%)
  - NUP98 mutations (5%)
  - IDH1/IDH2 mutations (4%)
  - CSF3R mutations (2%)
  - CBFA2T3-GLIS2 (2%)
  - DEK-NUP214 (1%)
  - RBM15-MKL1 (1%)
Figure 2:

Diagnosis

- ALK
- Cell cycle
- Other/unknown
- RAS-MAPK

Relapse

- ALK
- Cell cycle
- Other/unknown
- RAS-MAPK
Relapsed/refractory solid tumor, CNS tumor, or lymphoma

Biopsy at time of relapse

Actionable alteration identified

Matching study agent available

- MEK inhibitor
- BRAF inhibitor
- PI3K/mTOR inhibitor
- TRK inhibitor
- FGFR inhibitor
- ALK inhibitor
- EZH2 inhibitor
- PARP inhibitor

Stable disease, partial response, complete response

Continue until progression

Progressive disease

Another actionable mutation identified

Yes

Off-study

No

Progressive disease

Yes

Off-study

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