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Pharmacogenomics in Pediatric Oncology Research and Treatment

Leo Kager, MD and William E. Evans, PharmD

ABBREVIATIONS 6-MP, 6-mercaptupurine; ABCC1, ATP binding cassette subfamily C member 1; ABCC4, ATP binding cassette subfamily C member 4; ADR,adverse drug reaction; ALL,acute lymphoblastic leukemia; CEP72, centrosomal protein 72; CIPN,chemotherapy-induced peripheral neuropathy; COG, Children's Oncology Group; CPIC,Clinical Pharmacogenetics Implementation Consortium; EFS, event-free survival; EURAMOS, European American Osteosarcoma Group; FPGS, folylpolyglutamate synthase; GWAS, genome-wide association study; G6PD, glucose-6-phospate dehydrogenase; HGOS, high-grade osteosarcoma; *KMT2A*-r, *KMT2A*-rearrangements; MAPPYACTS, MoleculAr Profiling for Pediatric and Young Adult Cancer Treatment Stratification; MATCH, Molecular Analysis for Therapy Choice; MTX, methotrexate; MTXPGs, MTX-polyglutamates; NUDT15, nudix hydrolase 5; PharmGKB, pharmacogenomics knowledge base; PGx, pharmacogenomics; PGx-genes,pharmacogenes; SJCRH, St. Jude Children's Research Hospital; SLC19A1, Solute carrier family 19 member 1; SNV, single nucleotide variant; TLS, tumor lysis syndrome; TPs, thiopurines; TPMT, thiopurine S-methyltransferase; VCR, vincristine; VIP, very important pharmacogene; VIPN, vincristine-induced peripheral neuropathy

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With the use of risk-adapted chemotherapy based treatments, outcomes for children and adolescents with hematological malignancies have significantly improved in industrialized countries. For example, in acute lymphoblastic leukemia (ALL), the most common pediatric cancer, with a peak incidence in preschool-children, long-term survival steadily improved in US Children's Oncology Group (COG) clinical trials; 499 patients treated from 1970–1972 had a survival rate at 10 years of ~20% versus >90% in 11,806 patients treated from 2010–2015.[1] Similar or better results have been achieved in major single-institution clinical trials including the Total Therapy protocols at St. Jude Children's Research Hospital (SJCRH).[2] Yet, even with a 90% cure rate, more children die of ALL than any other pediatric cancer, and less toxic treatment is needed to improve quality of life for those who are cured. Risk-adapted chemotherapies coupled with surgery and/or radiotherapy are also essential for treating children and adolescents with most malignant solid tumors. However, the improvement in outcomes is unequal across cancer types. For example, in patients with localized Ewing sarcoma, intensification of chemotherapy in COG trials resulted in improved 5-year event-free survival (EFS) of 87% in recent trials, whereas no such improvements were achieved in patients with localized osteosarcoma in the European American Osteosarcoma Group (EURAMOS-1) and other osteosarcoma clinical trials, with unacceptably low 5-year EFS of ~60%.[3]

Overall, outcome (i.e., survival, long-term morbidities, and quality of life) differences in pediatric cancer patients fueled the development of strategies to reduce toxicity, especially in patients with excellent prognoses, to enhance treatment efficacy or to identify novel therapeutic targets in patients whose cancers do not respond sufficiently to current medications, giving rise to "precision oncology" and "precision follow-up care" strategies.

Pharmacogenomics (PGx) has been defined as "the study of genomic technologies to enable the optimization of drug dose and choice in individual patients to maximize efficacy and to minimize toxicity, and to enable the discovery and development of novel drugs"[4]; and is an essential strategy in precision oncology, and can help to improve outcomes in children with cancer. Recent advances in genome, transcriptome, and epigenome interrogation as well as *in silico* analytical technologies provide a basis to better characterize cancers and pathways important for the pharmacokinetics and pharmacodynamics of anticancer medications, and allow essentially agnostic genome/epigenome wide investigations to identify pharmacologically relevant relationships between genomic variants and well-defined pharmacological endpoints.[5] In addition, single-cell multiomics technologies and methods offer

enormous potential to characterize cancer cell states and activities by integrating different single omics methods that profile the transcriptome, genome, epigenome, proteome or metabolome. For example, in childhood ALL, the application of these methods have helped to identify genetic-drivers and subclones that may confer either primary resistance, or develop drug resistance under the selective pressure on leukemia cells via commonly used medications (e.g., methotrexate [MTX], thiopurines [TPs], or glucocorticoids).[6,7] Such insights hold the promise to individualize and improve treatment of children with ALL.

Drug exposure at the target site is influenced by demographic (age, sex, weight), clinical (liver-, and kidney function, co-medications), dosing (formulation, route, regimen), genomic/epigenomic and other (adherence, food, etc.) factors.[8] In cancer, not only germ-line variants in relevant pharmacogenes (PGx-genes) play a role, but also somatic variants in target cells that influence the disposition and efficacy of anticancer medications. Researchers from the SJCRH recently discovered such complex interactions in the context of MTX treatment, an important component of ALL, non-Hodgkin lymphoma and osteosarcoma therapy. Lopez-Lopez et al[9] showed that *in vivo* accumulation of the pharmacologically active MTX metabolites—MTX-polyglutamates (MTX-PGs)—differs among major ALL subtypes, with T-lineage ALL and B-lineage ALLs, which carry *ETV6-RUNX1* or *TCF4-PBX1* fusion genes have low MTXPGs, and B-lineage hyperdiploid ALL and *BCR-ABL1*-like ALL have higher MTXPG accumulation.[9] Approximately 42% of these differences can be explained by MTX infusion time and by ALL subtype specific expression of genes (*SLC19A1*, *ABCC1*, *ABCC4*, and *FPGS*), which encode key proteins for cellular MTX transport (Solute carrier family 19 member 1, influx transporter; ATP binding cassette subfamily C member 1 and member 4, MTX efflux transporters) and MTXPG synthesis (folylpolyglutamate synthase).[9] Overall, such information can help to personalize MTX therapy in children with ALL.

In contrast to such complex interactions, some PGx-genes encode proteins that have a very strong impact on drug effects (so called "very important PGx-genes [VIPs]"). Exhaustive information on PGx-genes are provided at the "Pharmacogenomics Knowledge Base" (PharmGKB) website (http://www.pharmgkb.org), which reports for example genotypes, molecular, and clinical knowledge integrated into pathway representations and "VIP" summaries (https://www.pharmgkb.org/vips). An archetypal example of a VIP is glucose-6-phospate dehydrogenase (G6PD) deficiency, a potential cause of a severe pharmacologically predictable (type A), and therefore preventable adverse drug reaction (ADR).

One important strategy to improve outcomes in children treated for cancer is to avoid severe, potentially life-threatening ADRs. Tumor lysis syndrome (TLS) is the most common disease related emergency in hematological cancers, and hyperuricemia, an important contributor to TLS, can be prevented/treated via rasburicase, an enzyme that catalyses the cleavage of uric acid. Rasburicase administration is therefore a standard in supportive care in children with ALL at diagnosis and during initial induction therapy. However, in patients with G6PD deficiency (~5% of the world population are affected), treatment with rasburicase results in oxidative damage to erythrocytes that can lead to acute hemolytic anemia, and even fatalities occurred after rasburicase administration in ALL patients with G6PD deficiency. Pre-emptive testing before the start of rasburicase is essential; and evidence-based guidelines, including information on G6PD genetic and activity tests, as well as WHO classification (classes I–IV) of variants, are available at the "Clinical Pharmacogenetics Implementation Consortium (CPIC)" website (https://cpicpgx.org/guidelines/cpic-guideline-for-g6pd/).[10]

One other important example of an acute severe ADR in childhood ALL is dose dependent hematotoxicity after treatment with TPs in thiopurine S-methyltransferase (TPMT) or nudix hydrolase 5 (NUDT15) (both of which catalyze the inactivation of TPs) deficient patients, and dose adaption based on *TPMT* and *NUDT15* genotypes can ameliorate the ADR, without compromising therapeutic efficacy.[11] Details on TPMT and NUDT15 are available at the PharmGKB website (https://www.pharmgkb.org/vip/PA166169909, https://www.pharmgkb.org/gene/PA134963132). Evidence-based guidelines on *TPMT*/*NUDT15* guided pre-emptive TP dose adjustments, which is a classic example of precision oncology, are available at the CPIC website (https://cpicpgx.org/guidelines/guideline-for-thiopurines-and-tpmt/).

Treatment with vincristine (VCR), an important component in pediatric oncology (e.g., ALL, Ewing sarcoma, rhabdomyosarcoma, nephroblastoma), can be associated with severe acute and chronic vincristine-induced peripheral neuropathies (VIPN). The St. Jude lifetime cohort study (SJLIFE) identified chemotherapy-induced peripheral neuropathy (CIPN) among 21.9% of childhood cancer survivors who were, on average, 25 years from diagnosis (https://sjlife.stjude.org/). A promoter variant in *CEP72* (encoding centrosomal protein 72kD), creates a binding site for a transcriptional repressor, thereby reducing the expression of CEP72 mRNA. The *CEP72* rs924607 "TT" single nucleotide variant (SNV) was found to be significantly associated with CIPN in children and adults with ALL, and the risk for persistent motor CIPN was independently associated with the CEP72 genotype.[12] In addition, children with ALL and homozygous "TT" genotypes have leukemic cells more sensitive to VCR. Testing for the rs924607 "TT" variant in children with, and survivors of, childhood ALL may identify individuals at greatest risk for VIPN. Such information can be used for both, VCR dose adjustments based on genotypes during ALL therapy, and to inform targeted strategies like physiotherapy and

rehabilitation in ALL survivors, to prevent the development of permanent impairments later in life.[12] In the ongoing SJCRH Total Therapy XVII trial, which enrolls newly diagnosed children with ALL and lymphomas, patients with the *CEP72* rs924607 "TT" variant (~16% of patients) are randomly assigned between a decreased dosage (1.0 mg/m^2) and conventional dosage (1.5 mg/m^2) of VCR during the continuation phase of treatment (https://clinicaltrials.gov/study/NCT03117751), to ascertain whether VCR dose reduction in patients with high-risk CEP72 genotype reducing peripheral neuropathy without compromising treatment efficacy.

One important issue is how to best integrate PGx knowledge into daily clinical routine. The combination of sophisticated electronic health record systems and clinical decision support systems that include PGx information offers a strong possibility.[13] The "Clinical Implementation of PGx—PG4KDS protocol" at SJCRH provides a proof-of-principle on the feasibility of such an approach, and as of June 2020, PG4KDS pharmacogenetic test results were being used in the health records for nearly 6000 patients at SJCRH (https://www.stjude.org/treatment/clinical-trials/pg4kds-pharmaceutical-science.html).

Prognosis is still poor for children with refractory disease or disease recurrence. The range of disease recurrence, however, varies considerably among pediatric cancers; from 10% to 15% in ALL to ~40% in osteosarcoma. Identification of the mechanisms of drug failure in resistant cancer cell clones is underway, and insights from such studies can help to develop strategies to prevent disease recurrence and develop more-effective therapies. PGx research is also of increasing importance in drug development, and 63% of US Food and Drug Administration approved drugs in the past decade had supportive human genomic evidence.[14] Genomic profiling of samples obtained from children and adolescents with cancer at diagnosis, remission, and relapse have identified different pathways in which somatic mutations are enriched or exclusively present at relapses. For example, in children with later ALL relapses, relapse specific variants were found in genes that encode proteins that play a role in the development of resistance to glucocorticoids (*NR3C1/2*, *CREBBP*, and *WHSC1*), TPs (*NT5C2*, *MSH2/6*, *PMS2*, and *PRPS1/2*) and MTX (*FPGS*). Interestingly, leukemia cells from very early ALL relapses (<9 months from diagnosis) harbored different signatures and only a few of these variants, suggesting that these relapses arise from the outgrowth of a priori multidrug resistant subclones, which were not eliminated via conventional therapy. These primary multidrug resistance signatures were mainly observed in rare, poor-prognosis ALL subtypes like infant ALL, which carry *KMT2A*-rearrangements (*KMT2A*-R) and ALLs, which carry the *BCR-ABL1* fusion genes.[6] Different treatment strategies in these intrinsically resistant leukemias are necessary, and recently the use of the bispecific T-cell engager molecule blinatumomab, which targets CD19, resulted in exceptional improved 2-year disease-free survival (81.6% with blinatumomab + standard therapy versus 49.4% with standard therapy only) in infants with *KMT2A*-R ALL.[15]

One example of a childhood cancer, in which intensification of conventional chemotherapy failed to improve outcome is high-grade osteosarcoma (HGOS), and patients are still treated with surgery and a chemotherapy regimen (MTX, doxorubicin, and cisplatin) established 30 years ago. HGOS is genomically complex with widespread and recurrent somatic copy-number alterations and structural rearrangements. Alejandro Sweet-Cordero's group used patient-derived HGOS xenografts, and discovered a high degree of response for "genome-matched" therapies, thereby demonstrating the utility of a targeted genome-informed approach in such a genomically complex cancer.[16] These interesting results await clinical testing, but might be—if proven to be successful—a "light at the end of the tunnel" for patients with HGOS, whose tumors relapse or do not respond to conventional therapy.

Many efforts are underway to identify molecularly informed therapeutic targets in refractory or relapsed pediatric cancers. For example, the US Pediatric Molecular Analysis for Therapy Choice (MATCH, NCT03155620) and the European MoleculAr Profiling for Pediatric and Young Adult Cancer Treatment Stratification (MAPPYACTS, NCT02613962) trials have identified druggable targets in a subset of patients.[17,18] In the MAPPYACTS trial, ~100 patients subsequently received a matched targeted therapy, mainly within clinical trials, showing the direct benefit patients receive with such an approach. Of note, in children with extracranial tumors, most of the druggable alterations were also identified in the blood, paving the way for future liquid biopsy research.[18]

Besides the elucidation of cancer cells genomic landscapes, also "pharmacotyping" (i.e., *in vitro* drug screens like the "MTT drug-resistance assay" or "image-based single-cell functional precision medicine approach") is of increasing importance. For example, *in vitro* drug testing was performed in 805 children with newly diagnosed ALL treated at SJCRH. The results were integrated with the level of *in vivo* minimal residual disease during therapy and molecular subtype of leukemia cells.[19] Based on the "pharmacotypes," 6 prognostic patient clusters, including a subset of T-cell ALL with poor prognosis, were identified. Interestingly, the T-cell subset was sensitive to targeted therapies, highlighting opportunities for further treatment personalization in childhood ALL.[19]

In conclusion, PGx has a great potential to improve outcomes in children with numerous types of cancers. Moreover, PGx studies also pave the way towards reducing toxicity and "precision follow-up care" in survivors treated for pediatric cancer, to further

improve both cure rates and quality of life for children who are cured.

Article Information

Affiliations. Department of Pediatrics and Adolescent Medicine (LK), St. Anna Children's Hospital, Medical University Vienna, Austria; St. Anna Children's Cancer Research Institute (CCRI) (LK), Vienna, Austria; Department of Pharmaceutical Sciences (WEE), St. Jude Children's Research Hospital, Memphis, TN.

Correspondence. Leo Kager; leo.kager@stanna.at

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