Diagnostic Yield From a Nationwide Implementation of Precision Medicine for all Children With Cancer

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ABSTRACT

PURPOSE Several studies have indicated that broad genomic characterization of childhood cancer provides diagnostically and/or therapeutically relevant information in selected high-risk cases. However, the extent to which such characterization offers clinically actionable data in a prospective broadly inclusive setting remains largely unexplored.

METHODS We implemented prospective whole-genome sequencing (WGS) of tumor and germline, complemented by whole-transcriptome sequencing (RNA-Seq) for all children diagnosed with a primary or relapsed solid malignancy in Sweden. Multidisciplinary molecular tumor boards were set up to integrate genomic data in the clinical decision process along with a medicolegal framework enabling secondary use of sequencing data for research purposes.

RESULTS During the study's first 14 months, 118 solid tumors from 117 patients were subjected to WGS, with complementary RNA-Seq for fusion gene detection in 52 tumors. There was no significant geographic bias in patient enrollment, and the included tumor types reflected the annual national incidence of pediatric solid tumor types. Of the 112 tumors with somatic mutations, 106 (95%) exhibited alterations with a clear clinical correlation. In 46 of 118 tumors (39%), sequencing only corroborated histopathological diagnoses, while in 59 cases (50%), it contributed to additional subclassification or detection of prognostic markers. Potential treatment targets were found in 31 patients (26%), most commonly *ALK* mutations/fusions (n = 4), RAS/RAF/MEK/ERK pathway mutations (n = 14), *FGFR1* mutations/fusions (n = 5), *IDH1* mutations (n = 2), and *NTRK2* gene fusions (n = 2). In one patient, the tumor diagnosis was revised based on sequencing. Clinically relevant germline variants were detected in 8 of 94 patients (8.5%).

CONCLUSION Up-front, large-scale genomic characterization of pediatric solid malignancies provides diagnostically valuable data in the majority of patients also in a largely unselected cohort.

ACCOMPANYING CONTENT

Data Supplement

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BACKGROUND

Pediatric cancer is a group of rare diseases, with approximately 350 new cases diagnosed in Sweden every year. During the past 50 years, multimodal treatment strategies have increased the survival rates and about 85% of children diagnosed with cancer are now expected to survive 5 years or more after diagnosis.[1] Nevertheless, of the children responding to treatment, 25% are still at risk of relapse. In fact, cancer is the most common cause of death among children between 1 and 14 years in Sweden.[2] Also, the burden of treatment-related long-term complications in childhood cancer survivors is still very high.[3,4]

Solid tumors make up around two thirds of pediatric malignancies and encompass a diverse set of diagnostic entities,

CONTEXT

Key Objective

Several recent studies have shown that broad sequencing methods are helpful for diagnosing and deciding on treatment for pediatric patients with cancer. However, most previous studies of these methods' clinical impact have been conducted at large tertiary referral centers, focused on patients with relapsing and/or treatment refractory cancers. The present study evaluated the clinical benefits of nationwide, decentralized implementation of whole-genome sequencing of tumor and germline DNA from patients with childhood cancer, performed already at the time of diagnosis.

Knowledge Generated

Somatic mutations providing clinically relevant information were detected in a majority (112/118) of sequenced tumors. Around 10% of patients also exhibited clinically relevant germline mutations. Most of the found somatic or germline mutations were of diagnostic or prognostic relevance, while few impacted treatment.

Relevance

Upfront whole genome sequencing of tumor and germline provides relevant diagnostic and prognostic information for most children affected by a malignant solid tumor.

making histopathological classification challenging. The advancement of molecular methods during the past few decades has led to the discovery of diagnostic, prognostic, and treatment-predictive genetic markers. Recent sequencing studies have demonstrated that (1) pediatric tumors possess fewer point mutations and structural variants, (2) are typically defined by smaller number of driver mutations, (3) show a different landscape of genes involved in cancer development, and (4) are also more often related to germline cancer predisposing variants compared with adult cancer.[5,6]

During the past few years, several large-scale precision oncology programs have been initiated to investigate the potential of molecular-driven precision medicine and to assess the clinical benefit of targeted therapies in children with cancer.[7,8] The patient cohorts reported from these programs are primarily children with high-risk cancer and/or relapsed/refractory cancer, patients admitted to single highly specialized pediatric oncology centers, and/or those with ultrarare types of cancers.[9-18] These reports have suggested that pathogenic variants would be detected in at least 50% of patients when multiple sequencing platforms were combined.[11-14] The Zero Childhood Cancer Program analyzed 252 tumors from 247 high-risk pediatric patients with cancer using germline and tumor whole genome sequencing (WGS) and RNA-Seq, complemented with methylome analysis in CNS tumors.[19] In that study, 94% of patients exhibited at least one germline or somatic aberration, 71% showed potential therapeutic targets, and 5.2% had a change in diagnosis. In the Genomes for Kids (G4K) project, including WGS, whole-exome sequencing, and RNA-Seq data from 309 children from a single center with newly diagnosed or relapsed/refractory cancers, approximately 86% of patients harbored diagnostic, prognostic, therapeutically relevant, and/or cancer predisposing variants.[15] Two very recent studies (from the INFORM Registry and the iTHER Program) have confirmed a high yield of druggable mutations in large cohorts enriched for relapsed/treatment refractory pediatric patients with cancer, leading to a choice of targeted therapy for a small but significant minority of patients.[17,18] In addition, the recently reported multicenter MAPPYACTS study found at least one genetic alteration leading to a targeted treatment suggestion in 69% of patients whose tumors were subjected to whole-exome sequencing, with 10% of these alterations considered ready targets for routine use.[20]

In contrast to these studies, enriched for high-risk patients from highly specialized single centers, Trotman et al[16] demonstrated the clinical benefits to individual patients when implementing WGS in the routine diagnostics for pediatric cancer in general as it refined (2/36) or changed (4/36) the diagnoses and revealed new therapeutic opportunities (8 of 36 patients) in a small patient cohort. However, unselected pediatric patients with newly diagnosed solid tumors are being underrepresented in the published literature. To our knowledge, few if any examples of a nationwide approach have been reported. Here, we present the first data from a prospective study (Genomic Medicine Sweden Childhood Cancer),[21] offering tumor and germline WGS and RNA-Seq to all children diagnosed with a solid malignancy in an entire country irrespective of diagnosis or risk group.

METHODS

Study Design, Patients, and Workflow

Between January 1, 2021, and March 1, 2022, pediatric patients (age <18 years) diagnosed with a primary or relapsed solid malignancy were offered to participate in the study (Data Supplement [Fig 1]). The study was approved as a multicenter study by the Swedish Ethical Review Authority. Written informed consent was obtained for all included study

subjects, directly collected from the patients age ≥15 years, parents, or from legal guardians for the younger patients. Inclusion criteria for the study were collection of informed consent, verification of at least 40% viable tumor cells in the parallel sample used for routine diagnostics, and that enough material was secured for sequencing analysis. Local logistics were adapted to current regional and local conditions while maintaining a common workflow (Fig 1). Regional multidisciplinary molecular tumor boards (MTBs) were set up to integrate genomic data results in the clinical decision process. Following MTB, somatic WGS and RNA-Seq findings of clinical value were reported as an addendum to the pathology report at each center. Significant germline variants were reported via the affiliated clinical genetics departments, and the affected families were offered genetic counseling. In addition to routine histopathology, CNS tumors were also subjected to classification by DNA methylation. DNA methylation has recently been evaluated on a national scale in Sweden[22] and was therefore considered routine diagnostics excluded from the present evaluation.

Sequencing and Data Analysis

DNA and RNA from the fresh tumor samples and matched normal blood samples were prepared according to standard methods. Libraries for WGS were prepared with TruSeq DNA PCR-Free library preparation according to the manufacturer's instruction. Samples were sequenced using the S4 flow cell on NovaSeq 6000 (Illumina, CA), aiming for a mean read depth of minimum 90× for tumor DNA and 30× for germline DNA. To detect somatic variants, we implemented a workflow where tumor and germline DNA are analyzed in parallel. Variants were filtered against a gene list containing 1,696 cancer driver genes, including cancer census genes (Cosmic v90) and genes commonly altered in pediatric tumors, as identified in previous studies (Data Supplement [Table 1A]).[5,6,23,24] In specific cases, all nonsynonymous exonic somatic variants were also reviewed manually. Libraries for RNA-Seq were prepared with TruSeq Stranded mRNA or Illumina Stranded mRNA Prep kit (Illumina, CA) and sequenced using the S4 std reagent kit on NovaSeq 6000 (Illumina), aiming for a coverage of 100 M r-p. Germline variants were filtered against a virtual panel of 50 actionable childhood cancer predisposition genes (Data Supplement [Table 1B]). Further details including germline analyses are provided in Supplementary Methods.

Ethics and Data Deposition for Secondary Use

The study was approved by the Swedish Ethical Review Authority (Permit Nos. 2020-03827 and 2021-05916-02). The generated raw, and analyzed, data from the WGS and RNA-Seq as well as methylation array analysis are deposited at the Swedish Childhood Tumor Biobank. These data are available for secondary use in research projects on pediatric cancer after application to the Swedish Childhood Tumor Biobank. The data access procedure involves a scientific and medicolegal evaluation by a nationally appointed committee.

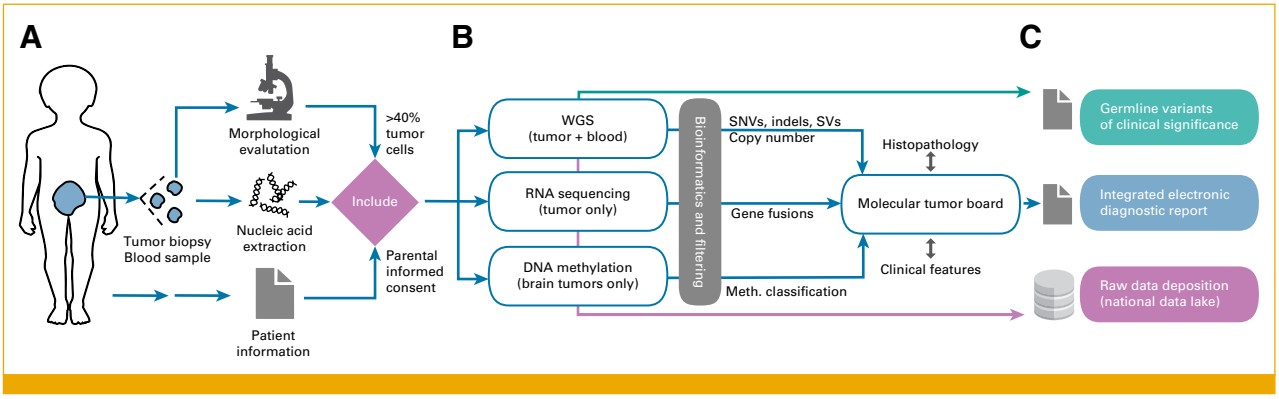

FIG 1. Workflow for whole-genome profiling of pediatric cancer. (A) Concomitant to diagnostic biopsy or tumor resection, a fresh tumor sample for molecular characterization and a peripheral blood sample were acquired. (B) DNA was extracted from tumor and peripheral blood leukocytes while RNA was extracted from tumor alone. Patients were included after informed consent and verification of at least 40% viable tumor cells in the parallel sample used for routine diagnostics. Tumor DNA was subjected to WGS (118 tumors) and DNA methylation analysis (CNS tumors only) while tumor RNA-Seq was performed in cases with sufficient material (52 tumors). (C) WGS data underwent bioinformatic analyses after which somatic SNVs and small insertions and deletions (indels) were filtered against each patient's constitutional (germline) variants and a panel of genes with known pathogenic importance in childhood cancer (Data Supplement [Table 1A]). SVs and copy number profiles were extracted in parallel while RNA-Seq data were used for fusion gene capture. For CNS tumors, DNA methylation analysis was performed as part of routine diagnostics (detailed data not included in the present study). For each tumor, genetic findings were assessed in relation to preliminary histopathological diagnosis and clinical features at a molecular tumor board, after which a final report was issued, integrating histopathological findings and molecular profiles. Germline DNA was also screened for mutations in established childhood cancer predisposition genes and actionable targets that might affect treatment. Raw data from sequencing and methylation profiles are planned for deposition in a national data lake under authority of the Swedish Childhood Tumor Biobank. SNV, single-nucleotide variants; SV, structural variants; WGS, whole-genome sequencing.

The controlled access to these research data is described in the informed consent forms signed by all study subjects and/or legal guardians.

RESULTS

During 14 months, a total of 118 solid tumors from 117 pediatric patients (age <18 years) were included for WGS. Informed consent was obtained for an additional 32 patients, but these had to be excluded because of inferior quality of fresh tumor samples. Tumors excluded due to inferior quality consisted primarily of lymphomas (n = 10), sarcomas (n = 6), and CNS tumors (n = 6; Data Supplement [Fig 1]). Seven tumors were relapses, while the remaining were primary tumors. From one patient, both primary tumor and relapse (tumor IDs 29 and 43) were analyzed by WGS within the given time frame. The average coverage for WGS of tumor DNA was 116×. Complementary RNA-Seq for fusion gene detection was performed for 52 tumors. A summary of the findings in each tumor is presented in the Data Supplement ([Table 2A]).

Geographic Distribution

While sequencing and bioinformatic analyses were centralized to three geographic nodes, all Sweden's six pediatric oncology centers contributed to patient enrollment (range, 5-31 patients/center). Differences in the number of enrolled patients among centers were well in proportion to the known geographic distribution of childhood cancer cases among Sweden's health care regions, with <10% difference between the proportion of sequenced tumors per center compared with the expected proportion for each center on the basis of annual patient burden (Data Supplement [Table 2B]).

Histopathological Distribution

Well in concordance with the annual incidence of pediatric solid tumors in Sweden, the most common entities among the included 117 patients were tumors of the CNS (49%), sympatho-adrenal tumors (12%), soft tissue sarcomas (10%), bone sarcomas (8%), and renal tumors (7%; Data Supplement [Table 3]). Lymphomas/histiocytoses were the only major diagnostic group where the proportion of included cases (five cases, 4%) differed more than 10% from the expected proportion (17%) on the basis of the annual population incidence.[1] The main reason for this was that some relatively frequent entities in this group, particularly Hodgkin lymphoma and Langerhans cell histiocytosis, have characteristically low fractions of malignant cells in diagnostic samples, which led to exclusion on the basis of insufficient (<40%) tumor cell fraction.

Types of Reported Somatic Mutations

Somatic mutations were detected in 112 of 118 tumors (95%). Of these, 106 tumors (90%) showed clinically significant genetic changes, that is, variants having a clear association to a specific diagnostic/prognostic subgroup or treatment response according to the scientific literature (Data Supplement [Tables 1A and 2A]).[25] Somatic copy number aberrations of clinical significance were reported in 98 of 118 tumors (83%), while clinically significant single-nucleotide variants and small insertions/deletions were detected in 47 of 118 tumors (40%). Of tumors subjected to RNA-Seq, pathogenic fusion transcripts were detected in 23 of 52 tumors (44%); in an additional 17 tumors, gene fusions were detected by WGS in the absence of RNA-Seq data. Thus, 40 of 118 tumors (34%) exhibited gene fusions of clinical significance. All fusion genes detected by conventional diagnostic methods, such as targeted fluorescent in situ hybridization (FISH) analysis or reverse transcriptase polymerase chain reaction (RT-PCR), were also detected by RNA-Seq or WGS. The clinical information value of WGS and RNA-Seq combined is presented in Figure 2.

Diagnosis Revised or Corroborated by Sequencing Data

For one tumor, sequencing led to a revised diagnosis. This was a TRK-positive spindle cell tumor tentatively classified as infantile fibrosarcoma, where RNA-Seq and WGS revealed an *SS18::SSX1* fusion mandating a revised diagnosis of synovial sarcoma (Fig 3). Of the tumor genomic profiles, 40% (47/118) served solely to corroborate histopathological diagnosis. For example, in a kidney tumor (tumor ID 9), histologically consistent with a clear cell sarcoma (Figs 4A-4D), WGS revealed an internal tandem duplication in *BCOR* confirming the diagnosis.[26] As another example, a cerebral tumor histologically consistent with ependymoma (tumor ID 24; Figs 4E-4G) was tentatively classified by DNA methylation profile as a tumor belonging to the *MN1::PATZ1* CNS tumor subgroup. Here, WGS demonstrated copy number aberrations in chromosome 22 involving *MN1* and *PATZ1* (Figs 4H-4J), while RNA-Seq confirmed an *MN1::PATZ1* fusion.[27]

Preliminary Diagnosis Refined on the Basis of Sequencing Data

Of the tumor genomic profiles, 50% (59/118) contributed to refining diagnosis by additional diagnostic subclassification through the detection of prognostic markers. For example, one patient (tumor ID 19) was diagnosed with a paratesticular tumor histologically consistent with rhabdomyosarcoma (RMS). The subtype, by clinical presentation and histology, was most consistent with spindle cell/sclerosing RMS. However, there were foci observed with high-grade pleomorphism, not typical of this diagnosis and possibly suggesting anaplastic RMS (Figs 5A-5D). WGS indeed revealed a complex copy number profile with *MDM2* amplification and a frame-shift deletion in *BCOR* (Figs 5E and 5F). Neither mutation in *MYOD1* nor *FOXO1* rearrangements were detected with WGS and RNA-Seq,

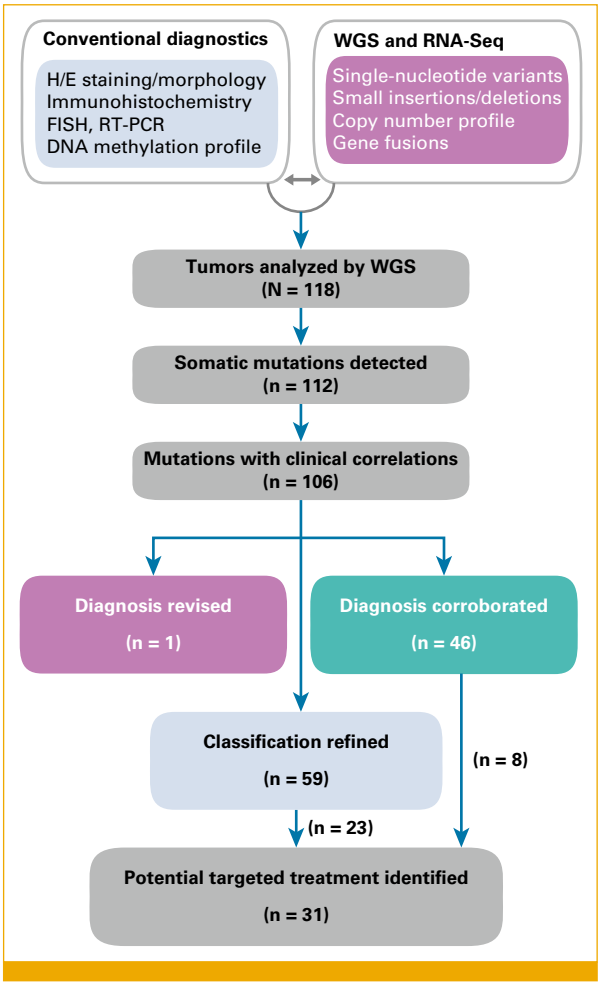

FIG 2. Clinical relevance of sequencing data. Information from RNA and DNA sequencing was compared with data from routine diagnostics, including already well-established molecular adjunctive methods, such as RT-PCR, FISH, NGS panel, and DNA methylation profiling (CNS tumors only). Of 118 sequenced tumors, 108 showed mutations with known clinical correlations, mandating revision of a preliminary diagnosis in one case (Fig 3), and corroborating diagnosis in the remaining cases. Further subclassification was possible based on sequencing data in 60 of 107 cases of which 29 exhibited mutations in genes that are targetable in clinical trials. The number of tumors in each category is denoted by n; 32 patients were excluded from analyses because of technically inferior biopsy material (Data Supplement [Fig 1]). FISH, fluorescence in situ hybridization; RT-PCR, reverse transcriptase polymerase chain reaction; WGS, whole-genome sequencing.

respectively, arguing against spindle cell RMS and alveolar RMS, respectively. The genetic profile was thus most consistent with *FOXO1* fusion-negative embryonal RMS. In fact, the high grade of complexity and focal pleomorphism argued for a diagnosis of anaplastic embryonal RMS.

Another example where the diagnosis was refined by genetic analysis was a pretreated tumor in the CNS (tumor ID 23). Histologically, the tumor consisted of a teratoma with a regional component of RMS (Figs 5G and 5H). WGS revealed hyperdiploidy, a 17p deletion and loss of heterozygosity for chromosomes 11 and 13 (Fig 5I). No *FOXO1* gene fusion could be detected, arguing against alveolar RMS. These findings were consistent with an embryonal RMS in a preexisting teratoma.

Identification of Potential Treatment Targets

Potential treatment targets (OnkoKB Level 1) or indirect markers for targeted treatment were found in 32 tumors from 31 patients (26%; Data Supplement [Table 4]), including *ALK* mutations/fusions (n = 4), mutations in the RAS/RAF/MEK/ERK pathway (n = 11), *ERBB2* amplification (n = 1), *FGFR1* mutations/fusions (n = 5), *IDH1* mutations (n = 2), a *KIT* mutation (n = 1), and *NTRK2* gene fusions (n = 2). All patients were discussed at MTBs. In total, four patients received treatment on the basis of WGS findings (Data Supplement [Table 2A]), including one relapsing epithelioid glioblastoma with *TRIM24::NTRK2* fusion receiving larotrectinib (tumor IDs 29 and 43), two *ALK*-mutated/amplified neuroblastomas receiving lorlatinib (tumor IDs 45 and 102), and an *EWSR1::KLF15* fusion sarcoma receiving pazopanib (tumor ID 53).

An example of how treatment targets could be identified in highly rearranged genomes was a neuroblastoma (tumor ID 102) where WGS revealed structural aberrations of multiple regions in chromosome arm 2p including *MYCN* and *ALK*; here, the *ALK* amplification was part of complex structural variations including rearrangement with the *DUSP3* untranslated region in 17q21, part of one of several segmental gains in 17q (Figs 6A-6F). The detection of *ALK* amplification made the patient eligible for ALK inhibitor treatment.

Another example where WGS provided clues to treatment, albeit indirectly, was an intramuscular tumor (tumor ID 53) metastasizing to regional lymph node and lungs. Histopathology showed a primitive tumor with rhabdomyogenic differentiation, while WGS revealed an *EWSR1::KLF15* fusion. These findings were at variance with previous reports describing this fusion in tumors with myoepitheliomatous or Ewing-like phenotype (Figs 6G-6K).[28-30] Initial chemotherapy according to the Cooperative Weichteilsarkom Study (CWS) for stage IV tumors (CEVAIE) was ineffective. On the basis of a previous report of a metastatic *EWSR1::KLF15* positive tumor responding to a multityrosine kinase inhibitor,[28] chemotherapy was discontinued and pazopanib monotherapy was initiated.

Actionable Germline Variants

Totally, 94 patients could be subjected to germline analysis after informed consent. In eight patients (8.5%), germline variants in childhood cancer predisposition genes were detected. Of these, seven were discovered from the analysis of 50 genes deeemed actionable (Data Supplement [Table 1B]),

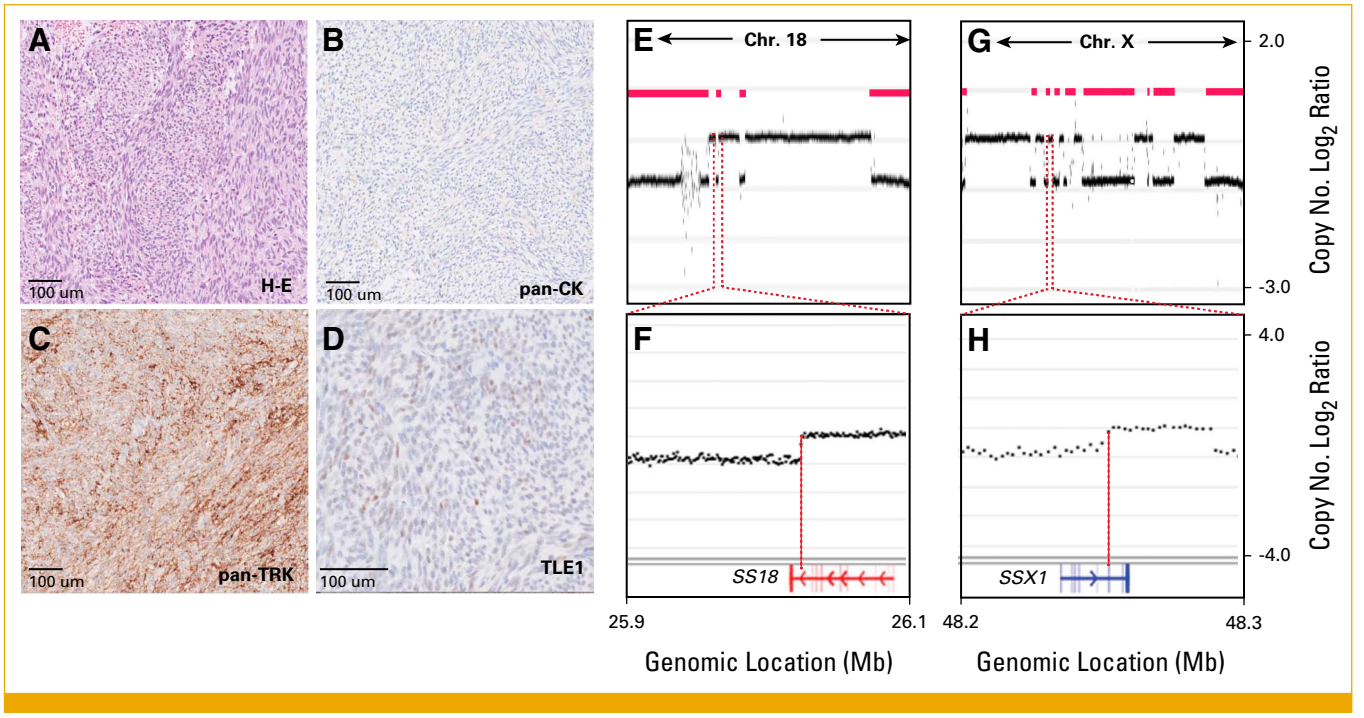

FIG 3. Diagnosis revised based on sequencing data. Histological features by (A) H-E staining of a tumor located at the cervical spine (tumor ID 7). The tumor consists of fascicles of monotonous spindle cells with a herring-bone growth pattern in the absence of epithelial features. (B-D) Pan-CK staining was negative, and TLE1 positivity was limited to occasional nuclei, while antibody for neurotrophic tyrosine receptor kinases 1-3 (pan-TRK) showed strong diffuse positivity, arguing against synovial sarcoma and leading to a preliminary diagnosis of infantile fibrosarcoma. (E-H) Copy number calling on the basis of WGS showed complex patterns of deletions in chromosomes 18 and X, also including breakpoints in the *SS18* and *SSX1* genes leading to a corresponding *SS18::SSX1* fusion at the RNA level. (E and G) Horizontal red lines demarcate deleted chromosomal segments while broken lines denote areas magnified from whole chromosomes view (F and H) to show single gene view. HE, hematoxylin-eosin; Pan-CK, Pan cytokeratin; WGS, whole-genome sequencing.

including *NF1* (n = 2), *PMS2* (n = 1), *RB1* (n = 1), *TP53* (n = 2), and *WT1* (n = 1); three of these mutations were known from previous investigations. One additional patient was known from previous studies to have xeroderma pigmentosum before cancer diagnosis due to a homozygous variant in *ERCC5*. As an example of direct utility of germline analysis, a patient (tumor ID 35) with a colorectal carcinoma at age 15 years was found to carry a homozygous germline variant in *PMS2*. This finding, together with a hypermutation profile at somatic mutation analysis, enabled a diagnosis of constitutional mismatch repair deficiency and made the patient eligible for immunotherapy and surveillance.[31,32] All patients with detected germline variants and their close family members were offered genetic counseling.

DISCUSSION

We describe a nationwide systematic implementation of WGS for precision diagnostics of children diagnosed with a primary or relapsed solid malignancy in Sweden. Our results from the first 118 tumors indicate that large-scale genomic characterization of pediatric cancer in general provides highly relevant information from a clinical perspective, especially by contributing to additional subclassification (50% of patients) and identifying drug targets (26%). This is well in line with previous results from a recent similar, but smaller and regional, cohort of largely unselected patients.[16] Furthermore, germline WGS analysis detected pathogenic or likely pathogenic variants in 8.5% of patients. This is consistent with previous reports where pathogenic germline variants in known cancer predisposition genes have been detected in up to 10% of children.[6,33,34] Our strategy of performing WGS already at presentation has the benefit of providing support to the initial diagnostic process by adding valuable data on somatic mutations. On the other hand, if relapse or progression occurs, it will be necessary to resequence the tumor to account for changes in the genetic landscape due to clonal evolution, adding financial costs.

An advantage of performing WGS compared with targeted sequencing is that it allows for the detection of copy number alterations and structural variants, which are frequently driver events in pediatric cancers.[5,6,9] We found copy number alterations that correlated with clinical subgroups in 83% (98/118) of analyzed tumors, underscoring the value of having a reliable bioinformatic pipeline for producing whole-genome profiles of copy number and allelic balance

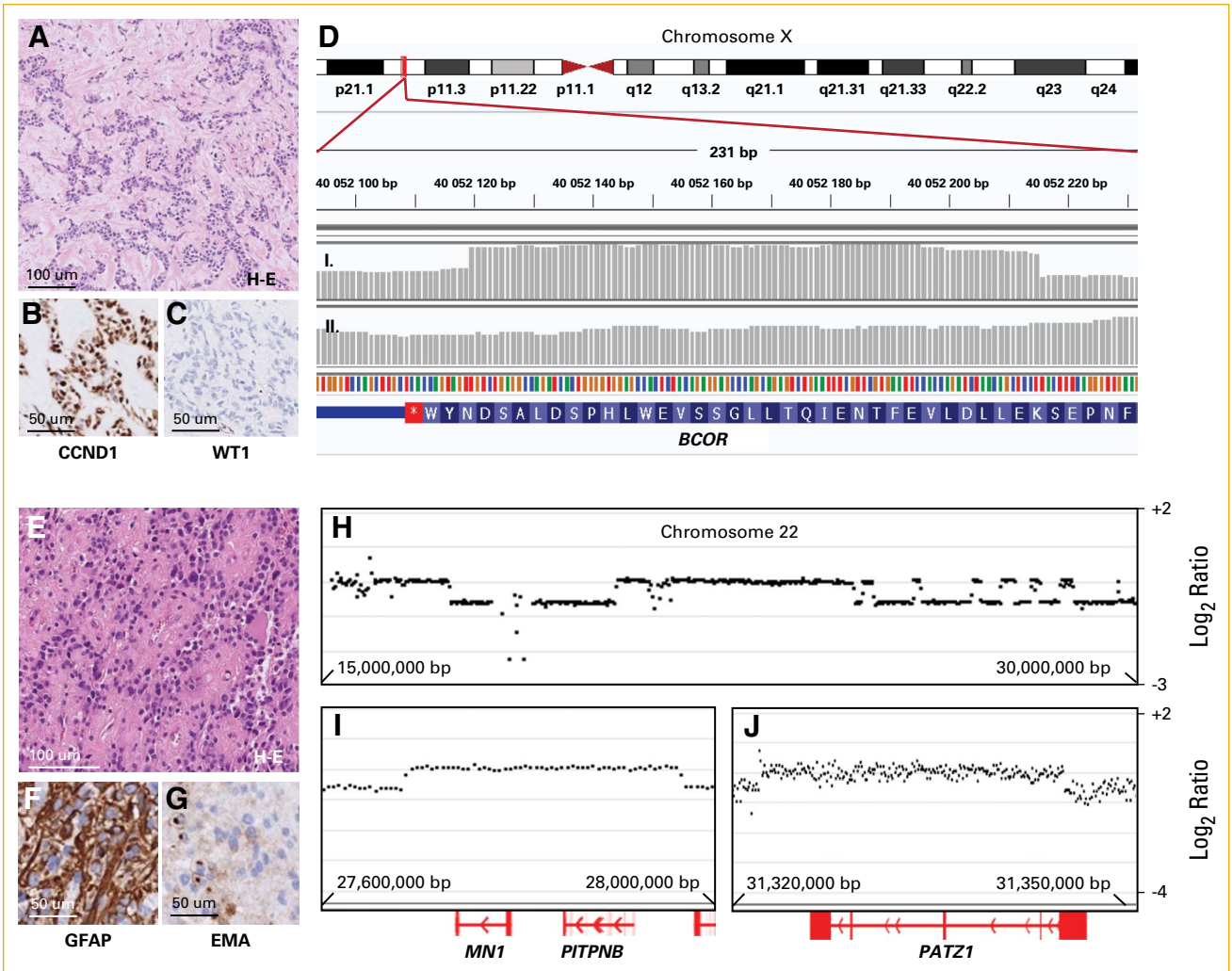

FIG 4. Diagnosis corroborated by sequencing data. Histological features by (A) H-E staining of a kidney tumor with clinical suspicion of Wilms tumor (tumor ID 9). H-E staining of tumor sections shows nests and cords of plump tumor cells, often laying in a reticular growth pattern against a sclerosing background. The cell borders were indistinct, and the nuclei showed fine chromatin without prominent nucleoli. (B) Cyclin D1 was expressed in tumor cells while (C) WT1 was absent, suggestive of CCSK. WGS demonstrated a somatic 105 bp in-frame insertion/internal tandem duplication in exon 15 (NM_001123385.1) of (D) the *BCOR* gene, (I) in the tumor sample, and (II) the lack of insertion in the corresponding germline sample. The insertion was supported by 87 of 107 generated sequencing reads (VAF 81%). The *BCOR* internal duplication corroborates a diagnosis of CCSK. (E) Histology of a cerebral tumor located near sulcus centralis (tumor ID 24) shows mildly pleomorphic cells and multiple perivascular pseudorosettes. (F) Immunohistochemistry for GFAP showed strong positivity and (G) EMA a dot-like cytoplasmic expression. The findings were suggestive of ependymoma, but DNA methylation classification suggested the tumor to be an *MN1::PATZ1* fusion positive CNS tumor. WGS revealed a complex pattern of deletions (H-J) in chromosome 22 leaving a normal copy number of *NM1* but with a breakpoint in the 5′region of *PATZ1*. RNA-Seq (data not shown) confirmed the presence of an in-frame *MN1::PATZ1* fusion. CCSK, clear cell sarcoma of the kidney; EMA, epithelial membrane protein; H-E, hematoxylin-eosin; WGS, whole-genome sequencing.

(B-allele frequency). Complementary RNA-Seq for fusion gene detection was performed in 52 tumors, selected based on the availability of material and access to the method at the sequencing site. The role of RNA-Seq was primarily to corroborate gene fusion candidates found by WGS. In absence of RNA-Seq data, corroboration was done by FISH or RT-PCR. A previous paper by Mody et al[14] also supported the use of RNA-Seq for pediatric cancer cases due to the clinical benefit especially in leukemia and sarcomas. However, in the present study, tumors of the CNS dominated the group where pathogenic fusion transcripts were found by RNA-Seq (12/23) or by WGS in the absence of RNA-Seq data (7/17); in total, gene fusions were found in 33% of the CNS tumors (19/58).

Focusing on the 31 tumors where we could detect potential treatment targets, the majority were CNS tumors (n = 14) or neuroblastoma/ganglioneuroblastoma (n = 3), most commonly with *ALK* mutations/fusions (n = 4) and RAS/RAF/MEK/ERK pathway mutations (n = 13). Besides detecting mutations directly linked to a dysfunctional druggable protein target, our approach was also useful to indicate indirect markers

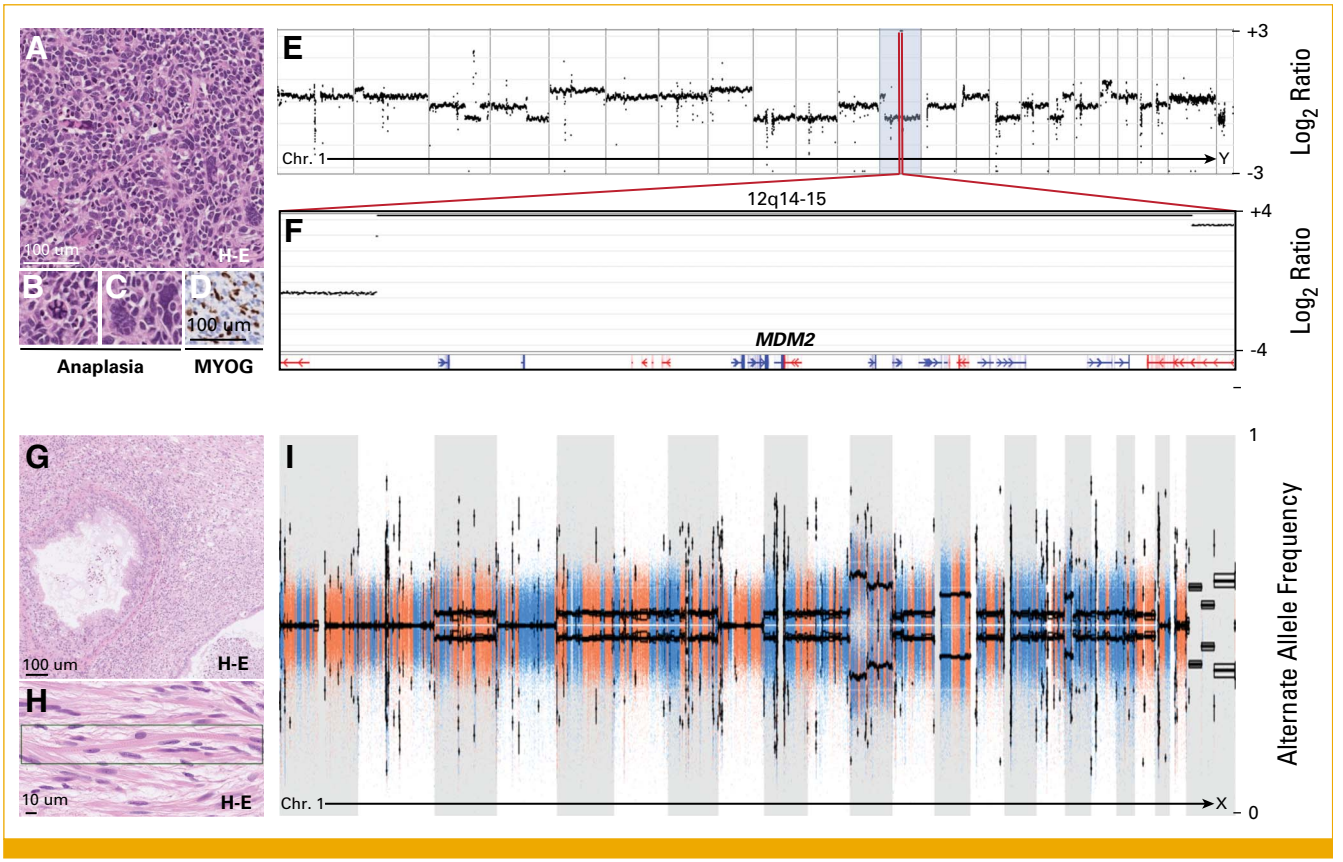

FIG 5. Diagnosis refined by sequencing data. (A) H-E staining of a paratesticular tumor (tumor ID 19). The tumor showed alternating hypercellular and hypocellular areas of spindle cells with a storiform growth pattern with a hyalinizing matrix; (B and C) there were areas with anaplastic features and atypical mitotic figures indicative of anaplasia. Tumor cells were positive for (D) desmin and myogenin (MYOG). The findings raised the suspicion of RMS with anaplasia; this subtype was supported by a copy number profile with aneuploidy and (E) multiple segmental chromosome aberrations, (F) including amplification of *MDM2*. Histological features by (G) H-E staining of a tumor located in the pineal gland (tumor ID 23). The tumor was dominated by a mix of bland stromal and glandular components, indicative of a teratoma. However, there was also a component of variable mature myogenic cells with (H) cross striations suggesting a rhabdomyoma or RMS. Alternate allele frequency plot on the basis of (I) WGS showing a hyperdiploid profile with few segmental changes in the absence of *PAX3/7::FKHR* fusions or myogenin mutation, most consistent with a component of ERMS in a preexisting teratoma. ERMS, embryonal RMS; H-E, hematoxylin-eosin; WGS, whole-genome sequencing; RMS, rhabdomyosarcoma.

for molecularly targeted therapy, such as immunotherapy. The therapeutic utility of detected potential treatment targets is limited today because few ongoing clinical trials of targeted drugs focus on children. Also, survival after standard therapy is overall very high, making the incentive for applying targeted drugs less pressing for children with cancer up-front; in the present study, only four patients were given treatment on the basis of the molecular findings during the study period. However, the standard treatment protocols, often consisting of multiagent chemotherapy combined with radiotherapy, come at the risk of lifelong side effects for many patients. Thus, there is a strong rationale in the end for gradually incorporating targeted drugs into first-line treatment protocols. Several recent studies have reported larger patient cohorts with inclusion of targeted treatment choices and survival data.[17,18] The present study is still in an early phase, having been ongoing for only 2 years. This fact prevents analysis of the benefits from precision oncology treatments at the present stage as survival time would be far too short for valid results. On the other hand, our study has the strength of demonstrating the diagnostic yield in a relatively unbiased pediatric cancer cohort, not enriching for high-risk/relapsed/treatment refractory patients as many of the other studies of precision oncology for children, where such patients usually make up the vast majority.[17,18] In the present study, relapsed patients were a minority (7/117) as expected when not selected for at inclusion.

A limitation of the present study is that it covers only the early pilot phase of the project, including the period when the system was initiated at the six participating centers. Of the approximately 260 children estimated to have presented with a solid malignancy in Sweden during the study period, only 149 were included (57%). Furthermore, the fact that clinical grade WGS and RNA-Seq still requires fresh tissue with a high tumor cell content, resulted in additional dropout as fresh material was too sparse or of insufficient quality for analysis in 21% of

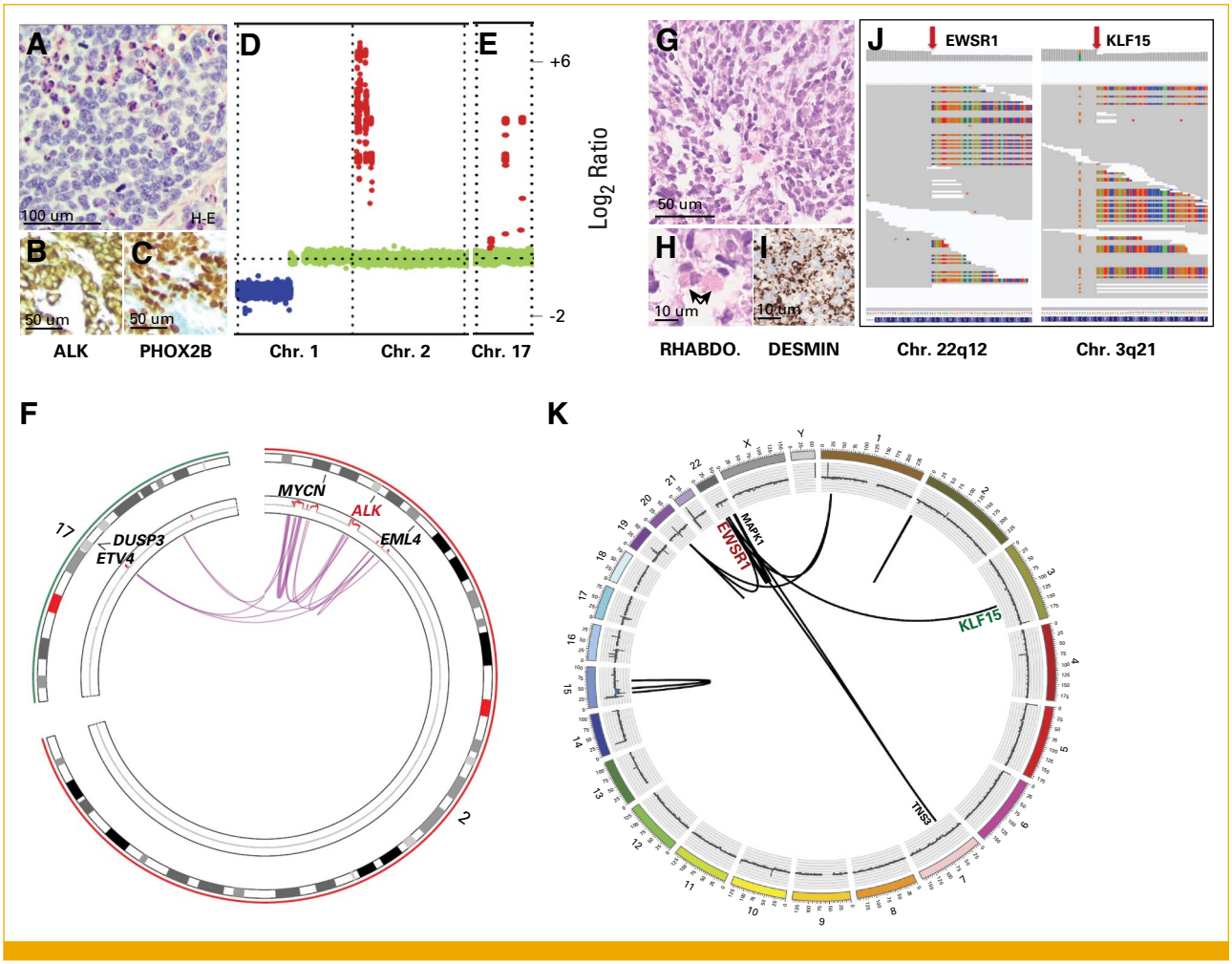

FIG 6. Direct and indirect identification of drug targets. (A) H-E staining of a small round blue cell tumor (tumor ID 102), with salt and pepper chromatin and rich presence of mitotic and apoptotic cells. (B) Tumor cells expressed ALK and (C) PHOX2B proteins, confirming a suspicion of neuroblastoma. Copy number calling from WGS data revealed deletion in (D) 1p and complex amplicons in 2p as well as (E) in 17q. (F) WGS also showed complex structural variants involving multiple genes in 2p and 17q, including *ALK*, indicating sensitivity for ALK inhibitors. Morphology of a neoplasm (tumor ID 53) in upper extremity (G) showing a primitive high-grade small-cell tumor with scattered cells with more excentric nuclei and granular cytoplasm suggestive of (H) rhabdomyosarcomatous differentiation (RHABD; arrows), supported by (I) immunoreactivity for desmin and Myf4 (not shown). (J) WGS analysis identified several structural rearrangements, one resulting in an *EWSR1::KLF15* fusion. Structural variant calling from (J) WGS data revealed a small deletion in 22q12.2 and 3q21.3, respectively, resulting in breakpoints (red arrows) within the coding regions of the *EWSR1* and *KLF15* genes, visualized as spanning split reads in the IGV(hg38). (K) Circos plot depicts all structural variants present in the tumor sample highlighting the rearrangement between *EWSR1* in 22q (red) and *KLF15* in 3q (green). RNA-Seq (data not shown) confirmed the presence of an in-frame *EWSR1::KLF15* fusion. H-E, hematoxylin-eosin; IGV, Integrative Genomics Viewer; WGS, whole-genome sequencing.

enrolled patients (Data Supplement [Fig 1]). This particularly affected lymphomas, of which many subtypes have low tumor cell content. Because the present cohort consisted of patients with a wide spectrum of different tumor types but a relatively small number of each tumor type, further statistical analysis could not be performed on diagnostic subgroups. However, the Genomic Medicine Sweden Childhood Cancer project is ongoing, with a steadily increasing inclusion rate and now also includes childhood leukemias. This will allow future health economic analyses including identification of which diagnostic groups that benefit the most from whole-genome profiling in clinical routine.

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REFERENCES

1. Swedish Childhood Cancer Registry Annual Report, Barncancerregistret, 2019. https://sbcr.se
2. Swedish Cause of Death Registry, National Board of Social Affairs and Health, 2021. https://www.socialstyrelsen.se/statistik-och-data/register/dodsorsaksregistret/
3. Barncancerrapporten. Stockholm, Sweden, Swedish Childhood Cancer Fund, 2019
4. Armstrong GT, Chen Y, Yasui Y, et al: Reduction in late mortality among 5-year survivors of childhood cancer. N Engl J Med 374:833-842, 2016
5. Ma X, Liu Y, Liu Y, et al: Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours. Nature 555:371-376, 2018
6. Gröbner SN, Worst BC, Weischenfeldt J, et al: The landscape of genomic alterations across childhood cancers. Nature 555:321-327, 2018
7. Sturm D, Capper D, Andreiuolo F, et al: Multiomic neuropathology improves diagnostic accuracy in pediatric neuro-oncology. Nat Med 29:917-926, 2023
8. Church AJ, Corson LB, Kao PC, et al: Molecular profiling identifies targeted therapy opportunities in pediatric solid cancer. Nat Med 28:1581-1589, 2022
9. Langenberg KPS, Looze EJ, Molenaar JJ: The landscape of pediatric precision oncology: Program design, actionable alterations, and clinical trial development. Cancers (Basel) 13:4324, 2021
10. Worst BC, van Tilburg CM, Balasubramanian GP, et al: Next-generation personalised medicine for high-risk paediatric cancer patients—The INFORM pilot study. Eur J Cancer 65:91-101, 2016
11. Chang W, Brohl AS, Patidar R, et al: MultiDimensional ClinOmics for precision therapy of children and adolescent young adults with relapsed and refractory cancer: A report from the Center for Cancer Research. Clin Cancer Res 22:3810-3820, 2016
12. Harttrampf AC, Lacroix L, Deloger M, et al: Molecular screening for cancer treatment optimization (MOSCATO-01) in pediatric patients: A single-institutional prospective molecular stratification trial. Clin Cancer Res 23:6101-6112, 2017
13. Khater F, Vairy S, Langlois S, et al: Molecular profiling of hard-to-treat childhood and adolescent cancers. JAMA Netw Open 2:e192906, 2019
14. Mody RJ, Wu YM, Lonigro RJ, et al: Integrative clinical sequencing in the management of refractory or relapsed cancer in youth. JAMA 314:913-925, 2015
15. Newman S, Nakitandwe J, Kesserwan CA, et al: Genomes for Kids: The scope of pathogenic mutations in pediatric cancer revealed by comprehensive DNA and RNA sequencing. Cancer Discov 11:3008-3027, 2021
16. Trotman J, Armstrong R, Firth H, et al: The NHS England 100,000 Genomes Project: Feasibility and utility of centralised genome sequencing for children with cancer. Br J Cancer 127:137-144, 2022
17. van Tilburg CM, Pfaff E, Pajtler KW, et al: The Pediatric Precision Oncology INFORM Registry: Clinical outcome and benefit for patients with very high-evidence targets. Cancer Discov 11:2764-2779, 2021
18. Langenberg KPS, Meister MT, Bakhuizen JJ, et al: Implementation of paediatric precision oncology into clinical practice: The Individualized Therapies for Children with cancer program 'iTHER. Eur J Cancer 175:311-325, 2022
19. Wong M, Mayoh C, Lau LMS, et al: Whole genome, transcriptome and methylome profiling enhances actionable target discovery in high-risk pediatric cancer. Nat Med 26:1742-1753, 2020
20. Berlanga P, Pierron G, Lacroix L, et al: The European MAPPYACTS Trial: Precision medicine program in pediatric and adolescent patients with recurrent malignancies. Cancer Discov 12:1266-1281, 2022
21. Fioretos T, Wirta V, Cavelier L, et al: Implementing precision medicine in a regionally organized healthcare system in Sweden. Nat Med 28:1980-1982, 2022
22. Schepke E, Lofgren M, Pietsch T, et al: DNA methylation profiling improves routine diagnosis of paediatric central nervous system tumours: A prospective population-based study. Neuropathol Appl Neurobiol 48:e12838, 2022
23. Mackay A, Burford A, Carvalho D, et al: Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. Cancer Cell 32:520-537.e5, 2017
24. Northcott PA, Buchhalter I, Morrissy AS, et al: The whole-genome landscape of medulloblastoma subtypes. Nature 547:311-317, 2017
25. Heim S, Mitelman F: Cancer Cytogenetics: Chromosomal and Molecular Genetic Aberrations of Tumor Cells (ed 4). Chichester, West Sussex; Hoboken, NJ, Wiley Blackwell, 2015
26. Aldera AP, Pillay K: Clear cell sarcoma of the kidney. Arch Pathol Lab Med 144:119-123, 2020
27. Alhalabi KT, Stichel D, Sievers P, et al: PATZ1 fusions define a novel molecularly distinct neuroepithelial tumor entity with a broad histological spectrum. Acta Neuropathol 142:841-857, 2021
28. Stevens TM, Qarmali M, Morlote D, et al: Malignant ewing-like neoplasm with an EWSR1-KLF15 fusion: At the crossroads of a myoepithelial carcinoma and a Ewing-like sarcoma. A case report with treatment options. Int J Surg Pathol 26:440-447, 2018
29. Bodis S, Kroiss S, Tchinda J, et al: Myoepithelial carcinoma of soft tissue with an EWSR1-KLF15 gene fusion in an infant. Pediatr Dev Pathol 24:371-377, 2021
30. Cajaiba MM, Jennings LJ, Rohan SM, et al: Expanding the spectrum of renal tumors in children: Primary renal myoepithelial carcinomas with a novel EWSR1-KLF15 fusion. Am J Surg Pathol 40: 386-394, 2016
31. Le DT, Uram JN, Wang H, et al: PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 372:2509-2520, 2015
32. Durno C, Ercan AB, Bianchi V, et al: Survival benefit for individuals with constitutional mismatch repair deficiency undergoing surveillance. J Clin Oncol 39:2779-2790, 2021
33. von Stedingk K, Stjernfelt KJ, Kvist A, et al: Prevalence of germline pathogenic variants in 22 cancer susceptibility genes in Swedish pediatric cancer patients. Sci Rep 11:5307, 2021
34. Kratz CP, Jongmans MC, Cave H, et al: Predisposition to cancer in children and adolescents. Lancet Child Adolesc Health 5:142-154, 2021