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Genomic Profiling of Pediatric Solid Tumors With a Dual DNA/RNA Panel: JCCG-TOP2 Study

Kayoko Tao[1,2,3] | Takako Yoshioka[4] | Miho Kato[5] | Kazuyuki Komatsu[6] | Shinichi Tsujimoto[7] | Kenichi Sakamoto[8] | Kazuki Tanimura[1,9] | Minako Sugiyama[1,10] | Masahiro Sekiguchi[11] | Yoshiko Nakano[11,12] | Yoshihiro Otani[13] | Yasushi Yatabe[14] | Akihiko Yoshida[14] | Hajime Okita[15] | Junko Hirato[16] | Kenichi Kohashi[17] | Yukichi Tanaka[18] | Shinji Kohsaka[3] | Takashi Kubo[2,19] | Kuniko Sunami[19] | Makoto Hirata[12] | Shuichi Tsutsumi[20] | Hiroyuki Aburatani[20] | Katsuyoshi Koh[21] | Masahiro Hirayama[22] | Shuhei Karakawa[23] | Yukayo Terashita[10] | Hiroyuki Fujisaki[9] | Takeshi Yagi[24] | Akihiro Yoneda[25,26] | Shinji Mochizuki[27] | Hiroyuki Shichino[27] | Tatsuya Suzuki[28] | Tetsuya Takimoto[5] | Koichi Ichimura[29,30] | Chitose Ogawa[1] | Kimikazu Matsumoto[31] | Hitoshi Ichikawa[2,32] | Motohiro Kato[11,31]

Correspondence: Hitoshi Ichikawa (hichikaw@ncc.go.jp) | Motohiro Kato (katom-tky@g.ecc.u-tokyo.ac.jp)

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ABSTRACT

To develop an optimized genomic medicine platform for pediatric cancers, a nationwide cancer genome profiling project was conducted from January 2022 to February 2023 in collaboration with the Japan Children's Cancer Group. This prospective observational study analyzed matched blood and FFPE tumor samples from patients aged 0–29 years with solid tumors. Genomic analysis used the TOP2 hybrid capture–enrichment system, targeting 737 and 455 genes in the DNA and RNA panels, along with allele-specific genome copy number alterations. A total of 210 patients from 50 institutions were enrolled across Japan (median age, 8 years; range, 0–25). Of these, 154 (77%) were enrolled at diagnosis or during/after initial treatment and 56 (27%) at disease progression or relapse. The TOP2 findings had great benefits in clarifying the diagnosis of pediatric solid tumors. Among the 204 patients with genomic results, 147 (72%) had potentially actionable findings, including diagnostic, prognostic, and therapeutic findings in 111 (54%), 61 (30%), and 64 (31%), respectively. Oncogenic fusions were noted in 45 (23%) patients. A copy number alteration was identified in at least one genomic region in 170 (83%) patients. Two patients exhibited a high tumor mutation burden. Seventeen (8%) patients harbored a germline pathogenic/likely pathogenic variant in cancer-predisposing genes. This study highlighted the feasibility of implementing a nationwide precision medicine platform and the clinical utility of the TOP2 system for pediatric cancers. The results support the integration of genomic data into the standard clinical care of pediatric patients with cancer, both at diagnosis and at relapse.

1 | Introduction

Annually, approximately 3000 children and adolescents aged < 20 years are diagnosed with cancer in Japan [1]. Pediatric cancer comprises a broad and heterogeneous range of rare cancers with distinct pathogenesis and molecular characteristics. Inherent diagnostic complexities and limited therapeutic options often compromise patient outcomes, for which comprehensive genomic profiling (CGP) holds immense potential to overcome diagnostic and therapeutic challenges.

Abbreviations: CGM, cancer genomic medicine; CGP, comprehensive genomic profiling; FFPE, formalin-fixed paraffin-embedded; JCCG, Japan Children's Cancer Group; MTB, molecular tumor board; PAF, potentially actionable findings; TOP, Todai OncoPanel.

For affiliations refer to page 12.

Cancer genomic medicine (CGM) was officially commenced in Japan in June 2019, with two CGP tests, namely, FoundationOne CDx and OncoGuide NCC Oncopanel System, approved for patients with (1) advanced and/or refractory cancer or (2) cancer lacking established standard therapy. These CGP tests were primarily designed to identify therapeutic targets in common adult cancers, warranting the development of a genome profiling system that is more optimal for precision medicine in pediatric cancer. Identifying genomic abnormalities to support diagnosis and predict prognosis is critical in pediatric oncology. Recognizing the unique characteristics of the pediatric cancer genome, a gene panel that can detect genome copy number alterations and structural aberrations such as gene fusion is desirable for this purpose. Given the prevalence of underlying genetic factors, screening germline variants in cancer-predisposing genes is also relevant [2].

To address the aforementioned needs, the Todai OncoPanel (TOP), a dual hybrid capture-based DNA/RNA panel, was developed to enable target enrichment from formalin-fixed paraffin-embedded (FFPE) specimens [3]. The TOP2, a further expanded panel, incorporates the pediatric cancer-relevant genes selected by a working team of pediatric cancer experts and has received national health coverage in Japan as of August 1st, 2023 (GenMineTOP Cancer Genome Profiling System).

Previous studies have demonstrated that the first-generation TOP system provides high analytical performance and clinical utility in adult cancers, with 120 of 198 cases (61%) yielding diagnostic or therapeutic insights [3, 4]. The Japan Children's Cancer Group (JCCG)-TOP2 study is a nationwide pediatric cancer genome profiling study assessing the feasibility of genomic testing in pediatric cancers—from sample collection to result reporting—while also gathering demographic and clinical data critical for interpretation. The study also evaluates the clinical utility of the TOP2 system. This report summarizes the advances in precision medicine achieved through the project, using data from 210 patients ranging from infants to young adults with diverse tumor types and disease stages, including newly diagnosed, progressive, and relapsed cases.

2 | Materials and Methods

2.1 | Study Design and Ethics

The JCCG-TOP2 is a multicenter prospective observational study that aimed to perform genomic screening in pediatric patients with cancer. The study was managed by the study office at the National Center for Child Health and Development (NCCHD) and the National Cancer Center (NCC) and was conducted following the principles of the Declaration of Helsinki. The study was approved by the central institutional review board (IRB) of the NCCHD (Approval no. 2021-167). Written informed consent was obtained from the patients, parents, or legal representatives, with assent from minors as appropriate. Germline findings were disclosed to the treating oncologist and the patient's family in accordance with the informed consent obtained at study enrollment. The consent form included an option for participants to indicate whether they wished to receive information about germline findings. Genetic counseling was offered to all families in whom a cancer predisposition syndrome was suspected or a pathogenic/likely pathogenic germline variant was identified.

2.2 | Patients and Samples

Eligible patients were individuals aged 0–29 years with histologically confirmed or suspected solid tumors. Patients who had undergone allogeneic stem cell transplantation or had tumors that developed from transplanted organs were excluded. A minimum of 20 unstained slides with 5 μm thickness and tumor content of ≥ 20%, along with 2 mL of peripheral blood, were collected for sequencing analysis. Patient samples were sent to the NCCHD from participating institutions, where the tumor content was re-evaluated, and tumor sections were centrally reviewed as part of the JCCG Observational Study of Childhood and Young Adult Solid Tumors. The overall workflow is shown in Figure S1.

2.3 | TOP2 Genomic Profiling

The tumor and peripheral blood samples were sent to the Konica Minolta REALM Inc. (Tokyo, Japan) and processed for sequencing in its quality-certified laboratory. The details on library preparation and targeted sequencing have been previously described [3, 4]. The TOP2 system evaluates (1) tumor mutation burdens (TMBs), (2) single nucleotide variants (SNVs), short insertions/deletions, and gene amplifications in 737 genes, (3) allele-specific genome copy number alterations, (4) fusion genes in 455 genes, and (5) exon skipping in five genes. Germline variants in 40 cancer-predisposing genes were reported according to the American College of Medical Genetics and Genomics [5] and the Kosugi group [6] guidelines. The transcripts per million values were reported for 27 transcribed genes. Genes in the TOP2 DNA and RNA panels are listed in Table S1. In this paper, the results reflect the genomic findings from the standard genomic report.

2.4 | Clinical Information and Pathology Findings

Clinical information and pathology findings of each patient were collected from the participating institution using the Research Electronic Data Capture (REDCap) system [7, 8] hosted at the Department of Data Management, NCCHD.

2.5 | Definition and Interpretation of Clinically Relevant Alterations

In the JCCG-TOP2 study, the clinical evidence level was defined in the categories of (1) diagnosis (Dx), (2) prognosis (Px), and (3) therapeutics (Tx) (Table S2). Clinical evidence levels were assigned to each genetic alteration, following the Association for Molecular Pathology/American Society of Clinical Oncology/College of American Pathologists guidelines [9] and the American College of Medical Genetics and Genomics and the Cancer Genomics Consortium consensus recommendation, in the categories of diagnosis and prognosis. For the treatment recommendation, the evidence-level criteria as per the Japanese Society of Medical Oncology, the Japanese Society of Clinical

Oncology, and the Japanese Cancer Association were used [10]. To evaluate genomic alterations, a two-step review process, involving the molecular tumor board (MTB) and expert panel (EP), was implemented (Figure S1). The weekly MTB, comprised of a multidisciplinary team, reviewed the genomic results, integrated clinical information and histological findings, and drafted genomic summary reports. The EP further reviewed genomic findings, where the attending physicians participated in the discussion from the participating institutions, and the final genomic summary reports were issued for both physicians and patients. Potentially actionable findings (PAF) were defined as genomic alterations with evidence levels corresponding to Dx2 or higher, Px2 or higher, and TxD or higher.

3 | Results

3.1 | Patient Characteristics

The JCCG-TOP2 study was conducted between January 2022 and February 2023, enrolling a total of 210 patients (male 111, female 99) from the 50 participating institutions across Japan (Figure S2). These institutions include designated core hospitals ($n=6$), designated hospitals ($n=13$), and cooperative hospitals ($n=27$), which are classified by the Ministry of Health, Labor and Welfare on the basis of their expertise in CGM [11, 12], and unassigned hospitals ($n=4$). Of the 210 enrolled patients, 205 provided both tumor and peripheral blood samples, and genomic data from 204 patients were analyzed. The patient characteristics and study consort diagram are shown in Table 1 and Figure 1, respectively. The median age at enrollment was 8 (range, 0–25) years. Moreover, 34 (16%) patients were enrolled at the initial diagnosis, 120 (57%) during the initial treatment, and 56 (27%) at disease progression or relapse.

TABLE 1 | Patient characteristics.

Enrolled patients ($n=210$)	
Male:Female	111:99
Age at diagnosis	Median 7 years (range 0–23 years)
Age at enrollment	Median 8 years (range 0–25 years)
Timing of study enrollment	
• At the initial diagnosis	34 (16%)
• During the first-line treatment	83 (40%)
• After the first-line treatment	37 (18%)
• At disease progression or relapse	56 (27%)
Patients whose samples were evaluable ($n=204$)	
Tissue biopsy sites	
• Primary site tumors	165 (81%)
• Metastatic site tumors	39 (19%)
Timing of tumor sample obtained	
• At the initial diagnosis	139 (68%)
• During or after the first-line treatment	26 (13%)
• At disease progression or relapse	39 (19%)

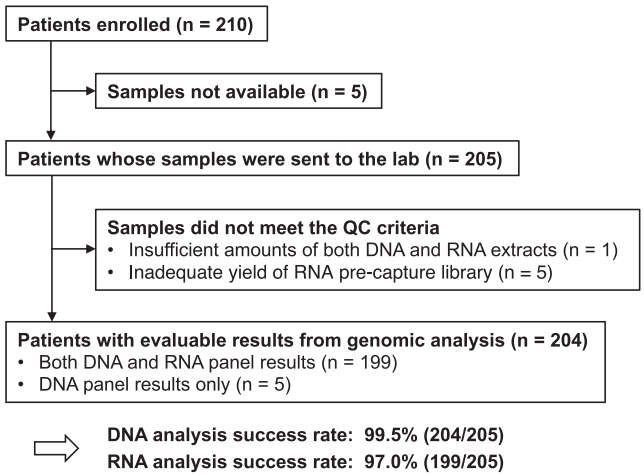

FIGURE 1 | JCCG-TOP2 study consort diagram. The figure shows the consort diagram of the 210 patients enrolled in the JCCG-TOP2 study. The letter "n" indicates the number of patients.

3.2 | Sample Quality Assessment

The median tumor content of 205 tumor samples evaluated at the central site was 70% (range, 20%–90%). Macrodissection was performed in 29 tumors, in which the tumor region was marked at the local and central sites in 23 and 6 tumors, respectively. In three tumors, the tumor region was remarked at the central site. The median ΔΔCt of the extracted tumor DNA was 3.4 (range, 0.4–6.2), and the median DV200 of the extracted tumor RNA was 84.2% (range, 28.1%–90.7%). The median storage period of the FFPE blocks was 82.5 days (range, 0–1041). More extended storage periods positively correlated with the ΔΔCT values of the tumor-derived DNA but negatively correlated with the DV200 value of the tumor-derived RNA and pre-capture tumor DNA/RNA library yield (Figure S3). Of the 205 samples, 6 failed to pass QC evaluation because of the insufficient quantity of the extracted DNA and RNA ($n=1$) and inadequate pre-capture RNA library yield ($n=5$) (Figure 1). Of the 205 patients, 204 (99.5%) and 199 (97.0%) had evaluable genomic data from DNA and RNA analyses, respectively. In 204 samples, the mean target coverage of the tumor DNA was 1198 reads, with an average of 99.9% of the targeted exons with coverage ≥100 reads, and the mean target coverage of peripheral blood DNA was 704 reads. The median turnaround time from the sample submission to issuing the genomic report was 57.5 (range, 41–139) days (Figure S4).

3.3 | Tumor Category and Diagnosis

The tumor categories and types in 204 analyzed patients are shown in Figure 2A. The details of their diagnosis are provided in Table S3. The major tumor categories included bone and soft

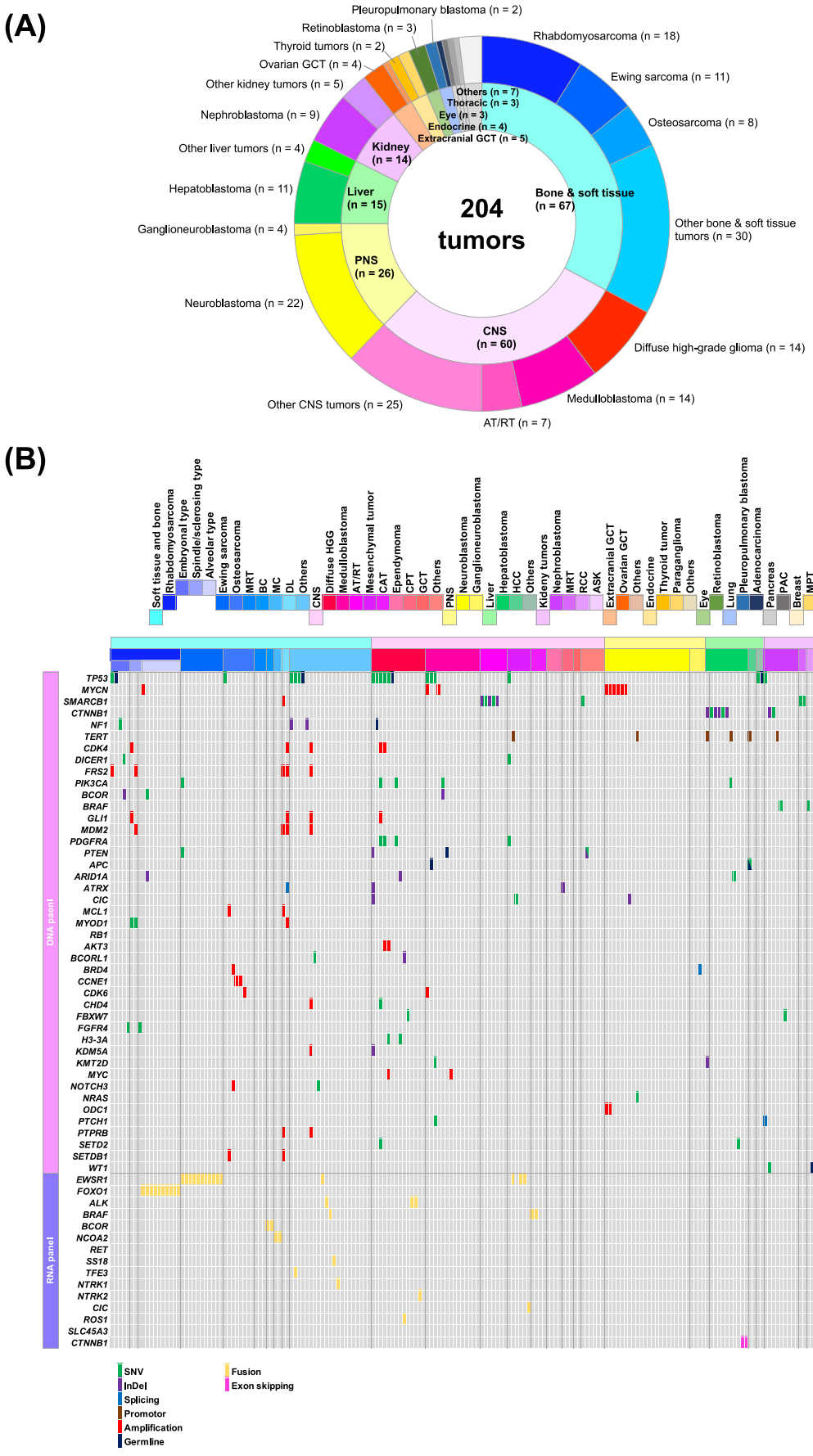

FIGURE 2 | Legend on next page.

FIGURE 2 | Tumor types and genomic alterations in 204 patients analyzed. (A) The pie chart shows the tumor categories (inner circle) and types (outer circle) of 204 analyzed patients. (B) The oncoplot includes genes altered in two or more patients by DNA analysis and fusion genes or exon skipping, which were detected in one or more patients by RNA analysis. The top bar shows the tumor categories and types in each patient. The number on the right side of the oncoplot indicates the frequency of gene alterations. AT/RT, atypical teratoid rhabdoid tumor; CNS, central nervous system; GCT, germ cell tumor; HGG, high-grade glioma; PNS, peripheral nervous system; SNV, single nucleotide variant. The letter "n" indicates the number of patients.

tissue tumors ($n=67$, 33%), central nervous system (CNS) tumors ($n=60$, 29%), peripheral nervous system (PNS) tumors ($n=26$, 13%), liver tumors ($n=15$, 7%), and kidney tumors ($n=14$, 7%).

3.4 | Genomic Alterations Identified With the TOP2 System

Figure 2B summarizes the representative genomic alterations identified in 204 patients, and all detected alterations, along with their evidence level and relevant molecularly targeted agents, are presented in Table S4. The DNA panel analysis of 204 samples detected SNVs, small insertions/deletions, and gene amplifications in 122 (60%), 57 (28%), and 30 (15%) patients, respectively. The most frequently altered genes included *TP53, MYCN, SMARCB1, CTNNB1*, and *NF1*. Copy number aberrations of whole chromosomes, chromosome arms, or segments were found in 170 (83%) patients; characteristic cases are shown in Figure 3. The RNA panel analyses of 199 samples detected fusion genes in 44 (22%) patients, of which 12 were therapeutically targetable fusions (Figure 4 and Table S5). Exon-skipping events were found in 2 (1%) patients (Figure 4). Based on TMB values available for 204 patients, the median tumor TMB was 0.5 (range, 0–30.5) mutations per megabase (Mb). Two (1%) patients with high-grade glioma (HGG) had a high TMB of ≥ 10 mutations per Mb: one had a germline mutation in a mismatch repair (MMR) gene *MLH1*, whereas the other had a somatic mutation in MMR genes *MSH2* and *MSH6*. Tumor samples from nine patients were exposed to temozolomide treatment; however, no significant difference was found in the TMB value when compared with patients not exposed to temozolomide (Mann–Whitney *U* test, two-tailed, $p=0.571$) (Figure S5).

In bone and soft tissue tumors ($n=67$) (Figures S6–S9), rhabdomyosarcoma (RMS) ($n=18$), Ewing sarcoma (EWS) ($n=11$), and osteosarcoma ($n=8$) were major tumor types. Disease-defining fusions were prevalent across these tumors (31/67, 46%) (Table S5). In RMS, tumor subtype-specific genomic alterations were detected: *MYCN* amplification in alveolar RMS, *MYOD1* L122R hotspot mutation in spindle/sclerosing RMS, and *TP53, NF1*, and *YAP1* alterations in embryonal RMS. Noteworthily, *FGFR4* V550E and N535K mutations identified in fusion-negative RMS were gate-keeper mutations, suggesting primary resistance to currently approved FGFR inhibitors (Figure S6) [13, 14]. In EWS, recurrent copy number alterations were identified, such as 1q gain, 8q gain, and 16q loss (Figure S7). The copy number alterations of 17p and aneuploidy were typically seen in osteosarcoma (Figure S8). *FN1::ALK* fusion was detected in an inflammatory myofibroblastic tumor (Figure 4A). One of the two tumors with spindle cell morphology harbored *KIAA1549::BRAF* fusion; the other had *SOX13::NTRK1* fusion (Figure S9 and Figure 4B).

In CNS tumors ($n=60$) (Figures S10–S12), medulloblastoma (MB) ($n=14$) and diffuse HGG ($n=14$) were the major tumor types. *TP53*, *PDGFRA*, *PTEN*, and *PIK3CA* were the most frequently altered genes. In MB, the genomic alterations that support the classification of the molecular subgroup were identified: an *APC* germline mutation and monosomy 6 in a WNT-activated MB case, a *PTCH1* mutation and 10q loss in a SHH-activated MB case, isodicentric 17q, and whole chromosomal aberration, such as gain of chromosome 7 and loss of chromosome 8 in group 3/group 4 MB cases (Figures 3A and S10). In pediatric gliomas (Figure S11), histone mutation *H3-3A* K28M was detected in diffuse midline glioma and *H3-3A* G35V in diffuse hemispheric glioma. Receptor tyrosine kinase gene fusions were identified among diffuse HGG, particularly in infant-type hemispheric gliomas (Figure 4C,D), whereas *BRAF* fusions were found in low-grade glioma (LGG) (Figure 4E). In atypical teratoid/rhabdoid tumors (AT/RTs), *SMARCB1* mutations and 22q loss/LOH were detected (Figure 3B). The tumor-specific fusions confirmed the diagnosis of EWS and *CIC*-rearranged sarcoma in mesenchymal CNS tumors (Table S5 and Figure S12).

In PNS tumors consisting of neuroblastoma ($n=22$) and ganglioneuroblastoma ($n=4$) (Figure S13), copy number aberrations such as *MYCN*/2p24.3 amplification, 17q gain, 1p loss, and 11q loss/LOH were recurrently found (Figure 3C,D). *ATRX* loss and *TERT* promoter mutations were also detected in *MYCN* non-amplified neuroblastoma. *ODC1* (2p25.1) and *ALK* (2p23.2–p23.1), located in proximity to the *MYCN*, were co-amplified with *MYCN*. Near-triploidy, a prognostic indicator for favorable outcomes, was also found in infant neuroblastoma cases (Figure 3E).

In liver tumors ($n=14$) (Figure S14), hepatoblastoma ($n=11$) was the primary tumor type, and *CTNNB1* and *TERT* were most frequently altered. The identified *CTNNB1* genomic alterations include hotspot missense mutations, in-frame deletions, and exon skipping (Figure 4F). *TERT* promoter mutation was seen in older patients. Copy number alterations, such as 1q gain and 11p LOH, were prevalent in hepatoblastoma. *APC* germline pathogenic variant was identified in a hepatocellular carcinoma case.

In kidney tumors ($n=14$) (Figure S15), nephroblastoma ($n=9$) was the major tumor type, in which the *CTNNB1* gene was altered in two patients. Nephroblastoma with *TP53* mutation exhibited diffuse anaplastic histology. *WT1* somatic mutation was seen in nephroblastoma, whereas *WT1* germline mutation was identified in a patient who developed renal cell carcinoma

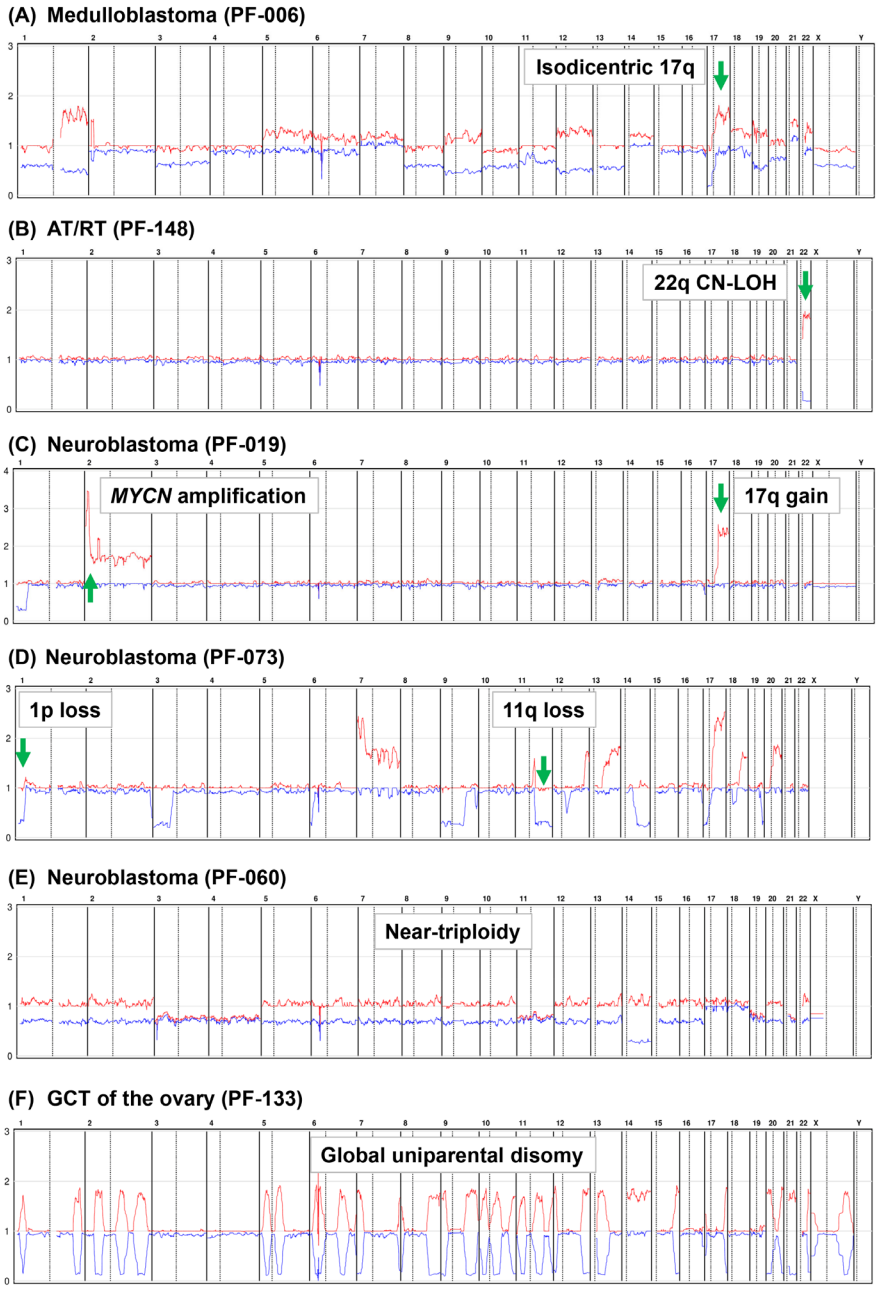

FIGURE 3 | Genomic copy number alterations identified in the TOP2 panel. Images depict genome-wide copy number plots seen in the study patients. (A) Isodicentric 17q in medulloblastoma. (B) 22q copy neutral loss of heterozygosity (CN-LOH) in atypical teratoid/rhabdoid tumor (AT/RT). (C) *MYCN* amplification and 17q gain in neuroblastoma. (D) 1p loss and 11q loss in neuroblastoma. (E) Near-triploidy in neuroblastoma. (F) Global uniparental disomy in ovarian germ cell tumor. The estimated copy numbers of major and minor alleles are shown as red and blue lines, respectively. The *y*-axis represents the copy number of each individual allele at a specific genomic locus, whereas the *x*-axis represents the genomic position along a chromosome.

(RCC) after dialysis. *BRAF* V600E mutation was noted in two patients, one with a composite of papillary renal carcinoma and metanephric adenofibroma and the other with a metanephric adenoma-epithelial nephroblastoma overlapping lesion. Given the high incidence of *BRAF* mutation in metanephric tumors and the difficulty in morphological distinction between metanephric tumors and renal malignancies, the oncogenic role of *BRAF* V600E mutation was unclear. *SMARCB1* loss-of-function mutations and/or *SMARCB1* locus deletions within chromosome 22 were detected in malignant rhabdoid tumors, whereas *DICER1* hotspot mutation was identified in anaplastic sarcoma of the kidney.

In germ cell tumors (GCTs) (CNS GCT, $n=2$; non-CNS GCT, $n=5$) (Figure S16), copy number alterations such as isochromosome 12p (i12p) and 1q and 21q gains were recurrently seen. *KIT* mutation was identified in dysgerminoma. Global uniparental disomies were found in immature teratoma of the ovary and ganglioneuroblastoma-like tumor derived from teratoma of the ovary with unknown oncogenic role (Figure 3F).

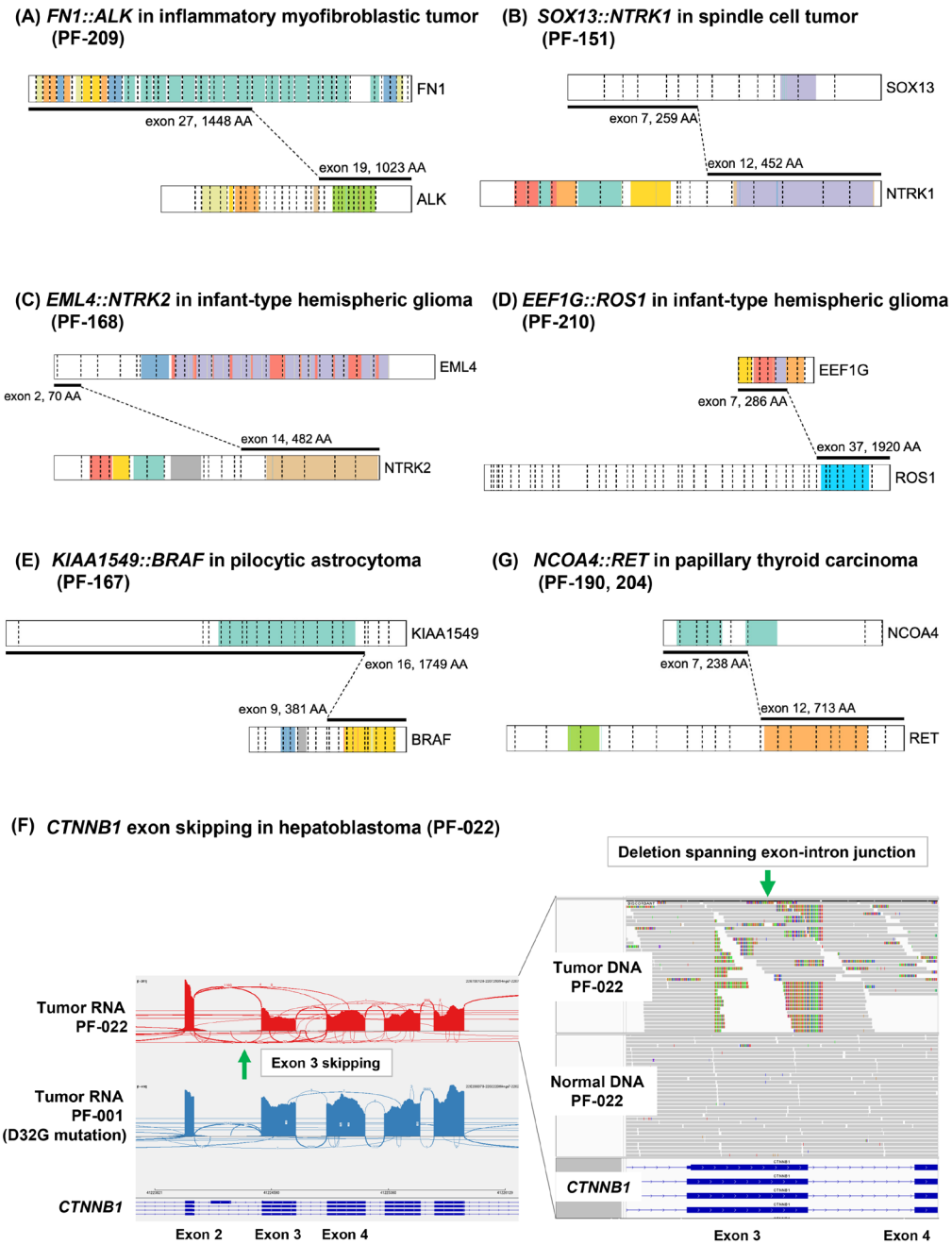

FIGURE 4 | Fusion genes and exon skipping detected in the TOP2 RNA panel. The images illustrate oncogenic fusions identified in the TOP2 system. (A) *FN1::ALK* in inflammatory myofibroblastic tumor. (B) *SOX13::NTRK1* in a spindle cell tumor. (C) *EML4::NTRK2* in infant-type hemispheric glioma. (D) *EEF1G::ROS1* in infant-type hemispheric glioma. (E) *KIAA1549::BRAF* in pilocytic astrocytoma. (F) The IGV Sashimi plot on the left shows a differential splicing pattern of the *CTNNB1* transcript in hepatoblastoma cases (PF-022 case with *CTNNB1* Exon 3 skipping; PF-001 case with *CTNNB1* D32G alteration). The image on the right is an IGV read alignment view in PF-022 tumor and normal tissue DNA, showing the deletion spanning 3′ exon-intron junction of *CTNNB1* exon3. (G) *NCOA4::RET* in papillary thyroid carcinoma.

In the rare tumor fraction not included above ($n = 17$) (Figure S17), *RB1* loss-of-function mutations were found in three retinoblastomas, and two of them had LOH at the 13q14 region. Somatic *DICER1* hot spot mutations were identified in two pleuropulmonary blastomas, and both had LOH at the 14q32 region. One teenager with a germline *APC* mutation had a lung adenocarcinoma with *EML4::ALK*. Two papillary thyroid carcinomas harbored *NCOA4::RET* fusion (Figure 4G).

3.5 | Clinical Utility of the TOP2 System

We evaluated the clinical utility of the TOP2 system by determining the proportion of patients with potentially actionable findings (PAFs) in diagnostic (Dx1/2), prognostic (Px1/2), and therapeutic (TxA–D) categories. Of the 204 patients, 111 (54%) received diagnostic confirmation, refinement, or reclassification based on genomic data, most notably through the detection of fusion genes or characteristic copy number alterations,

TABLE 2 | Clarification of diagnosis after the TOP2 test.

Pre-TOP2 diagnosis	Pre-TOP2 molecular work-up	TOP2 genomic findings	Post-TOP2 integrated diagnosis
Rhabdomyosarcoma	**RT-PCR:** Negative for *PAX3::FOXO1, PAX7::FOXO1*	*TP53* G244S	Embryonal rhabdomyosarcoma, anaplastic
Rhabdomyosarcoma	**RT-PCR:** Negative for *PAX3::FOXO1, PAX7::FOXO1*	*TP53* H214Ifs	Embryonal rhabdomyosarcoma, anaplastic
Spindle cell rhabdomyosarcoma	**RT-PCR:** Negative for *PAX3::FOXO1, PAX7::FOXO1*	*MYOD1* L122R	*MYOD1*-mutant spindle cell rhabdomyosarcoma
Spindle cell/ Sclerosing rhabdomyosarcoma	**FISH:** Negative for *FOXO1* split	*MYOD1* L122R	*MYOD1*-mutant spindle cell rhabdomyosarcoma
Spindle cell rhabdomyosarcoma	**RT-PCR:** Negative for *PAX3::FOXO1* nor *PAX7::FOXO1*	*KIAA1549::BRAF*	*BRAF* fusion-positive spindle cell tumor
Pleuropulmonary blastoma metastasis	**IHC:** Positive for Desmin, Myogenin **FISH:** Positive for *ALK* split positive.	*DICER1* E1813D	*DICER1*-associated Embryonal rhabdomyosarcoma
Spindle cell tumors	**IHC:** Positive for BCOR (focal), Pan-Trk (focal and weak) **RT-PCR:** Negative for *BCOR* ITD nor *YWHAE::FAM22*.	*MDM2* amplification *CDK4* amplification	Dedifferentiated liposarcoma
Spindle cell tumors	**IHC:** Positive for Pan-Trk. Negative for BCOR *and* CCNB3 **FISH:** Positive for *NTRK1* split. Negative for *NTRK3* split. **RT-PCR:** Negative for *ETV6::NTRK*.	*SOX13::NTRK1*	*NTRK*-rearranged spindle cell neoplasm
Small round cell tumor	**IHC:** Positive for BCOR and SS18-SSX	*SS18::SSX2*	Synovial sarcoma
Small round cell tumor	**IHC:** Positive for CCNB3.	*BCOR::CCNB3*	Sarcoma with *BCOR* genetic alteration
Small round cell tumor	**IHC:** Positive for BCOR. Negative for CCNB3 **FISH:** *MDM2* amplification	*CDK4* amplification *GLI1* amplification *MDM2* amplification	*GLI1*-altered mesenchymal tumor
Infant-type hemispheric glioma	**RT-PCR:** Negative for *ETV6-NTRK3, GOPC-ROS1, EEF1G-ROS* **Methylation profiling:** MC Infant-type hemispheric glioma score 0.95 match	*EML4::NTRK2*	Infant-type hemispheric glioma, *NTRK*-altered
Pediatric-type diffuse high-grade glioma, NOS, Infant-type hemispheric glioma, suspected	**IHC:** positive for ALK5A4	*ROCK2::ALK*	Infant-type hemispheric glioma, *ALK*-altered
Pediatric-type diffuse high-grade glioma, H3 wildtype and IDH-wildtype, NOS	**Pyrosequence:** wildtype of *IDH1* R132, *IDH2* R172, *H3F3A* K27, *H3F3A* G34, and *HIST1H3B* K27	*PDGFRA* D842V *MSH2* S77Cfs*4	Diffuse pediatric type high-grade glioma, H3-wildtype and IDH-wildtype, RTK1

(Continues)

TABLE 2 | (Continued)

Pre-TOP2 diagnosis	Pre-TOP2 molecular work-up	TOP2 genomic findings	Post-TOP2 integrated diagnosis
Diffuse pediatric type high-grade glioma, H3-wildtype and IDH-wildtype, suspected	**Pyrosequence:** wildtype of *IDH1* R132, *IDH2* R172, *H3F3A* K27, *H3F3A* G34, and *HIST1H3B* K27 **Methylation profiling:** MC Diffuse pediatric-type high grade glioma, RTK1 subtype, Score 0.72 no match	*PDGFRA* C290R *TP53* E286G *AKT3* amplification *CDK4* amplification	Diffuse pediatric type high-grade glioma, H3-wildtype and IDH-wildtype, RTK1
Glioblastoma with lipidized feature, IDH wildtype	**Pyrosequence:** wildtype of *IDH1* R132, *IDH2* R172, *H3F3A* K27, *H3F3A* G34, and *HIST1H3B* K27	*FMNL2::ALK*	Glioblastoma with lipidized features, IDH-wildtype, with *FMNL2::ALK* fusion, NEC
Intracranial malignant tumor with epithelioid features	**Pyrosequence:** wildtype of *IDH1* R132, *IDH2* R172, *H3F3A* K27, *H3F3A* G34, and *HIST1H3B* K27 **Methylation profiling:** MC Glioblastoma, IDH-wildtype, mesenchymal subtype, subclass B (novel) Score 0.44554, no match	*HIST1H3B* K37I	Intracranial malignant tumor with epithelioid features with *HIST1H3B* K37 mutation

particularly in bone, soft tissue, and CNS tumors (Table 2). We identified prognostically relevant genomic alterations in 61 patients (30%), while 64 patients (31%) harbored potentially therapeutically targetable alterations. Figure 5B summarizes the targeted therapies suggested by the MTB based on the genomic alterations. Inhibitors of BRAF/MEK, EZH2/EZH1/2, PI3k/AKT/mTOR, CDK4/6, and ALK were the most frequently recommended drug classes.

3.6 | Germline Findings

The DNA panel analysis of peripheral blood revealed that 17 (8.3%) patients harbored pathogenic or likely pathogenic germline variants in the 40 cancer-predisposing genes (Tables 3 and S1). *TP53* was the most frequently mutated gene, followed by *APC* and *NF1*. Among these 17 patients, 7 had previously undergone some form of genomic testing: 4 had confirmed germline variants from prior analyses, while 3 had potentially germline variants identified through tumor-only profiling. Only 3 of the 17 patients had a family history of cancer in first-degree relatives. Thus, pathogenic or likely pathogenic germline variants were found in 3 of 12 (25%) patients with first-degree relatives diagnosed with cancer, compared with 14 of 192 (7.3%) patients with no family history of cancer. As evidence of second-allele inactivation, the LOH status was confirmed in eight patients, and somatic second-hit mutation was noted in one patient.

3.7 | Contribution of TOP2 Findings to the Medical Decision-Making Process

Feedback surveys were completed by physicians for 197 patients (Figure S18). Genomic data obtained via the TOP2 system influenced clinical decision-making in 53 cases (27%). Specifically, the data informed therapeutic decisions in 29 patients (15%), facilitated diagnostic clarification in 16 patients (8%), and contributed to prognostic assessments in 4 patients (2%). The perceived clinical utility of TOP2 data was particularly notable at the time of initial diagnosis and during first-line treatment. Conversely, in 144 cases (73%), the genomic findings were deemed noncontributory. The primary reasons included the absence of actionable variants (107 patients, 54%), lack of access to targeted therapies (39 patients, 20%), prior knowledge of the genomic results (19 patients, 10%), and clinical deterioration that precluded the implementation of genomics-informed interventions (1 patient, 0.5%).

4 | Discussion

The JCCG-TOP2 study sought to establish and standardize a genomic platform optimized for pediatric cancers. The study utilized the TOP2 dual-panel system, achieving high success rates in both DNA and RNA analyses of FFPE samples. Consistent with earlier literature [2, 15], the pediatric cancer genome exhibits a low mutation burden. However, incorporating RNA panel analysis and allele-specific genome copy number estimation significantly facilitated the identification of oncogenic alterations unique to pediatric cancers. The fusion gene is a predominant oncogenic mechanism in pediatric cancers, particularly bone and soft tissue tumors and CNS tumors. RNA analysis also benefited fusion identification with an atypical genomic breakpoint and exon skipping, which were not detected in existing DNA panels. Furthermore, copy number alterations were prevalent, some of which were characteristic of the disease entity and clinically informative. The allele-specific copy number plots clarified the mechanism of biallelic inactivation events in tumor suppressor genes, providing support for assessing whether the identified germline events are involved in the tumorigenesis of a current disease or are merely incidental, secondary findings.

Several pediatric cancer molecular profiling studies have been reported, such as the Zero Childhood Cancer Program (Australia) [16, 17], the INFORM study (Germany) [18], the MAAPYACTS trial (France) [19], and the KiCS program

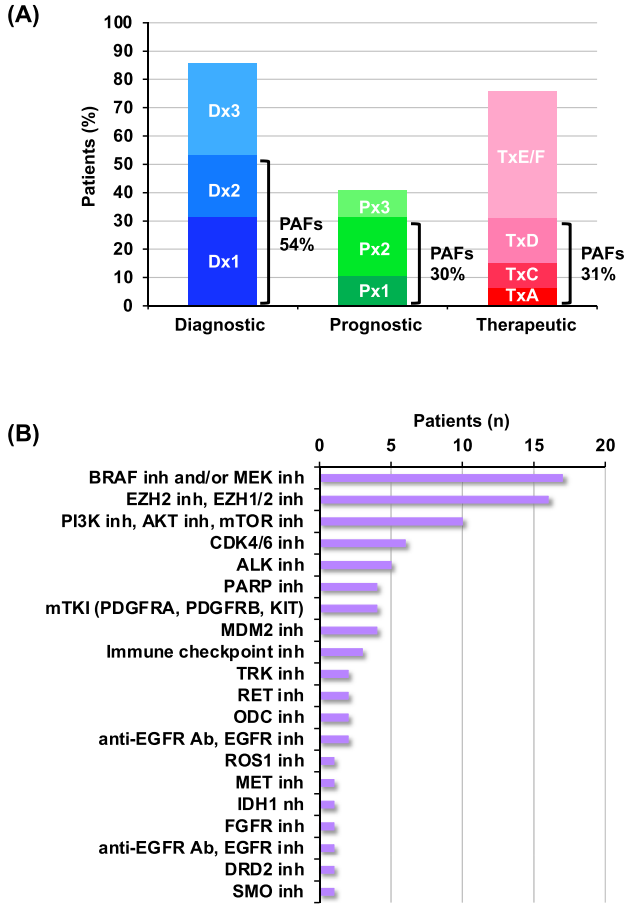

FIGURE 5 | Clinical utility of the TOP2 system. (A) Proportion of patients with potentially actionable findings (PAFs). The clinical utility of the TOP2 system was evaluated by calculating the proportion of patients with potentially actionable findings (PAFs) in diagnostic (Dx), prognostic (Px), and therapeutic (Tx) categories. PAFs were defined as genomic alterations with an evidence level equivalent to level Dx2 or higher for diagnostic findings, Px2 or higher for prognostic findings, and D or higher for therapeutic findings. Diagnostic, prognostic, and therapeutic PAFs were identified in 54%, 30%, and 31% of the patients, respectively. (B) Recommended drug class. The horizontal bar graph represents the number of patients the Molecular Tumor Board recommended for each drug class. The letter "n" indicates the number of patients. Ab, antibody; inh, inhibitor; mTKI, multi-tyrosine kinase inhibitor.

(Canada) [20], and the Molecular Characterization Initiative (MCI) (USA) [21]. The MCI profiled 3972 pediatric patients across 188 institutions, identifying gene fusions in 27% of CNS tumors and 40% of soft tissue tumors, with diagnostic refinement in 25% of cases. These findings underscore the value of large-scale data sharing and centralized profiling, particularly for rare and diagnostically challenging tumors such as pediatric CNS and soft tissue sarcomas, which, due to their morphological heterogeneity and overlapping histopathological features, benefit significantly from integrated genomic analysis. Most of these studies have comprehensively analyzed the pediatric cancer genome using fresh-frozen tumors, including whole-genome sequencing (WGS), whole-exome sequencing (WES), whole transcriptome sequencing (WTS), and methylome profiling, making the direct comparisons with our results difficult. The scope of the JCCG-TOP2 study is more similar to that of the iCAT/GAIN study [22] and the Pediatric MATCH [23] conducted in the USA, which use FFPE tumor samples obtained within routine clinical practice and perform targeted DNA panel sequencing. In the iCAT/GAIN study, genomic alterations with diagnostic, prognostic, or therapeutic significance were present in 61%, 16%, and 65% of the patients, respectively [22]. The Pediatric MATCH study reported that actionable alterations were detected in 31.5% of the first 1000 tumors screened, and treatment arm assignment and enrollment occurred in 28.4% and 13.1% of patients, respectively [23]. In a large U.S. genomic profiling study of 109,695 solid tumors across all age groups (median age 67 years; range 1–89), clinically relevant genomic alterations were identified in 4.5% of cases (range: 0%–92.3%) as diagnostic markers, 13.2% (range: 0%–76.1%) as prognostic markers, and 47.6% (range: 3.5%–79.7%) as treatment-predictive markers [24]. Although direct numerical comparisons are limited due to differences in study design and gene panel composition, the proportion of patients with diagnostically significant findings in the JCCG-TOP2 study was comparable to those reported in this study. However, the proportion of patients with therapeutically relevant findings was notably lower, likely reflecting differences in access to molecularly targeted therapies between pediatric and adult populations, as well as differences in the status of drug development and regulatory approval between Japan and the United States.

Pathogenic or likely pathogenic germline variants were found in 8.3% of patients, which is similar to the cohort's results, such as the Pediatric MATCH (7%) [25] and other recent international studies (10%–11%) [26–28]. The actual proportion in our study population may be slightly higher, in part due to the limited genes ($n = 40$) in the TOP2 panel, which does not include the genes responsible for rare pediatric cancer predisposition syndromes, such as *SMARCB1* for rhabdoid tumor predisposition syndrome and *DICER1* for DICER1 syndrome. Early and accurate diagnosis of cancer-predisposing conditions is essential for appropriate medical management, cancer surveillance, and family planning, demanding a validated germline reporting system and multidisciplinary approach. Further exploratory analysis is ongoing to investigate germline variants beyond the 40 reporting genes and gene expression profiling in the study patients, which will be reported in subsequent papers.

The study emphasized the importance of central pathology review and sample qualification, which contribute to the high sequencing success rate of FFPE tumor samples in a multi-institutional setting involving CGM-designated and non-designated hospitals. The integrative assessment of genomic and pathology findings is essential for the accurate interpretation and validation of genomic findings, particularly when heterogeneous architecture is seen within the tumor or when shared genomic alterations are found in distinct tumor types.

The study has some limitations. First, the median turnaround time was 57 days, which is relatively long compared to other molecular profiling efforts such as Pediatric MATCH (median 24 days) [23] and MCI (within 21 days) [21]. This is largely due to the centralized review process by the MTB and EP, as well as the preparation of genomic summaries. Subsequent workflow optimizations and increased familiarity with the system

TABLE 3 | Germline alterations identified in the TOP2 system.

Category	Tumor type	Altered gene	Variant type & zygosity	Second hit (somatic)	Family history in the 1st degree relatives
CNS	Medulloblastoma, SHH-activated	*PTEN*	Heterozygous SNV	LOH	
	Medulloblastoma, WNT-activated	*APC*	Heterozygous deletion	LOH	Yes
	Astrocytoma, IDH-mutant, grade 4	*MLH1*	Heterozygous SNV	Not identified	
	Diffuse high-grade glioma	*NF1*	Heterozygous deletion	LOH	
	Diffuse high-grade glioma	*TP53*	Heterozygous deletion	LOH	
	Ewing sarcoma	*PALB2*	Heterozygous SNV	Not identified	
Bone & soft tissue	Sarcoma, NOS	*TP53*	Heterozygous SNV	Not identified	
	Rhabdomyosarcoma, embryonal type	*TP53*	Heterozygous deletion	LOH	
	Spindle cell neoplasm	*NBN*	Heterozygous SNV	Not identified	
Eye	Retinoblastoma	*RB1*	Heterozygous SNV	SNV	
Breast	Malignant phyllodes tumor	*NF1*	Heterozygous SNV	LOH	
Kidney	Renal cell carcinoma	*WT1*	Heterozygous SNV	Not identified	
Liver	Hepatocellular neoplasm, NOS	*APC*	Heterozygous SNV	Not identified	
	Perivascular epithelioid cell neoplasms (PEComas)	*TP53*	Heterozygous SNV	Not identified	Yes
Lung	Lung adenocarcinoma	*APC*	Heterozygous SNV	Not identified	
Endocrine & neuroendocrine	Paraganglioma	*SDHB*	Heterozygous SNV	LOH	Yes
	Neuroendocrine tumor	*NF1*	Heterozygous SNV	LOH	

Abbreviations: CNS, central nervous system; hetero, heterozygous; LOH, loss-of-heterozygosity; NOS, not otherwise specified; PNS, peripheral nervous system.

contributed to reductions in this interval. Following the approval of TOP2 under national health coverage, genomic testing is now performed within the national genomic medicine framework. Genomic results are typically returned within 4–5 weeks. Secondly, given the small sample size, the results may not be representative of the entire pediatric population. Many genomic alterations were identified only once in the analysis of 204 tumors, despite being critical alterations in each tumor type. This highlights the importance of collecting and sharing pediatric cancer data to facilitate data-driven basic, translational, and clinical research. Although the study's inclusion criteria allowed enrollment up to 29 years of age, only 6 patients aged 20 years or older were included. This limited representation of young adults may be partly due to the fact that patient enrollment was conducted through JCCG institutions, which primarily treat pediatric populations.

The JCCG-TOP2 study demonstrated the significant clinical utility of TOP2 testing in pediatric cancers by improving diagnostic accuracy, facilitating biomarker-based risk stratification, and uncovering additional therapeutic targets that might have been missed in pre-existing DNA panels. These also imply the benefit of the upfront use of the TOP2 test in clinical practice. Consistent with previous studies, our findings support the conclusion that precision-guided treatment provides the greatest clinical benefit in high-risk cancers with strong evidence of targeted therapy and/or oncogenic definitive drivers—such as fusion genes—when initiated prior to disease progression [17, 18]. The accumulated evidence from the chronological analysis of tumor tissue or circulating tumor DNA has demonstrated tumor evolution and alterations in therapeutically targetable drivers [20, 29], supporting the resampling of progressing tumors. Nonetheless, the current Japanese universal health insurance policy allows for CGP testing only once per patient. This limitation reduces the clinical utility of CGP testing, making the determination of optimal timing critically important. Based on the results of the physician feedback survey (Figure S18), early testing may be particularly valuable in cases with diagnostically ambiguous or high-risk tumor features, whereas delayed testing may capture alterations associated with disease progression. In certain clinical scenarios, repeated CGP testing may significantly impact patient outcomes, underscoring the need for

case-dependent testing strategies and supporting the consideration of re-testing when clinically indicated.

The interdisciplinary collaboration for the JCCG-TOP2 study has yielded significant advancements in genomic medicine optimized for pediatric cancers. Further analysis of genomic data beyond the standard report is underway. The objective is to construct a pediatric precision medicine platform that will improve the quality of patient care and benefit every child everywhere.

Author Contributions

Kayoko Tao: conceptualization, data curation, formal analysis, investigation, project administration, visualization, writing – original draft. **Takako Yoshioka:** conceptualization, data curation, investigation, project administration, resources, writing – review and editing. **Miho Kato:** formal analysis, project administration, writing – review and editing. **Kazuyuki Komatsu:** investigation, writing – review and editing. **Shinichi Tsujimoto:** investigation, writing – review and editing. **Kenichi Sakamoto:** investigation, writing – review and editing. **Kazuki Tanimura:** investigation, writing – review and editing. **Minako Sugiyama:** investigation, writing – review and editing. **Masahiro Sekiguchi:** investigation, writing – review and editing. **Yoshiko Nakano:** investigation, writing – review and editing. **Yoshihiro Otani:** investigation, writing – review and editing. **Yasushi Yatabe:** data curation, investigation, writing – review and editing. **Akihiko Yoshida:** investigation, writing – review and editing. **Hajime Okita:** investigation, writing – review and editing. **Junko Hirato:** investigation, writing – review and editing. **Kenichi Kohashi:** investigation, writing – review and editing. **Yukichi Tanaka:** investigation, writing – review and editing. **Shinji Kohsaka:** data curation, methodology, writing – review and editing. **Takashi Kubo:** data curation, writing – review and editing. **Kuniko Sunami:** investigation, writing – review and editing. **Makoto Hirata:** investigation, writing – review and editing. **Shuichi Tsutsumi:** data curation, methodology, writing – review and editing. **Hiroyuki Aburatani:** data curation, methodology, writing – review and editing. **Katsuyoshi Koh:** resources, writing – review and editing. **Masahiro Hirayama:** resources, writing – review and editing. **Shuhei Karakawa:** resources, writing – review and editing. **Yukayo Terashita:** resources, writing – review and editing. **Hiroyuki Fujisaki:** resources, writing – review and editing. **Takeshi Yagi:** resources, writing – review and editing. **Akihiro Yoneda:** project administration, writing – review and editing. **Shinji Mochizuki:** project administration, writing – review and editing. **Hiroyuki Shichino:** project administration, resources, writing – review and editing. **Tatsuya Suzuki:** funding acquisition, supervision, writing – review and editing. **Tetsuya Takimoto:** conceptualization, project administration, writing – review and editing. **Koichi Ichimura:** conceptualization, data curation, project administration, writing – review and editing. **Chitose Ogawa:** conceptualization, data curation, project administration, writing – review and editing. **Kimikazu Matsumoto:** resources, supervision, writing – review and editing. **Hitoshi Ichikawa:** conceptualization, data curation, project administration, visualization, writing – original draft. **Motohiro Kato:** conceptualization, data curation, funding acquisition, investigation, project administration, resources, writing – original draft.

Affiliations

[1]Department of Pediatrics, National Cancer Center Hospital, Tokyo, Japan | [2]Department of Clinical Genomics, National Cancer Center Research Institute, Tokyo, Japan | [3]Division of Cellular Signaling, National Cancer Center Research Institute, Tokyo, Japan | [4]Department of Pathology, National Center for Child Health and Development, Tokyo, Japan | [5]Department of Childhood Cancer Data Management, National Center for Child Health and Development, Tokyo, Japan | [6]Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan | [7]Department of Pediatrics, Yokohama City University, Yokohama, Japan | [8]Department of Pediatrics, Shinshu University School of Medicine, Matsumoto, Japan | [9]Department of Pediatric Hematology and Oncology, Osaka City General Hospital, Osaka, Japan | [10]Department of Pediatrics, Hokkaido University Hospital, Sapporo, Japan | [11]Department of Pediatrics, The University of Tokyo, Tokyo, Japan | [12]Department of Genetic Medicine and Services, National Cancer Center Hospital, Tokyo, Japan | [13]Department of Neurological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan | [14]Department of Diagnostic Pathology, National Cancer Center Hospital, Tokyo, Japan | [15]Department of Pathology, Keio University School of Medicine, Tokyo, Japan | [16]Department of Pathology, Public Tomioka General Hospital, Tomioka, Japan | [17]Department of Pathology, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan | [18]Department of Pathology, Kanagawa Children's Medical Center, Yokohama, Japan | [19]Department of Laboratory Medicine, National Cancer Center Hospital, Tokyo, Japan | [20]Genome Science & Medicine Division, Research Center of Advanced Science and Technology, The University of Tokyo, Tokyo, Japan | [21]Department of Hematology and Oncology, Saitama Children's Medical Center, Saitama, Japan | [22]Department of Pediatrics, Mie University Graduate School of Medicine, Tsu, Japan | [23]Department of Pediatrics, Hiroshima University Hospital, Hiroshima, Japan | [24]Okinawa Prefectural Nanbu Medical Center & Children's Medical Center, Haebaru, Japan | [25]Department of Pediatric Surgery, National Center for Child Health and Development, Tokyo, Japan | [26]Department of Pediatric Surgery, National Cancer Center Hospital, Tokyo, Japan | [27]Department of Pediatrics, National Center for Global Health and Medicine, Japan Institute for Health Security, Tokyo, Japan | [28]Department of Hematology, National Cancer Center Hospital, Tokyo, Japan | [29]Department of Pathology, Kyorin University Faculty of Medicine, Tokyo, Japan | [30]Division of Brain Tumor Translational Research, National Cancer Center Research Institute, Tokyo, Japan | [31]Children's Cancer Center, National Center for Child Health and Development, Tokyo, Japan | [32]Department of Analytical Pathology, National Cancer Research Institute, Tokyo, Japan

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Conflicts of Interest

Aburatani: advisor remuneration and research funds from Konica Minolta Inc., endowed chair funded by Chugai Pharmaceutical Co.; Ichikawa: research funds from Chugai Pharmaceutical, Eisai, Healios, and Ono Pharmaceutical; Ichimura: advisor remuneration from Daiichi-Sankyo, research funds from Daiichi Sankyo, Riken Genesis, honoraria from Chugai Pharmaceuticals, Eisai, Nippon Kayaku, Bayer Yakuhin, Illumina, Inc., and Konica Minolta, endowed chair supported by Idorsia Pharmaceuticals Japan Ltd.; Kato: honoraria for lectures from Konica Minolta REALM, Chugai, Bayer; Kohsaka: research funds from the Boehringer Ingelheim, Chordia Therapeutics, Eisai, Konica Minolta, CIMIC, and H.U. Group Research Institute; Otani: honoraria from Eisai and Ono; Tao: honoraria from Konica Minolta and Sysmex, research fund from Boehringer, travel support from Konica Minolta; Yatabe: honoraria from AstraZeneca, research funds from Merk Biopharma, Chugai pharma, Konica Minolta, Optieum Biotechnologies, and Eizai; Yoshida: honoraria from Boehringer and Eisai; research funds from Daiichi Sankyo, Boehringer, and Chugai; Aburatani, Ichimura, Kohsaka, Sunami, and Yatabe: editorial board members of Cancer Science.

Consent

All patients provided written informed consent for study enrollment and sample collection according to the Declaration of Helsinki.

Ethics Statement

Approval of the research protocol in this study was granted by the NCCHD Ethics Committee Institutional Review Board (2021-167).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** JCCG-TOP2 study workflow. **Figure S2:** Number of patient registrations and designation status of institutions participating in the JCCG-TOP2 study. **Figure S3:** Effect of FFPE tumor sample storage period on RNA quality. **Figure S4:** Turnaround time. **Figure S5:** Tumor mutation burdens. **Figure S6:** Genomic alterations in rhabdomyosarcoma. **Figure S7:** Genomic alterations in Ewing and Ewing-like sarcoma. **Figure S8:** Genomic

alterations in osteosarcoma. **Figure S9:** Genomic alterations in other bone and soft tissue tumors. **Figure S10:** Genomic alterations in medulloblastoma. **Figure S11:** Genomic alterations in glioma. **Figure S12:** Genomic alterations in other central nervous system tumors. **Figure S13:** Genomic alterations in neuroblastoma and ganglioneuroblastoma. **Figure S14:** Genomic alterations in liver tumors. **Figure S15:** Genomic alterations in kidney tumors. **Figure S16:** Genomic alterations in germ cell tumors. **Figure S17:** Genomic alterations in other rare tumors. **Figure S18:** Physician feedback survey on the contribution of TOP2 findings to the medical decision-making process. **Table S1:** Gene list of the TOP2 panel. **Table S4:** Summary of genomic alterations identified in the JCCG-TOP2 study. **Table S2:** Evidence level defined in the JCCG-TOP2 study. **Table S3:** Summary of the diagnoses of the JCCG-TOP2 study patients. **Table S5:** Oncogenic fusions identified in the JCCG-TOP2 study. **Table S6:** Institutions participating in the JCCG-TOP2 study.