RESEARCH ARTICLE

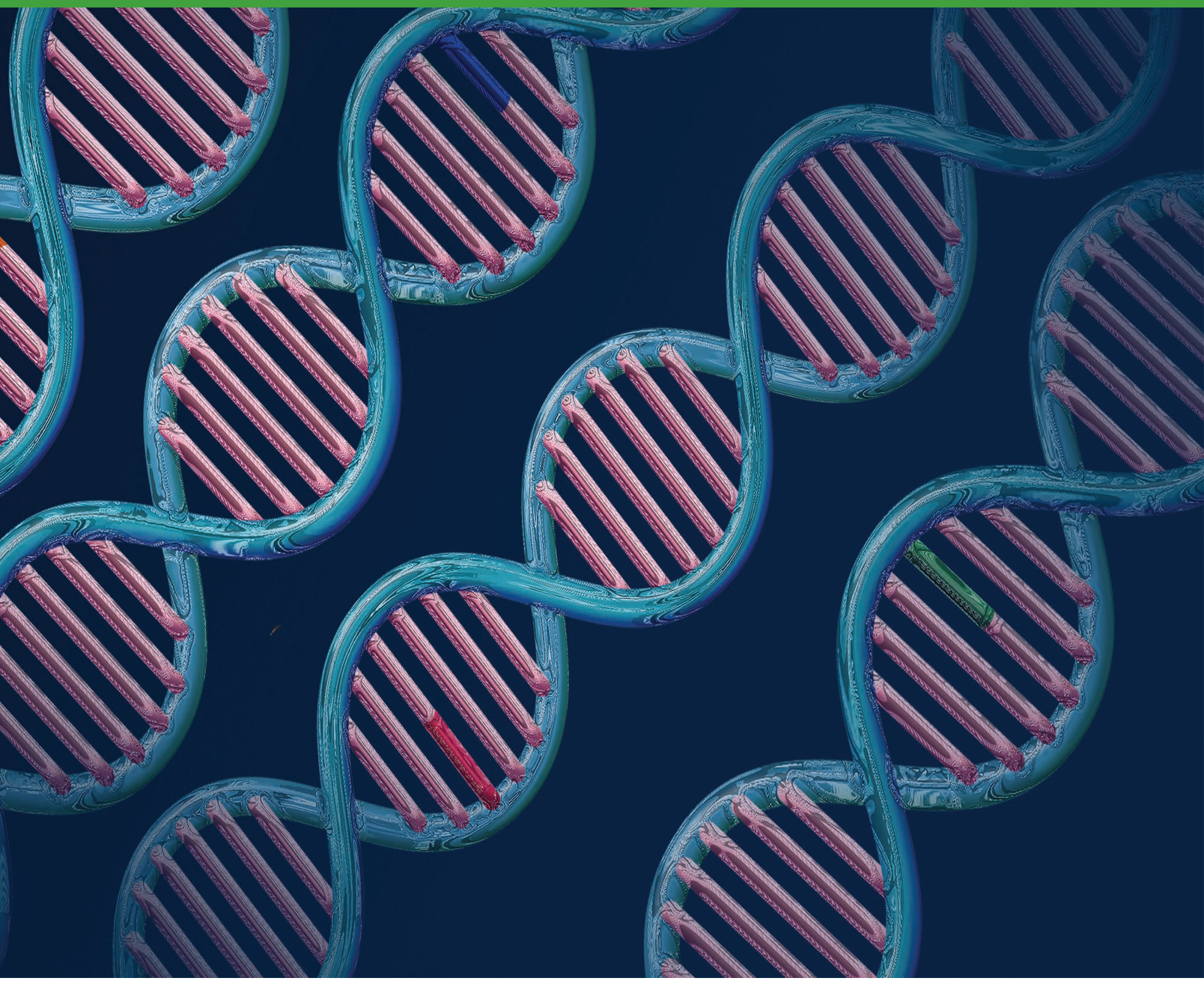
# The European MAPPYACTS Trial: Precision Medicine Program in Pediatric and Adolescent Patients with Recurrent Malignancies

Pablo Berlanga[1], Gaelle Pierron[2], Ludovic Lacroix[3], Mathieu Chicard[4], Tiphaine Adam de Beaumais[5], Antonin Marchais[6], Anne C. Harttrampf[1], Yasmine Iddir[4,7], Alicia Larive[8], Aroa Soriano Fernandez[9], Imene Hezam[1], Cecile Chevassus[8], Virginie Bernard[10], Sophie Cotteret[3], Jean-Yves Scoazec[3], Arnaud Gauthier[11], Samuel Abbou[1], Nadege Corradini[12], Nicolas André[13,14], Isabelle Aerts[15], Estelle Thebaud[16], Michela Casanova[17], Cormac Owens[18], Raquel Hladun-Alvaro[19], Stefan Michiels[8], Olivier Delattre[4,10,15], Gilles Vassal[5], Gudrun Schleiermacher[4,15], and Birgit Geoerger[1,6]

**ABSTRACT** MAPPYACTS (NCT02613962) is an international prospective precision medicine trial aiming to define tumor molecular profiles in pediatric patients with recurrent/refractory malignancies in order to suggest the most adapted salvage treatment. From February 2016 to July 2020, 787 patients were included in France, Italy, Ireland, and Spain. At least one genetic alteration leading to a targeted treatment suggestion was identified in 436 patients (69%) with successful sequencing; 10% of these alterations were considered "ready for routine use." Of 356 patients with follow-up beyond 12 months, 107 (30%) received one or more matched targeted therapies—56% of them within early clinical trials—mainly in the AcSé-ESMART platform trial (NCT02813135). Overall, matched treatment resulted in a 17% objective response rate, and of those patients with ready for routine use alterations, it was 38%. In patients with extracerebral tumors, 76% of actionable alterations detected in tumor tissue were also identified in circulating cell-free DNA (cfDNA).

**SIGNIFICANCE:** MAPPYACTS underlines the feasibility of molecular profiling at cancer recurrence in children on a multicenter, international level and demonstrates benefit for patients with selected key drivers. The use of cfDNA deserves validation in prospective studies. Our study highlights the need for innovative therapeutic proof-of-concept trials that address the underlying cancer complexity.

[1]Department of Pediatric and Adolescent Oncology, Gustave Roussy Cancer Campus, Université Paris-Saclay, Villejuif, France. [2]Unité de Génétique Somatique, Service de Génétique, Hospital Group, Institut Curie, Paris, France. [3]Department of Pathology and Laboratory Medicine, Translational Research Laboratory and Biobank, AMMICA, INSERM US23/CNRS UMS3655, Gustave Roussy Cancer Campus, Université Paris-Saclay, Villejuif, France. [4]INSERM U830, Laboratoire de Génétique et Biologie des Cancers, Research Center, PSL Research University, Institut Curie, Paris, France. [5]Clinical Research Direction, Gustave Roussy Cancer Campus, Université Paris-Saclay, Villejuif, France. [6]INSERM U1015, Gustave Roussy Cancer Campus, Université Paris-Saclay, Villejuif, France. [7]Equipe SiRIC RTOP Recherche Translationelle en Oncologie Pédiatrique, Institut Curie, Paris, France. [8]Biostatistics and Epidemiology Unit, Gustave Roussy Cancer Campus, INSERM U1018, CESP, Université Paris-Saclay, Villejuif, France. [9]Laboratory of Translational Research in Child and Adolescent Cancer, Vall d'Hebron Research Institute (VHIR)-UAB, Barcelona, Spain. [10]Institut Curie Genomics of Excellence (ICGex) Platform, Research Center, Institut Curie, Paris, France. [11]Department of Pathology, PSL



Research University, Institut Curie, Paris, France. [12]Department of Pediatric Oncology, Institut d'Hematologie et d'Oncologie Pédiatrique/Centre Léon Bérard, Lyon, France. [13]Department of Pediatric Hematology and Oncology, Hôpital de La Timone, AP-HM, Marseille, France. [14]UMR Inserm 1068, CNRS UMR 7258, Aix Marseille Université U105, Marseille Cancer Research Center (CRCM), Marseille, France. [15]SIREDO Oncology Center (Care, Innovation and Research for Children and AYA with Cancer), Institut Curie, PSL Research University, Paris, France. [16]Department of Pediatric Oncology, Centre Hospitalier Universitaire, Nantes, France. [17]Pediatric Oncology Unit, Fondazione IRCCS Istituto Nazionale Tumori, Milano, Italy. [18]Paediatric Haematology/Oncology, Children's Health Ireland, Crumlin, Dublin, Republic of Ireland. [19]Division of Paediatric Haematology and Oncology, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain.

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G. Schleiermacher and B. Geoerger are co-last authors.

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**Corresponding Author:** Birgit Geoerger, Department of Pediatric and Adolescent Oncology, Gustave Roussy Cancer Campus, Université Paris-Saclay, 114 Rue Eduard Vaillant, 94805 Villejuif, France. Phone: 33-1-42-11-46-61; Fax: 33-1-42-11-52-75; E-mail: birgit.geoerger@gustaveroussy.fr

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## INTRODUCTION

Cancer remains the primary cause of disease-related mortality in children and adolescents (1). Comprehensive molecular profiling of tumors through high-throughput technologies identifies molecular targets and predictive biomarkers. Together with improved understanding of tumor biology and development of targeted anticancer agents, these approaches have facilitated therapeutic approaches adapted to cancer molecular profiles. This "cancer precision medicine" approach has now been implemented to guide treatment in patients with advanced malignancy (2–5), including children.

MAPPYACTS (MoleculAr Profiling for Pediatric and Young Adult Cancer Treatment Stratification; ClinicalTrials.gov identifier: NCT02613962) is a European international prospective precision medicine trial aiming to define the molecular profile of recurrent/refractory malignancies in pediatric and young adult patients in order to suggest the most adapted salvage treatment in the setting of further relapse/progression. The main objective of MAPPYACTS was to identify the proportion of patients who receive matched targeted treatments based on their individual molecular tumor profile at recurrence using whole-exome sequencing (WES) and RNA sequencing (RNA-seq). Secondary objectives were to describe the safety and feasibility of the on-purpose procedure of collecting cancer tissue in a multicentric setting and outcome of patients treated with matched targeted agents. Circulating cell-free DNA (cfDNA) analysis was explored as one main ancillary study to determine the feasibility of noninvasive assessment of tumor-related genomic alterations in patients with extracerebral tumors.

## RESULTS

### Patient and Procedure Characteristics

From January 2016 to July 2020, 787 patients were included in 18 centers following informed consent. Figure 1 depicts the detailed study flow. Thirteen patients consented to the MAPPYACTS trial but did not undergo a procedure to obtain cancer tissue and were considered screening failures. Thus, 774 patients underwent a biopsy, surgical tumor resection, and blood or bone marrow sampling as study procedure for cancer tissue collection. The median age at inclusion was 11.6 years (5th–95th percentile range: 2.2; 19.8; range, 0.5–38.5), and 59% were male. Study procedure was performed at a median of the first relapse or progression (range, 1–10). The median time since initial cancer diagnosis was 1.8 years (5th–95th percentile range: 0.4; 9.0; range, 0.1–32.0). Main tumor types were sarcomas (290 patients, 37%), central nervous system (CNS) tumors (216, 28%), other solid tumors (181, 23%), leukemia (54, 7%), and lymphomas (33, 4%; Table 1; Supplementary Table S1).

The 774 patients underwent 833 procedures for cancer tissue sample acquisition. Among patients with solid tumors and lymphomas, the procedure was performed exclusively for MAPPYACTS in 62%. Fifty-three patients (7%) underwent a second procedure and six a third procedure, most due to insufficient material, unsuccessful tumor sequencing, or progression on targeted treatment. Biopsy was the most frequent procedure performed in 55% patients; procedures were done in 53% from metastatic sites. Sixty-seven procedure-related adverse events were reported in 55 surgical or biopsy procedures (6% of on-purpose and 9% of therapeutic/diagnostic ones), most frequently bleeding/hematoma and pneumothorax. Procedure-related adverse events were grade 1 (17), grade 2 (31), grade 3 (12), and grade 4 (7) in the 833 cases.

Molecular profiling was performed on 695 (84%) samples from 679 (88%) patients. For 138 samples (16%), no sequencing analysis was done, mainly because of low tumor cell content. Seven cases (1.1%) were considered secondary malignancies (all identified by pathology review performed on the sample, which served for sequencing). Six cases were classified as likely radiation-induced, and three were with a family history of cancer (Supplementary Table S2).

### Molecular Tumor Board and Clinical Molecular Tumor Board Recommendations

Successful tumor sequencing was seen in 632 samples from 624 patients with WES, RNA-seq, and/or panel sequencing analysis (in three cases; Fig. 1 and Table 1): 91% (628/691) using WES or 90% (550/614) RNA-seq. Lower tumor cellularity and DNA/RNA quality were the main reasons for unsuccessful tumor sequencing.

Profiling coupled with pathology review suggested a revision of the initial diagnosis in 12 patients (1.9%) and in eight cases through the identification of specific gene fusions (Supplementary Table S2). Genetic counseling was recommended for 51 of 674 patients with a potentially relevant germline WES finding (7.6%).

We defined genetic somatic or germline alterations as "potentially actionable" when the detected molecular alteration or affected pathway in the patient's tumor or germline analysis would be theoretically targetable by an approved or investigational agent, either directly or indirectly in the affected pathway. With this definition, "potentially actionable" therapeutic targets were identified among 436 of 632 (69%) samples in 432 of 624 (69%) patients. Of 1,144 "potentially actionable" findings, 533 were single-nucleotide variants (SNV; 484 somatic, 49 germline), 527 focal copy-number alterations (CNA; 212 amplifications/high-level gains, 315 deletions), 59 targetable gene fusions, and 25 elevated tumor mutational load (Figs. 2 and 3A and B).

One to seven (median: 2) treatment recommendations for targeted agents as single agent or in combination were given per patient involving mainly inhibiting agents of WEE1 ($n$ = 150), mTOR ($n$ = 123), CDK4/6 ($n$ = 105), MEK ($n$ = 95), PARP ($n$ = 64), BET ($n$ = 59), EZH2 ($n$ = 38), FGFR ($n$ = 31), and PD1/PDL1 ($n$ = 31). Suggested targeted agents alone or in combination for the oncogenic alterations are presented in Fig. 4.

To guide treating physicians when prioritizing targets, we used an algorithm based on the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) evidence scale (27) that we adapted to the pediatric cancer context. It considers biological relevance, clinical evidence, and drug availability within clinical trials of targeting the molecular alteration. Forty-four of the 432 patients with potentially actionable alterations (10%) had a recommendation for the specific treatment at relapse that we considered as "ready for routine use" and for which significant clinical activity had been reported (6–18), that is, gene fusions involving *ALK* ($n$ = 10), *BRAF*

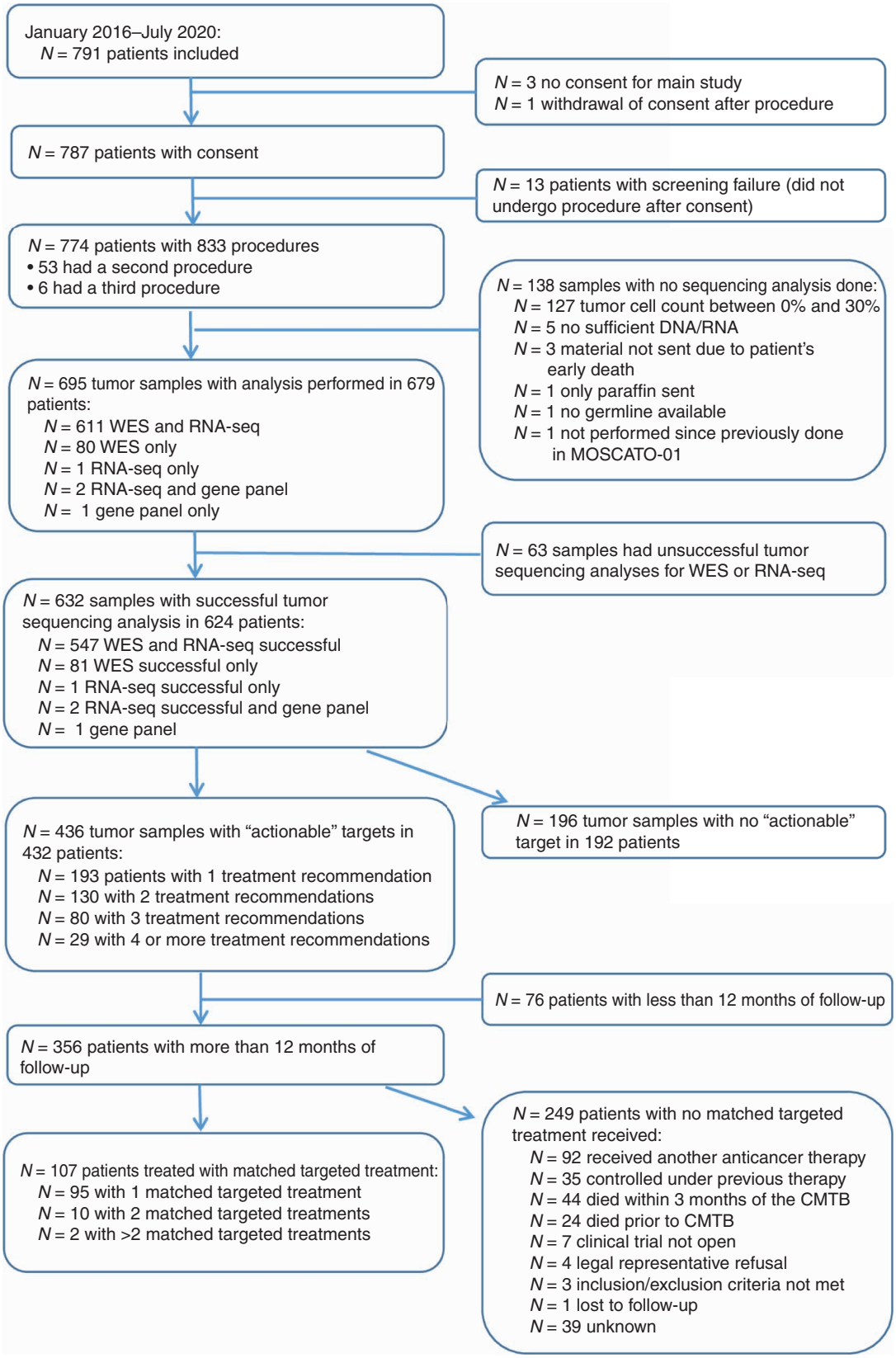


**Figure 1.** Study flow chart. Paired tumor and germline samples were analyzed in all patients with WES. CMTB, clinical molecular tumor board.

**Table 1. Characteristics of patients, sampling study procedures, molecular profiling results, and matched treatments received per tumor types in the MAPPYACTS trial**

| Diagnoses | Patients *n* = 774 | Study procedures *n* = 833 | Successful sequencing analysis *n* = 632 (74%) | ≥1 actionable alteration *n* = 436 (69%) | Ready for use alteration *n* = 44 (10%) | Investigational or hypothetical[a] alterations (in genes altered in >3 patients) | ≥1 actionable alteration and FU >12 months *n* = 356 (82%) | Targeted matched treatment *n* = 107 (30%) |
|---|---|---|---|---|---|---|---|---|
| **SARCOMAS** | 290 | 317 | 248 | 165 (68) | 5 (3) | | 139 | 39 (23) |
| Osteosarcoma | 79 | 90 | 69 (77) | 56 (83) | 0 | *TP53*, *CDKN2A/B*, *EGFR/ERBB2/3/4*, *MYC*, *RB1*, *TP53*, *AKT1/2/3*, *CDK4*, *IGF1R*, *MAP2K4*, *NOTCH1/2/3/4*, *PTEN* | 50 | 14 |
| Ewing sarcoma | 71 | 74 | 56 (76) | 23 (43) | 0 | *CDKN2A/B*, *TP53* | 21 | 2 |
| BCOR or CIC sarcoma | 6 | 6 | 4 (66) | 1 (25) | 0 | | 1 | 0 |
| Other bone sarcoma | 1 | 2 | 1 (50) | 0 (0) | 0 | | 0 | 0 |
| Rhabdomyosarcoma (RMS) | 70 | 77 | 62 (81) | 51 (85) | 0 | *TP53*, *FGFR1/2/3/4*, *CDKN2A/B*, *MYCN*, *CDK4*, *PI3KCA* | 44 | 14 |
| Alveolar RMS | 34 | 37 | 30 (81) | 22 (76) | 0 | *TP35*, *MYCN*, *CDK4* | 20 | 10 |
| Embryonal RMS | 30 | 34 | 27 (79) | 25 (96) | 0 | *TP53*, *FGFR1/2/3/4*, *CDKN2A/B* | 20 | 3 |
| RMS NOS | 6 | 6 | 5 (83) | 4 (80) | 0 | | 4 | 1 |
| Non-RMS soft-tissue sarcoma (NRSTS) | 63 | 68 | 56 (82) | 34 (52) | 0 | *SMARCB1*, *TP53*, *CDKN2A/B*, *NF1* | 25 | 9 |
| DSRCT | 9 | 9 | 7 (78) | 1 (12) | 0 | | 0 | 0 |
| MPNST | 5 | 5 | 5 (100) | 5 (100) | *ROS1-GOPC* (1) | *NF1* | 5 | 3 |
| Synovial sarcoma | 7 | 9 | 6 (67) | 1 (17) | 0 | | 1 | 1 |
| Undifferentiated sarcoma | 8 | 9 | 8 (89) | 6 (75) | *NTRK3-ETV6* (1), *NTRK1-LMNA* (1) | | 5 | 1 |
| Rhabdoid tumor | 10 | 10 | 9 (90) | 9 (100) | 0 | *SMARCB1* | 5 | 0 |
| Other NRSTS | 24 | 26 | 21 (81) | 12 (57) | *ALK-MYH9* (1), *COL1A1-PDGFB* (1) | | 9 | 4 |
| **OTHER SOLID TUMORS** | 181 | 199 | 138 (69) | 93 (67) | 1 (1) | | 69 | 20 (21) |
| Neuroblastoma | 104 | 117 | 73 (62) | 55 (75) | 0 | *MYCN*, 11q, *ALK*, *CDKN2A/B*, elevated mutational rate, *HRAS*, *TP53* | 37 | 8 |
| Carcinoma | 29 | 30 | 24 (77) | 11 (48) | *RET-CCDC6* (1) | *TP53* | 11 | 2 |
| Wilms tumor | 27 | 29 | 24 (83) | 16 (64) | 0 | *TP53* | 13 | 7 |
| Hepatoblastoma | 8 | 8 | 8 (100) | 3 (37) | 0 | | 2 | 1 |
| Other solid tumors | 13 | 15 | 9 (62) | 8 (89) | 0 | | 6 | 2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **CNS TUMORS** | 216 | 226 | 184 (82) | 125 (56) | 28 (22) | | 103 | 47 (38) |
| High-grade glioma | 59 | 64 | 56 (87) | 54 (96) | See subsections mid-line/non-midline | *TP53, CDKN2A/B, MLH1/MSH1/6*, elevated mutational rate, *PDGFRA*[a], *BRAF, NF1, PI3KCA, PIK3R1, KDR, KIT* | 45 | 13 |
| Midline | 24 | 26 | 24 (92) | 22 (92) | *NTRK1-BCAN* (1), *NTRK2-TLE4/KANK1/2* (3) | *TP53, CDKN2A/B* | 20 | 13 |
| Non-midline | 35 | 38 | 32 (84) | 32 (100) | *BRAF*$^{V600E}$ (4), *NTRK2-NACC2/TLE4* (2) | *TP53, CDKN2A/B*, elevated mutational rate, *MLH1/MSH1/6, PIK3CA, PTEN, PDGFRA*[a], *BRAF, NF1, PTEN* | 25 | 14 |
| Low-grade glioma | 23 | 24 | 17 (71) | 17 (88) | *BRAF-KIAA1549* (10), *NF1* germline (2), *ROS1-GOPC* (1) | | 12 | 2 |
| Medulloblastoma | 52 | 53 | 44 (83) | 24 (55) | *PTCH1* (4) mut/del, *TP53/SMO* wild-type | *TP53, EGFR/ERBB2/3/4, MYC* | 20 | 10 |
| Ependymoma | 34 | 36 | 30 (83) | 7 (23) | 0 | | 7 | 3 |
| Infratentorial | 23 | 25 | 20 (80) | 2 (10) | 0 | | 2 | 1 |
| Supratentorial | 11 | 11 | 10 (91) | 5 (50) | 0 | | 5 | 2 |
| Atypical teratoid rhabdoid tumor | 10 | 11 | 6 (55) | 6 (100) | *NTRK3-SPECC1L* (1) | *SMARCB1* | 6 | 3 |
| CNS germ cell tumors | 7 | 7 | 5 (71) | 4 (80) | 0 | | 3 | 0 |
| Choroid plexus carcinoma | 6 | 6 | 5 (83) | 4 (80) | 0 | | 2 | 0 |
| Other CNS tumors | 25 | 25 | 21 (84) | 7 (67) | 0 | | 5 | 2 |
| **LEUKEMIA** | 54 | 57 | 46 (81) | 38 (81) | 1 (3) | | 32 | 1 (1) |
| B-acute lymphoblastic leukemia | 20 | 20 | 18 (90) | 15 (83) | 0 | *CDKN2A/B, KRAS, NRAS* | 14 | 0 |
| T-acute lymphoblastic leukemia | 14 | 15 | 13 (97) | 12 (92) | 0 | *CDKN2A/B, NOTCH1/2/3/4* | 10 | 1 |
| Acute myeloid leukemia | 17 | 19 | 12 (63) | 9 (69) | *IDH1*$^{R132L}$ | | 6 | 0 |
| Other leukemia | 3 | 3 | 3 (100) | 2 (67) | 0 | | 2 | 0 |
| **LYMPHOMA** | 33 | 34 | 16 (47) | 15 (94) | 9 (60) | | 13 | 0 (0) |
| Hodgkin lymphoma | 6 | 6 | 0 (0) | 0 (0) | 0 | | 0 | 0 |
| Anaplastic large-cell lymphoma | 15 | 16 | 9 (56) | 9 (100) | *ALK-NPM1/TFG/TRAF1* (9) | | 8 | 0 |
| Other non-Hodgkin lymphoma | 12 | 12 | 7 (58) | 6 (86) | 0 | | 5 | 0 |

Abbreviations: DSRCT, desmoplastic small round cell tumor; FU, follow-up; MPNST, malignant peripheral nerve sheath tumor; NOS, not otherwise specified.

[a]Hypothetical evidence-level alterations.

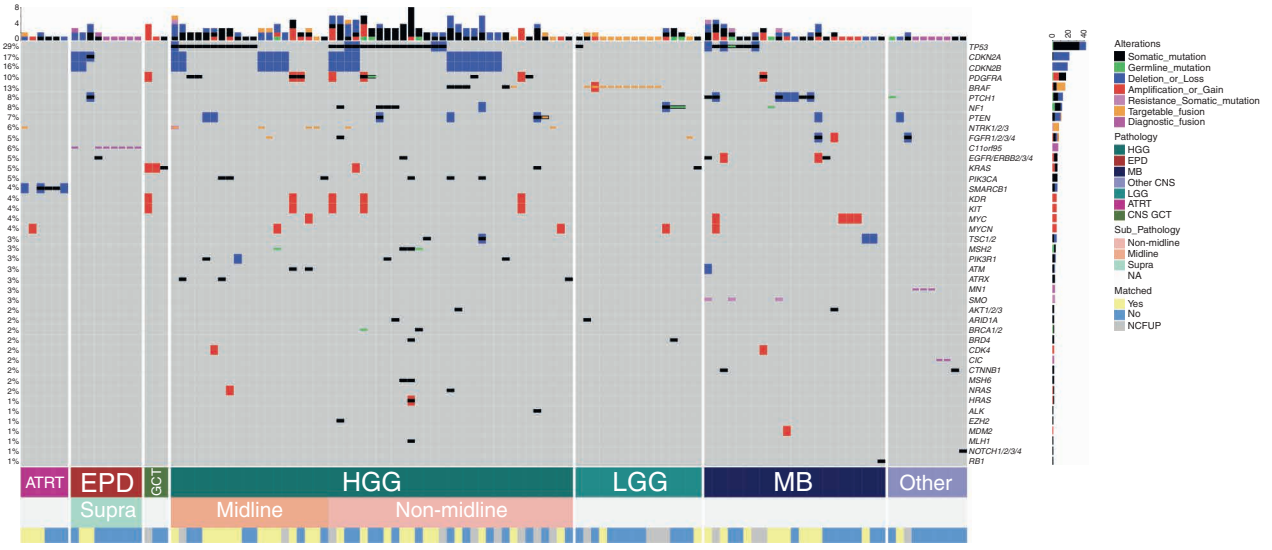


**Figure 2.** Oncomap of potentially actionable alterations and canonical fusions in CNS tumors in 118 patients with 119 samples. Tumor types with five or fewer samples are grouped together in "Other." Only alterations identified five or more times within the whole study are reported here. ATRT, atypical teratoid rhabdoid tumor; EPD, ependymoma; GCT, CNS germ cell tumor; HGG, high-grade glioma; LGG, low-grade glioma; matched, matched therapy received according to recommendations; MB, medulloblastoma; NA, not applicable; NCFUP, follow-up < 12 months; Other, other CNS tumors; subpathology, pathology subtype; supra, supratentorial.

(10), *NTRK1/2/3* (9), *ROS1* (2), *COL1A1/PDGFB* (1), and *RET* (1), pathogenic mutations in *BRAF* (4), *NF1* (2), and *IDH1* (1), or mutation or deletion in *PTCH1* (4) in selected tumor types (Table 1). Nineteen of them (42%) were previously unknown (Supplementary Table S3). Most alterations (909/1,144; 80%) were considered on the "investigational" level, which refers to oncogenic events for which the relevance of matched experimental treatments has been reported with limited activity or is being explored in the clinical setting. These alterations included gene amplifications, fusion, or activating mutations in *MYCN/MYC* (43), *KRAS/NRAS/HRAS* (42), *FGFR1/2/3/4* (27), *PIK3CA/PIK3R1/2* (23), *ALK* (15), and deletions or deleterious mutations in *TP53* (215), *CDK2NA/B* (157), *NF1* (33), *SMARCA4/SMARCB1* (27), *PTEN* (25), and *RB1* (11; Table 1). Ten cases were found with mutations in *NTRK* (3), *SMO* (3), *ALK* (3), and *NRAS* (1) that may be associated with treatment resistance; all except one had previously received the specific NTRK, SMO, ALK, or BRAF/MEK inhibitors, respectively, and benefited from the procedure at treatment failure.

These retained treatment suggestions were then discussed in the clinical molecular tumor board (CMTB) between experts in new drug development, experts in tumor diseases, and the treating physician considering the patient's history and other treatment options that may be of relevance to the patient.

## Patients' Treatment and Follow-up after CMTB Recommendations

Following CMTB recommendations, patients with a minimum follow-up of 12 months (censored in September 2020) were analyzed. Three hundred fifty-six of the 432 patients with "potentially actionable" alterations had a minimum follow-up of 12 months and 107 (30%) of them had received 122 matched targeted therapies (range, 1–4/patient). Fourteen (11%) therapies were considered "ready for routine use," 97 (80%) were "investigational," and 11 (9%) "hypothetical." Sixty-four (52%) treatments were administered as a single agent, 45 (37%) in combination with chemotherapy, and 13 (11%) combined with another targeted therapy. Sixty-eight (56%) of these treatments were performed within a phase I/II trial, 49 of them (72%) within AcSé-ESMART (NCT02813135).

Of the 249 patients with potentially actionable alterations, main reasons for not receiving the suggested matched treatment were death prior to the CMTB ($n$ = 24, 10%) or within the 3 months that followed ($n$ = 44, 18%), other nonmatched therapies ($n$ = 92, 37%), or effective ongoing therapy ($n$ = 35, 14%). Sixteen of the 44 patients (36%) with ready for routine use suggestions had received the recommended matched treatment previously—14 prior to the study procedure and two after the procedure and before the CMTB; in four of them, a second- or next-generation matched agent was administered after the CMTB.

## Outcome of Patients Treated with Matched Therapies

Disease response was not evaluable in four patients because matched targeted treatment was received after complete tumor resection, and data were missing for nine patients. In 109 cases with evaluable or measurable disease and response evaluation, 18 were reported to have had partial response [PR; 17% objective response rate (ORR); 95% confidence interval (CI), 10%–25%) and 27 stable disease (SD; 25%), leading to a 41% disease control rate (DCR; 95% CI, 32%–51%). Median treatment duration for the 45 patients with controlled disease was 129 days (range, 58–697 days).

For patients with ready for routine use alterations, ORR was 38% (5/13; 95% CI, 18%–65%); all treatments were administered as single agents. Lower-level evidence recommendations ("investigational" and "hypothetical") resulted in a 14% ORR (13/96; 95% CI, 32%–51%). The ORR for patients with


**Figure 3.** Oncomap of potentially actionable alterations and canonical fusions in sarcoma in 213 patients with 215 samples (**A**) and other solid tumors in 86 patients with 86 samples (**B**). Tumor types with five or fewer samples are grouped together in "Other." Only alterations identified five or more times within the whole study are reported here. aRMS, alveolar rhabdomyosarcoma; CA, carcinoma; DSRCT, desmoplastic small round cell tumor; eRMS, embryonal rhabdomyosarcoma; EWS, Ewing sarcoma; matched, matched therapy received according to recommendations; MPNST, malignant peripheral nerve sheath tumor; NA, not applicable; NBL, neuroblastoma; NCFUP, follow-up < 12 months; NOS, not otherwise specified; NRSTS, non-rhabdomyosarcoma soft-tissue sarcoma; OS, osteosarcoma; Other, other solid tumor; RMS, rhabdomyosarcoma; RT, rhabdoid tumor; SS, synovial sarcoma; subpathology, pathology subtype; US, undifferentiated sarcoma; WT, Wilms tumor.

investigational alterations was 14% (12/86; 95% CI, 7%–23%), and for those with hypothetical alterations, it was 10% (1/10; 95% CI, 0%–44%). This study population treated with a matched targeted agent and chemotherapy combination had an ORR of 18% (8/44; 95% CI, 8%–33%), those of single-agent matched targeted treatment of 13% (5/39; 95% CI, 4%–27%), and those of targeted therapy combinations of 0% (0/13; 95% CI, 0%–24%).

## Associated Ancillary Studies

### *Tumor Mutational Burden*

Tumor mutational burden (TMB) was recalculated retrospectively on harmonized raw data in the first 451 enrolled patients with solid malignancies and 452 successfully sequenced WES tumor samples. A median of 0.6 mutations per megabase (mut/Mb; range, 0–195.3 mut/Mb; Fig. 5) was found. Seven patients exhibited >10 mut/Mb [three high-grade glioma (one of them >100 mut/Mb), three neuroblastoma, one medulloblastoma], and three were in the context of a probable, previously unknown constitutional mismatch repair deficiency involving *MSH2*, *MLH1*, and *MSH6* genes. Five tumors displayed microsatellite instability (Supplementary Fig. S1).

### *cfDNA and Circulating Tumor DNA*

To explore the role of liquid biopsies and the possibility of detecting tumor-specific molecular alterations in advanced pediatric diseases, plasma samples at the time of tumor tissue collection were obtained in all cases. Among the first 500 enrolled patients, cfDNA extracted from the plasma of 234 patients with extracerebral solid tumors was analyzed (Supplementary Fig. S2). Nine had technical failure, and 225 were considered for further analysis. cfDNA quantities showed significant differences among different cancer types (ANOVA test: $P = 1.4e{-}03$), with the highest cfDNA quantities observed in the 43 patients with neuroblastoma compared with the 182 other tumors (Wilcoxon test: 3e–04; Fig. 6A). The circulating tumor DNA (ctDNA) content in cfDNA, as evaluated by bioinformatics methods, also varied significantly between cancer types (ANOVA test: $P = 2.35e{-}05$), with highest values in patients with neuroblastoma compared with other disease types: median 35% ctDNA content (range, 0%–95%) versus median 16% (range, 0%–86%), respectively (Wilcoxon test: 1.4e–08; Fig. 6B).

Among 190 patients for whom tumor WES analysis was deemed successful, cfDNA WES was considered successfully sequenced in 128 of 190 samples (67%) and was more frequent

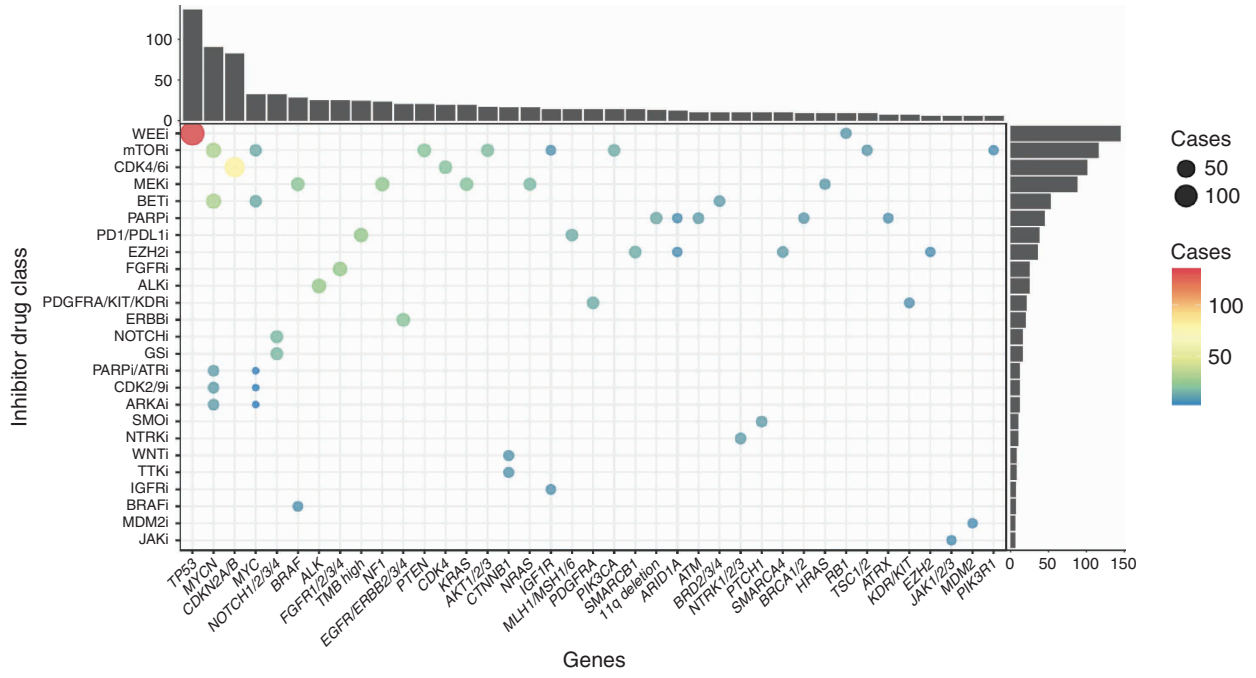


**Figure 4.** Matched targeted treatment recommendations per genetic alterations (only one alteration by gene and those occurring in more than five cases are represented). GSi, gamma-secretase inhibitor; PD1/PD1Li, programmed cell death protein 1/ligand immune checkpoint inhibitors; TMB, tumor mutational burden.

in patients with metastases than localized disease (66% vs. 47%, respectively, $\chi_2$ *P* value: 0.04). Among these 128 cfDNA samples, cfDNA WES showed both somatic CNAs and SNVs in 51 cases, whereas in the remaining 77 cases, only somatic SNVs were observed. Detailed analysis of the SNVs indicated that among the 128 cases, a median of 15 somatic SNVs (range, 0–103) were common to plasma and tumor, with a median of six somatic SNVs (range, 0–127) observed only in tumor and a median of one somatic SNV (range, 0–460) only in plasma, with no significant differences between different tumor types (Supplementary Fig. S3). In this patient group (190 patients with extracerebral solid tumors and successful tumor WES analysis), a total of 94 SNVs were considered potentially actionable. Of these, 71 SNVs observed in the tumor were also identified in plasma by cfDNA WES (76%; Fig. 6C and D). Importantly, among 14 of these patients, 35 somatic SNVs in potentially actionable genes were observed by cfDNA WES that were not detected by tumor WES (median per patient 1; range, 1–13). This included, among others, an *ALK* p.Arg1275Gln mutation seen only in the plasma (Fig. 6D).

Concerning the 35 patients for whom tumor WES was either not done ($n = 16$) or unsuccessfully sequenced ($n = 19$), cfDNA WES was considered successful in 11 patients (34%), with a total of 12 SNVs targeting actionable genes identified in five patients.

## DISCUSSION

MAPPYACTS reports the feasibility and outcome of an international precision medicine trial in pediatric and young adult patients performing on-purpose cancer tissue collection for molecular characterization of recurrent or refractory malignancies. The study is the first to explore the role of liquid biopsy for cfDNA analysis in this population.

Consistent with our preliminary monocentric experiences (19, 20) and other reports (19, 21–26), 69% of patients in MAPPYACTS had one or more genomic alterations in their tumor that we considered "potentially actionable" through targeted agents. Importantly, the study procedures proved safe, and 30% of the patients subsequently underwent a matched targeted treatment.

We show that 4% of all defined potentially actionable gene alterations in 10% of patients were considered "ready for routine use," and the matched single-agent targeted treatment resulted in a 38% ORR in patients receiving this treatment after the CMTB, which was significantly higher than in lower-level evidence treatments (14%). In addition, out of 12 evaluable patients who received such a matched treatment prior to MAPPYACTS inclusion, seven had a PR and two a CR (75% ORR), resulting globally in a 56% ORR (95% CI, 37%–73%) for matched therapies for this highest evidence–level category. This is in line with recent data of the INFORM registry (26). Most of them are identical to our highest-level alterations, albeit we have not considered for example *HRAS/KRAS* and *ALK* mutations because of the limited clinical activity of MEK and ALK inhibitors in patients with these alterations (35–38). Importantly, in half of our patients, the identified high-level evidence gene alterations were not identified in previous routine molecular diagnostics, underlining the importance of more comprehensive molecular profiling in matching rare genomic alterations with available effective targeted therapies. Search for high-level evidence gene alterations should now be part of front-line molecular diagnostics and will allow us to introduce active new targeted agents into standard treatment.

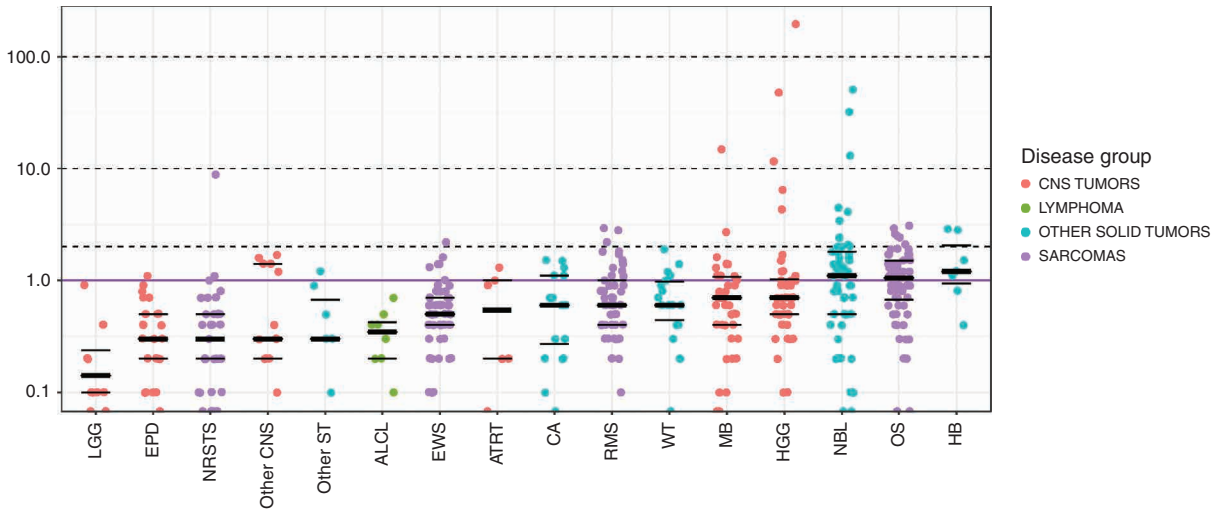


**Figure 5.** Tumor mutational load in main cancer types (only disease types with more than five cases are presented). Median mutational loads and quartiles 25 and 75 are shown as black solid lines for each tumor type. Samples with 2 to <10 mut/Mb are considered pediatric high, 10 to <100 hypermutators, and >100 ultrahypermutators. ALCL, anaplastic large cell lymphoma; ATRT, atypical teratoid rhabdoid tumor; CA, carcinoma; EPD, ependymoma; EWS, Ewing sarcoma; HB, hepatoblastoma; HGG, high-grade glioma; LGG, low-grade glioma; MB, medulloblastoma; NBL, neuroblastoma; NRSTS, non-RMS soft-tissue sarcoma; OS, osteosarcoma; Other ST, other solid tumors; RMS, rhabdomyosarcoma; WT, Wilms tumor.

An additional direct clinical impact through profiling and pathology review was found in 3.0% of patients with diagnostic modifications, mainly through detection of gene translocations in CNS tumors and sarcomas, or a secondary cancer diagnosis. In the INFORM registry, a change or refinement of diagnosis was identified in 8.2% of cases, which can in part be explained through the inclusion of samples at diagnosis (26). These observations again confirm the importance of performing selected gene DNA analysis as well as RNA-seq ± methylation in diagnostically challenging cases.

Furthermore, we detected mutations known to refer resistance to targeted therapies in 10 of our patients; nine of them had progressed on the specific targeted treatment, highlighting the clinical relevance of considering molecular profiling at targeted treatment failure to define resistance mechanisms and adapt future salvage strategies.

Overall ORR of patients in MAPPYACTS was 17% and 14% for lower-level evidence treatments, thus superior to the reported 4% ORR observed in pediatric phase I/II chemotherapy trials (27) and similar to the outcome in the targeted therapies era (28). The Zero Childhood Cancer Program reported a 31% ORR (11/35 patients); however, half of the patients were included at diagnosis, and the ORR for patients at the time of disease recurrence was not given (29).

This raises the debate on the definition of "actionable" or "potentially actionable" alterations, which has been used inconsistently throughout the programs. For this clinical research trial, taking into account the low numbers of high-level evidence alterations, we wished not only to limit the report to clearly defined oncogenic driver events with straightforward treatment recommendations but also to describe alterations in genes that could lead to a potential benefit according to preclinical findings or clinical data in adult cancers. Importantly, 96% of our reported "potentially actionable" oncogenic events matched with treatment suggestions were considered at an "investigational" (80%) or "hypothetical" (16%) evidence level. Albeit these alterations are well-known oncogenic events, their direct targeting, alone or in combination, has not been demonstrated with significant clinical activity, the alteration–treatment match is currently evaluated in clinical trials, or their match has been suggested in preclinical data, respectively. The high frequency of these often multiple alterations, absence of relevant clinical data, and lack of curative options in children and adolescents with relapsed/refractory malignancies led us to run a parallel project to tumor profiling, which explores targeted molecules or combinations in molecularly enriched patient populations. The "Secured Access–European proof-of-concept therapeutic Stratification trial of Molecular Anomalies in Relapsed or refractory Tumors" (AcSé-ESMART) platform trial (NCT02813135) started recruitment in July 2016, is currently open in five countries, and has recruited more than 190 patients, 78% of them referred through the MAPPYACTS trial, in addition to other European profiling programs. The primary objective is to detect signals of activity and potential new biomarkers. Treatment arms in AcSé-ESMART mainly address targeting of alterations in the cell cycle, the PI3K/mTOR pathway, homologous DNA repair, and immune checkpoint (30–32). TP53 alterations were considered investigational in MAPPYACTS based on data in ovarian cancer, suggesting that TP53 deficiency possibly associated with response to WEE1 inhibitors (33), and which we explored in combination with chemotherapy in the trial. The medical need for such clinical trials is reflected by the fact that 56% of the matched treatments in MAPPYACTS patients could be administered within the context of phase I/II clinical trials and 72% of them had been included in AcSé-ESMART. Preliminary data for the ongoing INFORM registry reported 28% (11% in a clinical trial) and the NCI–COG Pediatric Molecular Analysis for Therapeutic Choice (MATCH) trial (NCT03155620) 35% of patients treated with a matching drug (25, 34). The fact that most advanced cancers have no unique key drivers but rather

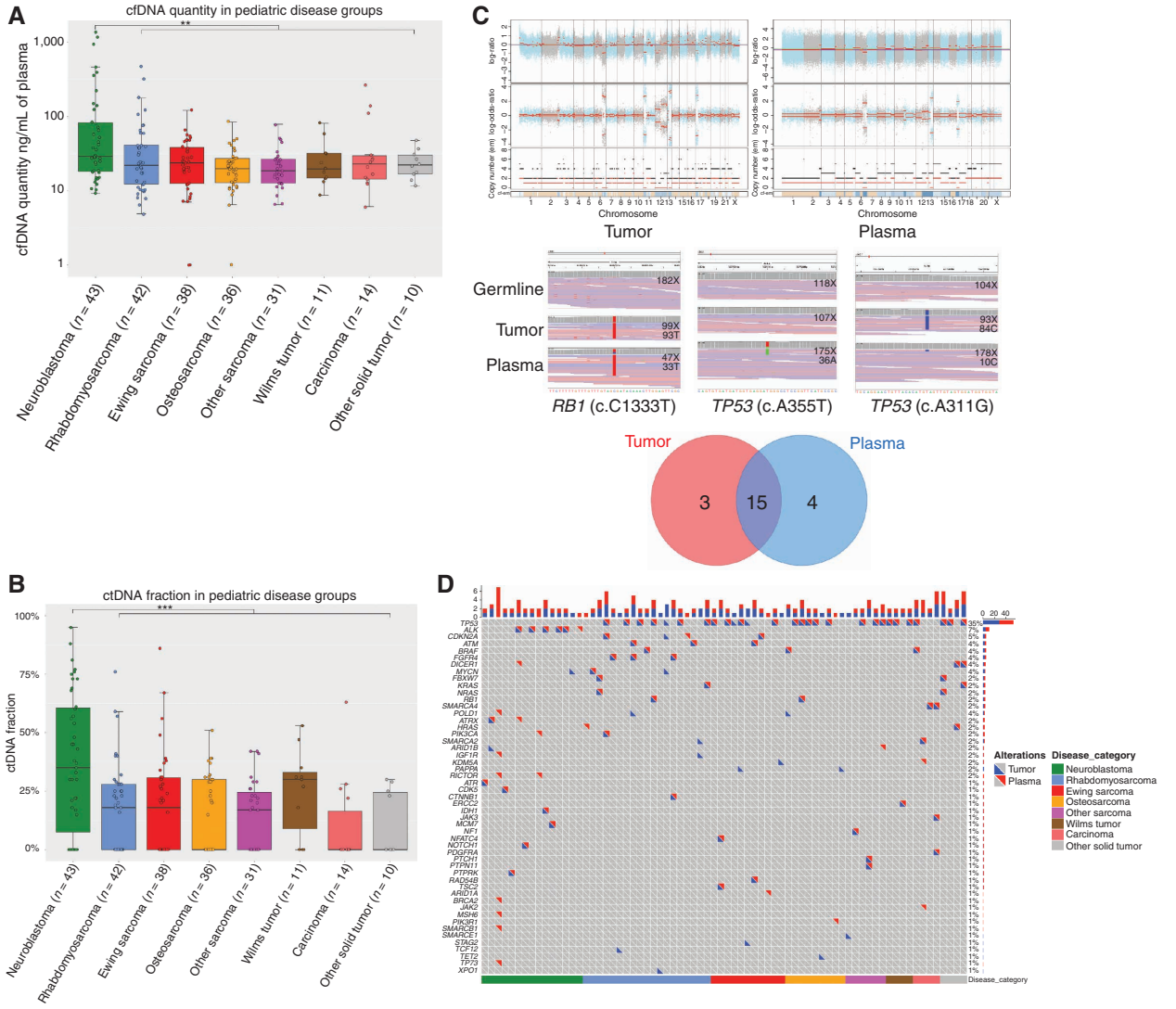


**Figure 6.** cfDNA and ctDNA ancillary study in 225 patients with extracranial solid tumors. **A,** cfDNA quantity per type of tumor (ng/mL of plasma, $\log_{10}$ scale). **, indicates statistically significant difference (Wilcoxon test: 3e−04). **B,** ctDNA fraction calculated by the Facet tool after WES for each type of tumor. ***, indicates statistically significant difference (Wilcoxon test: 1.4e−08). **C,** Example of a sequencing result for a patient with an alveolar rhabdomyosarcoma. On the top, copy-number profiles generated by the Facet tool, showing the same chromosome alterations identified in the tumor/plasma. Middle, *RB1* c.C1333T and *TP53* c.A311G mutations were identified in tumor and plasma, whereas *TP53* c.A355T was observed only in the plasma. Bottom, Venn diagram of SNVs detected in each type of samples with a good overlap (15 SNVs common between tumor and plasma). **D,** Oncoprint of SNVs in targeting potentially actionable genes detected in tumor (blue) and plasma (red) for 72 cases with both successful tumor and cfDNA analysis (only cases with at least one SNV in an actionable gene are reported here). Histograms on the top indicate the total number of SNVs in actionable genes in each case (blue, tumor; red, plasma); histograms on the right indicate the percentage of cases with alterations in the indicated actionable gene.

multiple oncogenic events in various cancer hallmarks (35) demonstrates the underlying cancer complexity to which current clinical trials are not yet perfectly adapted. Therefore, we wish to stress the importance of detailed comprehensive tumor board discussions for pondering the subjectivity of the treatment suggestions. Such discussions between physician-scientists, experts in new drug development and tumor diseases, as well as the treating physicians must consider the potential relevance of the selected targets based on current knowledge of tumor biology and outcome in clinical trials, the expertise on novel anticancer strategies, availability of the drug, availability of an open clinical trial, as well as other treatment options that may be relevant for the patient.

This consensus proposition must be shared in a comprehensive way with patients and parents so they may decide on the participation of such a treatment option given the uncertainty of their outcome. We insist that whenever possible these experimental treatments should be administered and explored within investigational clinical trials to improve knowledge and optimize safety for these patients. Furthermore, the time point of integration of advanced profiling in each patient's care is critical. MAPPYACTS is to date the only pediatric trial where the tumor sample acquisition intervention was performed within the study. Most of our patients were included at first relapse/progression and received tumor-specific first salvage treatment while waiting for the molecular profiling results.

Although theoretically it would be preferable to treat patients based on the sequencing results of the current relapse, avoiding further temporal tumor evolution, this pragmatic approach was deemed suitable for most patients and, as done in most other programs, with advanced molecular profiling (25, 26, 29).

MAPPYACTS further confirms the incidence of germline findings and cancer predisposition syndromes in 7% to 12% of pediatric patients, consistent with the data of others (19, 26, 36–38). The variation in numbers may relate to different predefined lists of known predisposition genes that were used. Furthermore, our data may suggest that TMB may be higher at disease recurrence than in newly diagnosed patients, as reported previously in a lower number of patients (39). Future comparison of paired primary and relapse tumor samples from the same patients will be necessary to confirm these findings and correlate these with treatment. All findings support the clinical approach of performing tumor profiling at recurrence or treatment failure.

MAPPYACTS is the first study to explore cfDNA analysis as a noninvasive technique to detect actionable alterations in recurrent pediatric and adolescent non-CNS solid tumors. Higher cfDNA quantities, and a higher content of ctDNA in cfDNA, were observed in patients with neuroblastoma compared with other disease types, confirming previous studies of the feasibility of ctDNA studies in these patients with different analytical techniques such as low-coverage whole-genome sequencing, WES, or droplet digital PCR (40). Other processes such as inflammation, systemic infection, or even pregnancy can lead to higher cfDNA content; however, the described pipeline aimed to identify tumor cell–specific genetic alterations specifically. Overall, cfDNA WES led to successful sequencing results in 62% of cases. Unsuccessful sequencing might be linked to low ctDNA content, which might be observed in patients with a low overall tumor burden. Different technical limitations might explain a low sequencing success rate in some cases. Given the approach by cfDNA WES, this study is limited to the detection of SNVs. Importantly, in 77 of 126 cases, although no CNAs could be identified, somatic SNVs could be detected, based on the higher sensitivity of WES for detection of SNVs (threshold for detection: 5% mutated allele fractions) than CNA (threshold: 20%; ref. 41). Furthermore, different capture techniques between the tumor versus plasma analysis hampered CNA analysis. However, the different capture techniques were accounted for in the bioinformatics pipeline, enabling the reliable calling of SNVs, with a detection limit of 1%, as reported previously (41).

Among all detected somatic SNVs in cases with successful cfDNA WES analysis, 57% were seen in both the cfDNA and the tumor, with 31% and 11% specific to the tumor and the cfDNA, respectively, possibly reflecting tumor heterogeneity. Tumor heterogeneity has been widely reported in pediatric cancers, with both spatial heterogeneity of the primary tumor itself, or genetic heterogeneity between the primary and metastatic sites. Based on our data, cfDNA analysis provides an important tool for the exploration of genetic heterogeneity in solid pediatric malignancies at the time of relapse. Altogether, somatic SNVs were detected in all analyzed tumor types, highlighting the importance of cfDNA WES, which might result in a different perspective of the disease. Although the cfDNA WES analysis was performed as an ancillary study and did not guide treatment recommendations, cfDNA WES identified 76% of all actionable alterations found in the matched solid non-CNS tumors. Our study underlines that in some instances, like neuroblastoma, where osteomedullary relapses are often not successfully sequenced for WES, or in patients without possibility of a new tumor biopsy, cfDNA extracted from plasma might lead to clinically relevant results. Furthermore, recent reports demonstrate the feasibility of cfDNA extracted from CSF for patients with CNS malignancies (42).

An additional ancillary research study in MAPPYACTS was the development of patient-derived xenografts (PDX). During the 4 years of the study, nine participating research laboratories have established 131 xenografts (43). The characterization of the PDX and their comparison with the primary tumors are ongoing. They represent a valuable tool for future research studies, and most of them have been shared with the Innovative Medicines Initiative (IMI2) ITCC-P4 project (https://www.itccp4.eu).

The MAPPYACTS trial was a dynamic process, undergoing continuous adaptation since commencing in 2016. CMTB interpretations and recommendations were made to the best of our knowledge at a given date, representing one limitation of the trial. Evidence levels for most targets will change based on current scientific knowledge, which is rapidly evolving and informed by ongoing research. Various and multiple oncogenic events that are not unique drivers in the diseases contribute to the complexity of cancers, and their biological relevance as well as their therapeutic targeting is mostly undefined. In addition, a limited understanding of the function of gene variants of unknown significance and a lack of insight about posttranslational and immunologic contexts are major limits of current precision medicine programs.

Our study further highlights these observations. The sequencing efforts on pediatric tumors at relapse underscore the low proportion of high-level evidence or "ready for routine use" alterations. This strongly underlines that high-throughput molecular profiling together with matched targeted therapies, innovative clinical trials, development of new technologies that allow cancer characterization beyond the genetic level, development of combination strategies, and analysis of the personal immune response of each patient are needed to overcome the current limitations.

In 2020, very high-throughput sequencing using WGS 80×, WES 150×, and RNA-seq has been introduced into the standard of care at relapse or treatment failure for children and adults in France through the Plan France Médecine Génomique 2025 (PFMG2025), launched by the Haute Autorité de Santé (HAS) and supported by the French Ministry of Health within the Plan Cancer 3. However, we believe that high-throughput molecular profiling and a better understanding of tumor biology, leading to matched targeted combination treatments, linking to clinical interventional studies, and defining novel technologies and innovative treatment strategies, will be needed to improve the survival of most high-risk patients. This will be explored within the MAPPYACTS 2 study that will complement the PFMG2025 project and include the validation of liquid biopsies and cfDNA analyses as well as studies on the health economic impact and psychosocial outcomes related to these analyses and treatments. In conclusion, MAPPYACTS underlines the feasibility of molecular profiling

at the time of pediatric cancer recurrence on a multicenter international level. Although selected high evidence–level alterations should be part of the initial diagnostic workup, cancer complexity justifies the continuous efforts and introduction of high-throughput sequencing and treatment recommendations as a standard of care for high-risk cancers. MAPPYACTS has identified future innovative diagnostic and treatment strategies, including the encouraging results of cfDNA analysis in solid extracerebral tumors, which are deserving of validation in further prospective studies.

## METHODS

### Study Population

Eligible patients were aged ≥6 months with recurrent/refractory solid tumors or leukemia, ≤18 years at cancer diagnosis, independent of the age at the time of disease recurrence, and potentially eligible for an early-phase clinical trial. In the setting of solid tumors, the lesion had to be accessible for biopsy or surgical resection. Patients were required to have adequate performance status, bone marrow, and organ function. Written informed consent was signed by the patient or parents/legal representatives, and assent of the minor child according to local laws to perform the procedure and molecular analysis of the tumor and blood sample, as well as optional ancillary studies, was obtained. Detailed inclusion/exclusion criteria are available in Supplementary Methods.

### Study Design and Procedure

The MAPPYACTS trial recruited from February 2016 until July 2020 in 15 French centers, Italy (from October 2017), Ireland (May 2018), and Spain (September 2019). Tumor biopsy or surgery of the recurrent/refractory malignancy, or bone marrow/blood sample if leukemia, was performed following written informed consent and inclusion in order to collect cancer tissue for molecular profiling specifically for this study. In parallel to the procedure, a blood sample for constitutional DNA extraction and cfDNA was collected. Molecular analyses were performed in the French National Cancer Institute (INCa)–labeled next-generation sequencing platforms Gustave Roussy, Institut Curie, and Centro Nacional de Análisis Genómico Barcelona. In the setting of unsuccessful tumor sample sequencing or progression during targeted treatment (Amendment 5), a new procedure could be offered and performed after new written consent was obtained.

The trial was approved by independent ethics committees and national medical authorities and conducted according to the principles of the Declaration of Helsinki.

### Adverse Event Reporting Related to the Study Procedure

All adverse events definitely/probably/possibly related to the MAPPYACTS study procedure were assessed by the local investigator and reported according to the Common Terminology Criteria for Adverse Events (CTCAE v4.0).

### Sample Analysis

Tumor cellularity in specimens from the sample used for nucleic acid extraction was determined by an experienced pathologist; those with ≥30% tumor cellularity were processed. Tumor DNA, RNA, and germline DNA from whole blood samples were extracted using the AllPrep DNA/RNA Mini Kit and DNeasy Blood and Tissue Kit.

### WES

Sequencing libraries were constructed according to standard procedures from 600 ng of tumor and paired constitutional DNA. WES was captured using the Agilent SureSelect V5 (50 Mb), Clinical Research Exome (54 Mb) kit, SureSelect XT human All exon CRE version 1 or 2, or Twist Human Core Exome Enrichment System. Sequencing of subsequent libraries was performed using Illumina sequencers (NextSeq 500 or HiSeq 2000/2500/4000) in 75-bp paired-end mode, aiming for a mean depth of coverage of 100×.

Bioinformatics processing was based on Illumina Pipeline (CASAVA1.8) or in-house pipelines consisting in alignment of sequencing reads on the hg19 human genome (Build37), determination of structural variants, CNAs, and variant detection, as well as determination of their functional impact (19, 41). In case of low cellularity, gene panel sequencing could be performed (19).

### RNA-seq

Libraries were prepared with TruSeq Stranded mRNA kit, PolyA mRNA capture with oligo dT beads 1 mg total RNA, fragmentation to approximately 400 bp, cDNA double-strand synthesis, and ligation of adaptors, library amplification, and sequencing (2, 19). For the optimized detection of potential fusion transcripts, an in-house designed metacaller approach was used (details on sequencing and bioinformatics in Supplementary Methods; refs. 16–20).

### Mutational Tumor Load

Sequencing library quality was estimated with fastqc and fastqscreen. Reads were mapped with BWA (v0.7.17 with parameters: -M -A 2 -E) onto the human reference genome assembly hg19/GRCh37. SNVs and small indels were called using GATK3 (Indel Realigner, Base Recalibrator), samtools (fixmate, markdup, mpileup), and Varscan (v2.3.9) from paired normal/tumors bam files. Variant annotation was performed with ANNOVAR using the public database released on November 7, 2019, from the 1000 Genomes Project, Exome Aggregation Consortium (ExAC), NHLBI-ESP project, and Kaviar. Functional prediction of variants was performed using the data set dbnsfp30a. Questionable somatic variants observed in less than three reads, with an allele frequency lower than 0.05, described in the 1000 Genomes and ExAC databases with a frequency higher than 0.05%, or nonexonic variants were excluded.

Somatic coding mutations were filtered according to their enrichment in the tumor samples compared with the paired normal samples as reported previously (30). Mutational load was calculated as the number of nonsynonymous somatic variants divided by the total length of targeted regions by the exome capture kits with a minimum coverage of 10×.

### cfDNA

A blood sample for study of cfDNA was collected on EDTA following inclusion in the MAPPYACTS study (mean delay between biopsy of the tumor and blood sample 3.4 days; range, 0–42 days). For patients with non-CNS solid tumors, cfDNA extracted from plasma was quantified using Qubit HS DNA assays for WES at 100× (41). ctDNA content in cfDNA was calculated using the Facets tools. cfDNA WES successful sequencing analysis was defined as a WES profile resulting in any CNA and/or at least three somatic SNVs also seen in the tumor (Supplementary Methods).

### Molecular Abnormality Reporting and Treatment Decision

Sequencing results were reviewed by a molecular geneticist, summarized in a report, and discussed with the physician-scientist core team during weekly MTBs. A successfully sequenced tumor sample was considered when WES identified somatic CNAs or somatic SNVs, when filtering on matched germline, or when RNA-seq identified tumor cell–specific fusion transcripts. SNVs were retained if pathogenic or likely pathogenic. For tumor suppressor genes, homozygous loss-of-function variants and focal deletions were considered; in genes like *TP53* and *ATM*, heterozygous loss-of-function was also reported. Furthermore, heterozygous chr11q deletions encompassing the homologous repair genes *ATM*, *CHK1*, *MRE11A*, and *H2AFX*

were also considered. For oncogenes, focal amplifications and high-level gains were retained. Germline findings were reported in cancer predisposition genes, as published by Zhang and colleagues (38).

In order not to restrict reporting to only a few recurrent molecular events and to provide treatment recommendations for the majority of patients, a wide definition of molecular alterations highlighted in the MTBs was applied. The term "potentially actionable" refers to a detected molecular alteration or affected pathway in the patient's tumor and/or germline analysis that theoretically would be targetable by an approved or investigational agent either directly or indirectly in the affected pathway. Prioritization was based on the clinical evidence of targeting the molecular alteration and considering accessibility of the treatment. Alterations were considered "ready for routine use" if the referred alteration–drug match had been associated with antitumor activity, defined as >30% ORR, or improved outcome in clinical trials in a given tumor type or similar indication (6, 8–14, 16, 18, 44–46). Oncogenic events for which the relevance of experimental treatments was reported with limited activity and/or is being addressed in clinical trials were considered "investigational," and those for which only preclinical data exist "hypothetical." Known solvent front, gatekeeper, or xDFG mutations were classified as "resistance" mutations.

MTB results were then discussed with the treating physician in weekly CMTBs, and recommendations were summarized in a CMTB report that ranked therapeutic options according to available evidence of the retained molecular alterations but included other treatments relevant for the patient at this disease stage. Germline abnormalities were discussed in consenting patients/families; oncogenetic counseling was suggested in case of clear pathogenicity.

### *Outcome Assessments*

For patients receiving recommended matched targeted therapy, tumor response assessed every 6 to 8 weeks from treatment start, according to the standard in each tumor entity [RECIST 1.1, Response Assessment in Neuro-Oncology (RANO), International Neuroblastoma Response Criteria (INRC); refs. 47–49], was recorded. ORR was defined as the proportion of patients who achieved a PR or CR and DCR as ORR and SD.

### *Data Sharing Agreement*

Sequencing data and basic clinical annotations have been deposited in European Genome-phenome Archive (EGA; hosted by the EBI and CRG) with the data set accession code EGAS00001005935. Further information about EGA can be found on https://ega-archive.org ("The European Genome-phenome Archive of human data consented for biomedical research"; http://www.nature.com/ng/journal/v47/n7/full/ng.3312.html). Additional, more detailed clinical data from the clinical trial can be requested by completing the data request form for Gustave Roussy clinical trials (https://redcap.gustaveroussy.fr/redcap/surveys/?s=DYDTLPE4AM) for validation by the trial steering committee and the sponsor prior to transfer of detailed clinical data.

## Authors' Disclosures

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## Authors' Contributions

**P. Berlanga:** Conceptualization, data curation, formal analysis, validation, investigation, methodology, writing–original draft, writing–review and editing. **G. Pierron:** Conceptualization, data curation, formal analysis, validation, investigation, methodology, writing–review and editing. **L. Lacroix:** Conceptualization, data curation, formal analysis, validation, investigation, methodology, writing–review and editing. **M. Chicard:** Data curation, formal analysis, validation, investigation, methodology, writing–original draft, writing–review and editing. **T. Adam de Beaumais:** Data curation, formal analysis, validation, investigation, project administration, writing–review and editing. **A. Marchais:** Data curation, software, formal analysis, validation, investigation, writing–review and editing. **A.C. Harttrampf:** Data curation, validation, investigation, writing–review and editing. **Y. Iddir:** Data curation, software, formal analysis, validation, investigation, writing–original draft, writing–review and editing. **A. Larive:** Data curation, software, methodology, writing–review and editing. **A. Soriano Fernandez:** Data curation, software, formal analysis, validation, investigation, writing–review and editing. **I. Hezam:** Conceptualization, supervision, project administration, writing–review and editing. **C. Chevassus:** Data curation, software, formal analysis, visualization, methodology, project administration, writing–review and editing. **V. Bernard:** Data curation, investigation, writing–review and editing. **S. Cotteret:** Data curation, software, formal analysis, validation, investigation, methodology, writing–review and editing. **J.-Y. Scoazec:** Data curation, formal analysis, validation, investigation, methodology, writing–review and editing. **A. Gauthier:**

Data curation, software, formal analysis, investigation, writing–review and editing. **S. Abbou:** Data curation, software, writing–review and editing. **N. Corradini:** Data curation, software, methodology, writing–review and editing. **N. André:** Data curation, validation, investigation, writing–review and editing. **I. Aerts:** Data curation, validation, investigation, writing–review and editing. **E. Thebaud:** Data curation, validation, writing–review and editing. **M. Casanova:** Data curation, validation, writing–review and editing. **C. Owens:** Data curation, validation, writing–review and editing. **R. Hladun-Alvaro:** Data curation, investigation, writing–review and editing. **S. Michiels:** Conceptualization, data curation, software, formal analysis, supervision, validation, investigation, methodology, writing–review and editing. **O. Delattre:** Data curation, investigation, writing–review and editing. **G. Vassal:** Conceptualization, resources, data curation, software, supervision, methodology, writing–review and editing. **G. Schleiermacher:** Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing. **B. Geoerger:** Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing.

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