Dokkyo Medical Journal
1(1): 56-62, 2022

DKMJ

Original

A Pilot Study of Comprehensive Genomic Profiling for Pediatric and Adolescent and Young Adult Solid Tumor Patients in Japan

Shotaro Matsudera[1,3], Yoshihito Kano[1], Yasuko Aoyagi[1], Kohki Tohyama[1], Kei Ogino[2], Kentaro Okamoto[2], Takeshi Yamaguchi[3], Shun Watanabe[3], Masanobu Nakajima[3], Shinji Morita[3], Takatoshi Nakamura[3], Kan Suzuki[3], Takashi Tsuchioka[3], Kazuyuki Kojima[3], Satoshi Miyake[1], Masatoshi Takagi[4], Sadakatsu Ikeda[1,5]

1) Department of Precision Cancer Medicine, Center for Innovative Cancer Treatment, Tokyo Medical and Dental University
2) Department of Specialized Surgeries, Tokyo Medical and Dental University
3) First Department of Surgery, Dokkyo Medical University
4) Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University
5) Moores Cancer Center, University of California

Summary

Introduction: Comprehensive genomic profiling (CGP) was widely adopted in Japan after its coverage by national healthcare insurance began in June 2019. We investigated the clinical utility of CGP in pediatric and adolescent young adults (AYA) solid tumor patients.

Materials and Methods: Between November 2017 and December 2019, 13 patients who progressed with or who were likely to progress with standard therapies were recruited to the PROFILE-F study to undergo CGP using either FoundationOne® CDx or FoundationOne® Heme.

Results: The median age was 28 years old. Tumor types were as follows: neuroblastoma (n = 1), Wilms' tumor (n = 1), rhabdomyosarcoma (n = 2), Ewing sarcoma (n = 1), gastric cancer (n = 1), rectal cancer (n = 1), osteosarcoma (n = 1), neuroendocrine tumor (n = 2), salivary gland carcinoma (n = 1), tracheal adenoid cystic carcinoma (n = 1), and thymic cancer (n = 1). In 92% of cases, at least one genomic alteration was identified, including *CDKN2A* (four cases), *TP53* (three cases), and *MYC* (two cases). Actionable aberrations were found in 10 cases (77%), and a clinical trial candidate was found in seven cases (54%). However, no patients were able to receive biomarker-matched therapy according to their genomic alterations.

Conclusions: Further efforts to increase basket trials and collection of clinical genomic data to predict response are necessary to advance precision cancer medicine and surgical management in pediatric and AYA populations.

Key Words: comprehensive genomic profiling, pediatric and AYA solid tumor patients, precision cancer medicine

Introduction

Treatment outcomes for children and adolescent young adult (AYA) with cancer have improved with the development of therapeutic methods, including surgery, chemotherapy, and radiation therapy. However, the treatment outcomes of pediatric and AYA patients with relapsed or refractory cancer remain poor[1].

Received October 9, 2021; accepted November 1, 2021
Reprint requests to: Shotaro Matsudera
matsudera.canc@tmd.ac.jp
Department of Precision Cancer Medicine, Center for Innovative Cancer Treatment, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo, Tokyo, 113-8510, Japan

Recently, technological innovations in clinical-grade next-generation sequencing (NGS) have contributed to the advancement of precision cancer medicine in clinical oncology. Personalized medicine for adult patients with advanced cancer is drawing attention worldwide[2]. For example, the development of molecular targeting drugs such as epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) inhibitors for lung cancer is remarkable[3-5]. A previous study showed that NGS assays have also been utilized to detect variants associated with a worse outcome in pediatric solid tumors, including *TP53* mutations and *STAG2* loss in Ewing sarcoma, *MYCN* amplification in neuroblastoma, 1q gain in Wilms' tumor, and *PAX3* gene rearrangements in alveolar rhabdomyosarcoma[6].

Tissue biopsy is often required for definitive diagnoses and risk classification of pediatric solid tumors. However, the clinical utility of comprehensive genomic profiling (CGP) for pediatric and AYA patients with relapsed or refractory solid tumors is still not established.

In this pilot study, we investigated the clinical utility of CGP for pediatric and AYA patients with solid tumors.

Materials and Methods

Patients

The Precision Cancer Medicine Registration Study of Omics Data from Genomic Information Analysis Leading to New Effective Therapy with FoundationOne CDx (PROFILE-F) was a prospective observational study conducted to characterize genomic aberrations and their clinical utility using genomic profiling. Thirteen pediatric and AYA cases with advanced solid tumors at our hospital who progressed or became resistant to standard systemic therapy were enrolled in this study. These patients underwent CGP between November 2017 and December 2019 under the PROFILE-F study. The PROFILE-F study was approved by the institutional review board of Tokyo Medical and Dental University (TMDU) (approval #G2018-002).

Comprehensive genomic profiling

CGP was performed using either FoundationOne® CDx or FoundationOne® Heme oncopanels (Foundation Medicine, Inc., Cambridge, MA, USA). Both are clinical-grade Clinical Laboratory Improvement Amendments (CLIA)-approved next-generation sequencing tests. FoundationOne® CDx analyzes 324 genes for single nucleotide variants, copy number alterations, indels, gene arrangements, tumor mutation burden (TMB), and microsatellite status (Table S1), while FoundationOne® Heme analyzes 406 genes and selected introns of 31 genes involved in rearrangements. Additionally, FoundationOne® Heme analyzes the RNA of 265 genes commonly rearranged in cancer to better identify known and novel gene fusions (Table S2). Therefore, FoundationOne® Heme is a more effective assay for pediatric patients with hematologic malignancies, sarcoma, and other solid tumors.

Definition of actionability

Actionable mutations are defined as genomic alterations that satisfy the following conditions: 1) mechanistically, the gene is associated with cancer and has data indicating therapeutic efficacy; and 2) a drug is available for human use either as an antibody or a small molecule compound with a low IC_{50} concentration[7].

Molecular Tumor Board

After genomic test results were obtained, each case was discussed by the Molecular Tumor Board, which consisted of specialists such as medical oncologists, pathologists, radiologists, bioinformaticians, genetic counselors, clinical research coordinators, and treating physicians. These members discussed actionable genomic alterations and treatment options on the basis of the patient's medical history, treatment history, family history, imaging findings, histopathological findings, and genetic test results[8].

Results

Patients' characteristics (Table 2)

Of 13 patients, seven were female, and the median age was 28 years old. Regarding the cancer types, two (15.4%) cases were rhabdomyosarcoma and two (15.4%) were neuroendocrine tumors, while there was one case each of neuroblastoma, nephroblastoma, Ewing sarcoma, osteosarcoma, gastric cancer, rectal cancer, salivary gland cancer, thymic cancer, and adenoid cystic carcinoma of trachea (Table 1). Eight patients under-

went FoundationOne® CDx and five patients underwent FoundationOne® Heme. The turnaround time, which is the period from the submission of the test to the return of the result, averaged 16.0 days (range, 8-54 days). Frequently altered genes were *CDKN2A* (30.8%, 4/13), *TP53* (23.1%, 3/13), and *MYC* (15.4%, 2/13) (Fig. 1). On average, 2.5 mutations were discovered per patient. Most of the alterations were single nucleotide variants (excluding variants of uncertain significance (VUS)) (37.5%) followed by amplifications (27.5%) (Fig. 2). TMB, which was defined as the number of somatic, coding, base substitution, and indel mutations per megabase of genome examined, averaged 2.8 mut/Mb (range, 0-11 mut/Mb).

Table 1 Patient characteristics

Characteristics	Total patients (*N*=13)
Age at diagnosis, years	
Median (CI 95%)	28 (3-39)
Gender	
Female	7 (53.8%)
male	6 (46.2%)
Type of cancer	
Rhabdomyosarcoma	2 (15.4%)
Neuroendocrine tumor	2 (15.4%)
Neuroblastoma	1 (7.7%)
Nephroblastoma	1 (7.7%)
Ewing sarcoma	1 (7.7%)
Osteosarcoma	1 (7.7%)
Gastric cancer	1 (7.7%)
Rectal cancer	1 (7.7%)
Salivary gland cancer	1 (7.7%)
Thymic cancer	1 (7.7%)
Adenoid cystic cancer (ACC)	1 (7.7%)

CI, confidence interval

Pharmacological interventions according to actionable genomic alterations (Fig. 3)

At least one genetic aberration was detected in 11 patients (84.6%). Actionable mutations were discovered in 10 patients (76.9%), and the median number of actionable alterations in our cohort was 2.0 (range, 0-6). Seven patients (53.8%) had clinical trial candidates. However, no patients were able to receive biomarker-matched therapy according to their genomic alterations.

Patients with suspected hereditary tumors

FoundationOne® CDx and Heme cannot compare tumor cells with normal cells. Thus, hereditary tumors (secondary findings) could not be diagnosed. In this study, germline aberrations were suspected in four patients (30.8%) (Table 3). We recommended genetic counseling to each patient. One patient (#11) with a

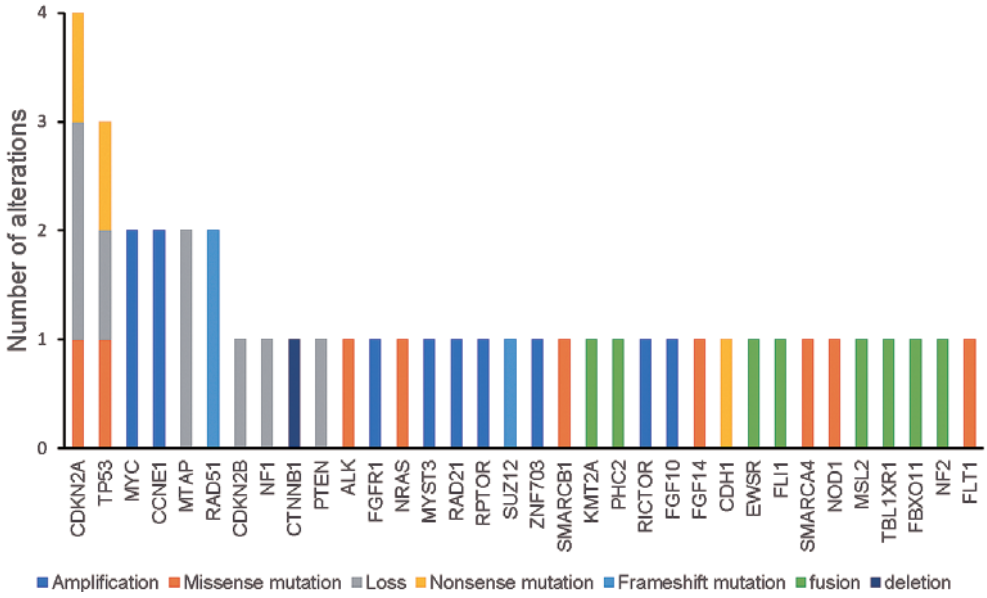

Figure 1 Percentage of individuals with the 34 most common alterations. The bar graph represents the percentage of individuals with the most common molecular alterations. Frequently altered genes were *CDKN2A* (30.8%, 4/13), *TP53* (23.1%, 3/13), and *MYC* (15.4%, 2/13). Only genes with pathogenicity by FoundationOne® report is described. Variant of unknown significance (VUS) is not involved.

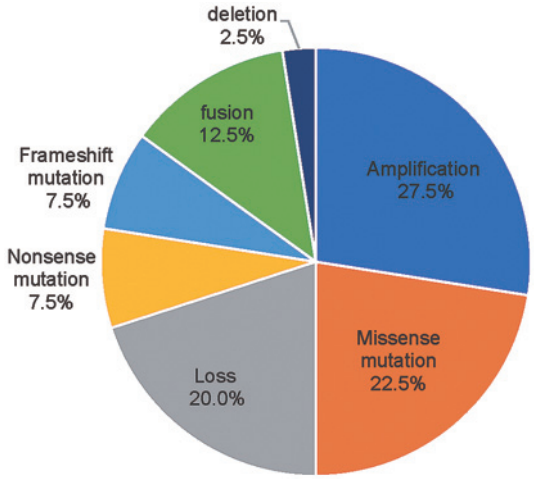

Figure 2 Pie chart displaying the different types of alterations. Most of the alterations were single nucleotide variants (37.5%), followed by amplifications (27.0%).

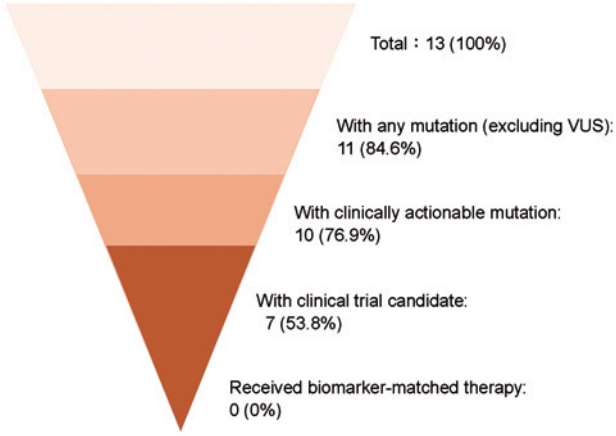

Figure 3 Schematic showing pharmacological intervention according to actionable genomic alterations. At least one genetic aberration was detected in 11 patients (84.6%). Actionable mutations were discovered in 10 patients (76.9%), and seven patients (53.8%) had clinical trial candidates. No patients were able to receive biomarker-matched therapy. VUS, variant of uncertain significance.

family history (his father had leukemia) was diagnosed with rhabdomyosarcoma in which a *TP53* inactivating mutation was detected. Therefore, we suspected Li-Fraumeni syndrome. Two patients (#2, #3) were unable to undergo genetic counseling due to their worsening general condition. One patient (#1) did not wish to proceed with genetic counseling.

Discussion

In this pilot study, we investigated the clinical utility of CGP for pediatric and AYA patients with solid tumors. A previous study reported that pediatric cancer patients have fewer point mutations than adult patients[1]. Profiling data of adult cancer patients are gradually accumulating, but there are few data sets of pediatric cancer patients. If genomic profiling data of pediatric cancer patients are accumulated in the future, biomarker-matched therapy such as entrectinib for neurotrophic receptor tyrosine kinase (NTRK) solid tumors will increase. Currently, the most common therapeutic method for pediatric cancer patients is multiple combination chemotherapy and radiation therapy, and side effects such as hair loss and nausea reduce quality of life. Further studies of the molecular biology of pediatric cancer patients will contribute to improved prognosis and quality of life.

Unfortunately, no patients were able to receive biomarker-matched therapy according to their genomic alterations. Seven patients (53.8%) were unable to receive biomarker-matched therapy despite having candidates for treatment. The reasons were as follows: two (15.4%) patients had worsening general conditions, and five (38.5%) patients were prioritized for standard chemotherapy.

One of the reasons is that fewer clinical trials in pediatric cancer were available in Japan compared with the USA. (four trials in Japan vs 145 trials in the USA, as searched in clinicaltrials.gov on July 14, 2021 using the following search criteria: condition or disease = pediatric cancer; recruitment status = recruiting and not yet recruiting studies; and country = Japan or United States.). Thus, clinical trials in pediatric patients may need to be conducted at the same time as clinical trials in adult patients. Better access to investigational drugs or liberal off-label use might improve the clinical outcome in Japan after CGP. A previous study reported that potentially actionable finding for 23 of the 58 patients (40%) were detected by genomic profiling and six of the 23 patients (26%) received matched targeted therapy[9].

ATM missense mutations were detected as VUS in the genetic report of one patient. However, it was revealed by the Molecular Tumor Board that this muta-

Table 2 Summary of 13 pediatric and Adolescent and Young Adult (AYA) patients with relapsed or refractory solid tumors

Research ID	Tumor type	Age (Years)	Gender	Panel	Potentially Actionable findings*	TMB (/Mb§)
#1	Rhabdomyosarcoma	19	Female	FoundationOne CDx	*NF1 loss*	11
#2	Thymic carcinoma	39	Female	FoundationOne CDx	*CDKN2A R87fs*33* *G103fs*58*	0
#3	Tracheal adenoid cystic carcinoma	38	Female	FoundationOne CDx	*SMARCB1 R377H*	0
#4	Pancreatic neuroendocrine tumor	30	Female	FoundationOne CDx	*MLL-PHC2 fusion*	9
#5	Cholangio-neuroendocrine tumor	38	Female	FoundationOne CDx	*none*	1
#6	Salivary gland carcinoma	38	Male	FoundationOne CDx	*PTEN loss* *RICTOR amp* *CDKN2A/B loss* *FGF10 amp* *FGF14 A236V* *MTAP loss* *TP53 loss*	9
#7	Gastric Cancer	28	Male	FoundationOne CDx	*CCNE1amp* *CDH1 Q23** *TP53 W146**	-
#8	Ewing sarcoma	19	Female	FoundationOne Heme	*EWSR1-FLI1 fusion* *CDKN2A/B R80*, P94L* *SMARCA4 R1243W*	2
#9	Wilms tumor	9	Male	FoundationOne Heme	*none*	2
#10	Neuroblastoma	3	Female	FoundationOne Heme	*ALK F1174L* *MYCN amp*	5
#11	Rhabdosarcoma	7	Male	FoundationOne Heme	*CCNE1 amp* *TP53 R273C*	2
#12	Rectal cancer	31	Male	FoundationOne CDx	*none*	-
#13	Osteosarcoma	20	Male	FoundationOne Heme	*NF2 rearrangement* *FLT1 W1260L*	4

§ TMB, tumor mutational burden

*Potentially Actionable findings, only genes with pathogenicity by FoundationOne® report is described. Variant of unknown significance (VUS) is not involved.

Table 3 Germline mutations suspected in four patients with relapsed or refractory solid tumors

Research ID	Tumor type	Age	Gender	Gene	Hereditary disease	VAF+ (%)	Counseling
#1	Rhabdomyosarcoma	19	Female	*NF1 loss*	NF1**	0	Not yet
#6	Salivary gland carcinoma	38	Male	*TP53 loss*	LFS***	0	Not yet
#7	Gastric cancer	28	Male	*CDH1 Q23**	HDGC****	7.07	Not yet
#11	Rhabdomyosarcoma	7	Male	*TP53 R273C*	LFS***	95.31	Done

** NF, neurofibromatosis type 1; *** LFS, Li-Fraumeni syndrome; **** HDGC, hereditary diffuse gastric cancer; +VAF, variant allele frequency

tion was an inactivating mutation on the basis of a previously published in vitro experiment[10]. We noted that if single nucleotide polymorphism information of the corresponding ethnicity is not used in the evaluation of genetic variants, there is an increased possibility of false-positive or false-negative results. Therefore, we need to be careful when interpreting the results of genomic profiling and the Molecular Tumor Board is considered to have an extremely important role.

It is important to determine whether the pediatric patient has a hereditary tumor. In this study, germline aberrations were suspected in four patients (30.8%). FoundationOne® CDx and OncoGuide® NCC oncopanels for CGP were widely adopted after its coverage by national healthcare insurance was provided from June 2019 in Japan. One of the differences between the two CGP panels is whether CGP is conducted on blood cells. OncoGuide® NCC oncopanel can identify germline variants by interrogating DNA from white blood cells. However, FoundationOne® CDx interrogates tumor tissue only, and hereditary tumors are "suspected" by identifying somatic variants, combined with family history and allele frequency. When the patient was a child, we consulted with their parents regarding genetic counseling and confirmation testing. The findings of hereditary tumors in pediatric and AYA cancer patients are especially important, and these patients often receive radiation therapy. However, radiation therapy should be carefully discussed with patients with *TP53* germline mutations (Li-Fraumeni syndrome) to minimize secondary tumors.

This study had a small sample size, and the clinical utility of CGP in pediatric cancer patients need to be validated in a larger study, ideally combined with prospective randomized therapeutic clinical trials.

Conclusions

In conclusion, this pilot study indicated CGP could identify actionable alterations in pediatric and AYA patients with solid tumors in the Japanese population. Further research should be conducted to characterize the clinical utility of CGP in a larger population and other settings, such as an early line of chemotherapy or a perioperative phase.

Acknowledgments

No public or private funding was used in this study. We thank Ms. Junko Yokobori, Ms. Mika Ohki, and Ms. Yumi Kobayashi for their exceptional support of this study as clinical research coordinators. Ms. Eriko Takamine, a certified genetic counselor, helped to organize the Molecular Tumor Board and the genetic counseling of patients who had potential germline mutations. We would like to extend our gratitude to Ms. Tomomi Urata, Ms. Rieko Furuya, and Ms. Akiko Noguchi for their administrative support. Finally, we thank H. Nikki March, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Disclosure Statement

The authors have no conflict of interest.

Authors contribution

S.M.: Collecting, analyzing data, and writing manuscript

S.M., Y.K., M.T., S.I.: Designed the study and reviewed the manuscript

Y.A., K.T., K.O., K.O.: Data analysis

S.W., T.Y., M.N., S.M., T.N., K.S., T.T., K.K., S.M.: Reviewed manuscript

All authors read and approved the final manuscript.

Funding

No public or private funding was used in this study.

Abbreviations

NGS, next-generation sequencing; AYA, adolescent and young adult; CGP, comprehensive genomic profiling; SNV, single-nucleotide variants; VUS, variants of uncertain significance; TAT, turnaround time

References

1) Mody RJ, Wu YM, Lonigro RJ, et al.: Integrative Clinical Sequencing in the Management of Refractory or Relapsed Cancer in Youth. Jama-Journal of the American Medical Association **314**: 913-925, 2015. https://doi.org/10.1001/jama.2015.10080.

2) Tsimberidou AM, Wen S, Hong DS, et al.: Personalized medicine for patients with advanced cancer in the phase I program at MD Anderson: validation and landmark analyses. Clin Cancer Res **20**: 4827-4836,

2014. https://doi.org/10.1158/1078-0432.CCR-14-0603.
3) Schwaederle M, Parker BA, Schwab RB, et al.: Precision Oncology: The UC San Diego Moores Cancer Center PREDICT Experience. Molecular Cancer Therapeutics **15**: 743-752, 2016. https://doi.org/10.1158/1535-7163.MCT-15-0795.
4) O'Bryant CL, Wenger SD, Kim M, et al.: Crizotinib: a new treatment option for ALK-positive non-small cell lung cancer. Ann Pharmacother **47**: 189-197, 2013. https://doi.org/10.1345/aph.1R002.
5) Paez JG, Jänne PA, Lee JC, et al.: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science **304**: 1497-1500, 2004. https://doi.org/10.1126/science.1099314.
6) Abbou SD, Shulman DS, DuBois SG, et al.: Assessment of circulating tumor DNA in pediatric solid tumors: The promise of liquid biopsies. Pediatr Blood Cancer **66**: e27595, 2019. https://doi.org/10.1002/pbc.27595.
7) Ikeda S, Tsigelny IF, Skjevik Å, et al.: Next-Generation Sequencing of Circulating Tumor DNA Reveals Frequent Alterations in Advanced Hepatocellular Carcinoma. Oncologist **23**: 586-593, 2018. https://doi.org/10.1634/theoncologist.2017-0479.
8) Schwaederle M, Parker BA, Schwab RB, et al.: Molecular tumor board: the University of California-San Diego Moores Cancer Center experience. The oncologist **19**: 631-636, 2014. https://doi.org/10.1634/theoncologist.2013-0405.
9) Pincez T, Clément N, Lapouble E, et al.: Feasibility and clinical integration of molecular profiling for target identification in pediatric solid tumors. Pediatr Blood Cancer **64**: e26365, 2017. https://doi.org/10.1002/pbc.26365.
10) Takagi M, Yoshida M, Nemoto Y, et al.: Loss of DNA Damage Response in Neuroblastoma and Utility of a PARP Inhibitor. J Natl Cancer Inst **109**: djx062, 2017. https://doi.org/10.1093/jnci/djx062.