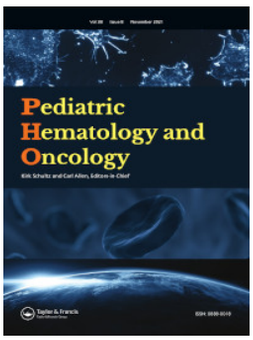

Taylor & Francis
Taylor & Francis Group

Pediatric Hematology and Oncology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ipho20

Microsatellite instability-high is rare events in refractory pediatric solid tumors

Taro Yoshida, Hideki Muramatsu, Manabu Wakamatsu, Rieko Taniguchi, Daisuke Ichikawa, Masato Nakaguro, Atsushi Natsume & Yoshiyuki Takahashi

To cite this article: Taro Yoshida, Hideki Muramatsu, Manabu Wakamatsu, Rieko Taniguchi, Daisuke Ichikawa, Masato Nakaguro, Atsushi Natsume & Yoshiyuki Takahashi (2021): Microsatellite instability-high is rare events in refractory pediatric solid tumors, Pediatric Hematology and Oncology, DOI: 10.1080/08880018.2021.1998266

To link to this article: https://doi.org/10.1080/08880018.2021.1998266

© 2021 The Author(s). Published with license by Taylor & Francis Group, LLC.

View supplementary material

Published online: 29 Dec 2021.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ipho20

PEDIATRIC HEMATOLOGY AND ONCOLOGY
https://doi.org/10.1080/08880018.2021.1998266

Taylor & Francis
Taylor & Francis Group

OPEN ACCESS Check for updates

Microsatellite instability-high is rare events in refractory pediatric solid tumors

Taro Yoshida[a], Hideki Muramatsu[a], Manabu Wakamatsu[a], Rieko Taniguchi[a], Daisuke Ichikawa[a], Masato Nakaguro[b], Atsushi Natsume[c,d] and Yoshiyuki Takahashi[a]

[a]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [b]Department of Pathology and Laboratory Medicine, Nagoya University Hospital, Nagoya, Japan; [c]Nagoya University, The Institute of Innovation for Future Society NIC Room 803, Furo-Cho, Chikusa-Ku, Nagoya, 464-8601, Japan; [d]Kawamura Medical Associates, Gifu, 501-3144, Japan

ABSTRACT

Microsatellite instability (MSI)-high status is associated with good responsiveness to immune checkpoint inhibitors. Although MSI-high status has been actively investigated in pediatric brain tumors, studies of other pediatric solid tumors are lacking. Among 334 consecutive pediatric patients with solid tumors, we retrospectively analyzed formalin-fixed paraffin-embedded tumor tissues of 36 of 74 patients (49%) who died of disease. We assessed the MSI status in these tissues using five multiplexed markers. The results revealed that none of the patients had an MSI-high status. These results indicate that MSI-high status is a rare event in pediatric patients with refractory/relapsed solid tumors.

Abbreviations: MSI: Microsatellite instability; MMR: Mismatch repair

ARTICLE HISTORY
Received 28 January 2021
Revised 6 July 2021
Accepted 17 October 2021

KEYWORDS
Microsatellite instability; mismatch repair gene; pediatric solid tumor

Introduction

Microsatellites are sets of repeating base sequences consisting of one or several bases within a chromosome. Generally, mismatch repair (MMR) proteins correct the base mismatches that occur during DNA replication. However, tumor cells with deficient MMR function accumulate genetic mutations, which lead to changes in the repeat counts at microsatellite sites; such a status is referred to as microsatellite instability (MSI)-high status. According to recent research, MSI-high status is associated with good responsiveness to immune checkpoint inhibitor therapy.[1]

MSI status has been well described in various solid tumors in adults; for example, one of the largest studies demonstrated that 1,188 of 12,019 (9.9%) patients with various tumor types exhibited an MSI-high signature.[2] The MSI-high status in pediatric patients has been actively investigated in patients with brain tumors. Marta et al, determined the

CONTACT Yoshiyuki Takahashi ytakaha@med.nagoya-u.ac.jp Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan

Supplemental data for this article is available online at https://doi.org/10.1080/08880018.2021.1998266

© 2021 The Author(s). Published with license by Taylor & Francis Group, LLC.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

MSI status in 71 pediatric patients with high-grade gliomas, and MSI-high was found in only 1 of 71 (1.4%) cases.[3] They also evaluated the MSI status in 36 patients with medulloblastoma and found that only 1 case of 36 (2.8%) had an MSI-high status.[4] However, determination of the MSI status in other pediatric solid tumors has been insufficient.

Treatment outcomes of pediatric tumor patients are better than those of adults, and most cases achieve long-term survival with existing treatments; however, new treatments are needed in some cases of recurrence and relapse. Immune checkpoint inhibitors are a promising treatment for adult tumors with MSI-high status and are also expected to be introduced in children. Therefore, in this study, we investigated the MSI status in pediatric patients with various solid tumors who died from their disease.

Methods

Patients

From April 2000 to May 2019, 334 pediatric patients with solid tumors were admitted to Nagoya University Hospital (Figure 1). Archived formalin-fixed paraffin-embedded (FFPE) samples from 40 of the 74 (54%) patients who died were obtained from the Department of Pathology and Laboratory Medicine, Nagoya University Hospital. Using hematoxylin and eosin staining, tumor tissue was confirmed to be present in these samples by a pathologist (MN).

DNA extraction

Archived FFPE samples were retrieved and deparaffinized, and the tumor and normal tissue areas were microdissected and collected into a microtube. DNA was extracted using a QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

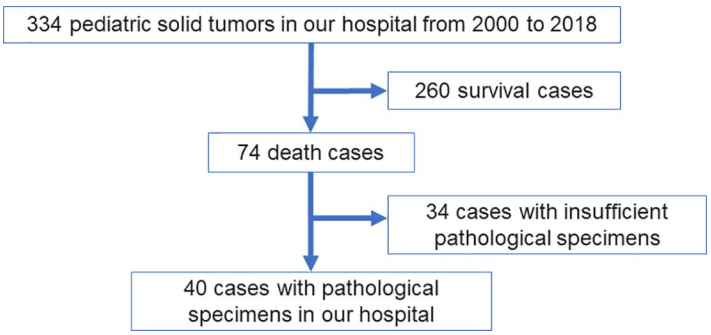

Figure 1. Flow chart of patient cohort. From April 2000 to May 2019, 334 patients with pediatric solid tumors were admitted to the Nagoya University Hospital. Seventy-four (22%) patients died, including 68 due to relapse or refractory tumor, 4 due to pulmonary complications after stem cell transplantations, and 2 due to infection after chemotherapies. Formalin-fixed paraffin-embedded tumor tissues from 40 (54%) of the 74 patients who died were retrospectively collected and assessed the MSI status.

Microsatellite instability analysis

Microsatellite instability (MSI) analysis was performed with multiplex polymerase chain reaction (PCR) using five quasi-monomorphic mononucleotide repeat markers (MONO27, NR21, NR24, BAT25, and BAT26), as previously described.[5] We performed capillary electrophoresis with a DNA sequencer using the amplified products of each microsatellite region. We used the quasi-monomorphic variation range width of the number of repetitions for the five types of microsatellite region markers, as previously described.[6] The MSI status of the tumor samples and positive control sample (DNA derived from a cell line of MSI-high colorectal cancer, EpiScope® Methylated HCT116 gDNA, TaKaRa Bio, Ohtsu, Japan) was defined as MSI-high (MSI-H, instability at two or more markers), MSI-Low (MSI-L, instability at one marker), or microsatellite stable (MSS, absence of instability).

Results

In all, 334 pediatric patients with solid tumors were admitted to Nagoya University Hospital from April 2000 to May 2019. The 5-year overall survival rate of the 334 cases was 77.5% (95% confidence interval; 72.3%–81.8%), and the median (range) follow-up time was 1,826 (81–6,416) days (Figure 2). Of the 334 pediatric patients with solid tumors, 74 died, including 68 due to relapse or refractory tumors, 4 due to pulmonary complications after stem cell transplantations, and 2 due to infection after chemotherapy treatment. The MSI status was assessed in FFPE tumor tissues from 40 of the 74 patients (54%) who died (Table 1). Of these 40 patients, 17 (43%) were male and 23 (58%) were female, and the median (range) age was 2 (0–19) years. The diagnoses included neuroblastoma in 14 cases, medulloblastoma in 6 cases,

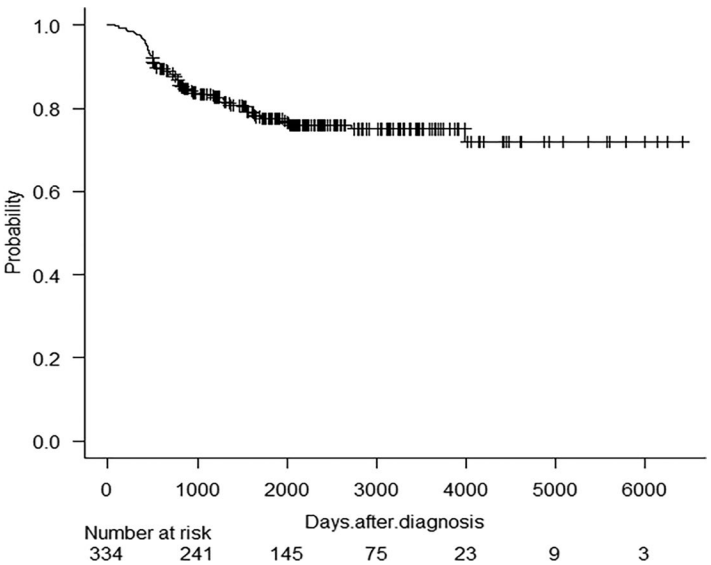

Figure 2. Overall survival of 334 cases with pediatric solid tumors. The Kaplan-Meier estimates of overall survival (OS). Five-year OS rate was 77.5% (95% confidence interval; 72.3%–81.8%) with median (range) follow-up of 1,826 (81–6,416) days.

Table 1. Patient characteristics with MSI analysis.

Case number	Sex	Age at diagnosis	Age at death	Diagnosis	Tumor site	Cause of death	Sample type	MSI
1	M	1	6	Neuroblastoma	Left adrenal gland	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
2	F	3	6	Neuroblastoma	Retroperitoneum	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
3	M	4	8	Neuroblastoma	Retroperitoneum	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
4	F	5	9	Neuroblastoma	Retroperitoneum	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
5	M	18	19	Neuroblastoma	Left adrenal gland	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
6	F	2	3	Neuroblastoma	Left adrenal gland	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
7	F	0	1	Neuroblastoma	Right adrenal gland	Treatment related mortality	Biopsy	MSS
8	F	4	6	Neuroblastoma	Left adrenal gland	Treatment related mortality	Biopsy	MSS
9	F	7	10	Neuroblastoma	Right adrenal gland	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
10	F	1	3	Neuroblastoma	Right adrenal gland	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
11	F	2	3	Neuroblastoma	Right adrenal gland	Treatment related mortality	Resected specimen	MSS
12	F	2	4	Neuroblastoma	Retroperitoneum	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
13	M	0	2	Neuroblastoma	Right adrenal gland	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
14	M	7	8	Medulloblastoma	Cerebellum	Infection	Resected specimen	MSS
15	F	2	3	Medulloblastoma	Cerebellum	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
16	F	12	14	Medulloblastoma	Right cerebral hemisphere	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
17	M	1	5	Medulloblastoma	Cerebellum	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
18	M	0	1	Glioblastoma	Cerebellum	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
19	F	1	2	Medulloblastoma	Cerebellum	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
20	M	0	0	Medulloblastoma	Cerebellum	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
21	F	1	2	Rhabdomyosarcoma	Retroperitoneum	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
22	F	13	14	Rhabdomyosarcoma	Anal muscle	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
23	M	11	14	Rhabdomyosarcoma	Left abdominal wall	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
24	M	1	2	PNET	Left frontotemporal lobe	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
25	F	8	9	PNET	Right chest cavity	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
26	F	4	5	Ewing sarcoma	Right clavicle	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
27	M	0	1	AT/RT	Pineal gland	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
28	F	1	3	AT/RT	Cerebellum	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
29	F	2	3	Retinoblastoma	Pineal gland	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
30	M	2	2	Pineoblastoma	Pineal gland	Recurrence/exacerbation of the underlying disease	Biopsy	MSS
31	F	1	1	PNET	Pineal gland	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS

32	M	14	19	Liver anaplastic sarcoma	Liver	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
33	F	2	3	Hepatoblastoma	Liver	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
34	M	11	11	Germ cell tumor	Pineal gland	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
35	M	14	18	Clear cell sarcoma of the kidney	Left kidney	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
36	M	4	7	Adrenal cortical cancer	Left adrenal gland	Recurrence/exacerbation of the underlying disease	Resected specimen	MSS
37	F	0	0	Neuroblastoma	Retroperitoneum	Recurrence/exacerbation of the underlying disease	Biopsy	NA
38	F	8	9	Ewing sarcoma	Thoracic vertebra	Recurrence/exacerbation of the underlying disease	Biopsy	NA
39	M	12	17	Malignant ameloblastoma	Mandible	Recurrence/exacerbation of the underlying disease	Biopsy	NA
40	F	15	17	Renal cell carcinoma	Left kidney	Recurrence/exacerbation of the underlying disease	Biopsy	NA

Abbreviations: AT/RT, atypical teratoid/rhabdoid tumor; PNET, primitive neuroectodermal tumor; MSI, microsatellite instability; MSS, microsatellite stable; NA, not available.

rhabdomyosarcoma in 3 cases, PNET in 3 cases, AT/RT in 2 cases, Ewing sarcoma in 2 cases, and one case each of glioblastoma, retinoblastoma, pineoblastoma, liver anaplastic sarcoma, hepatoblastoma, germ cell tumor, clear cell sarcoma of the kidney, adrenal cortical cancer, malignant ameloblastoma, and renal cell carcinoma. The causes of death included recurrence/exacerbation of underlying disease in 36 cases, treatment-related mortality in 3 cases, and infection in 1 case. The results demonstrated that 36 of the 40 cases were microsatellite stable and that none of the patients had an MSI-high status; however, this observation could not be confirmed in the remaining 4 patients due to poor sample quality. Supplemental Figure 1 shows the electrophoresis plots of the positive control and of one of the negative samples (Case number 34).

Discussion

Of the 334 consecutive pediatric patients with solid tumors who were treated at our institution, we analyzed the MSI status in 40 of 74 children who died of their disease. Unfortunately, MSI-high status, a biomarker that is correlated with treatment response to immune checkpoint inhibitors, was found to be a very rare event in these patients, who therefore require novel, more promising treatments. Although several case reports have been published on the efficacy of checkpoint inhibitors in pediatric MSI-high tumors,[7] a recent interim analysis of data from KEYNOTE-051,[8] a study of pembrolizumab treatment, reported a response in only 6% of pediatric solid tumors other than Hodgkin's lymphoma, despite the finding that the cohort was limited to PD-L1 immunostaining positivity. Our results and these findings suggest that surveillance of biomarkers for immune checkpoint inhibitors, ie, MSI status and PD-L1 immunostaining, may need to be reevaluated in the future in larger trials of pediatric solid tumors.

The outcomes of standard treatment with conventional chemotherapy for pediatric solid tumors are generally good.[9] The first limitation of this study is that we assessed the MSI status only in patients with poor prognosis for whom the use of immune checkpoint inhibitors may be clinically justified. This resulted in a relatively small sample size, especially that of cases of medulloblastoma (n = 6) and high-grade glioma (n = 1), for which sporadic MSI-high cases have been reported in previous studies.[3,4] The second limitation is that we did not evaluate other biomarkers used to predict response to immune checkpoint inhibitors, such as PD-L1 immunostaining, tumor mutation burden, presence of constitutional mismatch repair deficiency syndromes, and next generation sequencing-based MSI analysis.[10] In the future, multiple biomarkers need to be evaluated in a larger, unbiased cohort of pediatric solid tumor patients.

In conclusion, our study demonstrated that MSI-high status is a rare event in refractory/relapsed pediatric solid tumors. To efficiently identify pediatric patients who may be eligible for immune checkpoint inhibitor treatment, a better understanding of the potential biomarkers that predict response to immune checkpoint inhibitors in pediatric solid tumors is needed.

Funding

This study was supported by funding from the Nagoya Pediatric Cancer Fund.

ORCID

Taro Yoshida http://orcid.org/0000-0003-4385-8165
Hideki Muramatsu http://orcid.org/0000-0002-8801-0021
Manabu Wakamatsu http://orcid.org/0000-0003-0364-5886
Rieko Taniguchi http://orcid.org/0000-0001-6735-0214
Daisuke Ichikawa http://orcid.org/0000-0002-6196-7421
Masato Nakaguro http://orcid.org/0000-0001-6987-3043
Atsushi Natsume http://orcid.org/0000-0002-9113-0470
Yoshiyuki Takahashi http://orcid.org/0000-0001-8644-171X

References

1. Yamashita H, Nakayama K, Ishikawa M, et al. Microsatellite instability is a biomarker for immune checkpoint inhibitors in endometrial cancer. *Oncotarget*. 2018;9(5):5652–5664. doi:10.18632/oncotarget.23790. PMID: 29464025.
2. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409–413. doi:10.1126/science.aan6733. PMID: 28596308.
3. Viana-Pereira M, Lee A, Popov S, et al. Microsatellite instability in pediatric high grade glioma is associated with genomic profile and differential target gene inactivation. *PLoS One*. 2011;6(5):e20588. doi:10.1371/journal.pone.0020588. PMID: 21637783.
4. Viana-Pereira M, Almeida I, Sousa S, et al. Analysis of microsatellite instability in medulloblastoma. *Neuro Oncol*. 2009;11(5):458–467. doi:10.1215/15228517-2008-115. PMID: 19179424.
5. Buhard O, Suraweera N, Lectard A, Duval A, Hamelin R. Quasimonomorphic mononucleotide repeats for high-level microsatellite instability analysis. *Dis Markers*. 2004;20(4-5):251–257. doi:10.1155/2004/159347. PMID: 15528790.
6. Bando H, Okamoto W, Fukui T, Yamanaka T, Akagi K, Yoshino T. Utility of the quasi-monomorphic variation range in unresectable metastatic colorectal cancer patients. *Cancer Sci*. 2018;109(11):3411–3415. doi:10.1111/cas.13774. PMID: 30142704.
7. Cacciotti C, Choi J, Alexandrescu S, et al. Immune checkpoint inhibition for pediatric patients with recurrent/refractory CNS tumors: a single institution experience. *J Neurooncol*. 2020;149(1):113–122. doi:10.1007/s11060-020-03578-6. PMID: 32627129.
8. Geoerger B, Kang HJ, Yalon-Oren M, et al. Pembrolizumab in paediatric patients with advanced melanoma or a PD-L1-positive, advanced, relapsed, or refractory solid tumour or lymphoma (KEYNOTE-051): interim analysis of an open-label, single-arm, phase 1-2 trial. *Lancet Oncol*. 2020;21(1):121–133. PMID: 31812554. doi:10.1016/S1470-2045(19)30671-0.
9. Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA A Cancer J Clin*. 2020;70(1):7–30. PMID: 31912902. doi:10.3322/caac.21590.
10. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. Microsatellite instability detection by next generation sequencing. *Clin Chem*. 2014;60(9):1192–1199. doi: 10.1373/clinchem.2014.223677.PMID: 24987110.