INTERNATIONAL JOURNAL OF UROLOGY
International Journal of Urology (2022) 29, 741–747 doi: 10.1111/iju.14885

Original Article: Clinical Investigation

Phase II trial of nivolumab monotherapy and biomarker screening in patients with chemo-refractory germ cell tumors

Takashi Kawahara,[1] Koji Kawai,[2] Takahiro Kojima,[1] Yoshiyuki Nagumo,[1] Shotarou Sakka,[1] Shuya Kandori,[1] Hiromitsu Negoro,[1] Bryan J Mathis,[3] Kazushi Maruo,[4] Koji Miura,[5] Noriaki Sakamoto,[6] Nobuo Shinohara,[7] Shinichi Yamashita,[8] Kan Yonemori,[9] Takeshi Kishida,[10] Osamu Ukimura,[11] Kazuo Nishimura,[12] Yasuyuki Kobayashi[13] and Hiroyuki Nishiyama[1]

[1]Department of Urology, University of Tsukuba, Tsukuba, Ibaraki, [2]Department of Urology, International University of Health and Welfare, Narita, Chiba, [3]International Medical Center, University of Tsukuba Affiliated Hospital, [4]Department of Clinical Trial and Clinical Epidemiology, Faculty of Medicine, University of Tsukuba, [5]Tsukuba Clinical Research and Development Organization (T-CReDO), Faculty of Medicine, University of Tsukuba, [6]Department of Pathology, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, [7]Department of Urology, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, [8]Department of Urology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, [9]Department of Breast and Medical Oncology, National Cancer Center Hospital, Chuo-ku, Tokyo, [10]Department of Urology, Kanagawa Cancer Center, Yokohama, Kanagawa, [11]Department of Urology, Kyoto Prefectural University of Medicine, Kyoto, Kyoto, [12]Department of Urology, Osaka International Cancer Institute, Osaka, Osaka and [13]Department of Urology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Okayama, Japan

Abbreviations & Acronyms
AE = adverse event
AFP = alpha-fetoprotein
ECOG = Eastern Cooperative Oncology Group
GCT = germ cell tumor
hCG = human chorionic gonadotropin
IHC = immunohistochemistry
irRECIST = immune-related RECIST
MSI = microsatellite instability
mTOR = mammalian target of rapamycin
ORR = overall response rate
OS = overall survival
PD = progressive disease
PD-1 = programmed cell death protein-1
PD-L1 = programmed death ligand-1
PFS = progression-free survival
PR = partial response
RECIST = Response Evaluation Criteria in Solid Tumor
SD = stable disease
TMB = tumor mutational burden
WES = whole exome sequence

Correspondence: Hiroyuki Nishiyama M.D., Ph.D., Department of Urology, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan. Email: nishiuro@md.tsukuba.ac.jp

Received 5 December 2021; accepted 21 March 2022.
Online publication 23 April 2022

Objectives: Germ cell tumors are highly susceptible to chemotherapy; however, there is a lack of established treatments for consistently relapsing germ cell tumor. Therefore, in this phase II study, we evaluated the efficacy and safety of nivolumab for relapsed germ cell tumor.

Methods: Seventeen adult patients (median age 34 years) with refractory primary germ cell tumor after second-line or higher chemotherapy were enrolled. Nivolumab was administered over 30 min at 240 mg/body every 2 weeks until disease progression or intolerable adverse event occurrence. The primary endpoint was the overall response rate.

Result: We performed a biomarker analysis of programmed death ligand-1 expression and genomic sequencing. Tumor histology revealed nonseminoma and seminoma in 14 and three patients, respectively. Patients were pretreated with a median of three chemotherapy lines, and three patients received high-dose chemotherapy. The median number of nivolumab doses was 3 (range 2–46). One patient showed a partial response and three showed stable disease. Responses were durable in one patient with a partial response and one patient with stable disease (median 90 and 68 weeks, respectively). Nivolumab was well-tolerated, with only two Grade 3 adverse events observed. Programmed death ligand-1 expression was not associated with objective responses. Genomic sequencing revealed a high tumor mutation burden in a patient with a durable partial response. While a small subset of chemorefractory germ cell tumors may respond to nivolumab, programmed death ligand-1 is unreliable to measure response.

Conclusions: Tumor mutation burden is a potential biomarker for future testing of germ cell tumor response.

Key words: genomic sequencing, germ cell tumor, nivolumab, programmed death ligand-1, refractory disease.

Introduction

The high susceptibility of GCTs to chemotherapy indicates that up to 80% of patients with advanced GCTs can be cured by chemotherapy and surgery.[1] Even if GCTs relapse after initial chemotherapy, patients may be treated with second-line standard-dose chemotherapy or high-dose chemotherapy;[2,3] however, there is no established treatment for patients with consistently relapsing GCT. In such cases, the most effective treatment is a combination of gemcitabine, oxaliplatin, and paclitaxel paired with subsequent aggressive surgery. However, only

10–15% of patients achieve long-term survival with this regimen,[4] and a meta-analysis of single-agent chemotherapy showed that PFS and OS rates of patients with consistently relapsing GCT were only 1.0 and 4.7 months, respectively.[5] Moreover, molecularly targeted, single-agent therapies using tyrosine kinase or mTOR inhibitors have been shown to be ineffective.[6]

Given the low efficacy of existing chemotherapeutic and molecularly targeted drugs for unresponsive GCTs, several novel therapies are being developed. Checkpoint inhibitors targeting PD-1 and PD-L1 pathways are promising candidates for GCT treatment. Pembrolizumab, a humanized monoclonal antibody targeting PD-1, is widely used to treat melanoma and non-small cell lung cancer.[7,8] Treatment regimens featuring pembrolizumab improve the responses of tumors with a high PD-L1 expression.[7,8] As PD-L1 expression was reported in GCT patients,[9,10] these treatment regimens are also promising for GCT patients.

Several case reports and series have indicated such a GCT response to pembrolizumab or nivolumab, suggesting that PD-L1 is a prime target for therapy.[11–13] To our knowledge, there are no published clinical studies on the effect of nivolumab, another widely used anti-PD-1 antibody, on refractory GCTs.

Therefore, we conducted a phase II, multi-institute trial to elucidate the efficacy and safety of nivolumab monotherapy for patients with unresponsive GCT who relapsed after second-line or higher previous chemotherapy.

Methods

Study design

This was a multicenter, single-arm, open-label, phase II study. The study protocol and informed consent forms were reviewed and approved by the respective independent ethics committees, and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines of Japan. All patients provided written, informed consent before enrollment, and this trial was registered at the University hospital Medical Information Network Clinical Trials Registry (UMIN000028249).

Patients

Patients aged ≥18 years with relapsed or refractory primary GCTs (seminoma or nonseminoma) after previous second-line or higher chemotherapy were eligible for this study. The patients were recruited between July 2017 and December 2018. Patients with gonadal (testis or ovary primary site) or extragonadal primary GCT were included, but those with intracranial GCTs were excluded. The inclusion criteria were treatment with cisplatin-based chemotherapy, an ECOG performance status score of 0 or 1, and measurable metastases according to the RECIST version 1.1. Resistance to the most recent chemotherapy was confirmed by more than 20% increase in the number of metastases according to the RECIST criteria, new lesions, or two consecutive increases in tumor marker levels presenting at least 1 week apart. Other inclusion criteria were nonresectable GCT and adequate hematologic, renal, and liver functions. The main exclusion criteria were active intracranial disease, administration of systemic corticosteroids or immunosuppressants within 28 days, and previous anti-PD-1 or PD-L1 therapy.

Drug administration

Nivolumab was administered over 30 min at 240 mg/body every 2 weeks. Nivolumab was provided by Ono Pharmaceutical Co., Ltd., the sponsor of this investigator-initiated phase II trial, and three nivolumab administrations were defined as one treatment cycle. Treatments were continued until disease progression or unacceptable toxicity as determined by the clinical assessment of symptoms. All treatment-related AEs were recorded.

Response and toxicity evaluation

We performed physical examination and measured vital signs before each administration of nivolumab. Blood cell counts and serum chemistry were evaluated on days 8, 15, 29, and 43 of the first treatment cycle and days 1 and 43 of the second or any subsequent cycles. Tumor markers were evaluated on days 8, 15, 29, and 43 of the first treatment cycle, days 15, 29, and 43 of the second cycle, and days 15 and 43 of any subsequent cycles. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for AEs version 4.0. Computed tomography was performed every 6 weeks until 18 weeks, every 8 weeks until 50 weeks, and thereafter, every 12–20 weeks.

Biomarker analysis

We performed biomarker analysis, when tumor samples were available. Tumor samples were obtained from primary tumor sites or resected tumors after chemotherapy. Biomarker analysis included PD-L1 expression (PD-L1 IHC 28-8 pharmDx assay kit; Agilent Technologies, Santa Clara, CA, USA) and genomic sequencing (QIAseq Targeted DNA Panels; QIAGEN, Hilden, Germany).

PD-L1 IHC

PD-L1 expression was assessed in formalin-fixed, archived tumor samples using the commercially available PD-L1 IHC 28-8 pharmDx assay kit. IHC and biomarker evaluations were conducted according to the manufacturers' instructions. Briefly, PD-L1 protein positivity was defined as complete circumferential or partial linear plasma membrane tumor cell staining at any intensity. Patients were considered PD-L1 positive if the percentage of positive tumor cells was ≥1% of the total tumor cell expression. A minimum threshold of 100 viable tumor cells in each PD-L1-stained slide was required to determine the percentage of stained cells.

Next-generation sequencing

Tumor DNA from formalin-fixed paraffin-embedded tissue was extracted using the QIAamp DNA FFPE Tissue Kit

(QIAGEN) according to the manufacturer's instructions. DNA concentration was determined using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Tumor DNA concentrations ≥1.5 ng/μL, as quantified using a Qubit fluorometer, were further analyzed. Ten nanograms of DNA was used as a template to generate an amplicon library for sequencing. The QIAseq Human Comprehensive Cancer Panel (DHS-3501Z-12; QIAGEN) was used for library construction according to the manufacturer's instructions. Libraries were assessed using the Bioanalyzer High Sensitivity DNA Kit (5067–4626; Agilent Technologies) and placed in a MiSeq sequencer (Illumina, San Diego, CA, USA) to obtain 2 × 151-base reads. FASTQ files were imported into the CLC Genomics Workbench (ver.12.0; QIAGEN). Reads were mapped to the hg19 human reference genome and analyzed using the QIAseq Panel Analysis workflow. Germline variants were removed from the QIAseq variant call results using control peripheral blood sample exome sequencing data. Potential somatic mutations were selected according to the following criteria: allele frequency ≥5%, number of reads with variants ≥5, and coverage ≥30.

End points and statistical analysis

The primary endpoint was the ORR, considered as the best overall response, as measured using RECIST version 1.1 and an independent committee. Secondary endpoints were tumor response duration, ORR using irRECIST, PFS, and OS.

Nivolumab toxicity and tolerability were also assessed. Response and toxicity were evaluated in patients who received at least one nivolumab dose. PFS and OS were estimated using the Kaplan–Meier method. Statistical analyses were performed using JMP 14 software (SAS, Cary, NC, USA) and the data cutoff date was October 9, 2020.

As patients with relapsed or refractory primary GCTs after second-line or higher previous chemotherapies were eligible for this study, the response rate was required to be higher than normal to demonstrate significance. Therefore, the threshold response rate was set to 5% in view of the placebo response rate. In contrast, the expected response rate was set to 30% to reflect the rate of response to the combination of gemcitabine and oxaliplatin (17–46%). Accordingly, the minimum sample size required to achieve a power of 80% was calculated to be 14 patients. The minimum target number of enrolled participants was set to 16 to account for study dropouts.

Results

Patient and disease characteristics

Seventeen patients were enrolled in this study and their characteristics are summarized in Table 1.

The median age was 34 (range 18–60) years and only one patient was female. Tumors were histologically classified as nonseminomas in 14 patients and seminomas in three patients. The primary sites were the gonads in 12 patients (one patient had ovarian GCT), whereas the remaining five had extragonadal GCTs; three of these GCTs were mediastinal. The patients were heavily pretreated with a median of 3 (range 2–5) chemotherapy lines, including high-dose chemotherapy in three patients. Seven patients (41%) had received more than four chemotherapy lines previously. The median number of previous chemotherapy cycles was 12 (range 5–30) and 10 patients (59%) were treated with more than 10 chemotherapy cycles. Active metastatic sites before nivolumab therapy included the lungs in 14 patients, retroperitoneal lymph nodes in four, and other lymph nodes in eight; five patients had liver metastases and three had bone metastases. The following tumor markers were elevated: AFP only in six patients, hCG only in nine, and both markers in two. Nine patients (53%) had an ECOG performance status score of 0, whereas one patient had a score of 1.

Treatment administration

All 17 enrolled patients received at least two nivolumab doses. The median time from the most recent chemotherapy to nivolumab administration was 2.6 (range 1.1–17.5) months. The median nivolumab dose number was 3 (range 2–46).

Table 1 Patient charcteristics

	N	%
Patient	17	100
Tumor histology		
Seminoma	3	18
Nonseminoma	14	82
Primary tumor site		
Gonadal	12	71
Mediastinal	3	18
Others	2	12
International Germ Cell Cancer Collaborative Group risk at initial diagnosis†		
Intermediate	3	21
Poor	10	71
Unclassified	1	7
No. of previous lines of chemotherapy		
2 lines	4	24
3 lines	6	35
More than 4 lines	7	41
No. of previous courses of chemotherapy		
Median (range)	12 (5–30)	
<10 courses	7	41
More than 10 courses	10	59
Sites of active metastases		
Retoperitoneum	4	24
Other lymphadenopathy	8	47
Pulmonary	14	82
Non-pulmomary visceral metastases	8	47
Liver	5	29
Bone	3	18
No. of active metastatic sites		
1	4	24
2	5	29
More than 3 sites	8	47
Mean (range) of pretreatment markers		
AFP (ng/mL)	386 (1–3516)	
hCG (IU/L)	3268 (0.5–26 833)	
Lactate dehydrogenase (IU/L)	440 (178–2860)	
ECOG performance status		
0	9	53
1	8	47

†Testis and mediastinum origine.

Response

Clinical responses to nivolumab are summarized in Table S1. Figure 1 presents the clinical course of the one patient who achieved a PR with a median duration of 90.1 weeks. In the remaining 16 patients, the best response was SD in three patients with a median duration of 11.7 (range 5.9–68.4) weeks, whereas one patient achieved a durable SD with a duration of 68.4 weeks. Twelve patients showed PD, but one was not evaluable. The median number of nivolumab doses for patients with PD was 3 (range 2–10). As shown in Table S1, one patient with a durable PR received four lines (14 cycles) of chemotherapy before nivolumab but another patient with durable SD was also heavily pretreated with three lines (eight cycles) of chemotherapy and oral etoposide. There was no significant relationship among treatment response, histological types, and metastatic sites. The irRECIST criteria were evaluated in nine patients. The best response was irPR in one patient, followed by irSD in four, and irPD in four. The median PFS was 1.5 (range 0–23.6) months, the median OS was 4.1 (range 1.6–29.8) months, and the 1- and 2-year survival rates were 27% and 13%, respectively. The Kaplan–Meier OS curve is shown in Figure 2.

Adverse events

Nivolumab was tolerated by heavily pretreated patients with GCT. The observed AEs associated with nivolumab are listed in Table 2.

No Grade 4 AEs were observed. There were two Grade 3 AEs, hypophosphatemia and rapid tumor progression, the latter probably from the natural course of the disease; nevertheless, nivolumab-induced hyperprogression could not be completely excluded. Except for the patient with hyperprogression, no other patient discontinued nivolumab due to AEs.

Biomarker analysis

Informed consent for biomarker analysis was obtained from 13 patients. Of these patients, tumor samples of eight and 12 patients were available for genomic sequencing and PD-L1 IHC, respectively (Table S1). For PD-L1 IHC, two more samples were unevaluable due to insufficient viable tumor cells, leaving 10 patients evaluable for PD-L1 staining. Among these patients, seven were evaluated for primary tumors, and three were evaluated for metastatic sites after chemotherapy. All samples were obtained before and after chemotherapy, not before nivolumab treatment. IHC indicated that three patients (30%) were PD-L1-positive; among these patients, the best response was SD in one patient and PD in two patients. Two patients who experienced a durable PR (median 90.1 weeks) and SD (median 68.4 weeks) had tumors negative for PD-L1. Genomic sequencing revealed that all analyzed samples had nonsynonymous mutations. The median number of nonsynonymous mutations was 16 (range 8–81), and there were no significant differences between the three patients with SD (10.3 average mutations) and the four patients with PD (20.3 average mutations) (Tables S2 and S3). In contrast, the patient with a durable PR had as high as 81 mutations.

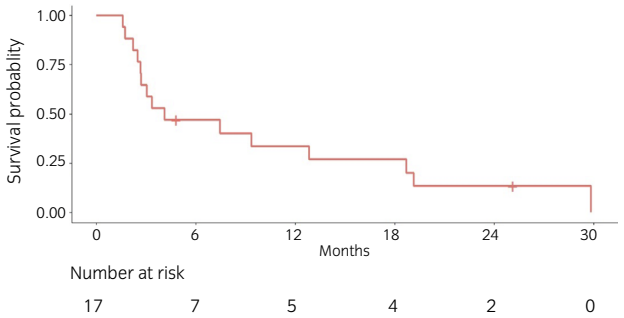

Fig. 2 Kaplan–Meier estimates of OS. The median OS was 4.1 (range 1.6–29.8) months, and the 1- and 2-year survival rates were 27% and 13%, respectively.

Discussion

Most patients with GCT can be cured with first-line standard chemotherapy and subsequent surgery, but some

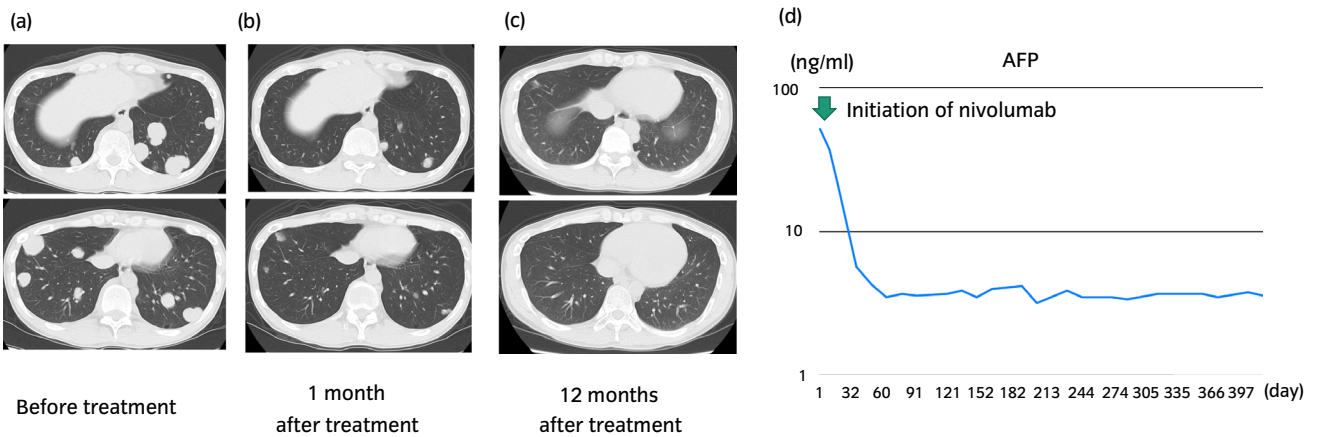

Fig. 1 Time course of radiologic findings and alpha fetoprotein levels in patients who achieved a PR. The response has continued to date for 94 weeks in this patient (data cut-off: 9 October 2020). Computed tomography scans before (a) and after (b, c, 1 and 12 months) initiation of nivolumab show PR of a lung metastasis. (d) Time course of alpha fetoprotein levels in this patient.

Table 2 Treatment-related AEs

AE	Grade 1 *n*	Grade 2 *n*	Grade 3 *n*	Any grade (%) *n*	(%)
Rash	2	0	0	2	11.8
Fever	1	1	0	2	11.8
Fatigue	1	0	0	1	5.9
Diarrhea	0	1	0	1	5.9
Muscle pain	1	0	0	1	5.9
Hypertension	0	1	0	1	5.9
Rapid tumor progression	0	0	1	1	5.9
Pnemonitis	1	0	0	1	5.9
Hypophosphatemia	0	0	1	1	5.9
Elevation of C-reactive protein level	0	1	0	1	5.9
Hypothyroidism	0	1	0	1	5.9
Dermatitis contact	0	1	0	1	5.9
Elevation of creatinine phosphokinase level	0	1	0	1	5.9

patients may relapse and require salvage therapy. Although such a standard- or high-dose salvage chemotherapy[2,3] can treat a substantial proportion of first-relapse patients, there is no established chemotherapy for patients who relapse after this. Additionally, the results of an early study of molecularly targeted, single-agent therapies using tyrosine kinase or mTOR inhibitors have not been promising.[6] In this setting, immunotherapy with anti-PD-L1 or anti-PD-1 antibodies can provide a target for a propotion of GCT patients; however, two GCT clinical studies indicated that these antibodies were not clinically efficient.[14,15] In contrast, a study using pembrolizumab, an anti-PD-1 antibody, demonstrated durable SD (19 and 28 weeks, respectively) in 2 of 12 patients with refractory GCT.[16] Recently, another phase II trial of pembrolizumab showed durable SD (range 4.5–10.9 months) in 3 of 12 heavily pretreated patients with GCT.[17] Although durable responses were observed in several patients, no partial or complete responses were observed in these two previous studies, disappointingly. Thus, we started a novel phase II trial to elucidate the efficacy and safety of nivolumab, another anti-PD-1 antibody, for patients with GCT relapsing after second-line or higher previous chemotherapies. Nivolumab was well-tolerated by heavily pretreated patients; one patient had a PR, whereas three had SD, with a median duration of 11.7 (range 5.9–68.4) weeks. Notably, the maximum response duration of patients with a PR and SD was 90.1 and 68.4 weeks, respectively. While the proportion of patients who benefitted from nivolumab was low, the present results indicate that nivolumab is worth considering if effective biomarkers for response prediction are available.

Regarding suitable biomarkers, PD-L1 expression in cancer cells is a logical choice for predicting tumor response to anti-PD-1 or anti-PD-L1 therapy as observed in previous studies.[8] However, several factors limit the use of PD-L1 expression as a biomarker for predicting refractory GCT response, as there might be heterogeneity between primary and metastatic lesions. PD-L1 expression may also fluctuate during treatment and thus PD-L1 expression may not be a reliable predictive biomarker, which is supported by our results.[18]

To search for other viable biomarkers, we utilized targeted, next-generation sequencing of 275 cancer-related genes, but not WES, to evaluate mutation profiles. The median number of mutations in all eight tested samples was 16, with no significant differences between the three patients with SD and four patients with PD. However, mutations were extremely high in the tumors of patients with a durable PR. The high TMB assessed by WES has been associated with favorable clinical outcomes after anti-PD-1/PD-L1 therapy for various cancers[19,20] due to the expression of mutation-mediated neoantigens.[21] Although limited information is available, the frequency of high-TMB GCT cases or cases with high GCT mutation rates is reportedly low.[21–23] To our knowledge, no study has assessed the correlation between TMB and clinical outcomes of anti-PD-1/PD-L1 therapy for GCTs.[22] Although our data are not derived from WES or targeted gene sequencing panels for TMB assessment, our results suggest that the mutation rate is a potential biomarker for predicting GCT response.

This study had some limitations. First, biomarker samples were not obtained from all patients enrolled in the study. Second, MSI information was lacking. However, although both high TMB and MSI expressions have been reported as effective biomarkers,[24] studies have reported that the frequency of high MSI expression is low in patients with GCT.[23,25] Thus, measuring both markers may be optimal to avoid overlooking potential candidates. Despite these limitations, our data showed that a small subset of patients with completely chemo-refractory GCT has a chance to benefit from anti-PD-1/PD-L1 therapy. Further development of an effective predictive biomarker is warranted.

Acknowledgments

The authors thank the study participants, research assistants, coordinators, and all other staff of the Tsukuba Clinical Research and Development Organization (T-CReDO) for their invaluable assistance with data collection. This work was supported by COI-NEXT (grant number JPMJPF2017). This study was sponsored by Ono Pharmaceutical Co., Ltd., who provided the study drug to the participants for free. The authors state that the funding or sponsoring agency had no role in study design, patient enrollment, data acquisition/analysis, manuscript drafting, or the decision to publish this study.

Author contributions

Takashi Kawahara: Data curation; investigation; methodology; writing – original draft; writing – review and editing. Koji Kawai: Conceptualization; data curation; investigation; methodology; project administration; resources; writing – original draft. Takahiro Kojima: Investigation; methodology; project administration; resources. Yoshiyuki Nagumo: Data curation; formal analysis; writing – original draft. Shotaro

Sakka: Investigation; writing – review and editing. Shuya Kandori: Investigation; project administration; resources. Hiromitsu Negoro: Investigation; writing – review and editing. Bryan J Mathis: Writing – review and editing. Kazushi Maruo: Data curation; formal analysis. Koji Miura: Data curation; formal analysis. Noriaki Sakamoto: Data curation. Nobuo Shinohara: Project administration; resources. Shinichi Yamashita: Project administration; resources. Kan Yonemori: Resources. Takeshi Kishida: Project administration; resources. Osamu Ukimura: Project administration; resources. Kazuo Nishimura: Project administration; resources. Yasuyuki Kobayashi: Project administration; resources. Hiroyuki Nishiyama: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing – original draft; writing – review and editing.

Conflict of interest

H. Nishiyama has a consultant/advisory role with Merck Sharp & Dohme, Lilly, Bayer Yakuhin, Janssen, and Chugai Pharma, and receives research funding from Ono, Astellas, Takeda, and Bayer. N.S. receives research grants from Astellas, Pfizer, Takeda, Sanofi, Ono, Eizai, and Taiho. K.N. receives research funding from Bayer. The remaining authors have no conflict of interest to declare. The funding source had no role in the design, practice, or analysis of this study.

Approval of the research protocol by an Institutional Reviewer Board

The protocol for this research project has been approved by a suitably constituted Ethics Committee of the institution, and it conforms to the provisions of the Declaration of Helsinki. Approval was obtained from the Committee of University of Tsukuba Hospital IRB (Approval No. 23).

Informed consent

Informed consent was obtained from all the subjects.

Registry and the Registration No. of the study/trial

This trial was registered at the University hospital Medical Information Network Clinical Trials Registry (UMIN000028 249).

Animal studies

N/A.

References

1 Hanna NH, Einhorn LH. Testicular cancer – discoveries and updates. *N. Engl. J. Med.* 2014; **371**: 2005–16.

2 Einhorn LH, Williams SD, Chamness A *et al*. High-dose chemotherapy and stem-cell rescue for metastatic germ-cell tumors. *N. Engl. J. Med.* 2007; **357**: 340–8.

3 Feldman DR, Sheinfeld J, Bajorin DF *et al*. TI-CE high-dose chemotherapy for patients with previously treated germ cell tumors: results and prognostic factor analysis. *J. Clin. Oncol.* 2010; **28**: 1706–13.

4 Oechsle K, Kollmannsberger C, Honecker F *et al*. Long-term survival after treatment with gemcitabine and oxaliplatin with and without paclitaxel plus secondary surgery in patients with cisplatin-refractory and/or multiply relapsed germ cell tumors. *Eur. Urol.* 2011; **60**: 850–5.

5 Feldman DR, Patil S, Trinos MJ *et al*. Progression-free and overall survival in patients with relapsed/refractory germ cell tumors treated with single-agent chemotherapy: endpoints for clinical trial design. *Cancer* 2012; **118**: 981–6.

6 Oing C, Alsdorf WH, von Amsberg G, Oechsle K, Bokemeyer C. Platinum-refractory germ cell tumors: an update on current treatment options and developments. *World J. Urol.* 2017; **35**: 1167–75.

7 Herbst RS, Baas P, Kim DW *et al*. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; **387**: 1540–50.

8 Carbognin L, Pilotto S, Milella M *et al*. Differential activity of nivolumab, pembrolizumab and MPDL3280A according to the tumor expression of programmed death-ligand-1 (PD-L1): sensitivity analysis of trials in melanoma, lung and genitourinary cancers. *PLoS One* 2015; **10**: e0130142.

9 Fankhauser CD, Curioni-Fontecedro A, Allmann V *et al*. Frequent PD-L1 expression in testicular germ cell tumors. *Br. J. Cancer* 2015; **113**: 411–3.

10 Cierna Z, Mego M, Miskovska V *et al*. Prognostic value of programmed-death-1 receptor (PD-1) and its ligand 1 (PD-L1) in testicular germ cell tumors. *Ann. Oncol.* 2016; **27**: 300–5.

11 Shah S, Ward JE, Bao R, Hall CR, Brockstein BE, Luke JJ. Clinical response of a patient to anti-PD-1 immunotherapy and the immune landscape of testicular germ cell tumors. *Cancer Immunol. Res.* 2016; **4**: 903–9.

12 Zschäbitz S, Lasitschka F, Jäger D, Grüllich C. Activity of immune checkpoint inhibition in platinum refractory germ-cell tumors. *Ann. Oncol.* 2016; **27**: 1356–60.

13 Chi EA, Schweizer MT. Durable response to immune checkpoint blockade in a platinum-refractory patient with nonseminomatous germ cell tumor. *Clin. Genitourin. Cancer* 2017; **15**: e855–7.

14 Necchi A, Giannatempo P, Raggi D *et al*. An open-label randomized phase 2 study of durvalumab alone or in combination with tremelimumab in patients with advanced germ cell tumors (APACHE): results from the first planned interim analysis (Apache). *Eur. Urol.* 2019; **75**: 201–3.

15 Mego M, Svetlovska D, Chovanec M *et al*. Phase II study of avelumab in multiple relapsed/refractory germ cell cancer. *Investig. New Drugs* 2019; **37**: 748–54.

16 Adra N, Einhorn LH, Althouse SK *et al*. Phase II trial of pembrolizumab in patients with platinum refractory germ-cell tumors: a Hoosier cancer research network study GU14-206. *Ann. Oncol.* 2018; **29**: 209–14.

17 Tsimberidou AM, Vo HH, Subbiah V, et al. Pembrolizumab in patients with advanced metastatic germ cell tumors. *Oncologist* 2021; **26**: 558.e1098.

18 Shen X, Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. *BMJ* 2018; **362**: k3529.

19 Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N. Engl. J. Med.* 2017; **377**: 2500–1.

20 Goodman AM, Kato S, Bazhenova L *et al*. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Mol. Cancer Ther.* 2017; **16**: 2598–608.

21 Loveday C, Litchfield K, Proszek PZ *et al*. Genomic landscape of platinum resistant and sensitive testicular cancers. *Nat. Commun.* 2020; **11**: 2189.

22 Necchi A, Bratslavsky G, Corona RJ *et al*. Genomic characterization of testicular germ cell tumors relapsing after chemotherapy. *Eur. Urol. Focus* 2020; **6**: 122–30.

23 Matsumoto T, Shiota M, Uchiumi T *et al*. Genomic characteristics revealed by targeted exon sequencing of testicular germ cell tumors in Japanese men. *Int. J. Urol.* 2021; **28**: 40–6.

24 Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11 348 patients. *Cancer Med.* 2018; **7**: 746–56.

25 Cárcano FM, Lengert AH, Vidal DO *et al*. Absence of microsatellite instability and BRAF (V600E) mutation in testicular germ cell tumors. *Andrology* 2016; **4**: 866–72.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Clinicopathological characteristics and sample information.

Table S2. Results of non-synonymous mutations analyzed using next-generation sequencing.

Table S3. Non-synonymous mutations in 100 representative genes among 8 patients.

Editorial Comment

Editorial Comment to Phase II trial of nivolumab monotherapy and biomarker screening in patients with chemo-refractory germ cell tumors

Despite excellent cure rates in metastatic germ cell cancer (GCC), around 15 to 20% of the patients relapse or progress after first line cisplatin-based chemotherapy and subsequent salvage treatment regimens. In this situation, the combination of classical cytotoxic agents such as gemcitabine, oxaliplatin, and paclitaxel can induce objective response rates of approximately 50%, but most of the patients will progress and inevitably succumb to their disease.[1] With the implementation of targeted cancer therapies, several attempts were made to introduce new treatment options for refractory GCC patients. For this purpose, tyrosine kinase inhibiters, immunomodulatory substances, mTOR inhibitors, and others were investigated, but unfortunately no substance revealed reliable antitumor activity.[2]

In 2017, pembrolizumab was the first checkpoint-inhibitor (CKI) tested in a small cohort of refractory GCC patients but failed to induce any clinical responses, and the trial was closed prematurely.[3] In another phase II trial, durvalumab yielded no responses as well, and the combination with tremelimumab, a CTLA-4 antibody, reached clinical responses in two of nine heavily pretreated patients. In summary, the use of CKIs seems to represent another unsuccessful attempt concerning the implementation of new agents for refractory GCC patients. One reason concerning the lack of efficacy of CKIs in GCC could be a low rate of somatic mutations resulting in the absence of potential neoantigens, which are crucial for T-cell response.

In the article by Kawahara *et al.*, the authors present data from a phase II trial investigating the safety and efficacy of nivolumab in a cohort of 17 adults with refractory germ cell tumors aligned with an analysis of programmed death ligand-1 (PD-L1) expression and genomic sequencing.[4] The overall clinical activity of nivolumab was sobering, but one patient revealed a durable partial response, associated with the proof of a high tumor mutational burden. The authors concluded that a small subset of patients with chemotherapy-refractory germ cell tumor still have a chance to benefit from anti-PD-1/PD-L1 therapy with tumor mutational burden as a potential biomarker to predict therapy response.

To our point of view this trial demonstrates that oncologists should disclaim the use of CKIs in unselected refractory GCC patients. A molecular biomarker screening however could be considered for these patients to identify potential targets which can be used for optimal treatment stratification. Concerning the future perspective of immunotherapy in GCC, newest clinical data from the 2022 AACR congress revealed promising results and suggest that CAR-T-Cells could represent a novel approach in the treatment of refractory GCC.

Acknowledgment

Open Access funding enabled and organized by Projekt DEAL.

Finn-Ole Paulsen M.D. and Christoph Seidel M.D.
Department of Oncology, Hematology and Bone Marrow Transplantation with Division of Pneumology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
c.seidel@uke.de

DOI: 10.1111/iju.14949

Conflict of interest

None declared.

References

1 Oechsle K, Kollmannsberger C, Honecker F *et al*. Long-term survival after treatment with gemcitabine and oxaliplatin with and without paclitaxel plus secondary surgery in patients with cisplatin-refractory and/or multiply relapsed germ cell tumors. *Eur. Urol*. 2011; **60**: 850–5.
2 Oing C, Giannatempo P, Honecker F, Oechsle K, Bokemeyer C, Beyer J. Palliative treatment of germ cell cancer. *Cancer Treat. Rev*. 2018; **71**: 102–7.
3 Adra N, Einhorn LH, Althouse SK *et al.* Phase II trial of pembrolizumab in patients with platinum refractory germ cell tumors: a hoosier cancer research network study GU14-206. *Ann. Oncol*. 2018; **29**: 209–14.
4 Kawahara T, Kawai K, Kojima T *et al.* Phase II trial of nivolumab monotherapy and biomarker screening in patients with chemo-refractory germ cell tumors. *Int. J. Urol*. 2022; **29**: 741–7.