Rapid Response to Pembrolizumab in a Chemo-Refractory Testicular Germ Cell Cancer with Microsatellite Instability-High

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Abstract: Testicular germ cell tumor (TGCT) is highly chemo-sensitive cancer; however, there is no established treatment for TGCT relapsed after multiple chemotherapy. Although pembrolizumab showed durable stable disease in some patients, no reliable biomarker for predicting response is available. High microsatellite instability (MSI) is rare in chemo-naïve TGCT. We report a TGCT patient with a rapid response to pembrolizumab. A 34-year-old Japanese male diagnosed with advanced TGCT underwent PCR-based testing of the primary site; it did not reveal MSI. He relapsed after four chemotherapy regimens: bleomycin, etoposide and cisplatin; paclitaxel, ifosfamide and cisplatin; vinblastine, ifosfamide and cisplatin; and irinotecan+nedaplatin with a total of 20 treatment cycles. Chemotherapy was thus discontinued. Re-examination by a CT-guided needle biopsy for progressing retroperitoneal lymph node (RPLN) metastases showed MSI-high; pembrolizumab was initiated. After only two doses, the human chorionic gonadotropin level decreased from 6500 to <1.0 IU/L. PET-CT showed shrinkage of the RPLN metastases with diminished metabolism. The patient is currently free from disease progression for 6 months from the start of pembrolizumab. This is the first report of refractory TGCT with MSI-high responding to pembrolizumab. We emphasize the utility of a metastatic-site biopsy to check the MSI status for refractory TGCT even when primary site is MSI-negative.

Keywords: pembrolizumab, testicular cancer, microsatellite instability

Introduction

Testicular germ cell tumor (TGCT) is the most common malignancy in young adult men. TGCT is a highly chemo-sensitive cancer, and up to 80% of patients with advanced disease can be cured by initial chemotherapy and surgery. Even if the disease relapses after initial treatment, patients have a chance of cure with second-line chemotherapy; however, there is no established treatment for patients who have further relapse. Given the low efficacy of existing chemotherapeutic and molecularly targeted drugs for chemo-refractory TGCT, investigations into novel therapies are ongoing. One of the promising candidate families is the immune check-point inhibitors (ICIs) targeting programmed cell death protein-1 (PD-1) and programmed cell death ligand-1 (PD-L1) pathways.

There are several case reports and case series on the clinical responses of germ cell tumor (GCT) patients to pembrolizumab or nivolumab, and these reports describe the use of ICIs as a prime target. Of the four contemporary GCT clinical studies using ICIs, two tested the effect of the anti-PD-L1 antibodies
durvalumab and avelumab. In both studies, ICI mono-
therapy resulted in no clinical response. The other two
studies tested the efficacy of pembrolizumab; no partial
or complete responses were observed in 24 refractory GCT
patients, but five patients achieved relatively long-
term stable disease (range 19 weeks–11 months). While
the proportion of patients who benefitted from pembro-
lizumab was low, the results showed that the drug is
certainly worth considering if effective biomarkers for
predicting the response are available.

With respect to suitable biomarkers, PD-L1 expression
on cancer cells is the logical choice for predicting the
response to anti-PD-1 or anti-PD-L1 therapy. However,
results from a Phase II study for pembrolizumab showed
that PD-L1 expression may not be a truly reliable predic-
tive biomarker for refractory GCT. A recent meta-
analysis of randomized control studies for various cancer
types suggested that PD-L1 expression status alone is an
insufficient predictive biomarker. As other biomarkers,
higher tumor mutational burden (TMB) and microsatellite
instability (MSI)-high status were associated with favor-
able clinical outcomes after anti-PD-L1 therapy for various
types of cancer, but high TMB is known to be
extremely rare in GCT. MSI-high is also rare, but several studies suggested the incidence is higher in che-
mo-refractory TGCT compared to the chemo-naïve primary
tumor.

Herein, we describe a TGCT patient with histologically
proven MSI-high status who had a rapid response to pembro-
lizumab. To our knowledge, this is the first report of
refractory GCT with MSI-high status responding to pembro-
lizumab. We also discuss a rationale of cell-free DNA (cfDNA) next-generation sequencing (NGS) for detecting
the MSI-high status in this clinical setting.

Case Report
A 34-year-old Japanese male with advanced testicular
cancer was referred to Chiba University Hospital in
December 2017 for chemotherapy. Computed tomography
(CT) revealed multiple lung metastases and retroperitoneal
lymph node (RPLN) metastases. Laboratory testing
showed a human chorionic gonadotropin (hCG) level of
39,400 IU/L, lactate dehydrogenase (LDH) 528 IU/L, and
alpha-fetoprotein (AFP) 3.9 ng/mL. The pathological diag-
nosis of the primary site was pure seminoma, and poly-
merase chain reaction (PCR)-based MSI testing using five
microsatellite markers (MONO-27, BAT25, BAT26, NR-
21, and NR-24) did not reveal MSI.

The patient received multiple chemotherapy using four
different regimens with a total of 20 treatment cycles:
bleomycin, etoposide and cisplatin, paclitaxel, ifosfamide
and cisplatin, vinblastine, ifosfamide and cisplatin, and
a combination of irinotecan+nedaplatin. The disease
nevertheless progressed after the last chemotherapy in
December 2019, and the patient’s hCG level increased to
19,764 IU/L. Because of the definitive chemo-refractory
disease, the chemotherapy was discontinued.

At this point, the patient’s MSI status was re-checked
by a commercially available cfDNA NGS assay, the
Guardant360 (Guardant Health, Redwood City, CA),
which resulted in positive MSI-high status. As other mole-
cular findings of interest, Guardant360 revealed an inser-
tion mutation and a nonsense mutation in AT-rich
interactive domain-containing protein 1A (ARID1A) gene, also it showed an insertion mutation BRCA2 gene.
Those gene alterations are potential marker for the efficacy
of Poly (ADP-ribose) polymerase (PARP) inhibitors, but
RARP inhibitors are not available for TGCT in clinical
setting. Therefore, treatment with pembrolizumab was
considered, but unexpectedly, the patient’s metastases
regressed without any additional treatment. As shown in
Figure 1, between January 2020 and June 2020, CT images
revealed that both lung and RPLN metastases sponta-
neously regressed, and the hCG level decreased from
84,920 to 1402 IU/L. However, the level had increased to
2407 IU/L at 2 months later.

The patient was then referred to our hospital in
September 2020 for further management. As shown in
Figure 2, his hCG level continuously increased. Positron
emission tomography (PET)-CT revealed the re-
enlargement of RPLN metastases with hypermetabolism,
but the lung metastases had completely disappeared. Due
to the positive result on the Guardant360 assay, we
decided to re-examine the patient’s MSI status by perform-
ing a CT-guided needle biopsy of the progressing RPLN
metastases.

As shown in Figure 3, the histological examination of
the biopsy specimens revealed mixed germ cell cancer,
and the VENTANA (SP-142) immunohistochemistry
assay (Roche Diagnostics, Indianapolis, IN) result was
positive for PD-L1 expression. In addition, MSI
testing using a quasi-monomorphic variation range
showed MSI in four (MONO-27, BAT25, BAT26 and
NR-21) of five microsatellite markers. The tumors were
thus classified as MSI-high. Immunotherapy with pembro-
lizumab was then initiated, and as shown in Figure 2, the
hCG level decreased from 6500 IU/L to <1.0 IU/L after only two doses of pembrolizumab. PET-CT showed the shrinkage of RPLN metastases with diminished metabolism. Since asymptomatic organizing pneumonia developed, pembrolizumab was discontinued after 6 doses administration. The patient is currently free from...
disease progression for 6 months from the start of pembrolizumab.

**Discussion**

In the present case, a CT-guided needle biopsy for RPLN metastases demonstrated the MSI-high status, suggesting that pembrolizumab might yield clinical benefit. The patient had a rapid response, as his hCG level decreased from 6500 to <1.0 IU/L after only two doses of pembrolizumab. Since the preceding histological examination of the patient’s primary tumor did not show MSI, the decision to re-examine the patient’s MSI status by needle biopsy for progressing metastatic site was not easy, as this procedure is not free from potential complications. The positive data obtained by the Guardant360 assay was an important clue for making the decision to re-examine the MSI status.

Pembrolizumab has provided improved patient responses in various types of cancer that have a high level of PD-L1 expression. In TGCT, two studies reported higher levels of PD-L1 expression compared to normal testes, and both studies showed that high PD-L1 expression was associated with unfavorable survival. To date, five patients with refractory GCT were reported to partially respond to anti-PD-1 therapy. Of them, four showed positive PD-1 expression. These findings suggested that PD-1 expression is an effective biomarker for refractory GCT, but Adra et al reported that two of 12 patients treated with pembrolizumab achieved radiographic stable disease, and both had negative PD-L1 staining. Two patients had positive PD-L1 expression, and progressive disease was their best response. A meta-analysis of randomized control studies for various cancer types also suggested that the PD-L1 expression status alone is insufficient as a predictive biomarker. PD-L1 expression may thus not be a reliable predictive biomarker for refractory GCT.

As other biomarkers, the higher TMB and MSI-high status are effective predictors for the response to anti-PD-1/PD-L1 therapy. The reported proportion of positive cases for both markers in TGCT are extremely low; however, several studies suggested that the MSI-high incidence is higher in chemo-refractory tumors compared to chemo-naïve primary tumors. Carcano et al reported that MSI was completely absent in 133 primary tumors examined before chemotherapy, whereas Honecker et al showed MSI-high in 26% of 35 chemo-resistant tumors of GCT patients; most had been treated by first-line or salvage high-dose chemotherapy. MSI-high status was observed in four of eight late relapsed patients after chemotherapy, but Necchi et al reported only one (2%) MSI-high case among 51 chemotherapy-treated patients. Several factors may be involved in this discrepancy, such as differences in technology or the degree of chemo-resistance. Histology of MSI-high tumors is another discussion point.

The reported incidences of MSI-high do not support a routine biopsy for progressing chemo-resistant tumors, because it is not free from potential complications. As in

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**Figure 3** Pathological section of RPLN metastasis biopsy. The biopsy specimen showed the infiltration of mixed germ cell cancer. Immunohistochemistry revealed strong positive staining for PD-L1. (A) Hematoxylin and eosin staining. (B) VENTANA (SP-142) immunohistochemistry assay, magnification: 200x.
the present case, the Guardant360 cfDNA NGS assay is useful for identifying a biopsy candidate to examine the MSI status in clinical practice. MSI detection by the Guardant360 is reported to be highly concordant with tissue-based testing.20,21 The FDA approved the Guardant360 as a diagnostic tool to guide treatment options for non-small-cell lung cancer.

Our patient had experienced a dramatic spontaneous regression of lung metastases before the progression of RPLN metastases. In TGCT, a spontaneous regression of metastatic sites is extremely rare, but spontaneous regression of the primary tumor, so-called burned out tumor, is a well-described phenomenon.27 Lymphoplasmacytic infiltrates in the scars are commonly observed in regressed tumors. In the present case, pathology of testicular tumor was pure seminoma, but biopsy of RPLN metastases revealed mixed germ cell cancer. Since pretreatment hCG level was extraordinary high considering pure seminoma, we suspected presence of non-seminoma elements in metastatic sites. This is probably result of differentiation from seminoma to non-seminoma in metastatic sites. As another explanation, there is a possibility that non-seminoma elements in primary site had spontaneously regressed. Although the mechanism underlying spontaneous regression is unclear, these findings may indicate that some TGCT cases have immunogenic activity that facilitates the response to anti-PD-1/PD-L1 therapy.

Conclusion

In summary, our patient’s case suggests that pembrolizumab is effective for refractory TGCT with MSI-high status, as other MSI-high cancers may be. We emphasize that a biopsy of metastatic sites to check the MSI status is one of the choices for refractory TGCT even when the primary site is negative for MSI. The cfDNA assay is an important clue for deciding whether to perform a biopsy for metastatic sites. Although further studies to examine concordance of MSI detection by the cfDNA with tissue-based testing are needed, the present case suggests that if MSI-H is identified with cfDNA that may be also reasonable rationale to proceed with immunotherapy for TGCT.

Ethical Approval

Institutional approval was not required to publish the case details.

Patient Consent for Publication

Written informed consent was obtained from the patient.

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Disclosure

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