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Zhao, Hongyuan

Ma, Weijie

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Case report

Durable clinical response to the multidisciplinary management of neurosurgery, radiation and chemoimmunotherapy in a patient with PD-L1/PD-L2/JAK2 (PDJ)-amplified, refractory triple-negative breast cancer

Hongyuan Zhao[1,2], Weijie Ma[1], Ruben C. Fragoso[3], Griffith R. Harsh IV[4], Arya Ashok[5], Tianhong Li[1,*]

[1] *Division of Hematology/Oncology, Department of Internal Medicine, University of California Davis School of Medicine, University of California Davis Comprehensive Cancer Center, Sacramento, USA*
[2] *Current address: Department of Thyroid & Breast Surgery, Affiliated Hospital of Zunyi Medical University, Zunyi, China*
[3] *Department of Radiation Oncology, University of California Davis School of Medicine, University of California Davis Comprehensive Cancer Center, Sacramento, USA*
[4] *Department of Neurological Surgery, University of California Davis School of Medicine, Sacramento, USA*
[5] *Tempus Labs, Inc., Chicago, USA*

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ABSTRACT

Patients with refractory metastatic triple-negative breast cancer (mTNBC) and symptomatic brain metastases have poor prognosis and are challenging to treat. The addition of an programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) inhibitor (pembrolizumab or atezolizumab) to first line chemotherapy has prolonged survivals in mTNBC patients with PD-L1-positive tumor and/or tumor-infiltrating immune cells. The clinical efficacy of the chemoimmunotherapy combination in patients with refractory mTNBC, especially brain metastasis, is unknown. Co-amplification of PD-L1, PD-L2, and Janus kinase 2 (*PD-L1/PD-L2/JAK2*) genes (*PDJ* amplification) is associated with high PD-L1 protein expression and a 65-87% response rate to PD-1/PD-L1 inhibitors in patients with lymphomas. But the utility of *PDJ* amplification as a biomarker predictive of response to PD-1/PD-L1 inhibitors is unknown for mTNBC patients. Here, we report a 46-year-old woman who had rapid tumor progression in the brain and lung within 3 months after chemotherapy, neurosurgery, and gamma knife stereotactic radiosurgery for brain metastasis. Next-generation sequencing of her brain metastasis specimen revealed 9 copies of *PDJ* amplification and a tumor mutational burden of 5 mutations per megabase. Although high *PDJ* mRNA expression levels were detected, PD-L1 protein expression was negative on tumor cells and 10% on tumor-associated immune cells. After the debulking brain tumor resection, she received pembrolizumab monotherapy, whole brain radiation, and then atezolizumab and nab-paclitaxel with good intracranial and extracranial responses for >16 months. To the best of our knowledge, this is the first report that *PDJ* amplification is associated with durable clinical response to the PD-1/PD-L1 inhibitor-containing, multidisciplinary management in a patient with refractory, PD-L1 protein-negative, *PDJ*-amplified mTNBC. Further study is warranted to understand the underlying mechanism and validate *PDJ* amplification as a biomarker for clinical response to PD-1/PD-L1 inhibitor-containing therapy in patients with mTNBC.

1. Introduction

About 10–30% of women with metastatic breast cancer develop brain metastasis (BM) and 5% develop leptomeningeal metastasis (LM)[1]. The incidence of BM varies across the molecular subtypes of breast cancer. Metastatic triple-negative breast cancer (mTNBC) has the highest incidence: 14% at the time of diagnosis of metastatic disease and about 50% during the full disease course[2]. Median survival after the diagnosis of BM is about 5 months in mTNBC and 10–18 months in the other subtypes[2]. Local treatment, usually surgery followed by radiation, is the standard of care. Efficacy of systemic treatment for BM of breast cancer has been limited due to poor drug delivery through the blood–brain barrier. Recently, HER2-targeted therapy has shown intracranial clinical activity in patients with human epidermal growth factor receptor 2 (HER2)-positive breast cancer[3,4], but there is no effective systemic treatment for TNBC patients with BM. In mTNBC patients with programmed cell death-ligand 1 (PD-L1)-positive tumor and/or tumor-infiltrating immune cells, the addition of an immune checkpoint inhibitor (pembrolizumab or atezolizumab) to first line chemotherapy has

* Corresponding author.
E-mail address: thli@ucdavis.edu (T. Li).

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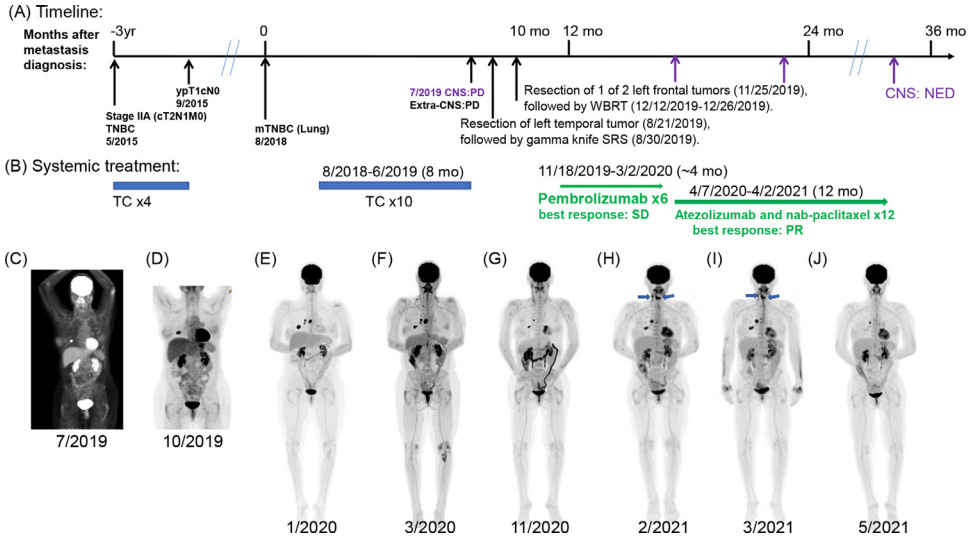


Fig. 1. Summary of key events during the patient's clinical course. (A) Timeline from diagnosis of metastatic disease. Arrows indicate key events. (B) Summary of systemic therapy. (C-J) Serial PET scans assess disease status. Blue arrows show increased radiotracer uptakes at the level of the hypopharynx and cervical lymph node.
TNBC, triple negative breast cancer; mTNBC, metastatic triple negative breast cancer; CNS, central nervous system; yr, year; m, month; wks, weeks; WBRT, whole brain radiation; SRS, stereotactic radiosurgery; TC, docetaxel and cyclophosphamide; PD, progressive disease; NED, no evidence of disease; SD, stable disease; PR, partial response; SRS, stereotatic radiosurgery; WBRT, whole brain radiotherapy.

improved their survival rates. However, the clinical efficacy of these chemoimmunotherapy combinations in patients with PD-L1 negative and refractory mTNBC, especially with BM, is not known. Here, we report a durable clinical response of >16 months to the multidisciplinary management of neurosurgery, radiation and chemoimmunotherapy in a 46-year-old female with PD-L1 immunohistochemistry (IHC)-negative, PD-L1/PD-L2/Janus kinase 2 (*PD-L1/PD-L2/JAK2) or PDJ*-amplified mTNBC with refractory brain, meningeal, and lung metastases.

2. Case report

Fig. 1 summarizes the clinical course. A non-Hispanic white female was initially diagnosed with a self-detected, clinical stage IIA (cT2N1) TNBC of the left breast at age 42 in May 2015. The patient had a radiographic partial response (PR) after 4 cycles of neoadjuvant chemotherapy with docetaxel and cyclophosphamide (TC). She had left breast lumpectomy and axillary lymph node dissection in September 2015, which revealed stage I [ypT1-cN0(0/13)] residual TNBC. She completed adjuvant radiation therapy in January 2016. In August 2018, she was found to have a 3.3-cm right middle lobe lung nodule during a workup for acute nephrolithiasis. A computed tomography (CT)-guided lung biopsy confirmed recurrent TNBC. She received TC as first-line systemic therapy for about 10 months during which a complete response (CR) was achieved. Unfortunately, a positron emission tomography / computed tomography (PET/CT) scan on July 3, 2019 showed enlargement of the right lung nodule and two new satellite lung nodules. At that time, she reported throbbing headache, short-term memory difficulty, blurred vision, and expressive dysphasia, all progressively severe over the preceding 6 months. A brain magnetic resonance imaging (MRI) on July 24, 2019 revealed a 2.7-cm mass in the left temporal lobe and significant vasogenic edema (Fig. 2A). She was treated with steroids for edema and levetiracetam for seizure prophylaxis prior to left temporal craniotomy on August 21, 2019, and Gamma Knife stereotactic radiosurgery (SRS) on August 30, 2019. Surgical pathology revealed poorly differentiated TNBC. Postoperatively, her memory and concentration improved, but headache, fatigue, confusion, blurred vision, occasional expressive aphasia, and leg weakness persisted.

She was referred to medical oncology for systemic therapy on September 30, 2019. In addition to her personal history of TNBC at age 42, her family history included maternal breast cancer in three generations (mother, maternal grandmother, great grandmother, and grandmother's sister), melanoma, colon cancer, and primary brain cancer (unknown histology). The patient did not have an Ashkenazi Jewish ancestry. She met the National Comprehensive Cancer Network (NCCN) criteria for BReast CAncer gene (BRCA) 1/2-related breast and ovarian cancer syndrome genetic testing[5] and underwent Invitae hereditary cancer panel testing (Invitae, San Francisco, CA), which revealed a pathogenic mutation, c.251G>A (p.Gly84Glu), in the *HOXB13* gene.

Tumor genomic profiling by the Tempus xT next generation sequencing (NGS) assay revealed 9 copies of *PDJ* gene co-amplification. Additional genomic alterations included *TP53* p.Y107* Stop gain - loss of function (LOF) (87.3% variant allele fraction, VAF), *ARID1A* p.S264* Stop gain –LOF (21.8%), copy number gain of *MYC, MYCL, RECQL4*. Fig. 3A illustrates the genomic amplifications and deletions for this patient. Details regarding the Tempus copy number analysis pipeline have been reported previously[6,7]. In addition to DNA sequencing, Tempus also performed whole-transcriptome RNA sequencing on the same specimen. Fig. 3B represents a density plot comparing RNA expression of this patient's sample and brain metastases of breast cancers in the Tempus database. RNA overexpression, relative to that seen in RNA sequencing of 117 BM of breast cancer in the Tempus clinico-genomic database, was observed for *PD-L1,PD-L2* and *JAK2* gene (with the patient in the 69th percentile, 83rd percentile, and 72nd percentile, respectively). This confirms that the detected DNA amplification was manifest in RNA overexpression of these genes. However, PD-L1 IHC staining was absent (<1%) on tumor cells and infrequent (10%) on tumor-associated immune cells in this patient (Fig. 3C). The tumor had intact DNA mismatch repair activity as determined by microsatellite instability assessment from NGS data and normal IHC stains for MLH1, PMS2, MSH2, and MSH6. Tumor mutational burden (TMB) was 5.0 nonsynonymous mutations per megabase (mut/MB), which was the 74th percentile of TMB values of brain metastases of breast cancers in the Tempus database.

On October 14, 2019, a PET/CT scan showed enlargement and increased fluorodeoxyglucose (FDG) avidity of the right middle lobe lung metastasis, a satellite nodule, and a right hilar lymph node. She started with her first dose of pembrolizumab monotherapy on November 18, 2019 when she had been having worsening confusion, lethargy, and general weakness for several days. A urgent MRI scan on November 24th, 2019 revealed two new large, partially solid, partially cystic left frontal

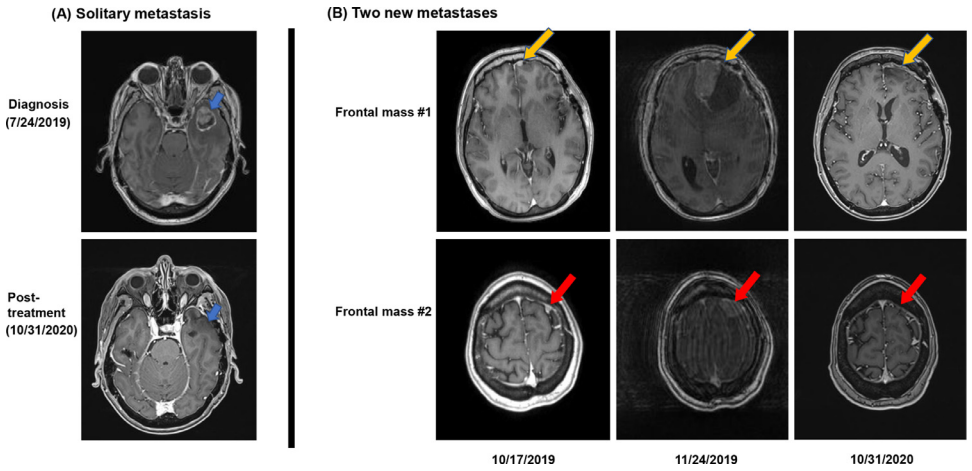


Fig. 2. MRI images of the brain. (A) The initial solitary brain metastasis was resected on July 24, 2019, and the surgical cavity was subsequently treated with radiosurgery. (B) Two new leptomeningeal metastases with dural enhancement detected on brain MRI on October 17, 2019: an anterior left frontal metastasis (top panel, gold arrow) and a left frontal vertex metastasis (bottom panel, red arrow). These masses quickly progressed over a short period of time as demonstrated on the brain MRI scan on November 24, 2019. The patient underwent surgery for one of the two left frontal lesions and subsequently completed a course of whole brain radiation. As seen on the scan on October 31, 2020, all BMs continue to show no evidence of active disease, even the unresected left vertex metastasis. BM, brain metastasis.

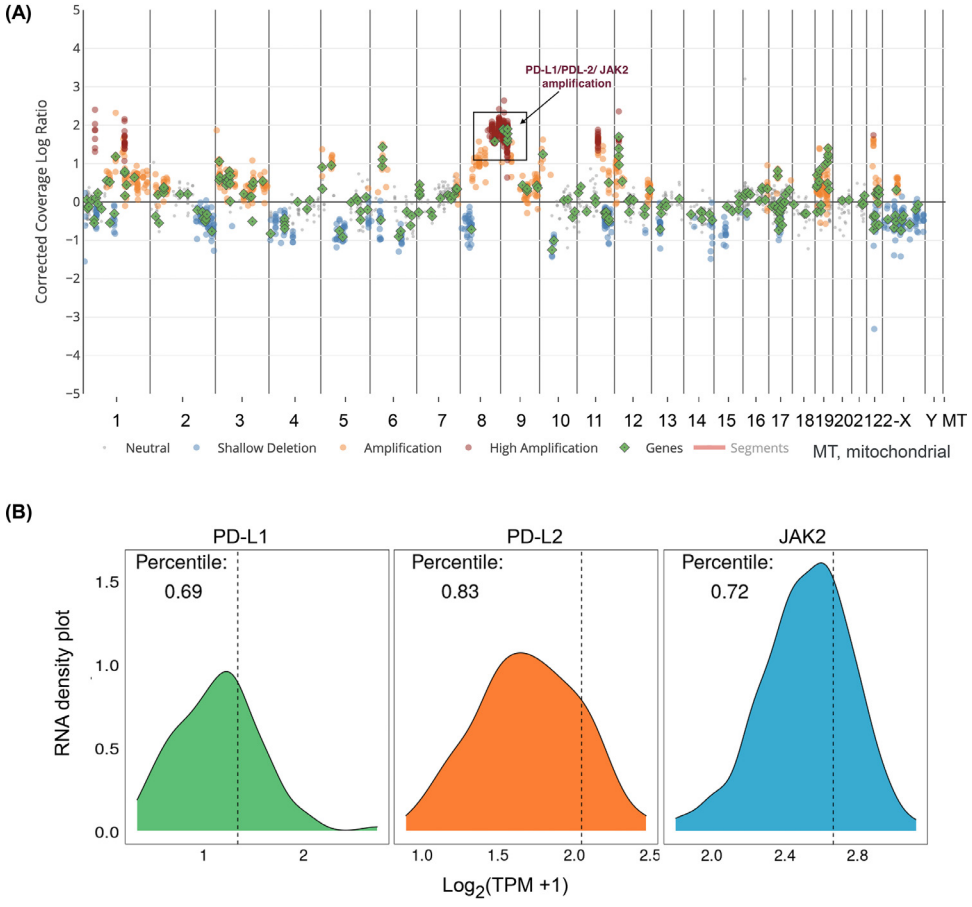
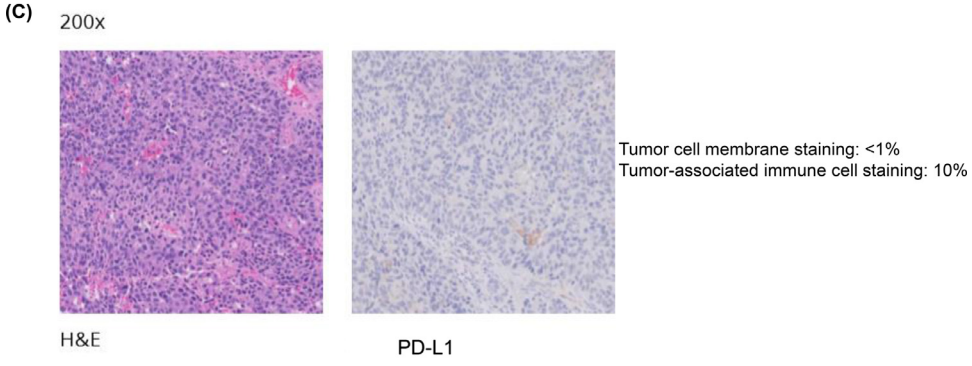


Fig. 3. DNA and RNA expression of *PDJ* amplification and PD-L1 protein expression. (A) DNA copy number plot of the genomic amplifications and deletions for all chromosomes of this patient. X-axis represents corrected coverage log ratio and y-axis represents chromosomes 1-22 and sex chromosomes. The dark red dots represent high amplification. Note, red dots clustered at chromosome 9 illustrating chromosome 9p24.1 amplification. (B) RNA density plot for PD-L1, PD-L2 and JAK2 expression. Note, this patient is represented as a dotted black horizontal line and compared to RNA expression data from BM obtained from breast cancer patients identified in the Tempus database. Patient is in the 69th percentile for PD-L1, 83rd percentile for PD-L2 RNA expression and 72nd percentile for JAK2. (C) PD-L1 IHC on tumor cells and tumor-associated immune cells. BM, brain metastasis; H&E, Hematoxylin and eosin stain; IHC, immunohistochemistry stain; MT, mitochondrial; TPM, Transcripts Per Kilobase Million.



masses [anterior (4.6 × 2.9 cm) and superior (4.1 × 2.3 cm)], significant perilesional edema, mass effect with rightward midline shift, subfalcine herniation, and compression and entrapment of the right lateral ventricle (Fig. 2B). The patient underwent urgent debulking of the majority of anterior frontal tumor on November 25, 2019. Surgical pathology was consistent with the previously reported mTNBC. Our patient had poor performance status with debilitating neurological symptoms after two brain tumor operations with partially unresected intracranial BM and LM. She was given a second dose of pembrolizumab monotherapy on December 9, 2019 and had significant clinical improvements in performance status and neurological symptoms before receiving whole brain radiotherapy (WBRT) (30 Gy between December 12 and December 26, 2019). She subsequently completed 4 more cycles of pembrolizumab monotherapy (from December 30, 2019 to March 2, 2020) with stable disease as the best tumor response. She had tumor progression in the right middle lobe mass and subcarinal and right hilar lymph nodes and new cervical lymph nodes. She received atezolizumab and nab-paclitaxel with partial response in the metastatic lymphadenopathy and right middle lobe nodule by Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST V1.1) after two cycles of treatment. Surveillance brain MRI scans at 6 weeks and every 3-months until now after radiation showed resolution of the parafalcine herniation and stable postsurgical and posttreatment changes of the left frontal and temporal lobes without any new metastases. She experienced several possible immune-related adverse events: grade 1 pneumonitis that resolved spontaneously, grade 2 colitis that improved to grade 1 without Imodium, and grade 2 joint discomfort that improved with supportive care. Of note, she was discontinued from the atezolizumab and nab-paclitaxel treatment after 12 cycles despite remaining PR on the PET/CT scan (Fig. 1H). The patient developed throat pain with bleeding, which correlated with the PET/CT scan findings showing increased radiotracer uptakes at the level of the hypopharynx and cervical lymph node. Biopsy of hypopharynx showed immune cell infiltration. After off treatment for about 2 months, a PET/CT scan showed mild tumor progression (Fig. 1I). The patient was started with antibody-drug conjugate sacituzumab govitecan-hziy (Trodelvy) since early April 2021 with an ongoing PR in lung metastases without any recurrent or new brain metastasis at the time of this report (Fig. 1J).

3. Discussion

Our study has several clinical implications. First, patients with mTNBC have limited treatment options and poor prognosis. Increasingly, tumor and germline genomic profiling and immune biomarker assays are used to select the treatment most likely to improve the duration and quality of life of patients with refractory mTNBC. While about 20% of TNBCs harbor germline *BRCA1/2* mutations, our patient lacked them. Broad germline mutation testing by NGS revealed a pathogenic mutation, c.251G>A (p.Gly84Glu), in the *HOXB13* gene. For males carrying a mutation in the *HOXB13* gene, the lifetime risk for prostate cancer is 33-60% (3 to 4.5 times higher than the average lifetime risk of about 12%). However, its clinical significance for patients with breast cancer is unknown and an area for further research. Tumor genomic profiling of brain tumor specimen from our patient identified 9 copies of the *PDJ* amplification, which resides in the chromosomal region 9p24.1. The original breast tumor specimen was not available for NGS and thus, we do not know if *PDJ* amplification was present in the original tumor.

The *PDJ* amplification at 9p24.1 upregulates PD-L1 expression, which has been the best-known biomarker for response to PD-1/PD-L1 inhibitors[8,9]. The *PDJ* amplification is detected in 63% of primary mediastinal large B-cell lymphomas and 50% of primary central nervous system large B-cell lymphomas. It is associated with high PD-L1 and PD-L2 IHC expression and a 65-87% response rate to PD-1/PD-L1 inhibitors[10]. The prevalence of PD-L1 amplification and its utility as a biomarker for response to PD-1/PD-L1 inhibitors are less known for solid tumors but are being prospectively evaluated in the Southwest Oncology Group (SWOG) 1609 clinical trial (NCI MATCH DART: Dual Anti-CTLA-4 & Anti-PD-1 blockade in Rare Tumors, NCT02834013). A retrospective review of 118,187 samples from over 100 types of solid tumor found *PD-L1* amplification (defined as ≥4 copies on NGS platforms) in 843 (0.7%)[10]. Most (84.8%) *PD-L1*-amplified tumors had a low to intermediate TMB, and *PD-L1* amplification did not always correlate with high PD-L1 expression by IHC. Of 9 patients treated with a PD-1 or PD-L1 inhibitor, the objective response rate for patients with solid tumors harboring *PD-L1* amplification was 66.7%, with a median progression-free survival (PFS) of 15.2 months (range, 1.2 to ≥24.1 months)[10]. Responders included 1 patient with glioblastoma (PFS, ≥5.2 months), 2 patients with head and neck squamous cell cancer (PFS, ≥9 and 15.2 months, respectively), 2 patients with metastatic basal cell cancer (PFS, 3.8 and ≥24.1 months, respectively), and 1 patient with urothelial cancer (PFS, ≥17.8 months). Median overall survival from the time of initiation of PD-1/PD-L1 inhibitors among patients with *PD-L1* amplification was not reached (range, 1.6 to ≥24.1 months). No breast cancer cases were included in this report.

The *PDJ* amplification at chromosome 9p24.1 has been detected in 1-2% of invasive breast cancer, of which about a third to half were TNBC in The Cancer Genome Atlas (TCGA) and Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) cohorts [11-13]. Its prevalence in TNBC was 15-18% in patient-derived xenograft samples by Fluorescence in situ hybridization (FISH) or whole genome copy number variation arrays[14]. Another study found that 22% (8 out of 36) surgical specimens from TNBC patients harbored *PDJ* amplification[12]. Thus, the *PDJ* amplification is likely a *de novo* genomic alteration. The frequency of the *PDJ* amplification increased after neoadjuvant chemotherapy[13], and is associated with aggressive behavior and poor outcome[15,16]. Further study is needed to determine the role of *PDJ* amplification in the tumorigenesis and progression of TNBC. TNBC patients with the *PDJ* amplification by oligonucleotide-based array of comparative genomic hybridization had worse outcomes than those TNBC patients without the *PDJ* amplification. The rate of disease-free survival at 5 years was 25% in TNBC patients with the *PDJ* amplification versus 66% in patients without the *PDJ* amplification. Overall survival at 5 years was 25% in TNBC patients with the *PDJ* amplification vs 69% in patients without the *PDJ* amplification (P=0.004)[15]. Tumors with the *PDJ* amplification could be a distinct molecular subtype of TNBC, and the *PDJ* amplification could be a biomarker for patients who might benefit from PD-1/PD-L1 inhibitors. One case report showed that a 62-year-old woman with mTNBC treated with pembrolizumab for 2.5 months before discontinuation due to hepatotoxicity had a 4.1-fold amplification of *PDJ* amplicon[13]. It is important to note that different research groups have utilized different cutoffs for *PDJ* amplification (such as ≥4 and ≥8 copies) and different assays to detect PD-L1 amplifications, such as FISH assay, oligonucleotide array, or NGS platform. The optimal cutoff for *PDJ* amplification as a predictive biomarker of response to PD-1/PD-L1 inhibitors needs to be defined. To the best of our knowledge, there is no report of the clinical efficacy of chemoimmunotherapy with a PD-1/PD-L1 inhibitor and chemotherapy in patients with *PDJ*-amplified mTNBC.

Patients with refractory mTNBC and symptomatic brain metastases are challenging to treat and have a life expectancy often measured in months or weeks[2]. Due to the neurological emergency of high intracranial pressure and herniation, the favorable clinical outcomes of our patient were due to the multidisciplinary management of neurosurgery, radiation and chemoimmunotherapy, which were all important and hard to dissect. When she received the first dose of pembrolizumab, she had been having the debilitating neurological symptoms which warranted the urgent debulking neurosurgery. After the surgery, the patient had residual, unresectable intracranial BM and LM. In addition to pembrolizumab, she received WBRT, which could be effective for treating both visible and microscopic intracranial BM. However, WBRT alone is usually not effective for treating LM with frequent recurrent and progressive metastasis through the cerebrospinal fluid[1]. Systemic

Table 1
Summary of clinical efficacy of immune checkpoint inhibitors for patients with mTNBC

Drug	Trial	Phase	Sample Size	Treatment setting	ORR	Median PFS (months; HR, 95% CI)	Median OS (months; HR, 95% CI)
Avelumab	JAVELIN Solid Tumor[29] (NCT01772004)	1b	168	Heavily pretreated metastatic or locally advanced breast cancer (with a median of three prior therapies)	3.0% (overall) and 5.2% (TNBC); 16.7% PD-L1$^+$ vs 1.6% PD-L1$^-$; TNBC Subgroup ORR: 22.2% PD-L1$^+$ vs 2.6% PD-L1$^-$	NA	NA
Pembrolizumab	KEYNOTE-012[30] (NCT01848834)	Ib	111	Patients with mTNBC whose tumor samples were screened for PD-L1 expression	18.5%	NA	NA
Pembrolizumab	KEYNOTE-028[31] (NCT02054806)	Ib	25	ER/HER2 advanced breast cancer with PD-L1-positive tumors	12.0%	12.0	clinical benefit rate: 20%
Pembrolizumab	ENHANCE 1[32] (NCT02513472)	1b/2	167	Patients with mTNBC (≤2 prior systemic therapies)	23.4%	NA	NA
Pembrolizumab	KEYNOTE-086[33] (NCT02447003)	II	170	Previously treated mTNBC	5.0% in the total and 5.0% in PD-L1$^+$	2.0	9.0
Pembrolizumab monotherapy/ single-agent chemotherapy	KEYNOTE-119[34] (NCT02555657)	III	622	Patients with centrally confirmed TNBC (1-2 prior systemic treatments)	CPS ≥10: 17.7% vs 9.2%; CPS ≥1: 12.3% vs 9.4%	CPS ≥10: 2.1 vs 3.4 (1.14, 0.82-1.59); CPS ≥1: 2.1vs 3.1 (1.35,1.08-1.68)	CPS ≥10: 12.7 vs 11.6 (0.78, 0.57-1.06); CPS ≥1: 10.7 vs 10.2 (0.86, 0.69-1.06)
Atezolizumab + nab-paclitaxel/ placebo + nab-paclitaxel	IMpassion-130[25] (NCT02425891)	III	910	First-line treatment for PD-L1$^+$ mTNBC	ITT: 56.0% vs 45.9%; PD-L1$^+$: 58.9% vs 42.6%	ITT: 7.2 vs 5.5 (0.80, 0.69-0.92); PD-L1$^+$: 7.5 vs 5.0 (0.62, 0.49-0.78).	ITT: 21.3 vs 17.6 (0.84, 0.69-1.02); PD-L1$^+$: 25.0 vs 15.5 (0.62, 0.45-0.86).
Atezolizumab/ placebo and paclitaxel	IMpassion131[35] (NCT03125902)	III	651	First-line treatment for locally advanced or mTNBC	PD-L1$^+$: 63% vs 55%; ITT: 54% vs 47%	PD-L1$^+$: 6.0 vs 5.7 (0.82, 0.60-1.12); ITT: 5.7 vs 5.6 (0.86, 0.79-1.05)	PD-L1$^+$: 22.1 vs 28.3 (1.12, 0.76-1.65); ITT: 19.2 vs 22.8 (1.11, 0.87-1.42)
Pembrolizumab/ placebo + chemotherapy	KEYNOTE-355[36,37] (NCT02819518)	III	566/ 281	First-line treatment for locally advanced or mTNBC	CPS ≥10: 53.2% vs 39.8%; CPS ≥ 1: 45.2% vs 37.9%; ITT: 41% vs 35.9%.	CPS ≥10: 9.7 vs 5.6 (0.65, 0.49-0.86); CPS ≥1: 7.6 vs 5.6 (0.74, 0.61-0.90), ITT: 7.5 vs 5.6 (0.82, 0.69-0.97)	NA

Abbreviations: CI, confidence interval; CPS, combined proportion score; ITT, intent-to-treat; mo, month; HR, hazard ratio; mTNBC, metastatic triple-negative breast cancer; NA, not available; ORR, overall response rate; OS, overall survivor; PD-1, Programmed cell death-1; PD-L1, programmed cell death-ligand 1; pCR, pathological complete response; PFS, progression free survival; TNBC, Triple-negative breast cancer

chemotherapy and targeted therapy are more effective than radiation for the treatment of LM[17], but there is no effective systemic treatment for TNBC patients with BM. Although we could not rule out the benefit of urgent debulking neurosurgery and WBRT in this current case, the lesson learned from this patient made us treat a non-small cell lung cancer (NSCLC) patient with only meningeal based brain metastasis with pembrolizumab alone without surgery or radiation. The patient achieved complete resolution of brain metastasis after 3 cycles of pembrolizumab therapy (*Personal communications*). Unlike the mTNBC patient in this case report whose brain tumor had no PD-L1 IHC expression, that NSCLC patient had high PD-L1 expression on brain metastasis. Additionally, our patient was not a good candidate for cytotoxic treatment after the surgery due to the poor performance status and debilitating neurological symptoms. Tumor genomic profiling of her tumor did not have any drug targets matched to targeted therapeutics. The identification of *PDJ* amplification in her tumor suggested the use of PD-1/PD-L1 inhibitors. She was excluded from the SWOG 1609 DART trial of nivolumab monotherapy (NCT02834013) due to the active BM and LM. A few studies have shown that pembrolizumab either alone or in combination with WBRT was safe and had modest clinical activity for BM in patients with PD-L1-positive melanoma[18,19] or NSCLC [20-23]. Furthermore, a durable clinical response of >23 months was observed in a NSCLC patient with high PD-L1 IHC and LM who received pembrolizumab and WBRT[24]. Our patient had rapid resolution of all her neurological symptoms and improvement in her performance status with the multidisciplinary management of debulking neurosurgery, pembrolizumab and WBRT, suggesting effective treatment for her BM and LM. She was tapered off steroids and stopped levetiracetam. However, our patient did not have a durable clinical response and was found to have progression in lung metastases after 6 cycles of pembrolizumab monotherapy. The addition of atezolizumab to nab-paclitaxel, but not paclitaxel, has been shown to improve progression-free survival (PFS)[25] and overall survival (OS)[26] as first line treatment in the PD-L1–positive, mTNBC patients. Table 1 summarizes the results of several published clinical trials with PD-1/PD-L1 inhibitors as monotherapy or in combination with chemotherapy in patients with mTNBC. The median PFS and OS in patients with PD-L1-positive mTNBC were 7-10 and 21-22 months, respectively, in first line setting, and 2-3.5 and 9-15 months, respectively, in refractory disease setting. Notably, patients with BM were not included in these trials. Thus, our mTNBC patient with refractory BM had a superior clinical response of >16 months to the multidisciplinary management described in this case report. Although the patient was discontinued from the chemoimmunotherapy due to the unusual immune-related adverse event (i.e., lymphocyte infiltration in the hypopharynx), the treatment was otherwise well tolerated and the patient had good performance status and organ function to receive new systemic therapy with sacituzumab govitecan with an ongoing PR in lung metastases without any recurrent or new brain metastasis at the time of this report.

Despite the positive results of IMpassion130, many patients do not benefit from treatment, and there is a large unmet need to tailor treatment through predictive biomarkers. Exploratory biomarker analysis for BM suggested that higher response rates were associated with higher levels of tumor-infiltrating lymphocytes (TILs) and CD8-positive T cells[27].

In a retrospective study of 84 BM of breast cancer, PD-L1 and PD-L2 expression were present in 53% and 36% of cases, respectively, and PD-1 expression on TILs correlated positively with the presence of $CD4^+$ and $CD8^+$ TILs[28]. Recent clinical trials have allowed patients with stable and progressive BM to enroll so the efficacy of these new agents against BM can be evaluated. Combining DNA and transcriptomic data can identify known and novel features of the transcriptome and provide a more comprehensive understanding of what is driving the patient's tumor. Since *PDJ* amplification was identified in this patient, it was important to determine the level of RNA expression of these genes. We found that the PD-L1 RNA overexpression did not correlate with the PD-L1 IHC expression. One potential explanation is that PD-L1 is being regulated via post-transcriptional mechanisms that affect mRNA stability. This supports that *PDJ* amplification alone may be an independent predictive biomarker of response to PD-1/PD-L1 inhibitors.

In conclusion, our patient with PD-L1 IHC-negative, *PDJ*-amplified, refractory, mTNBC with brain and meningeal metastases and lung metastases has achieved durable clinical response to the sequential treatment of partial surgical resection, pembrolizumab, WBRT and chemoimmunotherapy with atezolizumab and nab-paclitaxel. The uses of multidisciplinary management and biomarker-driven precision oncology were essential to the favorable clinical outcome.

Declaration of competing interest

Dr. Li has disclosed that she receives grant/research support from Pfizer, Hengrui, Merck, OncoImmune (Oncoc4), AstraZeneca, and Tempus. Dr. Ashok is an employee of Tempus, Inc. Other authors have disclosed that they have not received any financial consideration from any person or organization to support the preparation, analysis, results, or discussion of this article.

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Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Consent for publication

The patient provided consent for the case report. Only deidentified data and images are used in this case report.

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