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Combined Antiangiogenic Therapy and Immunotherapy Is Effective for Pancreatic Cancer With Mismatch Repair Proficiency but High Tumor Mutation Burden

A Case Report

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Abstract: Immunotherapy has been recommended as a second-line treatment only for high microsatellite instability or DNA mismatch repair deficiency advanced pancreatic cancer in National Comprehensive Cancer Network guidelines. Here, we report a case with a good response to immunotherapy in pancreatic cancer with mismatch repair proficiency. A 55-year-old woman diagnosed with pancreatic cancer cT4N1M1 (liver, lung) who harbored *ERBB2* mutations with high tumor mutation burden (TMB) underwent multiple therapies and survived 19 months. A partial response in pancreatic cancer was observed when the patient was treated with combined antiangiogenic therapy and immunotherapy after a series of ineffective treatments. Neutrophil-to-lymphocyte ratio (NLR), a predictive marker of efficacy of immunotherapy, confirmed that immunotherapy resulted in the partial response in pancreatic cancer. To our knowledge, this is the first to report advanced pancreatic cancer with mismatch repair proficiency had a good response to immunotherapy, and this is the first to report an association between high blood-based TMB or NLR and improved clinical outcomes in pancreatic cancer. Therefore, TMB may also be a biomarker for immunotherapy of pancreatic cancer, and NLR may be a prospective predictive marker for efficacy of immunotherapy in pancreatic cancer.

Key Words: pancreatic cancer, mismatch repair deficiency, mismatch repair proficiency, combined immunotherapy, tumor mutation burden, Neutrophil-to-lymphocyte ratio

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Pancreatic cancer is almost always fatal, with a 5-year survival rate of less than 5%. Because most patients are diagnosed with locally advanced pancreatic cancer or distant metastasis at diagnosis, only 15% to 20% of patients can be treated with surgery. The standard treatment regimen for advanced pancreatic cancer is chemotherapy with mainly gemcitabine, FOLFIRINOX (folinic acid (leucovorin)-fluorouracil-irinotecan-oxaliplatin) chemotherapy regimen and radiotherapy combined with chemotherapy. There are few targeted drugs for pancreatic cancer, and most of them are in clinical studies. Currently, erlotinib is the only targeted drug that has been accepted in the guidance of National Comprehensive Cancer Network (NCCN) for pancreatic cancer. Immunotherapy is still at the experimental stage in the treatment of pancreatic cancer,[1] and pembrolizumab was firstly included in NCCN guidelines as a second-line treatment only for high microsatellite instability (MSI-H) or DNA mismatch repair deficiency (dMMR) advanced pancreatic cancer in 2017. However, a rare subset of patients with dMMR pancreatic cancer has been reported to have partial responses (PRs) or complete responses to immunotherapy.[2–4] Immunotherapy in pancreatic cancer treatment is rarely successful probably because of the particular characteristic of pancreatic cancer itself, for example immunosuppressive tumor microenvironment,[1] low TMB,[5] or low probability of dMMR.[3,5]

Here we report a case with a good response to immunotherapy in pancreatic cancer with mismatch repair proficiency (pMMR) after a series of ineffective treatments. Neutrophil-to-lymphocyte ratio (NLR), a predictive marker of efficacy of immunotherapy, confirmed that immunotherapy resulted in PR in pancreatic cancer.[6–10] To our knowledge, this is the first to report advanced pancreatic cancer with pMMR had a good response to immunotherapy, and this is the first to report an association between high blood-based TMB (bTMB) or NLR and improved clinical outcomes in pancreatic cancer.

CASE REPORT

A 55-year-old woman experienced intermittent abdominal pain in March 2017, who had a history of hypertension (oral felodipine sustained-release tablets), gastric ulcer for 1 year, and hemorrhoids for many years. The color Doppler ultrasound of the abdomen suggested the possibility of pancreatic cancer, and the computed tomography (CT) scan suggested multiple liver and lung metastases. The positron emission tomography CT demonstrated a primary lesion in the pancreatic head, multiple lymph node metastasis with abnormal increased metabolism in hepatic portal vein, peritoneal back door-cavity gap, left side of left renal aorta, and lesser curvature of the stomach, multiple metastasis with abnormal increased metabolism in liver parenchyma, and multiple metastasis in the right lung (Fig. 1). Moderately differentiated liver adenocarcinoma was revealed by liver biopsy. The patient was diagnosed with pancreatic cancer cT4N1M1 (liver, lung).

Considering the patient's abdominal pain, radiotherapy combined with chemotherapy was performed. The patient was treated with radiotherapy for pancreatic lesion (70 Gy in 15 fractions) and 2 large metastatic lesions in the right lobe of the liver (60 Gy in 15 fractions and 50 Gy in 15 fractions respectively). Meanwhile, GS regimen (gemcitabine 1000 mg/m^2 day 1 [d1], day 8, and S-1 100 mg/d 1–14, qid [Q] 21d) was performed for 2 cycles. After that,

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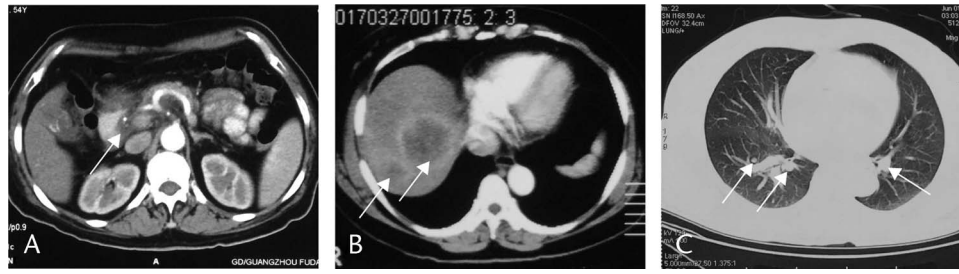

FIGURE 1. Pancreatic cancer with multiple metastases of liver and lung. Computed tomography scans of pancreas (A), liver (B), and lung (C) at diagnosis.

the patient's abdominal pain was relieved and she refused further chemotherapy because of grade II thrombocytopenia and fatigue. The results of genetic test revealed alterations in *ERBB2* with CNV amplification (20%) and mutations in exon19 (0.14%) and exon20 (0.12%), and subsequently, the patient received anti-*HER2*–targeted therapy with trastuzumab for 4 times (8 mg/kg d1, Q21d for the first time and later 6 mg/kg) plus erlotinib 100 mg/Qd, during which the level of carbohydrate antigen 19-9 decreased. However, after 3 months, targeted drugs were stopped for the reason that liver lesions without radiotherapy were enlarged according to positron emission tomography CT. Then, the patient received argon-helium knife cryoablation treatment for enlarged liver lesions once and liver TACE treatment twice. The systemic treatment was changed to lapatinib 750 mg/d, but after 15 days intermittent vaginal bleeding occurred. A CT scan of the pelvis suggested pelvic metastasis with ascites. The patient only took Yunnan Baiyao orally and the bleeding was slightly relieved. After a cycle of chemotherapy (oxaliplatin 85 mg/m^2 d1 plus irinotecan 180 mg/m^2 dl, Q14d), the patient refused to continue chemotherapy because of the physical decline, weight loss, leukocyte decline, and grade II thrombocytopenia. Subsequently, the patient was treated with combined anti-PD-1 and antiangiogenic therapy, that is, intravenous infusion of pembrolizumab for 8 times (100 mg d1, Q21d) plus oral lenvatinib for 5 months (20 mg/Qd). As a result, the lesion in primary tumor shrank by 27.3% and the lesion in metastatic liver tumor shrank by 33.0%. Meanwhile, the metastatic lung cancer (reduction by 13.2%) and metastatic tumor in pelvis (increase by 2.4%) achieved stable disease. The therapeutic effect was evaluated as PR in pancreatic cancer (reduction by 27.3%) and as an overall stable disease (reduction by 18.4%) (Fig. 2). After 5-month combined immunotherapy, the patient had a severe pulmonary infection, so pembrolizumab and lenvatinib were stopped and anti-infective therapy was used to control pulmonary infection. A CT scan revealed increased hepatic lesions and pleural effusion. Then, intravenous infusion of T-DM1 200 mg was used, but severe pulmonary infection occurred again after the first use of T-DM1; therefore, only anti-infection treatment was given next and pleural fluid and pelvic tumor increased. After pulmonary infection control, pelvic tumors were treated with interventional therapy once. The patient died on October 22, 2018, because of sudden deterioration. The timeline of therapy is presented in Figure 3.

METHODS AND RESULTS

Neutrophil-to-Lymphocyte Ratio

The clinical data of absolute neutrophil count and absolute lymphocyte count were obtained from peripheral blood test before, during, and after immunotherapy. Neutrophil-to-lymphocyte ratio (NLR) was calculated based on absolute neutrophil count and absolute lymphocyte count. The mean neutrophil count was 3432.0 (SD, 2099.8)/mm^3 (range, 750.0–7050.0), 2700.0 (SD, 584.6)/mm^3 (range, 2140.0–3730.0), and 5456.3 (SD, 1605.3)/mm^3 (range,

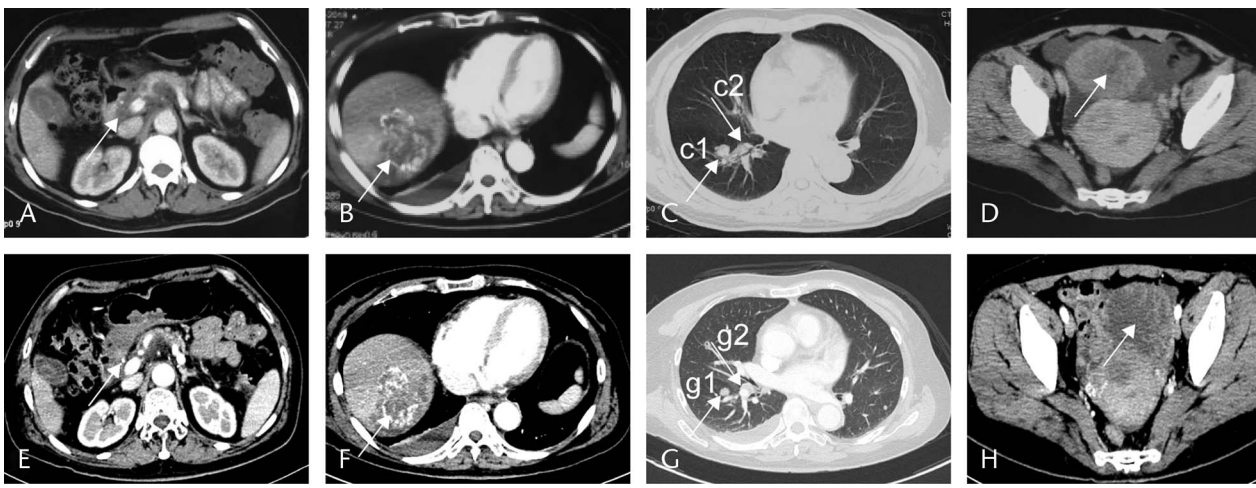

FIGURE 2. Tumors shrank after immunotherapy. Computed tomography scans of the pancreas (3.3 × 2.1 cm) (A), liver (11.2 × 7.5 cm) (B), lung (c1, 1.9 × 1.5 cm; c2, 1.9 × 1.5 cm) (C), and pelvis (8.4 × 6.7 cm) (D) before immunotherapy. Computed tomography scans of the pancreas (2.4 × 1.8 cm) (E), liver (7.5 × 5.9 cm) (F), lung (g1, 1.4 × 1.0 cm; g2, 1.9 × 1.5 cm) (G), and pelvis (8.6 × 6.4 cm) (H) after immunotherapy. Nodules are indicated by the arrows.

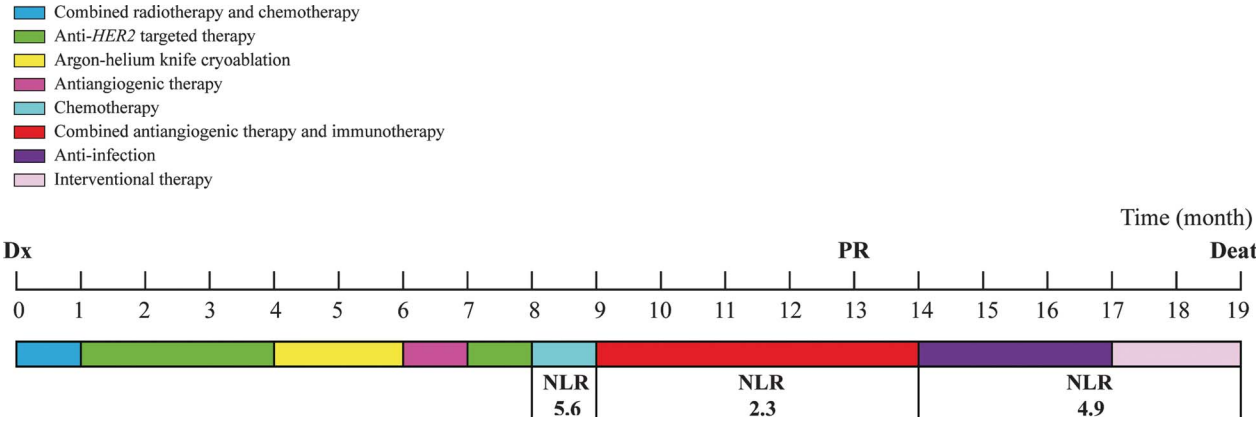

FIGURE 3. The timeline of therapy for the patient with pancreatic cancer. Neutrophil-to-lymphocyte represents the median NLR before, during, or after immunotherapy. Dx indicates diagnosis.

3730.0–8620.0), and the median neutrophil count was 3610.0/mm^3, 2620.0/mm^3, and 4845.0/mm^3 before, during, and after immunotherapy, respectively. The mean lymphocyte count was 618.0 (SD, 64.3)/mm^3 (range, 530.0–720.0), 1022.9 (SD, 86.6)/mm^3 (range, 930.0–1200.0), and 1013.8 (SD, 232.2)/mm^3 (range, 710.0–1400.0), and the median lymphocyte count was 610.0/mm^3, 1000.0/mm^3, and 1020.0/mm^3 before, during, and after immunotherapy, respectively. The mean NLR was 5.4 (SD, 2.9) (range, 1.2–9.8), 2.6 (SD, 0.6) (range, 2.1–3.7), and 5.9 (SD, 3.0) (range, 3.5v12.1), and the median NLR was 5.6, 2.3, and 4.9 before, during, and after immunotherapy, respectively.

Immunohistonchemistry

Immmunohistochemistry staining was performed using the automated Benchmark platform (Ventana Medical Systems, Tucson, Ariz), according to the manufacturer's instructions. Four-micrometer-thick sections were mounted onto slides and deparaffinized followed by antigen retrieval using cell conditioning solution and stained with the UltraView Universal DAB detection kit (Ventana Medical Systems). The following primary antibodies were used in this study: anti-*Ki-67*, anti-*EGFR*, anti-*Her-2*, anti-*Hepatocyte*, anti-*AFP*, anti-*CK20*, anti-*ck7*, anti-*Villin*, anti-*CDX-2*, anti-*COX-2*, anti-*PD-1*, anit-*PD-L1*, anti-*MLH1*, anti-*MSH2*, anti-*MSH6*, and anti-*PMS2*.

Immunohistochemical results showed *Ki-67* (+90%), *EGFR* (+), *Her-2* (+++), *Hepatocyte* (−), *AFP* (−), *CK20* (focal +), *ck7* (−), *Villin* (++), *CDX-2* (++), *COX-2* (++), *PD-1* (−), *PD-L1* (−), *MLH1* (+), *MSH2* (+), *MSH6* (+++), and *PMS2* (++).

Next-Generation Sequencing

Blood instead of tissues was used for next-generation sequencing (Shanghai Tongshu Biotechnology Co, Ltd, Shanghai, China) because of few tissues available. We performed cell-free DNA extraction from the blood sample using the MagMAX Cell-Free DNA Isolation Kit (A29319; Applied Biosystems, Waltham, Mass). We created targeted capture pulldown and a targeted library from native DNA using a targeted gene sequencing panel, which covers 156 genes and KAPA Hyper Prep Kit Illumina platforms (#KR0961; Kapa Biosystems, Wilmington, Mass), and generated paired-end sequence data using Illumina HiSeq machines. The sequence data, aligned to the human reference genome (NCBI build 37) using BWA, and sorted and removed PCR duplication using GATK 4.0.[11] Somatic mutation calling was performed using VarDict 1.5.8.[12] Somatic mutations existing in at least 2 of the results of the 3 software were selected as high confident mutations. Copy-number variations (CNVs) and loss of heterozygosity were analyzed using CNVkit 0.9.6.dev0.[13]

The results of next-generation sequencing showed alterations in *ERBB2* with CNV amplification (20%) and mutations in exon19 (0.14%) and exon20 (0.12%). Mutations were also occurred in *UGTIA1*, *GSTPI,* and *MTHFR*. TMB (14.9 mutations/Mb) was much higher than the average (2.5 mutations/Mb).

DISCUSSION

Pancreatic cancer has a poor prognosis, and the diagnosis and therapy of pancreatic cancer remain formidable challenges. However, multidisciplinary team is a promising solution for the treatment of pancreatic cancer. The patient with advanced pancreatic cancer reported here survived 19 months through multiple therapies including radiation therapy, chemotherapy, multidrug-targeted therapy, interventional therapy, and combined immunotherapy.

Erlotinib is currently the only targeted drug that has been included in the guidance of NCCN for pancreatic cancer. However, the median survival time was 6.24 months versus 5.91 months, and progression-free survival (PFS) was 3.75 months versus 3.55 months for the erlotinib and gemcitabine versus placebo and gemcitabine groups.[14] Furthermore, *EGFR* status was not associated with response or disease stability when therapy combining gemcitabine with erlotinib was performed.[14] In this case, there was no *EGFR* mutation. Based on the immunohistochemical results indicating strong positive expression of *Her-2* and the genetic test results indicating alterations in *ERBB2* with CNV amplification (20%) and mutations in exon19 (0.14%) and exon20 (0.12%), combined therapy with anti-*her-2* drug and erlotinib was adopted.[14,15] However, 11 weeks later, the disease progressed, which was consistent with the reported PFS approximately 3 months.[14,16] Recently, a phase 2b study of gemcitabine plus nimotuzumab reported that the median survival time was 8.6 months versus 6.0 months, and PFS was 5.3 months versus 3.6 months for the nimotuzumab and gemcitabine versus placebo and gemcitabine groups.[17] However, the phase 3 trial of gemcitabine plus nimotuzumab is required.

Inhibition of angiogenesis has been an established therapeutic strategy for many solid tumors, because angiogenesis is an important event in tumor growth and hematogenous metastasis. Several preclinical and clinical trials have been conducted to use antiangiogenic inhibitors in pancreatic cancer treatment.[18–21] Regrettably, the results showed that antiangiogenic therapies did not improve the efficacy of pancreatic cancer treatment.

Immunotherapy has emerged as a major therapeutic modality in oncology in recent years, especially in melanoma and lung cancer.

However, 50% to 80% of patients with tumors for which immune checkpoint inhibitors are indicated do not benefit from these drugs, and many patients experience severe adverse events.[22] Therefore, researchers hope to enhance and broaden the benefits of immunotherapy by combined immunotherapy. Among them, combined antiangiogenic and anti-*PD-1/PD-L1* therapy can stimulate the immune response and enhance the efficacy of immunotherapies by converting the intrinsically immunosuppressive tumor microenvironment to an immunosupportive one.[22,23] The Food and Drug Administration has granted the combination of the *PD-1* inhibitor pembrolizumab and the *VEGF/FGF* inhibitor lenvatinib a breakthrough therapy designation for the treatment of patients with advanced and/or metastatic renal cell carcinoma in January 2018, with 83% of objective response rate (ORR) and 100% of tumor control rate.[24,25] Moreover, the European Society for Medical Oncology 2016 reported a phase 1b trial of lenvatinib plus pembrolizumab with 54% of ORR and 100% of tumor control rate in 13 patients with solid tumors (nonsmall cell lung cancer [NSCLC]: n = 2; renal cell carcinoma: n = 8, endometrial cancer: n = 2; melanoma: n = 1).[26] The American Society of Clinical Oncology endometrial carcinoma with 48% of ORR and 96% of tumor control rate.[27] A trial of lenvatinib plus pembrolizumab in subjects with hepatocellular carcinoma is still ongoing. These trials suggested that combined therapy of pembrolizumab plus lenvatinib has a promising prospect. IMpower150 clinical trials have proved that the addition of atezolizumab to bevacizumab plus chemotherapy significantly improved PFS and overall survival among patients with metastatic nonsquamous NSCLC, regardless of PD-L1 expression and EGFR or ALK genetic alteration status.[28]

Pembrolizumab was included in NCCN guidelines as a second-line therapy for locally advanced or metastatic pancreatic cancer, but only for tumors with MSI-H or dMMR.[2,29] The dMMR in pancreatic cancer was reported to be a rare event, occurring at a frequency of 0.8%,[3] for whom single checkpoint inhibitor may be effective. However, the patient reported here was MSI stable (MSS) based on the immunohistochemical results (*MLH1* [+], *MSH2* [+], *MSH6* [+++], and *PMS2* [++]).

In addition to MSI and dMMR, TMB has also been generally accepted as a biomarker to predict responses to immunotherapy[30,31] and was included in the NCCN guidelines for NSCLC in 2019. Yarchoan et al[5] evaluated the relationship between TMB and the ORR with anti-*PD-1* or anti-*PD-L1* therapy in 27 tumor types. The results showed a significantly low TMB and a poor response to immunotherapy in pancreatic cancers. Chalmers et al found that MSI-H generally occurred as a subset of high TMB. Most MSI-H samples also had high TMB (83%), and 97% had TMB of 10 or more mutations/Mb. However, the converse was not true; only 16% of samples with high TMB were classified as MSI-H.[32] Thus, if MSI is used as the only predictor to determine whether to use immunotherapy or not, patients with low MSI (MSI-L) or MSS but high TMB may lose the opportunities to be well treated. Moreover, tissue-based TMB is limited because the availability of adequate tissue for molecular testing in patients with advanced disease can be challenging. Gandara et al has reported that bTMB can also be used as a predictor of clinical benefit in NSCLC patients treated with anti-PD-L1 drugs.[33] It is generally considered that TMB of more 20 mutations/Mb is high and TMB of less than 10 mutations/Mb is low. Of course, TMB values vary slightly from company to company depending on the package and technology used. The patient reported here was treated with pembrolizumab plus lenvatinib and achieved PR and PFS 5 m (Fig. 2), who had a high bTMB (14.9 mutations/Mb) but was MSS.

Recently, NLR was found to be a predictive marker for efficacy of immunotherapy in melanoma,[6] renal cell carcinoma,[7,10] and NSCLC.[8,9] It has been reported that NLR of more than 5 is a strong independent predictor of poor outcome in patients treated with immunotherapy.[6,9] It has been also reported that NLR of 3 or more is a prognostic factor for overall in patients with metastatic NSCLC treated with immunotherapy.[8] In our study, the median NLR was 5.6, 2.3, and 4.9 before, during and after immunotherapy, respectively, indicating the effectiveness of immunotherapy for this patient. Because the NLR is obtained from complete blood counts, it can be easily available without any additional procedures or costs.

Can TMB or NLR work as an effective predictor of clinical benefit in patients treated with immunotherapy? Can bTMB replace tissue-based TMB? Is immunotherapy more effective in patients with high TMB? How to optimize the combined therapy with pembrolizumab plus lenvatinib? These issues require further studies.

CONCLUSIONS

This is the first report that pMMR-advanced pancreatic cancer had a good response to immunotherapy, and this is the first to report an association between bTMB (and also NLR) and improved clinical outcomes in pancreatic cancer. High throughput sequencing technologies combined with bioinformatics analysis can provide precision treatment with targeted therapy or immunotherapy for pancreatic cancer patients. Despite low occurrence of TMB and dMMR in pancreatic cancers, patients with high TMB may have good responses to immunotherapy. Combined antiangiogenic and anti-*PD-1/PD-L1* therapy may be a better treatment for pancreatic cancers.

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