Conversion therapy with tislelizumab for high microsatellite instability, unresectable stage III gastric cancer: a case report

Dan Jian¹#, Chengyuan Qian¹#, Dong Wang¹, Qiang Ma², Li Wang³, Chunxue Li¹, Mingfang Xu¹, Nan Dai¹, Qian Chen¹, Juan He¹, Huan Zhang¹, Mingming Yuan¹, Rongrong Chen¹, Rui Chao⁵, Yan Feng¹^*

¹Cancer Center, Daping Hospital, Army Medical University, Chongqing, China; ²Department of Pathology, Daping Hospital, Army Medical University, Chongqing, China; ³Department of Gastric & Colorectal Surgery, Daping Hospital, Army Medical University, Chongqing, China; ⁴Geneplus-Beijing, Beijing, China; ⁵Department of Orthopaedic Surgery, Chongqing Emergency Medical Center, The Fourth People's Hospital of Chongqing, Chongqing University Central Hospital, Chongqing, China

#These authors contributed equally to this work and are co-first authors.

Correspondence to: Yan Feng, MD. Cancer Center, Daping Hospital, Army Medical University, No.10 Changjiang Zhilu, Daping Yuzhong District, Chongqing 400038, China. Email: fengyan19820729@sina.com; Rui Chao, MD. Department of Orthopaedic Surgery, Chongqing Emergency Medical Center, The Fourth People's Hospital of Chongqing, Chongqing University Central Hospital, No. 1 Jiankang Road, Yuzhong District, Chongqing 400014, China. Email: 956440096@qq.com.

Abstract: Gastric cancer (GC) is the fifth-highest ranked cancer for incidence and second for mortality from cancer worldwide. Conversion therapy has recently emerged as an alternative therapy for advanced/metastatic GC patients who are unable to undergo surgical resection at the time of diagnosis. Herein, we present the case of a patient with unresectable stage III GC of high microsatellite instability (MSI), high tumor mutation burden (TMB), and Epstein-Barr virus (EBV) positive. The patient received conversion therapy involving a combination of chemotherapy and immunotherapy regimens. After 3 courses of chemotherapy combined with tislelizumab, the patient underwent laparoscopic radical total gastrectomy. The pathological examination demonstrated that there was no cancerous tissue at the proximal or distal end of the tumor and no lymph node metastases in the lesser or greater curvature, indicating a pathologic complete response. Thereafter, the patient continued tislelizumab treatment to prevent postoperative carcinoma recurrence and metastasis, and to improve prognosis. In conclusion, our study confirmed that chemotherapy combined with immunotherapy is a promising conversion therapy for GC patients with locally unresectable lesions or distant lymph node metastasis, and these findings warrant large-scale clinical studies. This report highlights the clinical importance of next-generation sequencing technology in investigating therapeutic strategy to provide the maximal clinical benefit for patients with GC.

Keywords: Conversion therapy; case report; pathologic complete response; tislelizumab; unresectable gastric cancer (unresectable GC)

Submitted Jul 22, 2021. Accepted for publication Sep 18, 2021.
doi: 10.21037/atm-21-4295

View this article at: https://dx.doi.org/10.21037/atm-21-4295

Introduction

According to GLOBOCAN 2018 cancer statistics, gastric cancer (GC) is ranked fifth for incidence and second for mortality, with over 1,000,000 new cases and an estimated 783,000 deaths worldwide. The incidence rate is twice as high in men compared with women and markedly elevated in East Asia (1). Although the therapeutic effect of operative treatment for early-stage GC is acceptable, over 70%
of patients develop advanced-stage disease. The median overall survival (OS) time for those with advanced-stage GC is less than 1 year (2). Lauren classification and the WHO classification [2010] were commonly classification systems without prognostic value and without therapeutic effects (3). With the development of next generation sequencing, the TCGA study reported four major molecular subtypes to provide insights into the heterogeneity including Epstein-Barr virus (EBV), microsatellite instability (MSI), genomic stability (GS) and chromosomal instability (CIN) (4). The alternative classification of Asian Cancer Research Group (ACRG) stratified gastric cancer into tumors with MSI, including microsatellite-stable tumours showing epithelial to mesenchymal transition (MSS/EMT), MSS tumours with intact TP53 activity (MSS/TP53+) and MSS tumors with functional loss of TP53 (MSS/TP53–) (5). In addition, several studies defined gastric cancer molecular subtypes using immunohistochemistry (IHC) and EBV-encoded RNA in situ hybridization (EBER-ISH) (6,7). The clinicopathological and molecular characteristics of gastric cancer were associated with prognosis and used for the standardization of pathological definitions (8,9).

Multiple therapeutic regimens with immune checkpoint inhibitors (ICIs) have been developed to improve these dismal outcomes (10,11). A phase 2 study showed that first-line tislelizumab, a monoclonal antibody against programmed cell death-1 (PD-1), plus chemotherapy produced durable responses with manageable tolerability in patients with locally advanced/metastatic esophageal squamous cell carcinoma or gastric/gastroesophageal junction (G/GEJ) adenocarcinoma (12). Simultaneously, the use of biomarkers to predict tumor response to ICIs has been explored, including MSI (13) tumor mutation burden (TMB) (14), EBV (15), and expression of programmed cell death ligand-1 (PD-L1) (16). Previous studies showed that immune molecules, MSI and PD-L1 expression were considered as prognostic biomarkers in gastric cancer (17). The patients with MSI-high could be treated with PD-L1 antibody pembrolizumab for solid cancer to improve the prognosis (18). In MSI-H metastatic colorectal cancer, nivolumab provided durable disease control for improvement of clinical benefit (19). The expression of immune molecules including CD274, LAG3, and IDO1 inferred better prognosis in patients with MSI-high colon cancer (17). In KEYNOTE series of trials, high PD-L1 scores showed better overall survival in gastric cancer patients after treatment with immune checkpoint inhibitors (20). These biomarkers provide information about the state of the immune system in GC and may predict patient clinical outcomes. Studies have showed that the frequency of MSI and loss of heterocigosity (LOH) in neoplastic gastric carcinoma were 11.7% and 83%, respectively (21). Moreover, intestinal metaplasias are generally considered as pre-neoplastic gastric lesions (22). Studies have also showed that the frequency of MSI and LOH in preneoplastic gastric carcinoma were 17% and 54%, respectively (21). However, the appropriate time to incorporate immunotherapy into therapeutic plans for suitable patients is still under investigation.

Recently, conversion therapy has emerged as an alternative therapy for advanced/metastatic GC patients who are unable to undergo surgical resection at the time of diagnosis, such as those with locally unresectable lesions, distant lymph node metastasis, and signs or imaging manifestations of distant lesions (23,24). It has become increasingly common for surgeons to reevaluate surgical feasibility following palliative treatment in patients initially deemed unsuitable for surgical resection (25), making conversion therapy a promising therapeutic strategy for providing longer survival in patients with advanced GC after chemotherapy (26). Previous studies demonstrated that neoadjuvant chemotherapy followed by surgery provided dramatic survival benefit in gastric cancer (27). Biological and clinical factors involved in the impact on the prognosis of patients with incomplete pathological remission, including immune respond, altered gene signatures (28). Studies showed the anti-tumor functions of plasma B cells and myeloid-derived antigen-presenting cells were associated with incomplete pathologic response to neoadjuvant chemotherapy in breast cancer (29). Neoadjuvant chemotherapy significantly altered HER2 genomic signature of original breast cancer in patients with incomplete pathologic response (28). The high p53 expression in rectal cancer patients was correlated with incomplete pathological remission after neoadjuvant chemotherapy (30).

The development of treatment involving ICIs plus chemotherapy has greatly enhanced clinical benefits in patients with advanced GC (31). Studies (NCT03469557) have reported the safety and tolerability in the first-line treatment of tislelizumab plus chemotherapy for advanced gastric junction adenocarcinoma. The results showed that objective response rates and disease control rates were 46.7% and 80%, respectively in gastric/gastroesophageal junction adenocarcinoma (12). However, little research on the clinical value of a multimodal strategy of chemotherapy
combined with ICIs in conversion therapy for advanced GC has been reported (32,33). Additionally, the clinical population and molecular biomarkers of successful conversion therapy with chemotherapy and ICIs remain unclear.

Here, this study presented the case of a patient with unresectable stage III GC of high microsatellite instability (MSI-H), high tumor mutation burden (TMB-H), Epstein-Barr virus (EBV+) positive and unresectable stage III GC. These findings warrant large-scale clinical studies. There was no cancerous tissue at the proximal or distal end of the tumor and no lymph node metastases in the lesser or greater curvature, indicating a pathologic complete response (pCR) following treatment with sequential chemotherapy combined with tislelizumab and laparoscopic surgery. Responding to these observations, further investigation of tislelizumab with other novel combinations to build on the benefit of tislelizumab in patients with gastric cancer is warranted. We present the following article in accordance with the CARE reporting checklist (available at https://dx.doi.org/10.21037/atm-21-4295).

Case presentation

A 69-year-old man with a history of Parkinson’s disease complained of persistent epigastric pain and vomiting of dark red gastric contents. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal. Gastroscopy and positron emission tomography-computed tomography (PET-CT) with 18F-fluoro-2-deoxy-d-glucose (18FDG) revealed an ulcerative tumor with a 9.4 cm diameter extending from the lesser curvature of the gastric body to the lesser curvature of the gastric antrum under the cardia, and enlargement of scattered lymph nodes in hepatogastric space and retroperitoneum, with the largest being 2.0 cm in diameter (Figure 1A). An abnormal increase in phosphate-dependent glutaminase (PDG) metabolism was consistent with the manifestation of GC (Figure 1B,1C), corresponding to clinical stage T3-4N2M0 and Borrmann type III. The patient had an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 2. The patient’s serum level of carbohydrate antigen (CA) 19-9 was 5.16 ng/mL, and hemoglobin in venous blood was 85 ng/mL. Pathological examination of the gastric biopsy specimen indicated a poorly differentiated adenocarcinoma and negative HER2 expression (Figure 2A). The status of MSI-H was inferred from IHC results showing negative expression of MLH1 and PMS2 (Figure 2B,2C) and positive expression of MSH2 and MSH6 (Figure 2D,2E). The IHC test for PD-L1 by 22C3 pharmDx (Agilent) was positive (TPS 30%, CPS 40). Tests for EBV were positive.

To guide further therapy, the patient’s formalin-fixed and paraffin-embedded (FFPE) GC tissue was analyzed with target capture next-generation sequencing (NGS) using a 1,021-gene panel. The TMB value of 72.96 mutations per megabase (muts/Mb) was evaluated as TMB-H (the threshold for TMB-H is 9 muts/Mb). The status of MSI evaluated by MSIsensor (v0.5) was high, which supported the application of ICIs. Meanwhile, 75 single nucleotide variants (SNVs) were found in the GC tumor sample (Table S1). Mutations of ATR (5.0%), BRAC2 (5.1%), BRIP1 (6.5%), and CHEK1 (8.0%) in DNA damage response (DDR) pathways also suggested that the patient might benefit from PD-1/PD-L1 ICIs (34,35).

In view of the genetic testing results, the patient received celiac trunk angiography and celiac trunk perfusion chemotherapy combined with immunotherapy, which included paclitaxel-albumin (200 mg) transcatheter arterial infusion and paclitaxel-albumin (100 mg) intravenously on day 1 and tislelizumab (200 mg) intravenously every 3 weeks. Due to the patient’s poor physical condition, which was accompanied by general fatigue and soreness, oxaliplatin was not used in the first treatment cycle. Toxicity after the treatment mentioned above was moderate, involving mainly neutropenia caused by chemotherapy. The patient then received 2 cycles of paclitaxel-albumin (300 mg) and oxaliplatin (220 mg in the second cycle and 150 mg in the third cycle due to myelosuppression) intravenously on day 1, with tislelizumab (200 mg) intravenously every 3 weeks. Grade IV myelosuppression with fever was managed with recombinant human granulocyte colony-stimulating factor injection, recombinant human thrombopoietin treatments, and blood transfusions. After conversion therapy of chemotherapy combined with immunotherapy, abdominal CT revealed the body and antrum of the stomach were thickened and slightly enhanced (Figure 3). Nodular soft tissue shadow in hepatogastric space (the largest being 2.8 cm × 2.4 cm) and many rounded nonenhanced low-density shadows in the renal parenchyma (the largest being...
2.0 cm × 1.8 cm) were also observed. Further, the patient’s CA 19-9 level was within normal limits (20 U/mL).

A partial response was evaluated based on the Response Evaluation Criteria in Solid Tumours (RECIST v1.1) (36), and thus the patient underwent laparoscopic radical total gastrectomy with esophageojunostomy and laparoscopic cholecystectomy. An ulcerative mass of about 3.7×1.3×0.5 cm was observed in the resected gastric specimen, with subserosal invasion in the cut surface (Figure 4). The pathological examination demonstrated that there was no cancerous tissue at the proximal or distal end of the tumor and no lymph node metastases in the lesser or greater curvature (Figure 5A-5C), corresponding to the American Joint Committee on Cancer (AJCC) pathological stage ypT0N0. These findings indicated a pathologic complete response (pCR). Thereafter, the patient continued to receive tislelizumab to prevent postoperative carcinoma recurrence and metastasis, and to improve prognosis.

**Discussion**

We reported the case of a patient with stage III GC who was successfully treated with conversion therapy using chemotherapy and immunotherapy. To the best of our knowledge, this is the first report of a case that utilized gene testing to characterize the molecular biomarkers of primary tumor disease in order to develop a precision conversion therapy. Studies showed that molecular classification could directly associate gastric cancer with targeted therapies to overcome intertumoral heterogeneity for precision medicine (37). Clinical classification showed that ERBB2, FGFR2 and EGFR were observed as actionable
Figure 2 Hematoxylin-eosin staining and immunohistochemistry at diagnosis. (A) Hematoxylin-eosin staining section of the gastric biopsy specimen (original magnification, ×100). (B-E) MLH1, PMS2, MSH2, and MSH6 protein expression were analyzed by immunohistochemistry of the gastric biopsy specimen, respectively (original magnification, ×100).

Figure 3 The abdominal CT after conversion therapy. CT showed the changes of gastric body and antrum adenocarcinoma after chemotherapy plus tislelizumab (right) compared to that before conversion therapy (left). The circle stands the thick of the body and antrum of the stomach.

biomarkers in advance gastric cancer with intertumoral heterogeneity (38). Furthermore, ERBB2 cluster had better clinical benefit respond to anti-HER2 therapy in gastric cancer (37). This multiple therapeutic approach containing chemotherapy, tislelizumab, and subsequent surgery showed a pCR in the MSI-H, TMB-H, EBV+ unresectable stage III GC patient.

Palliative chemotherapy is a uniform standard treatment for unresectable GC (39). In general, advanced GC patients treated with sequential chemotherapy starting with first-
line platinum and fluoropyrimidine doublet chemotherapy have a median survival of less than 1 year (40). The approval of ICIs (pembrolizumab and nivolumab) in recent years is one of the most significant advances in the treatment of unresectable GC (41,42). Compared with chemotherapy alone, the novel approach of using PD-1/PD-L1 inhibitor with chemotherapy as a combined therapy has demonstrated outstanding antitumor activity and tolerability in first-line treatment for patients with metastatic GC (43). In the KEYNOTE-062 study, pembrolizumab in combination with standard chemotherapy was noninferior to chemotherapy for OS in untreated HER2 negative and PD-L1 positive (CPS ≥1) advanced GC (44). Our patient received tislelizumab, an ICI found to be structurally distinct from both pembrolizumab and nivolumab (45), which was used in combination with chemotherapy as conversion therapy. Two early phase studies (NCT02407990, CTR20160872) demonstrated that tislelizumab monotherapy is generally well tolerated and has promising antitumor activity in patients with advanced solid tumors, including GC (46). A phase II study (NCT03469557) showed that the overall response rate (ORR) was 46.7% with a median duration of response (DoR) of 12.8 months in a G/GEJ adenocarcinoma cohort who received tislelizumab plus oxaliplatin and capecitabine as first-line therapy (47). This result is consistent with the findings of the KEYNOTE-062 clinical trial which found ORR was 48.6% and progression-free survival (PFS) was 6.9 months (48). Taken together, these results support the use of tislelizumab in combination with chemotherapy as an effective therapeutic strategy for advanced GC patients.

Since only a small percentage of patients can benefit from ICIs, convincing biomarkers are needed to guide the precise use of PD-1 inhibitors. We presented a GC case with
locally-advanced, unresectable lesions who was treated with immunotherapy and chemotherapy. The prescription of conversion therapy was guided by 1,021-gene panel genetic testing. The patient's primary tumor biopsy revealed a high TMB value (72.96 muts/Mb). High mutational burden is associated with increased susceptibility to recognition by the immune system (49). GC cells develop an immune evasion system by upregulating the surface expression of PD-L1, which is overexpressed in 40–63% of GC cases (50). These findings have provided a rationale for immunotherapy in advanced GC. Patients with high TMB who received toripalimab as a monotherapy showed a significant superior OS of about 10 months longer than those with low TMB (14), which suggests that high TMB may be a predictive marker for OS improvement in advanced GC patients receiving ICIs.

The tertiary lymphatic structure (TLS) is an important part of the tumor microenvironment, which reflects the host's anti-tumor immune response. The hematoxylin and eosin-stained slides were used to measure histopathologically using the amounts of TLS (51). In gastric cancer, TLS-rich patients with revealed a better prognosis than TLS-poor patients (52). Moreover, CD103⁺ T cells in TLS had a better prognosis in gastric cancer patients (52). B cells in TLS are correlated with favorable prognosis in patients with gastric cancer (53). The effect of antitumor immunity on treatment of gastric cancer would be investigated in future.

In our patient's tumor sample, we detected 4 mutations in the DDR pathway. Patients with these mutations have a higher ORR and a longer PFS or OS, which supported the theory that ICIs could potentially be effective in this case (34,35). In 2014, The Cancer Genome Atlas (TCGA) categorized 295 cases into 4 distinct molecular subtypes based on 6 different molecular platforms: EBV+, MSI, CIN, and genomically stable (GS) GC (54). Separate follow-up studies have shown that EBV⁺ tumor is a special subgroup with CD8⁺ cytotoxic T-cell infiltration (55) and robust PD-L1 expression both in cancer cells and in immune cells (56), leading to a better prognosis after immunotherapy (57). In addition, an MSI-H GC tumor suggests a favorable response to ICIs, which may be related to immunosurveillance (58). Based on the results of genetic testing in addition to the PD-L1 IHC assay, we learned that TMB-H, EBV⁺, and MSI-H may be reliable biomarkers for immunotherapy in advanced GC patients.

Conversion therapy shows feasibility and efficacy for initially unresectable advanced GC when distant metastases are controlled by chemotherapy. Ramos et al. (25) retrospectively evaluated the efficacy of conversion therapy in 100 unresectable metastatic GC patients treated with docetaxel, cisplatin, and S-1 (DCS) chemotherapy. The clinical outcomes showed that DCS induced a high conversion rate (33%), R0 resection rate (84.8%), and pathological response rate (78.8%). Recent case reports have demonstrated that conversion surgery might help to control tumor progression and improve efficiency in responders after chemotherapy and nivolumab, resulting in longer survival periods of ~2 to 4 years (32,33). However, clinical significance and convincing biomarkers for conversion therapy remain uncertain for stage III GC patients with unresectable lesions. In the present case, we recommended the patient first undergo genetic testing. Based on the results of our tests for biomarkers, we selected tislelizumab combined with chemotherapy for the conversion therapy. The application of ICIs in frontline treatment followed by surgical intervention offered our patient with unresectable advanced GC the clinical benefit of pCR.

Conclusions

In conclusion, the patient presented in our case study achieved a pCR after conversion therapy involving chemotherapy, immunotherapy, and a curative resection, suggesting that tumor tissue genomic testing may have considerable benefits for unresectable advanced GC patients. The adoption of conversion therapy and radical R0 resection under the guidance of genetic testing may have great clinical application potential in the control of tumor progression for unresectable stage III GC patients. Further clinical investigations involving larger groups of stage III GC patients with unresectable lesion are required to investigate clinical utility of conversion therapy involving immunotherapy.

Acknowledgments

We owe thanks to the patient in our study and his family members. We acknowledge the staff of all centers for their assistance to this study.

Funding: This work was supported by Science and Technology Innovation Capability Improvement Project of Army Medical University (2019XLC3061).

Footnote

Reporting Checklist: The authors have completed the
CARE reporting checklist. Available at https://dx.doi.org/10.21037/atm-21-4295

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-4295). Dr. Zhang, Dr. Yuan and Dr. Chen are employees of Geneplus-Beijing. Dr. Feng reports funding support from Science and Technology Innovation Capability Improvement Project of Army Medical University (2019XLC3061). The other authors have no conflicts of interest to declare.

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. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Digklia A, Wagner AD. Advanced gastric cancer: Current treatment landscape and future perspectives. World J Gastroenterol 2016;22:2403-14.
3. Hu B, El Hajj N, Sittler S, et al. Gastric cancer: Classification, histology and application of molecular pathology. J Gastrointest Oncol 2012;3:251-61.
4. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:202-9.
5. Cristescu R, Lee J, Nebozhyn M, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med 2015;21:449-56.
6. Birkman EM, Mansuri N, Kurki S, et al. Gastric cancer: immunohistochemical classification of molecular subtypes and their association with clinicopathological characteristics. Virchows Arch 2018;472:369-82.
7. Ishii A, Itakura J, Akaike Y, et al. Epstein-Barr virus-associated gastric carcinoma with heterogeneous EBER positivity accompanied by distinctive morphological cellular changes. Pathol Int 2020;70:306-8.
8. Mariette C, Carneiro F, Grabsch HI, et al. Consensus on the pathological definition and classification of poorly cohesive gastric carcinoma. Gastric Cancer 2019;22:1-9.
9. Nshizirungu JP, Bennis S, Mellouki I, et al. Reproduction of the Cancer Genome Atlas (TCGA) and Asian Cancer Research Group (ACRG) Gastric Cancer Molecular Classifications and Their Association with Clinicopathological Characteristics and Overall Survival in Moroccan Patients. Dis Markers 2021;2021:9980410.
10. Joshi SS, Maron SB, Catenacci DV. Pembrolizumab for treatment of advanced gastric and gastroesophageal junction adenocarcinoma. Future Oncol 2018;14:417-30.
11. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science 2018;359:1350-5.
12. Xu J, Bai Y, Xu N, et al. Tislelizumab Plus Chemotherapy as First-line Treatment for Advanced Esophageal Squamous Cell Carcinoma and Gastric/ Gastroesophageal Junction Adenocarcinoma. Clin Cancer Res 2020;26:4542-50.
13. Ratti M, Lampis A, Hahne JC, et al. Microsatellite instability in gastric cancer: molecular bases, clinical perspectives, and new treatment approaches. Cell Mol Life Sci 2018;75:4151-62.
14. Wang F, Wei XL, Wang FH, et al. Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemo-refractory gastric cancer treated with toripalimab, a PD-1 antibody in phase Ib/II clinical trial NCT02915432. Ann Oncol 2019;30:1479-86.
15. Panda A, Mehnter JM, Hirshfield KM, et al. Immune Activation and Benefit From Avelumab in EBV-Positive Gastric Cancer. J Natl Cancer Inst 2018;110:316-20.
16. Jin S, Xu B, Yu L, et al. The PD-1, PD-L1 expression and CD3+ T cell infiltration in relation to outcome in advanced gastric signet-ring cell carcinoma, representing a potential biomarker for immunotherapy. Oncotarget

© Annals of Translational Medicine. All rights reserved. Ann Transl Med 2021;9(18):1489 | https://dx.doi.org/10.21037/atm-21-4295
17. Lee SJ, Jun SY, Lee IH, et al. CD274, LAG3, and IDO1 expressions in tumor-infiltrating immune cells as prognostic biomarker for patients with MSI-high colon cancer. J Cancer Res Clin Oncol 2018;144:1005-14.
18. Yamashita H, Nakayama K, Ishikawa M, et al. Relationship between Microsatellite Instability, Immune Cells Infiltration, and Expression of Immune Checkpoint Molecules in Ovarian Carcinoma: Immunotherapeutic Strategies for the Future. Int J Mol Sci 2019;20:5129.
19. Correction to Lancet Oncol 2017; 18: 1182-91. Lancet Oncol 2017;18:510.
20. Zeng Z, Yang B, Liao Z. Biomarkers in Immunotherapy-Based Precision Treatments of Digestive System Tumors. Front Oncol 2021;11:650481.
21. Roa JC, Araya JC, Villaseca MA, et al. Microsatellite instability and loss of heterozygosity in neoplastic and preneoplastic gastric lesions. Rev Med Chil 2003;131:1227-36.
22. Sugimoto R, Habano W, Yanagawa N, et al. Molecular alterations in gastric cancer and the surrounding intestinal metaplastic mucosa: an analysis of isolated glands. Gastric Cancer 2021;24:382-91.
23. Sato Y, Ohnuma H, Nobuoka T, et al. Conversion therapy for inoperable advanced gastric cancer patients by docetaxel, cisplatin, and S-1 (DCS) chemotherapy: a multi-institutional retrospective study. Gastric Cancer 2017;20:517-26.
24. Yoshida K, Yamaguchi K, Okumura N, et al. Is conversion therapy possible in stage IV gastric cancer: the proposal of new biological categories of classification. Gastric Cancer 2016;19:329-38.
25. Ramos MF KP, Pereira MA, Charruf AZ, et al. Conversion therapy for gastric cancer: expanding the treatment possibilities. Arq Bras Cir Dig 2019;32:e1435.
26. Chua C, Wisniewski T, Ramos A, et al. Multidisciplinary trauma intensive care unit checklist: impact on infection rates. J Trauma Nurs 2010;17:163-6.
27. Das M. Neoadjuvant chemotherapy: survival benefit in gastric cancer. Lancet Oncol 2017;18:e307.
28. Beitsch P, Whitworth P, Baron P, et al. Genomic Impact of Neoadjuvant Therapy on Breast Cancer: Incomplete Response is Associated with Altered Diagnostic Gene Signatures. Ann Surg Oncol 2016;23:3317-23.
29. Alistar A, Chou JW, Nagalla S, et al. Dual roles for immune metagenes in breast cancer prognosis and therapy prediction. Genome Med 2014;6:80.
30. El Otmani I, El Agy F, El Baradai S, et al. Analysis of Molecular Pretreated Tumor Profiles as Predictive Biomarkers of Therapeutic Response and Survival Outcomes after Neoadjuvant Therapy for Rectal Cancer in Moroccan Population. Dis Markers 2020;2020:8459303.
31. Song X, Qi W, Guo J, et al. Immune checkpoint inhibitor combination therapy for gastric cancer: Research progress. Oncol Lett 2020;20:46.
32. Matsumoto R, Arigami T, Matsushita D, et al. Conversion surgery for stage IV gastric cancer with a complete pathological response to nivolumab: a case report. World J Surg Oncol 2020;18:179.
33. Lin CP, Sung YC, Wong JU. Immunotherapy improves efficiency of conversion surgery for metastatic gastric cancer: A case report. Asian J Surg 2020;43:1039-40.
34. Boudadi K, Suzman DL, Anagnostou V, et al. Ipilimumab plus nivolumab and DNA-repair defects in AR-V7-expressing metastatic prostate cancer. Oncotarget 2018;9:28561-71.
35. Wang Z, Zhao J, Wang G, et al. Comutations in DNA Damage Response Pathways Serve as Potential Biomarkers for Immune Checkpoint Blockade. Cancer Res 2018;78:6486-96.
36. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
37. Ichikawa H, Nagahashi M, Shimada Y, et al. Actionable gene-based classification toward precision medicine in gastric cancer. Genome Med 2017;9:93.
38. Chao J, Lee J, Kim K, et al. A Pilot Study of Baseline Spatial Genomic Heterogeneity in Primary Gastric Cancers Using Multi-Region Endoscopic Sampling. Front Oncol 2020;10:225.
39. Arai H, Nakajima TE. Recent Developments of Systemic Chemotherapy for Gastric Cancer. Cancers (Basel) 2020;12:1100.
40. Smyth EC, Nilsson M, Grabsch HI, et al. Gastric cancer. Lancet 2020;396:635-48.
41. Bang YJ, Kang YK, Catenacci DV, et al. Pembrolizumab alone or in combination with chemotherapy as first-line therapy for patients with advanced gastric or gastroesophageal junction adenocarcinoma: results from the phase II nonrandomized KEYNOTE-059 study. Gastric Cancer 2019;22:828-37.
42. Kang YK, Boku N, Satoh T, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-
controlled, phase 3 trial. Lancet 2017;390:2461-71.

43. Fuchs CS, Doi T, Jang RW, et al. Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer: Phase 2 Clinical KEYNOTE-059 Trial. JAMA Oncol 2018;4:e180013.

44. Shitara K, Van Cutsem E, Bang YJ, et al. Efficacy and Safety of Pembrolizumab or Pembrolizumab Plus Chemotherapy vs Chemotherapy Alone for Patients With First-line, Advanced Gastric Cancer: The KEYNOTE-062 Phase 3 Randomized Clinical Trial. JAMA Oncol 2020;6:1571-80.

45. Feng Y, Hong Y, Sun H, et al. The molecular binding mechanism of tislelizumab, an investigational anti-PD-1 antibody, is differentiated from pembrolizumab and nivolumab. American Association of Cancer Research, 2019:abstract 4048.

46. Desai J, Deva S, Lee JS, et al. Phase IA/IB study of single-agent tislelizumab, an investigational anti-PD-1 antibody, in solid tumors. J Immunother Cancer 2020;8:e000453.

47. Lu S, Wang J, Yu Y, et al. Tislelizumab Plus Chemotherapy as First-Line Treatment for Locally Advanced or Metastatic Nonsquamous NSCLC (RATIONALE 304): A Randomized Phase 3 Trial. J Thorac Oncol 2021;16:1512-22.

48. Bang YJ, Van Cutsem E, Fuchs CS, et al. KEYNOTE-585: Phase III study of perioperative chemotherapy with or without pembrolizumab for gastric cancer. Future Oncol 2019;15:943-52.

49. Thomas A, Routh ED, Pullikuth A, et al. Tumor mutational burden is a determinant of immune-mediated survival in breast cancer. Oncoimmunology 2018;7:e1490854.

50. Wang X, Teng F, Kong L, et al. PD-L1 expression in human cancers and its association with clinical outcomes. Onco Targets Ther 2016;9:5023-39.

51. Song IH, Heo SH, Bang WS, et al. Predictive Value of Tertiary Lymphoid Structures Assessed by High Endothelial Venule Counts in the Neoadjuvant Setting of Triple-Negative Breast Cancer. Cancer Res Treat 2017;49:399-407.

52. Mori T, Tanaka H, Suzuki S, et al. Tertiary lymphoid structures show infiltration of effective tumor-resident T cells in gastric cancer. Cancer Sci 2021;112:1746-57.

53. Sakimura C, Tanaka H, Okuno T, et al. B cells in tertiary lymphoid structures are associated with favorable prognosis in gastric cancer. J Surg Res 2017;215:74-82.

54. Zhang W. TCGA divides gastric cancer into four molecular subtypes: implications for individualized therapeutics. Chin J Cancer 2014;33:469-70.

55. Zhang NN, Chen JN, Xiao L, et al. Accumulation Mechanisms of CD4(+)CD25(+)FOXP3(+) Regulatory T Cells in EBV-associated Gastric Carcinoma. Sci Rep 2015;5:18057.

56. Derks S, Liao X, Chiaravalli AM, et al. Abundant PD-L1 expression in Epstein-Barr Virus-infected gastric cancers. OncoTarget 2016;7:32925-32.

57. Kim ST, Cristescu R, Bass AJ, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med 2018;24:1449-58.

58. van Velzen MJM, Derks S, van Grieken NCT, et al. MSI as a predictive factor for treatment outcome of gastroesophageal adenocarcinoma. Cancer Treat Rev 2020;86:102024.

(English Language Editor: A. Muijlwijk)
Table S1 The 75 single nucleotide variants (SNVs) found in the GC tissue

| Gene      | Transcript   | c.       | p.         | Mutation frequency |
|-----------|--------------|----------|------------|--------------------|
| ARAF      | NM_001654.4  | c.1199G>A | p.R400H    | 13.90%             |
| MED12     | NM_005120.2  | c.5711C>T | p.A1904V   | 13.80%             |
| MLH1      | NM_000249.3  | c.791-1G>T | .          | 11.00%             |
| BCROR1    | NM_021946.4  | c.5036C>6 | p.P1681Qfs*20 | 11.00%          |
| ETV6      | NM_001987.4  | c.985G>A  | p.A329T    | 10.00%             |
| CHEK1     | NM_001114121.2 | c.1115G>A | p.R372Q    | 8.00%              |
| SOX9      | NM_000346.3  | c.1004G>A | p.W335*    | 7.80%              |
| RNASEL    | NM_021133.3  | c.172G>6 | p.W60Lfs*6 | 7.40%              |
| FLT4      | NM_182925.4  | c.1790C>T | p.T597M    | 7.40%              |
| CDH11     | NM_001797.2  | c.1196G>T | p.G399V    | 7.40%              |
| ROS1      | NM_002944.2  | c.6773T>C | p.I2258T   | 7.30%              |
| EP300     | NM_001429.3  | c.752A>G  | p.N251S    | 7.20%              |
| EPAS1     | NM_001430.4  | c.2254C>T | p.P752S    | 7.10%              |
| SOX9      | NM_000346.3  | c.1033C>4 | p.P346Rfs*37 | 7.10%         |
| JAK3      | NM_000215.3  | c.523C>T  | p.R175*    | 7.10%              |
| MTHFR     | NM_005957.4  | c.659C>T  | p.A220V    | 6.90%              |
| FAT1      | NM_005245.3  | c.9589C>A | p.L3197I   | 6.90%              |
| B2M       | NM_004048.2  | c.19T>3   | p.L7Ffs*50 | 6.90%              |
| PBRM1     | NM_018313.4  | c.4133G>A | p.G1378D   | 6.80%              |
| FAM175A   | NM_139076.2  | c.299A>G  | p.Y100C    | 6.80%              |
| MAGI2     | NM_012301.3  | c.1609A>G | p.M537V    | 6.80%              |
| NOTCH1    | NM_017617.3  | c.2644G>A | p.A882T    | 6.80%              |
| POLE      | NM_006231.2  | c.5312C>T | p.T1771M   | 6.70%              |
| AURKB     | NM_004217.3  | c.809A>G  | p.N270S    | 6.70%              |
| MPL       | NM_005373.2  | c.565G>A  | p.A189T    | 6.60%              |
| EPHB6     | NM_004445.3  | c.2130A>C | p.E710D    | 6.60%              |
| BRCA2     | NM_000093.9  | c.6700T>G | p.F2234V   | 6.60%              |
| RPTOR     | NM_020761.2  | c.928G>T  | p.G310C    | 6.60%              |
| SRC       | NM_198291.1  | c.1335G>T | p.K445N    | 6.60%              |
| BARD1     | NM_000465.2  | c.943C>T  | p.P315S    | 6.50%              |
| ATR       | NM_001184.3  | c.1817G>A | p.G606D    | 6.50%              |
| CDK12     | NM_016507.2  | c.2594T>3 | p.L866Cfs*2 | 6.50%           |
| BRIP1     | NM_032043.2  | c.688T>C  | p.S230P    | 6.50%              |
| CD74      | NM_001025159.2 | c.797G>A | p.R266H    | 6.40%              |
| ACTB      | NM_001101.3  | c.1022T>G | p.I341S    | 6.40%              |
| DDR1      | NM_001954.4  | c.352T>A  | p.Y118N    | 6.30%              |
| B2M       | NM_000408.2  | c.35T>C   | p.L12P     | 6.30%              |

Table S1 (continued)
| Gene   | Transcript | c.         | p.         | Mutation frequency |
|--------|------------|------------|------------|--------------------|
| NOTCH3 | NM_000435.2| c.5062T>A  | p.S1688T   | 6.30%              |
| PIK3CA | NM_006218.2| c.1634A>G  | p.E545G    | 6.20%              |
| PDGFβR | NM_002609.3| c.1712_1713delCT | p.S571Cfs*4 | 6.20%              |
| FAT2   | NM_001447.2| c.2304G>T  | p.E768D    | 6.20%              |
| MAP2K1 | NM_002755.3| c.199G>A   | p.D67N     | 6.20%              |
| SMAD4  | NM_005359.5| c.290G>A   | p.R97H     | 6.10%              |
| EPCAM  | NM_006218.2| c.1634A>G  | p.E545G    | 6.20%              |
| PDGFRB | NM_002609.3| c.1712_1713delCT | p.S571Cfs*4 | 6.20%              |
| FAT2   | NM_001447.2| c.2304G>T  | p.E768D    | 6.20%              |
| MAP2K1 | NM_002755.3| c.199G>A   | p.D67N     | 6.20%              |
| SMAD4  | NM_005359.5| c.290G>A   | p.R97H     | 6.10%              |
| EPCAM  | NM_006218.2| c.1634A>G  | p.E545G    | 6.20%              |
| PDGFRB | NM_002609.3| c.1712_1713delCT | p.S571Cfs*4 | 6.20%              |
| FAT2   | NM_001447.2| c.2304G>T  | p.E768D    | 6.20%              |
| MAP2K1 | NM_002755.3| c.199G>A   | p.D67N     | 6.20%              |
| SMAD4  | NM_005359.5| c.290G>A   | p.R97H     | 6.10%              |
| EPCAM  | NM_006218.2| c.1634A>G  | p.E545G    | 6.20%              |
| PDGFRB | NM_002609.3| c.1712_1713delCT | p.S571Cfs*4 | 6.20%              |
| FAT2   | NM_001447.2| c.2304G>T  | p.E768D    | 6.20%              |
| MAP2K1 | NM_002755.3| c.199G>A   | p.D67N     | 6.20%              |
| SMAD4  | NM_005359.5| c.290G>A   | p.R97H     | 6.10%              |
| EPCAM  | NM_006218.2| c.1634A>G  | p.E545G    | 6.20%              |
| PDGFRB | NM_002609.3| c.1712_1713delCT | p.S571Cfs*4 | 6.20%              |