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Case series

Tumor molecular profiling of responders and non-responders following pembrolizumab monotherapy in chemotherapy resistant advanced cervical cancer

Ngoi N.Y.L.[a], Heong V.[a,b], Lee X.W.[a], Huang Y.Q.[a], Thian Y.L.[c], Choo B.A.[d], Lim D.[e], Lim Y.W.[a], Lim S.E.[a], Ilancheran A.[f], Soong R.[b,e], Tan D.S.P.[a,b,*]

[a] Department of Hematology-Oncology, National University Cancer Institute, Singapore, 5 Lower Kent Ridge Rd, Singapore 119074, Republic of Singapore
[b] Cancer Science Institute of Singapore, National University of Singapore, 14 Medical Drive, Singapore 117599, Republic of Singapore
[c] Department of Diagnostic Radiology, National University Hospital, Singapore, 5 Lower Kent Ridge Rd, Singapore 119074, Republic of Singapore
[d] Department of Radiation Oncology, National University Cancer Institute, Singapore, 5 Lower Kent Ridge Rd, Singapore 119074, Republic of Singapore
[e] Department of Pathology, National University Hospital, Singapore, 5 Lower Kent Ridge Rd, Singapore 119074, Republic of Singapore
[f] Division of Gynecology-Oncology, Department of Obstetrics and Gynecology, National University Hospital, Singapore, 5 Lower Kent Ridge Rd, Singapore 119074, Republic of Singapore

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ABSTRACT

Optimal treatment for advanced cervical cancer after first line chemotherapy remains undefined. Immune checkpoint inhibition with pembrolizumab, a programmed cell death protein 1(PD-1) inhibitor, is under investigation. We analyzed the micro-environmental and molecular genetic profile of tumors from 4 patients with metastatic cervical cancer treated with off-label second-line pembrolizumab in an effort to identify predictive biomarkers. All patients received 2 mg/kg of pembrolizumab, 3-weekly until disease progression. Immunohistochemistry(IHC) for PD-1, PD-L1, CD3 and CD8, as well as next generation sequencing (NGS) for 50 cancer-related genes were performed on tumor samples. All patients tolerated treatment well with no discontinuation of treatment due to toxicity. One patient experienced dramatic and prolonged partial response, and remains stable on pembrolizumab with a progression free survival (PFS) of 21 months at the time of reporting of this series. Three patients experienced disease progression as best response. In the exceptional responder, there was no tumoral expression of PD-L1, however, combined positive score (CPS) for PD-L1 was 1 and we identified somatic mutations in *ERBB4*(R612W), *PIK3CA*(E542K) and *RB1*(E365K). In 2 patients, despite progressive disease defined by RECIST v1.1, symptom stabilization on pembrolizumab was observed. The tumors of both patients had PD-1 expression in ≥1% of stromal lymphocytes. All patients with response or clinical benefit had CPS for PD-L1 ≥ 1. NGS revealed *PIK3CA* mutations in 3 tumors. Pembrolizumab is a promising therapeutic option in advanced cervical cancer. Further evaluation of biomarkers may guide optimal patient selection.

1. Introduction

Cervical cancer is the second most common cancer among women in less developed countries (Torre et al., 2015). The GOG 240 trial transformed the treatment of advanced cervical cancer by demonstrating improved survival with bevacizumab added to standard chemotherapy (Tewari et al., 2014). Optimal treatment after progression on anti-angiogenic therapy remains unclear.

PD-1/PD-L1 (Programmed cell death protein 1/Programmed death-ligand 1) inhibition may be a viable therapeutic strategy in cervical cancers. PD-L1 expression has been reported in 95% of cervical intraepithelial neoplasia (CIN) and 80% of cervical squamous cell carcinomas (Mezache et al., 2015). A large proportion of squamous cell carcinoma of the cervix, and nodal metastases, have been characterized to harbor high levels of PD-L1+ antigen-presenting cells (APCs) and FOXP3+ regulatory T cells (Tregs) (Heeren et al., 2015). The PD-1:PD-L1 interaction in human papilloma virus (HPV)-associated head and neck squamous cell cancer (HNSCC) has also been shown to create an "immune-privileged" site for initial viral infection, and subsequent adaptive immune resistance once tumors are established (Lyford-Pike et al., 2013). This provides rationale for therapeutic PD-1/PD-L1 blockade in HPV-associated tumors, such as cervical cancer.

In the phase Ib KEYNOTE-28 study, 24 patients with advanced cervical cancer were treated with pembrolizumab, a PD-1 inhibitor. All

* Corresponding author.
E-mail address: david_sp_tan@nuhs.edu.sg (D.S.P. Tan).

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patients had PD-L1 expression in ≥1% of tumor or stromal cells by immunohistochemistry (IHC). At a median of 11 months follow-up, the confirmed objective response rate (ORR) was 17%, with partial response (PR) seen in 4 of 24 patients (Frenel et al., 2017). The role of pembrolizumab is currently being further investigated in the phase II KEYNOTE-158 trial (NCT02628067). Hitherto, there remains a lack of published data on relevant biomarkers in cervical cancer patients who have responded to, or are resistant to pembrolizumab therapy.

We report on 4 patients with recurrent or metastatic cervical cancer, treated with off-label pembrolizumab, after progression on initial platinum-based chemotherapy. One patient was an exceptional responder. We performed in-depth microenvironment and molecular genetic profiling of their tumors. Three patients received radiotherapy upon progression on pembrolizumab, in an attempt to reverse resistance to PD-1 inhibition by induction of an abscopal response.

2. Materials and methods

2.1. Patient selection

Ethical approval for molecular analysis of patient tumor samples was obtained from the National Health Group Review Board (2014/00131). Clinical data and tumor samples from four patients with metastatic cervical cancer, treated with off-label second-line pembrolizumab, at our institution from June 2015 to January 2017, were retrospectively analyzed. Patient responses were evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.1) with Computed Tomography (CT) or Fluorine-18 (F-18) fluorodeoxyglucose (FDG) positron emission tomography (PET) CT scans every 2 cycles. Progression-free survival (PFS) was defined as the interval from commencement of pembrolizumab until disease progression by RECIST v1.1.

2.2. Immunohistochemistry

IHC was performed using the Bond Polymer Refine Detection Reagent on the Bond-Max autostainer (Leica, Wetzlar, Germany). Antibodies used were 22C3 (pharmDx, Dako, Glostrup, Norway) at 1/100 dilution for PD-L1, NAT105 (Abcam, Cambridge, UK) at 1/100 for PD-1, 144B (Dako, Glostrup, Norway) at 1/600 for CD8, and LN10 (Leica) at 1/50 for CD3. CD8 and CD3 are T-cell specific markers, staining of which was intended to better define tumoral and stromal lymphocyte density. Combined positive score (CPS) was calculated using the ratio of PD-L1 staining tumor and immune cells, to total viable tumor cells (Bellmunt et al., 2017).

2.3. Next generation sequencing

DNA was extracted from 3 sections (5 μm each) of formalin-fixed paraffin-embedded (FFPE) tumor samples using the GeneRead DNA FFPE Kit (Qiagen, Hilden, Germany). Next generation sequencing (NGS) was performed using the Ampliseq Cancer Hotspot v2 Panel and the Ion Torrent Personal Genome Machine (Thermo Fisher Scientific, Waltham, MA) (Table S1). Variants with quality score > 200, in coding regions, non-synonymous and with minor allele frequency of < 5% in East Asian and South Asian populations in the 1000 Genomes Databases, were considered for report. The tumor from Patient 1 also underwent sequencing using the SmartGen NGS-467 assay (Precipio Diagnostics, New Haven, CT).

3. Results

3.1. Patient demographics

Patient demographics and clinical outcomes following pembrolizumab are summarized in Table 1. 2 patients (Patients 3 and 4) had upfront metastatic cancer and 2 (Patients 1 and 2) had relapsed disease. Patient 1 received trachelectomy for International Federation of Gynecology and Obstetrics (FIGO) stage IB1 cancer but relapsed 3 years after primary therapy, with widespread metastases. Patient 2 received chemo-radiation for FIGO stage IB2 cancer, with cisplatin (40 mg/m^2) weekly concurrent with 50 Gy external beam radiotherapy in 28 fractions, followed by an extended field boost of 9 Gy in 5 fractions to low para-aortic lymph nodes, and triple channel brachytherapy. She completed 3 out of 4 planned cycles of adjuvant chemotherapy with carboplatin dosed at area under curve (AUC) 5 and paclitaxel 175 mg/m^2 3-weekly. Residual FDG PET-avid disease in a single external iliac lymph node was surgically resected. She relapsed 6 months later with visceral and nodal metastases.

Patients 1, 2 and 3 received front-line cisplatin (50 mg/m^2), paclitaxel (175 mg/m^2) and bevacizumab (15 mg/m^2) 3-weekly, without maintenance bevacizumab. Patient 4 developed G4 hypersensitivity to paclitaxel during cycle 1. She was switched to cisplatin (50 mg/m^2 on day 1), gemcitabine (1000 mg/m^2 on day 1 and 8), 3-weekly. All patients received off-label pembrolizumab 2 mg/kg 3-weekly, in the second-line.

3.2. Patient outcomes following pembrolizumab

PR was observed in 1 patient (Patient 3), after 3 cycles of pembrolizumab, which was durable, with PFS of 21 months, at the time of reporting of this series. PD was observed in 3 patients, after 1.5 months each, and confirmed with repeat scan after a further 4 weeks. Nonetheless, 2 patients (Patient 2 and 4) had continued clinical benefit after time of declared radiological PD. This manifested in reduced pain over a symptomatic nodal site (Patient 2), as well as weight gain of 10% body weight and improvement of performance status from 1 to 0 (Patient 4). Both patients opted to continue on pembrolizumab for a total duration of 3.5 months (Patient 2) and 7.5 months (Patient 4), respectively before symptomatic disease progression necessitating discontinuation of treatment.

3.3. Adverse events on pembrolizumab therapy

No G3 or higher toxicities, based on Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0), were encountered. Patients experienced G1 fatigue (4/4 patients), G1 rash (1/4 patients) and G1 hypersensitivity (1/4 patients). Patient 3 developed G2 anorexia, fatigue, weight loss and vomiting, 14 months after commencing pembrolizumab. She was diagnosed with G2 cortisol insufficiency. Pituitary imaging revealed a partial “empty sella” appearance. Her adrenocorticotropic hormone and cortisol levels were low, suggesting pembrolizumab-induced hypophysitis with partial hypopituitarism. She started on physiological doses of hydrocortisone which resolved her symptoms. She chose to continue on pembrolizumab. No dose reduction was implemented at re-introduction of pembrolizumab, and she remained well on hydrocortisone replacement.

3.4. Efficacy of radiation with pembrolizumab upon progression to induce an abscopal effect

Patients 1, 2 and 4 received radiotherapy after progression on pembrolizumab. Patients 1 and 4 resumed pembrolizumab after radiotherapy (Table 1).

Patient 1 progressed after 2 cycles of pembrolizumab with worsening vertebral metastases causing spinal instability. She underwent spinal stabilization surgery followed by third-line platinum based chemotherapy. On PD after this line of treatment, she developed symptomatic liver metastases with pain and transaminitis. Two fractions of stereotactic beam radiotherapy, 14Gy each, were administered to the 2 largest hepatic lesions. Pembrolizumab 2 mg/kg was re-challenged 2 days after radiotherapy completion, with the goal of inducing an