Evaluation of PARP and PDL-1 as potential therapeutic targets for women with high-grade neuroendocrine carcinomas of the cervix

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HIGHLIGHTS

- Neuroendocrine carcinoma of the cervix is a microsatellite stable tumor.
- This cancer does not have significant PD-L1 expression.
- Significant PARP-1 expression may signal that neuroendocrine carcinoma of the cervix will respond favorably to PARP inhibition.

ABSTRACT

Objectives Women with recurrent high-grade neuroendocrine cervical cancer have few effective treatment options. The aim of this study was to identify potential therapeutic targets for women with this disease.

Methods Specimens from patients with high-grade neuroendocrine carcinomas of the cervix were identified from pathology files at MD Anderson Cancer Center. Immunohistochemical stains for PD-L1 (DAKO, clone 22-C3), mismatch repair proteins (MLH1, MSH2, MSH6, PMS2), somatostatin, and Poly (ADP-ribose) polymerase (PARP) were performed on sections from formalin-fixed paraffin-embedded tissue blocks. Nuclear PARP-1 staining was quantified using the H-score with a score of <40 considered low, 40–100 moderate, and ≥100 high.

Results Forty pathologic specimens from patients with high-grade neuroendocrine carcinomas of the cervix were examined (23 small cell, 5 large cell, 3 high-grade neuroendocrine, not otherwise specified, and 9 mixed). The mean age of the cohort was 43 years and the majority of patients (70%) were identified as white non-Hispanic. All 28 (100%) samples tested stained for mismatch repair proteins demonstrated intact expression, suggesting they were microsatellite stable tumors. Of the 31 samples tested for PD-L1 expression, only two (8%) of the 25 pure high-grade neuroendocrine carcinomas were positive whereas three (50%) of the six mixed carcinoma tumors tested positive. Of the 11 small cell specimens tested for PARP-1, 10 (91%) showed PARP expression with six (55%) demonstrating high expression and four (36%) showing moderate expression. Somatostatin staining was negative in 18 of 19 small cell cases (95%).

Conclusions Pure high-grade neuroendocrine cervical carcinomas were microsatellite stable and overwhelmingly negative for PD-L1 expression. As the majority of tumors tested expressed PARP-1, inclusion of PARP inhibitors in future clinical trials may be considered.

INTRODUCTION

Small and large cell neuroendocrine carcinomas of the cervix are high-grade malignancies that are histologically similar to their counterparts in the lung.[1] Extra-pulmonary neuroendocrine cancers can occur in multiple other visceral sites including the cervix, uterus, gastrointestinal tract, head, neck, and genitourinary tract.[2] High-grade neuroendocrine carcinomas from all sites typically have an aggressive clinical course with widespread disease being common at diagnosis, and even those with localized disease have a high risk for recurrence.[2 3] Multiple guidelines recommend a multi-modal treatment approach to newly diagnosed high-grade neuroendocrine cervical cancer, combining experience with adenocarcinoma and squamous cell carcinomas of the cervix with small cell carcinoma of the lung, with a role for platinum-based chemotherapy, radical surgery, and/or radiation depending on stage.[4–6]

For recurrent high-grade neuroendocrine cervical cancer there remains no standard therapeutic options and survival is poor.[7] Chemotherapy with topotecan, paclitaxel, and bevacizumab has shown some promise in improving outcomes for women with this disease; however, better therapeutic options for recurrent disease are needed.[7 8] Multiple targeted agents and immunotherapies have shown activity in other high-grade neuroendocrine tumors but their utility in cervical tumors has not been extensively evaluated.

Pembrolizumab, an anti-PD-1 antibody, has improved response rates in tumors from multiple sites that harbor significant mutational burden in mismatch repair genes (MLH1, MSH2, MSH6, PMS2) as well as in PD-L1 positive disease.[9 10] The US Food and Drug Administration (FDA) has recently approved

pembrolizumab for cases of recurrent or metastatic cervical cancer that test positive for PD-L1. In addition, pembrolizumab has been approved for the treatment of recurrent small cell lung cancer. The somatostatin receptor (SST) has been an effective target for neuroendocrine and carcinoid tumors of the gastrointestinal tract, which are consequently treated with somatostatin analogs. There are only rare reports of somatostatin receptor positivity in small cell cervical cancer.[11] Finally, high-grade neuroendocrine tumors from multiple different sites have shown potential sensitivity to PARP inhibitors. Molecular studies in recurrent high-grade neuroendocrine carcinomas of the prostate and lung have shown overexpression of PARP and inhibition of tumor growth with PARP inhibitors.[12 13]

Effective treatment options for recurrent high-grade neuroendocrine cervical cancer remain an unmet need. Active treatment regimens using targeted therapy based on immunohistochemical expression of pertinent biomarkers have been identified in high-grade neuroendocrine carcinoma of extra-cervical sites.[14] The goal of this study was to use immunohistochemistry to identify immune and molecular targets for potential therapeutic strategies in the treatment of recurrent high-grade neuroendocrine carcinoma of the cervix.

METHODS

Slides and formalin-fixed paraffin embedded tumor blocks from either biopsies or resection specimens of 40 cervical high-grade neuroendocrine carcinomas were identified from the pathology files between 2004 and 2016. This study was approved by the Institutional Review Board of the University of Texas MD Anderson Cancer Center. Clinical data from these patients were obtained from medical records including demographics such as age, race, body mass index, and stage of disease at diagnosis.

Immunohistochemical stains were performed with specific antibody staining of treated paraffin embedded tissue samples. Samples were first placed in the oven for 30 min before being loaded into the stainer for all markers except PD-L1. For PD-L1 staining, samples were first placed in the oven for 60 min before being loaded into the pre-treament system (PT Link). Antigen epitopes were unmasked by heat-induced epitope retrieval. Endogenous peroxidases were neutralized with hydrogen peroxide. Samples were then challenged with specific antibodies to the proteins of interest. Prior to secondary antibody challenge blocking the samples were bathed in blocking serum. Secondary biotin-conjugated antibodies were then added and reaction with diaminobenzidine tetrachloride was observed.

Separate stains were done for mismatch repair proteins (MLH1 Clone G168-728, Cell Marque), MSH2 (Clone FE11, Calbiochem), MSH6 (Clone 44, BD Biosciences), PMS2 (Clone A16-4, BD Biosciences), PD-L1 (DAKO, clone 22-C3), somatostatin (EP130 Cell Marque), and Poly (ADP-ribose) polymerase (PARP, RB1516P1, Thermo Scientific) on sections from these tissue blocks. Staining for mismatch repair proteins (n=28) was interpreted as either positive or negative in the presence of positive internal controls. PD-L1 (n=31) was interpreted as positive (combined positive score >1) when there was either partial or complete membrane staining in the tumor cells. The percentage of staining was also estimated. Somatostatin staining (n=19) was cytoplasmic and both percentage and intensity of staining were evaluated. Nuclear PARP-1 (n=11) staining was quantified using the H-score with a score of <40 considered low, 40–100 moderate, and ≥100 high, as previously established by the immunohistochemistry laboratory.[15]

Table 1 Demographics

Age, mean, years	43
Body mass index, mean, kg/m^2	27
Race/ethnicity, n (%)	
White	28 (70)
Black	2 (5)
Hispanic	3 (8)
Asian	5 (13)
Unknown	2 (5)
FIGO 2018 stage at diagnosis, n (%)	
IB1	14 (38)
IB2	6 (16)
IIA	3 (9)
IIB	4 (11)
IIIB	3 (8)
IIIC1	1 (3)
IV	6 (16)
Unknown	3
Histology, n (%)	
Small cell carcinoma	23 (58)
Large cell carcinoma	5 (13)
Mixed	9 (23)
High-grade neuroendocrine carcinoma	3 (8)

FIGO, International Federation of Gynecology and Obstetrics.

RESULTS

Demographic and stage data are summarized in Table 1. The nine patients with mixed histologies had components of both high-grade neuroendocrine carcinoma admixed with either squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma. Twenty-eight of the 40 specimens were tested for microsatellite instability. All of the 28 specimens tested demonstrated intact mismatch repair protein expression for each of the four proteins tested (MLH1, MSH2, MSH6, PMS2; Table 2). Thirty-one specimens were tested for PD-L1. Of these, only two (8%) of the 25 pure high-grade neuroendocrine carcinoma specimens tested positive, both of which were primary tumors, whereas three (50%) of the six mixed specimens tested positive. All three of the mixed specimens that tested positive for PD-L1 had adenocarcinoma as the non-neuroendocrine component and two of the three were in samples from recurrent tumors.

Somatostatin staining was positive in one (5%) out of 19 small cell samples and demonstrated only moderate staining. A total of 11 small cell specimens were tested for PARP-1 and 91% (n=10) tested positive with 55% (n=6) of positive samples demonstrating high expression and 36% (n=4) showing moderate expression.

Table 2 Biomarker staining

Biomarker	n (%)
MMR, all subtypes	
Intact	28 (100)
Deficient	0
PD-L1	
Pure neuroendocrine	
Positive	2 (8)
Negative	23 (92)
Mixed	
Positive	3 (50)
Negative	3 (50)
SST, small cell	
Positive	1 (5)
Negative	18 (95)
PARP-1, small cell	
High	6 (55)
Moderate	4 (36)
Low	0
Negative	1 (9)

H-score ≥100 is high and H-score 40–100 is moderate.
MMR, mismatch repair; PARP-1, Poly-(ADP-ribose) polymerase 1; PD-L1, programmed death-ligand 1; SST, somatostatin.

DISCUSSION

The results of the immunohistochemical staining of high-grade neuroendocrine cervical cancer in our study present potential therapeutic approaches for treating recurrent disease. Unlike well- and moderately-differentiated neuroendocrine tumors of the gastrointestinal system, high-grade neuroendocrine cervical cancers do not express the somatostatin receptor and therefore are unlikely to respond to somatostatin analogs. In addition, high-grade neuroendocrine cervical cancers are microsatellite stable without significant expression of PD-L1, raising concerns for potential lack of activity with PD-1/PD-L1 inhibitors in these tumors. In contrast, significant PARP expression was noted in an overwhelming majority of the samples tested. Therefore, there may be good rationale for using PARP inhibitors as part of potential therapeutic approaches for treating women with this disease.

Immunohistochemical testing of tissue samples for mismatch repair proteins (MLH1, MSH2, MSH6, PMS2) has an excellent predictive value for diagnosing microsatellite instability, although up to 11% of tumors with microsatellite instability are not detected using these markers.[16] While unlikely, there is a small possibility that our methods missed some microsatellite instability-high tumors.[17 18] Mismatch repair-deficient tumors have been shown to overexpress PD-1, suggesting a population of tumors susceptive to immune checkpoint blockade, and our lack of PD-1 expression supports the notion that these tumors are mismatch repair intact.[19] This was demonstrated in various microsatellite unstable tumors where PD-1 blockade with an anti-PD-1 antibody, pembrolizumab, led to partial disease response in over 50% of patients with complete response in 21%.[9]

The FDA recently approved pembrolizumab in recurrent cervical cancer that is PD-L1 positive based on the results from the KEYNOTE-158 study.[20] In that study, however, women with cervical cancers that were PD-L1 negative (combined positive score <1) saw no response to single agent pembrolizumab. As this current study shows, most patients with high-grade neuroendocrine cervical carcinomas will likely be PD-L1 negative by immunohistochemistry. For that reason, some might hypothesize that the single agent pembrolizumab is unlikely to have a significant therapeutic benefit in these patients. In a recent phase II basket trial of pembrolizumab in rare tumors there were no responses in seven women with small cell neuroendocrine carcinoma of the lower genital tract, which supports this hypothesis.[21] However, there is a case report of a patient with high-grade neuroendocrine cervical cancer responding to single agent nivolumab.[22] As therapeutic options for recurrent high-grade neuroendocrine carcinomas of the cervix are so limited, we still recommend testing for PD-L1 at the time of recurrence to expand potential regimens for these patients.[5]

In the KEYNOTE-158 study, all patients in the cervical cancer cohort had squamous, adeno-, or adenosquamous carcinomas and 84% tested positive for PD-L1 by immunohistochemistry. There were no patients with high-grade neuroendocrine carcinoma of the cervix.[20] In our study, only two (8%) of 25 patients with pure high-grade neuroendocrine histology tested positive for PD-L1 while three (50%) of six patients with mixed histology tested positive. In the patients with mixed histology, all three had adenocarcinoma as the non-neuroendocrine component in the primary tumor specimen, so one might assume that PD-L1 positivity was related to that portion of the tumor. However, pathologic specimens from two of the three patients who tested positive for PD-L1 came from biopsies of recurrent disease where the histologic component was high-grade neuroendocrine carcinoma.

Published guidelines recommend that clinicians apply strategies for treating small cell lung cancer to patients with high-grade neuroendocrine cervical cancers.[4] While tumor PD-L1 expression is the single factor most correlated with response to therapy, data from Checkmate-032 supports nivolumab as an active agent in small cell lung cancer despite lack of PD-L1 expression.[23 24] As mentioned, there has been at least one report of a woman with PD-L1 negative small cell cervical cancer responding to single agent nivolumab.[22] This supports the theory that there may still be a role for these agents in the treatment of recurrent high-grade neuroendocrine cervical cancer. Other factors in the tumor microenvironment, including tumor mutational burden and neoantigen expression, seem to play just as important a role in predicting the efficacy of these agents.[25–27] The combination of anti-PD-1 therapy with other agents such as ionizing radiation and DNA damage-inducing chemotherapeutics may upregulate PD-L1 expression which in turn may then offer an opportunity for immunotherapies in high-grade neuroendocrine tumors.[28]

The most promising results in this study may be the high expression of PARP in almost all the samples, with many of them having high expression. A study by Byers et al[13] demonstrated high PARP expression in pulmonary small cell carcinoma compared with large cell neuroendocrine carcinoma; furthermore, in that study small cell lung cancer cell lines also had high sensitivity to PARP inhibitors. Homologous recombination deficiency was not tested in our samples; while this is known to be a predictive biomarker for PARP

inhibitor efficacy in platinum sensitive ovarian cancer, in the ARIEL3 trial the authors noted some BRCA2 wild-type patients and patients with low loss of heterozygosity had clinical benefit with rucaparib.[29] The ARIEL3 data support the findings of ENGOT-OV16/NOVA that PARP inhibitor therapy can be beneficial without known homologous recombination deficiency.[28] Other biomarkers for PARP inhibitor efficacy, such as the helicase SLFN11, have also been shown *in vivo* to correlate with tumor sensitivity to PARP inhibitors.[30] In a recent randomized phase II study in small cell lung cancer, patients with tumors expressing SLFN11 had improved survival with the combination of temozolomide, an alkylating agent, and the PARP inhibitor veliparib compared with those who did not express SLFN11.[31] In this study we did not stain for SLFN11, but hope to do so in future projects.

PARP-1 has been shown to have a role in DNA repair and also in DNA methylation as well as having an effect on transcription factors.[32] Jiao et al found that treatment with PARP inhibitors led to increased PD-L1 expression in breast cancer cell lines and xenograft tumors.[33] The combination of PD-L1 blockers and PARP inhibitors in these models worked synergistically, improving efficacy over either therapeutic alone. Similarly, Sen et al showed that PARP inhibition significantly potentiates the effectiveness of PD-L1 inhibitors in small cell lung cancer cell lines and the combination produced complete responses in mouse models.[34] This suggests that, while the small cell cervical cancer samples we tested do not currently express PD-L1 or demonstrate microsatellite instability, PARP inhibitions may induce expression of PD-L1 creating an opportunity for the use of PD-1/PD-L1 inhibitors in combination with PARP inhibitors in this disease.

Our data are limited in that we have only studied the presence of these markers *in vitro*; however, one of the strengths of our paper is the use of patient tissue rather than derived cell lines. It would be of great interest to examine the *in vivo* effects of derived cell lines from these samples to see if they behave as expected in the presence of the PARP or PD-L1 inhibitors. We are also limited in the number of patients available to study; however, for such a rare disease our sample size is robust.

CONCLUSIONS

Despite numerous ongoing studies of extra-pulmonary small cell cancer, we believe this is the first to evaluate potential targets in the cervix. We found that pure high-grade neuroendocrine cervical carcinomas were microsatellite stable and overwhelmingly negative for PD-L1 expression. As the majority of tumors tested expressed PARP-1, inclusion of PARP inhibitors in future clinical trials may be considered. This immunohistochemical profile can help inform research avenues into new therapeutic options in recurrent high-grade neuroendocrine cervical cancer. It would be interesting to see if the dynamic changes seen in small cell lung cancer could be replicated in high-grade neuroendocrine cervical cancer.

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Contributors MRC analyzed data and drafted the manuscript. PR was involved in study design, data collection, analysis, and manuscript review. JF, GS, LMSS, NP, RTH and RC were involved with data collection, analysis, and manuscript review. LB and MF were responsible for data collection, analysis, study design, and manuscript review. All authors had approval of the final version of the manuscript.

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Competing interests LB has served as a consultant for Medivation, AbbVie, BioMarin Pharmaceutical, Lilly Humana, LUNGevity. She has served on Scientific Advisory Committees for AstraZeneca Pharmaceuticals, StemCentrx, Astex Therapeutics, GENMAB. MF has served as a consultant for Stryker, Genetench, and Ipsen and as a speaker for Stryker and gets research funding from Tesaro/GSK and Astra Zeneca.

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