

# Molecular Profiling Reveals Limited Targetable Biomarkers in Neuroendocrine Carcinoma of the Cervix

Adela Cimic, MD,\* Semir Vranic, MD, PhD,† David Arguello, PhD,‡  
Elma Contreras, CT (ASCP),‡ Zoran Gatalica, MD, DSc,§ and Jeffrey Swensen, PhD‡

**Abstract:** Neuroendocrine carcinoma of the cervix (NEC) is a rare and highly aggressive cervical malignancy. Given that no targeted therapy has been approved specifically to NEC, we investigated the presence of novel, potentially targetable biomarkers in a large cohort of NEC. Sixty-two NEC were molecularly profiled for biomarkers of targeted therapies including antibody-drug conjugates [delta-like canonical notch ligand 3 (DLL3), a trophoblast cell surface antigen 2 (TROP-2), and folate receptor 1 (FOLR1)], *NTRK1-3* gene fusions, and immune checkpoint inhibitors [programmed death-ligand 1 (PD-L1), tumor mutational burden, and microsatellite instability] using immunohistochemistry and DNA/RNA next-generation sequencing assays. A cohort of squamous cell carcinomas of the cervix (n = 599) was used for comparison for immune-oncology biomarkers. DLL3 expression was observed in 81% of the cases. DLL3 expression was inversely correlated with commonly observed pathogenic mutations in *PIK3CA* (17%) ( $P=0.018$ ) and *PTEN* (10%) ( $P=0.006$ ). Other more frequently seen pathogenic mutations (*TP53* 17%, *KRAS* 11%, and *CTNNB1* 5%) were not associated with DLL3 expression. TROP-2 expression was detected in only 1 case and no case expressed FOLR1. Although NTRK protein expression was observed in 21% of the cases, none of these had an *NTRK* gene fusion. PD-L1 expression (10%) and high tumor mutational burden (3%) were significantly less frequent in NEC compared with the squamous cell carcinoma cohort (79% and 11%, respectively). None of the NEC exhibited high microsatellite instability status. Despite frequent

DLL3 expression in NEC, a potential therapeutic benefit of DLL3-targeted drugs remains uncertain given the recent failure of the Rova-T therapeutic trial in small cell lung carcinomas. Small cohorts of NEC enriched in *PIK3CA/PTEN/AKT* and programmed cell death protein 1/PD-L1 alterations indicate therapeutic roles for their respective inhibitors.

**Key Words:** cervix, neuroendocrine carcinoma, molecular profiling, sequencing, targeted therapy

(*Appl Immunohistochem Mol Morphol* 2021;29:299–304)

Neuroendocrine carcinoma of the cervix (NEC) is a rare primary cervical cancer encompassing ~1% of all cervical malignancies.<sup>1–3</sup> These cancers tend to have a high propensity to spread both locally and distally, and affected patients usually present with advanced disease (eg, liver and lung metastases) at initial presentation.<sup>4,5</sup> The prognosis is very poor with a mean overall survival of ~40 months.<sup>1–3</sup> Similar to other subtypes of cervical carcinomas, NECs are also associated with high-risk human papillomavirus (HPV) infections (HPV16 and HPV18 as the most common).<sup>1,6</sup>

The current therapeutic approaches to NEC patients mainly derive from NECs of the lung.<sup>1</sup> These usually include radical hysterectomy followed by adjuvant chemotherapy (cisplatin/carboplatin and etoposide) or concurrent chemotherapy and radiation therapy for early disease; concurrent chemotherapy and radiation therapy that may be preceded by neoadjuvant chemotherapy and followed by adjuvant chemotherapy is used for locally advanced NEC, while palliative chemotherapy is the modality treatment for patients with metastatic NEC.<sup>1,3</sup>

Limited data are available regarding the targetable molecular features of NEC.<sup>7–11</sup> Given that no targeted therapy has been approved yet for NEC, we explored novel targetable biomarkers in a large cohort of NEC.

## MATERIALS AND METHODS

### Samples

Sixty-two NEC of the cervix were profiled for biomarkers of targeted therapies at Caris Life Sciences (Phoenix, AZ). All samples were submitted by referring pathologists and oncologists for molecular profiling purposes. >90% of the samples were provided from the pathology laboratories from

Received for publication May 21, 2020; accepted October 14, 2020.

From the \*Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY; †College of Medicine, QU Health, Qatar University, Doha, Qatar; ‡Caris Life Sciences, Phoenix, AZ; and §Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

S.V. are co-corresponding author.

A.C. and S.V. contributed equally.

The preliminary data from the study were presented at the European Society of Medical Oncology (ESMO) Congress 2019 that was held between September 27 and October 01, 2019, in Barcelona, Spain.

The publication of this study was funded by the Qatar National Library.

The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

D.A., E.C., and J.S. are employees of Caris Life Sciences. The remaining authors declare no conflict of interest.

Reprints: Jeffrey Swensen, PhD, Caris Life Sciences, 4610 South 44th Place, Phoenix, AZ 85040 (e-mail: jswensen@carisls.com).

Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

the United States. A cohort of squamous cell carcinomas (SCCs) of the cervix (n = 599) was used for comparison for immune-oncology biomarkers.

Before molecular testing, each NEC case underwent confirmation of the histologic diagnosis and a review of the diagnostic immunohistochemical (IHC) workup [eg, neuroendocrine markers (synaptophysin, chromogranin-A, CD56), cytokeratins (AE1-AE3, Cam5.2, CK5/6, CK7, and CK20), p16, p40, TTF-1] performed at the referring pathology laboratories. All reports were deidentified and remnant NEC samples provided by the referring laboratories. Given that the remnant tissues from previous samplings with no associated identifiers were used, this research was compliant with 45 CFR 46.101(b). Therefore, the present study was deemed exempt from Institutional Review Board approval and consent requirements were waived. The study was conducted in compliance with the guidelines for human studies provided by the World Medical Association Declaration of Helsinki.

All molecular assays were performed at a CLIA/CAP/ISO15189/NYSDOH certified clinical laboratory (Caris Life Sciences).

## IHC

IHC assays included stains against the following biomarkers: delta-like canonical notch ligand 3 (DLL3), a trophoblast cell surface antigen 2 (TROP-2), folate receptor 1 (FOLR1), neurotrophic receptor tyrosine kinase 1-3 (NTRK), and programmed death-ligand 1 (PD-L1). We summarized in Table 1 the information regarding the antibodies, thresholds for positivity, and subcellular localization of each biomarker.<sup>12,13</sup> All IHC assays were run with both positive and negative controls.

## HPV Detection [In Situ Hybridization (ISH)]

For HPV assessment, we used a cocktail of 3 mRNA probes for high-risk HPVs 16, 18, and 33. The HPV-ISH assay was performed following the manufacturer's instructions with the BenchMark automated slide staining system (Ventana Medical Systems).

## Next-generation Sequencing (NGS)

The NEC samples were profiled using massively parallel sequencing (NGS) of exons from 592 genes (SureSelect XT; Agilent, Santa Clara, CA, and the NextSeq instrument;

Illumina, San Diego, CA).<sup>13,14</sup> A full gene panel is available at: [www.carismolecularintelligence.com/wp-content/uploads/2017/03/TN0276-v5\\_Profile-Menu-Brochure.pdf](http://www.carismolecularintelligence.com/wp-content/uploads/2017/03/TN0276-v5_Profile-Menu-Brochure.pdf).

The tumor mutational burden (TMB) was assessed by calculating the number of nonsynonymous missense mutations, excluding common germline variants, per 1 Mbp of DNA. TMB was considered high if  $\geq 17$  mutations/Mbp were detected. The estimated threshold was based on a cohort of 599 cases of SCC of the cervix that was profiled at Caris Life Sciences. We applied the 80th percentile cutoff value for TMB assessment as suggested by Samstein et al.<sup>15</sup>

Microsatellite instability (MSI) was calculated from the NGS data by direct analysis of short tandem repeat tracts in the target regions of sequenced genes. The count only included alterations that resulted in increases or decreases in the number of repeats; high MSI was defined as  $\geq 46$  altered microsatellite loci. This threshold was established by comparing NGS with the polymerase chain reaction-based microsatellite fragments analysis results from  $\sim 2100$  samples.<sup>16</sup> MSI status was compared with that from a cohort of SCC of the cervix (n = 599).

Copy number alterations were assessed by comparing the depth of detected NGS sequence reads to reads from a diploid control. Genes having  $\geq 6$  copies were considered amplified.<sup>13,14,17-19</sup>

The ArcherDx FusionPlex Assay (ArcherDX, Boulder, CO) was used for the gene fusion assessment. The gene fusions panel (n = 54) is available at: [www.carismolecularintelligence.com/wp-content/uploads/2017/03/TN0276-v14\\_Profile-Menu.pdf](http://www.carismolecularintelligence.com/wp-content/uploads/2017/03/TN0276-v14_Profile-Menu.pdf).

## Statistical Methods

The  $\chi^2$  and Fisher exact tests were used to analyze the relationships between the examined variables. *P*-values  $< 0.05$  were considered significant. IBM SPSS Statistics (version 25) was used for statistical analysis (licensed to Qatar University).

## RESULTS

### Clinicopathologic Characteristics of the Cohort

The study group included 36 primary and 26 metastatic NEC of the cervix. Metastatic sites of cervical NEC included pelvis (and pelvic lymph nodes) (n = 6), liver (n = 5), uterus (n = 2), ovary (n = 1), vagina (n = 1), lung (n = 1), brain (n = 1),

**TABLE 1.** Overview of the Antibodies That Were Used in the Study

Antibody	Clone (Manufacturer)	Threshold for Positivity	Subcellular Localization
Delta-like canonical notch ligand 3 (DLL3)	SP347 clone (Ventana)	Any positivity $\geq 50\%$ (high expression)	Membranous/cytoplasmic
Trophoblast antigen 2 (TROP-2)	Anti-human Trop-2 (R&D Systems)	$\geq 10\%$ , 2+ intensity $> 50\%$ (high expression)	Membranous
Folate receptor 1 (FOLR1)	Clone 26B3.F2 (Biocare Medical)	<i>H</i> -score $\geq 1$ ( $\geq 20$ for high)	Membranous
Tropomyosin receptor tyrosine kinase (1-3) (Pan-TRK)	Clone EPR17341 (Abcam)	$\geq 1\%$ of tumor cells	Membranous/cytoplasmic and nuclear
Programmed death ligand 1 (PD-L1)	22C3 pharmDx Kit (Dako Agilent)	CPS $\geq 1^*$	Membranous/cytoplasmic

\*CPS = the number of PD-L1+ cells (cancer cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100; PD-L1 positivity is defined as CPS  $\geq 1$ .

CPS indicates Combined Positive Score.

**TABLE 2.** Comparison of Novel Predictive Biomarkers With Therapeutic Implications Between NECs of the Cervix and Other Common Cancers

Biomarker	Function	Mechanism of Action	Targeted Drug	Common Cancers	Diagnostic Assay	Status in NEC
DLL3	Notch ligand	Overexpression	Rovalpituzumab tesirine	SCLC	IHC	38+/47 (80%)
pNTRK	Nerve development and growth (activation by neurotrophins)	Protein overexpression due to gene fusion	TRK inhibitors (eg, entrectinib)	Pediatric sarcomas, thyroid, MASC, gliomas	IHC and Archer Fusion assay (NGS)	10+/47 (21%) positive by IHC <i>No TRK1-3 gene fusions</i>
FOLR1	Folate antimetabolites (eg, pemetrexed-therapy)	Overexpression	Imaging probes, drug conjugates, farletuzumab	NSCLC, breast, ovarian, CRC	IHC	0/47 (0%)
TROP-2	Transmembrane protein (intracellular calcium transducer)	Overexpression	Trop-2-targeted antibody-drug conjugates	Cervix (SCC), breast, CRC, esophagus, gliomas	IHC	1+/47 (2%)

CRC indicates colorectal carcinoma; IHC, immunohistochemistry; MASC, mammary analog secretory carcinoma; NEC, neuroendocrine carcinoma; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma; SCLC, small cell lung cancer.

peritoneum (n = 1), scapular region (n = 1), trunk (n = 1), and other distant lymph nodes (retroperitoneal, mediastinal, and supraclavicular) (n = 6). All cases were high-grade carcinomas. All cases were histopathologically composed of a monotonous population of small cells with hyperchromatic nuclei, frequent mitosis, nuclear molding, and scanty cytoplasm. Although the 2014 World Health Organization (WHO) classification<sup>20</sup> does not require the presence of any neuroendocrine markers, in all cases at least 1 neuroendocrine marker was positive to support the NEC diagnosis.

The mean patient's age with NEC was 43.6 years (range, 24 to 82 y). HPV results were available for 47 cases: 20 cases (43%) were positive by HPV-ISH, while the remaining 27 cases (57%) were negative. The HPV results were not associated with patients' age ( $P=0.38$ ) and tumor site (primary vs. metastatic NEC) ( $P=1.0$ ).

### Antibody-drug Conjugate (ADC) Targets in NEC

The results of ADC biomarkers are summarized in Table 2.

DLL3 expression was observed in 81% of the cases with 49% of cases expressing diffusely ( $\geq 50\%$  of positive cancer

cells) DLL3 protein (Fig. 1). Metastatic NEC tended to have higher DLL3 expression compared with the primary NECs ( $P=0.08$ ). DLL3 expression did not differ significantly in regards to HPV status but was inversely correlated with commonly observed pathogenic mutations in *PIK3CA* (17%) ( $P=0.018$ ) and *PTEN* (10%) ( $P=0.006$ ). Other more frequently seen pathogenic mutations (*TP53* 17%, *KRAS* 11%, and *CTNBI* 5%) were not associated with DLL3 expression.

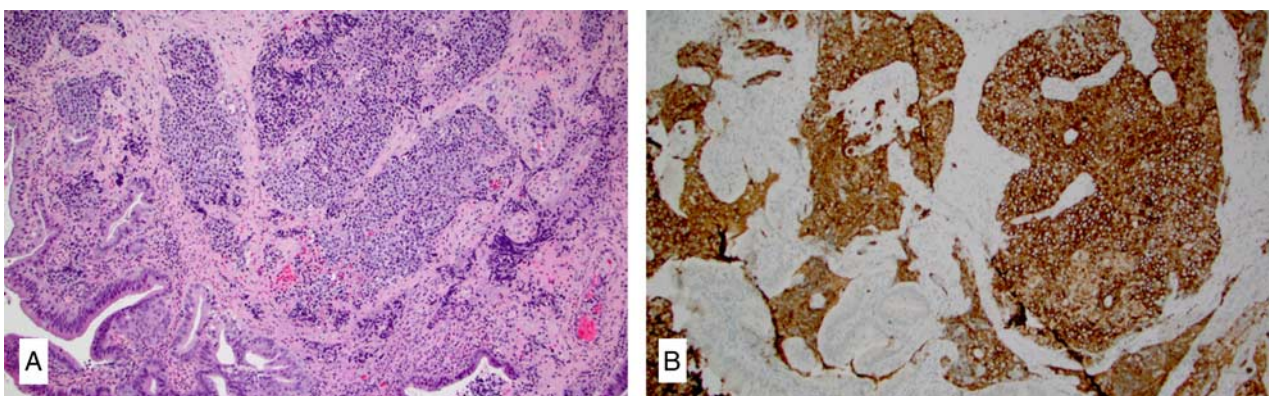
TROP-2 expression was positive in 1 case, while FOLR1 showed no expression in any of the tested cases.

### NTRK Status in NEC

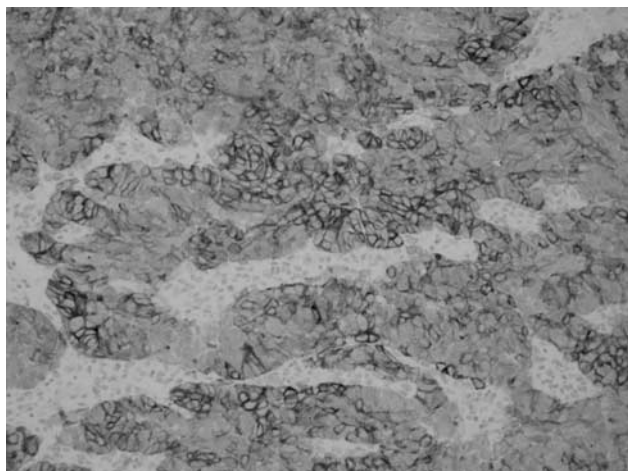
NTRK expression using pan-TRK antibody was positive in 10+/47 (21%) cases (Seven primary and 3 metastatic NEC). However, no *NTRK1-3* gene fusions were detected in any case including the 10 cases positive for NTRK by IHC (Table 2, Fig. 2).

### Genomic Profile of NEC

Pathogenic mutations were detected in 23 genes. The most commonly mutated genes included *PIK3CA* (11/62),



**FIGURE 1.** Hematoxylin and eosin (H&E) slide (A) of a case of NEC with diffuse and strong DLL3 positivity (B). The majority (81%) of NECs overexpressed DLL3 protein with ~50% of them having diffuse expression ( $> 50\%$  positive cells).



**FIGURE 2.** A diffuse (> 90% cancer cells) and NTRK expression in a case of cervical strong NEC; however, no *NTRK1-3* gene fusion was seen in this as well as in 9 additional cases that exhibited NTRK expression by immunohistochemistry.

*TP53* (11/62), *KRAS* (7/62), *PTEN* (6/62), *CTNNB1* (3/62), *BRAF* (2/62), *RBI* (2/62), *FGFR2* (2/62), and *HNF1A* (2/62), see Table 3 for complete results. *PIK3CA* and *PTEN* mutations were more prevalent in DLL3-negative NECs ( $P=0.018$  and  $0.006$ , respectively). *PTEN* mutations were also more commonly seen in HPV-negative NEC cases ( $P=0.04$ ). Four cases had concurrent *PIK3CA* and *PTEN* mutations. The mutational profiles did not differ significantly between primary and metastatic NECs.

Copy number alterations were rarely seen in NEC and included amplification of *MYCN* (2/31, 6%), *MCL1*, *MYB*, *MYC*, and *PCMI* (1/31, 3% each).

Gene fusions were not observed in any of the tested cases ( $n=31$ ).

### Immune-oncology Biomarkers in NEC

PD-L1 positivity (Combined Positive Score  $\geq 1$ ) and high-TMB ( $\geq 17$  mutations/Mbp) were significantly lower

**TABLE 3.** Genomic Profile of Neuroendocrine Carcinoma of the Cervix

Genomic Alterations	Affected Genes in Neuroendocrine Carcinomas of the Cervix
Mutations	<i>PIK3CA</i> (11/62, 17%) <i>TPPTEN</i> (6/62, 10%)* 53 (11/62, 17%) <i>KRAS</i> (7/62, 11%) <i>CTNNB1</i> (3/62, 5%) <i>BRAF</i> (2/62, 3%) <i>RBI</i> (2/62, 3%) <i>FGFR2</i> (2/62, 3%) <i>HNF1A</i> (2/62, 3%) <i>APC</i> , <i>AKT1</i> , <i>ATM</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>ERBB2</i> , <i>ERBB3</i> , <i>FBXW7</i> , <i>HRAS</i> , <i>KDM6A</i> , <i>KMT2C</i> , <i>KMT2D</i> , <i>RET</i> , <i>STK11</i> (single cases each, ~2%)
Copy number alterations	<i>MYCN</i> (2/31, 6%) <i>MCL1</i> , <i>MYB</i> , <i>MYC</i> , <i>PCMI</i> (1/31, 3%)

\*5/5 tested *PTEN*-mutated cases were human papillomavirus negative.

**TABLE 4.** Comparison Between NEC and Squamous Cell Carcinoma of the Cervix for 3 Predictive Biomarkers of Response to the Immune Checkpoint Inhibitors (Anti-PD-1/PD-L1)

I-O Biomarkers	n/N (%)	
	Neuroendocrine Carcinoma of the Cervix	Squamous Cell Carcinoma of the Cervix*
PD-L1 positivity	4+/39 (10)	77/98 (79)
TML-H	1/31 (3)	63/599 (11) <sup>†</sup>
MSI-H	0/31 (0)	6/599 (1) <sup>†</sup>

\*PD-L1 frequency based on KEYNOTE-158 clinical trial [led to FDA approval of pembrolizumab for cervical cancer (2018)]; PD-L1 was tested using 22C3 pharmDx Kit antibody (FDA approved test).

<sup>†</sup>The data for TMB and MSI status in squamous cell carcinoma were based on the Caris Molecular Profiling results.

FDA indicates Food and Drug Administration; I-O, immune-oncology; MSI-H, microsatellite instability-high; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TML-H, tumor mutational load-high was defined as  $\geq 17$  mutations/Mbp.

in NEC compared with SCC of the cervix (10% vs. 79% for PD-L1 and 3% vs. 11% for TMB-high, respectively). Similar to cervical SCCs, all tested NEC cases ( $n=31$ ) were MSI stable (Table 4).

### DISCUSSION

NEC is a rare but highly aggressive malignancy with limited therapeutic options, and treatments are extrapolated from studies in other sites. Molecular profiling studies on this cancer are also sparse but the importance of these studies cannot be underestimated given the huge potential benefit if targetable mutations allowed for treatment with emerging drugs.<sup>8,11</sup> Our study is the first to explore biomarkers of response to novel ADCs DLL3, TROP-2, and FOLR1 in an NEC cohort (the ADC drugs and their predictive biomarkers are summarized in Table 2). ADCs are considered one of the most promising anticancer treatments. The therapeutic approach with ADC is based on a monoclonal antibody to a cancer-specific antigen, conjugated to a cytotoxic drug via a chemical linker.<sup>21</sup> This allows cancer cells to be specifically targeted with cytotoxic drugs. Consequently, treatment with ADC substantially diminishes systemic exposure and toxicity.<sup>21</sup> DLL3 protein appears to be the only markedly overexpressed biomarker in our study. Notably, a high DLL3 expression was reported in Merkel cell carcinoma, a variant of highly aggressive cutaneous NEC and small cell lung carcinomas (SCLCs).<sup>22,23</sup> However, a potential therapeutic benefit of DLL3-targeted drugs in NEC remains uncertain given the recent termination of the Rova-T trial after it failed to demonstrate a therapeutic benefit for rovalpituzumab tesirine in patients with SCLC.<sup>24</sup> In our study, DLL3-negative NECs tended to harbor *PIK3CA* and *PTEN* mutations. Both mutations have been commonly described across cancers including NEC and other cervical malignancies,<sup>2,3,8,11</sup> leading to the upregulation of the PI3K-AKT-mTOR signaling pathway.

Interestingly, *PIK3CA* mutations were found in other HPV-associated malignancies such as oropharyngeal or anal cancer.<sup>25,26</sup> These mutations have also been associated with a favorable response to *PIK3CA* inhibitors, most notably in estrogen receptor-positive breast cancer.<sup>27,28</sup> Therefore, clinical trials exploring the therapeutic effects of *PIK3CA*/*mTOR* inhibitors in a subset of NECs are warranted. Similar to our findings, *KRAS* mutations were previously described in NEC<sup>8</sup> although another recent study<sup>29</sup> confirmed only *NRAS* but not *KRAS* mutations in a cohort of 30 NECs. The therapeutic utility of MEK inhibitors in *KRAS*-mutated NECs remains to be clinically explored.

Oncogenic fusions affecting the *NTRK1-3* genes have been identified in a wide range of human cancers.<sup>13,30</sup> Of cervical malignancies, *NTRK* gene fusions have been described in cervical sarcomas<sup>31,32</sup> and endocervical malignant peripheral nerve sheath tumor.<sup>33</sup> We have also previously reported an *NTRK1* gene fusion in 1/68 cervical SCCs.<sup>13</sup> *NTRK* gene fusions have been described in a small proportion of neuroendocrine malignancies from various anatomic sites including lung (large cell), pancreas, small bowel (with therapeutic response to entrectinib), and uterus.<sup>34,35</sup> Our recent study on breast NEC revealed no *NTRK* gene fusions.<sup>12</sup> The recognition of *NTRK*-rearranged cancers is clinically relevant given the recent availability of highly effective *NTRK* inhibitors. The most reliable predictive biomarker for *NTRK* inhibitors is a gene fusion identified by NGS or other equivalent assay (eg, fluorescence in situ hybridization, reverse transcriptase-polymerase chain reaction).<sup>36</sup> Since we did not identify *NTRK* gene fusions in any of the tested NEC cases including the IHC positive ones, it is unlikely that these patients would gain from the targeted treatment with *NTRK* inhibitors.

Immune checkpoint inhibitors (against programmed cell death protein 1/PD-L1) have markedly changed the cancer treatment paradigm contributing to better treatment options and improved outcomes for various cancers. The predictive biomarkers for immune checkpoint inhibitors include PD-L1 expression, high tumor MSI and TMB status.<sup>30,37–39</sup> The immune checkpoint inhibitor pembrolizumab/Keytruda has been approved for SCC of the cervix and SCLC. In the case of cervical carcinoma, pembrolizumab is indicated for treatment if the tumor tissue expresses PD-L1 (Combined Positive Score  $\geq 1$ ) as determined by a Food and Drug Administration (FDA)-approved test (22C3 pharmDx Kit antibody). Preliminary data based on 2 case studies revealed a substantial therapeutic benefit for nivolumab in 2 NEC patients.<sup>9,10</sup> Notably one of the successfully treated cases was PD-L1 negative, while the other had MSI-high NEC with a high TMB. Nevertheless, our results (PD-L1 expression and high TMB) indicate that  $\sim 13\%$  of NEC patients may be candidates for trials with immune checkpoint inhibitors.

In our study, we found that only 43% of the cases were positive for HPV by ISH. The reported rate of HPV in NEC varies and some studies reported HPV positivity in 85% of the cases.<sup>6,40</sup> It is important to emphasize that our ISH cocktail covered high-risk HPVs 16, 18, and 33,

while other high-risk HPV types were not covered. Without a history of HPV status at the time of a Pap test, it is difficult to estimate the prevalence of HPV and its importance for the development of NEC. Another limitation of our study is related to the fact that all cases were not explored for all molecular targets; the main reasons for this shortcoming include the lack of remnant tissue samples (for IHC assays) or a poor/insufficient DNA and/or RNA load (tumor mutation load and MSI analyses).

We conclude that patients with NEC have limited targeted therapeutic options. Despite frequent *DLL3* expression in NEC, a potential therapeutic benefit for *DLL3*-targeted drugs remains uncertain given the recent failure of the Rova-T trial in SCLC. A small cohort of NEC enriched in *PIK3CA*/*PTEN* pathway mutations, may benefit from *PIK3CA* inhibitors. Biomarkers of benefit for immune checkpoint inhibition (eg, PD-L1+ and high TMB) were present in  $\sim 13\%$  of patients, who consequently may be considered for this therapy.

## REFERENCES

- Gadducci A, Carinelli S, Aletti G. Neuroendocrine tumors of the uterine cervix: a therapeutic challenge for gynecologic oncologists. *Gynecol Oncol*. 2017;144:637–646.
- Patibandla JR, Fehniger JE, Levine DA, et al. Small cell cancers of the female genital tract: molecular and clinical aspects. *Gynecol Oncol*. 2018;149:420–427.
- Tempfer CB, Tischoff I, Dogan A, et al. Neuroendocrine carcinoma of the cervix: a systematic review of the literature. *BMC Cancer*. 2018;18:530.
- He Y, Zhao H, Li XM, et al. A clinical analysis of small-cell neuroendocrine carcinoma of the gynecologic tract: report of 20 cases. *Arch Gynecol Obstet*. 2019;299:543–549.
- Stecklein SR, Jhingran A, Burzawa J, et al. Patterns of recurrence and survival in neuroendocrine cervical cancer. *Gynecol Oncol*. 2016;143:552–557.
- Castle PE, Pierz A, Stoler MH. A systematic review and meta-analysis on the attribution of human papillomavirus (HPV) in neuroendocrine cancers of the cervix. *Gynecol Oncol*. 2018;148:422–429.
- Lyons YA, Frumovitz M, Soliman PT. Response to MEK inhibitor in small cell neuroendocrine carcinoma of the cervix with a *KRAS* mutation. *Gynecol Oncol Rep*. 2014;10:28–29.
- Frumovitz M, Burzawa JK, Byers LA, et al. Sequencing of mutational hotspots in cancer-related genes in small cell neuroendocrine cervical cancer. *Gynecol Oncol*. 2016;141:588–591.
- Sharabi A, Kim SS, Kato S, et al. Exceptional response to nivolumab and stereotactic body radiation therapy (SBRT) in neuroendocrine cervical carcinoma with high tumor mutational burden: management considerations from the center for personalized cancer therapy at UC San Diego Moores Cancer Center. *Oncologist*. 2017;22:631–637.
- Paraghamian SE, Longoria TC, Eskander RN. Metastatic small cell neuroendocrine carcinoma of the cervix treated with the PD-1 inhibitor, nivolumab: a case report. *Gynecol Oncol Res Pract*. 2017;4:3.
- Xing D, Zheng G, Schoolmeester JK, et al. Next-generation sequencing reveals recurrent somatic mutations in small cell neuroendocrine carcinoma of the uterine cervix. *Am J Surg Pathol*. 2018;42:750–760.
- Vranic S, Palazzo J, Sanati S, et al. Potential novel therapy targets in neuroendocrine carcinomas of the breast. *Clin Breast Cancer*. 2019;19:131–136.
- Gatalica Z, Xiu J, Swensen J, et al. Molecular characterization of cancers with *NTRK* gene fusions. *Mod Pathol*. 2019;32:147–153.
- Gatalica Z, Xiu J, Swensen J, et al. Comprehensive analysis of cancers of unknown primary for the biomarkers of response to immune checkpoint blockade therapy. *Eur J Cancer*. 2018;94:179–186.
- Samstein RM, Lee CH, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet*. 2019;51:202–206.

16. Vanderwalde A, Spetzler D, Xiao N, et al. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. *Cancer Med.* 2018;7:746–756.
17. Gargano SM, Senarathne W, Feldman R, et al. Novel therapeutic targets in salivary duct carcinoma uncovered by comprehensive molecular profiling. *Cancer Med.* 2019;8:7322–7329.
18. Vranic S, Senarathne W, Stafford P, et al. Biomarkers of targeted therapy and immuno-oncology in cancers metastatic to the breast. *Appl Immunohistochem Mol Morphol.* 2020;28:661–668.
19. Vranic S, Stafford P, Palazzo J, et al. Molecular profiling of the metaplastic spindle cell carcinoma of the breast reveals potentially targetable biomarkers. *Clin Breast Cancer.* 2020;20:326–331.e1.
20. Kurman RJ, Carcangiu ML, Herrington CS, Young RH, eds. *WHO Classification of Tumours of Female Genital Organs, Volume 6*, 4th ed. Lyon, France: IARC; 2014.
21. Khongorzul P, Ling CJ, Khan FU, et al. Antibody-drug conjugates: a comprehensive review. *Mol Cancer Res.* 2020;18:3–19.
22. Xie H, Kaye FJ, Isse K, et al. Delta-like protein 3 expression and targeting in merkel cell carcinoma. *Oncologist.* 2020.
23. Xie H, Boland JM, Maleszewski JJ, et al. Expression of delta-like protein 3 is reproducibly present in a subset of small cell lung carcinomas and pulmonary carcinoid tumors. *Lung Cancer.* 2019;135:73–79.
24. Morgensztern D, Besse B, Greillier L, et al. Efficacy and safety of rovalpituzumab tesirine in third-line and beyond patients with DLL3-expressing, relapsed/refractory small-cell lung cancer: results from the phase II TRINITY Study. *Clin Cancer Res.* 2019;25:6958–6966.
25. Nichols AC, Palma DA, Chow W, et al. High frequency of activating PIK3CA mutations in human papillomavirus-positive oropharyngeal cancer. *JAMA Otolaryngol Head Neck Surg.* 2013;139:617–622.
26. Casadei Gardini A, Capelli L, Ulivi P, et al. KRAS, BRAF and PIK3CA status in squamous cell anal carcinoma (SCAC). *PLoS One.* 2014;9:e92071.
27. Papa A, Pandolfi PP. The PTEN(–)PI3K axis in cancer. *Biomolecules.* 2019;9:153.
28. Yang J, Nie J, Ma X, et al. Targeting PI3K in cancer: mechanisms and advances in clinical trials. *Mol Cancer.* 2019;18:26.
29. Van Ta T, Nguyen QN, Truong VL, et al. Human papillomavirus infection, p16(INK4a) expression and genetic alterations in Vietnamese cervical neuroendocrine cancer. *Malays J Med Sci.* 2019; 26:151–157.
30. Yoshino T, Pentheroudakis G, Mishima S, et al. JSCO-ESMO-ASCO-JSMO-TOS: international expert consensus recommendations for tumour-agnostic treatments in patients with solid tumours with microsatellite instability or NTRK fusions. *Ann Oncol.* 2020;31:861–872.
31. Rabban JT, Devine P, Sangoi AR, et al. NTRK fusion cervical sarcoma: a report of 3 cases, emphasizing morphological and immunohistochemical distinction from other uterine sarcomas, including adenosarcoma. *Histopathology.* 2020;77:100–111.
32. Hodgson A, Pun C, Djordjevic B, et al. NTRK-rearranged cervical sarcoma: expanding the clinicopathologic spectrum. *Int J Gynecol Pathol.* 2020. [Epub ahead of print].
33. Wells AE, Mallen AM, Bui MM, et al. NTRK-1 fusion in endocervical fibroblastic malignant peripheral nerve sheath tumor marking eligibility for larotrectinib therapy: a case report. *Gynecol Oncol Rep.* 2019;28:141–144.
34. Sigal DS, Bhangoo MS, Hermel JA, et al. Comprehensive genomic profiling identifies novel NTRK fusions in neuroendocrine tumors. *Oncotarget.* 2018;9:35809–35812.
35. Sigal D, Tartar M, Xavier M, et al. Activity of entrectinib in a patient with the first reported NTRK fusion in neuroendocrine cancer. *J Natl Compr Canc Netw.* 2017;15:1317–1322.
36. Marchio C, Scaltriti M, Ladanyi M, et al. ESMO recommendations on the standard methods to detect NTRK fusions in daily practice and clinical research. *Ann Oncol.* 2019;30:1417–1427.
37. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science.* 2015;348:124–128.
38. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med.* 2015;372:2509–2520.
39. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet.* 2016;387:1909–1920.
40. Alejo M, Alemany L, Clavero O, et al. Contribution of human papillomavirus in neuroendocrine tumors from a series of 10,575 invasive cervical cancer cases. *Papillomavirus Res.* 2018;5:134–142.