Gynecologic Oncology 141 (2016) 588–591

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Sequencing of mutational hotspots in cancer-related genes in small cell neuroendocrine cervical cancer

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HIGHLIGHTS

- Hotspot mutations were found in 55% of patients with small cell cervical cancer.
- Druggable mutations were seen in 48% of patients with small cell cervical cancer.
- PIK3CA (18%), KRAS (14%), and TP53 (11%) were the most common mutations present.

ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form 30 March 2016
Accepted 1 April 2016
Availabe online 15 April 2016

Keywords:
Small cell
Large cell
Neuroendocrine
Cervical cancer
Somatic mutations
Personalized medicine

ABSTRACT

Objectives. Small cell cervical cancer is a rare malignancy with limited treatment options for recurrent disease. We sought to determine if tumor specimens of small cell cervical cancer harbor common somatic mutations and if any of these are actionable.

Methods. Using a registry of patients with neuroendocrine cervical cancer, we identified 44 patients with pure or mixed small cell cervical cancer who had undergone mutational analysis. Mutations had been detected using next generation sequencing of mutational hotspots in 50 cancer-related genes.

Results. Thirty-five mutations were identified in 24 patients (55%). Fifteen of these 24 patients (63%) had 1 mutation, 7 patients (29%) had 2 mutations, and 2 patients (8%) had 3 mutations. In all 44 patients, the most commonly seen mutations were mutations in *PIK3CA* (8 patients; 18%), *KRAS* (6 patients; 14%), and *TP53* (5 patients; 11%). No other mutation was found in >7% of specimens. Of the 24 patients who had a mutation, 21 (88%) had at least 1 alteration for which there currently exists a class of biological agents targeting that mutation. In the entire cohort of 44 patients, 48% had at least 1 actionable mutation.

Conclusion. Although no single mutation was found in the majority of patients with small cell cervical cancer, almost half had at least 1 actionable mutation. As treatment options for patients with recurrent small cell cervical cancer are currently very limited, molecular testing for targetable mutations, which may suggest potential therapeutic strategies, may be useful for clinicians and patients.

1. Introduction

Although the incidence of cervical cancer has steadily decreased in developed countries because of effective screening and human papillomavirus (HPV) vaccination, cervical cancer remains the second most prevalent cancer among women worldwide [1]. The vast majority (>95%) of cervical cancers are of the HPV-associated histologic subtypes of squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma [2]. Fewer than 1% of women with cervical cancer have a neuroendocrine tumor, which translates to approximately 100 to 200 cases of neuroendocrine cervical cancer diagnosed each year in the United States.

Neuroendocrine carcinoma of the cervix encompasses several histologic subtypes, including small cell, large cell, and carcinoid (low- and high-grade) tumors. Unlike the more common squamous and adenocarcinoma subtypes, which spread primarily by local extension, small and large cell neuroendocrine cervical cancers have a propensity to spread both locally and hematogenously, and affected patients frequently present with extrapelvic disease (e.g., liver and lung parenchymal metastases) at initial diagnosis [3]. In addition, even among patients with disease clinically limited to the cervix, the prevalence of regional nodal disease is substantially higher among patients with

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http://dx.doi.org/10.1016/j.ygyno.2016.04.001
0090-8258/

neuroendocrine cervical cancer than among patient with the more common histologic subtypes: up to 40% of newly diagnosed patients with stage IB1 small cell cervical cancer have nodal metastases [3–5]. Stage for stage, the survival of women with small cell carcinoma of the cervix compares poorly against the survival of women with the more common cervical cancer subtypes.

Because of the rarity of small cell carcinoma of the cervix, no prospective trials have been performed to determine optimal therapy for women with the disease. These tumors do, however, have pathologic appearances and clinical behaviors similar to those of small cell lung cancer. Therefore, almost all patients with small cell cervical cancer receive cisplatin and etoposide as part of their primary therapy, according to guidelines developed by professional societies and largely extrapolated from treatment protocols for small cell lung cancer [6,7]. In addition, because of the aggressiveness of small cell cervical cancer, most patients undergo multimodal therapy with consideration of surgery, radiation therapy, and/or chemotherapy. Fifty-eight percent of patients receive dual-modality treatment, and 9% receive all 3 treatment modalities [8]. Nevertheless, overall survival remains poor, despite multimodal treatment plans, with 5-year survival rates ranging from 13% to 25% for all patients and as low as 0% for women with advanced-stage disease (stages II–IV) [9].

Improving outcomes for women with small cell carcinoma of the cervix has proven difficult because of the rarity of this disease. For patients with recurrent disease, there are no standard treatment protocols, and both the Society of Gynecologic Oncology and Gynecologic Cancer InterGroup recommend individualized treatment because of the acknowledged lack of any clinical trials to guide therapy for these women [6,7]. As outcomes are poor and therapeutic regimens are uncertain, we sought to determine whether there were common somatic mutations that might inform targeted therapy or potential clinical trials for women with recurrent small cell cancer of the cervix. Specifically, we reviewed the results in a cohort of 44 patients with small cell carcinoma of the cervix who had next generation sequencing at our institution to identify mutations in a panel of 50 genes that are commonly altered and/or targetable with existing drug inhibitors.

2. Methods

Data presented in this manuscript were abstracted from the Neuroendocrine Cervical Tumor Registry (NeCTuR) of The University of Texas MD Anderson Cancer Center. This Institutional Review Board–approved registry collects a wide range of data on women with small and large cell cervical cancers. Women who have been diagnosed with this disease or family members of deceased patients consent to participate in the registry and then provide their medical records for entry. Participants are recruited through a Facebook support group (www.facebook.com/groups/scccsisters), our website (www.necervix.com), or word of mouth. This study is a retrospective review of all patients with confirmed small cell cervical cancer (pure or mixed) who underwent molecular testing of a tumor specimen at MD Anderson Cancer Center from January 1, 2013, to December 31, 2015. Patients with pure large cell or carcinoid tumors were excluded. All pathologic specimens were reviewed by a pathologist specializing in gynecologic malignancies to confirm the histologic diagnosis of small cell neuroendocrine cervical cancer. A total of 44 patients met these inclusion criteria. Forty-three patients were seen at least once at MD Anderson for treatment and/or treatment recommendations. One patient had pathology review and molecular testing at MD Anderson but was not seen by a gynecologic oncologist at MD Anderson.

For the somatic genomic analysis, DNA was extracted, purified, and quantified from formalin-fixed, paraffin-embedded archived tissue obtained from surgery or biopsy. Next generation sequencing was performed using the Ion Ampliseq Cancer Panel (Life Technologies, Grand Island, NY) [10]. Specimens required >20% tumor cell content for analysis. The initial 8 patients (18%) had mutation hotspots assessed in 46 cancer-related genes. In July 2013, an additional 4 genes were added to the testing panel (*EZH2*, *IDH2*, *GNA11*, and *GNAQ*), and the remaining 36 patients (82%) had evaluation of all 50 genes (Table 1). This 50 gene panel was standardized for clinical molecular testing across the entire institution. These 50 genes were originally chosen as they were either commonly mutated genes in malignancies or had targeted agents either developed or in development. Additional details regarding this platform's analytic sensitivity and genomic aberration coverage are provided in the supplemental methods, available online. Details of mutational analysis are also provided in the supplemental methods.

Descriptive statistics were used to summarize patient demographic and mutation data. Patients were considered to have an actionable mutation if there currently existed an agent (approved or in development) that targeted the mutation or abnormalities in the molecular pathway of the mutation [11].

3. Results

Forty-four patients with small cell cervical cancer had molecular testing for genomic alterations. Demographics for the entire cohort are shown in Table 2. The median age was 37.5 years (range, 24.7–63.6). Thirty-eight patients (84%) had pure small cell cervical cancer and 6 (14%) had mixed small and large cell cervical cancer. Twenty-six patients (59%) had clinical stage I disease.

Tumor for molecular evaluation was obtained from the cervix in 37 patients (84%), from a lymph node in 3 patients (7%), from the vagina in 2 patients (5%), and from the lung and from a subcutaneous lesion in 1 patient each (2%). In 37 patients (84%), tumor specimens were obtained prior to initiation of therapy; in the remaining 7 patients (16%), tumor specimens were obtained from persistent disease after treatment or at time of first recurrence.

All tumor samples yielded adequate DNA for genomic sequencing. Thirty-five mutations were identified in 24 patients (55%) (Table 3). Fifteen patients (63%) had 1 mutation, 7 patients (29%) had 2 mutations, and 2 patients (8%) had 3 mutations. In all 44 patients, the most commonly seen mutations were mutations in *PIK3CA* (8 patients), *KRAS* (6 patients), and *TP53* (5 patients). Of the 24 patients who had a mutation, 21 (88%) had at least 1 alteration for which there currently existed a class of biological agents targeting that mutation. In the entire cohort of 44 patients, 48% had at least 1 actionable mutation. Details of individual mutations are shown in Supplemental material Table 1.

The median follow-up time for the entire cohort was 16.6 months (range, 0.0–45.0). At this writing, 7 patients are undergoing active primary treatment, 10 patients are without evidence of disease after primary treatment, 14 patients are alive with disease being treated for recurrence, and 13 patients are dead of disease. Of the 37 patients who have completed primary treatment, 27 (73%) have had a recurrence.

4. Discussion

In this study of 44 patients with small cell cervical cancer, a rare disease, the most commonly mutated gene was *PIK3CA*, which was mutated in more than 18% of patients. Other mutations found in more than

Table 1
Gene panel for next generation sequencing.

ABL1	*EGFR*	***GNAQ***	*KRAS*	*PTPN11*
AKT1	*ERBB2*	*GNAS*	*MET*	*RB1*
ALK	*ERBB4*	*HNF1A*	*MLH1*	*RET*
APC	***EZH2***	*HRAS*	*MPL*	*SMAD4*
BRAF	*FGFR1*	***IDH2***	*NOTCH1*	*SMARCB1*
CDH1	*FGFR2*	*JAK2*	*NRAS*	*SRC*
CDKN2A	*FGFR3*	*JAK3*	*PDGFRA*	*STK11*
CSF1R	*FLT3*	*KDR*	*PIK3CA*	*TP53*
CTNNB1	***GNA11***	*KIT*	*PTEN*	*VHL*

NOTE: Genes in boldface were added to the original panel partway through the study period (see Methods section for details).