Evaluation of biomarker alterations in small cell cervical cancer identifies therapeutic options

J. K. Burzawa[1], S. Z. Millis[2], S. K. Reddy[2], I. Astsaturov[3], R. L. Coleman[1], J. Brown[1], M. Frumovitz[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Caris Life Sciences, Irving, TX, [3]Fox Chase Cancer Center, Philadelphia, PA

Abstract #5601

Objectives: Small cell cervical cancer (SCCC) is an extremely rare and aggressive form of cervical cancer, accounting for only 1% of all cervical cancer cases, or ~150 cases/yr. 70% of patients will recur, even when diagnosed with early stage disease and there are few therapeutic options in this setting. We evaluated tumor samples obtained from a large repository to determine prevalent targetable molecular aberrations in these rare tumors.

Methods: Seventy-eight SCCC samples were profiled, 53 of those on a commercial multiplatform, including a combination of gene sequencing (Sanger or NGS, up to 47 genes), amplification (CISH or FISH), and protein expression (IHC). Twenty-five samples were analyzed at a cancer center using a 50 gene NGS platform (CMS50). The profiles were compared to HPV+ cervical cancers (CC), neuroendocrine tumors (NET, all sites), and small cell lung cancers profiled at the same laboratory.

Results: TOP2A (85%) and TOPO1 (55%) had high overexpression, while ERCC1 had low expression (11%) in SCCC samples. SCCC tumors had higher protein expression of cKIT (26%) than HPV+ CC (3%, p<0.05). HER2 amplification was identified in 4.5% of SCCC and 8% of HPV+ CC. *EGFR* amplification was not seen in SCCC but was identified in 11% of HPV+ CC. Gene sequencing identified higher mutation rates for *TP53* (23%) and *KRAS* (18%) in SCCC compared to HPV+ CC (10% and 10%, respectively) but lower rates of *PIK3CA* (15% vs. 26%). Comparatively, small cell lung cancers had mutations in *TP53* in 57% of cases and in *KRAS* in 3% of cases. NGS evaluation of 51 cases also identified 3 *GNAS* and *RB1* mutations (6%), 2 *CTNNB1* and *SMAD4* mutations (4%), and single gene mutations in *BRCA1, PTEN, MET, APC, ATM, HNF1A*, and *FBXW7* (2% each).

Conclusions: Multiplatform tumor profiling identified high expression of TOP2A and TOPO1 in SCCC, which may explain sensitivity to etoposide and topotecan, respectively. Potential druggable mutations include *AKT1*, *KRAS*, *PIK3CA*, and *TP53*. We have identified one patient with a *KRAS* mutation treated with a MEK inhibitor who had a complete response and remains in remission at 14 months.

Comparison of SCCC to HPV-positive cervical cancer and small cell cancers of other organs

	IHC										ISH		Gene Sequencing					
	% Positive																	
Type of cancer \ Biomarker	BCRP	cKIT	cMET	ERCC1	ER	MRP1	PR	TOP2A	TOPO1	TUBB3	*EGFR*	*HER2*	*AKT1*	*GNAS*	*KRAS*	*PIK3CA*	*RB1*	*TP53*
SCCC	91	26*	5*	11*	2.3*	100	16.3	85.0	55	59*	0.0	4.5	6	6	18	15	6	23
Cervical, HPV+ [1]	38	3	22	36	20	86	8	89	56	26	11	8	1	3	10	26	1	10
NET, All [2]	58	7	22	34	22	89	11	80	61	26	7	0	1	3	7	28	2	12
SCLC[3]	43	63	6	21	0	86	15	91	63	84	11	0	0	0	3	1	11	57

* Indicates p<0.006 between SCCC and HPV+ cervical cancer

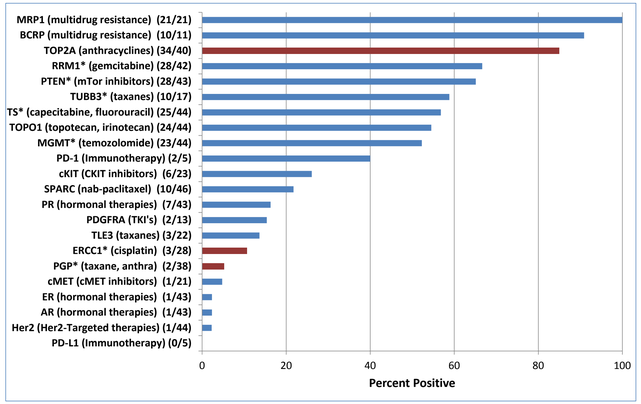
Table 1. Comparison of small cell cervical cancers to HPV-positive cervical cancer and small cell cancers arising in other organs. A subset of biomarkers identified that are most different between the different cancers is shown.

1. Feldman, R., Gatalica, Z., Reddy, S., Tewari, K., "Paving the Road to Personalized Medicine in Cervical Cancer: Theranostic Biomarker Evaluation in a 592-Specimen Library." Poster Session, SGO 2015.
2. Astsaturov, I, Cohen, S, Engstrom, PF, Millis, SZ, Profiling of 1,350 neuroendocrine tumors for identification of multiple potential drug targets, Poster Session, ASCO 2014.
3. Feldman, R., Astsaturov, I., Millis, S., Subramaniam, D., Liu, S.V., "Molecular Profiling in Small Cell Lung Cancer and Lung Neuroendocrine Tumors." Oral Session, Chicago Multidisciplinary Symposium in Thoracic Oncology 2014.

Results, Immunohistochemistry (IHC)

Figure 1. Levels of protein expression, reported as percent positivity/overexpression of total cases tested. For reference, PD-1 expression indicates presence of tumor-infiltrating lymphocytes (TILs), while PD-L1 expression indicates presence on tumor cells. Red lines indicate biomarkers associated with therapies currently used as standard of care.

** Underexpression or no expression (and not overexpression) is considered predictive of response to therapy shown in parentheses..*



Results, Gene sequencing

Table 2. Gene mutations/alterations. Mutations were found in 11 of 52 genes tested (22%) across the two platforms at Caris Life Sciences and MD Anderson.

	ABL1	*AKT1*	*ALK*	*APC*	*ATM*	*BRAF*	*BRCA1*	*BRCA2*	*CDH1*	*CDKN2A*	*cKIT*	*cMET*	*CSF1R*
Caris Life Sci.	(0/30)	(2/30)	(0/30)	(1/30)	(1/30)	(0/30)	(0/12)	(0/12)	(0/30)	nt	(0/30)	(1/29)	(0/30)
MD Anderson	(0/17)	(1/17)	(0/17)	(0/17)	(0/17)	(0/17)	(1/17)	(0/17)	(0/17)	(0/17)	(0/17)	(1/17)	(0/17)
Combined %	0	6.4	0.0	2.1	2.1	0.0	3.4	0.0	0.0	0.0	0.0	4.3	0.0

	CTNNB1	*EGFR*	*ERBB2*	*ERBB4*	*EZH2*	*FBXW7*	*FGFR1*	*FGFR2*	*FGFR3*	*FLT3*	*GNA11*	*GNAQ*	*GNAS*
Caris Life Sci.	(0/30)	(1/30)	(0/30)	(0/30)	nt	(1/30)	(0/30)	(0/30)	nt	(0/30)	(0/27)	(0/23)	(1/30)
MD Anderson	(2/17)	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(2/17)
Combined %	4.3	2.1	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	6.4

	HNF1A	*HRAS*	*IDH1*	*IDH2*	*JAK2*	*JAK3*	*KDR*	*KRAS*	*MLH1*	*MPL*	*NOTCH1*	*NPM1*	*NRAS*
Caris Life Sci.	(1/27)	(0/28)	(0/30)	nt	(0/30)	(0/30)	(0/30)	(5/34)	(0/30)	(0/30)	(0/30)	(0/30)	(0/30)
MD Anderson	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(0/17)	(4/17)	(1/17)	(0/17)	(0/17)	(0/17)	(0/17)
Combined %	2.3	0.0	0.0	0.0	0.0	0.0	0.0	17.6	2.1	0.0	0.0	0.0	0.0

	PDGFRA	*PIK3CA*	*PTEN*	*PTPN11*	*RB1*	*RET*	*SMAD4*	*SMARCB1*	*SMO*	*SRC*	*STK11*	*TP53*	*VHL*
Caris Life Sci.	(0/30)	(5/30)	(0/29)	(0/30)	(2/30)	(0/30)	(0/30)	(0/30)	(0/27)	nt	(0/30)	(7/30)	(0/28)
MD Anderson	(0/17)	(2/17)	(1/17)	(0/17)	(1/17)	(0/17)	(2/17)	(2/17)	(0/17)	(0/17)	(0/17)	(4/17)	(0/17)
Combined %	0.0	14.9	2.2	0.0	6.4	0.0	4.3	4.3	0.0	0.0	0.0	23.4	0.0

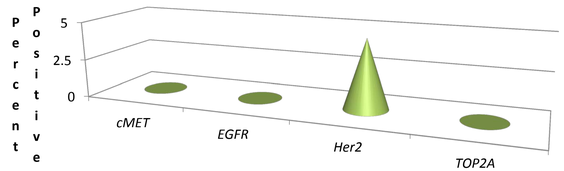
nt = not tested

Table 3 - Specific gene mutations. Representative mutations detected at Caris Life Sciences with corresponding protein changes are shown. The number of times detected is in parentheses.

Mutated Gene	Protein Change
TP53	R175H (1), H193R (1), E287X (1), R175H (1), R213X (2), V173M (1)
PIK3CA	E542K (1), E545K (2), D1017H (1), M1043I (1)
KRAS	G12D (2), G12V (3)

Results, *In situ* hybridization (ISH)

Figure 2. ISH (FISH or CISH) results showing percent amplification. Only *HER2* amplification was identified in SCCC. For reference, a *HER2:CEP17* signal ratio of >=2.0 was considered amplified while anything <2.0 was considered not amplified. Any *cMET* CISH with >= 5 copies was considered amplified. A *TOP2A:CEP17* signal ratio of >=2.0 was considered amplified. Finally, *EGFR* amplification was ≥ 4 copies in ≥ 40% of tumor cells.



Case Report, using biomarker/targeted therapy

52 year old female presents with a history of post-menopausal bleeding and abnormal Pap smear with endometrial cells.

- Initial diagnosis: high grade, neuroendocrine carcinoma with mixed small cell and large cell types, clinical stage IB1 (colposcopy, endometrial biopsy, cervical biopsy); follow-up PET/CT scans were negative for metastatic disease.
- Primary surgical treatment: robotic-assisted radical hysterectomy, bilateral salpingo-oophorectomy with bilateral pelvic lymph node dissection (negative margins and lymph nodes).
- Adjuvant chemo-radiation (4500 cGy in 25 fractions) concurrent with weekly cisplatin, then followed by an additional 4 cycles of adjuvant cisplatin and etoposide chemotherapy; follow-up revealed no evidence of disease by physical exam (PE) and CT scans of the chest, abdomen, and pelvis.
- Recurrence, however, was detected four months later. Biopsy confirmed neuroendocrine carcinoma.
- Prior identification of KRAS mutation (G12D) in tissue specimen from initial surgery; therefore, patient was then started on a MEK inhibitor, trametinib.
- Patient had complete response after three cycles and remained disease free for 14 months on therapy.

Conclusions

- Multiplatform tumor profiling identified high expression of TOP2A and TOPO1, which may explain the sensitivity to etoposide and topotecan.
- The high protein overexpression of drug pumps (i.e. BCRP and MRP1) highlight the difficulty in treating this disease.
- Lack of PD-L1 tumor-infiltrating lymphocytes suggests that immune-related monotherapies targeting the programmed death (PD) pathway may have limited utility in treating SCCC.
- SCCC has distinct differences from HPV+ cervical cancer, which may inform treatment options; differences include significantly higher protein expression of cKIT and PR but lower expression of ER, higher rates of *TP53* and *KRAS* mutations but lower rates of *PIK3CA* mutations.
- Although from different organs, SCCC shares more similarity to SCLC as evidenced by similar frequencies of *RB1* mutations; differences, however, include EGFR and HER2 amplification rates.
- Potential druggable mutations include *AKT1, KRAS, PIK3CA*, and *TP53*.
- Use of a targeted agent, based on patient's specific biomarker profile, may result in a positive outcome, as shown in the case study. Based on the 18% incidence of *KRAS* mutations in the population profiled, the *RAS/RAF* pathway may be an area of targeted focus.

References

1. Bellefqih S. et al, Small cell neuroendocrine carcinoma of the uterine cervix: report of six cases and a review of the literature. *Cancer Radiother.* 2014 Jun: 18(3):201-7.
2. Wright AA, et al. Oncogenic mutations in cervical cancer: genomic differences between adenocarcinomas and squamous cell carcinomas of the cervix. *Cancer*. 2013;119(21):3776-3783.
3. Atienza-Amores M. et al, Small cell carcinoma of the gynecologic tract: a multifaceted spectrum of lesions. *Gynecol Oncol.* 2014 Aug: 134(2):410-8.