Identifying therapeutic options in small cell cervical cancer by multiplatform evaluation of biomarker alterations

M. M. Frumovitz[1], J. K. Burzawa[1], R. L. Coleman[1], S. K. Reddy[2], I. Astsaturov[3], S. Z. Millis[2] and J. Brown[4]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Caris Life Sciences, Irving, TX, [3]Fox Chase Cancer Center, Philadelphia, PA, [4]The University of Texas, MD Anderson Cancer Center, Houston, TX

Abstract

Objectives: Small cell cervical cancer (SCCC) is an extremely rare and aggressive form of cervical cancer. Approximately 150 cases are diagnosed in the US each year, with few therapeutic options. We evaluated tumor samples in order to determine what percentage of patients may have targetable molecular aberrations.

Methods: Seventy-five SCCC samples were profiled, 50 using a commercial multiple platform, including a combination of gene sequencing (Sanger, next generation sequencing [NGS]), protein expression (IHC) and gene amplification (CISH or FISH), and 25 at a cancer center using a 50 gene NGS platform (CMS50). The results were compared to ~800 HPV+ cervical cancers, neuroendocrine tumors (NET) from all sites, and small cell lung cancers.

Results: TOP2A (85%), TOPO1 (55%), BCRP (91%), and MRP1 (100%) had high overexpression by IHC, while ERCC1 had low expression (11%). SCCC samples had higher protein expression of KIT (26%) than HPV+ cervical cancers (3%, p<0.05), but similar expression of KIT to small cell lung cancers (37%). HER2 amplification was identified in 4.5% of SCCC and 8% of HPV+ cervical cancers, while EGFR amplification was not seen in SCCC but was identified in 10% of HPV+ cervical cancers. Gene sequencing identified that SCCC samples had higher mutation rates for TP53 (23%) and KRAS (18%) compared to HPV+ cervical cancers (10% and 10%, respectively) but lower rates of PIK3CA (15% vs. 26%). Comparatively, small cell lung cancers had mutations in TP53 in 34% of cases and in KRAS in 5% of cases. NGS evaluation of 47 cases also identified 3 GNAS and RB1 mutations, 2 CTNNB1 and SMAD4 mutations, and single gene mutations in BRCA1, PTEN, MET, SMARCB1, APC, ATM, HNF1A, and FBXW7.

Conclusions: Multiplatform tumor profiling identified high expression of TOP2A and TOPO1 which may explain the sensitivity to etoposide and topotecan, while low levels of ERCC1 raise concern for platinum resistance. The high levels of drug resistance proteins (BCRP and MRP1) highlight the difficulty in treating these tumors. Potential druggable mutations include AKT1, KRAS, PIK3CA, and TP53.

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

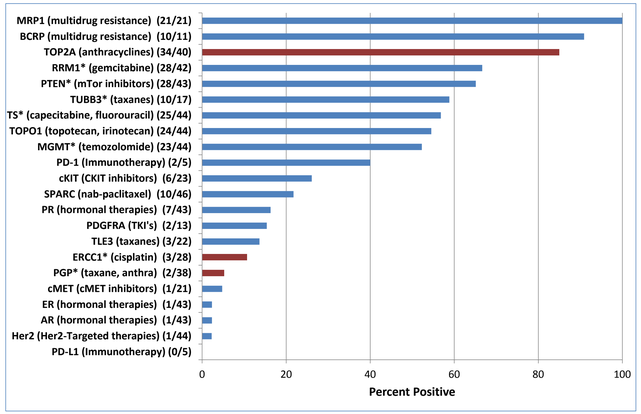
	IHC										ISH		Gene SEQUENCING					
	% Positive																	
Biomarker / Type of cancer	BCRP	cKIT	cMET	ERCC1	ER	MRP1	PR	TOP2A	TOPO1	TUBB3	EGFR	HER2	*AKT1*	*GNAS*	*KRAS*	*PIK3CA*	*RB1*	*TP53*
SCCC	91	26*	5*	11*	2.3*	100	16.3	85.0	55	59*	0.0	4.5	6	6	18	15	6	23
Cervical, HPV pos.	38	3	22	36	20	86	8	89	56	26	11	8	1	3	10	26	1	10
NET, All	58	7	22	34	22	89	11	80	61	26	7	0	1	3	7	28	2	12
Lung NET	42	37	2	17	1	84	23	48	43	82	8	0	0	0	5	3	11	34

* Indicates p<0.006 between SCCC and HPV+

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

Results, Immunohistochemistry

Figure 1. Levels of protein expression, reported as percent positive of total cases tested (PD-L1=presence of tumor infiltrating lymphocytes). Red lines indicate biomarkers associated with therapies currently used as standard of care.
*Expression of the biomarker below the threshold or loss of expression is considered predictive of response to therapy.



Results, Gene Mutations

Table 2. Gene alterations. Mutations were found in 11 of 52 genes tested (22%) across the two platforms. nt = not tested.

	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

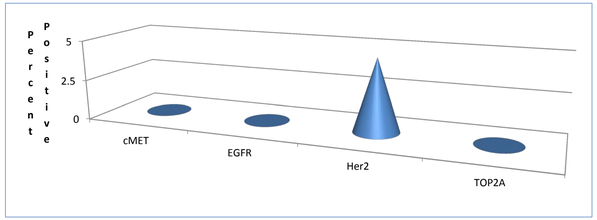
	PDGFRA	*PIK3CA*	*PTEN*	*PTPN11*	*RB1*	*RET*	*SMAD4*	*SMARCB1*	*SMO*	*SRC*	*STK11*	*TP53*	*VHL*
Caris Life Sci	(0/30)	(5/30)	(0/29)	(0/30)	(2/30)	(0/30)	(0/30)	(0/30)	(0/27)	nt	(0/30)	(7/30)	(0/28)
MD Anderson	(0/17)	(2/17)	(1/17)	(0/17)	(1/17)	(0/17)	(2/17)	(2/17)	(0/17)	(0/17)	(0/17)	(4/17)	(0/17)
Combined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

Table 3 - Specific Gene Mutations. Representative mutations with corresponding protein changes are shown.

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 (3), G12V (3)

Results, *in situ* hybridization

Figure 2. ISH (FISH or CISH) results expressed as percent cases positive for gene amplification. HER2 FISH: HER2/neu:CEP 17 signal ratio of >=2.0 is amplified and <2.0 is not amplified; 1.8-2.2 is equivocal. cMET CISH: >= 5 copies is amplified. TOP2A:CEP17 signal ratio of >=2.0 is amplified. EGFR: ≥ 4 copies in ≥ 40% of tumor cells.



Case Report, using biomarker targeted therapy

52 year old female, with postmenopausal bleeding and pap smear with endometrial cells

- Diagnosis: high grade neuroendocrine carcinoma with mixed small cell and large cell types, clinical stage IB1; PET/CT negative for metastatic disease (Colposcopy, endometrial biopsy, cervical biopsy).
- Primary treatment: robotic-assisted radical hysterectomy, bilateral salpingoophorectomy, and bilateral pelvic lymph node dissection (negative margins, lymph nodes).
- Adjuvant chemo-radiation (4500 cGy in 25 fractions) concurrent with weekly cisplatin, followed by an additional 4 cycles of adjuvant cisplatin and etoposide chemotherapy; result: no evidence of disease by physical exam and CT scan of chest, abdomen, and pelvis.
- Recurrence 4 mo later, biopsy confirmed neuroendocrine carcinoma.
- Prior identification of KRAS mutation (G12D) in tissue specimen from surgery; therefore, patient was started on an MEK inhibitor, trametinib.
- Patient had complete response after 3 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, while low levels of ERCC1 raise concern for cisplatinum resistance.
- The high levels of drug resistance proteins (BCRP and MRP1) highlight the difficulty in treating these tumors.
- Lack of PD-L1 tumor infiltrating lymphocytes suggests that immune therapies targeting the programmed death pathway may not be useful in treating small cell cervical cancers.
- SCCC has distinct differences from HPV+ cervical cancer, which may inform treatment options; differences include significantly higher expression of cKIT and PR and lower expression of ER, higher rate of *TP53* and *KRAS* mutations but lower rates of *PIK3CA* mutations.
- Although not identical, SCCC is more similar to lung NET's, including rate of cKIT, ER, and PR protein expression, and similar rate of *RB1* mutation; differences include TOPO2, *KRAS*, and *PIK3CA*.
- Potential druggable mutations include *AKT1, KRAS, PIK3CA*, and *TP53*.
- Use of a targeted agent, based on patient's specific biomarker profile may result in positive outcome, as seen in patient with identified *KRAS* mutation. Based on 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.