Comprehensive multiplatform biomarker analysis of 212 anal squamous cell carcinomas

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Abstract #3519

Background: Anal squamous cell carcinoma (ASCC or anal SCC) is a rare, HPV-associated malignancy accounting for 2% of lower digestive system cancers. Usually these malignancies are detected early and successfully managed with chemoradiation. Uncommonly, these cancers recur or present with metastases. In this setting, cisplatin and 5-fluorouracil represents the only endorsed regimen. Beyond standard therapy, few therapeutic options exist. The purpose of this study is to identify other novel, potential targets and therapeutic options for this disease, utilizing a multiplatform approach.

Methods: 212 squamous cell anal carcinoma specimens were tested via a multiplatform profiling service (Caris Life Sciences, Phoenix, AZ) consisting of gene sequencing (Sanger or next generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]) and gene amplification (CISH or FISH). Tissue from a metastatic site was submitted in 80.2% of the cases. Comparisons were made between primary and metastatic specimens. Documentation of positive HPV or HIV status was provided in six cases.

Results: IHC overexpression was noted in MRP1 (97.6%, 81/83), EGFR (89.7%, 35/39), TOPO1 (68.3%, 123/180), MGMT (67.2%, 125/186), and PTEN (46.9%, 90/192). PD-L1 expression was non-existent (0/24). EGFR and HER2 were amplified (ISH) in 7.4% (5/68) and 1.8% (2/111) of cases. High mutation rates were seen in biomarkers associated directly or indirectly with the PIK3CA/Akt pathway: PIK3CA (26.8%, 26/97), FBXW7 (11.8%, 8/68), PTEN (3.1%, 2/64), and Akt1 (1.5%, 1/68). PIK3CA exon 9 mutations represented 82% of all PIK3CA mutations. Point mutations in other genes were also identified, including a few co-occurring mutations.

Conclusion: Multiplatform tumor profiling identified several potential targets. Protein expression aberrations identified potential treatment options not routinely considered. Mutations in PIK3CA, Akt1, and FBXW7 and PTEN loss indicate potential for targeting the PI3 kinase pathway. Targeting the ErbB-family receptors, namely with anti-EGFR agents or newer generation pan-HER inhibitors, may represent another option, given EGFR and HER2 amplification as well as EGFR overexpression. Differences in anal carcinomas whose etiology is of viral origin may present different treatment options based on the driver mutations.

Results

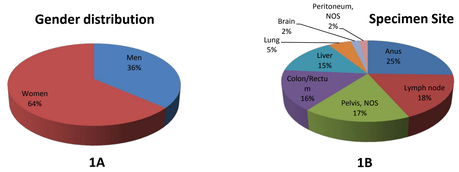
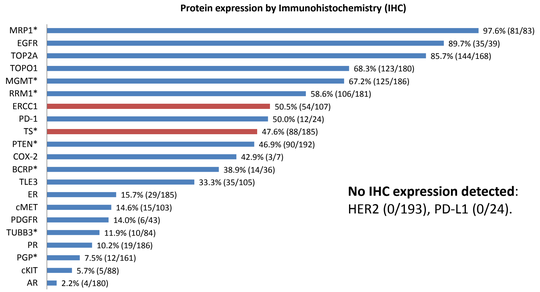


Figure 1. Distribution of cohort and patient specimens. Figure 1A (above) shows the number of men and women retrospectively analyzed. The average age was 58.9 years. Overall, 135 (63.7%) were women and 77(36.3%) were men. The age range was 31 – 89, with the mean age at 58.9 years. Figure 1B shows the specimen site of FFPE specimens. Most specimens collected involved locally advanced disease or neighboring lymph nodes in the pelvic region.

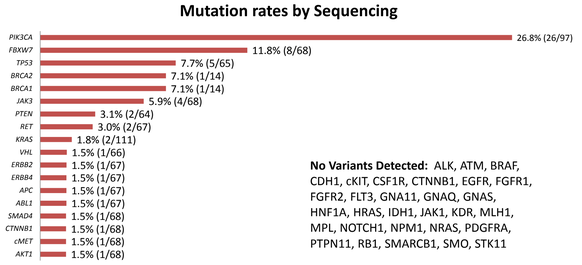


No IHC expression detected: HER2 (0/193), PD-L1 (0/24).

Biomarker	Associated Drug Class/Agent	Biomarker	Associated Drug Class/Agent	Biomarker	Associated Drug Class/Agent
AR	AR-targeted therapy (e.g. abiraterone)	ER/PR	anti-estrogen therapy (e.g. letrozole)	RRM1	nucleoside analogue (e.g. gemcitabine)
BCRP, MRP1, Pgp	various cytotoxic agents	ERCC1	platinum compounds (e.g. cisplatin)	TLE3, TUBB3	taxane (e.g. paclitaxel)
cKIT, PDGFR	kinase inhibitors (e.g. imatinib)	HER2	HER2-targeted therapy (e.g. trastuzumab)	TOP2A	anthracyclines (e.g. doxorubicin)
cMET	cMET-targeted therapy (e.g. crizotinib)	MGMT	alkylating agents (e.g. temozolomide)	TOPO1	camptothecins (e.g. irinotecan)
COX-2	COX-2 inhibitor (e.g. celecoxib)	PD-1, PD-L1	immunotherapy (e.g. pembrolizumab)	TS	pyrimidine analog (e.g. fluorouracil)
EGFR	HER-targeted therapy (e.g. cetuximab)	PTEN	mTOR inhibitors (e.g. everolimus)		

Figure 2. Distribution of protein expression by IHC. The bar chart above shows distribution of theranostic IHCs ranked from highest to lowest percentage. Bars marked in red correspond to biomarkers associated with agents on the NCCN Compendium. Biomarkers with an asterisk (*) are ones associated with treatment benefit to a specific drug and/or drug class when no or low expression is detected by IHC. For example, ERCC1 overexpression is associated with potential resistance to fluorouracil and capecitabine. The table below the bar graph lists drug and drug classes associated with that biomarker's protein expression. Absence of MGMT (32.8%, 61/186) implies a subset may derive potential benefit from alkylating drugs like temozolomide.

Results (cont.)



No Variants Detected: ALK, ATM, BRAF, CDH1, cKIT, CSF1R, CTNNB1, EGFR, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK1, KDR, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PTPN11, RB1, SMARCB1, SMO, STK11

Figure 3. Distribution of mutations by either Sanger or NGS. Several mutations were detected involving the PIK3CA/Akt pathway including *PIK3CA, PTEN*, and *AKT1*. In all, 59% (125/212) of anal squamous cell carcinomas showed dysregulation of the PIK3CA/AKT/mTOR based on IHC (i.e. PTEN) and sequencing (i.e. *AKT1, PIK3CA, PTEN*) analysis. Targeted agents along this pathway may be considered in future clinical trials design. The low incidence of *KRAS* mutations and the absence of *ras* mutations such as *HRAS* and *NRAS* is consistent with findings in the literature.

PIK3CA	Protein Change(s)	Percent
exon 9	E542K (x5), E545K (x16), E545Q (x1)	81.5%
exon 13	E726K (x1)	3.7%
exon 20	E1034Q (x1), H1047L (x1), H1047R (x2)	14.8%

Figure 4. *PIK3CA* mutations, distribution in ASCC. The figure above shows the mutations detected by either Sanger or NGS. In this ASCC cohort, *PIK3CA* exon 9 represented the most common site of mutations (81.5% of all *PIK3CA* mutations). In total, twenty-seven *PIK3CA* mutations were detected in twenty-six specimens.

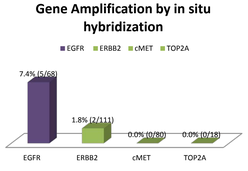


Figure 5 – Anal Cancer ISH Distribution. The bar chart on the left shows distribution in ISH (CISH and/or FISH). Higher percentages were detected in the HER-family of receptors, indicating a potential benefit to HER-targeted therapy. In addition, biomarkers showing no gene amplification such as *MET, TOPO2A*, and *ALK* indicate squamous cell carcinoma patients may not benefit from *MET*-targeted therapy (e.g. tivantinib, onartuzumab), anthracyclines, or *ALK*-targeted therapy (e.g. crizotinib) based on these ISH results alone. One specimen with *EGFR* amplification also had concurrent *ERBB2* (HER2) amplification. One other specimen had *EGFR* amplification along with a mutation in *KRAS*. Interestingly, all five specimens with *EGFR* amplification were from metastatic and not primary tumor samples.

Results (cont.)

Biomarker (Methodology)	Primary Tumor Specimens	Metastatic Specimens	p-value
ERCC1 (IHC)	70.4% (19/27)	43.8% (35/80)	0.0252
PTEN (IHC)	60.4% (32/53)	41.7% (58/139)	0.0239
TS (IHC)	63.8% (30/47)	42.0% (58/138)	0.0114

Figure 6 – Biomarker comparison of primary versus metastatic disease. The table highlights those biomarkers (and their corresponding testing methodology) where statistically significant ($p < 0.05$) differences were found between the (overall) primary versus metastatic specimens. No statistically significant differences were found in biomarkers tested by ISH or sequencing platforms (including *PIK3CA*). Although high ERCC1 and high TS (seen at higher rates in our primary specimens) indicate less benefit from cisplatin and fluoropyrimidines, we know these agents, combined with radiation, achieve high cure rates. Future studies differentiating between recurrent primaries versus untreated primaries may elucidate these findings. The high rate of PTEN loss again highlights the importance of the PIK3CA/AKT/mTOR pathway in this disease.

Conclusions

- To the best of our knowledge, this is the most comprehensive molecular profiling review of anal squamous cell carcinomas.
- Overexpression of drug pumps, especially MRP1, may explain why advanced disease is resistant to conventional cytotoxic therapy. In addition, overexpression of biomarkers like ERCC1, and TS may explain the limited benefits of platinum and fluorouracil-based therapy, respectively, in advanced stage disease. Although MGMT overexpression is high, nearly 1/3 patients lack MGMT expression, which might indicate a role for use of alkylating agents. Further, cMET overexpression (by IHC) may be relevant when considering emerging small molecule inhibitors of cMET. Caution is advised, though, given the lack of *MET* amplification.
- Our findings show novel therapies which may be considered when designing clinical trials. *EGFR* and *ERBB2* (HER2) amplification by ISH argues for using targeted therapy like trastuzumab or newer pan-HER agents in select patients. Our finding that EGFR amplification was exclusive to metastatic disease may guide patient selection when considering off-label use of targeted therapy.
- The high frequency of *PIK3CA* mutations warrants further investigation, as mutations can be targeted downstream in the PIK3CA/Akt/mTOR pathway. Although other mutations were rare, many were also targetable.
- The overall findings argue in favor of a role for comprehensive molecular profiling in advanced stage squamous cell carcinoma of the anus.

References

1. Gardini, AS, GL Frassineti, *et al.* (2014). "KRAS, BRAF, and PIK3CA status in squamous cell anal carcinoma." *PLoS ONE*. 9(3):e92071.
2. Gilbert, DC, J Summers, *et al.* (2013). "p16INK4A, p53, EGFR expression and KRAS mutation status in squamous cell cancers of the anus: correlation with outcomes following chemo-radiotherapy". *Radiother Oncol*. 109:146-151.
3. Klimant, E, M Markman, *et al.* (2013). "Management of two cases of recurrent anal carcinoma". *Case Rep Oncol*. 6:456-461.