Comprehensive multiplatform biomarker analysis of 199 anal squamous cell carcinomas

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Abstract

Background: Anal squamous cell carcinoma (ASCC) is a rare, HPV-associated malignancy accounting for 2.4% of digestive system cancers. In most cases, these malignancies are detected in the early stages and successfully managed with chemo-radiation. Uncommonly, these cancers recur or present with metastases. In this setting, cisplatin and 5-fluorouracil represent the only endorsed regimen. Once beyond standard therapy, few therapeutic options exist for those patients with aggressive disease. The purpose of this study is to identify other novel, potential targets and therapeutic options for this disease, utilizing a multiplatform approach.
Methods: In total, 199 anal squamous cell 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). 6 cases were documented as positive for HPV or HIV; status was not provided on the remaining 193.
Results: Key results are shown in the table below, as percent change/total cases.

Platform	Immunohistochemistry (IHC)								ISH		SEQ				
Biomarker	EGFR	ERCC1	MGMT	PD-1	PTEN	TOP2A	TOPO1	TUBB3	EGFR	HER2	AKT1	FBXW 7	KRAS	PIK3C A	TP53
	89	51	69	50	46	85	67	13	7	2	2	14	2	33	15
	NB: No mutations were found in 30 of 47 genes tested. Mutations in 5 genes were only seen in HPV or HIV positive cases, including ABL1,BRCA1/2,ERBB2/4.														

Conclusion: Multiplatform tumor profiling identified a low incidence of gene mutations. Protein expression aberrations identified potential treatment options not routinely considered, such as topoisomerase inhibitors and taxanes. Mutations in PIK3CA, Akt1, and FBXW7 as well as PTEN loss indicate potential for targeting the PI3 kinase pathway. Additionally, immunomodulatory agents may be a therapeutic option, based on the higher levels of PD-1. 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. It should be mentioned that differences in anal carcinomas whose etiology is of viral origin may present different treatment options based on the driver mutations.

Background

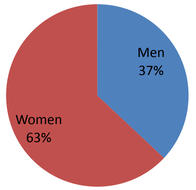
Anal squamous cell carcinoma is a rare, HPV-associated malignancy accounting for 2.4% of digestive system cancers. In most cases, these malignancies are detected in the early stages and successfully managed with chemo-radiation. Uncommonly, these cancers recur or present with metastases. In this setting, cisplatin and 5-fluorouracil represent the only endorsed regimen. Favorable case reports in the medical literature indicate cetuximab may be considered in advanced disease. Even then, little is known on what other therapies may be of clinical benefit. The purpose of this study is to identify other novel, potential targets and therapeutic options for this disease, utilizing a multiplatform approach.

Methods

In total, 199 anal squamous cell 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 (chromogenic in situ hybridization [CISH] or fluorescent in situ hybridization [FISH]). Six cases were documented as positive for HPV or HIV; status was not provided on the remaining 193.

Results

Figure 1 – Gender distribution. The pie chart shows the number of men and women retrospectively analyzed. Overall, 126 (63.3%) were women and 74 (36.7%) were men. The age range was 31 – 89, and the mean age was 58.7 years.



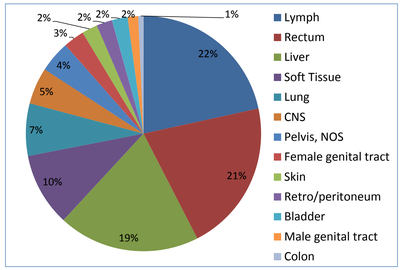


Figure 2 – Distribution of Submitted Metastatic Specimens. This pie chart shows the specimen site of FFPE samples identified as metastatic disease. Overall, at least 74% of cases had known metastatic disease based on the specimen submitted for analysis, with most metastatic specimens coming from the lymph node, rectum, or liver.

Results/Discussion

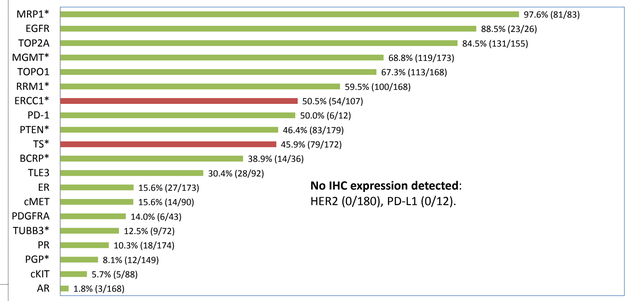


Figure 3 – Protein Over-expression in Anal Squamous Cell Carcinoma: IHC Distribution. The bar chart above shows distribution of theranostic IHCs ranked from highest to lowest percentage. Biomarkers with an asterisk (*) are ones associated with treatment benefit to a specific drug and/or drug class when no or low expression is detected by IHC. Bars marked in red correspond to biomarkers associated with agents that are on the NCCN Compendium. For reference, ERCC1 overexpression is associated with potential resistance to platinum-based therapy while TS overexpression is associated with potential resistance to fluorouracil and capecitabine. MGMT overexpression (69%) indicates a majority of anal cancers are resistant to alkylating agents. Still, a number of MGMT non-expressing anal cancers (31%) may derive benefit from agents like temozolomide. PD-1 expression but no PD-L1 expression was observed. The PD-1 expression alone may indicate that novel immune agents targeting PD-1/PD-L1 may be considered based on mechanism of action. However, further studies will be necessary to ultimately determine which patients derive the most benefit from agents like nivolumab and pembrolizumab.

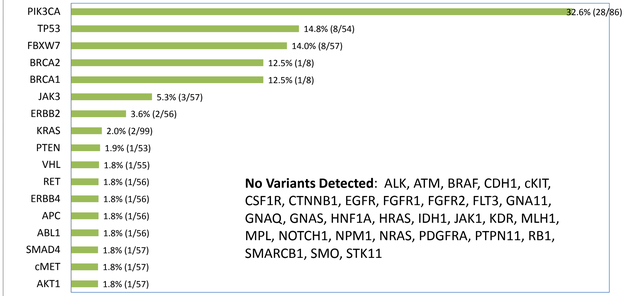


Figure 4 – Mutation Rates in Anal Squamous Cell Carcinomas. Several mutations were detected involving the PIK3CA/Akt pathway including PIK3CA, PTEN, AKT1, and FBXW7. In all, 60% of anal squamous cell carcinomas showed dysregulation of the PI3CA/AKT/mTOR based on IHC and sequencing analysis. Targeted agents along this pathway, then, may be worth considering in future clinical trials design. The presence of KRAS mutations indicates that a small group of patients receiving cetuximab-based regimen may derive no benefit, just as in colorectal cancer. Of note, the two specimens with KRAS mutations were identified with Sanger and came from the soft tissue of the mesentery and an inguinal lymph node. The absence of ras mutations such as HRAS and NRAS is consistent with findings in the literature.

Results/Discussion

Number of Co-mutations	*Number of Cases*	*Mutation Combinations*
4	1	APC + FBW7 + PIK3CA + SMAD4
2	11	ABL1 + ERBB4, BRCA1 + TP53, MET + PIK3CA, FBXW7 + PIK3CA, JAK3 + TP53, PIK3CA + RET

Figure 6 – Co-mutations in anal cancer. The table shows one specimen with four concurrent mutations, while eleven showed double mutations. All other specimens analyzed by either Sanger sequencing or NGS showed only one or no mutations.

ISH Test	Amplified	Total	Percent
EGFR	5	68	7.4%
HER2	2	99	2.0%
MET	0	69	0.0%
TOP2A	0	18	0.0%
ALK	0	3	0.0%

Figure 5 – Anal Cancer ISH Distribution. The table 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 cMET-targeted therapy (e.g. tivantinib, onartuzumab), anthracyclines, or ALK-targeted therapy (e.g. crizotinib). One specimen with EGFR amplification also had concurrent HER2 amplification. One other specimen had EGFR amplification along with a mutation in KRAS.

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 explain why the advanced-stage cancers derived less benefit from platinum and fluorouracil-based therapy, respectively. Although MGMT over-expression is high, a good percentage of patients may derive benefit form alkylating agents.
- Our results are consistent with published findings on anal squamous cell carcinomas, where the rarity of KRAS mutations allows consideration of cetuximab in conjunction standard radiation.
- Our findings show novel therapies which could be considered when designing clinical trials. EGFR and HER2 amplification by ISH argues for using targeted therapy like trastuzumab or newer pan-HER agents in select patients. 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 for comprehensive molecular profiling of this cancer in the advanced setting.

References

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