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## ABSTRACT

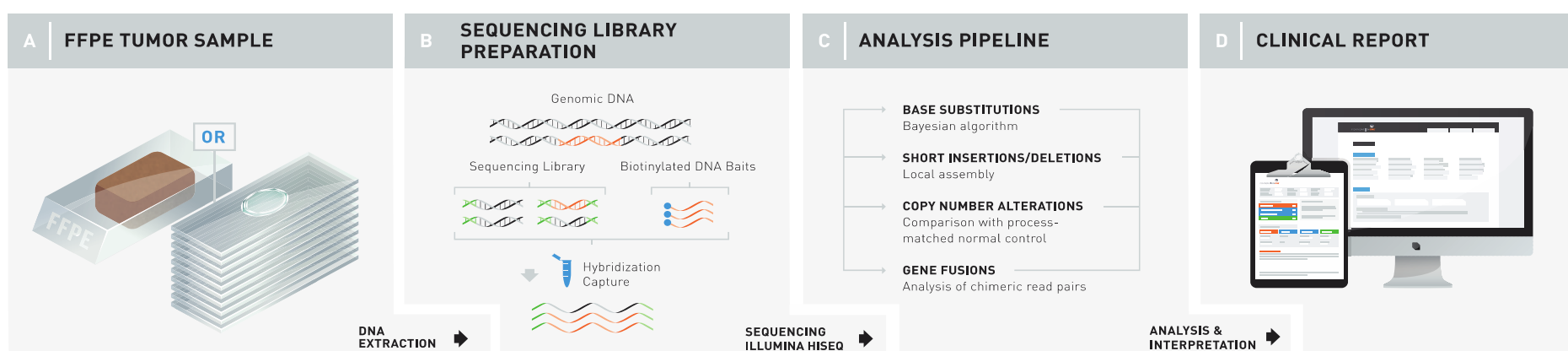
**Background:** We compared the genomic alteration (GA) profiles of metastatic squamous cell carcinoma (mSCC) of the penis (mPSCC), cervix (mCSCC), and skin (mSSCC) to study impact on the targeted and immunotherapy options for the men and women suffering from these refractory cancers.

**Methods:** 78 mPSCC, 604 mCSCC and 338 mSSCC underwent CGP using a hybrid-capture based assay. Tumor mutational burden (TMB) was determined on 0.83-1.14 megabase pairs (Mb) of sequenced DNA and reported as mutations/Mb (mut/Mb), and microsatellite instability (MSI) was determined on 114 loci.

**Results:** The HPV+/CDKN2A- status was significantly more frequent in the mCSCC than mPSCC or mSSCC (P<0.0001). The GA/tumor frequencies were similar for mCSCC and mPSCC, but significantly higher in mSSCC (P<0.0001). *TP53* mutations were more common in mSSCC (UV light exposure) and mPSCC (likely due to loss of an original HPV+ status). *TERT*, *NOTCH1* and *FAT1* GA were more frequent in mPSCC and mSSCC whereas *PIK3CA* GA were more common in mCSCC. mTOR pathway targets (GA in *STK11*, *FBXW7* and *PTEN*) were more common in mCSCC than mPSCC or mSSCC. MSI high status was extremely rare in all cases. TMB  $\geq 10/20$  mut/Mb frequencies were noteworthy in mPSCC and mCSCC but extraordinarily high in mSSCC.

**Conclusions:** Although mCSCC, mPSCC and mSSCC share a variety of clinicopathologic features, the 3 tumor types can be sharply differentiated on CGP. The *TP53*, *CDKN2A* and *HPV* status of the tumor types differ significantly with HPV+ higher in the mCSCC group. There are opportunities for targeted therapies in all groups predominantly identified in the mTOR pathway. The relatively high numbers of cases with significantly elevated TMB in all 3 tumor types suggest that immunotherapies would be beneficial in a large subset of patients.

## MATERIALS AND METHODS

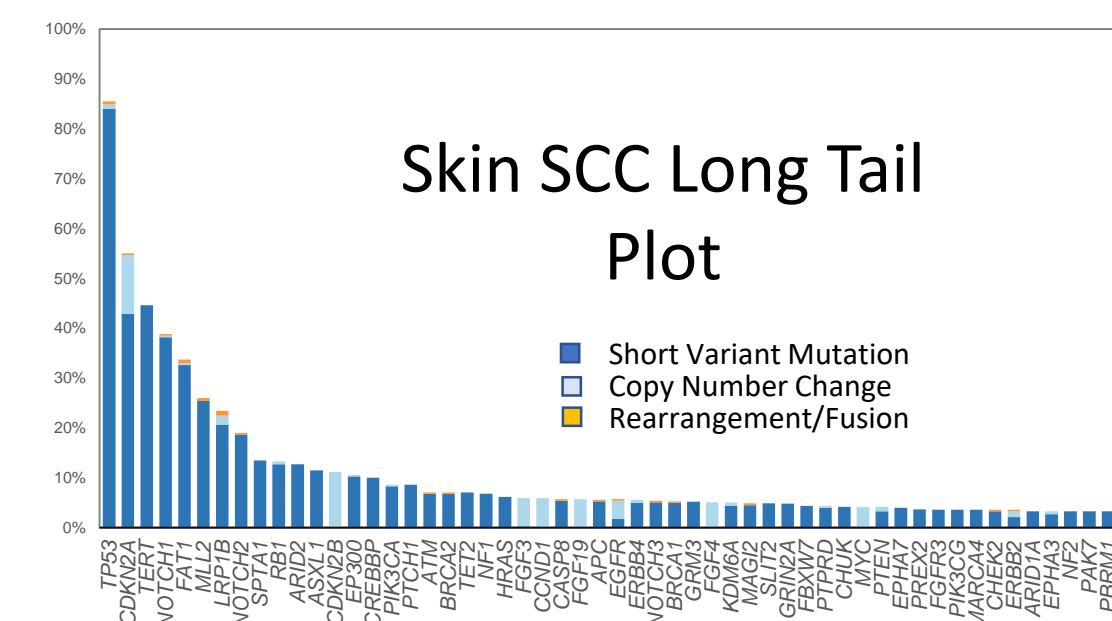
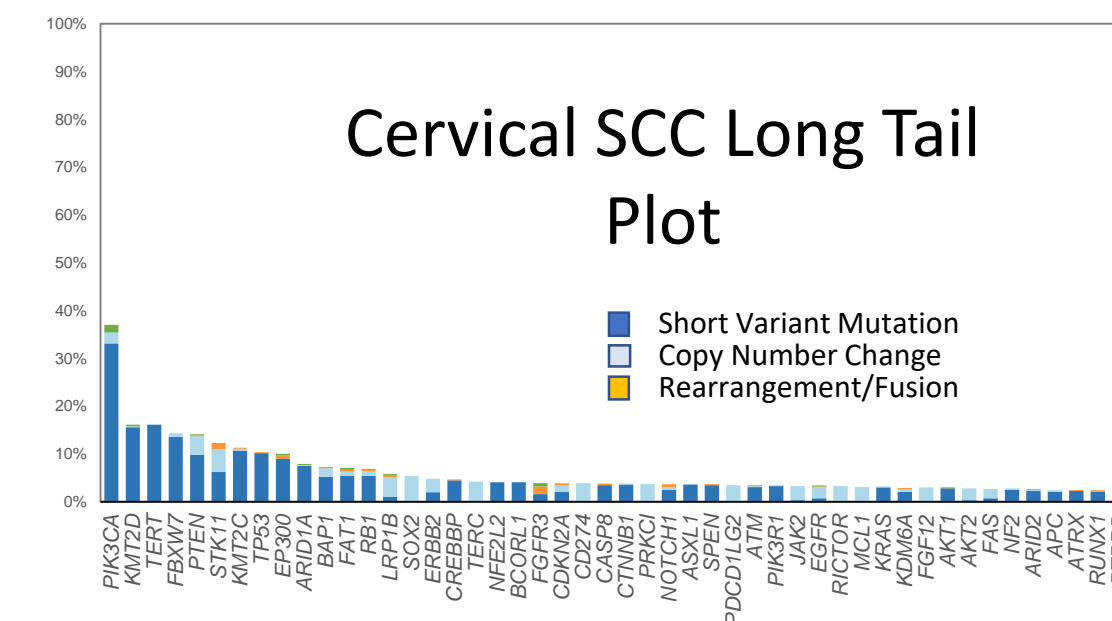
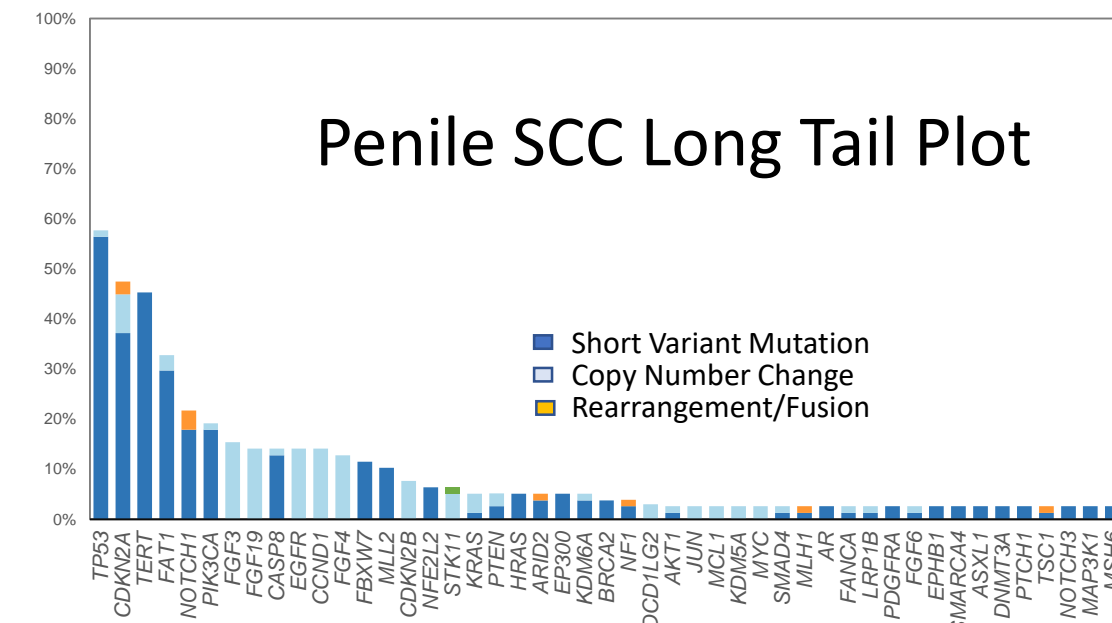


- $\geq 50$  ng DNA extracted from 40  $\mu$ m of FFPE sections
- Sequencing performed for up to 315 cancer-related genes and introns from 28 genes commonly rearranged in cancer
- Hybrid capture-based sequencing using adaptor ligation-based libraries
- Mean coverage depth >600X
- Base substitutions, insertions and deletions (short variants; SV), rearrangements, and copy number changes were assessed
- Tumor mutational burden (TMB) calculated from 0.83-1.14 Mb sequenced DNA, reported as mutations/Mb (mut/Mb), and microsatellite instability (MSI) was determined on 114 loci.

## RESULTS

- The HPV+/CDKN2A- status significantly more frequent in the mCSCC than mPSCC or mSSCC (P<0.0001)
- The GA/tumor frequencies were similar for mCSCC and mPSCC, but significantly higher in mSSCC (P<0.0001)
- *TP53* mutations were more common in mSSCC (UV light exposure) and mPSCC
- *TERT*, *NOTCH1* and *FAT1* GA were more frequent in mPSCC and mSSCC whereas *PIK3CA* GA were more common in mCSCC
- mTOR pathway targets (GA in *STK11*, *FBXW7* and *PTEN*) were more common in mCSCC than mPSCC or mSSCC
- MSI high status was extremely rare in all cases
- TMB  $\geq 10/\geq 20$  mut/Mb frequencies were noteworthy in mPSCC and mCSCC but extraordinarily high in mSSCC

	mPSCC (78 cases)	mCSCC (604 cases)	mSSCC (338 cases)
GA/case	5.8	4.9	9.6
<i>TP53</i>	58%	11%	86%
<i>CDKN2A</i>	47%	4%	55%
<i>TERT</i>	45%	16%	45%
<i>FAT1</i>	33%	7%	34%
<i>NOTCH1</i>	22%	4%	39%
<i>PTCH1</i>	3%	1%	9%
<i>PIK3CA</i>	19%	37%	9%
<i>CD274 amp</i>	2%	4%	2%
<i>PTEN</i>	5%	14%	4%
<i>FBXW7</i>	12%	14%	4%
<i>STK11</i>	6%	12%	3%
<i>MDM2</i>	1%	3%	3%
MSI-High	0%	2%	2%
TMB (median)	3.6	5.2	43.2
TMB $\geq 10/\geq 20$	18%/8%	24%/7%	71%/63%
HPV+	29%	69%	5%
<i>TP53</i> Mutated and HPV+	22%	7%	31%
<i>TP53</i> Mutated and HPV-	73%	93%	89%



## CASES

Primary site of an HPV-negative penile SCC in a 63 year old Caucasian male which progressed to metastatic disease. CGP revealed *EGFR* amplification and base substitution mutations in *TP53*, *APC*, *FAT1*, *MAP3K1* and *TERT*. In two studies, *EGFR* overexpression in SCC of the penis was prevalent with 92-100% (138/150; 18/18) of tumors testing positive. In addition, only rare *KRAS* mutations and no *BRAF* mutations were found, suggesting anti-*EGFR* targeted therapy may be appropriate for this cancer type. *EGFR* amplification is less common in other SCC's, with 10% of head and neck SCC and 7% of lung SCC reported positive. Two of 3 patients with *EGFR*-amplified SCC of the penis responded to anti-*EGFR* therapy. To date, however, the predictive capacity of *EGFR* amplification to predict responsiveness in penile SCC has not been confirmed.

Large cell keratinizing SCC of the cervix in a 50 year old African American woman which progressed to Stage IV disease. CGP revealed amplification in the *CD274* (*PD-L1*), *EGFR*, *PDCD1LG2* and *JAK2* genes. *CD274* encodes the programmed cell death ligand 1 (PD-L1), also known as B7-H1, which is a cell surface molecule important for regulating the activity of T-cells through binding to various T-cell receptors and helping tumor cells evade immune detection by natural killer cells. *PD-L1* amplification has been reported to be associated with increased expression. One genomic study of cervical cancer identified focal amplification of chromosomal region 9p24.1, including *CD274* and *PDCD1LG2*, in 21% of cases. On the basis of strong clinical evidence, *CD274* amplification and *PDL1* overexpression predict sensitivity to antibodies targeting PD-L1 or PD-1. Recent evidence has implicated *PD-L1* (*CD274*) amplification as the most predictive single biomarker predictor of immune checkpoint inhibitor efficacy.

## CONCLUSIONS

- mCSCC, mPSCC and mSSCC share clinicopathologic features
- However, the 3 tumor types can be sharply differentiated on CGP
- The *TP53*, *CDKN2A* and *HPV* status of the tumor types differ significantly with HPV+ higher in the mCSCC group
- There are opportunities for targeted therapies in all groups predominantly identified in the mTOR pathway
- The relatively high numbers of cases with significantly elevated TMB in all 3 tumor types suggest that immunotherapies would be beneficial in a large subset of these patients