Extra-Mammary Paget's Disease (EMPD) of the Skin: A Comprehensive Genomic Profiling (CGP) Study

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ABSTRACT

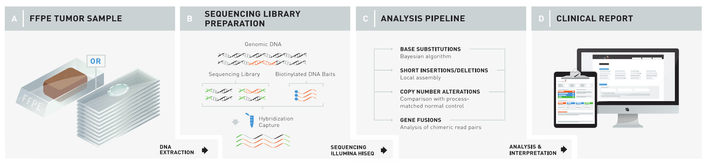
Background: EMPD is an intraepidermal adenocarcinoma which frequently progresses into invasive/metastatic disease.

Methods: 29 EMPD underwent hybrid-capture based CGP to evaluate all classes of genomic alterations (GA). Tumor mutational burden (TMB) was determined on 0.83-1.14 Mb of sequenced DNA and reported as mut/Mb. Microsatellite instability (MSI) was determined on 114 loci. PD-L1 expression was determined by IHC (Dako 22C3 antibody).

Results: All patients had locally recurrent and/or metastatic disease. The 20 (69%) male and 9 (31%) female patients had a median age of 70 years (range 45 to 101 years). The primary EMPD site, known in 90% of cases, involved the scrotum (6 cases), groin (6 cases), vulva (5 cases), anus (3 cases), perineum (2 cases), abdomen (2 cases), penis (1 case) and buttock (1 case), was used for sequencing in 15 (52%) cases and a metastatic site in 14 (48%) cases. The GA/tumor frequency was 5.0. The most frequent currently untargetable GA involved *TP53* (48%), *CDKN2A* (38%), *CDKN2B* (31%), *KMT2C* (28%), and *MYC* (14%). The most frequent potentially targetable GA were identified in *ERBB2* (35%), *ERBB3* (17%) and *PTEN* (14%) and rarely in *PDGFRA*, *CDK4/6*, *NF1/2*, and *ROS1* (fusion) all at 3%. The *ERBB2* GA included short variant (SV) activating mutations (21%), amplifications (10%) and multiple *ERBB2* GA in 3%. This finding is in contrast with mammary Paget's disease which classically features >90% of cases with *ERBB2* amplification. No EMPD cases were either MSI-High or stained positively for PD-L1 expression. The median and mean TMB were 5.2 mut/Mb and 6.3 mut/Mb, respectively; 4 (14%) had TMB ≥ 10 mut/Mb and 1 (3%) had TMB ≥ 20 mut/Mb.

Conclusion: EMPD is a rare source of relapsed/metastatic adenocarcinoma which features GA that are distinct from the more common disease originating on the nipple of the breast including a lower *ERBB2* GA frequency and lower relative frequency of *ERBB2* amplification versus SV mutations. A variety of mTOR pathway, cell cycle and kinase targets are also identified when EMPD undergoes CGP. However, the low TMB and absence of both MSI-High status and PD-L1 expression in EMPD cohort indicates a likely lack of benefit for immunotherapies for patients suffering from this rare form of malignancy.

MATERIALS AND METHODS



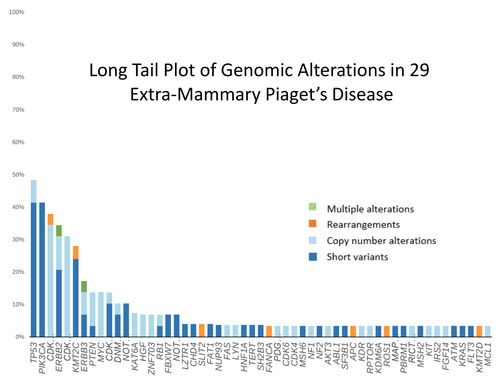
- ≥50 ng DNA extracted from 40 µm of FFPE sections
- Sequencing performed for up to 315 cancer-related genes and introns from 28 genes commonly rearranged in cancer
- Hybrid capture-based sequencing using adaptor ligation-based libraries
- Mean coverage depth >600X
- Base substitutions, insertions and deletions (short variants; SV), rearrangements, and copy number changes were assessed
- Tumor mutational burden (TMB) calculated from 0.83-1.14 Mb sequenced DNA
- PD-L1 expression was determined by IHC (Dako 22C3 antibody)

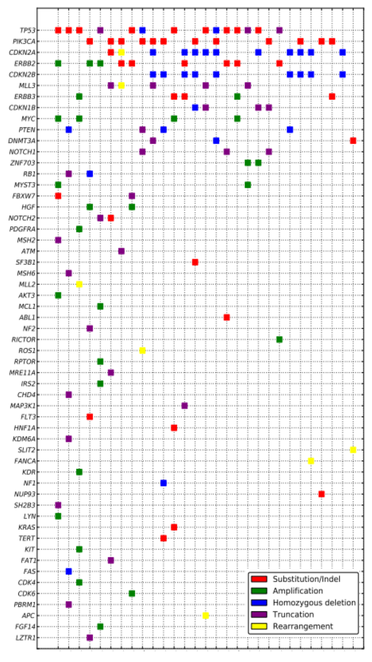
RESULTS

- All patients had locally recurrent and/or metastatic disease
- The 20 (69%) male and 9 (31%) female patients had a median age of 70 years (range 45-101)
- GA/tumor frequency was 5.0
- The most frequent currently untargetable GA involved *TP53* (48%), *CDKN2A* (38%), *CDKN2B* (31%), *KMT2C* (28%), and *MYC* (14%)
- The most frequent potentially targetable GA were identified in *ERBB2* (35%), *ERBB3* (17%) and *PTEN* (14%) and rarely in *PDGFRA*, *CDK4/6*, *NF1/2*, and *ROS1* (fusion) all at 3%
- *ERBB2* GA included short variant (SV) activating mutations (21%), amplifications (10%) and multiple *ERBB2* GA in 3%. This finding is in contrast with mammary Paget's disease which classically features >90% of cases with *ERBB2* amplification
- No EMPD cases were either MSI-High or stained positively for PD-L1 expression. The median and mean TMB were 5.2 mut/Mb and 6.3 mut/Mb, respectively; 4 (14%) had TMB ≥ 10 mut/Mb and 1 (3%) had TMB ≥ 20 mut/Mb.

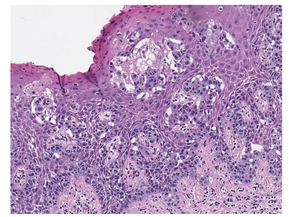
Site used for CGP	Cases
Scrotum	6
Groin	6
Vulva	5
Anus	3
Perineum	2
Abdomen	2
Penis	1
Buttock	1
Unknown	3
Primary Site	15 (52%)
Metastatic Site	14 (48)

Long Tail Plot of Genomic Alterations in 29 Extra-Mammary Piaget's Disease

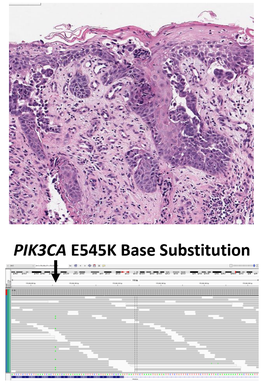




CASES



Biopsy of the vulva in a 64 year old Caucasian female showing intraepidermal adenocarcinoma characteristic of EMPD. This tumor was CK7, CEA and GATA-3 positive and CK20 and CDX2 negative. No underlying colorectal or urothelial carcinoma was detected. On CGP, this tumor was *ERBB2* amplified at 27 copies. Other alterations included amplifications of *FGF14, IRS2, MCL1* and *RICTOR*. Short variant mutations were also identified in *NOTCH2* Q2468fs*22 and *TP53* K291Q, S183*. ERBB2 amplification is widely described in Paget's Disease of the Breast where it reaches 100% of cases in some studies. In EMPD the frequencies of *ERBB2* amplification vary with the site of the disease with periumbilical location often cited as the most frequent site of "HER2 positive" EMPD followed by peri-anal and vulvar locations.



Vulvar biopsy in a 63 year old woman showing EMPD. This site of EMPD had been followed and progressed to invasive adenocarcinoma. On CGP, *ERBB2* amplification at 31 copies was detected along with *FLT3* S454L, *NF2* Q538*, *PIK3CA* E545K and *LZTR1* splice site 594-1G>C short variant mutations. The copy number revealed *HGF* amplification and *RB1* loss of exons 1-17. *PIK3CA* alterations were identified in 40% of the EMPD cases in this study and were all short variant mutations. *PIK3CA* encodes p110-alpha, which is the catalytic subunit of phosphatidylinositol 3-kinase (PI3K). The PI3K pathway is involved in cell signaling that regulates a number of critical cellular functions, including cell growth, proliferation, differentiation, motility, and survival. The E545K mutation is activating and known to be oncogenic. *PIK3CA* mutations have previously been reported in 24-31% of EMPD cases and are significantly associated with invasive tumors.

CONCLUSIONS

- EMPD is a rare source of relapsed/metastatic adenocarcinoma which features GA that are distinct from the more common disease originating on the nipple of the breast including a lower *ERBB2* GA frequency and lower relative frequency of *ERBB2* amplification versus SV mutations.
- A variety of mTOR pathway, cell cycle and kinase targets are also identified when EMPD undergoes CGP.
- Low TMB and absence of both MSI-High status and PD-L1 expression in EMPD cohort indicates a likely lack of benefit for immunotherapies for patients suffering from this rare form of malignancy.