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

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ABSTRACT

Background: EMPD is an intraductal 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 45-101). The primary EMPD site, known in 90% of cases, included the scrotum (6 cases), groin (6 cases), vulva (5 cases), axilla (3 cases), perineum (2 cases), abdomen (2 cases), penis (1 case) and buttck (1 case), was used for sequencing in 15 (52%) cases and a metastatic site in 14 (48%) cases. The GA was recurrent in 10 (31%) cases. The most frequent potentially targetable GA involved TP53 (48%), CDKN2A (38%), CDKN2B (31%), KMT2C (28%), and MYC (14%). The most frequent potentially targetable GA involved in EMPD were TP53 (35%), ERBB2 (17%) and PTEN (14%) and rarely in PDGFR, CDK4, NF1, and ROS1 (fusion) all at 1%.

EMPD included short variant (SV) activating mutations (21%), amplifications (10%) and multiple ERBB2 GA in 3%. This finding is in contrast with mammalian 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 most common adenocarcinoma 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.

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 frequently currently targetable GA involved TP53 (48%), CDKN2A (38%), CDKN2B (31%), KMT2C (28%), and MYC (14%). The most frequent potentially targetable GA involved in EMPD were TP53 (35%), ERBB2 (17%) and PTEN (14%) and rarely in PDGFR, CDK4, NF1, and ROS1 (fusion) all at 1%.
  • ERBB2 GA included short variant (SV) activating mutations (21%), amplifications (10%) and multiple ERBB2 GA in 3%. This finding is in contrast with mammalian 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.

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 CXX2 and CDX2 negative. No underlying colostral or urothelial carcinoma was detected. On CGP, this tumor was ERBB2 amplified at 27 copies. Other alterations included amplifications of FGFR4, IRF2, MLH2 and PIK3R. Short variant mutations were also identified in NOTCH2 Q2486F*,TP53 R291Q, S183*. ERBB2 amplification is widely described in Paget’s Disease of the Breast where it reaches 100% of cases in some series. In EMPD the frequencies of ERBB2 amplification vary with the site of the disease with perineal location often cited as the most frequent site of “HER2 positive” EMPD followed by penis and vulvar locations.

Vulvar biopsy in a 63 year old woman showing EMPD. This site of EMPD had been followed and progressed to invade adenocarcinoma. On CGP, ERBB2 amplification at 31 copies was detected along with FTS2 S454L, NF2 Q538*, PIK3CA E545K and LRT2R splice site S541-G+C short variant mutations. The copy number revealed HGF amplification and RPB1 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 p110alpha, 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.

MATERIALS AND METHODS

• >250 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)

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.

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Figure 1: Long Tail Plot of Genomic Alterations in 29 Extra-Mammary Paget’s Disease