original reports

Genomic Alterations as Potential Therapeutic Targets in Extramammary Paget's Disease of the Vulva

Marina Stasenko, MD[1]; Gowtham Jayakumaran, MS[1]; Renee Cowan, MD[1]; Vance Broach, MD[1]; Dennis S. Chi, MD[1]; Anthony Rossi, MD[1]; Travis J. Hollman, MD, PhD[1]; Ahmet Zehir, PhD[1]; Nadeem R. Abu-Rustum, MD[1]; and Mario M. Leitao Jr, MD[1]

abstract

PURPOSE To identify genomic alterations as potential therapeutic targets in extramammary Paget disease (EMPD) of the vulva.

METHODS We identified all patients with primary vulvar EMPD who were treated at our institution and underwent paired tumor-normal massively parallel sequencing of 410-468 cancer-related genes (MSK-IMPACT assay). EMPD of the vulva samples sequenced from 2014 to 2019 were reviewed and somatic mutations identified, with specific focus on mutations of potential therapeutic targets. Clinical data were abstracted from electronic medical records. Microsatellite instability (MSI) was assessed by MSIscore.

RESULTS Tumors of 26 patients with EMPD underwent genomic sequencing. At diagnosis, all patients had noninvasive or microinvasive (< 1 mm) disease; invasive disease eventually developed in 2 patients. Primary treatment was surgery for 19 patients (73%) and imiquimod topical therapy for 7 (27%). Seven patients had ≥ 2 surgeries as part of clinical course (1 patient had 5 vulvar resections). Samples had a median of 2 coding mutations in the genes analyzed (range, 0-29). The most common mutations were in *PIK3CA* (n = 9; 35%), *ERBB2* (4 mutations and 3 copy number alterations; 27%), and *TP53* (n = 7; 27%). MSIscore was available for 23 samples; all were microsatellite stable. After tumor genomic profiling, a patient who was initially treated with multiple resections and imiquimod was found to have a *PIK3CA* p.E542K mutation. She underwent PI3K-inhibitor treatment for 18 months before disease progression.

CONCLUSION EMPD of the vulva has a chronic and relapsing course, often requiring multiple surgical resections. Effective topical treatments are lacking. We identified targetable mutations (*PIK3CA* or *ERBB2*) in > 25% of a real-world clinical cohort. Additional prospective research implementing targetable therapies for EMPD treatment is warranted.

JCO Precis Oncol 4:1054-1060.

Extramammary Paget disease (EMPD) is a rare condition characterized by extreme pruritis and eczematoid-like lesions, most commonly of the skin of the external genitalia. EMPD of the vulva accounts for approximately 65% of EMPD. It occurs most often in White postmenopausal women and may be associated with underlying adenocarcinoma in 10%-30% of cases.[1]

The mainstay of treatment of noninvasive EMPD is surgical resection, with some patients requiring multiple resections over the course of the disease.[2] Microscopically positive margins after resection are frequent, and multiple studies have shown that disease recurrence is common regardless of margin status.[2-4] To spare patients repeated operations for recurrent disease, several nonsurgical modalities have been used to varying degrees of success. Among these, the topical immune-response modifier imiquimod, a toll-like receptor 7 (TLR7) agonist, has shown therapeutic promise as an alternative to surgery or as a perioperative adjunct.[1] Other treatment modalities, including radiation therapy and topical and systemic chemotherapy (eg, fluorouracil), have been used in inoperable disease, but their use is limited due to the lack of clinical data outside of case reports.[5] Given the frequency of recurrence and morbidity of repeated surgical excision, alternative, conservative treatment options would benefit these patients greatly.

In the search for alternatives to surgery or systemic therapy, prior studies have demonstrated that a subset of intraepithelial and invasive EMPD shows an overexpression of HER2 protein and *ERBB2* gene amplification, as well as oncogenic mutations in *PIK3CA* and *AKT1*,[6-11] which were associated with a more aggressive EMPD phenotype and poorer prognostic factors.

The goal of this study was to prospectively explore the molecular profile of primary noninvasive vulvar EMPD using massively parallel sequencing to identify

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on June 29, 2020 and published at ascopubs.org/journal/po on September 15, 2020: DOI https://doi.org/10.1200/PO.20.00073

CONTEXT

Key Objective

Extramammary Paget disease (EMPD) of the vulva is a rare, chronic disease leading to substantial morbidity. Mainstay of therapy is surgery, with patients frequently requiring multiple procedures during the course of disease. We sought to identify genomic alterations that may serve as potential therapeutic targets.

Knowledge Generated

The most common mutations noted were in *PIK3CA*, *ERBB2* (both point mutation and oncogenic gene amplification were seen), and *TP53*. All tumors were microsatellite stable. *PIK3CA* and *ERBB2* point mutations were classified as OncoKB level 3B: candidate predictive biomarkers for drug efficacy.

Relevance

These results suggest the frequent genomic alterations noted in EMPD in genes, including *PIK3CA* and *ERBB2*, may make these patients candidates for novel agents targeting these mutations.

potential therapeutic targets. We also report on a case of a patient with vulvar EMPD treated with targeted therapy on the basis of her tumor genomic mutations.

METHODS

Institutional review board approval for this retrospective analysis as well as for tumor molecular sequencing was obtained. Tumor and normal DNA were subjected to MSK-IMPACT—Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets—sequencing, which targets 410-468 cancer-related genes (the number of genes analyzed was dependent upon the time when the tumor was subjected to analysis and the version of test available).[12,13] Sequencing data analyses were performed, and mutations, copy number variations, and structural rearrangements were identified and annotated using validated bioinformatics approaches, as previously described.[14,15] These molecular alterations were further curated using OncoKB (Memorial Sloan Kettering Cancer Center, New York, NY), a precision oncology knowledge base, to identity clinically relevant cancer gene alterations.[16] For the quantification of microsatellite instability (MSI), MSIsensor was used, as previously described, and samples with an MSIsensor score $\geq$ 10 were deemed MSI-high.[14,17]

All pathology was confirmed by expert gynecologic pathologists. Electronic medical records were queried for all patients for demographics, clinical characteristics, treatment, and follow-up data. These features were integrated with the molecular findings. Research has shown that clinical outcomes are similar for patients with noninvasive and microscopically invasive (< 1 mm depth of invasion) disease, both groups were included in this report.[18]

RESULTS

Clinical Features

We identified 26 patients with EMPD of the vulva whose tumors had undergone genomic sequencing (Table 1). Median age at diagnosis was 62 years (range, 29-77 years). Seventeen patients (66%) were White, 4 (15%) were Black, and 5 (19%) were Asian. At the time of diagnosis, all patients had noninvasive or microinvasive (< 1 mm depth of invasion) tumors.

Primary treatment was surgery for 19 patients (73%) and imiquimod topical therapy for 7 (27%). Sixteen patients (62%) had recurrent disease after their initial treatment. Of these, 14 patients (87.5%) underwent a surgical resection at the time of diagnosis, whereas the other 2 (12.5%) were treated with imiquimod topical therapy. Seven patients (27%) had $\geq$ 2 surgeries as part of their clinical course, and 1 patient had undergone 5 vulvar resections.

Median time since initial diagnosis was 18.5 months (range, 3-226 months). Median follow-up time since tumor sequencing was 8 months (range, 0-26 months). Disease in 2 patients (8%) progressed to invasive adenocarcinoma, likely related to Paget disease, and both patients died of their disease.

Mutational Profiles of Vulvar EMPD

Molecular findings are summarized in Figure 1. The median coding mutation count was 2 (range, 0-29). Median allelic frequency of coding mutations in the samples ranged from 0%-32%. MSI was evaluated in 23 (88%) of the 26 samples. All 23 were microsatellite stable. Mutations were commonly seen in *PIK3CA*, *TP53*, and *ERBB2*.

Seven tumors (27%) harbored an oncogenic *TP53* mutation. Four tumors (15%) had an *ERBB2* mutation, and an additional 3 tumors (12%) had an oncogenic *ERBB2* amplification. The point mutations were all classified as OncoKB level 3B, which includes mutations that are candidate predictive biomarkers for US Food and Drug Administration–approved drugs being used off-label or in investigational agents.[16] Specific mutations that are characterized as OncoKB level 3B are summarized in Appendix Table A1.

Nine tumors (35%) harbored a *PIK3CA* mutation. The mutations were all classified as OncoKB level 3B. Of these, 3 tumors (33%) had the hotspot mutations p.E542K or

TABLE 1. Clinicopathologic Characteristics of Patients With Extramammary Paget Disease of the Vulva

Clinicopathologic Characteristic	No. (%)[a]
Median age at diagnosis, years (range)	62 (29-77)
Median time since diagnosis, months (range)	18.5 (3-226)
Median follow-up since tumor sequencing months (range)	8 (0-26)
Race	
White	17 (66)
Black	4 (15)
Asian	5 (19)
Primary treatment	
Surgical resection	19 (73)
Topical imiquimod	7 (27)
Depth of invasion, mm[b]	
< 1	24 (92)
≥ 1	2 (8)
Recurrence status	
Recurred	16 (62)
Did not recur	10 (38)
Treatment of initial recurrence (n = 16)	
Surgical resection	10 (63)
Topical imiquimod	4 (25)
Topical 5-flurouracil	1 (6)
Trial of small molecule targeting *PIK3CA*	1 (6)
Current status	
No evidence of disease[c]	10 (38)
Disease present	14 (54)
Deceased	2 (8)

[a]Unless otherwise indicated.

[b]The 2 patients with invasion ≥ 1 mm at the time of tumor sequencing were initially diagnosed with noninvasive extramammary Paget disease.

[c]No evidence of disease is defined as no clinical evidence of disease (including no obvious lesion on examination and no patient-reported symptoms).

p.E545K. All tumors harboring *PIK3CA* mutations were noninvasive, and in the time since diagnosis, only 1 patient (EMPD-04, whose tumor harbored a p.E542K mutation) had a recurrence requiring treatment.

Multiple copy number and structural variants were identified in 11 samples (69%). Most of these alterations were variants of uncertain significance, with a few predicted to be oncogenic based on OncoKB but clinically not actionable.

Targeted Treatment of Vulvar EMPD

One patient in whom an oncogenic *PIK3CA* mutation was identified elected to enroll in a phase II clinical trial for treatment with a novel agent targeting the mutation. She was initially diagnosed with vulvar EMPD at the age of 49 years and was treated with a wide local excision of the tumor. Margins were positive and there was no evidence of invasion. She then had a total vulvectomy 3 years later. Over the next 15 years, she was followed closely with multiple biopsies performed but did not require additional treatment. A symptomatic recurrence in 2015 prompted her to seek treatment, and treatment with topical imiquimod was initiated. Her treatment was limited to 7 months before she self-discontinued for substantial adverse effects and worsening disease.

The patient's tumor underwent sequencing, and a *PIK3CA* mutation, p.E542K, was identified. She enrolled in a trial of treatment with a molecule targeting the mutation and had a partial pathologic response with complete symptom resolution. Her disease was well controlled for 19 months before new symptomatic lesions led to drug discontinuation. The patient, once again, was treated with surgical resection. At 10 months since her surgery, she is experiencing minimal symptoms and has no new concerning lesions.

DISCUSSION

Noninvasive EMPD of the vulva is a chronic disease that causes substantial morbidity. The mainstay of treatment is surgical resection, with patients often requiring repeated excisions, leading to disfiguring cosmetic outcomes. Alternatives to surgery include imiquimod and, to a lesser, extent radiation therapy, photodynamic therapy, CO_2 laser, topical 5-fluorouracil, and topical bleomycin.[19] Topical 5-fluorouracil and bleomycin are highly toxic and are associated with poor response rates.[20] Localized radiation therapy can lead to complete regression in up to 80% of patients, but it is also associated with potential adverse effects.[21] Topical 5% imiquimod cream has been prospectively shown to be a feasible option for women with recurrent EMPD of the vulva, with a complete response rate of 55%-75%.[22,23] Even imiquimod, however, is associated with intolerable adverse effects that can lead to treatment discontinuation. Alternative treatments are needed for this disease.

Over the past decade, many cancers have been successfully treated with targeted therapies on the basis of tumor molecular profiling. Recent publications have reported a subset of EMPD tumors with an overexpression of HER2 protein and *ERBB2* gene amplification.[6-8] In fact, Barth et al[11] reported on a case of a patient with invasive EMPD who responded completely to single-agent trastuzumab. This patient's tumor was determined to be HER2 positive by immunohistochemistry; however, next-generation sequencing did not identify an *ERBB2* mutation or gene amplification. These findings led to an ongoing phase II trial of combining chemotherapy with trastuzumab in advanced EMPD (UMIN Clinical Trials Registry ID No. UMIN000021311). In our cohort, we identified patients with both *ERBB2* gene amplifications and mutations. The point mutations identified are all classified as OncoKB level 3B: candidate predictive biomarkers for drug efficacy. Early evidence suggests that

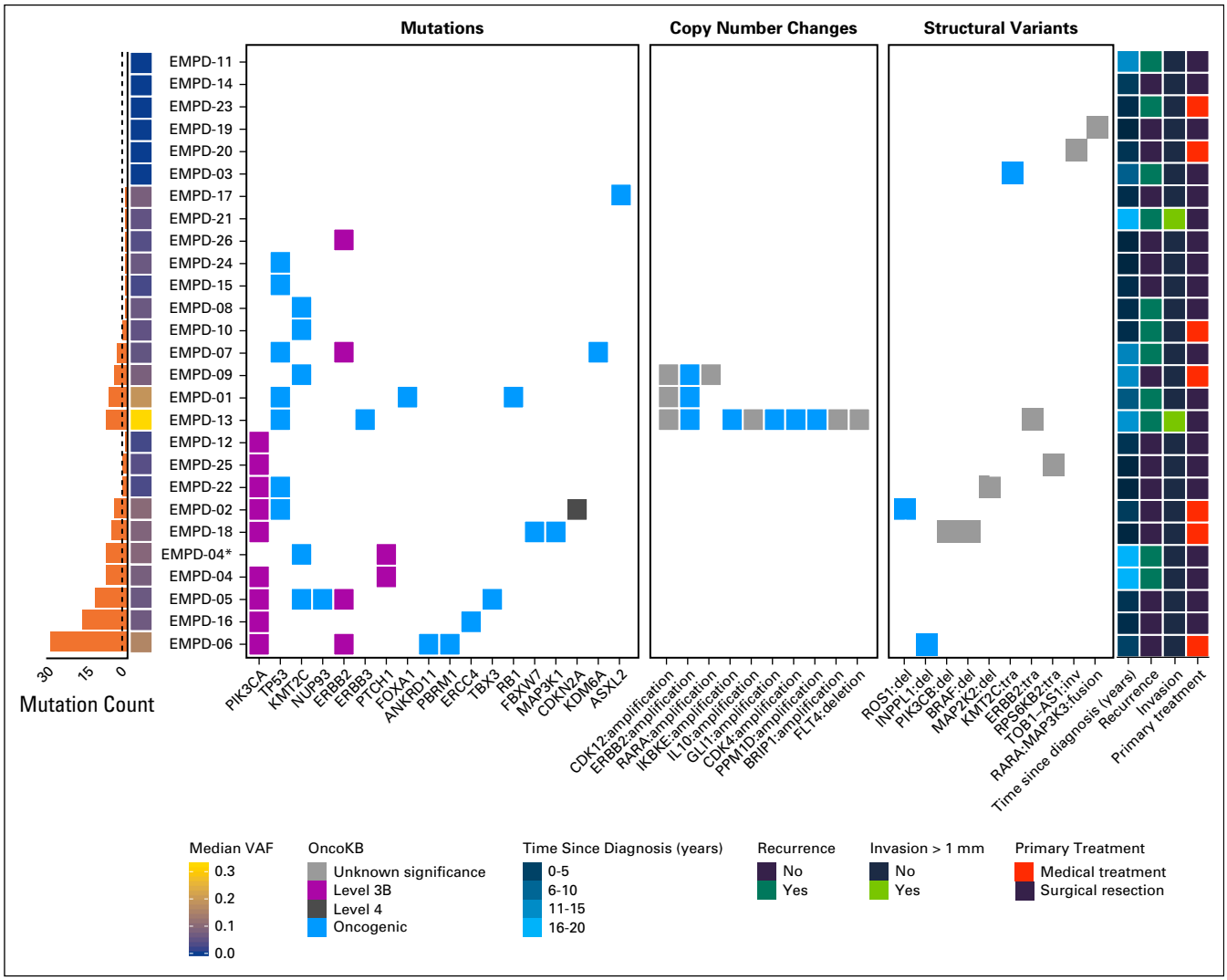

FIG 1. Vulvar extramammary Paget disease (EMPD) tumor sequencing. Tumor and normal DNA from 26 patients underwent MSK-IMPACT sequencing, targeting 410-468 cancer-related genes. All but 1 case (EMPD-21) were sequenced targeting for 468 genes. Mutations, copy number variations, and structural rearrangements were identified and annotated using validated bioinformatics approaches. These molecular alterations were further curated using OncoKB to identity putative clinically relevant cancer gene alterations. For copy number and structural rearrangement events, all variants are shown. For mutations, only those assigned an OncoKB level and/or classified as oncogenic are shown. Mutations of unknown significance are included in the mutation count presented in the bar plot on the left. The dotted line on the bar plot indicates the median mutation count of 2 across all the samples. Median variant allele frequency (VAF) in the samples ranged from 0%-32%. Relevant clinical features are mapped to the molecular results and are included on the right. (*) Metastatic sample for EMPD-04. Amp, amplification; del, deletion; inv, invasion; tra, translocation.

patients with EMPD with a somatic *ERBB2* mutation would benefit from HER2-targeting agents, similar to those with HER2 gene amplification.[24]

Another study of EMPD tumors from both male (79%) and female (21%) patients looked at mutations in 10 genes in the RAS/RAF, PI3K/AKT, and WNT pathways, and found mutant *RAS* and *RAF* genes in 19% of cases and oncogenic *PIK3CA* and *AKT1* mutations in 35% of cases.[9] Interestingly, the majority of EMPD tumors with a *PIK3CA* and *AKT1* mutation had an invasive disease phenotype. The authors postulated that activation of the PI3K/AKT pathway may be a precursor in the development of EMPD. Of note, the upregulation of the PI3K/AKT pathway is an important pathway in a multitude of cancers and may be associated with worse clinical outcomes and resistance to therapies.[25-28]

Exploring the genomic profile of EMPD in greater depth, Kiniwa et al[29] performed whole-exome sequencing on 3 tumors from patients with EMPD and identified recurrent somatic mutations in *TP53*, *PIK3CA*, and *ERBB2*.[29] This work contributes to our understanding of the molecular and genetic underpinnings of vulvar EMPD. Similar to previous reports, we identified a subset of patients with *PIK3CA* mutations and *ERBB2* mutations or amplifications.

Multiple PI3K inhibitors are in development, and here we have described a patient who received benefit from such a targeted agent. *ERBB2* inhibitors, including tyrosine kinase inhibitors and monoclonal antibodies, have demonstrated particular benefit in patients with breast cancer.[30] Although these medications are only approved for tumors with HER2 protein overexpression or *ERBB2* gene amplification, evidence suggests that patients with *ERBB2* mutation-positive cancers can benefit from *ERBB2* targeting agents.[24]

Similar to treatments aimed at tumor genetic mutations, immunotherapy drugs have become vital to the treatment of a variety of malignancies. Immunotherapy drugs are effective in DNA mismatch repair protein deficient or MSI-high tumors, and pembrolizumab, an antibody targeting anti-programmed cell death protein 1 (PD-1), has been approved by the Food and Drug Administration for use in these cancers. Previous studies have reported conflicting conclusions about programmed death-ligand 1 (PD-L1) expression in EMPD tumors or associated lymphocytes. Mauzo et al[31] noted PD-L1 expression in 14% of EMPD tumors and in 83% of the tumor-infiltrating lymphocytes. Conversely, Karpathiou et al[32] noted no expression of PD-L1 in any of the 22 EMPD tumors or associated immune infiltrates they examined. Furthermore, Tse et al[33] found that no EMPD case in their series was either MSI-high or stained positive for PD-L1.

Although there may be a subset of tumors that express PD-L1, all the tumors that underwent MSI testing in our study were microsatellite stable, and no tumor was MSI-high, likely limiting the utility of immunotherapy in these EMPD tumors. It is also notable that, to date, there is no published literature, to our knowledge, reporting on patients with EMPD responding to checkpoint inhibitors.

Our study is limited by the small number of cases included, making it difficult to draw definitive conclusions. However, vulvar EMPD is a rare disease and our data support findings of previous publications suggesting that some patients with poorly controlled disease may be able to benefit from novel, targeted agents. We believe that although noninvasive EMPD is not lethal, the severity of symptoms and the lack of effective nonsurgical treatment options warrant including patients with EMPD in basket trials evaluating drugs targeting molecular aberrations.

AFFILIATION

[1]Gynecology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY

CORRESPONDING AUTHOR

Mario M. Leitao Jr, MD, Gynecology Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065; Twitter: @leitaomd, @sloan_kettering; e-mail: leitaom@mskcc.org.

SUPPORT

This work was funded in part by the National Institutes of Health, National Cancer Institute Memorial Sloan Kettering Cancer Center (Support Grant No. P30 CA008748 [M.M.L.]).

AUTHOR CONTRIBUTIONS

Conception and design: Marina Stasenko, Renee Cowan, Nadeem R. Abu-Rustum, Mario M. Leitao Jr
Provision of study material or patients: Dennis Chi, Mario M. Leitao Jr
Collection and assembly of data: Marina Stasenko, Gowtham Jayakumaran, Dennis S. Chi, Anthony Rossi, Ahmet Zehir, Mario M. Leitao Jr
Data analysis and interpretation: Marina Stasenko, Gowtham Jayakumaran, Renee Cowan, Vance Broach, Anthony Rossi, Ahmet Zehir, Nadeem R. Abu-Rustum, Mario M. Leitao Jr
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Vance Broach
Open Payments Link: https://openpaymentsdata.cms.gov/physician/1162364

Dennis S. Chi
Leadership: C Surgeries
Stock and Other Ownership Interests: Bovie Medical, Verthermia, Intuitive Surgical, TransEnterix
Consulting or Advisory Role: Bovie Medical, Verthemia, Biom 'Up

Anthony Rossi
Honoraria: Allergan, Evolus, Biofrontera, LAM Therapeutics, Cutera, Regeneron
Research Funding: LEO Pharma (Inst), regen (Inst), Skin Cancer Foundation (Inst)
Travel, Accommodations, Expenses: Regeneron

Travis J. Hollman
Consulting or Advisory Role: Sanofi/Aventis
Research Funding: Bristol Myers Squibb

Ahmet Zehir
Honoraria: Illumina

Nadeem R. Abu-Rustum
Honoraria: Prime Oncology
Research Funding: Stryker/Novadaq (Inst), Olympus (Inst), GRAIL (Inst)
Travel, Accommodations, Expenses: Prime Oncology

Mario M. Leitao Jr
Honoraria: Intuitive Surgical
Consulting or Advisory Role: Intuitive Surgical, Johnson & Johnson/Ethicon
Research Funding: KCI
Travel, Accommodations, Expenses: Intuitive Surgical

No other potential conflicts of interest were reported.

REFERENCES

1. McDaniel B, Crane JS: Extramammary Paget disease, in StatPearls. Treasure Island, FL, StatPearls Publishing, 2019
2. Nitecki R, Davis M, Watkins JC, et al: Extramammary Paget disease of the vulva: A case series examining treatment, recurrence, and malignant transformation. Int J Gynecol Cancer 28:632-638, 2018
3. Black D, Tornos C, Soslow RA, et al: The outcomes of patients with positive margins after excision for intraepithelial Paget's disease of the vulva. Gynecol Oncol 104:547-550, 2007
4. Onaiwu C.O., Salcedo MP, Pessini SA, et al: Paget's disease of the vulva: A review of 89 cases. Gynecol Oncol Rep 19:46-49, 2016
5. Edey K.A., Allan E, Murdoch JB, et al: Interventions for the treatment of Paget's disease of the vulva. Cochrane Database Syst Rev 6:CD009245, 2019
6. Masuguchi S, Jinnin M, Fukushima S, et al: The expression of HER-2 in extramammary Paget's disease. Biosci Trends 5:151-155, 2011
7. Richter C.E., Hui P, Buza N, et al: HER-2/NEU overexpression in vulvar Paget disease: The Yale experience. J Clin Pathol 63:544-547, 2010
8. Tanaka R, Sasajima Y, Tsuda H, et al: Human epidermal growth factor receptor 2 protein overexpression and gene amplification in extramammary Paget disease. Br J Dermatol 168:1259-1266, 2013
9. Kang Z, Xu F, Zhang QA, et al: Oncogenic mutations in extramammary Paget's disease and their clinical relevance. Int J Cancer 132:824-831, 2013
10. Kang Z, Xu F, Zhang QA, et al: Correlation of DLC1 gene methylation with oncogenic PIK3CA mutations in extramammary Paget's disease. Mod Pathol 25:1160-1168, 2012
11. Barth P, Dulaimi Al-Saleem E, Edwards KW, et al: Metastatic extramammary Paget's disease of scrotum responds completely to single agent trastuzumab in a hemodialysis patient: Case report, molecular profiling and brief review of the literature. Case Rep Oncol Med 2015:895151, 2015
12. Cheng D.T., Mitchell TN, Zehir A, et al: Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn 17:251-264, 2015
13. Zehir A, Benayed R, Shah RH, et al: Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 23: 703-713, 2017 [Erratum: Nat Med 23(8):1004, 2017]
14. Smith ES, Da Cruz Paula A, Cadoo KA, et al: Endometrial cancers in BRCA1 or BRCA2 germline mutation carriers: Assessment of homologous recombination DNA repair defects. JCO Precis Oncol 3:1-11, 2019
15. Weigelt B, Bi R, Kumar R, et al: The landscape of somatic genetic alterations in breast cancers from ATM germline mutation carriers. J Natl Cancer Inst 110:1030-1034, 2018
16. Middha S, Zhang L, Nafa K, et al: Reliable pan-cancer microsatellite instability assessment by using targeted next-generation sequencing data. JCO Precis Oncol 2017:10.1200/PO.17.00084, 2017
17. Crawford D, Nimmo M, Clement PB, et al: Prognostic factors in Paget's disease of the vulva: A study of 21 cases. Int J Gynecol Pathol 18:351-359, 1999
18. Chakravarty D, Gao J, Phillips S, et al: OncoKB: A precision oncology knowledge base. JCO Precis Oncol 1:1-6, 2017
19. Wollina U: Extensive invasive extramammary Paget's disease: Surgical treatment. J Cutan Aesthet Surg 6:41-44, 2013
20. Lam C, Funaro D: Extramammary Paget's disease: Summary of current knowledge. Dermatol Clin 28:807-826, 2010
21. Son S.H., Lee JS, Kim YS, et al: The role of radiation therapy for the extramammary Paget's disease of the vulva; experience of 3 cases. Cancer Res Treat 37:365-369, 2005
22. Cowan R.A., Black DR, Hoang LN, et al: A pilot study of topical imiquimod therapy for the treatment of recurrent extramammary Paget's disease. Gynecol Oncol 142:139-143, 2016
23. Sawada M, Kato J, Yamashita T, et al: Imiquimod 5% cream as a therapeutic option for extramammary Paget's disease. J Dermatol 45:216-219, 2018
24. Bose R, Kavuri SM, Searleman AC, et al: Activating HER2 mutations in HER2 gene amplification negative breast cancer. Cancer Discov 3:224-237, 2013
25. Salvesen H.B., Carter SL, Mannelqvist M, et al: Integrated genomic profiling of endometrial carcinoma associates aggressive tumors with indicators of PI3 kinase activation. Proc Natl Acad Sci USA 106:4834-4839, 2009
26. Woenckhaus J, Steger K, Sturm K, et al: Prognostic value of PIK3CA and phosphorylated AKT expression in ovarian cancer. Virchows Arch 450:387-395, 2007
27. Ludovini V, Bianconi F, Pistola L, et al: Phosphoinositide-3-kinase catalytic alpha and KRAS mutations are important predictors of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in patients with advanced non-small cell lung cancer. J Thorac Oncol 6:707-715, 2011
28. Li SY, Rong M, Grieu F, et al: PIK3CA mutations in breast cancer are associated with poor outcome. Breast Cancer Res Treat 96:91-95, 2006
29. Kiniwa Y, Yasuda J, Saito S, et al: Identification of genetic alterations in extramammary Paget disease using whole exome analysis. J Dermatol Sci 94:229-235, 2019
30. Dean L: Trastuzumab (Herceptin) therapy and ERBB2 (HER2) genotype, in Pratt V, McLeod HL, Rubinstein WS, et al:(eds), Medical Genetics Summaries [Internet]. August 5, 2015. Bethesda, MD, National Center for Biotechnology Information, August 5, 2015
31. Mauzo SH, Tetzlaff MT, Milton DR, et al: Expression of PD-1 and PD-L1 in extramammary Paget Disease: Implications for immune-targeted therapy. Cancers (Basel) 11:E754, 2019
32. Karpathiou G, Chauleur C, Hathroubi S, et al: Expression of CD3, PD-L1 and CTLA-4 in mammary and extra-mammary Paget disease. Cancer Immunol Immunother 67:1297-1303, 2018
33. Tse, J, Elvin JA, Vergilio J, et al: Extra-mammary Paget's disease (EMPD) of the skin: A comprehensive genomic profiling (CGP) study. J Clin Oncol 37:9591, 2019 (15 suppl)

APPENDIX

TABLE A1. Genomic Alterations Identified in Paget's Disease of Vulva Tumor Samples

Sample ID	Chr	Pos	Ref	Alt	Gene	cDNAchange	AAchange	DP	AD	VF	Exon	VarClass	Variant_Caller
EMPD-05	17	37868208	C	T	*ERBB2*	c.929C>T	p.S310F	1172	24	0.02048	exon8	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-06	17	37880261	G	C	*ERBB2*	c.2305G>C	p.D769H	735	280	0.38095	exon19	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-07	17	37879658	G	A	*ERBB2*	c.2033G>A	p.R678Q	670	23	0.03433	exon17	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-26	17	37880261	G	T	*ERBB2*	c.2305G>T	p.D769Y	413	17	0.04116	exon19	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-04	12	56492623	G	A	*ERBB3*	c.2773G>A	p.E925K	1092	104	0.09524	exon23	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-05	12	56486842	G	C	*ERBB3*	c.1256G>C	p.G419A	844	69	0.08175	exon11	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-13	12	56482537	G	A	*ERBB3*	c.994G>A	p.E332K	381	156	0.40945	exon9	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-02	3	178952074	G	A	*PIK3CA*	c.3129G>A	p.M1043I	783	80	0.10217	exon21	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-04	3	178936082	G	A	*PIK3CA*	c.1624G>A	p.E542K	804	63	0.07836	exon10	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-05	3	178948136	G	A	*PIK3CA*	c.2908G>A	p.E970K	480	39	0.08125	exon20	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-06	3	178936091	G	A	*PIK3CA*	c.1633G>A	p.E545K	551	98	0.17786	exon10	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-12	3	178916854	G	A	*PIK3CA*	c.241G>A	p.E81K	1324	35	0.02644	exon2	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-16	3	178928079	G	A	*PIK3CA*	c.1357G>A	p.E453K	657	45	0.06849	exon8	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-18	3	178936091	G	A	*PIK3CA*	c.1633G>A	p.E545K	539	50	0.09276	exon10	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-22	3	178952074	G	C	*PIK3CA*	c.3129G>C	p.M1043I	496	17	0.03427	exon21	nonsynonymous_SNV	MUTECT_VARDICT
EMPD-25	3	178938934	G	A	*PIK3CA*	c.2176G>A	p.E726K	537	12	0.02235	exon14	nonsynonymous_SNV	MUTECT_VARDICT

Abbreviations: AAchange, amino acid sequence change; AD, sequence depth of variant allele at a genomic site; Alt, variant/alternate allele; Chr, chromosome; DP, overall sequence depth at a genomic site; Pos, genomic position/coordinate; Ref, reference allele; VarClass, variant classification; VF, variant allele frequency.

