

Supplemental Material

1. Supplemental Methods

Participants

We retrospectively enrolled all the 129 cases which were diagnosed as EMPD from three centers, including Peking University Cancer Hospital, Cancer Hospital of Chinese Academy of Medical Sciences, and Fujian Medical University Cancer Hospital between January 15, 2006 and July 5, 2022. Most of the patients underwent surgical treatment. We excluded patients younger than 18 years old and patients with missing essential clinicalpathological information. All the cases utilized for subsequent HER2 analysis consisted of formalin-fixed paraffin-embedded (FFPE) tissue and underwent stringent quality control. Morphological evaluation confirmed poor fixation quality of the FFPE tissue, and cases with insufficient remaining FFPE tissue for HER2 analysis are excluded from our cohort.

Detailed clinicopathological and survival data were collected for all cases. Unfortunately, the eighth edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual does not provide a TNM staging system specifically suitable for EMPD, resulting in the absence of staging information. Based on the extent of tumor invasion, intraepithelial EMPD, superficial infiltrating EMPD, and invasive EMPD are currently the most common classification to evaluate the progression of

EMPD. Intraepithelial EMPD refers to cases where the tumor is confined to the epidermis. A superficially invasive EMPD may involve tumor cells only invading the papillary dermis, while a deeply invasive EMPD may involve tumor cells invading the deeper layers of the body.

Survival information

In our study, disease-free survival (DFS) and overall survival (OS) were used to evaluate the survival time of EMPD. DFS refers to the time from the first surgical resection to local recurrence or progression, and OS refers to the time from the first surgery to death. The follow-up period was taken from the first day of treatment to September 25, 2022. The median follow-up period was 60 months (95% CI, 48-72 months).

HER2 status testing

All quality-controlled formalin-fixed paraffin-embedded (FFPE) samples were used to further analyze. HER2 immunohistochemical (IHC) staining was performed in 102 cases, of which all 44 cases from Cancer Hospital of Chinese Academy of Medical Sciences underwent fluorescence in situ hybridization (FISH), in addition, the two patients treated with DV from Peking University Cancer Hospital were also tested by FISH. A longitudinal cohort of EMPD patients with subsequent lymph node metastasis and distant metastasis was established. There were eight matched cases in which both primary and metastatic lesions were tested by IHC and seven matched cases tested by FISH, including one case with both lymph node metastasis and brain

metastasis.

HER2 IHC (VENTANA, anti HER2/neu rabbit monoclonal primary antibody) and FISH (PathVysion HER2/CEN17 FISH Probe Kit, JinLu Biotechnology Company, Shaoxing, China) were performed according to the manufacturer's instructions. HER2 IHC scoring and gene amplification evaluation were carried out according to the American Society of Clinical Oncology-College of American Pathologists (ASCO/CAP) 2018 guidelines and negative and positive quality-control was performed in every slide.

HER2 IHC staining was interpreted separately in each medical center, and then all sections were re-evaluated by CHCAMS as the central laboratory. All the pathologists who participated in the evaluation were trained in the interpretation standards and completed a ring study. If there are inconsistencies in the interpretation of certain cases, pathologists will discuss them until a consensus is reached. Furthermore, all FISH analyses of 46 cases are exclusively conducted in the central laboratory.

Statistical analysis

Correlations between HER2 status and clinicopathological parameters were evaluated using the Mann-Whitney U test or Kruskal-Wallis H test. Age is presented as the median and range. Survival analyses were performed using Kaplan-Meier estimates and Cox proportional hazards analysis [hazard ratio (HR) with 95% confidence

interval (CI). All tests were two-tailed, and significance was defined as $P < 0.05$. Data analysis was performed using SPSS v.26 (SPSS, Chicago, IL, USA), Prism 8 (GraphPad Software, San Diego, CA, USA) and R version 3.6.5 (<http://www.r-project.org/>).

2. Supplemental Results

2.1 Baseline clinicopathological characteristics of EMPD

The male-to-female ratio was 2.3:1 (90:39) and the median age was 65 (range, 40–90). The majority of cases occurred in anogenital areas (121/129, 93.8%), although rare sites such as the esophagus were included in our cohort. Intraepithelial and invasive EMPD accounted for nearly half proportions of the cases (53.5% vs 46.5%, respectively). Lymph node dissection was performed in 27.9% (36/129) of surgical cases. Around one-fifth of EMPD patients developed lymph node involvement (20.9%, 27/129), which was present in intraepithelial (4/27, 14.8%), superficially invasive (7/27, 25.9%), and deeply invasive EMPD (16/27, 59.3%, $P < 0.001$).

3. Supplemental Figures.

3.1 Figure S1. Extramammary Paget disease with HER2 expression in the primary and metastatic lesions

3.2 Figure S2. The clinical course of two patients with advanced EMPD received DV infusion.

4. Supplemental Tables.

4.1 Table S1. Baseline clinicopathological characteristics

4.2 Table S2. HER2 protein expression in extramammary Paget disease

4.3 Table S3. HER2 expression in eight matched cases of extramammary Paget disease

4.4 Table S4. Univariate analysis of overall survival and disease-free survival

4.5 Table S5. Multivariate analysis of overall survival and disease-free survival

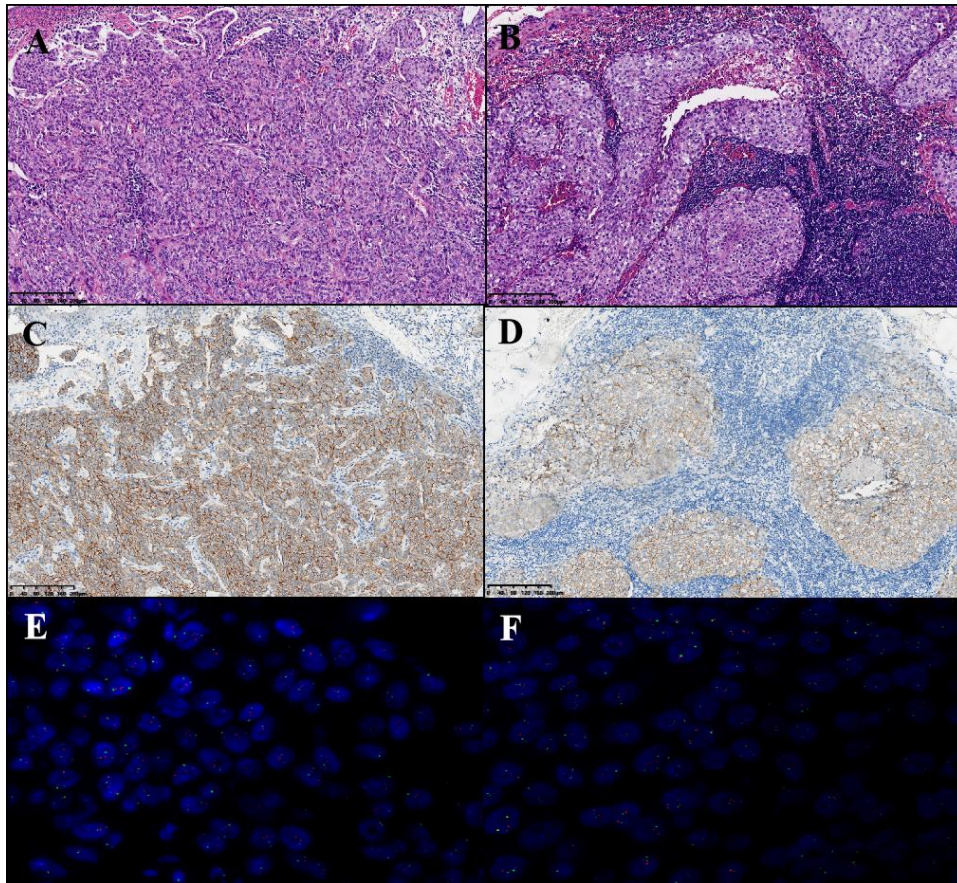


Figure S1. HER2 expression in the primary and metastatic lesions of extramammary Paget disease. Hematoxylin-Eosin staining shows similar morphological features at the scrotal tumor ($\times 100$) and metastatic lymph node (B, $\times 100$). Immunohistochemical stain of the primary tumor (C, $\times 100$) and metastatic lesion (D, $\times 100$) both shows HER2 2+ score, but with no amplification by fluorescence in situ hybridization (E-F).

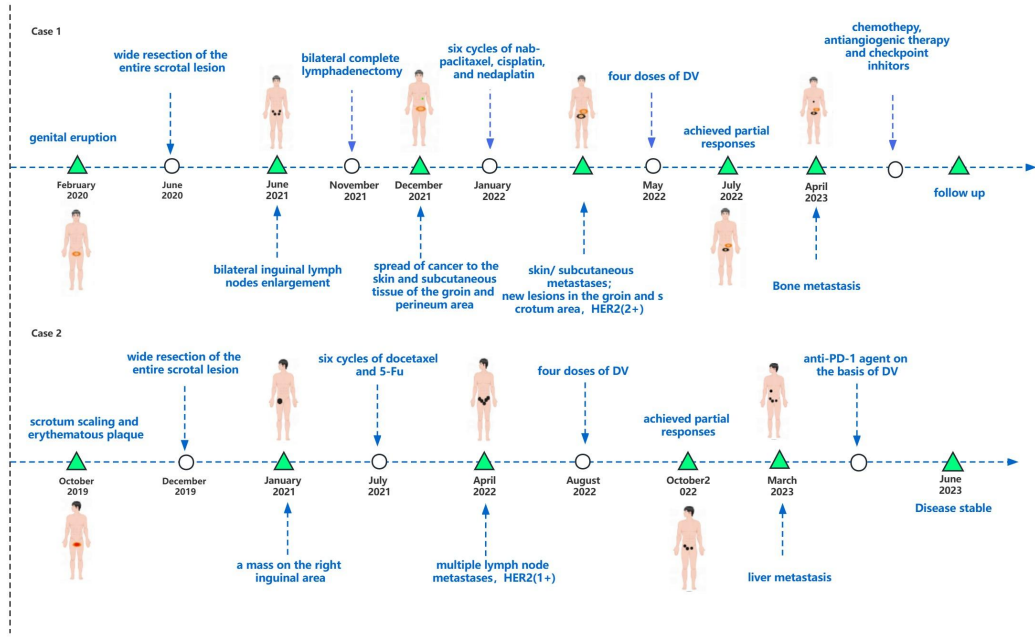


Figure S2. The clinical course of two patients with advanced EMPD received DV infusion.

Table S1. Baseline clinicopathological characteristics

Parameters	N (%)
Total(%)	129
Sex	
Male	90 (69.8)
Female	39 (30.2)
Age (years), median (range)	65 (40~90)
Location of Primary tumor	
Penoscrotal	78 (60.5)
Vulvar	35 (27.1)
Perianal	6 (4.7)
Inguinal	2 (1.5)
axillary	4 (3.1)
Other [†]	4 (3.1)
Specimens Type	
Surgical specimens	116 (89.9)
Biopsy specimens	13 (10.1)
EMPD Type	
Primary EMPD	124 (96.1)
Secondary EMPD [‡]	5 (3.9)
Pathology type	
Intraepithelial	69 (53.5)
Superficially invasive	32 (24.8)
Deeply invasive	28 (21.7)
Lesion Type	
Primary tumors	115 (89.1)
Recurrent tumors	8 (6.2)
Metastatic lesions	6 (4.7)
Vascular invasion	
Present	18 (14.0)
Absent	111 (86.0)
Lymph node dissection	
Present	31 (24.0)
Absent	98 (76.0)
Lymph node metastases	
Present	27 (20.9)
Absent	102 (79.1)
Distant metastases	
Present	8 (6.2)
Absent	121 (93.8)
Margin	
Positive	31 (24.0)
Negative	76 (58.9)
Unknown	22 (17.1)

EMPD: extramammary Paget disease.[†]Other sites included the esophagus (1), vagina(1) and thigh skin (2).[‡] Five cases of secondary EMPD all originated from colorectal.

Table S2. HER2 protein expression in extramammary Paget disease

Parameters	n (%)	HER2 n (%)				P value
		0	1+	2+	3+	
Total	102 (100.0)	7 (6.9)	48 (47.1)	38 (37.3)	9 (8.7)	
Sex						0.764
Male	69 (67.6)	6 (85.7)	30 (62.5)	26 (68.4)	7 (77.8)	
Female	33 (32.4)	1 (14.3)	18 (37.5)	12 (31.6)	2 (22.2)	
Age (years), median (range)	64 (40~90)	73 (50~90)	65 (40~81)	62 (47~88)	63 (46~82)	0.439
Location						0.915
Peroscrotal	54 (52.9)	3 (42.9)	26 (54.3)	20 (52.6)	5 (55.6)	
Vulvar	29 (28.4)	1 (14.3)	16 (33.3)	10 (26.3)	2 (22.2)	
Perianal	5 (4.9)	2 (28.6)	2 (4.2)	1 (2.6)	0 (0.0)	
Inguinal	2 (2.0)	0 (0.0)	1 (2.0)	1 (2.6)	0 (0.0)	
Axillary	4 (3.9)	1 (14.2)	1 (2.0)	1 (2.6)	1 (11.1)	
Other	8 (7.9)	0 (0.0)	2 (4.2)	5 (13.3)	1 (11.1)	
EMPD Type						0.226
Primary EMPD	98 (96.1)	6 (85.7)	46 (95.8)	37 (97.4)	9 (100.0)	
Secondary EMPD	4 (3.9)	1 (14.3)	2 (4.2)	1 (2.6)	0 (0.0)	
Pathology type						0.717
Intraepithelial	54 (52.9)	3 (42.9)	29 (60.4)	17 (44.7)	5 (55.6)	
Deeply invasive	23 (22.5)	3 (42.9)	6 (12.5)	13 (34.2)	1 (11.1)	
Superficially invasive	25 (24.6)	1 (14.2)	13 (27.1)	8 (21.1)	3 (33.3)	
Lesion Type						0.792
primary tumors	90 (81.1)	5 (71.4)	44 (84.6)	32 (76.2)	9 (90.0)	
Recurrent tumors	7 (6.3)	2 (28.6)	2 (3.8)	3 (7.1)	0 (0.0)	
Metastatic lesions [‡]	14(12.6)	0 (0.0)	6 (11.6)	7 (16.7)	1 (10.0)	
Lymph-node metastases						0.064
Present	21 (20.6)	0 (0.0)	7 (14.6)	13 (34.2)	1 (11.1)	
Absent	81 (79.4)	7 (100.0)	41 (85.4)	25 (65.8)	8 (88.9)	
Distant metastases						0.919
Present	8 (7.8)	0 (0.0)	5 (10.4)	2 (5.3)	1 (11.1)	
Absent	94 (92.2)	7 (100.0)	43 (89.6)	36 (94.7)	8 (88.9)	
Margin						0.388
Positive	28 (27.5)	5 (71.4)	12 (25.0)	8 (21.1)	3 (33.3)	
Negative	60 (58.8)	2 (28.6)	28 (58.3)	24 (63.2)	6 (66.7)	
Unknown	14 (13.7)	0 (0.0)	8 (16.7)	6 (15.7)	0 (0.0)	

[†]Other sites include 3 primary tumor and 5 metastatic lesions derived from scrotal tumors. [‡]HER2 testing was performed in eight cases with both primary and metastatic lesions, including one case with both lymph node metastasis and brain metastasis. Five individual metastases and nine matched metastases constituted all metastatic lesions. EMPD: extramammary Paget disease.

Table S3. HER2 expression in eight matched cases of extramammary Paget disease

Patient Case No.	Sex	Age	Primary tumor location	Diagnosis	IHC	FISH	Metastatic lesions location	Metastatic lesion type	IHC	FISH
P1	F	63	Vulvar	Deeply invasive	1+	N	Inguinal lymph nodes	Simultaneous	1+	N
P2	M	68	Penoscrotal	Superficially invasive	1+	N	Inguinal lymph nodes	Simultaneous	1+	N
P3	M	60	Penoscrotal	Deeply invasive	2+	N	Inguinal lymph nodes	Simultaneous	2+	N
P4	M	64	Penoscrotal	Intraepithelial	1+	N	Inguinal/Pelvic lymph nodes	Simultaneous	2+	N
P5	M	65	Penoscrotal	Superficially invasive	2+	N	Inguinal/Pelvic lymph nodes	Simultaneous	2+	N
P6	M	67	Penoscrotal	Superficially invasive	1+	N	Inguinal lymph nodes; brain	Simultaneous; Heterochronic	1+;1+	N
P7	M	69	Penoscrotal	Superficially invasive	3+	P	Inguinal lymph nodes	Heterochronic	3+	P
P8	M	56	skin of thigh	Deeply invasive	2+	/	Inguinal lymph nodes	Heterochronic	2+	/

Abbreviation: IHC, immunochemistry; FISH, fluorescence in situ hybridization; N, negative; P, positive.

Table S4. Univariate analysis of overall survival and disease-free survival

Variables	DFS		OS	
	HR (95% CI)	P value	HR (95% CI)	P value
Sex (Male vs Female)	0.412 (0.194-0.875)	0.021	0.750 (0.326-1.722)	0.497
Age	1.020 (0.992-1.050)	0.164	1.025 (0.989-1.062)	0.170
Location		0.164		0.472
Penoscrotal vs Vulvar	0.504 (0.234-1.083)	0.079	0.967 (0.412-2.269)	0.938
Penoscrotal vs Other	1.174 (0.545-2.526)	0.682	1.731 (0.695-4.309)	0.239
EMPD Type (Primary vs Secondary)	1.668 (0.402-6.925)	0.481	3.118 (0.727-13.364)	0.126
Pathology type		<0.001		0.013
Intraepithelial vs Deeply invasive	3.912 (2.139-7.157)	<0.001	2.977 (1.386-6.396)	0.005
Intraepithelial vs Superficially invasive	0.874 (0.406-1.880)	0.730	1.003 (0.416-2.418)	0.995
Vascular invasion (No vs Yes)	6.242 (3.391-11.49)	<0.001	2.485 (1.067-5.790)	0.035
Lymph node dissection (No vs Yes)	1.919 (1.097-3.359)	0.022	1.440 (0.686-3.026)	0.335
Lymph-node metastases (No vs Yes)	6.566 (3.611-11.940)	<0.001	3.776 (1.769-8.062)	0.001
Distant metastases (No vs Yes)	5.707 (2.623-12.419)	<0.001	5.187 (1.470-18.302)	0.011
HER2 status (zero vs expression)	1.740 (0.849-3.565)	0.130	1.392 (0.516-3.754)	0.514
Margin (Negative vs Positive)	0.790 (0.317-1.968)	0.612	0.757 (0.206-2.788)	0.676

Abbreviation: DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

Table S5. Multivariate analysis of overall survival and disease-free survival

Variables	DFS		OS	
	HR (95% CI)	P value	HR (95% CI)	P value
Sex (Male vs Female)	0.505 (0.234-1.089)	0.082	0.901 (0.385-2.111)	0.811
Pathology type (Intraepithelial vs invasive)	0.726 (0.374-1.552)	0.454	0.900 (0.380-2.128)	0.81
Lymph-node metastases (No vs Yes)	5.073 (2.226-11.557)	<0.001	2.701 (1.014-7.196)	0.047
Distant metastases (No vs Yes)	2.046 (0.776-5.394)	0.148	2.835 (0.663-12.122)	0.16
Vascular invasion (No vs Yes)	1.592 (0.668-3.794)	0.294	1.885 (0.662-5.369)	0.235

Abbreviation: DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.