Paget's disease: biomarkers differences between extra-mammary and mammary diseases

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Background

Extra-mammary Paget's disease (E-MPD) is a rare primary cutaneous carcinoma of uncertain etiology (apocrine, Toker cell, anogenital mammary-like glands) commonly arising in the vulva, while mammary Paget's disease (MPD) is a frequent manifestation of intra-epidermal dissemination of an underlying invasive breast carcinoma. The post-surgical recurrence rate in E-MPD is 20–40%, and metastatic E-MPD has a poor survival rate. While there is no standard systemic treatment, cases of E-MPD with amplified ERBB2/HER2 have been successfully treated with trastuzumab, alone or in combinations. However, HER2 amplification is overall rare in E-MPD, and most cases are triple (ER/PR/HER2) negative E-MPD.

Methods

12 patients (14 samples) with vulvar E-MPD and 10 patients with MPD (areolar) Paget's disease were available for a comprehensive molecular profiling at Caris Life Sciences (Phoenix, AZ).

Lesions were examined for mutations using NGS DNA sequencing (Illumina NextSeq) on 592 genes, gene copy number amplification (using NGS or *in situ* hybridization/ISH), protein expression (immunohistochemistry/IHC), and fusion events (NGS RNA sequencing, Archer FusionPlex) on 54 genes.

Tumor mutational burden (TMB) was calculated based on the total number of somatic non-synonymous missense mutations identified per megabase of genome coding area. Threshold for TMB-high was set at ≥10. Microsatellite (MS) instability was evaluated on over 7,000 known MSI loci in target regions. The threshold to determine MSI by NGS was determined to be 46 or more loci with insertions or deletions.

Biomarker results were compared between cohorts using Barnard's Unconditional Test.

Paget's Disease Patient Demographics

	Extra-mammary (n=12)	Mammary (n=10)
Age: Median (Mean)	62 (59)	66 (62)
Sample Site		
Local	N=11	N=10
Metastatic	N=3	N/A

Results

Table 1 – Summary of a comprehensive molecular profiling of Paget's Disease. *The observed differences between mammary and extra-mammary Paget's disease were not significantly different; bold – potential actionable (black) targets or response resistance (red).*

Biomarker Test	E-MPD	Mammary
ISH_TOP2A	**3/8**	**1/6**
IHC_TOP2A	**8/13**	**3/4**
CNV_PIK3CA	1/8	0/5
CNV_MDM2	0/8	1/5
CNV_MYC	1/9	0/4
CNV_ MLLT6	0/9	2/5
CNV_DDX5	0/9	1/5
CNV_ HMGA2	0/9	1/5
Next Gen SEQ_ BCOR	1/8	0/4
Next Gen SEQ_BRCA1	1/10	0/6
Next Gen SEQ_MUTYH	1/9	0/5
Next Gen SEQ_PIK3CA	**3/10**	**4/7**
Next Gen SEQ_RB1	1/10	0/7
Next Gen SEQ_TP53	3/7	1/6
Next Gen SEQ_SETD2	**2/9**	**0/5**
Next Gen SEQ_CDK12	0/9	1/5
Next Gen SEQ_CHEK2	0/9	1/5
Fusion_ARv7	**1/5**	**1/4**

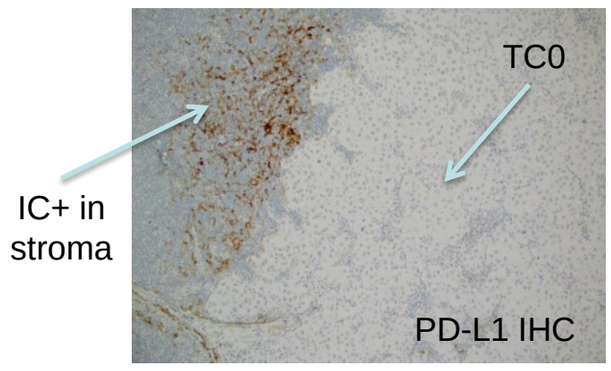

Figure 2 – PD-L1 in Paget's Disease. No cases of E-MPD expressed PD-L1 (SP142, Ventana) on tumor cells (TC0), but 2 cases had strong staining of immune cells (IC+), especially at the tumor-stromal interface. For other I-O biomarkers, TMB in E-MPD varied from 3-12 Mut/Mb (mean = 8); 3 cases were **TMB-high (>10 Mut/Mb).** No patients had MSI-H. One E-MPD patient (PD-L1: IC+, TMB-H) was treated with anti-PD1 therapy: stable disease (6months) followed by disease progression. Current therapy: Herceptin (HER2 amplified) and Docetaxel.

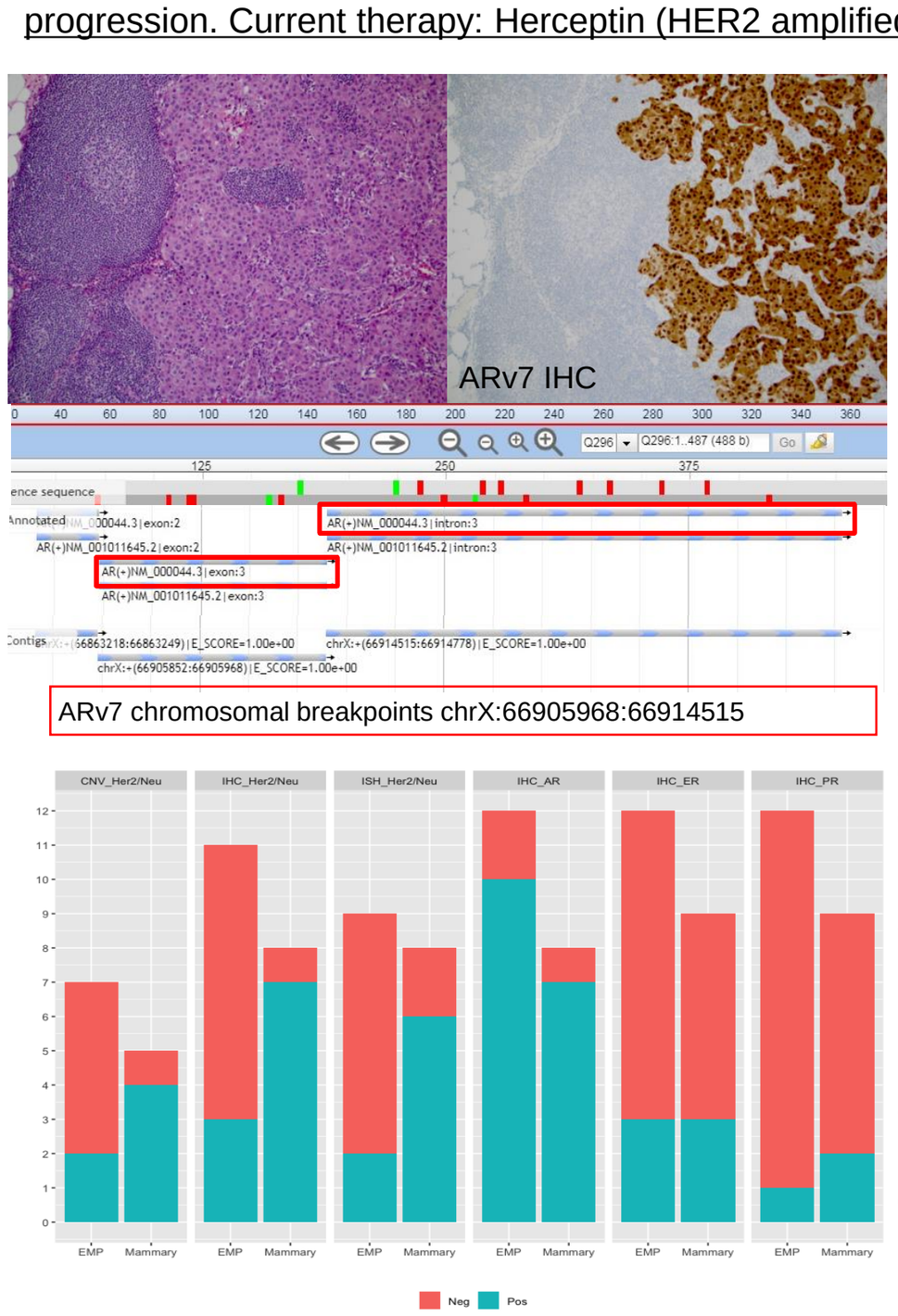

Figure 3 – Potential Novel Therapy Targets in Paget's Disease.

- Androgen receptor (AR) was frequently positive (>10%) in both cohorts (10/12 E-MPD and 7/8 MPD).

- **Androgen receptor variant transcript ARv7** was detected in one case each of E-MPD (1/5) and MPD (1/4) by RNA-seq. Both cases showed positive (nuclear) staining of ARv7 (ab198394).

- HER2 was positive (3+ IHC) and amplified (HER2/CEP17 ratio >2.0) at a significantly higher frequency in MPD (IHC 7/8, ISH 6/8) than in E-MPD (IHC 3/11, ISH 2/9) (p=0.011; p=0.049).

- **ER and PR** were rarely positive (>10%) in E-MPD (3/12 and 1/12, respectively).

Conclusions

Extra-mammary Paget's disease shows a distinct molecular profile from mammary Paget's disease, including lower HER2 over-expression (27% v. 88%) and amplification (22% v. 75%), but higher TOP2A amplification (38% v. 17%) .

Over-expression of AR in triple (Her2/ER/PR) negative E-MPD (n=5/6) also suggests a role for androgen inhibitor therapy, analogous to its use in triple negative breast cancer and prostate cancer.

Special note should be taken as ARv7 transcripts, resistance mechanism to AR-targeted therapies, are possible in this setting.