Sequential somatic mutations upon secondary anti-HER2 treatment resistance in metastatic ERBB2<sup>S310F</sup> mutated extramammary Paget’s disease

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ABSTRACT

Metastatic extramammary Paget’s disease is a rare adenocarcinoma with poor prognosis. Several reports of human epidermal growth factor receptor 2 alterations point to its pathogenic role in the disease. However, the occurrence of treatment resistance to anti-HER2 therapy demand the need for further knowledge. We report of a patient with metastatic penoscrotal extramammary Paget’s disease, with an ERBB2<sup>S310F</sup> mutation, in which near complete response was achieved upon treatment with trastuzumab and carboplatin. However, after 10 cycles of trastuzumab and carboplatin, widespread metastasis re-occurred. Analysis of a newly developing metastasis revealed additional genomic alterations including ERBB3<sup>A232V</sup> and PIK3CA<sup>G106V</sup> point mutations as well as MET and CDK6 amplification, providing a potential mechanism of acquired treatment resistance. Therefore, ERBB family inhibitor afatinib was initiated. Unfortunately, the patient succumbed to disease-related complications shortly after treatment initiation. This is the first report of ERBB2<sup>S310F</sup> mutated, metastatic extramammary Paget’s disease with secondary resistance to trastuzumab / carboplatin, potentially due to additional acquired genomic alterations. This case contributes to the growing evidence of HER2 in the pathogenesis of metastatic extramammary Paget’s disease and emphasizes the importance of repetitive, genomic analysis in rare diseases.

INTRODUCTION

Extramammary Paget’s disease (EMPD) is a rare intraepithelial adenocarcinoma, localized in apocrine gland-rich sites, most commonly perianal, vulvar and penoscrotal. The clinical presentation is discreet and eczematous in nature, often causing a delay in diagnosis. Most cases of EMPD are in-situ and have an excellent prognosis. In 5-25%, dermal invasion is visible, correlating with a poor prognosis. Due to the rarity of the disease, evidence-based management in both in-situ and metastatic disease is scarce. In the past, various combinatorial chemotherapeutic regimens were applied, with limited success. Recently, growing evidence suggests a central role of human epidermal growth factor receptor (HER) 2 in the disease. Yet incomplete responsiveness and the occurrence of treatment resistance to anti-HER2 treatment frequently occurs.
RESULTS

We report on an 80-year-old patient in excellent general condition (ECOG 0), who was referred to our clinic for further treatment of a chronic scrotal eczema. Upon physical examination, erythematous plaque with multiple nodules affecting the skin of the scrotum was seen, along with prominent inguinal lymphadenopathy (Figure 1A). Histologic analysis showed an ulcerated and invasive adenocarcinoma with Paget cells, consistent with the diagnosis of extramammary Paget’s disease. Furthermore, enlarged and metastatic active inguinal, iliacal and interaortocaval lymph-nodes were detectable on PET/CT-imaging (Figure 2A). Indeed, fine needle aspiration (FNA) confirmed the presence of lymph node metastasis. Fluorescence in situ (FISH) for HER2 amplification was negative and immune-histochemical staining for HER2 was 2+, classifying the patient as HER2 negative, according to current ASCO/CAP Guidelines [1]. Urological and gastroenterological examination did not reveal underlying malignancy of the prostate or gastrointestinal tract, respectively. After interdisciplinary discussion, the patient was opted for radiotherapy of the penoscrotal region and metastatic active lymph nodes (cumulative 60Gy), which was well tolerated.

6 months into follow-up, scrotal inflammation re-occurred (Figure 1B). Multiple mapping skin biopsies revealed apocrine adenocarcinoma in situ. PET/CT imaging showed new metabolic activity of the thoracic and retrocrural lymph-nodes, and a metabolically active nodulous in the superior right lobe of the lung (Figure 2B). FNA of the pulmonal nodule confirmed the presence of pulmonary metastasis. In order to identify targetable molecular alterations, next-generation sequencing (Oncomine Focus Assay) from a newly developing and metabolic active hilar lymph node metastasis was performed. Apart from the known ERBB2G2036R mutation, additional genomic alterations were identified, including ERBB3G422V and PIK3CAH1047R point mutations, and amplification of CDK6. In order to identify further potential treatable targets, Foundation One analysis was performed, revealing additional, equivocal MET amplification. Given the fact, that ERRB3 mutations have been associated with resistance to ERBB2 targeted treatment strategies, trastuzumab / carboplatin was discontinued and afatinib, an ERRB family Inhibitor, was initiated [3]. Unfortunately, 8 days after initiation of afatinib, the patient died of community acquired pneumonia.

DISCUSSION

Advances have been made in the treatment of metastatic extramammary Paget’s disease. The identification of ERBB2 amplifications and somatic mutations in EMPD has enabled disease specific, targeted treatment [4]. Indeed, 15-80% of all EMPD patients show immune-histochemical HER2 positivity, associated with a biologically aggressive phenotype [5]. Accordingly, anecdotal anti-HER2 treatment has significantly improved the outcome of metastasized EMPD, emphasizing its pathogenic role the disease. However, incomplete responsiveness and the occurrence of treatment resistance

Figure 1: Therapeutic efficacy assessed by clinical penoscrotal examination. Imaging of the penoscrotal region (A) at the initial presentation, (B) following recurrent disease after radiation therapy (C) after lapatinib treatment (D) best response during trastuzumab and carboplatin treatment, and (E) disease progression after 10 cycles of trastuzumab and carboplatin.
to anti-HER2 therapies demand the need for further knowledge.

The tyrosine receptor kinase HER2 causes increased MAPK/ERK and PI3K/mTOR pathway signaling, accelerating cell growth and survival. Historically, HER2 status is assessed by immuno-histochemistry (IHC) and fluorescence in-situ hybridization (FISH) for the detection of overexpression and amplification, respectively. However, nonamplified, activating ERBB2-mutations are not detected by IHC / FISH. Furthermore, only 30% of all ERBB2 alterations are amplifications, and nearly 2% of all tumors carry ERBB2 mutations [4, 6]. The activating S310F point mutation is the most common somatic mutation in ERBB2 and has been successfully targeted in EMPD [6, 7]. Given the similar beneficial response to anti-HER2 treatment in amplified and non-amplified ERBB2 alterations, patients with a potential benefit to anti-HER2 treatment may not be identified using IHC / FISH.

Here we report on the clinical efficacy of trastuzumab / carboplatin in an 80-year-old male patient with metastatic penoscrotal EMPD, harboring a somatic ERBB2 S310F mutation, with primary resistance to lapatinib. Upon disease progression, sequential genetic profiling revealed additional somatic point mutations in ERBB3 (p.A232V) and PIK3CA (p.G106V) as well as MET and CDK6 amplification. ERBB3 A232V is a missense, hotspot mutation within the extracellular domain, causing anchorage-independent growth and signaling when HER2 kinase activity is present [3]. Accordingly, in the presence of ERBB2 S310F, additional somatic MET and CDK6 may have caused a compensatory mechanism of resistance to ERBB2 targeted therapy. Indeed, ERBB3 mutations have been shown to cause resistance to ERBB2 targeted therapy [3]. PIK3CA G106V is a missense, activating hotspot mutation within the adaptors-binding domain of the catalytic subunit of the phosphoinositide 3-kinase, stimulating its lipid kinase activity [8].

This is the first description of acquired somatic alterations occurring after secondary treatment resistance in a patient with non-amplified, ERBB2 mutated, metastatic EMPD. Interestingly, these acquired genetic alterations may have caused treatment resistance and contribute to the understanding of commonly occurring secondary treatment failure of anti-HER2 treatments in metastatic EMPD. Furthermore, this case provides rationale for repetitive genomic analysis in treatment resistant EMPD lesions and contributes to the growing evidence of ERBB2 in the pathogenesis of metastatic extramammary Paget’s disease.

**MATERIALS AND METHODS**

DNA from FFPE tumor tissue was isolated using the automated Maxwell isolation system (Promega). Next generation sequencing was performed on formalin-fixed paraffin embedded (FFPE) samples, using the Oncomine Focus Assay (Life Technologies / Thermo Fisher) and the FoundationOne panel. Library preparation and sequencing was performed according to the manufacturers’ manuals.

**Author contributions**

All authors have contributed substantially to the manuscript.

**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.

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