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Case report

Response to anti-DKK1 therapy in uterine carcinosarcoma: A case report

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ABSTRACT

Targeted therapies are being increasingly used in clinical practice and trials. However, tumor heterogeneity among sites of metastatic disease can occur creating a conundrum when utilizing biomarker directed therapies. Here we demonstrate a patient with recurrent uterine carcinosarcoma whose local recurrence and metastatic recurrence had a varied response to paclitaxel in combination with DKN-01, a monoclonal antibody against DKK1, a modulator of Wnt/β-catenin and PI3K/AKT signaling pathways. This may be explained by differences in mutational profile found between the two sites. Our findings highlight the importance of analyzing tissue from the primary tumor as well as metastatic lesions, especially if there is a discrepancy in their response to treatment.

1. Introduction

Carcinosarcoma of the uterus (UCS) (previously known as malignant mixed Müllerian tumors [MMMT]) are rare, aggressive tumors that account for 2–5% of all uterine malignancies (Adachi et al., 2016). Despite its rarity, it is responsible for 15% of uterine cancer-related deaths. The 5-year overall survival (OS) for Federation of Gynecology and Obstetrics (FIGO) stage I/II is 30–46% and 0–10% for stage III/IV disease (Kanthan and Senger, 2011). UCS (aka malignant mixed müllerian tumor) is one of the most extreme examples of tumor heterogeneity, consisting of malignant epithelial (typically serous or endometriod) and mesenchymal (usually spindle cell or pleomorphic, but occasionally LMS) components (D'Angelo et al., 2009). As it is felt to be of epithelial origin, the National Comprehensive Cancer Network has classified UCS as an epithelial carcinoma for treatment and staging (Mccluggage, 2002; Kernochan and Garcia, 2009). Tumor heterogeneity poses significant challenges in clinical management as cytotoxic drugs seldom result in complete tumor cell death, allowing the resistant cells to drive disease recurrence. Understanding tumor heterogeneity on a molecular level could facilitate the development of more effective treatment strategies for UCS patients.

A major driving component of UCS heterogeneity and aggressive behavior is epithelial-to-mesenchymal transition (EMT). This phenomenon can enhance a cancer cell's metastatic potential, and metastatic lesions can have a different molecular profile and tumor microenvironment (TME) compared to the primary tumor (Marusyk and Polyak, 2010). Major signaling pathways that drive EMT in UCS are Wnt/β-catenin dependent (canonical) and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathways (Sagebiel et al., 2019; Cherniack et al., 2017). Mutations in Wnt/β-catenin dependent signaling (e.g. *CTNNB1*) occur frequently in cancer and result in constitutive signaling. Dickkopf-related protein 1 (DKK1) is overexpressed in tumors with active Wnt/β-catenin dependent signaling and has been linked to promoting tumor growth, metastasis and contributing to an immune suppressive tumor microenvironment (Kagey and He, 2017). Relative to other uterine histology, UCS has significantly increased DKK1 expression (Zhu et al., 2021). DKK1 can modulate both Wnt/β-catenin and PI3K/AKT signaling pathways, and elevated tumoral levels are a poor prognosis marker for many oncology indications. Therefore, neutralizing DKK1 activity may have therapeutic benefit in tumors with elevated DKK1 expression (Kagey and He, 2017).

DKN-01 is a humanized monoclonal antibody optimized for neutralizing activity against DKK1. In patients with previously treated advanced gastroesophageal adenocarcinoma, DKK1 tumoral expression was seen to be a predictor of DKN-01 response and survival. Those that were $DKK1^{HI}$ (*H-score $\geq$ 35) had an overall response rate (ORR) of 50% and disease control rate (DCR) of 80%, while $DKK1^{LO}$ patients had an ORR of 0% and DCR of 20% following treatment with DKN-01 in combination with pembrolizumab (Klempner et al., 2021). However, limited

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studies have investigated the effects of DKN-01 in UCS. Here we report a UCS patient with a *CTNNB1* mutation and elevated tumoral expression of DKK1 who experienced a rapid and sustained response to DKN-01 and paclitaxel combination therapy.

2. Patient presentation

A 46-year-old Caucasian female, gravida 1, Para 1–0-0–1, with FIGO Stage IVB UCS presented with enlarged uterus, complex pelvic masses, omental caking, large volume ascites, and metastatic nodules to the lung. A paracentesis was performed for both diagnostic and therapeutic purposes. The ascites fluid was positive for adenocarcinoma. A CT-guided biopsy was performed on her left lower lung lobe lesion that returned as metastatic UCS with heterologous elements, consistent with gynecologic origin.

3. Patient management

The patient was managed initially with 4 cycles of neoadjuvant paclitaxel (175 mg/m^2) and carboplatin (AUC = 6) chemotherapy given every 3 weeks. CT scan after initial chemotherapy revealed resolution of ascites and effusions, partial response of the peritoneal and omental disease, and persistence of the pelvic mass. She subsequently underwent interval cytoreductive surgery with removal of 30 cm pelvic mass, total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, pelvic and paraaortic lymph node dissection, and optimal cytoreduction to no gross residual disease. She resumed chemotherapy and completed 6 cycles of adjuvant paclitaxel/carboplatin. Overall, she tolerated the treatment well. CT scan upon completion of adjuvant therapy revealed no evidence of disease.

She remained without evidence of disease on routine surveillance until presenting approximately 10 months after the completion of therapy with a mass over the vaginal cuff with limited mobility on exam. CT of her chest, abdomen, and pelvis was performed, which showed vaginal cuff thickness, but no metastatic disease. Pelvic exam under anesthesia and Tru-Cut vaginal cuff biopsies revealed recurrent UCS. She was referred to radiation oncology for IMRT followed by HDR brachytherapy. She completed 4 cycles of weekly cisplatin (40 mg/m^2) and 4,400 cGy of IMRT before self-discontinuing therapy due to a family emergency.

Approximately 6 months later, she presented to the ED for an unrelated issue where a CT scan demonstrated stable vaginal cuff nodularity and new, numerous bilateral pulmonary metastases. CT-guided biopsy of the pulmonary metastases confirmed recurrence of the carcinomatous component of her known UCS. This biopsy underwent commercial molecular testing and was found to be microsatellite stable (MSS), PD-L1 negative, and have pathogenic *CTNNB1*, *PIK3CA*, and *PTEN* mutations.

She was then enrolled into a phase II clinical trial of DKN-01 in combination with weekly paclitaxel (NCT03395080). A pre-treatment biopsy of her vaginal cuff tissue was assessed for DKK1 expression by a RNAscope chromogenic in situ hybridization (CISH) assay. DKK1 expression was estimated to occur in 70% of the tumor cells and an H-score of 85 was calculated (Fig. 1). The pre-treatment did not yield sufficient tissue for molecular testing and an on-treatment biopsy obtained from the vaginal cuff after completion of one cycle was utilized instead. Similar to the lung lesion, the same *PIK3CA* and *PTEN* mutations were identified but a *CTNNB1* pathogenic mutation was not detected, possibly reflecting tumor heterogeneity. After 2 cycles of therapy, CT CAP showed a complete response in the lungs (Fig. 2) and a PR (60–70%) at the vaginal cuff (Fig. 2). She completed 8 cycles (8 months) of therapy until progression was seen at the vaginal cuff.

4. Discussion

Stabilizing mutations in *CTNNB1* result in constitutive activation of Wnt/β-catenin dependent signaling and enhanced DKK1 expression. Loss of function mutations in other signaling components of the Wnt signaling pathway such as APC and AXIN also result in activation of Wnt/β-catenin dependent signaling and likely increase DKK1 transcription. Given the role of DKK1 in tumor progression, it is reasonable to hypothesize that patients with DKK1^{HI} tumors will likely have a better response to DKK1 neutralizing therapy, DKN-01, compared to patients with DKK1^{LO} tumors.

Analysis of the patient in this case report highlights the role of tumor heterogeneity and DKN-01 response. Both of the patient biopsies (vaginal cuff and lung) demonstrated *PIK3CA* and *PTEN* mutations. However, there were conflicting findings of presence (lung) and absence (vaginal cuff) for *CTNNB1* mutation, suggesting variation in Wnt/β-catenin signaling and DKK1 expression. The DKN-01 response also varied between the two lesions, where complete response was seen in the lung while a partial response was seen in the vaginal cuff. These findings suggest that *CTNNB1* mutations correlate to improved DKN-01 response.

In patients with recurrent epithelial endometrial cancer (EEC), those harboring a Wnt activating mutation (Table 1) had a higher tumoral DKK1 expression and better disease control (67% vs 25%) and longer PFS (5.5 vs 1.8 mo, [HR 0.69; 0.30, 1.58]) following DKN-01 monotherapy than patients without these characteristics (Arend, 2020). In this patient, the partial response seen in the vaginal cuff could be due to the elevated DKK1 expression (H-score of 85), suggesting DKK1^{HI}

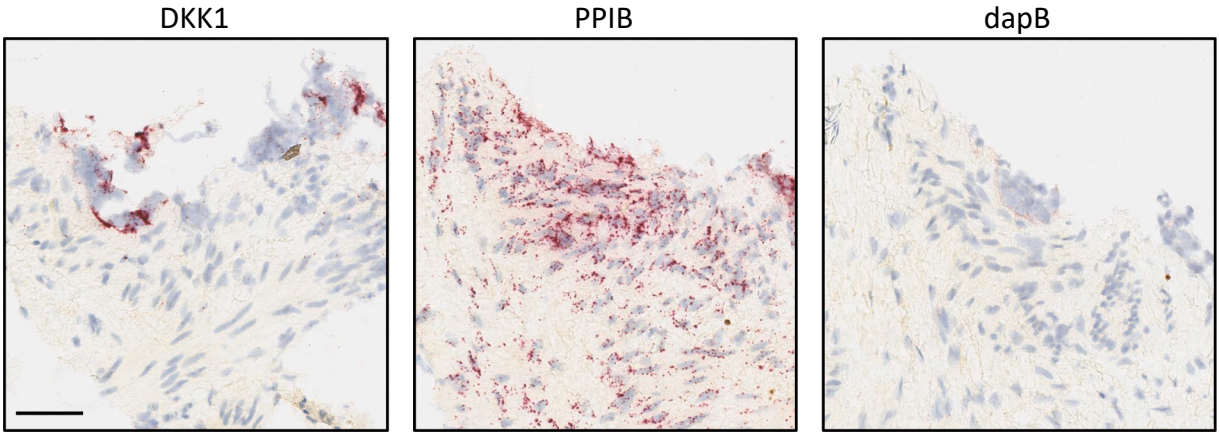

Fig. 1. DKK1 Tumor Expression DKK1 mRNA expression was assessed from a vaginal cuff biopsy using a RNAscope chromogenic in situ hybridization assay. An H-score (range 0 to 300) of 85 was calculated by estimating the number of low (1–3 dots per cell), medium (4–9 dots per cell) and high (10 + dots per cell) expressing tumor cells. H-score = (%low) + (%medium)*2 + (%high)*3. PPIB, positive control housekeeping gene. dapB, negative control bacterial gene. Scale bar: 50 μm.

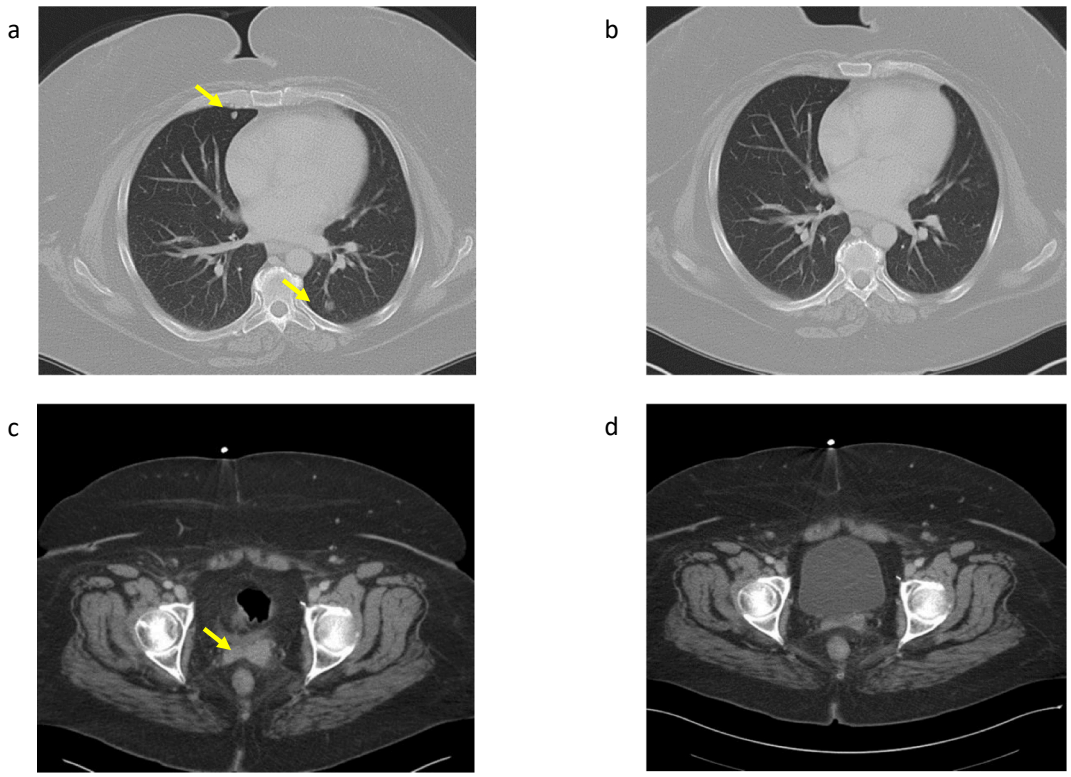

Fig. 2. Response to Therapy of Metastatic Lung Disease Representative section of chest computerized tomography showing (a) multiple bilateral pulmonary nodules (b) Complete response of lung lesions following treatment. (c) Locally recurrent vaginal cuff lesion (d) Vaginal cuff lesions with 60–70% partial response following treatment.

Table 1
Wnt Activating genes.

Gene	Genetic alteration
CTNNB1 (β-catenin)	Protein stabilizing alteration (missense mutation of S33, S37, T41 or S45; exon 3 missense mutation or inframe deletion of all or part of exon 3)
APC	Loss of function alteration (truncation or deletion)
AXIN1/2	Loss of function alteration (truncation or deletion)
RNF43	Loss of function alteration (truncation or deletion)
ZNRF3	Loss of function alteration (truncation or deletion)
RSPO2	Fusion protein (EIF3E-RSPO2)
RSPO3	Fusion protein (PTPRK-RSPO3)

tumors are sensitive to DKN-01. While we were unable to assess DKK1 expression in the lung lesion due to insufficient tissue for testing a CTNNB1 mutation was identified.

While the responses seen could be due to molecular tumor heterogeneity of the two different tumor sites, it could also be due to different tumor microenvironments (TMEs) surrounding the tumor sites. Previous studies have also highlighted the multiple functions of DKK1 in regard to immunity and inflammation (D'Amico et al., 2016; Chae et al., 2016; Malladi et al., 2016). Elevated DKK1 has been seen to be an immunosuppressive modulator and inhibit immune mediated antitumor response by activating myeloid derived suppressor cells and down regulating natural killer (NK) activating ligands on cancer cells (Klempner et al., 2021; D'Amico et al., 2016; Malladi et al., 2016). Future studies will also need to analyze TME of patient lesions to gain further understanding of the complexity surrounding their disease progression and therapeutic response.

Identifying biomarkers is crucial for predicting patient outcomes and to guide individual treatment options. However, biomarkers are susceptible to changes during disease progression as they reflect the biological properties of tumors. This susceptibility poses a challenge for adjuvant targeted therapies and therapeutic strategies for relapsed disease because they are often chosen on the basis of the initial diagnosis of the primary tumor, under the assumption that the target is maintained during disease progression. Divergent evolution of metastatic tumor cells and different TMEs could contribute to the change of expression of the biomarkers that were initially identified in the primary tumor. Therefore, treatment of metastatic disease according to the biomarkers expressed in the primary tumor may not always be optimal. Understanding tumor heterogeneity and considering the mechanisms behind it and how it affects the properties of the malignancy may facilitate the development of more-effective treatment strategies.

From this case report, we see that a UCS patient's primary and metastatic tumor response to DKN-01 varied, and this response is associated with a difference in mutational profiles between the two tumors. Our findings highlight the importance of analyzing not only tissue from the primary tumor, but also metastatic lesions independently especially if there is a discrepancy in their response to treatment. Furthermore, the results of this study support further exploration of UCS patient tumor(s) for DKK1 expression and/or genetic alterations that activate Wnt/β-catenin dependent signaling as potential biomarkers of response for DKN-01 in UCS. Given the aggressive nature of UCS and the lack of therapeutic options for these patients, it is paramount to implement analyses that will better predict patient response to therapies as well as identify potential therapeutic targets throughout disease progression.

Statement of Consent

The patient has expired and her family was not able to be reached for informed consent despite multiple attempts.

CRediT authorship contribution statement

A. ElNaggar: Methodology. **N. Zhang:** . **C.B. Scalise:** . **C. Sirard:**

Formal analysis. **M.H. Kagey:** Data curation, Methodology. **D. Vaena: . R. Arend:** Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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