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Case report

POLE-mutated clear cell cervical cancer associated with in-utero diethylstilbestrol exposure

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ABSTRACT

We report an extraordinary case of a woman, exposed to diethylstilbestrol in utero, who developed clear cell adenocarcinoma of the cervix with a concurrent polymerase-Ɛ (*POLE*) somatic mutation. The tumor exhibited the classic phenotypic characteristics of *POLE*-mutated tumors originating from other organs (e.g. the uterus or the colon) including increased tumor infiltrating lymphocytes and high PD-L1 expression and has remained in remission since completion of primary therapy for > 4 years. This case highlights the importance of next generation sequencing in unraveling the biology of rare tumors and supports that the presence of a *POLE* mutation and the associated ultramutated state confers a unique phenotype of higher immunogenicity and possibly improved prognosis in a tissue-agnostic manner, i.e. regardless of the type of cancer where the *POLE* mutation is present.

1. Introduction

Women exposed in utero to the synthetic estrogen diethylstilbestrol (DES) have a 40-fold increased risk of clear cell adenocarcinoma (CCA) of the vagina or cervix (Troisi et al., 2007). DES exposure during organogenesis fosters the tumorigenic effect, through disruption of Wnt signaling, SMAD/RUNX1 pathways, and Hox genes, which govern the appropriate differentiation of epithelia and development of the Mullerian tract (Block et al., 2000). This predisposes to Mullerian tract defects and the development of vaginal adenosis, from which CCA can arise. DES-related CCA of the vagina or cervix occurs in two age distributions, first peaking at a mean age of 20, and again around age 42 in a bimodal pattern (Huo et al., 2017).

We report a case of a woman, exposed to DES in utero, who developed CCA of the cervix with a concurrent polymerase-Ɛ (*POLE*) somatic mutation. The tumor exhibited the classic phenotypic characteristics of *POLE* mutated tumors originating from other organs (e.g. the uterus or the colon) including increased tumor infiltrating lymphocytes and high PD-L1 expression, and has remained in remission since completion of primary therapy for > 4 years. To our knowledge, *POLE* exonuclease domain mutations have not been reported before in clear cell cervical cancers in either DES-related or unrelated cases, and this case raises interesting questions regarding coexisting tumorigenic drivers as well as the hallmark clinical characteristics of *POLE*-mutated tumors regardless of site of origin.

2. Case report

The patient was diagnosed at 55 years old with FIGO Stage IB2 (per FIGO 2009 system), now FIGO Stage IB3 (per FIGO 2018 system), clear cell carcinoma of the cervix. She had presented to the hospital with vaginal hemorrhage leading to hypotension and presyncope, which was refractory to hormonal control. She was taken urgently for an exploratory laparotomy. Intraoperatively she was noted to have an enlarged uterus with a distended lower uterine segment and a polypoid mass protruding from the cervix, prompting a total abdominal hysterectomy. Pathology revealed a 6 cm clear cell carcinoma of the cervix, moderately-differentiated, extending circumferentially. Margins were close, with tumor invading the stroma to within 1.5 mm of the deep paracervical resection margin. There was no lymphovascular invasion, and subsequent staging PET/CT scan did not show any significant lymphadenopathy Notably, the patient reported that her mother took diethylstilbestrol during her pregnancy. She received adjuvant chemoradiation with cisplatin, in light of her large tumor and close margins, with parametrial and vaginal brachytherapy boost. This was followed by four cycles of carboplatin/paclitaxel as per the OUTBACK/GOG0274 regimen, due to concern for the aggressive behavior associated with the clear cell histology. She has remained in remission since

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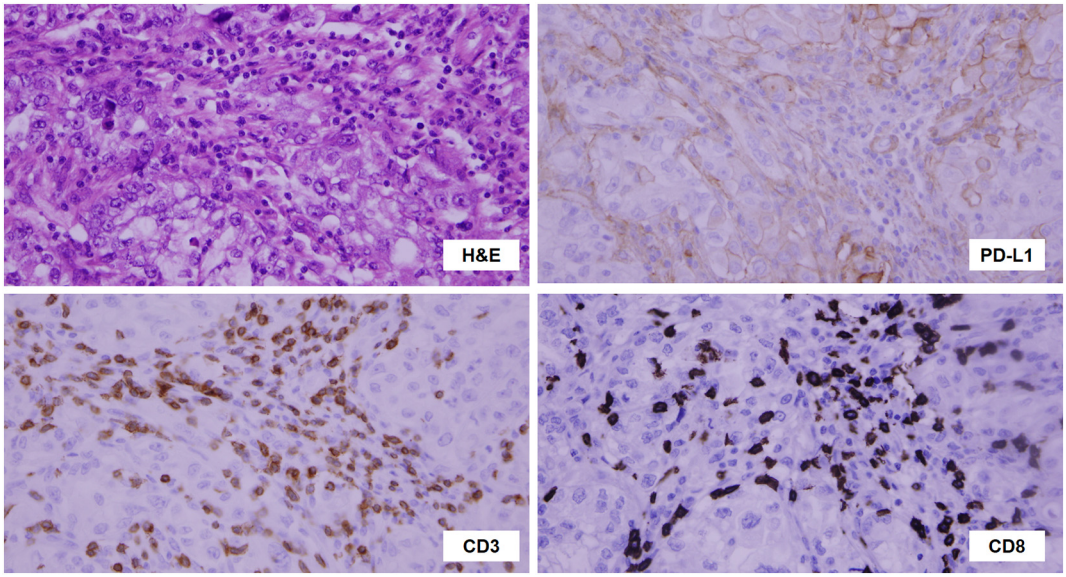

Fig. 1. PD-L1, CD3 and CD8 expression by immunohistochemistry (×40 magnification).

completing therapy; at the time of submission of this report, she is now 4 years disease-free. Her tumor was later tested on an in-house next generation sequencing panel ("Oncopanel") which evaluates somatic mutations, copy number variations, and structural variants in tumor DNA across exonic DNA sequences of 447 cancer genes and 191 regions across 60 genes for rearrangement detection (Wagle et al., 2012). Her tumor was found to have a well-described hotspot *POLE* P286R mutation. There were 201 additional mutations, including in *PIK3CA, ARID1A,* and *PTEN*, of which 28 mutations involved C > A transversions. There was a tier 2 missense mutation in *MLH1*, however IHC was intact for all mismatch repair proteins (*MLH1, MSH2, MSH6, and PMS2*). PD-L1 was positive, with staining in 10% of cells (Fig. 1), well above the criteria for the FDA-approved use of pembrolizumab in cervical cancer (combined proportionality score (CPS) ≥ 1). Tumor infiltrating lymphocytes were notably present, with a 1:4 ratio of CD4 to CD8 T-cells (Fig. 1).

3. Discussion

Diethylstilbestrol is a well-described cancer risk factor, due to its gene-modulating effects as a synthetic estrogen. CCA of the vagina or cervix even in a DES-exposed population, however, still remains relatively rare, with an absolute risk of 1.9–2.3 cases per 1000 based on a recent analysis of the University of Chicago CCA registry (Huo et al., 2017). This implies that there are other predisposing genetic or environmental factors that, in conjunction with DES exposure, facilitates development of vaginal or cervical CCA.

There is currently no data to suggest a correlation with or a mechanistic link between DES and *POLE* mutations, and no reported data describing the incidence of *POLE* mutations in DES-exposed patients specifically. *POLE* mutations in endometrial cancer, however, are well-described, occurring in 5–12% of endometrial carcinomas (Bosse et al., 2018; Kandoth et al., 2013). Hotspot mutations at P286R and V411 L in the exonuclease domain, along with other pathogenic variants, lead to loss of 3′-to-5′ proofreading ability during DNA replication, subsequent genomic instability, and the generation of an ultra-mutated phenotype with a unique pattern of C > A single nucleotide transversions (Kandoth et al., 2013). The *POLE*-mutated molecular subgroup is prognostically favorable in endometrial carcinoma, likely due to increased neoantigen burden (with predictions of > 8000 neoepitopes) promoting greater immunogenicity, as evidenced by increased number of peritumoral and tumor infiltrating lymphocytes and increased PD-1 and PD-L1 expression (Church et al., 2015; Howitt et al., 2015). *POLE* mutations within clear cell carcinomas are uncommon, seen in 1.7% of clear cell renal cell carcinomas in the TCGA PanCancer Atlas (Cerami et al., 2012; Gao et al., 2013), 6% of an NIH cohort of endometrial CCA (1 of 16 patients) (Cerami et al., 2012; Gao et al., 2013), and 7% (2 of 22 pts) in a separate endometrial CCA study (DeLair et al., 2017).

POLE mutations are generally associated with a microsatellite stable phenotype, likely reflecting a *POLE* mutation occurring early in tumor evolution. More recently, however, *POLE* mutations were also reported to be present in approximately 25% of tumors with microsatellite instability without MLH1 silencing (Billingsley et al., 2015; Konstantinopoulos and Matulonis, 2014). Thus, microsatellite instability may be a result of a *POLE* mutation leading to somatic inactivation of a mismatch repair protein. Large-scale analysis of hypermutation signatures across tumor types identified genetic clusters based on timing of *POLE* mutation and loss of mismatch repair. Presence of microsatellite stability or instability differed by how early loss of mismatch repair occurred in tumor evolution (Campbell et al., 2017), including as an event secondary to *POLE* mutation, in support of previous findings. Of interest, an older study of cervical clear cell carcinomas demonstrated somatic microsatellite instability in all of their DES-related cases (n = 8), though the mechanism of microsatellite instability was not further elucidated and thus cannot clearly be associated with either *POLE* mutation or DES exposure.

Prognosis for localized cervical cancer is favorable, with an estimated conditional 5-year overall survival (OS) of 91.7% per SEER 18 data. CCA of the vagina or cervix is considered a more aggressive histologic subtype, reflected in a decreased 5-year OS of 67% for DES-unrelated disease in an older study (Reich et al., 2000). In a more recent analysis of the Registry for Research on Hormonal Transplacental Carcinogenesis, authors reported a 5 year OS of 81.2% in patients with DES-unrelated vaginal or cervical CCA (Huo et al., 2018). Interestingly, DES-exposed CCA of the vagina or cervix was associated with a better 5 year OS of 86.1%, though the survival difference was significant only in the first five years after diagnosis, after adjusting for stage, histologic type, and age (Huo et al., 2018). After five years, the survival curve of DES-exposed patients approached that of non-exposed patients, leading to one hypothesis that DES-related disease may recur later, driving the survival curve down. Overall, data regarding whether adjuvant chemotherapy is beneficial for localized DES-related CCA, and if so, to what degree, is lacking. The larger question of adjuvant chemotherapy following chemoradiation is still being explored in cervical cancer in

the GCIG OUTBACK trial (clinicaltrials.gov identifier NCT01414608) for which data has not yet been reported.

Lastly, it is unknown to what extent *POLE* mutations affect the disease biology of CCA of the cervix. The high degree of TILs and counterbalanced positive PD-L1 expression in this case are concordant with the immunogenic environment commonly seen in *POLE*-mutated cancers of various histologies, reflecting an ultramutated status (Howitt et al., 2015; Bellone et al., 2017). It is thus possible that *POLE* mutation in cervical CCA may also be associated with a more favorable prognosis, as is seen in *POLE*-mutated endometrial cancer. This also provides a rationale to consider immunotherapy to treat recurrence of *POLE*-mutated cervical CCA, particularly as pembrolizumab is now FDA-approved for PD-L1 positive (CPS ≥ 1) recurrent cervical cancer based on results of the phase II KEYNOTE-158 trial (Frenel et al., 2017). There are case reports of good response to PD-1 or PD-L1 blockade in *POLE*-mutated tumors (Gong et al., 2017), and clinical trials exploring immune checkpoint blockade in *POLE*-mutated tumors are ongoing (clinicaltrials.govNCT02912572, NCT03150706, NCT03435107).

In conclusion, this case highlights the unusual situation of DES-related clear cell carcinoma of the cervix with a concurrent *POLE* mutation. The exact prevalence of *POLE* mutations in DES-related clear cell cervical cancers would be difficult to estimate as the individual relative rarities of CCA of the cervix and *POLE* mutations preclude the ability to study these conditions in a substantial, overlapping population. However, this case underscores the importance of next generation sequencing in unraveling the biology of rare tumors and supports that the presence of a *POLE* mutation and the associated ultramutated state confers a unique phenotype of higher immunogenicity and possibly improved prognosis in a tissue-agnostic manner, i.e. regardless of the type of cancer where the *POLE* mutation is present, suggesting that their management may also need to be tissue-agnostic.

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Prior presentations

N/A.

Disclaimers

N/A.

Conflict of interest statement

Drs. Lee and Lindeman have nothing to disclose. Dr. Matulonis reports personal fees from Astrazeneca, personal fees from Myriad Genetics, personal fees from Clovis, personal fees from Merck, personal fees from Eli Lilly, personal fees from Mersana, personal fees from Geneos, personal fees from Fuji Film, from 2X Oncology, personal fees from Cerulean, personal fees from Immunogen, all outside the submitted work. Dr. Konstantinopoulos reports other from AstraZeneca, other from Pfizer, other from Merck, all outside the submitted work.

Author contribution

All authors contributed to the concept, oversight, data collection, interpretation of data and writing of the manuscript. All authors approved the final version of the manuscript.

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