Endometrial cancer with an *EML4-ALK* rearrangement

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Abstract An 85-yr-old woman was diagnosed with endometrial adenocarcinoma, endometrioid type. Imaging studies showed a large tumor distending the endometrial canal without evidence of local invasion or extrauterine disease. A hysterectomy was performed, followed by microscopic examination of longitudinal tissue sections. Histopathological review showed only focal myometrial invasion, equivocal lymphovascular invasion, and negative bilateral sentinel lymph nodes (FIGO stage IA). A sample of the tumor was submitted for molecular testing (massively parallel sequencing on OncoPanel) and was found to harbor an inversion on Chromosome 2 resulting in an *EML4-ALK* gene fusion. Confirmatory immunohistochemistry showed ALK overexpression in just a portion of the tumor. Additional genomic characterization on a region of the tumor lacking ALK overexpression by immunohistochemistry was highly congruous with the genomic profile of the ALK-positive portion, showing similar patterns of copy-number variation and mutations in *TP53* and *KDM5C*, with no evidence for an *EML4-ALK* gene fusion, confirming that *EML4-ALK* rearrangement had occurred as a subclonal process. *EML4-ALK* fusions are driver events in 2%–5% of non-small-cell lung cancers; crizotinib is an approved targeted therapy for these patients. *EML4-ALK* rearrangements have not previously been reported in endometrial cancer.

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CASE PRESENTATION

An 85-yr-old woman was diagnosed with endometrial adenocarcinoma, endometrioid type, grade 3 (of 3), via endometrial biopsy performed for post-menopausal bleeding. Imaging studies showed a large tumor distending the endometrial canal without evidence of local invasion or extrauterine disease. Subsequent total laparoscopic hysterectomy bilateral salpingo-oophorectomy with surgical staging revealed a 5.8 cm, tan/white, exophytic mass involving the anterior aspect of the endometrial cavity. The polypoid nature of the tumor was most easily appreciated by microscopic examination of longitudinal tissue sections, where it appears folded over residual areas of benign endometrium (Fig. 1A). Further histopathological review showed only focal myometrial invasion, equivocal lymphovascular invasion, and negative bilateral sentinel lymph nodes (FIGO stage IA).

TECHNICAL ANALYSIS AND METHODS

A sample of the tumor ("sample 1"; estimated percentage of neoplastic cells was 70%) submitted for genomic characterization using OncoPanel (version 3)—a hybrid-capture and

Figure 1. (A) H&E slide of endometrial cancer specimen. (B) Genomic representation (IGV screenshot) of *EML4-ALK* fusion. (C) Immunohistochemical staining showing ALK overexpression in part of the specimen (shown boxed in A) only. (D) Plot of copy-number variation for specimen 1 (upper) and specimen 2 (lower) by color-coded chromosomes reveals a very similar pattern of copy-number alterations in the specimens. The vertical axis is the ratio of number of reads for this specimen and a panel of normals in log base 2 scale.

massively parallel sequencing assay. Testing was performed in a CLIA-certified laboratory as previously described (Sholl et al. 2016; Garcia et al. 2017). DNA was isolated with a kit (Qiagen) followed by ultrasonic fragmentation (Covaris), size selection, and quantification. Sequencing libraries were prepared using KAPA HTP library preparation kits (Roche) and hybridized to a biotinylated RNA bait set (Agilent SureSelect). The assay (OncoPanel) is designed to capture and sequence the full coding regions of 447 cancer genes; additional baits are tiled on intronic regions for 60 clinically relevant known rearrangement loci. Streptavidin-captured libraries were PCR-enriched, size selected, and sequenced on an Illumina HiSeq 2500 with 2×100 paired-end reads.

Sequence data were analyzed using a pipeline consisting of reconfigured publicly available tools and internally developed algorithms (RobustCNV, Anotate&Phase, Breakmer) as described previously (Sholl et al. 2016; Garcia et al. 2017). In brief, pooled sample reads were deconvoluted using Picard (version 1.13.0) and aligned using BWA to Human Genome Reference Consortium reference sequence b37 edition (duplicate reads were removed). Localized realignment around indel sites and recalibration of the quality scores was performed using the Genome Analysis Toolkit (McKenna et al. 2010; DePristo et al. 2011).

(GATK, version 3.3.0). MuTect v. 1.0.27200 (Cibulskis et al. 2013) and Variant Effect Predictor (VEP) v79 were used to identify and annotate mutations; SomaticIndelDetector (v1.65) was used to identify small insertions and deletions. Normal FFPE samples were used to filter sequencing and batch-level artifacts. As a matched normal was not available, SNPs present at >0.1% in Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (<http://evs.gs.washington.edu/EVS>; accessed May 30, 2013) were filtered unless also present in COSMIC at least twice. A custom R-based tool was used to call copy-number changes by calculating the fractional coverage of specified genomic intervals compared with the median fractional coverage obtained in a panel of 152 FFPE normal samples. Structural variant analysis was performed using Breakmer (Abo et al. 2014) with visualization of sequences of interest in Integrative Genomics Viewer (IGV) (Robinson et al. 2011).

OncoPanel performance metrics were established for the assay using genomically well-characterized clinical samples as previously described (Garcia et al. 2017). In summary, sensitivity for SNVs was 97.8% (95% CI: 86.5%–99.9%), and for rearrangements was 74% (95% CI: 53.4%–88.1%); specificity for SNVs was 100% (95% CI: 99.9%–100%), and for rearrangements was 100% (95% CI: 69.9%–100%). Mean target coverage was 436× (unique, high-quality, mapped reads) for sample 1, and 310× for sample 2. The mean percent target bases greater than 30× was 98% for both specimens.

VARIANT INTERPRETATION

Interestingly, sample 1 was found to harbor an inversion on Chromosome 2, involving a breakpoint within intron 19 of *ALK* and intron 21 of *EML4*. The resulting *EML4*-*ALK* fusion (Table 1 for genomic coordinates; Fig. 1B) contains the amino-terminal portion of *EML4* and the entire intracellular portion of the *ALK* protein—including the juxtamembrane domain and the catalytic tyrosine kinase domain—the latter of which is the presumed functional driver. Immunohistochemistry (IHC) showed *ALK* overexpression in just a portion of the tumor, with the marbled interface between *ALK*-positive and *ALK*-negative areas showing otherwise homogenous morphologic features (Fig. 1A, black rectangle, and 1C). Subsequent molecular testing on a region of the tumor lacking *ALK* overexpression by IHC (“sample 2”; 70% tumor) was highly congruous with the genomic profile of the *ALK*-positive portion (see Table 1): Both cases harbored mutations in *TP53* (I251S) and *KDM5C* (K498N), among others, and similar patterns of copy-number variation (other than the inversion on Chromosome 2) across regions of the genome covered in our assay (Fig. 1D). The *EML4*-*ALK* gene fusion was notably absent, however, confirming that *EML4*-*ALK* rearrangement had occurred as a subclonal process (roughly estimated using mapped reads over the locus at ~20%–30% allele burden in the tumor).

Table 1. Genomic findings

Gene	Chr	HGVS DNA ref	HGVS protein ref	Variant type	Predicted effect	Specimen 1		Specimen 2	
						Allele frequency	Target coverage	Allele frequency	Target coverage
<i>EML4</i> - <i>ALK</i>	2	t(2;2)(p21;p23.2) (Chr2:g.29447680-42554394)		Fusion	Sensitivity to <i>ALK</i> inhibitor	n/a	50×	Not detected	50×
<i>KDM5C</i>	X	c.1494G>T	p.K498N	Substitution	n/a	30%	635×	35%	406×
<i>TP53</i>	17	c.752T>G	p.I251S	Substitution	n/a	37%	201×	60%	105×

SUMMARY

This is the first report of an *EML4-ALK* rearrangement in endometrial cancer. *EML4-ALK* fusions are driver events in 2%–5% of non-small-cell lung cancers; moreover, crizotinib is an FDA-approved targeted therapy for lung cancer patients. Standard of care treatment for a FIGO Stage IA grade 3 endometrial cancer is vaginal brachytherapy, which was pursued in this case. It was noted that if the cancer recurs, ALK-directed adjuvant chemotherapy would be considered. Overall, the identification of this genomic result in an unexpected cancer type highlights the utility of using a comprehensive genomic approach to direct precision cancer therapy.

ADDITIONAL INFORMATION

Data Deposition and Access

Variants have been deposited as part of AACR Project GENIE (<https://synapse.org/genie>) and submitted to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) under accession number SCV000778842.

Ethics Statement

The patient provided written, informed consent (protocol 11–104). This study was approved by the institutional review board of the Dana-Farber Cancer Institute and the Partners Human Research Committee.

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Competing Interest Statement

The authors have declared no competing interest.

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Author Contributions

J.W.C. acquired clinical data and images, performed the literature search, and wrote the report. M.G.M. was responsible for the clinical management of the patient. B.J.Q. provided the final histopathological diagnosis of the lesion. L.E.M. interpreted the molecular findings, wrote the report, and supervised the project. All authors contributed to critical revision and final approval of the report.

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