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Genetic predictors of unexpected recurrence in low-risk endometrial cancer: A comprehensive genomic analysis reveals FGFR2 as a risk factor and a rare fatal POLE-mutated recurrence

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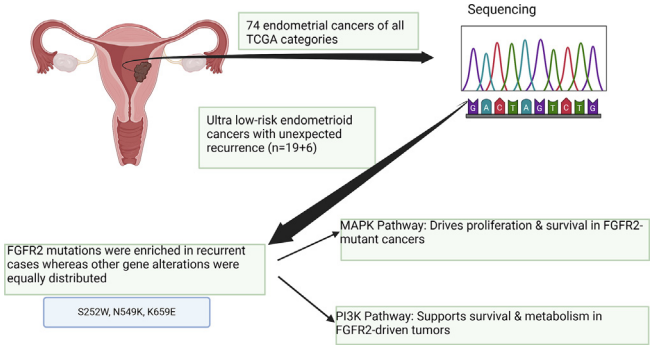
HIGHLIGHTS

- Recurrence cases showed frequent mutations in PTEN, PIK3CA, ARID1 A, and PIK3R1, with FGFR2 as a notable finding.
- FGFR2 mutations were found in 47.4 % of recurrence cases and absent in non-recurrent cases.
- FGFR2 mutations were significantly enriched in recurrence cases compared to TCGA ($p = 0.0039$).
- A rare POLE-mutated tumor, typically with favorable prognosis, recurred unexpectedly and resulted in a fatal outcome.
- FGFR2 mutations may drive oncogenesis via MAPK and PI3K/AKT pathways and are a potential therapeutic target.

GRAPHICAL ABSTRACT



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ABSTRACT

Objective. Endometrial cancer is the most common gynecological malignancy in high-income countries. While early-stage endometrial cancer generally has a favorable prognosis, a small proportion of low-risk patients experience unexpected recurrence. This study aimed to identify molecular factors contributing to recurrence in stage 1 A grade 1–2 low-risk endometrioid endometrial cancer.

Methods. We performed next-generation sequencing (NGS) on tumor samples from 19 patients who experienced recurrence despite favorable clinicopathological features and compared them with six control patients without recurrence. Results were also compared to a matched cohort of low-risk endometrial cancers from The Cancer Genome Atlas (TCGA) database.

Results. Mutations in PTEN, PIK3CA, ARID1A, and FGFR2 were the most frequent in the recurrence group. FGFR2 mutations were exclusive to the recurrence group (9/19, 47.4 %) and absent in the non-recurrent group (0/6), a difference approaching statistical significance ($p = 0.0571$). FGFR2 mutations were also significantly more prevalent in the recurrence cohort compared to the TCGA low-risk cohort ($p = 0.0039$). Prominent FGFR2 missense mutations included S252W, K659E, and N549K, which may drive oncogenesis and tumor progression. Among the recurrence group, a rare POLE-mutated tumor recurred unexpectedly and proved fatal, highlighting the potential for poor outcomes even in typically favorable molecular subtypes.

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Conclusion. FGFR2 mutations may play a role in tumor recurrence in a subset of low-risk endometrial cancers, underscoring the importance of molecular profiling in identifying patients at risk. FGFR2 represents a potential therapeutic target, warranting further validation in larger cohorts to establish its clinical utility.

1. Introduction

Endometrial cancer is the most common gynecological cancer in high-income countries and endometrioid cancer being the most common type. Most cases are diagnosed at an early stage, where adjuvant therapy is often not indicated, and the prognosis is highly favorable, with a 5-year overall survival exceeding 95 % [1]. However, a small subset of low-risk endometrial cancer patients experience unexpected recurrence, often with poor outcomes [2]. These cases challenge the existing paradigms of risk assessment and underscore the need for better predictive markers.

Endometrial cancer patients with early-stage disease, the risk of recurrence is associated with tumor histological grade, depth of myometrial invasion and lymphovascular space invasion (LVSI) [3,4]. Additionally, primary tumor diameter seemed to be significant risk factor for recurrence in women with low-risk endometrial cancer [5]. Despite these established clinicopathological features, current risk assessment models often fail to identify low-risk carcinomas that recur unexpectedly. The prognosis of recurrent endometrial cancer is poor [3]. The treatment of recurrent disease is based on histopathological and clinical features and involves most often radiation, chemotherapy, or hormonal therapy and lately immunological therapy has become to clinical use. Patients with local or distant metastasis have relative 5 year-survival decreased to 67.5 % and 16.9 %, respectively [1]. Current risk assessments have limitations identifying low risk carcinomas, which re-occur unexpectedly.

The Cancer Genome Atlas (TCGA) Research Network has provided a comprehensive genomic classification of endometrial cancer, stratifying tumors into four intrinsic molecular subtypes: POLE ultramutated (POLEmut), mismatch repair deficient (MMRd), mismatch repair proficient/no specific molecular profile (MMRp/NSMP), and p53 abnormal (p53abn) [6]. This classification, now integrated into the World Health Organization's framework [7] and clinical guidelines [8,9], has revolutionized the understanding of endometrial cancer's molecular landscape. It has also paved the way for more precise risk stratification and targeted therapeutic approaches.

Many genetic alterations including PIK3CA, FGFR2 and KRAS are associated with endometrial cancer [6]. The Catenin beta-1 (CTNNB1) gene plays also a role in tumorigenesis and recurrence in endometrial cancer and has been linked as a poor prognostic factor [6,10]. In addition, there have been reported specific molecular factors for the potential role for early stage metastatic endometrial cancer as well [10–13].

Low-risk endometrial cancer patients with recurrence have potentially biological tumor factors that contribute to an adverse prognosis and cause recurrence. Identifying these factors and molecular markers is essential to determine patients with a higher risk of recurrence within stage I endometrial cancer. In this study, we analyzed a cohort of endometrial cancer patients with purportedly ultra-low risk for recurrence, based on pathologic-anatomic findings, who unexpectedly relapsed. Next generation sequencing (NGS) was employed to uncover any common genetic factor that could explain the unusual outcome.

2. Materials and methods

A cohort of 759 endometrial cancer patients were operated in Turku University Hospital during the years 2008–2021. Patients were characterized by cancer histology, tumor grade, myometrial invasion, lymphovascular invasion and FIGO staging. From 759 patients we selected 74 patient representatives of four TCGA classes and their tissue block with adequate tumor percentage was sent to FoundationOne gene panel analysis. Seventy samples had enough tissue material for analysis. All the samples from 70 endometrial cancer, which consisted of 55 endometrioid, 10 serous and 5 clear cell carcinomas. They were classified according to TCGA to known subtypes [6]. The same samples were also analyzed using Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) with identical subtyping results [14].

From the larger cohort, we selected 25 patients who met the following criteria: endometrioid-type histology, histological grade 1 or 2, myometrial invasion <50 %, LVI negative and no adjuvant treatment. These included six control patients without recurrence and 19 patients with recurrence. This study was designed to investigate the molecular characteristics of ultra-low-risk endometrioid endometrial cancers that experienced unexpected recurrence. The recurrence rate in this cohort is therefore artificially high due to intentional selection of recurrent cases, and it does not reflect the recurrence rate of low-risk endometrial cancer in the general population. The primary aim was to characterize the molecular features associated with these rare events, rather than to estimate recurrence rates.

The samples were collected from formalin-fixed paraffin-embedded (FFPE) tissue blocks after pathologist's confirmation that the tumor tissue content was over 20 %. When the sample tumor cell percentage was <20 % the sections were trimmed to meet the test criteria. Next-generation sequencing (NGS) was performed using adapter-ligation and hybrid capture methods (FoundationOne CDxTM, Foundation Medicine, Cambridge, MA, US). This platform covers all exomes from 324 cancer-related genes and intron sequences of 28 genes frequently rearranged in cancer. The sequencing was conducted in Penzberg, Germany. The reported genomic alterations were analyzed in Cancer Genome Interpreter, CGI [15], Uniprot [16] and ClinVar [17] to stratify all putative driver and passenger mutations.

The identified alterations were finally divided into three categories. First, mutations which demonstrated to be oncogenic and known in endometrial or other cancer types. Second, mutations predicted as drivers (according to OncodriveMUT) which were categorized into Tier 1 and 2. According to COSMIC Cancer Gene Census (CGC) [18], Tier 1 alterations possess documented activity relevant to cancer and promotes oncogenic transformation. Tier 2 consists indications to have oncogenic potential but lack extensive evidence. Third category consisted of predicted passenger mutations. After analyzing 2962 mutations with CGI, a total of 2845 alterations were identified. Of those, we included only oncogenic and predicted driver mutations (1367/2845; 48, 0 %) and excluded passenger mutations.

As a validation cohort we used a database form The Cancer Genome Atlas (TCGA) in CBioPortal (https://www.cbioportal.org). The TCGA PanCanAtlas database consists of 209 endometrial cancers of which 146 are classified as low risk endometrioid endometrial cancer (Stage 1A G 1–2).

The study was approved by Auria Biobank's Scientific Steering Committee and the Institutional Review Board of Turku University Hospital (Auria Biobank permission number: 20151221).

2.1. Statistical analysis

IBM SPSS 27 statistics (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) was used to

calculate Fisher's exact test where association between two categorical variables were studied. Results were considered to be statistically significant at $p < 0.05$ (two-tailed). Kaplan-Meier curve was drawn to survival data and log-rank test was used to compare the groups.

3. Results

All 25 low-grade endometrioid patients had hysterectomy, and 24 patients had salpingo-oophorectomy, with one patient's ovaries preserved due to young age. Five patients also underwent lymphadenectomy due to an initial suspicion of high-grade carcinoma for endometrial cancer. Final histological samples confirmed stage 1A endometrioid endometrial adenocarcinoma grade 1–2, with no lymphovascular space invasion (LVSI). All patients underwent minimally invasive surgery, and none of the patients received adjuvant therapy following surgery. Only one patient (1/25) was operated in the central regional hospital, the rest in Turku University Hospital. The median follow up time was 5,2 years (range 2,1-13,7). Patients' demographics are presented in Table 1.

The median time to recurrence after operation was 21,9 months (range 4,4-132,5). The most common site was the vagina (8/19; 42 %), followed by vaginal cuff extensions (6/19; 32 %) and pelvic lymph node recurrence (7/19; 37 %) (Table 2). Some of these patients experienced a second recurrence after treatment, including para-aortic lymph node metastasis (2/19; 11 %), and liver metastasis (1/19; 5 %), and brain metastasis (1/19; 5 %). Pelvic lymphadenectomy was done for five (26 %) patients who later recurred, with recurrence sites including vaginal cuff (2/5), pelvic metastasis (1/5), para-aortic lymph node metastasis (1/5), and carcinosis with liver metastasis (1/5). One patient who had hysterectomy with ovary preservation due to young age (42 years at operation time) developed ovarian metastasis after 4.4 months postoperatively.

For recurrence treatment, four (21 %) patients received a single treatment modality (e.g. para-aortic radiotherapy, external pelvic radiotherapy or brachytherapy). The majority underwent combination treatments, with radiotherapy and hormone therapy being the most common (26 %). Of the 19 recurrent cases, four (21 %) underwent surgery, including vaginal cuff/pelvic tumor removal (11 %), adnexal/omental/pelvic/para-aortic lymph node removal (5 %), and brain tumor removal (5 %). Treatments for recurrences are detailed in Table 3. There were nine deaths in our cohort (9/25; 36 %), of which six (24 %) were related to endometrioid cancer.

Table 1
Comparison of clinicopathological characteristics of low-grade (Stage 1 A, Grade 1–2) endometrial cancer between study cohort and the TCGA PanCanAtlas cohort.

Characteristic	Study Cohort ($n = 25$)	TCGA PanCanAtlas ($n = 146$)
Age (years, median, range)	69 (42–82)	61 (31–87)
Age ≥ 60 years (%)	23 (92 %)	79 (54 %)
FIGO Stage 1 A (%)	25 (100 %)	146 (100 %)
Grade		
Grade 1	14 (56 %)	77 (53 %)
Grade 2	11 (44 %)	69 (47 %)
LVSI		
Positive	0	N/A
Negative	25 (100 %)	N/A
Lymphadenectomy		N/A
No	19 (76 %)	N/A
Pelvic	5 (20 %)	N/A
Pelvic + para-aortic	1 (4 %)	N/A
Primary tumor size (cm, median)	3.0	N/A
Adjuvant radiotherapy (%)	0	18 % (27/146)
Mean follow-up (months, range)	72 (24–167)	33 (0.4–188)
Status		
Alive	16 (64 %)	139 (95 %)
Deceased	9 (36 %)	7 (5 %)

Table 2
First recurrence sites in low-grade endometrioid cancer patients.

Recurrence Site	Number of Patients ($n = 19$)	Percentage
Vagina	8	42 %
Vaginal cuff	6	32 %
Para-iliac lymph nodes	7	37 %
Carcinosis	1	5 %
Ovary	1	5 %

Table 3
Recurrence treatments administered in the recurrence cohort.

Treatment Modality	Number of Patients ($n = 19$)	Percentage
Single treatment modality (para-aortic radiotherapy, external pelvic radiotherapy, or brachytherapy)	4	21 %
Radiotherapy and hormone therapy	5	26 %
Surgery	4	21 %
External radiotherapy and brachytherapy	2	11 %
Chemotherapy and external radiotherapy	2	11 %
Chemotherapy and hormone therapy	1	5 %
>3 different modalities (surgery, radiotherapy, chemotherapy, hormone therapy, and immunotherapy)	5	26 %

3.1. Genetic alterations in the recurrence group

Among 19 low-grade endometrioid recurrences, 509 alterations were identified using Foundation Medicine analysis (FMI). The most frequent alterations were in order of magnitude PTEN, PIK3CA, ARID1A, FGFR2 PIK3R1, NF1 and MSH3. The data was uploaded as "Protein Change Format: Gene Symbol" (e.g., NOTCH1:R1940C) into Cancer Genome Interpreter (CGI). A total of 271 protein-level alterations were identified, all of which were short variants, no copy number alterations detected. The most common mutations are shown in Fig. 1. A complete list of meaningful mutation and its protein change among 19 recurrence patients are listed in Supplementary Table 1.

ProMisE classification for the cohort is visualized in the accompanying heatmap (Fig. 2). Among the recurrence group, 11 patients (58 %) were MMRd, 7 (37 %) were MMRp, and 1 was POLE-mutated. FMI's molecular classification largely aligned with ProMisE results. One sample had a subclonal TP53-mutation by FMI but was classified as NSMP by ProMisE due to p53 immunohistochemistry (IHC). Two cases with POLE mutations were identified in the recurrence group. The first one

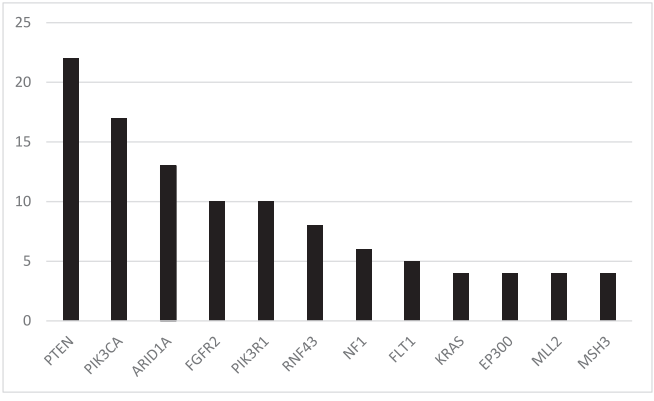

Fig. 1. The most frequent mutations identified in low-risk recurrent endometrioid cancers ($n = 19$), as determined by CGI analysis.

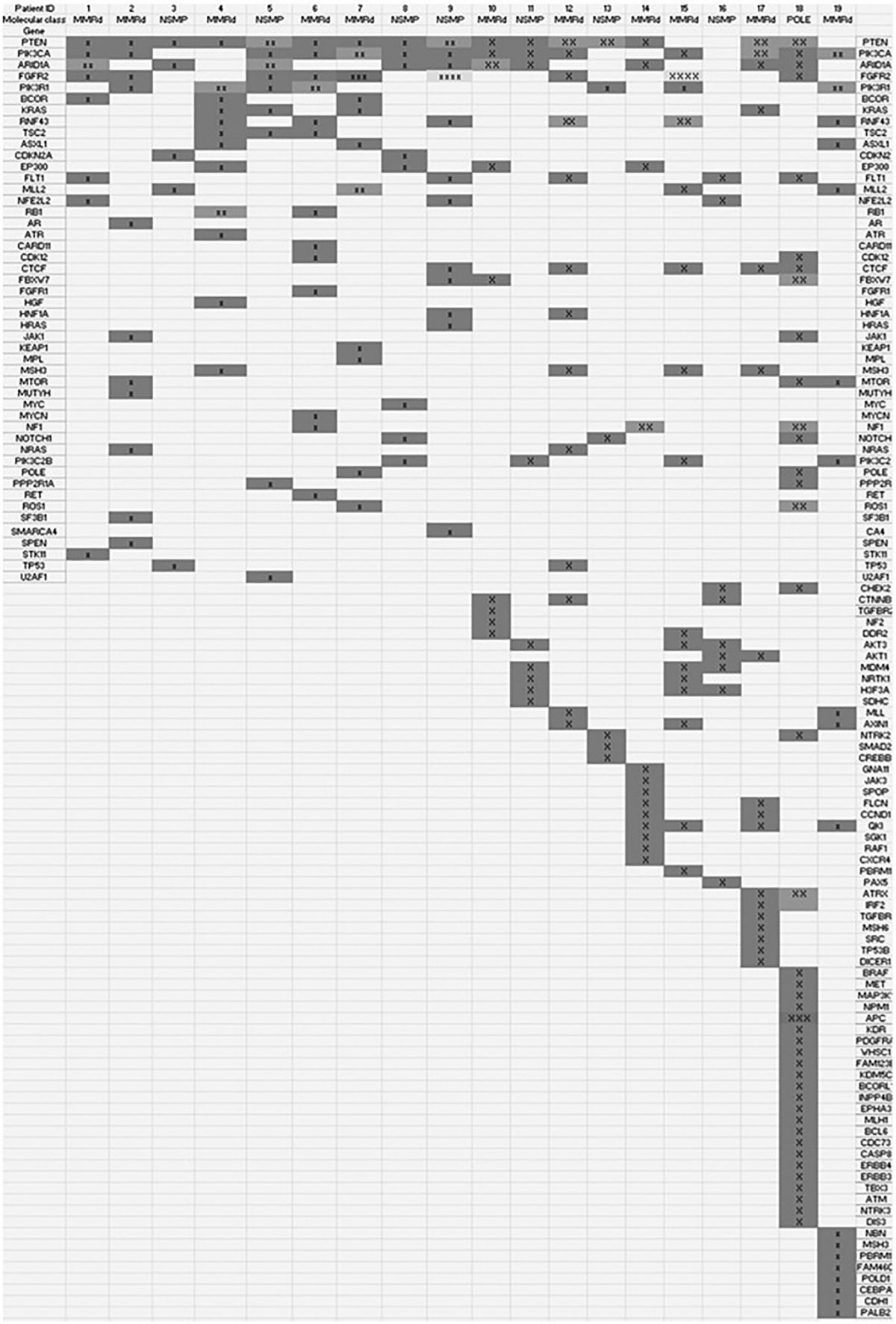

Fig. 2. Heatmap of mutations identified in 19 recurrent low-risk endometrioid uterine cancer patients.

(POLE: G1720S) was classified as an ambiguous, non-pathological variant, with the classification remaining MSI. The second case, discussed further below, involved a pathogenic POLE mutation along with an FGFR2 mutation.

A heatmap listing genetic alterations (excluding likely passengers) is displayed in Fig. 2. While no statistically significant point mutations differentiated the recurrence and non-recurrence groups, FGFR2 mutations were exclusively present in the recurrence group (9/19; 47.4 %), compared to none in the non-recurrence group (0/6; Fisher's Exact Test, $p = 0.0571$). Specific FGFR2 missense mutations, such as S252W, K659E, and N549K, were associated with gain-of-function alterations that potentially drive oncogenesis and tumor progression. Other gene alterations were distributed equally across both groups. No gene alterations were found to be protective.

Thirteen FGFR actionable mutations were identified, including eight unique driver mutations (one FGFR1 and seven FGFR2). Patients with FGFR2 mutations experienced recurrence earlier (median: 405 days; range 133–1818) compared to those without FGFR2 mutations (median: 774 days; range 195–3974), approaching statistical significance ($p = 0.057$). Progression-free survival (PFS) associated with FGFR2 mutations is shown in Fig. 3.

The FGFR2 mutation frequency in endometrioid endometrial cancer has previously been reported to range between 12 and 16 %, depending on patient age, with a tendency toward higher frequencies in higher-risk patients [19]. Due to the limitation that this study was a cohort study with a small non-recurrent control group, rather than a case-control study, we aimed to further evaluate FGFR2 mutation frequencies across all endometrioid endometrial cancer histologies in our larger cohort.

In addition to the 25 low-risk cases, we analyzed 30 patients with higher-risk endometrioid endometrial cancers, all of whom were treated with adjuvant therapy. FGFR2 driver mutations were identified in six of these higher-risk cases, including two cases that also harbored POLE mutations. The FGFR2 mutations were detected in patients with the following stages and grades: one patient with stage IA, grade 3 disease (13 %; 1/8 cases), two patients with stage IB, grade 1–2 disease (18 %; 2/11 cases), two patients with stage IIIA disease (33 %; 2/6 cases), one patient with stage IIIC1, grade 2 disease (100 %; 1/1 case), and one patient with stage IIIC2, grade 2 disease (50 %; 1/2 cases). No FGFR2 mutations were found in the single cases of stage IA disease with lymphovascular invasion (LVI+) or stage II, grade 3 disease.

The FGFR2 mutation frequency in this higher-risk group was 20 %, which is consistent with previous reports and clearly lower than the observed frequency in the recurrent low-risk group, where 9 out of 19 recurrent cases (47 %) harbored FGFR2 mutations. These findings suggest that even if a significantly larger non-recurrent control group were available, it would be unlikely to exhibit FGFR2 mutation frequencies exceeding 50 %. This supports the observation that FGFR2 mutations are specifically enriched in low-risk endometrial cancers that experience unexpected recurrence.

Loss of heterozygosity (LOH) testing to reveal potential defects in homologous DNA repair was successful in six recurrent and three non-recurrent cases. None of the tested cases was HRD-positive.

3.2. Validation

In the TCGA PanCanAtlas (created 3/2020; https://www.cbioportal.org) validation cohort of low-risk endometrioid endometrial cancer ($n = 146$), the most frequent alterations were PTEN (88 %), PIK3CA (56 %), ARID1A (52 %), CTNNB1 (44 %), and PIK3R1 (34 %). FGFR2 mutations were present in 24/146 cases (16.4 %), significantly less frequent than in our recurrence group (9/19; 47.4 %; Fisher's Exact Test, $p = 0.0039$). While limited follow-up data exists for some TCGA cases, differences in patient selection, diagnostic criteria, and follow-up practices between institutions limit the feasibility of directly comparing recurrence rates or progression-free survival between the two cohorts. Therefore, the primary comparison between the cohorts focuses on FGFR2 mutation frequency, which provides a more reliable cross-cohort measure of genomic differences.

3.3. Exceptional POLE mutation case

POLE mutated endometrial cancer is typically associated with an excellent prognosis, with rare disease-related deaths reported [20]. However, in our cohort, one patient with pathogenic POLE mutation (A456P) experienced a fatal outcome. At diagnosis, the 42-year-old had systemic lupus erythematosus (SLE) and was under immunosuppressive therapy for a kidney transplant. Following abnormal bleeding, a Grade 1

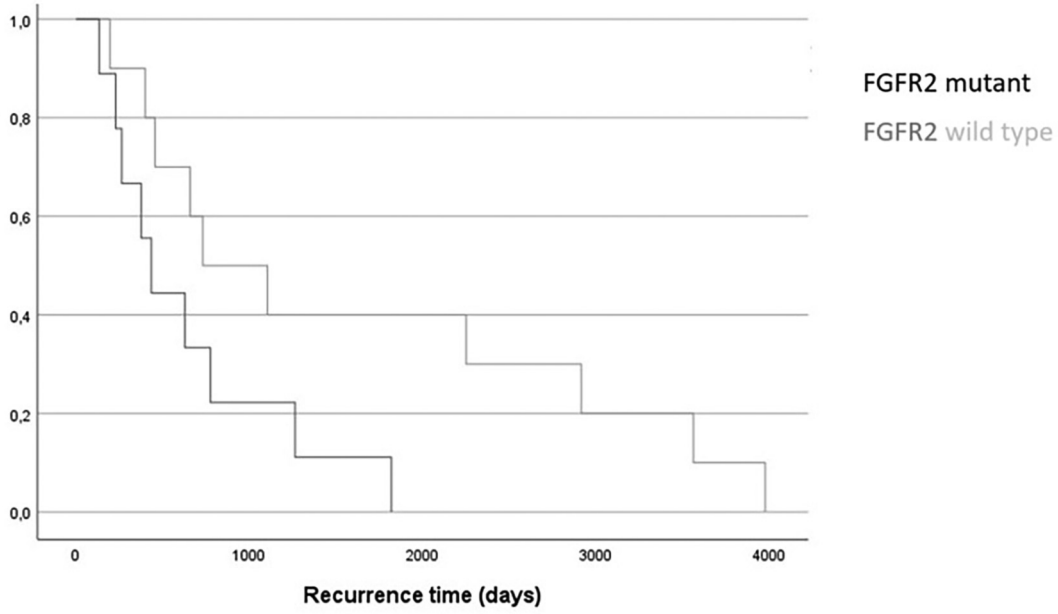

Fig. 3. Recurrence times for patients with FGFR2 mutations versus FGFR2 wild type among low-risk endometrioid cancers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Recurrence cohort: FGFR2 mutations and recurrence sites in 19 low-risk endometrioid cancer patients.

Patient	Stage	Grade	Molecular Class	Recurrence Time (days)	FGFR2 Mutations	Recurrence Site
1	1 A	2	MMRd	434	FGFR2:S252W	Vaginal cuff and para-iliac lymph node
2	1 A	2	MMRd	628	FGFR2:K659E	Inside vagina
3	1 A	1	NSMP	228	FGFR2:N549K	Inside vagina and para-iliac lymph node
4	1 A	1	MMRd	263	FGFR2:C382R, FGFR2:G301D, FGFR2:E360D	Inside vagina progressing to vaginal cuff and para-iliac lymph node
5	1 A	1	NSMP	774	FGFR2:A333G	Para-iliac lymph node
6	1 A	2	MMRd	375	FGFR2:N549K, FGFR2:R664W	Vaginal cuff
7	1 A	2	MMRd	1263	FGFR2:S252W	Inside vagina
8	1 A	1	NSMP	1818	FGFR2:N549K, FGFR2:R664W	Vaginal cuff; second recurrence to vaginal cuff and lung metastasis
9	1 A	1	NSMP	133	FGFR2:S702L	Ovarian metastasis; second recurrence to vaginal cuff and para-iliac lymph nodes
10	1 A	1	MMRd	4588	–	Inside vagina
11	1 A	1	NSMP	2647	–	Para-iliac lymph nodes
12	1 A	1	MMRd	1882	–	Inside vagina
13	1 A	1	NSMP	1825	–	Inside vagina
14	1 A	1	MMRd	750	–	Inside vagina
15	1 A	2	MMRd	4571	–	Vaginal cuff
16	1 A	1	NSMP	1528	–	Para-iliac lymph nodes
17	1 A	2	MMRd	4998	–	Carcinosis in abdomen, pleural area, intrahepatic metastasis
18	1 A	2	POLE	1448	–	Para-iliac lymph nodes
19	1 A	1	MMRd	1715	–	Vaginal cuff and pelvic side wall tumor

endometrioid adenocarcinoma with minimal myometrial invasion (1/17 mm) was diagnosed. The tumor size was 80 mm, and no LVSI was present. She had ovarian preservation due to young age, but later developed ovarian metastasis 4,4 months postoperatively. After reoperation and adjuvant treatment, she experienced bowel perforation twice and sepsis. The disease recurred in the pelvic lymph nodes, and the patient died of the disease 1,5 years after the second surgery.

3.4. Functional analysis of FGFR2 missense mutations and clinical outcome

It has previously been reported that FGFR 1–4 aberrations were found in 7.1 % of diverse 4853 diverse cancers, with 61 % being amplifications, most notably locating in FGFR1. In 80 cases of endometrial cancer, 11 % harbored FGFR abnormalities, predominantly FGFR2 [21]. In our present study, all genomic alterations in the FGFR2 were point mutations and no fusions were identified.

Two patients in our cohort harbored the S252W mutation, located in the extracellular domain of FGFR2. This alteration presents conflicting reports on its effects, with some studies suggesting loss of ligand specificity and increased activation, while others indicate a growth advantage and enhanced transformation activity compared to wild-type FGFR2 [22,23]. The first patient had recurrence in vaginal cuff and the other vaginal cuff and pelvic lymph nodes.

Another patient had the K659E mutation, a gain-of-function alteration positioned in the protein kinase domain. This mutation is associated growth advantage, transformation activity, and downstream signaling [23]. This tumor relapsed in the vagina.

Three patients had N549K mutation, also located in the protein kinase domain. This gain-of-function alteration is known to enhance growth advantage, transformation, and MAPK pathway signaling [22,24,25]. Two of these patients harbored also R664W mutation, which has uncertain function. While positioned in the protein kinase domain, R664W presents conflicting reports regarding its effects on growth advantage and autophosphorylation [23]. Among these three patients, one experienced recurrence in the vaginal cuff and obturator lymph nodes, another had an extension of a vaginal cuff tumor, and the third experienced recurrence in the vaginal cuff.

One patient with S702L mutation in exon 16 also harbored POLE mutation. The clinical significance of S702L remains unclear due to insufficient evidence. This patient's tumor recurred in the pelvic lymph nodes.

Only one patient had FGFR1 mutation (G301D) alongside an FGFR2 mutation (C382R). The recurrence in this case occurred in vaginal cuff and para-iliac lymph node.

The specific FGFR mutations and their recurrence sites are listed in Table 4.

4. Discussion

The outcome for endometrial adenocarcinoma is usually favorable, but the mortality rate for uterine cancer has increased more rapidly than the rate of incidence [26]. Treatment options for recurrent disease remain limited, and the prognosis of these patients continues to be poor despite recent advancements in targeted therapies.

In our cohort, MMRd recurrence cases accounted for 58 %, whereas the incidence of MMRd in endometrial cancer reported in the literature ranges between 17 % and 33 % [27]. The mutational landscape in endometrial cancer is broad and diverse. The reasons behind recurrences in low-risk cases remain poorly understood, but genomic profiling offers hope for identifying new insights. In endometrial cancer, multiple mutations can be identified, but the critical challenge lies in distinguishing actionable mutations from ambiguous ones. This distinction is particularly significant in p53abn and MMRp groups. While p53abn tumors are strong precursors for high-risk tumors with higher recurrence potential, MMRp is more heterogeneous and actionable mutations may play a crucial role in future recurrences. Conversely, in POLE groups, additional actionable mutations are unlikely to worsen outcomes. For example, multiple classifier groups such as POLEmut-p53abn are classified as POLEmut rather than p53abn, and MMRd-p53abn combinations are categorized as MMRd, with secondary mutations not impacting prognosis [28].

Following our comprehensive analysis, we identified 19 low-risk endometrial cancers with unexpected recurrence and examined their mutational profiles. The most common mutations among the recurrence cohort, according to CGI, were PTEN, PIK3CA, ARID1A, PIK3R1, and FGFR2 (Fig. 1). Our findings for driver mutations in low-stage recurrence versus non-recurrence groups align with previous studies [13,29], with a few notable exceptions.

One exceptional recurrence case in our cohort involved a patient with a pathogenic POLE mutation who experienced a fatal outcome. While POLE-mutated endometrial cancers are widely considered to have an excellent prognosis, with extremely low recurrence rates

reported in large prospective cohorts such as PORTEC-3 [30], rare exceptions may occur, particularly in the setting of additional clinical risk factors. In this case, the patient had systemic lupus erythematosus and was receiving immunosuppressive therapy for a prior kidney transplant, which may have contributed to the recurrence. Although this represents a single case, it highlights the need for individualized risk assessment, even in tumors typically associated with favorable outcomes.

Among our long list of genetic alterations, only FGFR2 mutations stood out as significantly enriched in relapsed cases compared to the low-risk TCGA control cohort and the non-recurrent cases in this study To address the small non-recurrent control group size, we further evaluated FGFR2 mutation frequencies separately in higher-risk endometrioid endometrial cancers treated with adjuvant therapy. In this higher-risk group, FGFR2 mutations were identified in 20 % of cases, consistent with previously published rates and substantially lower than the 47 % observed in the recurrent low-risk cohort. This additional analysis supports the notion that FGFR2 mutations are not uniformly associated with endometrial cancer risk but rather may contribute specifically to recurrence risk in low-risk cases where recurrence is unexpected.

Although recurrence in low-risk cases is rare and our sample size is small, FGFR2 alterations may play a role in tumorigenesis and progression in a subset of these cancers. Research has shown that FGFR2 mutations contribute to endometrial cancer development and progression [31]. Mutations such as C382R and N549K confer gain-of-function properties to FGFR2, enhancing cell proliferation, transformation, and oncogenic potential. These mutations disrupt normal regulatory mechanisms of cell growth, facilitating uncontrolled cell division and cancer progression. FGFR mutations often activate MAPK and PI3K/AKT signaling pathways, driving cancer cell proliferation and survival.

The FGFR2 (Fibroblast Growth Factor Receptor 2) gene plays a pivotal role in cell growth, differentiation, and repair. Alterations in this gene, including missense mutations and variations, have been extensively studied in various cancers, particularly endometrial cancer [19]. FGFR2 mutations occur in approximately 10 % to 16 % of endometrial carcinomas, particularly in the serous and endometrioid subtypes. These mutations are associated with aggressive disease and shorter progression-free survival (PFS) [19]. In previous studies, early-stage disease was categorized as stage I-II, without separate analysis of low-risk stage IA cases, as conducted in our study.

FGFR2 mutation has been linked to reduced disease-free survival (DFS) and cancer specific survival [29]. In the cohort of 386 patients having early-stage disease (stage I or II), FGFR mutation were significantly associated with shorted DSF (HR = 3.24, $p = 0.008$) and OS (HR = 2.00, $p = 0.025$). FGFR mutation also were more prevalent in well- and moderately-differentiated endometrioid tumors (grade 1:12 %, grade 2: 11 %, grade 3: 3 %) [32].

The GOG210 clinical trial included late-stage cases (stage III and IV) as well as early stage (I and II) recurrent cases and random samples from early-stage non-recurrent cases. In this study, FGFR2 mutation were more common in older patients and those with advanced-stage endometrial cancer, although this was not statistically significant ($p = 0.07$). However, progression was significantly more common in patients with FGFR mutation ($P < 0.001$). FGFR2 mutation was an independent prognostic factor for shorter PFS (HR1.9, p = 0.02) and endometrial cancer specific survival (HR2.1, $p = 0.002$) [19]. In our study, patients with FGFR2 mutations experienced earlier recurrence than those without, although the difference did not reach statistical significance ($p = 0.057$; Fig. 2).

FGFR2 alterations, particularly tumor driver mutations, are particularly intriguing, because some FGFR2 inhibitors have already received approval for treating other cancers or are under development (Committee for medicinal products for human use, European medicine agency) [33]. Preclinical studies suggest that endometrial cancer cells with FGFR2 alterations are sensitive to FGFR2 inhibition [31].

The heterogeneity of FGFR2 mutations, including R664W and S702L, highlights the complexity of targeting FGFR2 in cancer therapy. While some mutations confer a growth advantage or transformation potential, others exhibit ambiguous effects, complicating therapeutic strategies. Nonetheless, the identification of these mutations provides valuable biomarkers for the diagnosis, prognosis, and potential therapeutic targeting in endometrial cancer.

We acknowledge limitations of our study. First, the small size of the non-recurrent control group limits the statistical power to detect differences between recurrent and non-recurrent low-risk cases. However, the observed FGFR2 enrichment in recurrent cases, in combination with the lower FGFR2 frequency in both higher-risk cases and the TCGA low-risk cohort, suggests a biologically relevant association. Second, no formal sample size calculation was performed, as the study was inherently limited by the rarity of the target population. Despite these limitations, the findings point to FGFR2 mutations as a potential predictor of unexpected recurrence, warranting further validation in larger, prospectively collected cohorts.

In conclusion, our study highlights that while low-risk endometrial cancer generally has a favorable prognosis, unexpected recurrences remain a significant challenge. Genomic profiling revealed a spectrum of genetic alterations, with FGFR2 mutations prominently associated with recurrence. These mutations, characterized by gain-of-function alterations, may play a pivotal role in tumor progression and recurrence in a subset of low-risk cases. The potential for targeted therapies, such as FGFR2 inhibitors, offers a promising avenue for improving outcomes in patients with actionable FGFR2 mutations. These findings underscore the importance of integrating molecular profiling into clinical practice to enhance risk stratification and guide personalized treatment strategies for endometrial cancer.

CRediT authorship contribution statement

Tuukka Mettälä: Writing – original draft, Visualization, Validation, Project administration, Investigation, Formal analysis, Data curation. **Titta Joutsiniemi:** Writing – review & editing, Validation, Supervision, Project administration, Formal analysis. **Jutta Huvila:** Writing – review & editing, Validation, Supervision, Project administration, Formal analysis. **Sakari Hietanen:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests which may be considered as potential competing interests: T.M., T.J. and J.H. report no conflicts of interest. S.H. reports provision for materials from Roche Foundation Medicine (FMI).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2025.03.038.

References

[1] National Cancer Institute, Endometrial Cancer Treatment (PDQ®): Health Professional Version, PDQ Cancer Information Summaries [Internet], 2019.

[2] E. Vizza, G. Cutillo, V. Bruno, I. Sperduti, E. Mancini, E. Baiocco, B. Chiofalo, L. Cicchillitti, C. Certelli, A. Zampa, E. Piccione, G. Corrado, Pattern of recurrence in patients with endometrial cancer: a retrospective study, Eur. J. Surg. Oncol. (2020) https://doi.org/10.1016/j.ejso.2020.03.203.
[3] C.L. Creutzberg, W.L.J. Van Putten, P.C.M. Koper, M.L.M. Lybeert, J.J. Jobsen, C.C. Wárlám-Rodenhuis, K.A.J. De Winter, L.C.H.W. Lutgens, A.C.M. Van Den Bergh, E. Van De Steen-Banasik, H. Beerman, M. Van Lent, Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial, Lancet (2000), https://doi.org/10.1016/S0140-6736(00)02139-5.
[4] V. Loizzi, G. Cormio, M. Lorusso, D. Latorre, M. Falagario, P. Demitri, D. Scardigno, L.E. Selvaggi, The impact of lymph vascular space invasion on recurrence and survival in patients with early stage endometrial cancer, Eur. J. Cancer Care (Engl.) (2014), https://doi.org/10.1111/ecc.12115.
[5] K. Güngördük, Z.F. Cuylan, I. Kahramanoglu, T. Oge, O. Akbayir, M. Dede, S. Taşkln, N. Ozgul, T. Simsek, H. Turan, V. Gülseren, A. Ozdemir, M.M. Meydanll, A. Ayhan, Risk factors for recurrence in low-risk endometrial cancer: a case-control study, Oncol. Res. Treat. (2018), https://doi.org/10.1159/000488112.
[6] Cancer Genome Atlas Research Network, C. Kandoth, N. Schultz, A.D. Cherniack, R. Akbani, Y. Liu, H. Shen, A.G. Robertson, I. Pashtan, R. Shen, C.C. Benz, C. Yau, P.W. Laird, L. Ding, W. Zhang, G.B. Mills, R. Kucherlapati, E.R. Mardis, D.A. Levine, Integrated genomic characterization of endometrial carcinoma, Nature 497 (2013) 67–73, https://doi.org/10.1038/nature12113.
[7] Herrington C. Simon, WHO Classification of Tumours Female Genital Tumours, 5th ed. International Agency for Research on Cancer, 2020.
[8] W.-J. Koh, B.E. Greer, N.R. Abu-Rustum, S.M. Apte, S.M. Campos, J. Chan, K.R. Cho, D. Cohn, M.A. Crispens, N. Dupont, P.J. Eifel, A.N. Fader, C.M. Fisher, D.K. Gaffney, S. George, E. Han, W.K. Huh, J.R. Lurain, L. Martin, D. Mutch, S.W. Remmenga, R.K. Reynolds, W. Small, N. Teng, T. Tillmanns, F.A. Valea, N. McMillian, M. Hughes, Uterine neoplasms, version 1.2014, J. Natl. Compr. Cancer Netw. 12 (2014) 248–280, https://doi.org/10.6004/jnccn.2014.0025.
[9] N. Concin, X. Matias-Guiu, I. Vergote, D. Cibula, M.R. Mirza, S. Marnitz, J. Ledermann, T. Bosse, C. Chargari, A. Fagotti, C. Fotopoulou, A. Gonzalez Martin, S. Lax, D. Lorusso, C. Marth, P. Morice, R.A. Nout, D. O'Donnell, D. Querleu, M.R. Raspollini, J. Sehouli, A. Sturdza, A. Taylor, A. Westermann, P. Wimberger, N. Colombo, F. Planchamp, C.L. Creutzberg, ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma, Int. J. Gynecol. Cancer 31 (2021) 12–39, https://doi.org/10.1136/ijgc-2020-002230.
[10] K.C. Kurnit, G.N. Kim, B.M. Fellman, D.L. Urbauer, G.B. Mills, W. Zhang, R.R. Broaddus, CTNNB1 (beta-catenin) mutation identifies low grade, early stage endometrial cancer patients at increased risk of recurrence, Mod. Pathol. 30 (2017) 1032–1041, https://doi.org/10.1038/MODPATHOL.2017.15.
[11] T. Bosse, R.A. Nout, E. Stelloo, E. Dreef, H.W. Nijman, I.M. Jürgenliemk-Schulz, J.J. Jobsen, C.L. Creutzberg, V.T.H.B.M. Smit, L1 cell adhesion molecule is a strong predictor for distant recurrence and overall survival in early stage endometrial cancer: pooled PORTEC trial results, Eur. J. Cancer 50 (2014) 2602–2610, https://doi.org/10.1016/j.ejca.2014.07.014.
[12] E. Stelloo, R.A. Nout, E.M. Osse, I.J. Jürgenliemk-Schulz, J.J. Jobsen, L.C. Lutgens, E.M. Van Der Steen-Banasik, H.W. Nijman, H. Putter, T. Bosse, C.L. Creutzberg, V.T.H.B.M. Smit, Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC cohorts, Clin. Cancer Res. 22 (2016) 4215–4224, https://doi.org/10.1158/1078-0432.CCR-15-2878.
[13] M.R. Moroney, K.D. Davies, A.C. Wilberger, J. Sheeder, M.D. Post, A.A. Berning, C. Fisher, C. Lefkowits, S.R. Guntupalli, K. Behbakht, B.R. Corr, Molecular markers in recurrent stage I, grade 1 endometrioid endometrial cancers, Gynecol. Oncol. 153 (2019) 517–520, https://doi.org/10.1016/j.ygyno.2019.03.100.
[14] J. Huvila, K. Orte, P. Vainio, T. Mettälä, T. Joutsiniemi, S. Hietanen, Molecular subtype diagnosis of endometrial carcinoma: comparison of the next-generation sequencing panel and proactive molecular risk classifier for endometrial cancer classifier, Hum. Pathol. 111 (2021) 98–109, https://doi.org/10.1016/J.HUMPATH.2021.02.006.
[15] Website, Cancer Genome Interpreter, https://www.cancergenomeinterpreter.org; 2025.
[16] Website, Uniprot, https://www.uniprot.org;2025.
[17] Website, Clinvar, https://www.ncbi.nlm.nih.gov/clinvar/;2025.
[18] Z. Sondka, S. Bamford, C.G. Cole, S.A. Ward, I. Dunham, S.A. Forbes, The COSMIC Cancer gene census: describing genetic dysfunction across all human cancers, Nat. Rev. Cancer 18 (2018) 696–705, https://doi.org/10.1038/S41568-018-0060-1.
[19] Y.W. Jeske, S. Ali, S.A. Byron, F. Gao, R.S. Mannel, R.G. Ghebre, P.A. DiSilvestro, S.B. Lele, M.L. Pearl, A.P. Schmidt, H.A. Lankes, N.C. Ramirez, G. Rasty, M. Powell, P.J. Goodfellow, P.M. Pollock, FGFR2 mutations are associated with poor outcomes in endometrioid endometrial cancer: an NRG oncology/gynecologic oncology group study, Gynecol. Oncol. 145 (2017) 366–373, https://doi.org/10.1016/j.ygyno.2017.02.031.
[20] J.N. McAlpine, D.S. Chiu, R.A. Nout, D.N. Church, P. Schmidt, S. Lam, S. Leung, S. Bellone, A. Wong, S.Y. Brucker, C.H. Lee, B.A. Clarke, D.G. Huntsman, M.Q. Bernardini, J. Ngeow, A.D. Santin, P. Goodfellow, D.A. Levine, M. Köbel, S. Kommoss, T. Bosse, C.B. Gilks, A. Talhouk, Evaluation of treatment effects in patients with endometrial cancer and POLE mutations: an individual patient data meta-analysis, Cancer 127 (2021) 2409–2422, https://doi.org/10.1002/cncr.33516.
[21] T. Helsten, S. Elkin, E. Arthur, B.N. Tomson, J. Carter, R. Kurzrock, The FGFR landscape in cancer: analysis of 4,853 tumors by next-generation sequencing, Clin. Cancer Res. 22 (2016) 259–267, https://doi.org/10.1158/1078-0432.CCR-14-3212.
[22] A. Dutt, H.B. Salvesen, T.-H. Chen, A.H. Ramos, R.C. Onofrio, C. Hatton, R. Nicoletti, W. Winckler, R. Grewal, M. Hanna, N. Wyhs, L. Ziaugra, D.J. Richter, J. Trovik, I.B. Engelsen, I.M. Stefansson, T. Fennell, K. Cibulskis, M.C. Zody, L.A. Akslen, S. Gabriel, K.-K. Wong, W.R. Sellers, M. Meyerson, H. Greulich, Drug-sensitive FGFR2 mutations in endometrial carcinoma, Proc. Natl. Acad. Sci. USA 105 (2008) 8713–8717, https://doi.org/10.1073/pnas.0803379105.
[23] I.T. Nakamura, S. Kohsaka, M. Ikegami, H. Ikeuchi, T. Ueno, K. Li, T.S. Beyett, T. Koyama, T. Shimizu, N. Yamamoto, F. Takahashi, K. Takahashi, M.J. Eck, H. Mano, Comprehensive functional evaluation of variants of fibroblast growth factor receptor genes in cancer, NPJ Precis. Oncol. 5 (2021) 66, https://doi.org/10.1038/s41698-021-00204-0.
[24] P.K.-S. Ng, J. Li, K.J. Jeong, S. Shao, H. Chen, Y.H. Tsang, S. Sengupta, Z. Wang, V.H. Bhavana, R. Tran, S. Soewito, D.C. Minussi, D. Moreno, K. Kong, T. Dogruluk, H. Lu, J. Gao, C. Tokheim, D.C. Zhou, A.M. Johnson, J. Zeng, C.K.M. Ip, Z. Ju, M. Wester, S. Yu, Y. Li, C.P. Vellano, N. Schultz, R. Karchin, L. Ding, Y. Lu, L.W.T. Cheung, K. Chen, K.R. Shaw, F. Meric-Bernstam, K.L. Scott, S. Yi, N. Sahni, H. Liang, G.B. Mills, Systematic functional annotation of somatic mutations in cancer, Cancer Cell 33 (2018) 450–462.e10, https://doi.org/10.1016/j.ccell.2018.01.021.
[25] M.G. Gartside, H. Chen, O.A. Ibrahimi, S.A. Byron, A.V. Curtis, C.L. Wellens, A. Bengston, L.M. Yudt, A.V. Eliseenkova, J. Ma, J.A. Curtin, P. Hyder, U.L. Harper, E. Riedesel, G.J. Mann, J.M. Trent, B.C. Bastian, P.S. Meltzer, M. Mohammadi, P.M. Pollock, Loss-of-function fibroblast growth factor receptor-2 mutations in melanoma, Mol. Cancer Res. 7 (2009) 41–54, https://doi.org/10.1158/1541-7786.MCR-08-0021.
[26] P. Morice, A. Leary, C. Creutzberg, N. Abu-Rustum, E. Darai, Endometrial cancer, Lancet 387 (2016) 1094–1108, https://doi.org/10.1016/S0140-6736(15)00130-0.
[27] A. Favier, J. Varinot, C. Uzan, A. Duval, I. Brocheriou, G. Canlorbe, The role of immunohistochemistry markers in endometrial cancer with mismatch repair deficiency: a systematic review, Cancers (Basel) 14 (2022), https://doi.org/10.3390/cancers14153783.
[28] L.A. De Vitis, G. Schivardi, G. Caruso, C. Fumagalli, D. Vacirca, M.T. Achilarre, A. Aloisi, A. Garbi, V. Zanagnolo, G. Aletti, E. Guerini-Rocco, A. Mariani, A. Maggioni, M. Barberis, G. Bogani, N. Colombo, F. Multinu, I. Betella, Clinicopathological characteristics of multiple-classifier endometrial cancers: a cohort study and systematic review, Int. J. Gynecol. Cancer 34 (2024) 229–238, https://doi.org/10.1136/ijgc-2023-004864.
[29] M. Stasenko, N. Feit, S.S.K. Lee, C. Shepherd, R.A. Soslow, K.A. Cadoo, K. Alektiar, E.M. Da Silva, A.P. Martins Sebastião, M.M. Leitao, G. Gardner, P. Selenica, N.R. Abu-Rustum, B. Weigelt, J.J. Mueller, Clinical patterns and genomic profiling of recurrent € ultra-low risk' endometrial cancer, Int. J. Gynecol. Cancer 30 (2020) 717–723, https://doi.org/10.1136/ijgc-2020-001241.
[30] S.M. de Boer, M.E. Powell, L. Mileshkin, D. Katsaros, P. Bessette, C. Haie-Meder, P.B. Ottevanger, J.A. Ledermann, P. Khaw, R. D'Amico, A. Fyles, M.-H. Baron, I.M. Jürgenliemk-Schulz, H.C. Kitchener, H.W. Nijman, G. Wilson, S. Brooks, S. Gribaudo, D. Provencher, C. Hanzen, R.F. Kruitwagen, V.T.H.B.M. Smit, N. Singh, V. Do, A. Lissoni, R.A. Nout, A. Feeney, K.W. Verhoeven-Adema, H. Putter, C.L. Creutzberg, PORTEC Study Group, Adjuvant chemoradiotherapy versus radiotherapy alone in women with high-risk endometrial cancer (PORTEC-3): patterns of recurrence and post-hoc survival analysis of a randomised phase 3 trial, Lancet Oncol. 20 (2019) 1273–1285, https://doi.org/10.1016/S1470-2045(19)30395-X.
[31] A.T. Sengal, V. Bonazzi, D. Smith, C.P. Moiola, R. Lourie, R. Rogers, E. Colas, A. Gil-Moreno, S. Frentzas, N. Chetty, L. Perrin, P.M. Pollock, Endometrial cancer PDX-derived organoids (PDXOs) and PDXs with FGFR2c isoform expression are sensitive to FGFR inhibition, NPJ Precis. Oncol. 7 (2023), https://doi.org/10.1038/S41698-023-00478-6.
[32] S.A. Byron, M. Gartside, M.A. Powell, C.L. Wellens, F. Gao, D.G. Mutch, P.J. Goodfellow, P.M. Pollock, FGFR2 point mutations in 466 endometrioid endometrial tumors: relationship with MSI, KRAS, PIK3CA, CTNNB1 mutations and clinicopathological features, PLoS One 7 (2012), https://doi.org/10.1371/JOURNAL.PONE.0030801.
[33] Committee for Medicinal Products for Human Use (CHMP), https://www.ema.europa.eu/en/committees/committee-medicinal-products-human-use-chmp;2025 (accessed April 16, 2024).