https://doi.org/10.31925/farmacia.2024.5.20 **ORIGINAL ARTICLE**

IDENTIFICATION OF PHARMACOLOGICALLY RELEVANT MUTATIONS IN ENDOMETRIAL CANCER BY WHOLE-EXOME SEQUENCING OF FFPE TUMOUR SAMPLES

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Abstract

Endometrial cancer is a prevalent gynaecologic cancer, but it is far from being homogeneous. It shows an exceptionally diverse genetic background and requires highly personalised treatment plans. In this study, we performed whole-exome sequencing (WES) on formalin-fixed, paraffin-embedded (FFPE) tumour samples from 13 patients with histologically confirmed endometrial cancer in order to identify pharmacologically relevant mutations. This study focused on oncogenic and tumour-suppressor gene mutations that novel therapies might target. Variants in the PIK3CA, PTEN, KRAS, ARID1A, TP53 and mismatch repair genes (MLH1 and MSH2) were interesting. We identified 352 unique variants, with significant mutations observed in PIK3CA (8 patients), PTEN (6 patients), KRAS (4 patients) and ARID1A (5 patients). The mutations were classified as either pathogenic or likely pathogenic. These could be potential targets for existing or experimental therapies. We have found several mutations, possibly actionable by drugs like PI3K inhibitors, mTOR inhibitors and immune checkpoint inhibitors. This research demonstrates the massive potential of WES to reveal the genetic background of endometrial cancer that could help clinicians refine and personalise the treatment options of these patients.

Rezumat

Cancerul endometrial este o malignitate ginecologică frecventă, dar este departe de a avea un profil omogen. Prezintă un bagaj genetic excepțional de divers și necesită planuri de tratament personalizate. În acest studiu, am utilizat secvențierea întregului exom (WES) pe probe de tumoră fixate în formol, încorporate în parafină (FFPE) de la 13 paciente cu cancer endometrial confirmat histologic pentru a identifica mutații relevante farmacologic. Analiza a vizat oncogene cheie și gene supresoare tumorale care ar putea fi vizate de terapii noi. Variante din genele PIK3CA, PTEN, KRAS, ARID1A, TP53 și repararea nepotrivirii (MLH1 și MSH2) au fost de interes. Am identificat 352 de variante unice, cu mutații semnificative observate în PIK3CA (8 paciente), PTEN (6 paciente), KRAS (4 paciente) și ARID1A (5 paciente). Mutațiile au fost clasificate fie ca fiind patogene sau probabil patogene. Acestea ar putea fi ținte potențiale pentru terapiile existente sau experimentale. Am identificat mai multe mutații posibil acționabile de medicamente precum inhibitorii PI3K, inhibitorii mTOR și inhibitorii punctelor de control imun. Această cercetare demonstrează potențialul masiv al WES de a dezvălui bagajul genetic al cancerului endometrial care ar putea ajuta clinicienii să perfecționeze și să personalizeze opțiunile de tratament ale acestor paciente.

Keywords: endometrial cancer, personalised therapy, PI3K/AKT/mTOR pathway, mismatch repair deficiency

Introduction

One of the most frequent malignancies among the developed-world countries is endometrial cancer (EC). We are now observing an increasing number of cases across the globe, obesity being one of the leading causes of that fact [1]. Despite advances in surgery and adjuvant

chemotherapy, EC remains difficult to manage, particularly the recurrent cases. It is important to define the patient's unique molecular profile when using customised treatments, as there are unique genetic changes in each EC case.

Recent genomic studies have shown that there are distinct molecular subtypes of endometrial cancer, each with its own set of clinical results and response to treatment. These studies have been enabled by high-throughput sequencing technologies, especially by whole-exome sequencing (WES), which have allowed the investigators to perform deep-scale mutational profiling of endometrial cancer pathology and discern in great detail the spectra of driver mutations and pathways critical for tumorigenesis [3]. Since targeted therapies and precision oncology ideally require knowledge of mutation profiles, the detailed "mutation story" of endometrial cancer is very important for currently developing clinical applications [4]. A powerful tool, WES captures the coding regions of the genome, where most mutations that lead to disease are found. This makes WES appropriate for finding out oncogenic mutations. In EC, WES has shown that critical genes-like PIK3CA, PTEN, KRAS and ARID1A [5-7] are usually mutated. These genes are important in key cancer pathways, like PI3K/AKT/mTOR signalling and chromatin remodelling [8]. These studies show WES' potential in improving patient outcomes and finding targeted strategies.

Even with these advancements, WES is still not used for routine clinical practice, especially regarding the most commonly available specimens: formalin-fixed, paraffin-embedded (FFPE) tumour samples. Genomic analysis from these samples is problematic due to fixation-induced cross-linking and the degradation of DNA [9]. However, a recent robust methodology shows it is a reliable approach for genomic profiling [10].

For this investigation, we obtained WES data from FFPE tumour samples of 13 patients with EC. We aimed to find tumour mutations that might be relevant for personalised therapeutic regimens. We focused on mutations that were directly targetable with existing drugs or clinical trial eligibility, showing the potential of WES in treating EC in a personalised manner. We also wanted to investigate the potential of WES in delivering results that can be integrated into the decision-making process when treating endometrial cancer in a personalised way, as suggested by recent works [11, 12].

This paper presents extensive genomic data about the mutation landscape of EC, which has major implications for personalised therapy for this disease. We have also discussed how our findings on the use of WES can be integrated with what is already known about the mutation landscape of EC pathways to make a big step change in improving treatment outcomes for the massive number of women suffering from this disease worldwide.

Materials and Methods

Patient selection

For this study, a cohort of 13 female patients with histologically confirmed endometrial cancer was selected based on the inclusion and exclusion criteria shown in Table I, designed to ensure a representative and clinically relevant sample for WES analysis.

Table I

Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Confirmed diagnosis of endometrial cancer based on histopathological examination	Prior cancer treatment (neoadjuvant chemotherapy, radiotherapy, or hormonal therapy)
Age 40 and older	Secondary malignancies
According to the FIGO classification system, tumour stages range from early stage (stage I) to advanced stage (stage III-IV).	Poor sample quality: FFPE samples with inadequate quality (DNA degradation or contamination)
Availability of FFPE samples (sufficient tissue quantity and quality for genomic analysis)	Incomplete clinical data or insufficient follow-up data
Informed consent to participate in the study	

The study cohort of 13 patients varied in terms of the three features of the patient population that are critical to determining the effectiveness of treatment: age, tumour stage and tumour histology. The average age of patients was 60, and their ages ranged from 47 to 75. Most patients had endometrioid tumours, the most common form of endometrial cancer. Most patients had stage I disease, though some had more advanced tumours (stage II-IV).

Written informed consent was obtained from the participants. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (1975).

The study was approved by the Ethics Committee of the "Alessandrescu-Rusescu" National Institute of Mother and Child Health, Bucharest, Romania.

FFPE samples

For this study, we used FFPE tumour samples from the included patients. The standard protocols were followed to obtain sufficient quantity and quality DNA for the WES process. The samples were first fixed in 10% neutral-buffered formalin for 24 hours at room temperature to preserve tissue morphology and nucleic

acids. Next, we used the Leica Histocore Pegasus automated system (Leica Biosystems, Wetzlar, Germany) to carry out the dehydration process. The tissues were dehydrated in graded ethanol concentrations (70%, 80%, 95% and 100%) and cleared in xylene before infiltrating with paraffin. The subsequent process involved using the molten paraffin wax kept at a temperature of 60°C using the Leica Histocore Arcadia embedding system (Leica Biosystems, Wetzlar, Germany), in which the tissues were embedded. Each tumour block obtained ten sections of FFPE tissue (thickness ranging from 10 to 20 μm). The sections were then divided into two 1.5 mL Eppendorf tubes, with five sections each. This division was designed to keep the paraffin-embedded material in sufficient amounts for DNA extraction while minimising the risk of sample loss or contamination. We determined the tumour content in each FFPE sample to guarantee high-quality sequencing results. Only those sections with content greater than 30% were chosen for sequencing. The samples were sent to CeGaT GmbH (Tübingen, Germany) for the actual sequencing step, ensuring sample integrity during transport.

DNA extraction and quality control

DNA was extracted from FFPE tissue using MagMAX FFPE DNA/RNA Ultra Kit (Thermo Fisher Scientific, Waltham, MA, USA). Deparaffination of FFPE curls used a combination of CitriSolv Clearing Agent (1 mL) and heat (incubation at 50°C for 3 minutes). The samples were centrifuged at maximum speed for 2 minutes to pellet the tissue; the supernatant was carefully discarded. The tissue pellets were washed twice with 100% ethanol to remove any residual clearing agent and then dried under vacuum at 37°C for 25 minutes. Tissue was then digested with protease solution (10 μL protease in 100 μL digestion buffer per section up to 40 μm) at 55°C for 1 hour, followed by incubation at 90°C for 1 hour to reverse cross-links. Lysates were then subjected to magnetic bead-based separation. The DNA bound to the beads was washed twice with DNA wash buffer and then eluted in 50 μL of elution buffer. A NanoDrop spectro-photometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the concentration of the extracted DNA. An absorbance ratio (A260/A280) between 1.8 and 2.0 shows high purity and low protein contamination. Since a minimum of 50 ng of DNA is required for successful library preparation, we assessed the total yield and DNA concentration to ensure that it is enough for the next steps. We then checked the integrity of the DNA with the Agilent 2200 Tape-Station (Agilent Technologies, Santa Clara, CA, USA) with the Genomic DNA ScreenTape assay before we proceeded with the library preparation. A DNA Integrity Number (DIN) of 6.0 or higher was considered acceptable, showing that the DNA was of sufficient quality. All samples passed the quality control checks, resulting in DNA concentrations that showed a reassuringly wide range of 45.7 ng/μL to 134.0 ng/μL. This ensured more than enough genetic material for our purposes – library construction. We checked the quality of those libraries by looking at two essential elements: size and the absence of adapter dimers. The Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was utilised for that. The CeGaT Exome V5 kit (Twist Bioscience) was used for WES. For each sample, 50 ng of DNA was used to prepare the sequencing libraries. This involved fragmenting the DNA, ligating the adapters and enriching the exonic regions. The Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) was used for the sequencing, with paired-end reads of 2 × 101 base pairs. It generated an incredible amount of data: with an average depth across the samples of approximately 551.78 ± 27.96×, each sample generated between 181.9 million and 220.5 million reads, which yielded about 18.37 to 21.6 gigabases of sequencing data *per* sample (an average of 21.11 ± 1.04 gigabases). Analysis of the coverage showed that over 95% of the aimed exonic regions had at least 110× of depth, guaranteeing reliable variant detection. The trimmed FASTQ reads had a consistent length distribution, with more than 99% being 100 - 101 base pairs long. The Phred scores of readings were between 36 and 38, which means that the accuracy of the basic call was high. Q30, which means the number of bases with a Phred score of 30 or better, exceeded 89.78, giving confidence in the quality of the sequence. Finally, the distribution of the GC content was centred around 48 - 50%. The even distribution of reads along the exonic regions is a strong indicator that the sequencing was not biased with respect to the GC content. At the end of the process, the raw data was provided in the FASTQ format for further analysis.

Bioinformatics analysis

We have safely downloaded FASTQ files from the FFPE tumour samples from CeGaT GmbH. We then loaded them to the Geneyx Analysis platform (Geneyx Genomex Ltd., Israel), version 6, for bioinformatics processing and variant analysis. The platform uses integrated tools to assess key quality values, ensuring that sequencing data meets the standards for correctly calling variants. Following the quality control step, FASTQ files were processed using Geneyx alignment algorithms, which map the gross sequencing readings to the human reference genome (hg38). This step is critical for identifying sequences' precise genomic locations and subsequent calling of the variant. DRAGEN (Dynamic Read Analysis for GENomics) (Illumina, Inc., USA), version 3.7.5, is used by the Geneyx platform to identify unique nucleotide (SNV), inserts, deletions (indels) and structural variants in the genome's exonic regions.

Variant annotation and filtering

After calling the variants, all detected variants were annotated and filtered through the Geneyx Analysis

platform to determine the possible impact on protein function and to assess the probable combination of variants with disease. The platform uses the guidelines of the American College of Medical Genetics (ACMG) to classify variants into different categories: "pathogenic", "probably pathogenic", "variants with uncertain significance" (VUS), "probably benign" and "benign". This classification system is used by geneticists to determine which variants are responsible for a patient's disease. We have ensured the relevance of the patient's clinical status by crossing all variants with multiple databases, including ClinVar, gnomAD and COSMIC. Variants with a frequency of minor alleles (MAF) greater than 1% in the gnomAD database were classified as common and supposedly benign unless contradictory evidence has been found in other databases or scientific literature. This frequency threshold was chosen based on the principle that variants with a higher prevalence in the general population are less likely to cause rare diseases, such as cancer. We examined missense variants more carefully using in silico prediction tools. We used SIFT, PolyPhen-2 and CADD to judge better how likely these were to cause disease. A SIFT score of < 0.05 flags a variant as likely to be deleterious. PolyPhen-2 uses a similar flagging mechanism but has a threshold of > 0.85 for a "probably damaging" score. CADD uses a different scoring scale; it flags a variant as damaging if it assigns a PHRED-scaled score of > 15. If at least two tools predicted a variant was deleterious or classified as VUS, we flagged it for further investigation, especially if they occurred in genes relevant to endometrial cancer.

Data security and compliance

The analysis involved strict data security protocols, guaranteeing adherence to the GDPR and other pertinent data protection laws. All data were stored securely and at an appropriate access level in a cloud environment.

Results and Discussion

The analysis of WES data provided a comprehensive portrait of the genetic changes associated with this disease. We identified 352 unique variants among the study cohort, showing many genetic alterations, such as SNVs, insertions and deletions (indels) in key oncogenes and tumour suppressor genes. PIK3CA, PTEN and ARID1A were identified as frequently mutated genes. Despite their lower prevalence, mutations in the KRAS, FGFR2 and TP53 genes could be the basis for developing targeted therapies for specific patient subsets. The identified mutations were classified based on their predicted impact on protein function: 75 mutations were classified as high impact (including nonsense and frameshift mutations that likely result in truncated or nonfunctional proteins). High-impact mutations were observed in PTEN and ARID1A, known tumour suppressors. Several 210 mutations of moderate-impact mutations were found, almost all of which were missense mutations with the potential (and, in many cases, the demonstrated ability) to alter the protein landscape of the cell. These mutations were primarily found in genes well known for granting gain-of-function effects (and therefore becoming oncogenes) when they are mutated: PIK3CA and KRAS, for example. We also found 67 synonymous mutations; they supposedly do not alter the protein sequence but might affect mRNA stability or splicing. The clinical significance of the identified mutations was evaluated using the ClinVar and COSMIC databases, as well as *in silico* predictions: 120 mutations were classified as pathogenic or likely pathogenic, including well-documented mutations in PIK3CA, PTEN and KRAS that are known to cause cancer progression and are targets for existing therapies. In contrast, we found 185 mutations whose significance remains unclear and require further investigation for therapeutic value. Finally, 47 mutations were classified as benign or likely benign, suggesting they do not contribute to the oncogenic process.

Particularly in key oncogenes and tumour suppressor genes, the mutations classified as pathogenic or likely pathogenic are significant from the clinical point of view because they could be possible targets for the personalised therapy in EC patients.

The cohort's most frequently mutated oncogene was PIK3CA, with 8 out of 13 patients showing pathogenic or likely pathogenic mutations. Four patients had the well-known hotspot mutation c.3140A>G (p.His1047Arg)-a gain-of-function mutation that leads to constitutive activation of the PI3K/AKT/mTOR pathway. This pathway, in turn, promotes the growth and survival of the cells. The p.His1047Arg mutation has been associated with good responses to PI3K inhibitors. Thus, it is an appropriate target for the therapeutic intervention [13]. Another mutation, c.1633G>A (p.Glu545Lys), was identified in 2 patients; this mutation also contributes to the activation of the PI3Kpathway activation and has been linked to oncogenic transformation, being another potential target for PI3Kpathway inhibitors [14]. PIK3CA is a potent oncogene that encodes the p110α catalytic subunit of phosphoinositide 3-kinase (PI3K). PI3K, in turn, is a key enzyme in the phosphoinositide 3-kinase/AKT/mammalian target of the rapamycin (mTOR) signalling pathway, which is essential for cell survival and metabolism [15]. The presence of activating PIK3CA mutations (p.His1047Arg and p.Glu545Lys) suggests that patients may benefit from PI3K inhibitors. It was recently found that breast tumour specimens with mutations in PIK3CA were prone to respond to drugs that target the PI3K pathway, like alpelisib (PIK3CA, H1047R) and are currently approved in therapy [16]. Our results suggest the potential off-label use of PI3K inhibitors or participation in clinical trials investigating PI3K inhibition for EC with PIK3CA mutations. Moreover, investigational drugs of interest are dual

inhibitors that target both the PI3K and the mTOR pathways-buparlisib and dactolisib, for instance. They have shown the potential to provide new treatment options for patients with PIK3CA mutations and those previously unresponsive to treatment with single-agent PI3K inhibitors [17].

The PTEN tumour-suppressor gene PTEN had mutations in 6 of the 13 patients within this cohort, suggesting its involvement in the pathogenesis of EC. A well-documented pathogenic variant of the PTEN gene, c.389G>A (p.Arg130Gln), was found in 3 of the 13 patients. This type of mutation typically leads to loss of PTEN protein function [18]. Normally, PTEN functions as a negative regulator of the PI3K/AKT [19] and inactivation of the PTEN protein leads to uncontrolled cellular proliferation and survival. The nonsense mutation c.697C>T (p.Arg233) was identified in two patients, resulting in a nonfunctional PTEN protein. This is a key event in initiating many cancers [20]. PTEN inactivation leads to upregulation of PI3K/AKT/mTOR signalling [19]. This highlights the potential efficacy of inhibitors targeting components of the PI3K/AKT/mTOR pathway, such as AKT inhibitors (*e.g.*, capivasertib) [21] and mTOR inhibitors (*e.g.*, everolimus) [22] that are currently being evaluated in clinical trials for various cancers [19]. In addition, using therapies that combine different treatments and target multiple nodes of the PI3K/AKT/mTOR pathway may be a good way to overcome resistance mechanisms and find a lasting benefit in cancers with a mutation in the PTEN gene.

The ARID1A gene encodes a protein part of the SWI/SNF chromatin-remodelling complex. Our cohort identified ARID1A mutations in 5 out of 13 patients, and 3 were classified as pathogenic. Two patients had a frameshift mutation, c.2208del (p.Glu736Aspfs15), which led to a truncated protein and subsequent loss of the ARID1A gene function. Mutations of ARID1A are frequently associated with a poor prognosis and are considered markers of aggressive tumour behaviour [23]. The c.4432C>T (p.Arg1478) nonsense mutation was identified in 1 patient. This mutation causes early termination of the ARID1A protein, disrupting its role in chromatin remodelling and transcriptional regulation [24]. ARID1A is a subunit of the Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodelling complex that regulates the structure of chromatin and, in turn, gene expression [25]. When ARID1A is mutated, DNA damage repair defects result, making these tumours susceptible to synthetic lethality approaches. For example, an ARID1A deficiency renders some cancers sensitive to drugs that inhibit alternative pathways of DNA repair-most notably, to inhibitors of the ataxia telangiectasia and Rad3-related (ATR) protein (*e.g.*, berzosertib) [26] and to inhibitors of the poly(ADP-ribose) polymerase (PARP) protein (*e.g.*, olaparib) [27]. In addition, preclinical studies indicate that EZH2 inhibitors – such as tazemetostat – might work on cancers that lack ARID1A, targeting the faulty epigenetic instructions that result when the SWI/SNF complex does not function properly [28].

The RAS/RAF/MEK/ERK signalling pathway, which controls cell survival and proliferation, has KRAS in a central role [29]. Traditionally, small molecules to drug KRAS were thought impossible to find because of the high affinity of the protein for GTP/GDP and the absence of suitable pockets in the protein into which small molecules could fit and thereby inhibit the protein. We now know of inhibitors (sotorasib and adagrasib) that block a mutant form of the protein (KRAS G12C) in non-small cell lung cancer [30, 31]. While the specific KRAS mutations identified in this cohort do not include the G12C variant, ongoing research to target KRAS signalling could provide new therapeutic options for these patients. Moreover, targeting the downstream components of the RAS/RAF/MEK/ERK pathway with MEK inhibitors (*e.g.*, trametinib) and ERK inhibitors is being explored as a second alternative for KRAS-mutant tumours [32]. In our study, 4 out of the 13 patients had pathogenic mutations in the KRAS oncogene, suggesting the involvement of the RAS/MAPK signalling pathway in the pathogenesis of EC. The c.35G>A (p.Gly12Asp) mutation was found in 3 patients and is among the most common KRAS mutations, leading to activation of the RAS/MAPK pathway, which controls the cell proliferation and survival. Targeting KRAS mutations has been challenging, but new therapies, such as pan-KRAS inhibitors, are promising [33]. Despite being less common, the c.34G>T (p.Gly12Cys) mutation was seen in 1 patient and is associated with more aggressive disease phenotypes.

We found mutations in the TP53 gene in 3 of our patients. Interestingly, 2 patients had the same mutation, c.818G>A (p.Arg273His). This particular alteration is known to severely disrupt p53's DNA-binding capabilities and its ability to regulate the cell cycle and apoptosis. Other studies have associated this TP53 mutation with aggressive tumour phenotypes and poor prognosis [34, 35]. We found a different mutation (c.637C>T [p.Arg213]*) in one patient with a nonsense mutation, leading to a nonfunctional p53 protein. TP53 mutations can contribute to the resistance against therapy and also complicate treatment. They do so by not infrequently occurring in combination with other cancer-related mutations. Because of this, the therapies developed for EC must account for the effects of mutant p53 and the way these affect the cancer behaviour. One approach aims to restore the normal p53 function in cancer cells using small molecules. One such molecule, APR-246 (eprenetapopt), is currently being tested in clinical trials [36, 37]. Future studies might involve the study of the co-occurrence of TP53 mutations with other genomic alterations in EC [38].

Two of the thirteen patients in our study had mutations in the MMR (mismatch repair) genes. One patient had the c.793C>T (p.Arg265*) mutation in the MLH1 gene. This mutation results in a loss of function of the MLH1 protein, which is key for the MMR process. Nonfunctional MLH1 is strongly correlated with developing tumours high in microsatellite instability (MSI) and have an elevated chance of responding to immune checkpoint inhibitors [39, 40]. The splice site mutation MSH2 c.942+3A>T was found in a different patient. This defect is expected to alter the normal splicing of the MSH2 gene, leading to loss of function and MSI phenotype. Tumours with a high MSI status, driven by deficiencies in MLH1 and MSH2, show a high response rate to immune checkpoint inhibitors. Pembrolizumab and nivolumab were approved for treating these cancers [42] and are promising options for EC patients with these mutations. Table II presents a detailed summary of clinically significant somatic mutations identified through WES in EC patients and outlines their therapeutic implications. It lists both existing and investigational therapies that target the affected pathways and potential therapeutic targets.

Table II

Comprehensive overview of key somatic variants identified in endometrial cancer patients and their therapeutic implications

Gene	Variant (HGVSC)	Protein change (HGVSP)	Patients	Functional impact	Therapeutic implication	Existing/ Investigational therapies	Potential therapeutic target
PIK3CA	c.3140A>G	p.His1047Arg	4	Activating	Activation of PI3K/AKT/mTOR pathway	PI3K inhibitors (*e.g.*, alpelisib), dual PI3K/mTOR inhibitors	PI3K/AKT/mTOR pathway
PIK3CA	c.1633G>A	p.Glu545Lys	2	Activating	Activation of PI3K/AKT/mTOR pathway	PI3K inhibitors (*e.g.*, alpelisib), dual PI3K/mTOR inhibitors	PI3K/AKT/mTOR pathway
PTEN	c.389G>A	p.Arg130Gln	3	Loss of function	Loss of PI3K/AKT/mTOR pathway regulation	mTOR inhibitors (*e.g.*, everolimus), AKT inhibitors (*e.g.*, capivasertib)	PI3K/AKT/mTOR pathway
PTEN	c.697C>T	p.Arg233*	2	Loss of function	Loss of PI3K/AKT/mTOR pathway regulation	mTOR inhibitors (*e.g.*, everolimus), AKT inhibitors (*e.g.*, capivasertib)	PI3K/AKT/mTOR pathway
ARID1A	c.2208del	p.Glu736Aspfs15*	2	Loss of function	Defects in chromatin remodelling	ATR inhibitors, EZH2 inhibitors (*e.g.*, tazemetostat)	Chromatin remodelling, DNA damage response
ARID1A	c.4432C>T	p.Arg1478*	1	Loss of function	Defects in chromatin remodelling	ATR inhibitors, EZH2 inhibitors (*e.g.*, tazemetostat)	Chromatin remodelling, DNA damage response
KRAS	c.35G>A	p.Gly12Asp	3	Activating	Activation of RAS/RAF/MEK/ERK pathway	MEK inhibitors (*e.g.*, trametinib), emerging KRAS inhibitors	RAS/RAF/MEK/ERK pathway
KRAS	c.34G>T	p.Gly12Cys	1	Activating	Activation of RAS/RAF/MEK/ERK pathway	MEK inhibitors (*e.g.*, trametinib), emerging KRAS inhibitors	RAS/RAF/MEK/ERK pathway
TP53	c.818G>A	p.Arg273His	2	Loss of function	Impaired tumour suppression	APR-246 (eprenetapopt), WEE1 inhibitors	Cell cycle control, DNA damage response
TP53	c.637C>T	p.Arg213*	1	Loss of function	Impaired tumour suppression	APR-246 (eprenetapopt), WEE1 inhibitors	Cell cycle control, DNA damage response
MLH1	c.793C>T	p.Arg265*	1	Loss of function	Microsatellite instability (MSI)	Immune checkpoint inhibitors (*e.g.*, pembrolizumab)	DNA mismatch repair, Immunotherapy
MSH2	c.942+3A>T	--	1	Splicing disruption	Microsatellite instability (MSI)	Immune checkpoint inhibitors (*e.g.*, pembrolizumab)	DNA mismatch repair, Immunotherapy

Implications for personalised medicine

This study shows how impactful WES could be in managing EC patients. It allows for the kind of comprehensive mutation identification necessary for precision medicine that we have not had at our disposal in the past. The discovery of recurring mutations in genes like PIK3CA, PTEN, KRAS and TP53 underscores the importance of these oncogenic pathways in the genesis of EC [43]. These genes are essential to normal cellular functions, especially those related to cellular transformation, *i.e.*, growth, survival and apoptosis. Their variants may lead to the dysregulation of these processes. For example, we found PIK3CA mutations in 8 of the 13 patients. When PIK3CA is mutated, it activates the PI3K/AKT/mTOR pathway – a central hub in a signalling network that, under normal circumstances, promotes growth and inhibits apoptosis [8]. PI3K pathway inhibitors, like alpelisib, are potentially effective agents for precisely treating patients with such mutations. Alpelisib has already shown promise in treating breast cancers with PIK3CA mutations, and its efficacy in EC is an area of active investigation [13].

Six patients carried mutations in PTEN, which, as a tumour suppressor, normally inhibits the PI3K/AKT/mTOR pathway [44]. PTEN loss leads to excessive activation of the pathway, which promotes carcinogenesis and could associate resistance to targeted therapies. Nonetheless, this also creates an opportunity for combination treatments that aim at several targets along the pathway. For instance, we might combine PI3K inhibitors with mTOR or AKT inhibitors, which could help us overcome resistance mechanisms and elicit a more substantial and durable patient response [19]. From this perspective, WES can identify gene mutations across a pathway. This is useful when trying to understand how to combine therapies to provide a personalised treatment for a patient.

Mutations in KRAS were also identified. The KRAS gene is classically viewed as "undruggable" due to its lack of suitable binding sites for small molecules. However, this perception has started to change with the development of the first KRAS (G12C) inhibitors [31]. The KRAS mutations identified in our cohort do not include the G12C variant. However, ongoing research into pan-KRAS inhibitors and other KRAS signalling pathway strategies reinforces the importance of identifying KRAS mutations with WES [45]. Knowing about even rare or less common mutations that might become actionable in the future enables us to offer more targeted therapies to EC patients.

Another important takeaway from this study is that it reveals mutations in certain MMR genes, most notably MLH1 and MSH2. MMR-deficient tumours exhibit MSI, a condition that not only has prognostic implications in many cancer types but is also strongly associated with a high likelihood of response to various forms of immunotherapy, particularly immune checkpoint inhibitors such as pembrolizumab and nivolumab [40]. Identifying tumours deficient in the MMR system through WES can help allocate likely immunotherapy candidates, which could be difficult using only traditional histopathological analysis. Including WES in the diagnostic process guarantees that every patient likely to gain from immunotherapy is recognised. This is especially timely in EC, where microsatellite instability is relatively common [41].

The comprehensive mutational profiling performed by WES allows the patient to be matched more precisely to the clinical trials currently enrolled. This is especially true for patients with rare mutations, who do not have many standard treatment options. The actionable mutations, plus the ability to identify the most effective therapy for a given patient, give WES the potential to improve patient outcomes with cutting-edge therapies [46]. WES can potentially enhance patient outcomes and minimise the often-used trial-and-error approach in oncology. As WES becomes more common, maintaining and enhancing the interpretative quality of genomic data is essential. Oncologists and other healthcare providers must be ready to use genomic information within clinical decision-making frameworks.

Our study adds to the expanding literature on the genetic background of EC, especially for identifying pathogenic and pharmacologically relevant mutations [47, 48]. Multiple investigations have reliably pinpointed PIK3CA, PTEN and KRAS as the genes most frequently mutated in EC [49-51]. For our cohort, these findings hold. The Cancer Genome Atlas (TCGA) project undertook a thorough molecular profiling of EC and found that about 53% of EC samples had mutations in the PTEN gene [3]. Our small-study cohort found PIK3CA mutations in 8 and PTEN mutations in 6 patients out of 13. The crucial part played by the PI3K/AKT/mTOR pathway in EC is reinforced by our results. They indicate that targeted therapies directed at this pathway could prove beneficial. Similarly, our study's KRAS mutation percentage corresponds with earlier investigations that found about 35% of EC possessed KRAS mutations [52]. Looking at our cohort of 13 patients, we found that 4 had KRAS mutations, highlighting the relevance of this gene in EC cases.

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Although our results are consistent with earlier studies, they provide new perspectives regarding mutations in the ARID1A gene and their effects on potential therapeutic strategies. According to the TCGA project, ARID1A is mutated in about 26% of endometrioid EC and plays a significant role in chromatin remodelling and tumour suppression in this and other cancers [3]. Our study found that 5 of the patients we examined had mutations in the ARID1A gene. Of these, three were pathogenic. Our findings suggest that ARID1A-deficient tumours could be good candidates for epigenetic therapies, such as the EZH2 inhibitors currently tested in clinical trials [28]. Furthermore, the identification in our study of mutations in MMR genes – specifically MLH1 and MSH2 – that are actionable mirrors what other research has found, linking MMR deficiency with MSI and immunotherapy efficiency [41]. Previous studies have demonstrated that cases of EC with high MSI and frequent mutations in the MMR genes are likely to respond to immune checkpoint inhibitors [40, 41, 53, 54]. This is particularly the case for mutations in MLH1 and MSH2, which we found also in our patient cohort, supporting the use of immunotherapy in these patients.

However, our study identified many variants of uncertain significance (VUS). This is a recurring problem with WES analysis, which often leads to difficulties in interpreting genetic variants' functional importance. Although large-scale projects like TCGA have tried to catalogue alterations found in many different types of cancer [3], future investigations should concentrate on defining the function of these VUS to elucidate their role in cancer and to judge their worth as potential therapeutic targets.

The ongoing work to include WES in personalised medicine [46, 55] can draw on this study. We detailed the mutations found in EC patients, and, more importantly, we pinpointed those that are actionable and can steer targeted therapy. Our findings are consistent with previous research, which attests to the importance of WES in showing genetic changes that have clear relevance to patient care [56]. Finding new mutations of interest to pharmacology shows how WES can shed light on new therapeutic opportunities as the cancer genome progresses and the understanding of tumour biology improves. Although this analysis found certain mutations that could have implications for therapy, we would like to point out that the treatment suggestions are based on what is currently known rather than what was revealed by this particular study. The potential treatment significance of these mutations arises from studies that have linked comparable genetic alterations to targeted therapies or drug responses in other circumstances. Therefore, although our findings establish a basis for additional investigation, clinical validation is still necessary to define the effectiveness and relevance of these treatment recommendations in EC patients.

Limitations

This research examines EC's genetic background and WES's ability to provide personalised treatments. However, it has some clear limitations. The small sample size restricts how far-reaching the results can be applied. The very similar demographics of the study population do not demonstrate the potential for providing relevant treatments for a wide variety of EC patients. Using FFPE samples could be a challenge because of DNA degradation and difficulties that can lead to mistaken identity of variants. Also, we found many variants of uncertain significance whose clinical importance remains unclear; therefore, future research should involve larger groups and consider whole-genome sequencing for a more complete picture, especially since we focused only on coding regions, which could involve the missed potential of non-coding mutations. Moreover, the lack of long-term data limits our understanding of patient outcomes.

Future directions

We must extend our research into much larger, more diverse populations to ensure that the identified mutations are both significant and relevant. Future work should directly correlate the gene variants identified in our studies with the precision medicine impact they could have. We may also be able to integrate our work with transcriptomics and proteomics to reveal new treatment options and provide better insights into tumour biology. Of course, clinical trials must be conducted to verify the effectiveness of the therapy suggested by WES data.

Conclusions

Our study underscores the potential of whole-exome sequencing for identifying mutations in endometrial cancer that may direct therapeutic strategies. Key actionable mutations were found in various genes. PI3K inhibitors (*e.g.*, alpelisib) and mTOR inhibitors (*e.g.*, everolimus) target mutations in the PI3K/AKT/mTOR pathway, while EZH2 inhibitors (*e.g.*, tazemetostat) are promising for ARID1A mutations. Emerging inhibitors such as trametinib show new options for KRAS mutations, and immune checkpoint inhibitors such as pembrolizumab could be effective in certain tumours that show deficiencies in the mismatch repair process. In the era of precision oncology, targeted therapies are needed, and advanced

tools such as whole-exome sequencing could help enhance the standard of care for cancer patients.

Conflict of interest

The authors declare no conflict of interest.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.*, 2018; 68(6): 394-424.
2. Talhouk A, McAlpine JN, New classification of endometrial cancers: the development and potential applications of genomic-based classification in research and clinical care. *Gynecol Oncol Res Pract.*, 2016; 3: 14.
3. Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, Benz CC, Yau C, Laird PW, Ding L, Zhang W, Mills GB, Kucherlapati R, Mardis ER, Levine DA, Integrated genomic characterization of endometrial carcinoma. *Nature*, 2013; 497(7447): 67-73.
4. Soberanis Pina P, Lheureux S, Novel molecular targets in endometrial cancer: mechanisms and perspectives for therapy. *Biologics*, 2024; 18: 79-93.
5. Matrai CE, Ohara K, Eng KW, Glynn SM, Chandra P, Chatterjee-Paer S, Motanagh S, Mirabelli S, Kurtis B, He B, Sigaras A, Gupta D, Chapman-Davis E, Holcomb K, Sboner A, Elemento O, Ellenson LH, Mosquera JM, Molecular evaluation of low-grade low-stage endometrial cancer with and without recurrence. *Int J Gynecol Pathol.*, 2022; 41(3): 207-219.
6. McConechy MK, Ding J, Cheang MC, Wiegand K, Senz J, Tone A, Yang W, Prentice L, Tse K, Zeng T, McDonald H, Schmidt AP, Mutch DG, McAlpine JN, Hirst M, Shah SP, Lee CH, Goodfellow PJ, Gilks CB, Huntsman DG, Use of mutation profiles to refine the classification of endometrial carcinomas. *J Pathol.*, 2012; 228(1): 20-30.
7. Hollis RL, Thomson JP, Stanley B, Churchman M, Meynert AM, Rye T, Bartos C, Iida Y, Croy I, Mackean M, Nussey F, Okamoto A, Semple CA, Gourley C, Herrington CS, Molecular stratification of endometrioid ovarian carcinoma predicts clinical outcome. *Nat Commun.*, 2020; 11(1): 4995.
8. Slomovitz BM, Coleman RL, The PI3K/AKT/mTOR pathway as a therapeutic target in endometrial cancer. *Clin Cancer Res.*, 2012; 18(21): 5856-5864.
9. Steiert TA, Parra G, Gut M, Arnold N, Trotta JR, Tonda R, Moussy A, Gerber Z, Abuja PM, Zatloukal K, Röcken C, Folseraas T, Grimsrud MM, Vogel A, Goeppert B, Roessler S, Hinz S, Schafmayer C, Rosenstiel P, Deleuze JF, Gut IG, Franke A, Forster M, A critical spotlight on the paradigms of FFPE-DNA sequencing. *Nucleic Acids Res.*, 2023; 51(14): 7143-7162.
10. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, Gabriel S, Meyerson M, Lander ES, Getz G, Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol.*, 2013; 31(3): 213-219.
11. Madhavan S, Subramaniam S, Brown TD, Chen JL, Art and challenges of precision medicine: interpreting and integrating genomic data into clinical practice. *Am Soc Clin Oncol Educ Book.*, 2018; 38: 546-553.
12. Ilhan M, Battal A, Kaptaner B, Dogan A, FATIH Donmez F, Yilmaz M, Eroglu H, Exploring the ameliorative effects of Hypericum scabrum L. on a surgically-induced endometriosis rat model and its phytochemical profiling by LC-MS/MS. *Farmacia*, 2023; 71(4): 710-721.
13. Vasan N, Razavi P, Johnson JL, Shao H, Shah H, Antoine A, Ladewig E, Gorelick A, Lin TY, Toska E, Xu G, Kazmi A, Chang MT, Taylor BS, Dickler MN, Jhaveri K, Chandarlapaty S, Rabadan R, Reznik E, Smith ML, Sebra R, Schimmoller F, Wilson TR, Friedman LS, Cantley LC, Scaltriti M, Baselga J, Double PIK3CA mutations in cis increase oncogenicity and sensitivity to PI3Kα inhibitors. *Science*, 2019; 366(6466): 714-723.
14. Rivière JB, Mirzaa GM, O'Roak BJ, Beddaoui M, Alcantara D, Conway RL, St-Onge J, Schwartzentruber JA, Gripp KW, Nikkel SM, Worthylake T, Sullivan CT, Ward TR, Butler HE, Kramer NA, Albrecht B, Armour CM, Armstrong L, Caluseriu O, Cytrynbaum C, Drolet BA, Innes AM, Lauzon JL, Lin AE, Mancini GM, Meschino WS, Reggin JD, Saggar AK, Lerman-Sagie T, Uyanik G, Weksberg R, Zirn B, Beaulieu CL, Finding of Rare Disease Genes (FORGE) Canada Consortium, Majewski J, Bulman DE, O'Driscoll M, Shendure J, Graham JM Jr, Boycott KM, Dobyns WB, *De novo* germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. *Nat Genet.*, 2012; 44(8): 934-940.
15. Lai K, Killingsworth MC, Lee CS, Gene of the month: PIK3CA. *J Clin Pathol.*, 2015; 68(4): 253-257.
16. Chang DY, Ma WL, Lu YS, Role of alpelisib in the treatment of PIK3CA-mutated breast cancer: patient selection and clinical perspectives. *Ther Clin Risk Manag.*, 2021; 17: 193-207.
17. Peng Y, Wang Y, Zhou C, Mei W, Zeng C, PI3K/Akt/mTOR pathway and its role in cancer therapeutics: are we making headway?. *Front Oncol.*, 2022; 12: 819128.
18. Cavaillé M, Crampon D, Achim V, Bubien V, Uhrhammer N, Privat M, Ponelle-Chachuat F, Gay-Bellile M, Lepage M, Ouedraogo ZG, Jones N, Bidet Y, Sevenet N, Bignon YJ, Diagnosis of PTEN mosaicism: the relevance of additional tumour DNA sequencing. A case report and review of the literature. *BMC Med Genomics.*, 2023; 16(1): 166.
19. Janku F, Hong DS, Fu S, Piha-Paul SA, Naing A, Falchook GS, Tsimberidou AM, Stepanek VM, Moulder SL, Lee JJ, Luthra R, Zinner RG, Broaddus RR, Wheler JJ, Kurzrock R, Assessing PIK3CA and PTEN in early-phase trials with PI3K/AKT/mTOR inhibitors. *Cell Rep.*, 2014; 6(2): 377-387.
20. Gbelcová H, Gergely L, Šišovský V, Straka Ľ, Böhmer D, Pastoráková A, Sušienková K, Repiská V, Korbeľ M, Danihel Ľ, Priščáková P, PTEN mutations as predictive marker for the high-grade endometrial

cancer development in Slovak women. *Physiol Res.*, 2022; 71(Suppl 1): S125-S135.
21. Smyth LM, Batist G, Meric-Bernstam F, Kabos P, Spanggaard I, Lluch A, Jhaveri K, Varga A, Wong A, Schram AM, Ambrose H, Carr TH, de Bruin EC, Salinas-Souza C, Foxley A, Hauser J, Lindemann JPO, Maudsley R, McEwen R, Moschetta M, Nikolaou M, Schiavon G, Razavi P, Banerji U, Baselga J, Hyman DM, Chandarlapaty S, Selective AKT kinase inhibitor capivasertib in combination with fulvestrant in PTEN-mutant ER-positive metastatic breast cancer. *NPJ Breast Cancer*, 2021; 7(1): 44.
22. Ciołczyk-Wierzbicka D, Gil D, Zarzycka M, Laidler P, mTOR inhibitor everolimus reduces invasiveness of melanoma cells. *Hum Cell.*, 2020; 33(1): 88-97.
23. Lu S, Duan R, Cong L, Song Y, The effects of ARID1A mutation in gastric cancer and its significance for treatment. *Cancer Cell Int.*, 2023; 23(1): 296.
24. Odnokoz O, Wavelet-Vermuse C, Hophan SL, Bulun S, Wan Y, ARID1 proteins: from transcriptional and post-translational regulation to carcinogenesis and potential therapeutics. *Epigenomics*, 2021; 13(10): 809-823.
25. Blümli S, Wiechens N, Wu MY, Singh V, Gierlinski M, Schweikert G, Gilbert N, Naughton C, Sundaramoorthy R, Varghese J, Gourlay R, Soares R, Clark D, Owen-Hughes T, Acute depletion of the ARID1A subunit of SWI/SNF complexes reveals distinct pathways for activation and repression of transcription. *Cell Rep.*, 2021; 37(5): 109943.
26. Telli ML, Tolaney SM, Shapiro GI, Middleton M, Lord SR, Arkenau HT, Tutt A, Abramson V, Dean E, Haddad TC, Wesolowski R, Ferrer-Playan J, Goddemeier T, Grombacher T, Dong J, Fleuranceau-Morel P, Diaz-Padilla I, Plummer R, Phase 1b study of berzosertib and cisplatin in patients with advanced triple-negative breast cancer. *NPJ Breast Cancer*, 2022; 8(1): 45.
27. Xu S, Tang C, The role of ARID1A in tumors: tumor initiation or tumor suppression?. *Front Oncol.*, 2021; 11: 745187.
28. Bitler BG, Aird KM, Garipov A, Li H, Amatangelo M, Kossenkov AV, Schultz DC, Liu Q, Shih IeM, Conejo-Garcia JR, Speicher DW, Zhang R, Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. *Nat Med.*, 2015; 21(3): 231-238.
29. Kim HJ, Lee HN, Jeong MS, Jang SB, Oncogenic KRAS: signaling and drug resistance. *Cancers (Basel)*, 2021; 13(22): 5599.
30. Koga T, Suda K, Fujino T, Ohara S, Hamada A, Nishino M, Chiba M, Shimoji M, Takemoto T, Arita T, Gmachl M, Hofmann MH, Soh J, Mitsudomi T, KRAS secondary mutations that confer acquired resistance to KRAS G12C inhibitors, sotorasib and adagrasib, and overcoming strategies: insights from *in vitro* experiments. *J Thorac Oncol.*, 2021; 16(8): 1321-1332.
31. Skoulidis F, Li BT, Dy GK, Price TJ, Falchook GS, Wolf J, Italiano A, Schuler M, Borghaei H, Barlesi F, Kato T, Curioni-Fontecedro A, Sacher A, Spira A, Ramalingam SS, Takahashi T, Besse B, Anderson A, Ang A, Tran Q, Mather O, Henary H, Ngarmchamnanrith G, Friberg G, Velcheti V, Govindan R, Sotorasib for lung cancers with KRAS p.G12C mutation. *N Engl J Med.*, 2021; 384(25): 2371-2381.
32. Farnsworth DA, Inoue Y, Johnson FD, de Rappard-Yuswack G, Lu D, Shi R, Ma LIJ, Mattar MS, Somwar R, Ladanyi M, Unni AM, Lockwood WW, MEK inhibitor resistance in lung adenocarcinoma is associated with addiction to sustained ERK suppression. *NPJ Precis Oncol.*, 2022; 6(1): 88.
33. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ, Drugging the undruggable RAS: mission possible?. *Nat Rev Drug Discov.*, 2014; 13(11): 828-851.
34. Eischen CM, Genome stability requires p53. *Cold Spring Harb Perspect Med.*, 2016; 6(6): a026096.
35. Borowczak J, Szczerbowski K, Maniewski M, Zdrenka M, Słupski P, Andrusewicz H, Łysik-Miśkurka J, Rutkiewicz P, Bodnar M, Szylberg Ł, The prognostic role of p53 and its correlation with CDK9 in urothelial carcinoma. *Clin Transl Oncol.*, 2023; 25(3): 830-840.
36. Sallman DA, DeZern AE, Garcia-Manero G, Steensma DP, Roboz GJ, Sekeres MA, Cluzeau T, Sweet KL, McLemore A, McGraw KL, Puskas J, Zhang L, Yao J, Mo Q, Nardelli L, Al Ali NH, Padron E, Korbel G, Attar EC, Kantarjian HM, Lancet JE, Fenaux P, List AF, Komrokji RS, Eprenetapopt (APR-246) and azacitidine in TP53-mutant myelodysplastic syndromes. *J Clin Oncol.*, 2021; 39(14): 1584-1594.
37. Mishra A, Tamari R, DeZern AE, Byrne MT, Gooptu M, Chen YB, Deeg HJ, Sallman D, Gallacher P, Wennborg A, Hickman DK, Attar EC, Fernandez HF, Eprenetapopt plus azacitidine after allogeneic hematopoietic stem-cell transplantation for TP53-mutant acute myeloid leukemia and myelodysplastic syndromes. *J Clin Oncol.*, 2022; 40(34): 3985-3993.
38. Li Q, Li Z, Luo T, Shi H, Targeting the PI3K/AKT/mTOR and RAF/MEK/ERK pathways for cancer therapy. *Mol Biomed.*, 2022; 3(1): 47.
39. Latham A, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, Middha S, Hechtman J, Zehir A, Dubard-Gault M, Tran C, Stewart C, Sheehan M, Penson A, DeLair D, Yaeger R, Vijai J, Mukherjee S, Galle J, Dickson MA, Janjigian Y, O'Reilly EM, Segal N, Saltz LB, Reidy-Lagunes D, Varghese AM, Bajorin D, Carlo MI, Cadoo K, Walsh MF, Weiser M, Aguilar JG, Klimstra DS, Diaz LA Jr, Baselga J, Zhang L, Ladanyi M, Hyman DM, Solit DB, Robson ME, Taylor BS, Offit K, Berger MF, Stadler ZK, Microsatellite instability is associated with the presence of Lynch syndrome pan-cancer. *J Clin Oncol.*, 2019; 37(4): 286-295.
40. Kavun A, Veselovsky E, Lebedeva A, Belova E, Kuznetsova O, Yakushina V, Grigoreva T, Mileyko V, Fedyanin M, Ivanov M, Microsatellite instability: a review of molecular epidemiology and implications for immune checkpoint inhibitor therapy. *Cancers (Basel)*, 2023; 15(8): 2288.
41. Lizardo DY, Kuang C, Hao S, Yu J, Huang Y, Zhang L, Immunotherapy efficacy on mismatch repair-deficient colorectal cancer: from bench to bedside. *Biochim Biophys Acta Rev Cancer.*, 2020; 1874(2): 188447.
42. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, Wong F, Azad NS, Rucki AA, Laheru D, Donehower R, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Greten TF, Duffy AG, Ciombor KK, Eyring AD, Lam BH,

Joe A, Kang SP, Holdhoff M, Danilova L, Cope L, Meyer C, Zhou S, Goldberg RM, Armstrong DK, Bever KM, Fader AN, Taube J, Housseau F, Spetzler D, Xiao N, Pardoll DM, Papadopoulos N, Kinzler KW, Eshleman JR, Vogelstein B, Anders RA, Diaz LA Jr, Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*, 2017; 357(6349): 409-413.

43. Watanabe T, Nanamiya H, Kojima M, Nomura S, Furukawa S, Soeda S, Tanaka D, Isogai T, Imai JI, Watanabe S, Fujimori K, Clinical relevance of oncogenic driver mutations identified in endometrial carcinoma. *Transl Oncol.*, 2021; 14(3): 101010.
44. Wan L, Wang Y, Li J, Wang Y, Zhang H, Inhibition of the AKT/mTOR pathway negatively regulates PTEN expression *via* miRNAs. *Acta Biochim Biophys Sin.*, 2022; 54(11): 1637-1647.
45. Kim D, Herdeis L, Rudolph D, Zhao Y, Böttcher J, Vides A, Ayala-Santos CI, Pourfarjam Y, Cuevas-Navarro A, Xue JY, Mantoulidis A, Bröker J, Wunberg T, Schaaf O, Popow J, Wolkerstorfer B, Kropatsch KG, Qu R, de Stanchina E, Sang B, Li C, McConnell DB, Kraut N, Lito P, Pan-KRAS inhibitor disables oncogenic signalling and tumour growth. *Nature*, 2023; 619(7968): 160-166.
46. Ju HY, Ho JY, Kang J, Hur SY, Kim S, Choi YJ, Han MR, Whole-exome sequencing reveals clinical potential of circulating tumor DNA from peritoneal fluid and plasma in endometrial cancer. *Cancers (Basel)*, 2022; 14(10): 2506.
47. Mustea A, Ralser DJ, Egger EK, Ziehm U, Vivas S, Brock S, Jackson D, Condic M, Rauschendorf MA, Würfel P, Dombrowski F, Otten LA, Sun P, Laib A, Cordova MC, Hartmann R, Stein MA, Koensgen D, Stope MB, Determination of endometrial cancer molecular subtypes using a whole exome-sequencing based single-method approach. *J Cancer Res Clin Oncol.*, 2024; 150(7): 367.
48. Liang H, Cheung LW, Li J, Ju Z, Yu S, Stemke-Hale K, Dogruluk T, Lu Y, Liu X, Gu C, Guo W, Scherer SE, Carter H, Westin SN, Dyer MD, Verhaak RG, Zhang F, Karchin R, Liu CG, Lu KH, Broaddus RR, Scott KL, Hennessy BT, Mills GB, Whole-exome sequencing combined with functional genomics reveals novel candidate driver cancer genes in endometrial cancer. *Genome Res.*, 2012; 22(11): 2120-2129.
49. Malentacchi F, Turrini I, Sorbi F, Projetto E, Castiglione F, Fambrini M, Petraglia F, Pillozzi S, Noci I, Pilot investigation of the mutation profile of PIK3CA/PTEN genes (PI3K pathway) in grade 3 endometrial cancer. *Oncol Rep.*, 2019; 41(3): 1560-1574.
50. Oda K, Stokoe D, Taketani Y, McCormick F, High frequency of coexistent mutations of PIK3CA and PTEN genes in endometrial carcinoma. *Cancer Res.*, 2005; 65(23): 10669-10673.
51. Cheung LW, Hennessy BT, Li J, Yu S, Myers AP, Djordjevic B, Lu Y, Stemke-Hale K, Dyer MD, Zhang F, Ju Z, Cantley LC, Scherer SE, Liang H, Lu KH, Broaddus RR, Mills GB, High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. *Cancer Discov.*, 2011; 1(2): 170-185.
52. Le Gallo M, Bell DW, The emerging genomic landscape of endometrial cancer. *Clin Chem.*, 2014; 60(1): 98-110.
53. Rizzo A, Immune checkpoint inhibitors and mismatch repair status in advanced endometrial cancer: elective affinities. *J Clin Med.*, 2022; 11(13): 3912.
54. Antill Y, Buchanan DD, Scott CL, Mismatch repair and clinical response to immune checkpoint inhibitors in endometrial cancer. *Cancer*, 2022; 128(6): 1157-1161.
55. Khan S, Focșa IO, Budișteanu M, Stoica C, Nedelea F, Bohîlțea L, Caba L, Butnariu L, Pânzaru M, Rusu C, Jurcă C, Chirita-Emandi A, Bănescu C, Abbas W, Sadeghpour A, Baig SM, Bălgrădean M, Davis EE, Exome sequencing in a Romanian Bardet-Biedl syndrome cohort revealed an overabundance of causal BBS12 variants. *Am J Med Genet A.*, 2023; 191(9): 2376-2391.
56. Suhaimi SS, Ab Mutalib NS, Jamal R, Understanding molecular landscape of endometrial cancer through next generation sequencing: what we have learned so far?. *Front Pharmacol.*, 2016; 7: 409.