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Next-Generation Sequencing in Gynaecological Tumours: The Prognostic and Predictive Value of the Most Common Mutations Found in Ovarian, Endometrial, and Cervical Tumours: Literature Review and the University Medical Centre Utrecht Next-Generation Sequencing Data

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Keywords
Next-generation sequencing · Mutation · Gynaecological tumours · Ovarian tumours · Endometrial tumours · Cervical tumours · Biomarker · Prognostic value · Predictive value

Abstract
Objective: To investigate whether next-generation sequencing (NGS) in ovarian and endometrial tumours can discover mutations with a relevant prognostic or predictive value. ***Methods:*** After a literature search, selected studies were critically appraised using the Quality in Prognostic Studies tool. Data on mutation incidence and correlations with prognostic and predictive items were extracted from relevant studies and compared to our own cohort consisting of 28 patients analysed using NGS. ***Results:*** Eight out of 739 articles were found eligible, including different tumour types. Prevalence of mutations in the *KRAS* gene ranged between 5.34 and 58.8% in ovarian cancer. Two studies showed a significant correlation between *KRAS* mutations and an improved disease free- and overall survival. Clinical data were available for 17 of our patients, mostly cases of endometrial carcinomas. *KRAS*, *PIK3CA*, *CTNNB1*, and *TP53* were the most frequently mutated genes in endometrial carcinomas, and *PTEN* and *CTNNB1* correlated with a higher FIGO stage. ***Conclusion:*** In the ovary *KRAS* mutation is associated with type I ovarian tumours (low-grade serous, mucinous, endometrioid, and clear-cell) and may seem to have a more favourable prognosis. The prognostic value of *TP53* is still controversial. In endometrial tumours, *PTEN* shows a positive correlation with better prognosis. *PIK3CA* may have a correlation with poorer prognosis. *CTNNB1* mutations in endometrial carcinomas could predict a worse prognosis.

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Introduction

In the field of cancer research, the use of next-generation sequencing (NGS) has increased in the last decade. As cancer is driven by heritable or somatic mutations, DNA sequencing has an important role in detecting new ways of diagnosing, predicting, and treatment of diverse cancers [1]. Sequencing of mutations in diverse cancers shows the potential of discovering new diagnostic, prognostic, therapeutic, or predictive mutation statuses. For melanoma, breast, and colon carcinomas, NGS is widely used to investigate new therapeutic strategies and to better predict prognosis [2–4]. In gynaecological tumours, the role of specific mutations is not yet thoroughly investigated, despite the widespread incidence, as gynaecological tumours account for an estimated 16% of all newly diagnosed carcinomas in women worldwide. For ovarian and endometrial cancer, 239,000 and 320,000 cases were newly diagnosed, and 152,000 and 76,000 deaths, respectively [5].

Because of the high incidence and mortality rates, but the establishment of new targeted/immunotherapy options, it is important to keep investigating new markers for prognosis, therapeutic options, and predictors of disease-free survival (DFS) or overall survival (OS). In the last few years, studies concerning somatic mutations and their role in personalized, targeted medicine in gynaecological cancers have been studied [6, 7], but not as thoroughly as studies relating to breast and colon carcinomas.

The aim of this study is to explore whether NGS in ovarian and endometrial tumours can discover mutations with a relevant prognostic or predictive value in the clinical setting, by giving an overview of the evidence currently available regarding the prognostic and predictive value of mutations in ovarian and endometrial tumours and by adding new clinical data from a data set of the University Medical Centre Utrecht (UMCU).

Materials and Methods

Search Strategy

A literature search was conducted on November 6, 2015, in PubMed, Embase, and Cochrane Library. Search terms "gynaecological cancer," "next generation sequencing," and "biomarker" were combined in the search engines with the associated synonyms and Mesh and Emtree terms: "sequence analysis," "ovarian neoplasms," "uterine neoplasms," "endometrial neoplasms," "fallopian tube neoplasms," and "biological markers" (online suppl. Table 1; see www.karger.com/doi/10.1159/000479797 for all online suppl. material). Duplicates were removed, and remaining articles were screened on title and abstract, and selected or rejected according to predefined exclusion criteria (Fig. 1). Subsequently, the full text of the articles was screened. Further selection was based on an analysis of the relevance of the full text for the aim of this article, according to the criteria explained below.

Eligibility Criteria and Information Sources

Studies were found eligible for inclusion if they were written in English, were original research studies with new results on mutation analysis in any type of gynaecological cancers, and had a prognostic and/or predictive study design. Mutation analysis was required to be performed using next-generation and Sanger sequencing analysis.

Studies were excluded if they had no prognostic, predictive value linked to the mutation status, or when techniques other than sequencing were used to determine the mutation status. Studies on epigenetics, gene amplification, translocations, mRNA, or protein expression were also excluded (Fig. 1).

Quality Assessment of Individual Studies

Using criteria of the Quality in Prognosis Studies tool (QUIPS) [8], the methodological quality of the remaining articles was assessed [9].

According to the QUIPS assessment tool the following 6 items were scored as low, moderate, or high risk of bias: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting. Studies that scored a low risk of bias at 3 or more items were found to be of high quality, studies with 3 or more high risk of bias scores were found to be of low quality. Studies that scored in between these criteria were found to be of moderate quality.

Data Collection and Data Items of Included Studies

For the included articles the following items were extracted from each study, if available: study design, sample size, patient material – fresh frozen or formalin-fixed, paraffin-embedded –, tumour type, genes sequenced, sequence technique, mutation prevalence, statistical analysis, correlation with tumour grade, FIGO stage, histology, and survival outcomes.

Collection and Data Extraction Samples from the UMCU Database

All endometrial and ovarian cancer samples from our sequence database (UMCU, Department of Pathology) were extracted. Samples dated between October 30, 2013, and October 28, 2015. The following items of clinical data were extracted from the patient files: date of birth, tumour type, histology and grade, FIGO stage, treatment, response to treatment, progression-free survival (PFS) and OS.

DNA Extraction and Ion Torrent Personal Genome Machine Sequencing

The tumour percentage was determined by an experienced pathologist (S.M.W.). DNA was extracted from an area containing >10% tumour cells using ProtK and subsequent purification (Cobas, Roche). For library preparation, 20 ng DNA was amplified by multiplex PCR, targeting regions of interest using Ion AmpliSeq Cancer Hotspot Panels v2 (Thermo Fisher) or OAv2 [10] (see online suppl. Table 2 for included genes). An NGS library was prepared using the Ion Ampliseq Library Kit (Thermo Fisher) and enriched using the Ion PGM™ Template OT2 200 Template Kit

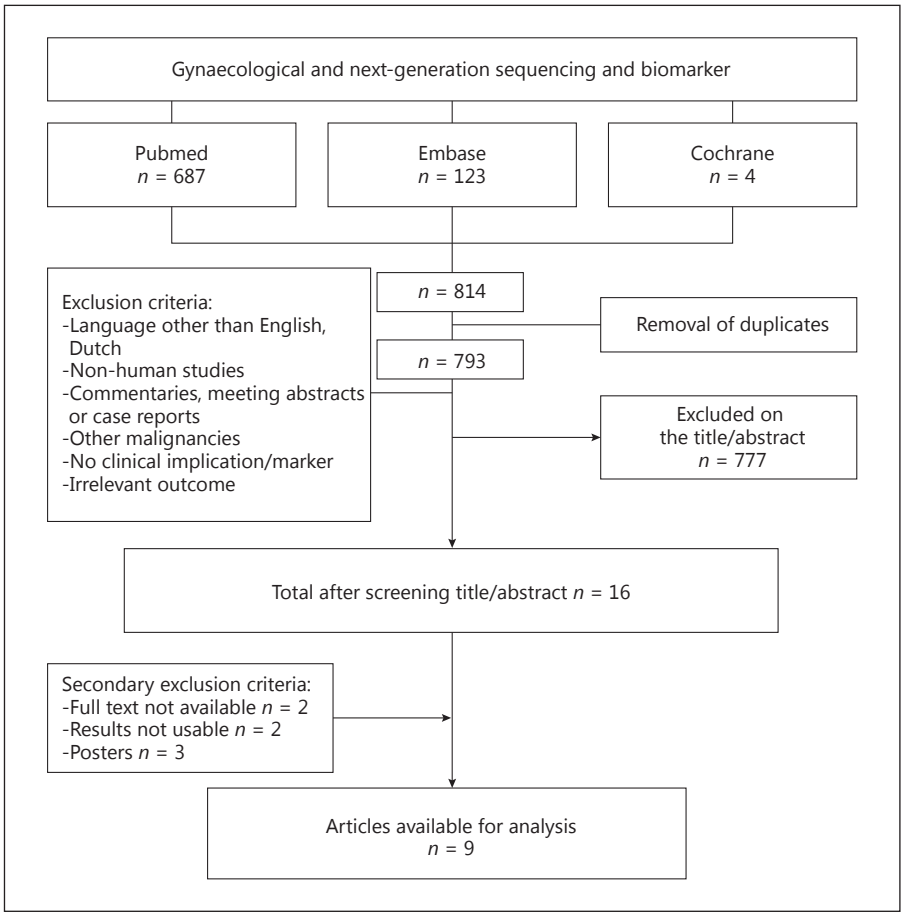

Fig. 1. Results of the literature search: flow chart of November 6, 2015.

(Thermo Fisher). Sequencing was performed using the Ion PGM™ Sequencing 200 kit v2 and the Ion Torrent PGM machine (Thermo Fisher). All procedures were performed as described by the manufacturer. The Torrent Variant Caller combined with an in-house data analysis pipeline provided filtering and annotation of sequencing data as described previously [11]. No germline, Lynch syndrome, or BRCA1/2 analysis was performed.

Synthesis of Results

Because of the heterogeneity of the cohorts, the analysis performed, and the results, a pooled analysis of the results was not possible. All individual results are shown. PFS was defined as from time of diagnosis until progression; OS was defined as from time of diagnosis until last contact.

Results

Search and Selection

The initial search yielded 793 unique articles. Sixteen articles were selected for full-text screening of which 2 were not available in full text, and 3 were posters. Another article had a discordant domain and 1 did not contain sequence data. Eight of the articles were considered eligible for final analysis after screening [12–19]. Six of them reported on ovarian cancer [12–15, 18, 19], and 2 papers investigated endometrial cancer [16, 17] (Fig. 1; Table 1).

Quality Assessment

The quality of all 8 studies was assessed using the QUIPS tool (Table 1). Seven were relevant to our study and the eighth [17] did not describe a prognostic or predictive outcome, but the determinant and domain were the same as in our research question. Therefore this paper was also included for further analysis.

The quality of the included papers ranged between good [14, 16, 17] and moderate [12, 13, 15, 18, 19]. Overall the articles were according to our research question and of fairly good quality. In particular the item "study confounding" scored insufficient, as the papers did not describe confounding factors, or a statistical analysis, whereby confounding factors were taken into account, was lacking (Table 1). Study attrition was scored moderate or high risk of bias, as in the concerned papers loss to

Table 1. Quality assessment of studies using the Quality in Prognosis Studies (QUIPS) assessment tool

Tumor type and study	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis and reporting
Ovarian cancer						
Despierre et al. [14], 2014	○	○	○	○	◐	○
Jia et al. [18], 2014	◐	◐	○	○	◐	◐
Anglesio et al. [12], 2013	◐	◐	○	○	○	○
Dobrzycka et al. [15], 2011	○	●	○	○	◐	◐
Bauerschlag et al. [13], 2010	◐	◐	◐	○	○	○
Wong et al. [19], 2010	◐	◐	○	○	◐	○
Endometrial cancer						
Garcia-Dios et al. [16], 2013	○	◐	○	○	○	○
Gatius et al. [17], 2011	○	◐	○	○	○	○

The articles included were assessed on quality using the QUIPS tool. A comprehensive description of each QUIPS item is provided by Hayden et al. [8]. Risk of bias: ○, low; ◐, moderate; ●, high.

follow-up was often not mentioned. All papers used well-defined prognostic factor measurements as well as outcome measurements. No studies were excluded based on the quality assessment.

Study Characteristics

The included studies comprised a total of 1,997 patients. Seven studies made prognostic correlations, and 3 had predictive conclusions as well (Table 2). As mentioned before, Gatius et al. [17] did not make any prognostic or predictive conclusions. Of the 6 papers concerning ovarian cancer, 1 made a distinction between low-grade serous carcinomas, high-grade serous carcinomas and serous borderline tumours [19]. The other 5 discriminated between type I tumours (low-grade serous carcinomas, mucinous, endometrioid, and clear-cell carcinomas) and type II tumours (high-grade serous carcinomas, undifferentiated cancers and carcinosarcomas). *KRAS* was the most frequently investigated gene, followed by *PIK3CA*. The material used for sequencing, formalin-fixed, paraffin-embedded [12, 17, 18], fresh frozen [15, 19], or both [13, 14, 16] differed among the studies (Table 2).

Literature Study Results

Ovarian Cancer

The prevalence of mutations in the *KRAS* gene ranged between 2.9 and 49% in ovarian cancer [12, 14]. *TP53* mutations detected by sequencing methods were found to be 57.5% in the study of Bauerschlag et al. [13] and 73% in high-grade serous carcinoma in the study of Wong et al. [19]. The mutation prevalences of the other studies are shown in Table 3.

KRAS mutations were associated with (1) histological type, (2) tumour grade, (3) response to therapy, and (4) clinical outcome. Most studies showed a correlation between *KRAS* mutation and mucinous tumour type. Mutation frequencies ranged from 23.5% [18] to 50% [14]. *KRAS* mutations were most common in type I tumours [18]: 27% of grade I tumours [14] and 19% of the low-grade serous carcinomas [19], although this could not be confirmed by another study [15]. Two studies showed a significant correlation between *KRAS* mutations and an improved DFS, PFS, and OS [12, 18] although Dobrzycka et al. [15] and Despierre et al. [14] did not find a significant correlation between all these parameters.

In the study of Despierre et al. [14] neither *KRAS* nor *PIK3CA* mutations had a significant effect on the response to platinum-based chemotherapy. Despierre et al. found that *PIK3CA* is most common in tumours with FIGO stage Ia–IIc, and that the prevalence is the highest in clear-cell carcinomas (46.2%). Bauerschlag et al. [13] did not report any data on *KRAS* mutations but looked into *TP53* mutations in ovarian cancer instead. They showed that the *TP53* mutation rate increases when cell differentiation decreases. No differences were found between early- and late-stage ovarian cancer and histological subtypes, and no correlation between *TP53* status, platinum response, 2-year survival and OS was seen.

Table 2. Study characteristics of selected articles

Study	Prognostic, predictive	Sample size	Tumour type	Genes tested	Material	Technique	Statistical analysis
Despierre et al. [14], 2014	Prognostic, predictive	262	Serous, mucinous, clear-cell, endometrioid, other ovarian tumours	*KRAS*, *PIK3CA*, *BRAF*, *NRAS*, *PTEN*, *FBXW7*, *AKT2*, *AKT3*, *FOXL2*	FF, FFPE	Sequenom MassARRAY	Friedman χ^2, Cochran-Mantel-Haenszel test, Kaplan-Meier curves, partial Spearman correlation coefficient
Jia et al. [18], 2014	Prognostic	109	Serous, mucinous, clear-cell, endometrioid, mixed, borderline ovarian tumours	*KRAS*	FFPE	Direct sequencing by biotechnologies	Pearson χ^2 test, Spearman correlation coefficient, Kaplan-Meier curves
Anglesio et al. [12], 2013	Prognostic, predictive	104	Mucinous, mucinous borderline ovarian tumours	*KRAS* (*NRAS*, *BRAF*, *ERBB2*)	FFPE	Sanger sequencing, ABI Prism 3130xl genetic analyser	Kaplan-Meier curves, log-rank statistic, Cox proportional hazards model
Dobrzycka et al. [15], 2011	Prognostic	126	Serous, mucinous, endometrioid, other ovarian tumours	*KRAS*	FF	ABI Prism 337 DNA sequencer	χ^2 test, Fisher exact test, Kaplan-Meier curves
Bauerschlag et al. [13], 2010	Prognostic, predictive	104	Serous, mucinous, clear-cell, endometrioid ovarian tumours	*TP53*	FF, FFPE	Dideoxy sequencing using a T7 sequencing kit	χ^2 test, Fisher exact test
Wong et al. [19], 2010	Prognostic	91	Low-grade, high-grade, borderline serous ovarian tumours	*BRAF*, *KRAS*, *TP53*	FF	SNP array, Affymetris Fluidic Station 450, Gene chip scanner 3000	Kaplan-Meier curves, log-rank statistic
Garcia-Dios et al. [16], 2013	Prognostic	1,063	Endometrial carcinoma type I, II	*PTEN*, *PIK3CA*, *KRAS*, *TP53*, *FBXW7*, *NRAS*	FF, FFPE	iPLEX technology, MassARRAY compact analyser	Binary logistic regression, Cox proportional hazards model
Gatius et al. [17], 2011	–	31	Endometrial carcinoma type I, II	*FGFR2*	FFPE	AB Prism 3100-Avant	Mann-Whitney and Spearman non-parametric test, Pearson linear correlation

FF, fresh frozen tumour tissues; FFPE, formalin-fixed, paraffin-embedded tumour tissues.

Wong et al. [19] reported a prevalence of *TP53* mutations in 73% of the high-grade serous carcinoma and 0% in low-grade or borderline tumours.

Endometrial Cancer

Garcia-Dios et al. [16] and Gatius et al. [17] investigated different genes in endometrial cancer. Gatius et al. reported an *FGFR2* mutation prevalence of 6.45% and found that these mutations only occur in endometrioid carcinoma. They described that *FGFR2* may be a target for therapeutic intervention. For other genes such as *PTEN*, *KRAS*, and *PIK3CA*, Gatius et al. [17] reported a prevalence of 48, 23, and 29%, respectively, whereas Garcia-Dios et al. [16] reported a prevalence of 15.4, 15.1, and 16.2%, respectively. Furthermore, a correlation between *TP53* mutation and type II tumours (serous, clear-cell carcinomas) and *PTEN* mutation with type I tumours (endometrioid carcinomas) was found [16]. *PIK3CA* correlated with grade 2–3 tumours. A correlation between mutation status and recurrence was not reported. Only *PIK3CA* hot spot mutation H1047R correlated significantly with a poorer prognosis.

Characteristics of UMCU Data

Our data set consisted of 20 tumour samples the mutation status of which was analysed by NGS. Of these, 17 consisted of endometrial cancers and 3 of cervical cancer (Fig. 2). From 14 patients we had clinical data consisting of tumour histology, grading, FIGO stage, treatment, and follow-up data (Table 4). Of the patients with clinical

Table 3. Study results of selected articles

Study	Tumour type	Genes	Patients with mutation, *n*	Mutation prevalence	Prognostic outcomes (correlation with FIGO stage/grade/histology/PFS/OS)	Predictive outcomes
Despierre et al. [14], 2014	OC	*KRAS*, *PIK3CA*, *BRAF*, *NRAS*, *PTEN*, *FBXW7*, *AKT2*, *AKT3*, *FOXL2*	29	Total sample 11%; *KRAS* 5.34%, *PIK3CA* 4.96%, *AKT2*, *AKT3*, *BRAF*, *FOXL2* <1%, *PTEN*, *FBXW7*, *NRAS* 0%	27.3% of grade I, 26.3% of FIGO stage Ia–IIc, 50% of mucinous tumours harboured *KRAS* mutation *PIK3CA* mutation occurred in 26.3% of FIGO Ia–IIc and 46.2% of the clearcell tumours No correlation with OS	*KRAS* and *PIK3CA* had no significant effect on the response to platinum-based therapy
Jia et al. [18], 2014	OC	*KRAS*	16	14.7%	*KRAS* mutation associated with lower grade 23.5% of the mucinous tumours harboured *KRAS* mutations Tumours harbouring *KRAS* mutations have probably a more favourable prognosis	–
Anglesio et al. [12], 2013	OC	*KRAS* (*NRAS*, *BRAF*, *ERBB2*)	57	58.8%	43.6% mucinous carcinomas and 78.8% mucinous borderline tumours harboured *KRAS* mutations *KRAS* mutations were associated with earlier stage[1] and improved PFS and OS	*KRAS* mutation concurrent to HER+ expression may predict lack of response to anti-EGFR therapy
Dobrzycka et al. [15], 2011	OC	*KRAS*	27	21.4%	61.1% of mucinous, 23.1% of endometrioid, 12.5% of serous OCs harboured *KRAS* mutations *KRAS* mutations were associated with grade 1 tumours *KRAS* mutations may predict an improved OS in mucinous carcinomas	–
Bauerschlag et al. [13], 2010	OC	*TP53*	27(42)[2]	26% (57.5%)	*TP53* mutation correlates with de-differentiation No correlation between *TP53* mutation, FIGO stage or histological subtypes	*TP53* mutation showed no correlation with the response to platinum-based chemotherapy, or 2-year survival
Wong et al. [19], 2010	OC	*BRAF*, *KRAS*, *TP53*	21	39.6%	LGSC: *KRAS* 19%, *BRAF* 2%, *TP53* 0% SBOT: *KRAS* 17%, *BRAF* 30%, *TP53* 0% HGSC: *KRAS*, *BRAF* 0%, *TP53* 73%	–
Garcia-Dios et al. [16], 2013	EC	*PTEN*, *PIK3CA*, *KRAS*, *TP53*, *FBXW7*, *NRAS*	?	*PIK3CA* 16.2%, *PTEN* 15.4%, *KRAS* 15.1%, *FBXW7* 3.0%, *TP53* 2.58%, *NRAS* 1.8%	*TP53* correlates with type II tumours, *PTEN* with type I *PIK3CA* correlates with grade 2/3 tumours No correlation with recurrence Hot spot mutation H1047R on *PIK3CA* correlated with poor prognosis	–
Gatius et al. [17], 2011	EC	*FGFR2* (*PTEN*, *KRAS*, *CTNNB1*, *PIK3CA*)[3]	2	*FGFR2* 6.45% (*PTEN* 48%, *KRAS* 23%, *CTNNB1* 17%, *PIK3CA* 29%)	–	–

OS, overall survival; DFS, disease-free survival; OC, ovarian carcinoma; LGSC, low-grade serous carcinoma; SBOT, serous borderline tumour; HGSC, high-grade serous carcinoma; EC, endometrial cancer; OS, overall survival; PFS, progression-free survival. [1] In mucinous tumours, when assessed within mucinous carcinomas, no association was demonstrated. [2] Authors defined 42 cases as mutated by different techniques, 27 of them were confirmed by sequencing. [3] Genes evaluated previously in the same cases.

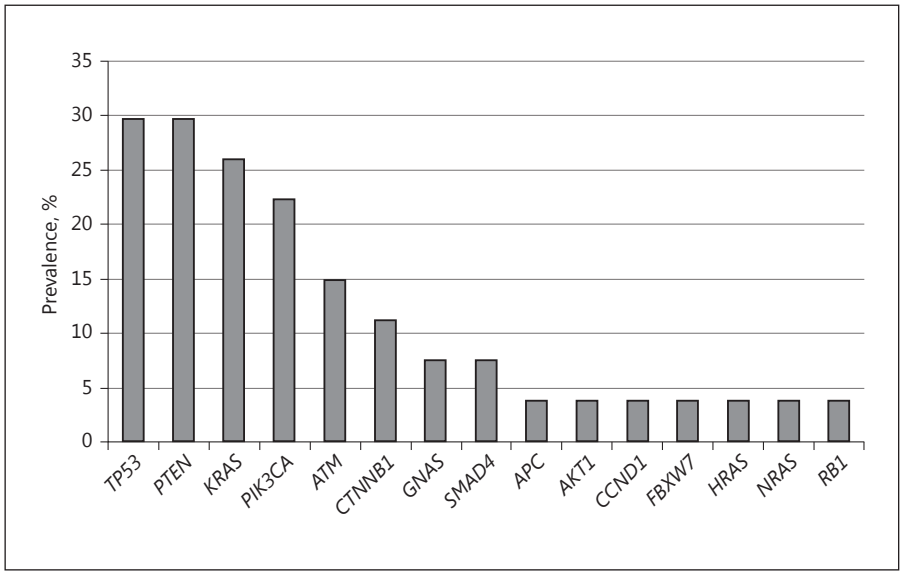

Fig. 2. Mutation prevalence in UMCU patients.

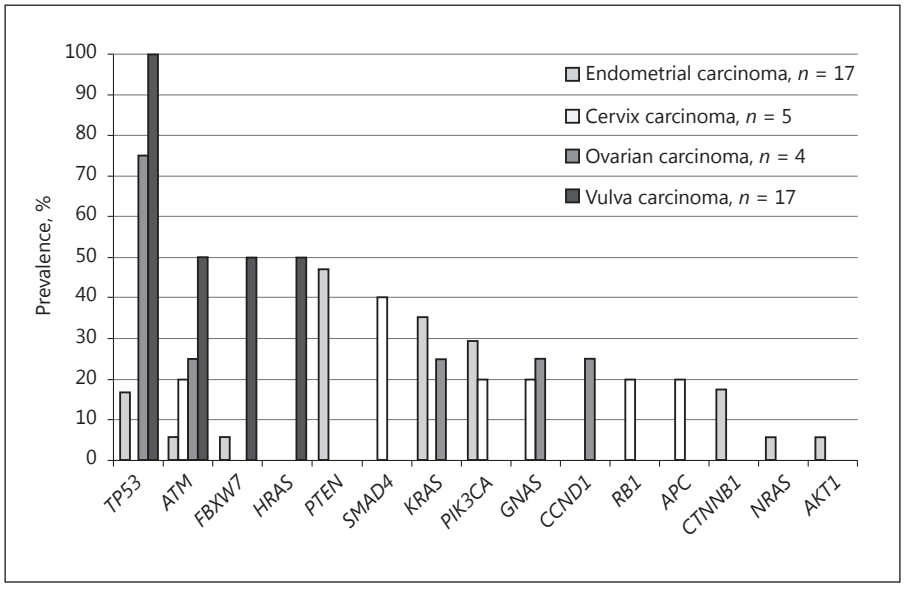

Fig. 3. Mutation prevalence of different genes per tumour type in UMCU patients.

data, 2 were diagnosed with ovarian cancer, 12 with endometrial cancer.

Results of UMCU Data

TP53 was mutated in 100% of the ovarian tumours (Fig. 2, 3). *CCND1* was mutated in 33% of the cases. Endometrial tumours comprised the largest group within the cohort and showed a *PTEN* mutation rate of 47%; *KRAS*, *PIK3CA*, *CTNNB1*, and *TP53* mutations showed a prevalence of 35, 29, 18, and 18%, respectively.

Clinical data were available in 12 cases (Table 4). In endometrial tumours, *TP53* mutations only occurred in FIGO stage Ia–b, 2 occurred in non-endometrioid carcinomas, the other sample with a *TP53* mutation was a mixed tumour. *PIK3CA* mutations were only identified in low FIGO stages Ia–c. Two of the 3 *KRAS* mutations occurred in FIGO stage Ia–b. Higher FIGO stage tumours correlated with *PTEN* and *CTNNB1* mutations. All *CTNNB1* mutations occurred in FIGO stage IIIa–b and 3 out of 4 *PTEN* mutations in FIGO stage IIIa. *PTEN* muta-

Table 4. Results of the UMCU cohort

Patient	Tumour type	Mutation	Histology	Grade	FIGO stage	Primary treatment	Response/ PFS	Next lines of therapy	Response/ PFS	OS
1	Ovarian	*TP53*, c.637C>T	Carcinosarcoma	–	IIIc	Optimal debulking, carboplatin/paclitaxel	CR, 11 months, ongoing	–	–	Alive
2[1]	Ovarian	*CCND1*, c.790C>T *TP53*, c.1004G>A	Clear-cell adenocarcinoma	–	IIb	Debulking, RTx, carboplatin/ paclitaxel	U, 24 months	2 times local surgery	U, 4 months, 6 months	Alive
3	Ovarian	*TP53*, c.242delC	Serous carcinoma	–	–	–	–	–	–	–
4	Uterus	*CTNNB1*, c.110C>T *PTEN*, c.540C>A	Endometrioid adenocarcinoma	2	IIIa	Hysterectomy with adnexa, RTx	NED, 2 months, ongoing	–	–	Alive
5	Uterus	*KRAS*, c.34G>T *PTEN*, c.389G>A	Endometrioid adenocarcinoma	2	IIIa	Hysterectomy with adnexa, RTx	U, 2 months, ongoing	–	–	Alive
6[1]	Uterus	*CCND1*, c.790C>T	Clear-cell adenocarcinoma	–	II	Debulking, RTx, carboplatin/ paclitaxel	U, 24 months	2 times local surgery	U, 4 months, 6 months	Alive
7	Uterus	*KRAS*, c.35G>A *PTEN*, c.951_954del4 *TP53*, c.637C>T	Endometrioid/ carcinosarcoma	2/3	Ib	Hysterectomy with adnexa and appendix	U	U	U	Alive
8	Uterus	*PIK3CA*, c.1633G>A *TP53*, c.560-1G>C	Clear-cell carcinoma	–	Ia	Hysterectomy with adnexa, para-aortal LND, omentectomy	NED, 20 months, ongoing	–	–	Alive
9	Uterus	*AKT1*, c.49G>A *CTNNB1*, c.110C>T *FBWX7*, c.1393C>T	Endometrioid	1	IIIb	Hysterectomy with adnexa, RTx	NED, 16 months, ongoing	–	–	Alive
10	Uterus	*KRAS*, c.35G>A	Endometrioid	1	Ia	Hysterectomy with adnexa	U, 19 months	RTx; medroxy-progesterone	RD, 8 months; SD, 5 months, ongoing	Alive
11	Uterus	*NRAS*, c.182A>G *PIK3CA*, c.1637A>G	Endometrioid	2	Ic	Hysterectomy with adnexa, RTx	NED, 96 months	*Colon carcinoma*		Alive
12	Uterus	*PIK3CA*, c.3140A>G *TP53*, c.577C>T	Carcinosarcoma	3	Ib	Hysterectomy, cisplatin/ epirubicin, RTx	NED, 36 months, ongoing	–	–	Alive
13	Uterus	*CTNNB1*, c.110C>T *PTEN*, c.540C>A	Endometrioid	2	IIIa	Hysterectomy with adnexa, RTx	NED, 12 months ongoing	–	–	Alive
14	Uterus	*KRAS*, c.35G>A *PTEN*, c.29G>A	Endometrioid adenocarcinoma	–	–	–	–	–	–	–
15	Uterus	–	Endometrioid adenocarcinoma	–	–	–	–	–	–	–
16	Uterus	*KRAS*, c.34G>T *PTEN*, c.389G>A	Adenocarcinoma	–	–	–	–	–	–	–
17	Uterus	*PTEN*, c.388C>G	Adenocarcinoma	–	–	–	–	–	–	–
18	Uterus	*PIK3CA*, c.1637A>G	Adenocarcinoma	–	–	–	–	–	–	–
19	Uterus	*KRAS*, c.35G>A *PIK3CA*, c.325_327del3 and c.3139C>T *PTEN*, c.520_528del9 and c.697C>T	Adenocarcinoma	–	–	–	–	–	–	–

UMCU, University Medical Centre Utrecht; PFS, progression-free survival; OS, overall survival; U, unknown; CR, complete remission; NED, no evidence of disease; RD, regression of disease; SD, stable disease; RTx, radiotherapy; LND, lymph node dissection. [1] Same patient, 2 primary tumours.

tions only occurred in grade 2 tumours and endometrioid carcinomas. No correlation was seen with treatment, PFS and OS. For ovarian carcinomas, no correlation between clinical data and mutation status was seen, most probably due to small sample sizes.

Discussion

In this paper we investigated whether NGS can discover mutations in gynaecological tumours with relevant prognostic or predictive value. A literature search was conducted in order to give an overview of the evidence currently available regarding the prognostic and predic-

tive value of mutations in gynaecological tumours. In an attempt to improve our knowledge of the value of the mutational status of gynaecological cancers, new clinical data on this subject from a data set of the UMCU was added.

Ovarian Carcinomas, TP53 *and* KRAS *Mutations*

In a large study of Spaans et al. [20], *PIK3CA*, *PTEN*, and *KRAS* were found the most frequently occurring mutations in gynaecological carcinomas in 22, 18 and 12%, respectively. This is comparable with the results of our study where 40, 30, and 25% of tumours harboured a *PTEN*, *KRAS*, or *PIK3CA* mutation, respectively. *TP53* mutations occurred in 30% of our samples (Fig. 2) but this gene was not included in the study of Spaans et al.

Different grades of ovarian cancer have been associated with different genetic alterations [21]. In type I tumours (low-grade serous, mucinous, endometrioid, clear-cell) *KRAS* and *BRAF* are often mutated [12, 14, 22] whereas *TP53* mutations are rare; type II tumours (high-grade serous, undifferentiated cancers, carcinosarcomas) on the other hand harbour often *TP53* mutations [22, 23]. *TP53* mutation frequencies have been described in high-grade tumours occurring in 58 [13], 73 [19], and 96% [24]. We identified a *TP53* mutation frequency in ovarian tumours of 100%, but note that the sample size was only 3.

Since the *TP53* mutation status is linked to increased genetic instability as DNA replication is no longer paused when errors occur, an adverse effect on the efficacy of platinum-based DNA intercalating substrates was suggested. Since resistance to platinum-based therapy is a problem in the treatment of ovarian cancer, *TP53* mutation status could serve as a predictive marker. Unfortunately, the link between *TP53* mutation status and response to platinum-based chemotherapy remains unclear. Reles et al. [21] showed a significant correlation between *TP53* mutation and platinum resistance, although its significance was lost in a multivariate analysis. In contrast, Bauerschlag et al. [13] could not detect a correlation using different methods to detect *TP53* mutations, thereby excluding the potential effect of mutation detection on the DNA, RNA, or protein level.

The association of *KRAS* mutations and prognosis is also not yet fully described in the literature [25, 26]. Jia et al. [18] and Anglesio et al. [12] reported a significant positive correlation between the *KRAS* mutation status and DFS/PFS/OS. Despierre et al. [14], however, showed no correlation. Because of the very small sample size of ovarian tumours in our data set, we cannot draw any conclusions.

Endometrial Carcinomas, PIK3CA, TP53, KRAS, PTEN, *and* CTNNB1 *Mutations*

In the subgroup of endometrial carcinomas a high prevalence of *PTEN*, *KRAS*, and *PIK3CA* mutations was detected in this study. The most frequently mutated gene was *PTEN* with a mutation frequency of 47% of the endometrial carcinomas, where the literature shows a prevalence ranging from 26 to 82% [17, 20, 27–30]. *PTEN* mutations occur frequently in premalignant lesions and in type I endometrial cancer [27, 30, 31]. In our study, it was noted that *PTEN* mutations occurred only in endometrioid carcinomas and grade 2 tumours, which is similar to the existing literature [16, 27, 28]. *PTEN* mutations show a positive correlation with a favourable prognosis [31]. Mutations in *PIK3CA*, a gene within the same signalling pathway as *PTEN*, are also linked to type I endometrial carcinomas [27, 28]. In our data set, *PIK3CA* mutations occurred in 29% of the cases, which is in line with previous reports ranging between 16 and 52% [16, 17, 32, 33, 34], where the lowest frequencies can be explained by targeted sequencing (hot spot only). For *PIK3CA* mutations, a correlation was described with a poorer prognosis as a result of the correlation with higher tumour grade [16, 17]. In our samples this correlation was not found. Konopka et al. [33] however describe a weak correlation between *PIK3CA* mutations and higher tumour grade. *KRAS* mutations occurred in 35% of our samples, which is slightly higher than previously reported rates of 10–31% [16, 17, 20, 27, 28]. *KRAS* mutations were mainly detected in low FIGO stage and endometrioid carcinomas (type I tumours). Garcia-Dios et al. [16] reported no difference in *KRAS* mutation between the FIGO stages but a higher prevalence with type I tumours as well, corresponding with previously reported papers [27, 28]. *CTNNB1* mutations in our data set were found in 18% of the cases, which correlates with previous studies [17, 20, 27, 35]. In our data set, *CTNNB1* mutations correlated with higher FIGO stage (IIIa–b) and grade 1–2 tumours; however, Saegusa et al. [36] described a correlation between *CTNNB1* and lower-grade tumours. A significant correlation between *CTNNB1* mutations and metastasis was described in their study, which corresponds to our finding of enrichment of *CTNNB1* mutations in high FIGO staged tumours. *TP53* mutations are associated with type II endometrial carcinomas [16, 27, 28]. In our data set, 2 *TP53* mutations occurred in type II endometrial carcinomas and 1 in a mixed tumour type. Our data therefore also suggest a correlation between *TP53* mutations and type II carcinoma, but note that this is based on only 3 tumours. No correlation between *TP53* and DFS

or OS could be demonstrated based on our findings [37, 38].

Online Database

In online supplementary Table 3 the 20 most common mutations in ovarian serous carcinomas and endometrial carcinomas according to the cBioPortal database [39] are shown. Not all of these genes were included in the gene panel used for sequencing in our cohort, as only the most common mutated genes were included. For ovarian cancer however, only 1 gene was found to overlap between our panel and the top 20 genes mutated shown in the database. For example, *KRAS* mutations are detected in 5.34–58.8% [12, 14], whereas in the database, *KRAS* is only mutated in 0.4% [39]. This difference can be explained by the tumour types included in the different studies. In the papers described above, *KRAS* mutations were mostly associated with mucinous tumours. The data used in the database of cbioportal.org are serous carcinomas. For endometrial cancers, the mutation prevalence reported in our study and that of the database are comparable.

Pathways

The affected genes in gynaecological tumours are part of several signalling pathways, including RAS-PI3K, NOTCH and Wnt signalling [24, 40, 41]. Not only somatic mutations, but alternative mechanisms like copy number aberrations and methylation events can cause changes in these pathways eventually resulting in neoplastic transformation, for example amplifications in the RAS/PI3K pathway in ovarian cancers [24].

In high-grade serous ovarian cancer, potential treatment options exist for 45% of the cases where RAS/PI3K signalling is altered due to copy number alterations and in a smaller part by mutations [24]. The homologous recombination pathway is also affected in about 50% of the tumours providing potential treatment options with PARP inhibitors, as already applied in the clinic for BRCA mutant platinum-sensitive ovarian cancers [42]. In type I ovarian tumours the RAS pathway is frequently affected, implying a potential role for MEK inhibitors. Although some patients show a complete response [43], others do not, implying the need for identifying a clear biomarker.

In endometrial cancers the PI3K/AKT pathway is most affected where mutations in *PTEN* and *PIK3CA* lead to activation of this anti-apoptotic pathway. AKT activation can result in activation of the mTOR signalling pathway providing a rationale for treatment with mTOR inhibitors [44]. In early clinical studies benefit is shown from mTOR inhibition, but there are no results from late-phase trials yet [45, 46]. Some endometrium tumours are highly mutated caused by a mutation in *POLE* [40]. As highly mutated tumours are shown to respond to immunotherapy, this might provide a treatment option for this subset of tumours [47].

Overall Remarks, Strengths, and Limitations

Overall limitations of our cohort study are both the small sample size of the data set and the heterogeneity of both the samples and reviewed articles included. We want to stress that the histology as mentioned in the original paper is used in this review, which might sometimes result in unclarity regarding the exact histological subtype. Besides that, a panel-based sequencing analysis was performed, so the investigated variations were limited and no information was gained about mutation load or gene signatures, possibly predictive for immunotherapy treatment.

DNA sequencing to find predictive/prognostic correlations is a valuable technique and (for some cancers) crucial in clinical decision-making. However, it should be noted that only looking at DNA aberrations as prognostic/predictive biomarkers is rather 1-dimensional and a limitation of our research method. To overcome this weakness, a broader view is needed and other techniques involving proteomics, metabolomics, and epigenetics should be coupled to genomics to provide a multidimensional approach and also biological explanations for the found genomic variants. The strengths of our study are the thorough literature search performed, and the critical appraisal of the studies included using the QUIPS tool [8]. The distinctive search combined with our cohort and the database verification supplement each other and give a firm overview about current evidence concerning the value of different mutations in prognostic and predictive terms.

Conclusion

In this study DNA sequencing results of a literature search and our own gynaecological patient population were described. In general, our findings show similarity to the reports in the literature. In summary, *PTEN*, *PIK3CA*, *KRAS*, and *TP53* were the most commonly mutated genes in gynaecological cancers. In ovarian tumours, *KRAS* is associated with type I ovarian tumours (low-grade serous, mucinous, endometrioid, and clear-cell) and may seem to have a more favourable prognosis.

TP53, on the other hand, shows a correlation with type II ovarian tumours (high-grade serous, undifferentiated cancers, carcinosarcomas). The predictive value, however, is still controversial. In endometrial tumours, *PTEN* is frequently mutated, especially in type I tumours (endometrioid carcinomas), and shows a positive correlation with better prognosis. PIK3CA is also correlated with type I tumours, and a correlation may exist with poorer prognosis. *CTNNB1* mutations in endometrial carcinomas could predict a worse prognosis based on the correlation with higher FIGO stage and metastasis. *TP53* mutations are correlated with type II tumours (serous, clear-cell carcinomas).

For conclusions regarding clinical relevance, and for the investigation of the prognostic and predictive value of the mutations found, a prospective trial with a high number of samples is needed, in order to perform a more thorough statistical analysis. Nevertheless, this review describes the presence of certain genetic variations, which can be the basis for further development of (targeted) therapies in these gynaecological cancers.

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