

Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative

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This study aimed to investigate whether molecular analysis can be used to refine risk assessment, direct adjuvant therapy, and identify actionable alterations in high-risk endometrial cancer. TransPORTEC, an international consortium related to the PORTEC3 trial, was established for translational research in high-risk endometrial cancer. In this explorative study, routine molecular analyses were used to detect prognostic subgroups: p53 immunohistochemistry, microsatellite instability and *POLE* proofreading mutation. Furthermore, DNA was analyzed for hotspot mutations in 13 additional genes (*BRAF*, *CDKNA2*, *CTNNB1*, *FBXW7*, *FGFR2*, *FGFR3*, *FOXL2*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PPP2R1A*, and *PTEN*) and protein expression of ER, PR, PTEN, and ARID1a was analyzed. Rates of distant metastasis, recurrence-free, and overall survival were calculated using the Kaplan–Meier method and log-rank test. In total, samples of 116 high-risk endometrial cancer patients were included: 86 endometrioid; 12 serous; and 18 clear cell. For endometrioid, serous, and clear cell cancers, 5-year recurrence-free survival rates were 68%, 27%, and 50% ($P = 0.014$) and distant metastasis rates 23%, 64%, and 50% ($P = 0.001$), respectively. Four prognostic subgroups were identified: (1) a group of p53-mutant tumors; (2) microsatellite instable tumors; (3) *POLE* proofreading-mutant tumors; and (4) a group with no specific molecular profile (NSMP). In group 3 (*POLE*-mutant; $n = 14$) and group 2 (microsatellite instable; $n = 19$) patients, no distant metastasis occurred, compared with 50% distant metastasis rate in group 1 (p53-mutant; $n = 36$) and 39% in group 4 (NSMP; $P < 0.001$). Five-year recurrence-free survival was 93% and 95% for group 3 (*POLE*-mutant) and group 2 (microsatellite instable) vs 42% (group 1, p53-mutant) and 52% (group 4, NSMP; $P < 0.001$). Targetable *FBXW7* and *FGFR2* mutations (6%), alterations in the PI3K-AKT pathway (60%) and hormone receptor positivity (45%) were frequently found. In conclusion, molecular analysis of high-risk endometrial cancer identifies four distinct prognostic subgroups, with potential therapeutic implications. High frequencies of targetable alterations were identified and may serve as targets for individualized treatment.

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Risk classification of endometrial carcinomas is based upon a combination of clinical and histopathological factors and is used in guiding adjuvant therapy. High-risk factors are (combinations of) advanced age, high-grade, non-endometrioid histology, extensive lymphovascular space invasion, and

more advanced disease stage. 15–20% of patients with endometrial cancer have high-risk disease and an aggressive clinical course. There has been widespread uncertainty among clinicians over the best treatment approach for this subgroup of patients, including extent of surgery, type and extent of radiotherapy, and chemotherapy. Two randomized trials PORTEC3 and GOG249 have recently completed an accrual and two trials are going on (GOG258 and the ENGOT-EN2-DGCC/EORTC55102 trial) to evaluate the role of adjuvant chemotherapy with or without pelvic radiation for patients with high-risk endometrial cancer. Significant inter-observer variability exists, even amongst expert gynecopathologists, when subtyping and grading endometrial.^{1–4} In most cases, tumor cell type and grade are diagnosed based on hematoxylin and eosin slides according to the WHO histopathological criteria.⁵ The use of immunomarkers for distinguishing subtype has increased during the past years. Identifying specific molecular alterations which determine tumor behavior and metastatic potential is needed to improve risk classification, inform treatment decisions, and identify targetable pathways in high-risk endometrial cancers. *TransPORTEC*, an international consortium related to the PORTEC3 trial, was set up to conduct such a translational research in high-risk endometrial cancer (www.msbi.nl/transportec).

Over the past decade, multiple groups have identified common molecular alterations of several important genes in endometrial cancer.^{6–11} These alterations have been fitted in the dualistic model of endometrial carcinogenesis discriminating endometrial cancers in the more indolent endometrioid cancers (type 1) and the more aggressive non-endometrioid cancers (type 2). Recently, comprehensive molecular profiling of 373 endometrial cancers suggested that the traditional dualistic model can be improved upon classification of endometrial cancers into four molecular subgroups with a potential prognostic significance: DNA polymerase epsilon (*POLE*) ultramutated, microsatellite instable hypermutated, copy-number low and copy-number high (serous-like and mostly *TP53* mutant).¹² At present such an extensive analysis is impractical and too expensive for a routine clinical utilization. However, testing for the surrogate markers of these subgroups (eg, p53 immunohistochemistry, microsatellite instability, and *POLE* proofreading mutation) may be cost effective and would be easy to apply in the current clinical practice. In this study, we aimed to investigate whether such molecular analyses can be used to detect prognostic subgroups in a series of high-risk endometrial cancers. Importantly, this pilot study includes clear cell tumors – a histological subtype not characterized by The Cancer Genome Atlas. In addition, we aimed to determine the frequency of molecularly targetable alterations in high-risk endometrial cancers,^{6,13–16} which is of

interest given the poor outcome of high-risk endometrial cancers with current management.

Materials and methods

Patient and Tissue Selection

Tumor tissues were selected from partner institutions within the *TransPORTEC* consortium, with the aim of obtaining a large series of high-risk endometrial cancers using inclusion criteria of the PORTEC3 study.¹⁷ In total, formalin fixed paraffin-embedded tumor samples from 116 patients that fulfilled these criteria were collected from five participating institutions: Leiden University Medical Center, The Netherlands ($n=14$); University Medical Center Groningen, The Netherlands ($n=46$); University College London, United Kingdom ($n=8$); St Marys Hospital Manchester, United Kingdom ($n=34$); and Gustave Roussy Paris, France ($n=14$). Paraffin-embedded tissue blocks containing representative tumor were selected. Hematoxylin-eosin-stained slides were viewed by experienced gynecopathologists (TB and VS) to select an area of tumor tissue containing at least 70% tumor cells.

Tissue Microarray Construction

Tissue microarrays were constructed from all samples with sufficient tumor volume ($n=114$) using a tissue microarray Master.¹⁸ Tissue microarrays contained 1-mm tumor and tumor/stroma cores of each sample, in triplicate, and were randomly distributed. Colon, normal endometrial, kidney, liver, ovary, placenta, skin, testis, tonsil, and fallopian tube samples were included in the tissue microarrays for orientation purposes and as internal positive controls. Immunohistochemistry was performed on whole slide and tissue microarray for the first 59 cases to validate the utility of tissue microarray for protein expression analysis in endometrial cancer. Comparison of the results showed a concordance of >80% for PTEN, ARID1a, p53, ER, and MLH1 (data not shown).

Immunohistochemical Analysis

Immunohistochemistry on tissue microarrays and whole slides (4 μ m) was performed as described previously.^{19,20} Details of the procedures and primary antibodies are described in Supplementary Table 1. As negative controls, slides were incubated in phosphate-buffered saline without primary specific antibodies. Two observers scored the tissue microarrays and whole slides independently. The observers were blinded for patient characteristics and outcome, and discrepancies were resolved at a multihead microscope. p53 was scored positive if >50% of the tumor cells showed a strong positive nuclear staining, or when discrete geographical

patterns showed >50% tumor cell positivity.²¹ 'Indefinite' cases in which no staining of the tumor was observed were sequenced for *TP53* mutations. PTEN staining was evaluated in three categories as negative, positive, and heterogenous.²² ARID1a was scored as negative, weak positive, or strong positive nuclear staining or as 'clonal loss'.²³ In the final analyses, 'clonal loss' was reclassified as 'loss of expression' as this pattern has been indicated to correspond with *ARID1a* mutations.²⁴ The ER, PR, and MLH1 scores for all three tissue microarray tumor cores were determined. ER and PR were scored positive when at least one tumor core showed any nuclear expression. MLH1 nuclear staining was scored positive or negative, if all cores were concordant. 'Clonal loss' of MLH1 was scored if one of the cores was discordant. Cases were scored 'failed' when two of the three cores could not be evaluated, because of the absence of the core, tumor, or internal control for staining.

DNA Isolation

Prior to DNA isolation, tumor DNA was enriched in the FFPE blocks by taking three 0.6-mm tissue cores from the tumor focus by using a tissue microarrayer (Beecher Instruments), to reach a tumor percentage >70%. Normal DNA was isolated from cores in the adjacent normal myometrium. In five cases, 10 sections (10 μ m) were used to microdissect fragments of tumor, for the enrichment of tumor DNA. DNA isolation was performed fully automated as described previously using the Tissue Preparation System (Siemens Healthcare Diagnostics).²⁵

Mutation Analysis

All samples were analyzed by using the Sequenom MassARRAY system and the GynCarta Assay version 2.0 (Sequenom) to test for the presence or absence of 159 hotspot mutations in 13 genes (*BRAF*, *CDKNA2*, *CTNNB1*, *FBXW7*, *FGFR2*, *FGFR3*, *FOXL2*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PPP2R1A*, and *PTEN*) as described previously.²⁶ This gene panel only covers 40% of PTEN mutations found in endometrial cancer and therefore loss of PTEN protein expression was analyzed by immunohistochemistry. Mutations in *POLE* exons 9 and 13, which together contain >90% of the pathogenic *POLE* exonuclease domain mutations, were detected by using Sanger sequencing.¹¹ Sanger sequencing for exon 5-8 of *TP53* was performed on those samples that showed an 'indefinite' p53 immunohistochemical staining pattern as described previously.⁹

Microsatellite Instability

The microsatellite status of each tumor was determined using the Promega microsatellite instability

analysis system (version 1.2, Promega), as described previously.⁹ Tumors with instability in two or more of these markers were defined as being high-frequency microsatellite unstable, whereas those with instability at one repeat or showing no instability were classified as being stable. Microsatellite unstable tumors in which no loss of MLH1 protein expression was observed were stained for MSH2, MSH6, and PMS2 to confirm loss of one of the mismatch repair proteins (Supplementary Table 1).

Methylation Specific PCR

Tumors with loss of *MLH1* protein expression were selected for further testing for methylation status of the 5' regulatory region of *MLH1*, using methylation-specific PCR, with primers that have been previously described.²⁷ Contamination of the carcinoma tissue by stromal or inflammatory cells was unavoidable and tumors with a partially methylated phenotype were scored as methylated.

Statistics

Rates of distant metastasis, recurrence-free, and overall survival were calculated with Kaplan–Meier method and log-rank test starting at the date of diagnosis. For analysis of overall survival, all deaths irrespective of cause were considered an event; for recurrence-free survival all recurrences (local, regional, and distant) were considered as an event. IBM SPSS software version 21.0 was used for all statistical analysis.

Results

Clinicopathologic Characteristics

Clinicopathological characteristics are shown in Table 1. In total, 18 (16%) clear cell, 12 (10%) serous, and 86 (74%) endometrioid (33 FIGO stage I grade 3, 18 stage II, 28 stage III, and 6 stage IV) endometrial cancers were included. Median follow-up was 28.6 months (range 0.3–165.5 months). For endometrioid, serous and clear cell cancers 5-year recurrence-free survival rates were 68%, 27%, and 50% ($P=0.014$), distant metastasis rates 23%, 64%, and 50% ($P=0.001$) and overall survival 67%, 18%, and 39% ($P=0.002$). The corresponding Kaplan–Meier curves are shown in Figure 1. In univariate analysis, age ($P=0.031$) was a prognostic factor for decreased recurrence-free survival, in contrast to grade ($P=0.988$) and deep myometrial invasion ($P=0.150$). However, it should be taken into account that the patients were selected on those high-risk factors. None of the clinicopathological factors (age, grade, and myometrial invasion) were prognostic for distant metastasis.

Table 1 Patient characteristics

N = 116	N	%
Age		
Mean, range	66	21–85
< 60	38	32.8
60–70	31	26.7
> 70	47	40.5
FIGO stage 2009		
I	42	36.2
II	21	18.1
III	41	35.3
IV	11	9.5
Unknown	1	0.9
Tumor type		
Endometrioid	86	74.1
Serous	12	10.3
Clear cell	18	15.5
Grade		
1	13	11.2
2	5	4.3
3	98	84.5
Depth of invasion		
< 50%	23	19.8
> 50%	87	75.0
Unknown	6	5.2
Lymphovascular space invasion		
Absent	40	34.5
Present	55	47.4
Unknown	23	18.1
Any adjuvant therapy		
Yes	82	70.7
No	10	8.6
Unknown	24	20.7
Adjuvant radiotherapy^a		
EBRT	55	47.4
VBT	1	0.9
EBRT+VBT	21	18.1
None	15	12.9
Unknown	24	20.7
Adjuvant chemotherapy		
Yes	16	13.8
No	76	65.5
Unknown	24	20.7

^aEBRT, external beam radiotherapy; VBT, vaginal brachytherapy.

Molecular Subgroups Within High-Risk Endometrial Cancers

The distribution and frequency of alterations differed substantially across the endometrial cancer subtypes, with the highest number of alterations in the endometrioid tumors (Supplementary Table 2-4). The co-occurrence of alterations was observed in a higher frequency in endometrioid ($n = 50$, 58%) and serous ($n = 7$, 58%) tumors compared with clear cell ($n = 6$, 33%) subtypes ($P = 0.062$). Combining the detected alterations resulted in the identification of

four molecular subgroups; (1) a group of p53-mutant tumors ($n = 39$, 34%); (2) microsatellite instable tumors ($n = 19$, 16%); (3) *POLE* proofreading-mutant tumors ($n = 14$, 12%) and (4) a group with no specific molecular profile (NSMP; $n = 44$, 38%) (Figure 2). A subset of tumors with endometrioid ($n = 20$, 23%) and clear cell morphology ($n = 8$, 44%) had a p53 mutant-like expression similar to all serous cancers. This group 1 (p53-mutant) tumors had relatively few alterations in *PPP2R1a*, *FBXW7*, and PI3K-AKT pathway. A p53 mutant-like expression was inversely correlated with a microsatellite instability. Only one endometrioid cancer showed a p53 mutant-like expression and the microsatellite instability with loss of MLH1 protein expression due to promoter hypermethylation. In group 3 (*POLE*-mutant), two endometrioid tumors showed a microsatellite instability lacking *MLH1* promoter hypermethylation. Furthermore, the group 3 (*POLE*-mutant) tumors were highly associated with *PIK3CA* hotspot mutations and showed an inverse relationship with p53 mutant-like expression. Group 2 (microsatellite instable) tumors consisted of 17 endometrioid and 2 clear cell cancers. All microsatellite instable tumors showed loss of protein expression of one or two mismatch repair proteins (MLH1, MSH2, MSH6, and PMS2). DNA promoter hypermethylation of *MLH1* was observed in 9 of the 12 microsatellite instable tumors with the loss of MLH1 protein expression. In the other cases, microsatellite instability could not be attributed to *MLH1* promoter methylation and microsatellite instability must therefore be the result of alternative mechanisms (eg, Lynch syndrome; Supplementary Table 4). In group 4 (NSMP), we did not detect microsatellite instability, p53 mutant-like expression or *POLE* proofreading mutations. These remaining tumors can be characterized mostly by endometrioid and clear cell morphology, high frequency of PI3K-AKT alterations, high levels of ER/PR expression and presence of *CTNNB1* mutations. Additionally, twelve tumors within this subgroup (27%) had none of the alterations tested.

Correlation of Molecular Subgroups with Clinical Outcome

Next, we analyzed the prognostic significance of the identified molecular subgroups and found that the four molecular subgroups were associated with different clinical outcomes. Both in group 3 (*POLE*-mutant; $n = 14$) and group 2 (microsatellite instable; $n = 19$) endometrial cancer patients, no distant metastasis occurred, compared with 5-year rates of distant metastasis of 50% and 39%, respectively, among group 1 (p53-mutant) and group 4 (NSMP; $P < 0.001$). Five-year recurrence-free survival was 93% and 95%, respectively, for group 3 (*POLE*-mutant) and group 2 (microsatellite instable) cases, vs 42% and 52% for group 1 (p53-mutant) and group 4 (NSMP; $P < 0.001$; Figure 3a). Overall survival at 5

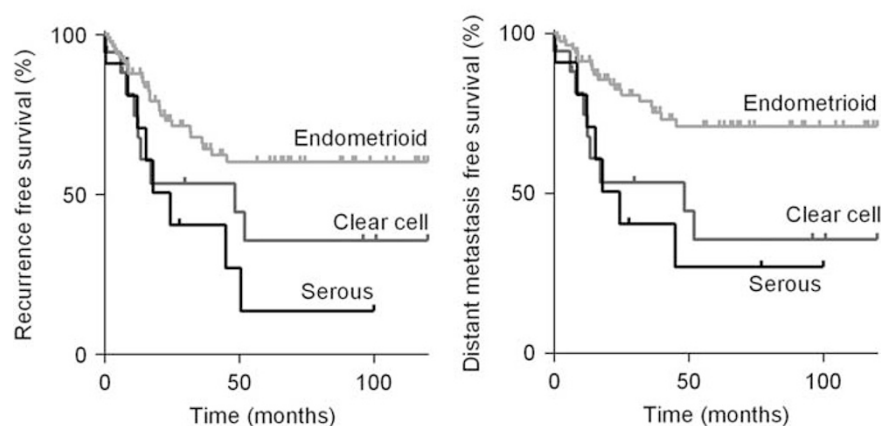


Figure 1 Recurrence and distant metastasis free survival of high-risk endometrial cancer patients stratified by tumor type. For endometrioid, serous and clear cell cancers 5-year recurrence-free survival rates were 68, 27, and 50% ($P=0.014$), distant metastasis rates 23, 64, and 50% ($P=0.001$) and overall survival 67, 18, and 39% ($P=0.002$).

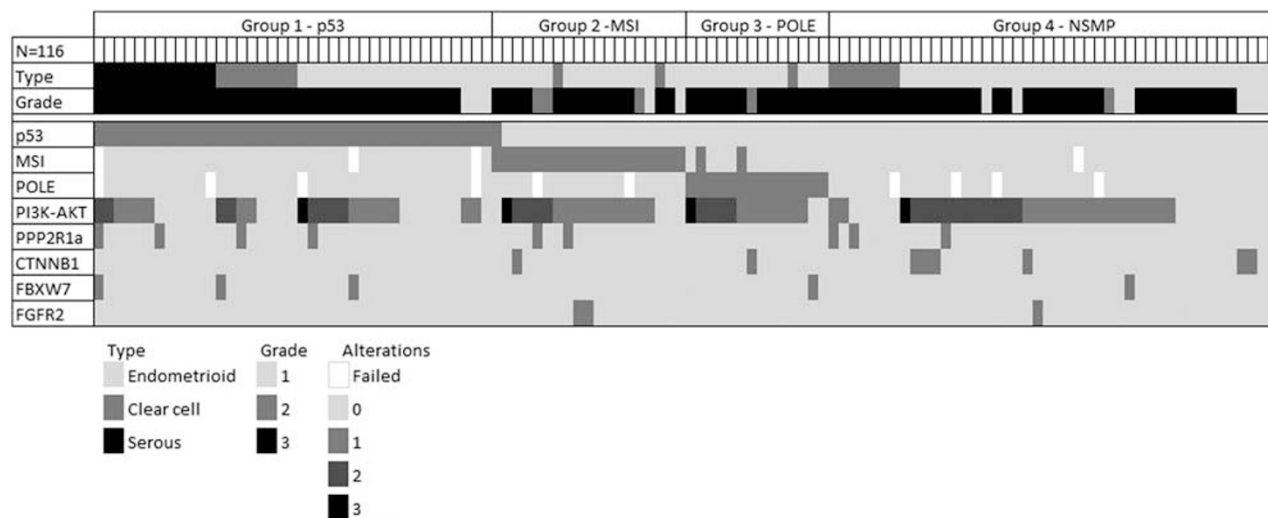


Figure 2 Molecular landscape of high-risk endometrial cancer. Routine analysis focusing on frequent hotspot mutations and known molecular drivers of endometrial cancer results in the identification of four molecular subgroups within high-risk endometrial cancer: (1) p53-mutant, (2) microsatellite instable, (3) *POLE* proofreading-mutant, and (4) with no specific molecular profile (NSMP). PI3K-AKT pathway alterations are hotspot mutations in *PIK3CA*, *KRAS*, *NRAS*, loss of *PTEN*, or loss of *ARID1a* expression.

years was 93% in group 3 (*POLE*-mutant) patients, 63% in group 2 (microsatellite instable; died from other causes), 40% in group 1 (p53-mutant) and 61% in group 4 (NSMP). Furthermore, *POLE*-mutant status was associated with a younger age, in contrast to p53-mutant status, which was associated with older age. After exclusion of the non-endometrioid tumors, our analysis was still able to discriminate endometrioid patients with good vs poor prognosis. Group 3 (*POLE*-mutant) and group 2 (microsatellite instable) cancer patients were both associated with a better recurrence and distant metastasis-free survival, as compared with group 1 (p53-mutant) and group 4 (NSMP) endometrioid cancer patients (Figure 3b). Interestingly, group 1 (p53-mutant)

endometrioid tumors and group 4 (NSMP) of high-risk endometrial tumors showed no differences in the clinical course.

Targetable Pathways within Molecular Subgroups

The frequencies of targetable alterations for each identified molecular subgroup is shown in Table 2. Group 1 (p53-mutant) and group 4 (NSMP) patients seem to have a poor outcome under the current treatment. Most cases within these two subgroups had alterations in the PI3K-AKT pathway (60%) or were hormone receptor-positive (45%) and potentially targetable with PI3K-AKT-mTOR inhibitors or

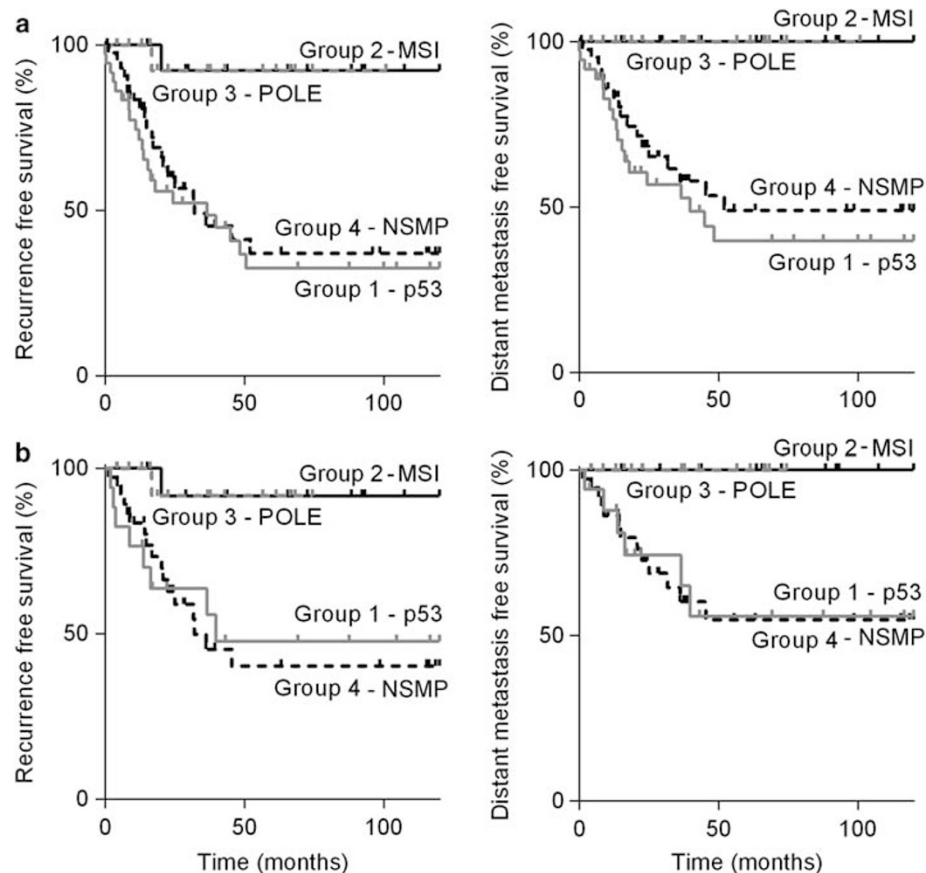


Figure 3 Clinical outcome of high-risk endometrial cancer patients stratified by the four molecular subgroups; (1) p53-mutant, (2) microsatellite instable (MSI), (3) *POLE* proofreading-mutant, and subgroup (4) with no specific molecular profile (NSMP). Recurrence-free and distant metastasis-free survival of all high-risk patients (**a**) and endometrioid high-risk patients (**b**) stratified by the four molecular subgroups. Both in group 3 (*POLE*-mutant; $n=14$) and group 2 (microsatellite instable; $n=19$) endometrial cancer patients no distant metastasis occurred, compared with group 1 (p53-mutant; $n=36$, 5-year distant metastasis 50%) and group 4 patients (NSMP; $n=44$, 39%; $P<0.001$). Five-year recurrence-free survival was 93% and 95% for group 3 (*POLE*-mutant) and group 2 (microsatellite instable) vs 42% (group 1—p53-mutant) and 52% (group 4-NSMP; $P<0.001$). Even after exclusion of the non-endometrioid cancer patients, group 3 (*POLE*-mutant) and group 2 (microsatellite instable) endometrioid cancer patients still shows improved recurrence and distant metastasis free survival ($P=0.004$ and $P=0.004$).

Table 2 Frequency of targetable alterations within the four subgroups; (1) p53-mutant, (2) microsatellite instable, (3) *POLE* proofreading-mutant and (4) with no specific molecular profile (NSMP).

Targetable alterations (%)	Group 1 p53 N= 39	Group 2 microsatellite instable N= 19	Group 3 POLE N= 14	Group 4 NSMP N= 44	Potential drugs
PI3K-AKT pathway					
PIK3CA	7 (18)	6 (32)	11 (71)	13 (30)	PI3K, AKT, mTOR inhibitors
PTEN ^a	16 (41)	8 (42)	6 (43)	14 (32)	
ARID1a ^a	5 (13)	7 (37)	1 (7)	11 (26)	
KRAS	3 (8)	2 (11)	0	4 (9)	
NRAS	1 (3)	0	1 (7)	0	
Hormone receptor positivity					
ER	23 (59)	14 (74)	9 (64)	33 (74)	Hormonal therapy
PR	16 (41)	11 (58)	7 (50)	25 (57)	
Other targetable genes					
FBXW7	3 (8)	1 (5)	1 (7)	1 (2)	HDAC inhibitors
FGFR2	0	2 (11)	0	1 (2)	FGFR inhibitors

^aAlterations based on immunohistochemistry.

hormonal therapies. In 6% of the cases, somatic mutations in *FBXW7* and *FGFR2* were identified, which could potentially be targetable with HDAC inhibitors or FGFR inhibitors (e.g. BGI398, AZD4547).

Discussion

This research shows that molecular subclassification of high-risk endometrial cancer can be effectively used to identify distinct subsets with prognostic significance. We found highly significant and clinically relevant differences in relapse and survival rates between the molecular subgroups, which can be used to determine adjuvant therapy in clinical practice. The technology required for this molecular classification is suitable for daily clinicopathological practice. This practical approach resulted in the confirmation of the four molecular subgroups proposed by The Cancer Genome Atlas, and additionally identified potentially targetable pathways for high-risk endometrial cancers. These results can be translated into clinical practice by tailoring adjuvant therapy and/or directing targeted treatment in future studies. The same molecular analyses have been shown to work successfully on pre-operative endometrial biopsy or curettages with high concordance with the hysterectomy specimen.¹⁹ The findings also illustrate that endometrial cancers currently classified as 'high-risk' are in fact a heterogeneous group of tumors with diverse molecular alterations and variable clinical outcome. Our data confirm that even in a selected cohort of high-risk patients serous and clear cell histology portends poor prognosis when compared with endometrioid tumors.²⁸ However, even after exclusion of the non-endometrioid tumors, molecular analysis can discriminate patients with a good and poor prognosis.

Importantly, our analyses further support previous studies that showed an association of *POLE* proof-reading mutations with younger age and favorable prognosis.^{11,29,30} It is noteworthy that in this cohort, the frequency of *POLE* proofreading mutation was 15%, consistent with its association with high tumor grade^{10,11,29} and higher than the 6% frequency detected in unselected low- and intermediate-risk endometrial cancers.^{10,11,29,30} In addition, we found that microsatellite instability was associated with a reduced risk of recurrence and distant metastases. The impact of microsatellite instability on the prognosis of women with endometrial cancer is controversial. Some studies reported that microsatellite instability is associated with a favorable prognosis,^{31,32} whereas in other studies a significant worse prognosis was found.^{33,34} Possible explanations for these discrepancies include cohort differences, lack of statistical power, and diversity of methodology. Further studies will be required to clarify the effects of *POLE* mutations and microsatellite instability on the biological behavior of

endometrial tumor cells and the associated mechanisms. Collectively our data suggest that *POLE* proof-reading mutations and microsatellite instability may be useful as biomarkers to identify patients, mostly with grade 3 and clinically high-risk disease who, in fact, have a good prognosis and may not require intensive postoperative radiotherapy or even chemotherapy. In addition, our data provide a rationale to develop treatment strategies that take into account these genetic alterations.

Within the molecular subgroups we identified potentially targetable pathways alterations. This is of particular interest for high-risk endometrial cancer patients with a poorer outcome under the current treatment regimens, such as the group 1 (p53-mutant) and group 4 (NSMP) cancer patients without p53 mutant-like expression, *POLE* mutation and/or microsatellite instability. Most of the cases in group 4 (NSMP) had alterations in the PI3K-AKT pathway or were hormone receptor-positive and potentially targetable with PI3K-AKT-mTOR inhibitors, anti-hormone therapies, or combination therapies with dual inhibitors (Table 2). A selected group of cancers may be targetable with HDAC inhibitors or FGFR inhibitors based on the mutation frequencies of *FBXW7* and *FGFR2*. Previously, higher frequencies of these targetable alterations were reported in the comparable p53-mutant/serous-like and microsatellite stable copy-number low subgroup.¹² The use of exome sequencing may explain in part the higher rate of detected mutations. However, the identified variants may also include non-pathogenic variants. Further studies are required to determine whether new therapies targeting these alterations improve the survival.

In the current analysis, we included a relatively large set of clear cell cancers ($n=18$). In our focused molecular analysis these cases were partly included not only within group 1 (p53-mutant/serous-like) but also in the other three subgroups of endometrioid cancers. Overlapping molecular features in clear cell cancers have been noted previously, including loss of ARID1a and p53 mutant-like expression.^{35–37} This questions whether clear cell cancer are molecularly distinct or whether these cancers are morphological variants of serous and endometrioid cancers harboring the same spectrum of molecular alterations. The data presented here are not conclusive yet and a broader unbiased molecular analysis will be required to answer this question.

This report on a subgroup of high-risk endometrial cancers was set up as an exploratory study and therefore results should be interpreted as such. The retrospective nature, limited sample size, differences in adjuvant treatment regimens and follow-up data are obvious limitations. Furthermore, with our focused approach analyzing known drivers and hotspot mutations we do not provide an unbiased molecular profile, but tested previously identified molecular clusters. This focused approach can be viewed as a strength of our study, in that it enhances

the clinical applicability. However, as a consequence, we did not identify novel molecular drivers in a group of tumors without any of the alterations tested.

In conclusion, we showed that relatively straightforward molecular analysis can be used to refine the risk assessment of endometrial cancer patients that are currently classified as high-risk based on clinicopathological factors. Our results indicate that group 3 (*POLE*-mutant) and group 2 (microsatellite instable) high-risk patients have a favorable prognosis and therefore the current risk assessment of these patients may be overestimated, possibly resulting in overtreatment. Group 1 tumors that have a p53-mutant like expression and group 4 tumors with no specific molecular profile are truly high-risk cancers. For truly high-risk patients, studies should be directed toward identifying targetable pathways. Our data provide a rationale to investigate not only the use of PI3K-AKT pathway inhibitors in this selected patient group but also hormonal treatment in those tumors with retained receptor expression remains an option. The molecular approach used in this work will be extended and tested in the International *TransPORTEC* Consortium Studies of the large randomized cohort of endometrial cancers of patients who participated in the PORTEC3 trial, with the advantages of clear, randomized treatment groups and complete follow-up data. This would result in a novel approach in which routine molecular analyses are incorporated in the workup of endometrial cancer to refine the risk assessment based upon clinical and histopathological factors and identify targetable alterations, resulting in the reduction of over- and undertreatment.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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