**Supplementary Data**

***High-throughput interrogation of PIK3CA, PTEN, KRAS, FBWX7 and TP53 mutations in primary endometrial carcinoma***

Diego A. Garcia-Dios\*, Diether Lambrechts\*, Lieve Coenegrachts, Ingrid Vandenput, An Capoen, Penelope M. Webb, Kaltin Ferguson, ANECS, Lars A. Akslen, Bart Claes, Ignace Vergote, Philippe Moerman, Johan Van Robays, Janusz Marcickiewicz, Helga B. Salvesen, Amanda B. Spurdle, Frédéric Amant

**SUPPLEMENTARY METHODS**

**Tumor collection**

The **Leuven Endometrial Cancer Study** (**LES**) is a hospital-based case-control study. Eligible cases, identified by active surveillance of electronic patient files at the Leuven University Hospital, were white women aged 25-93 years and diagnosed with endometrial cancer between August 1995 and September 2009. Clinical data for endometrial cancer patients were recorded during interview at the time of diagnosis and were retrieved from pathology reports. All medical records were reviewed by trained abstractors and pathology reports compatible with primary, invasive, epithelial endometrial adenocarcinoma of all stages (I –IV) and all grades were consulted. In the **Genk Endometrial Cancer study,** patients diagnosed with primary endometrial carcinoma in the Belgian region of Genk between November 1999 and December 2008 have been included. The **Australian National Endometrial Cancer Study** (**ANECS**; QIMR, Brisbane, Australia) is an Australian population-based case-control-family study of cancer of the uterine corpus, and details of recruitment and participation have been described previously [1]. Briefly, cases were women aged 18-79, newly diagnosed with histologically confirmed primary cancer of the endometrium between July 2005 and December 2007, and were identified through major national hospitals and state-based cancer registries. Case participation rate was 63%. Participants were interviewed to obtain epidemiological information. Information on tumor pathology characteristics was abstracted from clinical pathology reports. Tumor material was collected for cases where possible, including FFPE material as blocks or slides. Clinical information including FIGO stage, vital status and, if relevant, date of death was abstracted from medical records 3-4 years after diagnosis. In the **Bergen Endometrial Cancer study** (Haukeland University Hospital, Bergen, Norway) patients diagnosed with endometrial carcinoma in Hordaland County, Norway, during the 10-year period 1981-1990 have been included. Hordaland County has approximately 450,000 inhabitants, representing about 10% of the Norwegian population, and having a similar age-adjusted incidence rate of endometrial cancer [2]. Patient characteristics, histologic features, other tumor markers and the treatment for this group of patients have been reported previously [3]. FFPE tumor tissue was retrieved for the mutational study. None of the patients were lost due to insufficient follow-up. The Norwegian Data Inspectorate and the Regional Ethical Committee (Health Region III) has approved the research. In the **Sahlgrenska Endometrial Cancer study** (Gothenburg, Sweden), patients diagnosed with primary endometrial carcinoma in Gothenburg region, between October 2005 and December 2008 have been included. Region of Gothenburg has approximately 650,000 habitants, representing about 7.5% of the Swedish population, and having a similar age-adjusted incidence rate of endometrial cancer. Patient characteristics, histological features, other tumor markers and the treatment for this group of patients have been studied prospectively. Full clinical data were available, including follow-up and lymph node status, all grades and FIGO stages were considered. FFPE tumor tissue was retrieved for the mutational study and was provided in slides. None of the patients were lost due to insufficient follow-up. The Swedish Data Inspectorate and the Regional Ethical Committee (University of Gothenburg) has approved the study. All cases were women aged 25-92. All patients with previous gynaecologic malignancies or synchronous uterine and other intra-peritoneal tumors (n=25) were excluded. Patients with endometrial (complex) hyperplasia (n=7), mesonefric adenocarcinoma (n=2) or uterine sarcoma (n=39) were also not included. All studies predominantly consisted of women with European ancestry.

**Somatic oncogene profiling**

The COSMIC (Catalogue Of Somatic Mutations In Cancer) database [4] was screened for mutations in *KRAS*, *BRAF*, *NRAS*, *PIK3CA*, *PTEN*, *EGFR*, *FBXW7* and *TP53* in solid tumors, and the most frequent mutations in each gene were selected (Supplementary Table S1). Briefly, respectively, 21, 3, 20, 33, 11, 8, 15 and 18 somatic mutations were selected. The panel of hot spot mutations covered, respectively, 98%, 96%, 97%, 81%, 14%, 33%, 66% and 16% of all somatic mutations occurring in these genes, as reported in the COSMIC database [4]. DNA was aliquoted into 384-well plates and genotyped centrally at the Vesalius Research Center (Leuven, Belgium) to avoid discrepancies inherent to the use of different genotyping methods. The iPLEX technology on a MassARRAY Compact Analyser (Sequenom Inc., San Diego, USA) was used for mutation analysis, as reported previously [5]. Automated genotyping calls were generated using the MassARRAY RTTM software and were validated by manual review of the raw mass spectra. DNA samples were considered of sufficient quality when more than 75% of mutations were reliably genotyped such that each mutation was genotyped with a success rate exceeding 96%. Quality control was performed by genotyping 24 samples in duplicate, with a duplicate concordance of 99.7%.

**SUPPLEMENTARY REFERENCES**

[1] Spurdle A.B., Thompson D.J., Ahmed S., Ferguson K., Healey C.S., O'Mara T. et al. Genome-wide association study identifies a common variant associated with risk of endometrial cancer. *Nat Genet* ;**43**:451-4;2011

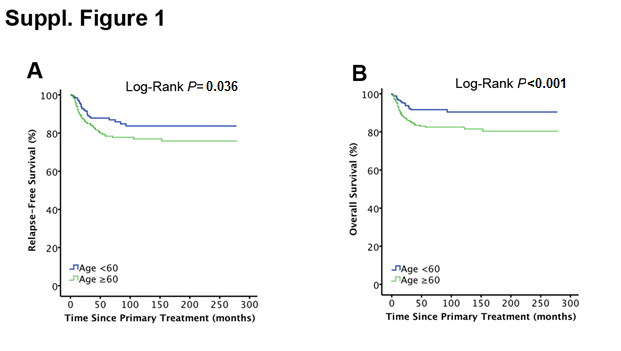
[2] Stefansson I.M., Salvesen H.B., Akslen L.A. Prognostic impact of alterations in P-cadherin expression and related cell adhesion markers in endometrial cancer. *J Clin Oncol* ;**22**:1242-52;2004

[3] Stefansson I.M., Salvesen H.B., Akslen L.A. Vascular proliferation is important for clinical progress of endometrial cancer. *Cancer Res* ;**66**:3303-9;2006

[4] Forbes S.A., Bindal N., Bamford S., Cole C., Kok C.Y., Beare D. et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res* ;**39**:D945-D950;2011

[5] De Roock W., Claes B., Bernasconi D., De S.J., Biesmans B., Fountzilas G. et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* ;**11**:753-62;2010

**SUPPLEMENTARY FIGURES**

****

**Supplementary Figure 1**

Kaplan-Meier curves for relapse-free survival (A) and overall survival (B) in patients with endometrial cancer according to age.

**Suppl Figure 2.tif**

**Supplementary Figure 2**

Kaplan-Meier curves for relapse-free survival (A) and overall survival (B) in patients with endometrial cancer according to FIGO stage.

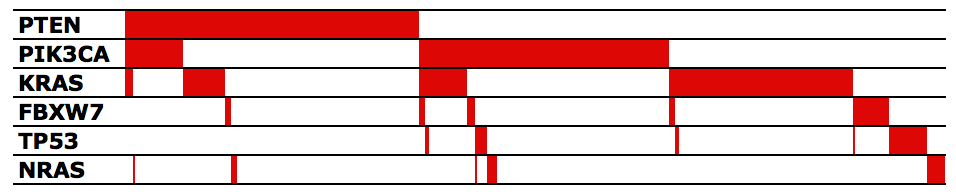
**SUPPLEMENTARY TABLES**

**Supplementary Table S1**

Overview of selected mutations in *KRAS, BRAF, PIK3CA, NRAS, P53, PTEN, FBXW7*, based on the COSMIC database. Frequency of mutations detected in our data set is presented as well. Total number of samples *n*=1,063 (mixed subtypes are excluded).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Amino acid change** | **Nucleotide change** | **Mutation coverage COSMIC (%)** | **Mutated samples Leuven** | **Mutation frequency Leuven (%)** | **Mutation frequency EC COSMIC (%)** |
| **KRAS** | p.G12S | c.34G>A | 4*.*29 | 4 | 2.53 | 4.00 |
| **(*n*=12,028)** | p.G12R | c.34G>C | 3*.*11 | - | - | 0.61 |
|  | p.G12C | c.34G>T | 11*.*97 | 16 | 10.13 | 8.61 |
|  | p.G12D | c.35G>A | 34*.*77 | 53 | 32.72 | 36.92 |
|  | p.G12A | c.35G>C | 6.00 | 17 | 10.76 | 13.53 |
|  | p.G12V | c.35G>T | 22*.*76 | 23 | 14.56 | 20.61 |
|  | p.G13D | c.38G>A | 13*.*45 | 39 | 24.07 | 10.77 |
|  | p.G13A | c.38G>C | 0*.*13 | - | - | 0.61 |
|  | p.G13V | c.38G>T | 0*.*13 | 1 | 0.63 | 0.31 |
|  | p.G13G | c.39C>A | 0*.*03 | 2 | 1.27 | - |
|  | p.G13G | c.39C>G | 0*.*01 | - | - | - |
|  | p.G13G | c.39C>T | 0*.*03 | 1 | 0.63 | - |
|  | p.A146T | c.436G>A | 0*.*18 | - | - | - |
|  | p.A59T | c.175G>A | - | - | - | - |
|  | p.Q61K | c.181C>A | - | - | - | - |
|  | p.Q61E | c.181C>G | 0*.*13 | - | - | - |
|  | p.Q61P | c.182A>C | - | - | - | - |
|  | p.Q61R | c.182A>G | 0*.*12 | - | - | - |
|  | p.Q61L | c.182A>T | 0*.*16 | - | - | 0.31 |
|  | p.Q61H | c.183A>C | 0*.*64 | 1 | 0.63 | 0.92 |
|  | p.Q61H | c.183A>T | - | 5 | 3.16 | - |
|  |  |  | **97.91%** | ***n*=162** | |  |
| **BRAF** | p.V600E | c.1799T>A | 95.60 | - | - | - |
| **(*n*=13,042)** | p.D594G | c.1781A>G | 0.13 | - | - | 0.22 |
|  | p.K601E | c.1801A>G | 0.24 | - | - | - |
|  |  | | **95.73 %** | ***n*=0** | |  |
| **PIK3CA** | p.G12D | c.35G>A | 0*.*03 | - | - | - |
| **(*n*=3,007)** | p.P17S | c.49C>T | 0*.*03 | - | - | - |
|  | p.R38H | c.113G>A | 0*.*13 | 4 | 2.31 | 0.31 |
|  | p.E81K | c.241G>A | 0*.*03 | 23 | 13.29 | 0.63 |
|  | p.R88Q | c.263G>A | 0*.*66 | 5 | 2.89 | 4.69 |
|  | p.R93W | c.277C>T | 0*.*09 | 13 | 7.51 | 0.63 |
|  | p.G106V | c.317G>T | 0*.*09 | 3 | 1.73 | 0.31 |
|  | p.R108H | c.323G>A | 0*.*13 | 9 | 5.20 | 0.31 |
|  | p.G118D | c.353G>A | 0*.*17 | 5 | 2.89 | 0.31 |
|  | p.P134S | c.400C>T | 0*.*03 | - | - | - |
|  | p.S158L | c.473C>T | 0*.*03 | - | - | - |
|  | p.H160N | c.478C>A | 0*.*03 | - | - | - |
|  | p.K179T | c.536A>C | 0*.*03 | - | - | - |
|  | p.K184E | c.550A>G | 0*.*03 | - | - | - |
|  | p.N345K | c.1035T>A | 0*.*92 | - | - | - |
|  | p.C420R | c.1258T>C | 0*.*69 | 2 | 1.16 | 0.31 |
|  | p.P539R | c.1616C>G | 0*.*49 | 2 | 1.16 | 0.31 |
|  | p.E542K | c.1624G>A | 12*.*12 | 26 | 15.03 | 5.31 |
|  | p.E545D | c.1635G>T | 1.10 | 1 | 0.58 | 1.56 |
|  | p.E542Q | c.1624G>C | 0*.*17 | - | - | - |
|  | p.E545K | c.1633G>A | 20*.*14 | 9 | 5.20 | 5.31 |
|  | p.E545Q | c.1633G>C | - | - | - | - |
|  | p.Q546K | c.1636C>A | 1*.*50 | 7 | 4.05 | 2.81 |
|  | p.Q546K | c.1636C>G | - | - | - | - |
|  | p.K567R | c.1700A>G | 0*.*06 | 3 | 1.73 | - |
|  | p.H701P | c.2102A>C | 0*.*13 | 2 | 1.16 | - |
|  | p.C901F | c.2702G>T | 0*.*09 | 2 | 1.16 | - |
|  | p.M1004I | c.3012G>T | 0*.*06 | - | - | - |
|  | p.G1007R | c.3019G>C | 0*.*09 | 1 | 0.58 | 0.31 |
|  | p.H1047L | c.3140A>T | 3*.*97 | 17 | 9.83 | 2.50 |
|  | p.H1047R | c.3140A>G | 37*.*76 | 36 | 20.81 | 19.06 |
|  | p.H1047Y | c.3139C>T | 0*.*93 | 2 | 1.16 | 2.81 |
|  | p.G1049R | c.3145G>C | 0*.*53 | 1 | 0.58 | 1.56 |
|  | p.G1049S | c.3145G>A | 0*.*26 | - | - | 0.31 |
|  |  | | **81.42%** | ***n*=173** | |  |
| **NRAS** | p.G12S | c.34G>A | 4*.*67 | 1 | 5.26 | - |
| **(*n*=2,403)** | p.G12R | c.34G>C | 0*.*58 | - | - | - |
|  | p.G12C | c.34G>T | 2*.*69 | 1 | 5.26 | - |
|  | p.G12D | c.35G>A | 12*.*57 | 3 | 15.79 | 25.0 |
|  | p.G12A | c.35G>C | 1*.*37 | - | - | - |
|  | p.G12V | c.35G>T | 2*.*11 | - | - | - |
|  | p.G13S | c.37G>A | 0*.*21 | 1 | 5.26 | - |
|  | p.G13R | c.37G>C | 2*.*32 | 3 | 15.79 | - |
|  | p.G13C | c.37G>T | 0*.*83 | - | - | - |
|  | p.G13D | c.38G>A | 6*.*82 | - | - | 25.0 |
|  | p.G13A | c.38G>C | 0*.*66 | - | - | - |
|  | p.G13V | c.38G>T | 2*.*19 | - | - | - |
|  | p.Q61K | c.181C>A | 20*.*64 | - | - | 25.0 |
|  | p.Q61E | c.181C>G | 0*.*37 | - | - | - |
|  | p.Q61P | c.182A>C | 0*.*79 | 1 | 5.26 | - |
|  | p.Q61R | c.182A>G | 28*.*92 | 4 | 21.05 | - |
|  | p.Q61L | c.182A>T | 5*.*83 | 1 | 5.26 | 25.0 |
|  | p.Q61H | c.183A>C | 1*.*29 | 4 | 21.05 | - |
|  | p.Q61H | c.183A>T | 2*.*12 | - | - | - |
|  | p.Q61Q | c.183A>G | 0*.*12 | - | - | - |
|  |  |  | **97*.*1%** | ***n*=19** | |  |
| **TP53** | p.G245R | c.733G>C | 0*.*05 | - | - | - |
| **(*n*=19,229)** | p.G245C | c.733G>T | 0*.*2 | - | - | - |
|  | p.G245S | c.733G>A | 1*.*13 | 1 | 3*.*33 | 1.48 |
|  | p.R175H | c.524G>A | 3*.*16 | 2 | 6.67 | 3.70 |
|  | p.R273C | c.817C>T | 1*.*64 | 11 | 36.66 | 5.93 |
|  | p.R273H | c.818G>A | 1*.*99 | 4 | 13.33 | 2.96 |
|  | p.R282W | c.844C>T | 1*.*55 | 3 | 10.00 | 2.22 |
|  | p.Y220C | c.659A>G | 0*.*80 | - | - | 0.74 |
|  | p.P250L | c.749C>T | 0*.*17 | - | - | - |
|  | p.R110L | c.329G>T | 0*.*07 | - | - | - |
|  | p.R110P | c.329G>C | 0*.*04 | - | - | - |
|  | p.R248W | c.742C>T | 1*.*97 | 2 | 6*.*67 | 7.41 |
|  | p.R248Q | c.743G>A | 2*.*21 | 4 | 13*.*33 | 5.19 |
|  | p.R248P | c.743G>C | 0*.*05 | - | - | - |
|  | p.R248L | c.743G>T | 0*.*30 | 3 | 10*.*00 | - |
|  | p.R249S | c.747G>C | 0*.*09 | - | - | - |
|  | p.R249S | c.747G>T | 1*.*23 | - | - | - |
|  | p.R249R | c.747G>A | 0*.*02 | - | - | - |
|  |  |  | **16*.*42%** | ***n*=30** | |  |
| **PTEN** | p.F241S | c.722T>C | 0*.*05 | - | - | - |
| **(*n*=1,893)** | p.E150Q | c.448G>C | 0*.*05 | - | - | - |
|  | p.K267fs\*9 | c.800delA | 2*.*38 | - | - | 2.40 |
|  | p.K62R | c.185A>G | 0*.*05 | - | - | - |
|  | p.N323fs\*21 | c.968delA | 1*.*49 | - | - | 1.71 |
|  | p.R173H | c.518G>A | 1*.*11 | - | - | 0.23 |
|  | p.R233\* | c.697C>T | 3*.*01 | 20 | 12.20 | 3.77 |
|  | p.Y65C | c.194A>G | 0*.*05 | - | - | - |
|  | p.R130\* | c.388C>T | 3*.*06 | 23 | 14.29 | 2.10 |
|  | p.R130G | c.388C>G | 3*.*12 | 119 | 72.56 | 7.43 |
|  | p.R130R | c.388C>A | 0*.*05 | - | - | 0.11 |
|  | [p.R130Q](http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=mut_summary&id=5033) | c.389G>A | - | 2 | 1.24 | 4.34 |
|  |  |  | **14*.*43%** | ***n*=164** | |  |
| **FBXW7** | p.R393\* | c.1177C>T | 1*.*82 | 4 | 12.50 | - |
| **(*n*=274)** | p.R465C | c.1393C>T | 18*.*25 | 3 | 9.38 | 8.33 |
|  | p.R465H | c.1394G>A | 13*.*14 | 8 | 25.00 | 8.33 |
|  | p.R479G | c.1435C>G | 1*.*09 | 1 | 3.13 | 8.33 |
|  | p.R479Q | c.1436G>A | 9*.*85 | 11 | 34.38 | 16.67 |
|  | p.R479L | c.1436G>T | 1*.*82 | 1 | 3.13 | - |
|  | p.V504I | c.1510G>A | 0*.*73 | - | - | - |
|  | p.R505C | c.1513C>T | 12*.*04 | 2 | 6.25 | - |
|  | p.S582L | c.1745C>T | 1*.*82 | 1 | 3.13 | - |
|  | p.Q98\* | c.292C>T | 0*.*36 | - | - | - |
|  | p.R224\* | c.670C>T | 1*.*09 | - | - | - |
|  | p.R278\* | c.832C>T | 2*.*19 | - | - | - |
|  | p.D480Y | c.1438G>T | 0*.*36 | - | - | - |
|  | p.R367\* | c.1099C>T | 1*.*09 | - | - | 8.33 |
|  | p.H379R | c.1136A>G | 0*.*36 | 1 | 3.13 | - |
|  |  |  | **66*.*01%** | ***n*=32** | |  |

**Supplementary Table S2**

Overview of mutually exclusive mutations present in endometrial tumors: Tumors carrying mutations in *PTEN, PIK3CA, KRAS, FBXW7, TP53* and *NRAS* are indicated in red. In total, 41.5% of the tumors had mutations in one of these genes.

**Supplementary Table S3**

Presence of *PTEN,* *PIK3CA, TP53, FBXW7* mutations according different demographic and histopathologic characteristics in only endometrioid tumors.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | ***PTEN*  mutations** | | | ***PIK3CA* mutations** | | | ***TP53* mutations** | | | ***FBXW7* mutations** | | |
| **Total cases analyzed = 970** | **Wild-type (*n*=807, 83.2%)** | **Mutant**  **(*n*=163, 16.8%)** | ***P* value** | **Wild-type**  **(*n*=812, 83.7%)** | **Mutant**  **(*n*=158 , 16.3%)** | ***P* value** | **Wild-type (*n*=955, 98.5%)** | **Mutant**  **(*n*=15, 1.5%)** | ***P* value** | **Wild-type (*n*=940, 96.9%)** | **Mutant**  **(*n*=30, 3.1%)** | ***P* value** |
| **Age (66 ± 10)**  >=60 (*n*=655, 67.5%)  <60 (*n*=314, 32.4%) | 544 (83.1%)  262 (83.4%) | 111 (16.9%)  52 (16.6%) | 0.878 | 552 (84.3%)  260 (82.8%) | 103 (15.7%)  54 (17.2%) | 0.283 | 641 (97.9%)  313 (99.7%) | 14 (2.1%)  1 (0.3%) | 0.072 | 640 (97.7%)  300 (95.5%) | 15 (2.3%)  14 (4.5%) | 0.246 |
| **Histopathological Grade**  Grade 1 (*n*=393, 40.5%)  Grade 2 (*n*=378, 39.0%)  Grade 3 (*n*=198, 20.4%) | 327 (83.2%)  318 (84.1%)  161 (81.3%) | 66 (16.8%)  60 (15.9%)  37 (18.7%) | 0.752 | 349 (88.8%)  303 (80.2%)  159 (80.3%) | 44 (11.2%)  75 (19.8%)  39 (19.7%) | 0.001 | 389 (99.0%)  373 (98.7%)  192 (97.0%) | 4 (1.0%)  5 (1.3%)  6 (3.0%) | 0.289 | 379 (96.4%)  373 (98.7%)  187 (94.4%) | 14 (3.6%)  5 (1.3%)  11 (5.6%) | 0.144 |
| **FIGO 2009**  **I** (*n*=750, 77.3%)  **II** (*n*=70, 7.2%)  **III** (*n*=125, 12.9%)  **IV** (*n*=18, 1.9%) | 637 (84.9%)  51 (72.9%)  98 (78.4%)  15 (83.3%) | 113 (15.1%)  19 (27.1%)  27 (21.6%)  3 (16.7%) | 0.041 | 633 (84.4%)  55 (78.6%)  105 (84.0%)  13 (72.2%) | 117 (15.6%)  15 (22.4%)  20 (16.0%)  5 (27.8%) | 0.590 | 740 (98.7%)  70 (100%)  120 (96.0%)  18 (100%) | 10 (1.3%)  -  5 (4.0%)  - | 0.663 | 730 (97.3%)  67 (95.7%)  121 (96.8%)  17 (94.4%) | 20 (2.7%)  3 (4.3%)  4 (3.2%)  1 (5.6%) | 0.785 |
| **Lymph Node Involvement**  Positive nodes (*n*=54, 5.6%)  Negative nodes (*n*=313, 32.3%) | 39 (70.9%)  259 (83.0%) | 15 (29.1%)  54 (17.0%) | 0.260 | 44 (81.5%)  255 (81.5%) | 10 (18.5%)  58 (18.5%) | 0.837 | 51 (94.5%)  311 (99.4%) | 3 (5.5%)  2 (0.6%) | 0.110 | 48 (88.9%)  303 (96.8%) | 6 (11.1%)  10 (3.2%) | 0.045 |
| **Recurrence**  Yes (*n*=122, 12.6%)  No (*n*=757, 78.0%) | 99 (81.1%)  631 (83.4%) | 23 (18.9%)  126 (16.6%) | 0.688 | 94 (77.0%)  631 (83.4%) | 28 (23.0%)  126 (16.6%) | 0.324 | 121 (99.2%)  744 (98.3%) | 1 (0.8%)  13 (1.7%) | 0.262 | 116 (95.1%)  737 (97.4%) | 6 (4.9%)  20 (2.6%) | 0.620 |

Percentages are given as row %. *P* values for age, grade, FIGO and type were calculated using a binary logistic regression for *PTEN*, *PIK3CA* *TP53* and *FBXW7* mutation status while adjusting for each of the other variables. *P* values for lymph node and recurrence were calculated separately because there were many missing data for these variables, using binary logistic regression while considering age, grade, FIGO and type as co-variates. Clinical variables were missing for a subset of individuals as follows: age (*n*=1), histopathological grade (*n*=1), FIGO (*n*=7), lymph node involvement (*n*=603), recurrence (*n*=91).