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## **The landscape of somatic mutations in Indonesian cervical cancer is predominated by the PI3K pathway**

Spaans, V.M.; Mahendra, I.N.B.; Purwoto, G.; Trietsch, M.D.; Osse, M.; Haar, N. ter; ... ; Jordanova, E.S.

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2 **predominated by the PI3K pathway**

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28

29 **Abstract**

30

31 *Objective.* To investigate the prevalence of somatic mutations in Indonesian cervical carcinoma  
32 patients in the context of histology and human papillomavirus (HPV) type.

33

34 *Methods.* In total 174 somatic hot-spot mutations in 13 genes were analyzed by mass  
35 spectrometry in 137 Indonesian cervical carcinomas.

36

37 *Results.* In 66/137 tumors (48%) 95 mutations were identified. *PIK3CA* was most frequently  
38 mutated (24%), followed by *FBXW7* (7%), *CTNNB1* (6%), and *PTEN* (6%). In squamous cell  
39 carcinomas more often multiple mutations per sample ( $p=0.040$ ), and more *PIK3CA* ( $p=0.039$ )  
40 and *CTNNB1* ( $p=0.038$ ) mutations were detected compared to adenocarcinomas. *PIK3CA*  
41 mutations were associated with HPV 16 positivity, *CDKN2A* mutations with HPV 52 positivity,  
42 and, interestingly, *PTEN* mutations with HPV negativity. Balinese tumor samples more often  
43 carried multiple mutations ( $p=0.019$ ), and more *CTNNB1*, *CDKN2A*, and *NRAS* mutations  
44 compared to Javanese samples.

45

46 *Conclusions.* Potentially targetable somatic mutations occurred in 48% of Indonesian cervical  
47 carcinomas. The landscape of mutations is predominated by mutations concerning the PI3K  
48 pathway, and we prompt for more research on developing therapies targeting this pathway,  
49 explicitly for the more advanced stage cervical carcinoma patients.

50

51 **Keywords**

52

53 cervical carcinoma, somatic mutation, *PIK3CA*, Indonesia, human papillomavirus, cancer  
54 genomics

55

56

57 **Introduction**

58

59 Today, around 85% of the global burden of cervical cancer occurs in the least developed  
60 countries of the world [1]. In Indonesia, cervical cancer is the second most common cancer in  
61 women, with estimated age-standardized incidence and mortality rates (ASR) of 17.3 and 8.2 per  
62 100,000 women per year, respectively. Herewith, the clinical (and economic) burden of this  
63 disease in Indonesia is substantial. By contrast, in the Netherlands, cervical cancer is the twelfth  
64 most common cancer in women, with an ASR for incidence and mortality of 6.8 and 1.6 per  
65 100,000 women per year, respectively [1].

66 Cervical cancer is caused by a persistent infection with high risk type human papillomavirus  
67 (HPV) [2]. Meta-analyses have shown that HPV type 16 and 18 are responsible for approximately  
68 73% of all cervical cancer cases worldwide, followed by HPV type 58, 33, 45, 31, and 52.  
69 However, considerable inter- and intraregional variation of HPV type distribution was described  
70 [3, 4]. We have previously investigated the HPV type distribution in the Indonesian population [5]  
71 and in Indonesian cervical cancer patients [6], and reported relatively high prevalence rates of  
72 HPV type 18 (1.3% population, 38% in cancer) and HPV type 52 (1.8% population, 14% in  
73 cancer), and a high percentage of multiple HPV infections (2.3% population, 14% in cancer).

74 However, with a worldwide overall HPV prevalence of 10% in healthy women, it is known that  
75 only a minority of women are prone to develop cervical cancer. The progression from initial  
76 infection to a persistent infection into premalignant lesions and eventually invasive cervical cancer  
77 is a multifactorial process, influenced by many life-style, environmental, cultural, political,  
78 geographical, and socioeconomic factors, such as smoking, parity, age, sexual behavior, and the  
79 quality of health care facilities [7]. The differences in incidence and mortality rates for cervical  
80 cancer between low-resource and industrialized countries are often ascribed to differences in  
81 these factors, and, predominantly, by the (lack of) implementation of cytological screening and/or  
82 vaccination programs [8].

83 In addition, recent studies have shown that various genetic and epigenetic events play an  
84 important role in the carcinogenesis of cervical cancer, such as copy number alterations, loss of

85 heterozygosity, tumor suppressor gene inactivation, or oncogene activation [9-13]. Insight into the  
86 molecular mechanisms driving tumorigenesis has become more and more relevant with the  
87 emergence of targeted drug therapies. Two well-known examples of successful targeting  
88 therapies are trastuzumab for *HER2* overexpressing mamma carcinoma patients, and  
89 vemurafenib for *BRAF* mutated melanoma patients [14, 15]. Disappointingly, for cervical cancer,  
90 no tumor-specific targeting drugs have proved to be successful yet, though diverse novel agents  
91 are enrolled in ongoing clinical trials [16] (<https://www.clinicaltrials.gov>). Furthermore, the  
92 presence or absence of certain somatic mutations in cervical cancer was suggested to be  
93 associated with different outcomes to adjuvant chemotherapy treatment and radiation sensitivity  
94 [17-19]. Knowledge concerning a tumor's genetic make-up may guide individualized treatment  
95 strategies.

96 Over the past few years, several research groups, including ours, have evaluated the genomic  
97 alterations of very small to quite large cohorts of cervical cancer patients [12, 20-26]. And very  
98 recently, The Cancer Genome Atlas (TCGA) Research Network published their integrated  
99 genomic and molecular characterization of cervical cancer [13]. However, in Indonesia, a high  
100 prevalence country for cervical cancer, genetic profiles were never investigated. Whilst preventive  
101 vaccines are introduced slowly and with the greatest difficulty [27], still most women present with  
102 advanced stage disease. The urge and need for alternative (targeted) adjuvant treatments is  
103 greatest in countries like Indonesia, where these treatments seem to be the most faraway though.  
104 In the present study, we analyzed the prevalence of somatic mutations in Indonesian cervical  
105 carcinoma patients, and placed this in the context of histology and HPV type. Furthermore, we  
106 discussed the similarities and differences in cervical cancer mutation profiles between Indonesian  
107 and Dutch cervical cancer patients.

108

## 109 **Methods**

110

### 111 *Patient samples*

112

113 This study was assessed by the Institutional Review Board. All samples were blinded for patient  
114 identification and used according to the Code of Conduct for responsible use of human tissue in  
115 the context of health research 2011 ([https://www.federa.org/sites/default/files/images/print\\_](https://www.federa.org/sites/default/files/images/print_version_code_of_conduct_english.pdf)  
116 [version\\_code\\_of\\_conduct\\_english.pdf](https://www.federa.org/sites/default/files/images/print_version_code_of_conduct_english.pdf)).

117 In total 142 cervical cancer specimens from Indonesia were available. Seventy-four cases derived  
118 from the outpatient clinic of the Dr. Cipto Manungkusumo National General Hospital, Jakarta,  
119 Java, Indonesia, and consisted of a consecutive cohort of patients diagnosed with invasive  
120 cervical cancer (2001-2002) as described previously [6]. An additional 10 Javanese cervical  
121 adenocarcinoma samples (2011) were provided from the Santosa Hospital, Bandung, Java,  
122 Indonesia. Fifty-eight cases derived from the Sanglah General Hospital, Denpasar, Bali,  
123 Indonesia, and consisted of two consecutive cohorts of patients diagnosed with invasive cervical  
124 cancer (27 cases from 2009, and 31 cases from 2011).

125 Of all included patients, formalin-fixed, paraffin-embedded (FFPE) material containing a  
126 representative part of the cervical tumor was available at the Leiden University Medical Center.  
127 Histological sections were reviewed for morphology by an experienced pathologist (GJF). When  
128 no glandular components were seen, sections were stained with Periodic Acid Schiff Plus and  
129 Alcian Blue to detect intracytoplasmic mucus. Cases were classified as squamous cell carcinoma  
130 (SCC), adenocarcinoma (AC), or adenosquamous carcinoma (ASC) according to the WHO 2014  
131 histological classification of tumors of the uterine cervix [28]. Three samples were excluded from  
132 further analysis due to poor fixation or unclear morphology.

133 All samples included in this study were typed for HPV using the SPF10 primer set and INNO-LiPA  
134 HPV genotyping extra line probe assay (Fujirebio Europe, Gent, Belgium) according to the  
135 manufacturers protocol.

136 For DNA isolation, three to five 0.6mm tissue cores were punched out of a marked tumor area of  
137 the FFPE tissue block containing >70% tumor. Of some FFPE blocks 10µm tissue sections were  
138 taken instead of cores as they contained >70% of tumor cells. DNA was isolated either manually,  
139 followed by a DNA purification step (NucleoSpin Tissue kit, Machery-Nagel, Germany), or using  
140 the automated Tissue Preparation System (Siemens Healthcare Diagnostics, NY, USA) [29].

141 After DNA isolation, the FFPE tissue blocks were returned to Indonesia to be stored in the  
142 respective local archives.

143

#### 144 *Mutation Genotyping*

145

146 The GyneCarta mutation genotyping panel (Agena Bioscience, San Diego) was used to detect  
147 174 known mutations in 13 validated oncogenes and tumor suppressor genes being *BRAF*,  
148 *CDKN2A*, *CTNNB1*, *FBXW7*, *FGFR2*, *FGFR3*, *FOXL2*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*,  
149 *PPP2R1A*, and *PTEN* [29].

150 All samples ( $N=142$ ), plus 28 (20%) samples in duplicate and 16/28 in triplicate, four negative  
151 controls ( $H_2O$ ), and two wild type leukocyte DNA samples were genotyped using the iPLEX  
152 technology system (Sequenom Inc., San Diego, USA) for matrix-assisted laser  
153 desorption/ionization time-of-flight mass spectrometry following the manufacturers' protocol [30].

154 Two investigators (VS, MT), blinded for tumor identification, analyzed the data independently  
155 using Mass Array Typer Analyzer software (TYPER 1.0.22, Sequenom, Hamburg, Germany) and  
156 Mutation Surveyor (Softgenetics, State College, Pennsylvania, USA). Two samples failed for all  
157 assays (one from Bali, one from Java) and were excluded from further analysis.

158

#### 159 *Statistics*

160

161 Statistical analyses were performed with IBM-SPSS Data Editor (version 20.0, Armonk, New  
162 York, USA) using the independent Students *t*-Test to compare numerical data and the Chi-  
163 squared test or Fisher's exact test to compare categorical and normally distributed data.

164 Pearson's correlation coefficients were used to detect bivariate correlations for HPV positivity or  
165 type and mutation status. Binary Logistic regression models were used to perform multivariate  
166 analyses for somatic mutation status, or gene specific mutation status, correcting for age, region,  
167 histological classification (block 1, method = Enter), and HPV type 16, 18, 52, and 45, and other

168 gene mutations (block 2, method = Backward Stepwise Conditional). All tests were two-tailed,  
169 and  $p$  values  $< 0.05$  were considered statistically significant.

170

## 171 **Results**

172

### 173 *Samples*

174

175 In total 137 samples were analyzed, 82 samples (60%) from Java, 55 samples (40%) from Bali.  
176 Tumor characteristics are summarized in table 1. Morphologically, 91 (66%) tumors were  
177 classified as SCC, 30 (22%) as AC, and 16 (12%) as ASC. The histological subtypes were  
178 unequally distributed amongst the two populations with relatively less SCC, and more AC and  
179 ASC in the Javanese cohort (table 1).

180 In total 120 (88%) samples were HPV positive, with HPV 16 as the most frequently detected HPV  
181 type (45%), followed by HPV 18 (29%) and 52 (12%). HPV 39 was the fourth most frequent HPV  
182 type, predominantly detected in the Balinese cohort, but occurred in 8/10 cases together with  
183 another high risk HPV type. HPV 16 was more frequently detected in SCC compared to AC and  
184 ASC (56%, 20%, and 25%, respectively,  $p=0.001$ ), whereas HPV 18 was more frequently  
185 detected in AC and ASC, compared to SCC (53%, 69%, and 14%, respectively,  $p=0.000$ ). HPV  
186 18 was more frequently detected in Javanese samples, which correlated with the higher  
187 frequency of AC and ASCs in this cohort (table 1).

188

### 189 *Mutation analyses*

190

191 In table 2, all detected mutations are listed, and in figures 1 and 2, the mutation spectrum is  
192 visualized for single (in grey) and multiple (in black) mutations per gene, for the total cohort, per  
193 region, or per histological subtype.

194 In total, 95 somatic mutations were identified in 66/137 cervical tumors (48%). In 45 tumors (33%)  
195 one mutation was detected, in 14 tumors (10%) two mutations were detected, in six tumors (4%)



196 three mutations were detected, and in one tumor four mutations were detected. Multiple  
197 mutations occurred within genes and between genes. *HRAS* mutations occurred significantly  
198 more often with a concomitant *CDKN2A* ( $N=2$ , OR 16.7, 95% CI 1.8-158.8) or *NRAS* mutation  
199 ( $N=2$ , OR 16.7, 95% CI 1.8-158.8).

200 In the Javanese cohort 44 mutations were detected in 34/82 tumors (42%), in the Balinese cohort  
201 51 mutations were detected in 32/55 tumors (58%) ( $p=0.055$ ). In the Balinese cohort significantly  
202 more tumors showed  $\geq 2$  mutations per sample compared to the Javanese cohort (14/55 (25%)  
203 vs. 7/82 (9%), respectively,  $p=0.019$ ). Comparing both cohorts per gene, significant differences  
204 were seen between Java and Bali for *CTNNB1* (2% vs. 11%,  $p=0.038$ ), *CDKN2A* (1% vs. 11%,  
205  $p=0.017$ ), and *NRAS* (1% vs. 11%,  $p=0.017$ ).

206 Comparing by histological subtype, we detected a significantly higher overall mutation frequency  
207 in SCC compared to AC (55% vs. 33%,  $p=0.040$ ), and a higher *PIK3CA* mutation frequency in  
208 SCC compared to AC (29% vs. 10%,  $p=0.039$ ). No significant differences were seen comparing  
209 SCC with ASC, or comparing AC with ASC, taking into account the small number of the ASCs  
210 ( $n=16$ ) in this study. Combining AC and ASC as one subgroup and comparing this with SCC,  
211 revealed that *CTNNB1* gene mutations occurred solely in SCC samples ( $N=8$  (9%),  $p=0.038$ ).

212 A correlation analysis was performed to detect associations between age and overall mutation  
213 status or gene specific mutation status. No association was found between age and any mutation,  
214 nor between age and a *PIK3CA* mutation. However, *CTNNB1* mutations were associated with a  
215 significantly higher age at time of diagnosis, with a mean age of 60,3 years in patients with  
216 *CTNNB1* mutated tumors, and a mean age of 47,7 years in patients with non-*CTNNB1* mutated  
217 tumors ( $p=0.001$ ).

218 The correlation analysis was repeated for FIGO stage (International Federation of Gynecology  
219 and Obstetrics). However, FIGO stages were only known for 66/82 (80%) Javanese tumors (23%  
220 stage 1b, 12% 2a, 35% 2b, 30%  $\geq 3a$ ), so this concerns a sub analysis for Javanese samples  
221 only. No correlation was found between FIGO stage and a positive mutation status or with  
222 multiple mutations. However, *PIK3CA* mutated tumors had significantly higher FIGO stages, with  
223 18/43 FIGO  $\geq 2b$  tumors mutated (42%) vs. 3/23 FIGO  $\leq 2a$  tumors mutated (13%) ( $p=0.025$ ). For

224 all other genes, the mutation rates were too low to perform meaningful statistical analysis,  
225 however, within the subgroup of 66 tumors, all mutations in *FBXW7*, *CTNNB1*, and *KRAS* were  
226 seen in FIGO 3b tumors ( $N=4, 2,$  and  $2,$  respectively), with no mutations in lower stage tumors.  
227 Subsequently, univariate analyses were performed for overall mutation status (having any  
228 somatic mutation) or gene specific mutation status, and HPV overall positivity (for any type) or  
229 HPV type specific positivity. Results are summarized in table 3. There was a significant  
230 correlation between a positive mutation status and a multiple HPV infection (16/21 (76%) vs. 5/21  
231 (24%),  $p=0.006$ ). Furthermore, having any somatic mutation was significantly associated with  
232 HPV 16 positivity (36/61 (59%) vs. 25/61 (41%),  $p=0.023$ ), and HPV 52 positivity (12/17 (71%),  
233 vs. 5/17 (29%),  $p=0.048$ ). *PTEN* mutations were associated with HPV negativity (4/17 (23%) vs.  
234 4/120 (3),  $p=0.009$ ). *KRAS* mutations were associated with an infection with multiple HPV types  
235 (3/21 (14%) vs. 1/99 (1%),  $p=0.017$ ). *PIK3CA* mutations were associated with HPV 16 positivity  
236 (23/61 (38%) vs. 10/76 (13%),  $p=0.001$ ), and inversely associated with HPV 18 positivity (5/40  
237 (12%) vs. 28/97 (29%),  $p=0.042$ ). *CDKN2A* mutations correlated with HPV 52 positivity (3/17  
238 (18%) vs. 4/120 (3%),  $p=0.041$ ).

239

#### 240 *Multivariate analysis*

241

242 Multivariate logistic regression analyses revealed that having any somatic mutation was  
243 associated with HPV 16 (OR 2.5, 95% CI 1.1-5.5), and HPV 52 (OR 4.4, 95% CI 1.3-14.7).  
244 Having a *PIK3CA* mutation was associated with HPV 16 (OR 7.9, 95% CI 2.3-27.1) and HPV 45  
245 (OR 12.0, 95% CI 1.6-89.1), not with histological subtype or age. Having a *CTNNB1* mutation  
246 was associated with age (OR 1.1, 95% CI 1.0-1.2), not with histopathology, nor with Balinese  
247 origin. Having a *CDKN2A* mutation was associated with HPV 52 (OR 30.8, 95% CI 1.9-489.3),  
248 with a concomitant *HRAS* mutation (OR 38.7, 95% CI 1.3-1157.8), and AC subtype (OR 27, 95%  
249 CI 1.1-674.4), but not with Balinese origin (OR 13.3, 95% CI 0.68-258.8). However, having a  
250 *NRAS* mutation was associated with Balinese origin (OR 10.7, 95% CI 1.0-113.3), and also with a  
251 concomitant *HRAS* mutation (OR 15.3, 95% CI 1.5-155.5). The other way around, *HRAS*

252 mutations were associated with a concomitant *CDKN2A* or *NRAS* mutation (OR 18.5 and 12.5,  
253 95% CI 1.4-252.2 and 1.2-135.4, respectively).

254

## 255 **Discussion**

256

257 In the present study, we have shown that potentially actionable somatic mutations occurred in  
258 48% of Indonesian cervical carcinomas. The landscape of mutations showed similarities as well  
259 as differences between two Indonesian cancer cohorts from Java and Bali, and several  
260 correlations were shown between somatic mutations and HPV (type) positivity.

261 With the emergence of tumor targeting drugs such as tyrosine kinase inhibitors, targeting the  
262 tumor based on its genomic profile rather than its histological background, it is important to study  
263 the prevalence of targetable oncogenic driver mutations throughout diverse ethnical cancer  
264 populations from diverse geographic areas. The prevalence of somatic mutations in cervical  
265 cancer was investigated previously in other cervical cancer cohorts worldwide from the US  
266 ( $N=80$ ) [21], Norway/Mexico ( $N=100/15$ ) [12], China ( $N=285$ ) [23], the Netherlands ( $N=301$ ) [20],  
267 France ( $N=29$ ) [25], Hong Kong ( $N=15$ ) [22], Guatemala/Venezuela/Mexico ( $N=280/40/325$ ) [24],  
268 to India ( $N=10$ ) [26], using varying techniques, from whole genome and/or exome sequencing  
269 [12, 13, 26], direct sequencing [23], to oncopanel analysis [20, 21, 25] or a combination of  
270 techniques [22, 24].

271 This is the first study to describe the prevalence of driver mutations in an Indonesian cervical  
272 cancer cohort. Indonesia is the world's largest, and most widely scattered archipelago, populated  
273 by more than 260 million people of more than 300 distinct native ethnic groups, and where  
274 cervical cancer is still the second most common cancer in women [1]. We analyzed a Javanese  
275 cohort, representing the largest ethnical Muslim population derived from the island Java, and  
276 compared this with a Balinese cohort, representing a relatively isolated Hindu population from the  
277 island Bali. We described the similarities and differences of the mutation spectrum for both  
278 cohorts (figure 1), and in multivariate analysis, a significantly higher mutation frequency of *NRAS*  
279 was seen in Balinese- (11%) compared to Javanese patients (1%). This is the first cervical cancer

280 cohort in which a *NRAS* mutation rate of 11% was described, and this may be of interest for  
281 future targeted therapies. *NRAS* plays a role in PI3K as well as MAPK signaling and is mutated in  
282 15-20% of melanomas. Studies concerning *NRAS* mutated melanomas suggested that combined  
283 targeting of both pathways may improve treatment [31].

284 Recently, we have reported on the mutation spectrum of a Dutch cervical cancer cohort [20],  
285 using the same mutation panel as in the present study [29], and therefore, comparisons between  
286 Indonesia, a high incidence country, and the Netherlands, a low incidence country, could be  
287 performed. A significantly higher overall mutation frequency, as well as a higher rate of multiple  
288 mutations per sample, and significantly more *FBXW7*, *CDKN2A*, *NRAS*, and *HRAS* mutations  
289 were seen in the Indonesian cohort compared to the Dutch cohort (supplementary table 1). It  
290 remains uncertain whether these differences are attributable to race/ethnicity/geography, or that  
291 they are based on differences in tumor characteristics or stage.

292 One limitation of the present study is the lack of some relevant clinicopathological characteristics  
293 of the Indonesian samples such as FIGO stage, tumor diameter, lymph node metastasis, and  
294 survival. However, FIGO stage data were known for 66 Javanese patients, and showed  
295 significantly more advanced stage disease compared to the Dutch cohort (Indonesian cohort  
296 20/66 (30%)  $\geq$  FIGO stage 3a, whilst Dutch cohort consisted of only stage 1b-2b tumors,  
297  $p < 0.001$ ). We presume, this could also be the case for the Balinese patients, as it is known that in  
298 Indonesia, patients often present with advanced stage disease. It is hypothesized that cancer,  
299 including cervical cancer, results from sequential mutations in specific oncogenes and/or tumor  
300 suppressor genes, and that the mutation frequency increases with advanced cancer stage [32].  
301 However, in the present study, we found no association between increasing FIGO stage and  
302 overall mutation frequency or multiple mutations, which is in line with other reports [12, 13, 21, 23,  
303 24]. Gene specifically, however, we do see that the occurrence of *PIK3CA* mutations is  
304 associated with higher FIGO stage tumors, which is in line with the Dutch cohort [20] and a  
305 recently published study by Verlaet et al., showing that *PIK3CA* mutations are considered a late  
306 event in cervical carcinogenesis, and a rare event in its precursor lesions [33].

307 *PIK3CA* was the most frequently mutated gene (24%) in the present Indonesian cervical cancer  
308 cohort, which is in line with previous reported frequencies in cervical cancer from the Netherlands  
309 (20%), France (27%), Latin America (28-33%), the U.S. (31%), and the TCGA data (26%) [13, 20,  
310 21, 24, 25]. However, lower frequencies were also described in Norway (15%) and China (12%)  
311 [12, 23]. And in a recent study from India, whole exome sequencing was performed on 10 FIGO  
312 stage 3b SCCs, with no *PIK3CA* mutations detected at all [26]. *PIK3CA* mutations lead to an  
313 altered production of the catalytic subunit p110 $\alpha$  of the enzyme phosphatidylinositol 3-kinase  
314 (PI3K), allowing the PI3K pathway to signal without regulation, leading to uncontrolled cell growth,  
315 proliferation and survival. The tumor suppressor *PTEN* was third most frequently mutated in  
316 Indonesian cervical cancer (6%), comparable with the mutation frequency of Dutch (4%) and  
317 Norwegian (6%) cervical cancer patients, and the TCGA data (8%) [12, 13, 20]. The function of  
318 *PTEN* is to dephosphorylate PI3K, and mutations lead to uncontrolled cell growth. *PIK3CA* and  
319 *PTEN* are the most frequently mutated genes in human cancers, and therapeutics targeting the  
320 PI3K pathway are being developed rapidly, and are today in diverse phases of (pre)clinical trials  
321 [34]. Though, for cervical cancer, therapies targeting the PI3K pathway are still scarce [35].  
322 In the Indonesian cohort, 97% of the *PIK3CA* mutated tumors were mutated in the helical domain,  
323 dominated by p.E545K, and followed by p.E542K; only 2 mutations in the kinase domain were  
324 detected (p.H1047L and p.H1047Y), which is in line with other studies [13, 24]. This is a  
325 distinctive feature of cervical carcinoma compared to other cancers with high frequencies of  
326 *PIK3CA* mutations, such as endometrial, ovarian, breast, and colorectal carcinoma, in which  
327 mutations in the kinase domain occur at least as frequent in the helical domain [24].  
328 Unfortunately, it is the kinase domain H1047R mutation that is explicitly associated with an  
329 increased response rate to PI3K/AKT/mTOR inhibitors [36]. In a study of Wang et al., 15/60  
330 locally advanced cervical SCCs had E542K or E545K mutations (there were no kinase domain  
331 mutations), and these patients showed a significantly worse response to cisplatin based  
332 chemoradiation [17]. Further research is necessary to develop therapies that can intervene  
333 cancers with specific *PIK3CA* helical domain mutations.

334 The p53-dependent tumor suppressor gene *FBXW7* also plays a role in the PI3K/mTOR pathway,  
335 and was the second most frequently mutated gene (6%) in Indonesian cervical cancer, which was  
336 significantly more frequent compared to our Dutch cohort (1%), but less compared to the study of  
337 Ojesina et al. (15%), and the TCGA data (11%) [12, 13, 20]. *FBXW7* mutations are hypothesized  
338 to be a late event in cervical cancer, which might explain the higher frequency in the Indonesian  
339 cohort [32]. *FBXW7* mutated tumor cell lines have shown to be sensitive to rapamycin treatment,  
340 and we urge for further research concerning *FBXW7* mutated cervical carcinomas [37].  
341 Furthermore, in this study we compared the mutation frequencies between histological  
342 subgroups, as we determined some differences between SCC, AC, and ASC in our previous  
343 study concerning Dutch cervical carcinomas [20]. In accordance with our study concerning Dutch  
344 carcinomas, also in Indonesia *PIK3CA* mutations and *CTNNB1* mutations were associated with  
345 the SCC subtype. However, in the TCGA data, *CTNNB1* mutations were only detected in three  
346 samples (1,7%) of which two were SCC subtype and one was a AC [13]. Remarkable, *KRAS*  
347 mutations were not associated with AC in the Indonesian cohort, which is in contrast with many  
348 other studies [12, 13, 20, 21].  
349 We also investigated the presence of HPV and its correlations with somatic mutations. In  
350 Indonesia, a different HPV type distribution amongst the population as well as in cervical cancer  
351 patients was described, especially with a significantly higher prevalence rate of HPV 52 [5, 6]. In  
352 a recent large, retrospective cohort study from Murdiyarto et al., 11.224 cytology swabs from  
353 Jakarta area were typed for HPV, and HPV 52 was the most prevalent HPV type in normal  
354 cytology (1%), and the second most common type in SCC (26%) [38]. It is unclear why no AC  
355 were included in that study. In the current cohort, again we showed a remarkable high prevalence  
356 of HPV 52 in the Javanese (12%) as well as in the Balinese cohort (13%). This is an important  
357 finding in the light of preventive strategies, because HPV 52 is not included in the available FDA  
358 approved HPV vaccines yet.  
359 Significant associations were identified between the presence of any somatic mutation and HPV  
360 16 positivity, based on the positive correlation between *PIK3CA* mutations and HPV 16 positivity.  
361 In the Dutch cervical cancer cohort this association was not found. Also Wright et al. investigated

362 associations between HPV type and *PIK3CA* or *KRAS* mutations in cervical carcinomas, but did  
363 not detect any [21]. Contrary, *PTEN* mutations were associated with HPV negativity, a feature  
364 that was also seen in the Dutch cohort, and described previously by Minaguchi et al.[39].  
365 However, the coverage of possible *PTEN* mutations by the mutation panel used was only 40%  
366 [29]. Therefore, additional techniques such as immunohistochemistry should be performed to  
367 identify the true mutation rate of *PTEN* in cervical cancer, to clarify its association with HPV. We  
368 also detected an association between any somatic mutation and HPV 52 positivity, based on the  
369 positive correlation between *CDKN2A* mutations and HPV 52 positivity. *CDKN2A* was mutated in  
370 11% of the Balinese cervical carcinoma patients, which is the highest frequency described in  
371 cervical cancer compared to other studies [20, 24]. Its correlation with HPV 52 is remarkable, and  
372 has not been described previously. Given the high prevalence rates of HPV 52 in Indonesia this  
373 feature certainly warrants for further investigation.

374 To conclude, we have presented the landscape of potentially actionable somatic mutations in an  
375 Indonesian cervical cancer cohort, and placed the results in the context of histology and HPV  
376 type. Most noticeable is the predominance of mutations concerning the PI3K pathway, in  
377 concordance with results from other countries. Although we realize that implementation of  
378 expensive targeting therapies in countries like Indonesia remains highly uncertain, we do prompt  
379 for more research to develop therapies that target this PI3K pathway, explicitly for more advanced  
380 stage cervical carcinoma patients.

381

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383

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386

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388

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390

391 **Conflict of Interest Statement**

392

393 The authors have nothing to disclose.

394

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396

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500

501 **Table legends**

502

503 **Table 1. Baseline characteristics**

504 Baseline characteristics of all 137 included cervical carcinoma patients from Indonesia, and for  
505 the Javanese and Balinese cohorts separately. *P* values in bold were considered to indicate  
506 statistical significance. Abbreviations: *N*, number; IQR, interquartile range; SCC, squamous cell  
507 carcinoma; AC, adenocarcinoma; ASC, adenosquamous carcinoma; HPV, human papillomavirus.  
508 \* Other, infrequent, HPV types detected were the high risk types HPV 31 (*N*=2), 33 (*N*=2), 35  
509 (*N*=1), 51 (*N*=1), 56 (*N*=1), 58 (*N*=1), 59 (*N*=2), 66 (*N*=1), and "X" (*N*=1), and the low risk types  
510 HPV 11 (*N*=1), and HPV 54 (*N*=1). The low risk HPV types occurred concomitantly with HPV 16  
511 and with HPV 33 and 52, respectively.

512

513 **Table 2. Mutation frequencies**

514 Mutation frequencies as detected in a cohort of 137 Indonesian cervical cancer samples. In total  
515 174 hot spot mutations in 13 genes were analyzed. Mutations are shown per gene and in order of  
516 frequency, mutations of genes that were not detected in any of the samples are not shown.  
517 *BRAF*, *FGFR2*, and *FOXL2* genes are not listed because no mutations were detected. *N*, number  
518 of samples with the mutation; %, percentage of mutated samples of 137 cervical cancer  
519 samples. <sup>a</sup> Three samples contained two *PIK3CA* mutations (2x E542K with E545K, and 1x  
520 E545K with H1047Y); <sup>b</sup> One sample contained two *CTNNB1* mutations (T41A with G34E); <sup>c</sup> One  
521 sample contained two *PTEN* mutations (R130fs\*4 with Q214\*).

522

523 **Table 3. Correlations between human papillomavirus infection and mutations**

524 Correlations between human papillomavirus (HPV) infection (any, multiple, or type specific), and  
525 somatic mutations (any, multiple, gene specific) are shown in number of HPV positive samples  
526 being mutated (percentage between brackets). Numbers and percentages in bold indicate  
527 statistical significant correlations. Two-sided *p* values were calculated by Chi-squared test or  
528 Fishers' exact test and only significant *p* values are annotated in the present table.

529 **Figure legends**

530

531 **Figure 1.** *Mutation spectrum per region*

532 Spectrum of somatic mutations detected in 137 Indonesian cervical cancer specimen (top panel)  
533 and with separate spectra for the Javanese and Balinese cohorts (middle and bottom panel,  
534 respectively) in *N*, number of mutated samples, and %, percentage of mutated samples within the  
535 cohort. The spectra are visualized from left to right in percentages, with black bars indicating  
536 samples with  $\geq 2$  mutations, and grey bars indicating samples with 1 mutation.

537

538 **Figure 2.** *Mutation spectrum per histological subtype*

539 Spectrum of somatic mutations detected in 137 Indonesian cervical cancer specimen (see also  
540 figure 1) separately visualized for squamous cell carcinomas (SCC, top panel), adenocarcinomas  
541 (AC, middle panel), and adenosquamous carcinomas (ASC, bottom panel) in *N*, number of  
542 mutated samples, and %, percentage of mutated samples within the cohort. The spectra are  
543 visualized from left to right in percentages, with black bars indicating samples with  $\geq 2$  mutations,  
544 and grey bars indicating samples with 1 mutation.

545

546 **Supplementary Information**

547

548 **Supplementary Table S1.** *Comparison of mutation frequencies between Indonesia and the*  
549 *Netherlands*

550 A cohort of 301 consecutive Dutch cervical carcinomas (166 squamous cell carcinomas, 55  
551 adenocarcinomas, and 80 adenosquamous carcinomas) was analyzed for somatic mutations  
552 previously using the Gynecarta mutation panel, as described by Spaans et al. [20]. Mutation data  
553 were compared with the current Indonesian cervical cancer cohort of 137 carcinomas.  
554

555 **Highlights**

556

- 557 • In 48% of 137 Indonesian cervical carcinomas  $\geq 1$  somatic mutation is present
- 558 • Most frequently mutated are *PIK3CA* (24%), *FBXW7* (7%), *CTNNB1* (6%), and *PTEN* (6%)
- 559 • Squamous cell carcinomas show more *PIK3CA* and *CTNNB1* mutations than
- 560 adenocarcinomas
- 561 • *PIK3CA* mutations correlate with HPV16, *CDKN2A* – with HPV52, *PTEN* – with HPV absence
- 562 • Prioritize research of PI3K-pathway targeting therapies in advanced cervical cancer
- 563

564 **Table 1.** Baseline characteristics

		Total N=137	Java N=82	Bali N=55	<i>p</i> value Java vs. Bali
Age in years, median (IQR)		47 (41-53)	46 (41-52)	49 (41-58)	0.057
Morphology, <i>N</i> (%)	SCC	91 (66)	45 (55)	45 (84)	<b>0.002</b>
	AC	30 (22)	25 (31)	5 (9)	
	ASC	16 (12)	12 (15)	4 (7)	
HPV positive, <i>N</i> (%)		120 (88)	77 (94)	43 (78)	<b>0.006</b>
>1 HPV type detected, <i>N</i> (%)		21 (15)	10 (12)	11 (20)	<b>0.006</b>
HPV type distribution, <i>N</i> (%)	HPV 16	61 (45)	33 (40)	28 (51)	0.218
	HPV 18	40 (29)	32 (39)	8 (15)	<b>0.002</b>
	HPV 52	17 (12)	10 (12)	7 (13)	0.926
	HPV 39	10 (7)	1 (1)	9 (16)	<b>0.001</b>
	HPV 45	6 (3)	5 (6)	1 (2)	0.401
	Other*	14 (10)	8 (7)	6 (11)	

565

566

**Table 2.** Mutation frequencies

Gene/mutation	<i>N</i>	%
<i>PIK3CA</i> <sup>a</sup>	33	24.1
p.E545K	27	
p.E542K	6	
p.E545D	1	
p.H1047L	1	
p.H1047Y	1	
<i>FBXW7</i>	9	6.6
p.R465H	4	
p.R465C	2	
p.R479Q	2	
p.R479L	1	
<i>CTNNB1</i> <sup>b</sup>	8	5.8
p.G34E	5	
p.G34R	2	
p.S33Y	1	
p.T41A	1	
<i>PTEN</i> <sup>c</sup>	8	5.8
p.R130fs*4	6	
p.R173H	1	
p.Q214*	1	
p.L318fs*2	1	
<i>CDKN2A</i>	7	5.1
p.W110*	3	
p.R58*	2	
p.P114L	2	
<i>NRAS</i>	7	5.1
p.G12S	2	
p.G13D	2	
p.G12D	1	
p.G12V	1	
p.Q61K	1	
<i>PPP2R1A</i>	7	5.1
p.R258H	7	
<i>KRAS</i>	5	3.6
p.G12A	2	
p.G12D	2	
p.G12V	1	
<i>HRAS</i>	5	3.6
p.G13S	3	
p.G12S	1	
p.G13D	1	
<i>FGFR3</i>	1	0.7
p.A391E	1	



570

**Table 3.** Correlations between human papillomavirus infection and mutations

HPV positive for: Gene mutation:	any type (N=120)	≥2 types (N=21)	type 16 (N=61)	type 18 (N=40)	type 52 (N=17)	type 39 (N=10)	type 45 (N=6)
Any mutation (N=66)	59 (49)	<b>16 (76)<sup>a</sup></b>	<b>36 (59)<sup>b</sup></b>	16 (40)	<b>12 (71)<sup>c</sup></b>	6 (60)	3 (50)
≥2 mutations (N=21)	18 (31)	5 (31)	12 (33)	5 (31)	3 (25)	2 (33)	0 (0)
<i>PIK3CA</i> (N=33)	32 (27)	7 (33)	<b>23 (38)<sup>f</sup></b>	<b>5 (13)<sup>g</sup></b>	5 (29)	0 (0)	3 (50)
<i>FBXW7</i> (N=9)	7 (6)	2 (10)	4 (7)	2 (5)	1 (6)	1 (10)	0 (0)
<i>CTNNB1</i> (N=8)	7 (6)	3 (14)	4 (7)	2 (5)	2 (12)	1 (10)	0 (0)
<i>PTEN</i> (N=8)	<b>4 (3)<sup>d</sup></b>	1 (5)	3 (5)	1 (3)	1 (6)	1 (10)	0 (0)
<i>CDKN2A</i> (N=7)	7 (6)	2 (10)	2 (3)	3 (8)	<b>3 (18)<sup>h</sup></b>	2 (20)	0 (0)
<i>NRAS</i> (N=7)	7 (6)	1 (5)	5 (8)	2 (5)	1 (6)	1 (10)	0 (0)
<i>PPP2R1A</i> (N=7)	6 (5)	1 (5)	4 (7)	2 (5)	0 (0)	0 (0)	0 (0)
<i>KRAS</i> (N=5)	4 (3)	<b>3 (14)<sup>e</sup></b>	2 (3)	2 (5)	2 (12)	0 (0)	0 (0)
<i>HRAS</i> (N=5)	5 (4)	1 (5)	2 (3)	2 (5)	1 (6)	1 (10)	0 (0)
<i>FGFR3</i> (N=1)	1 (1)	1 (5)	1 (2)	1 (3)	0 (0)	1 (10)	0 (0)
<i>p</i> value		<b><sup>a</sup>0.006</b>	<b><sup>b</sup>0.023</b>		<b><sup>c</sup>0.048</b>		
	<b><sup>d</sup>0.009</b>	<b><sup>e</sup>0.017</b>	<b><sup>f</sup>0.001</b>	<b><sup>g</sup>0.042</b>	<b><sup>h</sup>0.041</b>		

571

572

573 **Table S1.** Comparison of mutation frequencies between Indonesia and the Netherlands

Gene mutation:	Indonesia N=137	The Netherlands* N=301	p value
Any mutation	66 (48%)	103 (34%)	0.005
≥2 mutations	21 (15%)	13 (4%)	0.002
<i>PIK3CA</i>	33 (24%)	61 (20%)	ns
<i>FBXW7</i>	9 (7%)	3 (1%)	0.002
<i>CTNNB1</i>	8 (6%)	8 (3%)	ns
<i>PTEN</i>	8 (6%)	12 (4%)	ns
<i>CDKN2A</i>	7 (5%)	4 (1%)	0.019
<i>NRAS</i>	7 (5%)	1 (<1%)	0.001
<i>PPP2R1A</i>	7 (5%)	9 (3%)	ns
<i>KRAS</i>	5 (4%)	20 (7%)	ns
<i>HRAS</i>	5 (4%)	1 (<1%)	0.012
<i>FGFR3</i>	1 (1%)	2 (1%)	ns

574 \* Cohort described previously by Spaans et al. (2015) in PLoS ONE 10(7):e013670.

575

Region	Gene	N	%	Mutation Spectrum			
				25%	50%	75%	100%
Total N=137	Any Mutation	66	48.2				
	<i>PIK3CA</i>	33	24.1				
	<i>FBXW7</i>	9	6.6				
	<i>CTNNB1</i>	8	5.8				
	<i>PTEN</i>	8	5.8				
	<i>CDKN2A</i>	7	5.1				
	<i>NRAS</i>	7	5.1				
	<i>PPP2R1A</i>	7	5.1				
	<i>KRAS</i>	5	3.6				
	<i>HRAS</i>	5	3.6				
	<i>FGFR3</i>	1	0.7				
Java N= 82	Any Mutation	34	41.5				
	<i>PIK3CA</i>	24	29.3				
	<i>FBXW7</i>	4	4.9				
	<i>CTNNB1</i>	2	2.4				
	<i>PTEN</i>	3	3.7				
	<i>CDKN2A</i>	1	1.2				
	<i>NRAS</i>	1	1.2				
	<i>PPP2R1A</i>	2	2.4				
	<i>KRAS</i>	3	3.7				
	<i>HRAS</i>	1	1.2				
	<i>FGFR3</i>	1	1.2				
Bali N= 55	Any Mutation	32	58.2				
	<i>PIK3CA</i>	9	16.4				
	<i>FBXW7</i>	5	9.1				
	<i>CTNNB1</i>	6	10.9				
	<i>PTEN</i>	5	9.1				
	<i>CDKN2A</i>	6	10.9				
	<i>NRAS</i>	6	10.9				
	<i>PPP2R1A</i>	5	9.1				
	<i>KRAS</i>	2	3.6				
	<i>HRAS</i>	4	7.3				
	<i>FGFR3</i>	0	0.0				

576  
577

Histology	Gene	N	%	Mutation Spectrum			
				25%	50%	75%	100%
SCC N= 91	Any Mutation	50	54.9				
	<i>PIK3CA</i>	26	28.6				
	<i>FBXW7</i>	6	6.6				
	<i>CTNNB1</i>	8	8.8				
	<i>PTEN</i>	6	6.6				
	<i>CDKN2A</i>	5	5.5				
	<i>NRAS</i>	5	5.5				
	<i>PPP2R1A</i>	6	6.6				
	<i>KRAS</i>	2	2.2				
	<i>HRAS</i>	4	4.4				
	<i>FGFR3</i>	1	1.1				
AC N= 30	Any Mutation	10	33.3				
	<i>PIK3CA</i>	3	10.0				
	<i>FBXW7</i>	3	10.0				
	<i>CTNNB1</i>	0	0.0				
	<i>PTEN</i>	2	6.7				
	<i>CDKN2A</i>	2	6.7				
	<i>NRAS</i>	1	3.3				
	<i>PPP2R1A</i>	1	3.3				
	<i>KRAS</i>	2	6.7				
	<i>HRAS</i>	0	0.0				
	<i>FGFR3</i>	0	0.0				
ASC N= 16	Any Mutation	6	37.5				
	<i>PIK3CA</i>	4	25.0				
	<i>FBXW7</i>	0	0.0				
	<i>CTNNB1</i>	0	0.0				
	<i>PTEN</i>	0	0.0				
	<i>CDKN2A</i>	0	0.0				
	<i>NRAS</i>	1	6.3				
	<i>PPP2R1A</i>	0	0.0				
	<i>KRAS</i>	1	6.3				
	<i>HRAS</i>	1	6.3				
	<i>FGFR3</i>	0	0.0				