Clinical and genetic landscape of treatment naive cervical cancer: Alterations in PIK3CA and in epigenetic modulators associated with sub-optimal outcome

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Clinical and genetic landscape of treatment naive cervical cancer: Alterations in PIK3CA and in epigenetic modulators associated with sub-optimal outcome

Suzy Scholl a,⁎, Marina Popovic b, Anne de la Rochefordiere a, Elodie Girard a, Sylvain Dureau a, Aljosa Mandic b, Katarina Koprivsek b, Nina Samet c, Marius Craina d, Madalin Margan d, Sanne Samuels e, Henry Zijlmans e, Gemma Kenter e, Peter Hillemanns f, Sorin Dema d, Alis Dema d, Goran Malenkovic b, Branislav Djuran b, Anne Floquet b, Delphine Garbay g, Frédéric Guyon h, Pierre Emmanuel Colombo h, Michel Fabbro h, Christine Kerr h, Charlotte Ngo i, Fabrice Lecuru i, Eleonor Rivin del Campo i, Charles Coutant k, Frédéric Marchal l, Nathalie Mesguiez-Nebout m, Virginie Fourchotte a, Jean Guillaume Feron a, Philippe Morice b, Eric Deutsch n, Pauline Wimberger o, Jean-Marc Classe p, Noreen Gleeson q, Heiko von der Leyen r, Mathieu Minsat a, Coraline Dubot a, Pierre Gestraud a, Attila Kereszt s, Istvan Nagy s, Balazs Balint s, Els Berns (Petronella) t, Ekaterina Jordanova e, Nicolas de Saint-Jorre u, Alexia Savignoni a, Nicolas Servant a, Philippe Hupe a, Leanne de Koning a, Pierre Fumoleau a, Roman Rouzier a, Maud Kamal a

⁎ Corresponding author at: Department of Drug Development and Innovation, Institut Curie, 26, rue d’Ulm, Paris 75005, France.
E-mail address: suzy.scholl@curie.fr (S. Scholl).

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**Research in context**

**Evidence before this study**

The Cancer Genome Atlas Research Network (TCGA) published the molecular characterizations of its cervical cancer (CC) dataset, providing a wealth of information on tumour subtypes. However, clinical annotation of TCGA samples does not include complete data on tumour stage and treatment outcome. Therefore, it has not been possible so far to associate molecular data with response to treatment efficacy.

**Added value of this study**

BioRAIDS is the first prospectively collected extensive database from Central and Western EU countries. The aim of the European BioRAIDS study was to characterize newly diagnosed cervical cancer from a biological and a clinical view, in order to identify new pre-treatment prognostic factors and to provide a scientific rationale for novel treatment combinations. FIGO stage at inclusion and the revised staging (FIGO 2018) are compared with treatment categories and outcome. Close to 90% of BioRAIDS patients received chemoradiation, as compared to surgery, which was the primary treatment in the TCGA dataset.

**Implications of all the available evidence**

We identified genetic alterations in PIK3CA and in epigenetic modulators that were associated with a poor prognosis. Our data suggest that new treatment combinations may improve outcome in subsets of cervical cancer patients.

**1. Background**

Despite effective prophylactic vaccines, cervical cancer remains the second most commonly diagnosed cancer in women. It accounts for more than a quarter of a million deaths worldwide each year [1] with the highest impact in countries and regions with suboptimal access to health care. The etiology of >90% of cervical cancers is the persistent infection with high-risk human papillomavirus types (HPV) which inactivate normal cellular controls via the viral proteins E6 and E7, progressively leading to genomic instability and accumulation of molecular alterations. While prognosis is related to FIGO stage and the presence or absence of lymph node metastases [2], the role of specific molecular patterns that resist DNA repair interference (platinum compounds and chemoradiation) is poorly documented. The Cancer Genome Atlas Research Network [3] has published molecular characterizations of its cervical cancer (CC) dataset, but correlations with outcome based on specific treatments are awaited. While some patients can be treated with surgery up to stage IB2 and IIA, the gold standard of stages IB2-III cervical cancer treatment is chemoradiation. Two thirds of the “core” TCGA samples analyzed previously originated from patients in which surgery was reportedly the primary treatment.

For stages ≥IB2 at presentation, 30–40% of patients relapse within 2–3 years [4]. While improved therapeutic strategies over the last decade using IMRT (intensity modulated radiotherapy) to the pelvis and “preventive” para-aortic lymph node irradiation in patients with high risk disease and positive pelvic nodes, do make an impact on local control and outcome, there remains a high variability in treatment strategies. Image guided brachytherapy in a recently published multicenter cohort study (RetroEMBRACE) demonstrated excellent 3-year local control rates of 93% and 79% for patients with FIGO stage IIB and IIB disease, respectively. However, the 5-year actuarial disease-specific OS was 65% [5].

A recent report identified a subset of virally associated tumours that presented a molecular profile distinct from that of typical HPV+ tumours and exhibited poor treatment response, indicating molecular and clinical similarities with HPV- tumours [6]. Patient stratification based on molecular patterns associated with poor outcome is a first step towards targeted strategies. In the current manuscript, we report on the first prospectively collected extensive database from Central and Western EU countries with a focus on correlating molecular patterns with tumour pathophysiology, response to treatment and outcome.

**2. Methods**

2.1. Patient recruitment, samples collection and treatment

The EU funded RAIDs Network (Rational Molecular Assessment and Innovative Drug Selection, www.raids-fp7.eu) collected a prospective CC sample and dataset BioRAIDS: NCT02428842 (n = 419).

The clinical protocol together with tumour sampling procedures and quality control of samples and treatments in 18 European centers (seven European countries) have been previously published. [7,8]. A signed informed consent for the participation in the study protocol was a prerogative, prior to inclusion and sampling. Quality assurance and source data verification was performed according to a risk adapted approach and a pre-defined essential study data. Frozen and formalin-fixed, paraffin-embedded tumour samples, blood as well as frozen sera specimen were transferred to common repositories for centralized processing, pathology review, and quality assessments at Erasmus University, Rotterdam, prior to DNA sequencing, analysis and storage at SeqQomics Ltd., Hungary. Ethical review was conducted according to national requirements and in accordance with the Declaration of Helsinki [9,10]. All tumour and serum samples and clinical data were registered in a common database using a unique barcode system assuring data

*Findings: At a median follow up (FU) of 22 months, progression-free survival rates of this FIGO stage IB1-IV population, treated predominantly (87%) by chemoradiation, were 65% [95%: 60.2-71.1]. Dominant oncogenic alterations were seen in PIK3CA (40%), while dominant suppressor gene alterations were seen in KMT2D (15%) and KMT2C (16%). Cumulative frequency of loss-of-function (LOF) mutations in any epigenetic modulator gene alteration was 47% and it was associated with PIK3CA gene alterations in 32%. Patients with tumours harboring alterations in both pathways had a significantly poorer PFS. A new finding was the detection of a high frequency of gains of TLR4 gene amplifications (10%), as well as amplifications, mutations, and non-frame-shift deletions of Androgen receptor (AR) gene in 7% of patients. Finally, BPPA protein expression analysis defined three expression clusters. Interpretation: Our data suggests that patient population may be stratified into four different treatment strategies based on molecular markers at the outset.**

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privacy. A radiotherapy dummy run was performed before patient recruitment to homogenize contouring among centers (Rivin del Campo et al., Radioth Oncol 2017, Supplementary material). Treatment decisions were based on guidelines defined in the clinical protocol as detailed in Ngo et al., 2015 [7] and in Supplementary material. Since FIGO staging at the time of patient accrual did not take into account lymph node staging, for the purpose of the present analysis, the patient population is shown both as a function of initial FIGO staging as well as the new FIGO 2018 staging, upstaging early stage patients with lymph node positivity to stage IIIC and down staging patients with previous stage IVA to stage IIIC2.

2.2. HPV typing

All samples included in this study were analyzed for HPV type, using the SPF10 primer set and INNO-LiPA HPV genotyping extra line probe assay (Fujirebio Europe, Gent, Belgium) according to the manufacturers protocol. For DNA isolation, one to five 10 μm tissue sections were cut depending on the size of the tumour biopsy. DNA was isolated using the automated Tissue Preparation System (Siemens Healthcare Diagnostics, NY, USA).

2.3. WES & targeted sequencing and bioinformatics analyses

Paired-end whole exome sequencing of 87 samples and paired-end targeted gene panel sequencing (608 genes) of 96 paired tumour/normal samples was performed at SeqOmics (Hungary) on a HiSeq2500 platform to date. The sequencing was performed to reach an average depth of coverage of ~150× for WES and ~730× for targeted sequencing. The data were further processed by the Institut Curie bioinformatics pipeline. Somatic alterations (point mutations, insertions/deletions and copy number changes) were identified from the aligned sequences of matched-samples using dedicated tools (Supplementary methods).

Biologically relevant variants were selected regarding their functional impact per gene category (oncogene, tumour suppressor gene, or uncharacterized) and compared with patients’ outcome. Detailed information on genomic and bioinformatics analyses is available in the Supplementary Methods section.

The future integration of additional BioRAIDS data such as the ongoing longitudinal ctDNA analysis, of transcriptomics, methylose and TME characteristics will decipher the pathway complexity in more detail and hopefully assist innovative treatment orientations.

2.4. Reverse phase protein arrays (RPPA)

154 baseline samples, 103 post-treatment samples were processed. Arrays were labeled with 194 specific antibodies except for the negative control(s) where primary antibodies were omitted, using an Autostainer Plus (AGILENT). All primary antibodies used in RPPA have been previously tested by Western Blotting to assess their specificity for the protein of interest. Detailed information is available in the Supplementary Methods section [11,12].

2.5. Biostatistical analysis

Quantitative data were described as median with range. Qualitative data were presented as numbers and proportions leaving out missing data. Survival curves were constructed according to Kaplan-Meier and were compared using the log-rank test. Overall Survival (OS) and Progression-Free Survival (PFS) were calculated from the date of inclusion to the date of event, (disease related death for OS and relapse/or disease related death for PFS). Patients without event at the date of last follow-up were censored at this time. Univariate and multivariate Cox proportional hazard models were performed to determine prognostic factors associated with relapse or death. All analyses were performed using R version 3.4.4 software.

3. Findings

3.1. Patient population eligible for analysis

Four hundred and nineteen patients with histologically proven cervical carcinoma cases were prospectively recruited between 2013 and 2016 in 18 centers from seven European countries [7,8]. Forty two patients were not eligible for analysis due to: inclusion error [26], death before start of treatment [1], investigator/patient decision [6], second cancer [4], lost to follow up [3], or missing data [2]. At a median follow up of 22 months [min = 1.1 – max = 41.2], PFS data is available for 377 patients.

3.2. Staging procedures

As defined by protocol, the FIGO staging based on clinical examination was confirmed by magnetic resonance imaging (MRI) in all 377 patients (100%). Median tumour size by MRI was 48 mm [range: 9–167]. Tumour size ≤4 cm (23 patients) was present in 248/377 (65%) patients. Additional imaging was by CT scan in 230 patients (61%), positron electron tomography (PET) scan in 185 patients (49%). Lymphadenectomy for lymph node staging purposes was performed in a total of 130 (34%) patients, depending on center policy. According to the FIGO classification in use at the time of trial conduct 291 (77%), of the evaluable patients were considered stage I-II and 86 (23%) stage III-IV. If patients with positive lymph nodes are being reclassified according to the new FIGO 2018 classification [15], into IIIC1 (pelvic node positive) and IIIC2 (aortic node positive), 137 (36%) patients are to be considered stages I-III and 240 (64%) stages III-IV. PFS according to FIGO stages are illustrated in Supplementary Figs 1 & 2. Pretreatment PET-scan imaging was carried out in 13/56 (23%) of surgical patients, in 138/263 (52%) of chemoradiation patients and in 34/58 (74%) neoadjuvant chemotherapy (NACT) patients. Positive lymph nodes (by pathology or by imaging) were diagnosed in a total of 216/377 (57%) patients.

3.3. Treatment allocation

Primary treatment allocation as a function of restaging according to the FIGO 2018 classification is shown in Table 1. First line surgical management was the preferred management in early stage (IB1 and IB2) as well as in pelvic lymph node positive disease (IIIC1), whereas chemoradiation was the treatment of choice in higher stages (stages II and III). Only 15% (n = 56) of the population were allocated to first line surgery, while the majority of the patients (n = 263; 70%) were allocated to the chemoradiation (CR) group. The patients treated with NACT had stage III-IV advanced or metastatic disease, representing 15% (n = 58) of the population. In the chemoradiation group, 21 patients could not receive concomitant chemotherapy and 34 patients received less than the planned four to five cycles of chemotherapy for reasons of tolerance (see Supplementary data).

3.4. First line and follow on treatments received in the first 6 months

Surgery: While surgical resection was the first intention-to-treat in a limited population (n = 56), a surgical resection in the first 6 months period was deemed necessary in a total of 136 (36% of all) patients (see Supplementary data). Lymphadenectomy was carried out in 202 (53%) of all patients, as a staging procedure (34%), or for treatment (19%) purposes. (Additional information in Supplementary).

Radiotherapy: Treatment consisted in external beam radiation therapy (EBRT) with concomitant platinum based chemotherapy in 295 (78%) of the population as per protocol and previous publications [7,14]; in EBRT alone (n = 34) or in brachytherapy alone (n = 2). A total of 331/377 patients (87%) received radiotherapy either as primary or as follow on treatment after surgery or NACT. Following pelvic irradiation, an additional boost was administered to the tumour by
EBRT (n = 66) and/or by brachytherapy (n = 284). Twenty-two patients received no additional external radiotherapy boost. Forty-three received no brachytherapy. (Additional information in Supplementary).

Neoadjuvant Chemotherapy: A combination of Taxol and Carboplatin was the first treatment in 55 patients (Fig. 1). Additional loco-regional treatment was administered in 44/55 (80%) patients. Second line chemotherapy was administered as needed in advanced/refractory disease.

3.5. Survival based on clinical characteristics (Table 2)

Median overall survival is presently not reached; survival rates at 24 months are 85-2% [CI95%: 80-7–89-9], progression-free survival rates are 65-4% [CI95%: 60-2–71-1]. As expected, a highly significant correlation with outcome was seen for both FIGO 2014 and FIGO 2018 stages (Tables 1 and 2 and Supplementary Figs 1 & 2). We chose to integrate the new FIGO 2018 staging in the multivariate Cox regression model and the results of multivariate analysis identified FIGO 2018, ECOG performance index and BMI as correlated with PFS. Histological type was not related to outcome.

3.6. The genetic landscape of treatment-naive cervical cancer

In our cohort of 182 patients [out of the 377 evaluable BioRAIDs patients (48%)] analyzed, using next generation sequencing (NGS) all novel and previously confirmed significantly altered genes reported by The Cancer Genome Atlas [3] were detected in the RAIDs dataset; the most frequent alterations are represented in Fig. 1. PIK3CA mutations (Supplementary Fig 3) and/or gene amplifications were the most frequently diagnosed oncogenic alteration, present in 40% of BioRAIDs patients. The most frequently diagnosed suppressor gene alterations were loss-of-function (LOF) mutations in KMT2A-D (Lysine methyl transferase) genes leading to defective histone H3K4 methylation. The cumulative frequency of tumours harboring any alterations in the epigenetic pathway (involving KMT2C, KMT2D, KMT2A, KDM5C, EP300, CREBBP, ARID1A, ARID2, ATRX). When these pathway alterations were pooled in a “metagenes”, Kaplan Meyer progression free survival estimates (Fig. 2) confirmed a poorer PFS (p = 0.02) for patients with tumours harboring at least one molecular alteration in this metagenes as opposed to patients with no such alteration. While the medium number of variants increased slightly with FIGO stage this was not statistically significant, since the confidence intervals are very large (Supplementary Fig. 6).

Among the 154 tumours analyzed using RPPA, 136 patients were evaluable for outcome. While there was no difference in overall survival, our data showed a significant poorer PFS for the cluster “EMT” as compared to the other two clusters combined (Fig. 3) (p = 0.03). There was no correlation between WES analysis and RPPA data, likely due to the limited antibody targets used in RPPA as compared to a full exome analysis.

3.8. Interpretation

Patient stratification according to outcome categories based on genomic variants, needs a prospectively accrued, sufficiently large cohort of tumour samples together with supervised standard treatments. Chemoradia was the first line of treatment in ~2/3 of patients and chemoradia or radiation was administered in 87% of all patients if first line treatments were pooled with Supplementary treatments received during the first six months. This is in stark contrast with the predominant surgical treatment (70%) reported in other series [3,15]. In case of a pelvic lymph node detection prior to treatment, most centers perform primary chemoradia to avoid the morbidity of adding radical surgery to radiotherapy. In the BioRAIDs population, only 36% of patients had a surgical tumour resection, either as a first approach in small tumours (15%) or as a secondary resection (16%) in case of an incomplete or doubtful response to chemoradia. Some positive nodes are detected only during surgery leading to additional radiotherapy or chemoradia. Reclassifying BioRAIDs patients according to the new FIGO 2018 stage resulted in well differentiated PFS curves (Supplementary data).

Integrative bioinformatics analysis suggests 4 privileged treatments for tumours associated with resistance to present treatments directed to DNA repair interference.

1: PIK3CA activating mutations and amplifications, present in 40% of tumours, were frequently associated with LOF in epigenetic regulator genes. The cumulative prevalence of both (in 34% of the population), but not the PIK3 pathway alone as suggested before [16] was associated with a significantly poorer PFS. PI3K activity contributes to diverse functional roles in cellular metabolism, immune function and cell motility in cancer [17] and is associated with treatment resistance. In a patient group with PIK3 pathway mutations we previously reported a negative MET (p = 2.42e-11), high NOTCH (p = 4.14e-17) and low E-CADHERIN (p = 2.41e-06). Significant features of cluster 2 included high NBS1 (p = 5.85e-11), MRE11 (p = 6.09e-11), FANCD2 (p = 1.69e-09), phosphorylated FANCD2 (p = 6.37e-07), and HSP90alpha (p = 4.31e-06), as well as low levels of phosphorylated AKT (p = 5.48e-18), EGFR (p = 6.39e-11), HER2 (p = 1.96e-09), HER2 (p = 4.79e-09) and p70 S6K (p = 1.01e-05) compared to the other two clusters. Significant features of cluster 3 included phosphorylated forms of NFKB (p = 8.29e-09), p70 S6 kinase (p = 2.82e-04), AKT (p = 4.36e-04), p38 MAPK (p = 7.32e-03), ERK1/2 (p = 1.32e-02), and EGFR (p = 2.67e-02).

3.7. Molecular signatures predictive of outcome following standard therapy

When patients were clustered in quantiles of PFS (ONCOPRINT: Fig. 1), those patients who remained progression free at the time of analysis (PFS, yellow line), had visibly less alterations in genes involved in Tyrosine Kinase receptor/PI3K pathway and in suppressor functions related to the following epigenetic enzyme modifications (KMT2C, KMT2D, KMT2A, KDM5C, EP300, CREBBP, ARID1A, ARID2, ATRX). When these pathway alterations were pooled in a “metagenes”, Kaplan Meyer progression free survival estimates (Fig. 2) confirmed a poorer PFS (p = 0.02) for patients with tumours harboring at least one molecular alteration in this metagenes as opposed to patients with no such alteration. While the medium number of variants increased slightly with FIGO stage this was not statistically significant, since the confidence intervals are very large (Supplementary Fig. 6).

Table 1

<table>
<thead>
<tr>
<th>Stage Number</th>
<th>Stage Number (%)</th>
<th>Surgery</th>
<th>Chemo radiation</th>
<th>NACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB1</td>
<td>34</td>
<td>IB1</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>IB2</td>
<td>62</td>
<td>IB2</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>IA</td>
<td>28</td>
<td>IA</td>
<td>73</td>
<td>4</td>
</tr>
<tr>
<td>IIA</td>
<td>17</td>
<td>IIA</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>IIB</td>
<td>11</td>
<td>IIB</td>
<td>23</td>
<td>1%</td>
</tr>
<tr>
<td>IIB</td>
<td>42</td>
<td>IIB</td>
<td>3</td>
<td>1%</td>
</tr>
<tr>
<td>IIIA</td>
<td>31</td>
<td>IIIA</td>
<td>147</td>
<td>39%</td>
</tr>
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<td>IIIA</td>
<td>31</td>
<td>IIIA</td>
<td>147</td>
<td>39%</td>
</tr>
<tr>
<td>IVB</td>
<td>15</td>
<td>IVB</td>
<td>15</td>
<td>4%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>377</td>
<td></td>
<td>100%</td>
<td>56</td>
</tr>
</tbody>
</table>


impact on patient outcome by the addition of Cetuximab, an EGFR inhibitor [14] to chemoradiation. Recent evidence suggests that PIK3CA genetic alterations may induce centrosome amplification and increase tolerance to spontaneous genome doubling, thereby contributing to irreversible genomic changes in cancer [18]. While much effort has been devoted to targeting PIK3CA, tolerance to specific PI3K isoforms has been so far a rate limiting step. It was recently suggested that toxicities may be contained through intermittent dosing and nanoparticle delivery [17]. Many selective inhibitors for each PI3K isoform are in clinical trials and activity was shown associated with PI3K specific mutations [19].

2: Epigenetic LOF alterations were seen in 47% BioRAIDs tumours assessed so far. While epigenetic alterations (involving DNA methylation and covalent histone modifications) are increasingly reported in solid tumours, this is to our knowledge the first report emphasizing not only a frequent loss-of-function (LOF) mutations in KMT2A-D genes, leading to defective histone H3K4 methylation in cervical cancers, but also linking these to outcome. KMT2A-D have already been reported in many other solid tumours (reviewed by [20]) and have been associated with increased microsatellite instability (MSI) in colorectal carcinoma. Of interest to oncologists is that pan histone deacetylase inhibitors (HDACi), such as Vorinostat (Zolinza®), which affects the alterations regulation of histone and non-histone proteins by modifying their post-translational acetylation, is likely to be relevant for cervical cancer treatment. [21][ 22].

In preclinical models, Banerjee et al. [23] showed that Vorinostat significantly reduced E6 and E7 activity, abrogated viral DNA amplification and inhibited host DNA replication.
drugs are in clinical development across cancer types, they have a broad spectrum of epigenetic activities. Vorinostat (Zolinza®), initially marketed for the treatment of cutaneous T-cell lymphoma, and more recently Romidepsin (Istodax®) are approved drugs.

3: Immune checkpoint inhibition (ICI) has significantly changed cancer therapy showing impressive durable responses across cancer types. There is only limited data on ICI treatment of PDL-1 positive cervical cancer to date; a 17% partial response rate was documented in

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th></th>
<th>Multivariate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>IC95% (HR)</td>
<td>p-value</td>
<td>HR</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td></td>
<td></td>
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<tr>
<td>≥50</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tobacco consumption</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, current or past (&gt;1PA)</td>
<td>0·91</td>
<td>[0·63; 1·31]</td>
<td>0·62</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>1</td>
<td></td>
<td></td>
<td>0·01</td>
</tr>
<tr>
<td>≥25</td>
<td>0·63</td>
<td>[0·43; 0·91]</td>
<td>0·66</td>
<td>[0·45; 0·96]</td>
</tr>
<tr>
<td><strong>ECOG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>1–2</td>
<td>1·70</td>
<td>[1·04; 2·77]</td>
<td>0·04</td>
<td></td>
</tr>
<tr>
<td><strong>Histological type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0·11</td>
<td>[0·65; 1·88]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1·11</td>
<td>[0·30; 1·84]</td>
<td></td>
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<tr>
<td>Adenosquamous, clear cell, mixed + undifferentiated</td>
<td>0·75</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Hemoglobin (g/dl)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>0·61</td>
<td>[0·37; 1·01]</td>
<td>0·73</td>
<td>0·06</td>
</tr>
<tr>
<td>&gt;10</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>FIGO 2014</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1·87</td>
<td>[1·09; 3·22]</td>
<td>1·70</td>
<td>1·83</td>
</tr>
<tr>
<td>III</td>
<td>4·90</td>
<td>[2·66; 9·02]</td>
<td>3·25</td>
<td>3·25</td>
</tr>
<tr>
<td>IV</td>
<td>3·64</td>
<td>[1·83; 7·21]</td>
<td>3·46</td>
<td>3·46</td>
</tr>
<tr>
<td><strong>FIGO 2018 (integrates lymph nodes status under IIIC)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>2·14</td>
<td>[0·86; 5·35]</td>
<td>1·83</td>
<td>1·83</td>
</tr>
<tr>
<td>III</td>
<td>4·04</td>
<td>[1·76; 9·29]</td>
<td>3·25</td>
<td>3·25</td>
</tr>
<tr>
<td>IV</td>
<td>5·71</td>
<td>[2·23; 14·60]</td>
<td>3·46</td>
<td>3·46</td>
</tr>
<tr>
<td><strong>HPV type (based on hybridisation test)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clade 9 (HPV 16,31,33,35,52,58)</td>
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<tr>
<td>Clade 7 (HPV 18,39,45,59,68)</td>
<td>4·71</td>
<td>[0·96;2·24]</td>
<td>3·22</td>
<td>3·22</td>
</tr>
<tr>
<td>Others or Negatives</td>
<td>1·38</td>
<td>[0·76; 2·52]</td>
<td>1·30</td>
<td>1·30</td>
</tr>
</tbody>
</table>

Fig. 2. Progression free survival according to the presence/absence of alterations in a METAGENE composed of any of the following alterations in the PI3K pathway and/or in Enzymes involved in Epigenetic Signaling. The list of genes included in this analysis was predominantly (but not exclusively) oncogenes from the Tyrosine Kinase receptor/PI3K pathway: PIK3CA and PIK3CB mutations as well as PTEN in association with the following (predominantly suppressor) genes with loss of function alterations in Epigenetic enzymes: KMT2C, KMT2D, KMT2A, KDM5C, EP300, CREBBP, ARID1A, ARID2, ATRX.
Loss of function of CASP8 (caspase 8), edge], PDL-1 and PDL-2 amplification by Vorinostat may potentiate effects of ICI through upregulation of HDAC inhibitors, suggesting a potential synergy. Moreover, recent preclinical data further suggested that HDAC inhibition or miRNA which had not been planned in the present project upregulated genes might serve as biomarker for an inflammatory milieu by destabilizing the pRB and p53 family proteins. These features have been controlled by immunohistochemistry in a parallel project defining the tumour microenvironment (ongoing) with excellent correlation. EMT biomarkers may allow the identification of patients that could potentially benefit from ICI in the future. Additional data on gene methylation or miRNA which had not been planned in the present project upfront may shed light on the mechanism of enhanced gene expression. Moreover, recent preclinical data further suggested that HDAC inhibition by Vorinostat may potentiate effects of ICI through upregulation of PD-L1 and HLA-DR on tumour cells, suggesting that some RPPA clusters might be more sensitive to HDAC inhibition by Vorinostat than by mutation.

4. **LOF mutations linked to deficient DNA repair** were seen in ATRX (9%), BAP1 (5%), BRCA2 (5%) while the FANCB oncogene was mutated or amplified in 3% of tumours. RPPA also identified differences in expression and activation of DNA repair proteins among the three clusters, suggesting that some RPPA clusters might be more sensitive to PARP inhibitor than others. Treatment downstaging, through the use of a PARPi, rather than chemoradiation, may be envisioned in the context of a clinical trial. Compared to the omnipresent p53 and RB alterations in high grade serous ovarian cancer, the respective mutation frequencies 7% and 10% in CC were rare, consistent with the predominant HPV effects on the viral oncoproteins E7 and E6 to establish the permissive milieu by destabilizing the pRB and p53 family proteins rather than by mutation.

3.9. **Future prospects for clinical decision making**

While routine molecular diagnostic testing has yet to be introduced to guide personalized cervical cancer treatment, the BioRAIDs dataset defined activated genetic pathways and expression signatures that are associated with poor outcome based on the present standard therapies. IMRT and prophylactic irradiation to para-aortic lymph nodes in high risk patients has been shown to mitigate risk and improve outcome, yet, despite occasional abscopal effects, they cannot effectively target micro or macro metastases outside of the radiation field. Higher rates of abscopal effect in many tumour sites may be reached through the integration of radiation or chemoradiation with immunotherapy and combination trials (NICOL, PRIMMO) are ongoing.

Key biomarkers for CC diagnostics appear dominated by alterations in the PI3K pathway, in genes coding for epigenetic remodeling enzymes, for DNA repair and for inflammatory pathways. Pathway specific druggability, using distinct compounds to define the genomic correlates for drug specific sensitivity or resistance in cell lines and tumours as well as drug synergies and synthetic lethality need to be tested in future preclinical and clinical studies. For the purpose of translational studies, the continuous pretreatment collection of frozen tissues in advanced stage CC appears mandatory.

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Declaration of interests

- Suzy Scholl and Maud Kamal coordinated the project and wrote the manuscript.
- Marina Popovic, Anne de la Rocheфорде, Aliosa Mandic, Nina Samet, Marius Craina, Sanne Samuel, Madalin Margan, Henry Zijlmans, Peter Hillemanns, Sorin Dema, Akis Dema, Goran Malenkovic, Branislav Djuran, Anne Floquet, Delphine Garbay, Frédéric Guyon, Pierre Emmanu- ele Colombo, Michel Fabbro8, Christine Kerr8, Charlotte Ngo, Fabrice Lecuru, Charles Coutant, Frédéric Marchal, Nathalie Mesgouez-Nebout, Virginie Fourchette, Jean Guillaume Feron, MD, Philippe Morice, Pauline Wimberger, Jean-Marie Classe, Coraline Dubot, Noreen Gleeson and Gemma Kenter included patients.
- Katariina Koprivsek coordinated imaging.
- Elodie Girard, Pierre Gestraud, Nicolas Servant, Philippe Hupe and Balazs Balint performed bioinformatics analyses.
- Sylvain Dureau and Alexa Savignoni performed the biostatistical analyses
- Heiko von der Leyen coordinated the clinical trial in Eastern European countries
- Mathieu Minsat, Elenor Rivin del Campo, Anne de la Rocheфорде and Eric Deutsch coordinated radiotherapy procedures and included patients.
- Attila Kereszt and Istvan Nagy performed sequencing analyses.
- Mathieu Minsat, Eleonor Rivin del Campo, Anne de la Rochefordiere and Nicolas Servant, Philippe Hupe and Balazs Balint performed bioinformatics analyses.
- Pierre Fumoleau, Roman Rouzier participated to manuscription
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2019.03.069.

References


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