PIK3CA pathway mutations predictive of poor response following standard radio chemotherapy +/- Cetuximab in cervical cancer patients

Data from a randomized phase II trial of radio-chemotherapy +/- Cetuximab for locally advanced cervix carcinoma (EudraCT-2008-001053-18, NCT00957411)

Anne de la Rochefordiere¹, Maud Kamal¹; Anne Floquet²; Laurence Thomas³; Peter Petrow¹; Thierry Petit¹, Marius Pop³; Michel Fabbro, Christine Kerr⁴; Florence Joly, Emmanuel Sevin⁵, Sophie Maillard, Hervé Curé⁶, Béatrice Weber, Claire Brunaud⁷; Mathieu Minsat Laurence Gonzague⁸, Dominique Berton-Rigaud⁹, Maud Aumont⁹; Laurence Gladieff¹⁰, Karine Peignaux¹¹; Virginie Bernard¹; Quentin Leroy¹; Ivan Bieche¹, Audrey Margogne¹, AnaTereza Nadan¹, Virginie Fourchotte¹, Alhassane Diallo¹, Benard Asselain¹, Corine Plancher¹, Sébastien Armanet¹, Philippe Beuzeboc¹ and Suzy M Scholl¹.

¹Institut Curie, Paris, France; ²Institut Bergonnié, Bordeaux, France; ³Centre Paul Strauss, Strasbourg, France; ⁴Centre Val d’Aurelle – Paul Lamarque, Montpellier, France; ⁵Centre Regional Francois Baclesse, Caen, France; ⁶Institut Jean Godinot, Reims, France; ⁷Centre Alexis Vautrin, Nancy, France; ⁸Institut Paoli Calmettes, Marseille, France; ⁹Institut de cancérologie de l'Ouest - René Gauducheau, France; ¹⁰Institut Claudius Régaud, Toulouse, France; ¹¹Centre Georges-François Leclerc, Dijon

Corresponding author: Suzy M Scholl, MD, MRCOG, Department of Oncology, Institut Curie, 26 rue d’Ulm Paris, France 75248 Cedex 05

Email: suzy.scholl@curie.fr

Tel: +33 1 44 32 46 87; Cell: + 33 6 79 97 62 10

Funding: MERCK SERONO, ANR-10-EQPX-03, Fondation Cancer du Luxembourg

Disclosure: All the authors declared no conflict of interest

Running Title: PIK3CA-mutations predictive of bad response in cervix cancer

Abstract 258 words

Text 3165 words

Tables & Figures: 2 Tables & 4 Figures

4 supplementary tables
Abstract

**Background:** EGFR is frequently overexpressed in cervical cancer (CC), suggesting EGFR blockade as a promising treatment approach. Cetuximab, an anti EGFR antibody, used conjointly with radio-chemotherapy, was feasible in first line treatment of cervix carcinoma limited to the pelvis.

**Methods:** This randomized phase II trial enrolled 78 FIGO stage IB2-IIIB CC patients to either Cisplatin based radio-chemotherapy alone (Arm B, n=38) or conjointly with a 6 week course of weekly Cetuximab (Arm A, n=40). Brachytherapy was given to the pelvic mass. Primary endpoint was disease free survival (DFS) at 2 years. EGFR expression and targeted sequencing were performed in 54/78 patients.

**Findings:** Cetuximab over a 6 week period did not improve DFS at 24 months. At 31 months median follow-up, DFS was not significantly different (p=0.18). Complete response at 4-6 months was strongly predictive for excellent DFS (Log-Rank test; p<0.001). PIK3CA, KRAS and STK11 mutations were observed in 22%, 4% and 2% of patients respectively. No tumor with a PI3K-pathway mutation showed complete response (0/8 in Arm A and 0/6 in Arm B) whilst 14/52 (27%) tumors without mutations did (p=0.021). PI3K-pathway mutated tumors showed a trend towards poorer DFS (p=0.06) following Cetuximab (8/22) as compared to those following standard treatment only (6/18).

**Interpretation:** Similarly to Head and Neck cancer patients, CC patients showed no gain in DFS at 2 years following a combined treatment of Cetuximab with radio chemotherapy. While treatment tolerance and compliance were satisfactory, it remains to be demonstrated whether maintenance therapy with Cetuximab could be beneficial in selected patient groups.

**Translational Relevance Statement:**

Cetuximab, has proven beneficial in the treatment of patients with Kras wild-type metastatic colorectal cancer (mCRC) and in NSCLC patients. Our results show that the addition of Cetuximab to standard radio chemotherapy does not improve progression free survival of CC patients. Targeted sequencing on tumor material from our patient population showed patients with alterations in the PI3K pathway seem to have worst disease free survival. Mutations in the PI3K pathway seem therefore to be an important parameter to predict absence of response to Cetuximab.
Introduction

The treatment and the outcome of locally advanced, FIGO stage IB2-IIIB carcinoma to the cervix have not notably changed over the past 10 years (1). At the time the present trial was initiated, numerous publications suggested the importance of EGFR pathway activation in cervical cancers (CCs), as suggested by frequent EGFR gene overexpression and amplification, often in association with other EGFR family members (2) (3). More recently, EGFR gene amplification has been reported in as many as 20% of high-grade CIN and invasive carcinoma of a Chinese population from Nanjing (4) and was associated with intermediate-high levels of EGFR protein overexpression. In a large Dutch study, membrane staining of EGFR (p = 0.005) together with cytoplasmic staining of activated pEGFR (p = 0.016) were independent predictors of poor response to chemo-radiation (5). EGFR overexpression in CCs appeared to correlate with poor prognosis following standard therapy in some studies (6) (7) (8), but not in all (9) (10). Alterations in downstream signaling pathways such as the PI3K-pathway (11) are likely to modify the prognostic relevance of membrane EGFR.

While EGFR-TKI treatment (such as gefitinib or erlotinib) has been shown to be particularly effective in advanced non-small cell lung cancer (NSCLC) patients, whose tumors harbored activating EGFR mutations in the kinase domain (12) (13), no such mutations in exons 18-21, corresponding to the kinase domain, have been detected to date in CCs (14), (15, 16). Cetuximab, a chimeric monoclonal antibody that binds to EGFR, disrupting its dimerization (17), had been proven beneficial in the treatment of patients with squamous cell carcinoma of the head and neck (SCCHN) (18) (19), in Kras wild-type metastatic colorectal cancer (mCRC) (20) as well as in NSCLC (21) patients. In an intention-to-treat analysis in NSCLC (Flex trial), tumor cell expression of EGFR (<40% vs ≥40%) was not identified as a
prognostic factor in relation to survival in the overall analysis (22). In CC, a recent phase I/II clinical trial in treatment naïve patients, documented the feasibility of concurrent Cetuximab with cisplatin-based chemo-radiotherapy to the pelvis; while the need for extended field radiotherapy, (EFRT) in combination with Cisplatin and Cetuximab was reported to be insufficiently tolerated (23). Objectives of the present trial were to assess the tolerance and the efficacy of the combining Cetuximab with standard chemo radiotherapy, while also to assess outcome as a function of biological parameters.

Patients and Methods

This randomized phase II trial was approved by the French National review board and by the local ethics committee in September 2008 and was conducted in 11 French centers. It was regularly reviewed by a data safety management board (DSMB) at pre-determined time points.

Patient population

The trial recruited 78 stage IB2–IIIB CC patients between March 2009 and July 2011. All patients had provided written informed consent. Principal eligibility criteria were: ECOG (Eastern Cooperative Oncology Group) performance status (PS) ≤1, histologically proven squamous-cell cancer or adenocarcinoma, including International Federation of Gynecologists and Obstetricians (FIGO) stage IB2–IIIB disease, not amenable to curative treatment with upfront surgery. Extra-pelvic (stage IV) disease, a history of skin pathology, or chronic inflammatory disease according to Common Terminology Criteria for Adverse Events version 3·0 (CTCAE V3·0) were exclusion criteria.

Radiation, Cisplatin chemotherapy, and Cetuximab treatment
Eligible patients were treated with once-weekly cisplatin (40 mg/m²) chemotherapy combined with standard pelvic radiation therapy. In arm A, Cetuximab was administered intravenously at a loading dose of 400mg/m² the first week, followed by weekly doses of 250 mg/m² every week up to 6 weeks in association with chemo-radiation. No maintenance Cetuximab was administered. Endo-brachytherapy was performed with low dose rate, using Cesium¹³⁷ or pulse dose rate Iridium¹⁹² according to each center's technique, delivering a supplementary dose of 15-30 Gy to the cervix, the upper vagina and the uterus. Both external and intra cavitary radiotherapy (ICRT) were to be completed within eight weeks. Both Intensity modulated radiotherapy (IMRT) (two third of patients) and conformal RT (one third of patients) were permitted. The clinical target volume (CTV) of IMRT covered the pelvis and generally included a 0.5–1 cm margin to the gross tumor volume (GTV) radially, to the distal third of the vagina, the parametria and the regional lymph nodes. Radiation boosts up to 60Gy were administered for positive lymph nodes or 65 Gy to the parametrium.

**Patient assessments and follow-up**

Clinical and imaging assessments using CT scan and MRI were made 2 weeks after the last dose of study medication (approx. day 50), then one month after treatment completion (at 4-5 months) and scheduled every 4 months thereafter for the duration of the study period over two years. Tumor response was by MRI after treatment completion, according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria (24). For patients who underwent surgery (if feasible and in case of doubt about completeness of response) the response assessment was by pathology. A centralized blinded review of tumor imaging and pathology slides was performed. Patients were followed up every 4 months for 2 years.

**Statistical methods**
The statistical sample size was determined using a Fleming one step design under the hypothesis that the addition of Cetuximab to radio-chemotherapy would increase local regional control at 24 months from 50% (null hypothesis) to 75% (alternative hypothesis). Thirty eight patients per arm were required to detect a difference with a 96% power and accepting a 7% type I error. Patients were randomly assigned to either standard therapy (arm B, n=38) or standard therapy plus Cetuximab (arm A=40) using a technique of block permutations. Randomization was stratified by center and according to the pretreatment plan of surgery or no surgery following radio chemotherapy.

The primary endpoint was disease-free survival (DFS) at 2 years. Secondary endpoints were: complete response rates at 4-5 months, tolerance of the Cetuximab combination with radio-chemotherapy as defined by treatment-related SAE and AE rates occurring at any time, biological criteria (mutations, detection of HPV DNA, over expression of EGFR), time from randomization to death of any cause or last follow up. The rates of OS and DFS were estimated using the Kaplan–Meier method and compared with Log-rank tests. Cox proportional hazards regression models were used to perform multivariate analysis.

**Biological Methods**

*Mutational analyses: Targeted sequencing*

Fixed tumor material was available from 54/76 (72%) of patients and frozen samples from 27 patients (35%). Screening of hotspot mutations was performed by targeted sequencing using the Ion Ampliseq cancer panel V1 in conjunction with the Ampliseq library kit v2·0 and the Ion Torrent Personal Genome Analyzer (Life Technologies) for 54 patients for whom FFPE or frozen tumor samples were available.

*EGFR expression using IHC*
Immunohistochemical analyses were performed using antibody directed against EGFR (mouse monoclonal; clone 31G7, Invitrogen, France; code 28005; dilution 1:200). The antibody was tested using Leica BOND III automation using the BOND Polymer Refine Detection (Leica biosystems, Germany).

**HPV typing**

Total DNA was used for HPV typing. Sufficient material was available for 54 (69 %) of tumors which could be screened for the presence of HPV using generic GP5+/GP6+ primers by PCR. Specific primers were used for identification of HPV genotypes 6/11, 16, 18, 33 and 45.

**Results**

**Patients demographics:**

Cetuxicol opened to accrual in March 2009 and completed accrual in July 2011. There was a balanced distribution according to pre-treatment tumor size (MRI), parametrial involvement, PS, age, and smoking habits. There was no difference in pretreatment patient and tumor characteristics between both arms (Table 1). Two patients in arm A withdrew consent and were treated with standard therapy. 76 patients were evaluable for tolerance and outcome (Figure 1). One patient had FIGO Stage 1B1 disease, but remained in the intention to treat analysis. Salvage surgery was carried out in 24/38 (65%) of patients evaluable for response in arm A and 26/38 (70%) in arm B following radio chemotherapy.

**Treatment compliance:**

Compliance with protocol-specified radiation and drug therapy was satisfactory. In arm A, nine patients were not able to receive the full CDDP as per schedule; four patients received
only 4 or 5 weeks of Cetuximab mainly as a result of grade 3 hematologic intolerance; in arm B, 11 patients received only 4-5 weeks of CDDP, for reasons of hematological or renal tolerance. Overall, the median time between the start of RT-CT and the last day of treatment (brachytherapy, BT) was 53 days [range, 41-177] for all patients. The median doses of EBRT and brachytherapy received were similar in both arms (supplementary table 1).

**Treatment tolerance:**

This secondary endpoint was treatment-related serious adverse event (SAE) and adverse event (AE) rates as defined by protocol and occurring: 1° during the combination therapy and: 2° at any time. Both SAEs and AEs were scored according to the National Cancer Institute Common Terminology Criteria for AEs, version 3·0 (25). Over the course of treatment there were 24 unscheduled hospitalizations, 15/24 were considered to be treatment related. There were no treatment-related deaths AEs included all SAEs defined above, as well as grade 3/4 AE such as nausea, vomiting or diarrhea persistent for >2 weeks despite medical intervention or grade 3 neutropenia or leukopenia persisting for >7 days. These were more frequent in arm A, (P<0·001) but clinically manageable (AEs: supplementary table 2).

**Targeted sequencing controlled by Sanger Sequencing:**

Targeted sequencing of tumor DNA from 54 patients performed using the AmpliSeq 46 genes Cancer Panel on the Torrent PGM sequencer or using the SANGER technique for all PIK3CA and KRAS mutations. Hotspot mutations were detected in PIK3CA for 12 patients (22%), in KRAS for 5 patients (10%). The distribution was not significantly different (Table 2) between both arms. In addition, FBXW7 mutations were detected in 2 patients and BRAF, SMAD4, STK11, JAK3 and ATM in individual patients. Interestingly, one tumor with PIK3CA
mutation also had BRAF and KRAS mutations. Another PIK3CA mutated tumor also exhibited FBXW7 and SMAD4 mutations.

Treatment Efficacy

Primary endpoint

DFS at 24 months was 63% (95% CI: 49% - 80%) (14 events) in arm A and 76% (95% CI: 63% - 91%) (9 events in arm B). With a median follow up of 31 months, no significant difference of DFS was shown between the two arms (p=0.18) (Figure 2a). The 2-year OS was 83% for arm A (95% CI: 72% - 96% (7 events), and 87% for arm B (95% CI: 76% - 98%) (5 events).

DFS as a function of classical clinical and histological parameters

Outcome according to FIGO stage (p=0.19) was not statistically different (only 7 stage III patients), but pelvic lymph node involvement was highly significantly associated with poorer DFS (p=0.0069). Patients in the Cetuximab arm (A) who developed acne did mildly better but this result was not statistically significant. Patient age, smoking habits (>10), menopausal status and obesity did not correlate with a difference in outcome; neither did IMRT versus conformal radiotherapy. Squamous type histology patients had a better DFS than patients with non-squamous type histology, but not significantly so.

Clinical response evaluation:

Early response evaluation at the end of the 6 weeks chemo-radiation therapy seemed to be in favor the Cetuximab treatment arm (7 objective responses in arm A and 3 in arm B). At the
end of the complete treatment sequence and following central imaging review, (at 4-5 months) response evaluation by either MRI (in the absence of surgery) or by histopathology in the case of surgery was similar for both arms. (supplementary Table 3) Due to absent or poor quality MRI, seven patients were not evaluable for response (4 in arm A and 3 in arm B). 24/38 patients in arm A and 26/38 in arm B underwent salvage surgery. In arm A, 9/24 had residual disease on histology and 2 patients were node positive. In arm B, 12/26 patients had residual disease on histological assessment and 1 had positive nodes. A total of 16/38 patients in arm A and 15/38 in arm B have achieved a complete response; only one patient had progressed early on in each treatment arm. Complete response (by either MRI or histopathology) strongly correlated with excellent DFS (p=0.0002) (Figure 2b).

**Associations between clinical outcome data and biological parameters**

Clinical response was available on 52/54 patients analyzed by targeted sequencing. In this subgroup analysis 14/52 patients achieved a complete response and 38/52 did not. HPV could be assessed in 54 (69%) patients (table 1). Among tumors with an oncogenic HPV, 26 carried no detectable mutation while 14 were associated with a mutation.

- Among PI3K-pathway mutated patients, 0/14 achieved a complete response at the 4-5 months evaluation (p=0.042) (Table 2). Patients who had achieved both a complete response (and whose tumors showed no PI3K-pathway mutation) had a probability of a DFS at 24 months of 93% [80.3-100], contrasting with 50% [28.4-88] for those with either a PI3K-pathway mutation or an incomplete response or both (p=0.097) (Figure 3). Three out of eight patients with a PI3K-pathway mutation in arm A and three out of six in arm B had salvage surgery.
• IHC staining for EGFR was available in 39 patients (50%). EGFR protein expression, showed positive staining in ≥ 40% of tumor cells to be present in 8/23 patients of arm A and in 5/17 of arm B. EGFR expression was not related to response or outcome.

**DFS as a function of clinical response and of mutational data in the overall population and according to treatment arm.**

In both treatment arms, DFS was excellent if the patient had achieved a complete response at the 4-5 month evaluation while patients with an incomplete response had a significantly poorer outcome; with arm A being below arm B (Figure 2b). In a subgroup analysis of patients for whom mutational analysis was available (n=54) no deleterious effect was visible for patients treated with standard therapy, but following Cetuximab, patients with PIK3CA mutations showed a trend towards poorer DFS (Figure 4, p=0·06).

**Multivariate analysis.**

Complete response and low FIGO stage were independently associated with a reduced risk of recurrence (supplementary Table 4). Patients who had achieved a complete response (as assessed by either MRI or by histopathology in case of surgery) had a 91% reduced risk of recurrence (p=0·001). The subgroup analysis of patients with acne or specific tumor mutations was too small for valid conclusions.

**Discussion**

The addition of Cetuximab over a 6 week period in the initial treatment did not improve DFS in our population but was comparable to results from similar populations, such as the phase 2 RTOG 0417 trial (26, 27) (combination with Bevacizumab) and the phase 1/2 GOG trial with
Cetuximab. Disruption of receptor dimerization (17), is the postulated mechanism of action of the monoclonal antibody, Cetuximab, suggesting that EGFR overexpression +/- gene amplification might be the relevant biomarkers for activity. A recent meta-analysis in squamous cell carcinoma of head and neck (SCCHN) cancer patients (68 studies involving 6,781 patients) suggested that elevated EGFR expression and above all elevated gene copy number were predictive for poor survival (28). While recurrent/metastatic SCCHN and KRAS wild-type metastatic colorectal cancer (mCRC) patients have been documented to benefit from Cetuximab treatment with long lasting survival differences and a gain of almost 10% at 5 years (19); only recently, did the Flex trial in advanced NSCLC show a subgroup of patients with high EGFR expression (22), to have a better overall survival following the combined treatment of chemotherapy/Cetuximab (by 2.4 months; p=0.011) as compared to chemotherapy alone. A treatment interaction test assessing the difference in the HRs for overall survival between the EGFR expression groups suggested a predictive value for high EGFR expression (p=0.044). Similarly, a recent meta-analysis on NSCLC, showed the addition of Cetuximab to chemotherapy to significantly improve OS, PFS and response (29). In the present trial, Cetuximab was administered only over a 6 week period, while in most NSCLC trials the treatment duration was significantly longer, lasting up to 6-8 months. We cannot exclude that either the short duration of exposure to Cetuximab or the limited subpopulation which might have a significant EGFR overexpression/gene amplification would prevent to demonstrate such a correlation. Still, at the 7 weeks evaluation we observed 7 complete responses in arm A and 3 in arm B. Negative results were also reported recently from a phase III trial in patients with stage III or IV head and neck carcinoma (HNC) which, similarly to us, had tested the hypothesis that adding cetuximab to a radiation-cisplatin platform might improve progression-free survival (PFS) (30).
Of interest are our results showing alterations in the PI3K-pathway to be an important factor in impeding complete response to standard therapy and to Cetuximab since none of the tumors with one (or more) alterations, in the PIK3CA pathway showed a complete response following radio-chemotherapy. Our findings are in agreement with data showing pre-treatment alterations in gene expression in the PI3K/Akt signalling pathway (31) to correlate with a positive post-treatment PET scan in CC patients. PIK3CA mutations seemed to retain a higher negative impact on PFS in the Cetuximab treated patients. While the reason for this is not clear, similar results have been reported in metastatic colorectal cancer. KRAS mutations and PIK3CA/PTEN deregulation have been suggested to significantly correlate with resistance to Cetuximab (32) while the absence of mutations in KRAS, NRAS, BRAF and TP53 was correlated with improved outcome in patients treated with Cetuximab, oxaliplatin and UFT (33). Recent clinical evidence suggests reversible and adaptive transcriptional responses to drugs in this pathway (34). We did not detect any EGFR mutations in the present patient population in accordance with previous reports in CC (15) (14). EGFR mutations had similarly been undetectable in HPV positive SCCHN (T Seifert, ASCO 2013). Whilst EGFR mutations have been shown relevant for the clinical effect of small molecular inhibitors in advanced non-small-cell lung cancer (12) (13) they may not be relevant for the clinical effect of Cetuximab.

Molecular targeted therapies in CC remain presently inconclusive. Patients with KRAS and PIK3CA mutations (or PTEN loss) might benefit from therapies targeting downstream EGFR signalling such as PI3K and mTOR inhibitors. p-Akt has been suggested as a biomarker for poor prognosis (31). It also needs to be clarified how tumor cell alterations impact the tumor micro-environment to allow dual-targeting strategies, combining targeted anti-tumor drugs together with vaccines/antiviral strategies.
Conclusion and Perspectives

In conclusion, our results suggest that gene mutations may be an important parameter in predicting treatment response. It remains to be demonstrated whether a prolonged maintenance treatment with Cetuximab might have a significant impact in a targeted CC population whose tumors have EGFR gene amplification/overexpression and absent mutations in the PI3K pathway. For a better understanding of the driver mutations in CC, we initiated the RAIDs project (Rational Approach and Innovative Drug selection: RAIDs) with the aim to assess dominant mutational events with new generation sequencing (NGS) techniques, signalling proteins expression and activations with reverse phase protein array (RPPA), as well as studies on the tumor micro-environment in a prospective cohort of 700 patients. This project is supported by the European commission in the frame of the FP7 program (http://www.raids-fp7.eu/).
References


### Table 1. Patient and tumor characteristics

<table>
<thead>
<tr>
<th></th>
<th>Arm A Cetuximab + ST</th>
<th>Arm B ST</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>40*</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong> (median, min, max)</td>
<td>49·5 (23·74)</td>
<td>45·5 (25·73)</td>
<td>0.38</td>
</tr>
<tr>
<td><strong>History of Tabacco use</strong></td>
<td></td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td>+</td>
<td>21</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>19</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Post menopause</strong></td>
<td></td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td>+</td>
<td>19</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>21</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td><strong>History of Oral Contraception</strong></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>16</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>MV</td>
<td>22</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>Obesity (BMI≥30)</strong></td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>+</td>
<td>15</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>23</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>MV</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Histological tumor type</strong></td>
<td></td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>squamous</td>
<td>33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>non squamous</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><strong>FIGO Stage</strong></td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>IB1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IB2</td>
<td>13</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IIb</td>
<td>18</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IIIb</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Median clinical tumor size</strong></td>
<td>44 mm (range, 15-80)</td>
<td>40 mm (range, 20-80)</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Median MRI tumor size</strong></td>
<td>53·5 mm (range, 30-90)</td>
<td>47 mm (range, 24-85)</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Anemia at diagnosis (Hb≤10g/l)</strong></td>
<td>13</td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>

MV=missing value; *2 patients withdrew consent early in their treatment schedule
<table>
<thead>
<tr>
<th>Mutations</th>
<th>Tumor samples available for mutational analysis n=54 (%)</th>
<th>Arm A n=30</th>
<th>Arm B n=24</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA</td>
<td>12 (22)</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>BRAF</td>
<td>1 (3)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>KRAS</td>
<td>5 (10)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>STK11</td>
<td>1 (3)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>PIK3CA &amp; KRAS</td>
<td>14 (26)</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>PIK3CA &amp; KRAS &amp; STK11</td>
<td>14 (26)</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Complete Response n=14/52</th>
<th>Incomplete Response n=38/52</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA</td>
<td>0 (0%)</td>
<td>12 (23%)</td>
<td>0.042</td>
</tr>
<tr>
<td>No mutation detected</td>
<td>14 (27%)</td>
<td>26 (50%)</td>
<td></td>
</tr>
<tr>
<td>PIK3CA or KRAS</td>
<td>0 (0%)</td>
<td>14 (27%)</td>
<td>0.021</td>
</tr>
<tr>
<td>No mutation detected</td>
<td>14 (27%)</td>
<td>24 (46%)</td>
<td></td>
</tr>
<tr>
<td>One or several mutations*</td>
<td>0 (0%)</td>
<td>16 (31%)</td>
<td>0.009</td>
</tr>
<tr>
<td>No mutation detected</td>
<td>14 (27%)</td>
<td>22 (42%)</td>
<td></td>
</tr>
</tbody>
</table>

* PIK3CA-KRAS- STK11-FBXW7-SMAD4-ATM-BRAF-JAK3
Figure 4: DFS according to Mutational Status and Treatment Arm: supplementarylementary

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>7</th>
<th>6</th>
<th>2</th>
<th>2</th>
<th>2</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>21</td>
<td>17</td>
<td>16</td>
<td>16</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>18</td>
<td>14</td>
<td>13</td>
<td>11</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

$p = 0.06396$
Figure 3: DFS according to Mutational Status PI3KCA and response

Probability of relapse

No response (PI3KCA)
Response (no PI3KCA)
No response (no PI3KCA)

Time from randomization (months)

Number at risk

--- 12 11 7 6 6 3 1
--- 14 14 13 13 12 9 6
••• 26 24 17 15 14 8 4

p = 0.00658
Figure 2a: DFS according to Treatment Arm

Figure 2b: DFS according to Clinical Complete Response and Treatment Arm
Figure 4: DFS according to Mutational Status and Treatment Arm: supplementarylementary

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.
Clinical Cancer Research

PIK3CA pathway mutations predictive of poor response following Standard radio chemotherapy +/- Cetuximab in cervical cancer patients

Anne de la Rochefordiere, Maud Kamal, Anne Floquet, et al.

Clin Cancer Res  Published OnlineFirst February 27, 2015.

Updated version  Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-14-2368

Supplementary Material  Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2015/02/28/1078-0432.CCR-14-2368.DC1

Author Manuscript  Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.