Published in final edited form as:

Am J Clin Oncol. 2017 December ; 40(6): 590–597. doi:10.1097/COC.000000000000203.

c-Met Overexpression in Cervical Cancer, A Prognostic Factor and A Potential Molecular Therapeutic Target

Tamer Refaat, M.D., Ph.D., MSCI^{1,5,6}, Eric D. Donnelly, M.D.¹, Sean Sachdev, M.D.¹, Vamsi Parimi, M.D.², Samar El Achy, M.D., Ph.D.⁷, Prarthana Dalal, BS¹, Mohamed Farouk, M.D., PhD.⁶, Kim Natasha Berg, M.D.², Irene Helenowski, PhD³, Jeffrey Paul Gross, BS¹, John Lurain, M.D.⁴, Jonathan Strauss, M.D.¹, Gayle Woloschak, Ph.D.¹, Jian-Jun Wei, M.D.^{1,4}, and William Small Jr., M.D., FACRO, FACR, FASTRO⁸

¹Department of Radiation Oncology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA

²Department of Pathology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA

³Department of Preventive Medicine, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA

⁴Department of Obstetrics and Gynecology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA

⁵Developmental Therapeutics Institute, Northwestern University, Chicago, IL, USA

⁶Department of Clinical Oncology and Nuclear Medicine, Alexandria University, Alexandria, Egypt

⁷Department of Surgical Pathology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

⁸Department of Radiation Oncology, Stritch School of Medicine, Loyola University, Chicago, IL, USA

Abstract

Purpose—This study aimed to assess the association between pretreatment c-Met overexpression in local-regional advanced cervical cancer patients treated definitively with concurrent chemoradiation (CRT) and treatment outcomes including overall survival (OS), progression free survival (PFS), distant metastases control (DM), and local-regional control (LC).

Patients and Methods—This IRB approved study included cervical cancer patients treated definitively and consecutively with CRT. Evaluation of cytoplasmic immunoreactivity for c-Met was performed and scored semi-quantitatively by three pathologists, blinded to the treatment outcomes, and incorporated both the intensity and percentage of immunoreactivity in invasive

Corresponding author: William Small Jr., M.D., FACRO, FACR, FASTRO, Professor and Chairman, Department of Radiation Oncology, Cardinal Bernardin Cancer Center, Stritch School of Medicine Loyola University Chicago, 2160 S 1st Ave, Maguire Center, Rm 2932, Maywood, IL 60153 Phone: (708) 216-2559 Fax: (708) 216-6076.

Conflict of Interest: This work was not supported, funded, or sponsored by any extra-institutional source, nor are there any actual or potential conflicts of interest with the production and publication of this work. No author has a direct or indirect commercial financial incentive or any conflict of interest associated with publishing this article.

carcinoma (H-score). Treatment- outcomes were reviewed and reported. Outcomes were stratified by c-Met overexpression and tumor characteristics. OS, PFS, LC, and DC rates were obtained via the Kaplan-Meier method and differences between groups were evaluated by the log-rank test. Hazard ratios were obtained via Cox regression for both univariate and multivariate analyses.

Results—The 5-year OS, PFS, LC, and DC were 57.18%, 48.07%, 72.11%, and 62.85%, respectively. Ten (35.7%), and 18 patients (64.3%) had c-Met H-index > 30 and < 30, respectively. c-Met overexpression was significantly associated with worse 3-year and 5-year OS ($p = 0.003$), PFS ($p = 0.002$), LC ($p = 0.01$), and DC ($p = 0.0003$). Patients with c-Met overexpression had a hazard ratio of 6.297, 5.782, 6.28, and 18.173 for the risks of death, disease progression, local recurrence, and distant metastases, respectively.

Conclusion—c-Met overexpression could be a potential predictive marker and therapeutic target for local-regional advanced cervical cancer patients treated definitively with CRT.

Keywords

c-Met; overexpression; cervical cancer; concurrent chemoradiation; treatment outcomes

Introduction

Cervical cancer is a major cause of morbidity and mortality worldwide, with an estimated 529,800 newly diagnosed cases and 275,100 deaths in 2011. In the United States alone, it is estimated that there will 12,360 new cases in 2014.^(1, 2) Definitive concurrent chemoradiation therapy (CRT) is the standard of care for treatment of patients with local-regionally advanced cervical cancer^(3–9). However, almost all patients who develop recurrence still have a poor prognosis, despite advancements in salvage treatment.^(10–12) Identification of molecular biomarkers predictive of higher relapse risk after CRT is lacking. If patients who are likely to recur could be better identified then more individualized treatments could be of great benefit to this subset. Potential molecular markers would be helpful for determining prognosis and potentially for development of individualized target therapies.^(11, 12)

The c-Met oncogene plays an important role in cancer growth and metastasis, as well as development of drug resistance to targeted biological therapies.^(13–20) Baykal et al⁽¹⁴⁾ reported c-Met overexpression in 60% of early stage cervical cancer patients (FIGO stage IB disease, treated primarily with surgery). Other studies reported c-Met gene overexpression in up to 11% of patients with lung cancer,⁽¹⁵⁾ 10% of gastric cancers,⁽¹⁶⁾ and 4% of endometrial and esophageal cancers^(17, 18).

This study aimed to assess the association between pretreatment c-Met overexpression in local-regional advanced cervical cancer patients (treated definitively with CRT) and treatment outcomes including overall survival (OS), progression free survival (PFS), distant metastases control (DM), and local-regional control (LC).

Patients and Methods

The Institutional Review Board approved this retrospective study. The study aimed to evaluate the incidence and impact of c-Met oncogene expression on the treatment outcomes of local-regional advanced cervical cancer treated consecutively and definitively with CRT. Charts were reviewed of patients with local-regionally advanced cervical cancer who presented to our department between January 1983 and December 2009. Patients were treated with definitive cisplatin-based chemotherapy and external beam radiation therapy (EBRT) followed by a low-dose-rate (LDR) brachytherapy boost (BT). Inclusion criteria included (1) a histologically proven diagnosis of cervical cancer stages IB1 through IVA locally advanced cervical carcinoma, (2) Eastern Cooperative Oncology Group (ECOG) Performance Status 0–2, and (3) age > 18 years old. Women were excluded if treated with up-front surgery followed by adjuvant radiotherapy or CRT and also if they had prior radiotherapy for gynecologic or gastrointestinal diseases. The chart review yielded 129 eligible cervical cancer patients. Of the eligible patients, 28 had tissue available from the pathology core facility. All 28 eligible patients were treated from 2001 to 2008 utilizing the same radiation therapy technique.”

All patients had a cervical biopsy with pathologic confirmation of cervical carcinoma and underwent appropriate staging work-up. Concurrent cisplatin-based EBRT was to a total dose of 45 Gy, in 1.8 Gy/fraction, 5 fractions per week for a total of 5 weeks. Parametrial boost and nodal boosts were added at the treating physician’s discretion. As part of the definitive management, LDR Fletcher-Suit tandem and ovoid BT implant(s) were performed with Cs-137 sources, in one or two implants. The dose was prescribed according to the Manchester System with dose prescribed to point A based on patient and tumor characteristics. Doses to point B and the rectal and vaginal doses were also calculated. The brachytherapy treatment duration ranged from 40–70 hours, during which time the patients were admitted to the hospital. The details of pretreatment evaluation, treatment technique and treatment parameters of the entire cohort of 129 patients were described in detail in an earlier report.⁽⁸⁾

For the 28 patients whom cervical cancer tissue with available, we treatment outcomes including OS, PFS, LC, and DC. Outcomes were also stratified by c-Met overexpression as well as other relevant tumor characteristics.

Immunohistochemistry

c-Met (Clone C-28) immunohistochemistry was performed on formalin-fixed paraffin tissue microarray sections using the Dako EnVision™ polymer refine detection Horseradish peroxidase (HRP) (Dako, K4002) method. Four micron thick sections of Tissue Microarray (TMA) blocks (2 mm tissue cores were prepared from tumor and normal ectocervical tissue) were transferred to charged slides, deparaffinized and rehydrated prior to antigen retrieval in citrate buffer (pH 6) at 95 °C for 20 minutes. Immunohistochemistry staining was performed by use of the cMet (rabbit polyclonal, Clone C-28) antibody (diluted 1:50) (catalog No. sc-161-R; Santa Cruz Biotechnology, Santa Cruz, CA). Negative control sections were treated similarly, except the primary antibody was omitted. HRP conjugated polymer anti-rabbit secondary (DAKO, EnVision System HRP Labelled Polymer, USA), was used to

detect the primary antibody and 3,3'-diaminobenzidine (DAB) was used as the chromogenic substrates. Counterstaining was performed using Meyer's hematoxylin.

Evaluation of cytoplasmic immunoreactivity for c-Met was performed and scored semi-quantitatively by three pathologists (SE, VP, JJW), and incorporated both the intensity and percentage of immunoreactivity in invasive carcinoma (H-score).⁽²¹⁾ The immunointensity was defined as negative (0), weak (1+), moderate (2+), and strong (3+) (Figure 1). The H-score consisted of a sum of combination of immunoreactivity for intensity multiplied by percentages. The possible range of the H-score was 0–300. A score of 30 was used as a cut off value for positive staining based on the evaluating pathologist's recommendation and was verified by a receiver operating characteristic (ROC) curve showing sensitivity and specificity of 71% and 57%, respectively for H-score of 30 (Figure 2). The average H-score between the three investigators was used for statistical analysis. All pathologists were blinded to the treatment outcomes.

Statistical analysis

Continuous variables were summarized by means, standard deviations, medians, and ranges. Categorical variables were summarized by frequencies and percentages. OS, PFS, LC, and DC rates were obtained via the Kaplan-Meier method and differences between groups were evaluated by the log-rank test. Hazard ratios associated with these rates were obtained via Cox regression for both univariate and multivariate analyses.

Results

The mean patient age in this cohort was 51 ± 10 years. 53.57% of the patients had FIGO stage IIB disease. Almost two thirds of the patient had clinical node negative disease. Most of the patients (78.57%) had locally advanced disease (size ≥ 4 cm) at presentation. Detailed patients and tumor characteristics are presented in Table 1. Table 2 shows total radiation therapy dose delivered to points A and B, stratified by FIGO stage. Ten patients (35.7%) had c-Met H-index > 30 and 18 patients (64.3%) had c-Met H-index ≤ 30 .

Treatment outcomes

Tables 3 and 4 detail the treatment outcomes (OS, PFS, LC, and DC) for all patients stratified by Met expression (H-score index ≤ 30 versus > 30), as well as other patients and tumor characteristics. The 5-year OS, PFS, LC, and DC in the entire cohort were 57.18% (CI 34.06% – 74.82%), 48.07% (CI 25.50% – 67.52%), 72.11% (CI 49.96% – 85.73%), and 62.85% (CI 36.33% – 80.82%), respectively. Figure 3 and Table 3 show a significant association between c-Met overexpression (H index > 30) and worse 3-year and 5-year OS (p-value = 0.003), PFS (p-value = 0.002), LC (p-value = 0.01), and DC (p-value = 0.0003). The log-rank test demonstrated a significant association between smoking and OS (p = 0.04), PFS (p=0.02), LC (p=0.047), and DC (p=0.03). The hazard ratios (HR) and confidence intervals obtained from the proportional hazard model for comparing c-Met > 30 vs. c-Met ≤ 30 is presented in Table 3. Patients with a Met H-score index > 30 had a HR of 6.297 for the risk of death, 5.782 for risk of disease progression, 6.28 for risk of local recurrence, and 18.173 for risk of distant metastases compared to those with Met H-index

less than or equal to 30. There was also a significant association between OS and tumor stage ($p=0.03$), as well as treatment breaks (> 3 days throughout the treatment course) ($p=0.04$) as shown in Table 4. Additionally, there was a significant association between PFS and tumor stage ($p = 0.01$), tumor size ($p=0.052$), and treatment breaks (0.051). A multivariate analysis model that included age, FIGO stage, race, pretreatment anemia, treatment breaks, and body mass index, demonstrated that $c\text{-Met} > 30$ significantly impacted LC (HR 8.792, $p = 0.047$), and had a trend to impact OS (HR 5.95, $p=0.08$), and DM (HR 79.749, $p=0.07$). FIGO stages III and IV significantly impacted OS (HR 25.781, $p=0.02$), PFS (HR 18.713, $p=0.02$), and DM (HR 250.412, $p=0.02$).

Discussion

This study of 28 cervical cancer patients treated definitively with CRT followed by a boost with LDR BT demonstrated a strong association between $c\text{-Met}$ overexpression (H-score index >30) and poor OS, PFS, LC, and DC. Baykal C. et al. ⁽¹⁴⁾ reported that $c\text{-Met}$ oncogene overexpression was an important parameter for disease progression, recurrence, and survival in FIGO stage IB uterine cervix cancer patients who were treated primarily with surgery. This study provides further evidence that $c\text{-Met}$ overexpression not only impacts treatment outcomes for FIGO stage IB cervical cancer treated by surgery (Baykal et al. study), but also affects overall prognosis and treatment outcomes in advanced cervical cancer patients treated with definitive CRT.

In the setting of local-regional advanced cervical cancer, image-guided brachytherapy (IGBT) after EBRT is becoming more widely utilized. Results of MRI-based IGBT have demonstrated improved local control while minimizing treatment-induced adverse events. However, there still needs to be definitive clinical evidence of superior survival advantage compared to conventional two-dimensional historical planning.^(9, 22) Overall, treatment endpoints continue to be significantly worse in the setting of locally recurrent and metastatic cervical cancer. With a median OS up to 13 months, outcomes in these patients remain very poor. This is despite numerous clinical trials investigating different chemotherapeutic regimens before the era of individualized molecular medicine. Currently targeted therapies aiming to knock-down specific molecular targets have emerged as an important therapeutic approach that allow tailoring treatment towards the applicable patient on an individual level with an ultimate goal of better treatment outcomes.^(12, 23, 24) The Gynecologic Oncology Group (GOG) and the Radiation Therapy Oncology Group (RTOG) – now part of NRG - as well as various other groups have recognized the importance of identifying novel molecular therapeutic agents targeting specific pathways in order to improve the treatment outcomes in both the local-regional advanced and recurrent/metastatic settings. However, the majority of these studies have utilized bevacizumab in an attempt to interrupt angiogenesis with translation to a survival advantage. This potential target was reported in considerable number of reports including GOG Protocol 227-C⁽²⁵⁾, and recently in RTOG 0417 ⁽²⁶⁾, GOG 240⁽¹²⁾. The GOG 240 study, reported by Tewari et al., randomized 452 advanced cervical cancer patients to standard chemotherapy with or without bevacizumab at a dose of 15 mg per kilogram. Chemotherapy consisted of cisplatin 50 mg/m², plus paclitaxel at 135 or 175 mg/m² or topotecan at 0.75 mg/m² on days 1 to 3, plus paclitaxel 175 mg/m² on day 1 utilizing a 2-by-2 factorial design. The study did not show superiority of Topotecan-

paclitaxel over cisplatin-paclitaxel (hazard ratio for death, 1.20). However, the addition of bevacizumab to chemotherapy was associated with increased OS (17.0 months vs. 13.3 months; HR for death, 0.71; 98% confidence interval, 0.54 to 0.95; $P=0.004$ per a one-sided test) and higher response rates (48% vs. 36%, $P=0.008$). Bevacizumab resulted in higher incidence of treatment-induced hypertension grade 2 (25% vs. 2%), thromboembolic events grade 3 (8% vs. 1%), and gastrointestinal fistulas grade 3 (3% vs. 0%). The authors concluded that the addition of bevacizumab to combination chemotherapy in patients with recurrent, persistent, or metastatic cervical cancer was associated with an improvement of 3.7 months in median OS.⁽¹²⁾ Based on this, the FDA approved bevacizumab to treat patients with persistent, recurrent or late-stage (metastatic) cervical cancer on August 14, 2014. Although there is a growing body of evidence that targeting angiogenesis using bevacizumab might play an important role in optimizing treatment outcomes, identifying other molecular targets in different pathways in cervical carcinogenesis might add to therapeutic options and help improve treatment outcomes.

c-Met has been of increasing interest both as a predictive and prognostic biomarker in various tumor sites.^(14–18, 27–33) Hepatocyte growth factor (HGF), which is a plasminogen-like protein that was considered a humoral mediator of liver regeneration, has been shown to be the c-Met proto-oncogene product.^(13, 34) Pennacchietti S. et al.⁽³⁵⁾ studied various in vitro cell lines including cervical cancer to find that hypoxia promotes tumor invasion by sensitizing cells to HGF stimulation, which explains the molecular basis of c-Met overexpression. The authors reported that hypoxia activates transcription of the c-Met proto-oncogene, and since the hypoxic areas of tumors overexpress c-Met, inhibition of c-Met expression prevents hypoxia-induced invasive cancer growth. Leo C. et al.⁽²⁷⁾ investigated the expression of c-Met in cervical cancers in relation to intratumoral hypoxia levels and to clinico-pathological parameters, finding that although c-Met has been shown to be hypoxia-induced in vitro, it is not the mediator of deleterious effects of hypoxia for clinical outcome in cervical cancer. Cabozantinib, which is a tyrosine kinase inhibitor that targets the c-Met, VEGFR2, and RET pathways, was approved by the FDA on Nov. 29, 2012 for the treatment of medullary thyroid cancer based on a phase III, international, multicenter, randomized, double-blind clinical trial (the EXAM study).⁽³⁶⁾ The drug is currently being examined for efficacy in other solid tumors including breast cancer, castration resistant prostate cancer (CRPC), renal cell carcinoma, hepatocellular carcinoma, gastric or gastroesophageal junction cancer, melanoma, small cell lung cancer, ovarian cancer and primary peritoneal or fallopian tube carcinoma.⁽³⁷⁾

Interestingly, it was shown that Met gene amplification is one of the most relevant mechanisms involved in Epidermal Growth Factor Receptor – Tyrosine Kinase Inhibitors (EGFR-TKI) acquired resistance. Engelman et al.⁽²⁰⁾ correlated the resistance to EGFR-TKI agents in lung cancers to the focal amplification of the c-Met proto-oncogene, and proved that inhibition of c-Met signaling in these cancer cells restored their sensitivity to EGFR-TKI. c-Met amplification was detected in 4 of 18 (22%) lung cancer specimens that had developed resistance to EGFR-TKI. They concluded that amplification of c-Met causes EGFR-TKI resistance by driving ERBB3 (HER3)-dependent activation of PI3K, and therefore, c-Met amplification may promote drug resistance in other ERBB-driven cancers as well.⁽²⁰⁾ In a study by Bean et. al.,⁽¹⁹⁾ c-Met amplification was found in 21% of lung

cancer patients with acquired resistance to EGFR-TKI and only in 3% of untreated patients, confirming that c-Met could be a relevant therapeutic target for individuals with acquired resistance to EGFR-TKIs.

Would novel molecular therapy that targets both angiogenesis through VEGFR2 and c-Met oncogene add benefit to local-regional advanced, recurrent and/or metastatic cervical cancer patients? The answer to this question requires further controlled clinical trials to assess both the safety and efficacy of such agents with an ultimate goal of better and more effective individualized treatment options for patients. The limitations of our study include that fact that treatment outcomes were assessed through a retrospective chart review at a single institution with a relatively small number of patients reported. However, to our knowledge, this study is the first series to highlight the impact of c-Met overexpression on the treatment outcomes in advanced cervical cancer patients treated with definitive CRT.

Conclusion

c-Met overexpression is a potential predictive marker and therapeutic target for local-regional advanced cervical cancer patients treated definitively with CRT. Further controlled clinical trials are required for additional verification.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA: a cancer journal for clinicians*. 2011; 61(2):69–90. [PubMed: 21296855]
2. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA: a cancer journal for clinicians*. 2014; 64(1):9–29. [PubMed: 24399786]
3. Eifel PJ, Winter K, Morris M, Levenback C, Grigsby PW, Cooper J, et al. Pelvic irradiation with concurrent chemotherapy versus pelvic and para-aortic irradiation for high-risk cervical cancer: an update of radiation therapy oncology group trial (RTOG) 90-01. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2004; 22(5):872–80. [PubMed: 14990643]
4. Morris M, Eifel PJ, Lu J, Grigsby PW, Levenback C, Stevens RE, et al. Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. *The New England journal of medicine*. 1999; 340(15):1137–43. [PubMed: 10202164]
5. Pearcey R, Brundage M, Drouin P, Jeffrey J, Johnston D, Lukka H, et al. Phase III trial comparing radical radiotherapy with and without cisplatin chemotherapy in patients with advanced squamous cell cancer of the cervix. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2002; 20(4):966–72. [PubMed: 11844818]
6. Donnelly ED, Refaat T, Gentile M, Herskovic A, Boyle J, Helenowski I, et al. Evaluation of Outcomes in Patients With Carcinoma of the Cervix Treated With Concurrent Radiation and Cisplatin Versus Cisplatin/5-FU Compared With Radiation Alone. *American journal of clinical oncology*. 2013
7. Refaat T, Castelain B, Small W Jr, Elsaid A, Lotfy N, Lartigau E, et al. Concomitant Chemoradiotherapy With Image-guided Pulsed Dose Rate Brachytherapy as a definitive Treatment Modality for Early-stage Cervical Cancer. *American journal of clinical oncology*. 2013
8. Refaat T, Donnelly ED, Gentile M, Novak C, Yuan Y, Khedr GA, et al. Low-Dose-Rate Brachytherapy Boosting Concurrent Chemoradiation as a Definitive Treatment Modality for Cervical Cancer: Long-term Clinical Results of Outcomes and Associated Toxicity. *American journal of clinical oncology*. 2014

9. Refaat T, Nickers P, Lartigau E. Volume-based pulsed-dose-rate brachytherapy boosting concurrent chemoradiation as a definitive treatment modality in cervical cancer. *Brachytherapy*. 2014; 13(1): 80–7. [PubMed: 24246857]
10. Leitaó MM Jr, Chi DS. Recurrent cervical cancer. Current treatment options in oncology. 2002; 3(2):105–11. [PubMed: 12057073]
11. Eskander RN, Tewari KS. Targeting angiogenesis in advanced cervical cancer. *Therapeutic advances in medical oncology*. 2014; 6(6):280–92. [PubMed: 25364393]
12. Tewari KS, Sill MW, Long HJ 3rd, Penson RT, Huang H, Ramondetta LM, et al. Improved survival with bevacizumab in advanced cervical cancer. *The New England journal of medicine*. 2014; 370(8):734–43. [PubMed: 24552320]
13. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nature reviews Molecular cell biology*. 2003; 4(12):915–25. [PubMed: 14685170]
14. Baykal C, Ayhan A, Al A, Yuce K, Ayhan A. Overexpression of the c-Met/HGF receptor and its prognostic significance in uterine cervix carcinomas. *Gynecologic oncology*. 2003; 88(2):123–9. [PubMed: 12586590]
15. Cappuzzo F, Marchetti A, Skokan M, Rossi E, Gajapathy S, Felicioni L, et al. Increased c-Met gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2009; 27(10):1667–74. [PubMed: 19255323]
16. Hara T, Ooi A, Kobayashi M, Mai M, Yanagihara K, Nakanishi I. Amplification of c-myc, K-sam, and c-met in gastric cancers: detection by fluorescence in situ hybridization. *Laboratory investigation; a journal of technical methods and pathology*. 1998; 78(9):1143–53. [PubMed: 9759658]
17. Miller CT, Lin L, Casper AM, Lim J, Thomas DG, Orringer MB, et al. Genomic amplification of c-Met with boundaries within fragile site FRA7G and upregulation of c-Met pathways in esophageal adenocarcinoma. *Oncogene*. 2006; 25(3):409–18. [PubMed: 16186806]
18. Samuelson E, Levan K, Adamovic T, Levan G, Horvath G. Recurrent gene amplifications in human type I endometrial adenocarcinoma detected by fluorescence in situ hybridization. *Cancer genetics and cytogenetics*. 2008; 181(1):25–30. [PubMed: 18262049]
19. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. c-Met amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(52):20932–7. [PubMed: 18093943]
20. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. c-Met amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science*. 2007; 316(5827):1039–43. [PubMed: 17463250]
21. Budwit-Novotny DA, McCarty KS, Cox EB, Soper JT, Mutch DG, Creasman WT, et al. Immunohistochemical analyses of estrogen receptor in endometrial adenocarcinoma using a monoclonal antibody. *Cancer research*. 1986; 46(10):5419–25. [PubMed: 3756890]
22. Potter R, Georg P, Dimopoulos JC, Grimm M, Berger D, Nesvacil N, et al. Clinical outcome of protocol based image (MRI) guided adaptive brachytherapy combined with 3D conformal radiotherapy with or without chemotherapy in patients with locally advanced cervical cancer. *Radiotherapy and oncology: journal of the European Society for Therapeutic Radiology and Oncology*. 2011; 100(1):116–23. [PubMed: 21821305]
23. Greer BE, Koh WJ, Abu-Rustum NR, Apte SM, Campos SM, Chan J, et al. Cervical cancer. *Journal of the National Comprehensive Cancer Network: JNCCN*. 2010; 8(12):1388–416. [PubMed: 21147903]
24. Thigpen T, Shingleton H, Homesley H, Lagasse L, Blessing J. Cis-platinum in treatment of advanced or recurrent squamous cell carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. *Cancer*. 1981; 48(4):899–903. [PubMed: 7196794]
25. Monk BJ, Sill MW, Burger RA, Gray HJ, Buekers TE, Roman LD. Phase II trial of bevacizumab in the treatment of persistent or recurrent squamous cell carcinoma of the cervix: a gynecologic oncology group study. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2009; 27(7):1069–74. [PubMed: 19139430]

26. Schefter T, Winter K, Kwon JS, Stuhr K, Balaraj K, Yaremko BP, et al. RTOG 0417: efficacy of bevacizumab in combination with definitive radiation therapy and cisplatin chemotherapy in untreated patients with locally advanced cervical carcinoma. *International journal of radiation oncology, biology, physics*. 2014; 88(1):101–5.
27. Leo C, Horn LC, Einkenkel J, Hentschel B, Hockel M. Tumor hypoxia and expression of c-met in cervical cancer. *Gynecologic oncology*. 2007; 104(1):181–5. [PubMed: 16996116]
28. Jeffers M, Rong S, Vande Woude GF. Enhanced tumorigenicity and invasion-metastasis by hepatocyte growth factor/scatter factor-met signalling in human cells concomitant with induction of the urokinase proteolysis network. *Molecular and cellular biology*. 1996; 16(3):1115–25. [PubMed: 8622656]
29. Shimabukuro K, Ichinose S, Koike R, Kubota T, Yamaguchi M, Miyasaka M, et al. Hepatocyte growth factor/scatter factor is implicated in the mode of stromal invasion of uterine squamous cervical cancer. *Gynecologic oncology*. 2001; 83(2):205–15. [PubMed: 11606073]
30. Wong AS, Leung PC, Auersperg N. Hepatocyte growth factor promotes in vitro scattering and morphogenesis of human cervical carcinoma cells. *Gynecologic oncology*. 2000; 78(2):158–65. [PubMed: 10926796]
31. Takeuchi H, Bilchik A, Saha S, Turner R, Wiese D, Tanaka M, et al. c-c-Met expression level in primary colon cancer: a predictor of tumor invasion and lymph node metastases. *Clinical cancer research: an official journal of the American Association for Cancer Research*. 2003; 9(4):1480–8. [PubMed: 12684423]
32. Lengyel E, Prechtel D, Resau JH, Gauger K, Welk A, Lindemann K, et al. C-Met overexpression in node-positive breast cancer identifies patients with poor clinical outcome independent of Her2/neu. *International journal of cancer Journal international du cancer*. 2005; 113(4):678–82. [PubMed: 15455388]
33. Qian CN, Guo X, Cao B, Kort EJ, Lee CC, Chen J, et al. Met protein expression level correlates with survival in patients with late-stage nasopharyngeal carcinoma. *Cancer research*. 2002; 62(2): 589–96. [PubMed: 11809714]
34. Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, et al. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science*. 1991; 251(4995):802–4. [PubMed: 1846706]
35. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer cell*. 2003; 3(4):347–61. [PubMed: 12726861]
36. Elisei R, Schlumberger MJ, Muller SP, Schoffski P, Brose MS, Shah MH, et al. Cabozantinib in progressive medullary thyroid cancer. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2013; 31(29):3639–46. [PubMed: 24002501]
37. Roy S, Narang BK, Rastogi SK, Rawal RK. A Novel Multiple Tyrosine-kinase Targeted Agent to Explore the Future Perspectives of Anti-Angiogenic Therapy for the Treatment of Multiple Solid Tumors: Cabozantinib. *Anti-cancer agents in medicinal chemistry*. 2014

Figure 1.
C-Met expression by immunohistochemistry in human cervical carcinoma with varying degree of staining. A1 & A2: Cervical carcinomas with weak positive cytoplasmic staining, B1 & B2: Cervical carcinomas with moderate positive cytoplasmic staining, and C1 & C2: Cervical carcinomas with strong positive cytoplasmic staining. (50µm scale bar, 200-fold magnification).

Figure 2. Receiver Operating Characteristic (ROC) curve to detect the association between cMet overexpression and the local regional control.

Figure 3.
Overall Survival, Progression Free Survival, Local Control and Distant Control stratified by
Met expression (H index ≤ 30 versus > 30)

Table 1

Patients and tumor characteristics

Characteristics			
Age		51 ± 10 51 (32,74)	
		N	%
Race	White	14	50.00
	African American	7	25.00
	Hispanic	6	21.43
	Others	1	3.57
Smoking	Current Smoker	10	35.71
	< 20 p/y	2	20.00
	20–40 p/y	4	40.00
	> 40 p/y	4	40.00
	Ex-Smoker	5	17.86
	< 20 p/y	2	40.00
	20–40 p/y	2	40.00
	> 40 p/y	1	20.00
Non Smoker		13	46.43
Tumor Stage	IB1	1	3.57
	IB2	2	7.14
	IIA	0	0.00
	IIB	15	53.57
	IIIA	0	0.00
	IIIB	5	17.86
	IVA	3	10.71
	IVB	2	7.14
Nodal Status	Node negative	18	64.29
	Pelvic	4	14.29
	Para-aortic	1	3.57
	Both (Pelvic and PA)	5	17.86
Positive node location	Unilateral	9	32.14

Characteristics			
	Bilateral	18	64.29
	Unknown	1	3.57
Tumor Grade	1	1	3.57
	2	13	46.43
	3	8	28.57
	N/A	6	21.43
Tumor morphology	Exophytic	24	85.71
	Endophytic	3	10.71
	N/A	1	3.57
Tumor Histology	Squamous Cell Carcinoma	25	89.29
	Adenocarcinoma	3	10.71
Tumor Size in cm	< 4 cm	4	14.29
	4 cm	22	78.57
Pretreatment Anaemia	Yes (<12)	17	60.71
	No (≥ 12)	11	39.29
Body mass index	18.5	1	3.57
	18.5 – 24.9	9	32.14
	25.0 – 29.9	6	21.43
	30	12	42.86

Table 2

Points A and B total dose stratified by FIGO stage

FIGO stage	Dose in cGy	Mean	Median	Minimum	Maximum
IB	Point A	8289.00	8521.00	7606.00	8740.00
	Point B	6280.67	6335.00	6055.00	6452.00
IIB	Point A	8676.46	8632.00	8500.00	8962.00
	Point B	6236.15	6249.00	5574.00	6705.00
IIIB	Point A	8586.25	8575.00	8476.00	8719.00
	Point B	6195.25	6252.00	5619.00	6658.00
IVA	Point A	8872.50	8872.50	8500.00	9245.00
	Point B	6348.00	6348.00	5859.00	6837.00
IVB	Point A	9013.00	9013.00	9013.00	9013.00
	Point B	6720.00	6720.00	6720.00	6720.00

Table 3

Treatment outcomes (Overall Survival, Progression Free Survival, Local Control and Distant Control) for all patients and stratified by Met expression (H index ≤ 30 versus > 30)

	All patients (N=28; N events=10)	Met 30 (N=18; N events=3)	Met > 30 (N=10; N events=7)	p-value
3-yr OS	63.53% (CI 41.29% – 79.24%)	88.24% (CI 60.60% – 96.92%)	22.22% (CI 3.37% – 51.31%)	0.003
5-yr OS	57.18% (CI 34.06% – 74.82%)	77.21% (CI 41.83% – 92.61%)	22.22% (CI 3.37% – 51.31%)	
	All patients (N=28; N events=13)	Met 30 (N=18; N events=5)	Met > 30 (N=10; N events=8)	
3-yr PFS	61.04% (CI 39.51% – 76.91%)	81.93% (CI 53.77% – 93.80%)	23.33% (CI 3.60% – 52.88%)	0.002
5-yr PFS	48.07% (CI 25.50% – 67.52%)	71.69% (CI 39.00% – 88.90%)	11.67% (CI 0.64% – 40.07%)	
	All patients (N=28; N events=7)	Met 30 (N=18; N events=2)	Met > 30 (N=10; N events=5)	
3-yr LC	72.11% (CI 49.96% – 85.73%)	87.40% (CI 58.12% – 96.71%)	42.00% (CI 10.65% – 71.46%)	0.01
5-yr LC	72.11% (CI 49.96% – 85.73%)	87.40% (CI 58.12% – 96.71%)	42.00% (CI 10.65% – 71.46%)	
	All patients (N=28; N events=9)	Met 30 (N=18; N events=2)	Met > 30 (N=10; N events=7)	
3-yr DM	71.83% (CI 49.45% – 85.60%)	94.12% (CI 65.02% – 99.15%)	28.00% (CI 4.35% – 59.65%)	0.0003
5-yr DMFS	62.85% (CI 36.33% – 80.82%)	94.12% (CI 65.02% – 99.15%)	14.00% (0.74% – 45.51%)	
Hazard ratios obtained from proportional hazard model for comparing Met > 30 vs. Met 30.				
Outcome	HR	95% lower confidence limit	95% upper confidence limit	p-value
Death	6.297	1.616	24.539	0.008
Disease Progression	5.782	1.712	19.525	0.005
Local Recurrence	6.280	1.186	33.255	0.03
Distant Metastases	18.173	2.203	149.893	0.007

Table 4

Treatment outcomes (Overall Survival, Progression Free Survival, Local Control and Distant Control) stratified by patients and tumor characteristics.

Treatment Outcomes	Median in months	2-years (%)	3-years (%)	5-years (%)	Log-rank P value
Overall Survival (OS)					
OS according to tumor stage					
I	NA	100% (100%,100%)	50.00% (0.60%, 91.04%)	50.00% (0.60%, 91.04%)	0.03
II	NA	77.38% (44.93%, 92.11%)	77.38% (44.93%, 92.11%)	77.38% (44.93%, 92.11%)	
III	27 (7,127)	60.00% (12.57%, 88.18%)	30.00% (1.23%, 71.92%)	30.00% (1.23%, 71.92%)	
IVA	NA	66.67% (5.41%, 94.52%)	66.67% (5.41%, 94.52%)	66.67% (5.41%, 94.52%)	
IVB	11 (8,14)	0.00%	0.00%	0.00%	
OS according to Lymph node status					
Negative	NA	79.41% (53.97%, 91.75%)	72.79% (46.01%, 87.82%)	63.70% (34.58%, 82.56%)	0.08
Positive	18 (7,124)	33.33% (4.61%, 67.56%)	33.33% (4.61%, 67.56%)	33.33% (4.61%, 67.56%)	
OS according to tumor size					
< 4 cm	NA	100%	100%	100%	0.12
4 cm	NA	58.93% (34.30%, 77.00%)	52.38% (27.98%, 72.02%)	52.38% (27.98%, 72.02%)	
OS according to pretreatment anemia					
Yes (<12)	NA	75.00% (46.34%, 89.80%)	67.50% (38.35%, 85.11%)	67.50% (38.35%, 85.11%)	0.30
No (12)	42 (7,147)	57.14% (21.72%, 81.46%)	57.14% (21.72%, 81.46%)	42.86% (11.40%, 71.85%)	
OS according to treatment breaks					
Yes	NA	77.92% (45.90%, 92.32%)	77.92% (45.90%, 92.32%)	77.92% (45.90%, 92.32%)	0.04
No	27 (8,90)	57.14% (25.38%, 79.58%)	42.86% (12.64%, 70.67%)	28.57% (4.90%, 59.44%)	
Progression Free Survival (PFS)					
PFS according to tumor stage					
I	70 (42,97)	100% (100%,100%)	50.00% (0.60%, 91.04%)	50.00% (0.60%, 91.04%)	0.01
II	NA	77.92% (45.90%, 92.32%)	77.92% (45.90%, 92.32%)	77.92% (45.90%, 92.32%)	
III	19 (3,127)	20.00% (0.84%, 58.19%)	20.00% (0.84%, 58.19%)	20.00% (0.84%, 58.19%)	
IVA	57 (5,57)	66.67% (5.41%, 94.52%)	66.67% (5.41%, 94.52%)	0.00%	
IVB	6 (3,8)	0.00%	0.00%	0.00%	
PFS according to Lymph node status					

Treatment Outcomes	Median in months	2-years (%)	3-years (%)	5-years (%)	Log-rank P value
Negative	97 (19,127)	69.02% (43.37%, 84.83%)	69.02% (43.37%, 84.83%)	50.33% (22.34%, 73.01%)	0.23
Positive	8 (4,124)	33.33% (4.61%, 67.56%)	33.33% (4.61%, 67.56%)	33.33% (4.61%, 67.56%)	
PFS according to tumor size					
< 4 cm	NA	100%	100%	100%	0.052
4 cm	NA	49.33% (26.15%, 68.92%)	49.33% (26.15%, 68.92%)	39.46% (16.02%, 62.37%)	
PFS according to pretreatment anemia					
Yes	NA	63.03% (35.42%, 81.44%)	63.03% (35.42%, 81.44%)	50.42% (20.43%, 74.44%)	0.55
No	42 (4,127)	58.33% (22.98%, 82.07%)	58.33% (22.98%, 82.07%)	43.75% (11.87%, 72.57%)	
PFS according to treatment breaks					
Yes	NA	79.44% (48.79%, 92.89%)	79.44% (48.79%, 92.89%)	66.20% (29.87%, 86.87%)	0.051
No	27 (8,90)	38.10% (12.09%, 64.35%)	38.10% (12.09%, 64.35%)	25.40% (4.69%, 54.13%)	
Local-regional Control (LC) rate					
LC according to tumor stage					
I	NA	100%	100%	100%	0.08
II	NA	83.92% (49.40%, 95.73%)	83.92% (49.40%, 95.73%)	83.92% (49.40%, 95.73%)	
III	19 (4,128)	40.00% (5.20%, 75.28%)	40.00% (5.20%, 75.28%)	40.00% (5.20%, 75.28%)	
IVA	NA	66.67% (5.41%, 94.52%)	66.67% (5.41%, 94.52%)	66.67% (5.41%, 94.52%)	
IVB	NA	NA	NA	NA	
LC according to Lymph node status					
Negative	NA	74.77% (49.46%, 88.68%)	74.77% (49.46%, 88.68%)	74.77% (49.46%, 88.68%)	0.33
Positive	NA	66.67% (19.46%, 90.44%)	66.67% (19.46%, 90.44%)	66.67% (19.46%, 90.44%)	
LC according to tumor size					
< 4 cm	NA	100%	100%	100%	0.18
4 cm	NA	62.64% (36.58%, 80.45%)	62.64% (36.58%, 80.45%)	62.64% (36.58%, 80.45%)	
LC according to pretreatment anemia					
Yes	NA	80.88% (51.27%, 93.48%)	80.88% (51.27%, 93.48%)	80.88% (51.27%, 93.48%)	0.26
No	NA	58.33% (22.98%, 82.07%)	58.33% (22.98%, 82.07%)	58.33% (22.98%, 82.07%)	
LC according to treatment breaks					
Yes	NA	79.44% (48.79%, 92.89%)	79.44% (48.79%, 92.89%)	79.44% (48.79%, 92.89%)	0.40

Treatment Outcomes	Median in months	2-years (%)	3-years (%)	5-years (%)	Log-rank P value
	No	59.52% (23.51%, 83.04%)	59.52% (23.51%, 83.04%)	59.52% (23.51%, 83.04%)	
Distant Control (DC) rate					
DC according to tumor stage					
	I	NA	NA	NA	0.07
	II	NA	83.33% (48.17%, 95.55%)	83.33% (48.17%, 95.55%)	
	III	27 (7,127)	60.00% (12.57%, 88.18%)	30.00% (1.23%, 71.92%)	
	IVA	NA	NA	NA	
	IVB	15 (8,15)	0.00%	0.00%	
DC according to Lymph node status					
	Negative	NA	77.04% (49.72%, 90.72%)	68.48% (38.94%, 85.90%)	0.55
	Positive	NA	50.00% (5.79%, 84.49%)	50.00% (5.79%, 84.49%)	
DC according to tumor size					
	< 4 cm	NA	100%	100%	0.12
	4 cm	NA	61.39% (33.16%, 80.60%)	52.62% (24.68%, 74.48%)	
DC according to pretreatment anemia					
	Yes	NA	75.21% (40.72%, 91.37%)	75.21% (40.72%, 91.37%)	0.10
	No	NA	51.85% (16.41%, 78.77%)	51.85% (16.41%, 78.77%)	