Original Research Article

Glut-1 expression in small cervical biopsies is prognostic in cervical cancers treated with chemoradiation

Yada Kanjanapan a, Siddhartha Deb b,g, Richard J. Young c, Mathias Bressel d, Linda Mileskin a,g, Danny Rischina a,g, Michael S. Hofman a,g, Kailash Narayan a,g, Shankar Siva f,g

a Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia
b Department of Anatomical Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia
c Division of Cancer Imaging, Nuclear Medicine Department, Peter MacCallum Cancer Centre, Melbourne, Australia
d Translational Research Laboratory, Research Division, Peter MacCallum Cancer Centre, Melbourne, Australia
e Centre for Biostatistics and Clinical Trials, Peter MacCallum Cancer Centre, Melbourne, Australia
f Division of Radiation Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia
g University of Melbourne, Melbourne, Australia

ABSTRACT

Background/purpose: Chemoradiation (CRT) is standard therapy for locally advanced cervical cancer (LACC). However, there is a lack of biomarkers to identify patients at high relapse-risk. We examine metabolic (glucose transporter-1 [Glut-1]), hypoxic (hypoxia inducible factor [HIF-1α]; carbonic anhydrase [CA-9]) and proliferative (Ki-67) markers for prognostic utility in LACC.

Materials/methods: 60 LACC patients treated with CRT had pre-treatment biopsies. Immunohistochemistry was performed for Glut-1, HIF-1α and CA-9, to generate a histoscore from intensity and percentage staining; and Ki-67 scored by percentage of positive cells. For each biomarker, treatment response and survival was compared between low and high-staining groups by logrank testing and multivariate analyses.

Results: High Glut-1 expression was associated with inferior progression-free survival (PFS), (hazard ratio [HR] 2.8, p = 0.049) and overall survival (OS), (HR 5.0, p = 0.011) on multifactor analysis adjusting for stage, node positivity, tumour volume and uterine corpus invasion. High Glut-1 correlated with increased risk of distant failure (HR 14.6, p = 0.001) but not local failure. Low Glut-1 was associated with higher complete metabolic response rate on post-therapy positron emission tomography scan (odds ratio 3.4, p = 0.048). Ki-67 was significantly associated with PFS only (HR 1.19 per 10 units increase, p = 0.033). Biomarkers for hypoxia were not associated with outcome.

Conclusions: High Glut-1 in LACC is associated with poor outcome post CRT. If prospectively validated, Glut-1 may help select patients for more intensive treatment regimens.

© 2017 The Authors. Published by Elsevier Ireland Ltd on behalf of European Society for Radiotherapy and Oncology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in women worldwide [1]. Standard treatment in locally advanced disease is concurrent chemoradiation [2]. Clinical prognostic factors include tumour stage and nodal involvement [3], although metabolic response after chemoradiation has been shown to have even stronger prognostic value [4–7]. Additionally, post-therapy 18F-fluoro-deoxyglucose positron emission tomography (FDG-PET) response can help direct potentially curative salvage interventions in patients who fail primary therapy. However, post-therapy FDG-PET is unable to inform the design of investigations or therapeutic interventions before or during treatment.

There have been numerous putative pathobiological prognostic factors studied, including factors involving the angiogenesis, hypoxia, epidermal growth factor receptor and COX-2 pathways [8]. We set out to assess the prognostic significance of biomarkers for metabolism (glucose transporter-1 [Glut-1]), proliferation (Ki-67) and hypoxia (hypoxia-inducible factor-1α [HIF-1α] and carbonic anhydrase IX [CA-9]) in cervical cancer patients treated with chemoradiation. We hypothesised that these pre-treatment
Chemotherapy at a dose of 40 mg/m² weekly for 4–6 cycles. Within planned to between 40 and 45 grays (Gy) to the pelvis with a nodal brief, conventionally fractionated external beam radiotherapy was Amplification kit (amplifiers A and B, Roche Diagnostics) and /C176 Diagnostics) for 52 min followed by incubation with the Ki-67 anti- Benchmark Ultra (Roche Diagnostics, USA). Antigen retrieval was received from the PMCC institutional ethics board. This study is reported conforming to the REporting recommendations for tumour MARKer prognostic studies (REMARK) criteria[9]. This is a set of guidelines on assessing validity of tumour markers.

Irradiation techniques have been previously described[6]. In brief, conventionally fractionated external beam radiotherapy was planned to between 40 and 45 grays (Gy) to the pelvis with a nodal boost to 50–50.4 Gy as required, with conventional cisplatin chemotherapy at a dose of 40 mg/m² weekly for 4–6 cycles. Within 10 days of completion, a high-dose rate intracavitary brachytherapy boost was delivered twice weekly to a dose of 28 Gy in 4 fractions (or equivalent, to a total tumour dose of 80 Gy. All patients had histologically confirmed carcinoma of the uterine cervix, International Federation of Gynecology and Obstetrics (FIGO) stage Ib to IVa and ECOG performance status 0 or less.

A single post-therapy FDG-PET/CT was performed using the methods/techniques reported previously [6], between 3 and 6 months after completion of chemoradiation therapy, in accordance with The National Comprehensive Care Network (NCCN) 2016 guidelines. Metabolic changes post-therapy were scored as complete metabolic response (CMR) where there is no tracer uptake or background level of FDG-activity within the treated disease, partial metabolic response (PMR) where there is residual FDG-activity within the treated disease, and progressive metabolic disease (PMD) where there is increased intensity or distribution of FDG-avid disease, as previously published by our institution[10]. Clinical follow-up of patients including medical history and physical examination was performed at 4 weeks post-therapy, 3 monthly until 2 years post-therapy, 6 monthly in years 3, 4 and 5, then yearly thereafter.

**Immunohistochemistry**

Immunohistochemistry was performed on cut sections from formalin fixed paraffin embedded (FFPE) tumour tissue. 4 μm tissue sections were cut and de-waxed through histolene, and graded alcohols then into water. Antigen retrieval was performed using Dako high pH Target Retrieval Solution (Dako) for 3 min at 125 °C for Glut-1, HIF-1α and CA-9. Slides were then loaded onto a Dako autostainer (Dako) for the following incubations: primary antibody for Glut-1 (1:200, Dako), HIF-1α (1:50, Novus Biologicals) or CA-9 (1:4000, Novus Biologicals) for 60 min at room temperature; antibody detection with Envision+ (Dako) rabbit (Glut-1 and CA-9) or mouse (HIF-1α) antibody for 60 min at room temperature; colour reaction with 3,3’-Diaminobenzidine (DAB) for 10 min at room temperature. Staining for Ki-67 was performed on a Ventana BenchMark Ultra (Roche Diagnostics, USA). Antigen retrieval was performed in a high pH Ultra cell conditioning solution (CC1, Roche Diagnostics) for 52 min followed by incubation with the Ki-67 antibody (SP6, Cell Marque, diluted at 1/50), at 36 °C for 32 min. Amplification kit (amplifiers A and B, Roche Diagnostics) and UltraView Universal DAB detection kit (Roche Diagnostics) were used in accordance with the manufacturer’s instructions for on-board detection. Slides were then removed from the autostainer, counterstained with haematoxylin, mounted and coverslipped.

**Scoring criteria and cut-offs**

Scoring of membranous staining for Glut-1 and CA-9, or nuclear staining for HIF-1α was performed according to a previously used semi-quantitative system [11–15]. A histoscore (0–12) was generated by multiplying intensity (score 0–3) by a categorical percentage score (0 for No staining, 1 for <25%, 2 for 25–29%, 3 for 50–74% and 4 for ≥75% of cells). This method of assigning histoscore based on a combination of percentage and intensity of staining is a commonly utilized and accepted methodology of immunohistochemical scoring, including being used for Glut-1 in the melanoma setting [16]. Cohorts were dichotomised into low and high staining groups in equal proportion for statistical analysis, consistent with other similar studies [17,18]. This resulted in the following cut off values: for Glut-1 low is defined as score 0–3, high is 4–12; CA-9 low is defined as 0–2 and high is 3–12; for HIF-1α low is 0–2 and high is 3–12. Ki-67 scoring was performed by counting 1000 representative tumour cells and calculating the percentage of positive cells.

**Statistical analysis**

The primary objective was to evaluate the association between pre-treatment biomarkers and post-therapy PET metabolic response. The secondary objectives were to evaluate the association of these biomarkers with progression-free survival and overall survival. All time to event analyses were calculated from the date of commencement of radiotherapy to the date of the event. Death was a censoring event for time to local, time to nodal and time to distant failure. The impact of biomarkers on time to event outcomes were assessed using likelihood ratio test (Ki-67) and logrank test (all other biomarkers) on univariate analysis. Cox proportional hazards model was used to assess the impact of the biomarkers adjusting for possible confounders using known pre-treatment prognostic factors namely, tumour volume on magnetic resonance imaging (MRI), FIGO stage, node positivity and uterine corpus invasion. Our centre has previously published our findings that uterine corpus invasion in cervical cancer is correlated with overall survival [19], an observation collaborated by another group [20]. Time-to-event curves were described using Kaplan–Meier methods. The association between metabolic response and expression of biomarkers as dichotomous variables was performed using Barnard’s test. The association between Ki-67 as a continuous variable and metabolic response was examined by the Wilcoxon rank sum test.

**Results**

**Immunohistochemistry**

Sixty of 105 cases had tumour blocks available for immunohistochemical (IHC) biomarker analysis (Fig. 1). Baseline characteristics of the IHC study population are shown in Table 1. IHC results were obtained in 57 patients for CA-9, 59 for Glut-1 and 60 for HIF-1α staining. Fifty-eight cases were assessed for Ki-67 proliferation factors namely, tumour volume on magnetic resonance imaging (MRI), FIGO stage, node positivity and uterine corpus invasion. Our centre has previously published our findings that uterine corpus invasion in cervical cancer is correlated with overall survival [19], an observation collaborated by another group [20]. Time-to-event curves were described using Kaplan–Meier methods. The association between metabolic response and expression of biomarkers as dichotomous variables was performed using Barnard’s test. The association between Ki-67 as a continuous variable and metabolic response was examined by the Wilcoxon rank sum test.
FFPE: formalin fixed paraffin embedded

**Fig. 1.** Flow of cases through the study according to REMARK criteria.

### Table 1
Patient characteristics.

| Characteristic                      | Category       | Number | %    |
|------------------------------------|----------------|--------|------|
| FIGO stage                         | Ib             | 19     | 31.7%|
|                                   | II             | 27     | 45.0%|
|                                   | III            | 14     | 23.3%|
| Age                                | Median (range) | 54 (26–84)|      |
| Uterine corpus invasion            | Yes            | 39     | 65%  |
|                                   | No             | 21     | 35%  |
| Tumour volume (cc)                 | Median         | 35.9   |      |
|                                   | Interquartile range | 16–71 |      |
| Nodal status                       | Node positive  | 24     | 40%  |
|                                   | Node negative  | 36     | 60%  |
| No. of lymph nodes involved        | 1              | 8      | 13.3%|
|                                   | 2              | 7      | 11.7%|
|                                   | 3              | 5      | 8.3% |
|                                   | 4              | 4      | 6.7% |
| Histology                          | Squamous       | 58     | 96.7%|
|                                   | Adenosquamous  | 1      | 1.6% |
|                                   | Clear cell     | 1      | 1.6% |

There were no significant number of cases with small percentage but intense level of staining. We did not choose a specific cut-off such as >50% staining of 2+ or more for high level of staining; because there is currently no biological or clinical rationale supporting a specific cutoff. Furthermore, it has been common practice for other studies examining these biomarkers to assign high and low expression groups by the median value [17,18].

### Biomarkers as predictors of metabolic response

Of the 60 patients, 44 had CMR, 7 had PMR and 9 had PMD. The association between metabolic response (CMR vs non CMR) and expression of CA-9, HIF-1α and Glut-1 is described in Table 2. High Glut-1 expression was associated with a lower CMR rate, (odds ratio [OR] 0.30 [95% CI 0.08–0.97], p = 0.048). There was no evidence that the CMR rate is associated with CA-9 (p = 0.86), Hif-1α (p = 0.12) or Ki-67 (p = 0.08).

### Biomarkers and survival outcomes

The median follow-up for the 60 patients was 5.2 years (range 1.4–8.0 years). Glut-1 was significantly associated with progression-free survival (PFS) (HR 2.8 [95% CI 1.0–7.9], p = 0.049) and OS (HR 5.0 [95% CI 1.3–19.2], p = 0.011) (Figs. 2 and 3), on multivariable analysis adjusted for possible potential confounding factors namely, MRI volume, FIGO stage, node positivity and uterine corpus invasion (Table 3). On multivariable analysis, the HR of Ki67 (per 10 units increase) for PFS was 1.19 [95% CI 1.01–1.41], p = 0.033. Ki-67 was not significantly associated with OS. Hif-1α and CA-9 were not associated with survival.

### Patterns of failure

The estimated five-year local failure-free rate in all patients was 84% [95% CI (74–94%)], five-year nodal failure-free was 70% [95% CI (59–83%)], and five-year distant failure-free rates were 78% [95% CI (67–89%)]. None of the investigated biomarkers were associated with the risk of local failure, However, Glut-1 was significantly associated with risk of distant failure (HR 14.6 [95% CI 1.9–112.9], p = 0.001).

### Discussion

Glut-1, a glucose transporter protein, has been implicated as a mechanism for increased glycolytic metabolism of tumours. Its overexpression is a poor prognostic marker in a variety of tumours including non-small cell lung [21], colorectal [22], gastric [23] and oral squamous cell carcinoma [24]. With regards to cervical cancer, Airley et al. [25] found negative Glut-1 staining to be significantly associated with increased metastasis-free survival (p = 0.022), after adjustment for the effect of tumour stage, grade and patient age. This is in keeping with our observation of a significant association between high Glut-1 staining and risk of distant disease failure (p = 0.001).

Additionally, we found high Glut-1’s association with inferior progression-free (p = 0.049) and overall survival (p = 0.011). In Airley’s study however, Glut-1 staining was not significantly associated with disease-free survival. The two studies are similar with respect to the patient population of cervical squamous cell carcinoma treated with radiotherapy, although radiosensitising chemotherapy was also used in ours but not Airley’s study; and both had five years follow-up period. However, Airley et al. reported results based on absent versus present Glut-1 staining, whereas we dichotomised the cohort into low and high staining groups of approximately equal

### Table 2
Metabolic response according to CA-9, HIF-1α, Glut-1 and Ki-67 expression levels.

| Biomarker | Group | CMR | Non-CMR | OR (95% CI) | p-Value |
|-----------|-------|-----|---------|-------------|---------|
| CA-9      | Low   | 21 (75%) | 7 (25%) | 1 | 0.86 |
|           | High  | 21 (72%) | 8 (28%) | 0.88 (0.26–2.87) | 0.86 |
| HIF-1α    | Low   | 20 (65%) | 11 (35%) | 1 | 0.12 |
|           | High  | 24 (83%) | 5 (17%) | 2.64 (0.82–9.57) | 0.048 |
| Glut1     | Low   | 26 (84%) | 5 (16%) | 1 | 0.048 |
|           | High  | 17 (61%) | 11 (39%) | 0.30 (0.08–0.97) | 0.811 |
| Ki-67 (-10 units) | Median difference (95% CI) | −1.5 (−3.5 to 0.0) | 0.82 (0.65–1.01) | 0.081 |

CMR: complete metabolic response, OR: odds ratio.

* Negative number represents lower Ki67 on CMR.
case numbers. It may be that low and absent Glut-1 staining have a similar advantage in survival; therefore the association between Glut-1 and survival becomes diluted when examining only presence or absence of staining, as performed by Airley et al.

Post-therapy PET metabolic response has been shown to be a strong predictor of survival following chemoradiation for cervical cancer [5,6,26]. In this study, we have shown pre-treatment Glut-1 staining in cervical tumour biopsy correlates with CMR on PET post chemoradiation ($p = 0.048$). As previously reported, the presence of CMR was significantly associated with superior survival outcome [6]. Patients with a CMR had a 5-year OS of 95% [95% CI (89–100%)], compared with 21% [95% CI (8–57%)] in patients without a CMR ($p < 0.001$) [6]. Use of a pre-treatment biomarker is advantageous in informing treatment planning, and provides an opportunity for risk stratification of therapy.

Tumour hypoxia is recognized as an adverse prognostic factor in cervical cancer [27–29] treated with radiation alone and has been associated with resistance to radiation treatment [30,31]. In the setting of chemoradiation, a study in head and neck squamous cell carcinoma found significantly higher locoregional failure in patients with baseline tumour hypoxia as measured using $^{18}$F-Misonidazole PET [32]. Hypoxia inducible factor 1-alpha (HIF-1$\alpha$) protein levels are increased in response to decreased cellular oxygen concentration [33], and it has been examined as a surrogate for tumour hypoxia. There are studies supportive of HIF-1$\alpha$ as a prognostic factor for cervical cancer treated with radiotherapy; showing high HIF-1$\alpha$ expression to be significantly associated with progression-free [34,35] and cancer-specific survival [35] and risk of distant metastases [34]. Other studies, including ours, did not find HIF-1$\alpha$ expression to be prognostic for survival in similar populations [36,37]. Potential explanations for these differences include tumour heterogeneity in the level of oxygenation and hence HIF-1$\alpha$ expression; and HIF-1$\alpha$'s rapid degradation with restoration of normoxia, making HIF-1$\alpha$ more reflective of acute rather than chronic tumour hypoxia [36]. Oxygen probe studies have found only weak ($r = 0.40$) [37] to moderate ($r = 0.26$) [36] association between tumour HIF-1$\alpha$ expression and oxygenation. Additionally, HIF-1$\alpha$ has a short half-life and is therefore more transiently expressed, compared with CA-9 and Glut-1, resulting in increased potential false-negative results from HIF-1$\alpha$ staining. It is also possible that HIF-1$\alpha$ is up-regulated by factors other than hypoxia [38]. Another observation is that tumour size may modulate the prognostic implication of HIF-1$\alpha$ [36].

High levels of CA-9 expression have been demonstrated to predict for tumour hypoxia in cervical cancer by direct needle probe oxygenation measurements in Longcaster's study [18] studies. They

![Fig. 2. Progression free survival according to Glut-1 expression (histoscore).](image1)

![Fig. 3. Overall survival according to Glut-1 expression (histoscore).](image2)

| Endpoint | Biomarker | Group | 5 years estimate [95% CI] | Univariate analysis HR [95% CI] | p-Value | Multivariate analysis HR [95% CI] | p-Value |
|----------|-----------|-------|--------------------------|-----------------------------|---------|-----------------------------|---------|
| PFS      | CA-9      | Low   | 61 [45–82]               | 1                           | 0.83    | 1                           | 0.80    |
|          |           | High  | 66 [50–85]               | 0.9 [0.4–2.1]               | 0.14    | 0.9 [0.4–2.2]               | 0.17    |
|          | Hif-1$\alpha$ | Low | 52 [37–73]               | 1                           | 0.08    | 1                           | 0.049   |
|          |           | High  | 72 [58–91]               | 0.5 [0.2–1.2]               | 0.16    | 2.8 [1.0–7.9]               | 0.033   |
|          | Glut1     | Low   | 74 [60–91]               | 1                           | 1.12    | 1.19 [1.01–1.41]            | 0.014   |
|          |           | High  | 20 [35–72]               | 2.3 [0.9–5.4]               | 0.46    | 2.1 [1.2–3.7]               | 0.022   |
|          | Ki-67 ($\times 10$) | Continuous | 20 [12–33]               | 1.12 [0.96–1.30]            | 0.16    | 1.19 [1.01–1.41]            | 0.033   |
| OS       | CA-9      | Low   | 82 [69–98]               | 1                           | 0.58    | 1                           | 0.26    |
|          |           | High  | 72 [58–91]               | 1.5 [0.5–4.6]               | 0.61    | 2.0 [0.6–6.4]               | 0.81    |
|          | Hif-1$\alpha$ | Low | 73 [59–91]               | 1                           | 0.01    | 1                           | 0.011   |
|          |           | High  | 79 [66–96]               | 0.7 [0.3–2.1]               | 0.38    | 1.19 [0.98–1.46]            | 0.080   |
|          | Glut1     | Low   | 90 [80–100]              | 1                           | 4.6 [1.3–16.4] | 5.0 [1.3–19.2]               | 0.011   |
|          |           | High  | 60 [44–82]               | 1                           | 1.09 [0.90–1.30] | 0.38 | 1.19 [0.98–1.46] | 0.080 |

* Factors adjusted for included MRI volume, FIGO stage, node positivity, uterine corpus invasion.
found CA-9 expression to be prognostic for overall survival and risk of metastasis. Lee et al. also found CA9 to be associated with poorer disease-free survival, especially nodal spread [39]. In these studies, the prognostic significance of CA-9 has been most strongly demonstrated when comparing outcome between patients with absent versus any CA-9 staining; whereas in our study the comparison was between low and high staining.

Consistent with our results, Hedley et al. [17] did not find a significant association between CA-9 expression and patient survival, whether CA-9 was expressed as a continuous variable or dichotomised at the median. Potential explanations include variability in scoring criteria for staining between studies and intra-tumoural heterogeneity of CA-9 expression, leading to false-negative results when a single tumour biopsy was used per case. Furthermore, CA-9 staining did not correlate with needle probe oxygen (pO2) measurements in Hedley’s study. The authors raised the possibility of CA-9 expression being influenced by other biological factors, rather than being a pure surrogate for presence of tissue hypoxia. Collectively, our findings raise caution on the reliability of CA-9 and HIF-1α as clinical biomarkers for tumour hypoxia in the setting of small tumour biopsies. This is not to dispute tumour hypoxia per se is predictive of chemoradiation response and/or prognostic for survival; but CA-9 and HIF-1α may not be the best or most reliable surrogate markers of the hypoxic state in cervical cancer.

Ki-67 protein expression is regarded as a surrogate for mitoses and proliferation in many tumour types. Its correlation with chemoradiation response and prognostic significance in cervical cancer has been examined by various studies with contrasting findings [40–47]. Several series reported a lack of association between Ki-67 and treatment response or survival [40,41,43,46,47]. In our study, high Ki-67 was associated with worse PFS (p = 0.049) but did not reach statistical significance with OS (p = 0.08), although the hazard ratios were the same for OS and PFS (HR 1.19). Conversely, there are studies which report low tumour expression of Ki-67 was significantly associated poorer survival [42,44,45]. The survival advantage of high Ki-67 tumours was attributed to increased radiosensitivity, as determined using serum squamous cell carcinoma antigen level as a surrogate in the study by Suzuki et al. [45]. However, when we measured radiosensitivity directly through metabolic response on post-therapy PET scan, there was no significant association with Ki-67 level. Potential reasons for discrepancies in the different study findings include inter-observer variability in Ki-67 reporting. For example, Vosnik et al. [46] had median value of Ki-67 staining was 80% (range 30–100%), compared with our study’s median of 40% (range 3–95%). It is also possible intra-tumoural heterogeneity in tumour Ki-67 levels which may not be reflected through testing of a single cervical cancer biopsy specimen.

Limitations of our study include its relatively small sample size which may not allow detection of small differences in patient outcome between different biomarker levels that maybe present. We detected a statistically significant effect of Glut-1 on PFS and OS. However, given the small sample size and number of events, the confidence interval for the hazard ratio is wide, and we cannot estimate with adequate precision the effect size of Glut-1. A larger sample size and number of events are required to more precisely assess the degree of impact of Glut-1 on survival. The results of this retrospective study are hypothesis generating and should be confirmed on a prospective clinical trial of chemoradiation in cervical cancer [48].

In summary, we observed that high Glut-1 expression in pre-treatment cervical cancer biopsies is associated with worse survival and higher distant failure rate in patients undergoing chemoradiation. High Glut-1 was also significantly associated with shorter PFS and lower CMR rate on post-therapy PET. Our findings support Glut-1 as a promising pre-treatment biomarker of metastatic–relapse risk in advanced cervical cancer treated with chemoradiation.

Disclosure/Conflict of interest
Nil to declare by all authors.

Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ctro.2017.01.003.

References
[1] Jemal A, Bray F, Center MM, Ferlay J, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69–90.
[2] Landoni F, Maneo A, Colombo A, et al. Randomised study of radical surgery versus radiotherapy for stage IB–IA cervical cancer. Lancet 1997;350:335–40.
[3] Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010;17:1471–4.
[4] Grigsby PW, Siegel BA, Dehdashti F, Rader J, Zoberi I. Posttherapy [18F] fluordeoxyglucose positron emission tomography in carcinoma of the cervix: response and outcome. J Clin Oncol 2004;22:2167.
[5] Grigsby PW, Siegel BA, Dehdashti F, Mutich DG. Posttherapy surveillance monitoring of cervical cancer by FDG-PET. Int J Radiat Oncol Biol Phys 2003;55:907–13.
[6] Siva S, Herschtal A, Thomas JM, et al. Impact of post-therapy positron emission tomography on prognostic stratification and surveillance after chemoradiotherapy for cervical cancer. Cancer 2011;117:3981–8.
[7] Schwarz JK, Siegel BA, Dehdashti F, Grigsby PW. Metabolic response on post-therapy FDG-PET predicts patterns of failure after radiotherapy for cervical cancer. Int J Radiat Oncol Biol Phys 2012;83:185–90.
[8] Neuhous MG, Fojuncik J, Roosiek F, et al. Prognostic cell biological markers in cervical cancer patients primarily treated with (chemo) radiation: a systematic review. Int J Radiat Oncol Biol Phys 2011;79:325–34.
[9] McShane LM, Altman DG, Sauerbrei W, Taube SA, Ginn M, Clark GM. Reporting recommendations for tumor marker prognostic studies. J Clin Oncol 2005;23:9067–72.
[10] Mac Manus MP, Hicks RJ, Matthews JP, et al. Positron emission tomography is superior to computed tomography scanning for response-assessment after radical radiotherapy or chemoradiotherapy in patients with non-small-cell lung cancer. J Clin Oncol 2003;21:1285–92.
[11] Boddy JL, Fox SB, Han C, et al. The androgen receptor is significantly associated with vascular endothelial growth factor and hypoxia sensing via hypoxia-inducible factors HIF-1α, HIF-2α, and the prolyl hydroxylases in human prostate cancer. Clin Cancer Res 2005;11:7658–63.
[12] Couvelard A, Deschamps L, Rebour S, et al. Overexpression of the oxygen sensors PHD-1, PHD-2, PHD-3, and FIH is associated with tumour aggressiveness in pancreatic endocrine tumors. Clin Cancer Res 2008;14:6634–9.
[13] Soulieux EJ, Hurley H, Tian YM, Pugh CW, Gatter KC, Harris AL. Use of novel monoclonal antibodies to determine the expression and distribution of the hypoxia regulatory factors PHD-1, PHD-2, PHD-3, and FIH in normal and neoplastic human tissues. Histopathology 2005;47:602–10.
[14] Tan EY, Yan M, Campo L, et al. The key hypoxia regulated gene CAIX is upregulated in basal-like breast tumours and is associated with resistance to chemotherapy. Br J Cancer 2009;100:405–11.
[15] Yan M, Rayoo M, Takano EA, Fox SB. BRCA1 tumours correlate with a HIF-1alpha phenotype and have a poor prognosis through modulation of hypoxia enzyme profile expression. Br J Cancer 2009;101:1366–74.
[16] Maques O, Macia A, Moreto S, et al. Immunohistochemical analysis of T-type calcium channels in acquired melanocytic nevi and melanoma. Br J Dermatol 2016.
[17] Hedley D, Pintillie M, Woo JY, et al. Carbonic anhydrase IX expression, hypoxia, and prognosis in patients with uterine cervical carcinomas. Clin Cancer Res 2003;9:5666–74.
[18] Loncaster JA, Harris AI, Davidson SE, et al. Carbonic anhydrase (CA IX) expression, a potential new intrinsic marker of hypoxia: correlations with tumour oxygen measurements and prognosis in locally advanced carcinoma of the cervix. Cancer Res 2001;61:6394–9.
[19] Narayan K, Fisher R, Bernshaw D. Significance of tumor volume and corpus uteri invasion in cervical cancer patients treated by radiotherapy. Int J Gynecol Cancer 2006;16:623–30.
[20] Kim H, Kim W, Lee M, Song E, Loh JJ. Tumor volume and uterine body invasion assessed by MRI for prediction of outcome in cervical carcinoma treated with concurrent chemoradiotherapy. Jpn J Clin Oncol 2007;37:458–66.
[21] Younes M, Brown RW, Stephenson M, Gondo M, Cagle PT. Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. Cancer 1997;80:1046–51.
Kawamura T, Kusakabe T, Sugino T, et al. Expression of glucose transporter-1 (GLUT-1) in human gastric carcinoma: association with tumor aggressiveness, metastasis, and patient survival. Cancer 2001;92:634–41.

Kunelk M, Reichert TE, Benz P, et al. Overexpression of Glut-1 and increased glucose metabolism in tumors are associated with a poor prognosis in patients with oral squamous cell carcinoma. Cancer 2003;97:1013–24.

Airley RE, Loncaster J, Raleigh JA, et al. GLUT-1 and CAIX as intrinsic markers of hypoxia in carcinoma of the cervix: relationship to pimonidazole binding. Int J Cancer 2003;104:85–91.

Grigsby PW, Siegel BA, Dehdashti F, Rader J, Zoberi I. Posttherapy [18F] fluoro-deoxyglucose positron emission tomography in carcinoma of the cervix: response and outcome. J Clin Oncol 2004;22:2167–71.

Hockel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 2003;63:2695–700.

Loncaster JA, Harris AI, Davidson SE, et al. Carbonic anhydrase (CA IX) expression, a potential new intrinsic marker of hypoxia: correlations with tumor oxygen measurements and prognosis in locally advanced carcinoma of the cervix. Cancer Res 2001;61:6394.

Fyles A, Milosevic M, Hedley D, et al. Tumor hypoxia has independent predictor impact only in patients with node-negative cervix cancer. J Clin Oncol 2002;20:680.

Hockel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 1996;56:4509–15.

Fyles AW, Milosevic M, Pintilie M, Hill RP. Cervix cancer oxygenation measured following external radiation therapy. Int J Radiat Oncol Biol Phys 1998;42:751–3.

Rischin D, Hicks RJ, Fisher R, et al. Prognostic significance of [18F]-misonidazole positron emission tomography-detected tumor hypoxia in patients with advanced head and neck cancer randomly assigned to chemoradiation or with without tirapazamine: a substudy of Trans-Tasman Radiation Oncology Group Study 98.02... J Clin Oncol 2006;24:1098–104.

Juang BH, Semenza GL, Bauer C, Marti HH. Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O2 tension. Am J Physiol 1996;271:C1172–80.

Ishikawa H, Sakurai H, Hasegawa M, et al. Expression of hypoxia-inducible factor 1alpha predicts metastasis-free survival after radiation therapy alone in stage IIIIB cervical squamous cell carcinoma. Int J Radiat Oncol Biol Phys 2004;60:513–21.

Bachtieri B, Schindl M, Potter R, et al. Overexpression of hypoxia-inducible factor 1alpha indicates diminished response to radiotherapy and unfavorable prognosis in patients receiving radical radiotherapy for cervical cancer. Clin Cancer Res 2003;9:2234–40.

Hutchinson GJ, Valentine HR, Loncaster JA, et al. Hypoxia-inducible factor 1alpha expression as an intrinsic marker of hypoxia: correlation with tumor oxygen, pimonidazole measurements, and outcome in locally advanced cervical carcinoma. Clin Cancer Res 2004;10:8405–12.

Haugland HK, Vukovic V, Pintilie M, et al. Expression of hypoxia-inducible factor-1alpha in cervical carcinomas: correlation with tumor oxygenation. Int J Radiat Oncol Biol Phys 2002;53:854–61.

Salceda S, Caro J. Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J Biol Chem 1997;272:22642–7.

Lee S, Shin HJ, Han IO, et al. Tumor carbonic anhydrase 9 expression is associated with the presence of lymph node metastases in uterine cervical cancer. Cancer Sci 2007;98:329–33.

Cole DJ, Brown DC, Crossley E, Alcock CJ, Gatter KC. Carcinoma of the cervix uteri: an assessment of the relationship of tumour proliferation to prognosis. Br J Cancer 1992;65:783–5.

Levine EL, Renihan A, Gossiel R, et al. Apoptosis, intrinsic radiosensitivity and prediction of radiotherapy response in cervical carcinoma. Radiother Oncol 1995;37:1–9.

Nakano T, Oka K, Ishikawa A, Morita S. Immunohistochecmical prediction of radiotherapy response and local control in radiotherapy for cervical cancer. Int J Gynecol Pathol 2000;19:120–8.

Oka K, Arai T. MIB1 growth fraction is not related to prognosis in cervical squamous cell carcinoma treated with radiotherapy. Int J Gynecol Pathol 1996;15:23–7.

Pillai MR, Jayaprakash PG, Nair MK. Tumour-proliferative fraction and growth factor expression as markers of tumour response to radiotherapy in cancer of the uterine cervix. J Cancer Res Clin Oncol 1998;124:456–61.

Suzuki M, Tsukagoshi S, Saga Y, Ohtomo K, Sato I. Assessment of proliferation index with MIB-1 as a prognostic factor in radiotherapy for cervical cancer. Gynecol Oncol 2000;79:300–4.

Vosnik M, Laco J, Sirak I, et al. Prognostic significance of human papillomavirus (HPV) status and expression of selected markers (HER2, EGFR, VEGF, CD34, p53 and Ki67/MIB-1) on outcome after chemoradiotherapy in patients with squamous cell carcinoma of the uterine cervix. Pathol Oncol Res 2014;20:131–7.

Yamashita H, Murakami N, Asari T, Okuma K, Nakagawa K, et al. Correlation among six biologic factors (p53, p21(WAF1), MIB-1, EGFR, HER2, and Bcl-2) and clinical outcomes after curative chemoradiation therapy in squamous cell cervical cancer. Int J Radiat Oncol Biol Phys 2009;74:1165–72.

Mackay HJ, Wenzel L, Mileshkin L. Nonsurgical management of cervical cancer: locally advanced, recurrent, and metastatic disease, survivorship, and beyond. Am Soc Clin Oncol Educ Book 2015;35:e299–309.
Author/s: Kanjanapan, Y; Deb, S; Young, RJ; Bressel, M; Mileshkin, L; Rischin, D; Hofman, MS; Narayan, K; Siva, S

Title: Glut-1 expression in small cervical biopsies is prognostic in cervical cancers treated with chemoradiation

Date: 2017-02-01

Citation: Kanjanapan, Y., Deb, S., Young, R. J., Bressel, M., Mileshkin, L., Rischin, D., Hofman, M. S., Narayan, K. & Siva, S. (2017). Glut-1 expression in small cervical biopsies is prognostic in cervical cancers treated with chemoradiation. CLINICAL AND TRANSLATIONAL RADIATION ONCOLOGY, 2, pp.53-58. https://doi.org/10.1016/j.ctro.2017.01.003.

Persistent Link: http://hdl.handle.net/11343/253591

File Description: Published version

License: CC BY-NC-ND