ERCC1 is a prognostic biomarker in locally advanced head and neck cancer: results from a randomised, phase II trial

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Background: Cisplatin-radiotherapy is a preferred standard for locally advanced, head and neck squamous cell carcinoma (HNSCC). However, the cisplatin-attributable survival benefit is small and toxicity substantial. A biomarker of cisplatin resistance could guide treatment selection and spare morbidity. The ERCC1-XPF nuclease is critical to DNA repair pathways resolving cisplatin-induced lesions.

Methods: In a phase II trial, patients with untreated Stage III-IVb HNSCC were randomised to cisplatin-radiotherapy with/without erlotinib. Archived primary tumours were available from 90 of 204 patients for this planned substudy. Semi-quantitative ERCC1 protein expression (H-score) was determined using the FL297, 4F9, and 8F1 antibodies. The primary analysis evaluated the relationship between continuous ERCC1 protein expression and progression-free survival (PFS). Secondary analyses included two pre-specified ERCC1 cutpoints and performance in HPV-associated disease.

Results: Higher ERCC1 expression was associated with inferior PFS, as measured by the specific antibodies FL297 (HR = 2.5, 95% CI = 1.1–5.9, P = 0.03) and 4F9 (HR = 3.0, 95% CI = 1.2–7.8, P = 0.02). Patients with increased vs decreased/normal ERCC1 expression experienced inferior PFS (HR = 4.8 for FL297, P = 0.003; HR = 5.5 for 4F9, P = 0.007). This threshold remained prognostic in HPV-associated disease.

Conclusion: ERCC1-XPF protein expression by the specific FL297 and 4F9 antibodies is prognostic in patients undergoing definitive cisplatin-radiotherapy for HNSCC, irrespective of HPV status.
significantly improved OS, progression-free survival (PFS), and locoregional control (LRC) compared with radiotherapy alone in the sentinel clinical trial, Intergroup 0126 (Adelstein et al, 2003). Similarly, concurrent cisplatin is indicated in the high-risk adjuvant setting (Bernier et al, 2004; Cooper et al, 2004). Although local control and overall survival (OS) are improved with concurrent platinum-based chemoradiotherapy, disappointing local and distant failure rates of 50% and 15%, respectively, coupled with an absolute survival benefit of only 6.5% compared with radiotherapy alone suggest that only a subgroup benefits (Pignon et al, 2009). Given the considerable toxicities of cisplatin including nausea, hearing loss, nephrotoxicity, myelosuppression, and exacerbation of radiation effects such as mucositis and dysphagia (Henk, 1997; Adelstein et al, 2003; Trott et al, 2003), the capacity to pre-select patients who would benefit is paramount. Cisplatin [cis-diaminedichloroplatinum(II)] reacts with DNA to form adducts affecting either a single strand (the monoadduct or intrastrand crosslink) or two strands (the interstrand crosslink or ICL). These DNA lesions are, respectively, repaired by nucleotide excision repair (NER) or the distinct mechanism of ICL repair (Palom et al, 2002). ERCC1-XPF is a bipartite, structure-specific nuclease critical for both NER and ICL repair (De Silva et al, 2000). As such, ERCC1-XPF is the only enzyme required for removal of all cisplatin-induced DNA lesions. ERCC1 and XPF heterodimerise and stabilise each other in vivo; thus, expression levels tightly correlate (Niedernhofer et al, 2006), indicating that either protein may serve as a candidate biomarker for DNA repair capacity following cisplatin exposure.

In 2006, the International Adjuvant Lung Trial (IALT) bio-investigator group retrospectively reported that low vs high tumoural ERCC1 protein expression significantly predicted benefit from adjuvant cisplatin doublet chemotherapy in operable non-small cell lung cancer (NSCLC) (Olaus sen et al, 2006). Robust results from a large randomised phase III trial generated intense interest in further development of ERCC1 as a predictive biomarker for platinum benefit. Initial enthusiasm was tempered by recognition that the antibody used in IALT, 8F1, was not specific for ERCC1 (Niedernhofer et al, 2007). Although 8F1 is able to immunoprecipitate ERCC1-XPF, it also tags a spurious 45 kDa band on immunoblotting (Bhagwat et al, 2009), a cross-reaction that results in ERCC1-XPF-deficient cells being incorrectly termed ERCC1-deficient human fibroblasts as previously described (Niedernhofer et al, 2009). XP2YO cells were used because they have only a trace amount of ERCC1 and are an accepted reagent to assess antibody specificity for ERCC1 (Niedernhofer et al, 2007; Bhagwat et al, 2009). D10 and 8F1 were used as ERCC1-specific and non-specific control antibodies, respectively, as previously characterised (Bhagwat et al, 2009). A single pathologist (MA) blinded to outcomes evaluated all IALT slides sectioned at 4 μ thickness were autostained using standard immunohistochemistry (IHC) protocols on Leica Bond III immunostainers (Leica Microsystems Inc, Buffalo Grove, IL, USA) according to the manufacturer’s operating instructions. Three distinct ERCC1 antibodies were used, including an 8F1 monoclonal antibody (1:400 dilution, Neomarkers, Kalamazoo, MI, USA), a 4F9 monochoncal antibody (1:200 dilution, OriGene, Rockville, MD, USA), and an FL297 polyclonal antibody (1:50 dilution, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). Antigen retrieval was effected by heat-induced epitope retrieval (HER) using Tris-EDTA for 20 min. The slides were then incubated at room temperature for 15 min (8F1 and 4F9) or 60 min (FL297). Four micron-thick sections of normal and neoplastic lung tissue were included as external positive controls. Basal epithelial cells in normal tissue adjacent to each tumour served as an internal positive control. At the time of ERCC1 staining, the ultra-specificity of 4F9 had not yet been reported in the published literature (Ma et al, 2012). Thus, the 4F9 antibody was validated for specificity against ERCC1 by western blot, immunofluorescence, and IHC techniques in normal and XPF-ERCC1-deficient human fibroblasts as previously described (Niedernhofer et al, 2007; Bhagwat et al, 2009). XP2YO cells were used because they have only a trace amount of ERCC1 and are an accepted reagent to assess antibody specificity for ERCC1 (Niedernhofer et al, 2007; Bhagwat et al, 2009). D10 and 8F1 were used as ERCC1-specific and non-specific control antibodies, respectively, as previously characterised (Bhagwat et al, 2009).

ERCC1 evaluation. Pre-cut slides sectioned at 4 μ thickness were autostained using standard immunohistochemistry (IHC) protocols on Leica Bond III immunostainers (Leica Microsystems Inc, Buffalo Grove, IL, USA) according to the manufacturer’s operating instructions. Three distinct ERCC1 antibodies were used, including an 8F1 monoclonal antibody (1:400 dilution, Neomarkers, Kalamazoo, MI, USA), a 4F9 monoclonal antibody (1:200 dilution, OriGene, Rockville, MD, USA), and an FL297 polyclonal antibody (1:50 dilution, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). Antigen retrieval was effected by heat-induced epitope retrieval (HER) using Tris-EDTA for 20 min. The slides were then incubated at room temperature for 15 min (8F1 and 4F9) or 60 min (FL297). Four micron-thick sections of normal and neoplastic lung tissue were included as external positive controls. Basal epithelial cells in normal tissue adjacent to each tumour served as an internal positive control. At the time of ERCC1 staining, the ultra-specificity of 4F9 had not yet been reported in the published literature (Ma et al, 2012). Thus, the 4F9 antibody was validated for specificity against ERCC1 by western blot, immunofluorescence, and IHC techniques in normal and XPF-ERCC1-deficient human fibroblasts as previously described (Niedernhofer et al, 2007; Bhagwat et al, 2009). XP2YO cells were used because they have only a trace amount of ERCC1 and are an accepted reagent to assess antibody specificity for ERCC1 (Niedernhofer et al, 2007; Bhagwat et al, 2009). D10 and 8F1 were used as ERCC1-specific and non-specific control antibodies, respectively, as previously characterised (Bhagwat et al, 2009).
equivalent, and 3+ representing increased. Fifty neoplastic cells from three separate areas of each slide were counted. The percentage of cells staining for ERCC1 (see Figure 1) was assigned a weighted expression score as described (None = 0, 1–9% = 0.1, 10–49% = 0.5, and 50–100% = 1) (Olaussen et al., 2006; Handra-Luca et al., 2007). In tumours with heterogeneous ERCC1 staining, multiple representative areas were scored; staining intensity was assigned as a weighted sum rounded to the nearest quartile. For example, if 60% of cells stained for ERCC1 and the proportion of cells staining 3+ vs 2+ was 70:30, the H-score was calculated as weighted expression (1) × weighted intensity (2.75) = 2.75.

Although previous studies commonly used the H-score median split to define increased vs decreased ERCC1 expression (Handra-Luca et al., 2007; Hao et al., 2011), the use of an internal control allowed pre-definition of three categorical H-scores potentially applicable across study populations: ‘decreased expression’ (H-score ≤ 1.5), ‘normal expression’ (1.5 < H-score < 2.5), and ‘increased expression’ (H-score ≥ 2.5). These categorical thresholds were defined empirically: 1.5 corresponded to the mathematical point where the majority of tumour cells had decreased ERCC1 staining and, similarly, 2.5 corresponded to the mathematical point where the majority of tumour cells had increased staining.

Figure 1. Representative 3+ ERCC1 Staining for FL297, 4F9, and 8F1. Representative ERCC1 stains are presented for consecutive sections of a p16-negative hypopharynx tumour. Note that staining intensity cannot be compared among antibodies as it is referenced to an internal control designated 2+ (arrow in box C). (A) H&E stained invasive squamous cell carcinoma at × 20 magnification. (B) Negative control; tissue shows lack of non-specific ERCC1 staining. (C) 3+ ERCC1 staining for 4F9. (D) 3+ ERCC1 staining for 8F1. (E) 3+ ERCC1 staining for FL297.
p16 evaluation. Overexpression of the p16 cell cycle protein is the accepted surrogate for HPV infection, an established prognostic biomarker in oropharyngeal HNSCC (Ang et al, 2010). To assess p16, pre-cut slides sectioned at 4 μ thickness were auto-stained using standard immunohistochemistry (IHC) protocols on Leica Bond III immunostainers according to the manufacturer’s operating instructions. Antigen retrieval was effected by HIER using Tris-EDTA for 20 min. The slides were then incubated at room temperature for 30 min with an undiluted CINTec p16 antibody (MTM Labs AG, Ventana Medical Systems Inc., Tucson, AZ, USA). Four micron-thick sections of cervical intraepithelial neoplasia II tissue were included as external positive controls.

In accordance with standard grading criteria, p16-positivity was defined as tumours with ≥70% of neoplastic cells demonstrating strong and diffuse nuclear and cytoplasmic staining (Jordan et al, 2012). All available tumours were stained for p16, regardless of anatomic site. However, only p-16-positive oropharynx tumours were classified as HPV-associated.

Statistical analysis. The sample size for this analysis was determined by tissue availability. Assuming an exponential survival function and median PFS of 18 months for patients with higher risk of relapse/death (Ang et al, 2010), a study accruing 24 patients per year for 3.75 years (n = 90), with an additional 6 months of follow-up, would have ~91% power for a two-sided test to detect a hazard ratio of 3.0, if 50%-67% of patients have a lower risk of relapse. Power would be 82% to detect a hazard ratio of 2.5.

The primary statistical analysis evaluated the association between ERCC1 expression and PFS by Cox proportional hazards regression, stratified by randomisation strata and controlling for treatment arm. Formal hypothesis testing was planned only for the specific FL297 and 4F9 antibodies, to limit overall Type I error, with alpha set at 0.05. Although the primary statistical analysis considered ERCC1 H-scores as continuous variables, the three pre-specified ERCC1 expression categories were used for graphical display. Categorical ERCC1 expression also permitted preliminary investigation of two pre-defined cutpoints (decreased vs normal/increased; decreased/normal vs increased), for future investigation of ERCC1 expression as an integral prognostic biomarker. To minimise false discovery, no other cutpoints were tested.

Agreement among ERCC1 expression assays was assessed using Bland–Altman plots and summarised by the concordance correlation coefficient, using the SAS %CCC macro (Barnhart et al, 2002; Crawford et al, 2007). Associations between ERCC1 and p16 expression and other prognostic factors, and their independent or combined associations with PFS, were also explored. A Wilcoxon rank-sum test was used to evaluate ERCC1 expression in association with p16 status. Additional comparisons between categorical variables were conducted using z²-tests of association. Kaplan–Meier curves were used for graphical illustrations of associations between PFS and markers. Statistical tests were two-sided, and analyses were conducted using the SAS/STAT software version 9.3 (SAS Institute, Inc., Cary, NC, USA) and R version 2.15.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Clinical outcomes. In the parent trial, clinical outcomes did not differ according to the treatment arm, including the primary end point of CRR and the secondary end point of PFS (Martins et al, 2013b). The CRR according to standardised protocol criteria was 40% vs 52% on the standard vs experimental arm (P = 0.08). With a median follow-up of 26 months, there was no difference in PFS (HR = 0.9, P = 0.71). The primary biomarker analysis therefore did not control for treatment-by-marker interactions, which were not statistically significant and had little effect on parameter estimates or inference for ERCC1 effects.

Specificity of 4F9. The 4F9 monoclonal antibody was found to be specific for ERCC1. By western blot on XP2YO cell lysates, or by immunofluorescence and IHC on XP2YO cells, 4F9 demonstrated only background signal in the ERCC1-XPF-deficient cells (Figure 2).

ERCC1 expression. Sufficient analysable tissue was submitted from 90 patients consenting to optional tissue correlatives. Table 1 summarises the characteristics of patients with and without available tissue. Tissue assessment did not differ by treatment arm, sex, smoking status, or other demographic characteristics. There was a nonsignificant tendency for tissue to be available for patients with higher T-stage (P = 0.09), and patients classified with oral cavity or overlapping primary site were less likely to have tissue available as compared with pharyngeal or laryngeal sites (P = 0.009).

We evaluated marker prevalence for 8F1 and two specific anti-ERCC1 antibodies, FL297 and 4F9 (Table 2). IHC was performed for all three ERCC1 antibodies in 88 of 90 tumours, limited by tissue availability. The majority of tumours was p16 positive, including 50 oropharyngeal (50/64, 78%) and 6 non-oropharyngeal tumours (6/26, 23%). Approximately half of tumours demonstrated increased ERCC1 expression, relative to internal control, with each antibody. The 4F9 and FL297 assays showed good agreement with a concordance correlation coefficient (CCC) of 0.88 (95% CI 0.82–0.92). The 8F1 antibody demonstrated a higher percentage of tumours with decreased ERCC1 staining and had modest concordance with the 4F9 (CCC = 0.53, 95% CI 0.37–0.66) and FL297 (CCC = 0.44, 95% CI 0.26–0.59) antibodies. Concordance of ERCC1 assays is displayed by Bland–Altman plots in Figure 3.

ERCC1 and PFS. Table 3A presents the primary results from Cox proportional hazards models, examining the association between the three ERCC1 assays and PFS, stratified by randomisation strata and controlling for treatment arm. Higher ERCC1 expression was associated with a greater hazard of progression or death, as measured using the FL297 (HR = 2.5, 95% CI 1.1–5.9) and 4F9 assays (HR = 3.0, 95% CI 1.2–7.8), but not the 8F1 assay (HR = 1.4, 95% CI 0.8–2.5). To graphically illustrate the associations between ERCC1 expression and PFS, Kaplan–Meier plots using three pre-specified expression categories (decreased, normal, increased) are presented in Figure 4.

Exploratory analysis of two pre-specified categorical cutpoints for ERCC1 expression (decreased vs normal/increased or decreased/normal vs increased), as measured by the specific antibodies FL297 and 4F9, indicated that patients with increased tumoural expression were at significantly greater risk for progression or death compared with patients with decreased/normal expression (HR = 4.8 for increased FL297 ERCC1 expression, P = 0.003; HR = 5.5 for increased 4F9 expression, P = 0.007; Table 3B).

ERCC1 and known prognostic factors. Overexpression of the p16 cell cycle protein, a consequence of the degradation of retinoblastoma by the HPV E7 oncoprotein, is indicative of biologically relevant HPV infection and serves as a prognostic biomarker in oropharyngeal HNSCC (Ang et al, 2010). Associations between p16 status and ERCC1 were therefore analysed by the Wilcoxon rank-sum test for FL297 and 4F9 (Figure 5). For both antibodies, there was a significant trend for ERCC1 expression to be higher in p16-negative than in p16-positive tumours, although all categorical expression levels were observed in both. In the 33
p16-negative tumours, the proportion of (decreased/normal/increased) ERCC1 (FL297) expression was 12%, 27%, 61%, compared with 15%, 49%, 36% in 55 p16-positive tumours. Proportions were similar for 4F9. Associations between the two specific ERCC1 antibodies and PFS were not altered substantially by controlling for p16 status (p16-positive oropharyngeal tumour vs other; Table 3C). Data were too sparse to estimate an ERCC1-by-p16 interaction term.

The primary analysis (Table 3A) accounted for N-stage, a known prognostic factor, as part of randomisation strata. Another known prognostic factor, T-stage (T1/2 vs T3/4), did not contribute to predicting PFS in secondary models accounting for ERCC1 assays and randomisation stratum (models not shown).

An exploratory analysis of PFS by ERCC1 restricted to patients with p16-positive oropharyngeal tumours was performed to isolate whether ERCC1 may be prognostic in HPV-associated HNSCC as currently defined. Supplementary Figure 1 suggests that ERCC1 as detected by the specific antibodies FL297 and 4F9 remained significantly prognostic.

Figure 2. The 4F9 antibody is specific for ERCC1. Specificity of 4F9 is assessed in human skin fibroblasts isolated from either a normal individual (WT) or an individual with a mutation in XPF causing near-undetectable ERCC1 (XP2YO). (A) 4F9 is specific by western blot. Only a trace amount of ERCC1 is detected in XP2YO cells with either 4F9 or the specific anti-ERCC1 antibody D-10. In contrast, the non-specific 8F1 antibody recognises an additional band migrating slightly slower than ERCC1, present both in WT and ERCC1 deficient cells (arrows). Tubulin (loading control). (B) 4F9 is specific by immunofluorescence. Only background nuclear signal is observed in ERCC1-deficient cells either with 4F9 (white asterisks) or with the antibody D-10 while nuclear staining is readily observable in WT cells. In contrast, the nuclear signal persists in ERCC1 deficient cells when 8F1 is used, confirming the lack of specificity of this antibody. ERCC1 antibodies (red); DNA stain DAPI (blue). (C) Quantitation of average nuclear fluorescence intensity represented by boxplot; p (paired t-test); * indicates statistical significance. (D) 4F9 is specific by immunohistochemistry performed on formalin-fixed paraffin-embedded cells. Only background staining is observed in ERCC1-deficient cells (black asterisks). 4F9 (brown); haematoxylin counterstain (blue).
In a randomised clinical trial cohort of patients with locally advanced HNSCC treated with high-dose cisplatin-radiotherapy with or without erlotinib, ERCC1 protein expression level assayed by the specific ERCC1 antibodies FL297 and 4F9 was prognostic: patients with higher tumour expression experienced significantly inferior PFS. This relationship was significant both in proportional hazards regression with ERCC1 defined as a continuous variable and when the study population was divided into 'increased' vs 'decreased/normal' expression by a predefined binary cutpoint with p-values.<ref>

The ERCC1-XPF nuclease is the only DNA repair enzyme critical to both NER and ICL repair, thus is an attractive candidate biomarker for cisplatin resistance. The challenge in oncology has been validation of a measurement technique that correlates with DNA repair capacity, applies to available FFPE tissue specimens or peripheral blood, and predicts a relevant clinical outcome. Aspiring methodologies include single-nucleotide polymorphisms (SNPs) in the ERCC1-XPF gene, quantification of tumoural ERCC1 mRNA, and semi-quantitative ERCC1-XPF protein expression by IHC. Although SNPs are appealing due to ease of acquiring germline DNA through peripheral blood, no SNP has emerged as a consistent predictor of HNSCC risk or treatment response (Vaezi et al, 2011). ERCC1 mRNA is measurable with RT–PCR in FFPE specimens as a surrogate for ERCC1-XPF function and demonstrates promise in tailoring platinum chemotherapy in advanced NSCLC (Simon et al, 2012). However, because of post transcription processing, ERCC1 mRNA does not consistently correlate with protein expression(Britten et al, 2000; Zheng et al, 2007), and was not associated with response or survival in HNSCC patients undergoing cisplatin-radiotherapy (Hao et al, 2011). Because of lack of correlation with prognosis in antecedent studies, ERCC1 SNPs and mRNA were not evaluated here.

In HNSCC, the most promising measurement technique for predicting outcome from cisplatin-based therapy has been expression level of ERCC1-XPF protein by semi-quantitative IHC. Immunodetection faces several methodologic challenges including geographic variation in protein expression within a tumour (Taillade et al, 2007) pre-analytic variables such as collection, tissue processing/fixation protocols, and storage (Babic et al, 2010); and rates of interobserver agreement (Taylor and Levenson, 2006). In HNSCC, clinical development of an ERCC1-XPF biomarker also has been impaired by recognition of the non-specificity of 8F1 (Niedernhofer et al, 2007), used in the majority of early retrospective studies, and the inconsistent association of 8F1 with PFS. In the current study, we confirmed that 8F1 was not
prognostic in a randomised study population treated homogeneously with cisplatin-radiotherapy and do not recommend this antibody for further development. This recommendation is in line with the recent contradiction of the original IALT findings in NSCLC with 8F1 (Friboulet et al., 2013).

Another barrier to routine use of semi-quantitative IHC for ERCC1-XPF protein expression is that an optimal cutpoint for clinical classification has not been established. To date, retrospective studies commonly divided their population at the median to compare outcomes for high vs low tumour expression. While an illustrative technique, the median split from one study cohort may not be reproducible or valid in a subsequent cohort. Further challenging the movement of an ERCC1-XPF measurement technique into the integral biomarker setting is validation of a scoring methodology against a standardised control. In the current study, evaluation of diagnostic FFPE tissue posed challenges similar to the routine clinical setting. Patients were treated at eight academic and community centers; variations in collection and processing were assumed to influence ERCC1 immunodetection and to be confounding. For this reason, we developed a standard operating procedure using an internal control. We scored tumour cells with reference to non-neoplastic basal epithelial cells, assuming that pre-analytic variables influencing ERCC1 antigen detection were identical for tumour and non-neoplastic cells within the same specimen. Both specific antibodies were prognostic using this methodology, strengthening confidence in its application; however, external validation in a separate cohort is required.

Table 3. Cox proportional hazard regression for ERCC1 expression by PFS

|                      | ERCC1 (FL297) N = 88 | ERCC1 (4F9) N = 88 | ERCC1 (8F1) N = 90 |
|----------------------|----------------------|---------------------|---------------------|
| A. Continuous ERCC1 expression |                      |                     |                     |
| Hazard ratio (e.g. H-score of 3 vs 2 or 2 vs 1) | 2.5 (1.1–5.9) | 0.03* | 3.0 (1.2–7.8) | 0.02* | 1.4 (0.8–2.5) | 0.21 |
| B. ERCC1 expression categorised by pre-determined H-score cutpoints* |                      |                     |                     |
| Hazard ratio |                      |                     |                     |
| Normal/increased vs decreased | 3.4 (0.4–25.9) | 0.24 | 1.8 (0.4–8.1) | 0.42 | — | — |
| Increased vs normal/decreased | 4.8 (1.7–13.2) | 0.003* | 5.5 (1.6–18.9) | 0.007* | — | — |
| C. Categorised ERCC1, also controls for p16 (p16-positive oropharyngeal tumour vs other)* |                      |                     |                     |
| Hazard ratio |                      |                     |                     |
| Normal/increased vs decreased | 3.4 (0.4–25.5) | 0.24 | 1.9 (0.4–8.3) | 0.40 | — | — |
| Increased vs normal/decreased | 4.6 (1.6–13.2) | 0.004* | 5.2 (1.5–18.3) | 0.01* | — | — |

Models were stratified by randomization strata (including N-stage) and controlled for treatment arm.
*Exploratory analysis, excluding 8F1 antibody based on results of primary analysis (3A).
*Indicates statistical significance.

Figure 3. Bland–Altman plots comparing H-scores for three ERCC1 assays. Mean differences were centered around zero for all assays (solid lines), but 95% limits of agreement (dashed lines) were more narrow for the FL297 and 4F9 antibodies.
Two validated and specific antibodies, FL297 against ERCC1 (Hao et al., 2011) and SPM228 against XPF (Vaezi et al., 2011b), have been associated with PFS in small retrospective cohorts undergoing platinum-radiotherapy. The FL297 antibody is specific for ERCC1 and has been validated by multiple techniques (Bhagwat et al., 2009). However, an interpretive challenge with
FL297 is the variable degree of cytoplasmic staining (so-called ‘cytoplasmic bleed’) occurring in both normal and ERCC1-XPF-deficient cells (Bhagwat et al, 2009). Because scoring must be restricted to the nucleus, the exclusive site of ERCC1-XPF function, background cytoplasmic staining increases the difficulty of interpretation. In this study, we simultaneously evaluated FL297 and the newly available 4F9 clone that demonstrated excellent agreement and were similarly prognostic. However, the 4F9 antibody produced crisp nuclear staining with little to no cytoplasmic bleed. Moreover, 4F9 is a monoclonal antibody, which ensures consistent antigenicity and sustainable production over time. As detailed experiments confirmed that 4F9 is also specific for ERCC1, in line with a recent report of its ultra-specificity (Ma et al, 2012), we recommend 4F9 for further development based upon ease of clinical interpretation.

The primary analysis focused upon proportional hazards regression with ERCC1 expression as a continuous variable. We also explored two pre-specified cutpoints to facilitate future clinical application: decreased/normal vs increased expression and decreased vs normal/increased expression. Of note, the former classification significantly distinguished patients with superior vs inferior PFS, whereas the alternate classification did not. More precisely stated, patients with greater ERCC1 protein expression in tumour cells than adjacent normal basal epithelial cells demonstrated inferior PFS. Of interest, this cutpoint also corresponded closely with the median split, as utilised in retrospective reports (Hao et al, 2011; Vaezi et al, 2011b). This cutpoint is clinically applicable and prognostic with two specific antibodies. External validation and investigation of inter-observer agreement are appropriate next steps.

This study has several limitations. First, although enrolled prospectively onto a randomised trial and treated homogeneously with cisplatin-radiotherapy, tissue was collected optionally and investigation of inter-observer agreement are appropriate next steps.

The current study does not qualify ERCC1-XPF overexpression as a predictive biomarker of cisplatin resistance, as all patients received platinum. Investigating this hypothesis would require a randomised trial with stratification by ERCC1-XPF expression status, comparing cisplatin with a non-platinum alternative – such as docetaxel or cetuximab which radiosensitise through ERCC1-independent mechanisms (Huang et al, 1999; Milas et al, 1999). Such a trial could be justified should our findings, including the candidate cutpoint, be replicated in a separate cohort.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adelstein DJ, Li Y, Adams GL, Wagner Jr. H, Kish JA, Enslow JF, Schuller DE, Forastiere AA (2003) An intergroup phase III comparison of standard radiation therapy and two schedules of concurrent chemoradiotherapy in patients with unresectable squamous cell head and neck cancer. J Clin Oncol 21(1): 92–98.

Ang KK, Harris L, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, Westra WH, Chung CH, Jordan BC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML (2010) Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 363(1): 24–35.

Babic A, Loftin IR, Stanislaw S, Wang M, Miller R, Warren SM, Zhang W, Lau A, Miller M, Wu P, Padilla M, Grogan TM, Pestic-Dragovich L, McElhinny AS (2010) The impact of pre-analytical processing on staining quality for H&E, dual hapten, dual color in situ hybridization and fluorescent in situ hybridization assays. Methods 52(4): 287–300.

Barnhart HX, Haber M, Song I (2002) Overall concordance correlation coefficient for evaluating agreement among multiple observers. Biometrics 58(4): 1020–1027.

Bernier J, Domenge C, Ozaslan M, Matuszewska K, Lefebvre JL, Greiner RH, Giralt J, Maingon P, Rolland F, Bolla M, Cognetti F, Bourhis J, Kirkpatrick A, van Glabbeke M, European Organization for R, Treatment of Cancer T (2004) Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med 350(19): 1945–1952.

Bhagwat NR, Roginskaya VY, Acquafondata MB, Dhir R, Wood RD, Niederhofer LJ (2009) Immuno-detection of DNA repair endonuclease ERCC1-XPF in human tissue. Cancer Res 69(17): 6831–6838.

Britten RA, Liu D, Tessler A, Hutchinson MJ, Murray D (2000) ERCC1 expression as a molecular marker of cisplatin resistance in human cervical tumor cells. Int J Cancer 89(5): 453–457.
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