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Original Article

Hypoxia and angiogenic biomarkers in prostate cancer after external beam radiotherapy (EBRT) alone or combined with high-dose-rate brachytherapy boost (HDR-BTb)

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ABSTRACT

Purpose: To investigate angiogenic and hypoxia biomarkers to predict outcome in patients receiving external beam radiotherapy (EBRT) alone or combined with high-dose-rate brachytherapy boost (HDR-BTb) for localised prostate cancer.

Methods: Prostate biopsy samples were collected prospectively in patients entered into a phase 3 randomised controlled trial of patients receiving EBRT or EBRT + HDR-BTb. Univariate and multivariate analyses using Cox proportional hazards model were performed to identify associations between immunohistochemical staining of hypoxia inducible factor 1 alpha (HIF1α), glucose transporter 1 (GLUT1), osteopontin (OPN) and microvessel density (MVD) using CD-34 antibody with clinical outcome. The primary endpoint was biochemical relapse free survival (BRFS) and secondary endpoint was distant metastasis free survival (DMFS).

Results: Immunohistochemistry was available for 204 patients. Increased OPN (Hazard ratio [HR] 2.38, 95% Confidence Interval [CI] 1.06–5.34, $p < 0.036$) and GLUT1 (HR 2.36, 95%CI 1.39–4.01, $p < 0.001$) expression were predictive of worse BRFS. Increased GLUT1 expression (HR 2.22, 1.02–4.84, $p = 0.045$) was predictive of worse DMFS. Increased MVD (CD-34) (HR 1.82, 95%CI 1.06–3.14, $p = 0.03$) and OPN (HR 1.82, 95%CI 1.06–3.14, $p = 0.03$) but reduced GLUT1 expression (HR 0.40, 95%CI 0.20–0.79, $p = 0.009$) were predictive of improved BRFS in patients receiving EBRT + HDR-BTb.

Conclusion: Our data suggest angiogenic and hypoxia biomarkers may predict outcome and benefit of dose escalation, however further validation in prospective studies including hypoxia modification is needed.

Trial registration number ISRCTN98241100, registered with ISRCTN at http://www.controlled-trials.-com/isrctn/.

Prostate cancer is the most common malignancy affecting men in the UK and remains a significant health problem worldwide. Radical treatment in localised prostate cancer comprises external beam radiotherapy, brachytherapy and radical prostatectomy. Traditionally, patient T-stage, Gleason grade and PSA level have been used to stratify patients into risk groups to indicate the risk of recurrence following treatment [1]. Despite advances in radical treatment for localised prostate cancer, a significant proportion will develop local and/or distant relapse [2,3]. A relationship exists between radioresistance and tumour hypoxia [4,5] and hypoxia is associated with poor tumour local control, tumour relapse and metastases in various cancers [6,7–9,10]. Eppendorf microelectrode measurements have established the presence of significant hypoxia in prostate cancers [11] and a study investigating the presence of hypoxia using pimonidazole found greater hypoxia scores in patients with more aggressive prostate cancer [12]. A study of 57 patients with localised prostate cancer found hypoxic tumours had a significantly worse biochemical relapse free survival (BRFS) at 2 years [9]. Modification of radiotherapy regimens addressing dose delivery and methods of specifically targeting hypoxia may improve tumour control.

Several studies have demonstrated the advantage of dose escalation using conventional fractionation in prostate cancer correlating improved disease control with increasing radiation dose [13–15]. The low alpha/beta ratio of prostate cancer (estimated 1.5–3) [16,17] and high sensitivity to dose per fraction

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allows hypofractionation to achieve dose escalation [18]. Hypofractionation using high dose rate (HDR) brachytherapy has demonstrated good efficacy and low toxicity rates [16]. In a phase 3 trial randomising patients to dose escalation with an HDR brachytherapy boost (HDR-BTb) combined with external beam radiotherapy (EBRT) compared with external beam radiotherapy alone there was a significant improvement in BRFS in the dose escalation arm, which was statistically significant ($p = 0.04$) [18]. The multivariate analysis demonstrated that treatment group, risk category and androgen deprivation therapy (ADT) were significant covariates for risk of relapse.

The use of biomarkers indicative of hypoxia to determine which patients are at high risk of local and distant progression as well as response to radiotherapy would allow a more personalised approach for individual patients. Evidence suggests intrinsic markers of hypoxia and angiogenesis may be important predictors of radiotherapeutic outcome [19]. We hypothesise that increased expression of hypoxia and angiogenesis biomarkers may predict outcome in patients receiving radical radiotherapy and predict the benefit from dose escalation. In this study we investigate a panel of hypoxia and angiogenesis biomarkers as predictors of outcome and the benefit of dose escalation in a phase 3 randomised controlled trial of EBRT alone or EBRT + HDR-BTb for localised prostate cancer.

Methods

Patient population

Patients entered into the phase 3 randomised controlled trial of EBRT alone or EBRT + HDR-BTb for localised prostate cancer were included in this study [18]. The details of this study have been published previously [18]. Eligibility criteria included those with a histological diagnosis of prostate cancer (T1-T3) with no evidence of metastatic disease, PSA < 50 microgram/L, fitness to receive radical radiotherapy and a general anaesthetic. The primary endpoint was BRFS and secondary endpoints were overall survival (OS), acute and late urinary and bowel toxicity and quality of life. Median follow-up was 85 months. 108 patients received conformal EBRT alone (55 Gy in 20 fractions [F]) and 110 patients received EBRT + HDR-BTb (35.75 Gy in 13 fractions followed by an HDR-BTb of 2×8.5 Gy in 24 h).

Immunohistochemistry

Prostate biopsy samples were obtained prospectively on randomisation to the study, predominantly at trans-rectal ultrasound biopsy or at transurethral resection of the prostate. Paraffin blocks were used from which new sections were cut for immunohistochemical staining for hypoxia-inducible factor 1 alpha (HIF1α), glucose-Transporter 1 (GLUT1), Osteopontin (OPN) and microvessel density (MVD) using CD-34 antibody.

In order to validate cut-offs for increased (positive) and decreased (negative) marker expression for HIF1α, GLUT1 and OPN, forty cases of each stained sample were selected at random. Each case was scored for the percentage of uptake of the stain and the scores above the median value were defined as positive and below as negative. Scoring was conducted by two independent scorers and blinded to patient treatment and outcome.

MVD analysis (CD34) was performed using the 'hot-spot' method previously described by Weidner et al [20]. The areas of highest neovascularization were found by scanning the tumour sections at low power (×10). Once the area of highest neovascularisation was identified, individual microvessels were counted on three separate high-powered fields (HPF) (×40). For each specimen, the average vessel counts of the three separate HPF were calculated. The average vessel counts for the whole patient population were dichotomised around the median to give two groups defined as a high and low MVD. The scorer was blinded to patient treatment and outcome.

Statistical analysis

The primary endpoint was BRFS as defined as a rise of 2 μg/L or more above nadir PSA according to the RTOG-ASTRO 'Phoenix' definition. The secondary endpoint was distant metastasis free survival (DMFS).

Univariate and multivariate analyses were performed using Cox regression proportional hazard model. The assumption of proportionality of hazard was checked visually. Patients who had no evidence of biochemical relapse were censored on the date of their last PSA test. Factors tested within the multivariate analysis included age, overall treatment time, use of ADT, T-stage, PSA and risk category, allocation to treatment arm and immunohistochemical markers. Each factor was adjusted for treatment allocation ie EBRT or EBRT + HDR-BTb (and interaction between treatment allocation and predictor). Analyses were carried out using Stata version 13.1 for Windows (StatCorp LP). For multivariate analysis, MVD (CD34), GLUT1, and their interactions with EBRT + HDR-BTb as well as Gleason's grade, HIF1α, OPN, and T-stage were considered. Risk category did not hold proportional hazard assumption (and was not included it in the model even after merging low and intermediate categories). Stepwise forward selection and backward elimination method was used to arrive at the final model, which included PSA, OPN, GLUT1, and interaction between EBRT + HDR-BTb and PSA.

This trial study was performed in compliance with the Declaration of Helsinki and approved by the local research Ethics Committee.

Results

218 patients were entered into the original study and demographic data of these patients have been published previously [21]. There was insufficient tissue to perform all immunohistochemical staining in all patients. Immunohistochemistry for 204 patients was available. There were no significant differences found between the distribution of the biomarkers assessed in the two treatment groups (Table 1).

On univariate analysis of the whole cohort, increased expression of GLUT1 (Hazard Ratio [HR] 1.93, 95%CI 1.18–3.16, $p < 0.009$), HIF1α (HR 1.74, 95%CI 1.05–2.89, $p < 0.031$), OPN (HR 2.83, 95%CI 1.27–6.30, $p < 0.011$), and PSA (HR 1.04, 95%CI 1.02–1.06, $p < 0.0005$) were significant predictors of worse BRFS. Significant predictors for a worse DMFS were increased expression of OPN (HR 7.65, 95%CI 1.03–56.91, $p < 0.047$) and PSA (HR 1.04, 95%CI 1.01–1.07, $p = 0.003$) [See Table 2].

Table 1
Distribution of biomarker expression between treatment groups.

Biomarker*	EBRT alone n = 100	EBRT + HDR-BTb n = 104	Test for equality of proportions p value
HIF1α +ve	28	35	0.38
HIF1α –ve	59	59	0.74
GLUT1 +ve	27	29	0.89
GLUT1 –ve	67	69	0.92
OPN +ve	67	70	0.96
OPN –ve	13	23	0.09
MVD (CD34) +ve	34	41	0.42
MVD (CD34) –ve	40	47	0.45

* +ve refers to positive expression –ve refers to negative expression.

Table 2
Summary of univariate analysis of BRFS and DMFS for variables tested.*

Variable**	BRFS HR (95% CI)	BRFS *p* value	DMFS HR (95% CI)	DMFS *p* value
GLUT1 –ve	1.00		1.00	
GLUT1 +ve	1.93 (1.18,3.16)	0.009	2.09 (0.96,4.54)	0.064
HIF1α –ve	1.00		1.00	
HIF1α +ve	1.74 (1.05,2.89)	0.031	2.03 (0.91,4.52)	0.083
OPN –ve	1.00		1.00	
OPN +ve	2.83 (1.27,6.30)	0.011	7.65 (1.03,56.91)	0.047
T-stage 1	1.00		1.00	
T-stage 2	1.16 (0.65,2.07)	0.612	2.36 (0.77,7.17)	0.132
T-stage 3	1.114 (0.58,2.16)	0.749	2.60 (0.80,8.52)	0.114
MVD (CD34)	0.99 (0.57,1.72)	0.972	0.86 (0.35,2.12)	0.750
PSA	1.04 (1.02,1.06)	0.0005	1.04 (1.01,1.07)	0.003
Gleason's Grade	1.20 (0.94,1.53)	0.138	1.27 (0.87,1.86)	0.222

* Each variable has been adjusted for treatment allocation (and interaction between treatment allocation and variable) HR = hazard ratio, 95%CI = 95% confidence interval.
** +ve refers to positive expression –ve refers to negative expression.

On multivariate analysis, PSA (HR 1.04, 95%CI 1.02–1.06, $p < 0.0001$), increased expression of OPN (HR 2.38, 95%CI 1.06–5.34, $p < 0.036$) and increased GLUT1 expression (HR 2.36, 95%CI 1.39–4.01, $p < 0.001$) were significant predictors of a worse BRFS. Increased expression of GLUT1 (HR 2.22, 1.02–4.84, $p = 0.045$) and PSA (HR 1.04, 95%CI 1.01–1.07, $p = 0.006$) were significant predictors of a worse DMFS. Interaction between dose escalation and PSA levels showed an improved BRFS (HR 0.98, 95%CI 0.95–0.998, $p = 0.031$), i.e. for every ug/L of increase in PSA, those receiving HDR-BTb have a 2.4% risk reduction relative to those who receive standard treatment.

High MVD (CD-34) was predictive for an improved BRFS in patients who received an HDR-BTb (HR 1.82, 95%CI 1.06–3.14, $p = 0.03$) [Fig. 1]. Increased OPN expression was also predictive for improved BRFS in patients who received an HDR-BTb (HR 1.82, 95%CI 1.06–3.14, $p = 0.03$) [Fig. 2]. For patients with a low MVD (CD34) or OPN there was no statistically significant BRFS benefit following the HDR-BTb compared to EBRT alone. Reduced GLUT1 expression was predictive for an improved BRFS in patients receiving an HDR-BTb (HR 0.40, 95%CI 0.20–0.79, $p = 0.009$) [Fig. 3].

Discussion

Our results confirm that hypoxia and angiogenesis biomarkers are related to outcome and may predict a benefit from dose escalation in localised prostate cancer. We demonstrate increased expression of OPN and GLUT1 are independent predictors of a worse BRFS and increased expression of GLUT1 was an independent predictor of worse DMFS in patients treated with radical radiotherapy for localised prostate cancer. High MVD (CD-34), increased OPN and reduced GLUT1 expression were predictive for an improved BRFS in patients receiving dose escalation with an HDR-BTb.

In our study, OPN was found not only to predict outcome, but also a benefit in treatment. Of note there were a larger proportion of patients with higher OPN levels compared with lower OPN levels, however the proportion of patients were balanced between the treatment groups. Increased expression of OPN has been found to predict a shorter time to biochemical failure on univariate but not multivariate analysis in patients receiving radiotherapy for localised prostate cancer in one study [19]. This was not confirmed however in another study which failed to show a relation between plasma OPN levels and response to treatment, a paradoxical increase in OPN was seen in patients starting ADT with radiotherapy and although OPN levels were able to predict the likelihood of metastases it was not superior to serum PSA in defining risk [21]. These apparently conflicting results may reflect heterogeneity of the patient populations, the latter study including patients having surgery, radiotherapy, ADT and chemotherapy; variations in the patient cohorts, different cut-off points in measuring markers and differences between measuring OPN levels in tissue and plasma.

Our data also demonstrated that increased expression of GLUT1 was an independent predictor of a worse BRFS. One previous study also found that GLUT1 correlated significantly with a shorter time to biochemical recurrence after radical prostatectomy and was independent from the Gleason grade and stage of cancer [22]. Increased expression of GLUT1 was also found to be an independent predictor of a worse DMFS in our trial.

With respect to the expression of HIF1α, higher levels predicted worse BRFS on univariate analysis only. This is possibly due to the relatively small numbers. In one previous study, increased HIF1α expression has been shown to independently predict a worse BRFS following radiotherapy for localised prostate cancer [19], but in contrast another study reported that improved BRFS was seen in patients with increased HIF1α expression in intermediate and high-risk prostate cancer patients treated with radiotherapy with or without androgen deprivation therapy [23]. It seems the interaction between HIF1α and other covariates is complex and these differences reflect the heterogeneity of the patient populations.

There is little data on the predictive role of MVD in patients with prostate cancer receiving radiotherapy. One study used Factor VIII related antigen to assess MVD and found an increased MVD was associated with a significantly worse BRFS in localised prostate cancer patients receiving external beam radiotherapy [24]. In contrast we did not demonstrate high MVD (CD34) expression to be a predictor of outcome, and this is in keeping with findings from several other groups [25–28].

Although high MVD did not predict for a worse BRFS overall, it did identify patients who benefitted from dose escalation. Increased OPN expression and reduced GLUT1 expression also predicted for an improved BRFS in patients who received dose escalation. In contrast patients with increased GLUT1 expression had a trend towards a worse BRFS regardless of whether they received dose escalation or not. This suggests that high OPN and high MVD, both reflecting underlying hypoxia may signify mild to moderate hypoxia, which can be overcome with dose escalation. Whereas increased GLUT1 expression may reflect more severe hypoxia, which cannot be overcome with dose escalation and in these tumours hypoxia modification, may be more effective.

Following on from the immunohistochemistry based biomarkers discussed, a 28-gene hypoxia related prognostic signature for localised prostate cancer has recently been developed and independently validated in eleven prostate cancer cohorts [29]. This signature was found to predict biochemical recurrence in patients

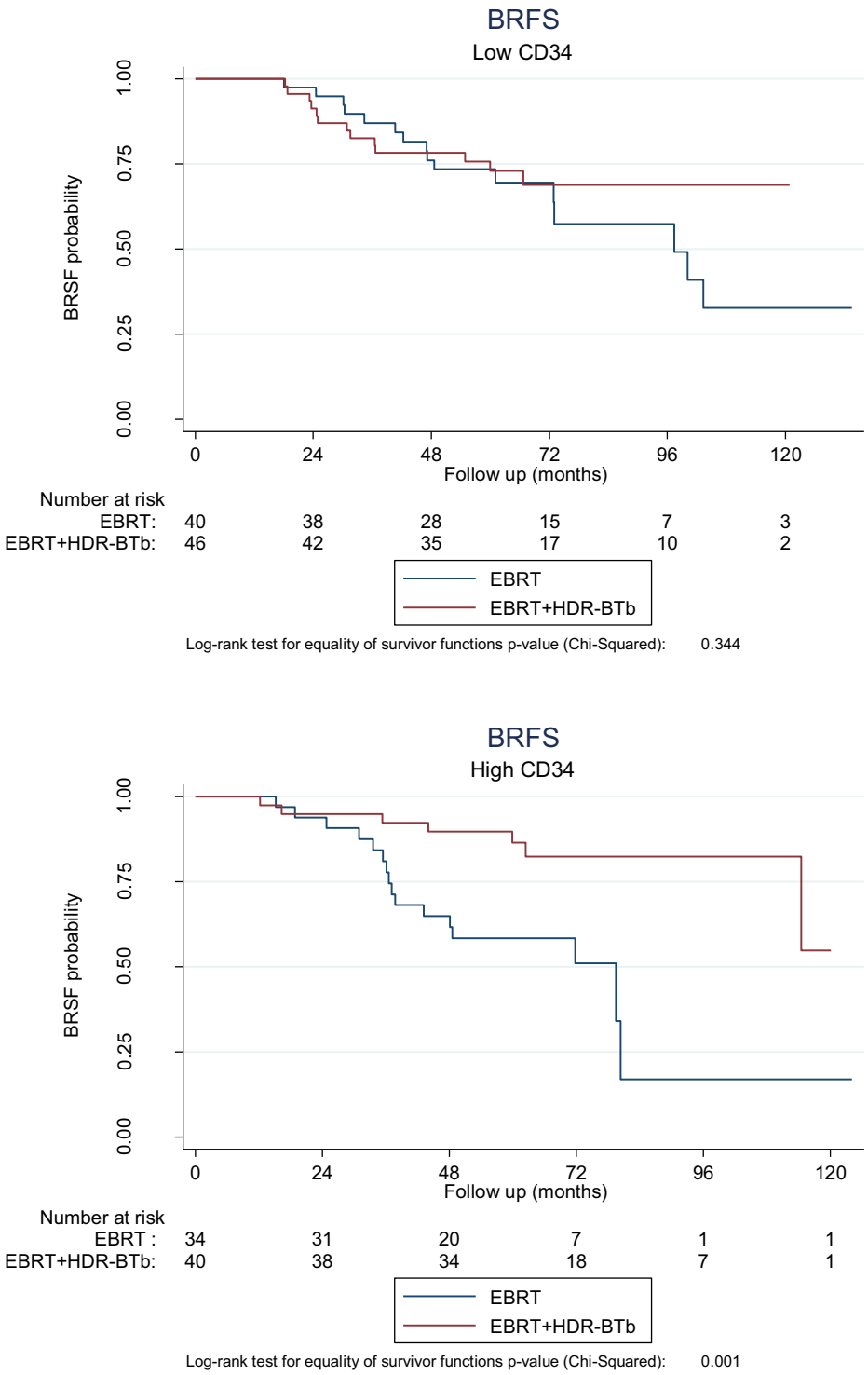

Fig. 1. Increased MVD (CD34) expression as a predictor of improved BRFS in dose escalation.

receiving post-prostatectomy radiotherapy and definitive radiotherapy alone. Furthermore, the signature predicted metastatic events in a pooled cohort. The findings from this study are in keeping with our results that hypoxia is an important predictive factor when treating prostate cancer patients with radiotherapy. Prospective validation of the signature in prostate cancer is awaited.

Hypoxia modification has been less well studied in prostate cancer than in other malignancies despite the demonstration that hypoxia is a consistent feature of these tumours [12,30,31]. Carbogen inhalation has been shown to improve oxygenation in prostate tumour xenografts and in human prostate cancers [32]. The use of hypoxic modification with carbogen and nicotinamide in radical radiotherapy for prostate cancer has been investigated in a phase II study (PROCON) the results of which are awaited. Further prospective validation of OPN, MVD and GLUT1 in a prospective study using hypoxic modification with dose escalated radiotherapy is now required. Hypoxia has been shown to predict not only for local control but also for selection of a more aggressive

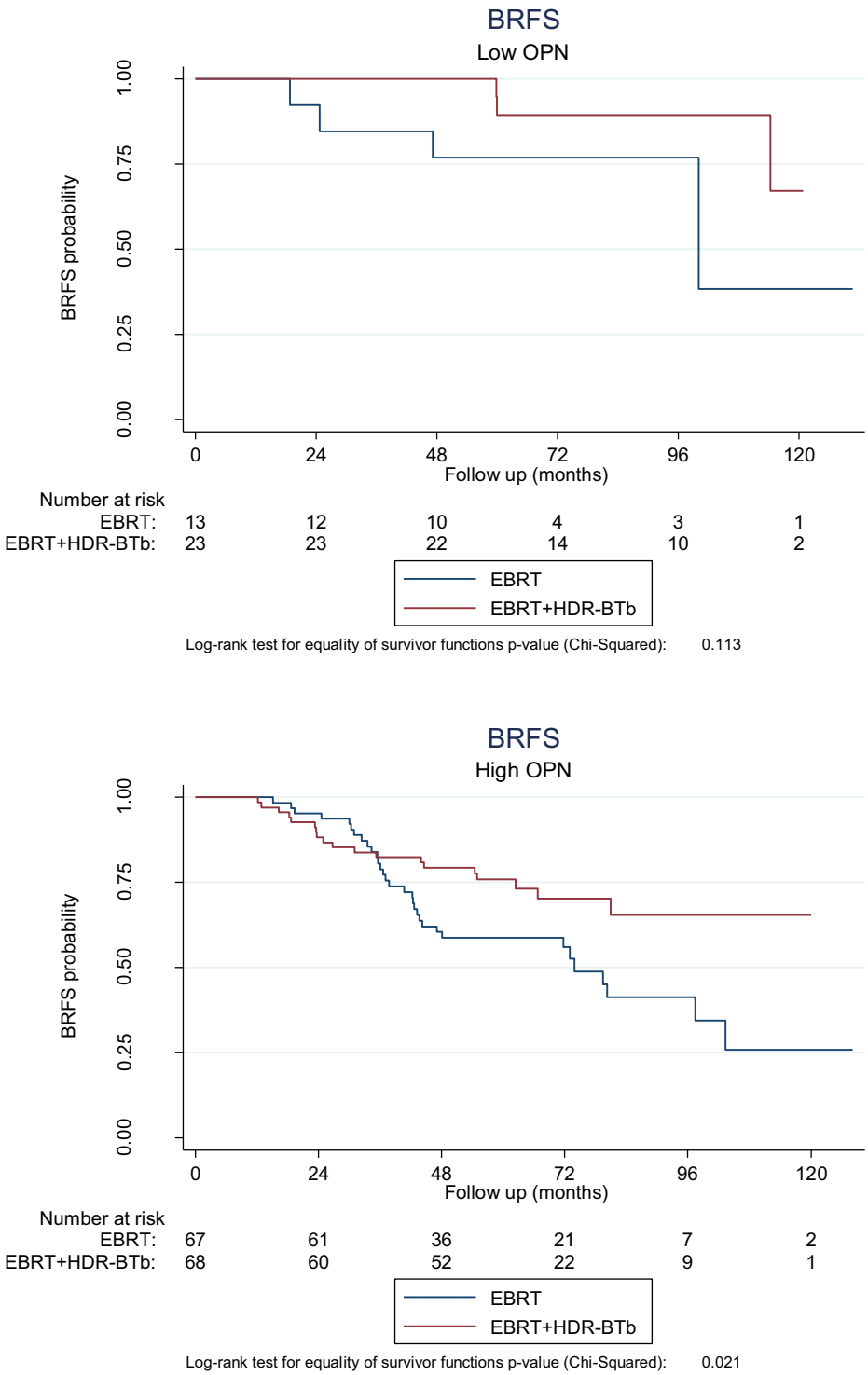

Fig. 2. Increased OPN expression as a predictor of improved BRFS in dose escalation.

phenotype with higher metastatic potential [12]. Increased expression of GLUT1 was found to be a significant predictor of a worse DMFS in this study however only 13% patients had documented metastasis at the time of this analysis.

One of the challenges is identifying hypoxic regions which may be missed when random biopsies are taken. This may be overcome with imaging. One study using 18F-misonidazole (18F-MISO) PET MRI in high-grade prostate cancer patients before and after ADT found 18F-MISO imaging showed variable uptake in prostate cancer associated with a higher Gleason score, lowering significantly after 3 months of ADT in high-grade lesions suggesting a modulation effect with ADT [33].

The main limitation of this study is related to the relatively small patient numbers and the fact that not all markers could be measured for all patients. However its strength lies in the analysis being based on a prospective randomised trial albeit predating the current era of external beam dose escalation and hypofractionation. We acknowledge that the doses delivered in this study would be considered by current standards as insufficient to eradicate prostate cancer. This in turn may have affected the results of the

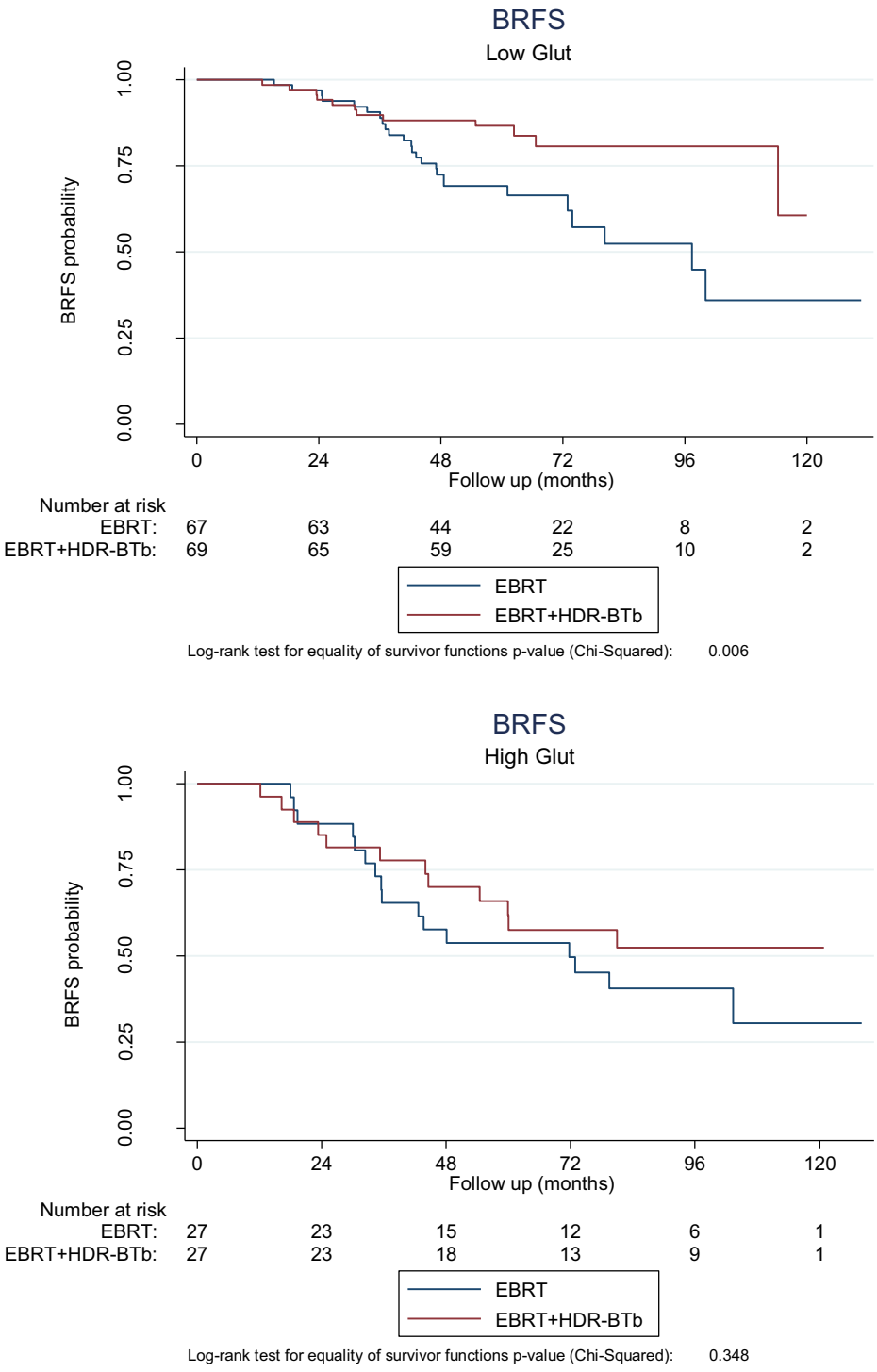

Fig. 3. Reduced GLUT1 expression as a predictor of improved BRFS in dose escalation.

biomarkers and evaluation of these markers in the current hypofractionated schedules such as 60 Gy in 20f or 64.6 Gy in 19f would be helpful.

In conclusion BRFS has been related to OPN, MVD and GLUT1 and DMFS to GLUT1 levels in tumour tissue. Increased levels of OPN and MVD but not GLUT1 predict for an advantage from dose escalation using HDR brachytherapy. We hypothesise that tumours with high levels of GLUT1 may benefit more from hypoxia modification. Further validation of this work in prospective studies including hypoxic modification will be of interest.

Conflicts of interest

No conflicts of interest.

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References

[1] D'Amico AV, Whittington R, Malkowicz SB, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. JAMA 1998;280:969–74.
[2] Crook J, Perry G, Robertson S, Esche B. Radiotherapy for localized prostate cancer: assessment of results by systematic biopsy and PSA. Can J Urol 1996;3:195–201.
[3] Kupelian PA, Elshaikh M, Reddy CA, et al. Comparison of the efficacy of local therapies for localized prostate cancer in the prostate-specific antigen era: a large single-institution experience with radical prostatectomy and external-beam radiotherapy. J Clin Oncol 2002;20:3376–85.
[4] Gray LH, Conger AD, Ebert M, et al. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol 1953;26:638–48.
[5] Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer 1955;9:539–49.
[6] Nordsmark M, Overgaard M, Overgaard J. Pretreatment oxygenation predicts radiation response in advanced squamous cell carcinoma of the head and neck. Radiother Oncol 1996;41:31–9.
[7] Fyles AW, Milosevic M, Wong R, et al. Oxygenation predicts radiation response and survival in patients with cervix cancer. Radiother Oncol 1998;48:149–56.
[8] Brizel DM, Dodge RK, Clough RW, Dewhirst MW. Oxygenation of head and neck cancer: changes during radiotherapy and impact on treatment outcome. Radiother Oncol 1999;53:113–7.
[9] Movsas B, Chapman JD, Hanlon AL, et al. Hypoxic prostate/muscle pO2 ratio predicts for biochemical failure in patients with prostate cancer: preliminary findings. Urology 2002;60:634–9.
[10] Hoskin PJ, Sibtain A, Daley FM, Wilson GD. GLUT1 and CAIX as intrinsic markers of hypoxia in bladder cancer: relationship with vascularity and proliferation as predictors of outcome of ARCON. Br J Cancer 2003;89:1290–7.
[11] Movsas B, Chapman JD, Horwitz EM, et al. Hypoxic regions exist in human prostate carcinoma. Urology 1999;53:11–8.
[12] Carnell DM, Smith RE, Daley FM, et al. An immunohistochemical assessment of hypoxia in prostate carcinoma using pimonidazole: implications for radioresistance. Int J Radiat Oncol Biol Phys 2006;65:91–9.
[13] Dearnaley DP, Jovic G, Syndikus I, et al. Escalated-dose versus control-dose conformal radiotherapy for prostate cancer: long-term results from the MRC RT01 randomised controlled trial. Lancet Oncol 2014;15:464–73.
[14] Al-Mamgani A, van Putten WL, Heemsbergen WD, et al. Update of Dutch multicenter dose-escalation trial of radiotherapy for localized prostate cancer. Int J Radiat Oncol Biol Phys 2008;72:980–8.
[15] Pollack A, Zagars GK, Starkschall G, et al. Prostate cancer radiation dose response: results of the M. D. Anderson phase III randomized trial. Int J Radiat Oncol Biol Phys 2002;53:1097–105.
[16] Hoskin P. High dose rate brachytherapy for prostate cancer. Cancer Radiother 2008;12:512–4.
[17] Bentzen SM, Ritter MA. The alpha/beta ratio for prostate cancer: what is it, really? Radiother Oncol 2005;76:1–3.
[18] Hoskin PJ, Rojas AM, Bownes PJ, et al. Randomised trial of external beam radiotherapy alone or combined with high-dose-rate brachytherapy boost for localised prostate cancer. Radiother Oncol 2012;103:217–22.
[19] Vergis R, Corbishley CM, Norman AR, et al. Intrinsic markers of tumour hypoxia and angiogenesis in localised prostate cancer and outcome of radical treatment: a retrospective analysis of two randomised radiotherapy trials and one surgical cohort study. Lancet Oncol 2008;9:342–51.
[20] Weidner N, Carroll PR, Flax J, et al. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 1993;143:401–9.
[21] Thoms JW, Dal Pra A, Anborgh PH, et al. Plasma osteopontin as a biomarker of prostate cancer aggression: relationship to risk category and treatment response. Br J Cancer 2012;107:840–6.
[22] Jans J, van Dijk JH, van Schelven S, et al. Expression and localization of hypoxia proteins in prostate cancer: prognostic implications after radical prostatectomy. Urology 2010;75:786–92.
[23] Weber DC, Tille JC, Combescure C, et al. The prognostic value of expression of HIF1alpha, EGFR and VEGF-A, in localized prostate cancer for intermediate- and high-risk patients treated with radiation therapy with or without androgen deprivation therapy. Radiat Oncol 2012;7:66.
[24] Hall MC, Troncoso P, Pollack A, et al. Significance of tumor angiogenesis in clinically localized prostate carcinoma treated with external beam radiotherapy. Urology 1994;44:869–75.
[25] Gettman MT, Bergstralh EJ, Blute M, et al. Prediction of patient outcome in pathologic stage T2 adenocarcinoma of the prostate: lack of significance for microvessel density analysis. Urology 1998;51:79–85.
[26] Gettman MT, Pacelli A, Slezak J, et al. Role of microvessel density in predicting recurrence in pathologic Stage T3 prostatic adenocarcinoma. Urology 1999;54:479–85.
[27] Rubin MA, Buyyounouski M, Bagiella E, et al. Microvessel density in prostate cancer: lack of correlation with tumor grade, pathologic stage, and clinical outcome. Urology 1999;53:542–7.
[28] Luczynska E, Gasinska A, Wilk W. Microvessel density and expression of vascular endothelial growth factor in clinically localized prostate cancer. Pol J Pathol 2013;64:33–8.
[29] Yang L, Roberts D, Takhar M, et al. Development and validation of a 28-gene hypoxia-related prognostic signature for localized prostate cancer. EBioMedicine 2018;31:182–9.
[30] Yip K, Alonzi R. Carbogen gas and radiotherapy outcomes in prostate cancer. Ther Adv Urol 2013;5:25–34.
[31] Parker C, Milosevic M, Toi A, et al. Polarographic electrode study of tumor oxygenation in clinically localized prostate cancer. Int J Radiat Oncol Biol Phys 2004;58:750–7.
[32] Alonzi R, Padhani AR, Maxwell RJ, et al. Carbogen breathing increases prostate cancer oxygenation: a translational MRI study in murine xenografts and humans. Br J Cancer 2009;100:644–8.
[33] Mainta IC, Zilli T, Tille JC, et al. The effect of neoadjuvant androgen deprivation therapy on tumor hypoxia in high-grade prostate cancer: an (18)F-MISO PET-MRI study. Int J Radiat Oncol Biol Phys 2018;102:1210–8.