SHORT COMMUNICATION

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IGF-1R associates with adverse outcomes after radical radiotherapy for prostate cancer

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Background: Activated type 1 insulin-like growth factor receptors (IGF-1Rs) undergo internalisation and nuclear translocation, promoting cell survival. We previously reported that IGF-1R inhibition delays DNA damage repair, sensitising prostate cancer cells to ionising radiation. Here we tested the clinical relevance of these findings.

Methods: We assessed associations between IGF-1R and clinical outcomes by immunohistochemistry in diagnostic biopsies of 136 men treated with 55–70 Gy external beam radiotherapy for prostate cancer, comparing results with publicly available transcriptional data in surgically treated patients.

Results: Following radiotherapy, overall recurrence-free survival was shorter in patients whose tumours contained high total, cytoplasmic and internalised (nuclear/cytoplasmic) IGF-1R. High total IGF-1R associated with high primary Gleason grade and risk of metastasis, and cytoplasmic and internalised IGF-1R with biochemical recurrence, which includes patients experiencing local recurrence within the radiation field indicating radioresistance. In multivariate analysis, cytoplasmic, internalised and total IGF-1R were independently associated with risk of overall recurrence, and cytoplasmic IGF-1R was an independent predictor of biochemical recurrence post radiotherapy. Insulin-like growth factor receptors expression did not associate with biochemical recurrence after radical prostatectomy.

Conclusions: These data reveal increased risk of post-radiotherapy recurrence in men whose prostate cancers contain high levels of total or cytoplasmic IGF-1R.

Insulin-like growth factors (IGFs) bind to type 1 IGF receptors (IGF-1Rs) on the surface of most cells, activating signalling effectors, including AKT, to promote normal embryonic and post-natal development (Baker *et al*, 1993). In tumours, IGF-1R signalling mediates proliferation, invasion and cell survival via protection from apoptosis (Chitnis *et al*, 2008). Activated IGF-1Rs undergo internalisation, and are then degraded or recycled back to the plasma membrane (Goh and Sorkin, 2013). We and others reported that some activated internalised IGF-1Rs translocate to the nucleus of human tumour cells, influencing gene expression (Aleksic *et al*, 2010; Sehat *et al*, 2010; Sarfstein *et al*, 2012; Warsito *et al*, 2012).

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The IGF axis is recognised as mediating resistance to anticancer therapies (King *et al*, 2014). The first report linking IGF-1R with radioresistance came from a study of breast cancer patients, where IGF-1R overexpression was associated with early recurrence within the irradiated site (Turner *et al*, 1997). High IGF-1R also associates with radioresistance in cervical cancer (Lloret *et al*, 2007), and we reported that IGF-1R is overexpressed in HPV-negative head and neck cancers that are characterised by resistance to chemo-radiation (Dale *et al*, 2015). Preclinical data support a link with radioresistance: we and others showed that IGF-1R targeting enhances chemo- and radiosensitivity in a range of tumour models *in vitro* and *in vivo,* independent of apoptosis induction (Macaulay *et al*, 2001; Tezuka *et al*, 2001; Cosaceanu *et al*, 2007; Isebaert *et al*, 2011; Riesterer *et al*, 2011). Subsequently, we reported that IGF-1R depletion or inhibition enhances the sensitivity of prostate cancer cells to ionising radiation (IR). This effect was associated with delayed resolution of IR-induced DNA double-strand breaks (DSBs) and inhibition of DSB repair by both non-homologous end-joining and homologous recombination (Turney *et al*, 2012; Chitnis *et al*, 2014). The aim of the current study was to investigate whether these findings have clinical relevance, by assessing IGF-1R expression in prostate cancers of men treated with radical radiotherapy. Our major findings are first that patients whose tumours express high total IGF-1R experience adverse outcomes, including increased risk of overall and metastatic relapse. Second, high internalised IGF-1R associates significantly with increased risk of biochemical relapse. This group includes patients experiencing recurrence within the irradiated field, supporting a link with clinical radioresistance.

MATERIALS AND METHODS

Clinical cases. Clinical data were extracted from records of patients treated with external beam radiotherapy in Oxford from 2000 to 2005, including age, date of diagnosis, presenting PSA, clinical tumour (cT) stage, Gleason grade and Gleason Sum score. Using follow-up data, including serial PSA monitoring, isotope bone scans, CT scans, MRI and PET-CT scans, patients were assigned to one of three groups: long-term remission, metastatic relapse, or biochemical relapse. The latter was defined using the ASTRO-Phoenix Consensus criteria (Roach *et al*, 2006) as $+2\,\mathrm{ng\,ml^{-1}}$ PSA rise above the post-radiotherapy nadir, without evidence of metastatic disease. This study was approved by the National Research Ethics Service (07/H0606/120).

Type 1 IGF receptor immunohistochemistry. Archival diagnostic formalin-fixed paraffin-embedded (FFPE) prostate biopsies were selected for this study. Routinely, left- and right-sided biopsies had been embedded in separate blocks, $\leqslant 6$ biopsies per block. For patients with unilateral tumour, the ipsilateral (i.e., cancer-containing) block was selected for study. For cases with bilateral tumour, the block selected was the most representative of Gleason grade. The chosen tumour-containing blocks were used for IGF-1R immunohistochemistry (IHC) as described (Aleksic *et al*, 2016). For details of method and scoring see Supplementary Information. In brief, IGF-1R signal in $\leqslant 6$ biopsies per case was scored blinded by uro-pathologist CV for intensity (0–3) and percentage positivity (0–4) in the malignant prostatic epithelium. This generated immunoreactive scores (intensity $\times$ percentage, range 0–12) for membrane, cytoplasmic and nuclear IGF-1R. Internalised IGF-1R (0–24) was represented by the sum of cytoplasmic and nuclear IGF-1R, and total IGF-1R (0–36) as the sum of membrane, cytoplasmic and nuclear IGF-1R. Where present, benign prostatic epithelium was also scored for IGF-1R content.

Statistical analysis. Clinical data were analysed using *t*-tests and Wilcoxon matched-pairs signed rank test for non-parametric data. The IGF-1R IHC scores were binarised by the median score and analysed as categorical variables by χ^2-test. Overall survival (OS) was defined as the follow-up time from radiotherapy until death. Patients not reported as dead were censored at their last known date alive. Recurrence-free survival (RFS) was defined as time from radiotherapy until any recurrence (biochemical or metastatic). Recurrence-free survival data are displayed using Kaplan–Meier curves, with the associated log-rank (Mantel–Cox) test. Cox proportional hazards models were fitted for IGF-1R for RFS and OS in both univariate and multivariate analyses. *IGF1R* expression data were extracted from (Taylor *et al*, 2010) and analysed for association with risk of biochemical recurrence by Wald test. Statistical analysis was performed using GraphPad Prism v6, STATA 11.2 (Stata Corporation, College Station, TX, USA) and R Statistical programming environment (v3.2.4, R package: survival v2.38-3. R Core Team: Vienna, Austria). All statistical tests were two-sided, and $P<0.05$ was considered significant.

RESULTS

From a database of $\sim$800 prostate cancer patients treated with radical radiotherapy from 2000 to 2005, we identified 136 with available FFPE tissue (Table 1), and in whom we could ascertain outcomes of remission (75 cases), metastatic recurrence (17) or biochemical recurrence (44) from imaging and serial PSAs. External beam radiotherapy had been 3D conformal CT planned, and treatment typically involved delivering a 55 Gy dose to the planning target volume in 20 fractions over 4 weeks (Table 1). Assuming an α/β ratio for prostate cancer of 1.8 Gy (Dearnaley *et al*, 2016), this dose/fractionation schedule is equivalent to 65.9 Gy in 2 Gy fractions. Radiotherapy was administered to all other patients in fractions of 2 Gy. Of the 136 patients, 57 (42%) had also received adjuvant endocrine therapy, generally for 6–48 months (Table 1). Median follow-up was 7.86 years (range 0.46–12.68 years). To assess the extent to which this cohort is representative, we assessed associations of adverse outcome with the principal prognostic factors: primary Gleason grade, stage, and presenting PSA (Heidenreich *et al*, 2014). Univariate analysis confirmed significant associations between risk of overall recurrence and Gleason grade (primary grade 4 *vs* 3), stage (1 *vs* 2 or 3) and PSA 10–20 *vs* <10, although increased risk in patients with PSA $\geqslant 20$ did not reach significance (Supplementary Table S1). Adjuvant endocrine therapy has been shown to improve disease-free survival and OS following radiotherapy for localised prostate cancer (Kumar *et al*, 2006; Bolla *et al*, 2010), but was associated with increased risk of overall recurrence in our series (Supplementary Figure S1A). This likely reflects the practice during 2000–2005 of offering endocrine therapy only to high-risk patients. Indeed, those receiving endocrine therapy had higher-grade tumours (median primary Gleason grade 4 *vs* 3) and a lower proportion of stage cT1 tumours (15 out of 57, 26% *vs* 30 out of 79, 38%) compared with patients not offered endocrine therapy.

We then evaluated the IGF-1R content of the prostate biopsies, detecting IGF-1R in 109 out of 136 (80.1%) of the cancers with variable subcellular localisation in the plasma membrane, cytoplasm and nuclei (Table 2; Figure 1A). All but 8 biopsies contained benign prostatic epithelium, of which 90 out of 128 (70.3%) contained detectable IGF-1R. Compared with benign glands from the same biopsies, IGF-1R expression was higher in the malignant prostatic epithelium ($P<0.001$; Figure 1B), confirming our previous report of IGF-1R overexpression in primary prostate cancer (Hellawell *et al*, 2002). The expression of IGF-1R did not associate with cT stage or presenting PSA, but higher-grade

Table 1. Demographic data

Median (range)	Patients, *N* (%)
Age	
69 (55–79)	136
PSA	
13.0 (1.3–414)	
Primary Gleason grade	
3	74
4	61
5	1
cT stage	
cT1	45
cT2	58
cT3a	28
Radiotherapy	
55 Gy in 20 fractions	120
64 Gy in 32 fractions	6
68 Gy in 34 fractions	1
70 Gy in 35 fractions	9
Adjuvant endocrine therapy	
Goserelin plus bicalutamide	57
Outcome	
Remission	75
Relapse	61
Metastatic recurrence	17
Biochemical recurrence	44
Death	26
Cancer IGF-1R	
Positive	109 (80.1)
Membrane	77 (56.6)
Cytoplasm	86 (63.2)
Nuclear	37 (27.2)
Internalised	96 (70.6)
Membrane only	13 (9.6)
Negative	27 (19.9)

Abbreviations: cT stage = clinical tumour stage at presentation; IGF-1R = type 1 insulin-like growth factor receptor; PSA = prostate-specific antigen. Demographic data of patients treated with radical radiotherapy for prostate cancer. Adjuvant endocrine therapy was administered concurrently and for median 6 months post radiotherapy; only 4 patients had <6 months endocrine therapy (range 2–48 months). Table also shows number (%) of tumours containing detectable IGF-1R overall and in each subcellular compartment.

Table 2. Association of IGF-1R with clinical factors

	Total IGF-1R		
	IGF-1R ⩽ 8	IGF-1R > 8	*P*-value
Clinical stage			
cT1	31	14	0.260
cT2	37	21	
cT3	14	14	
Primary Gleason grade			
Gleason 3	53	19	0.004
Gleason ⩾4	31	32	
PSA			
0–10	32	14	0.289
10–20	28	19	
>20	19	17	

Abbreviations: cT stage = clinical tumour stage at presentation; IGF-1R = type 1 insulin-like growth factor receptor; PSA = prostate-specific antigen. χ^2-test was used to test for differences in stage, Gleason score and PSA between high and low IGF-1R groups. There was significant association between total IGF-1R and Gleason grade, with higher-grade tumours (primary Gleason grade ⩾4) containing more IGF-1R than tumours with primary Gleason grade 3. There were no significant associations between cT stage or Gleason grade and IGF-1R scores in the plasma membrane, cytoplasm or nucleus, or with internalised (cytoplasmic plus nuclear) IGF-1R.

tumours (primary Gleason grade 4–5) contained significantly more IGF-1R than lower-grade tumours (primary Gleason grade 3, $P = 0.004$; Table 2).

Using Kaplan–Meier and univariate analyses, we tested for associations between clinical outcomes and IGF-1R expression and subcellular localisation. The risk of overall recurrence was significantly greater in men whose prostate cancers contained high total IGF-1R. There were also significant associations between overall recurrence and cytoplasmic or internalised (nuclear plus cytoplasmic) IGF-1R (Table 3; Figure 1C). Analysis of membrane and nuclear IGF-1R separately showed no significant association with outcome (Table 3). Of the 136 patients, 17 (12.5%) developed scan-confirmed metastatic disease, which associated significantly with high total IGF-1R (Table 3; Figure 1D). We also assessed associations with OS, as 26 out of 136 (19%) of the cohort had died, finding borderline association between risk of death and high total IGF-1R ($P = 0.059$; Table 3). Neither cytoplasmic nor internalised IGF-1Rs were associated with metastatic disease or OS (Table 3; Supplementary Figure 1B).

Biochemical recurrence was experienced by 44 (32%) patients, comparable with reported biochemical failure rates of 26–40% for low/intermediate risk disease and up to 70% for high-risk disease following radical external beam radiotherapy (Grossfeld *et al*, 2002; Morgan *et al*, 2007; Gabriele *et al*, 2016; Zargar *et al*, 2017). Biochemical recurrence showed borderline association with total IGF-1R ($P = 0.059$ by univariate analysis, Table 3; $P = 0.055$ by Kaplan–Meier analysis, Supplementary Figure S1C). Analysing IGF-1R scores by subcellular compartment (Table 3; Figure 1E), we identified significant associations between biochemical recurrence and IGF-1R in the cytoplasmic ($P = 0.002$ by univariate analysis and 0.001 by Kaplan–Meier) or internalised (nuclear plus cytoplasmic; $P = 0.034$ and 0.031, respectively) compartments. Patients experiencing biochemical recurrence include those with occult metastases and patients experiencing local recurrence within the radiation field. It is not possible to differentiate these two outcomes with certainty, because routine prostate biopsies are not performed in this situation (Morgan *et al*, 2007), and none of these patients underwent salvage prostatectomy. However, it is estimated that ⩾30% of post-irradiation biochemical recurrences are due to local recurrence, which is an indicator of clinical radioresistance (Bolla *et al*, 2010). In an attempt to further test associations of IGF-1R with local recurrence, we repeated the Kaplan–Meier analyses after excluding patients ($n = 5$) who experienced scan-confirmed metastatic recurrence within 2 years, and were therefore likely to have had occult metastases at the time of radiotherapy. This had no effect on the significance of associations identified between risk of biochemical recurrence and high cytoplasmic ($P = 0.001$, unchanged), internalised ($P = 0.030$ with 5 cases excluded and $P = 0.031$ for all 136 cases) or total IGF-1R ($P = 0.055$, unchanged; Supplementary Figure 1D).

Using multivariate analysis, tumour stage was independently associated with overall and biochemical recurrence (Table 4), as is predictable (Heidenreich *et al*, 2014), although this effect was apparent only when comparing clinical stage cT2 (not cT3) with cT1. Importantly, cytoplasmic, internalised and total IGF-1Rs were independent predictors of overall recurrence. Furthermore, cytoplasmic IGF-1R and stage (cT1 *vs* 2) were the only factors independently associated with the risk of biochemical recurrence (Table 4; Supplementary Table S2). In this cohort, primary Gleason grade (3 *vs* ⩾4) was not independently associated with overall or biochemical recurrence, but was the only factor independently predictive of metastatic recurrence (Table 4; Supplementary Table S2).

Finally, we utilised publically available data to test for associations between IGF-1R and outcome in men with prostate cancer treated by radical prostatectomy. Excluding 37 patients who had received previous radiotherapy, the cohort of Taylor *et al*

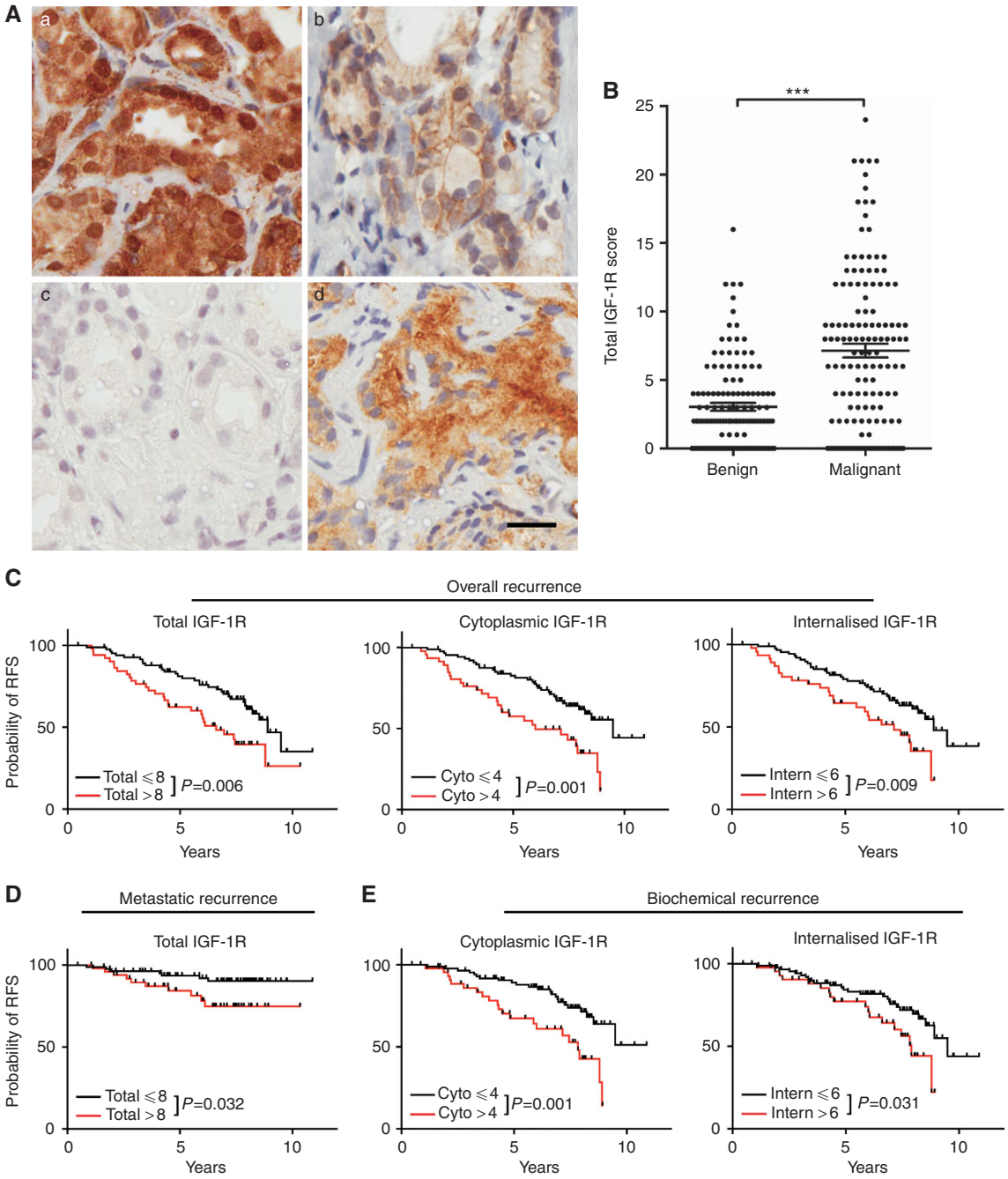

Figure 1. Type 1 IGF receptor associates with adverse outcomes in patients treated with radical radiotherapy for prostate cancer. (**A**) Examples of IGF-1R IHC in diagnostic biopsies of prostate cancer from patients treated with radiotherapy. (a) Gleason 3 + 4 = 7 cancer, IGF-1R score membrane 0, cytoplasm 12, nuclear 9; (b) Gleason 4 + 3 = 7 cancer, IGF-1R score membrane 6, cytoplasm 3, nuclear 0; (c) Gleason 3 + 3 = 6 cancer containing no detectable IGF-1R; (d) Gleason 4 + 5 = 9 cancer, IGF-1R score membrane 0, cytoplasm 12, nuclear 0. Scale bar, 20 μm. (**B**) Total IGF-1R score (bars, mean ± s.e.m.) in benign and malignant areas (***$P = 0.001$ by Wilcoxon matched-pairs signed rank test). (**C**–**E**) Kaplan–Meier plots with log-rank (Mantel–Cox) tests to assess association of total, cytoplasmic (Cyto) and internalised (Intern) IGF-1R with (**C**) all recurrences, (**D**) metastatic recurrence and (**E**) biochemical recurrence.

(2010) included 103 patients with microarray data on *IGF1R* expression, and clinical data on biochemical recurrence. In this surgical data set there was no significant association between *IGF1R* expression and biochemical recurrence (Supplementary Figure S1E). However, as noted previously, we found only borderline association of biochemical recurrence and total IGF-1R in the radiotherapy cohort (Table 3; Supplementary Figure S1C), and we are comparing semi-quantitative IHC scores with microarray analysis of *IGF1R* mRNA. Therefore, it will be important to assess the prognostic significance of IGF-1R at the protein level in a surgically treated cohort, to enable a more robust comparison.

DISCUSSION

The major findings of our study are the identification of significant associations between total IGF-1R and risk of overall and

Table 3. Univariate analysis

RFS: overall recurrence	Comparison	Relapse/total	HR	95% CI	*P*-value
Membrane IGF-1R	⩽1 >1	27/66 34/70	Baseline 1.31	 0.79–2.18	0.291
Cytoplasmic IGF-1R	⩽4 >4	33/89 28/47	Baseline 2.32	 1.39–3.87	0.001
Internalised IGF-1R	⩽6 >6	35/89 26/47	Baseline 1.97	 1.17–3.29	0.010
Nuclear IGF-1R	0 ⩾1	42/99 19/37	Baseline 1.14	 0.67–1.97	0.625
Total IGF-1R	⩽8 >8	32/85 29/51	Baseline 2.02	 1.22–3.36	0.007
RFS: biochemical recurrence					
Cytoplasmic IGF-1R	⩽4 >4	23/89 21/47	Baseline 2.62	 1.44–4.79	0.002
Internalised IGF-1R	⩽6 >6	26/89 18/47	Baseline 1.94	 1.05–3.58	0.034
Total IGF-1R	⩽8 >8	25/85 19/51	Baseline 1.79	 0.98–3.27	0.059
RFS: metastatic recurrence					
Cytoplasmic IGF-1R	⩽4 >4	10/89 7/47	Baseline 1.70	 0.64–4.48	0.284
Internalised IGF-1R	⩽6 >6	9/89 8/47	Baseline 2.03	 0.78–5.26	0.147
Total IGF-1R	⩽8 >8	7/85 10/51	Baseline 2.76	 1.05–7.26	0.040
Overall survival	**Comparison**	**Death/total**	**HR**	**95% CI**	***P*-value**
Cytoplasmic IGF-1R	⩽4 >4	18/89 8/47	Baseline 0.91	 0.39–2.11	0.827
Internalised IGF-1R	⩽6 >6	16/89 10/47	Baseline 1.36	 0.61–3.04	0.448
Total IGF-1R	⩽8 >8	12/85 14/51	Baseline 2.12	 0.97–4.61	0.059

Abbreviations: CI = confidence interval; HR = hazard ratio; IGF-1R = type 1 insulin-like growth factor receptor; RFS = recurrence-free survival. Univariate analysis of associations between IGF-1R and RFS and overall survival post-radiotherapy. Table shows IGF-1R immunoreactive scores, number experiencing recurrence or death/total number, HR for recurrence with 95% CI.

metastatic recurrence, and between cytoplasmic or internalised IGF-1R and biochemical recurrence, following radical radiotherapy but not surgery for prostate cancer. These associations were revealed by univariate and Kaplan–Meier analyses; multivariate analysis also indicated that cytoplasmic IGF-1R is an independent predictor of biochemical recurrence. Most of the patients in our series were treated pre-2006 and received radiotherapy at 55 Gy in 20 fractions, equivalent to 65.9 Gy in 2 Gy fractions. Although this seems a modest dose by current standards, this dose/fractionation schedule was widely used in the United Kingdom during the era in which these patients were treated. It remains to be seen whether IGF-1R retains predictive significance in patients treated at 74 Gy in 37 fractions as is current practice, or the biologically equivalent hypo-fractionated dose of 60 Gy in 3 Gy fractions (Dearnaley *et al*, 2014, 2016). Forty-two percent of patients in our series had received adjuvant endocrine therapy. This is relevant, given known crosstalk between IGF-1R, androgen receptor signalling and the DNA damage response, and reports that anti-androgen therapy enhances prostate cancer radiosensitivity in preclinical models and clinically (Pandini *et al*, 2005; Kumar *et al*, 2006; Bolla *et al*, 2010; Goodwin *et al*, 2013; Polkinghorn and Zelefsky, 2013; Zelefsky *et al*, 2013). In the current study, we found no evidence of better outcomes in the group receiving endocrine therapy, likely because this had been offered only to high-risk patients. It is possible that the use of adjuvant endocrine therapy in a subset of patients could have influenced the associations we found between IGF-1R and clinical outcomes.

In probing the relevance of IGF-1R subcellular localisation, we noted that the association of cytoplasmic or internalised IGF-1R with biochemical recurrence was stronger than for total IGF-1R. Type 1 IGF receptor internalisation is required for prolonged AKT activation (Romanelli *et al*, 2007), and for IGF-1R nuclear translocation, which contributes to IGF axis activity by promoting gene expression (Aleksic *et al*, 2010; Sehat *et al*, 2010; Sarfstein *et al*, 2012; Warsito *et al*, 2012). The lack of significant association with nuclear IGF-1R alone, and the stronger association of cytoplasmic IGF-1R than internalised (nuclear plus cytoplasmic) IGF-1R with overall and biochemical recurrence (Table 3) suggest that cytoplasmic IGF-1R is the major contributor here. Supporting this view, cytoplasmic IGF-1R was an independent predictor of biochemical recurrence in multivariate analysis (Table 4). Therefore, it is plausible that the association of biochemical recurrence with cytoplasmic IGF-1R could reflect increased IGF-1R activation in radioresistant tumours. This would be consistent with data we reported in cell lines, where suppression of IGF-1R activity enhanced radiosensitivity (Turney *et al*, 2012; Chitnis *et al*, 2014). In patients with PSA-detected localised prostate cancer, radiotherapy and surgery are reported to lead to comparable outcomes at median 10 years' follow-up (Hamdy *et al*, 2016). Several predictive biomarkers have been identified to predict outcomes post irradiation for prostate cancer (Wilkins *et al*, 2015), but a role for IGF-1R has not previously been highlighted in this context. Our data suggest that patients whose prostate cancers contain high cytoplasmic or

Table 4. Multivariate analysis

	Comparison	HR	95% CI	*P*-value
RFS: all recurrence				
Cytoplasmic IGF-1R	⩽4	Baseline		0.007
	>4	2.05	1.21–3.45	
Stage	cT1	Baseline		
	cT2	2.61	1.33–5.11	0.005
	cT3	1.84	0.81–4.19	0.145
Primary Gleason grade	3	Baseline		0.108
	⩾4	1.56	0.91–2.68	
RFS: all recurrence				
Internalised IGF-1R	⩽6			0.036
	>6	1.75	1.04–2.97	
Stage	cT1	Baseline		
	cT2	2.76	1.40–5.42	0.003
	cT3	1.90	0.72–4.34	0.130
Primary Gleason grade	3	Baseline		0.155
	⩾4	1.49	0.86–2.56	
RFS: all recurrence				
Total IGF-1R	⩽8	Baseline		0.037
	>8	1.77	1.04–3.02	
Stage	cT1	Baseline		
	cT2	2.88	1.47–5.65	0.002
	cT3	1.87	0.82–4.26	0.136
Primary Gleason grade	3	Baseline		0.315
	⩾4	1.33	0.76–2.34	
RFS: biochemical recurrence				
Cytoplasmic IGF-1R	⩽4	Baseline		0.009
	>4	2.29	1.23–4.26	
Stage	cT1	Baseline		
	cT2	2.62	1.19–5.74	0.016
	cT3	2.01	0.76–5.32	0.160
Primary Gleason grade	3	Baseline		
	⩾4	1.12	0.59–2.13	0.729

Abbreviations: CI = confidence interval; HR = hazard ratio; IGF-1R = type 1 insulin-like growth factor receptor; RFS = recurrence-free survival. Multivariate analysis to identify factors showing independent association with clinical outcomes post radiotherapy.

high total IGF-1R should be offered surgery or dose-escalated radiotherapy, hypotheses that warrant prospective evaluation. Taken together with preclinical data (Isebaert *et al*, 2011; Turney *et al*, 2012; Chitnis *et al*, 2014), these results also support evaluation of IGF axis inhibition as a novel route to radiosensitisation of prostate cancers that express high total or cytoplasmic IGF-1R.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

VMM and TA planned the study; DC treated the patients; RJB, ARW, LB, SL, GSH, FF and AS collected the clinical data; TA and CV performed and scored IHC; CH and VMM analysed IHC data; SH and FMB analysed gene expression data; and VMM wrote the manuscript. All authors approved the final version of the manuscript.

REFERENCES

Aleksic T, Chitnis MM, Perestenko OV, Gao S, Thomas PH, Turner GD, Protheroe AS, Howarth M, Macaulay VM (2010) Type 1 insulin-like growth factor receptor translocates to the nucleus of human tumor cells. *Cancer Res* **70**: 6412–6419.

Aleksic T, Worrall AR, Verrill C, Turley H, Campo L, Macaulay VM (2016) Improved immunohistochemical detection of type 1 insulin-like growth factor receptor in human tumors. *Immunochem Immunopathol* **2**: 114.

Baker J, Liu JP, Robertson EJ, Efstratiadis A (1993) Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* **75**: 73–82.

Bolla M, Van Tienhoven G, Warde P, Dubois JB, Mirimanoff RO, Storme G, Bernier J, Kuten A, Sternberg C, Billiet I, Torecilla JL, Pfeffer R, Cutajar CL, Van Der Kwast T, Collette L (2010) External irradiation with or without long-term androgen suppression for prostate cancer with high metastatic risk: 10-year results of an EORTC randomised study. *Lancet Oncol* **11**: 1066–1073.

Chitnis MM, Lodhia KA, Aleksic T, Gao S, Protheroe AS, Macaulay VM (2014) IGF-1R inhibition enhances radiosensitivity and delays double-strand break repair by both non-homologous end-joining and homologous recombination. *Oncogene* **33**: 5262–5273.

Chitnis MM, Yuen JS, Protheroe AS, Pollak M, Macaulay VM (2008) The type 1 insulin-like growth factor receptor pathway. *Clin Cancer Res* **14**: 6364–6370.

Cosaceanu D, Budiu RA, Carapancea M, Castro J, Lewensohn R, Dricu A (2007) Ionizing radiation activates IGF-1R triggering a cytoprotective signaling by interfering with Ku-DNA binding and by modulating Ku86 expression via a p38 kinase-dependent mechanism. *Oncogene* **26**: 2423–2434.

Dale OT, Aleksic T, Shah KA, Han C, Mehanna H, Rapozo DC, Sheard JD, Goodyear P, Upile NS, Robinson M, Jones TM, Winter S, Macaulay VM (2015) IGF-1R expression is associated with HPV-negative status and adverse survival in head and neck squamous cell cancer. *Carcinogenesis* **36**: 648–655.

Dearnaley D, Syndikus I, Mossop H, Khoo V, Birtle A, Bloomfield D, Graham J, Kirkbride P, Logue J, Malik Z, Money-Kyrle J, O'sullivan JM, Panades M, Parker C, Patterson H, Scrase C, Staffurth J, Stockdale A, Tremlett J, Bidmead M, Mayles H, Naismith O, South C, Gao A, Cruickshank C, Hassan S, Pugh J, Griffin C, Hall E. Investigators, CH (2016) Conventional versus hypofractionated high-dose intensity-modulated radiotherapy for prostate cancer: 5-year outcomes of the randomised, non-inferiority, phase 3 CHHiP trial. *Lancet Oncol* **17**: 1047–1060.

Dearnaley DP, Jovic G, Syndikus I, Khoo V, Cowan RA, Graham JD, Aird EG, Bottomley D, Huddart RA, Jose CC, Matthews JH, Millar JL, Murphy C, Russell JM, Scrase CD, Parmar MK, Sydes MR (2014) Escalated-dose versus control-dose conformal radiotherapy for prostate cancer: long-term results from the MRC RT01 randomised controlled trial. *Lancet Oncol* **15**: 464–473.

Gabriele D, Jereczek-Fossa BA, Krengli M, Garibaldi E, Tessa M, Moro G, Girelli G, Gabriele P, Consortium, E (2016) Beyond D'Amico risk classes for predicting recurrence after external beam radiotherapy for prostate cancer: the Candiolo classifier. *Radiat Oncol* **11**: 23.

Goh LK, Sorkin A (2013) Endocytosis of receptor tyrosine kinases. *Cold Spring Harb Perspect Biol* **5**: a017459.

Goodwin JF, Schiewer MJ, Dean JL, Schrecengost RS, De Leeuw R, Han S, Ma T, Den RB, Dicker AP, Feng FY, Knudsen KE (2013) A hormone-DNA repair circuit governs the response to genotoxic insult. *Cancer Discov* **3**: 1254–1271.

Grossfeld GD, Latini DM, Lubeck DP, Broering JM, Li YP, Mehta SS, Carroll PR (2002) Predicting disease recurrence in intermediate and high-risk patients undergoing radical prostatectomy using percent positive biopsies: results from CaPSURE. *Urology* **59**: 560–565.

Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C, Holding P, Davis M, Peters TJ, Turner EL, Martin RM, Oxley J, Robinson M, Staffurth J, Walsh E, Bollina P, Catto J, Doble A, Doherty A, Gillatt D, Kockelbergh R, Kynaston H, Paul A, Powell P, Prescott S, Rosario DJ, Rowe E, Neal DE, Group, PS (2016) 10-year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. *N Engl J Med* **375**: 1415–1424.

Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, Van Der Kwast T, Mason M, Matveev V, Wiegel T, Zattoni F, Mottet N, European Association Of, U (2014) EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. *Eur Urol* **65**: 124–137.

Hellawell GO, Turner GD, Davies DR, Poulsom R, Brewster SF, Macaulay VM (2002) Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. *Cancer Res* **62**: 2942–2950.

Isebaert SF, Swinnen JV, Mcbride WH, Haustermans KM (2011) Insulin-like growth factor-type 1 receptor inhibitor NVP-AEW541 enhances radiosensitivity of PTEN wild-type but not PTEN-deficient human prostate cancer cells. *Int J Radiat Oncol Biol Phys* **81**: 239–247.

King H, Aleksic T, Haluska P, Macaulay VM (2014) Can we unlock the potential of IGF-1R inhibition in cancer therapy? *Cancer Treat Rev* **40**: 1096–1105.

Kumar S, Shelley M, Harrison C, Coles B, Wilt TJ, Mason MD (2006) Neo-adjuvant and adjuvant hormone therapy for localised and locally advanced prostate cancer. *Cochrane Database Syst Rev*: CD006019.

Lloret M, Lara PC, Bordon E, Pinar B, Rey A, Falcon O, Molano F, Hernandez MA (2007) IGF-1R expression in localized cervical carcinoma patients treated by radiochemotherapy. *Gynecol Oncol* **106**: 8–11.

Macaulay VM, Salisbury AJ, Bohula EA, Playford MP, Smorodinsky NI, Shiloh Y (2001) Downregulation of the type 1 insulin-like growth factor receptor in mouse melanoma cells is associated with enhanced radiosensitivity and impaired activation of Atm kinase. *Oncogene* **20**: 4029–4040.

Morgan PB, Hanlon AL, Horwitz EM, Buyyounouski MK, Uzzo RG, Pollack A (2007) Timing of biochemical failure and distant metastatic disease for low-, intermediate-, and high-risk prostate cancer after radiotherapy. *Cancer* **110**: 68–80.

Pandini G, Mineo R, Frasca F, Roberts Jr CT, Marcelli M, Vigneri R, Belfiore A (2005) Androgens up-regulate the insulin-like growth factor-I receptor in prostate cancer cells. *Cancer Res* **65**: 1849–1857.

Polkinghorn WR, Zelefsky MJ (2013) Improving outcomes in high-risk prostate cancer with radiotherapy. *Rep Pract Oncol Radiother* **18**: 333–337.

Riesterer O, Yang Q, Raju U, Torres M, Molkentine D, Patel N, Valdecanas D, Milas L, Ang KK (2011) Combination of anti-IGF-1R antibody A12 and ionizing radiation in upper respiratory tract cancers. *Int J Radiat Oncol Biol Phys* **79**: 1179–1187.

Roach 3rd M, Hanks G, Thames Jr H, Schellhammer P, Shipley WU, Sokol GH, Sandler H (2006) Defining biochemical failure following radiotherapy with or without hormonal therapy in men with clinically localized prostate cancer: recommendations of the RTOG-ASTRO Phoenix Consensus Conference. *Int J Radiat Oncol Biol Phys* **65**: 965–974.

Romanelli RJ, Lebeau AP, Fulmer CG, Lazzarino DA, Hochberg A, Wood TL (2007) Insulin-like growth factor type-I receptor internalization and recycling mediate the sustained phosphorylation of Akt. *J Biol Chem* **282**: 22513–22524.

Sarfstein R, Pasmanik-Chor M, Yeheskel A, Edry L, Shomron N, Warman N, Wertheimer E, Maor S, Shochat L, Werner H (2012) Insulin-like growth factor-I receptor (IGF-IR) translocates to nucleus and autoregulates IGF-IR gene expression in breast cancer cells. *J Biol Chem* **287**: 2766–2776.

Sehat B, Tofigh A, Lin Y, Trocme E, Liljedahl U, Lagergren J, Larsson O (2010) SUMOylation mediates the nuclear translocation and signaling of the IGF-1 receptor. *Sci Signal* **3**: ra10.

Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Antipin Y, Mitsiades N, Landers T, Dolgalev I, Major JE, Wilson M, Socci ND, Lash AE, Heguy A, Eastham JA, Scher HI, Reuter VE, Scardino PT, Sander C, Sawyers CL, Gerald WL (2010) Integrative genomic profiling of human prostate cancer. *Cancer Cell* **18**: 11–22.

Tezuka M, Watanabe H, Nakamura S, Yu D, Aung W, Sasaki T, Shibuya H, Miura M (2001) Antiapoptotic activity is dispensable for insulin-like growth factor I receptor-mediated clonogenic radioresistance after gamma-irradiation. *Clin Cancer Res* **7**: 3206–3214.

Turner BC, Haffty BG, Narayanan L, Yuan J, Havre PA, Gumbs AA, Kaplan L, Burgaud JL, Carter D, Baserga R, Glazer PM (1997) Insulin-like growth factor-I receptor overexpression mediates cellular radioresistance and local breast cancer recurrence after lumpectomy and radiation. *Cancer Res* **57**: 3079–3083.

Turney BW, Kerr M, Chitnis MM, Lodhia K, Wang Y, Riedemann J, Rochester M, Protheroe AS, Brewster SF, Macaulay VM (2012) Depletion of the type 1 IGF receptor delays repair of radiation-induced DNA double strand breaks. *Radiother Oncol* **103**: 402–409.

Warsito D, Sjostrom S, Andersson S, Larsson O, Sehat B (2012) Nuclear IGF1R is a transcriptional co-activator of LEF1/TCF. *EMBO Rep* **13**: 244–250.

Wilkins A, Dearnaley D, Somaiah N (2015) Genomic and histopathological tissue biomarkers that predict radiotherapy response in localised prostate cancer. *Biomed Res Int* **2015**: 238757.

Zargar H, Lamb AD, Rocco B, Porpiglia F, Liatsikos E, Davis J, Coelho RF, Pow-Sang JM, Patel VR, Murphy DG (2017) Salvage robotic prostatectomy for radio recurrent prostate cancer: technical challenges and outcome analysis. *Minerva Urol Nefrol* **69**: 26–37.

Zelefsky MJ, Gomez DR, Polkinghorn WR, Pei X, Kollmeier M (2013) Biochemical response to androgen deprivation therapy before external beam radiation therapy predicts long-term prostate cancer survival outcomes. *Int J Radiat Oncol Biol Phys* **86**: 529–533.

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