Non-invasive evaluation of tumour hypoxia in the Shionogi tumour model for prostate cancer with $^{18}$F-EF5 and positron emission tomography

Donald T.T. Yap, Janet Woo, Aileen Kartono, Jonathan Sy, Thomas Oliver, Kirsten A. Skov, Cameron J. Koch*, Hans Adomat†, Wiesława H. Dragowska, Ladan Fadzli†, Thomas Ruth‡, Michael J. Adam‡, David Green‡ and Martin Gleave†

The Department of Advanced Therapeutics, BC Cancer Agency, Vancouver, BC, Canada, †The Department of Radiation Oncology, University of Pennsylvania, Philadelphia PA, USA, ‡The Prostate Centre at Vancouver General Hospital, and †Tri-University Meson Facility (TRIUMF), Vancouver, BC, Canada

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OBJECTIVE

To evaluate hypoxia non-invasively in androgen-dependent (AD), regressing (6-days after castration, RG) and androgen-independent (AI) Shionogi tumours, using the radiolabelled tracer for hypoxia, $^{18}$F-EF5, and positron emission tomography (PET).

MATERIALS AND METHODS

Groups of mice bearing AD, RG and AI Shionogi tumours were co-injected with $^{18}$F-EF5 and unlabelled EF5. The mice were imaged non-invasively with PET to examine the accumulation of $^{18}$F-EF5 in hypoxic regions of the tumour. The tumours were subsequently placed in a $\gamma$-counter, or disaggregated for flow cytometry, to determine the levels of $^{18}$F-EF5 and the percentage of hypoxic cells present in the tumour, respectively.

RESULTS

The mean (sd) levels of hypoxia in AD Shionogi tumours decreased significantly 6 days after androgen ablation as measured by flow cytometry, from 17.1 (4.77) to 1.74 (0.46)% (P=0.003). There were no significant differences in the levels of $^{18}$F-EF5 in the tissue between AD and RG tumours using region-of-interest analysis of PET images or $\gamma$-counting, although the differences were significant when measured by flow cytometry. However, mean (sd) levels of hypoxia in AI Shionogi tumours were significantly higher than in AD tumours regardless of the analysis method; PET, 10.5 (4.93) $\times 10^{4}$ Bq/cm$^{2}$ (P=0.017), flow cytometry, 42.98 (3.35)% (P<0.001), well count, 6.81 (1.17) $\times 10^{4}$ and 13.1 (1.99) $\times 10^{4}$ cpm/g, for AD and AI tumours, respectively (P<0.001).

CONCLUSIONS

Differences in hypoxia between AD and AI, but not RG, Shionogi tumours can be detected non-invasively with $^{18}$F-EF5 and PET. As prostate tumours are hypoxic and the oxygen levels can change with androgen ablation, noninvasive imaging of hypoxia with PET and $^{18}$F-EF5 might ultimately have a prognostic and/or diagnostic role in the clinical management of the disease.

KEYWORDS

prostate cancer, Shionogi tumours, androgen ablation, hypoxia, $^{18}$F-EF5, positron emission tomography

INTRODUCTION

Tumour hypoxia is a common characteristic of solid tumours, and results from dysfunctional vascular networks that do not deliver adequate supplies of oxygen, rapidly metabolizing or dividing cells that consume more oxygen than can be supplied, or more frequently a combination of both [1]. Hypoxic cells in a solid tumour have been known for decades to be resistant to radiotherapy [2]; the damage induced by ionizing radiation is often reversed in the absence of oxygen and so is less effective in hypoxic regions of tumours [3]. The effectiveness of chemotherapy is also modulated by the presence of hypoxic cells [3]; hypoxic cells often occur in areas that are beyond the diffusion limit of drugs from the nearest blood vessel, leading to suboptimal drug doses required for cell death. Moreover, chronically hypoxic cells have slower growth cycles and so might be less affected by standard cytotoxins, which are most effective when cells are actively proliferating [3]. The presence of resistant hypoxic cells will thus ultimately dominate the overall response of a tumour, even when the normoxic cell populations are killed. However, and potentially more important, tumour hypoxia is thought to exert a selective pressure that favours tumour progression [4], metastasis [5] and a poor clinical outcome [6].

Hypoxia in human prostate tumours has been detected using oxygen microelectrodes during surgery [7,8], and less invasively using positron-emission tomography (PET) with the tracer $^{18}$F-misonidazole [9]. The levels of hypoxia in prostate tumours were highly variable [9], although higher levels of hypoxia were associated with increasing disease stage [10].

These reports suggest that tumour hypoxia is an additional factor that should be considered in the management of prostate cancer, particularly as radiotherapy is a common treatment, used alone or in combination with androgen-deprivation therapy (ADT) [11–14].
Reducing tumour volume before radiotherapy by neoadjuvant, cytoreductive, androgen suppression has the potential to improve the therapeutic ratio, by decreasing the number of cancer cells requiring sterilization (improving local control) and the volume of normal tissue exposed to radiation, thereby decreasing complications. Furthermore, there might be synergistic interaction between hormonal therapy and radiation, as ADT is known to enhance tumour cell death through apoptosis [15]; thus ADT applied before the delivery of radiation substantially decreases the radiation dose required to control half of transplanted Shionogi tumours, compared to the dose required when the ADT is instituted after radiation [16]. In the clinic, recurrence rates are unacceptably high with radiation monotherapy, but several clinical studies have shown improved therapeutic benefits when radiation is combined with hormone therapy [11–13]. Indeed, combined hormone and radiation treatment is now considered standard therapy for managing patients with high-risk prostate cancer. However, several questions remain about the optimum timing (neoadjuvant vs adjuvant), total duration of therapy, and selection of appropriate patients (high-risk only vs all patients) for external beam radiation therapy.

More recent studies on hypoxia in the Shionogi tumour model showed that the extent of hypoxia in these tumours, measured with the hypoxia probe EF5 and flow cytometry [17], or with 18F-MRI [18], varied depending on the stage of disease progression and androgen dependence; levels of hypoxia in androgen-dependent (AD) tumours, regressing (after castration, RG) and androgen-independent (AI) tumours were variable, reduced and significantly increased, respectively [17,18].

Human LNCaP prostate tumours treated with the antiandrogen bicalutamide to block the androgen receptor have also been shown to up-regulate the expression of genes related to hypoxia [19], further supporting the idea that androgen ablation might increase hypoxia in androgen-deprived tumours. The intrinsic presence of hypoxia, or possible changes in hypoxic levels in prostate tumours during ADT, is of obvious clinical relevance for radiation treatment. More important perhaps, monitoring the levels of hypoxia in prostate tumours might also have prognostic or predictive value, as hypoxia is increasingly considered an important factor associated with tumour progression, disease outcome and metastatic potential in prostate cancer [20], and other tumour sites [21]. It might thus be important to assess the extent of hypoxia in prostate tumours during the clinical management of the disease, and to confirm whether ADT has a similar effect on hypoxic levels in human prostate tumours.

Our group has previously used unlabelled EF5 to examine hypoxia in tumour models for breast [22] and prostate cancer [17], because of its potential for measuring hypoxia non-invasively with PET using the labelled analogue [23]. We now report for the first time the use of 18F-labelled and unlabelled forms of EF5 to detect in situ tumour hypoxia non-invasively (with 18F-EF5 and PET) in the same Shionogi tumour when still AD, RG (6 days after bilateral orchidectomy), and regrowing in an AI manner. The PET results were correlated with the levels of radioactivity due to reduced 18F-EF5 adducts present in tumour tissue, and with the percentage of hypoxic cells containing unlabelled EF5 adducts detected using flow cytometry.

**MATERIALS AND METHODS**

Animal studies were conducted with the full approval of the University of British Columbia’s Animal Care Committee, operating under the auspices of the Canadian Animal Care Committee. The Toronto subline of the transplatable Shionogi SC-115 AD mouse mammary carcinoma was used in these studies [24]. The cell line was maintained by passage in mice; ≈5 × 106 cells were injected s.c. into adult male DD/S mice (25 g). When the Shionogi tumours reached 1–2 cm in diameter (usually 2–3 weeks after injection), orchidectomy was performed through the scrotal route under isoflurane anaesthesia. Groups of tumours were subsequently imaged with 18F-EF5 and PET at three distinct stages, i.e. before castration while still AD, while RG, and as tumours re-grew ≈4 weeks after castration when AI.

Tumour hypoxia was measured using the pentfluorinated analogue of the 2-nitroimidazole etanidazole (EF5), and the Cy5 tagged monoclonal antibody ELK5-31 specific for EF5 adducts in hypoxic cells. Unlabelled EF5 and the antibody were provided by the Koch group at the University of Pennsylvania, and used as previously reported [17,22]. Briefly, EF5 is a 2-nitroimidazole that is reduced by nitroreductases in viable cells, undergoes further reductions and binds to proteins present in hypoxic cells, but not when there is oxygen present [25]. Therefore only viable hypoxic cells are detected with this agent. The 18F-labelled analogue of EF5 was synthesized using previously published methods [23]; briefly 18F-fluorine gas was added across the double bond of the allyl precursor to EF5 in trifluoroacetic acid at 0 °C. The trifluoroacetic acid was then removed by evaporation under vacuum, and the crude product purified by HPLC before being made up in saline for injection.

Small-animal PET is a powerful tool for in vivo imaging of biological functions, with exquisite sensitivity; tracer concentrations as low as 10−4 to 10−10 M of radioisotopes are detectable. Most PET markers are small molecules or antibodies, labelled with a positron-emitting isotope (e.g. 18F, 11C, 68Cu), that bind selectively to the molecular target of interest in the biological system. The location of the radiotracer is subsequently identified by simultaneous detection of paired γ rays (511 keV) that originate from positron annihilation. Once the acquired data are corrected for physical effects and instrumentation-related inaccuracies, they are reconstructed into images that preserve proportionality between radioactive counts and tracer concentration. Thus the spatial and intensity distribution of the tracer can be determined non-invasively (within the limits of the scanner resolution). PET data can also be acquired in short consecutive intervals (dynamic scanning) to provide temporal information on the radiotracer distribution used to evaluate uptake rates of the tracer in the target of interest. The CTI microPET Focus120 scanner (Concorde Microsystems Inc., Knoxville, TN, USA) was used in these studies [26], and has a resolution of ≈1.3 mm, a radial field of view of 10 cm, an axial field of view of 7.6 cm and an absolute system sensitivity of 6.5%. The scanner acquires data in ‘list mode’, eliminating the need for predefined time sampling. Acquired data are corrected for detector dead-time, detection non-uniformity, attenuation, scatter in the object and acquisition of random events. Analytical (filtered back-projection) or statistical reconstruction algorithms (Fourier re-binning, followed by general or three-dimensional ordered subset expectation maximization) are available on this scanner. An independently acquired calibration scan provides the conversion factors from image
counts to radiotracer concentration, thus converting the images into quantitative, physical units. $^{18}$F-EF5 (2.220 MBq) was co-injected i.v. with unlabelled EF5 (30 mg/kg) dissolved in saline into tumour-bearing mice via the lateral tail vein. At 3 h after the injection, pairs of mice were anaesthetized (isofluorane, 5% induction, 1.5% maintenance), positioned side by side on the scanning platform and imaged for 30 min (non-dynamic imaging). A transmission scan using an external source ($^{60}$Co) to compensate for the body mass of the mice was taken after the scan. Immediately after scanning the mice were killed (CO$_2$, asphyxiation), the tumour harvested, weighed and divided into two portions. One portion was placed into a well-counter to determine the levels of activity (due to reduced $^{18}$F-EF5 in hypoxic cells) present per gram of tissue, and the second portion disaggregated for subsequent analysis with flow cytometry.

Three-dimensional PET images showing the location and levels of reduced $^{18}$F-EF5 in the mouse were reconstructed and viewed in transverse, sagittal and coronal projections (Fig. 1a). Regions of interest (ROI) on each tumour were drawn on three adjacent planes using appropriate software; the activity corresponding to levels of $^{18}$F-EF5 present within each ROI was normalized to the area defined by the ROI and subsequently averaged to determine the levels of $^{18}$F-EF5 present per unit area of tissue. The data thus obtained reflect the activity present within the tumour 3 h after injection.

Representative sagittal images for mice within each group are shown in Fig. 1b, indicating the levels of $^{18}$F-EF5 present in the tissue. Data for the time-activity curves (Fig. 1c) were obtained similarly except that the mice (two per tumour group) were scanned for 3 h from the time of injection to obtain the temporal distribution of the tracer.

For flow cytometry, briefly, the tumour was minced with scalpels while on ice, and subsequently enzymatically digested using trypsin (25 mg/mL), DNase (10 mg/mL), collagenase (4 mg/mL) and protease (S. griseus, Sigma Chem Co., St Louis, MO, USA; 25 mg/mL). After a series of washes, cells were fixed in 4% paraformaldehyde and blocked overnight to reduce nonspecific binding of ELK3–51, then resuspended with 50 µg/mL monoclonal Cy5-tagged ELK3–51. Samples containing $>$1 x 10$^5$ cells/mL were divided into two portions for staining with the antibody or carrier alone. The immunostained cell suspension was then stored in 1% paraformaldehyde until analysis by flow cytometry (Facsalibur, Becton-Dickson, Franklin Lakes, NJ, USA). Debris, characterized by low forward- and side-scatter, was gated out on the dot-plot distributions. Shionogi cells previously grown in culture and incubated with EF5 (200 µM, 3 h, serum-free medium) in air or a nitrogen atmosphere were fixed and stained with tumour samples to determine the Cy5 fluorescence thresholds for hypoxic and non-hypoxic cell populations. Cells from disaggregated tumours treated in this manner with fluorescence values above the thresholds, defined on the basis of hypoxic and non-hypoxic cells, were considered hypoxic and reported as a percentage of total viable tumour cells present in the suspension.

RESULTS

The values for individual tumours and group means for changes in the hypoxic levels of Shionogi tumours determined by flow cytometry before and after androgen ablation are shown in Figure 2a. The percentage of hypoxic cells in AD tumours was variable, at 3–20%; the overall mean, calculated from all 18 AD tumours was 14.2 (4.75)%). However, the number of hypoxic cells in the tumours...
FIG. 2. Shionogi tumours become more hypoxic after androgen ablation and re-grow as AI tumours. Flow cytometry analysis of tumour hypoxia in AD, RG and AI tumours is shown in (a). Empty triangles represent the percentage of viable hypoxic tumour cells for single tumours; the mean for each group is shown with the dash; the error bars indicate the SD associated with each set of results. Tumour hypoxia in RG tumours declined rapidly after interrupting androgen supplies. AI tumours were significantly more hypoxic than AD tumours (P < 0.001). The relationship between tumour size and hypoxia is shown for AD and AI tumours in (b) (empty and black squares, respectively). There was no significant correlation between tumour size and the percentage of hypoxic cells present (b).

FIG. 3. Determining tumour hypoxia in AD and AI Shionogi tumours with flow cytometry. The results from ROIs and using a γ-counter were comparable. The relative levels of tumour hypoxia in AD, RG and AI tumours were evaluated by disaggregating tumours and analysing cell suspensions with flow cytometry, by analysing ROIs in the tumour and by placing the excised tumour into a γ-counter after PET. In this series of experiments, only tumours that were scanned with PET were analysed; in a typical experiment, the tumour was excised after the PET scan and placed in a γ-counter before subsequent processing for flow cytometry analysis. Thus data from each of the analytical methods were derived from the same tumour within each of the AD, RG and AI tumour groups. The levels of tumour hypoxia in AD, RG and AI tumours derived from flow cytometry are shown in (a), b and c, respectively. Significant differences (P ≤ 0.005) between groups are indicated with a star. The number of AD, RG and AI tumours analysed in this way was 12, five and eight, respectively.

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<th>Time after androgen ablation, days</th>
<th>% Hypoxic cells (flow cytometry)</th>
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<td>-5</td>
<td>50</td>
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The results for ROIs and γ-counters were (a) AD, RG and AI tumours with flow cytometry. The percentage of hypoxic cells present (b) is shown with the dash; the error bars indicate the SD associated with each set of results. Tumour hypoxia in RG tumours declined rapidly after interrupting androgen supplies. AI tumours were significantly more hypoxic than AD tumours (P < 0.001). The relationship between tumour size and hypoxia is shown for AD and AI tumours in (b) (empty and black squares, respectively). There was no significant correlation between tumour size and the percentage of hypoxic cells present (b).

Activity in the tumours from 18F-EF5 show as turquoise-green areas within the tumour. On the basis of the colour scale used in these images, the activity present in AI tumours, which has a central green region within the turquoise (indicated by the red arrow), is more intense than in AD tumours, while RG tumours have variable amounts of activity present in the tumour (the image for this particular RG tumour shows low levels of activity; others had higher levels). The biological distribution of 18F-EF5 within AD, RG and AI tumours over 180 min is shown in Fig. 1c; the results indicate that the pattern of uptake in AD and AI tumours was similar, but that more 18F-EF5, normalized per unit area, accumulated in the set of AI tumours. However, the temporal distribution of 18F-EF5 in RG tumours was strikingly different and appeared to lag behind both AD and AI tumours; the final level of activity within the RG tumours was higher than in AD tumours.

Differences in the hypoxic levels of AD, RG and AI Shionogi tumours determined using EF5 and flow cytometry are shown in Fig. 3a; the cell suspensions used for flow cytometry in the three groups (12, 5 and 8, respectively) were derived from tumours that were...
scanned with $^{18}$F-EF5 and PET on the same day. AI tumours were significantly more hypoxic than AD tumours, at 42.98 (3.35)% and 17.11 (4.77)% ($P < 0.001$), whereas RG tumours were significantly less hypoxic than either AD or AI tumours, at 1.74 (0.46)% ($P = 0.003$ and $<0.001$) when comparing AD vs RG, and AI vs RG, respectively. AD and RG tumours had similar levels of $^{18}$F-EF5 when analysed using ROI analysis, at 5.72 (2.45) $\times 10^{-5}$ and 6.34 (2.12) $\times 10^{-5}$ Bq/cm$^2$, respectively (Fig. 3b) or using a γ-counter, at 6.81 (1.17) $\times 10^4$ and 6.34 (1.20) $\times 10^4$ cpm/g, respectively (Fig. 3c). Using ROI analysis, AI tumours, at 10.5 (4.93) $\times 10^{-5}$ Bq/cm$^2$, had significantly more $^{18}$F-EF5 than AD tumours ($P = 0.017$) but not when compared with RG tumours ($P = 0.176$). The activity present in AI tumours, measured using a γ-counter, at 13.1 (1.99) $\times 10^4$ was significantly higher than the activity present in AD and RG tumours ($P < 0.001$, AD vs AI; and $P = 0.006$, RG vs AI). The ratio of hypoxic cells between AI and AD tumours calculated from mean values for hypoxia determined with flow cytometry, ROIs and the γ-counter were 2.51, 1.83 and 1.66, respectively.

**DISCUSSION**

The Shionogi tumour model [27] has been used extensively to examine the effects of androgen ablation in prostate cancer. Shionogi tumours are strongly AD and regress quickly after androgen ablation, but most tumours re-grow independently of androgen, mimicking the behaviour of human prostate tumours after ADT. This model has also been used to examine the role of proteins implicated in cell survival after androgen ablation [24], and the effects of molecular factors such as claudin [28] and IGF-1–binding proteins [29], tumour size and timing of androgen ablation [30] on rates of AI progression in prostate cancer. Shionogi tumours are thus a well-established and suitable model for examining the effects of androgen ablation on tumour hypoxia.

The data in Fig. 2a show that the hypoxic levels in AD tumours were highly variable compared to RG and AI tumours; the spread of values in AD tumours was about twice that of AI tumours ($\approx$30% and $\approx$15%, respectively). However, after androgen ablation hypoxia levels decreased quickly, reaching a nadir at $\approx$6 days. Disrupting androgen supplies in Shionogi tumours is known to be a catastrophic event, resulting in increased apoptosis [29] and a large fraction of dead or dying cells, thus it is not surprising that tumour hypoxia decreases as the tumour’s overall oxygen requirements are decreased. However, the present results (Fig. 2a) indicate that after re-growth, AI tumours are significantly more hypoxic than AD tumours when measured with flow cytometry and EF5 ($P < 0.001$); similar conclusions were reached in a study where pO$_2$ levels were measured non-invasively in Shionogi tumours using $^{18}$F-MRI [18]. There were no significant correlations between tumour weight (used as a less subjective measure of tumour volume) and hypoxic levels in the present AD and AI tumours (Fig. 2b) and in other studies [18].

The classical model of hypoxia is based on tumour growth outstripping the development of the tumour vasculature, and thus a larger tumour mass would be expected to be more hypoxic; however, tumour hypoxia can also be the result of impaired vasculature or increased cellular oxygen consumption [1]. The reasons behind the observed, significantly higher levels of hypoxia in AI Shionogi tumours are unclear at present; studies with contrast-enhanced dynamic MRI indicate that blood flow in Shionogi tumours increases significantly when they become AI [31], suggesting that the delivery of oxygen supplies and/or functional vasculature is perhaps not a factor. Further studies to examine the role of tumour vasculature and metabolism are needed to resolve this question. Regardless of the reasons underlying the hypoxic levels in AI tumours, if these physiological changes also occur in human prostate tumours, then the need to monitor hypoxia in prostate cancer is acute if the benefits of radiotherapy, when combined with ADT, are to be optimized, as radiotherapy would be most effective when the tumour is well-oxygenated.

Various methods for detecting hypoxia in solid tumours and single cells [32] have been used, including polarographic O$_2$ microelectrodes in prostate tumours [7], the COMET assay [33], binding of reducible agents such as those containing metals [34], or preferential reduction of nitroimidazoles in hypoxic cells [35,36]. However, these techniques are highly invasive, as tumour biopsies or, in the case of microelectrodes, direct access to the tumour is required. Thus, there is great interest in the use of less invasive methods such as PET for evaluating in situ tumour hypoxia. PET tracers specific for hypoxia and under development, e.g. $^{18}$F-misonidazole, IAZAG, $^{18}$F-EF5 and $^{18}$Cu-ATSM are reviewed extensively in [37]. We chose to use $^{18}$F-EF5 in the present study to complement our previous work using the unlabelled form of the compound [17], and because with EF5 it is possible to examine hypoxia non-invasively [23] and with immunohistochemical methods [17,22] in the same tumour.

The transverse, coronal and sagittal views of a Shionogi tumour grown on the upper back of a mouse and imaged with PET and $^{18}$F-EF5 are shown in Fig. 1a; virtual slices through the tumour can be made to examine the distribution of the tracer through the tissue in the three projections. Representative images of Shionogi tumours at three stages (AD, RG and AI) are shown in Fig. 1b; the intensity of the colour scale applied to the images is proportional to the levels of $^{18}$F-EF5 present 3 h after injection. The uptake of $^{18}$F-EF5 in AD, RG and AI tumours over 3 h are also shown in Fig. 1b. The accumulation of $^{18}$F-EF5 in AD and AI tumours were similar except that overall levels of the tracer were significantly higher in AI tumours, but the uptake in RG tumours appeared to lag behind that in AD and AI tumours. The uptake of $^{18}$F-EF5 in AD and AI tumours reach a plateau around 1 h whereas in RG tumours, tracer accumulation appears to plateau only after 3 h. The delayed uptake of $^{18}$F-EF5 in RG tumours was probably due to the overall reduced viability of the tumour; regressing tumours are highly necrotic and undergo extensive reorganization of their vasculature, which would affect the rates at which the $^{18}$F-EF5 is delivered and removed in the tumour compared to AD and AI tumours. Thus, a direct comparison of the uptake of $^{18}$F-EF5 between RG and AD and AI tumours using similar scan parameters is unlikely to yield useful information at present.

A visual examination of the PET images indicates that the hypoxia levels were highest, and more evenly distributed, in AI than RG and AD tumours. The red arrow in the AI tumour shown in Fig. 1b indicates a green area within the tumour that has higher activity than the surrounding turquoise region. The high levels in the gut area (red) are possibly due to degradation of the compound under acidic conditions [23]; unfortunately,
In prostate cancer, oxygen levels measured and outcome [6] and metastatic potential [5]. Associations between low tissue oxygenation and the presence of reduced γ-counter readings that reflect the presence of reduced hypoxia increase with clinical stage [10], and the prostate/muscle hypoxia predicts biochemical failure after radiotherapy [7].

The relevance of hypoxia in radiotherapy and some forms of chemotherapy are well known [3]; moreover, its role as a predictive marker in cancer is becoming clearer, with the observed associations between low tissue oxygenation and outcome [6] and metastatic potential [5]. In prostate cancer, oxygen levels measured using pO2 electrodes show that levels of hypoxia increase with clinical stage [10], and that the prostate/muscle hypoxia predicts biochemical failure after radiotherapy [7].

Results from clinical trials evaluating combined ADT and radiotherapy imply that the duration and/or timing of androgen ablation with respect to radiation might also be important [11–14]. However, at present it remains unclear if hypoxia is a factor in these studies, as the trials were not designed to examine hypoxic levels in the tumours.

The presence of hypoxia in human prostate tumours is thus of immediate clinical relevance in the management of the disease with radiotherapy, particularly if ADT induces changes in hypoxic levels, as seen in animal models [17,20,38], and by inference, possibly in human trials [11,14]. Given also that the levels of tumour hypoxia are associated with disease stage, aggressiveness and increased metastatic potential in several solid tumour sites, including prostate [20], and that ADT in human prostate tumours might affect hypoxic levels, the ability to monitor changes and extent of hypoxia in prostate tumours would not only help to optimize the scheduling of ADT (neoadjuvant, or adjuvant) when used with radiotherapy, but potentially be used to assess tumour hypoxia as a prognostic marker in prostate cancer.

In conclusion, the present results show that the significantly different levels of hypoxia in AD and AI Shionogi tumours can be examined non-invasively using 18F-EF5 and PET. At present the discrepancy between direct and non-invasive measurements of hypoxia in RG tumours precludes the use of 18F-EF5 and PET to monitor changes in tumour hypoxia immediately after androgen ablation. Further studies examining how the uptake of 18F-EF5 in RG tumours is affected by changes in the tumour microenvironment, induced immediately by androgen ablation, are underway. However, the potential for using PET and 18F-EF5 to monitor changes in the same tumour before androgen ablation and when the tumour re-grows in an AI manner are very promising. As PET is a minimally invasive technique, is already used in the clinic, and 18F-EF5 is currently in human trials (Hahn et al. NCT00110032, personal communication) the opportunity is available to confirm the implied relationship between androgen ablation, or to non-invasively assess tumour hypoxia as an independent prognostic marker in prostate cancer over time.

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**CONFLICT OF INTEREST**

Cameron J. Koch is the inventor of EF5. Source of funding: Prostate Cancer Research Foundation of Canada.

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Correspondence: Donald T.T. Yapp, Advanced Therapeutics, BC Cancer Agency, 675 W 10th Ave, Vancouver, BC, V5Z 1L3, Canada. e-mail: dyapp@bccrc.ca

Abbreviations: PET, positron-emission tomography; ADT, androgen-deprivation therapy; AD(I), androgen-dependent (-independent); RG, regressing; ROI, region of interest.