



## Short Communication

## Reoxygenation during radiotherapy in intermediate-risk prostate cancer

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## ARTICLE INFO

## Article history:

Received 29 August 2018

Received in revised form 19 December 2018

Accepted 21 December 2018

Available online 12 January 2019

## ABSTRACT

Hypoxia is a major risk factor of prostate cancer radioresistance. We evaluated hypoxia non-invasively, using <sup>18</sup>F-Misonidazole PET/CT prior to radiotherapy and after a dose of 20 Gy in intermediate-risk prostate cancer patients. Decreased hypoxic volumes were observed in all patients, suggesting that radiotherapy induces early prostate tumor reoxygenation.

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## Keywords:

Dose painting radiotherapy

Radioresistance

Misonidazole

FAZA

IMRT

Hypoxia is a major factor of resistance to radiotherapy because it selects radioresistant mutant cells and reduced levels of oxygen decrease the amount of reactive oxygen species induced by irradiation [1]. In prostate cancer, hypoxic markers based on direct invasive measurement of oxygen levels [2,3] or hypoxia-related protein or gene expression [4,5] were shown to predict tumor aggressiveness and recurrence following irradiation. Because sampling some prostate areas using biopsies may not reflect the whole extent of hypoxia within the prostate, functional imaging of hypoxic regions may better reflect the intraprostatic heterogeneity of hypoxic regions. In two recent series of prostate cancer patients, FMISO detected a hypoxic signal in 33% to 63% of patients [6,7], confirming results of previous smaller series [8,9]. We showed that FMISO-positive volumes were partly located both in cancerous areas and in the normal gland [6]. FMISO may therefore represent a potent non-invasive tool to map hypoxia within the whole prostate.

Evaluating hypoxia using only one single image may be insufficient since hypoxia is a dynamic process and is influenced by treatment. Preclinical prostate cancer models [10] and clinical data in other tumors [11] suggest that reoxygenation frequently occurs during fractionated radiotherapy. To increase the dose to hypoxic

areas in the context of a dose-painting strategy, chronic and stable hypoxia may imply the use of a simultaneous-integrated boost strategy, while intermittent or decreasing hypoxia may imply boosting hypoxic regions prior to fractionated radiotherapy [9,12]. It is therefore important to evaluate hypoxia before and during the course of radiotherapy.

We hypothesized that FMISO could be used to evaluate radiotherapy-induced reoxygenation during the course of radiotherapy in intermediate-risk prostate cancer patients.

## Patients and methods

## Patient selection and treatment

The study was approved by the local Ethics Committee and registered in the NCI database (NCT01898065). Inclusion criteria were NCCN-defined intermediate-risk prostate cancer patients in whom high-dose radiotherapy to the prostate without hormone therapy was indicated. Our goal was to evaluate the influence of radiotherapy on prostate cancer hypoxia. Since prostate hypoxia is affected by hormone therapy (HT), we excluded high-risk prostate cancer patients who are routinely treated with RT + HT [13]. We also excluded low-risk tumors because the need for improved radiotherapy is not striking since most patients are now on active surveillance instead of radical treatment and the tumor is often not visible on prostate MRI. Intermediate-risk prostate cancer is the most frequent and also the more heterogeneous group with

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some patients who may benefit from active surveillance and others with a higher risk who should be treated similarly to high-risk prostate cancer patients. Moreover, in intermediate-risk prostate cancer, a hypoxic and genomic signature was able to discriminate patients at higher risk [5]. High-dose radiotherapy (78 Gy 39f) to the prostate was delivered without hormonal therapy similarly to the standard arm of the PROFIT trial [14].

#### <sup>18</sup>F-Misonidazole PET/CT

<sup>18</sup>F-Misonidazole was synthesized in Graz-Seiersber, Austria (IASON GmbH) or in Tours, France (Cyclopharma). Prior to radiotherapy, all patients underwent a FMISO1 PET/CT using a Siemens Biograph mCT40 (Siemens, Knoxville, TN, USA). For all patients, acquisition started immediately after intravenous injection of <sup>18</sup>F-Misonidazole (3 MBq/kg). Pelvic PET images were acquired 3.5 hours after injection, with an acquisition time of 10 min. In FMISO1-positive patients, a second FMISO2 PET/CT was acquired after 10 fractions (20 Gy) using the same imaging procedure. Quantification consisted of determining the maximum standard uptake value (SUV) and tumor to muscle (T/M) ratio of SUV of FMISO1 and FMISO2 for each volume of interest on PET scanning at 3.5 hours. Regions of interest (ROIs) were drawn over the *Gluteus Minimus* muscle and the hottest areas of FMISO uptake.

#### PET uptake delineation

All uptakes in FMISO images were semi-automatically delineated using the fuzzy locally adaptive Bayesian (FLAB) algorithm, developed specifically for PET image segmentation [15,16]. Uptakes of interest within the prostate were first visually located and then isolated to a volume of interest (VOI) that enclosed the tumor and its surrounding background, taking care to exclude nearby physiological uptakes (such as the urinary tract). This was performed using in-house software in which points are manually placed by the user around the target uptake, ensuring that the VOI contains the entire target uptake and some surrounding physiological background, thus providing the algorithm with sufficient data to perform parameters estimation and facilitate convergence of the iterative process.

#### Image coregistration

Image coregistration of FMISO was performed using a rigid, non-parametric, affine transformation (iPlan RTImage 4.1, Brainlab AG, Feldkirchen, Germany). To take into account bladder and rectal filling, automatic fusion was adjusted manually based on outer prostate volume and intraprostatic calcifications.

#### Statistical analysis

Results are expressed as median and range, or mean values of parameters  $\pm$  standard deviation (SD). Group differences were assessed using the Wilcoxon signed rank test. Survival curves were compared using a log-rank (Mantel-Cox) test. Statistical tests were performed with Stata 13.1 (Statacorp LP, College Station, TX, USA).

## Results

#### FMISO PET/CT prior to radiotherapy

In 9/27 patients (median age 76, median PSA 7.97 ng/ml) with intermediate-risk prostate cancer who underwent FMISO PET/CT, a FMISO1-positive volume was detected (median volume 1.1 ml [0.4–2.4]; median SUVmax 3.09 [1.74–5.39]; Median T/M ratio 4.4 [2.2–15.6] at 3.5 h). In two patients, two non-adjacent

FMISO-positive uptakes were detected. A complete description of the patient population and FMISO location within the prostate was published earlier [6].

#### FMISO PET/CT during radiotherapy

A second FMISO PET imaging was performed on the nine FMISO1-positive patients after they had received 20 Gy (10 fractions), except for one patient whose FMISO2 was performed after a dose of 32 Gy (16 fractions). During radiotherapy, FMISO2-positive volumes were no longer detected in five of nine patients. In the remaining four patients, a visible FMISO2-positive uptake was automatically delineated. Median T/M ratio of FMISO2 regions was 4.4 [1.6–10.7]. FMISO2-positive volumes were significantly smaller than FMISO1 volumes (median volume 0.7 ml [0.3–1.8],  $p = 0.0077$ ) (Fig. 1A).

#### FMISO1 vs FMISO2 uptake locations

FMISO2 uptakes of interest observed during radiotherapy were located within the same sextant as the uptakes of FMISO1 images in all four patients with persistent FMISO uptake (Fig. 1B). FMISO2 uptakes were all located within a 6 mm rim around the FMISO1 uptakes. In patients 17 and 26, 13% to 59% of the FMISO2 volume intersected with the FMISO1 volume respectively (Fig. 1C).

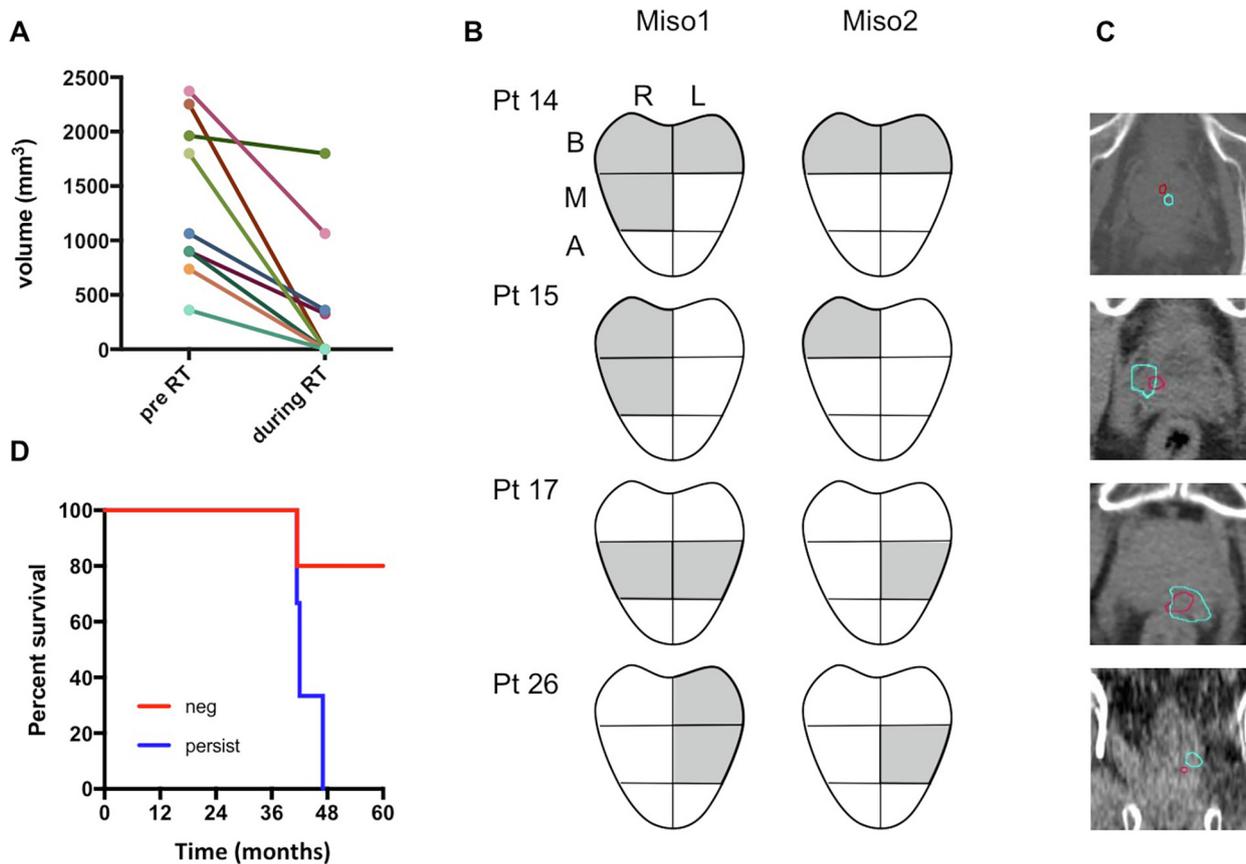
#### Survival of patients with negative vs persisting FMISO2 uptake

In an exploratory analysis, we retrospectively assessed biochemical relapse-free survival and the pattern of relapse was assessed using <sup>18</sup>F-Choline PET-CT. After a median follow-up of 65 months, biochemical relapse-free survival was poorer in patients with a persisting FMISO2 uptake as compared with patients with a negative FMISO2 ( $p = 0.046$ , Fig. 1D). One patient with a positive FMISO1 uptake but a negative FMISO2 relapsed both locally and in pelvic lymphnodes. Among the four patients with a persisting FMISO2 uptake, one patient died rapidly of lung cancer. The three other patients presented with a local relapse (patient 14 and 26) or a pelvic lymphnode relapse (patient 15).

## Discussion

Since hypoxia drives aggressiveness and radioresistance in prostate cancer, mapping hypoxia within the prostate using functional imaging may help personalize the treatment of prostate cancer patients. Our results showed that an FMISO-positive volume was undetectable in five of nine patients after two weeks of radiotherapy and that in the remaining four patients, the FMISO-positive residual volume was significantly smaller than before radiotherapy. The persisting FMISO volumes did not or only partially intersect with FMISO volumes prior to radiotherapy, but were located within the same prostate sextant as diagnostic prostate biopsies in all patients. It must be borne in mind that the prostate is a highly mobile and deformable organ: rigid coregistration of combined modality images of small hypoxic regions is therefore challenging.

These observations suggest that the prostate reoxygenates during the course of radiotherapy. Reoxygenation has been also observed in patients undergoing hormone therapy [17,18] or radiotherapy for other cancers [19,20] (Supplementary Table 1). Similarly, preclinical experiments showed that orthotopic prostate tumors reoxygenate during fractionated radiotherapy [10]. In prostate cancer patients undergoing serial magnetic resonance imaging during radiotherapy, changes in T(2) relaxation and apparent diffusion coefficient suggest that the whole prostate gland reoxygenates during radiotherapy [21]. However, radioinduced prostate



**Fig. 1.** (A) Evolution of FMISO volumes before and during radiotherapy. For patients with 2 FMISO-positive volumes, the volumes were summed. (B) Co-localization of FMISO1 and FMISO2-positive images. The prostate was divided into six sextants (3 levels: Apical -A-, Median -M- and Basal -B- and 2 sides Right -R- and Left -L-). (C) Rigid coregistration of FMISO1 GTV (blue) images with FMISO2 GTV (red) in prostate cancer patients with persisting FMISO uptake. Axial images are shown except for patient 26 where a coronal view is presented (D) Biochemical-relapse free survival (Phoenix definition) in patients with a negative (neg) vs positive (persist) FMISO2 uptake.

reoxygenation implies that hypoxia-driven dose-painting strategies need to adjust during radiotherapy or even consider initial boosting of hypoxic regions within the prostate rather than simultaneous integrated boosting during fractionated radiotherapy.

The presence of hypoxia was originally proven by direct oxygen measurements or the presence of intrinsic hypoxia markers such as HIF-1 $\alpha$ , GLUT1, CAIX, osteopontin (reviewed in [6]). Non-invasive  $^{18}\text{F}$ -FAZA PET imaging was unable to detect hypoxia [22]. Conversely, FMISO imaging showed some positivity in a proportion ranging from 33 to 100% of patients [8,9,18], but with a poor correlation between hypoxia-induced molecular events and FMISO images, possibly due to the fact that prostate biopsies might miss small intraprostatic hypoxic areas. Interestingly  $^{18}\text{F}$ -MISO uptake was coincidental with the highest Gleason score region [18]. A prostatectomy study in which patients receive FMISO and an extrinsic marker (e.g. pimonidazole) prior to surgery would be the best test to correlate hypoxia immunohistochemistry markers with FMISO images.

In various tumors, the individual dynamics of reoxygenation during irradiation plays a role in the overall tumor response (Supplementary Fig. 1). Reoxygenation is usually observed after at least 10 Gy during the second week of radiotherapy, similarly to our study. Tumors that reoxygenate early during radiotherapy have a more favorable prognosis than non-reoxygenating tumors [20]. Similarly, in our very small series of prostate cancer patients, an exploratory analysis suggests that lack of reoxygenation during radiotherapy may represent an adverse prognostic factor. This needs to be confirmed in a larger study.

Using  $^{18}\text{F}$ -Misonidazole, we were able to show that decreased hypoxic volumes were observed in all FMISO-positive patients,

suggesting that fractionated radiotherapy of prostate cancer may induce reoxygenation. We now need to evaluate FMISO as a novel predictive factor of radioresistance that may help not only personalize treatment decisions for intermediate-risk prostate cancer, but also modelize prostate cancer response to radiotherapy and determine the best dose-painting strategy boosting hypoxic regions within the prostate.

### Competing interests

The authors have declared that no competing interest exists.

### Acknowledgments

Funded by Programme d'Action Intégré de Recherche (PAIR PROSTATE 2010), Institut National du Cancer (INCA), Association pour La Recherche sur le Cancer (ARC), Ligue contre le Cancer, Région Bretagne, Région Pays-de Loire.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.radonc.2018.12.022>.

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