[^{18}F] Fluoromisonidazole PET in Rectal Cancer – a short review

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The World Health Organisation (WHO) predicts that the number of incidences of colorectal cancer worldwide will rise to 1.36 million for men and 1.08 million for women by 2035 [1]. Hypoxia in cancer cells leads to radioresistance [2,3]. One way to improve personalised radiotherapy involves targeting hypoxic regions within the tumour. Strategies to successfully identify hypoxia in rectal cancer have repeatedly failed due to the inability to distinguish tumours with severe or non-resolving hypoxia. Thus, it is crucial to develop non-invasive biomarkers of tissue hypoxia in such tumours through imaging. The invasive method of Eppendorf electrode [4] is considered the gold standard for measuring oxygen distribution in tumours and has been shown to correlate with response to RT. However, positron emission tomography (PET) is an imaging technique used to visualise and quantify pathophysiological processes of interest (for example, glucose metabolism or hypoxia) within a tissue via administration of a radiopharmaceutical (commonly known as tracer). [^{18}F]fluoromisonidazole ([^{18}F]FMISO) is a radiopharmaceutical used to identify hypoxia in various tumour types. Increased retention of [^{18}F] FMISO in tumour cells is suggestive of hypoxia and vice-versa. Therefore, the aim was to explore changes in [^{18}F]FMISO PET imaging parameters in human rectal tumours before and after 8-10 fractions (~2 weeks) of chemoradiotherapy (CRT) to predict clinical response.

Data Acquisition

Patients were recruited within an ethically-approved prospective observational study: modulation of *R*adiotherapy according to *HY*poxia: exploiting changes in the *T*umour *M*icroenvironment to improve outcome in rectal cancer (RHYTHM). All patients provided written informed consent for the study procedures. Patients with histologically confirmed invasive adenocarcinoma of the rectum having neoadjuvant chemoradiotherapy (CRT) (45Gy in 25 fractions over five weeks plus capecitabine chemotherapy (900mg/m^2 twice daily)), prior to planned curative rectal resection were recruited between October 2013 and April 2016. Patients were imaged on PET-CT between 0 and 45min, at 2h and 4h, both at baseline and two weeks into CRT. The patient cohort was divided into two groups. The first six patents did not receive an enema before the 4h PET-CT and were called the non-enema group. The last four patients received an MICROLAX® micro-enema before the 4h scan, called the enema group.

Image Analysis Methods

The tumour regions of interest (ROI$_{tumour}$) were manually delineated on magnetic resonance (MR) scans and transferred to the PET-CT using rigid registration in Eclipse radiation treatment planning software (Varian Medical Systems (version 10), Inc, Palo Alto, CA, USA). The ROI$_{tumour}$ were transferred to PMOD (PMOD Technologies (v3.6) Ltd., Zurich, Switzerland) to ensure exclusion of the bladder region using semi-automatic thresholding. All ROIs were propagated to the earlier scans using rigid registration in PMOD for the time series analysis of the PET data. Tumour-to-muscle (T:M) SUVmax and tumour-to-blood (T:B) SUVmax was used to analyse static PET images at 4h. The 0-45min dynamic PET scans were analysed using the Casciari pharmacokinetic model [5] to report relevant parameters including, hypoxia (Ka) and perfusion (F). American Joint Committee on Cancer (AJCC) 7.0 [6] was used to report pathological tumour regression grading and Shapiro-Wilk normality test was used to test the data normality.

Results and Discussion

Eight patients underwent total mesorectal excision. Five of the eleven patients were classed as good responders (AJCC 0/1 or good clinical response) and six as poor responders (AJCC 2/3 or poor clinical response). The median T:M SUVmax was 2.14 (IQR: 0.58) at baseline and decreased by 33% by two weeks. The corresponding median tumour hypoxia volume was 1.08 (IQR: 1.31) cm^3 and decreased by 95% by two weeks. The median T:B SUVmax was 2.46 (IQR: 1.50) at baseline and decreased by 29% by two weeks. The corresponding median tumour hypoxia volume was 5.68 (IQR: 5.86) cm^3 and decreased by 56% by two weeks. Using Casciari modelling, the median tumour F was 4.10 (IQR: 1.71) millilitres/grams/min (mlg^{-1}min^{-1}) at baseline and decreased by 29% to 2.48 (3.62) mlg-1min-1 by two weeks. In nine of the eleven patients scanned at baseline and two weeks into CRT, tumour perfusion decreased post-CRT in non-responders and increased in responders, except in one patient (Figure 1). The alterations in tumour perfusion trend with its response highlights the importance of changes in vasculature-related functional parameters during radiotherapy, its important role in understanding hypoxia, and its relation with outcome. None of

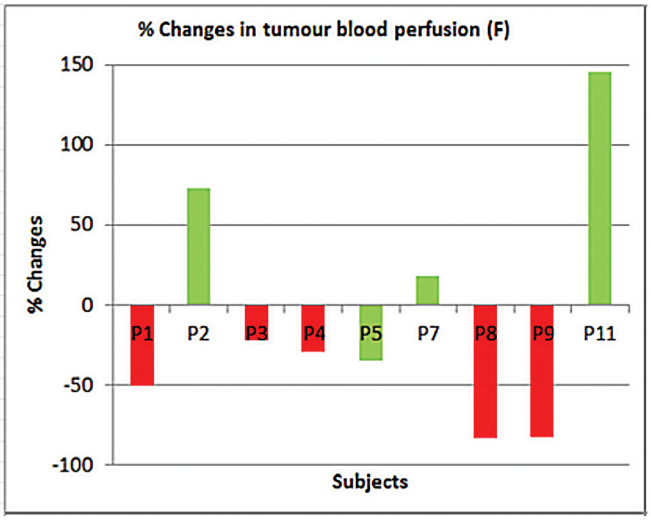

Figure 1: Percentage changes in tumour blood perfusion between baseline and after 2-weeks into chemoradiotherapy from fitting 0 to 45 min [^{18}F]FMISO PET data to the Casciari model in rectal cancer patients. The red bars show non-responders and green bars responders. [^{18}F]FMISO=[^{18}F]fluoromisonidazole; PET=positron emission tomography.

the other changes in PET imaging parameters between baseline and two weeks showed any clear trend with clinical outcome; see Greenhalgh et al. [7] and Puri et al. [13] for details.

At baseline, tumour F showed a weak relationship with T:M SUVmax and T:B SUVmax at 4h and none after two weeks of CRT, suggesting that these parameters from static PET may primarily exhibit chronic hypoxia. The Ka had a poor relationship with T:M SUVmax and T:B SUVmax at baseline and after two weeks of CRT, suggesting that the semi-quantitative and quantitative methods of measuring hypoxia from static and dynamic PET, respectively, are not equivalent.

There are two major challenges that affect the interpretation of [^{18}F]FMISO PET results in rectal tumour, including, but not limited to, the rate of renal excretion to the bladder, the activity concentration within the bladder and rectal lumen, their volumes at the time of imaging, their proximity to the tumour, the time the patient empties the bladder, how quickly the bladder refills, how well the enema works, the attenuation correction, and other factors.

1. Bladder Activity Accumulation: Accumulation of [^{18}F]FMISO in the bladder starts 10-15 min post-tracer injection due to urinary excretion at variable rates. This leads to a high activity concentration within the bladder by the end of the study (Figure 2) that can affect both delectability and quantification of the lesion due to a phenomenon called spill-in. Figure 3 shows a schematic diagram of the phenomenon of spill-in count from bladder inside the tumour due to scatter and random photons. The false lines of responses are accepted as true events, leading to an overestimation of activity in the surrounding region and causing an error. Tumour regions within very close proximity of the bladder also get further affected during image reconstruction by the spill-in of activity from bladder due the limited spatial resolution of the PET scanner. Catheterization of the bladder has been suggested, but it is uncomfortable for the patients and a potential source of infection. A comfortably full bladder is normally required for rectal cancer radiotherapy. Frusemide may be considered as aiding rapid urinary excretion and diluting the residual radioactivity in the bladder, but its feasibility has to be assessed. Another solution may be to reconstruct the PET images by restricting the counts from the bladder [8], but it is too early to see how this will be translated into the clinic.
2. Rectal Activity Accumulation: [^{18}F]FMISO is excreted through the biliary tract into the gastrointestinal tract as well as through the urine. When the rectal lumen contains high [^{18}F]

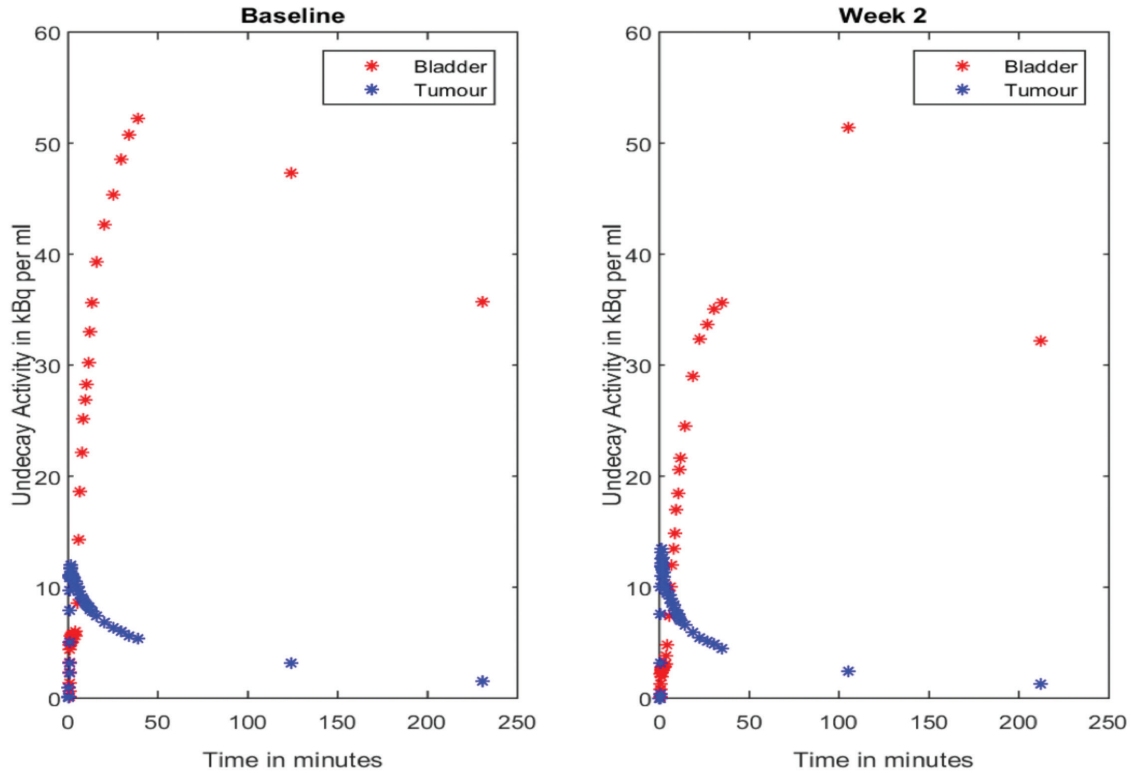

Figure 2: The undecay corrected averaged time activity curves of [^{18}F]FMISO PET at the bladder and tumour at baseline (in A) and after 8-10 fractions of chemoradiotherapy (in B) between 0 and 4h. Bettinardi et al [11] assessed random and scatter characteristics over a range of 0-60 kBq/ml within the PET field of view. However, it should be noted that we found a much higher activity concentrations (undecay corrected activity of up to 150kBq/ml directly related to random and scatter events) within individual human bladder that would lead to an increase in random and scatter in a non-linear fashion.

FMISO activity and is within close proximity to the tumour, the spill-in of activity from rectal lumen into the tumour is unavoidable due to the limited resolution of the PET scanner. The use of an enema prior to the 4h scan reduced this non-tumour luminal activity (Figure 4).

[^{18}F]FMISO is not taken up by the tumour in large amounts compared to other hypoxia tracers, leading to a lower tumour to background contrast. The PET tracers that show clearance to bladder or colorectal lumen may have similar issues for other tumours in the pelvic region, such as cervix and prostate cancer. A recent review [9] and two other studies [10,11] suggest similar difficulties with [^{18}F]FMISO PET in rectal cancer, but they did not take steps to mitigate these issues. Of these challenges, some are solvable, such as by using an enema to reduce spill-in from non-tumour accumulation of [^{18}F]FMISO. However, the problem of spill-in from bladder has not currently been solvable and may require considerably more investigation. Another PET study using ^{68}Ga-PSMA-11 in prostate cancer [14] suggested severe artefacts surrounding the bladder and the kidneys in PET images that are frequently used in clinical practice, and has investigated the impact of these artefacts on tumour quantification. They concluded that inaccurate scatter correction methods currently used in clinical routine tend to overestimate the scatter contribution.

Conclusion

This pilot study with only a small sample size does not support the hypothesis that a reduction in [^{18}F]FMISO uptake in rectal cancer is predictive of clinical response. There are two main problems, namely spill-in from non-tumour activity in the rectum, and from the bladder into the environs of the tumour. Careful consideration should be given to PET acquisition and reconstruction to minimise spill-in counts from the bladder. This preliminary review indicates fundamental difficulties in the interpretation of [^{18}F]FMISO PET for rectal cancer, limiting its clinical applicability.

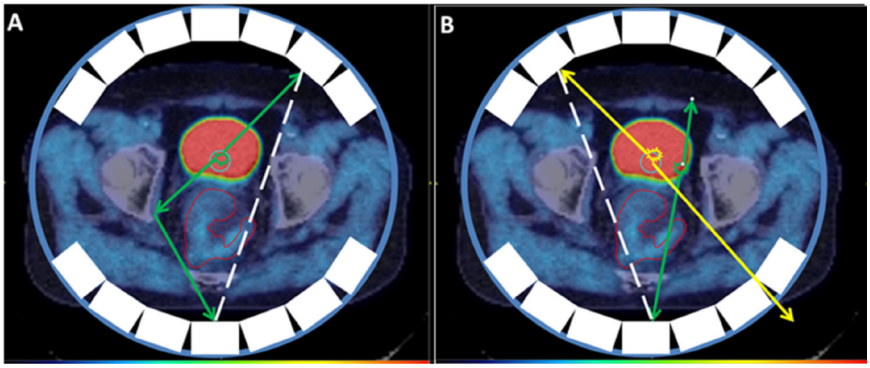

Figure 3: Artwork showing examples of scatter & random events originating from the activity inside the bladder as a potential cause of spill-in counts inside the tumour. The blue circular ring illustrates the PET scanner field of view, the white rectangular blocks represents the photon detectors, green and yellow arrows describe the path of the photons from two different annihilation events, red boundary represents tumour ROI and white dashed line shows the line of response (LOR). (A) An annihilation event occurs in the bladder and the path of a scattered photon is shown using green arrows. The two photons are detected between a pair of opposite detectors in 511 keV energy coincidence window and their LOR (white dashed line) passes through tumour, falsely contributing to the image as a true event. (B) Two annihilation events occur in the bladder and one of the photons in each annihilation gets lost (or undetected) and the other two photons (one from each event) are detected between a pair of opposite detectors in 511 keV energy coincidence window and their LOR (white dashed line) passes through tumour (red boundary), falsely contributing to the image as a true event. PET=positron emission tomography; LOR=line of response; keV=kilo electron volt; ROI=region of interest.

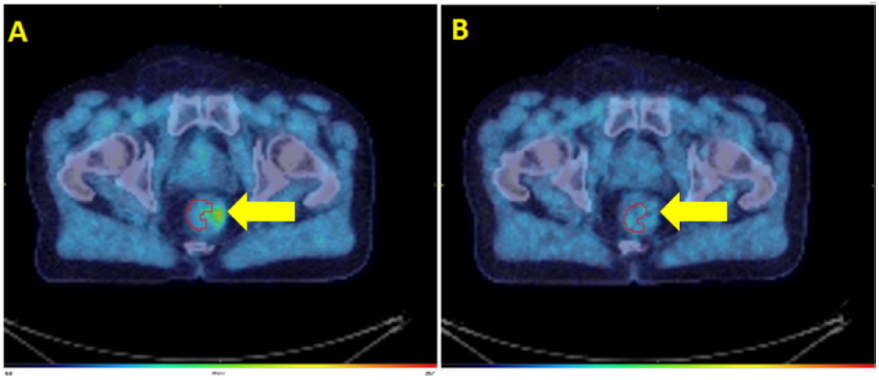

Figure 4: Example from the enema group showing the PET-CT scans at 2h (A) and 4h (B) for the same transaxial slice in the same subject. The red ROI marks the tumour and the yellow arrow shows the non-tumour activity in close proximity to tumour ROI. A and B highlights the fact that the non-tumour [18F]FMISO in the rectum in close proximity to the tumour is visible at 2h, but not after the enema given before the 4h scan. PET=positron emission tomography; CT=computed tomography; ROI=region of interest; [^{18}F]FMISO=[^{18}F]fluoromisonidazole.

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ETHICS, CONSENT AND PERMISSIONS

Informed consent was obtained from all participants included in the study. All involving human participants were in accordance with the local ethical standards and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The research ethics committee (REC) was the East of England – Essex Research Ethics Committee (reference number 13/EE/0123). The Radiotherapy & Imaging Trial Oversight Committee (RIOC) fulfilled the roles of the trial steering committee (TSC) and data and safety monitoring committee (DSMC).

EACR Travel Fellowship – Vicky Forster

Vicky Forster, an EACR member of nearly 8 years and an EACR ambassador has taken up a postdoctoral position in paediatric brain tumour research in Toronto, Canada after a successful EACR funded trip there last year. In January 2017, Vicky also featured on the Forbes 30 under 30 in Europe list for Science & Healthcare after being selected out of almost 1000 candidates!

I was incredibly lucky to be awarded an EACR Travel Fellowship to do a placement in the lab of Professor Rosanna Weksberg at SickKids in Toronto, Canada. My current postdoctoral work focuses on investigating mechanisms of neurotoxicity in childhood leukaemia patients treated with methotrexate, a common chemotherapy agent. The majority of patients undergo treatment with methotrexate and experience no significant toxicity, but some have severe symptoms such as paralysis and seizures. My work aims to investigate why these patients are so badly affected. The Weksberg lab in Toronto are part of a large, collaborative project which looks at long-term neurocognitive impact of methotrexate on leukaemia survivors, and are currently looking at changes in DNA methylation as a possible causative factor. Given the substantial overlap between our two research areas, we decided to collaborate and use my cell line models to see if there were any changes in global DNA methylation patterns after methotrexate dosing.

I sent my DNA samples ahead of me to be processed on the new methylation array so that the data would be available by the time I arrived in Toronto. When I arrived, my first few days were a fairly intensive crash course in learning to use the relevant software and techniques. For someone who is broadly a lab-based molecular biologist, the thought of spending an entire month doing bioinformatics was somewhat daunting! However I was incredibly lucky to have the support and expertise of members of the Weksberg lab and quickly learned the basics needed to begin analysing my data. I'm pleased to say that the data are excellent and will hopefully lead to a publication in the not-too-distant future. I attended lab meetings with the group and gave two presentations during my time there to get feedback and suggestions on my ongoing project. During my time, I also attended a number of high-quality seminars and presentations given by both external and internal speakers.

I really enjoyed experiencing Toronto as a city too. It's incredibly diverse and I lived in a wonderful neighbourhood with two very hospitable hosts who really made my stay comfortable and easy. I took the opportunity to get involved in various events including a meet up of the Toronto Women in Science and Technology (TWiST) and a half marathon in aid of SickKids which was covered by the local TV station! Doing the training runs was made exceptionally easy by running on the shores of Lake Ontario in the sunshine…and rewarding myself with ice-cream afterwards!

Toronto is an incredibly vibrant and varied place for top-quality medical research at numerous different research institutes, many clustered around a few blocks in the centre of the city. Experiencing this culture and community was invaluable for my professional development. As well as facilitating the valuable collaboration with the Weksberg lab, my visit ultimately led to an offer of a postdoctoral position at SickKids in the Tabori lab working in paediatric brain tumour research, which I began in early 2017. I'd like to thank the EACR once again for funding my Travel Fellowship and supporting me at a very exciting and pivotal stage of my career.