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Is there a role for an FDG derived biological boost in squamous cell anal cancer?

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Abstract

Aim

We aim to investigate the potential role for a biological boost in anal cancer by assessing whether subvolumes of high FDG avidity, identified at outset, are spatially consistent during a course of chemoradiotherapy (CRT).

Methods

FDG-PET scans from 21 patients enrolled into the ART study (NCT02145416) were retrospectively analysed. A total of twenty-nine volumes including both primary tumours and involved nodes >2 cm were identified. FDG-PET scans were performed prior to treatment and day 8 or 9 of CRT. FDG subvolumes were created using a percentage of maximum FDG avidity at thresholds of 34%, 40%, 50%, on the pre-treatment scans, and 70% and 80% on the subsequent scans. Both FDG-PET scans were deformably registered to the planning CT scan. The overlap fraction (OF) and vector distance (VD) were calculated to assess spatial consistency. FDG subvolumes for further investigation had OF >0.7, as this has been defined in previous publications as a “good” correlation.

Results

The median OF between the diagnostic FDG-PET subvolumes 34%, 40% and 50% of max SUV and subsequent FDG-PET subvolumes of 70% of max SUV were 0.97, 0.92 and 0.81. The median OF between the diagnostic FDG-PET subvolumes 34%, 40% and 50% and subsequent FDG-PET subvolumes of 80% were 1.00, 1.00 and 0.92. The median (range) VD values between diagnostic FDG-PET subvolumes 34%, 40% and 50% and subsequent FDG-PET subvolumes of 80% were 0.74mm (0.19-2.94) 0.74mm (0.19-3.39) and 0.71 (0.2-3.29) respectively. Twenty out of 29 volumes (69.0%) achieved a threshold of >0.7 between the FDG 50% subvolume on the diagnostic scan and the FDG 80% subvolume on the subsequent scan.

Conclusion

FDG avid subvolumes identified at baseline were spatially consistent during a course of CRT treatment. The subvolume of 50% of SUVmax on the pre-treatment scan could be considered as a potential target for dose escalation.

Keywords Biological boost, Anal cancer, FDG PET scan, Radiotherapy.

Introduction

Radical chemoradiotherapy (CRT) is the established standard of care for localised squamous cell carcinoma (SCC) of the anus delivering doses up to 66 Gy in 1.8 to 2.2Gy/fraction concurrently with chemotherapy [1]. CRT provides 3 year progression free survival (PFS) of 73% [2], however despite the widespread use of intensity modulated radiotherapy (IMRT), acute and chronic toxicity remains significant. Acute toxicities include radiation dermatitis, pain, gastrointestinal and genitourinary disturbance; these usually resolve 2-3 months following CRT. Late toxicities however can be lifelong and significantly affect quality of life; these include diarrhoea, faecal incontinence, dyspareunia and erectile dysfunction [3-6].

Treatment failure occurs most often in patients with locally advanced disease, with approximately 75% of relapses occurring at the site of the original tumour [7-10]. A tumour control probability model has suggested dose escalation may improve tumour control by potentially reducing local relapse in these patients [11]. PLATO (PersonaLising Anal cancer RadioTherapy dOse, ISRCTN88455282), the international anal cancer trial funded by Cancer Research UK; is investigating the role of dose escalation to the primary site of gross tumour in locally advanced disease [12]. Dose escalation to the whole gross tumour would likely add acute and late toxicity to the treatment; so the use of functional imaging to identify a smaller biological boost is of interest; as smaller volumes are recognised to minimise toxicity [13].

Over 90% of squamous cell anal tumours are 18F-flurodeoxyglucose (FDG) avid on Positron Emission Tomography (FDG-PET) scan [14] and there has been significant interest in the role of FDG-PET in anal cancer management. Trautmann et al. reported in a study of 21 patients, that the diagnostic FDG-PET identified additional nodes and metastasis not seen on MRI and

CT, having implication for prognosis and radiotherapy planning [15]. The metabolically active volume on diagnostic images have also correlated with local recurrence in small series of 23 and 45 patients respectively [16, 17]. It is likely metabolically tumour volume (MTV) correlates with lesion size therefore this finding is in keeping with the phase III data highlighting larger lesions do worse [18]. Kidd et al however identified a new finding that increased SUV at outset correlated with disease free survival and the presence of persistence or recurrence on subsequent FDG-PET's in 77 patients [19]. A number of single centre publications have reviewed between 23 and 110 patients with diagnostic FDG-PET and repeat FDG-PET completion of CRT at time points ranging from 3-36 weeks [20-22]. The metabolic response correlated in these studies with one or more of: local control rate, progression free survival, cancer specific survival or overall survival. Lastly, Hong et al looked at 23 patients with FDG-PET at outset and after 30Gy of radiotherapy [16]. They found that the interim scan MTV and total lesion glycolysis correlated with freedom from local progression.

All of the FDG-PET data in anal cancer to date has looked at predictive value of diagnostic or repeat FDG-PET, the authors are unaware of any other publication investigating the role of a biological boost in anal carcinoma. In other tumour types treated by concomitant CRT, such as non-small cell lung carcinoma (NSCLC), head and neck squamous cell carcinomas (HNSCC) and pancreatic adenocarcinoma; FDG-PET/CT scans have been reported to identify tumour subvolumes at greater risk of relapse [23-29]. There are ongoing trials investigating the use of biological boosts in HNSCC and NSCLC cancer [30-32].

The ART (Anal squamous cell carcinoma: Investigation of functional imaging during chemoRadioTherapy) study (NCT02145416) is a feasibility trial investigating the use of

functional imaging to predict response in anal cancer. All patients receive baseline FDG-PET/CT scans and further scans at fraction 8-10. We performed a post-hoc analysis on images obtained in this trial. Our aim is to investigate the feasibility of defining a biological boost in anal squamous cell carcinoma by assessing whether FDG avid subvolumes, identified at baseline, are spatially consistent in the second week of a course of CRT.

Materials and Methods

Patient Population

We identified 21 patients with paired scans performed at diagnosis and at fraction 8 or 9 of CRT, obtained from the ART study. 29 patients were recruited however 4 patients withdrew consent prior to any imaging and a further 4 patients withdrew consent following their initial scans due to ongoing social and medical issues. The ART study is a single arm, single centre imaging study, of patients receiving radical CRT for anal cancer within Oxford University Hospitals; evaluating changes in functional imaging during CRT treatment. ART recruited patients who were aged 18 years or above, had histologically confirmed invasive primary squamous carcinoma of the anus, had any tumour stage ≥T2N0 and were fit to receive radical CRT with curative intent. All patients had given informed consent in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Ethical approval incorporated post-hoc analysis of images to answer additional questions.

Chemoradiotherapy

CRT was delivered according to UK based guidance [33]. In summary patients received 50.4 or 53.2Gy to the primary tumour depending on TNM stage, 50.4Gy to involved nodes and 40Gy to prophylactic dose to non-involved nodes; all in 28 fractions using a simultaneous

integrated boost. Three patients were treated within the PLATO study, two with a dose of 61.6Gy to the primary tumour while maintaining 40Gy to the prophylactic nodes; the other patient with a reduced dose of 41.4Gy to the primary tumour and 34.5Gy to elective nodes in 23 fractions with simultaneous integrated boost. Treatment was delivered using IMRT or volumetric modulated arc therapy (VMAT), calculated using a type B algorithm, prescribing 100% to the median dose of the planned target volume (PTV) boost. Chemotherapy was Mitomycin 12 mg/m^2 Day 1 and Capecitabine 825 mg/m^2 orally twice a day on radiotherapy treatment days.

FDG-PET/CT scanning

All patients had a routine, full body, diagnostic FDG-PET/CT scan performed at baseline. As part of the ART study, 18 patients underwent a second FDG-PET/CT on fraction 8 and three on fraction 9 of their CRT.

All scans were performed using GE Discovery 690 scanners (GE healthcare, Buckinghamshire, UK). Patients were positioned supine with arms above the head, on a flat-top couch using radiotherapy immobilisation equipment to replicate their treatment position. After fasting for 6 hours and ensuring that the blood glucose was < 10 mmol/L, FDG was injected at a dose of 4 MBq/kg (up to 600 MBq). FDG-PET/CT acquisition commenced after an uptake time of 75 minutes. The initial FDG-PET/CT scanned the whole body. For the second FDG-PET/CT, 8 patients underwent an image limited to the pelvis, 13 patients had a further full body image.

Scans were performed in 3D with a scan time of 4 minutes at each bed position. For the CT phase, 120 kV automA (max 250 mA), noise index 25.0 0.5 s/rotation, pitch 0.984:1, 3.75 mm slice width was used. Attenuation corrected FDG-PET/CT images were used in the analysis.

Image analysis

The paired FDG-PET/CT images and the planning CT scans were imported into Mirada (Mirada Medical, Build 1.2.0.39, Oxford, UK). All data analysis was performed and checked by AS and RM. The planning CT scan and CT component of each FDG-PET/CT scan underwent a rigid registration followed by a deformable registration. The quality of both registrations was assessed visually by an experienced clinician and if the registration was judged poor, due to the FDG avidity lying outside the gross tumour, a rectangular region of interest (ROI) was drawn around the volume incorporating surrounding muscles and bones. The registration was repeated using that ROI. As the FDG-PET and CT components of the FDG-PET/CT scan share the same intrinsic frame of reference, this registered each FDG-PET image with the planning CT scan. To create the subvolumes, an elliptical ROI was drawn solely around the area of increased uptake within the anus on each FDG-PET scan, and tumour segmentation was performed as a percentage of the SUVmax within the elliptical ROI.

The method used to assess spatial consistency of volumes has been previously published by groups performing similar work in different tumour types [23, 24]. On the baseline FDG-PET, tumour segmentation was performed at 34%, 40%, 50%, 60% and 70% of the SUVmax (Pre34%, Pre40%, Pre50%, Pre60% and Pre70% respectively). On the subsequent scans subvolumes of 70% and 80% of the SUVmax were used (Sub70% and Sub80% respectively). The overlap fraction (OF) between the baseline and the second scan was calculated and expressed as a fraction of the volume of the second scan. Based on previous publications of this method, a mean OF of > 0.7 supported the use of a threshold as a target for a biological boost [23, 24]. Following this, the planning scan with attached subvolumes was loaded into Eclipse software (version 13.0, Varian medical systems, Palo Alto USA). The centre of mass

(COM) shifts were used to calculate a vector distance (VD) which was calculated using the following formula:

$$VD = \sqrt{(\Delta x)2 + (\Delta y)2 + (\Delta z)2}.$$

Figure 1 demonstrates the planning scan of Patient 14 with two volumes (Pre50% and Sub70%) highlighted. The Dice Similarity coefficient (DSC) was calculated for completeness however limitations of this method of analysis in this setting are highlighted in discussion.

Statistics

OF, VD and DSC between subvolumes of the baseline and subsequent scans were described using medians and means and full data reported.

Results

Twenty-one patients underwent two FDG-PET/CT scans between January 2015 and November 2017 at the two time points within the ART study and these images were used for this analysis. Areas of gross tumour of primary disease as well as nodal disease >2cm in the longest diameter were analysed, resulting in 29 volumes in total.

Patient characteristics

Table 1 describes the patient and tumour demographics.

Imaging results

The gross tumour volumes (GTVs), Pre50% and Sub70% volumes in cm^3 are given in Table 2. In terms of OF, the median OF of the Pre34%, Pre40% and Pre50% with the Sub70% subvolumes are all above 0.8. The median OF of the Pre34%, Pre40%, and Pre50% subvolumes with the Sub80% subvolumes are all above 0.9. The mean OF between the Pre34%, Pre40%, Pre50%, Pre60% and Pre70% and the Sub80% fell as the volumes reduced in size; from 0.86 with Pre34%, 0.79 with Pre40%, 0.70 with Pre50%, with Pre60% and

Pre70% both below 0.7. The median VD values between Pre34%, Pre40% and Pre50% and Sub80% were 0.74mm, 0.74mm and 0.71mm respectively. Figure 2 illustrates the median OF and VD for the different volumes. Full details on OFs and VD between volumes of high FDG avidity on the pre-treatment scan and the subsequent interim scan are shown in Tables 3 and 4 respectively. As expected the DSC values were poor. Figure 3 illustrates the median DSC for the different volumes assessed. DSC for all the subvolumes per node per patient are available in supplementary material.

Discussion

FDG uptake within tumours is heterogeneous. The first steps to delivering an FDG derived biological boost is to determine whether the areas of high avidity are spatially consistent during a course of radiotherapy. We report that in the majority of patients studied, the areas identified at baseline were indeed spatially consistent at fraction 8-9. This introduces the possibility of selectively boosting those areas at highest risk of relapse to improve local control. This has the potential to improve overall survival in this rare tumour type where dose escalation to the whole tumour is in use and under investigation; but acute and late toxicities may be dose limiting.

We have shown that areas of increased FDG uptake remain reasonably consistent between baseline and in the second week of radiotherapy treatment. Subvolumes of high FDG uptake before and during CRT showed good agreement. Both the mean and medians of the OF between the diagnostic FDG-PET subvolumes of 34%, 40% and 50% of SUVmax and subsequent FDG-PET subvolumes of 70% and 80% were all OF > 0.7. It was notable that agreement between those volumes was less successful in nodal disease compared to primary disease (mean OFs between the diagnostic FDG-PET subvolumes of 34% and 40%,

and subsequent FDG-PET subvolumes of 80% were 0.95 and 0.65 for nodal volumes but 1 for all primary tumours). This may be due to smaller volumes of nodal disease, where a slight inaccuracy in registration can have a significant effect on the results, therefore dose escalation to nodal sites might only be feasible with advanced image guidance or treatment adaptation.

Our approach to assessing volume overlap is based on a publication in NSCLC [23]**.** Aerts et al, recommended the FDG-PET subvolume of 50% of SUV max on the diagnostic FDG-PET/CT as a target for biological boost [34]. This was based on overlap between the diagnostic scans and post treatment residual disease therefore we acknowledge that our suggested boost volume demonstrates reproducibility rather than site of residual tumour or relapse as our second scan point is the second week of treatment. A study of pancreatic cancer [24], found that threshold of 40% of the SUVmax on baseline FDG-PET/CT identifies areas of residual metabolic activity seen on a post-CRT FDG-PET/CT, and this threshold could aid in the definition of a biological target volume.

Wilson et al and Aerts et al, detailed above, proposed that a mean OF > 0.7 supported the use of a threshold as a target for a biological boost. We have presented the median as the outcomes of interests are normally distributed however in order to allow comparison with previous literature we have also reported the mean (SD). Using a mean OF of > 0.7 defined in previous publications as a “good” correlation, the diagnostic FDG-PET subvolumes showed generally satisfactory results. When comparing the FDG-PET subvolumes of 34%, 40%, 50%, 60% and 70% of the SUVmax with the subsequent subvolumes of 80%; 24 of 29 (83%), 23 (79%), 20 (69%), 15 (52%) and 11 (38%) volumes achieved that threshold. Therefore, it would be reasonable to consider the diagnostic FDG-PET threshold of 50% of SUVmax as a possible target for dose escalation based on pre-treatment imaging, as it

achieved a mean OF of 0.7 used in other papers and was reasonably consistent with > 70% of cases showing good overlapping agreement. As the diagnostic FDG-PET threshold of 50% represented a mean of only 34% of the GTV, the reduction to volume would be substantial. Therefore boosting this reduced volume to a higher radiation dose is likely to result in reduced normal tissue toxicity than boosting the whole GTV, although this would require confirmation.

Our study has some limitations; the fact the study incorporates small numbers is representative of a rare disease. However even with small numbers our study serves to demonstrate the feasibility of defining a biological boost in anal cancer and suggests possible boost volumes for further investigation. There are multiple methods of reporting differences between two volumes [35]. We have primarily used the method previously published by Aerts et al. as they compared spatial position in volumes with changing sizes. Our assessment of the volumes using DSC suggests less spatial correlation. However these contrasting results between our primary methods of comparison and DSC were also seen in the study done by Calais et al [19], who highlighted that two volumes with a good superimposition but a large difference in size will yield low Dice and Jaccard indices. DSC values have been calculated for completeness however the poor DSC correlation should be taken in the context of its limitations when comparing different sized lesions. In our study, it was sometimes challenging to separate the tumour FDG activity from the background physiological uptake. All the subvolumes in our study were checked visually and areas of overlap with normal organs were removed. This may also be responsible for the only moderate overlap seen when like-for-like subvolumes were compared (Pre70% vs Sub70%), as such a comparison would leave no margin for small errors in registration and variations in background physiological FDG uptake. Results must be interpreted with caution in view of

the potential for errors with deformable registration. The differing field of view in the second FDG-PET/CT scans may raise some concerns. This occurred due to an amendment to the trial protocol in order to incorporate a further tertiary endpoint investigating the bone marrow toxicity of CRT. However SUV thresholds are calculated independently of non-pelvic organs using the patients' weight and activity in the primary tumour therefore this will not have affected results. Finally, the subvolumes used within this study are based on previous literature and we acknowledge the SUV threshold levels are somewhat arbitrary. The authors have based the study and the methods on best available evidence and we anticipate further investigation of biological boost volumes in a larger prospective format would be required to validate these methods, to guide patient selection and quantify the reduction in toxicity with smaller boost volumes in comparison to whole GTV.

Previous publications confirmed higher SUV correlates with worse outcome [16, 17, 19] therefore the next question that must be addressed is the hypothesis – Does local relapse occur at sites consistent with the high FDG avid subvolumes identified on diagnositic PET? Unfortunately, as FDG-PET/CT has only recently been incorporated into staging of anal cancer in the UK, and there is a relatively low loco-regional relapse rate; there are not sufficient numbers of patients with baseline and subsequent FDG-PET/CT to perform this analysis. Therefore, we must be clear that the proposed subvolumes are currently based on identifying a reproducible target, further work is required to confirm the proposed subvolumes are appropriate to cover sites of subsequent relapse using scans following treatment. We plan to perform this analysis initially on the ART cohort in due course and ideally subsequently in a larger number of patients as part of a prospective trial.

Lastly, investigation to date regarding the role of functional imaging to predict response is detailed in the introduction. The primary endpoint of the ART study is in fact based on the

hypothesis we can use functional imaging as an early predictor of response in order to perform treatment intensification, perhaps with a radiotherapy boost, on a selected group identified as poor responders. However the ability to select patients for dose escalation does not deter from the potential to use an FDG derived boost to these selected patients, as a method of improving local control while limiting toxicity.

In conclusion, on the basis of these findings we conclude that areas of high FDG avidity on diagnostic FDG-PET scans remain spatially consistent during a course of treatment. A subvolume of 50% of SUVmax on the diagnostic scan could be considered as the target for dose escalation. Despite the limitations of this small study, it demonstrates the potential of using a biological boost rather than whole GTV dose escalation; which may facilitate improved outcomes while minimizing the significant side effects of whole GTV dose escalation. This study suggests an exciting avenue for further investigation.

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Figure 1. Axial (A) and sagittal (B) slices through a planning CT scan with the Pre50% (black) and the Sub70% (white) volumes illustrated.

Figure 2. Illustration of median Overlap Fraction and Vector Distances between the different volumes assessed.

Figure 3. Illustration of the median Dice Coefficient of the different volumes assessed.

Tables

Table 1. Patient and tumour demographics.

Characteristic	n (%); median (IQR)
Gender	
Male	2 (10%)
Female	19 (90%)
Age (years)	60 (51,70)
T Stage	
T2	16 (76%)
T3	2 (10%)
T4	3 (14%)
N Stage	
N0	10 (48%)
N1	7 (33%)
N3	4 (19%)
M Stage	
M0	21 (100%)

Table 2. List of lesions with site, GTV and 50% SUV volume size.

	Site of lesion	GTV volume (cm^3)	Pre 50% SUV volume (cm^3)	Sub 70% SUV volume (cm^3)
Patient 1	Primary	34.7	5.6	0.7
Patient 2	Primary	29.8	2.7	1.8
Patient 3	Primary	14	5.3	0.5
Patient 4	Primary	69.8	36.9	5.8
Patient 4, Node 1	RT inguinal node	23.1	8.3	3.2
Patient 4, Node 2	RT external iliac node	6.2	3.5	1.2
Patient 4, Node 3	LT inguinal node	7.2	3.4	2.7
Patient 5	Primary	22.2	7.7	3.8
Patient 6	Primary	14	3.4	2
Patient 6, Node 1	LT inguinal node	18.3	5.8	0.3
Patient 6, Node 2	LT internal iliac node	13.3	5.5	2
Patient 6, Node 3	LT external iliac node	13.6	0.2	0.9
Patient 7	Primary	28.7	8.9	3
Patient 8	Primary	13.5	5.3	3.1
Patient 9	Primary	16.9	11.7	1.1
Patient 10	Primary	10.6	2.2	0.2
Patient 10, Node	RT mesorectal node	11.1	1.2	0.1
Patient 11	Primary	14.4	4.1	0.8
Patient 12	Primary	16.4	7	1.4
Patient 13	Primary	4.9	2.3	0.3
Patient 14	Primary	154.2	79.2	3
Patient 15	Primary	109.1	58	29
Patient 15, Node	RT inguinal node	5.8	4.7	1.3
Patient 16	Primary	23.1	8.9	1.7
Patient 17	Primary	32.6	6.9	2.1
Patient 18	Primary	9.7	0.5	0.5
Patient 19	Primary	17.5	4.6	1
Patient 20	Primary	6.4	1	0.7
Patient 21	Primary	63.5	14.4	6.2

RT – right; LT – left; GTV – gross tumour volume; Pre 50% SUV – volume created from 50% of maximum SUV on initial PET/CT scan; Sub 70% SUV – volume created from 70% of maximum SUV on PET/CT scan taken fraction 8 or 9 of radiotherapy.

Table 3: Overlapping fractions between volumes on the pre-treatment scan (Pre34%, Pre40%, Pre50%, Pre60% and Pre70% of the SUV max) and the subsequent interim scans (Sub70% and Sub80% of the SUV max).

Patient	Sub70%					Sub80%				
	Pre34%	**Pre40%**	**Pre50%**	**Pre60%**	**Pre70%**	**Pre34%**	**Pre40%**	**Pre50%**	**Pre60%**	**Pre70%**
Patient 1	1	1	0.86	0.71	0.43	1	1	1	1	1
Patient 2	0.50	0.28	0.11	0.56	0	0.60	0.40	0.20	0	0
Patient 3	1	0.80	0.60	0.40	0.20	1	1	0.50	0.50	0
Patient 4	1	1	1	0.95	0.66	1	1	1	1	1
Patient 4 Node 1	0.66	0.63	0.56	0.47	0.38	0.50	0.40	0.40	0.40	0.40
Patient 4, Node 2	0.33	0.25	0.17	0.83	0	0.33	0	0	0	0
Patient 4, Node 3	0.89	0.81	0.67	0.52	0.33	0.91	0.91	0.73	0.64	0.36
Patient 5	0.97	0.95	0.82	0.63	0.42	1	1	0.95	0.79	0.47
Patient 6	0.95	0.90	0.80	0.60	0.30	1	1	0.90	0.70	0.30
Patient 6, Node 1	1	1	1	1	0.67	1	1	1	1	1
Patient 6, Node 2	1	1	1	0.95	0.75	1	1	1	1	1
Patient 6, Node 3	0.22	0.11	0	0	0	0	0	0	0	0
Patient 7	0.97	0.97	0.93	0.83	0.83	0.92	0.92	0.92	0.92	0.92
Patient 8	0.84	0.77	0.65	0.52	0.35	0.93	0.86	0.71	0.57	0.43
Patient 9	1	1	1	1	0.91	1	1	1	1	1
Patient 10	1	1	1	1	1	1	1	1	1	1
Patient 10, Node	0	0	0	0	0	1	0	0	0	0
Patient 11	1	1	1	1	0.88	1	1	1	1	1
Patient 12	1	1	1	0.93	0.86	1	1	1	0.83	0.83
Patient 13	0	0	0	0	0	0	0	0	0	0
Patient 14	1	1	0.90	0.30	0	1	1	0.93	0.29	0.00
Patient 15	0.98	0.95	0.89	0.80	0.65	1	1	0.94	0.85	0.66
Patient 15, Node	0.92	0.92	0.85	0.77	0.69	1	1	1	1	0.75
Patient 16	1	1	1	0.94	0.94	1	1	1	1	1
Patient 17	0.76	0.71	0.67	0.57	0.38	0.80	0.80	0.70	0.70	0.40
Patient 18	0.60	0.40	0.20	0.20	0	1	1	0	0	0
Patient 19	0.90	0.80	0.60	0.30	0.10	1	0.75	0.75	0.50	0
Patient 20	1	0.86	0.71	0.43	0.14	1	1	0.67	0.33	0.33
Patient 21	0.98	0.97	0.81	0.39	0.07	1	1	0.94	0.50	0.06
Median	0.97	0.92	0.81	0.60	0.38	1.00	1.00	0.92	0.70	0.40

Table 4: Vector Distances between volumes on the pre-treatment scan (Pre34%, Pre40%, Pre50%, Pre60% and Pre70% of the SUV max) and the subsequent interim scans (Sub70% and Sub80% of the SUV max).

Patient	Sub70%					Sub80%				
	Pre34%	**Pre40%**	**Pre50%**	**Pre60%**	**Pre70%**	**Pre34%**	**Pre40%**	**Pre50%**	**Pre60%**	**Pre70%**
Patient 1	0.88	1.04	0.74	0.63	0.55	1.00	0.98	0.86	0.74	0.61
Patient 2	0.96	1.02	1.11	1.14	1.08	0.81	0.85	0.92	0.95	0.90
Patient 3	0.77	0.82	0.86	0.94	0.98	0.85	0.89	0.94	1.03	0.34
Patient 4	0.90	0.34	0.36	0.36	0.38	0.33	0.88	0.90	0.92	0.92
Patient 4 Node 1	0.81	0.73	0.62	0.44	0.42	1.22	1.14	1.02	0.86	0.82
Patient 4, Node 2	0.91	0.93	0.97	0.96	0.96	0.93	0.93	0.95	0.94	0.91
Patient 4, Node 3	0.50	0.53	0.51	0.51	0.55	0.55	0.57	0.55	0.55	0.58
Patient 5	0.33	0.31	0.28	0.30	0.32	0.36	0.34	0.32	0.36	0.39
Patient 6	0.95	0.93	0.89	1.02	1.08	1.00	0.99	0.95	1.08	1.13
Patient 6, Node 1	0.34	0.33	0.31	0.25	0.11	0.47	0.48	0.45	0.39	0.24
Patient 6, Node 2	1.47	1.93	2.03	1.46	1.94	2.94	3.39	3.49	3.51	3.41
Patient 6, Node 3	0.56	0.49	0.38	0.39	0.39	0.67	0.59	0.38	0.31	0.31
Patient 7	0.54	0.53	0.51	0.50	0.55	0.65	0.64	0.61	0.57	0.57
Patient 8	0.51	0.50	0.46	0.41	0.32	0.75	0.74	0.71	0.66	0.56
Patient 9	0.15	0.16	0.20	0.16	0.20	0.19	0.19	0.16	0.21	0.24
Patient 10	0.80	0.76	0.69	0.66	0.68	0.82	0.77	0.71	0.67	0.71
Patient 10, Node	0.31	0.31	0.33	0.33	0.36	0.37	0.37	0.40	0.39	0.42
Patient 11	0.67	0.62	0.60	0.60	0.59	0.63	0.58	0.54	0.51	0.51
Patient 12	1.12	1.16	1.23	1.29	1.39	1.13	1.17	1.24	1.30	1.36
Patient 13	0.55	2.43	2.49	2.85	3.28	2.54	2.51	2.57	2.93	3.36
Patient 14	0.25	0.55	0.56	0.59	0.80	1.57	1.57	1.57	1.60	1.69
Patient 15	0.63	0.23	0.19	0.19	0.21	0.51	0.47	0.42	0.36	0.33
Patient 15, Node	0.25	0.23	0.19	0.19	0.21	0.51	0.47	0.42	0.36	0.33
Patient 16	0.51	0.47	0.42	0.37	0.37	0.57	0.54	0.51	0.47	0.47
Patient 17	0.75	0.75	0.76	0.77	0.77	0.74	0.74	0.74	0.79	0.79
Patient 18	0.65	0.56	0.31	0.41	0.25	0.71	0.63	0.31	0.35	0.31
Patient 19	0.19	0.65	0.72	0.83	0.96	0.62	0.61	0.68	0.78	0.92
Patient 20	0.63	0.21	0.26	0.31	0.36	0.26	0.25	0.24	0.25	0.30
Patient 21	2.43	0.64	0.57	0.60	1.87	0.97	0.98	0.92	0.91	2.17
Median	0.65	0.62	0.60	0.59	0.55	0.74	0.74	0.71	0.74	0.61
Min-max	0.15 – 2.43	0.16 – 2.43	0.19 – 2.49	0.16 – 2.85	0.11 – 3.28	0.19 – 2.94	0.19 – 3.39	0.20 – 3.49	0.21 – 3.51	0.24 – 3.41

Illustrations fig 1

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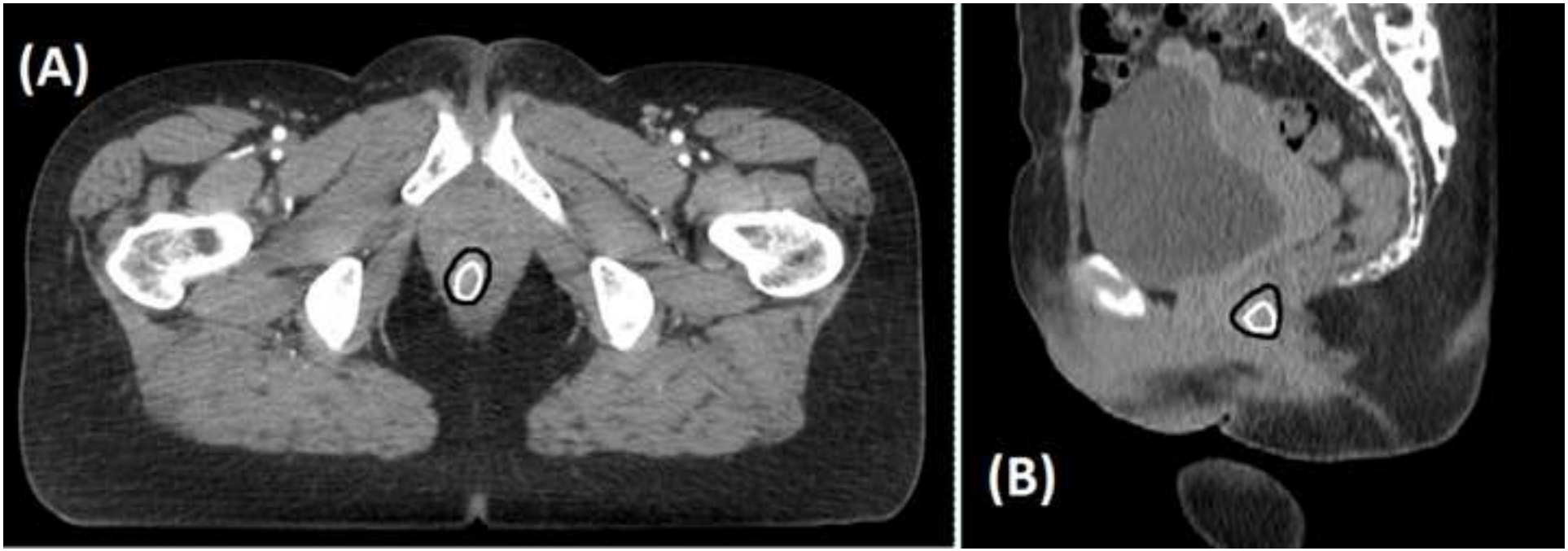

Illustrations fig 2

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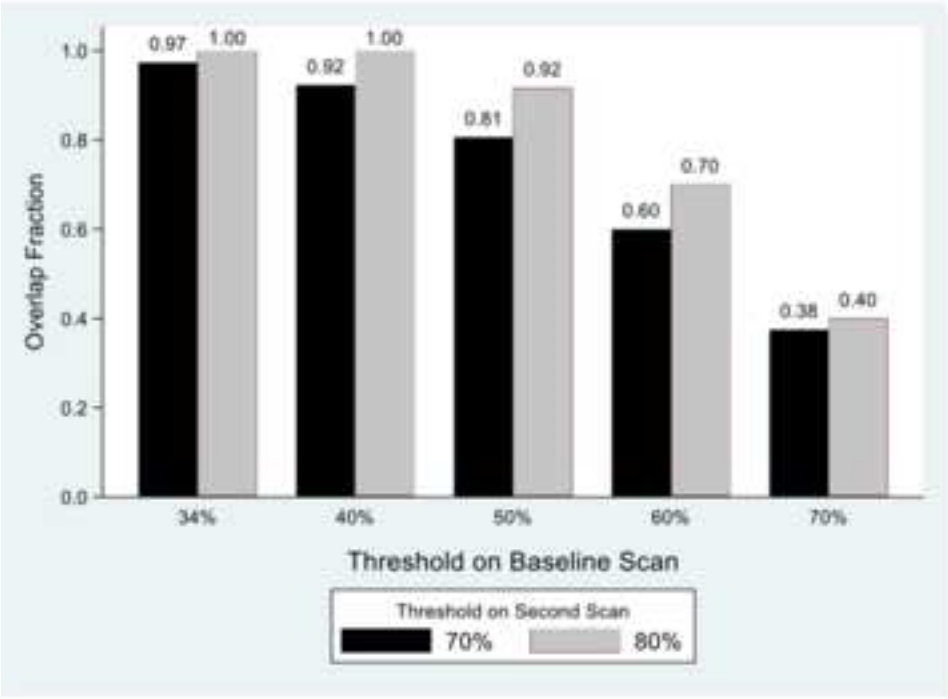

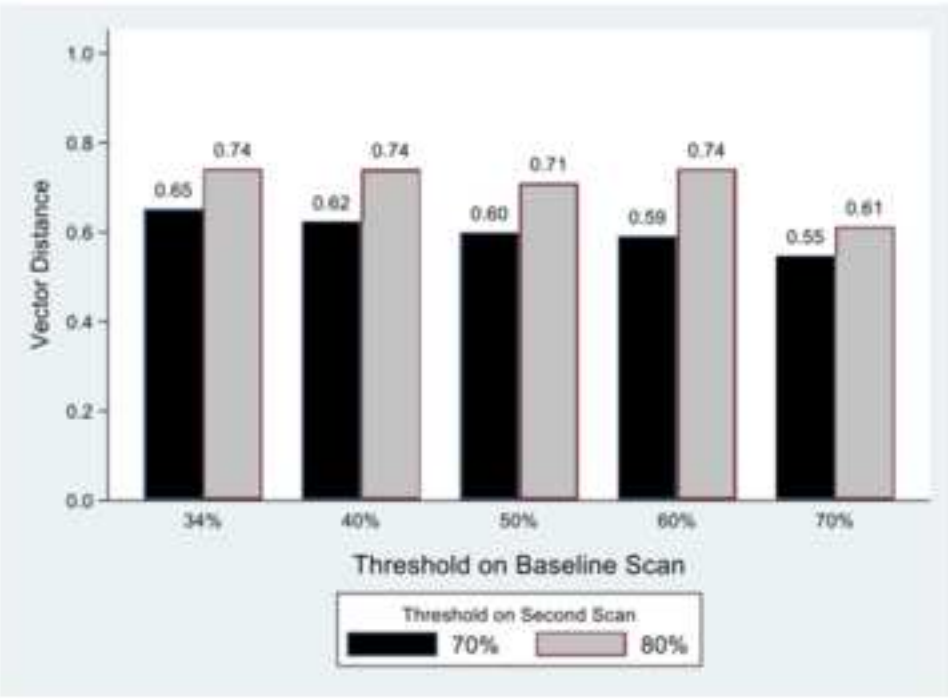

Illustrations fig 3

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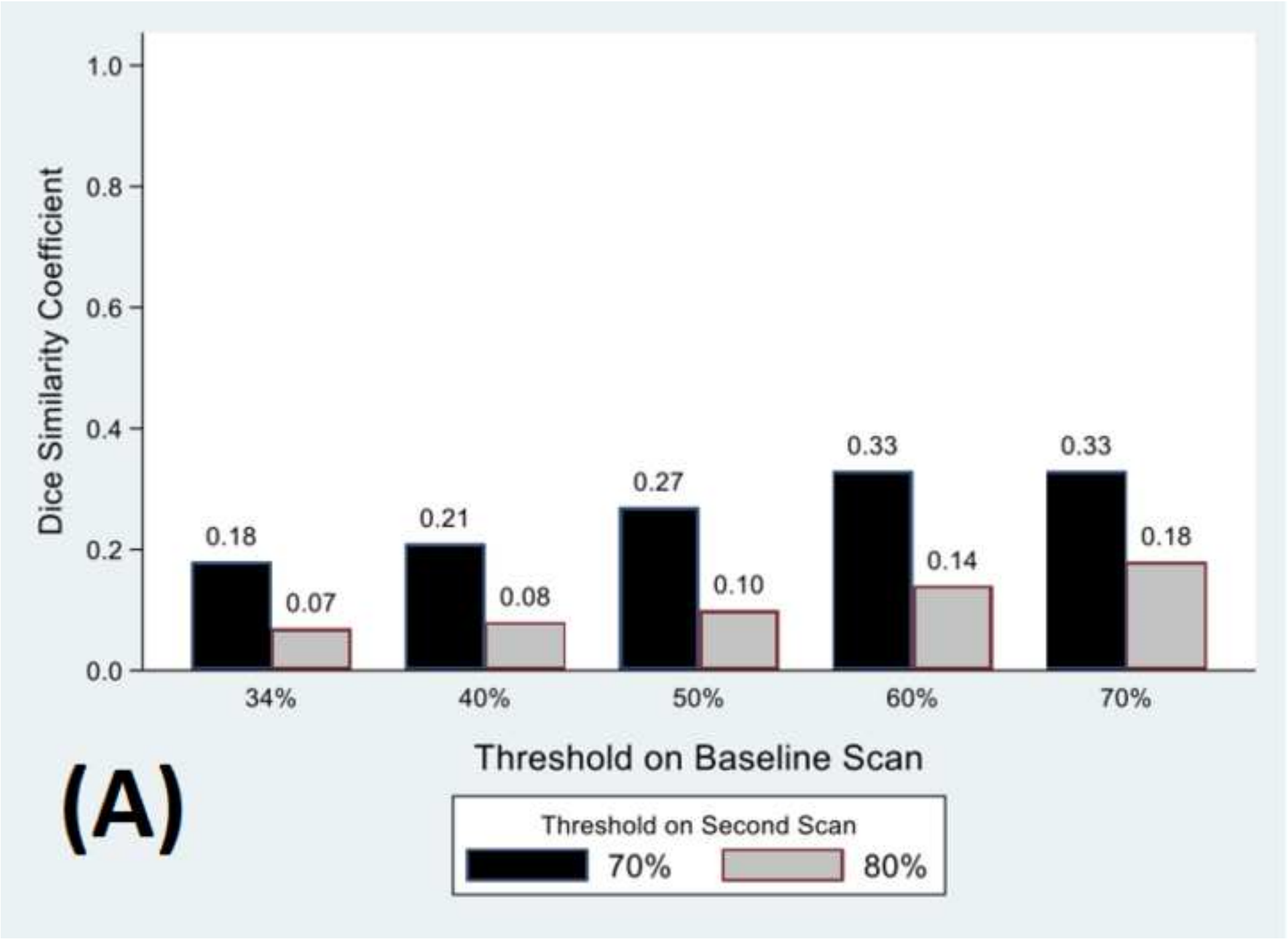

Abstract

Aim

We aim to investigate the potential role for a biological ~~radiotherapy~~ boost in anal cancer by assessing whether ~~the~~ subvolumes of high FDG avidity, identified at outset, are spatially consistent during a course of chemoradiotherapy (CRT).

Methods

FDG-PET scans from 21 patients enrolled into the ART study (NCT02145416) were retrospectively analysed. A total of twenty-nine volumes including both primary tumours and involved nodes >2 cm were identified. FDG-PET scans were ~~done~~performed prior to treatment and ~~at~~ day 8 or 9 of CRT. ~~Tumours~~FDG subvolumes were ~~segmented~~created using a percentage of maximum FDG avidity at thresholds of 34%, 40%, 50%, on the pre-treatment scans, and ~~at~~ 70% and 80% on the subsequent scans. Both FDG-PET scans were deformably registered to the planning CT scan. The overlap fraction (OF) and vector distance (VD) were calculated~~.~~ to assess spatial consistency. FDG subvolumes for further investigation had OF >0.7, as this has been defined in previous publications as a "good" correlation.

Results

The median ~~(interquartile range (IQR)) overlap fraction (~~OF~~) of~~ between the ~~Pre34% (~~diagnostic FDG-PET subvolumes 34%, 40% and 50% of max SUV and subsequent FDG-PET subvolumes of 70% of max SUV were 0.97~~ (0.76, 1.00)), Pre40% (~~, 0.92 ~~(0.71, 1.00))~~ and ~~Pre50% (~~0.81~~ (0.60, 1.00)) thresholds with the Sub70% thresholds were each above 0.8.~~. The median ~~(IQR) OF of the Pre34% (1.00 (0.~~OF between the diagnostic FDG-PET subvolumes 34%, 40% and 50% and subsequent FDG-PET subvolumes of 80% were 1.00, 1.00 and 0.92~~, 1.00)), Pre40% (1.00 (0.80, 1.00)) and Pre50% (0.92 (0.50, 1.00)) thresholds with the Sub80% thresholds were each above 0.9.~~. The median (range) VD values between ~~Pre34~~diagnostic FDG-PET subvolumes 34%, 40% and ~~Sub80~~50% and ~~Pre40% and the Sub80~~subsequent FDG-PET subvolumes of 80% were 0.74mm (0.19-2.94) ~~and~~ 0.74mm (0.19-3.39) and 0.71 (0.2-3.29) respectively. ~~Using a mean OF of > 0.7 defined in previous publications as a "good" correlation, 20~~Twenty out of 29 ~~of the Pre50% subvolumes~~ volumes

(69.0%) achieved ~~that threshold with the relevant Sub80% volumes.~~a threshold of >0.7 between the FDG 50% subvolume on the diagnostic scan and the FDG 80% subvolume on the subsequent scan.

Conclusion

~~Areas~~FDG avid subvolumes identified at baseline were spatially consistent ~~between baseline and the second week of~~during a course of ~~radiotherapy~~CRT treatment. The ~~threshold~~subvolume of 50% of SUVmax on the pre-treatment scan could be considered as a potential target for dose escalation.

Keywords Biological boost, Anal cancer, FDG PET scan, Radiotherapy.

Introduction

Radical chemoradiotherapy (CRT) is the established standard of care for localised squamous cell carcinoma (SCC) of the anus delivering doses up to 66 Gy in 1.8 to 2.2Gy/fraction concurrently with chemotherapy [1]. CRT provides 3 year progression free survival (PFS) of 73% [2], however despite the widespread use of intensity modulated radiotherapy (IMRT), acute and chronic toxicity remains significant. Acute toxicities include radiation dermatitis, pain, gastrointestinal and genitourinary disturbance; these usually resolve 2-3 months following CRT. Late toxicities however can be lifelong and significantly affect quality of life; these include diarrhoea, faecal incontinence, dyspareunia and erectile dysfunction [3-6].

Treatment failure occurs most often in patients with locally advanced disease, with approximately 75% of relapses occurring at the site of the original tumour [7-10]. A tumour control probability model has suggested dose escalation may improve tumour control by potentially reducing local relapse in these patients [11]. PLATO (PersonaLising Anal cancer RadioTherapy dOse, ISRCTN88455282), the international anal cancer trial funded by Cancer Research UK~~,~~; is investigating the role of dose escalation to the primary site of gross tumour in locally advanced disease [12]. Dose escalation to the whole gross tumour would likely add acute and late toxicity to the treatment; so the use of functional imaging to identify a smaller biological boost is of interest; as smaller volumes are recognised to minimise toxicity [13].

Over 90% of squamous cell anal tumours are 18F-flurodeoxyglucose (FDG) avid on Positron Emission Tomography (FDG-PET) scan [14] and there has been significant interest in the role of FDG-PET in anal cancer management. Trautmann et al. reported in a study of 21 patients, that the diagnostic FDG-PET identified additional nodes and metastasis not seen on MRI and

CT, having implication for prognosis and radiotherapy planning [15]. The metabolically active volume on diagnostic images have also correlated with local recurrence in small series of 23 and 45 patients respectively [16, 17]. It is likely metabolically tumour volume (MTV) correlates with lesion size therefore this finding is in keeping with the phase III data highlighting larger lesions do worse [18]. Kidd et al however identified a new finding that increased SUV at outset correlated with disease free survival and the presence of persistence or recurrence on subsequent FDG-PET's in 77 patients [19]. A number of single centre publications have reviewed between 23 and 110 patients with diagnostic FDG-PET and repeat FDG-PET completion of CRT at time points ranging from 3-36 weeks [20-22]. The metabolic response correlated in these studies with one or more of: local control rate, progression free survival, cancer specific survival or overall survival. Lastly, Hong et al looked at 23 patients with FDG-PET at outset and after 30Gy of radiotherapy [16]. They found that the interim scan MTV and total lesion glycolysis correlated with freedom from local progression.

All of the FDG-PET data in anal cancer to date has looked at predictive value of diagnostic or repeat FDG-PET, the authors are unaware of any other publication investigating the role of a biological boost in anal carcinoma. In other tumour types treated by concomitant CRT, such as non-small cell lung carcinoma (NSCLC), head and neck squamous cell carcinomas (HNSCC) and pancreatic adenocarcinoma; FDG-PET/CT scans have been reported to identify tumour subvolumes at greater risk of relapse [23-29]. There are ongoing trials investigating the use of biological boosts in HNSCC and NSCLC cancer [30-32].

The ART (Anal squamous cell carcinoma: Investigation of functional imaging during chemoRadioTherapy) study (NCT02145416) is a feasibility trial investigating the use of

functional imaging to predict response in anal cancer. All patients receive baseline FDG-PET/CT scans and further scans at fraction 8-10. We performed a post-hoc analysis on images obtained ~~on~~in this trial. Our aim is to investigate the feasibility of defining a biological boost in anal squamous cell carcinoma by assessing whether FDG avid subvolumes, identified at baseline, are spatially consistent in the second week of a course of CRT.

Materials and Methods

Patient Population

We identified 21 patients with paired scans performed at diagnosis and at fraction 8 or 9 of CRT, obtained from the ART study. 29 patients were recruited however 4 patients withdrew consent prior to any imaging and a further 4 patients withdrew consent following their initial scans due to ongoing social and medical issues. The ART study is a single arm, single centre imaging study, of patients receiving radical CRT for anal cancer within Oxford University Hospitals; evaluating changes in functional imaging during CRT treatment. ART recruited patients who were aged 18 years or above, had histologically confirmed invasive primary squamous carcinoma of the anus, had any tumour stage ≥T2N0 and were fit to receive radical CRT with curative intent. All patients had given informed consent in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Ethical approval incorporated post-hoc analysis of images to answer additional questions.

Chemoradiotherapy

CRT was delivered according to UK based guidance [33]. In summary patients received 50.4 or 53.2Gy to the primary tumour depending on TNM stage, 50.4Gy to involved nodes and

40Gy to prophylactic dose to non-involved nodes; all in 28 fractions using a simultaneous integrated boost. ~~One patient was~~ Three patients were treated within the PLATO study ~~and were randomised~~, two with a dose of 61.6Gy to ~~receive~~the primary tumour while maintaining 40Gy to the prophylactic nodes; the other patient with a reduced dose of 41.4Gy to the primary tumour and 34.5Gy to elective nodes ~~all~~ in 23 fractions with simultaneous integrated boost. Treatment was delivered using IMRT or volumetric modulated arc therapy (VMAT), calculated using a type B algorithm, prescribing 100% to the median dose of the planned target volume (PTV) boost. Chemotherapy was Mitomycin 12 mg/m^2 Day 1 and Capecitabine 825 mg/m^2 orally twice a day on radiotherapy treatment days.

FDG-PET/CT scanning

All patients had a routine, full body, diagnostic FDG-PET/CT scan performed at baseline. As part of the ART study, 18 patients underwent a second FDG-PET/CT on fraction 8 and three on fraction 9 of their CRT.

All scans were performed using GE Discovery 690 scanners (GE healthcare, Buckinghamshire, UK). Patients were positioned supine with arms above the head, on a flat-top couch using radiotherapy immobilisation equipment to replicate their treatment position. After fasting for 6 hours and ensuring that the blood glucose was < 10 mmol/L, FDG was injected at a dose of 4 MBq/kg (up to 600 MBq). FDG-PET/CT acquisition commenced after an uptake time of 75 minutes. The initial FDG-PET/CT scanned the whole body. For the second FDG-PET/CT, 8 patients underwent an image limited to the pelvis, 13 patients had a further full body image.

Scans were performed in 3D with a scan time of 4 minutes at each bed position. For the CT phase, 120 kV automA (max 250 mA), noise index 25.0 0.5 s/rotation, pitch 0.984:1, 3.75

mm slice width was used. Attenuation corrected FDG-PET/CT images were used in the analysis.

Image analysis

The paired FDG-PET/CT images and the planning CT scans were imported into Mirada (Mirada Medical, Build 1.2.0.39, Oxford, UK). All data analysis was performed and checked by AS and RM. The planning CT scan and CT component of each FDG-PET/CT scan underwent a rigid registration followed by a deformable registration. The quality of both registrations was assessed visually by an experienced clinician and if the registration was judged poor, due to the FDG avidity lying outside the gross tumour, a rectangular region of interest (ROI) was drawn around the volume incorporating surrounding muscles and bones. The registration was repeated using that ROI. As the FDG-PET and CT components of the FDG-PET/CT scan share the same intrinsic frame of reference, this registered each FDG-PET image with the planning CT scan. To create the ~~volumes~~subvolumes, an elliptical ROI was drawn solely around the area of increased uptake within the anus on each FDG-PET scan, and tumour segmentation was performed as a percentage of the SUVmax within the elliptical ROI.

The method used to assess spatial consistency of volumes has been previously published by groups performing similar work in different tumour types [23, 24]. On the baseline FDG-PET, tumour segmentation was performed at 34%, 40%, 50%, 60% and 70% of the SUVmax (Pre34%, Pre40%, Pre50%, Pre60% and Pre70% respectively). On the subsequent scans ~~thresholds~~subvolumes of 70% and 80% of the SUVmax were used (Sub70% and Sub80% respectively). The overlap fraction (OF) between the baseline and the second scan was calculated and expressed as a fraction of the volume of the second scan. Based on previous publications of this method, a mean OF of > 0.7 supported the use of a threshold as a target

for a biological boost [23, 24]. Following this, the planning scan with attached subvolumes was loaded into Eclipse software (version 13.0, Varian medical systems, Palo Alto USA). The centre of mass (COM) shifts were used to calculate a vector distance (VD) which was calculated using the following formula:

$$VD = \sqrt{(\Delta x)2 + (\Delta y)2 + (\Delta z)2}.$$

Figure 1 demonstrates the planning scan of Patient 14 with two volumes (Pre50% and Sub70%) highlighted. The Dice Similarity coefficient (DSC) was calculated for completeness however limitations of this method of analysis in this setting are highlighted in discussion.

Statistics

OF, VD and DSC between ~~thresholds~~subvolumes of the baseline and subsequent scans were described using medians ~~(interquartile range (IQR))~~ and means ~~(standard deviation (SD)).~~and full data reported.

Results

Twenty-one patients underwent two FDG-PET/CT scans between January 2015 and November 2017 at the two time points within the ART study and these images were used for this analysis. Areas of gross tumour of primary disease as well as nodal disease >2cm in the longest diameter were analysed, resulting in 29 volumes in total.

Patient characteristics

Table 1 describes the patient and tumour demographics.

Imaging results

The gross tumour volumes (GTVs), Pre50% and Sub70% volumes in cm^3 are given in Table 2. ~~The median (interquartile range (IQR)) overlap fraction (OF)~~In terms of OF, the median OF of the Pre34~~% (0.97 (0.76, 1.00)),~~%, Pre40% ~~(0.92 (0.71, 1.00))~~ and Pre50% ~~(0.81 (0.60,~~

~~1.00)) thresholds~~ with the Sub70% ~~thresholds were each~~subvolumes are all above 0.8. The median ~~(IQR)~~ OF of the Pre34~~% (1.00 (0.92, 1.00)),~~%, Pre40~~% (1.00 (0.80, 1.00))~~%, and Pre50% ~~(0.92 (0.50, 1.00)) thresholds~~subvolumes with the Sub80% ~~thresholds were each~~subvolumes are all above 0.9. The mean ~~(SD)~~ OF between the Pre34%, Pre40%, Pre50%, Pre60% and ~~the~~ Pre70% and the Sub80% ~~were~~fell as the volumes reduced in size; from 0.86 ~~(0.29),~~with Pre34%, 0.79 ~~(0.36),~~with Pre40%, 0.70 ~~(0.38), 0.60 (0.38)~~with Pre50%, with Pre60% and Pre70% both below 0.~~48 (0.42) respectively~~7. The median ~~(IQR)~~ VD values between Pre34%, Pre40% and Pre50% and Sub80% ~~and Pre40% and the Sub80%~~ were 0.74mm ~~(0.19-2.94) and~~, 0.74mm ~~(~~and 0.~~19-3.39)~~71mm respectively. Figure 2 illustrates the median OF and VD for the different volumes. Full details on OFs and VD between volumes of high FDG avidity on the pre-treatment scan and the subsequent interim scan are shown in Tables 3 and 4 respectively. As expected the DSC values were poor. Figure 3 illustrates the median DSC for the different volumes assessed. DSC for all the ~~thresholds~~subvolumes per node per patient are available in supplementary material.

Discussion

FDG uptake within tumours is heterogeneous. The first steps to delivering ~~a~~an FDG derived biological boost is to determine whether the areas of high avidity are spatially consistent during a course of radiotherapy. We report that in the majority of patients studied, the areas identified at baseline were indeed spatially consistent at fraction 8-9. This introduces the possibility of selectively boosting those areas at highest risk of relapse to improve local control. This has the potential to improve overall survival in this rare tumour type where dose escalation to the whole tumour is in use and under investigation; but acute and late toxicities may be dose limiting.

We have shown that areas of increased FDG uptake remain reasonably consistent between baseline and in the second week of radiotherapy treatment. ~~Areas~~Subvolumes of high FDG uptake before and during CRT showed good agreement~~, with~~. Both the ~~Pre34%~~mean and ~~Pre40%~~medians of ~~volumes largely corresponding with~~ the ~~Sub70% and Sub80%~~OF between the diagnostic FDG-PET subvolumes of ~~volumes (~~34%, 40% and 50% of SUVmax and subsequent FDG-PET subvolumes of 70% and 80% were all OF > 0.7~~).~~. It was notable that agreement between those volumes was less successful in nodal disease compared to primary disease (mean OFs between ~~thePre34% and Sub80%~~the diagnostic FDG-PET subvolumes of 34% and 40%, and subsequent FDG-PET subvolumes of 80% were 0.95 ~~vs. 1 respectively~~ and 0.65 for nodal ~~and~~ volumes but 1 for all primary ~~disease, and between the Pre40% and Sub80% subvolumes 0.65 vs. 1 respectively~~tumours). This may be due to smaller volumes of nodal disease, where a slight inaccuracy in registration can have a significant effect on the results, therefore dose escalation to nodal sites might only be feasible with advanced image guidance or treatment adaptation.

Our approach to assessing volume overlap is based on a publication in NSCLC [23]**.** Aerts et al, recommended the FDG-PET subvolume of 50% of SUV max ~~threshold~~ on the ~~pre-treatment~~diagnostic FDG-PET/CT as a target for biological boost [34]. This was based on overlap between the diagnostic scans and post treatment residual disease therefore we acknowledge that our suggested boost volume demonstrates reproducibility rather than site of residual tumour or relapse as our second scan point is the second week of treatment. A study of pancreatic cancer [24], found that threshold of 40% of the SUVmax on baseline FDG-PET/CT identifies areas of residual metabolic activity seen on a post-CRT FDG-PET/CT, and this threshold could aid in the definition of a biological target volume.

Wilson et al and Aerts et al, detailed above, proposed that a mean OF > 0.7 supported the use of a threshold as a target for a biological boost. We have presented the median as the outcomes of interests are normally distributed however in order to allow comparison with previous literature we have also reported the mean (SD). Using a mean OF of > 0.7 defined in previous publications as a "good" correlation, the ~~Pre-treatment volumes~~ diagnostic FDG-PET subvolumes showed generally satisfactory results ~~with 24 out of 29 of~~. When comparing the ~~Pre34% volumes (83%), 23 out of 29 (79%) of the Pre40% subvolumes, 20 out of 29 of the Pre50%~~FDG-PET subvolumes ~~(69%)~~of 34%, 40%, 50%, 60% and ~~15 out of 29~~70% of the ~~Pre60%~~SUVmax with the subsequent subvolumes of 80%; 24 of 29 (83%), 23 (79%), 20 (69%), 15 (52%) ~~achieving that threshold with the relevant Sub80% volumes. However, considering the Pre70% volume, only~~and 11 ~~subvolumes~~ (38%) volumes achieved ~~the 0.7 OF~~that threshold. Therefore, it would be reasonable to consider the ~~Pre50% SUVmax~~diagnostic FDG-PET threshold of 50% of SUVmax as a possible target for dose escalation based on pre-treatment imaging, as it achieved a mean OF of 0.7 used in other papers and was reasonably consistent with > 70% of cases showing good overlapping agreement. As the ~~Pre50% subvolume~~diagnostic FDG-PET threshold of 50% represented a mean of only 34% of the GTV, the reduction to volume would be substantial. Therefore boosting this reduced volume to a higher radiation dose is likely to result in reduced normal tissue toxicity than boosting the whole GTV, although this would require confirmation.

Our study has some limitations; the fact the study incorporates small numbers is representative of a rare disease. However even with small numbers our study serves to demonstrate the feasibility of defining a biological boost in anal cancer and suggests possible boost volumes for further investigation. There are multiple methods of reporting differences between two volumes [35]. We have primarily used the method previously

published by Aerts et al. as they compared spatial position in volumes with changing sizes. Our assessment of the volumes using DSC suggests less spatial correlation. However these contrasting results between our primary methods of comparison and DSC were also seen in the study done by Calais et al [19], who highlighted that two volumes with a good superimposition but a large difference in size will yield low Dice and Jaccard indices. DSC values have been calculated for completeness however the poor DSC correlation should be taken in the context of its limitations when comparing different sized lesions. In our study, it was sometimes challenging to separate the tumour FDG activity from the background physiological uptake. All the subvolumes in our study were checked visually and areas of overlap with normal organs were removed. This may also be responsible for the only moderate overlap seen when like-for-like ~~thresholds~~subvolumes were compared (Pre70% vs Sub70%), as such a comparison would leave no margin for small errors in registration and variations in background physiological FDG uptake. Results must be interpreted with caution in view of the potential for errors with deformable registration. The differing field of view in the second FDG-PET/CT scans may raise some concerns. This occurred due to an amendment to the trial protocol in order to incorporate a further tertiary endpoint investigating the bone marrow toxicity of CRT. However SUV thresholds are calculated independently of non-pelvic organs using the patients' weight and activity in the primary tumour therefore this will not have affected results. Finally, the ~~thresholds~~subvolumes used within this study are based on previous literature and we acknowledge the SUV threshold levels are somewhat arbitrary. The authors have based the study and the methods on best available evidence and we anticipate further investigation of biological boost volumes in a larger prospective format would be required to validate these methods, to guide patient

selection and quantify the reduction in toxicity with smaller boost volumes in comparison to whole GTV.

Previous publications confirmed higher SUV correlates with worse outcome [16, 17, 19] therefore the next question that must be addressed is the hypothesis – Does local relapse occur at sites consistent with the high FDG avid subvolumes identified on diagnositic PET? Unfortunately, as FDG-PET/CT has only recently been incorporated into staging of anal cancer in the UK, and there is a relatively low loco-regional relapse rate; there are not sufficient numbers of patients with baseline and subsequent FDG-PET/CT to perform this analysis. Therefore, we must be clear that the proposed ~~thresholds~~subvolumes are currently based on identifying a reproducible target, further work is required to confirm the proposed ~~thresholds~~subvolumes are appropriate to cover sites of subsequent relapse using scans following treatment. We plan to perform this analysis initially on the ART cohort in due course and ideally subsequently in a larger number of patients as part of a prospective trial.

Lastly, investigation to date regarding the role of functional imaging to predict response is detailed in the introduction. The primary endpoint of the ART study is in fact based on the hypothesis we can use functional imaging as an early predictor of response in order to perform treatment intensification, perhaps with a radiotherapy boost, on a selected group identified as poor responders. However the ability to select patients for dose escalation does not deter from the potential to use an FDG derived boost to these selected patients, as a method of improving local control while limiting toxicity.

In conclusion, on the basis of these findings we conclude that areas of high FDG avidity on ~~pre-CRT~~diagnostic FDG-PET scans remain spatially consistent during a course of treatment. A ~~threshold~~subvolume of 50% of SUVmax on the ~~pre-treatment~~diagnostic scan could be

considered as the target for dose escalation. Despite the limitations of this small study, it demonstrates the potential of using a biological boost rather than whole GTV dose escalation; which may facilitate improved outcomes while minimizing the significant side effects of whole GTV dose escalation. This study suggests an exciting avenue for further investigation.

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Figure 1. Axial (A) and sagittal (B) slices through a planning CT scan with the Pre50% (black) and the Sub70% (white) volumes illustrated.

Figure 2. Illustration of median Overlap Fraction and Vector Distances between the different volumes assessed.

Figure 3. Illustration of the median Dice Coefficient of the different volumes assessed.

CLINICAL ONCOLOGY

AUTHORSHIP RESPONSIBILITY, FINANCIAL DISCLOSURE & CONTRIBUTORSHIP

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4. Contributorship: Please list each authors' contribution to the above manuscript. Please provide details such as, the original idea for the research, which individual(s) undertook the collection of data, proportion of patients entered by an individual, responsibility for the first draft of the manuscript, etc.

.2.
Author 1 __Dr Sabbagh performed all the analysis for the paper and wrote a first draft of the manuscript

Author 2 __Dr Jacobs recruited and gathered data for the study and contributed to the running of the trial and manuscript

Author 3 Ms Cooke facilitated the correct images and radiotherapy while patients were on trial, aided with analysis and contributed to the manuscript._____
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Author 4 Ms Chu facilitated the correct images and radiotherapy while patients were on trial, aided with analysis and contributed to the manuscript._____

Author 5 __Dr Strauss contributed to the statistical analysis, and contributed to the paper_____
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Author 6 Mr Virdee contributed to the statistical analysis, and contributed to the paper_____
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Author 7 Prof Hawkins was a member of the TMG on the trial and contributed to the manuscript.

Author 8 Dr Aznar contributed to the trial design, analysis and manuscript.

Author 9 Dr Muirhead conceived of the trial, did some analysis, checked analysis and wrote up large parts of the manuscript.

Please also give details of persons (who may not be on the list of authors) who provided statistical advice for the data from the inception of the study and undertook statistical analyses.

Signatures

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Author 5 ___ ..... Date ____9/8/18_____

Author 6 _ Date ____9/8/18_____

Author 7 Date ____9/8/18_____

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***Conflict of Interest Statement**

The authors have no conflicts of interest to disclose.

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Supplementary data file

Click here to download Supplementary data file: Sabbagh et al_Supplementary Material.docx

Original Article

Is There a Role for an 18F-fluorodeoxyglucose-derived Biological Boost in Squamous Cell Anal Cancer?

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Abstract

Aims: To investigate the potential role for a biological boost in anal cancer by assessing whether subvolumes of high 18F-fluorodeoxyglucose (FDG) avidity, identified at outset, are spatially consistent during a course of chemoradiotherapy (CRT).

Materials and methods: FDG-positron emission tomography (FDG-PET) scans from 21 patients enrolled into the ART study (NCT02145416) were retrospectively analysed. In total, 29 volumes including both primary tumours and involved nodes >2 cm were identified. FDG-PET scans were carried out before treatment and on day 8 or 9 of CRT. FDG subvolumes were created using a percentage of maximum FDG avidity at thresholds of 34%, 40%, 50%, on the pre-treatment scans, and 70% and 80% on the subsequent scans. Both FDG-PET scans were deformably registered to the planning computed tomography scan. The overlap fraction and the vector distance were calculated to assess spatial consistency. FDG subvolumes for further investigation had an overlap fraction > 0.7, as this has been defined in previous publications as a 'good' correlation.

Results: The median overlap fractions between the diagnostic FDG-PET subvolumes 34%, 40% and 50% of maximum standardised uptake value (SUV_{max}) and subsequent FDG-PET subvolumes of 70% of SUV_{max} were 0.97, 0.92 and 0.81. The median overlap fraction between the diagnostic FDG-PET subvolumes 34%, 40% and 50% and subsequent FDG-PET subvolumes of 80% were 1.00, 1.00 and 0.92. The median (range) vector distance values between diagnostic FDG-PET subvolumes 34%, 40% and 50% and subsequent FDG-PET subvolumes of 80% were 0.74 mm (0.19–2.94) 0.74 mm (0.19–3.39) and 0.71 mm (0.2–3.29), respectively. Twenty of 29 volumes (69.0%) achieved a threshold > 0.7 between the FDG 50% subvolume on the diagnostic scan and the FDG 80% subvolume on the subsequent scan.

Conclusion: FDG-avid subvolumes identified at baseline were spatially consistent during a course of CRT treatment. The subvolume of 50% of SUV_{max} on the pre-treatment scan could be considered as a potential target for dose escalation.

Key words: Anal cancer; biological boost; FDG-PET scan; radiotherapy

Introduction (A head)

Radical chemoradiotherapy (CRT) is the established standard of care for localised squamous cell carcinoma of the anus, delivering doses up to 66 Gy in 1.8–2.2 Gy/fraction concurrently with chemotherapy [1]. CRT provides 3-year progression-free survival of 73% [2]. However, despite the widespread use of intensity-modulated radiotherapy, acute and chronic toxicity remain significant. Acute toxicities include radiation dermatitis, pain, gastrointestinal and genitourinary disturbance; these usually resolve 2–3 months after CRT. Late toxicities, however, can be lifelong and significantly affect quality of life; these include diarrhoea, faecal incontinence, dyspareunia and erectile dysfunction [3–6].

Treatment failure occurs most often in patients with locally advanced disease, with about 75% of relapses occurring at the site of the original tumour [7–10]. A tumour control probability model has suggested that dose escalation may improve tumour control by potentially reducing local relapse in these patients [11]. PLATO (PersonaLising Anal cancer RadioTherapy dOse, ISRCTN88455282), the international anal cancer trial funded by Cancer Research UK, is investigating the role of dose escalation to the primary site of gross tumour in locally advanced disease [12]. Dose escalation to the whole gross tumour would probably add acute and late toxicity to the treatment; so the use of functional imaging to identify a smaller biological boost is of interest, as smaller volumes are recognised to minimise toxicity [13].

Over 90% of squamous cell anal tumours are 18F-fluorodeoxyglucose (FDG) avid on positron emission tomography (PET) scan [14] and there has been significant interest in the role of FDG-PET in anal cancer management. Trautmann and Zuger [15] reported in a study of 21 patients, that the diagnostic FDG-PET identified additional nodes and metastasis not seen on magnetic resonance imaging and computed tomography (CT), having implication for prognosis and radiotherapy planning. The metabolically active volume on diagnostic images has also correlated with local recurrence in small series of 23 and 45 patients [16,17]. Metabolic tumour volume probably correlates with lesion size, therefore this finding is in keeping with the phase III data highlighting that larger lesions do worse [18]. However, Kidd *et al*. [19] identified a new finding that increased standardised uptake value (SUV) at outset correlated with disease-free survival and the presence of persistence or recurrence on subsequent FDG-PETs in 77 patients. A number of single-centre publications have reviewed between 23 and 110 patients with diagnostic FDG-PET and repeat FDG-PET completion of CRT at time points ranging from 3 to 36 weeks [20–22]. In these studies, the metabolic response correlated with one or more of: local control rate, progression-free survival, cancer-specific survival or overall survival. Finally, Hong *et al*. [16] looked at 23 patients with FDG-PET at outset and after 30 Gy of radiotherapy. They found that the interim scan metabolic tumour volume and total lesion glycolysis correlated with freedom from local progression.

All of the FDG-PET data in anal cancer to date have looked at the predictive value of diagnostic or repeat FDG-PET. The authors are unaware of any other publications

investigating the role of a biological boost in anal carcinoma. In other tumour types treated by concomitant CRT, such as non-small cell lung carcinoma (NSCLC), head and neck squamous cell carcinomas and pancreatic adenocarcinoma, FDG-PET/CT scans have been reported to identify tumour subvolumes at greater risk of relapse [23–29]. There are ongoing trials investigating the use of biological boosts in head and neck squamous cell carcinomas and NSCLC [30–32].

The ART (Anal squamous cell carcinoma: investigation of functional imaging during chemoradiotherapy) study (NCT02145416) is a feasibility trial investigating the use of functional imaging to predict response in anal cancer. All patients receive baseline FDG-PET/CT scans and further scans at fractions 8–10. We carried out a post-hoc analysis on images obtained in this trial. Our aim was to investigate the feasibility of defining a biological boost in anal squamous cell carcinoma by assessing whether FDG-avid subvolumes, identified at baseline, are spatially consistent in the second week of a course of CRT.

Materials and Methods (A head)

Patient Population (B head)

We identified 21 patients with paired scans carried out at diagnosis and at fraction 8 or 9 of CRT, obtained from the ART study. Twenty-nine patients were recruited. However, four patients withdrew consent before any imaging and a further four patients withdrew consent after their initial scans due to ongoing social and medical issues. The ART study is a single-arm, single-centre imaging study of patients receiving radical CRT for anal cancer within Oxford University Hospitals, evaluating changes in functional imaging during CRT treatment. ART recruited patients who were aged 18 years or above, had histologically confirmed invasive primary squamous carcinoma of the anus, had any tumour stage ≥ T2N0 and were fit to receive radical CRT with curative intent. All patients had given informed consent in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Ethical approval incorporated post-hoc analysis of images to answer additional questions.

Chemoradiotherapy (B head)

CRT was delivered according to UK-based guidance [33]. In summary, patients received 50.4 or 53.2 Gy to the primary tumour depending on TNM stage, 50.4 Gy to involved nodes and 40 Gy prophylactic dose to non-involved nodes, all in 28 fractions using a simultaneous integrated boost. Three patients were treated within the PLATO study, two with a dose of 61.6 Gy to the primary tumour while maintaining 40 Gy to the prophylactic nodes; the other patient was treated with a reduced dose of 41.4 Gy to the primary tumour and 34.5 Gy to elective nodes in 23 fractions with simultaneous integrated boost. Treatment was delivered using intensity-modulated radiotherapy or volumetric-modulated arc therapy, calculated using a type B algorithm, prescribing 100% to the median dose of the planned target volume boost. Chemotherapy was mitomycin 12 mg/m^2 day 1 and capecitabine 825 mg/m^2 orally twice a day on radiotherapy treatment days.

FDG-PET/CT Scanning (B Head)

All patients had a routine, full-body, diagnostic FDG-PET/CT scan carried out at baseline. As part of the ART study, 18 patients underwent a second FDG-PET/CT on fraction 8 and three on fraction 9 of their CRT.

All scans were carried out using GE Discovery 690 scanners (GE Healthcare, Buckinghamshire, UK). Patients were positioned supine with arms above the head, on a flat-top couch using radiotherapy immobilisation equipment to replicate their treatment position. After fasting for 6 h and ensuring that the blood glucose was < 10 mmol/l, FDG was injected at a dose of 4 MBq/kg (up to 600 MBq). FDG-PET/CT acquisition started after an uptake time of 75 min. The initial FDG-PET/CT scanned the whole body. For the second FDG-PET/CT, eight patients underwent an image limited to the pelvis, 13 patients had a further full-body image.

Scans were carried out in three-dimensions with a scan time of 4 min at each bed position. For the CT phase, 120 kV automA (maximum 250 mA), noise index 25.0 0.5 s/rotation, pitch 0.984:1, 3.75 mm slice width were used. Attenuation corrected FDG-PET/CT images were used in the analysis.

Image Analysis (B head)

The paired FDG-PET/CT images and the planning CT scans were imported into Mirada (Mirada Medical, Build 1.2.0.39, Oxford, UK). All data analysis was carried out and checked by AS and RM. The planning CT scan and CT component of each FDG-PET/CT scan underwent a rigid registration followed by a deformable registration. The quality of both registrations was assessed visually by an experienced clinician and if the registration was judged poor, due to the FDG avidity lying outside the gross tumour, a rectangular region of interest (ROI) was drawn around the volume incorporating surrounding muscles and bones. The registration was repeated using that ROI. As the FDG-PET and CT components of the FDG-PET/CT scan share the same intrinsic frame of reference, this registered each FDG-PET image with the planning CT scan. To create the subvolumes, an elliptical ROI was drawn solely around the area of increased uptake within the anus on each FDG-PET scan and tumour segmentation was carried out as a percentage of the SUV_{max} within the elliptical ROI.

The method used to assess spatial consistency of volumes has been previously published by groups performing similar work in different tumour types [23,24]. On the baseline FDG-PET, tumour segmentation was carried out at 34, 40, 50, 60 and 70% of the SUV_{max} (Pre34%, Pre40%, Pre50%, Pre60% and Pre70%, respectively). On the subsequent scans, subvolumes of 70 and 80% of the SUV_{max} were used (Sub70% and Sub80%, respectively). The overlap fraction between the baseline and the second scan was calculated and expressed as a fraction of the volume of the second scan. Based on previous publications of this method, a mean overlap fraction > 0.7 supported the use of a threshold as a target for a biological boost [23,24]. Following this, the planning scan with attached subvolumes was loaded into Eclipse software (version 13.0, Varian Medical Systems, Palo Alto, CA, USA). The centre of mass shifts were used to calculate a vector distance, which was calculated using the following formula:

$$\text{vector distance} = \sqrt{(\Delta x)2 + (\Delta y)2 + (\Delta z)2}.$$

Figure 1 shows the planning scan of patient 14 with two volumes (Pre50% and Sub70%) highlighted. The Dice similarity coefficient (DSC) was calculated for completeness. However, limitations of this method of analysis in this setting are highlighted in the discussion.

Figure 1 here

Statistics (B head)

Overlap fraction, vector distance and DSC between subvolumes of the baseline and subsequent scans were described using medians and means and full data reported.

Results (A head)

Twenty-one patients underwent two FDG-PET/CT scans between January 2015 and November 2017 at the two time points within the ART study; these images were used for this analysis. Areas of gross tumour of primary disease as well as nodal disease >2 cm in the longest diameter were analysed, resulting in 29 volumes in total.

Patient Characteristics (B head)

Table 1 describes the patient and tumour demographics.

Table 1 here

Imaging Results (B Head)

The gross tumour volumes (GTVs), Pre50% and Sub70% volumes in cm^3 are given in Table 2. In terms of overlap fraction, the median overlap fraction of the Pre34%, Pre40% and Pre50% with the Sub70% subvolumes were all above 0.8. The median overlap fraction of the Pre34%, Pre40% and Pre50% subvolumes with the Sub80% subvolumes were all above 0.9. The mean overlap fraction between the Pre34%, Pre40%, Pre50%, Pre60% and Pre70% and the Sub80% fell as the volumes reduced in size; from 0.86 with Pre34%, 0.79 with Pre40%, 0.70 with Pre50%, with Pre60% and Pre70% both below 0.7. The median vector distance values between Pre34%, Pre40% and Pre50% and Sub80% were 0.74, 0.74 and 0.71 mm, respectively. Figure 2 illustrates the median overlap fraction and vector distance for the different volumes. Full details on overlap fractions and vector distances between volumes of high FDG avidity on the pre-treatment scan and the subsequent interim scan are shown in Tables 3 and 4, respectively. As expected, DSC values were poor. Figure 3 illustrates the median DSC for the different volumes assessed. DSC for all the subvolumes per node per patient are available in supplementary material (see Appendix A).

Figures 2 and 3 here
Tables 2-4 here

Discussion (A head)

FDG uptake within tumours is heterogeneous. The first steps to delivering an FDG-derived biological boost is to determine whether the areas of high avidity are spatially consistent during a course of radiotherapy. We report that in most patients studied, the areas identified at baseline were indeed spatially consistent at fraction 8–9. This introduces the possibility of selectively boosting those areas at highest risk of relapse to improve local control. This has the potential to improve overall survival in this rare tumour type, where dose escalation to the whole tumour is in use and under investigation, but acute and late toxicities may be dose limiting.

We have shown that areas of increased FDG uptake remain reasonably consistent between baseline and in the second week of radiotherapy treatment. Subvolumes of high FDG uptake before and during CRT showed good agreement. Both the mean and medians of the overlap fraction between the diagnostic FDG-PET subvolumes of 34, 40 and 50% of SUV_{max} and subsequent FDG-PET subvolumes of 70 and 80% were all overlap fraction > 0.7. It was notable that agreement between those volumes was less successful in nodal disease compared with primary disease (mean overlap fractions between the diagnostic FDG-PET subvolumes of 34 and 40% and subsequent FDG-PET subvolumes of 80% were 0.95 and 0.65 for nodal volumes but 1.0 for all primary tumours). This may be due to smaller volumes of nodal disease, where a slight inaccuracy in registration can have a significant effect on the results. Therefore, dose escalation to nodal sites might only be feasible with advanced image guidance or treatment adaptation.

Our approach to assessing volume overlap is based on a publication in NSCLC [23]. Aerts *et al.* [34] recommended a FDG-PET subvolume of 50% of SUV_{max} on the diagnostic FDG-PET/CT as a target for biological boost. This was based on overlap between the diagnostic scans and post-treatment residual disease. Therefore, we acknowledge that our suggested boost volume shows reproducibility rather than site of residual tumour or relapse, as our second scan point is the second week of treatment. A study of pancreatic cancer [24] found that a threshold of 40% of the SUV_{max} on baseline FDG-PET/CT identifies areas of residual metabolic activity seen on a post-CRT FDG-PET/CT and this threshold could aid in the definition of a biological target volume.

Wilson *et al*. [24] and Aerts *et al*. [34], detailed above, proposed that a mean overlap fraction > 0.7 supported the use of a threshold as a target for a biological boost. We have presented the median as the outcomes of interests are normally distributed. However, in order to allow comparison with previous literature we have also reported the mean (standard deviation). Using a mean overlap fraction > 0.7, defined in previous publications as a 'good' correlation, the diagnostic FDG-PET subvolumes showed generally satisfactory results. When comparing the FDG-PET subvolumes of 34, 40, 50, 60 and 70% of the SUV_{max} with the subsequent subvolumes of 80%; 24 of 29 (83%), 23 (79%), 20 (69%), 15 (52%) and 11 (38%) volumes achieved that threshold. Therefore, it would be reasonable to consider the diagnostic FDG-PET threshold of 50% of SUV_{max} as a possible target for dose escalation based on pre-treatment imaging, as it achieved a mean overlap fraction of 0.7 used in other papers and was reasonably consistent, with >70% of cases showing good overlapping agreement. As the diagnostic FDG-PET threshold of 50% represented a mean of only 34% of the GTV, the reduction to volume would be substantial. Therefore, boosting this reduced volume to a higher radiation dose will probably result in reduced normal tissue toxicity than boosting the whole GTV, although this would require confirmation.

Our study has some limitations; the fact that the study incorporated small numbers is representative of a rare disease. However, even with small numbers, our study serves to

show the feasibility of defining a biological boost in anal cancer and suggests possible boost volumes for further investigation. There are multiple methods of reporting differences between two volumes [35]. We have primarily used the method previously published by Aerts *et al.* [34], as they compared spatial position in volumes with changing sizes. Our assessment of the volumes using DSC suggests less spatial correlation. However, these contrasting results between our primary methods of comparison and DSC were also seen in the study carried out by Calais *et al*. [19], who highlighted that two volumes with a good superimposition but a large difference in size will yield low Dice and Jaccard indices. DSC values have been calculated for completeness, but the poor DSC correlation should be taken in the context of its limitations when comparing different-sized lesions. In our study, it was sometimes challenging to separate the tumour FDG activity from the background physiological uptake. All the subvolumes in our study were checked visually and areas of overlap with normal organs were removed. This may also be responsible for the only moderate overlap seen when like-for-like subvolumes were compared (Pre70% versus Sub70%), as such a comparison would leave no margin for small errors in registration and variations in background physiological FDG uptake. Results must be interpreted with caution in view of the potential for errors with deformable registration. The differing field of view in the second FDG-PET/CT scans may raise some concerns. This occurred due to an amendment to the trial protocol in order to incorporate a further tertiary end point investigating the bone marrow toxicity of CRT. However, SUV thresholds are calculated independently of non-pelvic organs using the patients' weight and activity in the primary tumour. Therefore, this will not have affected results. Finally, the subvolumes used within this study are based on previous literature and we acknowledge that the SUV threshold levels are somewhat arbitrary. The authors have based the study and the methods on best available evidence and we anticipate that further investigation of biological boost volumes in a larger prospective format would be required to validate these methods, to guide patient selection and quantify the reduction in toxicity with smaller boost volumes in comparison with whole GTV.

Previous publications confirmed that higher SUV correlates with worse outcome [16,17,19]. Therefore, the next question that must be addressed is the hypothesis – Does local relapse occur at sites consistent with the high FDG-avid subvolumes identified on diagnositic PET? Unfortunately, as FDG-PET/CT has only recently been incorporated into staging of anal cancer in the UK, and there is a relatively low locoregional relapse rate, there are not sufficient numbers of patients with baseline and subsequent FDG-PET/CT to carry out this analysis. Therefore, we must be clear that the proposed subvolumes are currently based on identifying a reproducible target; further work is required to confirm that the proposed subvolumes are appropriate to cover sites of subsequent relapse using scans following treatment. We plan to carry out this analysis initially on the ART cohort in due course and ideally subsequently in a larger number of patients as part of a prospective trial.

Finally, investigation to date regarding the role of functional imaging to predict response is detailed in the introduction. The primary end point of the ART study is in fact based on the hypothesis that we can use functional imaging as an early predictor of response in order to perform treatment intensification, perhaps with a radiotherapy boost, on a selected group identified as poor responders. However, the ability to select patients for dose escalation does not deter from the potential to use an FDG-derived boost to these selected patients as a method of improving local control while limiting toxicity.

In conclusion, on the basis of these findings we conclude that areas of high FDG avidity on diagnostic FDG-PET scans remain spatially consistent during a course of treatment. A subvolume of 50% of SUV_{max} on the diagnostic scan could be considered as the target for dose escalation. Despite the limitations of this small study it shows the potential of using a biological boost rather than whole GTV dose escalation, which may facilitate improved outcomes while minimising the significant side-effects of whole GTV dose escalation. This study suggests an exciting avenue for further investigation.

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Appendix A. Supplementary data

[AQ]Supplementary data related to the article can be found at

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Fig 1. Axial (A) and sagittal (B) slices through a planning computed tomography scan with the Pre50% (black) and the Sub70% (white) volumes illustrated.

Fig 2. Illustration of median overlap fraction and vector distances between the different volumes assessed.

Fig 3. Illustration of the median Dice coefficient of the different volumes assessed.

Table 1

Patient and tumour demographics

Characteristic	*n* (%); median (IQR)
Gender	
Male	2 (10%)
Female	19 (90%)
Age (years)	60 (51,70)
T Stage	
T2	16 (76%)
T3	2 (10%)
T4	3 (14%)
N Stage	
N0	10 (48%)
N1	7 (33%)
N3	4 (19%)
M Stage	
M0	21 (100%)

IQR, interquartile range.

Table 2

List of lesions with site, gross tumour volume (GTV) and 50% standardised uptake value (SUV) volume size

	Site of lesion	GTV (cm^3)	Pre50% SUV volume (cm^3)	Sub70% SUV volume (cm^3)
Patient 1	Primary	34.7	5.6	0.7
Patient 2	Primary	29.8	2.7	1.8
Patient 3	Primary	14	5.3	0.5
Patient 4	Primary	69.8	36.9	5.8
Patient 4, Node 1	Right inguinal node	23.1	8.3	3.2
Patient 4, Node 2	Right external iliac node	6.2	3.5	1.2
Patient 4, Node 3	Left inguinal node	7.2	3.4	2.7
Patient 5	Primary	22.2	7.7	3.8
Patient 6	Primary	14	3.4	2
Patient 6, Node 1	Left inguinal node	18.3	5.8	0.3
Patient 6, Node 2	Left internal iliac node	13.3	5.5	2
Patient 6, Node 3	Left external iliac node	13.6	0.2	0.9
Patient 7	Primary	28.7	8.9	3
Patient 8	Primary	13.5	5.3	3.1
Patient 9	Primary	16.9	11.7	1.1
Patient 10	Primary	10.6	2.2	0.2
Patient 10, Node	Right mesorectal node	11.1	1.2	0.1
Patient 11	Primary	14.4	4.1	0.8
Patient 12	Primary	16.4	7	1.4
Patient 13	Primary	4.9	2.3	0.3

Patient 14	Primary	154.2	79.2	3
Patient 15	Primary	109.1	58	29
Patient 15, Node	Right inguinal node	5.8	4.7	1.3
Patient 16	Primary	23.1	8.9	1.7
Patient 17	Primary	32.6	6.9	2.1
Patient 18	Primary	9.7	0.5	0.5
Patient 19	Primary	17.5	4.6	1
Patient 20	Primary	6.4	1	0.7
Patient 21	Primary	63.5	14.4	6.2

Pre50% SUV, volume created from 50% of SUV_{max} on initial positron emission tomography/computed tomography (PET/CT) scan; Sub70% SUV, volume created from 70% of SUV_{max} on PET/CT scan taken at fraction 8 or 9 of radiotherapy.

Table 3

Overlapping fractions between volumes on the pre-treatment scan (Pre34%, Pre40%, Pre50%, Pre60% and Pre70% of the maximum standardised uptake value [SUV_{max}]) and the subsequent interim scans (Sub70% and Sub80% of the SUV_{max})

Patient	Sub70%					Sub80%				
	Pre34%	Pre40%	Pre50%	Pre60%	Pre70%	Pre34%	Pre40%	Pre50%	Pre60%	Pre70%
Patient 1	1	1	0.86	0.71	0.43	1	1	1	1	1
Patient 2	0.50	0.28	0.11	0.56	0	0.60	0.40	0.20	0	0
Patient 3	1	0.80	0.60	0.40	0.20	1	1	0.50	0.50	0
Patient 4	1	1	1	0.95	0.66	1	1	1	1	1
Patient 4, node 1	0.66	0.63	0.56	0.47	0.38	0.50	0.40	0.40	0.40	0.40
Patient 4, node 2	0.33	0.25	0.17	0.83	0	0.33	0	0	0	0
Patient 4, node 3	0.89	0.81	0.67	0.52	0.33	0.91	0.91	0.73	0.64	0.36
Patient 5	0.97	0.95	0.82	0.63	0.42	1	1	0.95	0.79	0.47
Patient 6	0.95	0.90	0.80	0.60	0.30	1	1	0.90	0.70	0.30
Patient 6, node 1	1	1	1	1	0.67	1	1	1	1	1
Patient 6, node 2	1	1	1	0.95	0.75	1	1	1	1	1
Patient 6, node 3	0.22	0.11	0	0	0	0	0	0	0	0
Patient 7	0.97	0.97	0.93	0.83	0.83	0.92	0.92	0.92	0.92	0.92
Patient 8	0.84	0.77	0.65	0.52	0.35	0.93	0.86	0.71	0.57	0.43
Patient 9	1	1	1	1	0.91	1	1	1	1	1
Patient 10	1	1	1	1	1	1	1	1	1	1
Patient 10, node	0	0	0	0	0	1	0	0	0	0
Patient 11	1	1	1	1	0.88	1	1	1	1	1
Patient 12	1	1	1	0.93	0.86	1	1	1	0.83	0.83
Patient 13	0	0	0	0	0	0	0	0	0	0
Patient 14	1	1	0.90	0.30	0	1	1	0.93	0.29	0.00
Patient 15	0.98	0.95	0.89	0.80	0.65	1	1	0.94	0.85	0.66
Patient 15, node	0.92	0.92	0.85	0.77	0.69	1	1	1	1	0.75
Patient 16	1	1	1	0.94	0.94	1	1	1	1	1
Patient 17	0.76	0.71	0.67	0.57	0.38	0.80	0.80	0.70	0.70	0.40
Patient 18	0.60	0.40	0.20	0.20	0	1	1	0	0	0
Patient 19	0.90	0.80	0.60	0.30	0.10	1	0.75	0.75	0.50	0
Patient 20	1	0.86	0.71	0.43	0.14	1	1	0.67	0.33	0.33
Patient 21	0.98	0.97	0.81	0.39	0.07	1	1	0.94	0.50	0.06
Median	0.97	0.92	0.81	0.60	0.38	1.00	1.00	0.92	0.70	0.40

Table 4

Vector distances between volumes on the pre-treatment scan (Pre34%, Pre40%, Pre50%, Pre60% and Pre70% of the maximum standardised uptake value [SUV_{max}]) and the subsequent interim scans (Sub70% and Sub80% of the SUV_{max})

Patient	Sub70%					Sub80%				
	Pre34%	Pre40%	Pre50%	Pre60%	Pre70%	Pre34%	Pre40%	Pre50%	Pre60%	Pre70%
Patient 1	0.88	1.04	0.74	0.63	0.55	1.00	0.98	0.86	0.74	0.61
Patient 2	0.96	1.02	1.11	1.14	1.08	0.81	0.85	0.92	0.95	0.90
Patient 3	0.77	0.82	0.86	0.94	0.98	0.85	0.89	0.94	1.03	0.34
Patient 4	0.90	0.34	0.36	0.36	0.38	0.33	0.88	0.90	0.92	0.92
Patient 4, node 1	0.81	0.73	0.62	0.44	0.42	1.22	1.14	1.02	0.86	0.82
Patient 4, node 2	0.91	0.93	0.97	0.96	0.96	0.93	0.93	0.95	0.94	0.91
Patient 4, node 3	0.50	0.53	0.51	0.51	0.55	0.55	0.57	0.55	0.55	0.58
Patient 5	0.33	0.31	0.28	0.30	0.32	0.36	0.34	0.32	0.36	0.39
Patient 6	0.95	0.93	0.89	1.02	1.08	1.00	0.99	0.95	1.08	1.13
Patient 6, node 1	0.34	0.33	0.31	0.25	0.11	0.47	0.48	0.45	0.39	0.24
Patient 6, node 2	1.47	1.93	2.03	1.46	1.94	2.94	3.39	3.49	3.51	3.41
Patient 6, node 3	0.56	0.49	0.38	0.39	0.39	0.67	0.59	0.38	0.31	0.31
Patient 7	0.54	0.53	0.51	0.50	0.55	0.65	0.64	0.61	0.57	0.57
Patient 8	0.51	0.50	0.46	0.41	0.32	0.75	0.74	0.71	0.66	0.56
Patient 9	0.15	0.16	0.20	0.16	0.20	0.19	0.19	0.16	0.21	0.24
Patient 10	0.80	0.76	0.69	0.66	0.68	0.82	0.77	0.71	0.67	0.71
Patient 10, node	0.31	0.31	0.33	0.33	0.36	0.37	0.37	0.40	0.39	0.42
Patient 11	0.67	0.62	0.60	0.60	0.59	0.63	0.58	0.54	0.51	0.51
Patient 12	1.12	1.16	1.23	1.29	1.39	1.13	1.17	1.24	1.30	1.36
Patient 13	0.55	2.43	2.49	2.85	3.28	2.54	2.51	2.57	2.93	3.36
Patient 14	0.25	0.55	0.56	0.59	0.80	1.57	1.57	1.57	1.60	1.69
Patient 15	0.63	0.23	0.19	0.19	0.21	0.51	0.47	0.42	0.36	0.33
Patient 15, node	0.25	0.23	0.19	0.19	0.21	0.51	0.47	0.42	0.36	0.33
Patient 16	0.51	0.47	0.42	0.37	0.37	0.57	0.54	0.51	0.47	0.47
Patient 17	0.75	0.75	0.76	0.77	0.77	0.74	0.74	0.74	0.79	0.79
Patient 18	0.65	0.56	0.31	0.41	0.25	0.71	0.63	0.31	0.35	0.31
Patient 19	0.19	0.65	0.72	0.83	0.96	0.62	0.61	0.68	0.78	0.92
Patient 20	0.63	0.21	0.26	0.31	0.36	0.26	0.25	0.24	0.25	0.30
Patient 21	2.43	0.64	0.57	0.60	1.87	0.97	0.98	0.92	0.91	2.17
Median	0.65	0.62	0.60	0.59	0.55	0.74	0.74	0.71	0.74	0.61
Minimum–maximum	0.15 – 2.43	0.16 – 2.43	0.19 – 2.49	0.16 – 2.85	0.11 – 3.28	0.19 – 2.94	0.19 – 3.39	0.20 – 3.49	0.21 – 3.51	0.24 – 3.41

Author queries

[16] Please uptake reference

AQ Please update web address for supplementary material

***Edited Research Highlights**

Highlights

- A biological boost at diagnosis is spatially consistent during chemoradiotherapy.
- Threshold of 50% SUV_{max} has been suggested as a potential boost volume.
- Biological boost in anal cancer should be further investigated in clinical trials.

Supplementary data file

Click here to download Supplementary data file: CLINONC 2018-380 supp info.docx