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Phase II study of a radiotherapy total dose increase in hypoxic lesions identified by F-miso PET/CT in patients with non-small cell lung carcinoma [RTEP5 study].

Keywords: positron emission tomography, fluoro-deoxy-D-glucose, f-misonidasole, hypoxia, lung cancer, radiotherapy dose

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Running title: Lung RT boost based on F-miso PET

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Statement of Translational Relevance

This phase II study has prospectively included 79 patients with localized non-small cell cancers candidate to concomitant chemoradiotherapy. Fifty four patients were evaluable. In 24/54 patients, the radiotherapy dose was increased up to 86 Gy in hypoxic areas identified on F-Miso PET/CT. The PET/CT images were acquired and delineated following a strict procedure validated a priori. We demonstrate that this approach is feasible in a multicentre setting. We also show that the presence of hypoxia is associated to a significantly worse outcome that could not be reversed with higher doses of radiotherapy. The series is the largest series published to date. As for the clinical aspect, a recent randomized trial (Bradley Lancet Oncol 2015) failed to demonstrate the benefit of escalated radiotherapy dose in large target volumes. Our data show that smaller volumes, identified on their functional characteristics, can be adequately targeted.

Abstract

Objectives. A multicenter phase II study investigated a selective radiotherapy (RT) dose increase to tumor areas with significant F-miso uptake in patients with non-small cell lung carcinoma (NSCLC).

Methods. Eligible patients had locally advanced NSCLC, no contra-indication to concomitant chemo-radiotherapy (CCRT). The F-miso uptake on PET/CT was assessed by trained experts. If there was no uptake, 66 Gy was delivered. In F-miso-positive patients, the contours of the hypoxic area were transferred to the radiation oncologist. It was necessary for the radiotherapy dose to be as high as possible while fulfilling dose-limiting constraints for the spinal cord and lungs. The primary endpoint was tumor response (CR+PR) at 3 months. The secondary endpoints were toxicity, disease-free survival (DFS) and overall survival (OS) at 1 year. The target sample size was set to demonstrate a response rate ≥40% (bilateral $\alpha = 0.05$, power $1-\beta = 0.95$).

Results. Seventy-nine patients were pre-included, 54 were included, and 34 were F-miso positive, 24 of whom received escalated doses of up to 86 Gy. The response rate at 3 months was 31/54 (57%, 95% confidence interval [43%-71%]) using RECIST 1.1 criteria (17/34 responders in the F-miso positive group). DFS and OS at 1 year were 0.86 [0.77 - 0.96] and 0.63 [0.49-0.74], respectively. DFS was longer in the F-miso negative patients ($p = 0.004$). The RT dose was not associated with DFS when adjusting for the F-miso status. One toxic death (66 Gy) and 1 case of grade 4 pneumonitis (>66 Gy) were reported.

Conclusion. Our approach results in a response rate ≥40% with acceptable toxicity. F-miso uptake in NSCLC patients is strongly associated with poor prognosis features that could not be reversed by RT doses up to 86 Gy.

Introduction

Radiotherapy is a major component in the treatment of non-resectable locally advanced non-small cell lung cancer[1] (NSCLC). Whereas concomitant radio-chemotherapy (CCRT) is the current standard for curative-intent treatment, the tumor control rate and survival probabilities remain disappointing. Improvements in radiotherapy techniques should yield better intra-thoracic control, a reduction in secondary distant dissemination, less normal tissue damage, and, as a consequence, reduced mortality caused by cancer, toxicity or worsening of pre-existing co-morbidities. The identification of the adequate target volumes and the delivery of sufficiently high total doses are closely linked. Phase II studies have shown that higher doses could only be delivered to smaller target volumes[2,3]. The RTOG 0617 randomized trial reported lower survival probabilities in the patients having received >60 Gy, possibly because the target volumes were too large[4]. Therefore, it is tempting to reduce the target volumes and escalate the radiotherapy dose only to the most aggressive parts of the tumor. For example, the dose could be selectively increased in the tumor areas with the highest ^{18}F-fluoro-2-deoxy-D-glucose (FDG) uptake[5,6]. Because oxygen is the most powerful radio-sensitizer[7], we hypothesized that the hypoxic areas in the tumor would be relevant targets for selective dose escalation.

In a phase II study, we used ^{18}F-misonidazole (F-miso), a PET/CT tracer for hypoxic cells, to identify and delineate hypoxic areas as biological target volumes (BTV) for escalated total dose radiotherapy associated with concomitant chemotherapy. A rigorous quality assurance protocol was set to assure that all positron emission tomography (PET/CT) images were acquired under reproducible conditions. The presence of F-miso uptake was assessed by consensus by trained experts[8]. The BTVs were centrally delineated. The primary endpoint was the tumor response at 3 months after CCRT. The secondary endpoints were acute and late toxicity, as well as disease-free and overall survival at 1 year.

Patients and Methods

Study design and patients

The design of the study is described in Figure 1. Between June 6, 2012, and March 19, 2015, the patients with NSCLC referred to the participating centers for CCRT were prospectively pre-included. Fifteen academic centers included patients into the study.

The main inclusion criteria were (i) age >18 years; (ii) histological proof of NSCLC with a measurable tumor (RECIST1.1); (iii) WHO performance status ≤1; (iv) eligible for curative-intent CCRT (no pleural, pulmonary or extra-thoracic metastases and no co-morbidity contraindicating CCRT); (v) adequate lung function (FEV ≥ 40% and DLCO / VA ≥ 50% of the predicted values, PaO_2 ≥ 60 mm Hg); (vi) a neutrophil count >1.5 x 10^9 cells/L, platelet count >100 x 10^9 /L and hemoglobin >10 g/dL; and (vii) estimated creatinine clearance >60 mL/min. All patients had to receive cisplatin-based chemotherapy as the induction treatment and concomitantly with radiotherapy. Inclusion was confirmed after completion of a radiotherapy plan confirming that the dose objective (a minimum dose of 60 Gy in 99% of the planning target volume) and the constraints (lungs, spinal cord) could be met.

The non-inclusion criteria were (i) histology other than primary NSCLC; (ii) a non-evaluable lesion (complete remission after induction chemotherapy); (iii) no uptake or metastases on the FDG_1-PET/CT performed after the induction chemotherapy and before CCRT; (iv) contraindication of curative-intent radiotherapy (tumor extension, performance status WHO ≥ 2, coexistent disease); (vi) synchronous cancer or previous malignancy within 5 years before inclusion; (vii) patient already participating in another clinical trial; (viii) confirmed or suspected pregnancy and lactating females; (ix) renal insufficiency contraindicating cisplatin treatment; (x) patients under legal protection; (xi) inability to comply to the follow-up procedures for geographical, social or psychological reasons; (xii) uncontrolled diabetes mellitus (blood glucose ≥ 10 mmol/L); and (xiii) patients unable to give informed consent.

The eligible patients had to have at least one FDG-avid lesion at FDG_1-PET/CT (after induction chemotherapy and before CCRT). These patients were then considered to have hypoxic lesion(s) if significant F-miso uptake was observed in the FDG-avid lesion(s) on a subsequent F-$miso_1$ PET/CT within 8 days. The evaluable population was formed from all the eligible patients who completed the protocol (a complete mandatory dataset is included at the end of the study). The patients who eventually withdrew their consent to participate were not evaluated.

The protocol and the consent form were approved by the "Comité de Protection des Personnes Nord-Ouest 1" (July 21st, 2011). All patients gave their written, informed consent. The study was registered in the Clinical Trials Protocol Registration System (NCT01576796; RTEP5 study). The clinical, biological, imaging and toxicities data were monitored by a certified clinical research unit.

PET imaging

The PET/CT machines were Biograph Sensation 16 (Siemens, Erlangen, Germany), Gemini (Philips, DaBest, Netherlands) or Discovery LS (General Electric Medical Systems; Milwaukee). For each patient, two FDG-PET/CT and two F-miso-PET/CT acquisitions were to be performed using the same machine and under the identical operational conditions. Quality control (QC) was centrally supervised to secure homogeneity in the image quality in all participating centers. The QC procedures and results are provided in the appendix.

The **FDG-PET Images** were acquired in treatment position (arms over the head, free breathing), at least 15 days after the last administration of chemotherapy. No chemotherapy was allowed between the PET/CT and the start of radiotherapy. Six to 8 bed positions per patient were acquired from the head to the upper third of the thighs. The images were acquired at a minimum of 60 ± 10 minutes (min) after the FDG injection. The patients were required to fast overnight or for at least 6 hours before the imaging to ensure that the serum glucose and endogenous serum insulin levels were low at the time of the FDG administration. The blood-glucose levels were measured before each FDG-PET acquisition. A total of 4.5 MBq/kg was administered intravenously after a rest period of at least 20 min. The first acquisition (FDG_1) after the induction chemotherapy started at time T_1 = 60 $\pm$ 10 min post-injection. The second FDG-PET (FDG_2) was performed during the 5^{th} week of radiotherapy at a total dose of 40-46 Gy as previously demonstrated[12]. The acquisition procedure followed the identical conditions as for FDG_1, specifically, time T2 = T1 $\pm$ 5 min.

The **F-miso-PET images** were acquired under identical conditions. Two to 3 bed positions per patient were acquired for the thorax. The images were acquired at a minimum of 240 ± 20 minutes (min) after the F-miso injection. A total of 4.5 MBq/kg was administered intravenously after a rest period of at least 10 min. A first F-miso-PET (F-miso$_1$) was scheduled after induction chemotherapy, 48 hours after the FDG$_1$. The second F-miso-PET (F-miso$_2$) was performed during the 5th week of radiotherapy, within 48 h after the FDG$_2$.

For all the FDG and F-miso acquisitions, the CT scan data were used for random coincidences, scatter and attenuation correction and anatomic localization. The PET images (FDG and F-miso) were fused with the CT scan images. The F-miso-PET images were finally smoothed with a Gaussian filter (full width at half maximum = 5 mm).

PET analysis

We previously showed[8] that the assessment of F-miso / FDG uptake (presence vs. absence) was reproducible in a multicenter setting. In this study, 3 independent experts (out of 9) reviewed the F-miso PET acquisitions and decided upon the presence or absence of uptake within 48 h.

Because the inter-observer agreement for the F-miso volume measurements was low[8], all images were centrally delineated in a single center (Rouen) by a nuclear physician and a radiation oncologist, via a dedicated network (Imagys Interface (QI/QO/QA/QC) and Keosys workstation, Nantes, France)[1]. For each patient, the CT of PET FDG and F-miso were first co-registered to the planning CT scanner (Oncoplanet, DosiSoft, France, v 1.4) with registration based on the the lesion. The volumes of interest (VOIs) for FDG (BTV_m) were defined as the sum of the pixels above 40% of the SUVmax inside the primary tumor or nodes[9]. The volumes of F-miso (BTV_h) were defined as sum of pixels with SUV ≥ 1.4 as previously validated**Error! Bookmark not defined.**.The co-registered FDG and F-miso PET/CT (DICOM), as well as BTV_m (FDG_{BTV}) and BTV_h (F-$miso_{BTV}$) (DICOM-RT) were transferred back to the local radiation oncologist by the same network.

In addition, the FDG and F-miso images on PET after the induction chemotherapy (PET_1) and during radiotherapy (PET_2 at 40-46 Gy) were used to calculate the maximum standard uptake values ($PET_{SUVmax1}$ and $PET_{SUVmax2}$), i.e., the highest-activity pixel value in the BTVs, and the percentages of variation in SUV_{max} (Δ%SUV_{max}) and BTV (Δ%BTV). The SUV_{mean} values yielded results similar to those of the SUV_{max} values and are not presented here.

Radio-chemotherapy protocol

The microscopic extension around the BTV_m (clinical target volume, CTV) was obtained either by isotropic expansion around the tumor (6 mm for squamous cell carcinoma, 8 mm for adenocarcinoma)[10] or by delineation of the FDG-PET/CT positive mediastinal nodes[11]. The isotropic CTV margin around the BTV_h was set to 5 mm. The margin for the planning target volume (PTV) was

[1] Keosis Imagys Interface (QI/QO/QA/QC) is 21-CFR part 11 compliant. The Keosys® company is ISO 9001 and ISO 13485 medical device compliant. The images were stored and archived a dedicated IIA class server.

10 mm around the CTV (possibly 15 mm in the cranio-caudal direction) to take into account internal movements and uncertainties in positioning.

All the dose calculations were corrected for heterogeneity. Intensity-modulated radiotherapy (IMRT) was not allowed. The total dose was prescribed by the International Commission for Radiation Units point (ICRU). The dose delivered in the PTV had to be within 95% and 107% of the prescribed dose. The target total dose was 86 Gy, provided that the maximum dose to the spinal cord was strictly <46 Gy and that no more than 30% of the total lung volume (excluding the GTV) received more than 20 Gy. As minor constraints, no more than 30% of the esophagus or the heart could receive more than 50 or 35 Gy, respectively.

The patients received 5 daily fractions of 2 Gy every week, with all the beams being treated daily. The shape of each beam was checked (electronic portal image) on the first fraction. The position of the isocenter was imaged daily (by orthogonal image or cone-beam CT-scanner). Concomitant chemotherapy was cis-platinum (50 mg/m² D1, D8, D29, D36) and etoposide (50 mg/m² D1 to D5, D29 to D33) or cis-platinum (80 mg/m² D1 and D22) and vinorelbine (15 mg/m² D1, D8, D22, D29). Cis-platinum could be replaced by carboplatin AUC 5 in case of renal insufficiency.

Follow-up procedures

The efficacy and toxicity assessments were planned at 3 months and 1 year after the end of treatment (clinical examination, CT scanner).

Endpoints

The primary endpoint was the tumor response on CT scan at 3 months (RECIST 1.1). Complete response (CR) was defined as no residual tumor image. Partial response (PR) was defined as a >30% reduction in maximal diameter. Progressive disease (PD) was defined as a >20 % increase in the maximal diameter, whereas variations between -30 % and +20 % were classified as stable disease (SD). The secondary endpoints were early and late toxicity (CTCAE) as well as disease-free and overall survival at 1 year from definitive inclusion.

Sample size

This open-label, single-arm, nonrandomized, multicenter phase II study followed a Gehan 2-step design. In the first step, 6 patients had to be evaluable 3 months after completion of treatment. If no complete response (CR) or partial response (PR) were observed, a response rate > 40 % would be excluded with 95 % power and accrual stopped. If at least one response was observed, the number of additional patients to be entered in step 2 was calculated assuming an a priori response rate (complete or partial) of 40 %, power $1-\beta = 95$ %, precision $\varepsilon = 10$ % and the number of responses in step 1, i.e., 19, 18, 15 and 8 additional patients if 1, 2, 3 or 4 responses in step 1, respectively.

The number of patients to include was calculated as follows to obtain 25 patients evaluable at 3 months (and 15 patients alive at one year, a 50% overall survival probability). Assuming 5 deaths / lost

for follow-up at 3 months, 30 patients with hypoxic lesions should be recruited and receive concomitant CCRT. Assuming that 50% of F-miso$_1$ PET/CT would demonstrate the presence of hypoxic lesions, 60 pre-included patients should have persistent FDG uptake on the post-induction chemotherapy FDG$_1$ PET/CT. We anticipated that 20% of the patients would have a negative FDG$_1$ PET/CT after induction chemotherapy[12]. Therefore, a total of 75 patients would have to be pre-included. The 30 patients without F-miso avid lesions would be monitored for one year (a secondary endpoint).

Statistical analyses

All analyses were conducted according to intent to treat, e.g., irrespective of the radiotherapy total dose that was actually delivered. Descriptive statistics (n, mean, SD minimum and maximum) were calculated for the quantitative variables. Frequency and percentages with 95 % confidence intervals (CI) were determined for the qualitative variables. Levene's test was used to assess the equality of variances before comparing the quantitative variables between two or more groups (ANOVA). The survival probabilities were compared with log-rank test. All the significance thresholds were set at 0.05 (two-tailed test). All the statistics were performed using SPSS software (version 20.0, IBM, Armonk, NY).

Results

Patient characteristics, flowchart and descriptive results

The study flow is shown in figure 1. Seventy-nine patients were pre-included, and 54 patients were definitely included. The reasons for non-inclusion were as follows: 8 inadequate procedures, 9 metastases and 3 with an absence of uptake on the FDG$_1$ PET/CT, 4 consent withdrawals, and 1 investigator's decision. Thirty-four patients were eligible for the experimental group (FDG$_1$+ and F-miso$_1$+).

The 54 definitively included patients were predominantly males (7F/47M), with a mean age (±SD) of 60.3 ± 7.7 years (Table 1). The histological subtypes were 26 (48 %) squamous cell carcinomas, 21 (39 %) adenocarcinomas and 7 (13 %) undifferentiated carcinomas. The disease stages were mostly IIIA and IIIB. The descriptive data of the 79 pre-included patients were not significantly different (data not shown).

In the experimental arm, 24/34 (71 %) patients received increased radiotherapy total doses (86 Gy: 5 patients, 80 Gy: 2, 76 Gy: 8, 74 Gy: 5, 72 Gy: 2, 70 Gy: 2). Because of organ-at-risk constraints, the dose was limited to 66 Gy in 10 patients. Among the 20 patients without F-miso uptake, 19 received 66 Gy, and one received 68 Gy.

PET description

The PET data are reported in Table 2. For the 54 included patients, the 54 FDG$_1$ and 54 F-miso$_1$ were available before the CCRT. In the 34/54 patients with hypoxia, 32/34 FDG$_2$ and 31/34 F-miso$_2$ could be

performed during the CCRT at 42 Gy (missing PETs because of medical and/or technical problems). The mean time intervals between injection and imaging were 66 (SD = 10) and 236 (SD = 6) minutes for the FDG and F-miso PET/CT, respectively. A total of 103 lesions (40 primary tumors and 63 nodes) were observed in the 54 patients. The per-patient and per-lesion analyses gave similar results. We present only per-patient PET data.

The patients with hypoxic lesions had significantly higher F-miso$_1$ SUV_{max} than the patients without hypoxia ($p < 0.001$). Similarly, the patients with hypoxia had higher FDG SUV_{max1} ($p = 0.02$) and larger FDG_{BTV1} tumor volumes ($p = 0.03$). The biological target volumes delineated on PET_1 were approximately 40% smaller with F-miso than with FDG (SD 54%), without statistically significant differences between the RT dose groups. For the 34 hypoxic patients who underwent FDG (n = 32) and F-miso (n = 31) during RCT, FDG SUV_{max}, FDG_{BTV} and F-miso SUV_{max} significantly decreased during RCT.

Toxicity

Acute and late toxicities are listed in Table 3a and 3b, respectively. There was one grade 4 acute pneumonitis case among the 24 patients who received escalated radiotherapy doses. Three acute grade 4 toxicities were observed in the patients having received 66 Gy (whatever their F-miso uptake). One death (hemoptysis) occurred before the evaluation at 3 months among the 10 patients with F-miso uptake and having received 66 Gy. No grade 4 or 5 late radiotherapy-related adverse events or acute / late cardiac toxicities were reported in the entire population. The causes of the 19 reported deaths are described below.

Tumor response and survival

The survival curves are presented in Figure 2. The tumor response was evaluated at 3 months (±7 days). The patient who died before evaluation at 3 months in the F-miso positive / 66 Gy group was considered as having a non-responding tumor. The response (CR+PR) rate at 3 months was 31/54 (57% with 95% confidence interval [43% - 71%]). The corresponding figures were 17/34 (50% [34% - 66%]) in the patients with F-miso uptake versus 14/20 (70% [48% - 85%] in the patients without uptake ($p = 0.25$). In the F-miso positive patients, the response rates were 12/24 (50% [31% - 69%]) after the escalated radiotherapy doses and 5/10 (50% [24% - 76%]) after 66 Gy.

At the date of point, 35 patients were alive (a median follow-up duration of 14 months (range 5 – 21 months)), and 19 were alive without disease (15 months (11 – 21)). Sixteen of the 19 deaths were due to cancer (9/10 in the high RT dose group and 3/4 in the 4/5 in the f-miso- group). The patient in the F-miso+/66Gy group who died at 3 months was discussed above. One patient in the high RT dose group was receiving nivolumab for progression under pemetrexed/bevacizumab maintenance. He died at home 17 months after inclusion, and the cause of death remains unknown (drug toxicity or tumor progression). One patient in the f-miso group, without previous documentation of a relapse, was admitted to a palliative care unit with cognitive impairment, fever and intestinal bleeding. He refused investigations and died at 18 months. The OS and DFS probabilities at 1 year for the entire group were 0.86 [0.77 – 0.96] and 0.63 [0.49 – 0.74]. Regarding the F-miso uptake, the OS at 1 year

was 0.81 [0.67 – 0.95] when positive and 0.95 [0.85 – 1.0] when negative (p = 0.12). The DFS at 1 year was 0.50 [0.32 – 0.65] and 0.85 [0.60 – 0.95], respectively (p = 0.004). The DFS was lower after radiotherapy doses larger than 66 Gy (0.50 [0.29 – 0.68] vs. 0.73 [0.54 – 0.86], p = 0.02). In the F-miso positive patients, the DFS was similar regardless of whether the radiotherapy dose was 66 Gy (0.50 [0.18 – 0.75]) or higher (0.50 [0.29 – 0.68]).

Discussion

Our purpose was to increase the total dose of radiotherapy in the hypoxic parts of non-small cell lung cancer in patients who were candidates for curative-intent chemo-radiotherapy. Hypoxia has been shown to strongly reduce the radio-sensitivity of tumor cells and to be associated with local failure[7]. In this study, a key issue was to timely provide the radiation oncologists with a reliable target, anatomically and functionally defined, in a prospective multicenter setting. We have demonstrated that hypoxic areas were identified using F-miso PET in 34 of 54 patients (15 centers) and that higher radiotherapy doses (70 to 86 Gy) could be delivered without excessive toxicity in 24 patients with hypoxic areas. There were no statistically significant differences in the tumor response rates at 3 months, and the overall survival at 1 year was similar among the 3 treatment groups. The DFS probability was significantly lower in the F-miso-positive patients, regardless of the radiotherapy dose. To our knowledge, we present the largest series of patients with NSCLCC receiving RT boosted based on the hypoxia PET/CT in multicentric and prospective conditions.

As a targeted treatment, radiotherapy critically depends on accurate delineation of the volumes to be irradiated. A conventional CT-scan is necessary for planning (Hounsfield units being correlated to electronic densities) and for drawing the anatomical contours of the tumor and the organs at risk. As for functional information (e.g., glucose metabolism with FDG), the PET/CT images must be either acquired in the treatment position or registered onto the planning CT. F-miso is one of several tracers that accumulate in hypoxic areas[13] and was selected for this study because it is commercially available. While this study was ongoing, the MAASTRO group demonstrated (using HX4) that hypoxia images were stable when PET/CT was repeated[14] and provided a representation of the tumor functional status that was different from FDG images[15]. A planning study of 10 patients by the same group showed that hypoxia images could be used to consider delivering heterogeneous doses to the tumor, specifically higher doses to hypoxic areas[16]. All the PET tracers of hypoxia yield a relatively low signal to noise ratio. Therefore, the initial step of this study was to validate a reproducible method to identify the tumors with hypoxic areas and delineate biological target volumes (BTV) for radiotherapy[8]. The patients with hypoxia were identified by at least 3 trained experts, and the delineation of all the BTVs was centralized in one center. An example of FDG and F-miso images with BTV are presented in Figure 3.

We did not gated our PET acquisitions on breathing movements. Since F-miso uptake is known to be low in lung tumors (SUVmax = 2.5 [± 0.7] in our study), good quality images cannot be obtained in respiratory gated mode, both for SUV measurement and for BTVh delineation. Our criteria for BTVh delineation was validated in free-breathing patients. We chose not to add further complexity to our design by requiring gated PET acquisitions and, for the sake of consistency, irradiations. In addition,

mobile tumors are usually small and located in the parenchyma while the majority of stage III tumors are large (and uptaking F-miso) and involve the mediastinum, i.e. are mostly fixed. Lin et al.[17] have suggested a low reproducibility of F-miso PET images performed within 48h.More recently, a preclinical study by Busk et al[18] showed a good reproducibility of PET faza images acquired within 48h (r=0.82; range 0.72-0.90), and Zegers et al. demonstrated the reproducibility of PET HX4 images in a human study[14]. Mathematical simulations based on microscopic tumor tissue sections compared F-miso, faza and HX4 and showed that F-miso provides a robust and reproducible signal four hours post-injection, with a lower contrast19. Our observation that F-miso avid tumors have much worse prognosis confirms that hypoxia imaged on a single PET acquisition is a strong prognostic indicator, making it a relevant target volume for selective radiotherapy dose increase.

Radiobiological and clinical data[7] suggest that total doses above 80 Gy are required to achieve tumor control in NSCLC. The RTOG 0617[4] randomized trial reported reduced overall survival probabilities in patients receiving 74Gy (versus 60Gy) in a target volume (median 90cc) defined on FDG-PET CT. Phase I-II studies have shown that doses in excess of 80 Gy could only be delivered to small tumors[2,3]. Our BTVs delineated on F-miso PET/CT are approximately 40% smaller than those delineated on FDG PET/CT. Our results indicate that F-miso uptake is associated with a worse outcome, regardless of the RT total dose. An increased F-miso uptake was correlated with other poor prognosis features (larger tumor size, higher FDG SUV_{max}), and hypoxia might not be the sole reason for treatment failure. The absence of F-miso uptake identifies a group of tumors with better prognosis. Our OS and DFS at 1 year compare favorably with those reported by RTOG 0617 (0.80 [0.74 – 0.85] and 0.49 [0.42 – 0.56], respectively) in their patients treated to 60 Gy[4]. Similar approaches are being evaluated in clinical trials increasing total dose to smaller sub-volumes that are considered at higher risk of failure (high FDG uptake sub-volumes on pre-radiotherapy FDG PET/CT (RTEP 7, NCT02473133), residual tumor at mid-treatment FDG PET/CT (RTOG 1106 NCT01507428, PET Boost NCT01024829).

An extensive discussion about radiotherapy dose and delivery is beyond the scope of the present paper. Briefly, our patients were irradiated with a 3D conformal technique. When our trial was designed, intensity-modulated radiation therapy (IMRT) was available in too few French centers. IMRT was used in approximately 50% of the RTOG 0617 patients with outcomes similar to 3D RT[4]. The dosimetry benefits of IMRT have not been confirmed in a randomized trial[20]. The dosimetry of protons is characterized by localized high-dose delivery and sharp fall-out (Bragg peak)[21]. No significant differences in tumor outcome were observed in a randomized comparison of 3D proton therapy vs. IMRT[22]. Radiotherapy in stereotaxic conditions is an accepted treatment for tumors up to 65cc, provided that strict OAR dose-volume constraints are met[23]. Our patients had mean F-miso-avid volumes of 33.5 cc, with large variability (SD 52.2 cc; range 1 – 234 cc). A few fractions additional could be delivered, intended as a concomitant boost, whilst the FDG-defined target volume is be treated conventionally (2 Gy per fraction), keeping the treatment duration around 6-7 weeks. The positive results of accelerated RT[24] suggest that tumor proliferation during RT might have contributed to the failure of escalated RT dosage delivered over a protracted treatment time to improve the outcome in NSCLC[4].

Conclusion

This prospective phase II study demonstrates the feasibility of delivering higher RT doses to smaller target volumes identified by F-miso uptake without exceeding the tolerance to the normal organs. The benefit of this approach, possibly with larger doses per fraction in stereotaxic conditions as a concomitant boost, remains to be investigated in a randomized trial.

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Author Disclosure Statement

All the authors has no conflict of interest

Table 1: Baseline characteristics of 54 included patients. SCC: squamous cell carcinoma; ADC: adenocarcinoma; SD: standard deviation. * Significantly different from 66 Gy group (p<0.0001).

	Hypoxia (n = 34) - Trial arm			**No hypoxia**	
	Total	Boost	66 Gy	**66 Gy**	**Total**
	n = 34	n = 24	n = 10	**n = 20**	**n = 54**
Sex F/M (n)	6/28	4/20	2/8	1/19	7/47
Age mean [SD]	59.5 [8.6]	60.5 [8.4]	57.2 [9.2]	61.4 [5.7]	60.3 [7.7]
Height (cm [SD])	169.7 [9.3]	171.1 [10.1]	166.6 [6.1]	170.3 [8.1]	170.0 [8.8]
Weight (Kg [SD])	73.2 [14.6]	71.5 [12.3]	77.2 [19.2]	76.5 [12.1]	74.4 [13.7]
Histology (n)					
SCC	17	14	3	9	26
ADC	11	6	5	10	21
Undifferentiated	6	4	2	1	7
Tumor stage (n)					
IB	1	1	-	1	2
IIA		-	-	1	1
IIB	2	1	1	0	2
IIIA	17	13	4	7	24
IIIB	13	8	5	11	24
IV	1	1	-	-	1
Mean RT Dose (Gy, [SD])	73.9 [6.7]	77.1 [5.2]*	66 [0]	66 [0.4]	71 [6.5]

Table 2: The PET data of the 54 included patients. The figures are means ± standard deviations. The P-values are for comparisons between the trial arm (n=34) and the no hypoxia groups (n=20) * significantly different from PET1 ($p<0.05$).

	Hypoxia (n = 34) - Trial arm			**No hypoxia**		
	Total	Boost	66 Gy	**66 Gy**	**Total**	
	n = 34	n = 24	n = 10	**n = 20**	**n = 54**	**P**
FDG-SUV_{max}						
PET1 (n=54)	14.5 [9.3]	13.8 [7.8]	16.4 [12.4]	8.4 [9.0]	10.0 [0.7]	0.021
PET2 (n=32)	9.4 [6.1]*	10 [7.1]*	8.1 [4.0]*	-	-	
Δ(%)	-32 [26]	-27 [29]	-44 [17]	-	-	
FDG_{BTV} (BTV_m) @ 40% SUV_{max} (cc)						
PET1 (n=54)	55.4 [72.2]	58.9 [84.8]	46.8 [24.9]	27.3 [23.9]	45.0 [60.3]	0.026
PET2 (n=32)	36.1 [44.6]*	39.9 [52.5]	27.4 [17.6]	-	-	
Δ(%)	-10 [226]	-27 [271]	-26 [41]	-	-	
F-miso-SUV_{max}						
PET1 (n=54)	2.5 [0.7]	2.4 [0.6]	2.7 [0.9]	1.4 [0.5]	2.1 [0.8]	<0.001
PET2 (n=31)	1.9 [0.5]*	1.8 [0.4]*	2.2 [0.6]	-	-	
Δ(%)	-17 [24]	-21 [20]	-8 [39]	-	-	
F-$miso_{BTV}$ (BTV_h) @ 1.4 SUV (cc)						
PET1 (n=54)	33.5 [52.2]	34.1 [58.1]	31.9 [37.9]	-	-	
PET2 (n=31)	20.9 [34.6]	18.9 [37.4]	25.4 [28.9]	-	-	
Δ(%)	-24 [75]	-20 [84]	-34 [44]	-	-	

Table 3a: Acute toxicity at 3 months for the 54 included patients

	Hypoxia (n = 34) – Trial arm						No hypoxia		
	Boost (n = 24)			66 Gy (n = 10)			66 Gy (n = 20)		
Acute Adverse events	G1&2	G3	G4&5	G1&2	G3	G4&5	G1&2	G3	G4&5
Asthenia	5			6	1		1		
Pain	2			1			4		
Thoracic pain	5			2					
Dysphagia	17	1		6	3	1	11	4	
Dyspnea	1			3	1		6		
Hemoptysis	1					1 (G5)	1		
Dry skin or pruritus	15			1	1		9		
Anorexia	3			2			3		
Pneumonitis		2	1 (G4)				3		
Cough or expectoration	16			6			11		
Hematological toxicities	2	1		1	1		4	2	1 (G4)
Chemotherapy toxicities	25	3		7	5		12	2	1 (G4)
Other toxicities	1						5		

Table 3b. Late toxicity for the 54 included patients (1 year)

	Hypoxia (n = 34) – Trial arm						No hypoxia		
	Boost (n = 24)			66 Gy (n = 10)			66 Gy (n = 20)		
Late Adverse events	G1&2	G3	G4&5	G1&2	G3	G4&5	G1&2	G3	G4&5
Asthenia	2			1			2		
Pain	1			1	1				
Thoracic pain	1			1					
Dysphagia									
Dyspnea	5			3			4		
Dry skin or pruritus	1						1		
Pneumonitis							1		
Peripheral neuropathy							1		
Cough or expectoration	5			5			1		
Chemotherapy Toxicities	3						1		
Others toxicities	2						1		

Figure 1: Study design/study flow. CCRT: radio-chemotherapy; Gy: Gray. Gy: Grays; CR: complete response; PR: partial response; SD; stable disease; PD: progressive disease (RECIST 1.1)

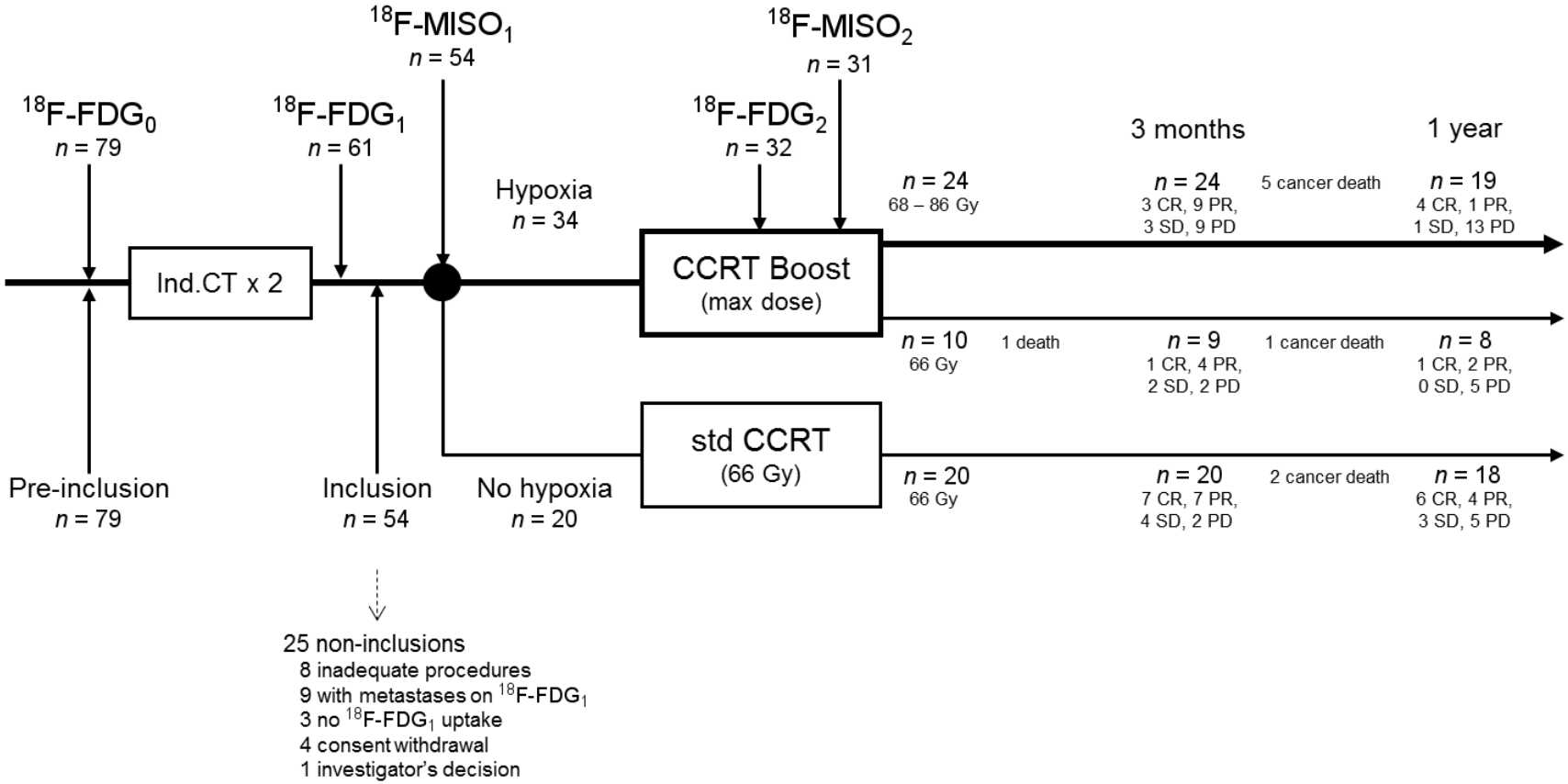


Figure 2: The overall survival (left) and disease free survival (right), for the entire population (A and B) as well as separation for the F-miso PET result (C and D), the dose radiation (E and F), and both the F-miso PET and dose radiation (G and H).

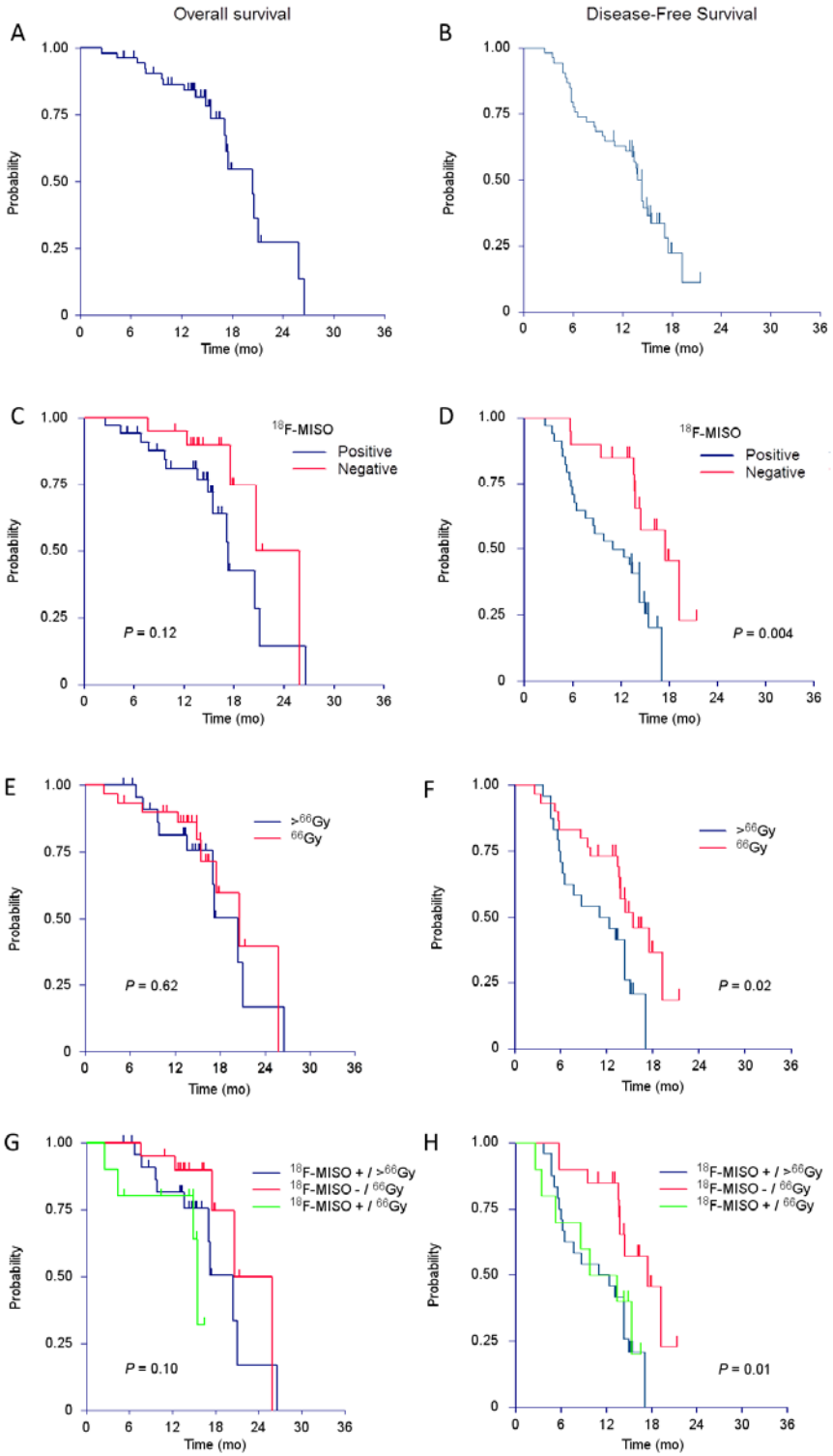
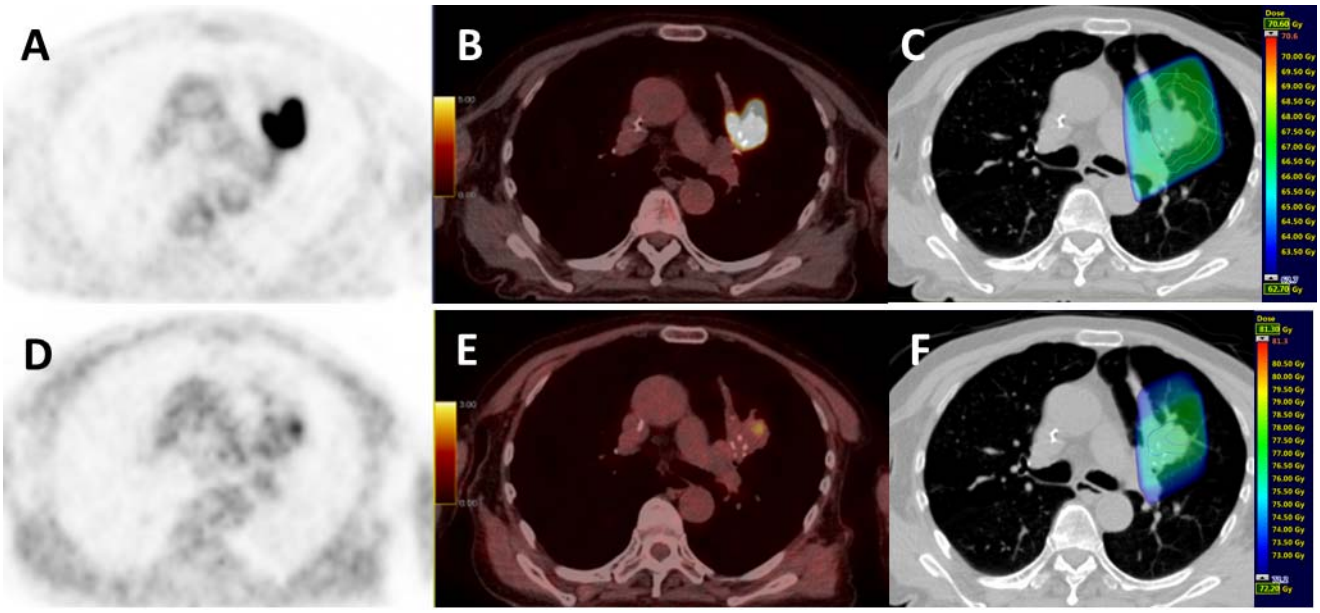


Figure 3: Example of a patient with an upper left lung NSCLC: A: FDG; B: FDG PET/CT; C: Planning radiotherapy based on FDG (66Gy) with BTV_m (GTV), CTV and PTV; D: PET F-miso; E: F-miso PET/CT; F: boost based on the F-miso PET (76Gy) with BTV_h (biological hypoxic target volume) and PTV boost.



Appendix: PET quality control (QC)

Written technical QC procedures were submitted to each center. Staff and equipment questionnaires for each center had to be filled out on a webserver by a physicist. An electronic logbook was implemented for each center to report quality control and scheduled/corrective maintenance results. The data were (i) quality controls required by the manufacturer, (ii) quarterly image uniformity and cross-calibration, (iii) image quality control, at baseline and after the manufacturer's maintenance, and (iv) quarterly Hounsfield Units (HU) calibration for CT.

Image uniformity was evaluated with a cylindrical phantom filled with homogeneous FDG solution. Relative standard deviations (the ratio of standard deviations from the mean value of several regions of interest) were computed on all slices (except the first and last slices to avoid border effect) and had to remain ≤10%. Considering all the centers, the overall mean value was 6.3% (maximum: 9.5%, standard deviation: 1.8%).

To evaluate the calibration between the dose-calibrator and PET system, images of a cylindrical phantom filled with a known FDG concentration were acquired. The relative error between the actual and measured concentrations (the average value computed on each slice) had to be ≤10%. Considering all the centers, the overall mean value was 1.9% (maximum: 6.7%, standard deviation: 1.7%).

The image quality was evaluated with NEMA IEC Body phantom. VA50[2] was measured for each sphere of the phantom. Relative error for each sphere volume VA50 was then used as a metric. Except for the two smallest spheres, standard deviation estimated at all the centers remains low (Figure Annexed).

In Hounsfield units, the mean value measured in a water phantom must be 0 ± 4HU.

The PET imaging centers completed more than 500 reports on the webserver, with good homogeneity in the QC results. Ten PET imaging centers applied and were accredited by EARL.

Figure annexed: Quality controls: for each sphere, the mean value of the relative error computed for each volume VA50, all centers included.

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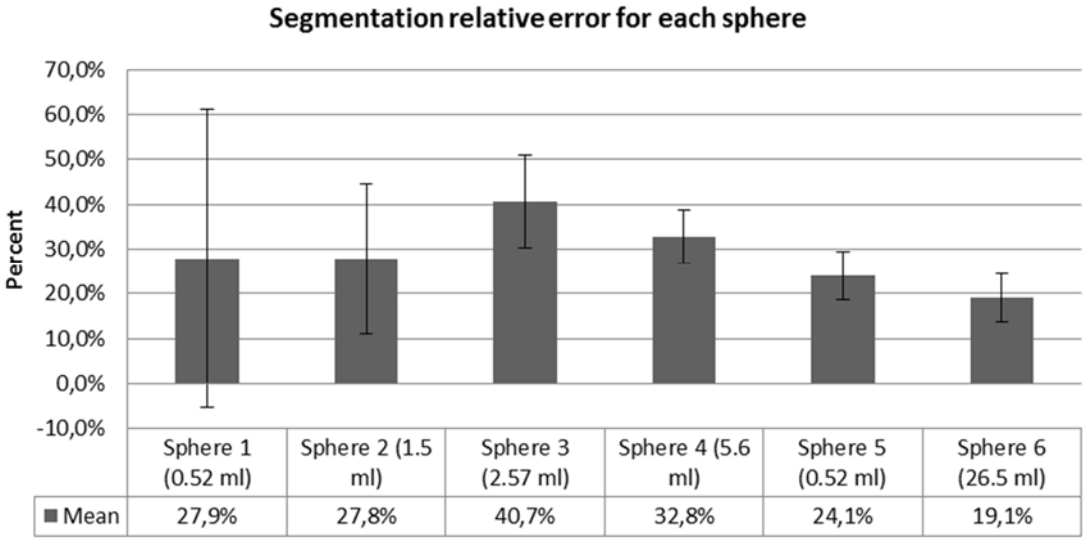
Segmentation relative error for each sphere
Percent
70,0%
60,0%
50,0%
40,0%
30,0%
20,0%
10,0%
0,0%
-10,0%
Sphere 1 (0.52 ml)
Sphere 2 (1.5 ml)
Sphere 3 (2.57 ml)
Sphere 4 (5.6 ml)
Sphere 5 (0.52 ml)
Sphere 6 (26.5 ml)
Mean
27,9%
27,8%
40,7%
32,8%
24,1%
19,1%

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