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FULL PAPER

^{18}F -FDG PET/CT imaging in rectal cancer: relationship with the *RAS* mutational status

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Objective: Treating metastatic colorectal cancer with anti-EGFR monoclonal antibodies is recommended only for patients whose tumour does not harbour mutations of *KRAS* or *NRAS*. The aim of this study was to investigate the biology of rectal cancers and specifically to evaluate the relationship between fluorine-18 fludeoxyglucose (^{18}F -FDG) positron emission tomography (PET) intensity and heterogeneity parameters and their mutational status.

Methods: 151 patients with newly diagnosed rectal cancer were included in this retrospective study. All patients underwent a baseline ^{18}F -FDG PET/CT within a median time interval of 27 days of tumour tissue sampling, which was performed before any treatment. Standardized uptake values (SUVs), volume-based parameters and texture analysis were studied. We retrospectively performed *KRAS* genotyping on codons 12, 13, 61, 117 and 146, *NRAS* genotyping on codons 12, 13 and 61 and *BRAF* on codon 600. Associations between PET/CT parameters and the mutational status were assessed using univariate and multivariate analysis.

Results: 83 (55%) patients had an *RAS* mutation: 74 *KRAS* and 9 *NRAS*, while 68 patients had no mutation (wild-type

tumours). No patient had *BRAF* mutation. First-order features based on intensity histogram analysis were significantly associated with *RAS* mutations: maximum SUV (SUV_{max}) (p -value = 0.002), mean SUV (p -value = 0.006), skewness (p -value = 0.049), SUV standard deviation (p -value = 0.001) and SUV coefficient of variation (SUV_{cov}) (p -value = 0.001). Both SUV_{cov} and SUV_{max} showed an area under the curve of 0.65 with sensitivity of 56% and 69%, respectively, and specificity of 64% and 52%, respectively. None of the volume-based (metabolic tumour volume and total lesion glycolysis), nor local or regional textural features were associated with the presence of *RAS* mutations.

Conclusion: Although rectal cancers with *KRAS* or *NRAS* mutations display a significantly higher glucose metabolism than wild-type cancers, the accuracy of the currently proposed quantitative metrics extracted from ^{18}F -FDG PET/CT is not sufficiently high for playing a meaningful clinical role.

Advances in knowledge: *RAS*-mutated rectal cancers have a significantly higher glucose metabolism. However, the accuracy of ^{18}F -FDG PET/CT quantitative metrics is not as such as the technique could play a clinical role.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cause of cancers worldwide in both males and females.¹ In terms of mortality, it ranks third in the USA and fourth in the world.^{1,2} CRC usually develops from adenomatous polyps and results from a succession of genetic alterations, leading to inactivation of tumour suppressor genes and DNA repair genes and activation of oncogenes.^{3,4} The *RAS*–*RAF*–*MAPK* pathway is one of the main intracellular signalling roads that

activate the growth and division cell mechanisms, a cascade triggered by the binding of the epidermal growth factor on its membrane epidermal growth factor receptor (EGFR).⁵ Downstream in the cascade, *RAS* proteins are essential for intracellular signal transmission. Mutations of the genes that encode *KRAS* and *NRAS*, described in about 30–40% and 10% of CRC, respectively, are responsible for the constitutive activation of this pathway independently of EGFR binding.^{6–12} Thus, targeted treatments with anti-EGFR

monoclonal antibodies (cetuximab and panitumumab) are recommended only for metastatic patients whose tumours do not harbour mutations of *KRAS* or *NRAS*, also called “wild-type” tumours (WT).^{6,13–15} Furthermore, *RAS* and *RAF* mutations are known to be mutually exclusive and seem to have the same tumorigenic effects.¹⁶

Several studies have explored the association between the tumoral metabolism evaluated by fluorine-18 fludeoxyglucose (¹⁸F-FDG) positron emission tomography (PET) and the presence of mutations in various types of cancers, including thyroid cancer,^{17,18} lung cancer^{19–24} and CRC,^{25–27} but with conflicting results. The aim of the present study was to clarify in a large cohort the overall concordance rate between the most common CRC mutations influencing the therapeutic strategy and the glucose metabolism and thus investigate whether ¹⁸F-FDG PET/CT could play a role in the prediction of the mutational status of rectal cancers in clinical routine.

METHODS AND MATERIALS

All procedures were performed in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study to use rectal cancer biopsies.

This retrospective study was approved by the ethics committee of our institution under protocol B707201420492. 151 patients with newly diagnosed rectal or rectosigmoid junction cancers diagnosed between September 2009 and January 2014 were included in the study. The median age was 66 years (range: 32–92 years), and there were 95 males and 56 females. According to the seventh edition of the American Joint Committee on Cancer staging classification, there were 7 (4.6%) patients with Stage I, 16 (10.6%) patients with Stage II, 99 (65.6%) patients with Stage III and 29 (19.2%) patients with Stage IV disease.

Mutational status analysis

All sample specimens analyzed were retrieved from the biobank of our institution, where they were kept for scientific research after patients provided their informed consent. These samples were obtained prior to any treatment from biopsies in 144 patients and from surgical resections in 7 patients. Each formalin-fixed, paraffin-embedded (FFPE) tumour tissue section was assessed by a fully trained pathologist, who confirmed the diagnosis and the histological type of rectal cancer and ensured that the tissue sections used for DNA extraction were significantly infiltrated by the tumour, according to the threshold of detection of our genotyping test. DNA was extracted from FFPE tumour tissue sections using a Maxwell® 16 FFPE Plus LEV DNA Purification Kit (Promega, Madison, WI). PCR and pyrosequencing were performed for *KRAS* (codons 12, 13, 61, 117 and 146), *NRAS* (codons 12, 13 and 61) and *BRAF* (codon 600). For *KRAS* codons 12 and 13 and *BRAF* codon 600, previously described methods were used.^{28,29} All other “positions” (codons) were screened following a home-made genotyping procedure, described in the [supplementary material](#). For each patient, positions were tested in a stepwise manner

according to their prevalence until a first mutation was found. We then stopped the screening.

Fluorine-18 fludeoxyglucose positron emission tomography/CT acquisition

All patients underwent ¹⁸F-FDG PET/CT before any treatment for tumoral staging or target volume delineation, within a median time interval of 27 days (range: 1–87 days) of the sample. ¹⁸F-FDG PET/CT was acquired at our institution on two cross-calibrated three-dimensional combined PET/CT scanners (Philips Medical Systems, Cleveland, OH), 70 ± 15 min after the injection of 282 ± 54 Mbq of ¹⁸F-FDG. Patients fasted for at least 6 h before the injection, and median glycaemia was 101 mg dl⁻¹. A low-dose CT (5-mm slice thickness; tube voltage: 120 kV; and tube current–time product: 50–80 mAs depending on the patient weight) was performed without the injection of the i.v. contrast agent, followed by the PET emission scan of 90 s per bed position performed from the upper thigh to the base of the skull. Images were reconstructed on a 144 × 144 matrix, leading to voxels of 4 × 4 × 4 mm³, using an iterative list mode time-of-flight algorithm and corrections for attenuation, dead time, random and scatter events were applied. There was no change of scan protocol during the period of patient inclusion.

Fluorine-18 fludeoxyglucose positron emission tomography/CT analysis

Only the primary lesion was included in the analysis, which was performed by an experienced nuclear physician unaware of the mutational status and the patient outcome. A volume of interest (VOI) was drawn manually around the tumour and special care was given to avoid the inclusion of other structures, particularly the bladder and adjacent lymph nodes. An automatic segmentation was performed inside these VOIs using a fuzzy locally adaptive Bayesian algorithm.^{30–32} Metabolic tumour volume (MTV), maximum standardized uptake value (SUV_{max}) and mean SUV (SUV_{mean}) were measured inside these segmented VOIs, and the total lesion glycolysis (TLG) was calculated according to the formula: $TLG = SUV_{mean} \times MTV$.³³

Several textural features were studied to provide a measure of tumour heterogeneity, using a home-made software implemented with the cross-platform Python language. The algorithms used to measure these parameters are given in the [supplementary data](#). We used a quantization into 64 grey levels for all the analyses, as previously recommended.³⁴ We calculated several first-order features based on histogram analysis, which identifies the intensity distribution on the original image: SUV_{min}, SUV_{max}, mean SUV_{mean}, SUV standard deviation (SUV_{sd}), SUV coefficient of variation (SUV_{cov}), skewness and excess kurtosis. Local features were measured using grey-level co-occurrence or with neighbourhood intensity difference matrices. Grey-level co-occurrence quantifies the relationships between pairs of voxels, providing several second-order textural features. We considered angular second moment, contrast, entropy, correlation, homogeneity and dissimilarity.^{35–37} Neighbourhood intensity difference matrices quantifies the differences between each voxel and its 27 neighbours, providing several other second-order textural features: coarseness, contrast acquired with neighbourhood intensity difference matrices (contrast_{NGTDM}) and busyness.^{35,38}

Finally, we included two regional textural features calculated in the intensity–size–zone matrix that study characteristics of homogeneous zones: intensity variability and size–zone variability.³⁵

Statistical analysis

Results were expressed as mean and standard deviation. The Spearman correlation coefficient was used to measure the relation between two parameters. For each parameter, we compared the three groups (*KRAS* mutated, *NRAS* mutated and WT) by the non-parametric Kruskal–Wallis test. The same test was used to look for associations between ¹⁸F-FDG PET/CT parameters and the presence of mutations (*RAS*-mutated tumours vs WT). We then performed the same analysis focusing on a subgroup of tumours with an MTV >10 cm³. We then carried out a stepwise multivariate logistic regression analysis including only parameters with univariate *p*-values ≤ 0.2. For significant parameters associated with the presence of any mutations, we determined the optimal cut-off maximizing the couple sensibility/specificity using receiver-operating characteristic analysis. Finally, we searched whether the mutation rate was different between rectal and rectosigmoid locations, using Fisher's exact test.

Results were considered statistically significant at the 5% critical level (*p*-value < 0.05). All statistical analyses were carried out using the SAS® statistical package v. 9.4 (SAS Institute Inc., NC).

RESULTS

151 patients with 151 rectal cancers were included in the study. We were able to detect a mutation in 83 (55%) patients (*KRAS*: *n* = 74; *NRAS*: *n* = 9; and *BRAF*: *n* = 0), while 68 patients were considered as wild type. *KRAS* mutations were found on codons 12 (*n* = 51), 13 (*n* = 13), 61 (*n* = 5), 117 (*n* = 1) and 146 (*n* = 4) and *NRAS* mutations were found on codons 12 (*n* = 2), 13 (*n* = 4) and 61 (*n* = 3). There was no difference of mutation rate between rectal (65 WT/75 *RAS*-mutated) and rectosigmoid (4 WT/8 *RAS*-mutated) locations (*p*-value = 0.55). We did not detect any patient with a *BRAF* mutation in codon 600. Clinical features are summarized in Table 1 and all the ¹⁸F-FDG PET/CT parameters results are shown in Table 2.

The SUV_{min} (*p*-value = 0.005), SUV_{max} (*p*-value = 0.002), SUV_{mean} (*p*-value = 0.006), skewness (*p*-value = 0.049), SUV_{sd} (*p*-value = 0.001) and SUV_{cov} (*p*-value = 0.001) were significantly higher in rectal tumours with *RAS* mutation than in WT (Figure 1). The volume-based parameters and the other textural analysis features were not associated with the presence of any kind of mutation. No significant difference was found between the two populations in terms of age (*p*-value = 0.76), gender (*p*-value = 0.50), T stage (*p*-value = 0.07), N stage (*p*-value = 0.75), metastatic disease (*p*-value = 0.68) or tumoral stage (*p*-value = 0.96). These results are presented in Table 3. The multivariate analysis showed that only SUV_{cov} was

Table 1. Study patient characteristics

Characteristics	Entire cohort <i>N</i> = 151	Mutated <i>N</i> = 83 (55%)	Wild type <i>N</i> = 68 (45%)
Age (years)	65.8 ± 11.7	66.0 ± 11.9	65.4 ± 11.7
Gender M/F	95/56 (62.9/37.1%)	50/33 (60.2/39.8%)	45/23 (66.2/33.8%)
T stage			
T1	5 (3.3%)	0 (0.0%)	5 (7.4%)
T2	25 (16.6%)	16 (19.3%)	9 (13.2%)
T3	111 (73.5%)	62 (74.7%)	49 (72.1%)
T4	10 (6.6%)	5 (6.0%)	5 (7.4%)
N stage			
N0	23 (15.2%)	12 (14.5%)	11 (16.2%)
N1	85 (56.3%)	49 (59%)	36 (52.9%)
N2	43 (28.5%)	22 (26.5%)	21 (30.9%)
M stage			
M0	122 (80.8%)	66 (79.5%)	56 (82.4%)
M1	29 (19.2%)	17 (20.5%)	12 (17.6%)
Stage			
I	7 (4.6%)	4 (4.8%)	3 (4.4%)
II	16 (10.6%)	8 (9.6%)	8 (11.8%)
III	99 (65.6%)	54 (65.1%)	45 (66.2%)
IV	29 (19.2%)	17 (20.5%)	12 (17.6%)

F, female; M, male.

There was no significant difference between mutated and wild-type groups for any of these parameters.

Table 2. Fluorine-18 fludeoxyglucose positron emission tomography intensity, volume-based and textural analysis feature results ($n = 151$)

Parameters	Mean	SD	Min	Max
SUV _{min}	5.4	2.5	1.3	19.9
SUV _{max}	16.0	8.2	3.8	51.1
SUV _{mean}	8.8	4.3	2.7	33.8
Skewness	0.63	0.28	0.04	1.72
SUV _{sd}	2.3	1.3	0.5	8.3
Excess kurtosis	-0.14	0.82	-1.24	4.40
SUV _{cov}	0.26	0.03	0.18	0.33
MTV (cm ³)	29.9	25.3	2.9	139.1
TLG	262.9	244.6	10.5	1644.8
ASM	0.0036	0.0031	0.0008	0.0223
Contrast	289.1	111.3	80.4	735.3
Entropy	5.95	0.66	3.83	7.38
Correlation	0.33	0.12	0.02	0.60
Homogeneity	0.15	0.03	0.10	0.24
Dissimilarity	13.2	2.8	6.7	22.0
Coarseness	5.08	1.40	1.55	9.49
Contrast _{NGTDM}	1.30	1.02	0.22	9.04
Busyness	0.11	0.08	0.03	0.45
Intensity variability	11.3	10.2	2.0	61.3
Size-zone variability	0.0046	0.0037	0.0004	0.0238

ASM, angular second moment; cm³, cubic centimetres; contrast_{NGTDM}, contrast acquired with neighbourhood intensity difference matrices; Max, maximum; Min, minimum; MTV, metabolic tumour volume; SD, standard deviation; SUV_{cov}, standardized uptake value coefficient of variation; SUV_{max}, maximum SUV; SUV_{mean}, mean SUV; SUV_{min}, minimum SUV; SUV_{sd}, SUV standard deviation; TLG, total lesion glycolysis.

significantly associated with the presence of mutations (p -value < 0.001 for tumoral stage).

Considering *KRAS* and *NRAS* mutations, there was no difference in terms of age (p -value = 0.59), gender (p -value = 0.99), T stage (p -value = 0.19), N stage (p -value = 0.23), metastatic disease (p -value = 0.99) and tumoral TNM stage (p -value = 0.82). Intensity, volume-based and textural features were also similar between these two groups of mutated rectal cancers.

The same analyses were then performed including only the lesions with MTV >10 cm³ ($n = 128$). In this population, SUV_{max} (p -value = 0.033), skewness (p -value = 0.014), SUV_{sd} (p -value = 0.033) and SUV_{cov} (p -value = 0.003) remained higher in *RAS*-mutated than in WT tumours. Only SUV_{min} and SUV_{mean} were not significantly different on *RAS*-mutated tumours. All the volume-based and the other textural features were similar between groups (p -value > 0.05). These results are presented in Table 4.

The area under the curve (AUC) was obtained for the parameters that stood out in the univariate analysis. SUV_{cov} was the feature with the highest AUC (0.65) and presented a sensitivity and specificity of 56% and 64%, respectively, for

a cut-off of 0.25. SUV_{max} had an AUC of 0.65 and sensibility and specificity of 69% and 52%, respectively, for a cut-off of 15.5.

Correlations among positron emission tomography parameters

Several PET parameters were correlated with each other, and the Spearman's correlation coefficients are listed in the [supplementary data](#).

DISCUSSION

Determination of the *RAS* and *BRAF* mutational status of CRC is essential in the management of metastatic diseases, as the presence of one of these mutations forbids treatment with anti-EGFR monoclonal antibodies. There is currently no definite answer with regard to the relationship between ¹⁸F-FDG uptake and mutational status of CRC. In a population of 121 patients with colon or rectal cancers, Chen et al²⁵ showed that SUV_{max} and TW40% (PET tumour width determined on a segmented volume obtained using a threshold of 40% of the SUV_{max}) were predictors of *KRAS* mutations in exon 2 (codons 12 and 13), while MTV and TLG were not. In a subgroup of rectal cancers, however, only TW40% was significantly higher in the mutant group, not SUV_{max}. Kawada et al²⁶ studied a population of

Figure 1. Means and standard deviation of minimum standardized uptake value ($\text{SUV}_{\min}$), maximum SUV ($\text{SUV}_{\max}$), mean SUV (SUV_{mean}), skewness, SUV standard deviation (SUV_{sd}) and coefficient of variation (SUV_{cov}) for *RAS*-mutated tumours, *KRAS*-mutated tumours, *NRAS*-mutated tumours and wild-type tumours (WT) ($n = 151$). * indicates the significant difference between groups and WT.

51 patients with colon and rectal cancers independently of the location, looking for the mutation of *KRAS* exon 2 and *BRAF*. They showed that $\text{SUV}_{\max}$ and tumour/liver activity ratio were significantly higher in the mutants group than in the wild-type group ($\text{SUV}_{\max} 17.3 \pm 7.1$ vs 12.1 ± 5.7). Finally, Krikelis et al²⁷ did not find any correlation between $\text{SUV}_{\max}$ and *KRAS* exon 2 mutation in a group of 44 patients with metastatic CRC. However, $\text{SUV}_{\max}$ was measured in the metastasis with the highest ^{18}F -FDG uptake, whereas the *KRAS* status was analyzed in the primary cancer. The mutational status between the primary tumour and the metastatic lesions may be different in up to 36%³⁹ even though a recent meta-analysis showed a high concordance rate between *KRAS* status of primary and corresponding metastasis. 9% of patients with WT primary tumour who received treatment had mutant *KRAS* metastasis, whereas 11.3% of the patients with mutant *KRAS* primary tumour had WT metastasis and did not receive treatment.⁴⁰ The development

of non-invasive techniques that would allow determining the mutational status of metastasis is consequently all the more a real challenge to personalized cancer treatment. Recently, Kawada et al⁴¹ showed that metastatic lesions of CRC had a higher $\text{SUV}_{\max}$ when they were *KRAS* mutated. However, this was observed in lesions >10 mm (p -value = 0.03), and there was no difference when the series included smaller lesions. They found that an $\text{SUV}_{\max}$ cut-off of 6.0 conferred the best accuracy with an area under the curve of 0.70 and sensitivity and specificity of 68% and 74%, respectively.

Our analysis was not limited to the $\text{SUV}_{\max}$, as in most related studies. We also evaluated volume-based parameters (MTV and TLG) and several textural features, which have been shown to be robust and reproducible,^{42,43} and have provided encouraging results as predictive or prognostic factors for various types of cancers.^{34,35,44–46} Our results showed that mutated rectal cancers

Table 3. Parameter distributions according to RAS-mutated status ($n = 151$)

Parameters	Wild type ($n = 68$)	RAS mutated ($n = 83$)	KRAS mutated ($n = 74$)	NRAS mutated ($n = 9$)
Age	65.4 $\pm$ 11.7	66.0 $\pm$ 11.9	66.3 $\pm$ 11.9	63.9 $\pm$ 13.0
Gender (M/F)	66%/34%	60%/40%	61%/39%	56%/44%
Stage I/II/III/IV	4%/12%/66%/18%	5%/10%/65%/20%	5%/11%/62%/22%	0%/0%/89%/11%
SUV _{min}	4.81 $\pm$ 2.05	5.94 $\pm$ 2.75 ^a	5.97 $\pm$ 2.79 ^a	5.73 $\pm$ 2.53 ^a
SUV _{max}	13.74 $\pm$ 6.77	17.80 $\pm$ 8.83 ^a	17.93 $\pm$ 8.98 ^a	16.73 $\pm$ 7.93 ^a
SUV _{mean}	7.73 $\pm$ 3.43	9.61 $\pm$ 4.70 ^a	9.66 $\pm$ 4.77 ^a	9.22 $\pm$ 4.40 ^a
Skewness	0.57 $\pm$ 0.24	0.68 $\pm$ 0.31 ^a	0.68 $\pm$ 0.30 ^a	0.68 $\pm$ 0.36 ^a
SUV _{sd}	1.93 $\pm$ 1.00	2.58 $\pm$ 1.38 ^a	2.60 $\pm$ 1.39 ^a	2.45 $\pm$ 1.40 ^a
Excess kurtosis	-0.27 $\pm$ 0.63	-0.03 $\pm$ 0.93	-0.05 $\pm$ 0.91	0.07 $\pm$ 1.21
SUV _{cov}	0.25 $\pm$ 0.03	0.26 $\pm$ 0.03 ^a	0.26 $\pm$ 0.03 ^a	0.26 $\pm$ 0.04 ^a
MTV (cm ³)	31.7 $\pm$ 28.3	28.4 $\pm$ 22.7	29.5 $\pm$ 23.6	19.3 $\pm$ 7.8
TLG	254.5 $\pm$ 265.1	269.9 $\pm$ 227.9	282.6 $\pm$ 237.3	165.6 $\pm$ 70.0
ASM	0.0035 $\pm$ 0.0026	0.0036 $\pm$ 0.0034	0.0036 $\pm$ 0.0035	0.0040 $\pm$ 0.0026
Contrast	282.2 $\pm$ 99.8	294.7 $\pm$ 120.2	293.9 $\pm$ 119.6	301.3 $\pm$ 132.8
Entropy	5.96 $\pm$ 0.67	5.93 $\pm$ 0.66	5.96 $\pm$ 0.67	5.73 $\pm$ 0.53
Correlation	0.33 $\pm$ 0.12	0.32 $\pm$ 0.13	0.32 $\pm$ 0.13	0.31 $\pm$ 0.12
Homogeneity	0.153 $\pm$ 0.024	0.153 $\pm$ 0.027	0.153 $\pm$ 0.027	0.153 $\pm$ 0.031
Dissimilarity	13.11 $\pm$ 2.55	13.32 $\pm$ 2.95	13.29 $\pm$ 2.93	13.51 $\pm$ 3.29
Coarseness	5.28 $\pm$ 1.53	4.91 $\pm$ 1.27	4.92 $\pm$ 1.27	4.83 $\pm$ 1.39
Contrast _{NGTDM}	1.22 $\pm$ 0.70	1.37 $\pm$ 1.23	1.36 $\pm$ 1.24	1.48 $\pm$ 1.14
Busyness	0.103 $\pm$ 0.071	0.122 $\pm$ 0.081	0.13 $\pm$ 0.08	0.08 $\pm$ 0.04
Intensity variability	11.26 $\pm$ 10.51	11.29 $\pm$ 9.92	11.76 $\pm$ 10.36	7.43 $\pm$ 3.36
Size-zone variability	0.0046 $\pm$ 0.0034	0.0046 $\pm$ 0.0040	0.0045 $\pm$ 0.0041	0.0051 $\pm$ 0.0033

ASM, angular second moment; cm³, cubic centimetres; contrast_{NGTDM}, contrast acquired with neighbourhood intensity difference matrices; F, female; M, male; MTV, metabolic tumour volume; SUV_{cov}, standardized uptake value of coefficient of variation; SUV_{max}, maximum SUV; SUV_{mean}, mean SUV; SUV_{min}, minimum SUV; SUV_{sd}, SUV standard deviation; TLG, total lesion glycolysis.

^ap-value < 0.05 in comparison with wild-type tumours.

have a significantly higher glucose metabolism than WT. All the intensity features of the lesions were significantly higher when a mutation was present, regardless of the tumour size. All quantitative measurements are affected by the partial volume effect according to the lesion size and the system resolution. Furthermore, considering the textural features, a significant relationship exists with the volume, but it has been suggested that above the threshold of 10 cm³, they provide additional information compared with MTV.³⁴ We thus analyzed the larger lesions, *i.e.* with an MTV superior to 10 cm³, and the results were quite similar except for SUV_{min} and SUV_{mean}, which became irrelevant. Our results confirm the significant correlations and the trend observed in previously studies but in a larger and more homogeneous population of 151 patients with only rectal and rectosigmoid junction cancers. In particular, SUV_{cov}, defined as SUV_{sd} divided by SUV_{mean}, was the only feature significantly associated with presence of the mutations from a multivariate standpoint. The coefficient of variation is one the most basic element of tumour heterogeneity expressing the

variability of ¹⁸F-FDG uptake in the entire tumour based on intensity histogram, which is quite simple to measure using routine software. SUV_{cov} seems to have an important place in rectal cancers. Interestingly enough, Bundschuh et al⁴⁷ showed that SUV_{cov} at baseline was the best parameter to predict response to neoadjuvant radiochemotherapy in locally advanced rectal cancer. A recent publication confirms the significant association between the ¹⁸F-FDG PET textural analysis and the tumoral regression of locally advanced rectal cancer after neoadjuvant treatment, but without studying the SUV_{cov} specifically.⁴⁸

Our analysis was limited to PET quantitative metrics. Image-derived features extracted from other modalities (CT, dynamic contrast-enhanced CT and MRI) may have clinical value in rectal cancer.⁴⁹ In addition, it was recently shown that extracting and combining features from both PET and CT components of PET/CT could provide additional value⁵⁰ and such an approach may be explored in the present cohort in a future work.

Table 4. Parameter distributions according to RAS-mutated status for lesions with metabolic tumour volume (MTV) > 10 cm³ (n = 128)

Parameters	Wild type (n = 56)	RAS mutated (n = 72)	KRAS mutated (n = 65)	NRAS mutated (n = 7)
Age	64.8 ± 10.8	66.8 ± 11.39	67.4 ± 11.1	60.5 ± 12.9
Gender (M/F)	70%/30%	65%/35%	66%/34%	57%/43%
Stage I/II/III/IV	0%/9%/71%/20%	1.5%/10%/65%/23.5%	1.5%/11%/63%/24.5%	0%/0%/86%/14%
SUV _{min}	5.13 ± 2.07	5.95 ± 2.78	6.02 ± 2.85	5.30 ± 2.09
SUV _{max}	14.82 ± 6.84	18.07 ± 9.00 ^a	18.36 ± 9.19 ^a	15.42 ± 6.91
SUV _{mean}	8.29 ± 3.44	9.62 ± 4.77	9.75 ± 4.88	8.44 ± 3.70
Skewness	0.55 ± 0.24	0.69 ± 0.32 ^a	0.69 ± 0.31 ^a	0.67 ± 0.41 ^a
SUV _{sd}	2.08 ± 1.02	2.57 ± 1.39 ^a	2.62 ± 1.42 ^a	2.11 ± 1.10
Excess Kurtosis	-0.30 ± 0.62	0.04 ± 0.98 ^a	0.02 ± 0.94 ^a	0.21 ± 1.36 ^a
SUV _{cov}	0.25 ± 0.02	0.26 ± 0.03 ^a	0.26 ± 0.03 ^a	0.25 ± 0.03 ^a
MTV (cm ³)	36.8 ± 28.6	31.8 ± 22.5	32.8 ± 23.4	22.6 ± 4.5
TLG	300.5 ± 270.8	300.7 ± 228.9	313.1 ± 237.0	185.2 ± 58.2
ASM	0.0025 ± 0.0011	0.0026 ± 0.0010	0.0025 ± 0.0010	0.0028 ± 0.0005
Contrast	257.9 ± 80.9	260.5 ± 81.5	262.7 ± 83.6	239.8 ± 58.9
Entropy	6.18 ± 0.48	6.13 ± 0.43	6.15 ± 0.45	5.98 ± 0.19
Correlation	0.36 ± 0.10	0.35 ± 0.11	0.35 ± 0.11	0.36 ± 0.08
Homogeneity	0.160 ± 0.024	0.160 ± 0.024	0.160 ± 0.024	0.160 ± 0.025
Dissimilarity	12.54 ± 2.24	12.53 ± 2.24	12.58 ± 2.28	12.07 ± 1.89
Coarseness	5.65 ± 1.40	5.23 ± 1.00	5.20 ± 1.03	5.46 ± 0.66
Contrast _{NGTDM}	0.99 ± 0.38	1.01 ± 0.38	1.02 ± 0.39	0.95 ± 0.25
Busyness	0.12 ± 0.07	0.13 ± 0.08	0.14 ± 0.083	0.09 ± 0.037
Intensity variability	12.96 ± 10.86	12.57 ± 10.06	13.00 ± 10.46	8.57 ± 2.86
Size-zone variability	0.0033 ± 0.0018	0.0034 ± 0.0016	0.0033 ± 0.0016	0.0036 ± 0.0009

ASM, angular second moment; cm³, cubic centimetres; contrast_{NGTDM}, contrast acquired with neighbourhood intensity difference matrices; F, female; M, male; SUV_{cov}, standardized uptake value of coefficient of variation; SUV_{max}, maximum SUV; SUV_{mean}, mean SUV; SUV_{min}, minimum SUV; SUV_{sd}, SUV standard deviation; TLG, total lesion glycolysis.

^ap-value < 0.05 in comparison with wild-type tumours.

Compared with previous studies that focused only on the codons 12 and 13, we investigated the presence of *KRAS* mutations on codons 61, 117 and 146, as well as *NRAS* mutations in codons 12, 13 and 61. Such mutations have recently been described in approximately 20% of *KRAS* exon-2 WT and they influence the response to targeted therapy in a similar fashion.^{12,15,51} This explains the high rate of *KRAS*-mutated tumours in this study as compared with similar previous studies, but our results are concordant with the overall rate of *RAS* mutations recently described in a pooled analysis of randomized controlled trials in CRC.⁵² The rate of *RAS*-mutated tumours was not significantly different between rectal and rectosigmoid locations, in agreement with the results of Yamauchi et al.⁵³ On the other hand, no *BRAF* mutation was observed in our population, which is not surprising considering that the mutation rate is significantly lower in rectal than in colon cancers.^{11,53}

The absence of *RAS* mutation in metastatic CRC is associated with a better prognosis in association with the possibility to

efficiently treat those patients with cetuximab.^{6,7,9,10} On the other hand, the prognostic value of baseline ¹⁸F-FDG PET parameters in rectal cancer remains debated, whether it is for predicting the pathological response to neoadjuvant treatment or the survival.^{47,54–57} Our population is rather large but nonetheless too heterogeneous in terms of stages and treatments to meaningfully assess survival. In any case, the trend towards higher metabolism in mutated tumours warrants further investigation in order to test whether the combination of the two parameters could improve patient prognostication in a more homogeneous group.

These results, although statistically significant, have little clinical relevance however, as we could not define an optimal cut-off value that would reliably differentiate mutated and non-mutated cancers. For the SUV_{max}, the optimal cut-off was 15.5, with a sensitivity of 69% and a specificity of 52%, leading to major overlaps across groups. Consequently, we do not believe that ¹⁸F-FDG PET/CT could play a clinical role for assessing the mutation status or even guide issue sampling.

CONCLUSION

Although rectal cancers with *KRAS* or *NRAS* mutations display a significantly higher glucose metabolism than wild-type cancers,

the accuracy of currently available quantitative metrics extracted from ^{18}F -FDG PET/CT is not as such as the technique could play a meaningful clinical role.

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