Lack of Association between *KRAS* Mutations and ^{18}F-FDG PET/CT in Caucasian Metastatic Colorectal Cancer Patients

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Abstract. *Background: Although Kirsten rat sarcoma (KRAS) gene mutational testing is essential for the optimal design of therapeutic strategies for colorectal cancer, it is not always feasible or reliable. In this retrospective study, we examined whether ^{18}F-Fluorodeoxyglucose positron-emission tomography/computed tomography (^{18}F-FDG PET/CT) scans can serve as a surrogate examination for KRAS mutational testing. Patients and Methods: KRAS codon 12 and 13 mutational status was tested in 44 colorectal primary tumors and was compared with the ^{18}F-FDG PET/CT maximum standardized uptake value (SUV_{max}) values of the respective metastatic lesions. Glucose transporter-1 (GLUT1) mRNA levels were also measured in colorectal primary tumors. Results: No statistically significant correlation between ^{18}F-FDG PET/CT SUV_{max} values and KRAS mutation status was found (parametric t-test: p=0.4753; non-parametric Kruskal–Wallis test: p=0.51). This result cannot be attributed to the effect of differing GLUT1 mRNA levels, as shown by multivariate analysis. Conclusion: Our study failed to promote ^{18}F-FDG PET/CT uptake as a surrogate examination for KRAS mutation testing.*

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Despite recent advances in the therapeutic management of colorectal cancer, the median survival for patients with metastatic disease remains modest (1). However, a significant chance for cure still exists, provided that a minimal disease burden is diagnosed early. In such a case, 5-year survival rates up to 30% are achieved by a multi-disciplinary approach combining both metastasectomy and systemic therapy (2). ^{18}F-Fluorodeoxyglucose positron-emission tomography/ computed tomography (^{18}F-FDG PET/CT) has evolved as a critical component of this management algorithm; preoperative PET is reported to modify or preclude curative-intent resection of liver metastases in 25% of cases, thus sparing patients of redundant operations (3).

Kirsten rat sarcoma *(KRAS)* gene mutational testing is essential for the optimal design of therapeutic strategies in colorectal cancer. Importantly, *KRAS* mutation status holds both positive and negative predictive value for the use of antibodies to epidermal growth factor receptor (EGFR) (4). However, *KRAS* testing is not always feasible or reliable (5), thus depriving patients with colorectal cancer of a major benefit.

It is long-acknowledged that one of the hallmarks of cancer is altered tissue metabolism (6). Tumor cells are characterized by glucose transporter-1 (*GLUT1*; official gene name: *SLC2A1*) overexpression compared with the corresponding normal cells, with a subsequent increase in their glycolytic activity (7). This differential metabolic profile is actually the biological basis of PET/CT imaging technology. Notably, there is growing evidence of a linkage between *KRAS* mutations and enhanced *GLUT1* expression, resulting in even higher intra-tumoral GLUT1 levels (8, 9).

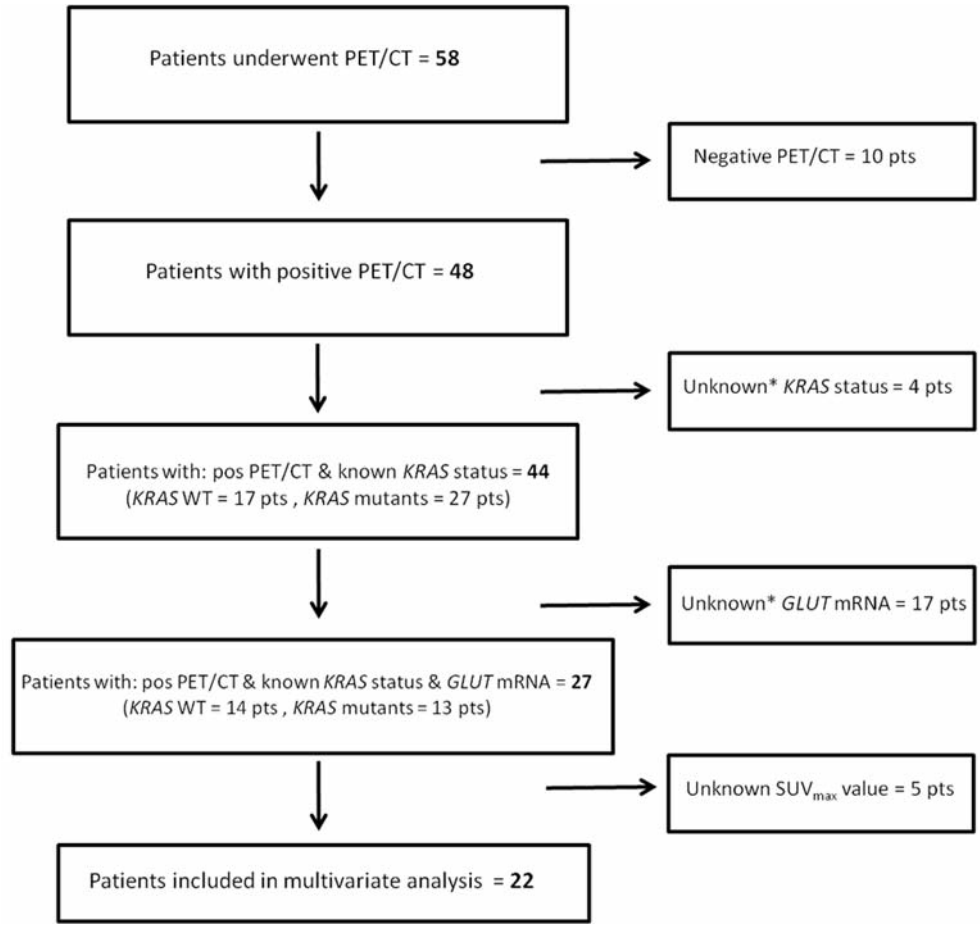

Figure 1. *REMARK diagram of the study. PET/CT: positron-emission tomography/computed tomography; GLUT1: Glucose transporter-1; KRAS: Kirsten rat sarcoma; WT: Wild-type; pos: positive; pts: patients; SUV_{max}: maximum standardized uptake value; Note: *Lack of available tumor tissue.*

In the present retrospective study, a considerable number of patients with metastatic colorectal cancer underwent PET/CT scans and *KRAS* mutation testing of their primary tumors. Our aim was to examine whether a) the intensity of ^{18}F-FDG PET/CT uptake correlates with *KRAS* mutation status, after adjusting for the effect of GLUT1 expression, and b) if standardized uptake value (SUV) can serve as a surrogate for *KRAS* mutational testing. To the best of our knowledge, this is the first time that such a hypothesis was studied in a population of Caucasian patients with colorectal cancer.

Patients and Methods

Patients. The study population consisted of 58 patients with metastatic colorectal cancer, either initially diagnosed at stage IV or relapsed following management of early disease. Metastases were confirmed with conventional imaging studies (CT or magnetic resonance imaging scans); no biopsies of metastatic lesions were performed. From February 2009 to May 2011, all patients underwent ^{18}F-FDG-PET/CT scans, which were diagnostic (positive) in 48 of them. *KRAS* mutation status was unknown for four patients, thus a total of 44 patients were eligible for analysis (REMARK diagram, Figure 1).

In parallel, a pilot set of 28 archival, paired primary and metastatic colonic cancer samples, different from those of the main study population, was utilized in order to examine whether *GLUT1* expression levels vary between primary tumor and the corresponding metastatic sites. These samples belonged to the Hellenic Cooperative Oncology Group tissue bank.

The present study was approved by the Hellenic Cooperative Oncology Group Protocol Review Committee and the Institutional Review Board of Papageorgiou General Hospital (Approval number 904/10-1-14). The study complied with the REMARK recommendations for tumor marker prognostic studies using biological material (10). All patients gave their informed consent for the provision of biological material for future research purposes.

All patients' characteristics are listed in Table I.

PET/CT procedure and technical details. A standard whole-body ^{18}F-FDG PET/CT protocol was utilized in all patients. The patients were asked to fast for six hours before the imaging study. Oral contrast material was given to all patients upon arrival and before

Table I. *Patients' characteristics, Kirsten rat sarcoma (KRAS) mutation type, Glucose transporter-1 (GLUT1) expression [Relative quantification (RQ) values], and positron-emission tomography/computed tomography (PET/CT) findings.*

			KRAS		*GLUT1*		*PET/CT*				
ID	Gender	Age (years)	Status	Mutation type	Tested	RQ	Site 1	Site 2	Site 3	SUV_{max}	CEA (ng/ml)
1	M	49	Mut	G12A	Y	41.08	Liver	na	na	8.9	1.9
2	M	62	Mut	G12V	Y	38.92	Lung	na	na	3	2.3
3	M	59	Mut	G12V	N	na	Liver	na	na	8	7.91
4	M	78	Mut	G13D	Y	38.59	Lung	Peritoneal	na	3.4	96
5	M	73	Mut	G12V	Y	40.72	Liver	Peritoneal	Lymph nodes	8.5	2.7
6	M	70	Mut	G12D	Y	39.38	Liver	na	na	13	4.6
7	F	62	Mut	G12A	Y	40.65	Presacral	Precoccygeal	na	12	126.6
8	M	62	Mut	G12V	N	na	Lung	Sigmoid	na	11.8	1.1
9	M	55	Mut	G13D	Y	38.67	Liver	Lymph nodes	na	16.6	3.4
10	M	56	Mut	G12V	Y	40.00	Lung	na	na	7.1	1.7
11	M	67	Mut	G12A	N	na	Lung	na	na	2.8	1.7
12	M	64	Mut	G12D	Y	40.02	Liver	na	na	5.5	2.1
13	M	66	Mut	G12D	Y	40.09	Lung	na	na	11.3	1.5
14	M	55	Mut	G12V	Y	39.42	Liver	na	na	12.4	25.4
15	M	49	Mut	G12V	N	na	Liver	na	na	11.6	1
16	M	67	Mut	G12V	N	na	Liver	na	na	12	2.4
17	F	41	Mut	G12V	N	na	Liver	na	na	7.3	4.3
18	F	30	Mut	G12A	Y	40.88	Peritoneal	Stoma	na	8.9	4
19	M	55	Mut	G12A	N	na	Lung	Lymph nodes	na	12.4	23
20	F	39	Mut	U	N	na	Lung	Lymph nodes	na	7.4	2
21	M	72	Mut	U	N	na	Liver	Rectum	Lymph nodes	18	6
22	M	58	Mut	U	N	na	Liver	na	na	8.6	16
23	F	60	Mut	U	N	na	Liver	na	na	7	21
24	M	38	Mut	G12V	Y	41.70	Negative	na	na	U	2.4
25	F	61	Mut	G12D	Y	40.39	Negative	na	na	U	3.5
26	M	59	Mut	U	N	na	Negative	na	na	U	2.5
27	M	74	Mut	G12D	Y	43.98	Negative	na	na	U	U
28	F	68	Mut	G12V	Y	38.82	Negative	na	na	U	U
29	F	59	Mut	G13D	Y	38.82	Sacrum	na	na	3.2	U
30	F	54	Mut	U	N	na	Peritoneal	na	na	14.2	U
31	M	70	Mut	U	N	na	Peritoneal	Sigmoid	na	25	U
32	F	47	Mut	G13D	N	na	Lymph nodes	na	na	6.3	U
33	F	76	WT	na	Y	38.22	Liver	Lymph nodes	na	7.4	308
34	F	40	WT	na	Y	40.65	Liver	Local relapse	na	5.7	146
35	F	44	WT	na	N	na	Lung	na	na	3.8	2.7
36	F	74	WT	na	Y	40.18	Liver	Local relapse	na	14.6	0.7
37	M	69	WT	na	Y	39.56	Liver	na	na	4.6	1.8
38	M	60	WT	na	Y	40.65	Presacral	Iliac bone	Adrenal	15	46
39	F	73	WT	na	Y	38.99	Lung	Lymph nodes	Vertebra	5.7	1
40	F	48	WT	na	Y	40.40	Peritoneal	Lymph nodes	na	16.5	12
41	M	67	WT	na	Y	42.83	Lung	na	na	3.5	1.4
42	M	53	WT	na	Y	38.55	Liver	Peritoneal	Lymph nodes	6.6	1.3
43	M	72	WT	na	N	na	Liver	na	na	9.1	5.2
44	M	59	WT	na	N	na	Liver	na	na	7.5	15
45	M	63	WT	na	Y	41.05	Negative	na	na	U	0.6
46	F	58	WT	na	N	na	Negative	na	na	U	1.3
47	F	64	WT	na	Y	40.18	Negative	na	na	U	1.3
48	F	59	WT	na	Y	39.37	Negative	na	na	U	U
49	F	63	WT	na	Y	39.32	Lung	Peritoneal	Lymph nodes	6.8	U
50	M	60	WT	na	Y	39.76	Rectum	Lymph nodes	na	14.3	U
51	F	57	WT	na	Y	40.82	Pancreas	na	na	4.5	U
52	M	75	WT	na	Y	39.69	Peritoneal	na	na	3.9	U
53	M	56	WT	na	Y	40.08	Spleen	Lymph nodes	na	14	U
54	M	61	U	na	N	na	Presacral	na	na	3.8	U
55	F	42	U	na	N	na	Liver	na	na	14	U
56	F	56	U	na	N	na	Pararectal	na	na	10.8	U
57	F	79	U	na	N	na	Negative	na	na	U	U
58	F	70	U	na	N	na	Liver	na	na	9.8	U

SUV_{max}: Maximum standardized uptake value; CEA: carcinoembryonic antigen; M: male; F: female; Mut: mutated; WT: wild-type; Y: yes; N: no; U: unknown; na: not applicable.

Table II. *Distribution predilection and absolute frequency of metastatic lesions based on ^{18}F-Fluorodeoxyglucose positron-emission tomography/computed tomography findings.*

Lesion	Absolute frequency in the study population
Liver	22
Pulmonary nodules	12
Abdominal LN	9
Peritoneal metastases	9
Common and internal iliac LN	6
Pararectal/precoccygeal/presacral tissue	5
Mediastinal LN	3
Bones	3
Rectal lesion	2
Sigmoid lesion	2
Cervical/supraclavicular LN	2
Local relapse	2
Relapse around stoma	1
Adrenal gland	1
Pancreas	1
Spleen	1

LN: Lymph nodes.

the initiation of the imaging procedure. The serum glucose concentration, measured prior to ^{18}F-FDG administration, was less than 150 mg/dl in all patients. Image acquisition started one hour after the intravenous injection of a dose of 5 MBq/kg ^{18}F-FDG. All acquisitions were made with an integrated PET/CT scanner (Discovery ST; GE Medical Systems, Waukesha, WI, USA). A whole-body image from the mid femur to the base of the brain was obtained, typically divided into six bed positions. The PET emission images were acquired for a 4-min acquisition period at each bed position. The imaging system enabled the simultaneous acquisition of 47 transverse PET images per field of view, using 3.27 mm intersection spacing, for a total of 15.7 cm transverse field of view. PET resolution was approximately 6.1 mm full width at half maximum near the center of the field of view. A four-detector row helical CT-scanner (140 kV and 80 mA) was also included in the PET/CT system. The resulting CT images were used not only in image fusion but also in the generation of an attenuation map for attenuation correction. PET scan was acquired in the two-dimensional mode. The field of view and pixel size of the reconstructed images were 50 cm and 3.91 mm, respectively, with a matrix size of 128×128. The reconstruction method used was filtered back projection with Hanning filter.

Standard whole-body PET/CT images were reviewed on a Xeleris workstation (GE Healthcare, Milwaukee, WI, USA) in transverse, coronal, and sagittal planes, along with maximum intensity projection images. For visual analysis, ^{18}F-FDG PET uptake was considered abnormal if located outside the normal anatomic structures or if having intensity greater to the background blood-pool activity or adjacent normal tissue. In addition, SUV of the lesions was measured on the standard whole-body PET/CT in a semi-quantitative factor. SUV was calculated using the following formula: $SUV=C_{dc}/(d_i/w)$, where C_{dc} is the decay-corrected tracer tissue concentration (Bq/g), d_i is the injected dose (Bq) and w is the patient's body weight (g). The maximum SUV (SUV_{max}) was recorded for each lesion after applying regions of interest (ROI) in the transaxial attenuation-corrected PET slices, around the pixels showing the greatest accumulation of ^{18}F-FDG.

For diagnostic reasons, the lymph nodes were divided into the following groups: cervical, mediastinal, abdominal, and iliac. SUVmax was defined from the lesion with the highest ^{18}F-FDG uptake of each organ or lymph node group.

Two experts, a nuclear medicine physician and a radiologist, interpreted visually the PET/CT scans. The evaluation included the calculation of the overall per patient sensitivity of ^{18}F-FDG PET/CT.

KRAS mutation analysis. DNA extraction from archival formalin-fixed paraffin-embedded tumor samples and KRAS genotyping for the seven most common *KRAS* mutations on codons 12 and 13 (c.34G>A p.G12S; c.34G>C p.G12R; c.34G>T p.G12C; c.35G>A p.G12D; c.35G>C p.G12A; c.35G>T p.G12V; c.38G>A p.G13D; according to CCDS 8702.1 and NM_004985.3) were performed as previously described (11). Briefly, manual macrodissection was performed in order to include the highest possible rate of tumor cells in the molecular sample; DNA was extracted with the QIAamp DNA mini kit (Qiagen, Hilden, Germany); DNA quality was tested with multiplex Polymerase Chain Reaction (PCR); *KRAS* mutations were analyzed with Taqman-MGB assays in a 7500 Real-Time PCR System (Applied Biosystems/Life Technologies, Paisley, Scotland, UK). Assays involved duplex real-time PCR reactions for the wild-type (Victoria Blue-labeled probe) and mutant (Fluorescein amidite-labeled probe) target; assay sequences are available upon request. Samples were tested in duplicates along with positive (mutant samples) and negative (wild-type sample and no template) controls. Samples were considered eligible for analysis according to percentage tumor cell content and wild-type curve cycle threshold (C_T), while for the determination of mutation status, previously validated deltaC_T (mutant C_T – wild-type C_T) cut-offs were applied (11).

With this method, all 44 samples yielded informative results for *KRAS* codon 12 and 13 mutation status (REMARK diagram, Figure 1). Furthermore, cycle-sequencing (sense and antisense) was also applied to these samples for *KRAS* exons 2 and 3; Taqman-MGB results were validated and no further mutations were observed.

GLUT1 mRNA expression levels. RNA extraction from formalin-fixed paraffin-embedded sections was performed upon macrodissection with Trizol-LS and Superscript III for reverse transcription, as previously described (12). GLUT1 expression was assessed with pre-made TaqMan® Gene Expression Assays (assay Hs00892681_m1; NM_006516.2; exons 8-9; amplicon 76bp) against β-glucuronidase (GUSB) as endogenous reference (assay #4333767F; Applied Biosystems). Samples were run in duplicate 10 μl reactions (50 ng template/reaction) in a 7900HT real-time PCR system and were evaluated under default conditions. Positive and negative controls included a commercially available reference RNA (#4307281; Applied Biosystems) and no-template samples, respectively. Relative quantification of GLUT1 was assessed as 40-dCT, whereby dC_T=(GLUT1 C_T–GUSB C_T). Samples were considered eligible for analysis for GUSB C_T values less than 36, and dC_T difference of less than 0.8 between duplicates.

A total of 128 samples were used for RNA extraction and *GLUT1* expression evaluation; out of these, 110 (85.9%) were found the eligible for analysis. In particular, these included

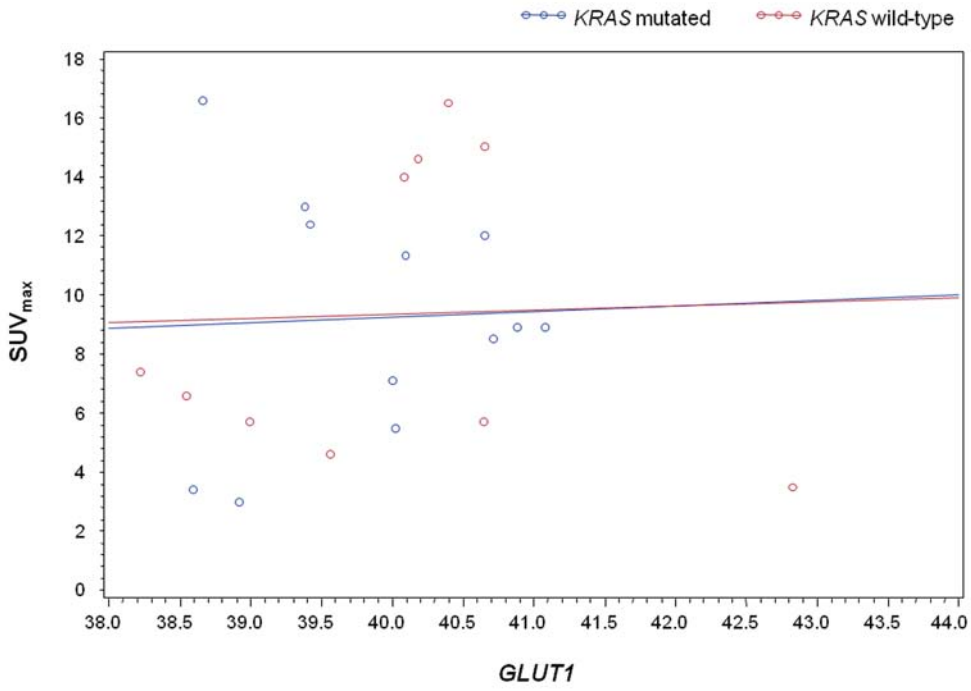

Figure 2. *Correlation of maximum standardized uptake value (SUV_{max}) and Glucose transporter-1 (GLUT1) mRNA expression correlation adjusted by Kirsten rat sarcoma (KRAS) mutation status.*

Table III. *Age, Glucose transporter-1 (GLUT1) mRNA, maximum standardized uptake value (SUV_{max}) and serum Carcinoembryonic antigen (CEA) levels in KRAS-mutated vs. KRAS wild-type populations in the study.*

	KRAS				Total	
	Mutant		Wild-type			
	N	Median	N	Median	N	Median
Age (years)	27	59.0	17	60.0	44	60.0
GLUT1 expression (RQ value)	13	40.0	14	39.9	27	40.0
SUV_{max}	23	8.9	13	7.4	36	8.6
CEA (ng/ml)	23	3.4	12	4.0	35	3.4

RQ: Relative quantification; KRAS: Kirsten rat sarcoma.

28 matched primary–metastatic tumor pairs and six normal colonic mucosa samples (pilot study for assessing *GLUT1* mRNA levels); and 48 primary tumor samples (study group). A 2-fold difference was considered as the cut-off for comparing relative *GLUT1* expression between primary tumors and metastatic sites, corresponding to 1.75-fold difference in relative quantification values between matched samples.

Statistical analysis. Continuous variables are presented as average values with the corresponding standard deviations, while categorical data are presented as frequencies and percentages.

Comparisons of categorical with continuous variables were made using the parametric *t*-test and the non-parametric Kruskal–Wallis test, as appropriate. The Fisher's exact test was used for testing associations between categorical variables. Spearman's rho was used to assess correlations among continuous variables. In univariate analysis, the significance level was set at 5%. All tests were two-sided.

Multivariate regression analysis was conducted in order to assess the effect of *GLUT1* expression on SUV_{max} values by *KRAS* levels.

The SUV_{max} values obtained from ^{18}F-FDG PET/CT scans were evaluated with regard to *KRAS* gene mutation status and *GLUT1* mRNA levels of the corresponding primary tumors. The statistical analysis complied with the reporting recommendations for tumor marker prognostic studies (10) and was performed with the use of SAS version 9.3 (SAS, Institute Inc., Cary, NC, USA).

Results

Study population, KRAS, GLUT1, and PET/CT descriptives. Detailed information for each patient in the study is presented in Table I. The study group consisted of 44 patients (28 males and 16 females), with age ranging from 30 to 78 years (mean=59.7±11.0 years). Mutations in *KRAS* gene were detected in 27 primary tumors, while *KRAS* was wild-type in the remaining 17. Specific *KRAS* mutation type distribution was as follows: G12V: 9 cases; G12A: 5 cases; G12D: 3 cases; G13D: 4 cases; G13D: 4 cases; unknown: 6 cases. Out of the 44 primary tumors of the study, 27 samples (61.4%) were tested for *GLUT1* mRNA levels; in 17 cases this was not possible due to exhausted material (REMARK diagram, Figure 1). The distribution of lesions in positive PET/CT scans is shown in Tables I and II. In three patients, the CT component (low-dose CT) of the PET/CT scan revealed suspicious pulmonary nodules showing no ^{18}F-FDG uptake, probably due to their small size (<1 cm); these scans were considered as negative and the respective patients were excluded from the study (REMARK diagram, Figure 1). The SUV_{max} value of the lesions with ^{18}F-FDG uptake ranged from 2.8 to 25 (mean=9.6±4.9). Carcinoembryonic antigen (CEA) values at the time of the PET/CT imaging ranged from 0.7 to 308 ng/ml (mean=25.7±60.1 ng/ml). Table III provides the variables under study in the *KRAS*-mutated *vs*. *KRAS* wild-type population.

Concordance of GLUT1 expression levels between primary and corresponding metastatic tumors. A pilot set of 28 archival, paired primary and metastatic colonic cancer samples, different from those of the main study population, was utilized in order to assess the association of *GLUT1* mRNA levels between primary and metastatic tumors. The scope was to use *GLUT1* levels of the primary tumors as a surrogate for the matched metastatic ones.

The intra-class correlation coefficient (ICC) was estimated in order to assess the concordance of the *GLUT1* mRNA levels between the primary and the corresponding metastatic colonic cancer samples. In our study, the ICC was 0.61, which is interpreted as a moderate level of concordance (ICC ranges from 0: absence of concordance to 1: maximum concordance). Since application of the ICC (random effects approach) showed that there was no significant difference, *GLUT1* expression levels of the primary tumors can, therefore, be used as surrogate for *GLUT1* mRNA levels of the corresponding metastatic lesions.

No association between GLUT1 mRNA expression and KRAS mutation status. Thirty-four primary tumor samples were tested in order to examine the possible association between *GLUT1* mRNA expression and *KRAS* mutation status. The two-sample *t*-test was used and a complete absence of correlation between *GLUT1* levels and *KRAS* status emerged (mean *GLUT1* value and standard deviation for *KRAS*-mutated *vs*. wild-type tumors=40.1 *vs*. 40.0 and 1.23 *vs*. 1.14, respectively; p=0.76).

Table IV. *Multivariate analysis to control for the effect of Glucose transporter-1 (GLUT1) mRNA expression on Kirsten rat sarcoma (KRAS) mutation status*.

Model	Effect	*p*-Value
A: Simple	*GLUT1*	0.8677
	KRAS	0.9523
B: Interaction	*GLUT1*	0.8711
	KRAS	0.9783
	GLUT1 × *KRAS*	0.9794

No correlation between SUV_{max} values and KRAS mutation status. Forty-four cases were tested and univariate analysis demonstrated that SUV_{max} values did not differ in a statistically significant manner between *KRAS* wild-type and *KRAS*-mutated tumors. Both parametric and non-parametric tests gave *p*-values greater than 0.05 (p=0.4753, parametric *t*-test) (variances equal); p=0.50998, non-parametric Kruskal–Wallis test. However, there was a trend for higher SUV_{max} values in *KRAS*-mutated tumors compared to *KRAS* wild-type ones (median SUV_{max} 8.9 *vs*. 7.4, respectively, Table III). Consequently, no correlation of SUV_{max} values with KRAS mutation status was established.

GLUT1 mRNA levels have no effect on SUV_{max} values in KRAS-mutated nor in KRAS wild-type tumors. In a subset of 22 samples, multivariate analysis was utilized in order to control for the effect of *GLUT1* levels in primary tumors on SUV_{max} values of metastatic lesions, either as a simple variable in a model or as an interaction with *KRAS* mutation status. In both cases, the *p*-values for the effect were greater than 0.05. Thus, the lack of correlation between SUV_{max} values and *KRAS* mutation status cannot be attributed to an effect of variation in *GLUT1* mRNA level (Table IV).

Figure 2 illustrates the pattern of the relationship between SUV_{max} and *GLUT1* expression between the two categories of *KRAS* (mutated *vs*. wild-type). In this scatter plot, the regression lines show the direction of the relationships in each case, which do not differ significantly.

Discussion

Testing for *KRAS* mutations has evolved to be a prerequisite for the design of treatment algorithms in metastatic colorectal cancer. Efficacy of monoclonal antibodies against EGFR, such as cetuximab and panitumumab, is largely dependent on the

absence of activating *KRAS* mutations in the tumor, while a detrimental effect is evident once they are used in patients with *KRAS*-mutant tumor (13). However, despite the widespread use of *KRAS* testing, a number of issues limit its utility and credibility (5). Metastatic lesions are often inaccessible and biopsy cores are frequently unsuitable, as low-cellularity specimens are inappropriate for exhaustive molecular examination. Moreover, contraindications for invasive biopsies are commonly present and intratumoral heterogeneity of the primary tumor questions the reliability of *KRAS* results (14).

The rationale of the present study was to take advantage of a commonly used imaging method, such as PET/CT, and evolve it into a surrogate which could compensate for the inherent flaws of *KRAS* testing. The objectivity of SUV measurement could potentially provide further advantage in this effort.

Recent studies have revealed that oncogenic signaling and tumor cell metabolism are closely interrelated (6). Metabolic profiling of tumor cells reveals accelerated rates of glucose uptake (15) and the association of glycolytic fueling with activated oncogenes, such as rat sarcoma (*RAS*) and myelocytomatosis (*MYC*) (16). The relationship between tumor growth and glucose metabolism may be explained in terms of adaptation to hypoxia through up-regulation of GLUTs, as well as the translocation and increased enzymatic activity of hexokinase (17). These metabolic changes seem to confer a universal advantage on many different cancer types, by increasing cell ability to survive, proliferate, and invade under hypoxic conditions.

Positive PET/CT signal acquisition in colorectal cancer is the result of an increased expression of glucose transporters, mainly of GLUT1 (18). Studies in implanted human colonic cancer cell lines suggest that GLUT1 activity is the critical factor for ^{18}F-FDG accumulation in colonic tumors, undermining the role of hexokinase (19). GLUT1 expression in colonic cancer has been reported to be positively correlated with SUV_{max} in a statistically significant way (18, 20). Nevertheless, in our study no link between GLUT1 levels and SUV_{max} was found. This is in line with the report of Hong and Lim, where GLUT1 in colorectal adenocarcinomas was not significantly associated with SUV_{max} (21).

In the present study, GLUT1 levels were not measured in the metastatic lesions, since no biopsies of metastases were performed. Bibliographic data are conflicting regarding differential expression of GLUT1 between primary tumors and the corresponding metastases. While in squamous carcinomas of the head and neck area, recurrent tumors were characterized by higher GLUT1 levels measured immunohistochemically (22), comparable GLUT1 expression has been reported in lung cancer and corresponding metastatic liver tumors (23). To address this issue, we conducted a parallel pilot study, measuring GLUT1 expression in 28 primary and matching metastatic colonic tumors; this colonic tumor set was different from that of the main study. As a result, a non-significant differential GLUT1 expression was found between primary tumors and their corresponding metastases, thus justifying GLUT1 measurement in the primary tumors as a surrogate for the respective metastatic ones.

There is substantial recent pre-clinical evidence that *KRAS* mutations are associated with enhanced tumoral expression of GLUT1. Studies in isogenic colorectal cancer cell lines indicate a significant increase in glucose uptake, caused by GLUT1 up-regulation, which is prominent in all cells with mutant *KRAS* alleles, providing them with a growth advantage in low glucose environments (9). Importantly, the increase in *GLUT1* expression and resultant metabolic changes were stable phenotypes, rather than transient responses to low glucose levels, as they persisted under normoglycemic conditions. This pre-clinical evidence was reproduced in *KRAS*-mutated clinical lung cancer specimens in a Japanese study (8). The aforementioned data support the long-standing theories, that RAS-family oncogenes modulate glucose metabolism by increasing GLUT1 expression (24, 25). In contrast with this, our study did not show any correlation of GLUT1 levels with *KRAS* mutations. This finding is in line with an earlier Japanese study, which reported no association between codon 12 *KRAS* mutations and GLUT1 expression in colonic cancer samples (26).

In addition, in the present study we did not find a statistically significant connection between *KRAS* status and ^{18}F-FDG uptake, despite the tendency for higher SUV_{max} values in *KRAS*-mutated cases. This is concordant with similar studies performed in cholangiocarcinoma (27), lung (28) and head and neck cancer (29), but contradicts a recent report in colorectal tumors, in which immunohistochemistry was used to assess GLUT1 expression (30).

There are several speculations for the conflicting bibliographical evidence. Given the much higher incidence of GLUT1 overexpression in colonic cancer (90%) (31) compared to KRAS mutation (40%) (32), it can be deduced that *GLUT1* gene transcription is regulated by additional driving factors. Hypoxia-alone can boost GLUT1 levels through HIF1A (33); moreover, the expression of certain oncogenes such as *c-MYC* and sarcoma (*SRC*), as well as elements of the PI3K/AKT pathway, has been associated with activation of glycolysis (24, 34-36). Besides, the mechanisms underlying ^{18}F-FDG accumulation into cancer tissues are complex, including both tumor-related (*e.g.* histological differentiation, vascular factors, and tumor size) and non-tumor-related components (*e.g.* diabetes mellitus and inflammation) (37). Furthermore, other RAS-family mutations, apart from those in exons 2 and 3 of the *KRAS* gene, may regulate glucose metabolism. Finally, other GLUT proteins (*e.g.* GLUT3) and enzymes downstream of hexokinase (*e.g.* pyruvate dehydrogenase-kinase 1) may be involved in 18F-FDG accumulation in cancer cells (38).

To the best of our knowledge, this is the first study in a Caucasian population of patients with colorectal cancer to explore the association of KRAS mutation status with the intensity of the ^{18}F-FDG PET/CT uptake of metastatic lesions. Qualities that strengthen the importance of our study are, among others, the adequate size of the study population for a hypothesis-generating study; the review of PET/CT images by two physicians, both a nuclear medicine and a radiologist; the assessment of *GLUT1* mRNA instead of protein expression and the incorporation of *GLUT1* mRNA levels into the multivariate analysis. On the other hand, only codons 12 and 13 of *KRAS* gene were tested, since the study was designed before the emergence of the predictive value of RAS family members in colorectal cancer (13). Hexokinase expression was not assessed in our study, since there are data undermining its role in ^{18}F-FDG accumulation compared to GLUT1 (30, 38).

In conclusion, our study failed to promote ^{18}F-FDG PET/CT uptake as a surrogate for *KRAS* mutation testing. Molecular pathogenesis of colorectal cancer, tumor metabolism and other factors regulating ^{18}F-FDG accumulation are interrelated in a highly sophisticated way, not yet fully-understood. Hopefully, our study will serve as a guide to elucidate the complex glucose metabolism pathways in colorectal cancer.

Conflicts of Interest

All Authors declare they have no conflicts of interest.

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