^{18}F-FDG PET/CT Is an Early Predictor of Pathologic Tumor Response and Survival to Preoperative Radiochemotherapy with Bevacizumab in High Risk Locally Advanced Rectal Cancer

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ABSTRACT

There is an unmet need for predictive biomarkers of the clinical benefit of antiangiogenic drugs. The aim of the present study was to prospectively evaluate the value of ^{18}F-FDG-PET/CT performed during and after preoperative chemoradiotherapy with bevacizumab for the prediction of complete pathologic tumor regression and survival in magnetic resonance imaging (MRI)-defined high-risk locally advanced rectal cancer (LARC) patients.

Methods Sixty-one patients treated in a non-randomized phase II study (BRANCH) with concomitant or sequential (4 days before chemoradiotherapy) administration of bevacizumab with preoperative chemoradiotherapy were included. ^{18}F-FDG PET/CT was performed at baseline, 11 days after the beginning of chemoradiotherapy (early) and before surgery (late). Metabolic changes were compared with pathological complete (TRG1) vs incomplete (TRG2-5) tumor regression, progression-free survival (PFS), cancer specific survival (CSS) and overall survival (OS). Receiver operating characteristic (ROC) curves were calculated for those ^{18}F-FDG-PET/CT parameters that significantly correlated with TRG1.

Results Early total lesion glycolysis (TLG-early) and its percent change compared to baseline (ΔTLG-early) could discriminate TRG1 from TRG2-5. Only ROC analysis of ΔTLG-early showed an area under curve (AUC) > 0.7 (0.76), with an optimal cutoff at 59.5% (80% sensitivity, 71.4% specificity) for identifying TRG1. Late metabolic assessment could not discriminate between the two groups. After a median follow-up of 98 months (range 77–132) metabolic responders (ΔTLG-early ≥ 59.5%) demonstrated a significantly higher 10-year PFS (89.3% vs 63.6%, p=0.02) and CSS (92.9% vs 72.6%, p=0.04) compared to incomplete metabolic responders.

Conclusion Our results suggest that early metabolic response can act as a surrogate marker of the benefit of antiangiogenic therapy and provide further support for the use of early ^{18}F-FDG-PET/CT evaluation to predict pathological response and survival in the preoperative treatment of patients with LARC. ΔTLG-early showed the best accuracy in predicting TRG and may be particularly useful in guiding treatment modifying decisions during preoperative chemoradiotherapy based on expected response.

Keywords ^{18}F-FDG-PET/CT; Rectal cancer; Chemoradiotherapy; Bevacizumab; TLG

INTRODUCTION

Although, preoperative multimodality treatment advances have remarkably improved the outcomes of patients with locally advanced rectal cancer (LARC), there is still a clear need to optimize the management of these patients (*1*). The evidence that LARC represents a widely heterogeneous group of tumors with different prognostic features has prompted to pursue different risk-adapted treatment strategies in order to maximize benefit and minimize toxicity of treatment (*1*).

Moreover, the increased awareness of negative effects on quality of life consequent to long-term morbidity of surgery (*2*) and the excellent outcomes associated with pathological complete response, have led to explore organ preservation strategies in selected patients with good response to neoadjuvant treatment (*3*).

An intriguing conservative strategy that has become very popular recently is the “watch and wait” approach. This strategy omits surgery when a complete clinical response is obtained after preoperative treatment and provides “true” organ-sparing. However, concerns about the safety of this approach have been raised (*2*) considering that the current selection criteria rely exclusively on clinical assessment, which may be of limited accuracy after preoperative treatment (*4,5*). A promising role in the prediction of pathological tumor response has been advocated for metabolic imaging with ^{18}F-2-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG-PET) in LARC (*6*). Interestingly, growing evidence has shown that this functional imaging is able to reliably predict treatment response early, during preoperative treatment (*7*). Our group has recently reported that early metabolic change, accurately predicts pathological response and long-term outcome in LARC (*8*). The early identification of response has great clinical importance because it offers the opportunity for response-guided tailoring of preoperative treatment and subsequent surgery, allowing to refer non-responders to alternative treatment and good responders to a conservative surgical approach.

We have recently reported the final results of a non-randomized phase 2 study, to assess the safety and efficacy of traditional “concomitant” versus experimental “sequential” (4 days before chemoradiotherapy) administration of bevacizumab, with preoperative chemotherapy, in magnetic resonance imaging (MRI)-defined high-risk LARC patients (BRANCH Trial) (*9*). The primary endpoint, pathological complete tumor regression (TRG1), was reached with the sequential schedule and the final TRG1 rate was 50% (95% CI 35%–65%). Since there is an unmet need for predictive biomarkers of the clinical benefit of antiangiogenic drugs, we have also explored the potential predictive role of early metabolic response.

MATERIALS AND METHODS

Patient Selection

PET imaging was performed as part of the phase II BRANCH trial (Clinicaltrials.gov number: NCT01481545) evaluating preoperative chemoradiotherapy in patients with pathologically confirmed untreated MRI-defined high-risk LARC as reported previously (9). These included patients with tumors with concomitant resectable distant metastases and/or T4 features, any T N1-2 tumors and T3N0 tumors located in the lower third of the rectum and/or whose radial margin was ≤ 5 mm from the mesorectal fascia (MRF). The study was conducted under a protocol approved by the local Ethics Committee and was in accordance with the Helsinki Declaration.

Treatment and Follow-up

Chemotherapy, given during radiotherapy (45 Gy over 5 weeks), consisted of three biweekly cycles of infusional oxaliplatin (100 mg/m2) followed by raltitrexed (2.5 mg/m2) on day 1, levo-folinic acid (250 mg/m2) and a bolus of 5-fluorouracil (800 mg/m2) on day 2. Bevacizumab 5 mg/kg was administered 2 weeks before the start of chemoradiotherapy and on day 1 of each cycle (concomitant schedule) or 4 days prior to the first and second cycle of chemotherapy (sequential schedule). Two additional cycles of chemotherapy with one bevacizumab infusion were allowed after the end of chemoradiotherapy in patients with distant metastases. Total mesorectal excision (TME) was planned 8 weeks after the last day of radiotherapy. Four months of post-operative adjuvant FOLFOX4 regimen was planned only in patients with ypN+ or circumferential resection margin (CRM) ≤ 1 mm on pathology or for patients having distant metastases at baseline resected with R0/R1 status. Clinical examination, CEA serum levels, whole body CT and pelvic MRI, were performed every 4 months for the first 2 years of follow up, every 6 months for the next 3 years and annually thereafter.

Pathological Analysis

The surgical specimens were evaluated and scored according to the Mandard score (*10*), by two experienced pathologists who were unaware of PET findings. In case of discrepancy between the two pathologists, the worse TRG score was assigned. Based on these findings, patients were classified as pathological tumor complete responders (TRG1) or incomplete responders (TRG2-5).

^{18}F-FDG-PET/CT Analysis

^{18}F-FDG-PET/computer tomography (^{18}F-FDG-PET/CT) imaging was performed before (baseline), 11 days after starting chemoradiotherapy (early) and within one week prior to surgical resection (late).

Patients, fasted for at least 6 h with blood glucose levels below 150 mg/dL, were administered 300-400 MBq of ^{18}F-FDG and scanning was started 60 min after injection. A General Electric Discovery DST-600 scanner (GE Healthcare, Milwaukee, WI, USA) routinely subject to daily and periodical quality control according to EANM guidelines (*11*) was utilized for scanning (3 min per bed position emission). CT based attenuation corrected images were reconstructed with the ordered subsets expectation maximization (OSEM) algorithm (2 iterations, 16 subsets). Emission data were corrected for decay, dead time and random coincidences. Data were normalized for injected dose and patient body weight. Image analysis was performed in all cases with the same semi-automatic region-of-interest (ROI) drawing software package where a three-dimensional region was drawn around the area of increased uptake. Threshold values were adjusted in order to encompass the area of increased uptake visually. For each tumor volume, the following parameters were calculated: a) SUV = (measured activity concentration [Bq/mL])/(injected activity [Bq]/body weight [kg] 1000); b) SUV-max= the maximum pixel value measured in the visualized lesion; c) SUV-mean= the average SUV values in the ROIs; d) TLG (Total Lesion Glycolysis)= SUV-mean x metabolic tumor volume (mm^3). Metabolic response was calculated by measuring early and late changes relative to baseline. Thus for all indicators a Δ value for early and late studies was calculated as follows [(baseline value – early or late value)/baseline value) × 100]. Correlations between these changes (Δ-early or Δ-late) and pathological tumor responses were determined.

Statistical Analysis

The primary end point of the BRANCH trial was the rate of TRG1 and the sample size was established applying Simon's two-stage design as previously reported in details *(9)*. The study also included biomarker studies and among these the predictive role of early metabolic response as assessed by ^{18}F-FDG-PET/CT as a prospective secondary endpoint. All quantitative values have been expressed as medians and ranges (minimum and maximum) and proportions with their 95% confidence interval (CI). Receiver operating characteristic (ROC) curves were calculated for the ^{18}F-FDG-PET/CT parameters that were found to be significantly correlated with TRG1. The area under the curve (AUC) was used to assess accuracy. Only when a test reported an AUC > 0.7 the maximum product of sensitivity and specificity was chosen as the best cut-off value of the parameter for the

prediction of TRG1. To evaluate the independence of the cut-off value in predicting TRG1 a multivariate analysis was performed with the most relevant clinical variables: age, gender, Eastern Cooperative Oncology Group (ECOG) performance status (0 vs. 1 or 2), Gunderson risk (12), distance from the anal verge (≤ 5 cm vs. > 5 cm), distance from the MRF (≤ 2 mm vs. > 2 mm) and baseline CEA serum level (≤ 5 UI/L vs. > 5 UI/L). The odds ratio (OR) and 95 % CI were used to report the results. Progression-free survival (PFS) was defined as the time from initial treatment until tumor progression or relapse, death for any cause or last follow-up. Patients who were progression free at the closeout date had their time to progression censored to that date. Overall survival (OS) was defined as the time from initial treatment until death for any cause or to last follow-up. Cancer-specific survival (CSS) was defined as the time to cancer related death or to the last follow-up. Patients who were alive at the closeout date had their OS and CSS censored to that date.

PFS, OS and CSS rates were estimated with their 95% CI using the Kaplan-Meier method and compared with the log-rank test. For survival analysis, in the eight patients with distant metastases, metabolic response of metastatic lesions according to previously described criteria (TLG reduction >50%) (*13*)) was also taken into account when defining patients as responders and incomplete responders based on ^{18}F-FDG-PET/CT.

The Cox regression model was used to assess the role of the cut-off value of ^{18}F-FDG-PET/CT parameters in predicting PFS, OS and CSS. Hazard ratios (HR) were derived from Cox regression analysis. A univariate analysis assessed the correlation of pre- and post-surgical characteristics with PFS, OS and CSS. Multivariate analysis was performed according to a backward elimination of factors showing a $p < 0.10$ in the univariate analysis. In all statistical tests a p value < .05 was considered significant. All statistical analyses were performed using SPSS software (version 22, SPSS Inc.).

RESULTS

Patient Characteristics and Pathology Results

A total of 61 consecutive patients, including eight patients with concomitant distant resectable metastases (5 liver, 1 lung and 2 lymph node metastases) were evaluated with ^{18}F-FDG-PET/CT. One patient refused surgery and of the remaining patients, 25 showed a TRG1 response, while 35 were TRG2-4. The median interval between the end of chemoradiotherapy and TME was 9 weeks for both TRG1 (range, 7–13) and TRG2-4 (range,

7–15). No significant differences in baseline disease characteristics were observed between TRG1 and TRG2-4 (Table 1).

Relationship between ^{18}F-FDG-PET/CT Parameters and Pathological Response

The median times between the start of preoperative chemoradiotherapy and the early PET scan and between the completion of chemoradiotherapy and the late PET were, respectively, 11 days for both TRG1 (range 9 – 21 days) and TRG2-4 (range 9 – 14 days) and 59 days (range 50 – 89 days) for TRG1 and 58 days (range 43 – 96 days) for TRG2-4. Among the ^{18}F-FDG-PET/CT parameters analyzed, early change of SUV-max showed borderline predictive value for response, but a significant differences between TRG1 and TRG2-4 were seen only in the early PET assessment, for day 11 TLG and for ΔTLG-early (Table 2). However, the AUC for day 11 TLG was not sufficiently accurate to establish an optimal cut-off (AUC 0.68, 95% CI 0.53-0.82, Supplemental Fig.1), whereas for ΔTLG-early the AUC was 0.76, (95% CI 0.64-0.89, p=0.001) with an optimal cut-off of 59.5% (80% sensitivity, 71.4% specificity) in identifying TRG1 (Fig.1). In Figs. 2 and 3 two representative cases of metabolic response in pathological complete and incomplete responders, are shown. Among the 30 patients with a ΔTLG-early ≥ 59.5%, 20 (67%) were classified as TRG1, 9 (30%) as TRG2 and 1 (3%) as TRG3, positive predictive value of 67% (PPV, probability of correct identification of TRG1). On the contrary, among the remaining 30 patients with a ΔTLG-early < 59.5%, only 5 (17%) were TRG1, 13 (43%) were TRG2 and 12 (40%) were TRG3-4, negative predictive value 83% (NPV, probability of correct identification of TRG2-4). It should be noted that two false-negative subjects (TRG1 with ΔTLG-early < 59.5%), received two additional cycles of chemotherapy after the end of chemoradiotherapy and prior to surgery because they had distant metastases.

Interestingly, the relationship between ΔTLG and TRG observed early was not maintained in the late evaluation (Table 2). Moreover, the pathological primary tumor stage was ypT0-2 in all but one (97%) patient with ΔTLG-early ≥ 59.5% (henceforth referred to as “metabolic responders”) and in 23 of 30 (77%) with ΔTLG-early < 59.5% (incomplete metabolic responders). Lymph node involvement was found in 7 (23%) metabolic responders and in 11 (37%) incomplete metabolic responders.

Overall, pathological complete responses were observed in 16 of 30 (53%) metabolic responders and in only 5 of 30 (17%) incomplete metabolic responders. Primary tumor resection was complete in all metabolic

responders, whereas a positive CRM was found in two incomplete metabolic responder patients. (Supplemental Table 1).

Multivariate analysis showed a strong and independent correlation of ΔTLG-early in predicting TRG1 (Table 3).

Metabolic Changes and Long-term Outcomes

With a median follow-up time of 98 months (range 77–132) 15 patients showed cancer progression (1 local recurrence; 6 local and distant recurrence; 4 distant recurrence; 4 progression of distant metastases) and 12 patients had died at the time of analysis. The overall estimated 10-year PFS, OS and CSS were 75.4% (95% CI 63.3%-84.5%), 78.7% (95% CI 66.9%–87.1%), and 82.0% (95% CI 70.5%-89.6%), respectively (Supplemental Fig. 2).

Survival analysis was performed to compare metabolic responders and incomplete responders. Two of the eight patients with distant metastases at enrollment showed discordant metabolic response between the primary tumor, showing marked metabolic response (ΔTLG-early 71% and 72%), and the metastatic lesions, that did not show significant metabolic response (TLG reduction <50%). These patients were classified as incomplete metabolic responders. In the remaining 6 patients metabolic response of the primary tumor (2 responders and 4 incomplete responders), was not affected by metastatic lesions.

Only 5 (18%) out of 28 metabolic responder patients received adjuvant chemotherapy; in 3 patients (11%) a recurrence occurred. On the contrary, 13 out of 33 (39%) incomplete metabolic responders (including the patient who refused surgery), received adjuvant chemotherapy; in 12 (36%) patients, recurrence or cancer progression was documented. Metabolic responders had a significantly longer PFS compared to incomplete responders (10-year PFS 89.3%, 95% CI 72.8%-96.3%, vs 63.6%, 95% CI 46.6%–77.8%, log-rank test, p=0.02, Fig. 4). In the univariate analysis, only ΔTLG-early and ypTNM categories showed a significant association with PFS (HR=0.26, p=0.02 and HR=0.15, p=0.04, respectively, Table 4). A clear trend toward reduced risk of recurrence or cancer progression was observed for the TRG category. At multivariate analysis, none of these factors showed prognostic significance (Table 4). In relation to OS, 3 metabolic responders (11%) and 10 incomplete metabolic responders (30%) died and all but two deaths, one for each subgroup, were cancer related. Metabolic responders had a longer OS, although this difference was not statistically significant (10-year OS 89.3%, 95% CI 72.8%-96.3%, vs 69.3%, 95% CI 52.7%–82.6%, log-rank test, p=0.06, Supplemental Fig. 3). However, metabolic

responders showed significantly longer CSS compared to incomplete responders, (10-year CSS 92.9% 95% CI 77.4%-98% vs 72.6% 95 % CI 55.8%-84.9%, log-rank test, p=0.04, Fig. 4). In the univariate analysis only ΔTLG-early showed a significant association with CSS (HR=0.23, p= 0.04, Table 4). At multivariate analysis, no factor showed prognostic significance.

Metabolic Changes and Bevacizumab Scheduling

The primary endpoint of the BRANCH study was the rate of TRG1. This was reached only using the sequential-schedule of bevacizumab (TRG1 rate 50%, 95% CI 35%–65%). On the contrary, for the concomitant-schedule accrual was stopped early since the number of TRG1 (2 out of 16 patients) was statistically inconsistent with the preplanned first-stage analysis. In line with these results, we observed a significant difference in ΔTLG-early between the two different bevacizumab schedules (concomitant schedule, median 35%, range -129% to 83% vs sequential schedule, median 68%, range -31% to 90%; p= 0.0022, Fig. 5). On the contrary, no differences were observed in ΔTLG-late values (concomitant schedule, median 86%, range -24% to 100% vs sequential schedule, median 91%, range -4% to 100%; p = 0.39, Fig 5).

DISCUSSION

This prospective study corroborates our previous evidence that early metabolic change accurately predicts pathological response and long-term outcome in LARC, showing a greater accuracy compared to late assessment (*8*). We found that only early metabolic response to preoperative chemoradiotherapy can discriminate LARC patients with TRG1 from those with TRG2-4 response. Looking for convenient parameters, we found that only ΔTLG-early showed an accurate AUC of 0.76 at ROC analysis, with an optimal cut-off of 59.5% to distinguish TRG1 from TRG2-4. Moreover, among pre-surgical parameters only ΔTLG-early showed a strong and independent correlation in predicting TRG1 in the multivariate analysis. In addition, the PPV was 67%, while the NPV was 83%. It is important to once again note that two false-negative subjects received two additional cycles of chemotherapy in the waiting period because they had distant metastases, highlighting the potential value of intensifying treatment in selected non-responding patients. Importantly, we showed that the relationship between ΔTLG and TRG observed early is lost in the late evaluation. Similarly, the borderline predictive value for response given by the more traditionally utilized SUV-max measurements is lost in the late

assessment. It should be noted that in the present study we used TRG1 instead of TRG1-2 as the reference for the evaluation of the predictive value of metabolic response. Most studies in this setting, as well as our previous experience, address major pathological response (TRG1-2). We confirm the difficulties of ^{18}F-FDG-PET/CT in distinguishing between TRG1 and TRG2 given the very low spatial resolution and the very subtle differences at the cellular level (14,15). Indeed, among the patients with a ΔTLG-early ≥ 59.5% there were 9 patients (30%) with TRG2 and overall all but one patient (97%) were classified as TRG1-2 and pT0-2. On the contrary, only 5 patients with TRG1 were wrongly classified as non-responders. In this group, 2 patients had received additional chemotherapy as reported above, leading to speculate that TRG1 may have been a consequence of additional chemotherapy and that ^{18}F-FDG-PET/CT could allow clinicians to modify/intensify treatment based on PET findings. However, the low PPV for identifying TRG1 suggests the need to combine early ^{18}F-FDG-PET assessment with other imaging modalities at different time points, such as DW-MRI or DCE-MRI, to improve the selection of patients for organ-preserving strategies (*16,17*). It is interesting to note that our data are consistent with another recent series (*18*) in which early reduction of SUV-max was the only parameter used to discriminate TRG1 from TRG2-5 although long-term outcome was not reported. In this regard, our findings support the use of TLG as a better composite parameter accounting for tracer avidity and metabolic tumor volume for this type of assessment as observed by several authors (*19,20*).

A point of strength of this study is the long- term follow-up (median 98 months, minimum 77 months) which is hardly ever seen in other studies on this matter. Metabolic responder patients showed significantly higher 10-year PFS probability (89.3%) compared to incomplete responders (63.6%), with a 74% reduction in risk of recurrence. Of note, none of the other pre-surgical parameters showed a significant association with PFS in the univariate analysis, while of the post-surgical factors only ypTNM was statistically correlated to PFS. Furthermore, metabolic responders show better 10-year OS (89.3% vs 69.3%), although this difference was not statistically significant, and significantly improved 10-year CSS (92.9%) compared to incomplete responders (72.6%), with a 77% reduction of the risk of cancer death. Overall, these results confirm the prognostic value of early metabolic response. However, the low number of events (recurrence, cancer progression or cancer death) limit the statistical power of the analysis for this as well as other prognostic factors in the multivariate analysis. The correlation between the early ^{18}F-FDG-PET changes and long-term outcomes may also be particularly helpful in selecting patients for adjuvant treatment, in light of the debate between pros and cons of adjuvant

treatment following preoperative chemotherapy in LARC (*21*). In this regard, early metabolic response showed a significant ability to predict outcome despite a lower percentage of patients (18% vs 39%) receiving adjuvant treatment in our series.

Finally we would like point out that, to the best of our knowledge, this is the first prospective study indicating a relevance of the use of early ^{18}F-FDG-PET in the prediction of pathologic response and outcome in LARC patients treated with a chemoradiotherapy regimen with bevacizumab. In this setting, early metabolic assessment allowed a higher rate of prediction of TRG1 in the sequential compared to the standard concomitant bevacizumab administration scheme. Again, this capability was lost in the late metabolic assessment. These findings highlight the importance of assessing early functional changes compared to conventional RECIST criteria which are inadequate in addressing early changes. Early PET response may provide the sought after and as of yet undefined functional biomarker for response or resistance to antiangiogenic therapy (*22*,*23*).

CONCLUSIONS

The results of this study provide further support for the use of early PET response assessment as a clinical tool in management of patients with LARC, showing that ΔTLG-early can be utilized to predict pathological response and outcome. Our results show that early ^{18}F-FDG-PET/CT could be particularly useful for identifying early incomplete pathological response, allowing clinicians to modify/intensify the treatment approach through radiotherapy dose escalation or additional chemotherapy in the waiting period. At the same time, early identification of patients likely to achieve complete pathologic response could complement morphological imaging findings and allow better selection of patients for organ-preserving strategies. Interestingly our results also indicate the potential of early ^{18}F-FDG-PET/CT as a surrogate marker of the benefit of antiangiogenic therapy. These hypothesis generating findings warrant further evaluation in larger properly powered, and possibly multicenter studies to validate this approach.

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FIGURE LEGENDS

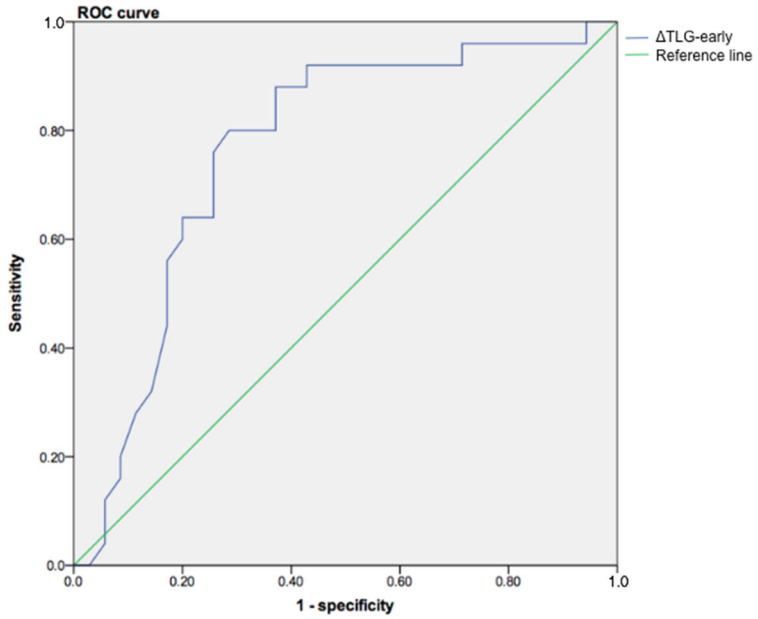

Figure 1 ROC curve for ΔTLG-early. The curve shows the accuracy of using ΔTLG-early (AUC 0.76, 95% CI 0.64-0.89; p=0.001), with a cut-off ≥ 59.5% (80% sensitivity, 71.4% specificity) for predicting TRG1 vs TRG2-4.

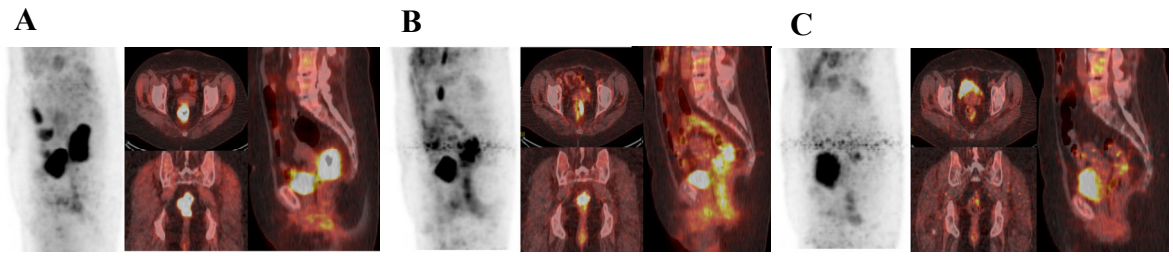

Figure 2 ^{18}F-FDG FDG-PET/CT images in a patient with complete pathological response. (**A)** Baseline ^{18}F-FDG uptake in a cT3 lesion (TLG 470.40). (**B**) Early significant decrease in tumor TLG (TLG 65.86; ΔTLG-early 86%). (**C**) Late near complete disappearance of the tumor (TLG 14.11; ΔTLG-late 97%). Pathological analysis showed complete tumor regression (ypT0 N0, TRG1). Neither local nor distant recurrence occurred during 83 months of follow-up.

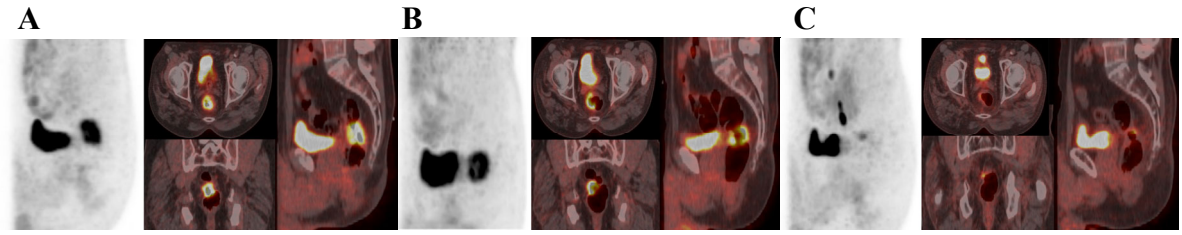

Figure 3 ^{18}F-FDG FDG-PET/CT images in a patient with incomplete pathological response. (**A**) Baseline ^{18}F-FDG uptake in a cT3 lesion (TLG 57.6). (**B**) Early slight decrease in tumor TLG (TLG 48.38; ΔTLG-early 16%). (**C**) Late near complete disappearance of the tumor (TLG 8.06; ΔTLG-late 86%). Pathological analysis showed incomplete pathological response (ypT3N1, TRG3). Pelvic recurrence and death occurred after 35 and 53 months from initial treatment, respectively

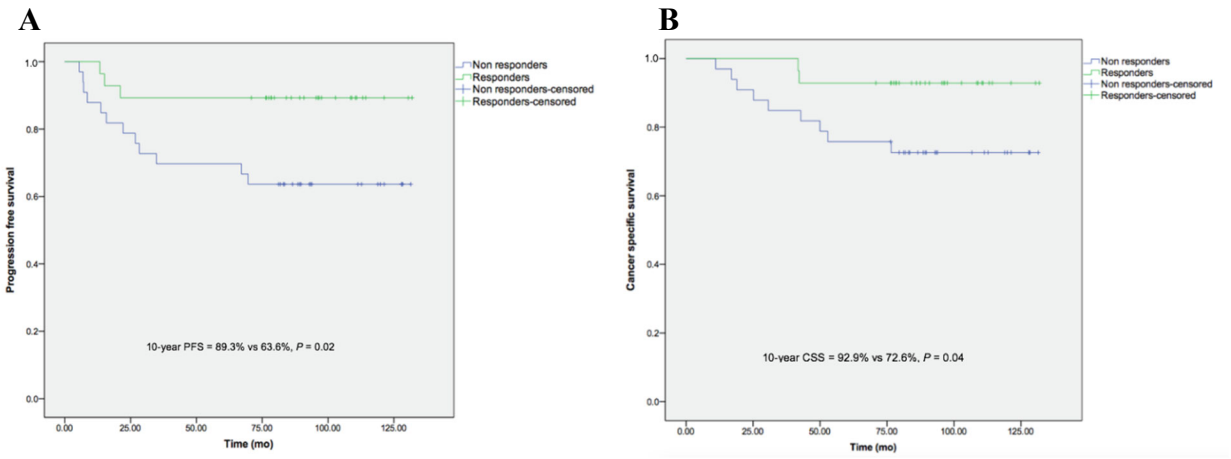

Figure 4 Kaplan-Meier curves for Progression free survival (A) and Cancer specific survival (B) according to metabolic response

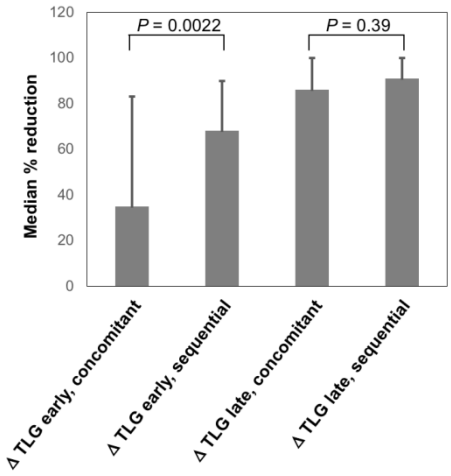

Figure 5 Relationship between ΔTLG-early and ΔTLG-late with bevacizumab scheduling.

Table 1 Patient and tumor characteristics

Characteristics	All patients* n= 61 (%)	TRG1 n= 25	TRG2-4 n= 35	P† value
Gender				0.40
Male/female	37 (61)/24 (39)	16/9	20/15	
Age				0.40
Median (range)	59 (43–74)	59 (47–74)	60 (43–69)	
ECOG performance status				0.20
0 vs 1 or 2	30 (49)/31 (51)	10/15	20/15	
Gunderson risk				0.21
Intermediate: T3 N0	4 (6)	0	4	
Moderately high: T3 N1/T4 N0	20 (33)/2 (3)	8/1	12/0	
High: T3 N2/T4 N1–2/anyTanyNM1	24 (39)/3 (5)/8 (13)	9/1/6	15/2/2	
Distance from the anal verge				0.34
≤ 5 cm (low-lying tumor)	34 (56)	15	18	
> 5 cm (mid/upper tumor)	27 (44)	10	17	
Distance from the Mesorectal Fascia (MRF)				0.40
≤ 2 mm	32 (52)	15	16	
> 2 mm	25 (41)	10	15	
Not evaluated‡	4 (7)	0	4	
Baseline CEA serum level				0.60
≤ 5 UI/L	38 (62)	16	22	
> 5 UI/L	23 (38)	9	13	

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*one patient with tumor T4N0 refused surgery; †Chi-square or Mann-Whitney test; ‡No MRI performed due to the presence of metal prosthesis

Table 2 FDG-PET/CT parameters in relation to TRG1 (tumor complete responders) and TRG2-4 (tumor incomplete responders)

Parameters	All patients* n= 61 (range)	TRG 1 n= 25 (range)	TRG 2-4 n= 35 (range)	P† value
Median SUV_{max}				
Baseline	12.1 (2.7-49.5)	12.2 (3.6-49.5)	10.9 (2.7-27.2)	0.87
Day 11 (early)	8.3 (1-30.6)	8.2 (1-30.6)	8.6 (3.7-19.3)	0.26
Pre-surgery (late)	4 (0-14.1)	3.9 (0-10.3)	4.1 (0-14.1)	0.46
ΔSUV-early, %	27 (-243 to 73)	40 (-16 to 73)	24 (-243 to 73)	0.06
ΔSUV-late, %	66 (-83 to 100)	72 (-69 to 100)	66 (-83 to 100)	0.94
Median SUV_{mean}				
Baseline	5.75 (1.6-13.9)	5.9 (1.7-11.2)	5.7 (1.6-13.9)	0.87
Day 11 (early)	4.8 (1.5-12)	4.7 (1.5-12)	4.9 (2.2-9.7)	0.38
Pre-surgery (late)	2.9 (0-7.9)	2.9 (0-5.7)	2.9 (0-7.9)	0.80
ΔSUV-early, %	19 (-123 to 61)	27 (-36 to 61)	15 (-123 to 59)	0.19
ΔSUV-late, %	56 (-125 to 100)	59 (-125 to 100)	56 (-80 to 100)	0.94
Median TLG				
Baseline	155.3 (13.4-980.4)	107.2 (13.4-980.4)	167 (36.9-572)	0.78
Day 11 (early)	65.2 (2.6-840.7)	41 (2.6-672)	82.4 (21.8-840.7)	**0.02**
Pre-surgery (late)	17.3 (0-193.9)	13.9 (0-193.9)	22.9 (0-153.8)	0.18
ΔTLG-early, %	58 (-129 to 90)	74 (-31 to 86)	42 (-129 to 90)	**0.001**
ΔTLG-late, %	90 (-24 to 100)	93 (-4 to 100)	86 (-24 to 100)	0.30

*one patient with tumor T4N0 refused surgery; †Mann-Whitney test;

Table 3 Multivariate analysis for the identification of TRG1

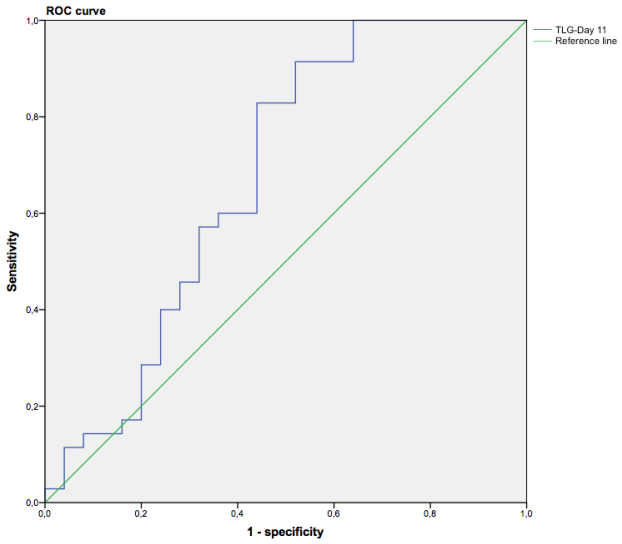
Variable	Multivariate Analysis		
	OR	95% IC	P value
Sex (Male vs Female)	0.52	0.12-2.19	0.38
Age (≤65 vs > 65)	0.66	0.15-2.79	0.57
ECOG performance status (0 vs 1-2)	0.45	0.12-1.70	0.24
Gunderson risk (Intermediate/Moderate vs High)	0.46	0.11-1.86	0.27
Distance from the anal verge (> 5 cm vs ≤ 5 cm)	0.29	0.06-1.27	0.10
Distance of Mesorectal Fascia (MRF) (> 2 mm vs ≤2 mm)	1.34	0.32-5.59	0.68
Baseline CEA serum level (≤ 5 UI/L vs > 5 UI/L)	1.53	0.35-6.75	0.56
ΔTLG-early % (≥ 59.5 vs < 59.5)	10.864	2.54-46.34	**0.001**

Table 4 Univariate and multivariate analysis of prognostic factors for PFS and CSS

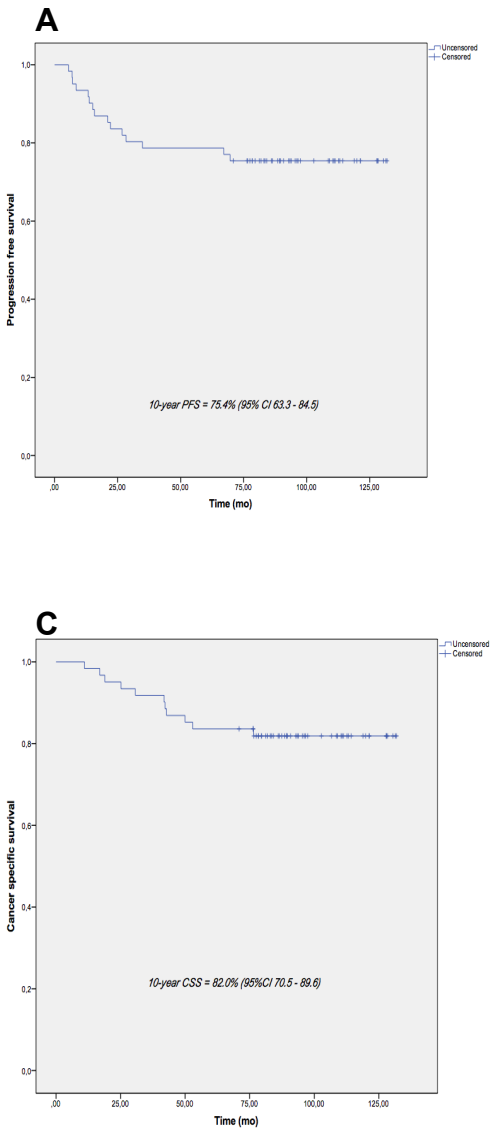
Variable	PFS						CSS					
	Univariate Analysis			Multivariate Analysis			Univariate Analysis			Multivariate Analysis		
Presurgical	**HR**	**95% IC**	**P value**	**HR**	**95% IC**	**P value**	**HR**	**95% IC**	**P value**	**HR**	**95% IC**	**P value**
Sex (Male vs Female)	0.75	0.27 – 2.06	0.57				0.78	0.24 – 2.56	0.68			
Age (≤65 vs > 65)	0.85	0.31 – 2.36	0.76				1.23	0.37 – 4.02	0.73			
ECOG performance status (0 vs 1-2)	0.52	0.18 – 1-46	0.21				0.45	0.13 – 1.54	0.19			
Gunderson risk (Intermediate/Moderate vs High)	0.68	0.23 – 2.01	0.49				0.50	0.13 – 1.89	0.29			
Distance from the anal verge (> 5 cm vs ≤ 5 cm)	0.41	0.13 – 1.28	0.11				0.50	0.13 – 1.96	0.20			
Distance of Mesorectal Fascia (MRF) (> 2 mm vs ≤2 mm)	0.56	0.19 – 1.66	0.29				0.43	0.11 – 1.62	0.20			
Baseline CEA serum level (≤ 5 UI/L vs > 5 UI/L)	0.70	0.25 – 1.9	0.47				1.05	0.31 – 3.60	0.93			
ΔTLG-early % (≥ 59.5 vs < 59.5)	0.26	0.07– 0.9	**0.02**	0.43	0.1 – 1.74	0.24	0.23	0.05 – 1.09	**0.04**	0.44	0.08 - 2.36	0.34
Postsurgical												
TRG (1 vs 2-4 or M1)	0.25	0.05 – 1.13	0.05	1.07	0.13 – 8.8	0.94	0.17	0.02 – 1.39	0.06	0.27	0.03 - 2.53	0.25
yTNM (T0N0M0 vs T1-4 and/or N+ or M1)	0.15	0.02 – 1.19	**0.04**	0.22	0.01 – 3.62	0.29	0.24	0.03 – 1.91	0.14			

Analysis for yCRM was not done because only 2 cases had a margin positive (≤ 1 mm)

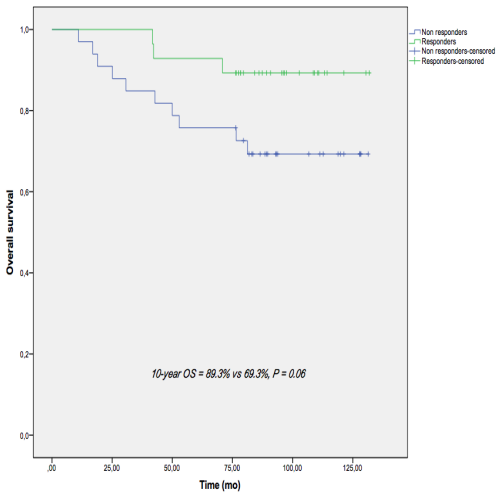
SUPPLEMENTARY MATERIAL



Supplemental Figure 1 ROC curve for TLG Day 11. The curve shows the accuracy of using TLG Day 11 (AUC 0.68, 95% CI 0.53-0.82; p=0.021) for predicting pathological tumor complete response (TRG1 vs TRG2-4). The optimal cut-off value was a TLG Day 11 of 42.3% (82.9% sensitivity, 56% specificity).



Supplemental Figure 2 Kaplan-Meier curves for Progression free survival (A), Overall survival (B) and Cancer specific survival (C).



Supplemental Figure 3 Kaplan-Meier curve for Overall survival according to metabolic response

Supplemental Table 1 Pathological findings in relation to metabolic responder,

Pathological Results*	ΔTLG-early ≥ 59.5% n=30	ΔTLG-early < 59.5% n=30	p† value
TRG1	20	5	
TRG2-4	10	25	**0.000086**
ypT0N0	16	5	
ypT1-4 or N1-2	14	25	**0.003**
ypT0-2	29	23	
ypT3-4	1	7	**0.02**
ypN0	23	19	
ypN1-2	7	11	0.26
yCRM+	0	2	
yCRM-	30	28	0.15

*one patient with tumor T4N0 refused surgery; †Chi-square test;
yCRM+= circumferential resection margin ≤ 1 mm;
yCRM-= circumferential resection margin > 1 mm;

Supplemental Table 2 Univariate and multivariate analysis of prognostic factors for OS

Variables	Univariate Analysis			Multivariate Analysis		
	HR	95% IC	P value	HR	95% IC	P value
Presurgical						
Sex (Male vs Female)	1.02	0.33 – 3.11	0.97			
Age (≤65 vs > 65)	0.88	0.29 – 2.62	0.81			
ECOG performance status (0 vs 1-2)	0.68	0.23 – 2.04	0.49			
Gunderson risk (Intermediate/Moderate vs High)	0.39	0.10 – 1.41	0.13			
Distance from the anal verge (> 5 cm vs ≤ 5 cm)	0.51	0.16 – 1.68	0.26			
Distance of Mesorectal Fascia (MRF) (> 2 mm vs ≤2 mm)	0.49	0.15 – 1.60	0.23			
Baseline CEA serum level (≤ 5 UI/L vs > 5 UI/L)	0.95	0.31 – 2.91	0.93			
ΔTLG-early % (≥ 59.5 vs < 59.5)	0.31	0.09 – 1.15	0.06			
Postsurgical						
TRG (1 vs 2-4 or M1)	0.31	0.07 – 1.44	0.11			
ypTNM (T0N0M0 vs T1-4 and/or N+ or M1)	0.44	0.09 – 2.01	0.27			

Analysis for yCRM was not done because only 2 cases had a margin positive (≤ 1 mm)

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^{18}F-FDG PET/CT Is an Early Predictor of Pathologic Tumor Response and Survival to Preoperative Radiochemotherapy with Bevacizumab in High Risk Locally Advanced Rectal Cancer

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