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# Metabolic response evaluation for colorectal liver metastases and correlation to pathologic response and tumour markers

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**Abstract**

**Background:** Tumour metabolic response to chemotherapy is increasingly recognised as a prognostic indicator for colorectal cancer liver metastases (CRCLM). However, its clinical role and the underlying biological mechanism of its prognostic ability are unclear. This study compares metabolic to pathologic response for CRCLM, and correlates metabolic response to tumour expression of six key biomarkers.

**Methods:** Thirty-seven patients who had PET imaging before and after preoperative chemotherapy prior to liver resection for CRCLM were included. Metabolic response was assessed according to the PET Response Criteria in Solid Tumours (PERCIST) and correlated to recurrence-free (RFS) and overall survival (OS). PERCIST was compared to tumour regression grading (TRG), CT response, tumour necrosis and mucin, and immunohistochemical expression of Ki-67, HIF-1±, VEGF, p53, p16 and vimentin. Area under the receiver operating curve (AUC), Kaplan-Meier survival, Spearman's correlation (rs) and multivariate Cox regression analyses were used.

**Results:** PERCIST correlated significantly to 2-year mortality (AUC=0.162, $p<0.01$) and 2-year recurrence (AUC=0.284, $p=0.03$). Metabolically responsive tumours conferred a better OS ($p=0.01$) and RFS ($p=0.03$). TRG did not stratify for outcome. Metabolic response was significantly correlated to Ki-67 and p16 expression ($rs = 0.559$ and $rs = -0.549$, respectively). Multivariate analysis revealed only PERCIST to be correlated to death and recurrence.

**Conclusion:** Preoperative PERCIST assessment of CRCLM was more prognostic than pathologic and CT response assessment. Metabolic non-response correlated with tumour proliferation and loss of tumour suppression.

## Introduction

Long-term outcome in patients with colorectal cancer liver metastases is dependent upon a combination of local and systemic disease control. Control of systemic micrometastases appears to be a critical determinant for long-term recurrence-free survival. Increasingly, preoperative evaluation of this control by chemotherapy response evaluation is used to improve risk stratification[1–4].

Increased tumour metabolism has been established as a Cancer Hallmark[5]. Our recent study indicated that metabolic response of tumour to preoperative chemotherapy with $^{18}$F-fluorodeoxyglucose positron emission tomography (PET) for patients undergoing resection of colorectal liver metastases evaluated systemic disease control[6]. Metabolic response as quantified by maximum standardized uptake value ($SUV_{max}$), total glycolytic volume (TGV) and metabolic tumour volume (MTV) was superior to CT size response and clinical-pathological prognostic scores in predicting outcome.

Metabolic response has long been assumed to reflect pathological response to therapy as determined by tumour regression grade (TRG)[7,8](Table 1). However, the prognostic impact of metabolic response may reflect a number of mechanisms other than tumour regression. Resistance to chemotherapy as evidenced by

metabolic non-response may be due to pre-existing or induced alterations in other complementary oncogenic mechanisms such as angiogenesis, sustained proliferative signalling, evasion of growth suppressors, and epithelial-mesenchymal transition (EMT)[5].

The aims of this study were to examine the prognostic value of metabolic response according to the PET response criteria in solid tumours (PERCIST)[9] in comparison to pathological response for resected colorectal cancer liver metastases, and also to assess the relationship between PERCIST and various tumour suppressor (p16 and p53), angiogenic (VEGF, HIF1±), proliferative (Ki67) and EMT (vimentin) markers in order to explore the mechanism for the prognostic ability of metabolic response.

## Materials and Methods

### Patients

This study included all patients who had PET imaging before and after preoperative chemotherapy for colorectal cancer liver metastases at the Austin Hospital in Melbourne, Australia between January 2004 and December 2011. All patients underwent subsequent resection of the liver metastases with curative intent. Ethics approval was obtained (Austin HREC# H2012/04676).

Patient data was retrieved from the prospectively maintained hepato-pancreato-biliary database.

PET Protocol and Tumour Metabolic Response Assessment

All patients underwent PET imaging in accordance with the standard PET acquisition protocol[10] on a dedicated PET/CT system (Phillips, Cleveland, USA). The scans were then analysed on the MedView software (MedImage, Ann Arbor, Michigan, USA).

Tumour metabolism and response was assessed in accordance with PERCIST[9](Table 1). In short, this quantifies the chemotherapy response of the lesion with the most intense $^{18}$F-FDG uptake by $SUL_{peak}$, which is the highest mean standardized uptake variable corrected for lean body mass (SUL) of a 1cm$^3$ spherical region of interest (ROI).

CT Tumour Response Evaluation

Response Evaluation Criteria in Solid Tumours (RECIST, version 1.1[11]) was used to classify radiological response (Table 1).

Tumour Pathological Response, Necrosis and Mucin Evaluation

Tumour regression grading (TRG) was scored according to the Mandard five-point system[12,13](Table 1). Tumour necrosis and mucin were graded according to the following criteria: 0, none; 1+, less than 25%; 2+, 25-50%; 3+, greater than 50%.

## Immunohistochemistry

HIF1±, VEGF, p16, p53, vimentin and Ki67 immunohistochemical expression were evaluated by computer-assisted scoring in the resected tumour specimens (Figures S1a and b). Please refer to the supplemental document for further details.

## Statistical Analysis

Outcome parameters were overall survival (OS) and recurrence-free survival (RFS). Time-points were calculated from the date of liver resection until death or the first evidence of tumour recurrence discovered by either PET or CT imaging. Area under the receiver-operating-characteristic curve (AUC) analysis was determined for 2-year mortality and recurrence. Kaplan-Meier survival curves were generated from survival data using a log-rank test. Spearman's correlation coefficients ($r_s$) and Pearson correlation coefficients ($r$) were used to assess correlation between ordinal data and continuous variables respectively. Univariate and multivariate Cox proportional hazards regression (forward step-wise method) was used to examine the correlation between the various demographic, clinical-pathological and metabolic parameters to OS and RFS. Statistical significance was regarded as $p$ value<0.05.

## Results

A total of 37 patients who had PET imaging before and after preoperative chemotherapy were included. All patients underwent resection of liver metastases with curative intent following completion of planned chemotherapy. Three patients were retrospectively found to have pre-existing extra-hepatic lesions on review of pre-operative imaging (2 lung, 1 ovarian metastases). Three patients were found to have positive resection margins on final pathology. All patients were included in the analysis. The median follow-up period was 39 months. Two-year follow-up was 100% and at two years, the OS was 78.4% while RFS was 48.6%. Demographic and clinical details are summarized in Table 2.

Metabolic Response

Post-chemotherapy PET scans were performed after a median of 18 days from the last dose (range 1-64 days). PERCIST response is summarized in Table 2. Metabolic response was inversely discriminative for 2-year mortality and recurrence on AUC analysis (AUC=0.162, $p<0.01$ and AUC=0.284, $p=0.03$, respectively). Patients with significant metabolic response (complete and partially responsive tumours) had a significantly improved OS compared to those patients who had insignificant metabolic response (stable or progressive disease) ($p=0.01$)(Figure 1a). Similarly, RFS was significantly improved in patients with significant metabolic response compared to patients with stable or progressive disease ($p=0.03$). When stratified to PERCIST criteria, increasing metabolic response was associated with a step-wise increase in OS ($p<0.01$)(Figure 1b) and

RFS ($p<0.01$). No patients who had complete metabolic response died during the follow-up period.

Radiological Response

RECIST response did not correlate with OS ($p=0.66$) or RFS ($p=0.92$).

Pathological Response

TRG findings are summarized in Table 1. TRG was not discriminatory for 2-year mortality or recurrence with AUC analysis. Dichotomisation of the data revealed that only 5 patients (13.5%) had significant tumour regression (TRG 1 and 2). Those with significant tumour regression had improved RFS compared to patients with insignificant tumour regression (TRG 3, 4 and 5) ($p=0.01$)(Figure 1c), but did not stratify significantly for OS ($p=0.1$, Figure 1d). No patients with significant tumour regression died during the follow-up period.

Metabolic Response without Tumour Regression

Due to the poor stratification in patients with insignificant tumour regression, the prognostic ability of metabolic response was assessed separately in this subgroup (TRG3-5). Metabolic response remained inversely predictive for 2-year mortality on AUC analysis (AUC=0.179, $p=0.01$) but not for recurrence. On Kaplan-Meier

analysis, increasing metabolic response maintained significant stratification for improved outcome (OS, p<0.001; RFS, p=0.001).

Tumour Necrosis and Mucin

Tumour necrosis and mucin grading is summarized in Table 2. There was no correlation between necrosis (OS, p=0.70; RFS p=0.86) or mucin grading (OS, p=0.34; RFS, p=0.84) with outcome.

Immunohistochemistry

The interquartile ranges of immunohistochemical expression are shown in Table 2. Tumour VEGF, HIF1±, p53, and vimentin expression did not correlate to outcome on AUC analysis.

The nuclear expression of p16 inversely correlated with 2-year mortality (AUC=0.222, p=0.03) but not with recurrence by AUC analysis. When p16 expression was dichotomized between the highest quartile of expression and the lowest three quartiles of expression (cut-off nuclear % = 25.5), Kaplan-Meier survival analysis revealed a 100% survival for patients with high p16 expression compared to 65% survival for patients with decreased p16 expression at 3 years (p=0.019).

No patients with the lowest quartile of Ki67 expression (<40.1%) died during the follow-up period. Dichotomization at this cut-off revealed a significant

improvement in OS with low expression at three years on Kaplan-Meier analysis (p=0.03), but there was no difference in RFS.

Correlation of Metabolic Response to Pathological Markers

Significant metabolic response showed moderate correlation with tumour regression ($r_s$=0.380, p=0.022) but not to RECIST ($r_s$=0.345, p=0.057). Tumours which were less metabolically responsive had increased necrosis ($r_s$=0.354, p=0.047). Neither tumour necrosis or mucin grades correlated significantly to the absolute $SUL_{peak}$ values at any time-point or to tumour regression grading. Both tumour regression ($r_s$=-0.559, p=0.001) and metabolic response ($r_s$ =-0.549, p=0.001) inversely correlated to Ki67 expression. Metabolic response was significantly associated with p16 expression ($r_s$=0.559, p=0.001). No other significant correlation was noted between metabolic response with pathological or immunohistochemical markers.

Between immunohistochemical markers, p16 expression showed a moderate inverse correlation to Ki-67 expression ($r_s$=-0.403, p=0.006). HIF1± and VEGF expression were also significantly correlated ($r_s$=0.508, p=0.001). Tumour metabolism as quantified by $SUL_{peak}$ before and after preoperative chemotherapy was significantly correlated with HIF1± expression ($r$=0.493, p=0.007; $r$=0.523, p=0.004 respectively) but not with VEGF expression.

Cox Regression Analysis

On univariate cox regression analysis (Table 3), PERCIST metabolic response was inversely associated with death and recurrence. TRG did not predict outcome but dichotomized tumour regression was inversely correlated to recurrence but not death.

Multivariate analysis was performed including the variables that showed significantly correlation on univariate analysis (Table 3). Only metabolic response correlated significantly to both death and recurrence. The presence of extrahepatic disease was significantly predictive for recurrence but not death.

Discussion

This study demonstrates the significant prognostic ability of metabolic response (by PERCIST) to preoperative chemotherapy for colorectal metastases. Preoperative metabolic response was more predictive of outcome compared to histological tumour regression. An important outcome of this study not previously reported is that metabolic response correlated significantly with p16 and Ki-67 expression. This highlights the interplay of metabolic changes in tumour with the biology of metastatic disease.

The concept of chemotherapy response assessment as a prognostic indicator is well established. RECIST has been used extensively but is limited in the preoperative setting by the time required for significant change in tumour size[7].

Tumour metabolism on PET imaging is quantifiable in a variety of ways[6,9,10]. This study showed metabolic response as quantified by PERCIST to be the strongest prognostic indicator. Metabolic response was the only factor predictive for death and recurrence on multivariate analysis.

Pathological response is the benchmark in the assessment of chemotherapy response[1,8,13]. However, preoperative liver tumour biopsy is avoided due to the risk of intraperitoneal seeding[14] and is limited by the potential for sampling error. Tumour regression grading was not prognostic as the majority of patients had insignificant tumour regression. The stratification for outcome in these patients by PERCIST suggests that metabolic response is not merely a surrogate for pathological response.

The expression of six immunohistochemical biomarkers were correlated to tumour metabolic response. These six biomarkers were chosen based on the Warburg effect[15] and the subsequently proposed biological pathways interweaving tumour metabolism with hypoxic adaptation, angiogenesis, proliferation, cell cycle regulation and invasiveness[16–19].

As part of an adaptive response to tumour hypoxia, HIF1± levels are increased, which in turn potentiates downstream mechanisms which confer a survival advantage to the cancer cells. Tumour metabolism switches from oxidative to glycolytic with increased glucose uptake[20]. In addition, HIF1± activates VEGF, stimulating angiogenesis. Accordingly, a significant correlation was observed between tumour HIF1± and VEGF expression in this study. There was also a

positive correlation between HIF1± and tumour metabolism before and after chemotherapy, but not to metabolic response.

HIF1± also mediates EMT via several interactive oncogenic pathways[19]. EMT is observed by upregulation of mesenchymal markers including vimentin[19], which has been associated with poor prognosis[21]. This was not confirmed in this study where vimentin expression was not correlated to outcome or tumour metabolism.

The Ki-67 proliferation index is an important prognostic indicator in colorectal cancer[22] and previously correlated to tumour metabolism[23]. Interestingly, this correlation was not seen in this study but a significant inverse relationship was observed between Ki-67 expression and metabolic response.

The tumour suppressors p53 and p16 are important cell cycle regulators commonly mutated in colon cancer[24,25]. Decreased p16 expression has been linked to increased tumour aggressiveness and poor outcome[25]. Other studies have correlated tumour metabolism with p53 and p16 expression[26,27]. This study found no correlation between p53 and tumour metabolism or outcome. High p16 expression was predictive for survival and correlated to metabolic response.

Our findings link metabolic response inversely to tumour proliferation and impaired tumour suppression and suggest that the prognostic impact of metabolic response assessment may not be confined to the observation of tumour regression. It is not known is whether these biological processes are inter-related or simply mechanisms observed in parallel.

The modern management of colorectal liver metastases distinctively implements the principles of local (surgical/ablative) and systemic (chemotherapeutic/tumour biology) cancer control. Preoperative metabolic response to chemotherapy, assessed in the macroscopic tumour, which is ultimately resected, likely indicates systemic control of unresected micrometastases.

This retrospective study is limited as the timing of the PET scans were not standardised, but PERCIST remained predictive for OS and RFS (pd0.02) on sub-analyses excluding patients with PET scans within 1 and 2 weeks of the last dose of chemotherapy.

## Conclusion

Evaluation of the metabolic response of colorectal liver metastases by PERCIST offers a readily available and non-invasive preoperative assessment of systemic disease control. It can risk-stratify patients being prepared for liver surgery to better inform management decisions. Metabolic response evaluation may be used to identify patients with favourable tumour biology who would best benefit from increasingly advanced surgical strategies to achieve local disease control (eg. portal vein embolization/ligation, multi-stage hepatectomy). Future studies will focus on delineating the entwined relationship between metabolic response and tumour biology.

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Supplementary Documents

Doc S1: Details regarding immunohistochemical staining and scoring technique

Figure S1

a) Representative example of Ki-67 immunohistochemical staining showing tumour expression with 42.5% nuclear positivity (20x magnification)

b) Ki-67 staining with representative computer scoring algorithm (blue is negative, yellow is weak positive, orange is positive and red is strong positive, 20x magnification)

Table 1 – Tumour Response Evaluation by PET, CT and Pathological Response Grading

| | |
|---|---|
| PET Response Criteria in Solid Tumours (PERCIST)[9] | |
| Complete Response (CR) | Complete resolution of tumour metabolism compared to background parenchyma |
| Partial Response (PR) | e30% decrease in $SUL_{peak}$* |
| Stable Disease (SD) | less significant change in tumour metabolism compared to PRR or PRD |
| Progressive Disease (PD) | e30% increase in $SUL_{peak}$ |
| CT Response Criteria in Solid Tumours (RECIST)[11] | |
| Complete Radiological Response (CRR) | Disappearance of target lesion(s) |
| Partial Radiological Response (PRR) | e30% decrease in the sum of the longest dimension of all target lesions measured |
| Stable Radiological Disease (SRD) | less significant change in size of lesions compared to PRR or PRD |
| Progressive Radiological Disease (PRD) | e20% increase in the sum of the longest dimension of all target lesions measured |

| Pathological Tumour Regression Grading (TRG)[1213] | |
|---|---|
| Grade 1 | Absence of tumour cells replaced by fibrosis |
| Grade 2 | Rare tumour cells scattered throughout fibrosis |
| Grade 3 | More residual tumour cells but fibrosis predominates |
| Grade 4 | Residual cancer cells predominate over fibrosis |
| Grade 5 | No signs of regression |

* $SUL_{peak}$ is the highest mean standardized uptake variable corrected for lean body mass in a $1cm^3$ spherical region of interest

Table 2 – Demographic, Clinical, Metabolic, Pathologic and Immunohistochemical Data

| Demographics | number or mean [range] (%) |
|---|---|
| n | 37 |
| Age (years) | 58.8 (23-76) |
| Sex (M:F) | 22 (59.5%) : 15 (40.5%) |
| Clinical Characteristics | |
| Chemotherapy | |
| FOLFOX | 34 (91.9%) |
| FOLFIRI | 2 (5.4%) |
| FOLFOX + cediranib | 1 (2.7%) |
| Number of liver metastases | 2 (1-5) |
| Size of largest metastasis (mm) | 21 (4 - 170) |
| Bilobar disease | 15 (40.5%) |
| Operation | |
| Subsegmental resection | 3 (8.1%) |
| Segmental resection | 19 (51.4%) |
| Hemihepatectomy or greater | 15 (40.5%) |
| Metabolic Response (PERCIST) | |
| Significant metabolic response | 19 (51.4%) |
| Complete response | 5 (13.5%) |

| | |
|---|---|
| Partial response | 14 (37.8%) |
| Insignificant metabolic response | 18 (48.6%) |
| stable disease | 13 (35.1%) |
| progressive disease | 5 (13.5%) |
| CT Tumour Size Response (RECIST) | |
| Significant radiological response | 18 (48.6%) |
| Complete radiological response | 5 (13.5%) |
| Partial radiological response | 13 (35.1%) |
| Insignificant radiological response | 19 (51.5%) |
| Stable radiological disease | 8 (21.6%) |
| Progressive radiological disease | 11 (29.7%) |
| Tumour Regression Grading | |
| Significant tumour regression | 5 (13.5%) |
| TRG 1 | 2 (5.4%) |
| TRG 2 | 3 (8.1%) |
| Insignificant tumour regression | 32 (86.5%) |
| TRG 3 | 9 (24.3%) |
| TRG 4 | 19 (51.4%) |
| TRG 5 | 4 (10.8%) |
| Immunohistochemical Staining (interquartile values) | |
| HIF1a - H score | 153 / 180 / 198.5 |
| VEGF - H score | 101.5 / 143 / 170 |
| Ki67 - % nuclear staining | 40.1 / 49.8 / 55.1 |

| | |
|---|---|
| p53 - % nuclear staining | 2.4 / 42.8 / 64.1 |
| p16 - % nuclear staining | 3.3 / 12.5 / 25.5 |
| vimentin - % peritumoral stromal staining | 12.3 / 24.3 / 35.7 |

Table 3: Univariate and Multivariate Cox Regression Analysis

| Features | | Death | | | Recurrence | | |
|---|---|---|---|---|---|---|---|
| | n | HR | 95%ci | p | HR | 95%ci | p |
| Demographic | | | | | | | |
| Age > 60 | 17/37 | 0.282 | 0.078 - 1.015 | 0.053 | 0.718 | 0.293- 1.761 | 0.470 |
| Sex (Female) | 15/37 | 0.916 | 0.317- 2.647 | 0.871 | 0.787 | 0.325- 1.906 | 0.595 |
| Clinical | | | | | | | |
| CEA | 37 | 0.997 | 0.988- 1.006 | 0.547 | 0.999 | 0.997- 1.002 | 0.666 |
| Number of Metastases | 37 | 1.463 | 0.977- 2.192 | 0.065 | 1.208 | 0.896- 1.630 | 0.215 |
| Extrahepatic Disease | 3/37 | 18.164 | 3.958- 83.367 | <0.001 | 4.801 | 1.357- 16.992 | 0.015 |
| Size of Largest Metastasis | 37 | 1.001 | 0.986- 1.016 | 0.883 | 0.997 | 0.987- 1.008 | 0.607 |
| Bilobar Disease | 15/37 | 2.523 | 0.832- 7.649 | 0.102 | 1.066 | 0.491- 2.316 | 0.871 |
| Positive Resection Margin | 3/37 | 1.74 | 0.456- 6.634 | 0.418 | 1.069 | 0.320- 3.570 | 0.913 |
| Resection Margin <1cm | 14/37 | 3.082 | 0.858- | 0.084 | 1.315 | 0.602- | 0.492 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 11.067 | | | 2.876 | |
| Metabolic | | | | | | | |
| Pre-chemo SULpeak | 37 | 0.95 | 0.762-1.185 | 0.652 | 0.896 | 0.757-1.060 | 0.201 |
| Post-chemo SULpeak | 37 | 1.181 | 0.935-1.492 | 0.163 | 0.95 | 0.766-1.179 | 0.643 |
| Metabolic Response (PERCIST) | 37 | 3.902 | 1.794-8.486 | 0.001 | 2.14 | 1.266-3.619 | 0.005 |
| Insignificant Metabolic Response (SD, PD) | 14/37 | 4.65 | 1.291-16.752 | 0.019 | 2.299 | 1.056-5.001 | 0.036 |
| Radiological | | | | | | | |
| CT Tumour Size Response (RECIST) | 37 | 0.98 | 0.949-1.021 | 0.294 | 0.97 | 0.623-1.531 | 0.891 |
| Pathologic | | | | | | | |
| Tumour Regression Grading | 37 | 0.73 | 0.635-1.912 | 0.730 | 1.199 | 0.846-1.699 | 0.309 |
| Insignificant Tumour Regression (TRG 3, 4 & 5) | 26/37 | 27.723 | 0.047-16361 | 0.307 | 8.182 | 1.098-60.966 | 0.04 |
| high mucin (grades 2 and 3) | 6/37 | 0.764 | 0.164-3.550 | 0.731 | 0.836 | 0.284-2.458 | 0.744 |
| high necrosis (grades 3-5) | 14/37 | 1.857 | 0.588-5.863 | 0.291 | 0.818 | 0.352-1.900 | 0.641 |
| Immunohistochemical | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| high HIF1a (quartiles 3 and 4) | 17/37 | 1.09 | 0.344-3.455 | 0.884 | 1.013 | 0.441-2.324 | 0.976 |
| high VEGF (quartile 4) | 17/37 | 0.747 | 0.199-2.812 | 0.666 | 0.475 | 0.160-1.413 | 0.181 |
| high Ki67 (quartiles 2-4) | 25/37 | 35.713 | 0.146-8735 | 0.203 | 1.785 | 0.667-4.774 | 0.249 |
| high p16 (quartile 4) | 7/37 | 0.031 | 0.000-10.884 | 0.246 | 1.138 | 0.453-2.858 | 0.783 |
| high p53 (quartiles 3 and 4) | 18/37 | 1.251 | 0.396-3.954 | 0.703 | 1.646 | 0.727-3.729 | 0.232 |
| high Vimentin (quartiles 3 and 4) | 17/37 | 0.329 | 0.087-1.243 | 0.101 | 0.927 | 0.420-2.044 | 0.851 |
| Multivariate Analysis | | | | | | | |
| extrahepatic disease | 3/37 | 3.966 | 0.695-22.617 | 0.121 | 4.318 | 1.152-16.189 | 0.030 |
| Metabolic Response (PERCIST) | 37 | 4.98 | 1.109-22.352 | 0.036 | 2.012 | 1.193-3.393 | 0.009 |
| Insignificant Metabolic Response (SD, PD) | 14/37 | 2.962 | 0.215-40.855 | 0.417 | 2.973 | 0.490-18.037 | 0.236 |
| Insignificant Tumour Regression (TRG 3, 4 & 5) | 26/37 | -- | -- | -- | 5.081 | 0.640-40.351 | 0.124 |

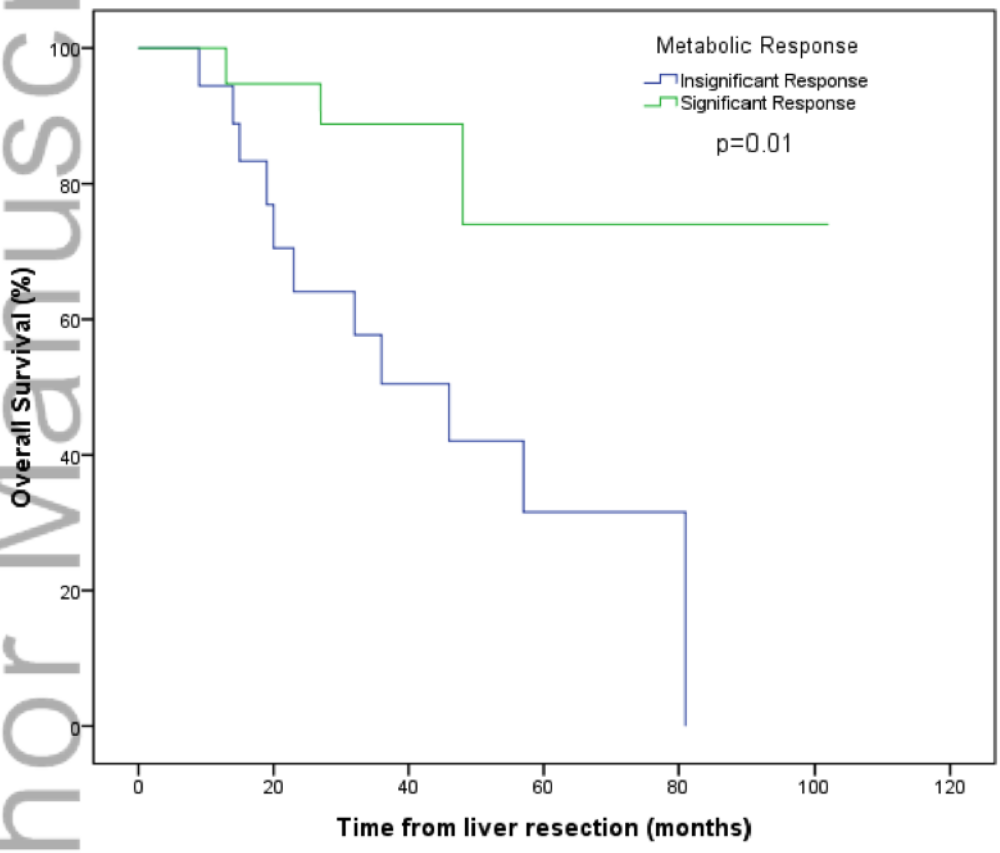


Figure_1a.tiff

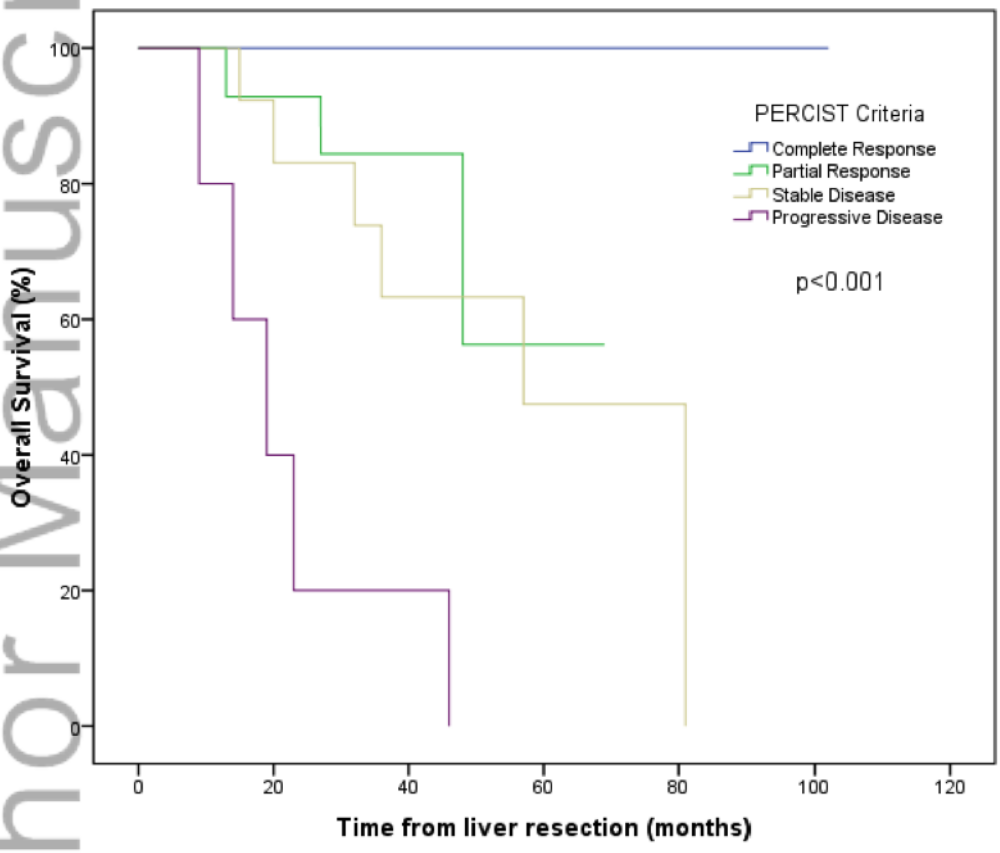


Figure_1b.tiff

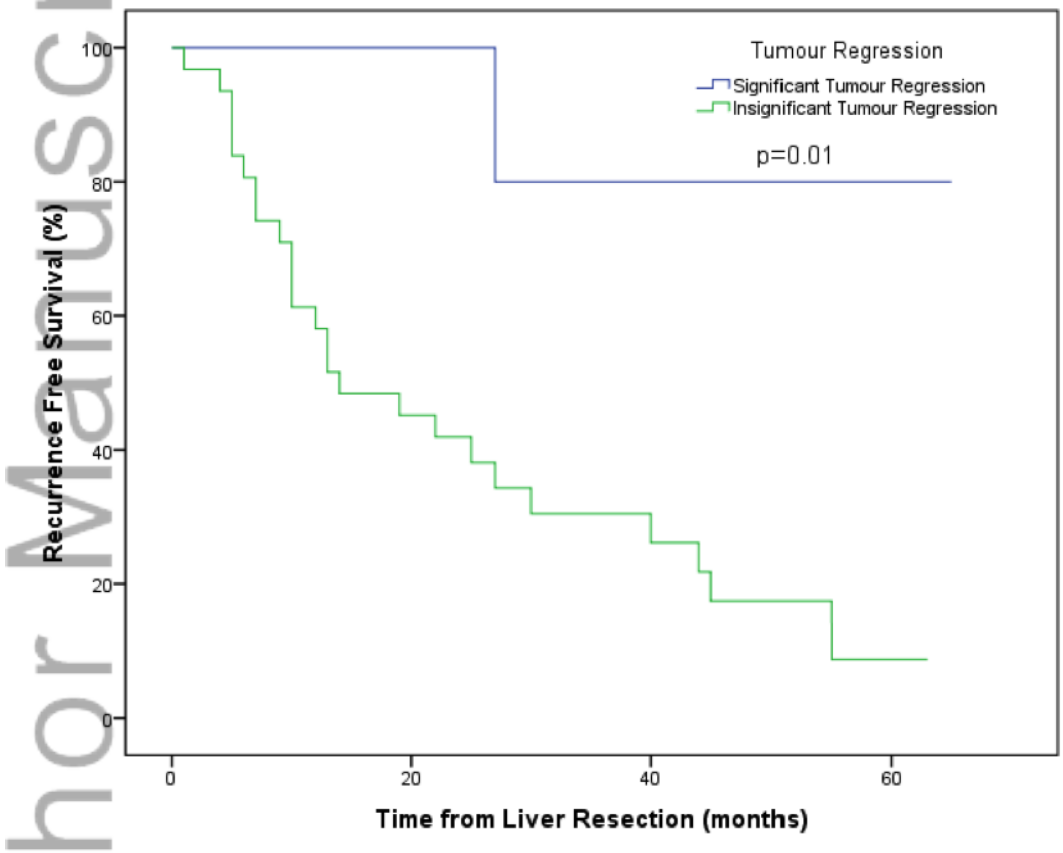


Figure_1c.tiff

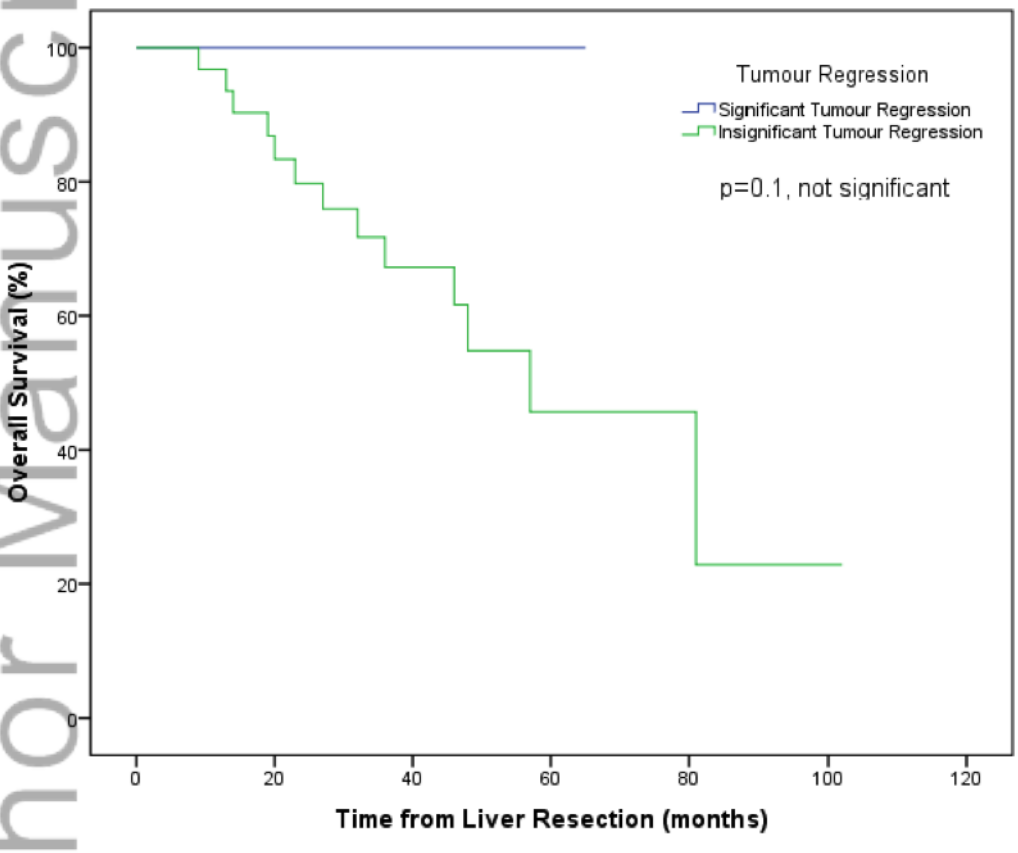


Figure_1d.tiff