ANGLIA RUSKIN UNIVERSITY

CHANGING CONCEPTS AND TRENDS IN RECTAL CANCER PATIENTS

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ABSTRACT
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Neoadjuvant chemoradiotherapy (CRT) followed by total mesorectal excision (TME) is the gold standard treatment for locally advanced rectal cancer with threatened circumferential resection margin (CRM). This strategy considerably improves loco-regional control but fails to improve overall survival (OS) due to distant failure. In addition there is a lack of consensus on optimal timing of surgery and the management of patients with complete clinical response after CRT who could benefit from either less invasive or wait and watch approaches. This shifting paradigm has placed greater recent interest in quantification of imaging biomarkers such as textural analysis (TA) linked to underlying intra-tumour heterogeneity associated with adverse outcomes. This could help to select patients with predicted poor prognosis for personalized intensive therapy. The aims of this thesis were: firstly, to assess the short and long-term effects of delayed surgery after CRT and secondly to investigate the prognostic potential of TA based on conventional magnetic resonance imaging (MRI) in stage II-III locally advanced rectal cancer. Thirdly, the potential of functional parameters (standardized uptake value [SUV], apparent diffusion coefficient [ADC]) quantified on pre-treatment integrated positron emission tomography and MRI (PET/MR) system to predict pathological response to CRT (independent sample t-test) and survival was assessed.

TA using a filtration-histogram technique of MR images was undertaken using TexRAD, a proprietary software algorithm. Regions of interest enclosing the largest cross-sectional area of tumour area were manually delineated on the axial images. Cox-multiple regression analysis determined which univariate features (clinical, textural, radiological and histological) on Kaplan-Meier survival analysis independently predicted OS, disease free survival (DFS) and recurrence-free survival (RFS). The time interval to surgery did not predict the survival outcomes. Male gender independently predicted DFS and RFS while CRM predicted RFS for the entire cohort (n-112). In a subset of the cohort (n-56) pre-treatment TA, extramural venous invasion (EMVI) on MRI independently predicted OS while TA and threatened CRM on MRI predicted DFS. For OS; EMVI on MRI and for DFS; TA and CRM involvement on MRI were the independent post-treatment factors. Only TA independently predicted RFS on pre- or post-treatment analyses. Both SUV and ADC values were not predictive of outcomes.

Delayed surgery after CRT does not lead to worse survival outcomes. Along with local or distant failures, male gender and pathological CRM are associated with worse survival. MR based TA of rectal cancers can predict outcome before undergoing surgery and could potentially select patients for individualized therapy.

Key words: Rectal cancer, Chemoradiotherapy, MRI, Textural analysis, PET/MRI, Tumour regression

Abbreviations

ADC	Apparent diffusion coefficient
AJCC	American joint committee on cancer
APC	Adenomatous polyposis colic
APR	Abdominoperineal resection
AR	Anterior resection
BGO	Bismuth germinate
CIMP	CpG island hypermethylation
CIN	Chromosomal instability
CRC	Colorectal cancer
CRM	Circumferential resection margin
CRT	Chemoradiotherapy
CT	Computed tomography
DCE	Dynamic contrast enhanced
DFS	Disease free survival
DWI	Diffusion-weighted imaging
EANM	European Association of nuclear Medicine
EMVI	Extra mural venous invasion
5-FU	5-Fluorouracil
FAP	Familial adenomatous polyposis
FDG	Fluorodeoxygenase
FDG-PET	Fluorine-18 fluorodeoxyglucose positron emission tomography
FDR	First degree relative
FOBT	Faecal occult blood test
FOLFLOX	Folinic acid (FOL) +Fluorouracil (F) + Oxaliplatin (OX)
FOV	Field of view
FSE	Fast spin echo
GLCM	Gray level co-occurrence matrix
HNPCC	Hereditary non-polyposis colorectal carcinoma
IBD	Inflammatory bowel disease
IR	Inversion recovery
KM	Kaplan Meier
LACR	Locally advanced rectal cancer
LOR	Line of response
MMR	Mismatch repair
MPP	Mean of positive pixels
MRI	Magnetic resonance imaging
MRTA	Magnetic resonance based textural analysis
MRTA	Magnetic resonance imaging based textural analysis
MSI	Microsatellite instability
NHS	National health services
NOC	N-nitroso compounds
OS	Overall survival
PET	Positron emission tomography
pTRG	Pathological tumour regression grading
RECIST	Response evaluation criteria in solid tumours
RFS	Relapse free survival
ROI	Region of interest
SD	Standard deviation
SE	Spin echo
SPECT	Single-photon emission computed tomography
SUV	Standardized uptake value

TA	Textural analysis
TE	Echo time
TEMS	Transanal endoscopic microsurgery
TKI	Tyrosine kinase inhibitor
TME	Total mesorectal excision
TR	Repetition time
TRG	Tumour regression grading
UFT	Tegafur-uracil
ULCH	University London college hospital
XELOX	Capecitabine (XEL) + Oxaliplatin (OX)

Presentations and publications

Jalil, O, Ganeshan, B, Afaq, A, Arulampalam, T, Boone, D, Groves, ***A, MRI Tumor Heterogeneity as a Potential Prognostic Imaging Biomarker in Patients with Rectal Cancer Treated with Neoadjuvant Chemoradiotherapy.*** Radiological Society of North America 2014 Scientific Assembly and Annual Meeting, Chicago IL.

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Omer Jalil, Tan Arulampalam, Darren Boone, Bruce Sizer, Roger W. Motson. ***Long term Results of Laparoscopic Surgery Following Long Course Chemoradiotherapy for Locally Advanced Rectal Cancer.*** Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) 2016 Annual meeting, Boston, USA

Omer Jalil**,** Asim Afaq, Balaji Ganeshan, Uday B Patel, Darren Boone , Ashley Groves, Bruce Sizer, Tan Arulampalam**. *Magnetic resonance based texture parameters as potential imaging biomarkers for predicting long term survival in locally advanced rectal cancer patients treated with chemoradiotherapy.*** *Colorectal Disease, accepted 08/06/2016 doi:10.1111/codi.13496*

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1 Chapter 1: Introduction and literature review

1.1 Overview

The management of rectal cancer has changed dramatically in the last two decades from a surgically managed disease into a multimodality treatment model over the last decade. Currently the standard management of locally advanced rectal cancer, where tumour invades or extends close to the mesorectal fascia, is neoadjuvant chemoradiotherapy (CRT) followed by total mesorectal excision (TME) (van Gijn, et al., 2011). In parallel, laparoscopic rectal cancer surgery has developed so that it is feasible to utilize this technique with its proven short term benefits for pelvic dissection (Motson, et al., 2011). Though, the current management with the use of high resolution magnetic resonance imaging (MRI) in staging and selecting patients for CRT has shown considerable improvement in loco regional control. But, this is not the case for systemic control and these strategies may not necessarily improve overall survival (Lange, et al., 2013).

In addition, there remain many unanswered questions, especially with regards to the management of locally advanced rectal cancer. There is a lack of consensus on the optimal time interval to surgery after finishing chemoradiotherapy in such patients (Sizer, et al., 2009). Currently, the time interval to surgery after completion of CRT is arbitrarily fixed at of 6-8 weeks (Glimelius, 2014) without regard for on-going tumour response which may occur after the conventional 6 to 8 week window (Johnston, et al., 2009). Furthermore, restaging of irradiated rectal tumours is challenging because of difficulty of morphological MRI in differentiating fibrosis from viable residual tumour (van der Paardt, et al., 2013). In addition, a proportion of such patients would achieve complete clinical response and could benefit from either wait and watch approach or less invasive local excision surgery (Habr-Gama, et

al., 2004). But there is a poor correlation of clinical complete response with true pathologic complete response (Zmora, et al., 2004).

This shifting paradigm has placed greater recent interest in quantification of imaging biomarkers linked to underlying intra-tumour heterogeneity associated with adverse outcomes in terms of treatment failure and drug resistance (Ganeshan and Miles, 2013) This could help to select patients with predicted poor prognosis for personalized intensive therapy. Heterogeneity can be quantified on imaging non-invasively using textural-analysis (TA). TA assesses the distribution of pixel grey-level intensity, coarseness and regularity in digital images (Castellano, et al., 2004). In the last decade or so, TA has been employed in oncological studies of lung (Kido, et al. 2002), brain (Skogen, et al. 2013), renal (Goh, et al., 2011) and breast (Ahmed, et al., 2013) to act as a diagnostic, prognostic and treatment response assessment imaging biomarker. Though, the potential of CT based textural analysis as a prognostic tool has been investigated for colorectal cancer (Ganehsan, et al., 2007 and Ng, et al., 2013) but there is a lacking evidence for the potential of MRI based TA (MRTA) in predicting survival in locally advanced rectal cancer.

Conventional radiological anatomical response assessment based on percentage reduction in tumour length such as the Response Evaluation Criteria in Solid Tumours (RECIST) (Machida, et al. 2008 and Therasse, et al. 2000) is well established. However change in tumour size does not always reflect treatment response. Sometimes size my increase due to necrosis, cytotoxic oedema or haemorrhage resulting from treatment rather than disease progression (Tuma, 2006). In addition this method of assessment is not appropriate for predicting response for cytostatic rather than cytocidal cancer therapies (Wahl, et al. 2009). In such cases tumour response cannot be reliably predicted. Because of the limitations of conventional imaging, functional MRI techniques such as diffusion-weighted imaging (DWI) and positron emission tomography (PET) reflecting tumour microenvironment are currently the subject of investigation for a potential prognostic and tumour response assessment tool

for various cancers. However the evidence in the literature is either based on the studies either using PET or DWI techniques separately or combining data indirectly from independent PET and MR images. The major limitation with such studies is that since scans are not performed simultaneously, there is a potential for errors in lesion co-registration especially for organs such as bowel which may change location and shape over short periods (Hofmann, et al., 2008). In recent years with technological advancement and development of PET detectors that could function in the presence of strong magnetic field, state of art integrated PET/MR imaging technique is now available and carries a huge research potential.

The main objectives of this research project was to investigate a gap in the knowledge of locally advanced rectal cancer with regards to short and long-term effects of delayed laparoscopic surgery after CRT, prognostic potential of TA based on conventional MRI and to assess the ability to functional parameters quantified on integrated PET/MRI to predict pathological response to CRT. This gap in the knowledge and the objectives were investigated through three clinical studies in the thesis.

- The main objective of the first clinical study was to determine both the short and long-term effect of delayed TME beyond 12 weeks in laparoscopic setting after long course CRT in locally advanced rectal cancer including patients with threatened CRM and T4 stage.

- The main objective of the 2nd clinical study in the thesis was to investigate whether MRI based textural analysis in addition to morphological MRI and histopathological parameters can predict survival outcomes in rectal cancer. Textural analysis of pre-treatment MRI, 6-week post CRT MRI was carried out to develop imaging biomarkers that can predict long term prognosis in locally advanced rectal cancer patients treated with neoadjuvant CRT.

- The main objective of the third study was to investigate whether pre-treatment integrated PET-MR functional features correlated with histological response in locally advanced rectal cancer treated with long course CRT. In addition, a potential correlation of PET and functional MRI features in the setting of integrated PET/MRI system was evaluated. Moreover, association of clinical, histological and functional imaging parameters with disease free survival was also evaluated for these patients.

1.2 Thesis outline

Chapter 1 This introductory chapter provides an overview of rectal cancers including its epidemiological background, aetiology, different treatment modalities, staging with particular reference to MR staging and restaging after long course CRT and issue relating to optimal timing of surgery. This also provides an introduction for chapter 4.

Chapter 2 The concept of textural analysis, basic principles of MRI, functional imaging modalities (PET, DWI) and integrated PET/MRI are introduced in this chapter along with their current use for various oncological applications with special reference to rectal cancer. This also provides an introduction to the chapters 5 and 6.

Chapter 3 Details of methodology, methods and materials employed for the clinical studies in chapters 4-6 are discussed here. This includes the inclusion and exclusion criterion for locally advanced rectal cancer, image acquisition and analysis protocols and statistical analysis.

Chapter 4 In this chapter, the outcomes for the patients with MRI defined poor risk histologically confirmed locally advanced rectal adenocarcinomas treated with neoadjuvant long course CRT followed by delayed surgery are discussed and critiqued in the light of relevant evidence in literature.

Chapter 5 This chapter is based on the second objective of the thesis. The results of the use of MRI based textural analysis as a prognostic imaging biomarker and an independent predictor of survival along with morphological conventional MRI parameters in patients with locally advanced rectal cancer are discussed.

Chapter 6 In this chapter, a pilot study addressing the third objective and research questions of the thesis is discussed. This study investigated whether pre-treatment integrated PET-MR functional features could predict pathological tumour regression and survival in the study population

Chapter 7 provides the summaries and conclusions of the clinical studies along with the limitations and future potential fields in rectal cancer research.

1.3 Rectal cancer

Rectal cancer is a malignant tumour arising from the epithelium of the rectal mucosa and constitutes one third of all the colorectal cancers (CRC) (Bowles, et al., 2013). More than 90% of all the colorectal carcinomas are adenocarcinomas. Conventional adenocarcinoma is characterized by glandular formation, which is the basis for histological tumour grading (Fleming, et al., 2012). Mucinous adenocarcinomas are a histological subtype of CRC in which the tumour cells secrete abundant extracellular mucin involving more than 50% of the tumour volume. It is well documented that up to two-third of all the mucinous colorectal adenocarcinomas arise from rectum which is the most common site (Secco, et al., 1994). Other rare types of colorectal carcinomas include neuroendocrine, squamous cell, adenosquamous, spindle cell and undifferentiated carcinomas. The World Health Organisation (WHO) classification of type and grade of CRC (Bosman, et al., 2010) is shown in Table 1-1-1

Table 1-1-1 Histological types and grades of colorectal carcinoma according to WHO classification. (Adopted from Bosman, et al. 2010 Note: G1: well differentiated G2: moderately differentiated G3: poorly differentiated G4: undifferentiated (Jass, and Sobin, 1989)

Histological type	Description	Grading system (G1-G4)
Adenocarcinoma	Glandular epithelium	1-3
Mucinous adenocarcinoma	More than 50% extracellular mucin	1-3
Signet ring cell carcinoma	More than 50% signet ring cells(intracytoplasmic mucin)	3
Squamous cell carcinoma	Exclusive squamous differentiation	1-3
Adenosquamous carcinoma	Adenocarcinoma and squamous cell carcinoma (mixed)	1-3
Small cell carcinoma	Similar to small cell carcinoma of the lung (neuroendocrine)	4
Undifferentiated carcinoma	No glandular or other features to indicate definitive differentiation.	4

1.4 Epidemiology

Understanding the magnitude of the clinical problem that CRC presents requires analysis of its epidemiology. According to the report of World Health Organization- International Agency for Research on Cancer, CRC is the third most common cancer in man and second in women world-wide (Ferlay, et al., 2013). CRC shows geographic variation in its distribution throughout the world and is mainly a disease of western world; the highest rates are in Australia/New Zealand and Western Europe and the lowest in Africa (except Southern Africa) and South-Central Asia (Haggar and Boushey, 2009). This difference might be due higher intake of dietary fats especially animal fats and less consumption of diets rich in fibre such as fruit and vegetables in the Western world (Gingras and Beliveau, 2011). In 2013, 41,112 new cases of CRC were registered in the UK (age-standardized incidence rate of 71 new cases per 100,000 persons) and it was the fourth most common cancer, accounting for

12% of all new cases (Cancer Research UK, 2016). The commonest anatomical site is in rectum and rectal cancer accounts for approximately one third of CRC. The proportions of rectal cancer cases are higher in males than females. In 2010-2012, the average numbers of new rectal cancer cases registered per year in the UK were 11567. Of those, 7327 were males (31.5%) and 4240 were females (23.1%) (Cancer Research UK, 2015). The lifetime probability of being diagnosed with an invasive CRC in the UK is 1 in 14 for men and 1 in 19 for women. The incidence of CRC is strongly related to age and it rises with increasing age in both sexes. Almost 83% of cases were diagnosed in people aged 60 or above in the UK in 2011-2013 (Cancer Research UK, 2016).

In addition to morbidity, CRC is also a major cause of mortality world-wide. CRC is the fourth commonest cause of cancer related mortality and accounts for 8% of all the deaths from cancer worldwide (Ferlay, et al., 2013). CRC is the second leading cancer killer both in United States (Haggar and Boushey, 2009) and Europe (Ferlay, Parkin and Steliarova-Foucher, 2010). The nationwide UK figures are representative of disease burden globally. According to Cancer Research UK 2012-dataset, CRC is also the second most common cause of cancer related mortality in the UK, with the crude mortality rates of 28 per 100,000 men and 23 per 100,000 women. Around 40% of CRC deaths are due to rectal cancers alone according to this dataset (Cancer Research UK, 2014).

1.5 Aetiology of colorectal cancer

1.5.1 Hereditary syndromes

Both genetic and environmental factors have been implicated in the development of CRC. Up to 90% of CRC are termed sporadic (Acheson and Scholefield, 2002) and arise from changes in the somatic cells. The hereditary syndromes account for fewer than 10% of CRC predominantly in younger patients (Lynch and de la Chapelle, 2003). The two commonest hereditary syndromes are hereditary non-polyposis colorectal carcinoma (HNPCC) and familial adenomatous polyposis (FAP) (Jang and Chung, 2010). Other hereditary syndromes

include Peutz-Jeghers syndrome and juvenile polyposis syndrome characterized by development of hamartomatous polyps as primary lesions. In HNPCC, also called Lynch Syndrome, affected individuals can develop colonic adenomas with greater frequency than the general population but polyposis is rare (Jasperson, et al., 2010). The lifetime risk of developing CRC is 50%– 80% and there is preponderance for an early age with 70% of males and 57% of females diagnosed with CRC before the age of 50 years (Stoffel, et al., 2009). Tumours tend to be right-sided (Weitz, et al., 2005) and have classical histological features such as being poorly differentiated, mucinous and have large numbers of tumour-infiltrating lymphocytes. Endometrial cancer is the most common extra-colonic malignancy associated with Lynch syndrome (Stoffel, et al., 2009). Other cancers associated with Lynch syndrome include gastric, ovarian, biliary, urinary tract, small bowel, brain and pancreatic. Characteristic features of FAP include the development of hundreds to thousands of colonic adenomas (50% of patients by age 15 years, 95% by age 35 years). Polyps undergo malignant change inevitably by the age of 40 (Weitz, et al., 2005) unless prophylactic colectomy is performed. An important variant is attenuated FAP with 10 to 100 colorectal adenomas. There may, in addition, be extracolonic manifestations, for example desmoid tumours and osteomas of the skull (Gardner syndrome) (Jasperson, et al., 2010). Hyperplastic polyposis (HPP) is an infrequent condition, characterized by presence of multiple hyperplastic polyps throughout the colon that are typically discovered in routine screening. These lesions carry significant cancer risk and histologically appear as sessile serrated adenomas (Legget, et al., 2001).

1.5.2 Pathogenesis

Understanding of the molecular mechanisms in pathogenesis of colorectal cancer is important to identity biological makers to improve cancer surveillance, assess response to a therapy and their correlation with long term outcomes. About 20% of all patients with this cancer are estimated to have some component of familial risk caused by alterations in single genes regulated by environmental factors that are less penetrant but more common than

those associated with hereditary colorectal cancer (Lynch and de,la, 2003). Pathogenesis of CRC can be classified into three distinct molecular pathways of genomic instability, proposed by Ogino and Goel (2008). These pathways include chromosomal instability (CIN) pathway, DNA mismatch repair pathway associated with epiphenomenon of microsatellite instability (MSI), and epigenomic instability or CpG island hypermethylation (CIMP) pathway. Figure 1-1 shows multiple pathways in pathogenesis of colorectal cancer. Table 1-1-2 shows molecular classification of colorectal carcinoma.

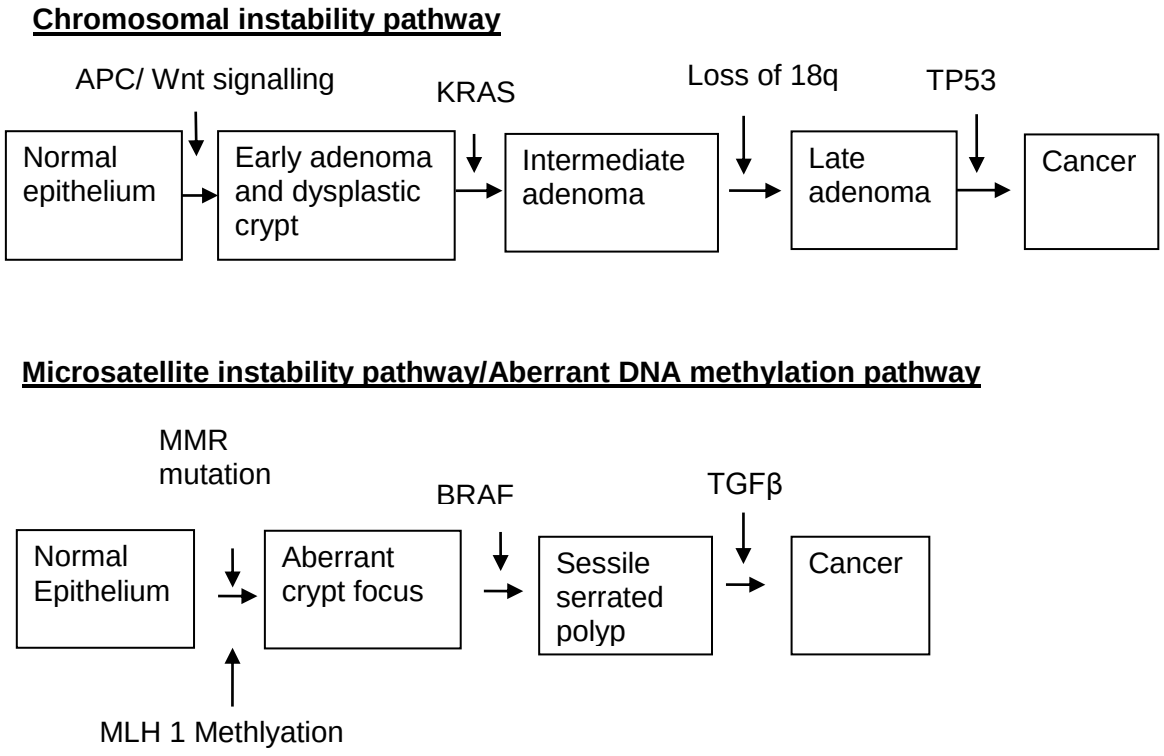

Figure 1-1 Multiple pathways in colorectal cancer pathogenesis

Table 1-1-2 Molecular classification of colorectal carcinoma. Adapted from (Cunningham, et al., 2010)

	Chromosomal instability pathway	**Mismatch repair pathway**	**Aberrant DNA methylation pathway**
	Hereditary or Sporadic	Hereditary	Hereditary or Sporadic
MSI	MSS	MSI-H	MSI-H or MSI-L
CIN	Present	Absent	Absent
CIMP	Negative	Negative	CIMP-H
KRAS Mutation	+++	±	---
BRAF	---	---	+++
MLH1 status	Normal	Mutation	Methylated

CIMP = CpG island methylator phenotype. MSS = microsatellite stability. MSI = microsatellite instability. MSI-H = high-level microsatellite instability. MSI-L= low-level microsatellite instability. +++ = present. +/− = might or might not be present. −−− = absent

1.5.2.1 Chromosomal Instability pathway (CIN)

CIN accounts for approximately 60% of CRC (Walther, Houlston and Tomlinson, 2008) and is characterized by aneuploidy and chromosomal gains and losses. These tumours can be inherited, as typified by familial adenomatous polyposis (FAP) due to germline mutations in the adenomatous polyposis colic (APC) gene which is found on chromosome 5q 21-22, or they can occur sporadically. The evolution of CRC proceeds in a classic adenoma cancer sequence proposed by Fearon and Vogelstein (1990), with inactivation of tumour suppressor APC gene initiating adenomas. This is followed by activation of proto-oncogene, KRAS, mutations in the transforming growth factor- b (TGF- b), allelic loss of chromosome 18q and finally TP53 inactivation, causing the transformation of normal cells into cancer cells (Figure 1-1). APC is a key negative regulator of β-catenin, a component of WNT signalling pathway. B-catenin is an intracellular protein which, when activated, translocates to the nucleus and stimulates cell proliferation by transcriptional activation of target genes. In the absence of

Wnt legend, the APC protein halts cellular proliferation and promotes apoptosis by phosphorylating β-catenin, leading to its ubiquitination and degradation through the proteosome pathway. In the case of an inactivating mutation, APC-mediated β-catenin degradation is lost and nuclear concentrations of β-catenin remain high, which results in the formation of the adenoma (Sepulveda and Lynch, 2013). KRAS induces tumour proliferation through EGFR signalling pathway. Mutations in KRAS gene occur in 35-45% of tumours of colon and rectum and resistant to benefits from anti-EGFR antibody therapy. Testing for KRAS mutations is recommended for metastatic CRCs who are candidate for anti-EGFR antibody therapy (Engstrom, et al., 2009).

1.5.2.2 Microsatellite instability pathway

Microsatellite instability is a measure of the inability of the DNA nucleotide mismatch-repair (MMR) system to correct errors that often occur during DNA replication, which is controlled by genes MLH1, MSH2, and MSH6 (Abdel-Rahman, et al., 2006). Deficient MMR leads to altered length of short nucleotide repeats in tumour DNA compared to normal DNA, a phenomenon termed microsatellite instability (MSI) (Boland and Goel, 2010)). Mutations in MMR genes are the underlying genetic defect in the majority of HNPCC Syndrome (Beggs and Hodgson, 2008). Sporadic tumours are characterised by proximal location, mucinous histology, poor differentiation, and lymphocytic infiltration (Cunnigham, et al., 2010). Unlike CIN pathway, MSI is associated with a better prognosis in CRC (Guastadisegni, et al., 2010).

1.5.2.3 Aberrant DNA methylation pathway

DNA segments with abundant guanine and cytosine bases (CpG islands) become methylated leading to a distinct the CpG island methylator phenotype (CIMP). Because the basic DNA sequence is not altered, this is considered an epigenetic rather than a genetic change and leads to stable differential states of gene expression (Laird, 2005). In most sporadic cases, microsatellite instability occurs when the promoter region of the genes in the

mismatch-repair system (often MLH1) is silenced by hypermethylation of CpG islands (Toyota, et al., 1999). Promoter CpG methylation is associated with a serrated pathway of colorectal carcinogenesis (Jass, 2005) distinct from the classic adenoma–carcinoma pathway described by Fearon and Vogelstein (1990). The BRAF oncogene is a downstream effector of KRAS in the EGFR-dependent signalling cascade. BRAF mutations have been reported in 5-10% of sporadic disease and have been linked to serrated carcinoma pathway of colorectal tumorigenesis (Sepulveda and Lynch, 2013).

1.5.3 Risk factors for colorectal cancer

1.5.3.1 Non-modifiable risk factors

Factors increasing the risk of developing colorectal cancer can be modifiable or non-modifiable. The non-modifiable risk factors include inflammatory bowel disease (IBD) and history of CRC in first degree relatives in addition to hereditary and genetic factors as described above (Lynch and Smyrk, 1999). The increased risk for cancer in IBD is predominantly acquired and is associated with the site, extent and duration of inflammation. Patients with ulcerative colitis affecting only the rectum, a condition termed ulcerative proctitis, rather surprisingly do not have significantly increased risk for developing rectal cancer (Ekbom, et al., 1990). In a meta-analysis of 116 studies comprising of 54478 patients, the overall prevalence of CRC in UC was estimated to be 3.7%, increasing to 5.4% in those with pancolitis (Eaden, et al., 2001) Whereas CRC is rarely encountered before 7 years of colitis, the risk rises thereafter, with a cumulative risk for CRC in a patient with UC of 2% at 10 years, 8% at 20 years, and 18% at 30 years (Eaden, et al., 2001).Crohn's colitis is also associated with increased risk of colorectal cancer; the relative risk is similar to that for ulcerative colitis (Itzkowitz and Harpaz, 2004). The presence of a family history of colon cancer does increase this risk still further for the individual with IBD; this shows that, as for sporadic colon cancer, both genetic and acquired factors are important. Molecular and genetic alterations occur more rapidly in IBD-CRC and in an unconventional sequence. In IBD-associated cancers evidence for dysfunctional signalling in the WNT signal transduction

pathway is found relatively infrequently and occurs late in the dysplasia–cancer sequence conversely, p53 mutations occur as a relatively early genetic change in IBD-associated cancers (Rhodes and Campbell, 2002). Having a first-degree relative (FDR) with CRC over the age of 50 can increases the risk up to two to three times that of an average risk individual (Jasperson, et al., 2010). Having one FDR with CRC under age 45 years, or having two FDRs affected with CRC confers a 3–6-fold CRC risk compared to the general population (Johns and Houston, 2001).

1.5.3.2 Modifiable risk factors

The modifiable risk factors are related to diet and life style. Recent meta-analysis by Johnson, et al., (2013) shows independent significant association of increased body mass index, red meat intake and cigarette smoking with colorectal cancer. Low physical activity, low vegetable consumption, and low fruit consumption showed significant inverse association with CRC. Different mechanisms are believed to be responsible for causative relationship between these risk factors and CRC. N-nitroso compounds (NOCs) are formed in processed meat food and a high intake of red meat is related to the endogenous formation NOCs in the gastro-intestinal tract. Decarboxylation of amino acids by gut bacteria yields amines and amides that can be nitrosated in the large bowel. Many NOCs, including nitrosamines and nitrosamides, are alkylating agents and can react with DNA and are carcinogenic in laboratory animals and, thus, may be a risk factor for some cancer entities, for example, colon cancer (Santarelli, Pierre and Corpet, 2008). A quantitative analysis of 56 observational studies found that each 5 kg m^2 increase of body mass index is associated with 18% increased risk of colorectal cancer. The association is stronger for colon than rectal cancer, for men than women, for premenopausal than postmenopausal women, for North American than European populations (Ning, Wang and Giovannucci, 2010). The exact mechanism of the correlation between obesity and colorectal cancer is not entirely clear but hormonal factors seem to play a significant role. Adiposity relates to hyperinsulinemia which reduces insulin-like growth factor (IGF)-binding proteins levels and thereby increases free

circulating IGF-I Concentrations. Both insulin and IFG-I are important determinants of cell proliferation and apoptosis and thus may promote carcinogenesis (Giovannucci, 2001). Adipose tissue also behaves as an active endocrine organ secreting cytokines such as interleukin-6 (IL-6), free fatty acids, and tumour necrosis factor-[alpha] (Giovannucci, et al., 2010). This induces a chronic proinflammatory response possibly promoting carcinogenesis. Inactivity increases the risk of colon and high levels of physical activity may reduce the risk of colon cancer by as much as 50% (Colditz, et al., 1997). Physical activity acts on cancer risk independent of its effects on body weight in a dose–response relationship. In a population based cohort study by White, Jacobs and Daling (1996), moderate or high intensity recreational activity (two or more times per week vs. none) was associated with a decreased risk of colon cancer (RR = 0.70, 95% CI=0.49-1.00). Physical activity may reduce circulating levels of insulin, hormones, and other growth and it decreases gastrointestinal transit time, physical activity can also minimise contact time between the colonic mucosa and potential carcinogens in the stool (Stein and Colditz, 2004).

1.5.4 Chemoprevention

In general, it takes 10-15 years to progress from normal colorectal epithelium, via the stage of an adenoma, to colorectal cancer (Stryker, et al., 1987). This time span provides a window of opportunity for chemoprevention, especially in high-risk patients with a history of an adenoma or cancer. A meta-analysis of placebo controlled double blind trials found a statistically significant 17% decrease in the relative risk of adenoma in general population for aspirin vs. placebo, which corresponded to a 6.7% absolute risk reduction (Cole, et al., 2009). Chemoprevention with calcium supplements has shown to be effective in preventing adenomas within those people who have previously undergone polypectomy (Carroll, et al., 2010. Studies have shown protective association between 5-aminosalicylates use and CRC or CRC and dysplasia in UC (Velayos, Terdiman, and Walsh, 2005). They can decrease epithelial cell turnover and promote apoptosis through COX-2 dependent (inflammatory) and independent (non-inflammatory) pathways by interfering with the Wnt/β-catenin signalling

pathway. 5-ASA's have also shown to have antioxidant and free radical scavenger properties and can reduce DNA oxidative stress and microsatellite instability (Stolfi, et al., 2008).

1.6 Screening for CRC

Before, treatment of symptomatic CRC is discussed; first the current state of knowledge regarding screening for asymptomatic disease needs to be outlined. Colorectal cancer like breast and cervical cancer enjoys typically long natural history and almost always proceeded by precancerous lesions such as adenomatous polyps. Early detection and removal of these lesions reduces the incidence of CRC as shown by the US National Polyp Study (Winawer et al., 1993). Specific screening strategies for CRC should be targeted to populations by considering its level of risk. A Population is considered to be of average risk for the development of colorectal cancer once they reach the age of 50. Those who have symptoms or who are at higher risk should be managed according to their perceived risk (Winawer, et al., 2003). Today there is a range of options for CRC screening in the average-risk population, with current technology falling into 2 general categories: stool tests, which include tests for occult blood (FOBT) or exfoliated DNA; and structural exams, which include flexible sigmoidoscopy (FSIG), colonoscopy, double-contrast barium enema, and computed tomographic colonography (Levin, et al., 2008). These tests may be used alone or in combination to improve sensitivity or, in some instances, to ensure a complete examination of the colon if the initial test cannot be completed. However, the only two tests which have been evaluated in randomized trials and demonstrated to reduce mortality of CRC are FOBT and FSIG. Stool tests are best suited for the detection of cancer, although they also will deliver positive findings for some advanced adenomas (Bretthauer, et al., 2010). Guaiac-based faecal occult blood test's efficacy has been confirmed in major prospective trials, with a reduction of 16% in relative risk for CRC mortality (Hewitson, et al., 2008). To improve the specificity of guaiac-faecal occult blood test, immunochemical faecal occult blood has been

evaluated and demonstrated to perform better (Van Rossum, et al., 2008) but it is more expensive and lacks evidence based on randomized trials. A newer stool DNA test checks for the presence of mutations associated with colorectal neoplasia, while this test is commercially available in United States since 2003 but it is not adapted for screening (Di Lena, Travaglio and Altomare, 2013) because it is not widely available, and the results not very accurate or reliable enough (Ahlquist, et al., 2008). The structural examination of distal colon in the form of FSIG can achieve the dual goals of detecting adenocarcinoma as well as identifying adenomatous polyps. The results of largest randomized trial to date (UK flexible sigmoidoscopy screening trial) demonstrated that after 11 years of follow-up, the incidence of colorectal cancer and mortality in the intervention group was reduced by 33% and 43% respectively (Atkin, et al., 2010). There are no prospective randomized controlled trials of screening colonoscopy for the reduction in incidence or mortality of CRC; however, because colonoscopy is used to evaluate other positive screening tests, there is evidence to indicate that colonoscopy and polypectomy result in incidence reductions in randomized controlled trials of other screening tests. The University of Minnesota randomized controlled trial of FOBT observed a 20% reduction in incidence of CRC, which the authors attribute to colonoscopy and polypectomy in patients with a positive FOBT (Church, et al., 2004).

1.7 Anatomy of rectum

Understanding the anatomy of rectum is essential to appreciate the principles of its treatment. The definitions of rectum and low rectal cancer are highly variable. The rectum has been defined as “the portion of the intestinal tract extending from the rectosigmoid junction to the anorectal ring (Lowry, et al., 2001). As a rule, one third of the rectum is located intraperitoneally and two thirds extraperitoneally. Martling, et al. (2001) defined the rectum as 15 cm from the anal verge as measured by rigid endoscopy and attributed low rectal cancer within 5 cm from the anus. This definition would be used in the study proposed as it is more practical and rigid sigmoidoscopy is carried out routinely for the objective assessment and local staging of tumour.

A cuff of peri-rectal fat surrounds the rectum in a circumferential manner and extends to the entire length of rectum except the last distal one centimetre. Peri-rectal fat is less abundant anteriorly than posteriorly. A distinct circumferential visceral layer termed as fascia propria of rectum separates the peri-rectal fat from parietal endopelvic fascia. This visceral fascia propria enclosing the peri-rectal fat containing rectal lymphatic and blood vessels is termed as mesorectum. Figure 1-2 depicts the mesorectal fascia on axial MRI. Autonomic nerve plexus containing both sympathetic and parasympathetic nerve fibers to pelvic viscera are present in an avascular plane between visceral fascia propria of rectum and endopelvic parietal fascia (Mahadevan, 2011). Mesorectum along with fascia propira forms the plane of a landmark surgical technique called “Total mesorectal excision” (TME) described by Heald, et al., (1993). TME is an anatomical approach to rectal cancer surgery that involves enbloc resection of rectal tumour along with excision of the mesorectal tissue to the level of levators outside the mesorectum in an avascular plane between fascia propria of rectum and parietal endopelvic fascia (Lowry, et al., 2001). By operating within this plane enables the surgeon to perform radical resection of rectal cancer but at the same time allows preservation of autonomic nerve fibers which are essential for pelvic visceral functions. Lateral or radial surgical resection margin also called the circumferential margin is the least distance between the resection margin and radial extend of rectal tumour microscopically (Lowry, et al., 2001). Its prognostic importance in rectal cancer was initially described in 1986 by Quirke, et al. Involvement of circumferential resection margin (CRM) is associated with poor prognosis (Quirke, et al., 1986). Involvement of mesorectum by rectal cancer enhances the risk of positive CRM. Determination of CRM involvement is one of the most important criteria of locally advanced rectal cancer that will make them suitable to neoadjuvant chemoradiotherapy and hence to down stage them. The details of imaging and histopathological criteria of determining the CRM involvement and its prognostic importance are discussed in sections 1.8.1 and 1.9.4.1. The blood supply to the rectum comes from superior rectal artery which is the continuation of inferior mesenteric artery. The blood supply to lower one third of rectum comes from inferior haemorrhoidal artery, a branch of

internal iliac artery. Like the rest of colon, the lymphatic drainage of rectum follows its blood supply. Lymph nodes within the mesorectum receive the initial lymph from the rectum and are divided into two types: N1, lymph nodes located close the rectal wall and centrally placed N2 lymph nodes. From these rectal nodes, the lymph form upper 2/3 of the rectum drains into the nodes located around the origin of inferior mesenteric artery. Lower 1/3 of the rectum also drains into inferior mesenteric lymph nodes along with the bilateral internal iliac nodes located along the pelvic side wall (Mahadevan, 2011). Chemoradiotherapy (CRT) with curative intent as well as radical surgery must therefore address these lymph node groups in treating rectal cancers.

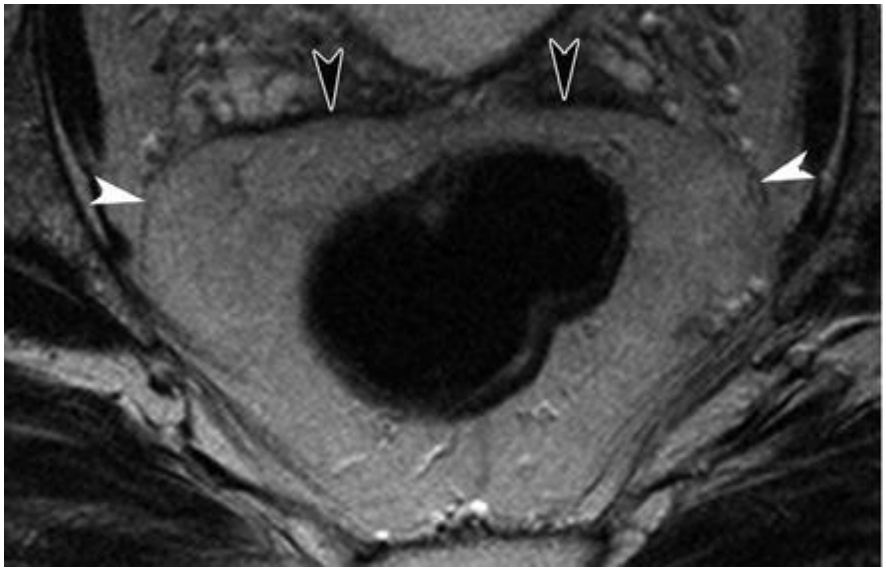

Figure 1-2 MRI axial image: Normal anatomy of mesorectum. Mesorectal fascia is shown as hypointense layer (white arrowheads) surrounding hperintense mesorectal fat. Meosrectal fat is more abundant posteriorly than anteriorly (black arrowheads). Adapted from Lafrate et al., (2006)

1.8 Staging

After establishing the diagnosis of rectal cancer histologically, the next step in the management of rectal cancer is to determine its staging. Staging determines the site, size and local infiltration of primary tumour along with involvement of lymph nodes and distant metastases. Staging is not only important from the management planning point of view but it

also signifies prognosis, evaluates the results of treatment and facilitates the exchange of information between treatment centres (Compton and Greene, 2004). The staging of rectal cancer has evolved over the years. It was originally classified by Dukes (1932) and further modified by Astler and Coller (1954) (Table 1-3).

Table 1-1-3 Dukes and Modified Astler-Coller (MAC) classification of colorectal cancer (Adapted from Wu, 2007).

Dukes' Classification	**Description**	**Modified Astler-Coller classification**
A	Invasion into but not through the bowel wall	Lesions limited to the mucosa
B	Invasion through the bowel wall	Type B_1—Lesions extending into the muscularis propria, but not penetrating it, with negative nodes Type B_2—Lesions penetrating the muscularis propria, with negative nodes
C	Involvement of lymph nodes	Type C_1—Lesions extending into the muscularis propria, but not penetrating it, with positive nodes Type C_2—Lesions penetrating the muscularis propria with positive nodes

Duke's three stages are based on mural involvement and the presence of regional nodal involvement on histopathological assessment, although the number of regional nodes involved was not considered. The Dukes' staging system does not incorporate preoperative clinical information which is essential for planning treatment. In 1987, cancer staging system based on local tumour depth of invasion (T), the presence and number of nodal metastases (N), and the presence of distant metastases (M) was introduced by the American Joint Committee on Cancer (AJCC) and International Union for Cancer Control (UICC) (Hutter, 1987). In addition to the histological assessment, the TNM staging system allows assessment of T, N, and M categories through endoscopy, imaging and physical examination as well. Though TNM staging was initially developed to predict prognosis but it

has assumed additional roles to determine optimal therapy, assessment response to therapy and entry into clinical trials (Quirke, et al., 2007). Table 1-1-4 shows the TNM classification of CRC tumors based on the AJCC cancer Staging Manual, 7th edition (Edge, et al., 2010 and Cunnigham, et al., 2010).

Assessment of the invasion depth (T stage) and lymph node involvement (N stage) are vital components of preoperative staging in rectal cancers. Both have been show independent marker for poor prognosis. The extent of tumour penetration through the rectal wall (T status) corresponds directly to the rate of recurrence and indirectly to the overall 5 year survival. In a pooled analysis of 5 randomized trials by Gunderson et al., (2004), there was a progressive decrease in overall survival at 5 years with increasing T stage (T1-2, 75%; T3, 60%; T4, 47%; $P < .001$). A similar trend was seen for disease recurrence. Increasing T stage (T1-2, T3, and T4) was associated with a steady increase in rates of both local (7% v 12% v 16%; $P < .001$) and distant relapse (22% v 34% v41%; $P < .001$). The presence of lymph node metastasis and metastatic lymph node ratio in a resected specimen shows a similar impact on overall survival and disease relapse regardless the depth of tumour invasion (Peng, et al., 2008). Once the T, N and M status of a rectal cancer is determined, they are combined to form a clinic-pathological stage grouping (Table 1-1-5) to be assigned to an individual tumour for the advantage of facilitating treatment decisions (Kim, et al., 2011). Only patients with stage II and III rectal cancer were included in the clinical studies. Locally advanced rectal cancers treated with neoadjuvant chemoradiotherapy usually requires restaging before undergoing surgery to assess response to the treatment and then further treatment is determined on the final histopathological TNM staging. Different prefixes to the TNM staging are applied and suggested in reporting rectal cancer staging to highlight the different imaging modalities used for investigation and the point at which the staging takes place (Edge, et al., 2010 and Moran, et al., 2008). These include; cTNM indicating the clinical classification, pTNM indicating the pathological classification, y prefix indicating the

stage assessed after neoadjuvant treatment (for example ypTNM), mr indicating the staging assessed on MRI.

Table 1-1-4 The UICC TNM Classification of CRC tumours. Based on the AJCC cancer Staging Manual, 7th edition. Adapted from Cunningham, et al., (2010).

T - Primary tumour
TX Primary tumour cannot be assessed
T0 No evidence of primary tumour
Tis Carcinoma in situ: intraepithelial or invasion of the lamina propria
T1 Tumour invades submucosa
T2 Tumour invades muscularis propria
T3 Tumour invades through muscularis propria into pericolorectal tissues
T4a Tumour directly invades other organs or structures and or perforates visceral peritoneum
T4b Tumour directly invades or is adherent to other organs or structures
N - Regional lymph nodes
NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Metastasis in 1-3 regional lymph nodes; N1a Metastasis in one and N1b Metastasis in two to three regional lymph nodes
N2 Metastasis in 4 or more regional lymph nodes; N2a Metastasis in 4–6 and N2b Metastasis in 7 or more regional lymph nodes
M - Distant metastases
MX Distant metastases cannot be assessed
M0 No distant metastases
M1 Distant metastases

Table 1-1-5 Clinical stages in rectal cancer and 5-year survival (Adapted from Edge, et al., 2010 and Weitz, et al., 2005).

Group Staging	T	N	M	Dukes	MAC	5-year survival
0	Tis	N0	M0	-	-	
I	T1	N0	M0	A	A	80-95%
	T2	N0	M0	A	B1	
IIA	T3	N0	M0	B	B2	72-75%
IIB	T4a	N0	M0	B	B2	65-66%
IIC	T4b	N0	M0	B	B2	
IIIA	T1-T2	N1/N1c	M0	C	C1	55-60%
	T1	N2a	M0	C	C1	
IIIB	T3-T4a	N1/N1c	M0	C	C2	35-42%
	T2-T3	N2a	M0	C	C1/C2	
	T1-T2	N2b	M0	C	C1	
IIIC	T4a	N2a	M0	C	C2	25-27%
	T3-T4a	N2b	M0	C	C2	
	T4b	N1-N2	M0	C	C3	
IV	Any T	Any N	M1	-	-	0-7%

1.8.1 Rectal Cancer staging-Modalities

Digital rectal examination, MRI and Endoscopic rectal ultrasound are used in local staging of rectal cancer. For the operating surgeon, digital rectal examination helps in determining the level of tumour from anal verge, and the pelvic floor and also the size of tumour, and its fixity. It is an essential first step and a rough guide in evaluating the tumour resectibility and sphincter preservation (Nicholls, et al., 1985). Rigid sigmoidoscopy is important in localizing especially those tumours beyond the reach of an examining finger. Currently, MRI and endoscopic rectal ultrasound are preferred imaging modalities for the local staging but both have strengths and limitations in terms of their accuracy in determining T and N status (Morino, et al., 2015).

Endoscopic rectal ultrasound is more accurate in staging T0, T1 and T2 tumours but it tends to over stage tumours than under stage especially the T3 tumours (Blomqvist, et al., 2000).

However this technique is operator dependent and has steep learning curve and therefore, its accuracy ranges from 63-94% for T staging and 64-76% for N staging (Yeung, et al., 2009). In addition, Brown, et al. (2004) demonstrated the limitations of endoscopic ultrasound in identifying the mesorectal facial involvement which as described in section 1.7, is crucial for predicting CRM involvement. The mesorectal fascia represents the potential CRM in patients undergoing TME. Involvement of or close proximity of a tumour to the CRM (1mm of less) has been shown to be an independent predictor of local failure when determined by pathological assessment (Quirke, et al., 1986). In a prospective study by Adam, et al., (1994) local recurrence after a median 5 year follow up was significantly higher (78%) for patients who had tumour involvement of the CRM than for those without such involvement (10%). MRI is the modality of choice for the staging of locally advanced rectal cancers because it is more accurate than endoscopic ultrasound for determining CRM involvement. A study by the Mercury group (2006) showed that high resolution MRI accurately predicts whether the surgical resection margins will be clear or affected by tumour. The accuracy for prediction of a clear margin was 91% with a negative predictive value of 93% (proportion of MR based negative CRM patients that were true negative on histology). This compared with an accuracy of 77% and negative predictive value of 98% in patients who had received preoperative chemoradiotherapy or long course radiotherapy. In addition, MRI is also useful for accurate detection of the presence of extramural vascular invasion which is associated with poor prognosis (Brown, et al., 2003). When first introduced in 1980s, rectal cancer staging using body coil MRI in initial studies was not accurate (Hodgman, et al., 1986). Though the introduction of an endorectal coil positioned directly over the lesion improved the accuracy of T staging but it had an endocoil insertion failure rate of up to 40% due to stenotic cancers (Hunerbein, et al., 2000). With the introduction of phase arrayed multielement surface coils and high performance magnetic field gradients, the resultant higher spatial and contrast resolution scanning increases the diagnostic accuracy. A study by Zhang, et al., (2008) using 3-T field strength and eight-channel phased array coil MRI showed 92 % accuracy for T staging and 79% for N staging compared to

histopathological staging. In addition, MRI correctly predicted sphincter sparing procedure in 97% of cases. Similarly study by Brown, et al., (2003) using high resolution MRI imaging showed higher weighted agreement between MRI and histological assessment of T (94%) and N staging (85%). Hence, MRI has become a gold standard for the staging of locally advanced rectal cancer and was also the imaging modality used in the staging and selecting locally advanced rectal cancer patients in the thesis studies as discussed in sections 3.1.1 and 3.2.2

1.9 Treatment of rectal cancer

Once the diagnosis is established and staging is determined, a decision regarding further management such as to carry out immediate surgery or neoadjuvant CRT is made. Factors such as tumour site, fixity in pelvis, grade, and histological type and TNM status of tumour are considered in making such decisions. The management of rectal cancer has changed dramatically in the last two decades from a surgically managed disease into a multimodality treatment model over the last decade (van Gijn, et al., 2011). In the subsequent sections, the treatment of early rectal cancer is described briefly but the treatment of locally advanced rectal cancers is discussed in details because the population of the thesis studies comprises of latter cancer type.

1.9.1 Early rectal cancer (T1-2 N0 M0)

Surgery is a mainstay for the curative treatment of early stage rectal cancer and includes local excision as well as classical surgery. Though radical surgery produces superior oncological outcome but at the expense of considerable morbidity, whereas local excision is associated with significantly higher rates of local recurrence due to occult nodal involvement (Endreseth, et al. 2005) but offers a good compromise in poor-risk patients with lower morbidity and mortality (Abir, Alva and Longo, 2004). Local excision by transanal endoscopic microsurgery (TEMS) can adequately treat patients with early rectal cancer confined to the rectal wall (Moore et al., 2008). However local excision on its own is an inadequate

treatment for T1 rectal cancer with adverse features such as poorly differentiated cancer, lymphovascular invasion, tumour size more than 3 cm and depth of submucosal invasion (Maeda, Koide and Katsuno, 2014). Local excision in such cases is combined with radiotherapy and chemotherapy or salvage resection (Valentini, et al., 2009). Local excision alone for T2 cancer is not recommended because it compromises survival and associated with high local recurrence of up to 30% and is best treated with radical surgery alone (Mellgren, et al., 2000).

1.9.2 Locally advanced rectal cancer (T3-T4 and/or N+)

Management of locally advanced rectal cancer follows two pathways. The conventional pathway is immediate surgery followed by adjuvant chemoradiotherapy based on the histological features and lymph node involvement in the resected specimen. However this has largely been replaced by neoadjuvant radiotherapy with or without chemotherapy and has become the standard treatment for rectal cancers with threatened CRM. Neoadjuvant pelvic radiation is administered in two ways: short-course and long course radiotherapy. The differences in two approaches lie in the fractionation and timing of surgery post radiotherapy. In general, short-course radiotherapy delivers a total radiation dose of 25 Gy in five fractions followed by surgery 1 week later. Though, this approach reduces the risk for local recurrence but because of short time interval to surgery, does not cause significant tumour shrinkage and hence is only recommended resectable rectal cancers (ACPGBI guidelines, 2007). Long-course radiotherapy delivers a total radiation dose of 50.4 Gy in 28 fractions followed by surgery 4 to 8 weeks later (Minsky, 2012). Long course therapy is typically administered with concurrent 5-fluorouracil based chemotherapy (Fleming, Påhlman and Monson, 2011). The aims of this approach are to downstage the tumour and achieve a histologically free CRM that results in decreasing the local recurrence.

1.9.2.1 Neo-adjuvant vs. adjuvant CRT

The use of neo-adjuvant CRT is preferable to adjuvant CRT in the management of locally advanced rectal cancer. The evidence of superiority of CRT in the pre-operative setting comes from a landmark trial by the German Rectal Cancer Study Group neoadjuvant vs. adjuvant long course CRT (Sauer, et al., 2004). In this trial, 823 rectal cancer patients with T3-4M0 and/or node-positive stage were randomly assigned to either preoperative or post-operative CRT group. The results of the study showed significant improvement in 5 year local recurrence in preoperative group (6% vs.13% P=0.006). In a sub group of patients who were likely to undergo abdominoperineal resection on staging, higher sphincter preservation rate was achieved (39% vs.20% P=.004) in the preoperative CRT group. Ten year follow up shows persistent improvement in local control in pre-operative group but no effect on overall survival (Sauer, et al., 2012). Another randomized multicentre trial, MRC CRO7, favoured neo-adjuvant short course radiotherapy to selective adjuvant CRT. The relative risk of local recurrence was significantly reduced in the patients receiving short-course radiotherapy by 61% (HR 0.30, p<0.0001) (Sebag-Montefiore, et al., 2009).

1.9.2.2 Short-course vs. long course neo-adjuvant CRT

In the last decade or so, the search for the best and optimal neo-adjuvant CRT or radiotherapy regimen has resulted in various trials which evaluated the two approaches of short and long-course radiotherapy in the pre-operative setting. The former approach was evaluated in trials by the Scandinavian countries. The Swedish Rectal Cancer Trial in 1997 randomly assigned the rectal cancer patients with cT1-3 stage to either short course radiotherapy followed by surgery and surgery alone groups. TME was not mandatory as inclusion criteria for the trial. The 5 year local recurrence rate was found to be significantly lower (11%) in the combined radiotherapy and surgery group than surgery alone group (27%) (p< 0.001). The other important finding of the trial was the significant increase in the survival rate of up to 21% in the combined modality treatment group (p=.002). Folesson, et

al, (2005) reported on-going benefits in terms of survival and local control for the same trial after longer follow-up of 13 years in radiation group. Though this is the first and the only trail to show the improved overall survival rate with radiation therapy but this may be attributed to difference to in local recurrence between the two groups. Non standardization of TME surgery may account for higher local recurrence of 27% in surgery alone group. This issue was addressed by the Dutch trial (Kapiteijn, et al., 2001) that used the same design but surgery was standardized as TME for both groups. Significant improvement in local control had been reported in radiation group on both initial and long term follow up (van Gijn, et al., 2011) but without difference in overall survival between the two groups. However, short-course radiotherapy did not offset the disadvantage of an involved CRM in this trial. Exclusion of the patients with CRM involvement in the analysis, improved 10-year overall survival was seen in patients with stage III but negative CRM compared to patients in the surgery alone group (50% vs. 40%, p=0.032). Both the trials above had patient population with significant number of early stage rectal cancers.

The approach of long-course chemoradiotherapy evolved in parallel to the short course approach and is more popular in the North America and some European countries. Both the trials of short-course radiotherapy mentioned above had patient population with significant number of early stage rectal cancers. The landmark, German Rectal Cancer trial (Sauer, et al., 2004), described in the section 1.9.2.1 changed the management for $T_{3-4} \pm N_{1-2}$ stage rectal cancer patients to preoperative CRT with concurrent chemotherapy. A Polish rectal study group carried out the first small randomized trial (n=312) comparing preoperative short course radiotherapy with long course CRT in resectable stage III/IV rectal cancers (Bujko, et al., 2006). Rectal cancers in the long course chemoradiotherapy group were on average 19mm smaller (P=0.001), achieved significantly higher rates of completer pathological response (16% vs. 0.7% P < .001) and lower positive circumferential margins (4.4% vs. 12.9% P=.017). Despite these significant findings there was no difference in sphincter preservation rate, post-operative complications, local recurrence and 4-year overall survival

between the two groups. Another randomized multicentre trial of 326 patients with cT3N0-2M0, comparing the two approaches conducted by the Trans- Tasman Radiation Oncology Group (TROG) showed no difference in survival or late toxicity (Ngan, et al., 2012). Though the difference in cumulative local recurrence was 3% at 3-years in favour of long-course but not statistically different. Similarly despite a large observed difference of local recurrence favouring long-course in sub-set of 79 patients with distal cancers <5 cm from anal verge (12.5% vs. 3%), the results were not significant (Ngan, et al., 2012). However, the results of the trial were limited by small number of patients and relatively short follow-up. The results of the Dutch trial and the trials comparing the two approaches (Polish and TROG trials) indicate the advantage of long course CRT for more advanced rectal cancers where down staging of cancer is desirable to enable complete surgical resection.

1.9.3 The role of chemotherapy in CRT regimens

Evaluation of the trials above shows that long-course chemoradiotherapy confers a better local control advantage on the advanced rectal cancers. Another advantage of long-course chemoradiotherapy is that it can safely be combined with systemic chemotherapy. The rationale for adding a chemotherapeutic agent to preoperative CRT regimens is that it enhances the effect of radiotherapy by acting as radiosensitizers and induces tumour down staging. This enables surgeons to perform sphincter preserving surgery in distal rectal cancers and enhances rate of pathological complete response. In addition early incorporation of these agents might address concurrent systemic disease (Ceelen, 2012). The French randomized control trial (Gerard, et al., 2012) compared the results of preoperative long course radiotherapy with combined long course CRT. Adjuvant chemotherapy was given to patients in both arms. Patients in combined therapy group experienced significant lower 5-year local recurrence compared to those in radiotherapy alone group (8.1 vs. 16.5% P=.004). Multicentre European Organization for Research and Treatment of Cancer (EORTC) trial of similar design randomized patients to receive preoperative long course chemoradiotherapy or radiotherapy alone (Bosset, et al., 2006).

However this trial also randomized patients to receive adjuvant chemotherapy to demonstrate the impact of chemotherapy administration in preoperative, post-operative or both settings. The results indicated that patients who did not receive chemotherapy either pre or post operatively had worse local recurrence rates. There was no difference in local recurrence rates among the other groups. Both these trials showed significantly higher pathological complete response in combined modality group. However despite these findings, there was no difference in overall survival between the study groups. By taking into consideration of all the above evidences, it can be concluded that rectal cancer patients who are at higher risk of local recurrence such as threatened CRM are best treated with neoadjuvant long course CRT.

1.9.4 Radical surgery and total mesorectal excision

1.9.4.1 Total Mesorectal excision

Though complete clinical response following long course CRT has led to non-surgical management for locally advanced rectal cancers in selected cases but surgical resection is still a gold standard for curative treatment. The main aim of surgical treatment is complete eradication of primary tumour along with mesorectal fat. Until early 1980s, a 5-cm rule for proximal and distal longitudinal resection margins was followed to prevent anastomotic recurrences (Kosinski, et al., 2012). Later studies demonstrated that distal intramural spread usually is limited to within 2.0 cm of the tumour except for metastatic and poorly differentiated rectal cancers. In a study by Wolmark and Fisher (1986), comparison of distal resections margins of less than 2 cm, 2–2.9 cm, and greater than 3 cm showed no significant differences in survival or local recurrence. The technique of Total Mesorectal Excision (TME) described by Heald, et al. (1993) has dramatically improved locoregional tumour control and overall survival in rectal cancer surgery (Heald, et al., 1998). The hallmark of TME technique is to achieve CRM clearance to rectal tumour. There is a strong agreement that higher local recurrence rates, higher distant metastases rates and poorer survival are seen when the CRM is involved or less than 1 mm (Nagtegaal and Quirke,

2008). In their review of more than 17,500 patients, Nagtegaal and Quirke demonstrated that following neoadjuvant therapy (both radiotherapy and radiochemotherapy) the predictive value of the CRM for local recurrence is significantly higher than when no preoperative therapy has been applied. The role of MRI in accurately predicting the CRM involvement and selecting patients for CRT is already discussed in section 1.8.1. Retrospective study by Hida, et al., (1997) of lymph node metastases detected in the mesorectum distal to carcinoma of the rectum suggested that the longest distal spread from the primary tumour to the metastatic node was 2 cm in carcinoma of the recto sigmoid, 4 cm in carcinoma of the upper rectum, and 3 cm in carcinoma of the lower rectum. Therefore, in proximal rectal cancer, mesorectal excision 5 cm below the lower border of the tumour is suffice to avoid low anastomosis resulting in poorer bowel functions for the patients.

1.9.4.2 Sphincter-sparing and non-sphincter sparing radical resections

There are two types of radical resections; sphincter-sparing (anterior resection) and non–sphincter-sparing (Abdominoperineal) resections. Sphincter-Preserving radical resections include anterior resection, low anterior resection and ultra-low anterior resection. The radical resection that preserves a portion of rectum with colorectal anastomosis is called anterior resection (Kosinski, et al., 2012). Low anterior resection is a colorectal resection with anastomosis of colon to the rectum below the peritoneal reflection (Lowry, et al., 2001). For more distal ultra-low anterior resection approach is used where entire rectum is removed with coloanal anastomosis. Intersphincteric resection with coloanal anastomosis represents the extreme form of sphincter sparing surgery in which part, or all, of the internal sphincter is resected. This approach is used for the rectal cancers that are confined to the rectal wall and within 2 cm of the sphincter complex (Mulsow and Winter, 2011). TME must be excised for up to 5cm distal to the lower edge of the tumour for the upper rectal cancers and for mid-low rectal cancer it should be excised up to the level of pelvic floor

Non-sphincter-sparing procedure includes Abdominoperineal resection (APR) that results in a permanent end colostomy. APR involves complete removal of distal colon, rectum, and anal sphincter complex using both abdominal and perineal approaches (Perry and Connaughton, 2007). Rectal tumours which are 2-3 cm from anal verge and fixed to levators and sphincter are best dealt with APR especially in experienced hands. In upper (10-15cm) and middle (5-10cm) rectal cancers TME has considerably reduced the local recurrence and survival but similar trend is not observed for low rectal cancers (0-5cm from anal verge) (Kapitijen, et al., 2001). In a prospective randomized trial, the Dutch Colorectal Cancer Group compared low anterior resection and APR for low rectal cancer performed by the surgeons who had been trained in TME. APR patients had a survival of 38.5%, compared with 57.6% for those undergoing sphincter sparing surgery ($p = 0.008$). Positive circumferential margins and tumour perforations were also significantly more common in APR patients (Nagtegaal, et al., 2005). The poorer results of APR were attributed to the plane of resection that followed the thinning mesorectum at the level of levators and anal sphincters. This surgical technique based on principles of TME results in surgical dissection along the tapering mesorectum through the sphincter muscle or rectal tube leading to higher rate of CRM involvement and higher perforation rate. The resected specimen with this conventional technique tapers (Morson's waist) at the level of levators that increases the risk of CRM involvement. An alternative approach described by Holm, et al. (2006) for locally advanced rectal cancers is characterized by dissection in extralevator plane that avoids "waisting" of the specimen. The resulting specimen is cylindrical and risk of CRM involvement is reduced.

1.9.4.3 Laparoscopic vs. open surgery

Laparoscopic approach in colonic cancer has proven to have both short and long term advantages in randomized trials. Short term benefits include reduced incidence of post-operative pain, pulmonary complications and surgical morbidity (Schwenk, et al., 2005). In addition, this approach is oncologically safe and enjoys similar long-term outcomes as for

the open approach. The 5-year follow-up of the patients in The UK Medical Research Council CLASSIC trial that randomized conventional and laparoscopic assisted surgery in colorectal cancer found no difference for overall survival, disease free survival and local or distant recurrences between the two groups ((Jayne, et al., 2007). Similarly, the COLOR trial, that only included patients with colonic cancer excluding rectal cancer found no difference in 3-year disease free survival between the open and laparoscopic colectomy groups (Bonjer, 2009).

Laparoscopic approach in rectal cancer is technically more demanding than that for colonic cancer because of the narrow and confined operative field within the pelvis. In the English CLASSIC trial, concerns were raised on this approach for the patients with low rectal who underwent laparoscopic low anterior resection and were found to have higher rates of positive CRM (12%) compared with open low anterior resection (6%) but this did not translate into higher local failure (Guillou, et al., 2005). In order to address the concerns for short and long-term oncological safety of laparoscopic approach specifically for rectal cancer, a randomized phase three COLOR II trial was carried out between 2004 and 2010 and randomized 1103 patients into laparoscopic and open surgery groups. There was no difference in terms of clinical short term outcomes such as involvement of resection margin, perioperative morbidity and completeness of mesorectum (van Der Pas, et al., 2013). The same group reported long-term outcomes for these patients recently and found no difference in 3-year locoregional recurrence (5% in each group), 3-year OS (87% vs. 84%) and DFS (75% vs. 71%) between the laparoscopic and open groups respectively (Bonjer, et al., 2015).

The COLOR II trail did not include rectal cancer patients with clinical stages, T4 and T3 within 2 mm of CRM and laparoscopic resection in such locally advanced rectal cancer patients becomes more challenging especially after neo-adjuvant CRT because of tissue oedema and fibrosis (Motson, et al. 2011). The COREAN trial addressed the feasibility of

laparoscopic approach in cT3N0-2 mid and low rectal cancer patients who had received neoadjuvant long course CRT by randomizing them to open or laparoscopic surgery (n-340 patients). There was no difference between the two groups for both the short-term outcomes in terms of CRM involvement, quality of TME excision specimen, number of harvested lymph nodes and peri-operative morbidity (Kang, et al., 2010) and long- term outcomes in terms of local recurrence and 3-year DFS (Jeong, et al., 2014). The results of these studies indicate that oncological outcomes in laparoscopic rectal surgery are comparable to those of conventional surgery. However the evidence based on these studies is not applicable to all patients with rectal cancer especially patients with threatened CRM and T4 stage.

1.9.5 Histological response to neo-adjuvant CRT

As I have discussed in the above section 1.9.2.2 that the treatment of locally advanced rectal cancer has evolved into multimodality treatment in the form of neoadjuvant CRT followed by TME. The main objectives of long-course CRT are to down stage tumours, enable R0 resection (completely excised tumour without any microscopic [R1] or macroscopic [R2] involvement of margins, see section 3.1.4.2) and improve locoregional control and survival outcomes. Down staging of tumour on histological assessment has shown to be independent prognostic factor in predicting long term survival in rectal cancer patients after long course CRT (Dhadda, et al., 2011). In addition to substantial downsizing, up to 25% of patients show complete pathological response characterized by absence of viable tumour cells in the resected specimen (Smith, et al., 2010 and Garcia-Aquilar et al., 2011). Results of the pool analysis of neoadjuvant CRT trials by Mass, et al. (2010) showed that 5 year disease free survival was significantly higher for patients with pathological complete response than for the patients without the response (83.3% vs. 65.6% p <0.0001).The effect of pathological complete response on long-term outcome was not affected or modified by clinical T or N category, administration of adjuvant chemotherapy, distance from anal verge, or type of surgery.

1.10 Optimal time interval between long course CRT and Surgery

The combined modality treatment in locally advanced rectal cancer has considerably decreased the local recurrence as evident from the results of pivotal German trial discussed in section 1.9.2.1 (Sauer, et al., 2005). However, this approach does not translate into improvement in overall survival in these patients. In addition, pathological complete response has emerged as new and valid surrogate marker of DFS (Mass, et al. 2010). Various treatment strategies can achieve higher percentages of complete pathological response. Continuous infusion of concurrent chemotherapy compared to boluses and increased radiation doses can achieve significantly higher pathological complete response in approximately 50% of cases (Mohiuddin, et al., 2000). Currently there is no consensus on the optimal timing of surgery after finishing long course CRT. Another strategy is to delay the interval between CRT and surgery to allow more time for the tumour to shrink and thus increasing the possibility of pathological down staging. In the large randomized trials discussed in the section 1.9.2.1 (Sauer, et al., 2004) and the section 1.9.3 (Bosset, et al., 2006 and Gerard, et al., 2006), the median time interval to surgery was 5-6 weeks. Randomized data on the time interval between the completion of chemoradiotherapy and surgery is very limited. Lyons R90-01 is the only trail that randomized patients to have surgery at 2 week and 6-8 week time intervals after long course CRT. Patients in longer interval group experienced significant greater down staging as compared to the patients who had surgery within two weeks (26% vs. 10% (Francois, et al. (1999). As a result of this trial, delaying the surgery up to 6 weeks after radiotherapy completion was established as the standard of care.

However the retrospective analyses of case-series and analysis of non-randomized trials later showed the relevance of the time interval to increasing clinical and pathological down staging of rectal tumour. Further delaying the surgery beyond conventional 4 to 6 week window can lead to greater tumour response to long course chemoradiotherapy in a time

dependent fashion. In a retrospective pilot study by Johnston, et al. (2009) at the Colchester University Hospital, an on-going radiological tumour response for up to 12 weeks after completion of long course CRT was demonstrated when assessed by performing serial MRIs. There was significant decrease in T-stage from 6% on MRI performed at 6 week interval to 41.2% on the MRI performed just before surgery (P <0.001). Similarly, Moore, et al. (2004) observed a non-significant trend towards increased pathological complete response in their retrospective analyses of 157 rectal cancer patients treated with long course CRT. The rate of pathological complete response increased from 9% in the interval of 30-40 days, to 16% in 41-49 days and to 23% in >49 days. A retrospective cohort study showed significant increase in complete pathological response for the patients operated beyond 7 weeks after long course CRT than for the patients operated earlier than 7 weeks (35% vs. 17% P=0.03) (Tulchinsky, et al., 2008). In a prospective nonrandomized study by Garcia-Aguilar, et al. (2011), 25.4% of patients demonstrated complete pathological response at 11 week as compared to 18% at 6 week time interval (P<0.05). A recent meta-analysis of 13 studies (n-3584 patients) that mainly included retrospective or prospective case series and one non-randomized phase II trial, demonstrated that an interval beyond classical 6-8 weeks to surgery after finishing CRT, results in significantly improved pathological complete response from 14% to 20% (RR=1.42 CI: 1.19 -1.68, p<0.0001) (Petrelli, et al., 2016).

As it is evident from the discussion above that with the delay of surgical interval after CRT, there is a corresponding increase in tumour regression but there is little evidence about its short-term effect on surgical morbidity and long-term oncological outcomes. Conventional time interval of 4-6 weeks is optimal enough to allow for the resolution acute radiation reaction before surgery. By prolonging the time interval to surgery beyond this conventional time period can theoretically make TME technically more difficult because of radiation induced pelvic fibrosis. This might result in increase in surgical complications. In the meta-analysis (Petrelli, et al., 2016), the data on post-operative complications was available for 7

studies out of 13 in total. No difference was observed in the R0 resection, sphincter-preservation, wound and anastomotic complications for the patients operated beyond 8 weeks. None of the studies included in the meta-analysis individually reported any significant influence of time interval on the post-operative complications. Instead, longer time interval post CRT could decrease perioperative morbidity as reported by Kerr, Norton and Glynne-Jones (2008) in their retrospective analysis of 189 patients. This study demonstrated that shorter interval by 1 week (median interval 10 weeks) independently predicted anastomotic leakage (OR 0·97, 95% CI 0·94 to 1·00) and perineal wound complications (OR 0.97, 95% CI 0.95 to 0.99).

As for surgical morbidity, the meta-analysis (Petrelli, et al., 2016) did not demonstrate any difference in long-term OS and DFS rates between the shorter and longer interval (beyond 8 weeks) groups (6- studies, n-1360 patients). None of the studies comparing shorter and longer time interval to surgery after CRT has demonstrated any significant positive influence on overall survival despite achieving increasing rate of pathological complete response in the longer interval group. Long-term results of the Lyons R90-01 trial showed no significant difference in overall survival rate between the short-interval and long-interval groups (69% vs. 66% P=0.880) (Glehen, et al., 2003). Conversely, in the retrospective analysis of 102 patients treated with neo-adjuvant radiotherapy alone, the time interval to surgery from diagnosis longer than 16 weeks was significantly associated with decrease in OS and metastasis free survival (OR 2.05, P=0.05) (Supitot, et al., 2006). This highlights the detrimental effect on patient survival if surgery is delayed beyond 12 weeks as there is a potential risk for the subclinical tumour in situ to grow and spread in that time interval. Conversely, the retrospective study by Habr-gama, et al., (2008) showed the deferral of surgery for up to 12 weeks and beyond was safe. There was no difference in disease free survival overall survival between the patients operated before or after 12 weeks.

1.11 Conclusion of Literature Review of the first chapter and research question for the first clinical study of the thesis

The optimal management of locally advanced rectal cancer has evolved into neoadjuvant long course CRT followed by TME. Laparoscopic surgery has also evolved in parallel to this paradigm shift in the management of locally advanced rectal cancer. Preoperative long course CRT improves local control and results in down-staging. Delay in surgery beyond 8 weeks after CRT results in more pronounced tumour regression and pathological complete response. However there is no consensus on optimal time interval to surgery after long course CRT. It varies between < 2 weeks in a randomized study to 8-12 weeks in non-randomized studies. Delaying the surgery beyond the conventional 6-8 week window does not lead to increase in sphincter preservation, surgical complications or local recurrence. It is also not detrimental to overall survival as shown in the meta-analysis (Petrelli, et al., 2016) but individual retrospective studies shows conflicting evidence. In addition, the effect of delaying surgery beyond the 12 week interval on surgical morbidity and oncological outcomes is very limited. Though the laparoscopic surgery is shown to be feasible in stage III rectal cancer in the two randomized trials, COLOR II (van Der Pas, et al., 2013) and COREAN (Keng, et al., 2010) but the evidence based on these studies is not applicable to all patients with rectal cancer especially patients with threatened CRM and T4 stage. One of the aims of this thesis is to address these questions. With this background knowledge and a potential gap in knowledge, the objective of the first study in the thesis is:

- To determine the both short and long-term effects of delayed TME beyond 12 weeks in laparoscopic setting after long course CRT in locally advanced rectal cancer including patients with threatened CRM and T4 stage.

2 Role of MRI and functional imaging (MRI, PET or PET/MRI) in rectal cancer

The role of MRI in staging and hence selecting patients with locally advanced rectal cancer with threatened CRM for neoadjuvant CRT to attain down-staging and hence resectibility is already discussed in section 1.8.1. The eligibility criteria for the inclusion of rectal cancer patients in the first clinical study (Chapter 4) were based on high risk MRI features as discussed in section 3.1.1. The 2nd study in the thesis (Chapter 5) investigated whether MR based textural features of locally advanced rectal cancers could predict survival outcomes. In the first section of this chapter, the basic principles of morphological MRI, concept of textural analysis and its current use in various oncological applications were discussed and this forms the introduction of for the chapter 5. In the second section of this chapter, quantification of imaging biomarkers on functional MRI techniques along with PET markers is discussed. The second section forms the basis for the introduction and literature review of the 3rd clinical study in the thesis (Chapter 6).

2.1 Magnetic Resonance Imaging

2.1.1 Basic principles of MRI

The hardware of MRI system can be categorized into three parts; main magnet, gradient system and radiofrequency coils. The main magnet produces a strong, constant and static magnetic field (Moser, et al., 2009). A typical clinical scanner has a magnetic field of 1.5 Tesla which is thirty thousand times stronger than the earth's magnetic field (Liney, 2006). The role of gradient system is in spatial localization of MR signal which is described later in the section 2.1.1.4. Electromagnetic pulse is generated by a transmitter coil which surrounds the whole or a part of the body. A body coil is usually built into the construction of the magnet (Liney, 2009). Coils can be classified as volume coil (area to be examined inside

the coil), surface coils (applied over limited region of interest) or phased-array coils (multiple surface coils in conjunction with each other) (Welker, et al., 2001).

The basic concept behind MRI is the spinning nucleus around its own axis which produces a tiny magnetic field (Liney, 2006). To produce an image MRI makes use of body's natural magnetic properties. It uses the hydrogen atom which is the simplest and most abundantly found in the human body. The nucleus of the hydrogen atom contains single proton that spins around its axis and behaves like a small bar magnet. In the normal circumstances, these millions of spins generate no net magnetic field because of random orientation of their axes. However, when these spins are exposed to a strong magnetic field (β0) such as that of MRI scanner as illustrated in Figure 2-1; these spins align themselves longitudinally and thus produce longitudinal magnetization (MHz) in parallel to the direction of the magnetic field. This alignment is not static and in addition to spinning on their own axes, the nuclei rotate around the direction of the magnetic field. This process is called precession which occurs at a characteristic speed called Larmor frequency which is the hall mark of MRI imaging (Weishaupt, 2006). The Larmor frequency is directly proportional to the strength of magnetic field and is given by Larmor equation: $\omega o = \beta o * \gamma$. Where ωo is the Larmor frequency in megahertz (MHz), βo is the strength of the magnetic field in Telsa (T) and γ is the gyromagnetic ratio, a constant fixed to a specific nucleus.

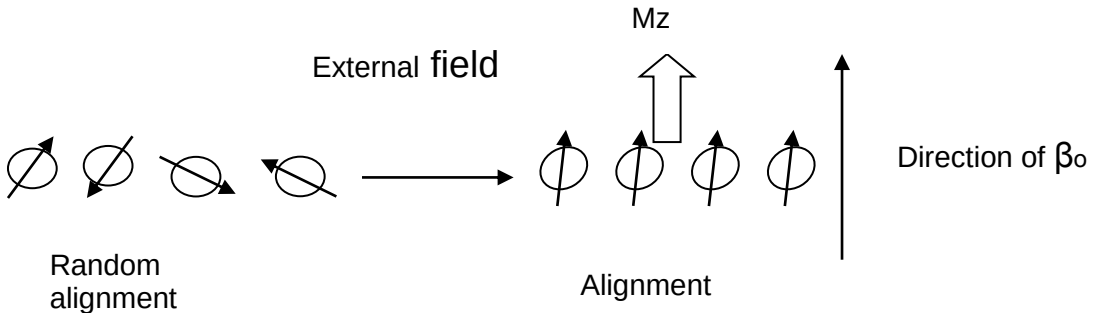

Figure 2-1 In the presence of magnetic field, randomly aligned protons align themselves in the direction of magnetic field and produce net magnetization vector Mz.

2.1.1.1 Excitation of Spin System

Energy is introduced into this stable spin system in the form of radiofrequency pulse at a frequency similar to that of Larmor frequency and at 90 degrees to the direction of βo. This causes resonation of the hydrogen nuclei and they precess in phase with each other. This phenomenon is called phase coherence. This causes the energy absorption by protons and excitation of spin system which results in tilting of longitudinal magnetic vector MHz through 90 degrees towards the transverse plane perpendicular to the direction of βo and thus produces transverse magnetization Mxy (Figure 2-2) (Weishaupt, 2006). The precession of transverse magnetization vector induces a current in a receiver coil that gives rise to MR signals. Receiver coils are located in the transverse plane and are used around the body part in question to act as aerials for the detection of the emitted signal. The intensity of the received signal is then plotted on a grey scale and cross sectional images are built up (Berger, 2002).

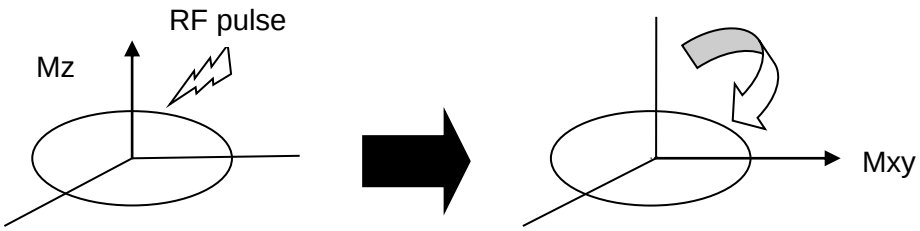

Figure 2-2 Application of a 90 degree RF pulse tilts longitudinal net magnetization Mz in xy plane

2.1.1.2 T1 and T2 relaxation times

The signal intensity and hence contrast image of MRI depends both upon intrinsic properties of biological tissues and extrinsic parameters controlled by system operator. Proton density and relaxation time of protons after excitation are intrinsic features of biological tissues and contribute to the image contrast in MRI (Sharma, 2009). When the RF pulse is switched off , the MR signals rapidly decreases in intensity due to two independent relaxations processes that results in decrease in transverse magnetization. (Berger, 2002). The first is called T1

relaxation that results when protons move from high energy stat to low energy state by emitting their energy into the surrounding tissues. Hence T1 relaxation is also called spin lattice relaxation. This results in decay of transverse magnetization and realignment of protons along the βo. The second is called T2 relaxation that results in the decay of transverse magnetization because of dephasing of precessing protons. There is an overall cumulative loss of phase coherence due to exchange of energy in between neighbouring spins intrinsic magnetic fields (spin-spin interaction).These two relaxations are independent of each other. T2 relaxation occurs within the first 100-300 msec. While complete decay of transverse magnetization due to T1 relaxation occurs within (0.5–5 sec) (Weishaupt, 2006). T1 increases with an increase in the magnetic field strength (B0) and T2 remains relatively unchanged with increasing magnetic field strength (B0) (Sharma 2009).

2.1.1.3 Repetition Time and Echo Time

These relaxation times can be altered by extrinsic system operator mechanisms such as repetition time (TR) and echo time (TE). TR is the time interval between the application of first RF pulse and the beginning of the successive pulse. It basically affects the length of relaxation period between excitation pulses and effects T1 relaxation time. By selecting a short TR, tissues with short T1 would regain their longitudinal magnetization during that TR and thus would emit a large signal and hence would appear bright on the image. On the other hand tissues with long T1 would have achieved less longitudinal magnetization and appear dark on the image. So a short TR would give strong T1 weighted image. Echo time affects the T2 relaxation and is the interval between the application of excitation pulse and the collection of MR signal. (How does MRI Work). Since T2 is much shorter than T1 relaxation time, so a longer echo time is selected so that tissues with Longer T2 would produce higher signal intensities and thus appear bright on T2 weighted images. Tissues with a short T2 would appear dark because of their signal decay at the time of echo collection. By selecting a short TE (avoids T2 weighting) and a longer TR (nullifies T1 effect),

a contrast based on differences in proton densities of the tissues is produced (Weishaupt, 2006 and Westbrook, 2010).

2.1.1.4 Gradient Coils

In order to achieve the selective excitation of the slice to be imaged and localization of the site of origin of MR signals, the magnetic field is made inhomogeneous by use of three orthogonal sets of gradient coils built in to the bore of the magnet along X, Y and Z axis (Figure 2-3) (McRobbie, 2007). Larmor frequency and precessional phase of protons thus alters in a linear fashion along the gradient and each slice now has its unique frequency (Weishaupt, 2006). This forms the basis for spatial encoding i.e. the localization of spatial position of a signal in three dimensions. Depending on the plane of the image, each gradient performs one of three functions that are essential for spatial encoding. These functions are slice selection in a desired image plane, frequency encoding (spatial identification of signal along the long axis of image) and phase encoding (spatial identification of a signal along the short axis) (Westbrook, 2010). This three dimensional information about the image signal is stored as a raw data in MRI imaging system in the form of matrix voxels called k-Space. The k-space data is further processed through Fourier transformation to form an MRI image (Bitar, et al., 2006).

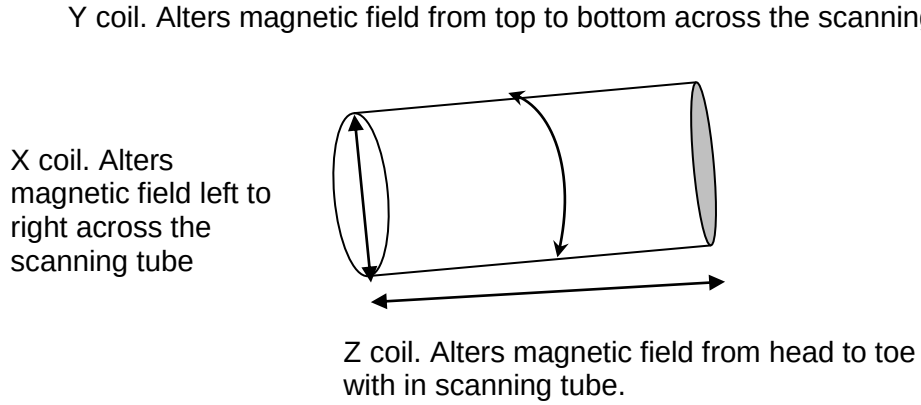

Figure 2-3 X, Y & Z gradient axis

2.1.1.5 Pulse sequences Mechanisms

MRI pulse sequence is a set of radio frequency and gradient pulses of fixed duration and separation applied to acquire T1 and T2 weighted sequences (Liney, 2010). Two basic but important MRI pulse sequences relevant to staging of rectal cancer are spin echo (SE) and inversion recovery (IR) sequences. In order to understand the mechanism of these sequences, it is important to know another form of relaxation time termed as T2*.In addition to dephasing due to pure spin-spin energy transfer, proton spins lose phase coherence due to inhomgeneities of external magnetic field generator itself, differences in magnetic properties of neighbouring tissues or tissue-air interface. This type of decay in MRI signal is called free induction delay and is designated as T2*. Unlike T2 relaxation, T2* is time independent and typically shorter than T2 (Westbrook, 2010).

2.1.1.5.1 Spin Echo Sequence (SE)

The loss of MR signal due to T2* can be avoided by using spine echo sequence. In this sequence, 90° excitation pulse is applied to the slide selected in the first instance. After its application, protons start losing phase coherence due to static magnetic field inhomgeneities (T2*). So half way through the echo time, a second 180° excitation pulse is delivered to rephrase the spins. The regenerated signal is measured at total echo time (Weishaupt, 2006). The spin echo sequences produce good quality image and is considered as gold standard for image contrast and weighting. Standard spin echo sequences take long scan times and are prone to motion artefacts. Due to advancement in MRI technology, fast or turbo spine echo (FSE/TSE) is used where multiple 180 degree rephrasing pulses are applied per repetition time allowing multiple phase encoding per TR (Westbrook, 2010).

2.1.1.5.2 Inversion Recovery Sequence (IR)

Inversion recovery sequence is essentially a standard SE sequence preceded by an additional 180° inverting pulse that flips the longitudinal vector in opposite direction to that of βo. As this negative longitudinal vector begins to relax towards βo, the 90° excitation pulse

of SE is applied within the time interval T1 (inversion time) after the inversion pulse. A final 180° rephasing pulse similar to SE sequence is applied to produce echo signals. The main determinant of contrast in this sequence is T1 inversion time. Two widely used inversion recovery sequences in clinical practice are Short T1 inversion recovery (STIR) and fluid-attenuated inversion recovery (FLAIR). STIR is used to suppress fat signals by delivering a 90° excitation pulse at T1 inversion time when net magnetization vector of fat is just passing through the traverse plane called the null point for the fat. Since at this point fat has got no longitudinal magnetization so 90° excitation pulse induces no transverse magnetization and thus no signals from the fat tissue. The duration of T1 for fat suppression is usually between 100-180 ms based on field strength .FLAIR sequence is mainly used in brain MRI where using long T1 suppresses signals from cerebrospinal fluid to differentiate brain tumours, oedema and fat (Weishaupt, 2006 and Westbrook, 2010).

2.1.2 Application of MRI in restaging of rectal Cancer

The initial clinical staging of a rectal cancer is determined by the combination of endoscopic, clinical and radiological findings. As already discussed in section 1.8.1 that how MRI evolved over the years since its introduction and has become the imaging modality of choice for the initial staging and assessment of proximity of the tumour to the CRM, with accuracy rates up to 94% (Brown et al, 2003). Restaging of rectal cancer after CRT is important as it gives valuable information about the tumour response to CRT. Down staging of tumour on histological assessment has shown to be independent prognostic factor in predicting long term survival in rectal cancer patients after long course CRT (Dhadda, et al., 2011). Restaging of rectal cancer can change the management strategy for the locally advanced rectal cancers. Restaging helps to identify patients who achieve complete clinical response characterized by absence of tumour on physical examination, biopsy and radiological assessment. A study by Habr-Gama et al. (1998) has reported a cCR of up to 30% to CRT in distal rectal cancer patients. Surgery with its associated morbidity and mortality can be avoided in such patients with excellent long term overall and disease free survival results

(Habr-Gama et al 2004). Down staging enables surgeons to perform sphincter sparing operations for distal cancer once considered standard of care for such tumours.

However accuracy of MRI in restaging locally advanced cancer after long course CRT has been questioned. The factors responsible for low accuracy include fibrosis, desmoplastic reaction, oedema, inflammation, and viable tumour nets at a fibrotic scar from a previous tumour (Kim, et al., 2010). The low accuracy of post treatment MRI is related to both over-staging and under-staging of tumour. The mercury study group (2006) reported that accuracy of MRI in predicting clear circumferential margin decreases from 91% (284/311, 95% CI: 88% to 94%) in patients who underwent primary surgery to 77% (75/97, 95% CI: 69% to 86%) in patients with neoadjuvant CRT or long course radiotherapy. In their retrospective study, Allen, et al. (2007) reported overall accuracy of 60% (18/30, 95% CI: 42 t0 78) for T stage and 70% (21/30, 95% CI: 54 to 86) for N stage on T2-weighted MRI post CRT when compared with findings on histological assessment of resected specimen. The N stage accuracy increased to 87% when nonmucinuous adenocarcinomas were separately assessed. Similar study by Chen, et al. (2005) reported overall accuracy of T stage as 52% (24/50) and for N stage as 68% (16/50). Both these studies showed that most of the inaccurate T staging occurs as result of over staging of superficial tumours T0-T2 and T3<5mm. The overstaging is due to the limited capability of MR imaging to allow differentiation between viable tumour, residual fibrotic nontumor tissue, and desmoplastic reaction (Kim, et al., 2010). Another study by Suppiah, et al. (2009) showed even poorer accuracy of 45% for T staging. The accuracy for N staging was consistent with other studies i.e. 71%. In all these studies, patients had pre-treatment MRI and post treatment MRI followed by surgery. However the results of these studies may be flawed as speculated by Arulampalam, et al, (2010). In the study by Suppiah, et al. (2009), the post treatment MRI was performed after median time interval of 4½ weeks but definitive surgery was actually carried out 8 weeks after completion of CRT. In the study by Allen, et al. (2007) the median time interval of performing post treatment scan was 38 days since CRT completion date and

surgery was delayed for further 43 days since the treatment scan. Arulampalam, et al, (2010) argue that the gap between the post treatment MRI and surgery could undermine the results of these studies. The study by Johnston, et al. (2009) reported an on-going clinical and symptomatic response in the gap between post treatment MRI and the surgery. While reviewing the data for locally advanced rectal cancer patients in their institution they observed that a subset of 17 patients had undergone MRI a third time. Each of these 17 patients had three high resolution MRI scans; MRI 1 before treatment, MRI 2 6-7 Weeks after CRT and MRI 3 immediately before definitive surgery. MRI 3 was performed 15–37 (mean 29) days after MRI 2, 3–21 (mean 10) days before surgery. This studied showed that there was significant decrease in T staging of the cancer from 6% on MRI 2 to 41.2 % on the MRI 3. There was high correlation between the T -stage on the third MRI scan and the pathological T-stage (82% 14/17). The second MRI overstaged 7 out of the 17 cases, giving an agreement between this MRI and the subsequent pathology of 58.8%. Three out of the 17 patients showed complete response on histopathology. This had been correctly classified in two cases on MRI 3, with MRI 3 overstaging the third case as T1N0. Interestingly, staging on MRI for these three cases had been reported as T3a for two and T3b for one. There was no further response on N staging on the third MRI and the correlation between the scan and pathological assessment was 88%. The author attributes these high correlation findings between the third MRI and histology, to the resolution of tissue oedema and easier interpretation of fibrosis with the increasing time frame post CRT, together with the on-going response to treatment. Though this study is limited by a small number of patients but their findings in the study were significant in terms of their magnitude. In the first study of thesis, serial MRIs after CRT were used to optimize the timing of surgery after long course CRT in locally advanced rectal cancer. Because of the time dependent response post CRT, some of these patients who achieved complete clinical and radiological response could be considered for weight and watch approach or could undergo minimally invasive surgery such as TEMS. The first study in the thesis will also try to establish the proportion of such patients in the entire cohort.

2.1.3 MRI as an imaging biomarker

Though the current combined modality management of locally advanced rectal cancer combined with use of high resolution MRI has shown considerable improvement in loco regional control. However, this is not the case for systemic control and these strategies may not necessarily improve overall survival (Lange, et al., 2013). Furthermore, restaging of irradiated rectal tumours is challenging because of difficulty of morphological MRI in differentiating fibrosis from viable residual tumour van der Paardt, et al., 2013). In addition, a proportion of such patients would achieve complete clinical response and could benefit from either wait and watch approach or less invasive local excision surgery (Habr-Gama, et al., 2004). But there is a poor correlation of clinical complete response with true pathologic complete response (Zmora, et al., 2004). This shifting paradigm has placed greater recent interest in quantification of imaging biomarkers associated with adverse outcomes.

Quantification of imaging biomarker on MRI can be divided into those based on conventional imaging and those based on functional imaging techniques. In this section, only the imaging biomarkers linked to conventional or morphological MRI are discussed and described. The quantification of imaging biomarkers on functional MRI techniques along with PET markers is discussed in section 2.2 . The role of conventional MRI in TNM staging of rectal cancer is already discussed in detail in the section 1.8.1. In addition, high accuracy of MRI in predicting threatened CRM ((Brown et al., 2003) and hence selection of these patients for neoadjuvant CRT before surgery has become a gold standard in rectal cancer staging (Mercury study group, 2006). Subsequent studies and planned subgroup analysis of high quality imaging, histopathological and surgical data by the Mercury study group validated additional imaging biomarkers correlating with prognostic outcomes. Extra mural venous invasion (EMVI) defined as the presence of cancer cells in the vessels beyond the muscularis propria is another manifestation of locally advanced rectal cancer and is an established marker of poor prognosis in rectal cancer (Freedman, Macaskill and Smith,

1984). Traditionally, it has been assessed on histological analysis of resected specimens. Pre-treatment high resolution MRI can detect EMVI and has a potential of prognostic imaging biomarker. The study by Brown et al. (2003) demonstrated that pre-operative MRI correctly predicted EMVI in 15 of 18 patients who had positive EMVI on histopathological analysis (κ=0.68). In a separate study by the same group (Smith, et al., 2008), the sensitivity (probability of MRI to detect EMVI among those with positive EMVI on histology) and specificity (fraction of patients with negative EMVI status on MRI who would have negative EMVI on histology) of MRI-predicted EMVI in 94 rectal and sigmoid cancer patients were 62% and 88% respectively on comparison with histological analysis. Relapse free survival (RFS) at 3 years for the patients with positive EMVI status was 35% compared to 74% for the patients with negative EMVI status ($p< 0.001$).

One of the advantages of long course CRT over short course is that it induces tumour regression. There are number of pathological tumour regression grading (pTRG) criteria validated in rectal cancer and are mainly based on the qualitative assessment of proportion of fibrosis relative to remaining cancer tissue (Thies and Langer, 2013). Pathological TRG has been demonstrated to be an independent predictor of 5-year DFS in rectal cancer patients treated with CRT (Vecchio, et al., 2005). In the study by Rödel, et al. (2005), prognostic assessment of histological tumour regression grading in a cohort of 385 rectal cancer patients treated with long course CRT was carried out. It was found that 5-year DFS in patients with complete regression, intermediate regression and no regression was 86%, 75% and 63% respectively (p=.006). On multivariate analysis, the histopathological T and N stages were the independent prognostic factors for DFS. The Mercury study group (2006) applied the same principles of pTRG to MRI assessment of TRG (mrTRG) to determine the degree of tumour replacement by fibrosis characterized by low signal intensity (Patel, et al., 2011). This study showed that mrTRG independently predicted OS (HR 4.4, 95% CI: 1.65 to 11.7, p=.003) and DFS (HR 3.28, 95% CI: 1.22 to 8.80, p=.019) on multivariate analysis.

2.1.4 Textural Analysis

Besides quantification of MRI biomarkers such as CRM, EMVI and TRG, there has been a greater recent interest in quantification of imaging biomarkers linked to underlying intra-tumour heterogeneity associated with adverse outcomes in terms of treatment failure and drug resistance (Ganeshan, et al., 2013). Heterogeneity can be quantified on imaging non-invasively using textural-analysis (TA). The interpretation of radiological images is based on the naked eye examination. Yet there are features in the images that can yield a greater degree of information by analysing its textural properties. TA assesses the distribution of pixel grey-level intensity, coarseness and regularity in digital images (Castellano, et al., 2004).

There are four steps involved in analysis of medical images for computer aided diagnosis described by Sharma, et al. (2008). 1) Image filtering, 2) segmentation, 3) feature extraction, and 4) analysis of extracted features. The main aim of image filtration is to suppress unwanted photon noise and to enhance image features important from further analysis point of view. Application of band pass filters in statistical textural analysis is used to highlight different spatial scales. One such filter is Laplacian of Gaussian band pass filter. Different filter values or width will enhance specific structures corresponding to that filter value, while structures less that this filter value will become blurred (Ganeshan, et al., 2009). Lower filter values (filter 0.5-1.0) will highlight structures with fine textures, and higher filter values highlight structures with medium (filter 1.5-2.0) and coarse (filter 2.5) textures in the filtered image (Davnall, et al., 2012).

Segmentation is the process that divides the image into various regions of similar properties based on their texture, grey-level, colour or contrast. Digital images used in clinical practice are usually stored in the computer as a two dimensional array and is made up of mutually related small picture elements called pixels. Each pixel has a value that represents the grey-level intensity of that picture. According to Haralick (1979), pixels grey-level intensities and

their spatial relationship gives image a fine, smooth, coarse or grainy texture. Tuceryan and Jain (1998) defined image texture as a function of the spatial variation in grey-level pixel intensities. Texture analysis, thus is the evaluation of the position and intensity of pixels, in digital images (Castellano, et al., 2004). Texture features produced as a result of the analysis are in fact the complex mathematical parameters computed from the distribution of pixels. These features thus represent the underlying texture type.

Feature extractions and analysis relates to patterns recognition and their quantifications. There are different techniques for textural analysis that can be categorized into four main types: structural, model based, statistical and transform methods (Tuceryan and Jain, 1998). The description of each of these techniques is beyond the scope of my thesis. More commonly used methods in analysis of medical imaging are statistical and transform methods and will be mainly discussed here. Statistical methodology is the most widely used and it measures the distribution and relationships of grey-level values in the image. Texture parameters derived from these methods are ranked into first, second and higher order parameters. First and second order parameters are more commonly used in medical image. First order statistical parameters include histogram of an image and its variance and are dependent on the individual grey-level value of a pixel without taking into the consideration of spatial interaction between the pixel values. Parameters derived on the basis of histogram analysis, include mean, standard deviation, uniformity (in-homogeneity) entropy (irregularity of intensity distribution), skewness (asymmetry of the histogram), and kurtosis (flatness of the histogram) (Davnall, et al., 2012). First order statistics are not suitable if image has got more than one texture or non-random spatial distribution of pixels (Prats-Montalban, De Juan and Ferrer, 2011). Second order statistical parameters analyse the grey-level distribution of pixel pairs in the image at a random location and orientation relative to each other. Gray level co-occurrence matrix (GLCM) proposed by Haralick (1979) is the most widely used texture feature. A GLCM matrix contains a number of rows and a number of columns equal to number of gray level intensities that shows the frequency of a pixel location

relative to each other pixel for a given distance and angle (Prats-Montalban, De Juan and Ferrer, 2011). Haralick (1979) described up to 14 textural features that can be computed from GLCM, however more commonly used are variance, contrast (difference between the highest and smallest values of pair of pixels considered), Entropy (disorderliness of the matrix), dissimilarity (heterogeneity of the grey levels), homogeneity (uniformity of the matrix), Correlation(e linear relationship between the grey levels of pixel pairs) and energy(consistency/orderliness of textural information) (Mridula, Kumar and Patra 2011). Another second order statistics is Run length matrix (RLM) that measures the runs of pixels with same grey level intensity in a particular direction. The average of these run lengths is a measure of coarseness of a texture. More small runs with similar grey level intensities will form a smooth texture as compared to long runs with different grey level intensities that would form a coarse texture.

Higher order statistics estimate properties of three or more than three pixel values occurring at specific locations relative to each other. Amadasun and king (1989) categorized them into coarseness (measures edge density) contrast (measures intensity difference between neighbouring regions), busyness (measures spatial frequency of intensity changes) and complexity (measures sharp edges and lines). In Transform based analysis, textural features are defined by spatial frequencies. Fine textures are rich in high frequencies, whereas coarse textures are rich in low frequencies. They include Fourier, and Wavelet transforms. Fourier transform gives a global sense of the frequency characteristics of an image but lacks spatial localization and hence not very popular. This problem can be overcome by using wavelet filters that allows the analysis of frequency content of an image at various resolution scales with minimal loss of information (Prats-Montalban, De Juan and Ferrer, 2011). Another popular model-based method in medical imaging process is based on the concept of fractal geometry. This concept is based on the natural objects having statistical self-similar fractal sets at different scales. In other words the variations in the object have the same distribution as the whole over a range of different scales. Fractional dimension is a measure

of variation at different scales and determines the roughness of surface. For an image, the fractal dimension is related to the variation in image intensity at different scales or pixel ranges (Tuceryan and Jain, 1998). In general features based on statistical methods have more discriminatory powers in image quantification than transform methods (Conners and Harlow, 1980).

2.1.4.1 Application of textural analysis in medical imaging

Textural analysis of medical imaging is not a new idea. In early 1970, it was first applied to plain radiographs (Harlow and Eisenbeis, 1973). Chen, Chang and Huang (1999) used it to characterize solid breast nodules on ultrasonography. In the last decade, there has been renewed interest in textual analysis of medical imaging due to advances in computer technology and development of new textural analysis algorithms and increasingly applied to CT, MRI and PET imaging. In oncological imaging based studies, textural analysis has emerged as diagnostic, prognostic and treatment response assessment tool.

2.1.4.2 Tumour Heterogeneity

Heterogeneity is a well-known feature of tumours and is associated with adverse outcomes in terms of treatment failure and drug resistance (Mroz and Rocco, 2013). Intratumour heterogeneity can be related to both genetic and histopathological variations such as cellularity, angiogenesis, extravascular extracellular matrix, and areas of necrosis (Davnall, et al., 2012). Gerlinger, et al., (2012) in their study showed that intratumour genetic heterogeneity varies both in space and over time. They also demonstrated that single or random biopsy of tumour may not represent the full extent of intratumour heterogeneity due to sampling error. Therefore it is important to identify the imaging biomarkers that can be correlated with worse histopathological features such as tumour grade, hypoxia and angiogenesis. Heterogeneity can be quantified on imaging using textural analysis which provides the non-invasive method of assessment. The study by Ganeshan, et al., (2013) identified the biological correlates for tumour hypoxia and angiogenesis on the basis of

textual analysis of CT images. This study showed significant association between medium to coarse texture scale and angiogenesis and hypoxia makers in primary non-small cell lung cancers.

2.1.4.3 Lesion detection and characterization

Texture analysis helps in characterizing lesion into benign or malignant based on their texture differences. In the study by Kido, et al., (2002), fractal analysis of gray-scale images of <2cm small periphery nodules, showed that fractal dimensions for organizing pneumonias and tuberculomas were greater than those of bronchogenic carcinomas ($p < 0.0001$) and hamartoma ($p < 0.0001$). Similarly the study by Gibbs and Turnbull (2003) showed significant differences for second order co-occurrence matrices such as contrast, variance and sum entropy between benign and malignant breast lesions when applied to high resolution contrast enhanced MRI images. These findings supported the general perception that benign lesions are homogenous compared to malignant lesions. They also showed, combining textural analysis with other parameters such as lesion size, age and time to maximum enhancement, can achieve diagnostic accuracy of 0.92 ± 0.05. Other studies showed the potential of textural analysis in differentiating malignant and benign lymph nodes in rectal cancer (Cui, et al., 2011) and differentiating colon cancer and normal colonic mucosa (Goh, et al., 2009).

2.1.4.4 Treatment response

Imaging biomarkers based on Textural analysis also helps in improving the predictive response to a cancer treatment. In a study of 39 patients with metastatic renal cell cancer receiving tyrosine kinase inhibitors (TKI), analysis of changes in CT textural parameters after 2 cycles of TKI were better predictor of response than conventional response evaluation criteria in solid tumours(Goh, et al., 2011). Percentage change in coarse texture uniformity of ≤ 2 % was an independent factor that correlated with shorter time to progression. O' Connor et al. (2011) studied 10 patients with 26 liver metastasis and

showed that tumour heterogeneity measured by fractal dimension on pre-treatment MRI predicted shrinkage in tumour volume after 5 cycles of anti-angiogenic and cytotoxic chemotherapy. In a recent study of 100 breast cancer patients who received chemotherapy , textual analysis (GLCM-Matrices) of dynamic contrast enhanced-MR images showed significant differences for the contrast (p value=0.042) and difference in variance(p value=0.043) parameters between responders and non-responders (response determined by greater than or less than 50% change in largest diameter)(Ahmed, et al., 2013). Higher values of contrast (a measure of local image variation) and difference in variance (a measure of variation in the difference in gray levels between pixel pairs) found in the study supported the fact that heterogeneous tumours will respond poorly to the chemotherapy. These differences were more significant at 1-2 minute post contrast image time and no significant differences were observed in pre contrast images. Textural analysis of fluorodeoxygenase (FDG) uptake heterogeneity on pre CRT ^{18}F-FDG PET images of patients with oesophageal carcinoma was assessed by Tixier, et al., (2011). Co-occurrence matrices strongly differentiated non responders from partial responders.

2.1.4.5 Prognostic tool

To date few studies have explored the potential of textural analysis as a prognostic tool for cancer survival. These studies are confined to CT or PET-CT based textural analysis. Ganeshan, et al. (2012) carried out two separate pilot studies on non-small cell lung cancer (NSCLC) (54 patients) and oesophageal cancers (21 patients). The studies showed that histogram based textural analysis of pixel distribution (first order statistics) of unenhanced CT images obtained using PET-CT examinations was significant independent predictor of poor survival for both NSCLC (P=0.001) and oesophageal cancer (P=0.0006). In a separate study by the same group (Ng, et al., 2012); textural analysis of primary colorectal cancers (57 patients) was done to determine its relation with overall survival. Textural analysis showed that at a filter value of 1.0, entropy, uniformity, kurtosis, skewness, and standard deviation of pixel distribution were separate independent predictors of poorer 5-year overall

survival. These results differ from the other studies as it showed that primary tumours with less heterogeneity at fine-texture level showed association with worse prognosis. But this study assessed whole-tumour volume rather than a single axial level and contrast enhanced CT images were analysed unlike previous studies. The author postulates that this may also be due increased vascular permeability of tumour cells that itself has shown to be associated with advance tumour stage and worse survival in colorectal cancer patients (Yonenaga, et al., 2005). Increased vascular permeability leads to greater cell packing resulting in uniform distribution of vascularisation and greater parenchymal enhancement. In another study, coarse uniformity texture of liver in patients with non-metastatic colorectal cancer was shown to be effective marker of survival than hepatic perfusion CT (Miles, et al., 2009).

2.1.5 MRI based textural analysis and colorectal cancer

Textural analysis has also emerged as a promising imaging biomarker in MRI over the last 10 years or so. Majority of the studies are confined to lesion detection and its differentiation involving breast, brain and prostate tumours (Davnall, et al., 2012). A few studies assessed the feasibility of textural analysis in predicting tumour response to neoadjuvant treatment. O' Connor, et al., (2011) investigated 10 patients with 26 CRC liver metastases who had dynamic-contrast enhanced MRI before starting bevacizumab and FOLFOX-6. They demonstrated that pre-treatment heterogeneity measurement of tumour microvasculature was able to predict the response. Conversely, pre-treatment tumour volume did not determine the subsequent change in volume after the treatment. To date, there has been very little work in exploring the potential of MR based TA as an imaging biomarker in rectal cancer. In a recent prospective study of 15 consecutive rectal cancer patients treated with CRT, textural analysis of pre- and mid-treatment T2w -MR images was carried out to assess its correlation with pathological tumour response to treatment. (De Cecco, et al., 2015). The study demonstrated that the textural parameter, kurtosis was significantly lower (p=.01) in patients with pathological complete response in comparison with those with either partial or no response on pre-treatment scan. The higher kurtosis in partial and no-responder group in

this study reflects higher heterogeneity (Miles, Ganeshan and Hayball, 2013) and thus poorer response to CRT.

2.1.6 Technical limitations and challenges

Though the textural analysis of medical images has been in use for over a couple of decade but wide spread application of this technique is limited due to lack of standardized approach and due to difference in acquisition parameters and reconstruction modes. In longitudinal studies measuring the change in parameters due to neoadjuvant treatment, acquisition parameters in image segmentation and scanner characteristics on serial scans should be kept the same so that the measurement of heterogeneity reflects the underlying tumour biology rather than scanner variations. In their analysis of 200 PET/CT images of various cancers, Galavis, et al. (2010) studied 50 statistical first, second and third order parameters for their variability due to different image reconstruction modes. Entropy (first order), energy, low grey RLM, and maximal correlation coefficient showed small variations (≤ 5%). Forty features including high order statistics such as coarseness, contrast and busyness exhibited large > 30% variations. Second order entropy, sum entropy and high grey level run emphasis showed intermediate variations (10% ≤ range ≤ 25%). However robustness of textural analysis methodology used can resist changes in textual analysis due to variations in acquisition parameters. This was demonstrated in a multicentre study where textural analysis was performed in three centres with the same MRI machine but using their own routine acquisition protocols. Analysis was performed on T1 and T2-weighted images from 10 healthy individuals and 63 patients with histologically proven intracranial tumours. This study found no significant differences in textural classification among three acquisition sites (Herlidou-Même, et al., 2003). In this study the authors conducted initially a preliminary phantom study to assess the effectiveness of textural analysis methodology in relation to changes in acquisition parameters(varying slice thickness, and excitation to change size to modify to signal to noise ration and voxel resolution) and found no significant differences in textural classification among test objects. Another technical consideration of importance is

the demarcation of region of interest on the images that can induce inter and intra-observer variation. However this can be reduced by using semi-automated approaches to delineate the tumour (Ganeshan and Miles, 2013).

2.1.7 Conclusion of Literature Review of the MRI and textural analysis and research question for the second clinical study of the thesis

This literature review highlights the principle of conventional or morphological MRI and issues in restaging of irradiated rectal tumours because of difficulty of conventional MRI in differentiating fibrosis from viable residual tumour (van der Paardt, et al., 2013). Though the combined modality treatment and use of high resolution MRI in selecting and staging locally advanced rectal cancer has considerable improved loco-regional control but there is no improvement in OS because of distant failure (Lange, et al., 2013). In addition, individual tumours show intra-tumour heterogeneity due to regional variations in microvasculature, cellular density and metabolic activity. Intra-tumour heterogeneity is linked to tumour aggressiveness in terms of poor prognosis and resistant to treatment. This shifting paradigm has put a great emphasis on quantification of non-invasive imaging biomarkers linked to tumour heterogeneity (Ganeshan, et al., 2010). The CRM involvement, EMVI and quantification of TRG on conventional MRI have proven to be important prognostic factors. In addition quantification of tumour heterogeneity in the form of textural analysis allows extraction of additional features on imaging without needing for the patients to undergo further scans or invasive biopsies. It has been increasingly applied to various imaging modalities in the last decade or so and emerged as a non-invasive imaging biomarker. To date there is no study that assessed the potential of MR based textural analysis in predicting survival outcomes in rectal cancer. This information could be used in future to tailor treatment in individual cases and could prove to be an important step towards personalization of patient management. In addition early identification of patients with poor predicted prognosis increases cost effectiveness by switching patients to alternative treatments and avoiding toxicity of chemotherapy drugs and radiation.

- The main objective of the 2nd clinical study in the thesis is to investigate whether MR based textural analysis in addition to morphological MRI and histopathological parameters can predict survival outcomes in rectal cancer.

2.2 Functional imaging in rectal cancer and integrated PET/MRI

As discussed in section 1.9.2.1, neoadjuvant CRT combined with total mesorectal excision has become a standard treatment of locally advanced rectal cancer after paradigm changing trials by the German rectal cancer study group (Sauer, et al., 2004) and the Dutch cancer society (Kapiteijin, et al., 2001). Long term follow-up in such patients showed that though this strategy has significantly reduced the local recurrence but no difference in overall survival was observed because of distant failure (Sauer, et al., 2012 and van Gijn, et al., 2011). The long term outcome of these landmark trials tells us that there is still much room for additional research to improve rectal cancer management in terms of improving OS. Tumour response assessment has emerged as an attractive end point (Glynne-Jones, et al., 2006) and is also important because this information can be used for treatment planning and carries prognostic significance. In the study by Rödel, et al. (2005), prognostic assessment of histological tumour regression grading in a cohort of 385 rectal cancer patients treated with long course CRT was carried out. It was found that 5-year DFS in patients with complete regression, intermediate regression and no regression was 86%, 75% and 63% respectively (p=.006). Complete pathological response as discussed in section 1.9.5, is present in 15-27% of patients treated with neoadjuvant chemoradiotherapy and is associated with significant increased disease free survival with low local and distal recurrence rates as show in the systematic review and meta-analyse by Mass, et al. (2010) and Martin, Heneghan and Winter (2012) respectively. In a separate meta-analysis by Lee, Hsieh and Chuang (2013) it was shown that patients with partial tumour regression had a superior DFS than those with poor response and pooled HR was decreased by 50% (HR 0.49, 95% CI 0.28–0.85). This has placed greater emphasis on pre-operative imaging modalities to identity

patients that could be predicted to have either worse or better tumour regression grades. This in turn could help to select patients for personalized treatment in the form of more intensive therapy in patients with predicted poor response.

Because of the limitations of conventional imaging as discussed in section 2.1.2, functional MRI techniques such as DWI and PET reflecting tumour microenvironment are currently the subjects of investigation for a potential prognostic and tumour response assessment tool for various cancers. In this section, principles of PET imaging formation, factors affecting PET imaging, commonly used PET radiotracers and PET instrumentation are discussed. In addition, principles of MRI modality-DWI and integrated PET/MRI are also discussed. This section also forms the basis for the introduction and literature review of the 3rd clinical study in the thesis (Chapter 6). In this study the potential of integrated PET-MRI features to predict histological tumour response was investigated.

2.2.1 Principles of PET imaging

Positron emission tomography (PET) is a functional imaging modality that uses molecular probes to image and measure biochemical processes in vivo (Basu, et al., 2011). In essence, it is a nuclear imaging method based on physical properties of radioactive substances called radiopharmaceuticals that emit positrons when they undergo radioactive decay upon administration to patients. Positron emitter molecules contains proton rich but nutrient deficient nucleus. The relative dearth of neutrons in the nucleus results in protons that are in close approximation with each other and repel one another. This results in making their nuclei unstable that achieve stability by transforming protons into neutrons and emits a positively charged electron i.e. positron during this process. Positron on release travels a very short distance (termed as range) not more than a mm or two in the surrounding matter until it interacts with negatively charged electron. The range of positron is dependent on the energy with which it is emitted and density of a matter. Upon their collision, these two electrons are annihilated that results in release of two high energy (511-Kev) gamma

photons traveling at 180˚from one another. Specialized ring of opposing PET detectors are used to detect these photon pairs simultaneously within a narrow time frame of few nanoseconds. The simultaneous detection of the two photons is called coincident detection and is the basis for obtaining a tomographic image. The straight path along which these two γ rays are detected is called line of response (LOR) (Workman and Coleman, 2006). This principle of PET imaging is illustrated in Figure 2-4.

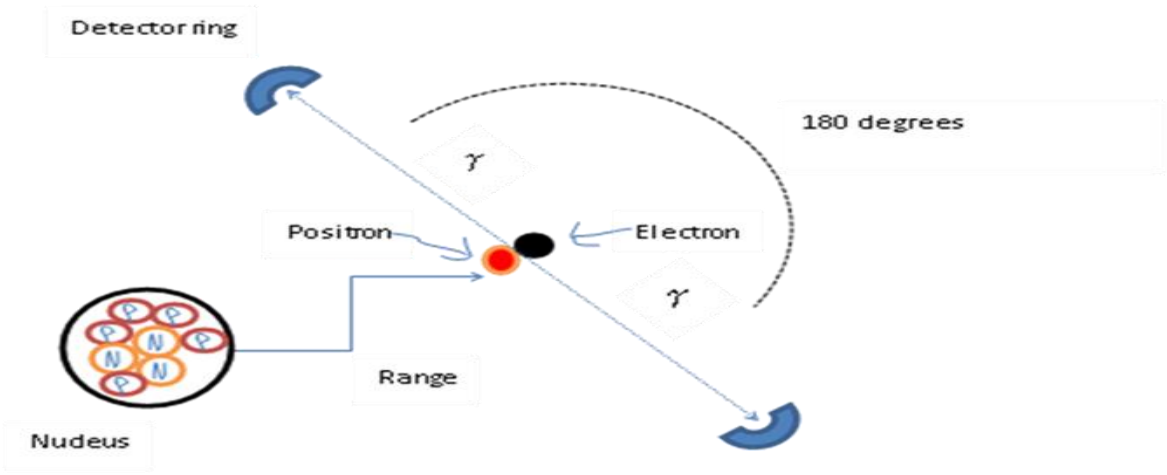

Figure 2-4 Electron–positron annihilation, producing two 511 keV photons leaving in opposite directions.

2.2.2 Factors affecting the quality of PET imaging

2.2.2.1 Attenuation

There are several physical factors that degrade the quality of images and introduce image reconstruction bias affecting the accuracy of a PET scan. However some can be corrected. Attenuation is one of the most important factors affecting the accuracy of quantification in PET Images. The intensity of photon signal tends to decrease as it passes through tissue based on the density and thickness of tissue. Attenuation refers to some of photon travelling through the tissue may stop completely before reaching the detectors termed as absorption (Figure 2-5a). This results in overall loss of detection of true coincidence events leading to increased image noise. In addition attenuation causes non-uniformities in the reconstructed image leading to image artifacts and distortion. Since both photons must exit the body to

make a coincident event, so attenuation depends only on the total thickness or total length along the path of photons independent of depth of source of annihilation event. Attenuation correction has traditionally been performed by rotating a rod source of positron emitter such as germanium-68 along the field of view first without and then with the patient in the imaging position. This results in a blank and transmission scans respectively and attenuation correction factor is derived from the ratio of coincidence counts in these two scans. This then can be used to correct the PET data for emission scan and to create an attenuation-corrected image of the patient (Zanzonico, 2012). The development of combined PET/CT scanner system has been evolutionary in imaging technology and is more diagnostically useful than dedicated PET system. The CT part of PET/CT scanner is a transmission map that is used to generate attenuation corrected image. This not only reduces acquisition time but also allows precise anatomical localization of functional abnormalities (Workman and Coleman, 2006).

2.2.2.2 Random coincidence

Random coincidence event occurs due to two photons generated from different annihilation event and gets inappropriately detected by detector pair and positioned within the time window used to define a true coincidence. This phenomenon is illustrated in (Figure 2-5b). There are two ways in which random events can be detected and subtracted from the acquired data. The random events can either be estimated from mathematical assumption that random events are proportional to square of single events or from direct measurements of delayed events acquired in out of time window (Townsend, 2004).

2.2.2.3 Scattering

Scattering event refers to change in the direction of one or both photon's path due to interaction with tissues along the path and as a consequence loses energy before reaching the detectors resulting in in-correct line of response (Figure 2-5c). Scatter comprises of a large proportion of acquired data especially in 3D mode. Scatter can be corrected by

applying energy threshold but in reality scatter is difficult to assess and additional sophisticated correction scatter models have been developed to minimize the residual scatter bias (Townsend, 2004).

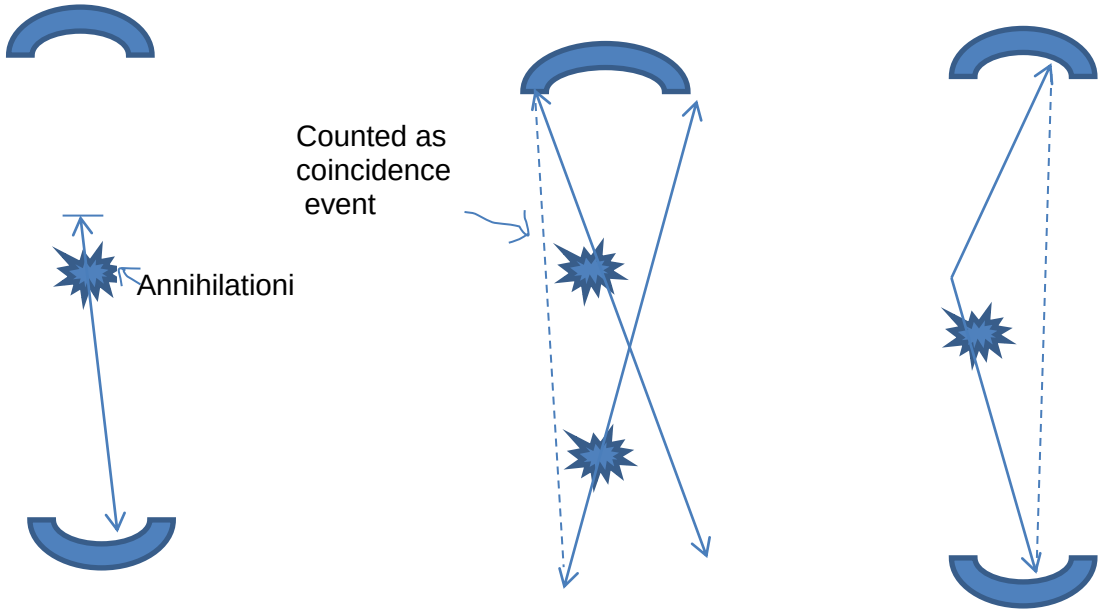

Figure 2.5a - Attenuation Figure2.5b - Random coincidence Figure 2.5c - Scatter

Figure 2-5 Factors affecting the quality of PET imaging

2.2.2.4 Spatial Resolution

There are three factors that affect the spatial resolution in PET imaging (Shukla and Kumar, 2006). Firstly, positron on release carry kinetic energy and travel a finite distance (range) through the surrounding material before it annihilates with an electron. This introduces an intrinsic degree of misposition error in spatial localization. Secondly, positron does not stop completely just before it combines with an electron and always have residual momentum. As a result the two annihilation photons are not emitted exactly at 180 degree from each other and have directional component by 0.5 degree. This induces a degree of error in spatial localization based on the diameter of detector ring and is about 2mm for 90cm ring detector. The third factor that determines the spatial resolution is the size of detector rings and a pair of opposite detectors receiving the annihilated photons in coincidence constitutes a channel. These channels are usually 0.4-0.6cm wide and reduction in size of detectors decreases the variation in delineating specific spatial location.

2.2.3 PET radioisotopes and radiopharmaceuticals

Radionuclides that are commonly used in PET imaging are isotopes of atoms that are naturally present in biological molecules such as carbon, oxygen and nitrogen. These radionuclides have fewer neutrons than their stable counterparts. Table 2-1 lists the commonly used PET radionuclides along with their value of the ranges and the energies.

Table 2-1 Common PET Radioisotopes (Blokland, et al. 2002, Workman and Coleman, 2006)

Radionuclides	Number of protons (Atomic Number)	Mass number (Number of protons + neutrons)	Half-life (minutes)	Maximum Positron energy (MeV)	Mean range (mm)	Non-radioactive stable counterparts with mass number
Fluorine - 18	9	18	110	0.64	0.2	Fluorine -19 (9 protons and 10 neutrons)
Oxygen - 15	8	15	2	1.72	1.5	Oxygen – 16 (8 protons and 8 neutrons)
Nitrogen-13	7	13	10	1.19	1.4	Nitrogen -14 (7 protons and 7 neutrons)
Carbon - 11	6	11	20	0.96	0.3	Carbon – 12 (6 protons and 6 neutrons)

Positron emitting radionuclides are not found naturally and have to be synthesized. A cyclotron is a device that can facilitate this process. It is essentially a particle accelerator. Charged particles made from an ion source are introduced into a large evacuated container. Two high-voltage electrodes accelerate these particles that are kept in a circular pathway by an application of strong magnetic field at ring angle to the electrical field. A beam of protons is produced and cyclotron shoots them at the target atoms and results in these protons

ending up in the nucleus of target atom and making them unstable by lowering the neutron to proton ratio (Bomanji, Costa and Ell, 2001). Radiolabelling is a chemical reaction in which radionuclides once formed are incorporated into a pharmaceutical agent or a desired molecule to form a radiopharmaceutical. These radiopharmaceuticals are then administered into a patient and get distributed according to its pharmacokinetic. Since many of positron emitters have small molecular weights relative to the desired molecule or agent used for labelling, consequently they have no or minimal impact on behaviour of these molecules in the body (Li and Conti, 2010). Also very small amount of radiopharmaceuticals are administered in diagnostic imaging so they have a negligible pharmacologic effect. Radioactive biomolecules of sugars, amino acids and nitrogen bases have been developed. Some of the examples of PET pharmaceutical are shown in Table 2-2 (Dunphy and Lewis, 2009).

Table 2-2 Example of radiopharmaceuticals (Dunphy and Lewis, 2009)

Radiopharmaceutical	**Structural analogue**	**Measured parameter**
(18F)Fluorodeoxyglucose (FDG)	Glucose	Glucose metabolism
(18F)Fluorothymidine (FLT)	Thymidine	DNA synthesis
(18F)Fluoromisonidazole(FMISO)	nitroimidazoles	Hypoxia
(11C)Methionine (MET)	Methionine	Amino acid metabolism
(18F)Fluoroestradiol (FES)	Estradiol	Estrogen receptor status
(11C)Choline	Choline	Up regulation of choline kinase associated with cancer

The selection of PET nuclides is based on their physical and chemical features, half-lives, availability and the biological process that needs to be analysed. In general most radionuclides have short half-lives and limited availability. Ideally the half-life of PET nuclides should be long enough to allow radiolabelling and imaging procedure. Radionuclides such as nitrogen-13 (10 minutes) and oxygen-15 (2 minutes) have very short half-lives that not only limit their production in only PET centres with on-site cyclotron facility but also limit their clinical applicability. 18-FDG in the most widely used PET nuclide because of its longer half-

life that allows its distribution by commercial vendors and can be used directly at PET sites without on-site cyclotron (Wadsak and Mitterhauser, 2010). The positron energy of 18-F nuclide is low resulting in short tissue range. This leads to lower radiation dose and higher resolution (Basu, et al. 2011). In addition, 18-FDG is a structural analogue of glucose molecule and is very similar to it in terms of biochemical behaviour. Since most pathological settings are associated with changes in glucose metabolism and for this reason, 18-FDG is used as major work horse in oncology, cardiology and neurology PET imaging. Glucose undergoes glycolysis as a first step in generating energy at a cellular level. There is a typical increase in the expression of glucose transport receptors (GLUT) in tumour cells which in turn leads to increased glucose uptake and increase glycolytic rate in these cells than normal tissue. Being a structural analogue, 18-FDG behaves similar to glucose but only to a point. It is phosphorylated by hexokinase enzyme to become a FDG-6-phosphate. Since FDG-6-Phosphate is not a suitable substrate to undergo further metabolism by glucose-6-phosphatse enzyme so it becomes trapped within cells. Tumour cells being more metabolically active will accumulate more [^{18}F] FDG and hence leads to more uptake of radiotracer which is a basis for differentiating pathological tissue from benign tissue with FDG-PET imaging (Groves, 2007). Figure 2-6 shows metabolic trapping of fluorodeoxygenase.

Despite its major oncological applicability, FDG-PET imaging is not without limitations. The major limitation of FDG is its nonspecific uptake by both neoplastic cells and the other cells associated with increased metabolic activity such as inflammation and infection. Although, viable cancer cells form the majority of the FDG signal but a part of uptake by infiltrating immune cells such as activated macrophages induces a false-positive PET signal and has significant implications in the analysis of PET images (Wahl, et al., 2011). In addition, tumours enclosed in organs with higher normal physiological uptake such as brain, muscle and bladder cannot be properly delineated. Moreover, some malignancies such as prostate and renal cancers and hepatomas are not associated with enhanced glucose metabolism

and therefore, are poorly differentiated by FDG-PET imaging (Wadsak and Mitterhauser, 2010).

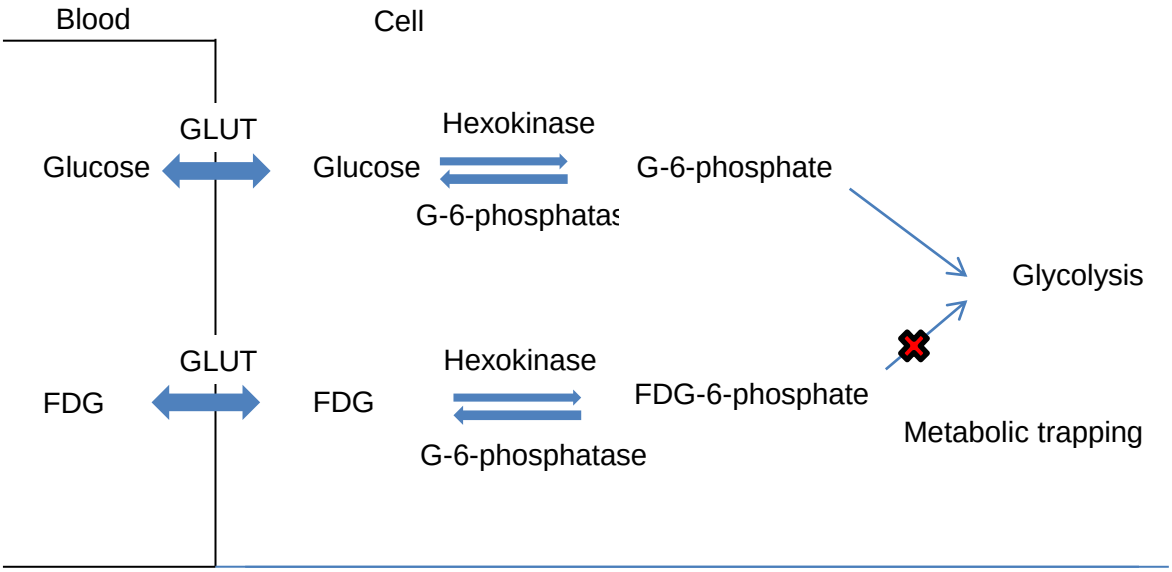

Figure 2-6 Metabolic trapping of fluorodeoxygenase

2.2.4 PET instrument

2.2.4.1 Photon detection and scintillation detectors

The main aim of photon detection is to measure the total energy delivered by emitted high energy photons (511 KeV) as it passes through the detector. In order to achieve the highest accuracy and sensitivity in detecting the photon's energy, detectors need to have four specialized features; stopping power, light output, decay time of light and good energy resolution (Shukla and Kumar, 2006). The stopping power relates to the detectors ability to efficiently absorb photon's total energy and is inversely proportional to the average distance traversed by photons before they deliver energy in the detector. This distance relies on high effective atomic number and density of the detector's material. In addition the detector should have an ability to produce higher light output to allow the better spatial and energy resolution. The time taken by the light to decay determines the accuracy with which coincident photons can be detected by a pair of detectors. As the proportion of photons striking a detector increases, the chance of missing a photon increases due to detector dead time that puts limits on count rates. A shorter decay time is desirable as it allows faster generation of the signal and counting higher photon rates. The use of fast detectors with

narrow coincidence time window reduces the likelihood of detecting accidental coincidence events.

The detection elements that are used in most PET scanners are scintillation crystals which are a few millimetres in size. These small individual crystals are tightly arranged into blocks, typically 6 x 6 or 8 x8 coupled to four photo-multiplier tubes. After their interaction with photons, the scintillation crystals release visible or near ultraviolet light to photo-multiplier tubes that detect and measure the scintillation photons. The photo-multiplier tubes have multiple channels coated with photocathode inside a common vacuum envelope and each channel provides essentially an independent photo detectors. The photocathode, when struck with light from the crystals emits electrons that are amplified into logical output electronic signals. These electronic pulses are then fed into energy discriminating circuit called pulse height analyser that sorts out the pulses according to their energy. Only the pulses within a predetermined pulse energy window are included in the image (Zanzonico, 2012). Sodium iodide doped with thallium (NAI) and bismuth germinate (BGO) are the first generation scintillators used in conventional PET scanner. BGO detectors were associated with high stopping power and sensitivity but poor light output compared to NAI detectors that had very high light output. In addition these detectors had longer decay time limiting their performance at high count rates. Latest generation of very high performance scanners use Lutetium Oxyorthosilicate that possesses high light output similar to NAI and high sensitivity similar to BGO detectors as well as the very fast decay time (Basu, et al., 2011).

2.2.4.2 PET image formation

Once the coincident events are determined along their lines of responses between pairs of parallel and opposite detectors, next step is to store the data set of a large number of these events. Data associated with each line of response is characterized by angle of orientation along y-axis and by the shortest distance between the detector and centre of field of view along the x-axis (Figure 2-7a). If multiple LORs passing through the same point at different

angles are plotted, the graph obtained is termed as a sinogram resembling a sine wave plot turned on its side (Figure 2-7b). All the detector pairs that observe this interaction will have LOR data stored on this curve. A sinogram is essentially a matrix of pixels that represent the number of counts measured along each LOR. Each horizontal row of pixels represents the number of counts at a specific projection angle (Fahey, 2002). Sinogram data is then corrected for variations due to factors such as scatter and attenuation. This data is then used to reconstruct cross-sectional images using various algorithms. Although it is beyond the scope of this thesis to give details of reconstruction algorithm but it is important to mention the two techniques of reconstruction that are filtered back projection or iterative reconstruction technique. Filtered back projection is a traditional reconstruction algorithm that has been used in nuclear medicine and is still used in single-photon emission computed tomography (SPECT) and CT reconstruction. Since this technique is known to amplify noise in the image, so it has been replaced by iterative method in new generation PET systems as a more accurate and superior method of quantification (Jha, et al., 2014).

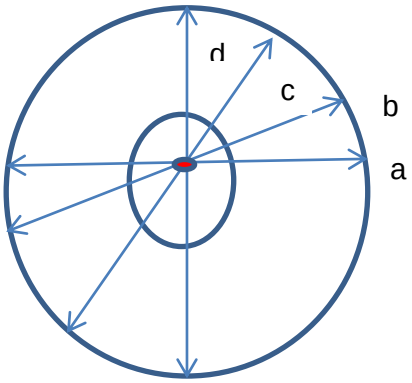

Figure 2.7a - Four LORs labelled a-d are shown passing through a point source shown in red (adapted from Fahey, 2002)

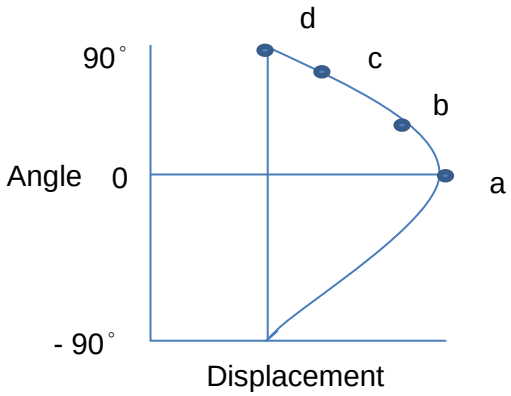

Figure 2.7b - Sinogram formation. Each LOR is a function of its angular orientation versus its displacement from the centre of field of view.

Figure 2-7 Principles of PET image formation

2.2.4.3 Data acquisition mode

PET data can be acquired in both 2D multi-slice and 3D modes. In a typical PET system, the scanner has retractable lead or Tungsten septa placed in between each ring. These septa

are positioned for 2D multi-slice mode and essentially separate the imaging planes. Detection of photons in one imaging plane being in coincidence with photons detected in other imaging planes is prohibited. Use of the septa reduces the scattered events from 30-40% of all collected events to approximately 10-15%. Retracting the interplane septa in 3D acquisition mode increases the sensitivity by a factor of 10. However the disadvantage of this mode is an increase in the amount of scatter and random coincidences and the need for complicated and more time consuming 3D reconstruction algorithms. In addition the sensitivity in 3D mode is much higher at the axial centre of the scanner than at either end requiring more position for the whole body scanning. Despite of all these factors, majority of studies are performed in this mode due to improved image quality and low signal to noise ratio (Fahey, 2002 and Smith, 2010).

2.2.4.4 Quantification of PET imaging data

PET imaging data can be quantified and analysed using different methods. A semi-quantitative method for assessing glucose metabolism by means of standardized uptake values (SUV) is discussed here and was used in the data analysis of this study. Measurement of SUV is relatively easy, clinical practicable and accessible in all clinical acquired PET scans. SUV is defined as a proportion of the FDG concentration in a ROI to a total dose injected corrected to patient's body weight (Strauss and Conti, 1991). Either an absolute value such as SUV ± max (maximal value in ROI) or serial measurements ΔSUV (relative changes in SUV max) have been used for various oncological and clinical trial purposes (Boellaard, 2011). Quantitative PET has been recognized as a prognostic and response monitoring tool. SUV max was shown to be an independent prognostic factor ($p=0.01$) for diseases specific survival and predictive of treatment response along with performance status of patient and staging in inoperable non-small cell lung cancer patients treated with radiotherapy (Borst, et al., 2005). In another prospective case series study of 152 patients with metastatic colorectal cancer, significant 2 and 3 year survival benefit was observed in patients with lower than median uptake SUV ($p=0.017$). The survival

disadvantage was regardless of the subsequent treatment in the form of curative surgery or chemotherapy (De Geus – Oei, et al., 2006). Based on the outcome of these studies, it can be hypothesized that FDG uptake indicates biological aggressiveness of underlying tumours.

2.2.4.5 Principles of diffusion weighted imaging-MRI

A variety of MRI pulse sequences comprising of radiofrequency and gradient pulses and providing a range of contrast has already been discussed in details in the section 2.1.1.5. The image formed on the bases of these pulse sequences is principally based on the rate of decay of protons from high energy state to low energy state after excitation by a radiofrequency pulse to a static magnetic field (Berger, 2002). In comparison, DWI technique produces image contrast by measuring the diffusion of water molecules with in tissues at cellular level. Water molecules are in constant state of random flow between intracellular, extracellular and interstitial tissue compartments limited by partially permeable cell membranes. The apparent diffusion coefficient of water molecules is altered by various pathological conditions either due to destruction of cell membranes (e.g. cell lysis), changes in cellular density (e.g. inflammation or tumour) or due to cellular swelling (e.g. ischemia)(Bammer, 2003). Diffusion weighted sequence is achieved by applying two additional dephasing and rephasing gradients of equal magnitude but in opposite direction to the conventional spin echo pulse sequence over a set interval. In tissues with impeded water movement due to higher cellular density (e.g. tumours), relatively stationary water molecules will dephase and rephase equally during that interval. Therefore it has little effect on the overall T2 decay and thus produces stronger signal. However in tissues with low cellular state and free diffusion, movement of moving water molecules during that interval would lead to incomplete rephasing and results in signal loss from that spatial location (Charles-Edwards and deSouza, 2006).

2.2.4.6 Image analysis

The sensitivity of a DW-MRI to quantify diffusion is represented by its b-value that can be adjusted by altering the amplitude and application time of gradient pulses as well as the time interval (diffusion time) between their application. The higher this interval, the more sensitive an image is to small changes in water movement at cellular level (Boone, Taylor and Halligan, 2013). It is important to note that image contrast in DWI-MR sequences depends not only on diffusion but also on relaxation times of water molecules. To abolish the effects other than that of diffusion, DWI-MR signal is acquired at least with two b-values, typically one b-value of 0 (without diffusion weighting) and the second higher b-value depending on tissue or organ being evaluated. By acquiring images at multiple b-values, more accurate quantitative calculation of DWI over time termed as apparent diffusion coefficient (ADC) can be achieved. The ADC infect represents a slope of graphical plotted line between logarithm of signal intensity of tissue along the y-axis versus b values along the x-axis. The ADC values are displayed as a parametric map by assigned ADC to each voxel and then recorded by drawing ROIs on the map (Qayyum, 2009). The calculation of ADC is an automated process by a software application on a scanner workstation.

2.2.4.7 Clinical application of DWI in response assessment of rectal cancer

Radiological anatomical response assessment based on percentage reduction in tumour length such as the Response Evaluation Criteria in Solid Tumours (RECIST) (Machida, et al., 2008 and Therasse, et al., 2000) is well established. However change in tumour size does not always reflect treatment response. Sometimes size my increase due to necrosis, cytotoxic oedema or haemorrhage resulting from treatment rather that disease progression (Tuma, 2006). In addition this method of assessment is not appropriate for predicting response for cytostatic rather than cytocidal cancer therapies (Wahl, et al. 2009). In such cases tumour response cannot be reliably predicted. Functional MRI techniques such as DWI have the ability to combine morphological, physiological and pathological changes in order assess tumour response.

The potential of pre-treatment tumour ADC in predicting of response to chemoradiation has been investigated previously in patients with locally advanced rectal cancer. In a pilot study of 14 patients with clinically advanced non-metastatic rectal adenocarcinoma, Dzik-Jurasz, et al. (2002) found a strong negative correlation between mean pre-treatment tumour ADC and percentage MR regression in tumour size after chemotherapy (r= −0·67, p=0·01) and after chemoradiation (r= −0·83, p=0·001). This study showed that low ADC in responders may represent the amount of necrosis and loss a nonviable part of treated cancer. In a separate study of 9 patients with locally advanced rectal cancer treated by CRT (Hein, et al., 2003) it was found that mean ADC decreased continuously in the 2nd week (p=0.028), 3rd week (p=0.012) and 4th week of treatment (p=0.008). Subsequent histopathological evaluation revealed that there was an increased interstitial fibrosis in the tumour region. Limiting effect of fibrosis to free diffusion was hypothesized to be the factor for decrease in ADCs. Jung, et al. (2012), explored the potential of DWI to predict histological response in rectal cancer and found that in histopathological responders, per-treatment ADC was significantly lower as compared to non-responders (p = 0.034). The results also showed a significant increase of mean ADC after neoadjuvant CRT in responders (p <0.005) suggesting effective tumour response (e.g. tumour lysis) and change from high to low cellular tissue state and increase in free diffusion. Curvo-semedo, et al. (2012) explored the potential of ADC quantified on initial staging MRI as a non-invasive imaging biomarker of rectal tumour invasiveness. Significant lower ADC values were found in rectal cancers with mesorectal fascia (p=0.013) and lymph node involvement (p=0.011) on MRI, poor histological differentiation (p=0.025). Though lower ADC values were noticed in patients with EMVI but the difference was not significant. A recent meta-analysis by li, et al. (2014) of 1564 patients showed superiority of DW-MRI over FDG-PET or FDG-PET/CT in predicting histological tumour regression in patients with locally advanced rectal cancer treated with neoadjuvant CRT. The pooled sensitivity (true positives i.e. histological responder) 85% (95% CI: 75-91) vs. 81% (95% CI: 74-86) and negative predictive value (proportion of patients correctly identified who didn't respond to CRT on histology) 91% (95% CI: 80-95)

vs. 80% (95% CI: 68-89) were significantly higher than those of FDG-PET or FG-PET/CT (=p <0.05) This suggest that DWI-MRI is a valid technique to evaluate tumour regression in rectal cancers and hence this modality was used in this study as well.

2.2.5 Multimodality molecular imaging-PET/MRI

Recent introduction of high resolution PET combined with other modalities has revolutionized the practice of clinical imaging. Combined PET/CT scanners in the same gantry increases the sensitivity and specificity of PET for the detection of lesion by enabling direct correlation of region of increased uptake of radiotracer with their anatomical location on the CT scan. In addition X-rays from the CT component of PET/CT scanners are used to construct an attenuation map of the body which is imperative in accurate interpretation of PET images (Collins, 2007). However the scanning with CT carries an inherent theoretical risk of radiation exposure especially if acquired with full diagnostic protocols and poor soft tissue resolutions in the absence of oral or intravenous contrast. Conversely MRI technique does not carry the risk of radiation and also produces high spatial resolution and contrast imaging. In addition, functional MRI techniques such as diffusion and perfusion imaging could compliment the functional information obtained from PET (Habib and Rameshwar, 2009). In recent years with technological advancement and development of PET detectors that could function in the presence of strong magnetic field, state of art integrated PET/MR imaging technique is now available.

In the last decade indirect PET/MRI studies have been conducted (combining data from independent PET/CT images software fused with independent MRI images and shown the potential use of PET/MRI biomarkers in various oncological applications. In a retrospective study of 37 patients with suspected live metastasis, diagnostic accuracy between 18F-FDG PET/CT, dynamic contrast enhanced MRI, and retrospectively fused PET and MRI (PET/MRI) was compared. The results showed that the sensitivity of PET/MRI and contrast-enhanced MRI was significantly higher than that of PET/CT (Donati, et al., 2010). The major

limitation with such studies is that since scans are not performed simultaneously, there is a potential for errors in lesion co-registration especially for organs such as bowel which may change location and shape over short periods (Hofmann, et al., 2009). In the pilot study of the thesis, PET and DWI parameters on integrated PET/MRI platform were assessed for their potential in predicting histological response of rectal cancers to neoadjuvant treatment.

2.2.5.1 Oncological applications of integrated PET/MRI

There is limited evidence on integrated PET/MRI in oncologic applications and there was no study found to date on the correlation of histological TRG with imaging features for rectal cancers on integrated system. Other pilot studies comprising of small patient population have demonstrated the feasibility of PET/MRI in diagnosing and staging cancers. A study by Paspulati, et al. (2015) was carried out to determine the feasibility of PETMRI in colorectal cancer (n- 12 patients with 4 rectal cancers) by comparing its diagnostic and staging potential with PET/CT. The correlations of main outcome measures (maximal and mean standard uptake value (SUVmax and SUVmean) and the longest and shortest tumor diameters) were high between PET/MRI and PET/CT with no statistical differences. In another feasible study of 32 patients with different cancer lesions by Drzezga, et al. (2012) comparing interrelated PET/CT and PET/MRI did not reveal a significant difference (p=0.5) between the number of lesions detected on PET/MR and the number detected on PET/CT. In addition this study showed as reliable and as comparable anatomical location of suggestive PET lesions in PETMRI as that of PET/CT. However quantitative evaluation based on SUV showed significant difference between the two imaging modalities in mean SUV measured with higher lesion contrast in PET/MR implying suitability of PET/MRI in quantitative evaluation of therapy response.

The real theoretical advantage of adding MRI to PET comes from functional element of MRI such as DWI and contrast-enhanced images. There is no valid data that would infer superiority of combining PET and MRI in initial staging of rectal cancer over conventional

staging modalities such as pelvic MRI and or TRUS. A retrospective study of 23 patients by Kam, et al. (2010) correlated the primary staging of rectal cancer on PET/MRI fusion images with histopathological staging. The results did not seem to add much benefit to conventional rectal cancer staging modalities. Fusion PET/MRI correctly staged 57% of T3 and 75% of T tumours respectively. For N staging, sensitivity was low and only 44%. In all the cases with absent positive lymph nodes on MRI, PET was never positive. Hence, TNM staging of rectal cancers included in this study was assessed on conventional MRI.

2.2.6 Conclusion of Literature review of the functional imaging (MRI-DWI, PET/MRI) and main objective of the third clinical study of the thesis

This chapter highlights the principles of functional imaging modalities of PET and MRI and its application in oncology especially with reference to rectal cancer. Although various exploratory studies are present in the literature that investigated the role of functional MR-DWI, PET, PET-CT and combining PET and MRI data in identifying responders to neoadjuvant treatment in rectal cancers but there is a lacking evidence on the role of integrated PET-MR in this area. With this gap in the knowledge, the third study of the thesis discussed in chapter 6 was carried out. The main objective of this study was to investigate whether pre-treatment integrated PET-MR functional features correlated with histological response in locally advanced rectal cancer treated with long course CRT. In addition, a potential correlation of PET and functional MRI features in the setting of integrated PET/MRI system was evaluated. Moreover, association of clinical, histological and functional imaging parameters with disease free survival was also evaluated for these patients.

3 Methodology, methods and materials

In this chapter research methodologies used for the three clinical studies (Chapters 5-7) included in the thesis have been discussed. Firstly, a brief background and essential principles that need to be considered in conducting a social research are discussed with relevance to this thesis and then methodologies for the clinical studies included in the thesis are presented.

3.1 Methodology of the study of Outcomes in a Cohort of patients with delayed surgery after long course CRT

Consideration of underlying philosophical perspectives is critical in conducting social research as this will govern the choice of research design and methodology (Crotty, 1998). Research in health care is mainly based upon empirical approach and is based on scientific, logical and methodological principles and conducted in a way that is objective and value free. It is mainly of quantitative nature and is conducted within the domain of positivist paradigm. It involves the description of population of interest and measurement of its characteristics (variables) and the variability of these observations. It takes into account the probability or chance that might be responsible for the variations among the variables (Bunniss and Kelly, 2010). Interpretivism is a contrasting epistemological position to positivism and is concerned with that how people interpret the world around them and requires the social scientist to see perceive things from the individual's point of view through interviews, unstructured surveys and focus groups (Bryman, 2012). The main objective of this thesis was to determine the effect of delayed surgery in locally advanced rectal cancer after the long course chemoradiotherapy (section 1.11). A deductive approach was used to hypothesize that delaying the surgery after long course chemoradiotherapy does not adversely affect outcomes. Since the data collected was mainly numerical so a quantitative research methodology based on a positivist paradigm was employed. A research design provides a structural framework for technique of the data collection and analysis i.e. research methodology (Bryman, 2012). The studies included in the thesis are observational

studies based on single arm case series without a control group. Non randomized clinical studies will always have low internal validity because it is impossible to be confident that all important confounding elements have been determined and appropriately controlled for. Though patients are not, strictly speaking, allocated in truly observational studies but such studies are still prone to selection bias because clinicians propose treatments to patients which are apparently going to benefit them based on their demographic and prognostic information (MacLehose, et al. 2000). In terms of hierarchy of evidence (Evans, 2003), findings of case series study design are ranked low but like randomized controlled trials, results derived from such studies depend upon their quality by ensuring that bias is attenuated and effect on outcomes shown are likely to be true (Dalziel, et al. 2005). Although randomized controlled trials are considered to be the most valid study design to evaluate the effect of an intervention, up to 30% of NICE health technology assessments have included information from the case series studies (Dalziel, et al. 2005). Such studies are often useful to illustrate the outcomes of novel treatments, in discovery of unexpected benefits or risks of a treatment and in generating new hypothesis to be tested in clinical trials (Vandenbroucke, 2001). Moreover, these studies are easier to conduct, need less time and financial resources than the randomized controlled or cohort studies (Chan and Bhandari, 2011). Chapter 4 in this thesis is based on a retrospective cohort of 112 consecutive patients diagnosed with rectal adenocarcinoma and treated with long course CRT with curative intent from 01/2004 to 06/2012 at the department of surgery, Colchester General Hospital. The goal of this study was to assess a new clinical and radiological approach to optimize timing of surgery after long course CRT and to assess the safety and feasibility of the laparoscopic approach in patients with rectal cancer who undergo long course CRT. The timing of surgery after long course CRT is a controversial issue with no clear consensus. At present, the standard practice is to wait 6-8 weeks after the completion of CRT for resection of tumour (Foster, et al. 2013). An earlier study from Colchester General Hospital involved small number of patients (n-46) and showed that there is an on-going time dependent tumour response beyond this period when assessed on serial MRIs as a part of an established protocol in the

hospital (Johnston, et al. 2009; section 1.10). As a result of that, laparoscopic surgery was postponed for the responding patients up to almost three months or beyond without resulting in wider CRM and greater tumour downgrading (Motson, et al., 2011). Based on the positive findings of these preliminary case series studies from Colchester University Hospital, this retrospective consecutive case series was carried out to determine the long term effect on clinical and oncological outcomes of delaying the surgery beyond the traditional 6-8 weeks in the colorectal department of Colchester General Hospital.

3.1.1 Patient Selection

The number of patients in the thesis reflects the selection of patients considered suitable for long course CRT. The indication of long course CRT in this series was restricted to stage II-III MRI defined poor risk histologically confirmed locally advanced rectal adenocarcinomas originating within 15cm from the anal verge. Patients who were administered with palliative CRT were excluded. Features of poor risk were defined as below:

- T3 tumours with > 5 mm infiltration into perirectal fat,
- T4 tumours,
- multiple enlarged lymph nodes in the mesorectum,
- threatened or involved CRM

Involvement of or threatened CRM on histology (presence of tumour cells within ≤ 1mm of resection margin) is associated with higher positive predictive value (85%) for local recurrence (Quirke, et al. 1986). In addition CRM involvement is also a powerful predictor of both developing distant metastasis (HR-2.8) and poor survival (HR-1.7) (Nagtegaal and Quirke, 2008). Similarly T3 tumours extending more than 5mm extramurally into perirectal fat show poorer cancer specific 5 year survival than the tumours with 5mm or less of perirectal fat infiltration regardless of nodal status (54% vs. 85% 5 year survival rate, p value < 0.0001) (Merkel, et al. 2001). The role of MRI in rectal cancer staging has been discussed in section 1.8.1. High resolution MRI can predict CRM involvement with great accuracy (Mercury study group, 2006) and differentiate between good prognosis tumours (Taylor, et

al. 2011) from the one with poor features. Based on this evidence, MRI has become a gold standard for rectal cancer staging and hence this imaging modality was used to select poor risk patients eligible for long course CRT. All patients were assessed for eligibility of long course CRT in the multidisciplinary team meeting comprising of a surgeon, oncologist, radiologist and pathologist. All patients had the first staging MR scan before starting the long course CRT. Patients with metastatic rectal cancers at the time of diagnosis assessed by the CT of chest, abdomen and pelvis were excluded.

3.1.2 Treatment

Chemotherapy consisted of 240 mg/m²/day oral Tegafur–uracil (UFT) on days 1–28, given with leucovorin 90 mg/day or iv bolus 5-FU 300 mg/m2 days 1, 8, 15, 22, and 29 with leucovorin 20 mg/m². Chemotherapy was administered concurrently with two-phase, conformal, external beam radiotherapy of 45–50.4 Gy in 25–28 fractions over 5 weeks. Clinical reassessment was done at two week intervals from 4 weeks post completion of CRT with a second MRI scan, performed approximately 6–7 weeks after CRT completion to optimise the timing of surgery at maximal response. Clinical reassessment was done with the combination of clinical examination, rigid sigmoidoscopy and examination under anaesthesia. A CT scan of the chest, abdomen, and pelvis was also repeated to exclude the development of distant metastases. Following a multidisciplinary meeting review, the patients with documented down staging or no response proceeded to surgery. For the patients that appeared to show partial response had their surgery deferred and a third MRI was performed 4 weeks after the second (post CRT) MRI was performed to gain a maximal response. Selective patients who achieved complete clinical and radiological response were not immediately operated with their consent and were kept under strict follow up using Habr-Gama's "watch and wait" approach (Habr-Game, et al., 2004). "Watch and wait" included complete physical, digital and endoscopic rectal examinations every 4-8 weeks during the first year with a low threshold for the surgical intervention if any indication occurred. CT scan of chest abdomen and pelvis and MRI scan every 6 months during the first year and then

once yearly were also performed for such cases. If the complete clinical response was sustained for a year without recurrence then patients were considered complete responders and were kept under routine oncology follow up without surgery.

Total mesorectal excision (section 1.9.4.1) was performed laparoscopically in the majority of cases to assess the safety and feasibility of the laparoscopic approach in patients with rectal cancer who underwent long course CRT. The choice of a surgical procedure in the form of either anterior resection or abdominoperineal resection was at surgeon's judgement but a suggestion by multidisciplinary team meeting was also taken into consideration. All operations were carried out by experienced colorectal surgeons and their senior trainees under direct supervision. Operative and technical specifications were standardized (Motson, et al., 2011). Patients undergoing anterior resection were given bowel preparation a day before surgery. Patients received 20 mg of enoxaparin at 1800 on the day before surgery and 1.5 g of cefuroxime and 500 mg of metronidazole. Enhanced Recovery Programme based on the recommendations of enhanced recovery after surgery (ERAS) group (Fearon, et al., 2005) was standardized for all the patients undergoing elective surgical resections. Pneumoperitoneum was achieved using the standard Colchester technique with a blunt 5-mm reusable trocar placed in the right flank, which accommodated a 5-mm 30º laparoscope (Motson, 1994). A 5-mm port was placed in the epigastrium just to the right of the midline and a further 10-mm port was placed in the right iliac fossa. Another 5-mm port was placed in the left flank for retraction and splenic flexure mobilisation. The 10-mm right iliac fossa port was exchanged later for 10/12 disposable port (Ethicon Endosurgery, UK) if the bowel was to be transacted using an Endoscopic stapling device. Dissection was performed using the Harmonic Scalpel (Ethicon Endosurgery, Cincinnati, OH). Before any dissection, the small intestine was displaced into the right upper abdomen by a combination of lateral tilt and reverse- Trendlenburg position. A medial to lateral dissection was performed commencing at the sacral promontory and continued in a cranial direction toward the origin of the inferior mesenteric artery, which was divided using Laparoclips (Covidien, Gosport,

UK). The left ureter was identified. Dissection proceeded along the fascia of Toldt to the lateral peritoneal attachments and cranially toward the spleen. The inferior mesenteric vein (IMV) was preserved until the lateral dissection was completed and then divided between laparoclips. Where necessary the splenic flexure was mobilized via an approach through the lesser sac above the pancreas with dissection continued laterally to join up with the previous lateral dissection. Laparoscopic TME was performed down to the pelvic floor. Autonomic nerves were identified and preserved. The rectal tube was transacted using a linear stapler cutter (Ethicon Endosurgery). The segment to be resected was extracted through a 5–6-cm vertical transumbilical incision with a wound protector in situ (3M Steri-DrapeTM Wound Edge Protector). Postoperative adjuvant chemotherapy was administered to the patients based on the histopathological analysis of the resected specimens in the MDT meeting. Patients with involved lymph nodes or R+ resections received adjuvant chemotherapy after their recovery from the surgery.

3.1.3 Follow up

Patients were followed up postoperatively at 4-6 week for the detection of potential complications related to surgery such as wound healing and then every 3 months for the first year and then every 6 months for the detection of recurrent disease. Follow up included clinical examination, assessment of carcinoembryonic antigen, colonoscopy, and periodic radiological imaging by ultrasound study or CT/PET/MRI to detect local or distant recurrence. The follow up time interval was calculated from the date of diagnosis to death, last contact or date of conclusion of the study (21.03.2014 20:00 hours) whichever came first.

3.1.4 Outcomes measured

3.1.4.1 Survival end points

Overall survival (OS), disease free survival (DFS) and relapse free survival (RFS) were measured as survival end points. The ultimate goal of cancer therapies has been to lengthen

the overall survival. Moreover up to 80% of recurrences occur within 2 years of curative surgical resection for colorectal cancers (Waldron and Donovan, 1987). DFS has been increasingly used as survival end point in colon cancer trials.

3.1.4.2 Histopathological end points

The histopathological information was retrieved from the pathology database at the Colchester general hospital. All the reports contained a standard data set of pathology results including the information regarding the CRM involvement, attainment of curative resection (R0) and pathological complete response according to rectal carcinoma guidelines of Royal College of Pathologists (Loughrey, Quirke and Shepherd, 2014). These early histopathological endpoints were selected for the analysis because of their prognostic importance. R classification was used in this study to specify whether tumour was completely excised or not. Based on its prognostic importance, the R classification was adopted both into TNM classification of malignant cancers and the American Joint Committee on Cancer (AJCC) Manual for staging of cancer (Wittekind, et al., 2009). When a tumour was completely excised, it was classified as R0, that with residual microscopic disease at margins was classified as R1 and R2 included a tumour with macroscopic margin involvement. The presence of tumour at the resection margins greatly influences the outcome. Presence of residual tumour was associated with significant decrements in 5-year survival as demonstrated by Hermanek, et al. (1995) in their multicentre observation study of over 1100 patients of colorectal cancers (R0 - 55% (95% CI: 52-58%) vs. R1 and R2 - 7% (95% CI: 3-11%). In addition to these traditional definitions of residual disease, an alternative criterion for the residual disease with respect to CRM in rectal cancer has evolved. Studies have shown that CRM is valid and reproducible early endpoint that predicts both local regional and distant failure (Glynne-Jones, et al., 2006). Involvement of CRM (lateral or radial margin) margin has been demonstrated to be a single most prognostic factor in predicting local recurrence in rectal carcinoma (Adam, et al., 1994). Involvement of CRM or CRM positive defined as minimal distance between tumour and CRM ≤ 1 mm is associated

with high local recurrence rate in 85% of cases compared to low risk of 3% in CRM negative tumour with a minimal distance between the tumour and CRM > 1 mm (Quirke, et al., 1986). In addition, positive CRM after neoadjuvant chemotherapy is also associated with poorer 3 year-DFS rate of only 9% compared to 52% in CRM negative cases (Mawdsley, et al. 2005). A CRM of ≤ 1 mm was categorized as R1 resection in this thesis. Complete pathological response rate was defined as absence of viable tumour cells in a resected specimen (Smith, Waldron and Winter, et al., 2010) and has been shown to be a potential early surrogate endpoint for predicting DFS. A pooled analysis of 14 datasets comprising of total 3105 rectal cancer patients treated with chemoradiation and TME, demonstrated significantly improved disease-free and overall survivals for the patients with pathological complete response after chemoradiation (Mass, et al., 2010).

3.1.4.3 Operative Complications

Laparoscopic rectal surgery is considered to be a major undertaking due to confined space in pelvis, intimate anatomical relations of rectum and different nature of pathological tumour spread in both longitudinal and circumferential manner (Chand, et al., 2012). It gets more challenging after CRT due to distortion of tissue planes resulting from scarring and fibrosis in irradiated tissues (Motson, et al. 2011). As a result significantly higher complication rates are associated with rectal cancer surgery (Law, et al., 2007). In addition, development of postoperative complication adversely affects the long term oncological outcomes in terms of higher local recurrence and worse survival rates (Ptok, et al., 2007 and Law, et al., 2007). The study included in the thesis assessed the feasibility and quality of laparoscopic TME in locally advanced rectal cancer patients treated with neoadjuvant CRT in terms of R0 resection of distal margin and CRM, local recurrence rate and number of lymph node harvested in the resection specimen. Postoperative complications were recorded and categorized into major (Grade III or above) and minor complications (grade I-II) based on Clavien-Dindo classification for surgical complications (Dindo, Demartines and Clavien, 2004 Appendix - 1) to determine their influence on overall and disease free survival. This

classification system is the most widely used system worldwide to define and grade post-operative complication because it is simple, reproducible and flexible (Clavien, et al., 2009). Postoperative mortality was defined as death occurring during the hospital stay or within 30 days post-surgery (Clavien, et al., 2009).

3.1.5 Data collection and ethical considerations

Guidance on the ethical approval of this study was sought from the Colchester University Hospital Trust Research and Development department. It was advised that ethical approval of retrospective review of patient data was not required because of the historical nature of data (Appendix - 2). Instead, permission, guidance and advice were sought form the trust internal audit department to proceed with the study. NHS Data was accessed in line with the Trust policy respecting patient confidentiality and all the studies in the thesis were performed according to guidance of Helsinki Declaration as emended in Edinburgh Scotland in October 2010 (Sierra, 2011). Medical data was collected anonymously so that patients could not be identified by assigning linkage numerical codes to the each patient. A list of consecutive patients undergoing CRT for the study period was retrieved from the radiation physics department based at Essex County hospital. All the patients in the list were assessed against the eligibility criteria by referring to their oncological and surgical record accessed through respective databases. Further information regarding MR images, details of operation, histopathological outcomes, postoperative complications and follow up was gathered by looking into patient's medical record and radiological PACS and pathology databases.

3.1.6 Statistical analysis

Survival analysis requires for each patient a well-defined point in time when the patient is observed, and there must also be a well-defined end-point when the observation ends (Bradburn et al., 2003). Censoring relates to subjects who form part of a cohort but who never sustain the event of interest (Flynn, et al., 2012). All Standard definitions based on the

consensus of an expert panel proposed by Punt, et al., (2007) were used in this thesis as follows. All observations were censored at the dates of last follow-up of patient, end of the study or loss to follow up (section 3.1.3). Overall survival was defined as “time from the date of diagnosis to death from any cause”. Recurrence either local or distant and second primary cancer were not considered as events and ignored. Disease free survival was defined as “time from the date of diagnosis to any event, irrespective of the cause”. Recurrence, second primary same or other cancer and death from any cause were considered as events. Relapse free survival was defined as “time to any event except for second primary same or other cancers that were ignored”. Local recurrence was defined as evidence of recurrent tumour mass within the pelvis or in the perineum after a surgical resection (Bujko, et al., 2006). Recurrence anywhere else was considered as distant.

Descriptive and inferential analyses were performed using the computer program R (R Development Core Team, 2013). Descriptive statistics included gender, age, time interval to surgery after neoadjuvant L long course CRT, type of operation, laparoscopic vs. open surgery, ypCRM involvement, complete pathological response, complete response (radiological or pathological), R resection, adjuvant chemotherapy, post-operative morbidity and mortality (both 30 and 60 day), recurrence and median follow-up. Continuous variables were expressed either as means with standard deviation (SD) or medians with interquartile range (IQR) based on their normal distribution. Survival analysis was performed based on binary outcomes relating to any event that may be absent or present as describe in the above paragraph. The simplest and most widely used survival technique i.e. Kaplan-Meier survival curve based on the probabilities of occurrence of event at a certain point of time was used to calculate the estimates of overall survival, DFS and relapse free survival (Goel, Khanna and Kishore, 2010). The Kaplan-Meier technique is limited as it cannot calculate the actual effect size or adjust for potential confounders affecting survival outcomes (Flynn, 2012). Because of the retrospective and non-randomized nature of the study, it is prone to confounding variables that can limit the true estimate of survival times (Ho, et al., 2008). To

decrease this risk, a multivariate survival model, the Cox Proportional Hazard Model was used to estimate the effect of different covariates on all the three survival times. Covariates included were sex, age, time interval (weeks) to surgery after long course CRT, complete response minor and major complications, anastomotic leak, CRM involvement, lymph node involvement, ratio of lymph node involved, type of operation, adjuvant chemotherapy, local and distal recurrence. The significance of these covariates in adversely affecting the survival outcomes has already been discussed in the above sections (1.8.1, 1.9.4.2, 1.9.5 and 1.10). Survival analysis was performed by R package survival (Therneau, 2013), R package prodlim (Thomas, 2013), and R package rms (Harrell Jr, 2013). For a Cox Proportional Hazards Model approach to the analysis, the relative hazard for any two subjects should be independent of time, which is the proportional hazards assumption (Bellera, at al., 2010). This has been checked by plotting the Schoenfeld residuals against time and testing the Pearson-product moment correlation between the residuals and time for each of the covariates. This was achieved using function cox.zph from the R package survival. The proportional hazards assumption was met, therefore data were not transformed. The output form the Cox model was Hazard ratio (HR) where HR >1 indicated increased risk of an event associated with that covariate and HR <1 indicated a reduced risk for that covariate. The associated 95% confidence intervals were calculated to indicate statistical uncertainty of HR estimate (where it crossed 1, this indicated that there was no statistically significant difference).

Searches of Cox regression models for survival end points with time interval to surgery after neoadjuvant CRT were performed using the stepAIC function from the R package MASS (Venables and Ripley, 2002). This selected the covariates (lymph node involvement, time interval to surgery after long course CRT, major complications, CRM involvement and distal recurrence) using the Akaike Information Criterion (AIC). This allowed the subsequent comparison of the models with all the covariates and the selected covariates using the same data. The likelihood ratio test for comparing the models with selected covariates that include

and exclude time interval to surgery after long course CRT was also performed to determine whether the covariate ,time interval to surgery after long course CRT adjusted for selected covariates was statistically significant or not.

3.2 Methodology for MRI Textural Analysis Study

The role of MRI in staging and restaging after neoadjuvant CRT has already been discussed in sections 1.8.1 and 2.1.2 respectively. The trial by the Mercury group showed the tumour regression grade assessed by MRI post CRT correlates well with overall survival and DFS (Patel, et al., 2011). The main objective of this study was to determine whether textural features of rectal cancer on MRI can predict long term survival in patients treated with long course CRT. Texture analysis measures intra-tumour heterogeneity that has been shown to be associated with poorer prognosis secondary to intrinsic aggressive tumour biology or treatment resistance (Davnall, et al., 2012). From the data-set collected in the first thesis study, 56 consecutive patients during 01/2006 - 06/2011 time periods were selected to perform textural analysis. This time period was selected because of inconsistency of available imaging formats for the earlier patients and to achieve a minimum of a 3 year follow-up time period for all the patients. This enabled to calculate three year DFS that has been demonstrated as a valid surrogate for 5 year OS with coefficient of correlation >0.90 in a meta-analysis of 18 randomized trials involving 13000 patients with resectable colorectal cancer (Sargent, et al., 2005).

3.2.1 MR protocols and acquisition parameters

Clinical reassessment was performed twice-weekly from 4 weeks post completion of CRT with a second MRI scan, performed approximately 6–7 weeks after CRT completion to optimise the timing of surgery at maximal response. CT scans of the chest, abdomen, and pelvis were also repeated to exclude the development of distant metastases. Following a multidisciplinary meeting review, patients with no response or substantial down staging, a date for surgery was arranged. Those with partial response were further followed on and a

second MRI was performed 4 weeks after the first staging MRI, to assess if further response occurred. The scanner and acquisition parameters of MRI pose a technical challenge for clinical implementation of texture analysis as changes in these parameters lead to greater non-linear influence on signal intensity that could confound the texture analysis results (Davnall, et al. 2012). In order to avoid this, the series of patients selected for this study had their MRIs performed with the same GE Sigma Genesis 1.5-T (software version 9.0) whole-body system using a torso coil (phased array). All patients underwent the same imaging protocol without the intravenous contrast. The imaging protocol was based on the recommendations of 2012-consensus meeting of the European Society of Gastrointestinal and Abdominal Radiology in the clinical management of rectal cancer (Beets-Tan, et al., 2012). No bowel preparation or bowel relaxants were given. The imaging protocol included an initial localizing scan followed by a sagittal T2-weighted fast spin-echo (FSE) sequence scan to identify the primary tumour. Parameters for the sagittal T2-weighted FSE were recovery time (TR) 5020 and echo time (TE) of 89; field of view (FOV) 260mm; number of excitations (NEX) 4; and slice thickness/gap 4/1mm. An axial T2-weighted FSE sequence was used to image the whole pelvis from the iliac crest to the symphysis pubis to identify the pelvic side wall and nodal disease. Parameters for this axial sequence were TR/TE 3420/85; FOV 400mm; NEX 2; slice thickness/gap 5/1mm. An oblique axial T2 weighted FSE high-resolution sequence was performed with slices positioned perpendicular to the long axis of the rectal tumour to enable accurate tumour staging. The parameters of this high-resolution axial sequence are TR/TE 5200/95; FOV 200mm NEX 6; slice thickness/gap, 3/0.3mm with the number of slices dependent on the tumour size. Finally, an oblique coronal T2 weighted FSE high resolution sequence was performed perpendicular to the oblique axial sequence. In the context of low rectal cancer, additional oblique acquisitions were angled to the long axis of the anal canal to show the relationship of the levator ani muscles to the tumour to demonstrate the potential resection margin. The parameters of this sequence are TR/TE, 5000/93; FOV 200mm; NEX 6; slice thickness/gap 3/0.3mm with the number of slices dependent on the tumour size. All sequences used a matrix of 256/256. Acquisition times

were approximately 4.26 min for sagittal T2 weighted FSE; 3.26 min for axial T2 weighted FSE and 13.42 min for each of the high resolution series.

3.2.2 Image Interpretation

To limit intra-observer variations in interpretation, MRI scans were reviewed by two independent radiologists. Both the radiologists had more than 10 years' experience of gastrointestinal radiology reporting and were blinded to clinical outcomes to avoid measurement bias. The image interpretation was standardized and reporting was done according to a proforma formulated on the basis of validated and published criteria of the Mercury study group (Appendix – 3) (Patel, et al., 2011). The discrepancy in reporting was resolved by discussion and consensus when required. T staging of tumour at baseline and post treatment (ymrT) was based on interpretation of local extent of persistent tumour signal intensity relative to the layers of bowel wall on T2 weighted images (Patel, et al., 2011). Nodal stage baseline and post treatment was based on interpretation of lymph node size, morphology, border characteristics and signal intensity (Koh, et al., 2008). A node was regarded as positive if unequivocally larger than expected for a regional node, if the node was of abnormally rounded shape, if it had an irregular border or mixed signal intensity. Extramural vascular invasion was defined on MRI as intermediate signal intensity apparent within vessels with accompanying nodular expansion of the vessel or irregular vessel contour was recorded on baseline and post treatment MRI (Smith, et al., 2008).

3.2.3 MRI tumour regression grading (mrTRG)

Both the pathological and MRI based tumour regression grading were evaluated. For pathological tumour regression, the grading system described by Dworak, Keilholz and Hoffmann for rectal cancer in 1997 was used. This grading system was based on the amount of radiation induced fibrosis in relation to residual tumour and classified tumour regression into five grades shown in Table 3-1. This grading system is widely used in rectal cancer (Rödel, et al., 2005). Fokas, et al., (2014) revisited and assessed the prognostic

significance of pTRG based on Dworak system for the 386 patients treated in the land mark trial by the German study group (Sauer, et al., 2004). They were able to demonstrate that TRG along with ypN status were the only independent prognostic factors in predicting 10-year cumulative incidence of distant metastasis (p=.035) and DFS (p=.039). TRG based on similar principles of pathological TRG described by Dworak, Keilholz and Hoffmann (1997) has been validated and applied to MRI assessment of TRG by Patel, et al., (2011). Scans were reviewed to determine the degree of tumour replacement by fibrotic stroma. Table 3-2 details the MRI tumour regression grading system.

Table 3-1 Pathological tumour regression grading (Dworak, Keilholz and Hoffmann, 1997 and Fokas, et al., 2014)

Grade 0	No tumour regression
Grade 1	Minimal regression (Predominantly tumour with fibrosis in less than 25% of tumour mass
Grade 2	Moderate regression (Predominantly tumour with fibrosis in up to 50% of tumour mass
Grade 3	Good regression (Predominantly fibrosis)
Grade 4	Total regression (No viable tumour cells, only fibrotic mass)

Table 3-2 MRI based tumour regression grading (Patel, et al., 2011)

Grade 1.	Complete radiological response.	No evidence of treated tumour.
Grade 2.	Good response.	Dense hypointense fibrosis. Minimal residual tumor.
Grade 3.	Moderate response.	>50% fibrosis/mucin and intermediate signal representing residual tumour.
Grade 4.	Slight response.	Minimal fibrosis/mucinous degeneration, mostly tumour.
Grade 5.	No response.	Tumour has same appearance as baseline.

3.2.4 MRI length analysis/mr RECIST

Maximum tumour length was measured on sagittal images baseline and post treatment. Complete disappearance of tumour was defined as a complete response. Partial response to treatment was defined as at least a 30% decrease in tumour length, taking as reference the baseline tumour length. Progression of disease was defined as at least a 20% increase in tumour length, stable disease was defined as neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progression of disease (Machida, et al. 2008 and Therasse, et al., 2000).

3.2.5 MR Textural Analysis (MRTA)

MR images were retrieved from the radiology PACS system at the Colchester University hospital and burned onto encrypted discs in DICOM format. These discs were taken to the University College Hospital, London for the texture analysis. There are different methods for performing texture analysis but statistical based methods already discussed in details in section 2.1.4 are most commonly used for the analysis of medical images (Holli, et al., 2010) and hence this method was used for this study as well. These methods also produce higher discrimination indexes than the other methods (Castellano, et al., 2004). Statistical based methods measure intra-tumour heterogeneity by evaluating grey level intensity and position of pixels within the image. First order statistical features derived from the histogram analysis of pixel intensities within the region of interest were evaluated.

T2-weighted pre-treatment and 6-week post CRT MRI was used for MRTA. Regions of interest (ROIs) enclosing the largest cross-sectional area of rectal tumour area were manually delineated on the axial images. This was done under the supervision of a GI radiologist with 7 years' experience who was blinded to clinical outcome. The ROIs underwent textural analysis under the supervision of an imaging scientist with 9 years of experience in texture analysis using proprietary commercially available TexRAD research software (version 3.3, TexRAD Ltd www.texrad.com, part of Feedback Plc, Cambridge,

UK)(Yip, et al. 2014). MRTA comprised an image filtration-histogram approach where texture within the ROI was quantified following Laplacian of Gaussian (LoG) band-pass spatial scale filter (SSF) to highlight features ranging from 2mm (fine) to 6mm (coarse) in radius; 3mm-5mm in radius corresponds to medium-texture scales. Thus, band-pass image filtration was used to essentially extract and enhance image features of different sizes and intensity variation corresponding to fine, medium and coarse texture scales within the ROI delineating the rectal cancer. Histogram analysis comprised quantifying first-order statistics of mean grey level intensity, standard-deviation, and entropy, mean of positive pixels (MPP), kurtosis and skewness of the rectal ROI. An article by Miles, et al. 2013 has described the above parameters in detail (Table 3-3) and what these parameters mean in terms of image features i.e. reflect in varying degree - the number, intensity and variability of objects or features of high and low signal intensity in this study within the rectal cancer. These parameters have further shown to be associated with underlying histological features reflecting tumour heterogeneity such as solid cancerous tissue, necrosis, angiogenesis, hypoxia and fibrosis (Sieren, et al., 2011 and Ganeshan, et al., 2013), predicting response to neoadjuvant CRT (De Cecco, et al., 2015) and survival(Ng, et al., 2013 and Yip, et al. 2014) as a potential imaging biomarker.

Table 3-3 Definitions of histogram parameters (Miles, et al., 2013)

Parameter	**Definition**
Mean	The average value of pixels within the region of interest
Standard deviation (SD)	Measurement of dispersion from the mean value. A low SD indicates homogeneity of an image.
Skewness	Asymmetry of histogram. A negative value indicates long tail on the left and a positive value indicates the tail on the right side is longer.
Entropy	Irregularity of gray-level distribution
Kurtosis	A measure of flatness of the histogram. A positive value indicates more peaked histogram than a normal distribution and a negative value indicates flatter histogram than a normal distribution.

3.2.6 Follow up

Details of follow up are described in section 3.1.3.

3.2.7 Data analysis

Tumours were categorized into “favourable” and “unfavourable” responders to enable binary comparison by multivariate analysis. Based on known histopathological outcomes according to ypT stage, “favourable” mrT and ymrT stages were defined as stages T0, T1, T2 and T3a with “unfavourable” defined as mrT and ymrT stages T3b, T3c, T3d or T4. Stage T3a and T2 tumours have similar outcomes and therefore classified as “favourable” (Patel, et al., 2011). “Favourable” mrN, ymrN and ypN were defined as N0, while node positivity was unfavourable. “Favourable” mrEMVI, ymrEMVI was defined as no EMVI, while presence of EMVI was unfavourable. Favourable MRI tumour regression grade was defined as grades 1, 2 & 3 with unfavourable defined as stages 4 and 5. This binary division was chosen since Dworak pTRG 0-2 included patients with predominant tumour and minimal/no fibrosis whereas pTRG3-4 included patients with 50% or greater fibrotic stroma. Similarly favourable pTRG was defined as stages 2, 3 and 4 while unfavourable pTRG was defined as TRG stages 0 and 1. For length analysis partial response was categorised as favourable, while stable or progression of disease was unfavourable.

3.2.8 Statistical analysis

All Standard definitions of survival endpoints based on the consensus of an expert panel proposed by Punt, et al., (2007) were used and has already been discussed and explained in section 3.1.6. Univariate Kaplan Meier survival analysis was employed to identify which texture parameter predicted survival outcomes (OS, DFS and RFS) which further involved to identify the best "optimal" cut-off (via an iterative process) at which the good and poor survival patient groups are best separated (lowest p value from Log-rank test which assesses the difference between the Kaplan-Meier curves) for each parameter. P value of less than .05 was considered to be significant difference. Due to small numbers, significant

textural parameters yielding less than 10 patients per group for comparison were not reported and hence censored (Weiss, et al., 2014). Multi-variate Cox regression analysis (Forward-Wald) was used to determine which of the significant univariate textural markers, clinical, histological and radiological parameters were significant independent predictors of outcomes. Analysis was performed separately for pre and post-treatment variables. Statistical analysis was performed using R software (version 2.14.2; R Foundation for Statistical Computing, Vienna, Austria) and SPSS (version 20).

3.3 Methodology Integrated PET/MRI imaging biomarkers to predict histological tumour regression and 3 year DFS

3.3.1 Patient selection

This pilot study was a part of a wider tumour angiogenesis study investigating radiological-pathological and prognostic correlation across various cancers initiated by Institute of nuclear medicine at University College London Hospital (UCLH). Institutional ethical approval was obtained for this prospective study from the ethical committee of ULCH and Research and Development departments of the hospitals in East London and Essex County. Research activities were carried out by taking into account of ethical principles laid down in the Declaration of Helsinki as international guidance for research involving human subjects (Sierra, 2011). Patients with non-metastatic operable locally advanced rectal cancer stage II and III eligible for long course CRT were included in the study. Pre-operative imaging was performed as part of a wider PET/CT PET/MR cross validation study. Exclusion criteria were as below:

- Patients that are under 45.
- Patients allergic to CT/MRI dyes (contrast mediums)
- Patients that are unable to give informed consent
- Patients with severe claustrophobia
- Pregnancy
- Severe uncontrolled Diabetes

- Renal impairment with estimated glomerular filtration rate < 50 ml/min/1.73m2
- Contraindications of MRI such as cardiac pacemaker, metallic and cochlear implants
- Patients not able to undergo 2 scanning procedures

Patients were identified from the multidisciplinary meetings held in the local hospitals of East London and Essex Counties. Patients were then contacted either by telephone or in person at the time of their outpatient appointments. Informed consent of the patients was taken by explaining and giving them an information leaflet (Appendix 4). Patients, who were willing to participate after fully understanding the patient information sheet, were consented by signing the paper consent form (Appendix 5). PET and MRI safety questionnaires were filled in to rule out contraindications to MRI (Appendix 6 and 7). Patients were then booked to go for the preoperative integrated PET/MRI scanning at nuclear department of university college hospital, London.

3.3.2 Imaging protocol

3.3.2.1 Instrumentation

All scans were carried out on a fully integrated single gantry PET/MRI system (Siemens, Biograph mMR installed in 2012) in the Department of Nuclear Medicine at ULCH. MRI component comprised of state of the art 3-T magnet (63cm in length and 60cm in bore) and high performance whole body gradient system (159cm in length, 45mT/m and 200 T/m/s slew rate). The PET component setup between gradient and coils was equipped with compatible PET photodetectors (avalanche photodiodes) (Delso, et al., 2012). The transverse spatial resolution, sensitivity and axil FOV for the PET scanner was 4.3 at 1cm, 15.0 kcps/MBq and 25.8 cm respectively (Drzezga, et al., 2012).

3.3.2.2 Attenuation correction

Several different approaches have been used for attenuation correction in PET/MR images (Wagenknecht, et al., 2013) but the details of all of them are beyond the scope of this thesis. Attenuation correction has traditionally been performed by either doing transmission scan in

PET-only scanners or by CT part of PET/CT scanners and has already been described in section 2.2.2 . These traditional approaches to attenuation correction are not possible in integrated PET/MRI system. MR image intensities are based on proton density and T1 and T2 relaxation mechanisms (described in section 2.1.1.2) and have no direct correlation with photon attenuation in regard to ionizing radiation in CT or PET images (Wagenknecht, et al., 2013). For example, bone and air that cause highest and lowest attenuation in PET respectively do not contribute a MR signal (Hofmann, et al., 2008). The sequence-based attenuation correction approach was used in this study. Two-point Dixon fat- and water-weighted images were used for the segmentation of whole body MR images into four tissue classes: soft tissue, fat, lung and background (Al-Nabhani, et al., 2014). Lung regions were segmented as air in the inner part of the body through connected component analysis that groups pixel in the image into components sharing similar intensity values. Misclassified voxels such as air artifacts in pelvis (because of absence of signals in cortical bone) and in blood vessels including heart and aorta (because of blood flow) were corrected by application of spatial morphological closing filter (Martinez-Möller, et al., 2009). One known limitation of Dixon based attenuation correction is absent signal within cortical bone. As a result of that, bone tissues was not attenuated and regarded as soft-tissue. This could underestimate the SUVs either in lesions within bone or close to it such as brain tumours (Samarin, et al., 2012).

3.3.2.3 Workflow

Imaging was performed as part of a wider PET/CT and PET/MR cross validation study. All the scans were performed according to a protocol based on the guidelines by the European Association of Nuclear Medicine (EANM) (Boellaard, et al., 2010). The protocol included initial patient preparation that aimed to lower tracer uptake in normal organs such as muscle, bladder, heart and kidneys while enhancing uptake in the tumour tissue and keep radiation exposure as low as possible. Patients were asked to fast at least 6 hours before the scan and venous blood sample was taken to measure blood glucose levels ensuring a value

below 150 mg/dl. Patients were injected intravenously based on their body weight with F-FDG and then rested quietly avoiding unnecessary movements for approximately 90 minutes to allow pharmaceutical uptake and avoid active muscles uptake (Schelbert, et al., 1998). After the uptake period, patients were urged to urinate to reduce bladder activity and then positioned in the scanner. All patients underwent whole-body PET/CT first and then transferred onto the 3T PETMR scanner as soon as was practical. The standard PET/MRI protocol used in the study is shown in Table 3-4. After the application of phased array surface coil, MR sequence of approximately 20 seconds; coronal (2-point Dixon) 3-dimensional volumetric interpolated breath-hold examination (VIBE), for attenuation-correction purposes was acquired. This was followed by acquisition of diagnostic T2-weighted MR Sequences; small field of view, axial non-angled, turbo spin echo sequence centred on the rectal tumour was acquired. DWI images were also acquired using 3 b-values of 0, 400, and 800. ADC map was generated automatically from these on a pixel based basis. PET acquisition took place concomitantly and the PET data was reconstructed using ordered-subset expectation maximization with 21 subsets and 3 iterations and a Gaussian filter of 5 mm in full width at half maximum (pixel size 2mm; 2-mm slice thickness; 127 slices). PET data was acquired simultaneously for 5 minutes.

Table 3-4 Imaging protocols for integrated PET/MRI

	T2- Turbo spin echo (TSE)	**Diffusion weighted imaging**
Repetition time (ms)	4560	14726
Echo time (ms)	104	90
Echo train length	25	1
Section thickness	3 mm	5
Interslice gap	3.6mm	5
Field of view	~22cm	~32cm
Matrix	384x384	224x256
Fat suppression	No	Y
Bandwidth (Hz/Px)	200	1698
NEX	3	2
b-values		0, 400, 800

3.3.2.4 Data analysis

PET/MR images were analysed with a dedicated software workstation for display and interpretation of multimodality DICOM images (OsiriX MD v7.0) by a PET accredited radiologist with 4 years of experience in reporting oncological PETMR studies (Rosset, Spadola and Ratib, 2004). The observer was blinded to clinical outcomes of the patients. Tumour location and extent is confirmed by visual review of all the images acquired, and with fusion of the PET & MR dataset as required. Quantitative analysis was performed by measuring the SUVs derived by attenuation corrected PET images using a 3D spherical volume of interest. Two types of standardized uptake values were measured for correlation with response to treatment; maximum SUV ($SUV_{max)}$ and peak SUV (SUV_{peak}). SUV_{max} reflected the highest metabolic activity of the tumour (highest image pixel) within the 3D ROI and is the most commonly used value in response assessment studies. However being a relatively single pixel ROI, SUV_{max} is subjected to image noise leading to inaccuracies in its measurement and is thus less reproducible (Boellaard, et al., 2004). A more robust alternative measurement, SUV_{peak} has been proposed based on a circular volume of interest ($1cm^3$) with centred on the hottest part of tumour (Wahl, et al. 2009). Because of its larger size, SUV_{peak} avoids the image noise seen with SUV_{max}. ADC measurements were obtained using the single axial slice on the ADC map with the largest cross-sectional tumour dimension and expressed as mean (ADC_{mean}) and minimum (ADC_{min}) absolute values. Region of interest was drawn free-hand, carefully excluding areas of clear artefact as seen on the DWI images with high b value-800 (Schaarschmidt, et al., 2015)(Ippolito, et al., 2015).

3.3.3 Neoadjuvant chemoradiotherapy

All the patients included in the study have had long course CRT preoperatively delivered at their local hospitals. The CRT protocols differed slightly from one hospital to another as shown in Table 3-5.

Table 3-5 Long course chemoradiotherapy protocols

Hospital	Neoadjuvant radiotherapy (external beam) dose	Concurrent chemotherapy agent
Hospital 1	45 Gy in 25-27 fractions over 5 weeks	Capacitabine
Hospital 2	50.5 Gy in 28 fractions over 5 weeks	Capacitabine
Hospital 3	45–50.4 Gy in 25–28 fractions over 5 weeks	Tegafur–uracil (UFT)

3.3.4 Surgical technique

Total mesorectal excision was performed at the respective hospitals following neoadjuvant treatment and the choice of a surgical procedure in the form of either anterior resection or abdominoperineal resection was at surgeon's judgement but a suggestion by multidisciplinary team meeting was also taken into consideration.

3.3.5 Follow-up

Patients were followed up according to the local colorectal cancer surveillance protocols at their respective hospitals. The follow up time interval was calculated from the date of diagnosis to death, last contact or date of conclusion of the study (28.01.2016), whichever came first. The information regarding follow-up details including recurrences were gathered by carrying out physical visit to the respective hospitals and sitting together with the colorectal advanced nurse practitioners. With the help of the specialist nurses, information was retrieved from the local hospital cancer databases and MDT letters.

3.3.6 Histopathological Assessment

The histopathological information was retrieved from the respective institutional pathology databases. All the reports contained a standard data set of pathology results including the information regarding the CRM involvement. For the assessment of pathological tumour regression grades, lead consultants in the histopathology departments at respective

hospitals were contacted and requested to assess the tumour regression grades on the post-operative pathology specimens. Different classification systems are available to quantify tumour regression in rectal cancers but they are all based on a single reproducible parameter of the degree of tumour replacement by radiation induced fibrotic stroma (Thies and Langer, 2013). The classification systems used in this study differ between the patients because of the differential preference of the local histopathologists. Two systems were used in the study were Dworak (a five-tier system, TRG 0–4) (Dworak, Keilholz and Hoffmann, 1997) and 4 tier system endorsed by Royal college of pathologists (TRG 1-4) (Loughrey, Quirke and Shepherd, 2014) (Table **3-6**). In addition to a difference in the number of tiers, the Dworak grading system differed from the royal college of pathology because of the reverse order of its numerical scheme. For the statistical analysis, patients were divided into two groups of good and bad responders based on the tumour regression grades. Good responders were defined as Dworak TRG 3-4 and royal college of pathology TRG 1-2 while bad responders were defined as Dworak TRG 0-2 and royal college of pathology TRG 3-4.

Table 3-6 Tumour regression systems

Royal college of pathologist system	**Dworak system**
1. No viable tumour cells (fibrosis or mucus lakes only	0. No regression
2. Single cells or scattered small groups of cancer cells	1. Predominantly tumour with fibrosis <25% of tumour mass
3. Residual cancer outgrown by fibrosis	2. Fibrosis 25%-50% of tumour mass
4. Minimal or no regression (extensive residual tumour).	3. Fibrosis >50% of tumour mass
	4. No vital tumour cells detectable

3.3.7 Outcomes measured

The primary outcome measured was pathological tumour regression as described in the above section and secondary outcome measured was DFS. Disease free survival was defined as “time from the date of diagnosis to any event, irrespective of the cause”.

Recurrence, second primary same or other cancer and death from any cause were considered as events (Punt, et al. 2007).

3.3.8 Statistical analysis

Descriptive analysis was performed initially. Categorical variables were summarized and presented as frequency and percentages while mean ± SD were determined for continuous variables. Continuous variables including PET and DWI parameters were tested for their normal distribution using Kolmogorov-Smirnov and Shapiro Wilks W tests (Ghasemi and Zahediasl, 2012). Both the tests indicated that the functional imaging parameters (SUV_{max}, SUV_{peak}, ADC_{mean} and $ADC_{min)}$ were normally distributed. Thus the parametric independent sample t test (Sedgwick, 2012) was used to compare the means of functional imaging parameters for the two groups of histopathological responders (good vs. bed). The potential correlations between the functional PET features (SUV_{max}, SUV_{peak}) and DWI features (ADC_{mean} and ADC_{min} (reflection of highest tumour cellularity) was evaluated using Pearson's correlation coefficient (r) (Bewick, Cheek and Ball, 2003). Classification system by Salkin shows strength of correlation based on r values (r=0.8-1.0, very strong correlation; r=0.6-0.8, strong correlation; r=0.4-0.6, moderate correlation; r=0.2-0.4, weak correlation; r=o.o-2, no relationship (Grueneisen, et al., 2014). A p value was set at <0.05 to consider a significant difference. Univariate Kaplan-Meier survival analysis was employed to identify which clinical (age, sex), pre-treatment MRI (EMVI status and CRM involvement), histopathological (ypT stage, ypN stage, ypCRM involvement, ypEMVI status and pathological responders) and functional PET/MRI parameters (SUV_{max}, SUV_{peak}, ADC_{mean} and ADC_{min}) predicted DFS. Tumours were categorized into "favourable" and "unfavourable" responders to enable binary comparison (Patel, et al., 2011). Based on known outcomes according to ypT stage, favourable ypT stage was defined as stages T0-T2 with unfavourable defined as stages T3-T4. Favourable mrN and ypN were defined as N0, while node positivity was unfavourable. "Favourable" mrEMVI, ypEMVI was defined as no EMVI, while presence of EMVI was unfavourable. "Optimised Kaplan- Meier" analysis was employed via an iterative process to

identify the best "optimal" cut-off at which the good and poor survival patient groups are best separated (best p value) for the PET (SUV_{max}, SUV_{peak}) and DWI (ADC_{mean}, ADC_{min}) parameters. Differences between Kaplan-Meier curves were evaluated by using a log-rank test with a p value of less than .05 considered to indicate significant difference. Statistical analysis was performed using R software (version 2.14.2; R Foundation for Statistical Computing, Vienna, Austria) and SPSS (version 20).

4 Outcomes in a cohort of patients with delayed surgery after long course CRT

In this chapter, outcomes for the patients with MRI defined poor risk histologically confirmed locally advanced rectal adenocarcinomas treated with neoadjuvant long course CRT followed by delayed surgery are described. The details of the eligibility criteria, treatment, methodology and follow-up are discussed in section 3.1. Data was collected on demographics, treatment regime, operative data, complications, mortality and overall, disease free and relapse free survival. There were 112 patients identified on the basis of eligibility criteria. The basic characteristics of patients are shown in Table 4-1.

Table 4-1 Base line characteristics of patients (n=112)

Male Female	70 (63%) 42 (37%)
Age, median (IQR)	64 (58-71)
Time interval (weeks) to surgery after long course chemo-radiotherapy, median (IQR)	12 (10-14)
Anterior resection APR Hartman's TEMS Found inoperable at surgery No surgery	59 (52.6%) 32 (28.5%) 4 (3.5%) 3 (2.6%) 5 (4.4%) 9 (8%) (wait and watch- 4, disease progression-4, unfit for surgery-1)
Laparoscopic TME Laparoscopic converted to open Open TME	87 (84%) 11 (12.6%) 8 (8%)
R1 or R2 resection	8 (8%, ypCRM involvement-7, distal margin involvement-1)
Complete pathological response	19 (19%)
Complete response (clinical, radiological and pathological)	26 (23%)

Adjuvant chemotherapy	20 (20%)
Major complications	33 (32%)
Minor complications	13 (13%)
Complications type	
Anastomotic leak	14/59 (24%)
Perineal wound infection	6/32 (19%)
Surgical site infection	9 (9%)
Postoperative mortality	
30 day	3 (3%)
60 day	4 (4%)
Local recurrence	11/102 (10%)
Distal recurrence	25/102 (22%)
Median follow up, months (IQR)	41 (27-61)
Deaths recorded during follow up	43 (38%)
Median follow up for survivors ,months	56 (range, 19-100)

4.1 Surgical outcomes

The total number of patients treated with long course CRT with curative intent was 112. Different outcomes of the patients following CRT is shown in a flow diagram (Figure 4-1). Twelve patients did not have any surgery after CRT as a part of their initial management plan due to multiple reasons; 7 had complete clinical and radiological response and were treated with a wait and watch approach, 4 experienced disease progressions on repeat staging and one patient was deemed anesthetically unfit. The remaining 100 patients underwent planned surgery after neoadjuvant CRT. However 5 patients were found to be inoperable at the time of surgery either on laparotomy (n-3) or examination under anaesthesia (n-2). TME was carried out in 93 patients (laparoscopic-85, open=8). Two patients underwent TEMS. The median time interval to surgery was 12 weeks (IQR, 10 -14). Three further patients initially treated with wait and watch approach had to undergo local resection during the follow-up. One underwent TEMS on the basis of a suspicion of local recurrence but did not have recurrence. Two patients developed local recurrence after 20 and 30 months respectively and underwent successful laparoscopic TME.

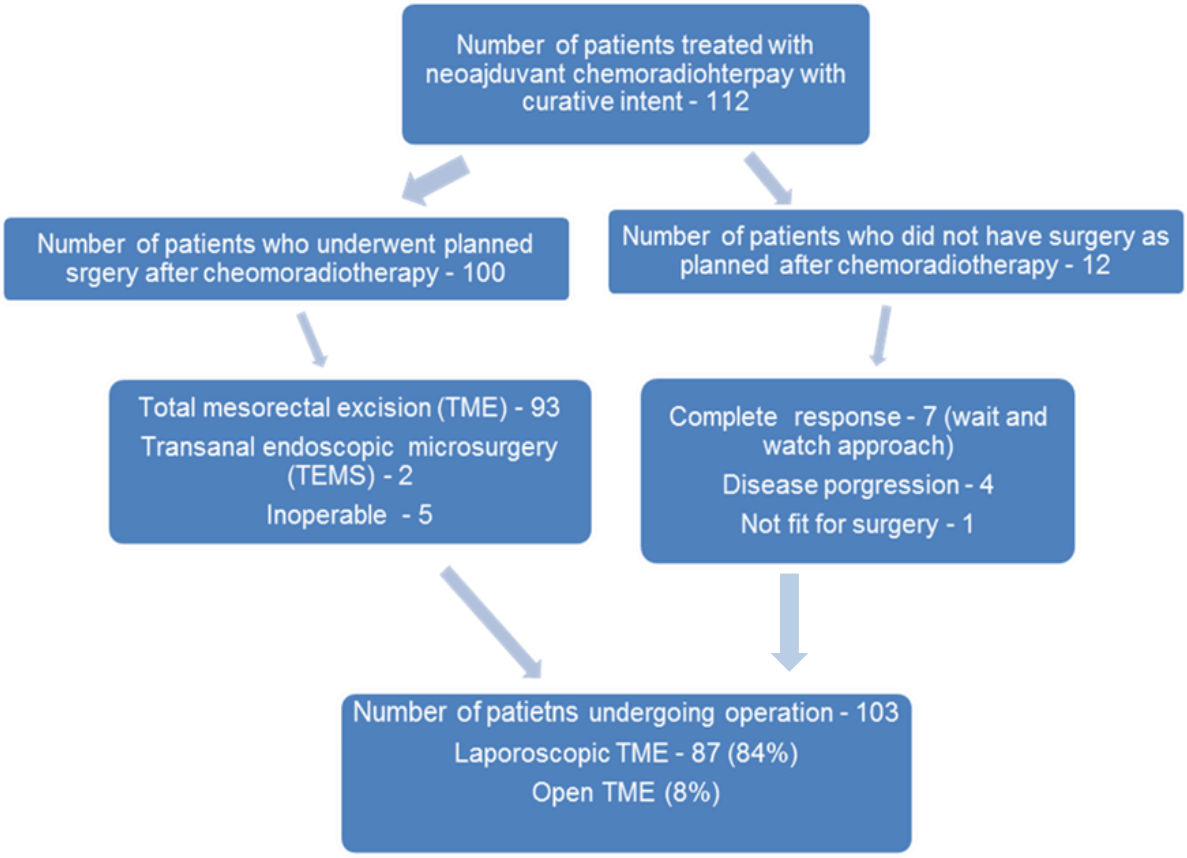

Figure 4-1 Flow diagram showing different outcomes of patients treated with neoadjuvant chemoradiotherapy

Surgical morbidity is quoted for these three patients as well along with the 100 patients undergoing planned surgery (n-103). Anterior resection was carried out in 59 cases (53%) while 32 patients (29%) underwent APR. The 30-day post-operative mortality was 2.9% (3 patients). This included one patient who had an anastomotic leak and died of multiple organ failure following re-operation; one patient who had a post-operative myocardial infarction and one patient who developed Adult Respiratory Distress Syndrome. An additional patient died within 60 days of primary surgery making the 60 day mortality rate 3.8%. This patient underwent laparotomy for small bowel obstruction and developed prolonged ileus and died of aspiration pneumonia. Major complications occurred in 32% of patients (33/103). These complications included those requiring interventions (surgical, radiological or endoscopic), life threatening complications requiring ITU support or death of patient. Minor complications occurred in 13% of patients. Surgical site infection (defined according to criteria of the

'Centres for Disease Control and Prevention' (Horan, et al. 1992) occurred in 15 patients (14%), 8 patients with incisional wound infection and 7 patients with intra-abdominal collections. Two patients with intra-abdominal abscess underwent laparotomy and wash out, three had radiological drainage and the remainder were treated with antibiotics. The anastomotic leak rate was 24 % (14/59). Seven patients with an anastomotic leak underwent surgical intervention and 1 patient had CT guided drainage. Four patients were managed conservatively (two of these patients were found to have a small asymptomatic leak on water soluble enema prior to reversal of defunctioning ileostomy). Two patients with a leak had an examination under anaesthesia and drainage of abscess cavity through rectum/vagina. One of these patients developed chronic pelvis sepsis leading to fasciitis of gluteal tissues resulting in fasciotomy but developed further sepsis and died. In addition to the 7 patients with an anastomotic leak requiring surgical intervention a further seven patients had further surgery within 30 days: 3 laparotomies for small bowel obstruction secondary to adhesions, 2 laparotomies and washouts for intra-abdominal collections, 1 haematoma and 1 wound dehiscence. The rate of perineal wound break down following abdominoperineal resection was 19% (6/32). Three patients developed rectovaginal fistula. Two were managed conservatively and one had a colostomy.

4.2 Histological outcomes

R0 resection was achieved in 92 % (90/98) of cases who underwent surgery with curative intent after long course CRT. Complete pathological response was seen in 19 such cases (19%).

4.3 Recurrence

Recurrence was calculated for the patients who either underwent a planned macroscopic complete resection (n-95) or patients with complete clinical response treated with a 'wait and watch' approach (n=7). The 10 patients that were excluded were; 5were inoperable at the time of surgery, 4 had disease progression on repeat staging and 1 was unfit for surgery. Overall recurrence was 31% (32/102). The local recurrence rate was 11% (11/102). The 5-

year cumulative local recurrence rate was 16% (Figure 4-2). The majority of local recurrences occurred during the first two years and no patient experienced local recurrence after 42 months. Distant metastasis was observed in 25% of cases (n=25/102)

4.4 Survival Outcomes

4.4.1 Kaplan-Meier survival analysis

A Kaplan-Meier overall, DFS and RFS survival time curves were estimated using data from 112 patients (Figure 4-3, Figure 4-4and Figure 4-5. The median follow up time was 41 months (IQR, 27 – 61) and the total number of deaths recorded was 43 (38.4%). The median follow up for the survivors was 56 months (range 19-100). The median OS and DFS were 94 (95% CI: 59.8 to NA) and 60 (95% CI: 36.5 to NA) months respectively. The recurrence-free time curve did not fall to the level required to allow the estimation of the median time to recurrence, and so this and its confidence limits were not estimated. The incidence of 5 year-OS, DFS and RFS was 58% (95% CI: 47 to 68), 49% (95% CI: 39 to 59) and 67% (95% CI: 59 to 78) respectively. All the survival curves flattened after 5 years indicating that the patients who survived to 5 years continued extended survival (Figures 4- 3, 4- 4 and 4- 5).

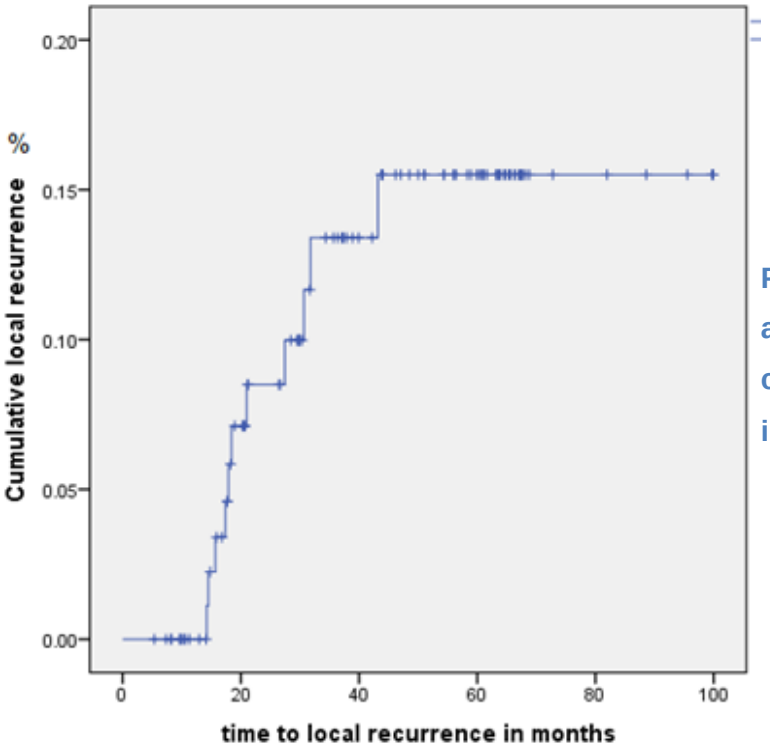

Figure 4-2 Cumulative absolute local recurrence per cent rate as a function of time in months following surgery

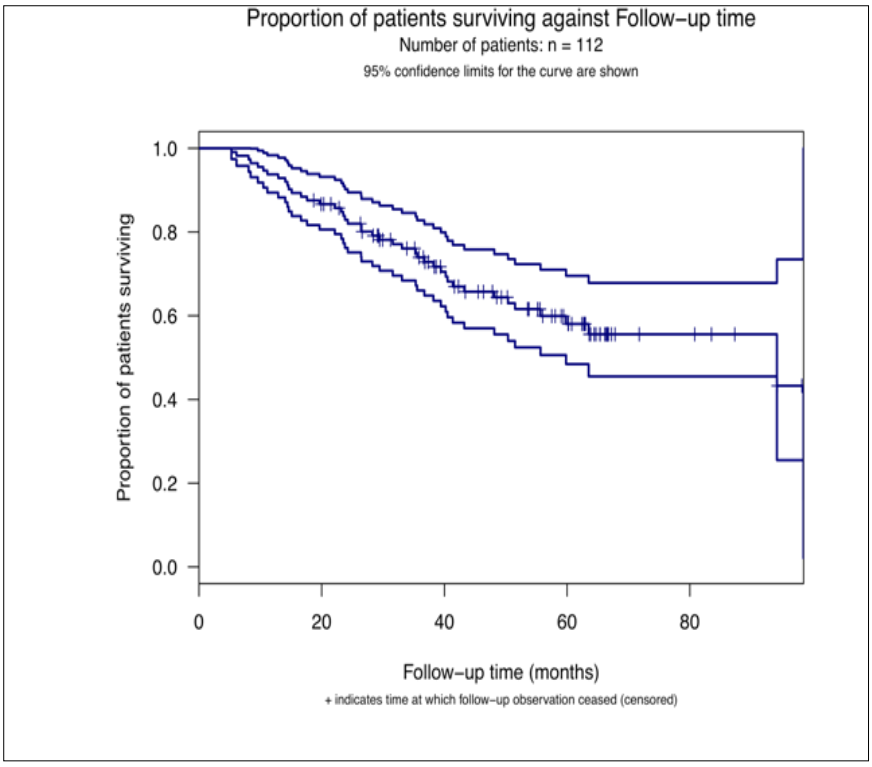

Figure 4-3 Overall Survival: proportion of patients at follow up

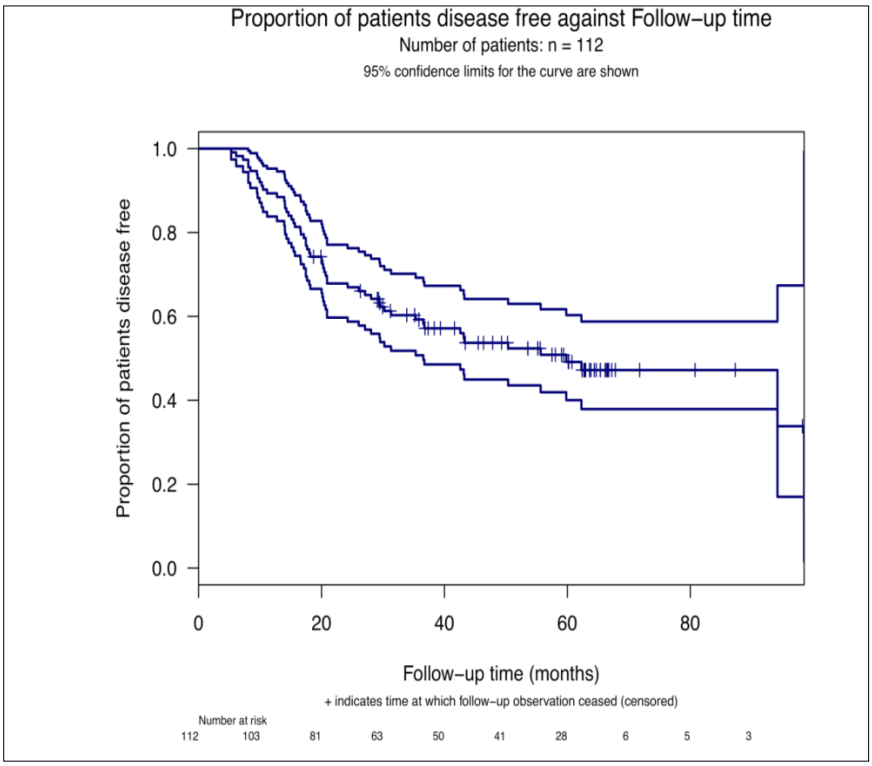

Figure 4-4 Disease free survival: proportion of patients at follow up

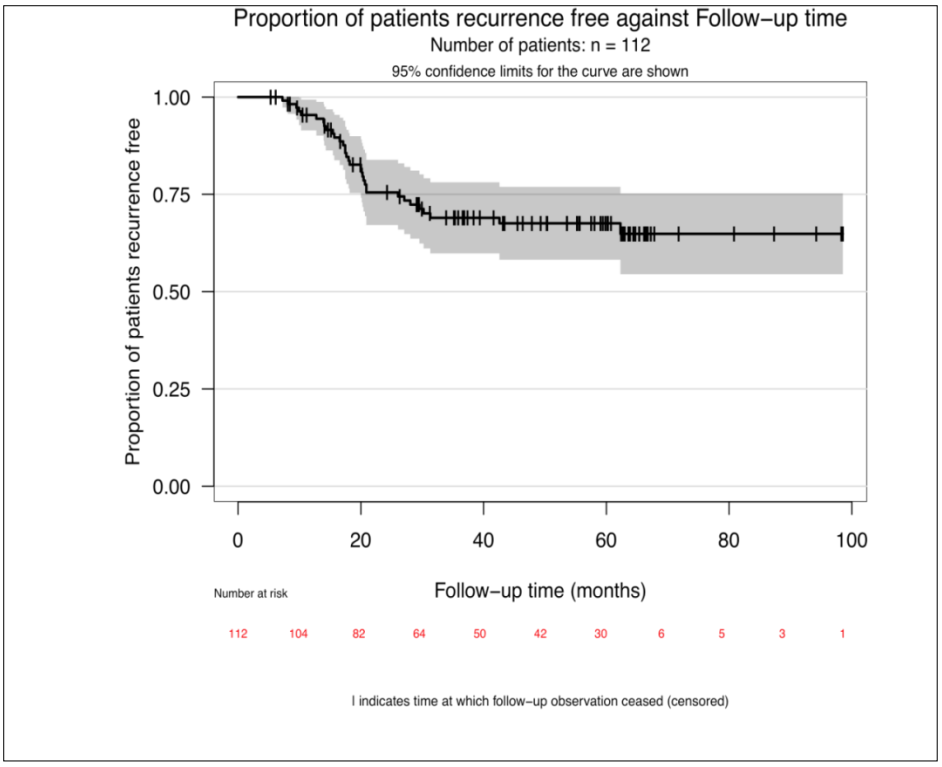

Figure 4-5 Recurrence free survival: proportion of patients at follow up

4.5 Cox proportional univariate predictor model with the covariate, time interval to surgery after long course CRT

There was complete case data for 97 of 112 cases for the survival analysis with the covariate, time interval as the only predictor. Fifteen were excluded because of different reasons (complete clinical response-7, disease progression-4, not fit for surgery-1, missing data-3). A Cox proportional hazards model was used to analyze the 97 cases. From the likelihood ratio test the covariate, time interval to surgery after long course CRT was not statistically significant for either of OS (p= 0.813), DFS (p= 0.506) and RFS times (p= 0.619). The hazard ratios estimated on a Cox proportional hazards model with time interval to surgery as the only predictor were 1.011 (95% CI: 0.923 to 1.107), 1.027 (95% CI: 0.951 to 1.109 p=0.499) and 1.024 (95% CI: 0.933 to 1.126 p=0.614) for OS, DFS and RFS respectively.

4.6 Cox proportional multivariate predictor models

From 97 above patients, a further 8 were excluded (inoperable-5, missing data-3) when defining the complete-cases data set for the survival analysis with all the covariates; lymph node involvement, time interval to surgery post long course chemo-radiotherapy, gender, age at diagnosis, minor complications, major complications, anastomotic leak, CRM involvement, complete response (pathological and clinical response), total number of lymph nodes yielded in a specimen, ratio of lymph nodes involved, type of operation (anterior resection, APR, Other), adjuvant chemotherapy, local and distal recurrence. The time interval to surgery did not correlate significantly with the survival outcomes on multivariate analysis as well (Table 4-2). The estimated hazard ratio for the time interval covariate, adjusted for all the other covariates, was 1.054 (95% CI: 0.914 to 1.215, p= 0.469), 1.090 (95% CI: 0.968 to 1.227, p=0.167) and 1.131 (95% CI: 0.938 to 1.363, p=0.196) for OS, DFS and RFS respectively. Among all the covariates, it was not surprising to find that only distal recurrence was significantly associated with worse survival outcomes for all three parameters (p value <0.001). Male gender showed statistically significant worse correlation with both DFS (HR 3.374, 95% CI: 1.294 to 8.796, p value= 0.013) and RFS (HR 8.983, 95% CI: 1.604 to 50.308, p value=0.013) while CRM involvement predicted worse RFS (HR 15.275, 95% CI: 1.702 to 137.052, p=0.015). On multivariate analysis, hazard ratio for the covariate 'complete response' showed beneficial effect on OS (HR 0.701) and DFS (HR 0.786) indicating that such patients were less likely to suffer an event at any given point in time but this relationship was not significant (Table 4-2).

Table 4-2 Estimated hazard ratios for Overall Survival, DFS and RFS with classical 95% confidence limits, with all the covariates, using the complete-cases data set n=89 with all the covariates

Predictor and category	Reference category	Overall Survival				Disease free survival				Recurrence free survival			
		Hazard ratio	95% confidence limits		p-value	Hazard ratio	95% confidence limits		p-value	Hazard ratio	95% confidence limits		p-value
			Lower	Upper			Lower	Upper			Lower	Upper	
Nodes involved [Yes]	[No]	3.169	0.503	19.962	0.219	3.438	0.527	22.443	0.197	0.349	0.030	4.096	0.402
Time interval to surgery		1.054	0.914	1.215	0.469	1.090	0.968	1.227	0.157	1.131	0.938	1.363	0.196
Sex [Male]	[Female]	2.398	0.835	6.884	0.104	3.374	1.294	8.796	**0.013**	8.983	1.604	50.308	0.013
Age		1.027	0.975	1.082	0.313	1.024	0.981	1.068	0.285	1.027	0.966	1.093	0.395
Minor complications[Yes]	[No]	1.035	0.274	3.913	0.960	1.236	0.364	4.200	0.734	0.607	0.112	3.289	0.562
Major complications[Yes]	[No]	2.162	0.642	7.278	0.213	1.990	0.731	5.421	0.178	0.868	0.121	6.243	0.888
Anastomotic leak [Yes]	[No]	0.910	0.236	3.516	0.891	0.927	0.279	3.080	0.901	1.159	0.163	8.257	0.883
CRM involvement [Yes]	[No]	3.297	0.591	18.409	0.174	3.264	0.681	15.648	0.139	15.275	1.702	137.052	**0.015**
Complete pathological and clinical response [Positive]	[No]	0.786	0.196	3.149	0.734	0.701	0.212	2.314	0.560	1.239	0.210	7.320	0.813

Total number of lymph nodes		0.966	0.858	1.087	0.563	0.972	0.859	1.099	0.647	1.144	0.941	1.391	0.177
Ratio of lymph nodes		0.040	0.000	268.159	0.473	0.004	0.000	62.679	0.261	19.721	0.001	436563.903	0.559
Type of operation													
[APR]	[Ant]	0.169	0.015	1.974	0.156	0.938	0.095	9.273	0.956	0.577	0.041	8.111	0.683
[Other]	[Ant]	0.171	0.006	5.273	0.313	0.346	0.013	8.894	0.521	0.012	0.000	1.409	0.069
Adjuvant chemotherapy [Yes]	[No]	0.858	0.260	2.829	0.802	0.498	0.156	1.588	0.238	0.212	0.032	1.393	0.106
Local [Yes]	[No]	1.479	0.330	6.636	0.609	1.503	0.464	4.864	0.496	5.367	0.898	32.071	0.065
Distal [Yes]	[No]	4.798	1.925	11.956	**0.001**	14.639	5.730	37.401	**0.001**	270.349	38.619	1892.554	**0.001**

4.7 Discussion

The main objective of the study was to determine the effect of delaying the surgery up to 12 weeks and beyond on the short and long term outcomes in locally advanced rectal cancer patients treated with CRT. The median time interval to surgery was 12 weeks. The surgical approach is this study was predominantly laparoscopic and up to 92% of TMEs were performed with this technique (Table 4-1). Laparoscopic surgery for locally advanced rectal cancer is complex and associated with considerable morbidity. Radiotherapy leading to tissue scarring and fibrosis make it more challenging to define tissue planes. More than 90% of patients had clear CRM in this series which is one of the most important prognostic factors in rectal cancer surgery as discussed in sections 1.8.1 and 2.1.3. Despite including patients with threatened CRM and cT4 lesions, the rate of clear CRM was almost similar to the reported results in COLOR II (93%) (van der Pas, et al., 2013) and COREAN trials (96%) (Kang, et al., 2010). Both these trials, as discussed in section 1.9.4.3, excluded patients with cT4 lesions and COLOR II trial also excluded the patients with threatened CRM. The rate of major complications (32%) in our series is in range of 25-37.6% reported in the other laparoscopic rectal cancer studies (Scheidbach, et al., 2002, Leroy, et al., 2004, Laurent, at al., 2007 and Lujan, et al., 2009). However the rate of anastomotic leak (24%) was higher than the reported rate of 6-17% in the aforementioned studies. This difference may be due to different population group as these studies also included patients with stage I cancers that did not require pre-operative CRT and excluded patients with T4 locally advanced rectal cancer. Male gender (17% vs. 8%, p=0.009), neoadjuvant radiotherapy (31% vs. 9%, p=value 0.005) and low anastomosis (24% vs. 4%, p <0.001) have been shown to be an independent risk factors for higher anastomotic leak rate in rectal cancer surgery (Matthiessen, et al., 2004). Morino, et al., (2003) analysed a consecutive series of 100 patients who had laparoscopic TME in the form of anterior resection for low and mid-rectal tumours. The indications of TME also included adenomas (13%) in addition to rectal cancer. Only 38 patients have had neoadjuvant CRT before undergoing TME. They reported

conversion rate of 12%, overall morbidity of 36% and clinical anastomotic leak rate of 17%. However in the sub-group analysis, leak rate was even higher for the patients treated with neoadjuvant CRT (21%, 8/38) than the patients without neoadjuvant CRT (12.5%). The higher leak rate in this cohort could be due fact that all the patients and majority (90%, 52/59) in the cohort have had neoadjuvant long course CRT and low anterior resection with defunctioning ileostomy respectively. Anastomotic leak rate has been mainly defined in the literature on the basis of clinical or symptomatic leaks ignoring those patients with subclinical or asymptomatic leak detected by contrast imaging in the absence of sign and symptoms (Matthiessen, et al., 2004). Using the same criteria and excluding 2 patients in our series with radiological leak detected on contrast enema prior to reversal of stoma, it could be acceptable to conclude the rate of symptomatic leak of 20% in our series which is line with the published data for the high risk group included in this series. One of the criteria for measuring the feasibility of the procedure is unplanned conversion rates from laparoscopic to open surgery. In this cohort, the conversion rate was 12.6% which is within the range (0-34%) of the most recent data in the meta-analysis comparing laparoscopic and open TMEs (Ohtani, et al., 2011). The conversion rates in the randomized COLOR II (van der Pas, et al., 2013) and COREAN (kang, et al., 2010) trials were 17% and 1% respectively.

The delay in the surgery did not lead to statistically worse survival outcomes. The hazard ratios for the time interval to surgery post CRT for OS, DFS and RFS were 1.011 (95% CI: 0.923 to 1.107), 1.027 (95% CI: 0.951 to 1.109 p=0.499) and 1.024 (95% CI: 0.933 to 1.126 p=0.614). The results indicate that with the help of serial MRIs, surgery can safely be delayed without compromising long term outcomes in selective patients who show partial response on initial repeat staging after CRT. The patients with partial response were further followed on and a second MRI was performed 4 weeks after the first staging MRI, to assess if further response occurred. In addition, with this approach, patients who achieve complete clinical and radiological response could avoid radical surgical resection and potentially be treated with either minimal invasive local excision such as TEMS or using Habr-Gama's

“watch and wait” approach. In this study, 9% of cases (n- 9/112) were treated with either wait and watch approach with the patients’ consent (n-7) or TEMS (n-2).

The incidence of 5 year-OS, DFS and RFS in this study was 57%, 49% and 67% respectively which is lower than that reported in other series (Table 4-3). The landmark trial by Saur, et al., (2004) demonstrated 5-year overall survival and DFS rate of 76% and 68% respectively in the pre-operative group receiving chemo-radiotherapy followed by surgery 6 weeks later . In the NSAB-03 trial, the 5-year OS and DFS were 74.5% and 64.7% respectively in the preoperative arm receiving chemo-radiotherapy (Roh, et al., 2009). In the trial by Park, et al., (2011), 5-year OS and DFS were 83% and 73% respectively. This discrepancy in the results among these randomized trials as well as between the current study could be attributed to differences in timing of surgery, definition of survival outcomes or adjuvant chemotherapy administration (Table 4-3). The definition of DFS in the German Cancer Study trial (Sauer, et al., 2004) matched our definition of RFS the incidence of which is similar to the results presented here. The time interval to surgery in these trials was up to 8 weeks compared to median time interval of 12 weeks in this study. Though, this extended time interval did not impact on the survival outcomes but there is a theoretical concern that tumours with poor response to CRT could regrow and repopulate within the time interval to surgery as demonstrated by Tarnawski, et al., (2002) in head and neck cancers. But this factor may not have any major impact on the outcome of this study. Firstly there is no convincing evidence on the re-growth of either primary irradiated tumours or lymph nodes in 10-12 week time interval for rectal cancer (Glimelius, 2014). Secondly, the patients with either no response or substantial down staging on the first staging MRI, were immediately operated and only the patients with partial response were further followed on with serial MRIs and clinically to assess if further response occurred. None of the other studies with few exceptions demonstrated any impact of time interval to surgery on survival. Long term results of the Lyon trial that randomized patients to have surgery either 2 weeks or 6-8 weeks after radiotherapy did not show any survival difference between the two groups

(Glehen, et al., 2003). Contrary to this Supitot, et al., (2006) analyzed 102 patients with $T_{2–4}$, N_{0-1}, M_0 rectal cancers that received preoperative RT in their institution and found that that an interval of more than 16 weeks between diagnosis and surgery correlated significantly with poorer OS (OR=2.59, p=0.005) and metastasis-free survival (OR=2.05, p=0.05) on univariate analysis but did not predict survival outcome on multivariate analysis. However in this study, radiotherapy without concurrent chemotherapy was given as neoadjuvant therapy whereas evidence suggests that neoadjuvant CRT is more effective than radiation treatment alone in achieving a better tumour response (Bosset, et al., 2006) which could overcome the prognostic importance of a long time interval to surgery after CRT. One noticeable difference as compared to the other studies shown in Table 5-3 was that only 20% of the patients received adjuvant chemotherapy in the thesis study. However, it was mandatory for all the patients in the neoadjuvant arm of the randomized trials. There is a limited data to support the advantage administering adjuvant chemotherapy in patients treated with neoadjuvant long course CRT. The evidence favoring this approach is primarily extrapolated from the proven benefit shown in the studies employing adjuvant radiotherapy in the management of locally advanced rectal cancer before the era of neoadjuvant combined modality treatment (Petersen, et al. 2012). A meta-analysis of four trials randomizing rectal cancer patients treated with neoadjuvant CRT to adjuvant chemotherapy did not demonstrate improvement in overall survival (HR 0.97, 95% CI: 0.81 to 1.17), DFS (HR 0.91, 95% CI: 0.77 to 1.07) or distal recurrences (HR 0.94, 95% CI: 0.78 to 1.14) (Breugom, et al., 2015).

The finding of a lack of detrimental effect of longer time interval on survival outcomes in this study is relevant form the point of view that by delaying surgery after CRT can lead to greater down staging due to time dependent response (Johnston, et al., 2009). Complete pathological response was seen in 19 patients (19%) and complete response (clinical and pathological) was seen in 26 patients (23%) in our study. This rate of pathological complete response is in keeping with that of a meta-analysis of 13 non-randomized and several retrospective studies comprising of 3584 patients (Petrelli, et al., 2016). This meta-analysis

demonstrated that pathological complete response increased from 14% in shorter interval group (< 8 weeks) to 20% in longer interval group (> 8 weeks) (RR 1.42 95% CI: 1.19–1.68, p< 0.0001). The attainment of higher rate of pathological complete response is an attractive end point because it has shown to be associated with improved survival in the pool analysis of several studies by Mass, et al., (2010). They demonstrated that the 5 year disease free survival was significantly higher for patients with pathological complete response than for the patients without pathological complete response (83.3% vs. 65.6% p <0.0001).The effect of pathological complete response on long-term outcome was not affected or modified by clinical T or N category, administration of adjuvant chemotherapy, distance from anal verge, or type of surgery. In this study the hazard ratio for the covariate, complete response was in the favour of OS (HR 0.701) and DFS (HR 0.786) indicating that such patients were less likely to suffer an event at any given point in time. However this relationship was not significant which could be due to small number of patients in this cohort.

The only covariates which were associated with worse survival other than local or distant failures were male gender and pathological CRM. On multivariate analysis, male gender was significantly associated with worse DFS and RFS (p=0.01) while CRM involvement was associated with RFS only (p=0.01) (). Survival disadvantage of male compared to female in colorectal cancer has also been observed in a large population based study where 5 year age adjusted survival was higher in women compared to men (64.5% vs. 61.9% p <0.0001) (Majek, et al., 2013). The prognostic importance of CRM involvement in rectal cancer was initially described in 1986 by Quirke; et al. and is associated with poor prognosis. In a prospective study by Adam, et al., (1994) local recurrences after a median 5-year follow up was significantly higher (78%) for patients who had tumour involvement of the CRM than for those without such involvement (10%).

Table 4-3 Outcomes comparison with other series for the patients with locally advanced rectal cancer treated with neoadjuvant long course chemo-radiotherapy followed by total mesorectal excision

Outcome measures	Colchester series (n-112)	German rectal cancer study (n-799) (Sauer, et al., 2004)	NSABP trial R-03 (n-254) (Roh, et al., 2009)	Korean trial (n-240) Park, et al., 2011)
Neoadjuvant therapy	45–50.4 Gy in 25–28 fractions + Tegafur uracil	50 Gy/28 fractions + fluorouracil	45 Gy/25 fractions with a 5.40 Gy boost + fluorouracil and leucovorin	50 Gy/25 fractions + capecitabine
Proportion of patients receiving adjuvant chemotherapy	Selective (20%)	Mandatory for all the patients	Mandatory for all the patients	Mandatory for all the patients
Time interval to surgery	12 weeks	6 weeks	Within 8 weeks	4-6 weeks
Length of follow up	42 months (range, 19-100 months)	45 months (range, 5 to 101)	8.4 years (range, 10.9 months to 12.9 years)	52 months
Post-operative complications	32%	36% (p=0.68)	25%	16%
Anastomotic leak	24%	11%	Not stated	4%
Complete pathological response	19%	2% (p<0.001)	15%	18/105 (17%)
5 year-OS	58%	76% (p=0.80, HR 0.96, 95% CI, 0.70 to 1.31)	74.5%	83% (p=.620)
5- year DFS	49%	68% (p=0.32, HR 0.87, 95% CI 0.67 to 1.14)	64.7 (p=.011, HR 0.629, 95% CI 0.439 to 0.902)	73% p=.865
5-year RFS	67%	Not stated	Not stated	Not stated
5-year cumulative incidence of local recurrence	16%	6% (p=0.006)	10.7% (*p*=.693, HR 0.86, 95% CI 0.41 to 1.81)	5% (p=.392)

4.8 Conclusion

This study clearly demonstrates that the delay in surgery after long course CRT does not lead to worse survival outcomes and achieves higher percentage of complete responder. But this did not translate into significant improvement in survival outcomes. Instead, distant failure was the single most significant factor that predicted worse OS, DFS and RFS. The incidence of short-term outcomes such as clear CRM and surgical morbidity in this high risk group was comparable to the series including low-high risk rectal cancer patients. This demonstrates the safety and feasibility of laparoscopic TME in locally advanced rectal cancer including patients with threatened CRM and T4 lesions.

5 MRI based texture parameters as potential imaging biomarkers for predicting long term survival in locally advanced rectal cancer patients

In this study the role of MR based textual analysis as prognostic imaging biomarker and an independent predictor of survival in patients with locally advanced rectal cancer was investigated. The details of methodology including patient selection criteria are described in section 3.2. The brief description of the methodology is given as below.

Consecutive patients with primary, non-metastatic, locally advanced rectal adenocarcinoma treated with long course CRT with curative intent from 01/2006 to 06/2011 in our institution were included. Textural analysis (TA) using a filtration-histogram technique of T2-weighted pre- and 6-week-post CRT MRI was undertaken using TexRAD, a proprietary software algorithm by manually delineating a region of interest around the largest tumour cross-sectional area. The filtration-step extracted features at different anatomical scales (fine, medium, and coarse) followed by quantification of statistical features (mean intensity, standard-deviation, entropy, skewness, kurtosis and mean of positive pixels – MPP) using histogram analysis. Univariate Kaplan-Meier analysis was used to assess the ability of the textural biomarkers, clinically employed radiological and histological features (TN-staging, EMVI, CRM involvement, tumour height from anal verge, pathological complete response, TRG and response evaluation criteria in solid tumours [RECIST]) to predict overall survival (OS), disease-free survival (DFS) and recurrence-free survival (RFS). Cox-multiple regression analysis determined which univariate markers were independent predictors of survival.

5.1 Baseline characteristics

Base line characteristics of patients are described in Table 5-1. The details of T-sub staging, nodal status, CRM involvement, EMVI status and TRG on pre and post treatment MRI scans are shown in Table 5-2. The study population consisted of 56 patients (34 male, 22 female) with median age of 64 ± 8.8. TME was carried out in 51 patients. Three patients were found to be inoperable at the time of surgery, one underwent TEMS and one patient has had disease progression on repeat staging. Complete pathological response (T0N0) was observed in 21% of patients (n-12). Overall recurrence was observed in 23% of patients (n-13). The rate of distal and local recurrence (defined in section 3.1.6) was 20% and 5% respectively. The median follow up for the entire cohort was 47.2 ± 18.2 months (range 6-87). Thirty six (36/50, 64%) patients were alive and censored when data was analysed. The median follow up for these 36 patients was 56±11.6 months (range 31-87). The mean overall survival was 65.7 months (95% CI, 57.9 -73.823) and 5 year cumulative survival time was 64%. The mean DFS and 5 year cumulative DFS was similar i.e. 60 months (95% CI, 51.2-69.219). The mean RFS time was 70.8 months (95% CI, 62.4 – 79.2). All the relapses occurred by 21 months at which time the cumulative survival time was 75%.

Table 5-1 Base line Characteristics of Patients

Gender Male Female	 34 (61%) 22
Age (median ± SD)	64±8.82
Time interval to surgery after completing long course chemo-radiotherapy (median ± SD) weeks	13±3.42
Operation Type Anterior resection APR Hartmann's TEMS	 33 (59%) 16 (28%) 2 (4%) 1 (2%)

Found inoperable at surgery	3 (5%)
No surgery (disease progression)	1 (2%)
Laparoscopic TME	47 (84%) (4 converted to open)
Open TME	4 (7%)
Height of tumour from anal verge(cm)	
>5	39 (70%)
<5	14 (25%)
ypCRM involvement	6 (11%)
yp T stage	
T0	14 (25%)
T2	14 (25%)
T3	20 (36%)
T4	4 (7%)
yp N stage	
N0	36 (64%)
N1	14 (25%)
N2	2 (4%)
Complete pathological response T0N0	12 (21%)
R0 resection	
Yes	46 (82%)
No	6 (11%)
yp tumour regression grade(0-4)	
0	3 (5%)
1	12 (21%)
2	10 (18%)
3	1 (2%)
4	14 (25%)
NA	4 (7%)
Not documented	12 (21%)
yp tumour regression grade	
Good responders (TRG 2-4)	25 (47%)
Bad responders (TRG 0-1)	15 (27%)
Adjuvant Chemotherapy	
Yes	11 (20%)
No	42 (75%)

Major post-operative complication Yes No	 17 (30%) 35 (63%)
Anastomotic leak Yes No	 6 (18%) 27
Overall Recurrence	13 (23%)
Local Recurrence	3 (5%)
Distal Recurrence	11 (19%)

Table 5-2 Pre and Post treatment Magnetic resonance imaging parameters

	Pre-treatment MRI	**Post-treatment MRI**
T0		3 (5%)
T1		2 (4%)
T2	4 (7%)	7 (13%)
T3a	3 (5%)	1 (2%)
T3b	11 (20%)	13 (23%)
T3c	12 (21%)	14 (25%)
T3d	10 (18%)	5 (9%)
T4	14 (25%)	9 (16%)
N0	14 (25%)	40 (71%)
N1	24 (43%)	12 (21%)
N2	16 (29%)	0
Circumferential resection margin (CRM) threatened	31 (55%)	24 (43%)
Median tumour height from anal verge (cm)	8.4	8.7
Tumour regression grade (mrTRG 1-5) 1 2 3 4 5		 6 (11%) 17 (30%) 13 (23%) 16 (29%) 2 (4%)
Tumour regression (mrTRG) Good responders (1-3) Bad responders (4-5)		 36 (64%) 18 (32%)

Complete responder T0N0		6 (11%)
Extramural vascular invasion (EMVI)		
Yes	14 (25%)	8 (14.2%)
No	40 (71%)	44 (79%)

5.2 Survival Analysis

5.2.1 Overall Survival

5.2.1.1 Pre-treatment variables

Pre-treatment MRTA was significant univariate markers of OS (best was MPP at fine texture-scale, p=0.008, Table 5.3). Amongst other pre-treatment MRI characteristics, positive mrEMVI status (p=0.017, Table 5.4) and threatened mrCRM (p=0.036, table 5.4) were significant univariate markers. The clinical variable, major complication also predicted worse OS (p=0.002) but as this was post-operative rather than pre-treatment or post-treatment factor, so was not included in multivariate analysis. Using multi-variate analysis, the pre-treatment textures (MPP at fine texture-scale, HR: 6.9, 95% CI: 2.43 – 19.55, p<0.001, mean at medium texture-scale, HR: 5.73, 95% CI: 1.62 – 20.21, p=0.007) and mrEMVI positive status (HR: 2.96, 95% CI: 1.04 – 8.37, p=0.041) were the only independent predictors of OS (Table 5.5, Figures 5.1-a, 5.1b and 5.1c respectively).

5.2.1.2 Post-treatment variables

Texture feature, skewness at fine texture-scale, was the only univariate marker of OS on post-treatment MRTA (p=0.034, Table 5.3). Positive ymrEMVI status (p=0.002, Table 5.4), threatened ymrCRM (p=0.027, Table 5.4) and poorer ymrTRG (p=0.002, Table 5.4) predicted worse OS. Among the histological variables, only ypCRM involvement (p=0.007, Table 5.4) predicted OS. On multivariate analysis, positive ymrEMVI status (Table 5.5, Figure 5.1-d) was the only independent predictor of OS (HR: 4.23, 95% CI: 1.41- 12.69, p=0.01)

5.2.2 Disease free survival

5.2.2.1 Pre-treatment variables

Similar to OS, pre-treatment MRTA was significant univariate markers for DFS. Best textural feature was mean at medium-texture (p= 0.007, Table 5.3 and Figure 4.2a). Amongst the other pre-treatment MR characteristics only threatened mrCRM was associated with poorer DFS (p=0.006, Table 5.4). On multivariate analysis, MPP at fine texture-scale (HR: 3.36 95% CI: 1.36 – 8.31, p=0.008), mean at medium texture-scale (HR: 4.53, 95% CI: 1.58 – 12.94, p=0.003), and threatened mrCRM (HR: 3.1, 95% CI: 1.01 – 9.46 p=0.046) were the only independent predictors of DFS (Table 5.5, Figures 5.2a, 5.2b and 5.2c respectively).

5.2.2.2 Post-treatment variables

Post-treatment MRTA was significant univariate markers of DFS (best was kurtosis at medium texture-scale, p=0.009, Table 5.3). Amongst other post-treatment MRI characteristics, positive mrEMVI status (p=0.017, Table 5.4), threatened mrCRM (p=0.019, Table 5.4) and mrTRG (p=0.02, Table 5.4) showed significant association with DFS. The only histopathological parameter showing association with DFS was pathological complete response (p=0.035, Table 5.4). On multivariate analysis, Kurtosis at medium texture-scale (HR: 3.97, 95% CI: 1.44 – 10.94, p=0.007) and ymrCRM involvement (HR: 3.36 95% CI: 1.21 – 9.32, p=0.02) were the only independent predictors of DFS (Table 5.5, Figures 5.2d and 5.2e respectively).

5.2.3 Relapse free survival

5.2.3.1 Pre-treatment variables

Similar to OS and DFS, pre-treatment MRTA was significant univariate markers for RFS as well (Table 5.3). Amongst the texture features best were standard deviation and entropy at coarse-textures (p=0.011) and MPP at fine and medium-textures (p=0.011). Amongst the pre-treatment MR characteristics, threatened mrCRM (p=0.016, Table 5.4) showed

significant association with worse RFS. Using multivariate analysis, texture parameters of MPP at fine texture-scale (HR: 8.90, 95% CI: 2.39 –33.13, p= 0.001) and kurtosis at medium texture-scale (HR: 7.78 95% CI: 2.08 - 29.05, p=0.002) were the only independent predictors of RFS (Table 5.5, Figures 5.3a and 5.3b respectively).

5.2.3.2 Post-treatment variables

Post-treatment MRTA was also a significant univariate markers of RFS (best was entropy at coarse-texture, p=0.002, Table 5.3). Amongst the histopathological parameters, ymrN-Stage (p=0.024, Table 5.4), ypCRM involvement (p=0.009, Table 5.4) and pathological complete response (p=0.034, Table 5.4) were significant predictors of RFS. On multivariate analysis, only texture parameters, entropy at coarse texture-scale (HR: 8.6, 95% CI: 1.89 – 39.86, p=0.005) and kurtosis without filtration (HR: 4.27, 95% CI: 1.40- 13.03, p=0.01) were the only independent predictors of RFS (Table 5.5, Figures 5.3c and 5.3d respectively).

Table 5-3 MR Textual analysis-Significant parameters predicting OS, DFS, and RFS on univariate analysis

Textural parameter	Filter value	Threshold value	Number of patients above and below the threshold value		Mean Survival	95% Confidence interval	P value
Overall Survival: Significant pre-treatment texture parameters							
Mean	3	<-8.26000	Poor	27	45.42	38.49-52.35	0.03
			Good	29	72.87	62.53-83.21	
MPP	2	<63.73500	Poor	17	40.78	29.28-52.28	0.008
			Good	39	72.23	63.53-80.93	
	3	<75.29500	Poor	19	43.51	32.54-54.48	0.029
			Good	37	71.63	62.70-80.56	
	4	<82.32500	Poor	22	45.60	35.83-55.36	0.019
			Good	34	74.32	65.64-83	
Overall Survival: Significant post-treatment texture parameters							
Skewness	2	>0.33500	Poor	36	38.96	27.30-50.62	.034
			Good	18	65.71	55.09-76.33	
DFS: Significant pre-treatment texture parameters							
Mean	2	<-3.54000	Poor	25	39.20	30.63-47.76	0.031
			Good	31	68.61	57.38-79.85	
	3	<-8.26000	Poor	27	38.24	30.14-46.34	0.007
			Good	29	71.31	60.04-82.59	
	4	<-14.94500	Poor	27	39.49	31.24-47.74	0.027

			Good	29	69.29	57.75-80.82	
	6	<-37.01500	Poor	28	40.23	32.11-48.35	0.043
			Good	28	68.65	56.81-80.48	
MPP	2	<64.45000	Poor	18	37.14	25.38-48.91	0.022
			Good	38	66.87	56.66-77.09	
	3	<75.29500	Poor	19	38.78	27.20-50.35	0.045
			Good	37	66.35	55.97-76.74	
	4	<84.73000	Poor	24	40.32	30.20-50.44	0.022
			Good	32	69.74	59.17-80.31	
	5	<93.57000	Poor	28	42.21	32.76-51.66	0.047
			Good	28	69.68	58.48-80.88	
	6	**<**102.46000	Poor	28	42.21	32.76-51.66	0.047
			Good	28	69.68	58.48-80.88	
Skewness	2	<0.21000	Poor	28	42.35	33.22-51.48	0.044
			Good	28	69.28	57.78-80.78	
DFS: Significant post-treatment texture parameters							
MPP	2	>69.58500	Poor	37	43.04	34.91-51.17	0.032
			Good	17	74.01	61.17-86.86	
Skewness	2	>0.33500	Poor	18	38.96	27.30-50.62	0.034
			Good	36	65.71	55.09-76.33	
Kurtosis	3	<-0.11000	Poor	20	36.87	28.50-45.25	0.042
			Good	34	65.73	54.49-76.97	
	4	<-0.42500	Poor	18	34.73	25.96-43.52	0.009

			Good	36	67.04	56.34-77.75	
RFS: Significant pre-treatment texture parameters							
Mean	3	<-870000	Poor	26	43.44	35.13-51.75	0.0169
			Good	30	80.75	72.34-89.17	
	4	<-14.94500	Poor	27	44.10	36.01-52.19	0.026
			Good	29	80.47	71.77-89.17	
Standard Deviation	0	<39.73000	Poor	21	47.36	35.99-58.73	0.032
			Good	35	77.69	68.85-86.54	
	2	<137.50999	Poor	38	64.17	52.90-75.44	0.034
			Good	18	64.54	58.73-70.35	
	4	<151.41000	Poor	28	50.57	39.79-61.35	0.018
			Good	28	80.71	72.28-89.14	
	5	<164.86499	Poor	32	61.52	48.81-74.22	0.017
			Good	24	63.29	57.59-69	
	6	<162.29000	Poor	31	50.88	40.70-61.07	0.011
			Good	25	82.72	74.92-90.52	
Entropy	0	<5.18500	Poor	33	52.67	43.14-62.19	.034
			Good	23	81.74	72.66-90.82	
	4	<6.33500	Poor	32	61.52	48.81-74.22	0.016
			Good	24	63.29	57.59-69	
	5	<6.34500	Poor	32	61.52	48.81-74.22	0.016
			Good	24	63.29	57.59-69	
	6	<6.34500	Poor	31	50.88	40.70-61.07	0.011

			Good	25	82.72	74.92-90.52	
MPP	2	<63.04500	Poor	16	42.82	29.42-56.22	0.011
			Good	40	77.33	69.02-85.63	
	5	<118.22000	Poor	31	50.88	40.70-61.07	0.011
			Good	25	82.72	74.92-90.52	
	6	<99.05000	Poor	27	47.93	38.13-57.73	0.019
			Good	29	80.56	71.95-89.16	
Skewness	2	<0.45500	Poor	38	50.89	42.91-58.87	0.037
			Good	18	84.08	75.48-92.68	
Kurtosis	4	<0.09500	Poor	17	40.17	30.92-49.43	0.047
			Good	39	76.45	67.56-85.34	
RFS: Significant post-treatment texture parameters							
Standard deviation	5	<128.57999	Poor	18	56.21	39.42-73	0.018
			Good	36	60.95	54.61-67.29	
	6	<158.10500	Poor	30	61.13	48.31-73.95	0.021
			Good	24	63.53	57.38-69.69	
Entropy	3	<6.17500	Poor	28	61.62	48.32-74.92	0.042
			Good	26	61.85	55.13-68.56	
	4	<6.18000	Poor	26	57.96	44.17-71.75	0.005
			Good	28	64.11	58.67-69.54	
	5	<6.15000	Poor	25	56.73	42.60-70.87	0.003
			Good	29	64.27	59.03-69.50	
	6	<6.1600	Poor	24	55.41	40.93-69.89	.002

			Good	30	64.42	59.37-69.46	
Kurtosis	0	>0.75000	Poor	17	45.55	32.89-58.21	.034
			Good	37	76.17	67.11-85.23	

Table 5-4 Clinical, MR and histopathological parameters significantly predicting OS, DFS an RFS on univariate analysis

Parameters		n=	**Mean OS (95% CI)**	**p-value**	**Mean DFS (95% C1)**	**P-value**	**Mean RFS(95% CI)**	**P-value**
Clinical parameters								
Age	<65 years	29	50.76 (42.85-58.66)	.271	46.32(37.08-55.57)	.444	51.79(42.56-61.02)	.150
	≥65 years	27	69.34 (58.06-80.62)		63.53(51.07-75.98)		77.14(66.89-87.39)	
Sex	Female	22	52.45 (44.17-60.72)	.633	47.14 (37.22-57.06)	.711	52.06 (42.15-61.97)	.360
	Male	34	65.72 (56.57-77.59)		61.76(50.18-73.33)		74.29(64-84.58)	
Adjuvant chemotherapy	Positive	11	55.95(42.76-69.14)	.628	52.41(36.44-68.38)	.948	52.41(36.44-68.38)	.321
	Negative	42	69.17(60.34-77.99)		62.72(52.64-72.80)		72.54(63.27-81.81)	
Major complication	Positive	17	39.96(30.67-49.24)	**.002**	35.76(25.72-45.80)	.007	42.73(31.94-53.52)	.132
	Negative	35	77.41(70.06-84.76)		71.35(61.53-81.17)		74.27(64.75-83.8)	
Anastomotic leak	Positive	6	49.92(34.96-64.88)	.174	47.60(28.82-66.37)	.280	52.73(38.49-66.98)	.795
	Negative	27	74.87(65.04-84.69)		70.18(58.41-81.94)		72.40(61-83.79)	
Pre-treatment MRI parameters								
mrT Stage	MrT1-T3a	7	62.37(53.19-71.553)	.256	54.10(38.48-69.71)	.493	54.10(38.48-69.71)	.989
	mrT3b-T4	47	65.37(56.51-74.20)		60.46(50.61-70.31)		70.66(61.44-79.89)	
mrN stage	mrN0	14	54.388(43.34-65.43)	.799	47.96(35.59-60.33)	.714	55.67(43.89-67.45)	.895
	mrN1&2	40	66.66(57.35-75.97)		62.12(51.51-		69.93(59.97-	

					72.72)		79.90)	
mrEMVI status	Positive	14	46.11(33.66-58.57)	**.017**	42.85(28.91-56.79)	.097	51.55(36.84-66.27)	.221
	Negative	40	72.56(63.91-81.22)		65.87(55.65-76.08)		73.49(64.16-82.82)	
Height	<5cm	14	46.81(37.95-55.67)	.315	41.58(30.61-52.54)	.226	50.36(39.59-61.13)	.973
	≥5cm	39	66.96(58.78-75.14)		65.19(54.75-75.64)		71.37(61.55-81.18)	
mrCRM status	Clear	23	77.56(67.68-87.44)	.**036**	76.93(66.50-87.36)	**.006**	82.42(74.27-90.58)	**.016**
	Threatened	31	52.18(43.85-60.51)		44.02(34.40-53.65)		51.41(41.38-6.45)	
Post-treatment MRI parameters								
ymrT stage	ymrT1-T3a	13	64.30(58.42-70.19)	.056	57.36(47.32-67.40)	.194	60.21(51-69.41)	.306
	ymrT3b-T4	41	62.95(53.41-72.43)		58.11(47.41-68.81)		67.89(57.50-78.27)	
ymrN stage	ymrN0	41	70.78(61.74-79.83)	.171	65.03(54.91-75.15)	.278	75.42(66.56-84.28)	.024
	ymrN1&2	12	49.94(39.02-60.86)		42.87(28.50-57.24)		42.87(28.50-57.24)	
ymrEMVI status	Positive	8	38.30(23.85-52.74)	**.002**	33.26(18.27-48.25)	**.017**	46(27.62-64.40)	.236
	Negative	44	72.47(64.18-80.76)		66.14(56.38-75.90)		73.22(64.28-82.15)	
Height	<5cm	13	48.39(39.29-57.49)	.661	42.72(31.06-54.39)	.445	49.50(38-61)	.895
	≥5cm	38	66.97(57.31-76.63)		62.97(52.24-73.71)		70.89(60.85-80/93)	
ymrCRM status	Clear	29	76.08(67.09-85.06)	**.027**	71.40(60.26-82.54)	.**019**	75.79(65.57-86)	.141

	Threatened	24	49.80(40.01-59.59)		43.44(32.82-54.07)		52.72(41.54-63.90)	
mrTRG status	mrTRG1-3(Good responders)	36	63.98(58.05-69.91)	**.002**	57.82(49.83-65.81)	**.022**	61.55(54.06-69.04)	.205
	mrTRG 4-5 (Bad responders)	18	50.31(35.86-64.76)		47.08(31.51-62.64)		62.21(45.39-79.04)	
mrRECIST tumour response	Partial response	36	69.40(59.98-78.81)	.319	62.12(51.21-73.03)	.625	71.96(61.83-82.10)	.417
	Stable disease	16	51.32(38.69-63.95)		47.99(34.09-61.90)		53.99(40.50-67.49)	
Histopathological parameters								
ypT stage	ypT0-T2	13	64.30(58.42-70.19)	.056	57.36(47.32-67.40)	.194	60.21(51-69.41)	.306
	ypT3-T4	41	62.92(53.41-72.43)		58.11(47.41-68.81)		67.89(57.50-78.27)	
ypN stage	ypN0	36	73.02(64.21-81.82)	.126	67.67(57.31-78.04)	.142	74.24(64.70-83.78)	.138
	ypN1-2	16	48.91(38.37-59.45)		42.84(30.63-55.04)		47.10(34.62-59.59)	
ypCRM involvement -	Clear	46	72.30(64.33-80.26)	**.007**	66.19(56.79-75.58)	.058	73.92(65.33-82.51)	**.009**
	Threatened	6	35.15(23.16-47.14)		30.61(16.21-45.02)		30.61(16.21-45.02)	
Complete response (ypT0N0M0)	Positive	12	82.45(70.91-93.98)	.073	82.45(70.91-93.98)	.035	All cases censored	.034
	Negative	40	56.23(49.43-63.02)		49.43(41.18-57.68)			
pTRG	Good responder (pTRG 2-4)	25	72.81(61.73-83.90)	.934	71.63(59.7-83.55)	.949	82.22(73.74-90.70)	.159
	Bad responder(p0-1)	15	61.21(51.24-71.18)		58.37(46.26-70.48)		58.37(46.26-70.48)	
pTRG	Complete	14	77.81(63.96-91.65)	.354	77.81(63.96-	.301	All cases	.072

	response (pTRG 4)				91.65)		censored	
	Incomplete or no response	26	60.36(52.79-67.92)		57.40(48.09-66.72)			

Table 5-5 Parameters significantly predicting OS, DFS and RFS on multivariate analysis

Pre-treatment multivariate analysis				
Survival endpoints	**parameters**	**p-value**	**Hazard ratio**	**95% confidence interval**
OS	Mean (SSF-3)	0.007	5.73	1.62 – 20.21
	MPP(SSF-2)	<0.001	6.9	2.43 – 19.55
	mrEMVI status	0.041	2.96	1.04 – 8.37
DFS	Mean(SSF-3)	0.003	4.53	1.58 – 12.94
	MPP(SSF-2)	0.008	3.36	1.36 – 8.31
	mrCRM status	0.046	3.1	1.01 – 9.46
RFS	MPP(SSF-2)	0.001	8.90	2.39 – 33.13
	Kurtosis(SSF-4)	0.002	7.78	2.08 - 29.05
Post-treatment multivariate analysis				
OS	ymrEMVI status	0.01	4.23	1.41-12.69
DFS	Kurtosis(SSF-4)	0.007	3.97	1.44– 10.94
	ymrCRM status	0.02	3.36	1.21 – 9.32
RFS	Entropy(SSF-6)	0.005	8.6	1.89 – 39.86,
	Kurtosis(SSF-0)	0.01	4.27	1.40- 13.03

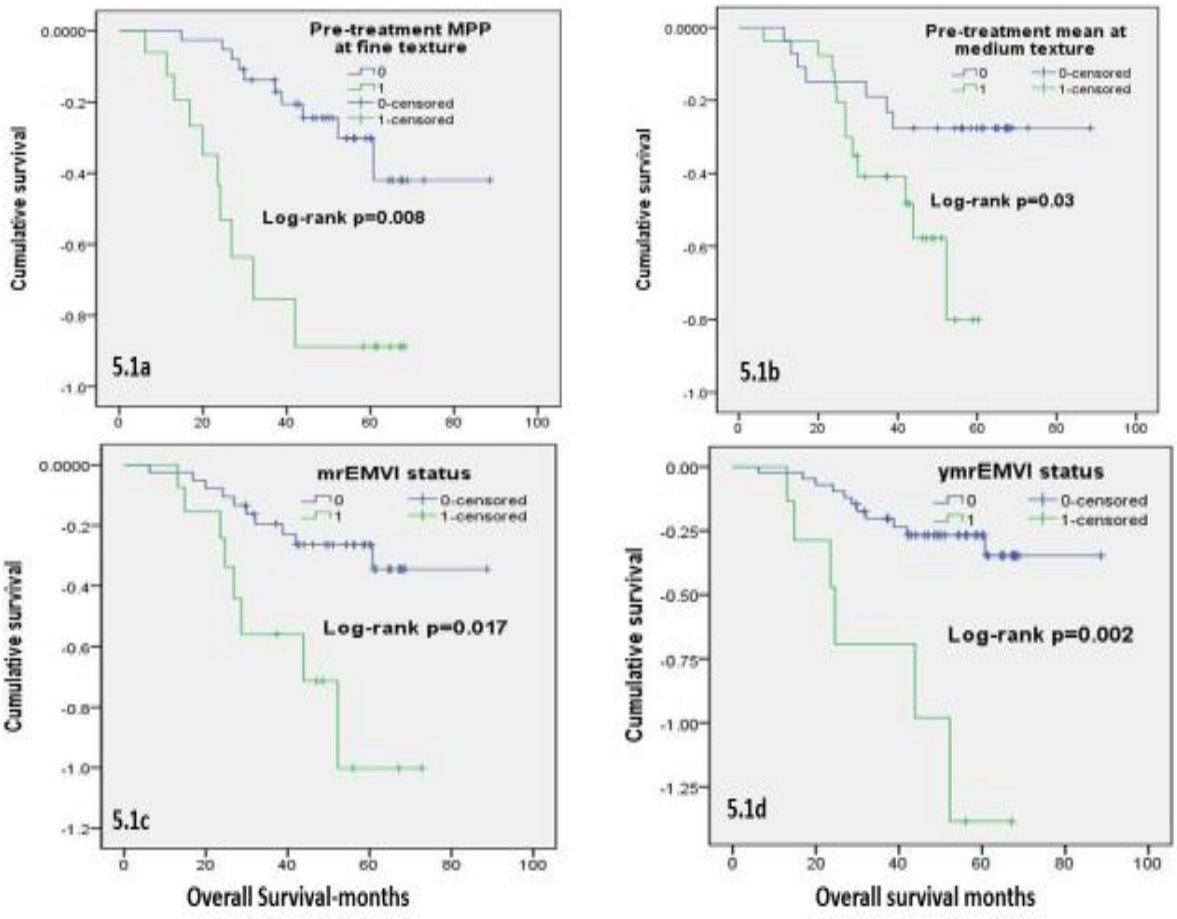

Figure 5-1 Kaplan-Meier curves show a significance difference in survival for (a) pre-treatment mean positive pixel (MPP) at fine texture (b) pre-treatment mean at medium texture (c) mrEMVI status and (d) ymrEMVI status with their log- p values.

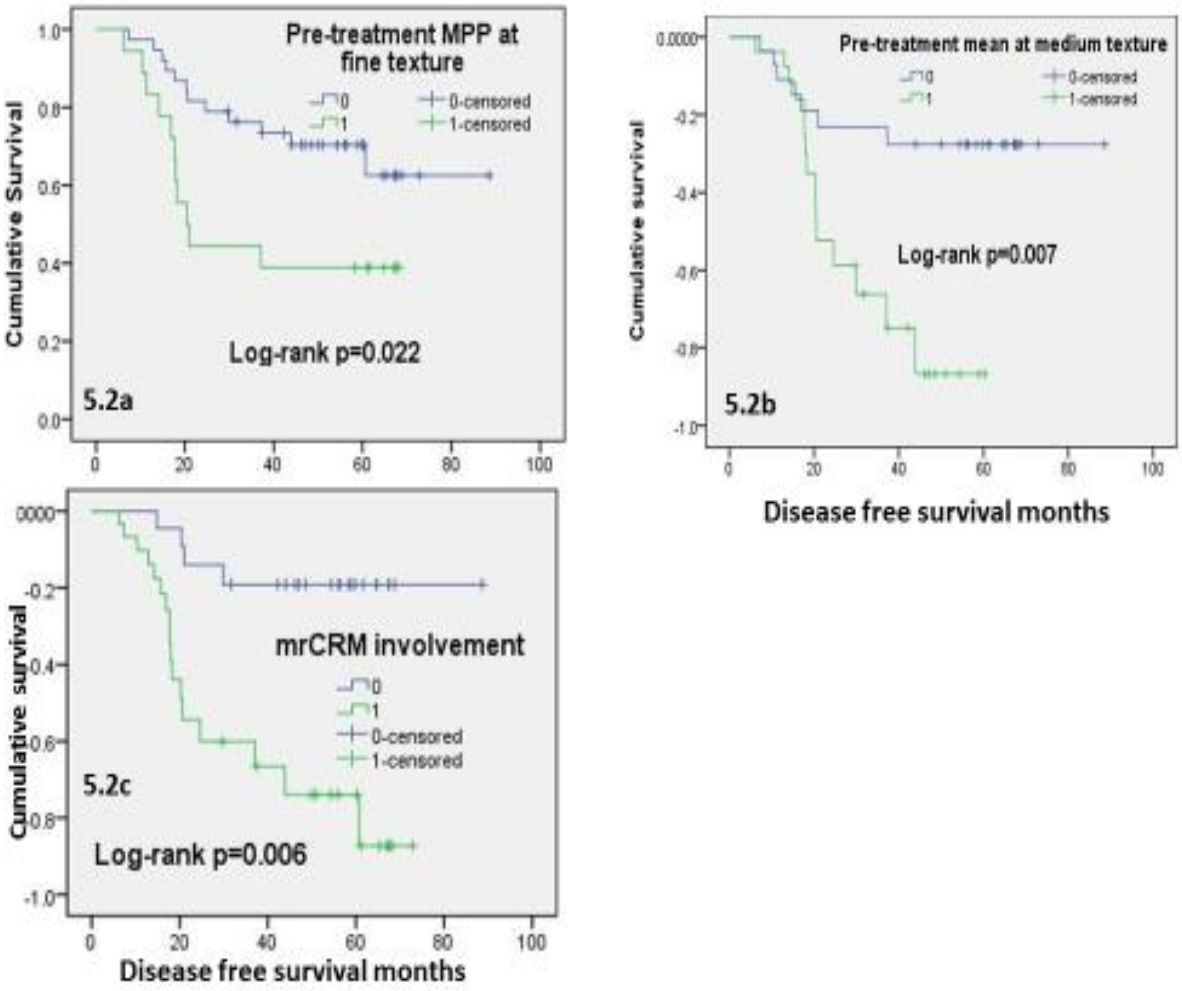

Figure 5-2 Kaplan-Meier curves show a significance in DFS for (a) pre-treatment mean positive pixel (MPP) at fine texture (b) pre-treatment mean at medium texture (c) mrCRM involvement

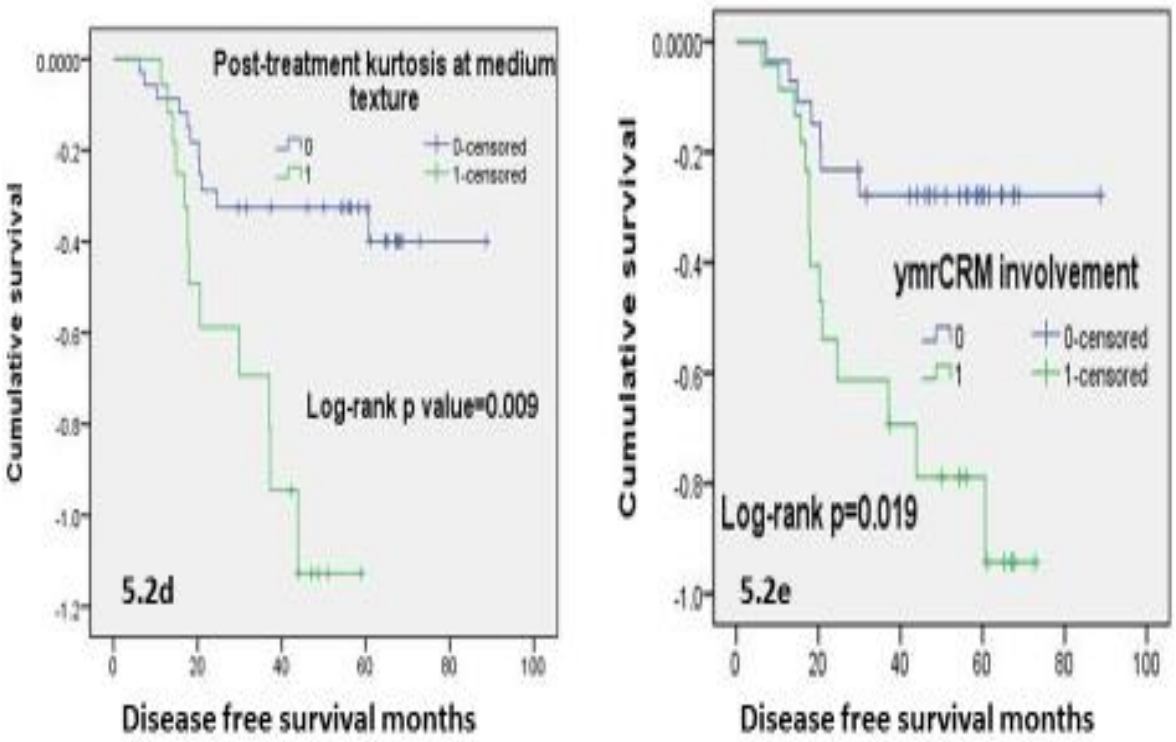

Figure 5- 2 Kaplan-Meier curves show a significance in DFS for (d) post-treatment kurtosis at medium texture (e) post-treatment ymrCRM involvement

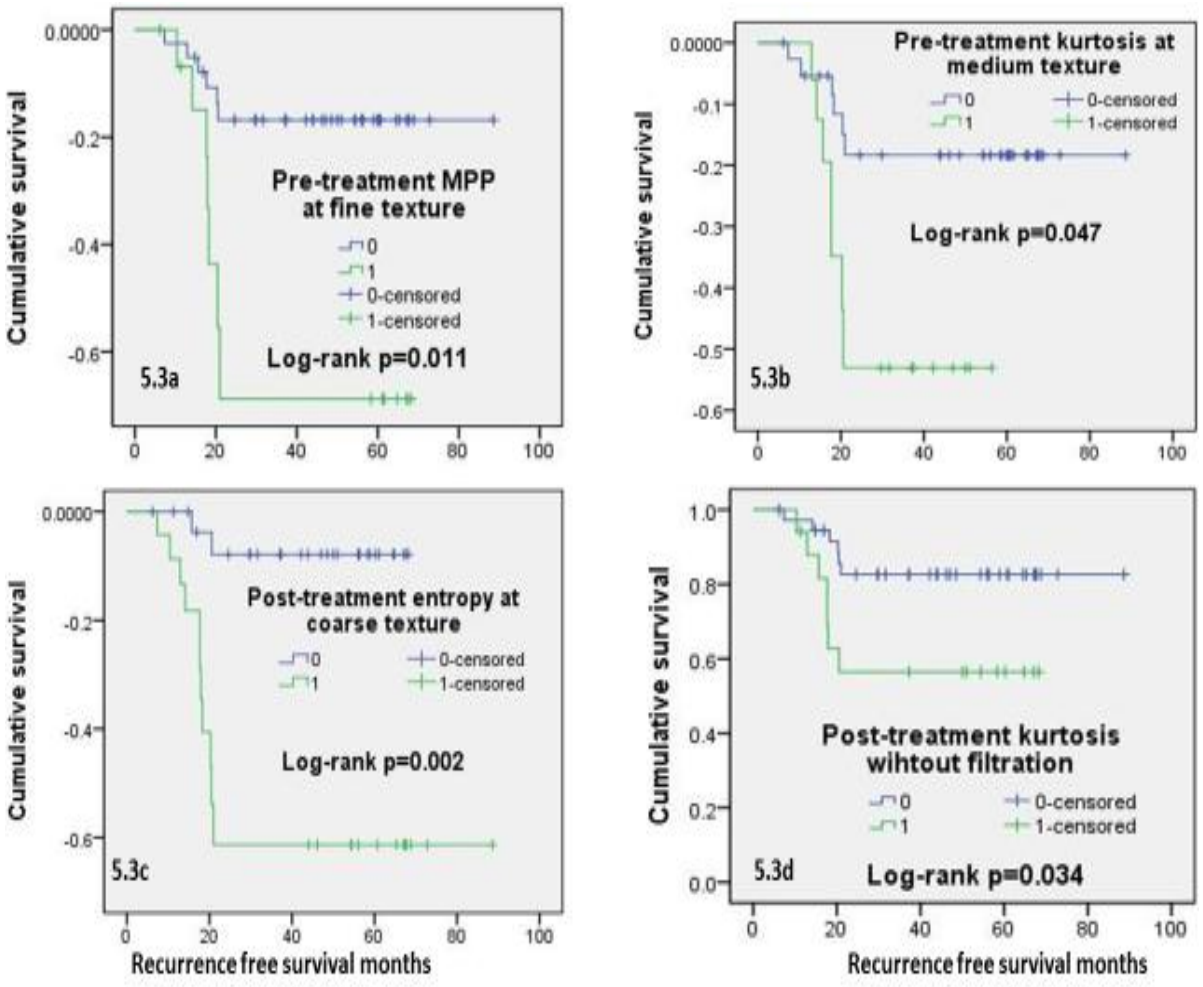

Figure 5-3 Kaplan-Meier curves show a significance difference in recurrence free survival for (a) pre-treatment mean positive pixel at fine texture (b) pre-treatment kurtosis at medium-texture (c) post-treatment entropy at coarse-texture and (d) post-treatment kurtosis without filtration

5.3 Discussion

This is the first study to assess the prognostic significance of texture variables in addition to morphological MRI and pathology findings in rectal cancer undergoing chemo radiotherapy. The results of this study demonstrate the potential use of MRTA on T2w images to predict survival outcome. On pre-treatment MRTA; lower MPP at fine-texture was the independent predictor for all three survival outcomes, negative and lower mean at medium-texture was independent predictor of OS and DFS and Kurtosis at medium-texture was independent predictor of RFS only. Intra-tumour heterogeneity has been attributed to various factors such as hypoxia, necrosis, angiogenesis and genetic variations (Nelson, et al., 2004 and Russnes, et al., 2011). Both hypoxia and necrosis reflect increase numbers of dark tumour regions which tend to give negative mean (Miles, et al., 2013). The negative mean correlating with worse outcomes in this study suggests highlighted dark areas of rectal tumours exceeding the number of hyperdense bright areas reflecting angiogenesis MPP considers only pixels greater than 0 and reduces the impact of dark areas on the mean histogram value. MPP has been correlated negatively with hypoxia in colorectal cancers exhibiting K-RAS mutations (Ganeshan, et al., 2012). Lower than threshold MPP values in predicting inferior outcomes are consistent with possibility of predominance of hypoxic areas in rectal cancer rather than angiogenic in this study. The role of hypoxia in rendering solid cancers resistant to radiotherapy and its strong association with cancer propagation and hence poorer survival outcome is well established (Hockel, et al., 1996).

For RFS none of the variables other than texture features predicted worse RFS on both pre and post-treatment multivariate analysis. On pre-treatment analysis MPP along with lower kurtosis at medium texture and on post-treatment analysis lower entropy at coarse texture along with higher kurtosis without filtration were independent predictors. For DFS lower '-ve kurtosis' at medium texture along with ymrCRM involvement independently predicted DFS. Kurtosis is inversely related to the number tumour areas being highlight by filter irrespective

of whether the opposing areas are dark or bright but increases in the presence of greater intensity variations in highlighted areas (Miles, et al., 2013). Lower –ve kurtosis at medium texture would reflect highlighted areas of same attenuation at this texture scale such as hypoxia and necrosis. The finding of lower kurtosis at medium texture predicting poorer DFS and RFS on post-treatment MRTA may suggest more focal radiation induced inactive fibrosis which has previously been associated with inferior outcomes in lung cancers (Takahashi, et al., 2011). There is only one study in the literature that assessed the relationship of contrast enhanced CT texture features with OS in colorectal stage I-IV cancer (Ng, et al., 2013). In this study, it was demonstrated that texture features reflecting less heterogeneity were associated with poorer survival and the author attributed the findings to hypo-vascularity (Ng, et al., 2013). Somewhat similar findings in our study could be hypothesized to lack of vascular enhancement in these tumours. Heterogeneity at medium and coarse texture scale in colorectal cancer has been attributed largely to angiogenesis (Ganeshan, et al., 2012). Because MR-scans in this study were without contrast, less heterogeneity could be hypothesized to lack of vascular enhancement in these tumours. However, the diverse relations between textural features and survival outcomes in this study would reflect dissimilarities in the associations between angiogenesis and hypoxia among NSLC, CRC and rectal cancer alone.

Pre- and post-treatment MR EMVI status was independent predictors of OS on multivariate analysis. These results matched with those of recent study in the literature by Chand, et al. 2015. In this database patients with ymrEMVI-positivity had significantly worse DFS at 3 years (42.7%) compared with ymrEMVI-negative tumours (79.8%). MRI CRM status at pre- and post CRT was noted to be significant on multivariate analysis for DFS, while mrTRG and ymrEMVI were also significant on univariate analysis. This is similar to previous datasets from Patel, et al., (2011) (where mrTRG was significant on multivariate analysis for OS and DFS) and Taylor, et al., (2014)(where involvement of CRM on preoperative MR-based staging was the only significant parameter that independently predicted OS, DFS, and LR on

multivariate analysis compared to AJCC-TNM based criteria). Significant univariate histopathological parameters such as ypCRM, pathological complete response and ypTRG fail to predict survival independently on multivariate analysis. This study suggests that high resolution pre- and post-treatment MR based assessment of CRM and EMVI status along with MRTA are superior and independent imaging markers for predicting survival than the standard TNM based criteria.

5.4 Limitations of the study

However results of this study should be interpreted in the context of the study design and limitations. There is lack of validated published histological correlates of tumour heterogeneity for different texture scales and for pre and post treatment analysis in rectal cancers. Thus our contemplated affiliations of texture features in this study are hypothetical and hence study is hypothesis generating rather than hypothesis confirming. The studies exploring the potential of TA in predicting survival outcomes for CRC has been carried out for CRCs rather that rectal cancer alone and all are CT based texture features. In addition this data is based on small population from a single centre. This is the first exploratory study with regards to MRTA in rectal cancer survival. Due to small number, using the same data to identify optimal cut off for each marker to divide the population into good and bad prognosis group could lead to overstatement of significant results. In terms of technical considerations, acquisition parameters with MRI can introduce higher signal intense variability as compared to CT or PET which in theory could affect reproducibly of the results.

5.5 Conclusion

Intra-tumour heterogeneity quantified as textural analysis on MRI may indirectly reflect tumour hypoxia and necrosis which are associated with treatment resistance and adverse outcomes. Morphological MRI evaluations as well as MR Textural analysis have a complementary role identifying locally advanced rectal cancer patients with poor prognosis.

Treatment for this group could be tailored with for example, more intensive individualized neoadjuvant treatment before undergoing surgery and administration of adjuvant chemotherapy.

6 Integrated PET/MRI imaging biomarkers to predict histological tumour regression and 3 year DFS

The rationale for using neoadjuvant CRT in locally advanced rectal cancers is to induce tumour down staging. Histological assessment of down staging and regression has shown to be an independent prognostic factor in predicting long term survival in such patients (section 1.9.5). As a result of that tumour response assessment has emerged as an attractive end point and is also important because this information can be used for treatment planning and carries prognostic significance. Though MRI based restaging could help to identify patients who achieve complete clinical response but its accuracy is limited due to therapy induced fibrosis, desmoplastic reaction, oedema, inflammation, and viable tumour nets at a fibrotic scar from a previous tumour (section 2.1.2). This has placed greater emphasis on pre-operative imaging modalities to identity patients that could be predicted to have either worse or better tumour regression grades. Pre-treatment values can be argued to more valuable if it could predict response to neoadjuvant therapy. It is more important to be able to predict who is going to respond to neo-adjuvant therapy rather than simply documenting change post therapy because whatever side effect or disadvantage neoadjuvant therapy carries, it would have already been inflicted by the time of post therapy scan.

The potential of textural analysis on conventional MR images to predict survival was explored in the study discussed in the last chapter. Although various exploratory studies are present in the literature that investigated the role of functional MRI such as diffusion weighted imaging (DWI), PET, PET-CT and combining PET and MRI data in identifying responders to neoadjuvant treatment in rectal cancers but there is a lacking evidence on the role of integrated PET-MR in this area.

The main objective of this study was to investigate whether pre-treatment integrated PET-MR functional features correlated with histological response in locally advanced rectal cancer treated with long course CRT. In addition, a potential correlation of PET and functional MRI features in the setting of integrated PET/MRI system was evaluated. Moreover, association of clinical, histological and functional imaging parameters with disease free survival was also evaluated for these patients. The details of methodology including patient selection criteria are described in section 3.3. The brief description of the methodology is given as below.

Patients with non-metastatic operable locally advanced rectal cancer stage II and III eligible for long course chemoradiotherapy were recruited from the hospitals in Essex and East London to undergo pre-treatment integrated PET/MRI scanning at nuclear department of university college hospital, London. Quantitative analysis of PET/MRI images was performed by measuring maximum SUV (SUV_{max}) and peak SUV (SUV_{peak}) reflecting metabolic activity of the tumour and ADC_{mean} and ADC_{min} (reflection of tumour cellularity). Quantitative analysis was performed by measuring the SUVs derived by attenuation corrected PET images using a 3D spherical volume of interest. Region of interest was drawn free-hand, carefully excluding areas of clear artefact as seen on the DWI images with high b value-800 to quantify ADC values (section 3.3.2.4). For the statistical analysis, patients were divided into two groups of good and bad responders based on the tumour regression grades. Independent sample t test was used to compare the means of functional imaging parameters for the two groups of histopathological responders (good vs. bed). The potential correlations between the functional PET features (SUV_{max}, SUV_{peak}) and DWI features (ADC_{mean} and ADC_{min} was evaluated using Pearson's correlation coefficient. Univariate Kaplan-Meier survival analysis was employed to identify which clinical (age, sex), pre-treatment MRI (EMVI status and CRM involvement), histopathological (ypT stage, ypN stage, ypCRM involvement, ypEMVI status and pathological responders) and functional PET/MRI parameters (SUV_{max}, SUV_{peak}, ADC_{mean} and ADC_{min}) predicted DFS.

6.1 Results

Baseline characteristics of patients are shown in Table 6-1. Total number of patients recruited for the study was 16 with 11 (69%) males. Median age of the patients was 62 years. All the patients completed neoadjuvant CRT and underwent curative resection but two patients. One patient has had complete clinical response on restaging and was treated with wait and watch approach and the second patient has had disease progression after the neoadjuvant treatment and died before undergoing resection. Three patients (19%) had complete response (1-clinical response and 2- complete pathological response). In terms of pathological tumour regression, 11 (69%) patients were categorized as bad responder and 5 patients (31%) as good responder in response to CRT. The rate of both local and distal recurrences was 19%. The patient who died before undergoing surgery was considered to have local recurrence. The median follow- up time period was 29 months (range 3-45). The overall 3-year DFS was 45% for the entire cohort.

There was no significant statistical difference between the mean values of PET parameters (SUV_{max} and SUV_{peak}) and DWI parameters (ADC_{max} and ADC_{min}) across the two groups of good and bad pathological responders (**Error! Reference source not found.**).The average alues of SUV_{max} and SUV_{peak} were 13.73 vs. 19.2 (p=0.164) and 10.3 vs. 13.93 (p=0.161) in the two groups of responder vs. non-responder respectively. The average values of ADC_{mean} and ADC_{min} were 1174mm²/s vs. 1055 mm²/s (p=0.346) and 743.6 mm²/s vs. 584.18 mm²/s (p=0.303) for the two groups respectively.

The Pearson's correlation coefficients (r) between PET and DWI parameters for all the rectal cancers are shown in Table 6-3. The correlation between SUVmax/ADCmean was -0.406(p=0.150), SUVmax/ADCmin, -0.312(p=0.278), SUVpeak/ADCmean, -0.280(p=0.331) and SUVpeak/ADCmin,-0.239(p=0.410)

On Univariate KM survival analysis, only ypN stage significant predicted worse 3-year DFS (P=.025). None of the PET/MRI parameters including SUV_{max}, SUV_{peak}, ADC_{mean} and ADC_{min} could significantly predict DFS (p=0.069, p= 0.069, p=0.176 and p=0.06 respectively (Table 6-4).

Table 6-1 Base line characteristics of patients

Total number of patients	16
Gender Male Female	 11 (69%) 5 (31%)
Age median ± SD	62 ± 7.5
Pathological tumour regression Good responder Bad responder	 5 (31%) 11 (69%)
Complete responder	3 (19%)
CRM involvement on MRI Yes No	 8 (50%) 8
EMVI status on MRI Yes No	 11 (69%) 5 (31%)
ypT0-T2 stage ypT3-T4 stage	7 (44%) 8 (50%)
ypN0 stage ypN1-2 stage	6 (56%) 9 (38%)
ypCRM involvement Yes No	 2 (13%) 13 (81%)
ypEMVI status Yes No	 5 (31%) 10 (63%)
Local recurrence Distal recurrence	3 (19%) 3 (19%)
Follow-up months median ± SD (range months)	29 ± 10 (3 – 45)

Table 6-2 Comparison of mean values for functional imaging parameters across the two groups of responder and non-responder to neoadjuvant treatment

Variable	Responders (Mean ± SD)	Non responders (Mean ± SD)	P value (Independent T test)
SUV_{max}	13.73 ± 4.16	19.2 ± 6.79	0.164
SUV_{peak}	10.3 ± 3.46	13.93 ± 4.29	0.161
ADC_{mean}	1174 ± 360.78	1055.76 ± 137.197	0.346
ADC_{max}	1707.8 ± 515.32	1760.36 ± 364.73	0.817
ADC_{min}	743.6 ± 316.71	584.18 ± 77.93	0.303

Table 6-3 Pearson's correlation coefficients between PET and DWI parameters

		ADC_{mean}	**ADC_{max}**	**ADC_{min}**
SUV_{max}	Pearson Correlation	-.406	-.316	-.312
	P value	.150	.271	.278
SUV_{peak}	Pearson Correlation	-.280	-.216	-.239
	P value	.331	.459	.410

Table 6-4 Clinical, histopathological and PET/MRI parameters predicting DFS on univariate analysis

Parameters		**n=**	**Mean DFS (95% CI)**	**p-value**
Clinical Parameters				
Age	<65 years	9	30.55 (23.33 – 37.77)	.657
	≥65 years	7	35.91 (24.58 – 47.2)	
Sex	Male	11	37.88 (30.89 – 44.87)	.363
	Female	5	26.7 (14.04- 39.35)	
Pre-treatment MRI parameters				
mrEMVI status	Positive	11	33.24 (24.13 – 42.35)	.663
	Negative	5	25.60(22.22 – 28.97)	
mrCRM status	Clear	8	33 (22.01 – 43.98)	.978
	Threatened	8	29.5(22.83 – 36.16)	
Histopathological parameters				
ypT stage	ypT0-T2	7	37.48 (28.55 – 46.41)	.885
	ypT3-T4	8	32.62 (25.73 – 39.51)	
ypN stage	ypN0	6	All cases censored	.025
	ypN1-2	9		
ypCRM involvement -	Clear	13	36.08 (29.19 – 42.98)	.464
	Threatened	2	22.5 (16.26 – 28.73)	
ypEMVI status	Positive	5	30.80 (22.86 – 38.73)	.759
	Negative	10	37.14 (29.58 – 44.70)	
pTRG	Good responder	5	38.80 (27.93 – 49.66)	.400
	Bad responder	11	29.54 (22.16 -36.92)	

Functional imaging parameter						
	Threshold value	**Number of patients above and below the threshold value**		**Mean Survival**	**95% Confidence interval**	**P value**
SUV_{max}	>17.21	Poor	8	23.62	14.44-32.80	.069
		Good	6	34	34 - 34	
SUV_{peak}	>12.58	Poor	8	23.62	14.44 – 32.80	.069
		Good	6	34	34	
ADC_{mean}	<1253.519	Poor	13	All cases censored		.76
		Good	3			
ADC_{min}	>432.5	Poor	12	All cases censored		.06
		Good	4			

6.2 Discussion

The main objective of this study was to assess the association with pathological tumour regression and pre-therapy functional imaging features derived from integrated PET/MRI scan. Though the results failed to prove any significant association but the mean values of pre-treatment rectal tumour mean ADC and minimum ADC were higher in the responder group than in the non-responder group. Pre-treatment ADC values have been investigated as a predictor of response to CRT in rectal cancers but there is conflicting evidence in this regard. The results in the thesis study are in keeping with those of the studies by Ha, et al. (2013) and Monguzzi, et al. (2013) where mean ADCs for rectal cancer before neoadjuvant CRT were higher but not significantly in responders than non-responders (p=0.631 and p=0.276). However in both these studies, post-treatment ADC values were also evaluated and there was a significant difference between the two groups with significantly greater increase in post-treatment ADC values in tumours with favourable response. In a separate study by Elmi, et al. (2013), the mean pre-treatment ADC value was marginally higher in significance in responders (p=0.035) compared to non-responders but conversely, Sun, et

al. (2010) in their study demonstrated that rectal tumours with lower pre ADC_{mean} values are more like to undergo pathological T down-staging after CRT (p=0.013). Comparison of per- and post-treatment ADC values along with relative change in predicting response to CRT in rectal cancer was done in the meta-analysis of 16 studies by Xie, et al. (2015). In predicting a good response, the post-treatment ADC value proved to have highest specificity (p<0.001) but there was no difference in sensitivity between the three values (p=0.380, 0.192 and 0.214 respectively). For completer pathological response, post-treatment ADC value was shown to have the lowest sensitivity (p<0.001). In the thesis study, only pre-treatment ADC values were evaluated.

Pre-treatment values can be argued to more valuable if it could predict response to neoadjuvant therapy. It is more important to be able to predict who is going to respond to neo-adjuvant therapy rather than simply documenting change post therapy because whatever side effect or disadvantage neoadjuvant therapy carries, it would have already been inflicted by the time of post therapy scan. Though the pre-treatment mean ADC values were higher in the responder group in the thesis study but these values are typically higher in poor responders (Xie, et al., 2015). Higher pre-treatment ADC values in non-responding tumours are hypothesized to be related to tumour necrosis and hypoxia which renders them resistant to chemoradiation (Koh, et al., 2007). This difference could be due different underlying biology of rectal cancers in the thesis study such as more differentiated tumours associated with higher pre-treatment ADC values (Hayashida, et al., 2006) in the responder group. Another possible explanation for the overlapping of ADC values may be variations in the quantitative measurements and image analysis. Due to conflicting evidence in the literature, validation studies to correlate tumour heterogeneity factors and DWI-parameters are required.

Similar to ADC values, mean values of PET parameters (SUV_{max} and SUV_{peak}) were higher in the non-responder group but the difference was not statistically significant. This may well

be due to small number of patients in this pilot study which may also account for the non-significance of values between the two groups. The patients in the study did not undergo repeat PET/MRI staging due to the nature, cost of the study and difficulty in getting the patients to travel again to central London after a stressful five week course of chemoradiation followed by restaging at their local hospitals. Though significant reduction in SUV values have been demonstrated in pathological responders in several studies (de Geus-Oei, et al. 2009) but other studies have shown conflicting evidence. In the study by Perez, et al. (2014), a statistical significant correlation was found between low baseline SUV_{max} assessed on integrated PET/CT and complete pathological response (p=0.043) compared to SUV_{max} (p=0.23) at 6 and 12 weeks (0.15). In a separate study by Martoni, et al. (2011), median baseline SUV was significantly higher in non-responding group. Both the post-treatment SUV and percentage change did not show statistical difference between the two groups. In addition, quantitative assessment of SUVs after the therapy could lead to bias because radiation itself could cause inflammation leading to FDG uptake. In the systematic review by Joye, et al. (2014), a pooled analysis of individual data of 14 studies on DWI and 25 studies on FDG PET/CT was carried out. The results of the analysis were not encouraging enough to safely identify responders and non-responders. A low overall mean positive predictive value was observed for both functional imaging parameters in predicting pathological complete response (54% for DWI and 39% for FDG PET/CT). There are many limitations to aforementioned studies and reviews as most of the data is from the retrospective ultra-specialized single centre studies with small population groups as well as heterogeneity within the studies in terms of patient selection, neoadjuvant treatment, imaging protocols and analysis. In addition to these limitations the data on these studies in based on PET or PET/CT and MRI-DWI parameters. The thesis study was different from those in literature as the functional imaging parameters were measured on combined integrated PET/MRI system. CT component of PET carries an inherent risk of poor soft tissue resolutions in the absence of oral or intravenous contrast. Conversely MRI technique does not carry the risk of radiation and also produces high spatial resolution and contrast imaging.

Though the findings in the study lacked statistical power but the results of this study could theoretically be accurately correlating with intra-lesional characteristics due to integrated nature of PET and MRI systems.

In addition to determining the potential of imaging parameters in predicting pathological tumour response, the potential of PET/MRI parameters along with known clinical, radiological and pathological variables in predicting 3-year DFS was also assessed. Because of prospective nature of the study, long term follow-up information was not possible. Three year-DFS has been demonstrated as a valid surrogate for 5 year OS with coefficient of correlation >0.90 in a meta-analysis of 18 randomized trials involving 13000 patients with resectable colorectal cancer (Sargent, et al., 2005). The meta-analysis also revealed that most relapses occur within 2 years of surgery. The median follow-up for the thesis study was 29 months. Hence DFS was chosen as endpoint rather than OS. The only variable that predicted DFS among all the variable was ypN stage (p=0.25) (Table 6-4, Figure 6-1). Lymph node involvement is a biological predictor of survival for survival after surgery in rectal cancer treated with neoadjuvant radiotherapy (Chang, et al., 2009). The analysis of Surveillance, Epidemiology and End Results (SEER) US population based data had shown progressive decrease in survival for any T stage category with increasing lymph node involvement (Gunderson, et al. 2010). In the thesis study, the patients with no lymph node involvement (ypN0) were disease free until the conclusion of the study. Kaplan-Meier survival curves for the PET/MRI functional parameters SUV_{max} (Figure 6-2a), SUV_{peak} (Figure 6-2b) and ADC_{min} (Figure 6-3) showed good separation between the good and bad survivor groups (p=0.06) but it was not significant difference. The patients with higher SUV_{max}, SUV_{peak} ADC_{min} and lower ADC_{mean} values than cut-off had poor survival.

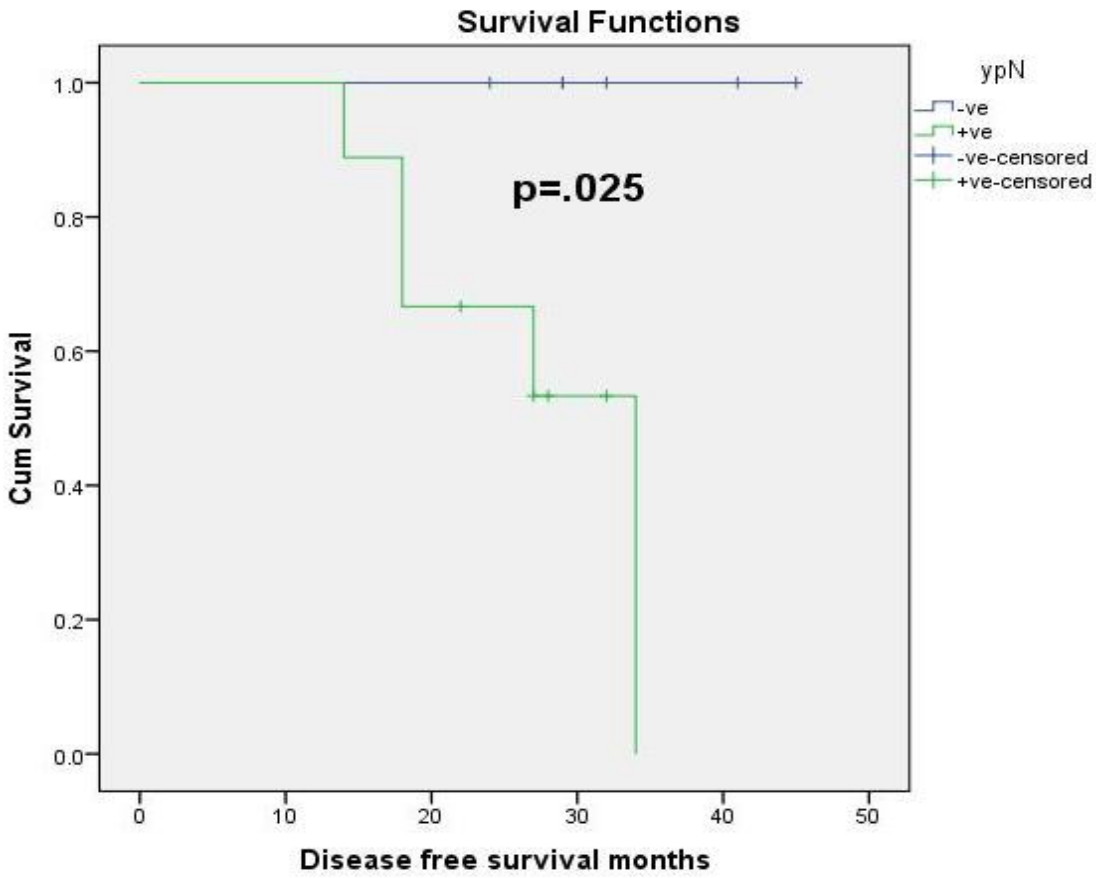

Figure 6-1 Kaplan-Meier curves show a significance difference in disease free survival (DFS) for ypN involvement

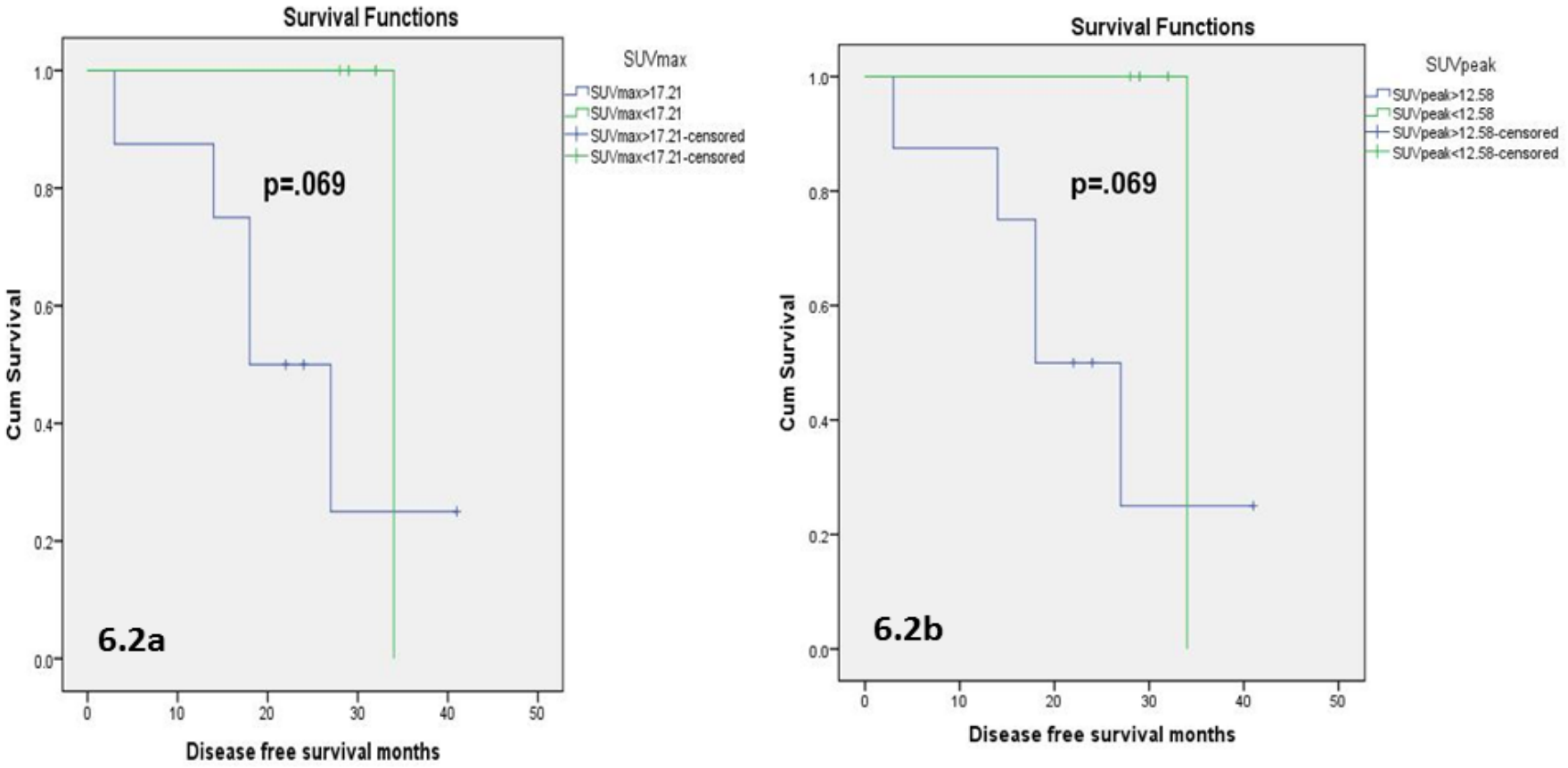

Figure 6-2 Kaplan-Meier curves show a non-significance difference in disease free survival (DFS) for (a) SUVmax and (b) SUVpeak

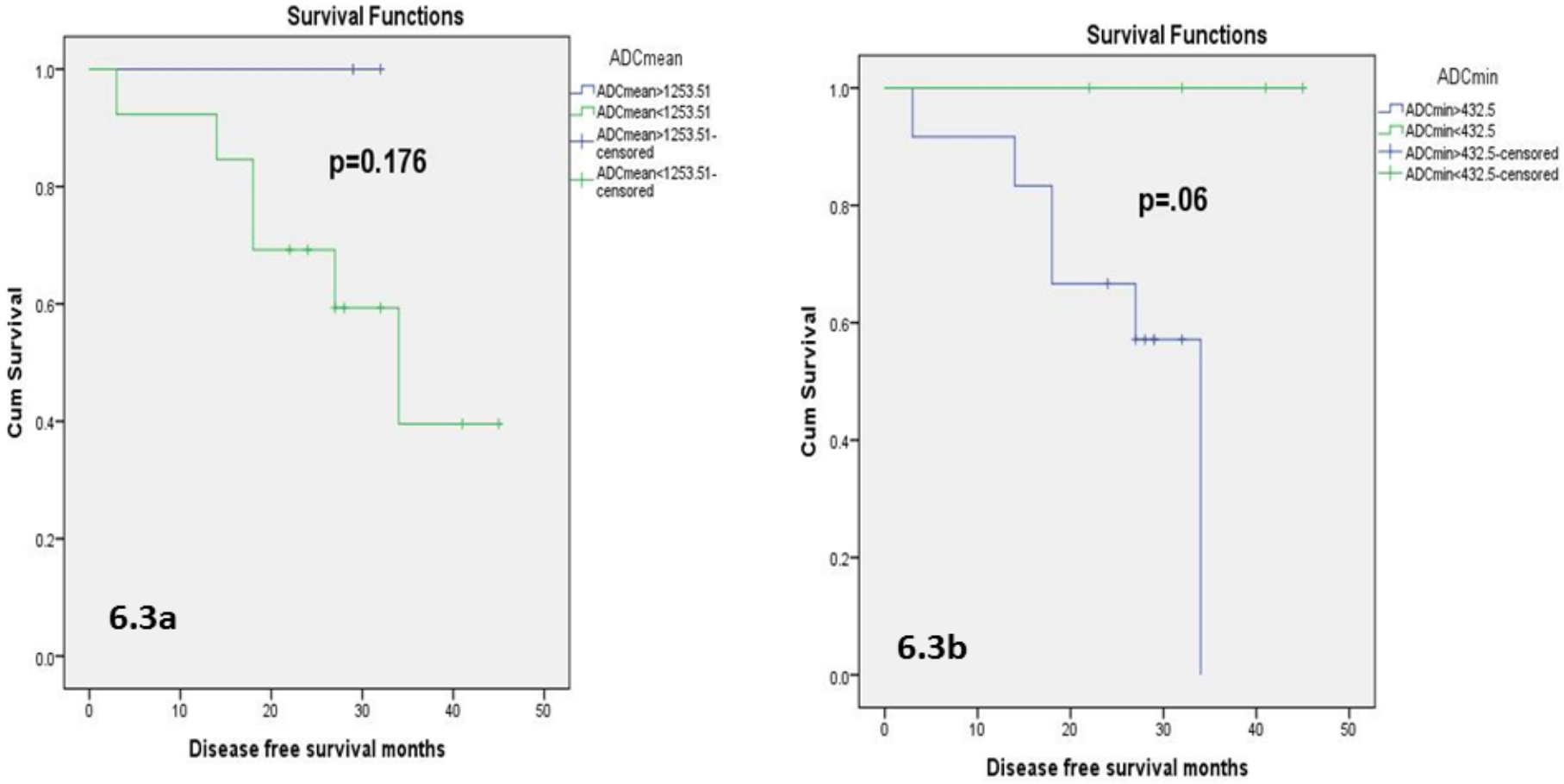

Figure 6-3 Kaplan-Meier curves show a non-significance difference in disease free survival (DFS) for (a) ADCmean and (b) ADCmin

The evidence of prognostic significance of PET or DWI-MRI parameters in the literature is mainly limited to stage IV colorectal lesions with liver metastasis and there is a lack of specific evidence on the prognostic significance of these parameters specifically for rectal cancer. In a retrospective study of 163 patients by Jang, et al. (2012), median SUV_{max} values did not show prognostics significance for DFS (p=0.52) in resectable stage1-IV primary colorectal cancers. In the study by Tam, et al. (2013), ADC values of liver lesions did not predict either progression free survival or OS in patients with colorectal liver metastasis. In a separate study involving patients undergoing liver resection for colorectal metastasis, survival was significantly higher for patients with low SUV_{max} (p=0.0095 at cutoff of 10) on univariate analysis (Riedl, et al., 2007). In another study of patients with colorectal liver metastasis, pre-treatment higher than median SUV_{max} and lower ADC_{mean} values of liver lesions were significant predictors of poor OS (Heijmen, et al., 2015). In the thesis study, only resectable stage III rectal cancers were included and instead of median values, Optimised Kaplan Meier" analysis was employed to determine the cut-off point using a minimal or best p-value approach (Weiss, et al., 2014). Patients with poor survival have had non-significant lower ADCmean values in the thesis study. A Lower ADCmean value has been shown to be significantly associated with aggressive tumour profile in rectal cancer such as poor histological tumour differentiation and presence of nodal disease (Curvo-Semedo, et al., 2012). Both these features are associated with adverse outcomes in colorectal cancer (Nanni, et al., 2002). Another interesting but non-significant finding in thesis study was that patients with higher ADC_{min} values than the cut-off had poor survival. This finding is not consistent with that of previous studies involving central nervous system tumours. Lower pre-therapy ADC values had been demonstrated to be predictive of worse survival rates in contrast enhanced intracranial tumours such as lymphoma (Barajas, et al. 2010) and glioblastoma (Yamasaki, et al., 2010). Low ADC values are associated with increased cell density and conversely areas with reduced cell density such as necrosis are associated with an increased ADC values (Herneth, Guccione and Bednarski, 2003). Higher

pre-ADC_{min} values in poor survivors in the thesis study could be hypothesized due to preponderance of necrotic areas in these tumours that has been shown to be an independent prognostic factor in colorectal cancer irrespective of pathological staging (Richards, et al., 2012). In the thesis study, rectal tumour ADC values were obtained using the single axial slice on the ADC map with the largest cross-sectional tumour dimension. The size of ROI can significantly influence the ADC values in rectal cancer. Variations in the reporting of ADC values could be due to the choice of method employed to mark ROIs such as whole tumour volume (Sun, et al., 2010), single tumour slice with the largest cross-sectional diameter (Dzik-Jurasz, et al., 2002) or small sample of solid tumour (Kim, et al., 2011). ROIs encompassing only solid tumour portion are likely to omit areas of necrosis leading to lower ADC values. In their study, Lambregts, et al., (2011), demonstrated that the significantly higher rectal tumour ADC values were obtained from ROIs involving the whole-volume and single-slice than the small tumour sample ROI on pre-treatment MRI. In addition, there were no significant variations rectal tumour ADC values obtained either from whole tumour volume or single slice method.

In colorectal cancers, increasing tumour size and hence cellular density has been demonstrated to be associated with higher SUV values (p=.0004) (Gu, et al., 2006). The tumour zones with minimum ADC value reflect the highest cellular and proliferative tumour zone (Lee, et al., 2011). Because SUV_{max} reflects the most metabolically active zone of tumour lesions, a potential correlation was investigated between the ADC_{min} and SUV_{max} as well as other between other functional parameters of PET and DWI-MRI parameters. The results indicated negative correlation between the ADC and SUV values but none of the results were significant. A significant negative correlation between SUV and ADC values have been demonstrated in previous studies involving rectal cancer (Khong, et al., 2011 and Er, et al., 2014) but in these studies the measurements were quantified on subsequent PET/CT and DWI-MRI scans performed after few days apart. In contrary to this, in the thesis study the datasets for both SUV and ADC were acquired at the same time on an integrated

PET/MRI scanning system, thus reducing the potential risk of artifacts due to misregistration and biological changes. A significant and negative correlation between the SUV_{max} and ADC_{min} was demonstrated in a study of 19 patients with primary cervical cancer on integrated PET/MRI scanning ($r=-0.692$, $p<0.001$) (Grueneisen, et al., 2014). The non-significance of the results in the thesis study could be due to small sample size and due to heterogeneity in quantifications of ADC as compared to other studies. Because, ADC quantifications are potentially sensitive not only to choice of b-values and field strength (Malyarenko, et al., 2013) but measurements differ among different vendors and coil systems (Sasaki, et al., 2008).

6.3 Limitations of the study

The study is subject to many biases. Firstly, the study sample comprised of small number of patients that could have been the reason that no significance difference was present between the responders and non-responder in terms of functional imaging biomarkers. Patients were recruited from the different hospitals with slight variations in the regimens of long course CRT protocols. This could have resulted in differential tumour response to neoadjuvant treatment leading to selection and treatment biases. Grading system to quantify pathological tumour response also differed among the pathologists in these hospitals leading to measurement bias. Functional imaging parameters were measured by one experienced radiologist leading to potential intra-observer variations. The results of this study should also be interpreted in light of the fact that there is a lack of standardization protocols for image sequence acquisition, image registration and data analysis leading to apparent considerable differences in ADC values of similar cancer type. There is also a lack of validated histopathological correlates with functional imaging parameters especially on integrated PET/MRI platform. Randomized, multicentre prospective larger studies with longer follow-up are required to investigate prognostic significance of functional imaging parameters.

6.4 Conclusion

To my best knowledge this was the first study that investigated the potential correlation of functional imaging parameters on integrated PET/MRI system with pathological tumour response and their impact on prognosis in non-metastatic resectable locally advanced rectal cancers. This study indicated that there was no statistical difference for pre-treatment ADC and SUV values measured on integrated PET/MRI between good and bad histological responder groups of rectal cancer patients treated with neoadjuvant CRT. In addition, both ADC and SUV did not predict survival and only nodal disease involvement predicted survival among all the clinical, radiological and pathological variables.

7 Conclusion of thesis and future directions

7.1 Summary of findings in the first study on the cohort of patients with delayed surgery after long course CRT

There is a lack of consensus on optimal management of timing of surgery after long course CRT for rectal cancers. Studies have shown that there is an on-going tumour response to CRT after the conventional 6 to 8 week window. On the other hand concerns have been raised that a longer time interval to surgery after CRT can make TME technically more difficult because of radiation induced pelvic fibrosis and may also be detrimental to patient survival as it may allow the tumour in situ to grow and spread. It is against this background that the first study in the thesis was carried out to determine the effect of delayed surgery after long course CRT in rectal cancer patients on patient outcomes specifically mortality, morbidity, local and distant recurrence of tumour.

1. There is very limited evidence in the literature with intervals beyond 12 weeks, whereas majority of patients were operated beyond 12 weeks after finishing neo-adjuvant treatment in this study. Nevertheless, the delay in surgery after long course CRT did not lead to worse survival outcomes.

2. Delaying the surgery beyond 12 weeks demonstrated higher complete response (clinical and pathological) in this study but it did not translate into improved survival.

3. In addition, through this study I was able to demonstrate the feasibility of the laparoscopic approach in the setting of delayed surgery after CRT in locally advanced rectal cancer patients. Based on the comparable results of operative morbidity and mortality in this study, it can be argued it is feasible and safe to use a

minimally invasive approach for surgical resection of rectal cancer following CRT in a specialist unit with structured training.

4. On multivariate analysis, male gender independently predicted DFS and RFS, while threatened CRM independently predicted RFS. It was not surprizing to find out that distant failure was the only independent factor that predicted worse OS as well as DFS and RFS.

7.2 Summary of findings in the 2nd study on prognostic potential of MRI based texture analysis as a potential imaging biomarkers for predicting long term survival in locally advanced rectal cancer patients

The objectives of neoadjuvant therapy in locally advanced rectal cancer are to attain loco-regional control, tumour regression, R0 resection and to increase overall survival. Though CRT followed by TME achieves most of these goals but it does not necessarily improve OS as demonstrated in the first study where distant failure rate was double the rate of local failure. This result is in agreement with the evidence in the literature. Furthermore, restaging of irradiated rectal tumours is challenging because of difficulty of morphological MRI in differentiating fibrosis from viable residual tumour. In addition, a proportion of such patients would achieve complete clinical response and could benefit from either wait and watch approach or less invasive local excision surgery. This shifting paradigm has placed greater recent interest in quantification of imaging biomarkers linked to underlying intra-tumour heterogeneity associated with adverse outcomes in terms of treatment failure and drug resistance. With this background and based on the outcome of the first study, I carried out MR based textural analysis on a sub-set of entire cohort of pre and 6-week post-treatment images to correlate with survival outcomes.

1. This is the first study of its kind that clearly demonstrated the prognostic significance of texture features in addition to morphological MRI in rectal cancer undergoing CRT. For OS, MRTA along with mrEMVI were independent predictors pre-treatment analysis while on post-treatment analysis, the only independent predictor was mrEMVI. For DFS, both MRTA and mrCRM were the independent predictors on the both analyses. Interestingly, only MRTA was an independent predictor on both pre- and post-treatment analysis for RFS.

2. Another interesting fact is that none of the pathological parameters such as TN staging, ypCRM, tumour regression and pathological complete response stood out as independent predictors on multivariate analysis. This negative finding in the study strongly favours the argument that it is more important to find markers such as based on imaging that could predict prognosis rather than post-surgery because whatever morbidity or mortality that surgery carries, it would have already been inflicted.

3. This study clearly signifies the potential importance of quantification of pre and post CRT imaging biomarkers derived from TA for rectal cancer patients before undergoing surgery. These markers along with established morphological MR features such as EMVI and CRM could prove to very valuable in selecting patients predicted to have worse survival outcomes for personalized treatment in the form of additional chemotherapy before undergoing definitive surgery.

7.3 Summary of findings of the third study on Integrated PET/MRI imaging biomarkers to predict histological tumour regression

Tumour response assessment is important because of its prognostic significance. Complete and partial responses are associated with significant increased survival with low local and distal recurrence rates (Mass, et al., 2010 and Martin, Heneghan and Winter, 2012). This

has placed greater emphasis on pre-operative imaging modalities to identity patients that could be predicted to have either worse or better tumour regression grades. This in turn could help to select patients for personalized treatment in the form of more intensive therapy in patients with predicted poor response. With this background the potential of integrated PET-MRI features to predict histological tumour response was investigated in the third study of thesis. There is limited evidence on integrated PET/MRI in oncologic applications and to the best of my knowledge it was the first study to investigate correlation of histological TRG with imaging features for rectal cancers on integrated system.

1. No significant association was demonstrated between pre-treatment rectal tumour functional imaging parameters (ADC and SUV values) and tumour regression groups.

2. Interestingly pre-treatment meanADC was higher in patients with good tumour regression grades but these values are typically higher in poor responders (Xie, et al., 2015).

3. Higher pre-treatment ADC values in non-responding tumours are hypothesized to be related to tumour necrosis and hypoxia which renders them resistant to chemoradiation (Koh, et al., 2007). Conversely, more differentiated tumours are associated with higher pre-treatment ADC values (Hayashida, et al., 2006) and such tumours are more likely to respond to systemic treatment. In this study of 16 patients, all patients but one had good-moderately differentiated rectal cancer.

4. Though the findings in the study lacked statistical power, the results of this study could theoretically be accurately correlating with intra-lesional characteristics due to integrated nature of PET and MRI systems. There is a less chance of potential for

errors in lesion co-registration especially for organs such as the bowel which may change location and shape over short periods with this technique.

5. Because of the prospective nature of the study, only short-term follow-up of these patients was possible. The only variable that predicted 3-year DFS was ypN stage. Kaplan-Meier survival curves for the PET/MRI functional parameters SUV_{max}), $SUV_{peak,}$ and ADC_{min} showed good separation between the good and bad survivor groups (p=0.06) but it was not a significant difference probably due to the small number of patients.

7.4 Future directions

The evidence available so far on delaying the surgery is based on retrospective studies including the study in this thesis using variable CRT protocols and time intervals. Though the delay of 12 weeks and beyond did not impact on the survival outcomes in this thesis and the surgical morbidity was comparable to the evidence in the literature but prospective randomized control trials are needed to develop a consensus on optimal timing of surgery in the delayed setting. An on-going phase II multicentre randomized 6 vs. 12 trial launched by Royal Marsden NHS Foundation Trust (www.clinicaltrials.gov/show/NCT01037049) was started in 2009 to determine the primary end point whether greater tumour regression is observed when surgery is delayed to 12 weeks. Secondary outcome measures are surgical morbidity and 5-year local and distal recurrence rates. Another similar French trial (GRECCAR6) is in progress randomizing patients between 7 and 11 weeks (www.clinicaltrials.gov/show/NCT01648894). The results of these trials will help in determining the optimal time interval to operate at maximal response time.

Textural analysis and functional PET and MRI parameters are evolving as a potential imaging biomarker in cancer imaging in the last decades or so. The observed correlations

between survival and texture analysis in the study should be confirmed in prospective studies comprising of large number of representative populations. An extensive analysis is required to assess the combined effectiveness of textural parameters, DWI and PET features in predicting tumour response and prognosis. In addition there is a lack of validated published histological correlates of tumour heterogeneity and functional imaging parameters especially on integrated PET/MRI platform for colorectal cancers and especially rectal cancers. Moreover, there is a lack of standardization protocols for image sequence acquisition, image registration and data analysis leading to apparent considerable differences in SUV and ADC values of similar cancer types. Hence, randomized, multicentre prospective larger studies with longer follow-up are required to investigate prognostic significance of functional imaging parameters.

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Appendices

Appendix 1 Clavien-Dindo Classification of surgical complications. Adopted from Clavien, et al., (2009). The Clavien-Dindo classification of surgical complications: five-year experience. *Annals of Surgery*. 2009, 250 (2):187-96

Grade		Definition
Grade I		Any deviation from the normal course without the need for pharmacological treatment or surgical, endoscopic and radiologic interventions Allowed therapeutic regimens are: drugs as antiemetic, antipyretics, analgesics, diuretics, electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside
Grade II		Requiring pharmacological treatment with drugs other than such allowed for grade I complications Blood transfusions and total parenteral nutrition are also included
Grade III		Requiring surgical, endoscopic or radiological intervention
	III a	Intervention not under general anaesthesia
	III b	Intervention under general anesthesia
Grade IV		Life-threatening complication (including CNS complications)* requiring IC/ICU management
	IV a	Single organ dysfunction (including dialysis)
	IV b	Multiorgan dysfunction
Grade V		Death of a patient

**Brain haemorrhage, ischemic stroke, subarachnoid bleeding, but excluding transient ischemic attacks.*

CNS, central nervous system; IC, intermediate care; ICU, intensive care unit.

Appendix 2 Ethical approval letter

Colchester Hospital University NHS
NHS Foundation Trust

Colchester General Hospital
Research and Development (R&D) Department
Villa 3
Turner Road
Colchester
CO4 5JL

Tel: 01206 744242
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20th August 2013

Omer Jalil
Clinical Research Fellow
General Surgery (Colorectal)
Colchester General Hospital

Dear Omer

Re: Retrospective audit of rectal cancer patients who had long course chemoradiotherapy followed by surgery

Please accept this letter as confirmation that Trust R&D approval is not required for Audit Projects. Guidance and permission should be obtained from the Trust Audit Department in line with trust policy.

Let me take this opportunity to wish you well with your project.

Yours faithfully

Mr Ayres Caldeira
R&D Manager

Appendix 3 MRI report proforma

Patient study Number
Reader 1 2

	Initial MRI	MRI 6 weeks	MIR 12 weeks if applicable
Date of scan			
T stage Subgroup			
N stage	N0 N1 N2	N0 N1 N2	N0 N1 N2
Distance to tumour to anal margin mm			
CRM status	YES/NO	YES/NO	YES/NO
Tumour regression grade 1-5		1 2 3 4 5	1 2 3 4 5
Length of tumour RECIST mm			
EMVI	YES/NO	YES/NO	YES/NO

Progression: Yes No

Standard for reporting T-Stage Subgroups:
T3a ≤ 1 mm extramural spread beyond the muscularis propria
T3b - 1 to 5 mm beyond
T3c > than 5 mm and ≤ 15 mm beyond
T3d > than 15 mm beyond.
T4a - invasion of organs/muscles i.e. sphincters, levators, bladder
T4b - invasion of peritoneum

MR Tumour regression grade-Criteria
TRG-1 absence of any tumour signal
TRG-2 Small amounts of residual tumour visible but with a predominant fibrotic low signal intensity
TRG-3 Mixed areas of low signal fibrosis and intermediate signal intensity present but without predominance of tumour.
TRG-4 Predominantly tumour signal intensity remains with minimal fibrotic low signal intensity
TRG-5 No fibrosis evident, tumour signal visible only

Good responders: TRG 1-3
Poor responders: TRG 4-5

Nodal stage criterion
Nodal stage post treatment based on interpretation of lymph node border characteristics and signal intensity. A node was regarded as positive if either an irregular border or mixed signal intensity was demonstrated

Appendix 4 Patient information sheet

University College London Hospitals NHS
NHS Foundation Trust

- Institute of Nuclear Medicine -

Patient Information Sheet (Version 12) February 2013

Tumour Angiogenesis in Non-small cell Lung, Colorectal, Breast, Oesophageal, Thyroid, Urogenital, Brain, Bone, Head and Neck cancer and Lymphoma: Radiology-Pathology and Prognostic Correlation

You are invited to participate in this study, which is looking to examine blood vessels in tumours. It is purely voluntary to participate in the study and if you do not wish to be involved your treatment will not be disadvantaged in any way. Please take your time to decide.

1. What is the purpose of the study? - To investigate the use of PET/CT and PET/MR to determine blood vessels in cancers
2. Why have I been chosen? - During this period of time, patients with types of lung, breast, colorectal, oesophageal, brain, bone, head and neck, thyroid, hepatocellular, urologenital cancers, and lymphoma are being asked to volunteer.
3. Who is organising the Study? - Lister/QEII Hospitals and University College Hospitals.
4. What will happen to me if I take part? - You will have a PET/CT (see below) scan and a PET/MR scan . Immediately after the PET/CT scan we will perform a short extra scan of your tumour.
5. What do I have to do? - You will undergo a PET/CT and PET MRI scan (see below).

For many cancer patients this is not research but standard routine medical care. In others the role of PET/CT scanners is unclear. We will make it clear to you whether your PET/CT scan is research or routine.

Before you have the PET CT scan you will need to fast 6 hours (you can drink water). 45 minutes before the scan we put a cannula (a small plastic tube) into a vein in your arm. The cannula will be used to give you the injection of the radioactive sugar called FDG, and we may need to take some samples of blood during your scan. This will be about 2 tablespoons of blood.

You will need to lie still in order for the radioactive sugar to make its way around your body.

You will then need to lie still on the scanning table for about 45 minutes whilst we perform a whole body scan. At the end of your PET/CT scan you will need to wait a further 5 to 10 minutes on the table whilst we perform the additional scan (CT), which is for research only. For this, we will give you a small injection (you will not need an additional needle, as you will have had a cannula/drip already in place for your PET/CT scan) of CT dye in to your blood at this time. This may make you feel warm. We will also take a small blood samples from a vein with a small needle.

You will then have a PET MR scan. You will need to lie still on the scanning table for 45 minutes for this scan. The entire procedure will take about 4 hours.n Any unused blood samples will be stored in secured freezers (Biochemical Medicine department, 1st floor, Whitfield Street Laboratories or other suitable equivalent facilities) and may be used for future research, provided that any such research has been granted ethical approval We would like all patients to undergo a PET MRI scan. PET MRI scans are a combination of PET and MRI technology It is explained more thoroughly below. All of these scans will happen on the same day.

We would also like you to return to have the PET CT scan again, using a different radioactive tracer/dye. This will give us additional information about the inflammation. There are several tracers/dye we could use: Rubidium, Fluciclatide, Fluorothymidine, HX4 or F-

Miso. You will not have CT dye with this scan. We will only carry out a second PET CT scan if you are happy to have another scan. If you return for a second PET CT scan using a different tracer/dye, the procedure will be fully explained to you prior to booking the appointment.
Patients with colorectal urogenital, and oesophageal cancer will receive a small injection of buscopan (hyoscine butylbromide) to relax the bowel. This medication is used routinely, but can cause dry mouth, blurred vision or a feeling of faintness. These side effects usually lasts less than an hour. Patients with high pressure in the eye (acute glaucoma) will not be given buscopan.
If you have an operation to remove your tumour, either at UCLH or at a different hospital, we will use a small sample of it to stain for blood vessels and other tumour growth factors. We may also wish to keep a separate sample as a gift for future ethically approved projects, including genetic research. The tumour sample will be identifiable to the researcher as yours, so that we may compare the results of the staining, with the results of your scan.
We are also interested in patients' experience of PET-CT and MRI scans, therefore we will ask you to complete questionnaires regarding anxiety, and your experience of the scan.
6. What is a PET/CT scanner? This scanner combines two scans. A CT scan shows if there are structural abnormalities, and the PET scan shows any rapidly growing tissue such as cancer tissue.
7. What is a PET MR scanner?
This is a state of the art imaging scanner. This equipment is able to look at the radioactive injection and image the lungs at the same time. There is no additional radiation involved. The PET-MR scanner uses magnets to produce the images. You will be lying inside an open tunnel for up to 45 minutes. The scanner will make some loud noises in order to create the pictures. Because the scanner is loud and noisy, you will be given earplugs and headphones to protect your ears. You will also be able to listen to music. You can bring your own CD or iPod if you want to.
You will be asked to hold your breath during the scans for up to 20 seconds, you will also be given an injection of a dye (contrast agent) through a small tube in one of your veins, this will highlight the blood vessels in the area being scanned. You may receive some oxygen while you are having your MRI scan.
The MRI component measures different structures and densities to CT scans and this difference may be useful in providing improved structural information about cancer.
PET/MRI scans are not suitable for patients that have had certain types of metallic implants such as pacemakers.
8. What are the risks involved?
As part of the PET/CT scan you will receive an injection of what is known as a 'tracer'. A tracer known as FDG (Fluorodeoxyglucose) is routinely administered prior to PET/CT scans. We also have ethical approval to use a range of novel tracers, including 82Rubidium, 18F-MISO, HX4, ML10, FLT, choline or tracers targeted to molecules on the surface of cells called "integins", because they integrate the cells with those in their environment (eg Fluciclatide). The only known significant risk from the use of these tracers is from radiation. The risk from the radiation dose you will receive is small, about the same radiation you will get from the environment over 4-6 years. This is known as background radiation. However some people believe that small amount of radiation can cause a small increase in cancer after many years (e.g. in 20 years time). The increased risk is small if it exists at all. This risk becomes less as you get older. Thus we only perform the study on patients above 45.
Given that some of these tracers are novel in use, there are currently no known side-effects other than the risk from radiation. If any further information about the use of these tracers in this study and their possible risks becomes available, we will amend the protocol and patient information sheet to take these risks into account, or we will stop the use of the tracer altogether, pending Sponsor confirmation and ethical review.
9. What are the benefits for entering the study? Your doctors might gain extra information about your tumour, which may help them make a more accurate prognosis and improve treatment. You may be helping future patients with a similar illness to your own.

You may help scientists understand the biology of cancer better
10. What if new information becomes available? If we discover the answer to our question earlier we will stop the trial.
11. What happens at the end of the study?
The result of your study will be made available to your referring doctor.
12. Will my records be kept private? All scans images and reports in our departments are strictly confidential and access is restricted only to the relevant health care professionals. Although the results of our research may be published, no individuals are ever named. Your records will be kept at the University College London Teaching Hospital Trust. The overall responsibility for your records being kept private will be Professor Groves (Chief Investigator). Anonymised data collected during the study may be sent to associated researchers of GE Healthcare, which may be sent outside of the European Economic Area where the laws do not protect your privacy to the same extent as the UK law. The company will take all reasonable steps to protect your privacy.
13. Will my GP be informed? The combined results of all scans will be communicated to your doctor. In turn your doctor may convey information to your GP.
14. Are there compensation mechanisms available if things go wrong? - Yes our departments and individuals are all fully insured.
15. Are some patients excluded from the trial? Yes. Patients that are under 45. Patients allergic to CT/MRI dyes (contrast mediums) Patients that cannot understand this information sheet.
if you have a Pacemaker and certain metallic implants. Some patients with severe kidney problems will also be excluded
Your participation in the trial is entirely voluntary. You are free to decline to enter or to withdraw from the study any time without having to give a reason. If you choose not to enter the trial, or to withdraw once entered, this will in no way affect your future medical care. All information regarding your medical records will be treated as strictly confidential and will only be used for medical purposes. Your medical records may be inspected by competent authorities and properly authorised persons, but if any information is released this will be done in a coded form so that confidentiality is strictly maintained. Participation in this study will in no way affect your legal rights'.
16. What if there is a problem?
Every care will be taken in the course of this study. However, in the unlikely event that you are injured by taking part, compensation may be available.
If you suspect that the injury is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation. After discussing with your research doctor, please make the claim in writing to Professor Ashley Groves who is the Chief Investigator for the research and is based at The Institute of Nuclear Medicine, University College Hospital, 235 Euston Road, London NW1 2BU. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.
Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff or about any side effects (adverse events) you may have experienced due to your participation in the research, the normal National Health Service complaints mechanisms are available to you. Please ask your research doctor if you would like more information on this. Details can also be obtained from the Department of Health website: http://www.dh.gov.uk" 05/Q0505/34 – PIS (V12) February 2013 4

Appendix 5 Patient consent form

University College London Hospitals NHS
NHS Foundation Trust

- Institute of Nuclear Medicine -

Centre Number: **ID number: 05/Q0505/34**

Patient Identification Number for this study:

INFORMED CONSENT FORM - (V10) July 2012

Title of project:

Tumour Angiogenesis in Non-small cell Lung, Colorectal, Breast, Oesophageal, Thyroid, Urologenital, Head and Neck cancer and Lymphoma: Radiology-Pathology and Prognostic Correlation

Please initial box

	I confirm that I have read and understood the information sheet dated July 2012 (version 10) for the above study and have had the opportunity to ask questions.	□□□
2	I confirm that I have had sufficient time to consider whether or not want to be included in the study	□□□
3	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	□□□
4	I agree that a tumour sample may be stored for use in future ethically approved research studies.	□□□
5	I agree to take part in the above study.	□□□
6	'I understand that data (scan images, and details of treatment) collected during the study may be looked at by individuals from GE Healthcare (in an anonymous form), from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data.'	□□□

Appendix 6 General safety recruitment checklist for PET- MRI

NAME	Date of birth	Hospital No	Contact No	AMG Group Contact

Tumour Type and Location:_____ Referral Source:_____
Date AMG research group informed of patient: _____
Patient given PIS Date: _____ Initial patient contact date: _____
Study vetted by: _____ Date of patients MDT /Clinic appt: _____
Checklist completed by:_____ Date:_____ Date checklist emailed:_____

The following details must be obtained before contacting PET-CT / PET-MR
Patient availability (3 dates minimum) Date 1) _____ Date 2) _____ Date 3)_____

- MR safety questionnaire verbally completed
  YES/NO

If the patient answers YES to any question please contact PET-MR to discuss.

- Previous imaging requested via IEP
  YES/NO

Reference no: _____

- For colorectal patients: Colonoscopy report requested
  YES/NO
- Patient discussed with PET-CT Date:_____ PETCT staff member:_____

	Brain MR	Liver MR	Local Tumour Staging	Staging CT	Other
Clinical Indications					
Vetted By					
Tick as required					

- Patient discussed with PET-MR Date:_____ PETMR staff member:_____

	Injection	PET-CT	PET-MR	Staging CT	Staging MR
Date					
Time					

CT & MRI contrast

- Does the patient have a known contrast allergy?
  YES/NO
- Does the patient have an iodine allergy?
  YES/NO
- Is the patient asthmatic?
  YES/NO
- Does the patient suffer from kidney problems?

  YES/NO

- Patients most recent eGFR/Creatinine received?
  YES/NO

  Result _____eGFR/Creatinine (delete as appropriate)

- Is the patient diabetic?
  YES/NO
- If yes, how is their diabetes managed? Please circle as appropriate:

Diet controlled / Insulin / Oral medication specify type: _____
If patient is on Metformin, please discuss with AMG medics to decide if patient is suitable to participate.

Name of Dr patient discussed with: _____ Signature of Doctor:_____ Date:_____
Patient suitable to participate in research : Yes/No
Reason:_____

Appendix 7 MRI safety questionnaire

MRI scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the MRI scan room with any metal **in or around your body and clothing**.

NAME	Date of birth	Height	Weight

Please answer the following questions carefully, and ask if anything is not clear.

All information is held in the strictest confidence.

please circle YES or NO

1. Do you have a heart pacemaker? **These may stop working near the MRI scanner.** YES/NO
2. Have you ever had any surgery on your heart?
   YES/NO
3. Have you ever had any surgery on your head, brain, spine or eyes?
   YES/NO
4. Have you had any surgery in the past 2 months?
   YES/NO
5. Do you have any foreign bodies inside you? (e.g. implants, devices, shrapnel)
   YES/NO
   If yes, please list_____
   _____
6. Have you ever had any metal particles in your eyes? (e.g. from welding or metal work)
   YES/NO
7. Could you be pregnant? (Women Only)
   YES/NO
8. Before entering the MRI scan room you must remove all metal objects, ***including coins, jewellery, body-piercings, hearing aids, dentures containing metal, dental braces, artificial limbs or callipers.***
   Do you agree to remove all of the above before entering the MRI scan room?
   YES/NO
9. Is there anything else you think we should know about in relation to your MRI scan?
   YES/NO
   If yes, please give details_____

10. Do you wear dentures, a dental plate or a brace?
YES/NO
11. Have you had blackouts, epilepsy or fits in the past 2 months?
   YES/NO
12. Do you have any tattoos or trans-dermal patches (i.e nicotine or pain relief patches)
YES/NO
13. Are you wearing coloured contact lenses?
   YES/NO
14. An MRI contrast agent (dye) is often required to give us the best information from your MRI scan. Do you consent to an injection of contrast agent (dye) if required?
   YES/NO
   If yes, please complete the MRI Contrast section overleaf

* If you are having an MRI scan of your abdomen or pelvis we may need to administer drugs that have a short term effect on your body as part of your scan. Please complete the relevant questions related to your MRI scan on the following page.

2) MRI of abdomen/pelvis – Buscopan/ Glucagon (antispasmodic)

Your bowel is constantly moving. This can cause blurring of the MRI images. Buscopan or Glucagon is injected into a vein or muscle to slow down your bowel motion for about 30 minutes and improve the quality of your MRI scan. It is rare to have an allergic reaction to these drugs. However, it is important that we know if you have any of the conditions mentioned below. If you would like any more information about Buscopan/Glucagon please ask one of the radiographers.

Do you suffer from glaucoma (high pressure in the eyes)?
YES/NO

Do you have a family history of glaucoma?
YES/NO

Do you any problems with your heart? If yes please give details...
YES/NO

Are you diabetic?
YES/NO

Do you have Myasthenia Gravis?
YES/NO

Are you driving home after your scan?
YES/NO

For Radiographer use only: Checked By_____

By_____

Anonymised data from your MRI scan may be used for teaching/research purposes.

I have read, understood and answered all the relevant questions above.

Signature…… Date……

Checked by (MRI Authorised Person)……..……
Date……

For clinical staff use only: IV Access record

Date & time of insertion		Site		Gauge	
Gloves worn		Skin preparation		Sterile dressing applied & dated	
Number of attempts		Inserted by		Volume of saline flush	
Time of removal		Removal by			