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Neoadjuvant chemoradiotherapy in oesophageal cancer : response, outcome and the role of surgery

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Neoadjuvant Chemoradiotherapy

In Oesophageal Cancer:

Response, Outcome And The Role Of Surgery

Presentation Of A Thesis By

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To my parents, Christopher and Helga Furlong, in Liebe und Dankbarkeit.

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iii DECLARATION

I, Dr Heidi Furlong, declare that this thesis, which I submit to the Royal College of Surgeons in Ireland (RCSI) for examination in consideration of the award of a higher degree of Doctor of Medicine (MD), is my own personal effort. Where any of the content presented is the result of input or data from a related collaboration, this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text. I agree that the library of the RCSI may maintain a copy of this thesis, which may be taken out on loan.

Signed Heidi Furlong

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CHAPTER I: INTRODUCTION

1.1 OESOPHAGEAL CANCER

Oesophageal cancer is the eighth most common cancer and sixth most common cause of death from cancer worldwide[1]. It is estimated that 16,980 people will be diagnosed with and 14,710 men and women will die of cancer of the oesophagus in the USA in 2011[2]. Despite improvement in five-year survival rates from 4% in the 1970s[3, 4], the prognosis of oesophageal cancer remains poor and current European overall five-year survival rates are at best 11%[5].

1.1.1 GEOGRAPHICAL DISTRIBUTION

There is a significant variation in geographical incidence. Approximately 83% of all cases and 86% of the deaths occur in developing countries[1]. A 15-fold variation in incidence is observed in males between high-risk southern Africa and low-risk western Africa and 20-fold variation in females between southern Africa and Micronesia/Polynesia[1]. The area with the highest reported incidence for oesophageal cancer is the so-called Asian 'oesophageal cancer belt', which stretches from eastern Turkey through north-eastern Iran, northern Afghanistan and southern Russia to northern China[6, 7]. High rates have also been reported for South-east and South Africa, parts of South America and Western Europe[1, 6, 7]. Squamous cell carcinoma is the most prevalent histological type worldwide but the incidence of adenocarcinoma is rapidly increasing in first world countries[8, 9].

There is a wide variation in incidence not only between countries but also in different ethnic groups and populations within a particular country. For example, in the USA, the incidence of adenocarcinoma is almost four times higher in white men than in black men, while the incidence of squamous cell carcinoma is almost six times higher in black men than in white men[10].

1.1.2 OESOPHAGEAL CANCER IN IRELAND

Oesophageal cancer is the tenth most common male and fourteenth most common female cancer (excluding non-melanoma skin cancers) in Ireland[11]. Between 2000 and 2004, there were on average 296 male and 183 female cases of oesophageal cancer (excluding cardia cancer) diagnosed in Ireland each year[11]. During the same period, there were 296 male and 174 female deaths per year (giving an incidence:mortality ratio of 0.98)[11]. This is a frightful indictment on the efficacy of current therapeutic strategies. It is also similar to the number of motor-vehicle related deaths in Ireland each year[12] which receives a considerably more media and legislative attention. Oesophageal cancer accounts for only 2% of all cancers in Ireland, but 4% of cancer deaths[11] and the incidence rates are 1.2 to 3 times higher in Ireland than in the United States or the European Union[13]. Between 1994 and 2009, the incidence rate for squamous cell carcinoma increased by 0.9% and 1.4% annually for females and males respectively, while the incidence rate for adenocarcinoma increased by 2.2% and 3.0% for females and males respectively[14]; a trend seen in most developed countries.

In Ireland, the majority of cases of oesophageal cancer occur in elderly patients. Roughly 70% of cases occur in patients over the age 65 and 50% in the over 70s[11]. The

relative 5-year survival in Ireland is a dismal 14%, but this compares favourably with international data[5, 13]. In Ireland, the number of cases of cancer of the oesophagus is projected to increase by 69% (2% annually) for females and 187% (6% annually) for males between 2005 and 2035[15].

1.1.3 GENDER DISTRIBUTION

Oesophageal cancer is two to four times more common in males in most regions[1, 6], but can vary tremendously. For example the male to female sex ratio is 7:1 in Eastern Europe complared with 3.5:1 in the USA[16]. In the high-risk areas of Asia and Africa, however, the sex ratio is much closer to unity[8].

1.1.4 AGE DISTRIBUTION

The risk of oesophageal cancer increases with age, with less than 3% being diagnosed under the age of 45[4]. The mean age at diagnosis in the USA is 67 in males and 73 in females, or 68 overall[4]. Approximately 75% of the people who die from oesophageal cancer are over the age of 65[13].

1.1.5 CHANGING TRENDS

During the past several decades important changes have occurred in the epidemiologic patterns of oesophageal cancer. Until the 1970s, squamous cell carcinoma accounted for the vast majority of oesophageal cancers diagnosed and continues to do so in developing countries[8, 9]. Since the 1970s, however, the incidence of squamous cell carcinoma has remained stable or decreased in most western countries while that of

adenocarcinoma has rapidly become the dominant histology in developed countries such as the UK, USA and in Europe[7-10, 17, 18].

1.1.6 PATHOLOGY AND ETIOLOGY

1.1.6.1 PATHOLOGY

Adenocarcinoma and squamous cell carcinoma account for approximately 90% of oesophageal cancers with rarer tumours such as melanomas, leiomyosarcomas, carcinoids and lymphomas making up the remainder[19]. The majority of adenocarcinomas are found in the distal one third of the oesophagus and cardia, whereas squamous cell carcinomas are usually located between the middle and distal thirds[20, 21]. The proximal one third of the oesophagus is a relatively uncommon site of disease[19].

Although most clinical studies have not differentiated between the two major histological types, increasing evidence supports the concept that they differ in terms of pathogenesis, epidemiology, tumour biology, and prognosis. Current series suggest that the prognosis of adenocarcinoma is better than that of squamous cell carcinoma, particularly in earlier disease[21-26]. One reason may be that lymphatic spread occurs less frequently in Barrett's-associated cancer than in squamous cell carcinoma[24, 27]. In acknowledgement of these differences, the most recent AJCC cancer staging system[28] provides separate stage groupings for adeno- and squamous cell carcinoma (**Table 1, Table 2, Table 3**)

1.1.6.2 ETIOLOGY

1.1.6.2.1 SMOKING AND ALCOHOL CONSUMPTION

Smoking is one of the major risk factors associated with both squamous cell carcinoma and adenocarcinoma of the oesophagus. The risk of oesophageal cancer correlates directly with the quantity of cigarettes smoked per day and the duration of smoking[29, 30]. This is thought to be related to the resulting contact of nitrosamines with oesophageal mucosa[31]. Alcohol multiplies the effect of tobacco consumption but also independently increases the risk of squamous cell carcinoma in the absence of smoking[32].

1.1.6.2.2 RADIOTHERAPY

Previous radiotherapy to the mediastinum, as for the treatment of breast and lung cancers, lymphoma and other neoplasms, predisposes patients to both adenocarcinoma and squamous cell oesophageal carcinoma, which typically develop ten or more years after radiation therapy exposure[33, 34].

1.1.6.2.3 MEDICATIONS

A number of common medications, such as calcium channel blockers, tricyclic antidepressants and certain asthma medications such as theophylline and beta agonists, promote gastro-oesophageal reflux by relaxing the lower oesophageal sphincter. One study examined the role of medications as risk factors for the increasing incidence of oesophageal and gastric cardia adenocarcinomas[35]. They found that the

increase in incidence of these tumours were not likely to be related to the use of lower oesophageal sphincter-relaxing drugs as a group but did suggest that persons treated for long-standing asthma may be at increased risk of oesophageal adenocarcinoma[35], possibly reflecting the fact that gastro-oesophgeal reflux disease and asthma are inextricably linked[36]. A later study, however, has suggested that the widespread use of lower oesophageal sphincter-relaxing drugs may have contributed to the increase in incidence of oesophageal adenocarcinoma[37].

1.1.6.2.4 DIETARY FACTORS

Studies in high risk areas of northern Iran and China have identified a number of dietary risk factors for oesophageal cancer. These include opium use, nutritional deficiencies particularly zinc, diets deficient in fruit and vegetables, certain foods which contain high levels of mycotoxins and nitrosamines and thermal injury from consumption of very hot beverages[38, 39]. In South Africa, where oesophageal cancer is the most common cancer in black males, one major contributory factor is thought to be the consumption of imported maize which has replaced sorghum as the main staple diet[40]. The ingestion of this maize when contaminated with fungus, especially *Fusarium moniliforme,* and the resultant mycotoxins has been implicated in the increase in incidence of oesophageal cancer in this population[40].

1.1.6.2.5 BARRETT'S OESOPHAGUS

Barrett's oesophagus is the eponym used to describe the change from the normal stratified squamous epithelium of the lower oesophagus to a polarised, columnar-lined epithelium with intestinal-type differentiation. Norman R. Barrett (1903–1979), was a

distinguished thoracic surgeon in London who wrote an article in 1950 entitled *"Chronic Peptic Ulcer of the Oesophagus and 'Oesophagitis'"*[41]. In fact Barrett did not fully understand what he was describing and he did not recognise intestinal features (goblet cells) in the columnar-lined oesophagus. In 1953 Phillip Allison, a thoracic surgeon from Leeds in England, published an article entitled *The Oesophagus Lined with Gastric Mucous Membrane*[42]. Magnanimously, the authors suggested that the term "Barrett ulcers" be used to describe ulcer craters in the columnar cell-lined oesophagus and the term *Barrett's Oesophagus* became enshrined as the eponym thereafter.

Intestinal metaplasia develops in the context of chronic gastro-oesophageal reflux disease and bile reflux. Barrett's metaplasia represents the first step of the metaplasia-dysplasia-adenocarcinoma sequence, but despite the initial almost universal acceptance that intestinal metaplasia is a prerequisite for the development of oesophageal adenocarcinoma, there is continued debate, with some authors finding it unlikely that adenocarcinoma would develop in its absence and others strongly disagreeing[43-46].

Overall, Barrett's is associated with an approximate 0.12–1% annual progression rate to oesophageal adenocarcinoma[47-49] but accurate estimates of the annual incidence of high-grade dysplasia and adenocarcinoma in patients with Barrett's oesophagus have been difficult to establish, due to the considerable variation in reported rates. In two of the more recent reviews, the pooled incidences of adenocarcinoma were estimated to be up to 6 cases per 1000 person-years, with much higher incidence estimates of up to 10 cases per 1000 person-years for high-grade dysplasia[50, 51]. Several studies have

demonstrated a higher incidence of adenocarcinoma associated with longer-segment Barrett's oesophagus, especially when greater than 6–8 cm[52-55], but has also been shown to be similar in short-segment Barrett's by large UK based study[56] and others[44]. Patients with known Barrett's oesophagus are thought to have 30 to 60 times the risk of developing oesophageal adenocarcinoma than the general population[57-60] but data from a recent study calls into question the rationale for ongoing surveillance in patients who have Barrett's oesophagus without dysplasia[49]. Hvid-Jensen et al analysed the data of all 11,028 patients with Barrett's oesophagus in Denmark during the period from 1992 through 2009 and conducted follow-up for a median of 5.2 years. During the study period, only 7.6% of adenocarcinomas diagnosed nationwide were diagnosed in patients known to have Barrett's oesophagus. The authors acknowledged Barrett's oesophagus as a strong risk factor for oesophageal adenocarcinoma, but found that the absolute annual risk of 0.12%, or 1 case of adenocarcinoma per 860 patient-years, was several times lower than the assumed risk of 0.5%, which forms the basis for current surveillance guidelines[61, 62].

Surveillance programs have yet to show any effect on survival[50, 63-67]. In fact, due to the low risk of malignant progression of Barrett's oesophagus, most patients with Barrett's die due to causes other than oesophageal adenocarcinoma[50]. The results of the most recent large studies and meta-analyses[49-51] call attention to the questionable rationale and cost-effectiveness of surveillance of Barrett's oesophagus and thus highlight the need for valid risk stratification to allow focus on the minority of patients that are likely to benefit from surveillance.

1.1.6.2.6 OBESITY

Adenocarcinoma has become the predominant tumour type in the Western world[7-10, 17]. The most likely explanation for this rapid increase in incidence seems to be the increasing prevalence of Barrett's oesophagus as a consequence of gastro-oesophageal reflux, which, in turn, is becoming more common with the increasing incidence of obesity[68-70]. The exact biological mechanisms by which obesity increases the risk of oesophageal cancer remain unknown and are likely to be multifactorial.

It has been suggested that obesity increases intra-abdominal pressure and gastro-oesophageal reflux[71]. Several studies have shown an association between obesity and gastro-oesophageal reflux and its complications [68-70, 72], although one study found this hypothesis to be true only in women[73], while another found it to be true chiefly in males[74].

Adipose tissue has long been considered to be primarily responsible for energy storage and was thought to be metabolically passive[75]. It is now known that along with its role in energy homeostasis, adipose tissue also functions as an intricate endocrine and immune organ which secretes a wide variety of cytokines, hormones, and other biochemically active substances which regulate insulin sensitivity and glucose homeostasis, hypothalamic activity, central sympathetic output, vascular tone, and reproduction, through endocrine, autocrine and paracrine effects[75]. Abdominal visceral adipose tissue in particular is now known to be metabolically active and to secrete a variety of molecules important in the pathogenesis of glucose intolerance and insulin

resistance, cardiovascular risk factors such as dyslipidemia and hypertension[76]. It is thought that the altered immunological, metabolic and endocrine environment present in obesity facilitates pro-inflammatory and pro-tumourigenic pathways thought to play a crucial role in the development of oesophageal adenocarcinoma[77].

1.1.7 CLINICAL PRESENTATION

The most common symptoms of oesophageal cancer are dysphagia, which occurs in three quarters of patients[20] and weight loss which is present in two thirds[78]. It may also present with a range of other symptoms such as odynophagia, vomiting, heartburn, regurgitation, epigastric pain, gastrointestinal bleeding, vomiting, dyspepsia and nausea. While 8% of patients with oesophageal cancer can present with hiccups as their initial symptom[79], some patients are entirely asymptomatic and are diagnosed on surveillance endoscopy for Barrett's oesophagus.

One of the reasons for the poor prognosis of oesophageal cancer is the advanced stage of disease at diagnosis in most patients[80] with one third of patients having metastatic disease at presentation[78]. One of the explanations for this is the aggressive biological nature of this disease, resulting in rapid dissemination. Another, and more modifiable reason, is the lack of awareness, especially among the public, of the symptoms of oesophageal cancer. FitzGerald et al[81] found that only 12% of patients questioned were aware of the main symptoms of oesophageal cancer. Grannell et al[82] reported that only 17 per cent felt that cancer was a probable explanation for dysphagia compared with 80 per cent who would consider cancer a likely cause of breast lump. Rothwell at al[83] reported that delay in patient presentation and resultant definitive

treatment (median 15 weeks) was multi-factorial. Not only did lack of patient awareness of the disease lead to delayed presentation to the general practitioner, but inefficient management by both family doctors and hospital services were implicated emphasising the importance of "fast-tracking" patients with the sinister symptoms and highlighting the need for increased awareness of oesophageal cancer.

1.1.8 DIAGNOSIS

Oesophageal cancer is an aggressive disease and only a quarter to one third of cases are diagnosed while the cancer is still confined to the primary site; one third are diagnosed after the cancer has spread to regional lymph nodes or directly beyond the primary site; and a third are diagnosed after the cancer has already metastasised[4, 78].

Patients presenting with dysphagia and weight loss should undergo urgent investigation. A barium swallow may show a suspicious ulcer or stricture but definitve diagnosis of oesophageal cancer, however, can only be made on endoscopy and biopsy. On endoscopy, macroscopic evaluation of the abnormality, accurate documentation of the level of the tumour and sufficient biopsies (we would suggest at least ten) are key factors. Histological analysis then confirms malignancy. In the presence of a macroscopic abnormality or clinical suspicion, endoscopy must be repeated if biopsies do not confirm malignancy. The definition of malignancy versus high-grade dysplasia, however, is contentious and what is diagnosed as malignant in Japan may be defined as high-grade dysplasia in the West[84].

Not all patients present symptomatically and many early cancers are detected on surveillance endoscopy for Barrett's oesophagus. The endoscopist must be vigilant in patients with both long- and short-segment Barrett's oesophagus and in those with dysplasia as these patients are at higher risk of oesophageal cancer[44, 53-56]. Indeed up to half of patients with severe dysplasia have co-existent invasive carcinoma[85].

1.1.9 STAGING AND RESTAGING

Patients are staged at diagnosis and should be restaged following treatment. The stage determines whether the intent of the therapeutic approach will be curative or palliative.

1.1.9.1 STAGING

A number of different staging systems are used to classify oesophageal tumours. The TNM staging system assesses tumours in three ways: extent of the primary tumour (T), absence or presence of regional lymph node involvement (N), and absence or presence of distant metastases (M). Once the T, N, and M are determined, a stage of I, II, III, or IV is assigned, with stage I being early and stage IV being advanced disease. The histologic grade assigned to a tumour reflects its biologic activity and is graded as well, moderately, poorly or undifferentiated.

The American Joint Committee on Cancer (AJCC) was established in 1959 to formulate and publish evidence-based systems of classification of cancer, including staging and end results reporting, to be used by health professionals to guide management and

determine prognosis of cancer patients. The *AJCC Cancer Staging Manual* and *Handbook* is currently in their 7th editions and the latest commentary of oesophageal cancer staging was published in 2010[28]. The definition of TNM staging is outlined in **Table 1**. The individual adenocarcinoma and squamous cell carcinoma groupings are outlined in **Table 2** and **Table 3** respectively.

Table 1: Definition of TNM Adapted From AJCC Cancer Staging Manual 7th Edition[28].

DEFINITION OF TNM		
Primary Tumour (T)		
TX		Primary tumour cannot be assessed
T0		No evidence of primary tumour
Tis		High grade dysplasia (HGD), formerly known as in situ
T1		Tumour invades lamina propria or submucosa
	T1a	Tumour invades mucosa or lamina propria
	T1b	Tumour invades submucosa
T2		Tumour invades muscularis propria
T3		Tumour invades adventitia
T4		Tumour invades adjacent structures
	T4a	Resectable cancer invades adjacent structures such as pleura, pericardium, diaphragm
	T4b	Unresectable cancer invades adjacent structures such as aorta, vertebral body, trachea
Regional Lymph Nodes (N)		
NX		Regional lymph nodes (i.e. any perioesophageal lymph node from cervical to celiac nodes) cannot be assessed
N0		No regional lymph node metastasis
N1		1-2 positive regional lymph nodes
N2		3-6 positive regional lymph nodes
N3		≥7 positive regional lymph nodes
Distant Metastasis (M)		
MX		Distant metastasis cannot be assessed
M0		No distant metastasis
M1		Distant metastasis
Histologic Grade (G)		
G1		Well differentiated
G2		Moderately differentiated
G3		Poorly differentiated
G4		Undifferentiated
Cancer Location		
Upper thoracic		20-25cm from incisors
Middle thoracic		>25-30cm from incisors
Lower thoracic		>30-40cm from incisors
Oesophagogastric Junction		Includes cancers whose midpoint is in the distal thoracic oesophagus, oesophago-gastric junction, or within the proximal 5cm of the stomach (cardia) that extend into the oesophago-gastric junction or distal thoracic oesophagus (Siewert III). These stomach cancers are stage grouped similarly to adenocarcinoma of the oesophagus

Table 2: Adenocarcinoma Stage Groupings Adapted From AJCC Cancer Staging Manual 7th Edition[28].

Adenocarcinoma				
AJCC Stage	Tumour (T)	Node (N)	Metastases (M)	Grade (G)
0	is (HGD)	0	0	1
IA	1	0	0	1-2
IB	1	0	0	3
	2	0	0	1-2
IIA	2	0	0	3
IIB	3	0	0	Any
	1-2	1	0	Any
IIIA	1-2	2	0	Any
	3	1	0	Any
	4a	0	0	Any
IIIB	3	2	0	Any
IIIC	4a	1-2	0	Any
	4b	Any	0	Any
	Any	N3	0	Any
IV	Any	Any	1	Any

Table 3: Squamous Cell Carcinoma Stage Groupings Adapted From AJCC Cancer Staging Manual 7th Edition[28].

Squamous Cell Carcinoma					
AJCC Stage	Tumour (T)	Node (N)	Metastases (M)	Grade (G)	Location
0	is (HGD)	0	0	1	Any
IA	1	0	0	1	Any
IB	1	0	0	2-3	Any
	2-3	0	0	1	Lower
IIA	2-3	0	0	1	Upper, middle
	2-3	0	0	2-3	Lower
IIB	2-3	0	0	2-3	Upper, middle
	1-2	1	0	Any	Any
IIIA	1-2	2	0	Any	Any
	3	1	0	Any	Any
	4a	0	0	Any	Any
IIIB	3	2	0	Any	Any
IIIC	4a	1-2	0	Any	Any
	4b	Any	0	Any	Any
	Any	N3	0	Any	Any
IV	Any	Any	1	Any	Any

The most clinically useful methods of staging are endoscopic ultrasonography (EUS), computerised tomography (CT), ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) and laparoscopy, although all of these are known to have their limitations, especially in detecting small tumour deposits.

1.1.9.1.1 ENDOSCOPIC ULTRASONOGRAPHY

Endoscopic ultrasonography (EUS) is often used to determine the depth of tumour invasion (T stage) and the presence of malignant regional and celiac lymph nodes in patients with oesophageal cancer (N stage). EUS, however, has a limited depth of penetration of approximately 5 cm and metastases in distant lymph nodes or organs can often be overlooked with this form of imaging[86]. A recent meta-analysis comparing the diagnostic performances of various staging techniques, however, found that EUS was significantly more sensitive but less specific than computerised tomography (CT) and ^{18}F-fluoro-2-Deoxy-D-Glucose Positron Emission Tomography (FDG-PET) for the detection of regional lymph node metastases but was shown to be particularly useful for the exclusion of regional lymph node metastases[87]. EUS may be combined with fine needle aspiration cytology (FNAC) to establish if questionable lymph nodes contain malignancy.

1.1.9.1.2 COMPUTERISED TOMOGRAPHY

Computerised Tomography (CT) is commonly used to determine the degree of involvement and whether malignant lymph nodes or distant metastases are present. However, in N staging, CT relies largely on "size criteria" which reduces its sensitivity and specificity in its ability to distinguish between lymph nodes enlarged by metastases or by a benign process, or detect tumour in a normal sized lymph node and also in detecting tumour deposits[88, 89]. The sensitivity of CT for detection of distant metastases ranges between <50% and >90%[90].

1.1.9.1.3 ^{18}F-FLUORO-2-DEOXY-D-GLUCOSE POSITRON EMISSION TOMOGRAPHY

^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) is used to detect the presence of malignant lymph nodes or distant metastases. Detection of tumour deposits by FDG-PET is based on an altered tissue glucose metabolism and has been shown to detect additional sites of metastatic disease at initial evaluation[91]. Tumour deposits less than 1cm in diameter may not be detectable by FDG-PET[92] and it is difficult to discriminate between lymph nodes adjacent to the primary oesophageal cancer and the primary tumour itself with FDG-PET due to its limited spatial resolution[88, 93].

Despite these limitations, a recent meta-analysis found that the diagnostic performance of FDG-PET was significantly higher than that of CT for distant metastases[87]. FDG-PET has been shown to detect metastatic disease in approximately 20% of patients who are considered as having only loco-regional disease on CT[90]. The accuracy for correct identification of recurrence in oesophageal cancer is also higher for FDG-PET than for CT scan[90]. Additionally, CT and EUS have been reported to be less effective than FDG-PET imaging at predicting long-term survival[94, 95].

1.1.9.1.4 PET/CT

Due to the limitations of CT and FDG-PET outlined above, integrated FDG-PET/CT scanners have been developed and are now commonly used. The introduction of integrated PET/CT has improved accuracy over the use of PET and CT imaging

conducted separately[96, 97]. PET/CT significantly improves the sensitivity, accuracy and negative predictive value of FDG-PET imaging in the assessment of locoregional lymph nodes[96]. The accuracy of PET/CT can lead to up and down-staging of patients resulting in a change in management in up to 17% of patients due to the detection of occult metastases or earlier disease than suspected with conventional imaging modalities[98]. Another study reported that the tumour length-SUV index could differentiate patients with unresectable disease and those who are potentially curable with a specificity of 90% and a sensitivity of 93%; and by combining this index with visual analysis, specificity could be increased to 96%[99].

1.1.9.1.5 LAPAROSCOPY

Diagnostic laparoscopy has been used to determine resectability and to avoid unnecessary surgery in patients with advanced oesophageal cancer. Diagnostic laparoscopy has been found to be particularly useful for detecting and confirming nodal involvement and distant metastatic disease that potentially would alter treatment and prognosis in patients with oesophageal cancer. One study by Heath et al[100] found that 76% of patients with abnormal-appearing nodes at laparoscopy were confirmed by biopsy to have node-positive disease, whereas 78% of patients with normal-appearing regional or celiac nodes, were confirmed by biopsy to be tumour free. In this study, laparoscopy changed the treatment plan in 17% of patients. Another larger series by de Graaf et al[101] found that sensitivity of laparoscopy for resectability was 88% and that staging laparoscopy avoided unnecessary laparotomy and changed patient management in 20.2% of patients. Laparoscopy was found to be most useful in adenocarcinoma, distal oesophageal and oesophago-gastric junction tumours and gastric cancers and was found to be probably unnecessary in lesions of

the upper two-thirds of the oesophagus[101]. The addition of peritoneal lavage and cytology to laparoscopy has been shown to detect both peritoneal macro- and micro-metastases so small as to evade the resolution of all current imaging techniques[102].

1.1.9.2 RE-STAGING

1.1.9.2.1 ENDOSCOPY

Endoscopy is an easily performed, well tolerated and a readily available investigation. Studies using endoscopy to determine luminal response in re-staging patients following neoadjuvant chemoradiotherapy have produced varying results. Brown et al[103] reported that an endoscopically normal lumen correlated with a 50% likelihood of a complete pathological response but neither biopsy of the lumen nor CT scanning were performed which may have reduced the accuracy of these assessments. The findings of a study from the Memorial Sloan-Kettering Cancer Center[104] were more disappointing where a negative endoscopy and biopsy was only 31% predictive of a complete pathological response. In this paper, however, only 71% of patients were biopsied following chemoradiotherapy, and neither the number of biopsies or the experience of the endoscopist were not commented upon.

1.1.9.2.2 ENDOSCOPIC ULTRASONOGRAPHY

There is one report in the literature which suggests that endoscopic ultrasonography was more accurate than CT in staging patients post-neoadjuvant chemoradiotherapy, with an accuracy of 77–81%[105] but routine restaging endoscopic US has not gained traction in the literature[106, 107], largely due to its inability to distinguish inflammation

and fibrosis from residual cancer[107]. In one study T stage accuracy was only 29% with a sensitivity of positive nodes of only 52%[106].

1.1.9.2.3 COMPUTERISED TOMOGRAPHY

CT is useful post neoadjuvant therapy for ruling out solid organ metastases and has up to a 78% accuracy in detecting nodal disease but was not found to be as accurate as PET/CT in reliably detecting nodal or residual disease or complete responders post neoadjuvant chemoradiotherapy[108-110].

1.1.9.2.4 ^{18}F-FLUORO-2-DEOXY-D-GLUCOSE POSITRON EMISSION TOMOGRAPHY

Studies evaluating tumour response with PET during and at the completion of neoadjuvant therapy have yielded encouraging results[91, 109, 111-118]. These studies suggest that changes in FDG uptake in response to therapy correlate with the pathological response as well as predicting the risk of local recurrence and survival. Levine et al[117], performed an FDG-PET at diagnosis and following chemoradiotherapy in 31 patients with oesophageal cancer. They found that the standardised uptake value (SUV) decreased significantly more in those patients who responded (pathological complete response or microscopic residual disease) than in those who did not (p=0.05).

1.1.9.2.5 PET/CT

PET/CT has been found to be unhelpful for restaging post-chemoradiotherapy due to the response to inflammation meaning that micro-deposits of tumour may remain undetected[108, 109]. Another prospective study, however, identified FDG-PET/CT as being

more accurate than EUS-FNA and CT scan for predicting nodal status and complete response following neoadjuvant therapy in patients with oesophageal cancer[110]. In this study FDG-PET/CT and CT alone were found to provide targets for biopsy, but results were often found to be falsely positive[110].

1.1.10 TREATMENT STRATEGIES

A multidisciplinary approach, including the input from family practice physicians, surgeons, gastroenterologists, radiologists, medical and radiation oncologists and specialist nurses, is necessary to improve the outlook for patients with this disease. Many treatment strategies are available and each newly diagnosed patient requires tailored therapy according to their stage of disease and overall health.

1.1.10.1 SURGERY ALONE

Techniques of curative oesophageal resection range from endoscopic mucosal ablation and mucosal resection to radical en bloc oesophagectomy.

1.1.10.1.1SURGICAL RESECTION

Surgery alone has long been the preferred treatment modality for loco-regional control of oesophageal cancer. The natural history of this disease, however, is to disseminate early and over 80 percent of patients undergoing potentially curative resection have micrometastases in their bone marrow at the time of resection[119]. Few patients with oesophageal cancer present early enough, or are fit enough, to undergo and to benefit from surgical resection. Only about 20% of oesophageal cancer patients have operable

localised disease (stages 0, I and II)[120] and only the minority of patients are considered suitable for resection[78, 121].

Despite advances in surgical techniques and aftercare, the mortality rate of surgery remains formidable. A recent SEERs database[122] reported a 14% mortality rate for resection in North America, and the morbidity of resection remains significant[123]. It incurs a considerable impairment of quality of life[124, 125]. Survivors are exposed to a lifetime risk of the complications of oesophago-gastric resection; recurrent laryngeal nerve injury will result in vocal cord paralysis and aspiration[126, 127], resection of the lower oesophagus and the lower oesophageal sphincter will result in reflux oesophagitis[128-130], vagotomy will result in "early" and "late" dumping[131, 132] and delayed gastric emptying[133] and post-operative anorexia, odynophagia and eating difficulties result in nutritional consequences[134].

A 1980 review by Earlam et al of 122 papers described the surgical care of 83,783 cases of squamous cell carcinoma of the oesophagus and revealed a dismal 5-year survival rate of 4%[3]. A decade later, a similar review article by Müller et al[135] quoted a 5-year survival rate of 10%. Overall, the current survival following surgery alone remains poor. While most series report a 5-year survival rate of around 20% to 40%[136-139], even following *en-bloc* resection, these data are drawn from hospital series of resectable patients with early disease. The current overall 5-year survival of all patients, including those treated by surgery, based on community data is a dismal 5%[140].

Super-selection of patients who are fit enough for surgery and have early disease, which may represent as few as 5.4% of patients undergoing resection[141], can lead to a decrease in mortality and post-operative morbidity and an increase in survival[142, 141]. It is clear that even those with early T1b disease frequently succumb to loco-regional recurrence or metastases[142,141]. Super-selection of patients for surgery can produce flattering long-term survival rates but cannot lead to an increase in the overall survival rate of oesophageal cancer and has little impact on the disease as a whole.

An R0 resection is defined as one in which all margins are histologically free of tumour and is the aim of all *en-bloc* resections, especially in those with early disease. Even in patients with early disease, however, Bosset et al[143] found a significantly lesser rate of curative resection in those who had surgery alone versus those who had neoadjuvant chemoradiotherapy. Similarly, Burmeister et al[123] described significantly fewer R0 resections in the surgery alone group versus those who had neoadjuvant chemoradiotherapy. Since R0 resectability impacts favourably on overall survival, the lower rate of R0 resection in those undergoing surgery alone is concerning, particularly in those staged as having early disease.

One of the traditional arguments in favour of surgery alone over neoadjuvant chemoradiotherapy for oesophageal cancer is that the latter increases the morbidity and mortality of oesophageal resection but the literature on this contention is conflicting. In their randomised controlled trial comparing neoadjuvant chemoradiotherapy and surgery to surgery alone, Burmeister et al[123] reported surgical complications in 55% of patients undergoing primary surgery compared with 49% in those receiving neoadjuvant chemoradiotherapy. There was an equal rate of

anastomotic leaks (5%) and a similar rate of cardiac complications in both groups. There were more pulmonary complications (28 vs. 20%) and an increase rate in anastomotic strictures (24 vs. 19%) in the surgery alone group compared with the chemoradiotherapy group. The mortality rate and median length of stay was the same in both groups. Similarly Berger et al[144], Lin et al[145], Kane et al[146], Kelsen et al[147] and Medical Research Council Oesophageal Cancer Working Group[148] reported no increase in morbidity or mortality following induction therapy.

Conversely, studies such as those of Eguchi et al[149] described an increase in morbidity and mortality in those who received chemotherapy before surgery versus those who had surgery alone. In their study, this was increased further in the group that received two cycles versus those who had only one cycle. There was also an increase in morbidity and mortality associated with preoperative chemoradiotherapy, especially when a higher dose of radiotherapy was administered. Hagry et al[150] and Bosset et al[143] also described an increase in morbidity and mortality in those undergoing preoperative chemoradiotherapy.

While a few studies show an increase in morbidity and mortality in those undergoing neoadjuvant chemoradiotherapy before resection, the weight of evidence seems to suggest that preoperative therapy does not impact negatively on morbidity or mortality while permitting a greater incidence of R0 resection.

Whilst surgery alone may be curative in that small subset of patients with true early disease, these patients cannot be identified with current pre-operative staging

techniques. Instead, it must be assumed that all patients with disease that has spread beyond the mucosa have systemic micrometastases, and other modalities are required to treat such patients who present at a more advanced stage of disease and for those who are unfit for surgery.

1.1.10.1.2 ENDOSCOPIC RESECTION AND ABLATION TECHNIQUES

Because of the malignant potential of Barrett's oesophagus, screening endocopy is performed to allow the detection of dysplasia before it progresses to adenocarcinoma. Traditionally, an oesophagectomy would be offered to patients with high-grade dysplasia but more recently endoscopic ablation and resection techniques have been developed. Endoscopic ablation techniques such as radiofrequency ablation, electrosurgery, and photodynamic therapy have been developed to destroy the neoplastic tissue and allow healthy squamous epithelium to re-grow with some success[151-154] with outcomes comparable to surgery[155]. However, there are concerns that residual areas of metaplasia may remain hidden beneath the newly grown squamous epithelium which may advance to invasive carcinoma[151, 156] and that this technique may not adequately treat foci of invasive carcinoma that may have been missed on initial pre-treatment evaluation biopsies.

Endoscopic mucosal resection is an alternative endoscopic technique with which the neoplastic epithelium is excised, allowing for both more accurate histopathologic diagnosis and curative therapy. This technique has been used safely and effectively in high-grade dysplasia and small intramucosal carcinomas[157-161] and can be comparable to surgery[162] with 5-year survival rates of up to 98% reported[160]. However, as with

surgery, since so few patients present with suitably early disease[141], this technique is unlikely to impact on the overall treatment and survival of oesophageal cancer sufferers.

1.1.10.2 CHEMOTHERAPY ALONE

Palliative chemotherapy is widely used as an alternative or additional treatment, with the intent to control tumour growth, improve quality of life and prolong survival in those with metastatic oesophageal cancer. Both squamous-cell and adenocarcinoma of the oesophagus are responsive to chemotherapy and in 15 to 55 percent of patients, undergoing various chemotherapeutic regimes, shrinkage of the tumour by at least 50 percent may occur[163-169]. The response to chemotherapy is usually short-lived, however, and survival rarely exceeds one year. There is a lack of evidence that chemotherapy improves survival and/or quality of life for these patients[19]. Despite the numerous phase II trials, only two randomised controlled trials comparing chemotherapy versus best supportive care have been published[170, 171]. These trials had conflicting results, had small patient numbers (156 and 24 respectively), and used different types of chemotherapy. There is a need for a randomised phase III trial comparing chemotherapy versus best supportive care to assess the impact of palliative chemotherapy on quality of life and survival.

1.1.10.3 RADIOTHERAPY ALONE

As the long-term survival for surgery alone is so poor, many felt that it could be equalled or improved on by radiotherapy alone, especially since radiotherapy alone was so effective in head and neck cancer.

Oesophageal cancer treatment with radium was first described by Exner in 1904[172] but the results for radiotherapy alone, however, have traditionally been disappointing. In a review of 49 early series involving more than 8400 patients treated with radiotherapy alone, survival rates at one, two, and five years were 18, 8, and 6%, respectively[173].

Trials comparing radiotherapy alone with surgery alone and chemoradiotherapy are difficult to interpret, since many involve patients with advanced and irresectable disease, widely varying doses of radiotherapy are used and many were performed before the modern radiotherapy era. Better results are reported in later studies from single institutions in well-defined patient populations, especially in early disease, using more modern radiotherapy protocols[174, 175] with up to 59% 5-year survival[174] but are limited by small numbers of patients[174, 175].

Badwe et al[176] compared radiotherapy alone with surgery alone in a randomised controlled trial. In this trial, survival in the surgery arm was significantly better than in the radiotherapy arm (p=0.002), although again the small number of patients recruited confounds interpretation. Previous to this, an MRC prospective randomised trial of radiotherapy versus surgery for operable squamous cell carcinoma of the oesophagus was discontinued at 18 months following recruitment of only 31 patients in 16 centres[177].

Okawa et al[178] conducted a randomised controlled trial comparing radiotherapy alone with radiotherapy and intraluminal brachytherapy for oesophageal squamous cell carcinoma and described an overall 5-year survival rate of 20%. There was no

statistically significant improvement in survival between the two groups, except on subgroup analysis of those with a tumour less than 5cm in length, where the addition of brachytherapy incurred a significant survival advantage (p=0.025).

There are more phase III trials comparing chemoradiotherapy with radiotherapy, but results for radiotherapy alone are disappointing with 5 year survival rates in the region of 0-14.5%[179, 180].

A Cochrane review of 19 randomised trials comparing chemoradiotherapy alone with radiotherapy alone for localised oesophageal carcinoma[181] demonstrated an absolute survival benefit for chemoradiotherapy at years one and two of 9 and 4% respectively. Additionally, there was an absolute increase in local recurrence for radiotherapy. According to this review, concomitant radiotherapy alone was inferior to chemoradiotherapy, when a non-operative approach was selected.

1.1.10.4 DEFINITIVE CHEMORADIOTHERAPY

The disappointing rates of survival and local control associated with single modality therapy, and the observation that at least one quarter of surgical specimens have shown complete tumour eradication following neoadjuvant chemoradiation[182] and the need for more effective non-surgical management led to the development of definitive chemoradiotherapy regimes for oesophageal cancer.

The landmark RTOG trial compared concurrent chemoradiotherapy ((5-fluorouracil) 5-Fluorouracil, Cisplatin and 50 Gy radiotherapy) to radiotherapy alone (64 Gy) in patients with locoregional thoracic oesophageal cancer (90% squamous cell carcinoma)[179]. This trial was stopped after interim analysis demonstrated a significant advantage for chemoradiotherapy with a significant reduction in both locoregional and distant failure for chemoradiotherapy. Long-term follow-up of this trial[183] demonstrated a 5-year survival rate for chemoradiotherapy of 26% compared with 0% following radiotherapy alone.

Minsky et al[184] conducted a trial to compare the local/regional control, survival and toxicity of combined-modality therapy using high-dose (64.8 Gy) versus standard-dose (50.4 Gy) radiation therapy for the treatment of patients with oesophageal cancer. They enrolled 236 patients with stage T1 to T4, N0/1, M0 squamous cell carcinoma or adenocarcinoma who had been selected for a non-surgical approach. This study showed that the higher radiation dose did not increase survival or local/regional control.

A Cochrane review examined 19 randomised controlled trials (eleven concurrent and eight sequential chemoradiotherapy studies) comparing combined chemotherapy and radiotherapy (without surgery) with radiotherapy alone in localised carcinoma of the oesophagus[181]. This work concluded that concurrent chemoradiotherapy provided a significant reduction in mortality, with an absolute survival benefit at years one and two for chemoradiotherapy of 9% and 4% respectively and an absolute reduction of local recurrence rate of 12%. The results of sequential chemoradiotherapy studies,

however, showed no significant benefit in survival or local control but significant toxicities[181].

It is uncertain whether definitive chemoradiotherapy can achieve treatment outcomes comparable to surgery, since there is only one small randomised controlled trial to date comparing chemoradiotherapy alone and surgery alone[185]. In this trial, involving eighty patients, a two- or three-stage oesophagectomy with two-field dissection was performed in the surgery alone group (N=44) and patients in the chemoradiotherapy group (N=36) received 5-FU, Cisplatin and concurrent 50-60 Gy radiotherapy. Although it failed to reach statistical significance, standard oesophagectomy or chemoradiotherapy offered similar early clinical outcome and survival. Similar results were noted by a Japanese group who performed a non-randomised retrospective comparison between definitive chemoradiotherapy and radical surgery in 82 patients with resectable oesophageal squamous cell carcinoma[186]. Thirty-three patients were treated with chemoradiotherapy and forty-nine with surgery and were followed up for a median of 36 months. The patients in the chemoradiotherapy group received 5-FU, Cisplatin and 50.4 Gy radiotherapy and those in the surgery group were treated by oesophagectomy with radical node dissection. Eighteen patients in the surgery alone group went on to have post-operative chemotherapy. The overall survival rates and disease-free survival rates at 3-years were 48% and 44% in the CRT group and 65% and 59% in the surgery group, respectively. Although this non-randomised study lacked statistical significance, it showed that chemoradiotherapy could result in survival comparable with conventional surgery. Another similar study of 98 patients showed a trend favouring definitive chemoradiotherapy over surgery in the treatment of

oesophageal carcinoma, even though those receiving chemoradiotherapy had more advanced disease[187].

Bedenne et al[188] reported on a randomised trial in which patients with locally advanced tumours and who were responding to induction therapy (two cycles of 5-fluorouracil and Cisplatin and either conventional or split-course concomitant radiotherapy) were randomised to chemoradiation alone or chemoradiation followed by surgery. Two hundred and fifty-nine patients who responded to treatment and who had no contraindication to either therapy were randomly assigned to surgery or continuation of chemoradiation. This study found that chemoradiation alone and chemoradiation followed by surgery were equivalent in both terms of survival and quality of life in responders. These results are consistent with the results from the study by Stahl et al[189] in which 172 patients with oesophageal cancer were randomly assigned to either chemoradiation with surgery or chemoradiation without surgery. Median survival time was 16.4 months with surgery compared with 14.9 months without surgery, and 2-year survival rates were 39.9% and 35.4%, respectively (p=0.007).

Definitive CRT is now used in the USA in nearly as many patients as undergo surgery (30 vs. 34%)[80] and is now being offered in several centres for patients with potentially resectable tumours[183, 187, 190]. A recent phase II trial of chemoradiotherapy for stage I oesophageal squamous cell carcinoma conducted in Japan demonstrated a complete response rate of 87.5% with a 4-year survival of 81% and thus verified the effectiveness of chemoradiation in very early disease[191].

Larger-scale randomised trials comparing radical chemoradiotherapy with surgery alone in both adenocarcinoma and squamous cell carcinoma are necessary but may pose difficulties in recruitment of patients and treating clinicians alike.

1.1.10.5 NEOADJUVANT CHEMOTHERAPY

As the majority of resected patients succumb to cancer[80, 135, 192], most likely due to the persistence of micrometastases undetectable by current staging modalities[119], the elimination of microscopic disease has become a key consideration. Giving chemotherapy pre-operatively urgently addresses this putative microscopic burden of disease before it can become any greater.

A number of trials have investigated whether neoadjuvant chemotherapy followed by surgery leads to an improvement in cure rates (**Table 4**), but the results have been conflicting and subsequent meta-analyses have also failed to reach a consensus with some, including the largest of the randomised trials, demonstrating a survival advantage[148, 193, 194] and some not[195, 196].

The largest randomised controlled trial to date, the MRC trial[148], randomised 802 patients with squamous cell (31%) and adenocarcinoma (66%) of the oesophagus, in 42 European centres, to two cycles of neoadjuvant chemotherapy with Cisplatin and 5-fluorouracil (5-FU). This trial found that the overall survival was better in the neoadjuvant chemotherapy group (p=0.004) than in the surgery alone group. Long-term follow up of this trial confirmed that neoadjuvant chemotherapy improved survival in operable oesophageal cancer (p=0.03) with a 5-year survival of 23% for neoadjuvant chemotherapy compared with 17% for surgery alone[197].

The most recent meta-analysis examined eleven randomised controlled trials involving 2019 patients comparing neoadjuvant chemotherapy with surgery alone[198]. This meta-analysis concluded that neoadjuvant chemotherapy may offer a survival advantage compared to surgery alone for resectable thoracic oesophageal cancer of either histological subtype, but that further research was necessary. The authors found that there was no evidence of a difference in rate of resections, tumour recurrence, or post-operative morbidity with the addition of neoadjuvant chemotherapy and proposed that the most beneficial chemotherapy combination appeared to be Cisplatin and 5-Fluorouracil based.

Table 4: Randomised Controlled Trials Comparing Neoadjuvant Chemotherapy And Surgery With Surgery Alone In Oesophageal Cancer.

Author	Year	Tumour Type	Treatment	No. of Patients	Survival Advantage (p Value)
Schlag[199]	1992	SCC	C/5FU	22	NS
			Surgery	24	
Nygaard[200]	1992	SCC	CB	44	NS
			Surgery	41	
Maipang[201]	1994	SCC	BVC	24	NS
			Surgery	22	
Law[202]	1997	SCC	C/5FU	74	NS
			Surgery	73	
Kelsen[147]	1998	AC/SCC	C/5FU	213	NS
			Surgery	227	
Ancona[203]	2001	SCC	C/5FU	47	NS
			Surgery	47	
MRC[148]	2002	AC/SCC	C/5FU	400	<0.01
			Surgery	402	

C/5FU= Cisplatin/5-Fluorouracil
CB= Cisplatin/bleomycin
BVC= Bleomycin/Vindesine/Cisplatin
NS= Not statistically significant

1.1.10.6 NEOADJUVANT RADIOTHERAPY

In many patients who have early recurrent disease, the disease recurs locally. It is reasonable therefore to attempt to "sterilise" the tumour bed prior to resection. Neoadjuvant radiotherapy has been explored as a possible means of reducing local spread, thereby, improving survival. By down-staging the tumour, it was hoped to increase tumour resectability and improve survival.

Five prospective randomised controlled trials have investigated the effects of preoperative radiotherapy using varying doses of radiotherapy[200, 204-207] (**Table 5**). All but one trial examined the role of neoadjuvant radiotherapy in squamous cell carcinoma alone.

A meta-analysis of these five trials with long-term follow-up data suggested that neoadjuvant radiotherapy may provide a small survival advantage for patients with potentially resectable cancer of the oesophagus[208]. This meta-analysis found that there was no clear evidence that neoadjuvant radiotherapy was detrimental in terms of survival. Any small benefit derived from preoperative radiotherapy could be offset, however, by the increased morbidity, cost, and duration of treatment associated with giving radiotherapy pre-operatively. It was concluded in this meta-analysis, therefore, that neoadjuvant radiotherapy could not be routinely recommended outside of controlled clinical trials.

Table 5: Randomised Controlled Trials Comparing Neoadjuvant Radiotherapy And Surgery With Surgery Alone In Oesophageal Cancer.

Author	Year	Tumour Type	Treatment	No. of Patients	Radiotherapy Dose	Survival Advantage
Launois[204]	1981	SCC	NART Surgery	67 57	39-45Gy	NS
Gignoux[205]	1988	SCC	NART Surgery	102 106	33Gy	NS
Wang[206]	1989	SCC	NART Surgery	104 102	40Gy	NS
Nygaard[200]	1992	SCC	NART Surgery	48 41	35Gy	NS
Arnott[207]	1992	SCC & AC	NART Surgery	90 86	20Gy	NS

NART=Neoadjuvant radiotherapy
NS=Not statistically significant

1.1.10.7 NEOADJUVANT CHEMORADIOTHERAPY

The aim of combining neoadjuvant chemotherapy and radiotherapy is to exploit the radiosensitising effects of chemotherapy to reduce the tumour size and maximise local control[179], in addition to acting against micrometastases and leading to better curative resection rates and improvement in survival[123, 143]. Neoadjuvant chemoradiotherapy has recently become the focus of interest in an effort to prolong survival and reduce recurrence rates in patients with oesophageal cancer.

Patients who have a complete pathological response to neoadjuvant chemoradiotherapy are known to have a significant survival advantage over incomplete responders[209-212]. There have been significant advances in increasing the complete response rates of between 43 and 87.5%[191, 213] depending on disease stage and regimen

employed, with the highest complete response rates reported for patients with earliest disease stage[191].

To date, there have been many randomised controlled trials comparing neoadjuvant chemoradiotherapy and surgery (multimodal therapy) with surgery alone (**Table 6**). The results have been conflicting and subsequent meta-analyses (**Table 7**) and even meta-analysis of meta-analyses[214] have also failed to reach a consensus with some, demonstrating a survival advantage[182, 215-222] and some not[123, 143, 200, 211, 213, 214, 223, 224]. Most such randomised trials of neoadjuvant chemoradiotherapy versus surgery alone do not have enough power to show smaller yet worthwhile survival improvements. It has also been suggested that that due to the substantial variation of outcomes after surgery alone - due to case selection, variation in staging techniques, advances in peri-operative care and surgical expertise - the benefits achieved by a moderately effective neoadjuvant therapy might be obscured[222]. As such, conclusions from trials showing benefit from neoadjuvant therapy have been criticised because outcomes in the control (i.e. surgery alone) group have been regarded as suboptimal[225-230].

Traditionally, meta-analysis has been used to increase the precision of the comparisons of such trials and the estimation of treatment benefit. Interpretation of these meta-analyses has been limited, however, due to treatment heterogeneity (e.g. use of different chemotherapeutic agents and concurrent versus sequential radiotherapy), grouping of different tumour types together, small patient numbers and short-term follow-up. The trend is, however, that neoadjuvant chemoradiotherapy seems to improve outcome in oesophageal cancer with the most recent meta-analysis showing an absolute survival benefit at 2 years of 8.7%, with similar survival benefits in

adenocarcinoma (p=0.02) and squamous cell carcinoma (p=0.004)[222]. The complete results of the most recent randomised controlled trial must be awaited and may shed new light on the current controversy[221].

Table 6: Randomised Trials Comparing Neoadjuvant Chemoradiotherapy And Surgery (Multimodal Therapy) With Surgery Alone In Oesophageal Cancer

Trial	No. of Patients	Tumour Type	Chemotherapy	Radiotherapy	CPR Rate	Conclusion
Nygaard 1992[200]		SCC	Cisplatin	35 Gy	-	
▪ Multimodal	47		Bleomycin	Sequential		NS
▪ Surgery	41					
Apinop 1994[223]		SCC	Cisplatin	40 Gy	27%	
▪ Multimodal	35		5-FU	Concurrent		NS
▪ Surgery	34					
Le Prise 1994[224]		SCC	Cisplatin	20 Gy	9.8%	
▪ Multimodal	41		5-FU	Sequential		NS
▪ Surgery	45					
Walsh 1996[182]		AC	Cisplatin	40Gy	25%	
▪ Multimodal	58		5-FU	Concurrent		Multimodal superior p<0.01
▪ Surgery	55					
Bosset 1997[143]		SCC	Cisplatin	37 Gy	21%	
▪ Multimodal	143			Split course		NS
▪ Surgery	139					
Urba 2001[211]		AC	Cisplatin	45 Gy	28%	
▪ Multimodal	50	SCC	5-FU	Hyperfractionated		NS
▪ Surgery	50	Mixed	Vinblastine			
Lee 2004[213]		SCC	Cisplatin	45.6 Gy	43%	
▪ Multimodal	51		5-FU	Hyperfractionated		NS
▪ Surgery	50					
Burmeister 2005[123]		AC	Cisplatin	35 Gy	16%	
▪ Multimodal	128	SCC	5-FU	Concurrent		NS
▪ Surgery	128	Mixed				
Tepper 2008[215]		AC	Cisplatin	50.4 Gy	40%	
▪ Multimodal	30	SCC	5-FU	Concurrent		Multimodal superior p=0.002
▪ Surgery	26					
Mariette 2010[231]		AC	Cisplatin	45 Gy	-	NS
▪ Multimodal	97	SCC	5-FU	Concurrent		
▪ Surgery	98					
Van der Gaast 2010[221]*		AC	Paclitaxel	41.4 Gy	33%	
▪ Multimodal	273	SCC	Carboplatin	Concurrent		Multimodal superior p=0.011
▪ Surgery	86					

AC = Adenocarcinoma
SCC = Squamous Cell Carcinoma
5-FU = 5 Fluorouracil
CPR = Complete Pathological Response
NS = not statistically significant
*Preliminary results

Table 7: Meta-Analyses of Neoadjuvant Chemoradiotherapy And Surgery (Multimodal Therapy) Versus Surgery Alone In Oesophageal Cancer

Author	Year	No. of Trials	No. of Patients	Conclusion
Urschel[216]	2003	9	1116	Multimodal superior p=0.038*
Kaklamanos[193]	2003	5	669	Multimodal superior‡
Fiorica[217]	2004	6	764	Multimodal superior p=0.03*
Malthaner[230]	2004	8	1008	Trend towards multimodal superior at 1 year‡
Greer[232]	2005	6	738	Multimodal superior‡ p=0.07†
Geh[218]	2006	26	1335	Increasing radiotherapy increases CPR p=0.006
Gebski[219]	2007	10	1209	Multimodal superior p=0.002**
Graham[233]	2007	6	733	Multimodal superior: QALY
Lv[220]	2009	14	1737	Multimodal superior† p=0.015
Sjoquist[222]	2011	13	1932	Multimodal superior p<0.0001

*3-year survival benefit over surgery alone
**2-year survival benefit
†5-year survival benefit over surgery alone
‡Not statistically significant
CPR = Complete pathological response
QALY = Quality-adjusted life-years

1.1.10.8 ADJUVANT CHEMOTHERAPY AND ADJUVANT RADIOTHERAPY

Not all patients undergoing resection will require chemotherapy or radiotherapy. Clearly if the tumour is confined to the mucosa, local involvement is unlikely and adjuvant treatment unnecessary. Oesophageal cancer, however, can spread early to lymph nodes and adjacent structures and all too frequently surgery alone is not enough to offer a positive long-term outcome for patients with this disease.

Variations of post-operative or adjuvant treatments have been explored extensively in the literature and are summarised below. Such adjuvant treatments however are infrequently used in clinical practice.

1.1.10.8.1 ADJUVANT CHEMOTHERAPY

Adjuvant chemotherapy is provided after surgery to eliminate systemic micrometastases which are present in over 80% of patients at resection[119]. This approach over surgery alone, however, is not supported in two randomised trials[234, 235]. Another randomised trial evaluated adjuvant chemotherapy versus adjuvant radiotherapy following curative oesophageal resection and found no difference in 3-year survival between the groups[236].

1.1.10.8.2 ADJUVANT RADIOTHERAPY

The rationale for post-operative radiotherapy is that this may help eliminate residual local disease following incomplete resection, or following retention of residual microscopic disease.

One randomised trial compared neoadjuvant radiotherapy with adjuvant radiotherapy following curative oesophageal resection[237]. No difference in the survival rate was detected, but there was increased morbidity with neoadjuvant radiotherapy.

Other randomised trials compared surgery and adjuvant radiotherapy with surgery[237-241]. Overall, there was no significant difference in the risk of mortality with post-

operative radiotherapy and surgery at one year compared with surgery alone. Although the rate of local recurrence with radiotherapy was lower in three trials[238, 239, 241], two of the trials noted this benefit was achieved at the expense of increased morbidity[238, 239].

1.1.10.9 ADJUVANT CHEMORADIOTHERAPY

To date, no randomised trial has evaluated adjuvant chemoradiotherapy versus surgery alone, despite the success of neoadjuvant chemoradiotherapy regimes. A survival benefit has been suggested, however, when modern adjuvant chemoradiotherapy is compared with historical experience[242, 243]. In a recent series, Rice et al[244] sought to determine whether chemoradiotherapy improved outcome after oesophagectomy. In this review the addition of post-operative adjuvant chemoradiotherapy to oesophagectomy alone doubled survival time, time to recurrence and recurrence-free survival in patients with locoregionally advanced (T3-4, N1, or M1a) oesophageal carcinoma.

One prospective randomised trial compared the outcomes of adjuvant radiotherapy with adjuvant chemoradiotherapy[245]. This study did not demonstrate a survival advantage of radiotherapy administered concurrently with chemotherapy compared with chemotherapy alone.

1.1.10.10 SALVAGE SURGERY

Definitive chemoradiotherapy is being used more frequently especially in patients with early disease. Often surgery is not intended as part of the treatment plan for patients undergoing definitive chemoradiotherapy, but salvage oesophagectomy may be offered in an attempt to cure those with an incomplete response or recurrent disease. Five-year survival rates of up to 25–35% can be achieved by salvage oesophagectomy after local failure of CRT[246], but it is a formidable procedure and there must be careful patient selection.

1.1.10.11 PALLIATIVE THERAPY

At least one third of patients with oesophageal cancer have metastases at presentation and many without metastases are not fit for further treatment[78]. Self-expanding metal stent placement, external beam radiotherapy, intraluminal radiotherapy (brachytherapy), laser therapy, blood transfusion and nutritional optimisation are some of the commonly used palliative modalities to improve dysphagia and other symptoms of oesophageal cancer[247, 248].

As outlined in section 1.1.10.2, palliative chemotherapy is also used to control tumour growth, improve quality of life and prolong survival in patients with metastatic oesophageal cancer. Although shrinkage of the tumour by 50 percent may occur[163-169], there is a lack of evidence that this treatment modality improves quality of life and survival beyond one year is rare[19].

1.2 MICROMETASTASES

Approximately 20 to 30% of patients with early stage epithelial cancers who have curative surgery develop overt metastases within 5 to 10 years[249-252]. Similarly, in oesophageal cancer, surgery alone can be curative for patients with true loco-regional disease but the majority of patients currently staged as having tumour confined to the oesophagus and regional lymph nodes succumb to oesophageal cancer[80, 135, 253]. Even the most selective surgical series cannot achieve long-term survival in the majority of their patients. This late metastatic relapse seems to be mainly due to a phenomenon described as early dissemination of tumour cells from the primary tumour, occurring at an unknown time prior to surgery. These micrometastatic cancer cells reside mainly in the bone marrow after their dissemination, and are believed to carry the potential to develop into overt and usually fatal metastases.

At present, the sensitivities of conventional histopathological, biochemical and radiological staging techniques is sub-optimal for the detection of minimal residual disease and latent metastases[254]. To improve detection of disseminated epithelial malignancy, immuno-histochemical and molecular methods have been employed that search for epithelial cell-specific proteins in non-epithelial tissue. These are not yet incorporated into routine clinical practice however, for many reasons, most notably due to the lack of standardisation and automation of the technology and techniques employed.

Micrometastases within the bone marrow have been shown to indicate a poor prognosis in patients with epithelial tumours. The detection of micrometastases in tumour types such as colon[255], lung[256] and breast[257] cancers is indicative of poorer outcome. The presence of micrometastases is also associated with the depth of penetration of the primary tumour[258, 259], degree of cytological differentiation[260], and increased tumour microvessel density[261], recognised to correlate with poor survival in oesophago-gastric cancer[262]. The degree to which micrometastases represent true residual disease or cell shedding and metastatic potential, however, is unclear[263, 264].

These epithelial deposits are easily identified within the bone marrow, as cytokeratin-positive cells[119, 265] and have been identified in the majority of patients presenting with oesophageal cancer[119, 266]. Several studies have investigated the prognostic significance of bone marrow micrometastases in oesophageal cancer with variable results[119, 267-269]. This may reflect the marrow site which has been studied. Most have examined iliac crest marrow, which is a site remote from the tumour source. O'Sullivan et al[119] examined marrow flushed from the resected rib segment at thoracotomy and found that micrometastases were present in 88% of 50 patients with oesophageal cancer. This study established that haematogenous spread of these metastatic cells was independent of histological type of tumour or nodal status. These micrometastatic cells were found to be viable, tumourigenic (in nude mice) and resistant to neoadjuvant therapy. Ryan et al[269] found viable tumour cells in more than half of all marrow cultures from patients who had recieved chemoradiotherapy and surgery, also suggesting resistance of these cells to chemoradiation. Thorban et al [268] prospectively studied 225 patients with squamous cell carcinoma of the oesophagus. This study showed a significant survival difference between patients with and without epithelial

cells in bone marrow ($p<0.001$) and found that bone marrow status was an independent prognostic factor.

There is accumulating evidence that the detection of micrometastases may provide independent prognostic information and these micrometastases may be used as targets in the development of novel modes of treatment[252, 254], but their exact role in oesophageal cancer and its prognosis currently remains to be determined.

1.3 HISTOLOGICAL PREDICTORS OF RESPONSE AND RESISTANCE

Chemoradiotherapy holds the most promise for positive outcomes in oesophageal cancer care. Randomised trials have shown a survival advantage for neoadjuvant chemoradiotherapy over surgery alone[182, 215, 221]. Chemoradiotherapy can induce a complete pathological response in over one quarter of patients[182, 211, 215, 221, 223] with more recent regimens achieving even higher complete response rates[191, 213]. The chief shortcoming of neoadjuvant chemoradiotherapy it that is still not possible to know, in advance of treatment, which patients will respond and which patients will not benefit or indeed be harmed by the treatment[143].

There is an urgent need, therefore, for markers of response or resistance to treatment, especially considering the now widespread use of neoadjuvant therapy in oesophagal cancer care. Those patients deemed to have tumours responsive to chemoradiotherapy could be identified prospectively and be considered for neoadjuvant treatment with or without surgery. Those patients predicted to respond poorly could be spared the potential morbidity, inconvenience, time and financial burden of undergoing such treatment, and may opt for alternative treatment regimes or palliative measures alone.

Much work has been done in this area using histological indices, clinical parameters, radiological imaging, and a wide range of tissue and serum markers[270]. Whilst many methods have shown potential, no one technique has come to the fore or has been adopted into routine clinical practice. Immunohistochemistry is a convenient and

inexpensive technique used in the routine diagnostic laboratory which is simple to conduct and straightforward interpret. Individual tissue markers have therefore been extensively studied in human cancers and indeed in oesophageal cancer and have shown potential for clinical application[270-286]. To date, however, they have not been shown to be sufficiently accurate on their own, and comparison of studies is difficult due in part to differing techniques, different tumour types, variability of results and lack of standardisation.

If suitable immunohistochemical markers are identified, they may provide invaluable information for patients and and their multidisciplinary team.

1.3.1 P53, METALLOTHIONEIN AND VEGF

Response and resistance of cancer cells to chemotherapy and/or radiotherapy may be influenced by their propensity to undergo apoptosis which, when induced by chemoradiotherapy, involves various biological processes such as DNA repair, altered drug metabolism, inflammation and alteration of the cell cycle[287, 288]. The molecular markers p53, metallothionein and vascular endothelial growth factor (VEGF) all play a central role in this process and may be detected by immunohistochemical means in tumours.

1.3.1.1 P53

The p53 gene, and the protein it expresses (also known as protein 53, or tumour protein 53), is amongst the most widely investigated genes and proteins in humans. p53 has many anti-cancer mechanisms[289] and is often referred to as the "guardian of the genome"[290]. It can activate DNA repair proteins when DNA has sustained damage. It can induce growth arrest by holding the cell cycle at the G1/S regulation point on DNA damage recognition allowing DNA repair before allowing it to continue the cell cycle. It plays a role in genetic stability. It can inhibit angiogenesis and can initiate apoptosis, or programmed cell death, if the DNA damage proves to be irreparable. If p53 is damaged or defective, tumour suppression is severely reduced. More than 50 percent of human tumours contain a mutation or deletion of the p53 gene[291]. p53 has been the subject of investigation in many human cancers, including oesophageal cancer and it has been implicated, at least in part, in therapeutic resistance and prognosis.

1.3.1.1.1 P53 EXPRESSION AND PROGNOSIS

The protein that p53 encodes has been found to be one of the prognostic indicators in various cancers such as prostate[292], breast[293] and lung[294], where accumulation of the protein correlated with a poor prognosis. There is growing evidence that abnormalities of p53 expression in oesophageal cancer might have a relationship with survival.

Studies evaluating the prognostic significance of p53 expression have focused primarily in oesophageal squamous cell carcinoma[271-279] and to a lesser extent adenocarcinoma[280-283] with few including both tumour types[284, 285] and the results have been conflicting[271-286]. Several studies have identified p53 as a good prognostic indicator for tumour invasiveness and propensity to metastasise or recur[274, 275, 286] and improved survival has been demonstrated in tumours with negative expression for p53 over those positive for p53 expression[271, 273-275, 277, 278, 282, 285, 286]. In contrast however, several other studies have not found p53 to have prognostic significance[272, 276, 280, 284] with one study suggesting that failure to find a significantly shorter disease free survival in p53 positive tumours may actually reflect the ability of neoadjuvant chemoradiotherapy to improve outcome[280].

1.3.1.1.2 P53 AND RESPONSE AND RESISTANCE TO CHEMORADIOTHERAPY

Response or resistance of cancer cells to chemotherapy and or radiotherapy may be influenced by their propensity to undergo apoptosis, or programmed cell death. p53 is one of the most important regulators of this process[289] and therefore it is possible that

p53 expression may play a central role in treatment resistance. Several studies have explored this concept, with mixed results.

Seitz et al[278] examined p53 expression in squamous cell carcinoma and identified a significant association between p53 over-expression and a lower complete response rate. Krasna et al[283] also found that p53 protein expression in pre-treatment endoscopy specimens may predict response to trimodality therapy and survival in these patients, but this study was limited by small numbers. Sunada et al[279] found p53 positivity or negativity, in association with other markers, predictive of sensitivity to definitive chemoradiotherapy (5-fluorouracil, cisplatin and 60Gy radiotherapy) in squamous cell carcinoma. In contrast with Seitz et al[278], Sarbia et al[277] found that tumours without p53 expression showed a trend towards more frequent response to treatment than p53 positive tumours, but this failed to achieve statistical significance.

Similarly, in oesophageal adenocarcinoma, Duhaylongsod et al[280] studied p53 immunoreactivity in 42 patients with adenocarcinoma who underwent neoadjuvant chemoradiotherapy. In this study, p53 positivity significantly correlated with the presence of residual disease after neoadjuvant cisplatin, 5-flourouracil and radiotherapy ($p=0.01$). This study also showed a trend towards significance between p53 over-expression and lymph node metastasis. With greater numbers, the correlation between p53 and lymph node metastasis may have shown significance and thus could be a very useful marker of response and down-staging to treatment.

1.3.1.1.3 P53 EXPRESSION AND BARRETT'S OESOPHAGUS

Barrett's oesophagus is a well recognised risk factor for oesophageal carcinoma and there has been much investigation into role of p53 in the progression of Barrett's oesophagus to adenocarcinoma. p53 expression is significantly higher in patients with Barrett's oesophagus (up to 50%) versus controls (1-10%) ($p < 0.005$)[295]. Duhaylongsod at al[280] examined p53 expression in 42 patients with oesophageal adenocarcinoma, 22 of whom had Barrett's metaplasia and found that the frequency of p53 expression occurred equally if Barrett's metaplasia was present or absent. A recent study by Binato et al[296] indicated that over-expression of p53 could be associated with the development and progression to oesophageal adenocarcinoma in patients with gastro-oesophageal reflux disease. An extensive review of the significance of p53 in Barrett's oesophagus concluded that p53 function plays a major and common role in the transition of Barrett's metaplasia to dysplasia to cancer[297].

1.3.1.2 METALLOTHIONEIN

The metallothioneins are a family of low molecular weight, cysteine-rich proteins, which have a high affinity for metal ions[298]. Metallothioneins are known to be involved in many pathophysiological processes, including metal ion homoeostasis, protection against oxidative damage and cell proliferation and apoptosis[299, 300]. Over-expression of metallothionein has been described in a variety of human tumours, in relation to different stages of tumour development, progression and metastasis[301] and is also known to be involved in chemo-resistance and radiotherapy resistance[302].

1.3.1.2.1 METALLOTHIONEIN EXPRESSION AND PROGNOSIS

Metallothionein, like p53, has been considered as a potential prognostic marker in various carcinomas. Over-expression of metallothionein correlates significantly with a poorer prognosis in breast carcinoma[303-305], and more aggressive and advanced tumours, such as pancreatic carcinoma[306] and malignant melanoma[307]. In oesophageal squamous cell carcinoma, over-expression of metallothionein has been shown to correlate with metastatic tumour activity and proliferative potential[308]. Expression of metallothionein in tumours from patients with oesophageal cancer treated with neoadjuvant chemoradiotherapy has been shown to have prognostic significance especially in association with other markers[279, 309] but results from another study showed no such association with prognosis[310].

1.3.1.2.2 METALLOTHIONEIN EXPRESSION AND RESPONSE AND RESISTANCE TO CHEMORADIOTHERAPY

The ability of metallothionein to inhibit apoptosis[311] and its free radical scavenging property[299] are thought protect tumour cells from radiation and chemotherapeutic agents. Thus, metallothionein is implicated in chemo-resistance and radiotherapy resistance[302]. Its over-expression has been linked with resistance to cisplatin in many tumour types such as small cell lung[312], prostatic[313], hepatocellular[314] and testicular cancer[315]. It has also been implicated in oesophageal squamous cell carcinoma[309, 316, 317], where cisplatin forms the cornerstone of the most successful treatment regimes[182, 215].

1.3.1.2.3 METALLOTHIONEIN EXPRESSION AND BARRETT'S OESOPHAGUS

Li et al[318] reported that metallothionein expression was significantly increased with histological progression towards adenocarcinoma. This study also suggested that metallothionein may contribute to cytoprotection, thereby inhibiting apoptosis and leading to carcinogenesis of Barrett's oesophageal cells. Another, but far smaller study, demonstrated that there was no association between the metallothionein levels in Barrett's epithelium and the presence of inflammatory cells, metaplasia or dysplasia[319]. This group concluded that metallothionein is a marker of progression from normal to Barrett's epithelium but is not increased in oesophageal adenocarcinoma.

1.3.1.3 VASCULAR ENDOTHELIAL GROWTH FACTOR

Vascular endothelial growth factor (VEGF) belongs to a sub-family of growth factors that are important signalling proteins involved in angiogenesis. VEGF is one of the most powerful and specific inducers of new vasculature in malignant neoplasms and plays a vital role in inhibiting tumour cell apoptosis[320]. This angiogenesis has an important role in metastasis and tumour growth[321]. It has anti-apoptotic activity[320] and has been implicated in treatment resistance[322].

1.3.1.3.1 VEGF EXPRESSION AND PROGNOSIS

VEGF expression has been shown to correlate with poor prognosis in many cancers such as breast[323, 324], lung[325, 326] and colon[327]. The data in oesophageal cancer mainly refers to squamous cell carcinoma, where a positive correlation between VEGF expression and presence of local lymph node metastases, depth of tumour invasion

and the presence of distant metastases has been demonstrated[328-333]. Low levels are associated with improved long-term outcome[334].

1.3.1.3.2 VEGF EXPRESSION AND RESPONSE AND RESISTANCE TO CHEMORADIOTHERAPY

Tumour microcirculation and vessel permeability have a strong influence on tissue oxygenation, drug delivery and radio-sensitisation of cancer cells[335]. For these reasons VEGF expression in oesophageal tumours has been explored as a means of predicting response to chemoradiotherapy. Research has shown that levels of VEGF in pre-treatment biopsies are significantly higher in non-responders than in individuals who respond to chemoradiotherapy[336, 337]. Similarly, weak VEGF immunoreactivity in pre-treatment biopsies is associated with a higher incidence of complete tumour regression and improved long-term survival after neoadjuvant chemoradiotherapy with 5-fluorouracil, cisplatin and 36 Gy radiotherapy[334]. Interestingly, circulating VEGF levels in patients with oesophageal cancer are unchanged following neoadjuvant chemoradiotherapy, which may explain in part the failure of chemotherapeutic regimens to deal with the circulating micrometastatic burden, even in the setting of a complete local response[338].

1.3.1.3.3 VEGF EXPRESSION AND BARRETT'S OESOPHAGUS

Angiogenesis is one of the key processes in tumourigenesis and growth of cancers. The acquisition of angiogenic properties may identify a subset of pre-invasive lesions such as in Barrett's oesophagus, as suggested for colon carcinoma[339]. Angiogenic factors, such as VEGF, therefore, may prove to be useful prognostic markers for the management of pre-neoplastic lesions and adenocarcinomas in Barrett's oesophagus.

One study by Couvelard et al[340] quantified vascularisation in a large surgical series of Barrett's adenocarcinoma and associated pre-neoplastic lesions. Using immunohistochemistry, they examined the expression of VEGF and correlated these results with clinico-pathological data and prognosis. This study showed that while high-grade dysplastic Barrett's mucosa presented with a higher microvessel density compared with non-dysplastic Barrett's mucosa, and while expression of VEGF correlated with vascularisation, it had no independent prognostic relevance.

In conclusion, of all the treatment strategies, chemoradiotherapy holds the most promise for positive outcomes in oesophageal cancer and is a rapidly advancing area of oncological research. Predicting who will respond to treatment, which regimes are most effective and who, if anyone, can benefit from surgery remain to be uncovered.

CHAPTER II: AIMS

The aims of this body of work were:

1 To review the currently available treatment strategies for oesophageal cancer in the literature.

2 To review prognostic and predictive indicators for oesophageal cancer.

3 To study the long-term results of two randomised controlled trials of neoadjuvant chemoradiotherapy and surgery versus surgery alone in oesophageal adeno- and squamous cell carcinoma and determine if the short-term survival advantage of neoadjuvant chemoradiotherapy previously shown in adenocarcinoma is durable and extends to squamous cell carcinoma.

4 To determine the value of endoscopy and biopsy in predicting complete pathological response and outcome following chemoradiotherapy.

5 To determine whether the addition of rib-micrometastatic status to luminal response to neoadjuvant chemoradiotherapy would more accurately predict long-term survival in oesophageal cancer.

6 To examine the response, survival and outcome of patients over the age of 70 with oesophageal cancer who have undergone chemoradiotherapy.

7 To examine the role of p53, VEGF and metallothionein as predictive markers for response to neoadjuvant chemoradiotherapy and outcome in oesophageal cancer.

CHAPTER III: STUDY 1

IS THE SHORT TERM SURVIVAL ADVANTAGE OF NEOADJUVANT CHEMORADIOTHERAPY SUSTAINED? LONG-TERM FOLLOW-UP OF TWO RANDOMISED TRIALS.

3.1 ABSTRACT

Introduction. Oesophageal cancer is a systemic disease at presentation in the majority of patients necessitating systemic treatment. Neoadjuvant chemoradiotherapy provides a complete pathological response (CPR) in over 25% of patients and a short-term survival advantage in meta-analyses but its overall role is disputed because of the small numbers of patients enrolled into randomised trials, the heterogeneity of treatment protocols and the short follow-up of all such studies.

Aim. To study the long-term results of two randomised trials of neoadjuvant chemo-radiotherapy and surgery versus surgery alone.

Methods. Between 1990 and 1997 two randomised trials were undertaken in one institution on 211 patients. Patients with adenocarcinoma (AC)(n=113) or squamous carcinoma (SCC)(n=98) were separately randomised to identical protocols of chemoradiotherapy of two courses chemotherapy, on weeks 1 and 6 (fluorouracil, 15mg/kgx5 days and cisplatin, 75mg/m^2 day 7) and radiotherapy (40Gy) before surgery (multimodal therapy (MMT)) or to surgical monotherapy (SM).

Results. Follow-up ranged up to 206 months (median 163 months). Of the 211 patients, 58 and 46 patients were randomised to the MMT limb and 55 and 52 to SM in the AC and SCC trials respectively. The CPR rates were 25% and 30% for AC and SCC respectively, incurring a survival advantage overall (p=0.03). Twice as many patients in the MMT group were lymph node negative as the surgical monotherapy group (74% vs. 36%) (p=0.002) reflecting significant downstaging in this group and had a survival advantage (AC: p<0.001, SCC: p=0.041). In the AC trial, 12 patients who had received MMT were alive 10 years or longer compared to 2 that had SM. In the SCC trial the

respective figures were 5 and 2. MMT conferred a long-term survival advantage over SM in both trials (AC $p<0.001$) and SCC ($p=0.036$). A survival advantage for MMT was also seen on intention to treat analysis in both trials (AC $p<0.004$, SCC $p<0.02$).

Conclusion At least 25% of patients had a CPR to this treatment protocol. The survival advantage previously identified in AC at 3 years persisted long-term, and extended to SCC suggesting that neoadjuvant chemoradiotherapy be considered the standard of care for patients with locoregionally-advanced disease.

3.2 INTRODUCTION

The first ever randomised trial of therapy was carried out by the British Medical Research Council in 1947 to examine the effect of streptomycin for the treatment of tuberculosis[341]. The addition of streptomycin to the traditional treatment of bed-rest was associated with a survival advantage in the short-term and hailed as a medical breakthrough. A subsequent study, however, by Fox et al[342] revealed that this short-term survival advantage was lost with time. This led to the development of multidrug therapy which provided a sustained treatment advantage. Short-term benefit is no guarantee of long-term success in infection or indeed cancer therapy.

Surgery has long been considered the best hope for cure for locoregionally advanced oesophageal cancer but the morbidity and mortality rates associated with oesophagectomy has restricted its role to a minority of patients with limited disease and who are fit for resection. Although improvement in surgical technique has led to a decrease in post-operative morbidity and hospital mortality, it has not resulted in an increase in overall survival rates[3, 5, 135, 343]. Reported 5-year survival rates for surgery alone of 40-50% reflect more on patient selection than any dramatic advance in the curative potential of more extensive surgery[137, 138, 140] and still only translate to a 5% overall survival even when an R0 resection has been achieved[136, 140, 344].

The natural history of oesophageal cancer is to disseminate early and present with systemic spread[345] with only about 20% of patients having localised disease[120]. When data for entire communities is scrutinised, it is clear that only a small minority of patients with oesophageal cancer present early enough, or are fit enough, to undergo

and benefit from surgical resection, ranging from 30%[78, 121], to as low as 10% in some studies[120, 140, 142, 346]. Even when we confine our attention to patients undergoing potentially curative resection, the great majority of patients have bone marrow micrometastases at the time of resection[119] precluding cure. In one review of almost ten thousand patients, only 5.4% had pTis or T1 disease[141]. Despite early disease, even in this small minority, local and distant recurrence dominates after resection[142] and the majority succumb within 5 years[141].

As the disease is systemic in the majority at presentation, systemic therapy is mandatory. When given preoperatively, systemic treatment has the advantage of addressing the systemic component of the disease earlier than if given after surgery and post-operative recovery. A combined modality approach allows additive or synergistic effects to be exploited to both intensify the effect on the local disease and reduce subsequent distant failures due to resurgent metastatic disease. When administered with radiotherapy, some chemotherapeutic agents have the added advantage of enhancing the local effect of radiotherapy[347], maximizing tumour cell kill thereby down-staging the tumour[179, 181] and permitting a higher rate of R0 resections[123, 143]. However randomised controlled trials[123, 143, 182, 211, 213, 215, 221, 223, 224, 231, 348] and subsequent meta-analyses[193, 217-220, 222, 230, 232] have not definitively established a consensus on management strategy.

We have previously reported a randomised trial showing a short-term survival advantage for neoadjuvant chemoradiotherapy in adenocarcinoma, but we expressed our reservations about the durability of this advantage[182]. Other criticisms of this trial included the short duration of follow-up[225, 226] and the poor results of surgery alone[225-230].

3.3 AIMS

The aim of this study was to examine long-term results of two randomised trials of neoadjuvant chemoradiotherapy and surgery versus surgery alone, one of which has not previously been reported in full[182], and to determine whether the short-term survival advantage identified for neoadjuvant therapy for adenocarcinoma is sustained and extends to squamous cell carcinoma.

3.4 PATIENTS AND METHODS

The recruitment of patients and conduction of these original trials do not form part of the work submitted for this thesis. The work completed for this thesis consisted instead of completing the long-term follow-up of the patients involved in the original trials and the analysis of data to compare the outcome of multimodal therapy and surgery alone on long-term survival. The short-term data of the adenocarcinoma trial was published in 1996 by Walsh et al[182], but the squamous cell trial data has to date never been published in full.

3.4.1 PATIENTS

Patients with oesophageal adenocarcinoma (AC) or squamous cell carcinoma (SCC) were separately enrolled into two randomised controlled trials to compare the outcome of neoadjuvant chemoradiotherapy and surgery (multimodal therapy, MMT) with the outcome of surgery alone for oesophageal AC and SCC.

The randomised trials were approved by the St James's Hospital Ethics Committee and informed consent was obtained from all patients. Individual patient data was entered into a prospectively-accrued oesophageal cancer database containing demographic, clinical, operative, pathological and follow-up data.

For the purposes of this study, all patients were followed-up until the date of death or last clinical interaction. Follow-up and cause of death, if applicable, was determined by telephone communication with their General Practitioner, review of patient records or searches in the Archives of the National Death Registry Offices, Dublin, Ireland. Follow-up was calculated from the date of randomisation.

3.4.2 INCLUSION CRITERIA

Patients who met all of the following criteria were included in the original trials: biopsy proven AC or SCC of the oesophagus (excluding cervical oesophagus requiring laryngectomy), age less than 76 years, leukocyte count of greater than 3500/mm^3, platelet count of greater than 100,000/mm^3 and serum creatinine concentration below 1.4 mg/dL. Patients with evidence of distant metastases, an Eastern Cooperative Oncology Group (ECOG) performance status of 3 or 4, previous chemotherapy or radiotherapy, previous malignancy (excluding skin cancer) or co-morbidities contraindicating surgery were excluded from this study. There was no restriction on the length of tumour or on the presence or location of lymph node metastases.

3.4.3 PREOPERATIVE TUMOUR STAGING

Tumour staging was determined by physical examination, chest x-ray and abdominal ultrasound scanning. Computerised Tomography (CT) of the thorax and abdomen was performed only in selected cases due to the limitation of availability at this time. Where symptoms indicated, a bronchoscopy or isotope bone scan was obtained.

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3.4.4 CHEMORADIOTHERAPY

Identical concurrent chemoradiotherapy was given to patients randomised to the multimodal therapy arms of both trials.

3.4.4.1 CHEMOTHERAPY

The chemotherapy regime consisted two cycles of 5-fluorouracil and cisplatin as described previously[182]. These were administered during weeks one and six. On days one to five of each course, patients received an infusion of fluorouracil (15mg/kg of body weight/day) over a period of 16 hours. Cisplatin (75mg/m^2 of body surface area) was infused over eight hours on day seven.

3.4.4.2 RADIOTHERAPY

Concurrent radiotherapy was commenced on day one of the first cycle of chemotherapy and administered on days 1-5, 8-12 and 15-19, a total of 15 days, each patient receiving a total of 40Gy external beam radiation. All patients underwent treatment with megavoltage therapy units with 4- or 8-MV photons (Cobalt model

SEM100, Fairy Engineering, or Phillips model SL75-5 or Dynaray model 10, Radiation Dynamics, respectively). Radial and longitudinal margins of the tumour were defined endoscopically and radiologically and the treatment fields extended 2-3cm and 5cm beyond the radial and longitudinal margins respectively.

Prior to 1994, al patients were treated with parallel-opposed fields (anteroposterior and posteroanterior) with a midline dose of 40Gy in 15 fractions. This was then modified to a three-field approach (anterior and left- and right-posterior oblique fields), thereby reducing exposure of the spinal cord to radiation. Using a computerised treatment-planning system (AECL/Theratronics Therplan), a dose of 40Gy (+/-10%) in 15 fractions was delivered to the entire treatment volume giving 2.67Gy per fraction in both regimes. There was no correction for transmission of radiation to the lungs during either treatment delivery method. The patients assigned to surgical monotherapy had neither preoperative chemotherapy nor radiation therapy.

3.4.5 SURGERY

In the surgical monotherapy (SM) group, surgery was performed on average one week post-randomisation and eight weeks after treatment was commenced in the MMT group, with a delay if the leukocyte count was less than 2500/mm^3 or platelet count was less than 100,000/mm^3. Five operative approaches were employed. Tumours of the upper and middle oesophagus were resected with a three-stage operation whereby gastric mobilisation was performed via a midline laparotomy, the oesophagus was mobilised via a right thoracotomy and the anastomosis was performed in the neck. The Lewis-Tanner operation (right-sided thoracotomy and laparotomy) was employed

for tumours in the lower third of the oesophagus. Cardia tumours were resected using an abdominal approach and a left thoracotomy. In patients who had poor respiratory function, a transhiatal approach was used with the anastomosis fashioned in the neck. Selected patients had an abdominal approach with gastrectomy and distal oesophagectomy.

3.4.6 PATHOLOGICAL STAGE

Cancer staging was based on pathologic findings referenced to the classification of the American Joint Committee on Cancer guidelines for oesophageal cancer[349]. Following chemotherapy and radiotherapy patients were staged based on the location and extent of any residual disease. If no residual disease was identified in the resected specimen or in the lymph nodes, this was defined as a complete pathological response (CPR) (stage 0). If there was residual tumour in the mucosa or submucosa in the absence of disease in the lymph nodes, it was classified as stage 1. If any residual deposits involved the muscularis propria or adventitia in the absence of tumour in the lymph nodes, it was classified as stage 2a. Stage 2b was defined as the absence of residual tumour in the oesophagus but with tumour in the lymph nodes. If the tumour breached the oesophageal wall the wall and lymph nodes were positive for tumour, this was defined as stage 3. Stage 4 referred to distant metastasis beyond the locoregional lymph nodes.

3.4.7 STATISTICS

Statistical analyses were performed with using the statistical package SPSS version 15.0 for Windows (SPSS, Inc., Chicago IL). Continuous variables were expressed as median ± standard deviation or mean ± standard error of the mean as appropriate and were compared using a two-sample t-test. Categorical variables were compared using a c^2 test, with Fishers exact test used where appropriate. Survival probabilities for clinical, pathological, and treatment variables were estimated using the Kaplan–Meier method[350] and pairwise comparisons were made using a log–rank test. The effects of treatment modality (neoadjuvant chemotherapy and external-beam radiation therapy followed by surgical resection *vs.* surgical monotherapy), tumour histology, size and stage, the presence of positive lymph nodes on survival were examined using logistic regression, and optimal cut-offs were determined using the maximal chi^2 method. Significant univariate factors were included in a Cox proportional hazards regression model to establish independent predictors of survival. Further substratification analysis was performed using the Mantel-Haenszel test[351]. P values of less than 0.05 were considered statistically significant.

3.5 RESULTS

3.5.1 DEMOGRAPHIC DATA

Beginning in May 1990, 113 patients with AC and 98 patients with SCC of the oesophagus were enrolled into two randomised trials in a single institution comparing neoadjuvant chemoradiotherapy and surgery (multimodal therapy, MMT) with surgery alone.

In the AC trial, 158 patients were assessed for eligibility; 45 of whom were excluded due to not meeting the inclusion criteria, patient choice and other or unknown reasons (**Figure 1**). Fifty-eight patients were randomised into the MMT arm and received neoadjuvant chemotherapy and external-beam radiation therapy prior to surgical resection; 55 patients were randomised into the surgical monotherapy (SM) arm and received primary surgery as the sole treatment modality.

In the SCC trial, 147 patients were assessed for eligibility; 49 of whom were excluded due to not meeting the inclusion criteria, patient choice and other or unknown reasons (**Figure 2**). Forty-six patients were randomised into the MMT arm, and 52 patients into the SM arm. The AC and SCC trials were concluded in September 1995 and February 1997 respectively; the first because interim analysis of the data identified a statistically significant difference between the groups and the latter when both senior surgical authors ceased work in this hospital.

The median age of patients in the AC trial was 65 years (range 37-75 years); with a median age in the MMT group of 65 years (range 47-75 years) and in SM group also of 65 years (range 37-75 years). In the SCC trial the median age of patients was 66 years (range 33-75 years); median age in the MMT group was 65 years (range 40-73 years) and that in the SM group was 67 years (range 33-75 years). Seventy-three percent (n=83) and 51% (n=50) were male in the AC and SCC trials respectively. Follow-up ranged from 0.25-205 months and from 0.25-206 months in the AC and SCC trials respectively (overall median 163 months). Overall, the age and sex profiles did not differ significantly between the groups (p=0.643 and p=0.182 respectively). A summary of the demographic data is displayed in **Table 8**.

Table 8: Demographics And Operative Approach

		Adenocarcinoma			Squamous Cell Carcinoma		
		Multimodal Therapy	Surgical Mono-therapy	Total	Multimodal Therapy	Surgical Mono-therapy	Total
		No. of Patients (%)	No. of Patients (%)		No. of Patients (%)	No. of Patients (%)	
Sex	Male	39 (67)	44 (80)	83	19 (41)	31 (60)	50
	Female	19 (33)	11 (20)	30	27 (59)	21 (40)	48
	Total	58 (100)	55 (100)	113	46 (100)	52 (100)	98
Age at Diagnosis	Median (years)	65	65		65	67	
	Range (years)	47-75	37-75		40-73	33-75	
	Aged >70 years	15 (26)	15 (27)	30	10 (22)	18 (35)	28
	Aged <70 years	43 (74)	40 (73)	83	36 (78)	34 (65)	70
	Total	58 (100)	55 (100)	113	46 (100)	52 (100)	98
Surgical Approach	Laparotomy and left thoracotomy	14 (30)	17 (31)	31	3 (8)	4 (8)	7
	Lewis-Tanner	20 (42)	22 (40)	42	14 (35)	22 (44)	36
	Transhiatal	0 (0)	2 (4)	2	2 (5)	0 (0)	2
	Three stage	13 (27)	11 (22)	24	21 (53)	24 (48)	45
	Abdominal	1 (2)	2 (4)	3	0 (0)	0 (0)	0
	Total	48 (101*)	54	102	40 (101*)	50 (100)	90

*due to rounding to nearest percentage

3.5.2 DEVIATIONS FROM PROTOCOL

There were a total of eleven incidences of deviation from protocol in the AC trial, ten in the MMT group and one in the SM group. In the SCC trial there were a total of eight protocol deviations, six in the MMT group and two in the SM group. These are outlined in **Table 9**. For the purposes of this study, these patients were excluded from further analysis in order to analyse the true outcome of therapy. Survival analysis based on intention to treat is performed in **Section 3.5.5.4.**

Table 9: Deviations From Protocol

Age(years)/Sex	Chemoradiotherapy	Surgery	Comment	Follow-up (months)
MULTIMODAL THERAPY				
Adenocarcinoma				
75/F	None	No	Died of probable myocardial infarct before treatment commenced	0.25
68/M	Not completed	Yes	Complete dysphagia developed	0.5
69/M	Not completed	Yes	Pericarditis developed on treatment	8
70/F	Not completed	No	Deterioration in performance status	5
63/M	Not completed	Yes	Upper gastrointestinal haemorrhage during treatment	11
73/M	Not completed	No	Fatal haemorrhage from tumour bed, no tumour at post mortem	1
75/F	Completed	No	Deterioration of performance status	3
74/M	Completed	No	Developed lung metastases	3
60/M	Completed	No	Myocardial infarction after treatment	65
40/M	Completed	No	Developed lung metastases	10
Squamous Cell Carcinoma				
72/F	None	Yes	Patient choice	78
52/M	Completed	No	Disease progression on treatment	8
68/F	Completed	No	Development of lung metastases	8
71/F	Completed	No	Complete remission on endoscopy, patient choice	28
72/M	Completed	No	Patient choice	18
53/M	Completed	No	Development of lung metastases	7
SURGICAL MONOTHERAPY				
Adenocarcinoma				
64/M	N/A	Yes, emergency	Iatrogenic perforation, delayed referral	0.5
Squamous Cell Carcinoma				
53/F	N/A	No	Tumour invading bronchus	1
55/M	N/A	No	Tumour invading bronchus	4

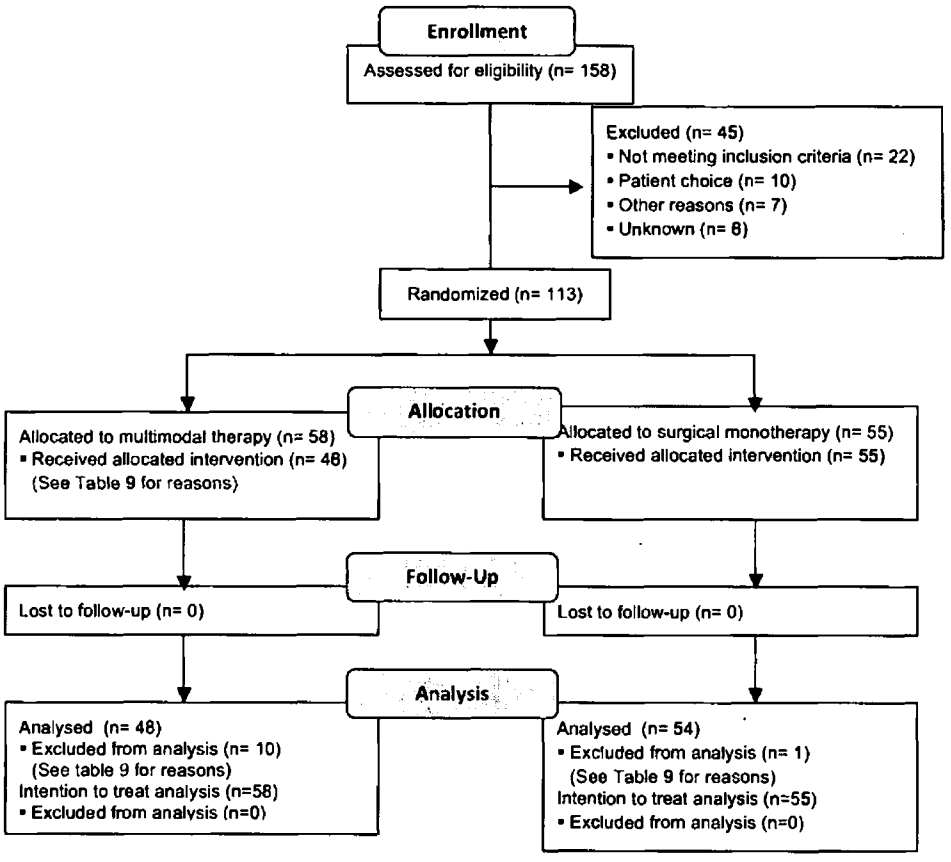


Figure 1: A Flow Diagram Depicting The Passage of Patients Through The Adenocarcinoma Randomized Trial Of Multimodal Therapy Versus Surgical Monotherapy

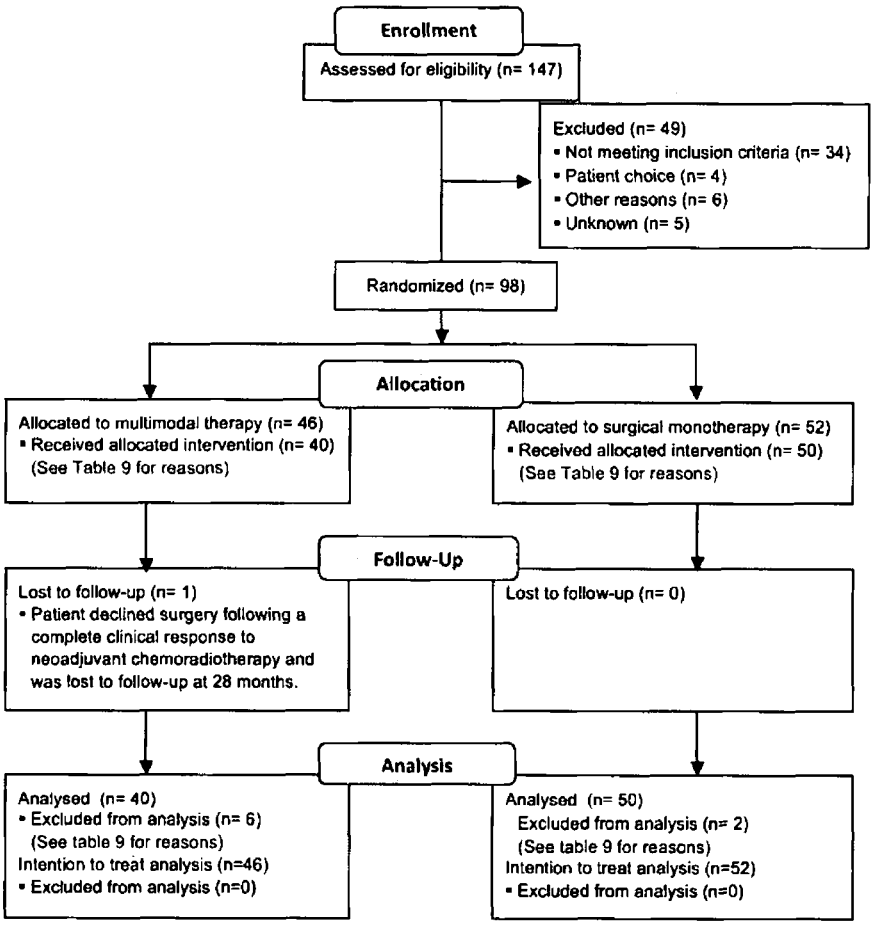


Figure 2: A Flow Diagram Depicting The Passage of Patients Through The Squamous Cell Carcinoma Randomized Trial Of Multimodal Therapy Versus Surgical Monotherapy

3.5.3 RESPONSE TO CHEMORADIOTHERAPY

Significant down-staging was seen in the patients treated with MMT in both trials. There was a complete pathological response (CPR) rate of 25% (12/48) in the AC and 30% (12/40) in the SCC trials respectively.

In the AC, trial 65% (31/48) of patients receiving MMT who underwent resection were node negative (N0) compared to 20% (11/54) undergoing SM ($p<0.001$). In the SCC trial, 85% (34/40) of patients receiving MMT were node negative compared to 52% (26/50) undergoing SM ($p<0.001$). The AJCC staging of both tumour types at the end of treatment is outlined in **Table 10**.

When both tumour types are analysed together, a significant downstaging was seen overall in the patients treated with MMT. There was an overall CPR rate of 27% (24/88). Furthermore 74% of the MMT group (65/88) were node negative compared to 36% (37/104) in the SM group ($p=0.002$).

Table 10: Pathological Stage At End Of Treatment.

		ADENOCARCINOMA		SQUAMOUS CELL CARCINOMA	
		Multimodal Therapy	Surgical Monotherapy	Multimodal Therapy	Surgical Monotherapy
		No. of patients	No. of patients	No. of patients	No. of patients
AJCC Stage	0	12	0	12	0
	1	1	2	6	1
	2a	18	8	14	19
	2b	7	2	1	0
	3	10	37	5	27
	4	0	5	2	2
	Unknown	0	0	0	1
	Total	48	54	40	50
Nodal Status	Positive	17	43	6	23
	Negative	31	11	34	26
	Unknown	0	0	0	1
	Total	48	54	40	50

3.5.4 HOSPITAL MORTALITY RATE

There was no statistical difference in post-operative mortality noted between the treatment arms in either trial (AC;p=0.254, SCC;p=0.434). There was an overall hospital mortality rate of 11% (21/192).

There were four in-hospital mortalities in the AC trial resulting in an overall in-hospital mortality of 4% (4/102), including one patient who had a CPR. Of the three mortalities in the MMT group, one patient died of an anastomotic leak, one of post-operative haemorrhage and one of a chylothorax. In the SM group, one patient died from post-operative chylothorax.

In the SCC trial, there were 17 in-hospital mortalities resulting in an overall in-hospital mortality of 19% (17/90), including 2 patients who had had a CPR. Of the nine patients in the MMT group, two patients died of post-operative haemorrhage, two of

anastomotic leak, one of disseminated intravascular coagulation, one of cerebral metastases, one of subphrenic abscess and two of multi-organ failure. Of the eight in-hospital mortalities in the SM group, one died of a cerebro-vascular accident, three of respiratory failure, one of post-operative haemorrhage, one of ischaemic stomach and sepsis, one of anastomotic leak and one of multi-organ failure.

The hospital mortality of those with a CPR was 13% (3/24).

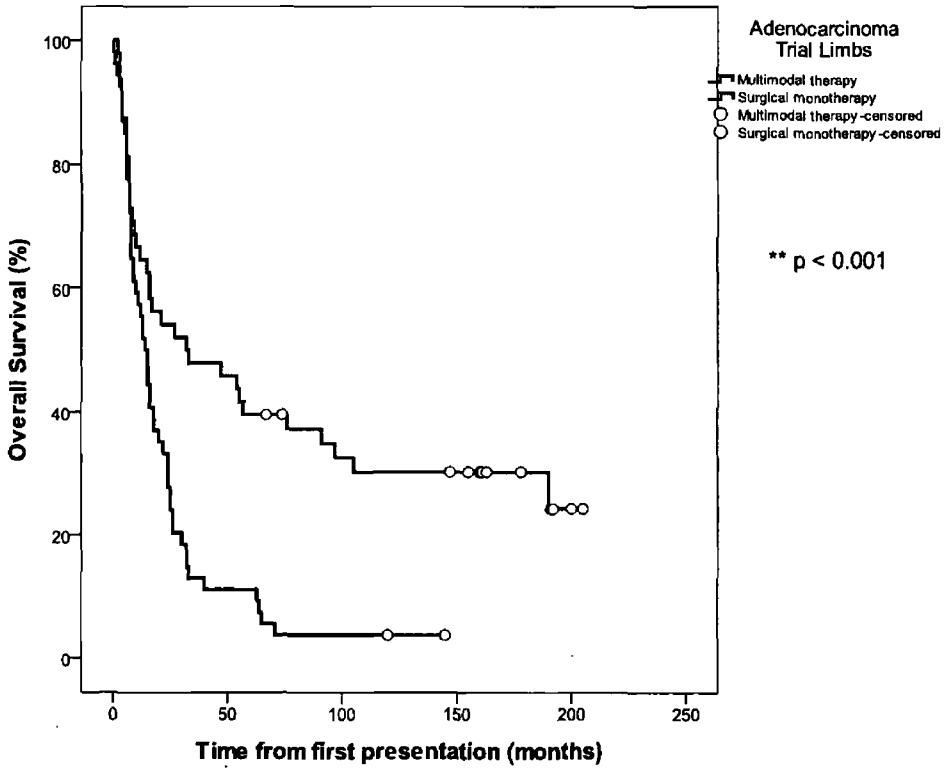
3.5.5 SURVIVAL

3.5.5.1 ADENOCARCINOMA

AC patients who received MMT had a statistically significant survival advantage over those who received SM ($p<0.001$) (**Figure 3**) with a median survival of 33 (range 0.1-203) months and 23 (0.25-145) months respectively. In the AC trial MMT group, the overall three, five and ten-year survival was 48 (n=23), 40 (n=19) and 27% (n=13) respectively. The three, five and 10-year survival rate of the 12 patients who had a CPR was 75% (n=9), 58% (n=7) and 25% (n=3) respectively. This compares with 42% (n=14), 33% (n=12) and 19% (n=7) respectively in the 36 patients with an incomplete response and 13% (n=7), 11% (n=6) and 4% (n=2) in the 54 patients in the SM group. Thus patients with adenocarcinoma who had downstaging to a CPR had a significant survival advantage over incomplete responders ($p=0.01$) (**Figure 4**). Overall, those who were lymph node negative had improved survival over those who were lymph node positive ($p<0.001$) (**Figure 5**). Patients with AC who received MMT and who were node negative had a significantly longer median survival time of 67 months compared with 16 months for SM patients who were lymph node negative ($p=0.005$). In lymph node-positive patients, those who had MMT had similar survival compared with those who had SM

with a median survival of 12 and 16 months respectively but this did not reach statistical significance (p=0.266).

At conclusion of this study, 14 patients of the AC cohort were alive at 205, 200, 192, 191, 178, 163, 163, 161, 160, 155, 147, 147, 145 and 120 months post diagnosis, 12 of whom received MMT and two had SM.



Months	0	50	100	150	200	250	Alive at 5 years (%)	Alive at 10 years (%)
MMT	48	22	14	11	2	0	19/48 (40%)	13/48 (27%)
SM	54	6	2	0	0	0	6/54 (11%)	2/54 (4%)

Figure 3: Kaplan–Meier Plot *Of Overall Survival Of* Patients *With Adenocarcinoma:* Multimodal Therapy Versus Surgical Monotherapy. The Corresponding Table Indicates Number Of Patients At Risk At Each Time Point.

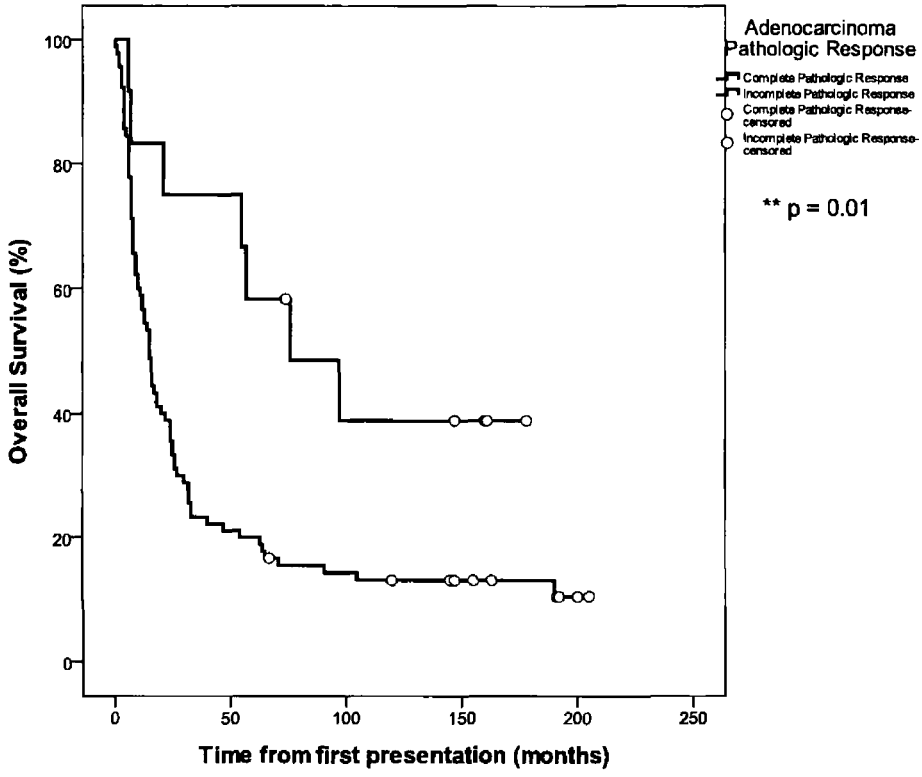


Figure 4: Kaplan–Meier Plot Of Overall Survival Of Patients With Adenocarcinoma: Complete Pathological Response Versus Incomplete Pathological Response.

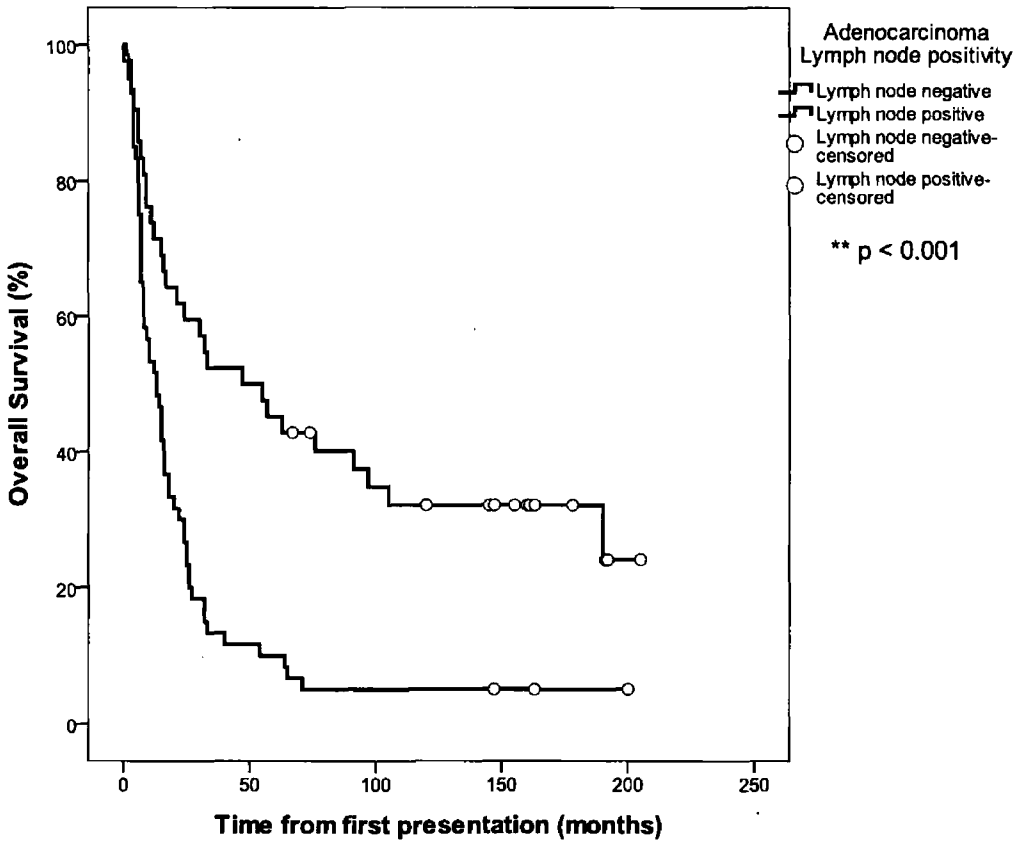
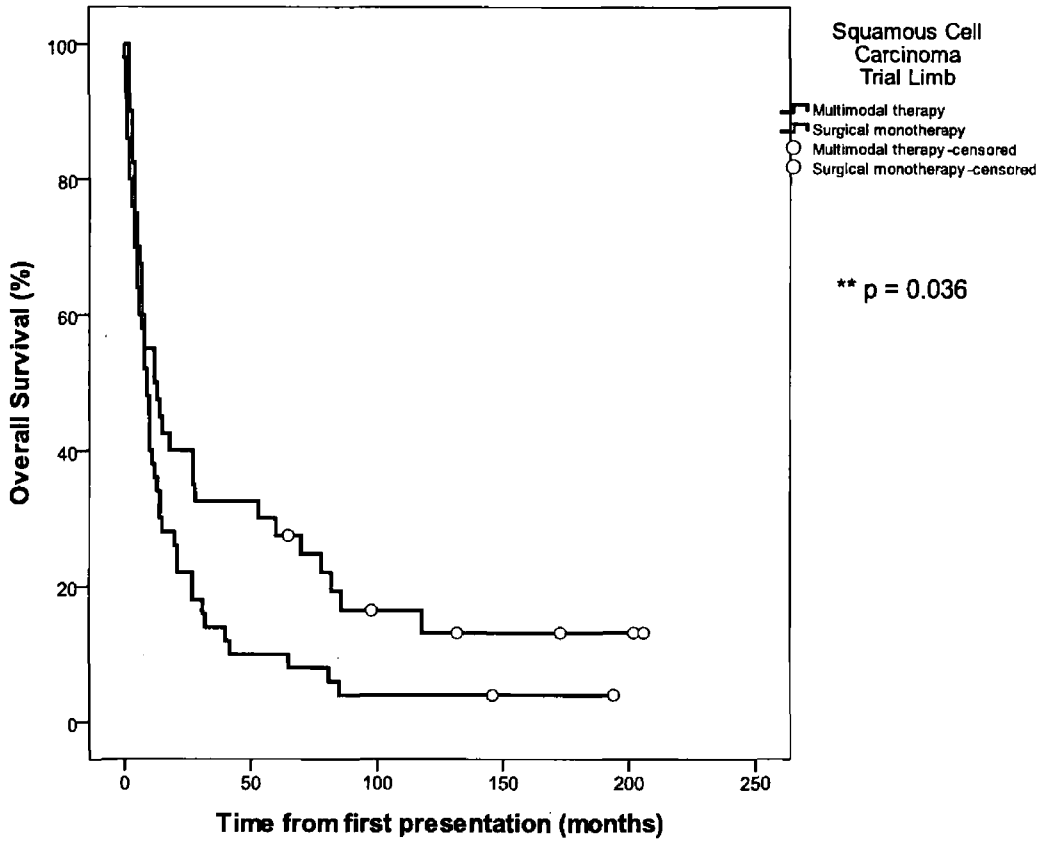


Figure 5: Kaplan–Meier Plot Of Overall Survival Of Patients With Adenocarcinoma: Lymph Node Negative versus Lymph Node Positive.

3.5.5.2 SQUAMOUS CELL CARCINOMA

SCC patients who received MMT had a statistically significant survival advantage over those who received SM (p=0.036), with median survivals of 13 (range 2-206) and 9 (range 0.2-194) months respectively (**Figure 6**). In the SCC trial MMT group, the overall three, five and ten year survival was 33% (n=13), 30% (n=12) and 10% (n=4) respectively. The three, five and 10-year survival rate of the 12 patients with a CPR was 50% (n=6), 42% (n=5) and 17% (n=2) respectively. This compares with 25% (n=7), 25% (n=7) and 7% (n=2) respectively in the 28 patients with an incomplete response and 14% (n=7), 10% (n=5) and 4% (n=2) in the surgery alone group (p=0.099). Overall, patients who were lymph node negative had improved survival (p=0.041) (**Figure 7**). Patients with SCC who received MMT and who were node-negative had a similar median survival time of 14 months compared with 8 months for SM (p=0.138). In lymph node positive patients, those who had MMT had a median survival of 6.5 compared with 9 months in the SM group.

Of the SCC cohort at the conclusion of this study, 7 patients were alive at 206, 202, 194, 173, 146, 132 and 98 months post diagnosis, 5 of whom received MMT and two had SM.



Months	0	50	100	150	200	250	5-Year Survival (%)	10-Year Survival(%)
MMT	40	13	5	3	2	0	12/40 (30%)	4/40 (10%)
SM	50	5	2	1	0	0	5/50 (10%)	2/50 (4%)

Figure 6: Kaplan–Meier Plot Of Overall Survival Of Patients With Squamous Cell Carcinoma: Multimodal Therapy versus Surgical Monotherapy. The Corresponding Table Indicates Number Of Patients At Risk At Each Time Point.

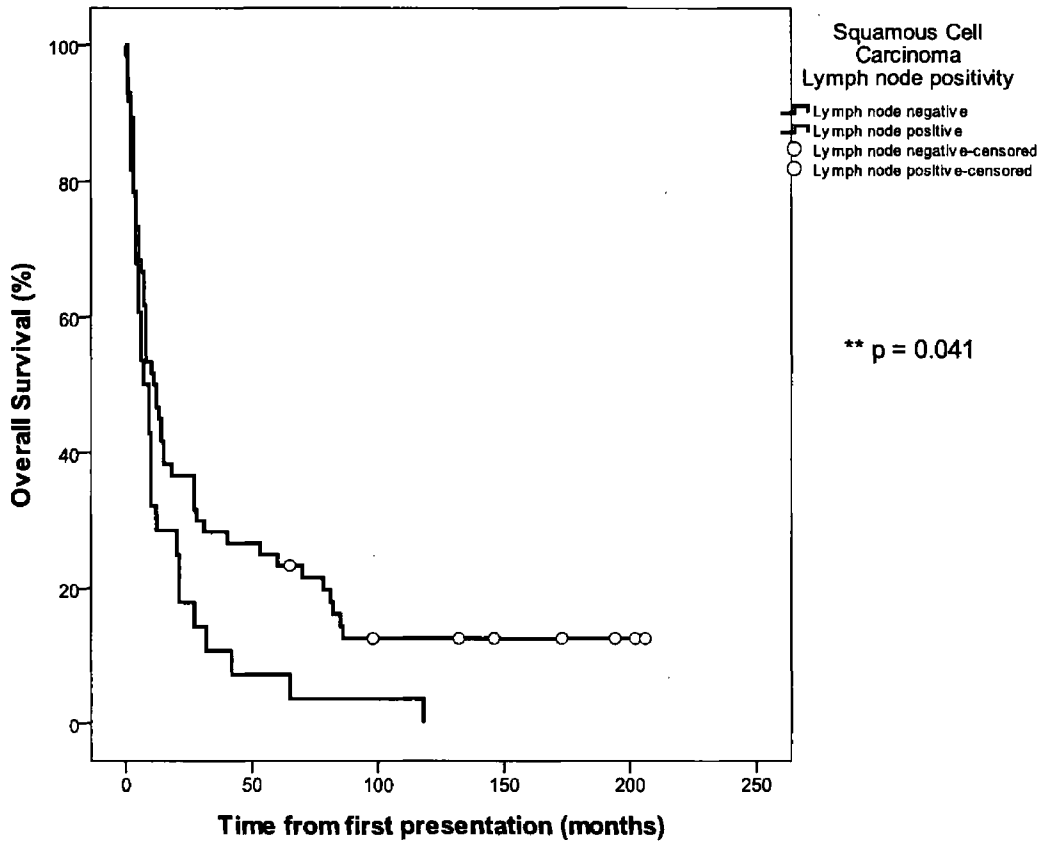
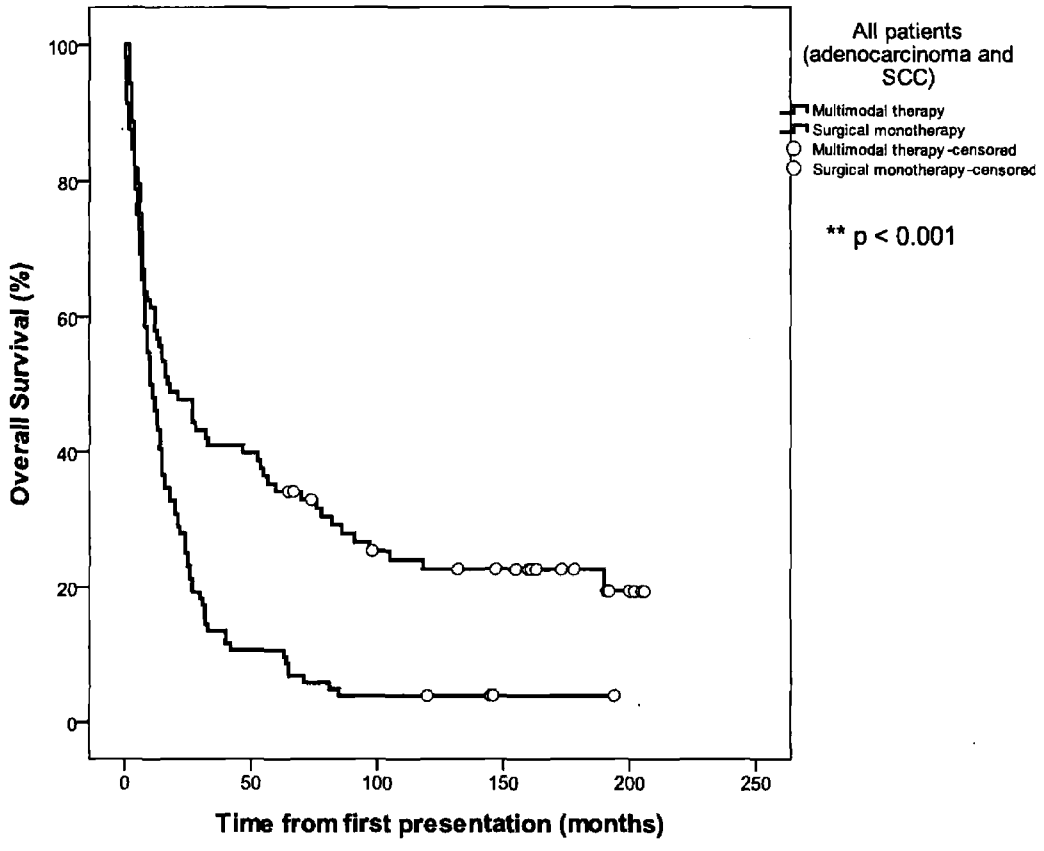


Figure 7: Kaplan–Meier Plot Of Overall Survival Of Patients With Squamous Cell Carcinoma: Lymph Node Negative Versus Lymph Node Positive.

3.5.5.3 COMBINED ANALYSIS OF ADENOCARCINOMA AND SQUAMOUS CELL CARCINOMA

Overall, patients that received MMT had a statistically significant survival advantage over those that received SM ($p<0.001$) (**Figure 8**). The overall three, five and ten year survival for all groups was 26% (50/192), 22% (42/192) and 11% (21/192), with a median of 13 months (range 0.1-206). In the MMT group as a whole, the overall three, five and ten-year survival was 41% (36/88), 35% (31/88) and 19% (17/88) respectively. The three, five and 10-year survival rate of the 24 patients who had a CPR was 58%, 50% and 21% respectively based on treatment received. This compares with 33%, 30%

and 14% respectively in the 64 patients with an incomplete response and 13%, 11% and 4% in the SM group (p=0.003) (**Figure 9**).



Months	0	50	100	150	200	250	5-Year Survival (%)	10-Year Survival (%)
MMT	88	35	19	14	4	0	31/88 (35%)	17/88 (19%)
SM	104	11	4	1	0	0	11/104 (11%)	4/104 (4%)

Figure 8: Kaplan–Meier Plot Of Overall Survival Of Patients With Adenocarcinoma And Squamous Cell Carcinoma Combined: Multimodal Therapy Versus Surgical Monotherapy. The Corresponding Table Indicates Number Of Patients At Risk At Each Time Point.

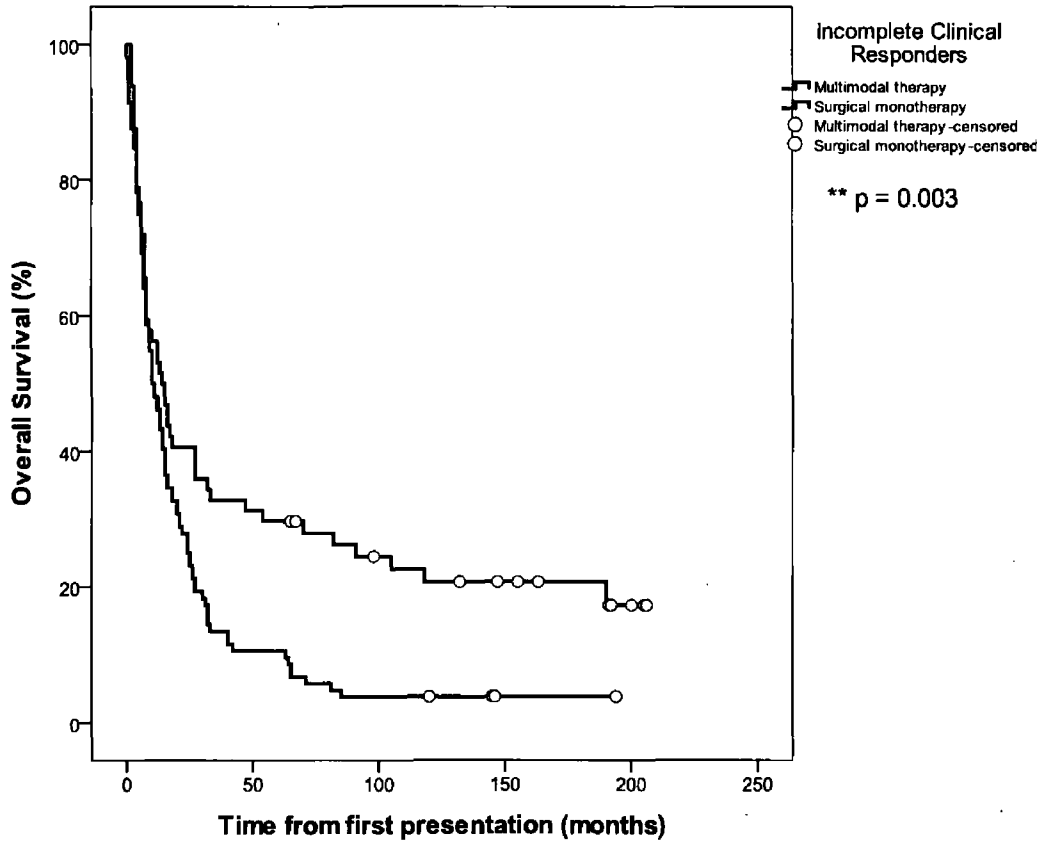


Figure 9: Kaplan–Meier Plot Of Overall Survival Of Patients With Adenocarcinoma And Squamous Cell Carcinoma Combined Who Had An Incomplete Pathological Response to Multimodal Therapy, compared with Patients Who Underwent Surgical Monotherapy .

When both trials are pooled and patients who were lymph node negative after neoadjuvant therapy were compared with those who were lymph node negative following SM the 3, 5, and 10 year survivals were 48% (31/65), 42% (27/65), and 22% (14/65) respectively compared with 21% (8/37), 19% (7/37) and 11% which was statistically significant suggesting a systemic effect for neoadjuvant therapy (p=0.009).

Patients who received MMT and who had node-negative disease had a significantly longer median survival time of 32 months compared with 11 months for SM (p=0.009) (**Figure 10**). In lymph node positive patients, those who had MMT had a median

survival time of 8 months compared with 10 in the SM group, with no difference in survival (p=0.18).

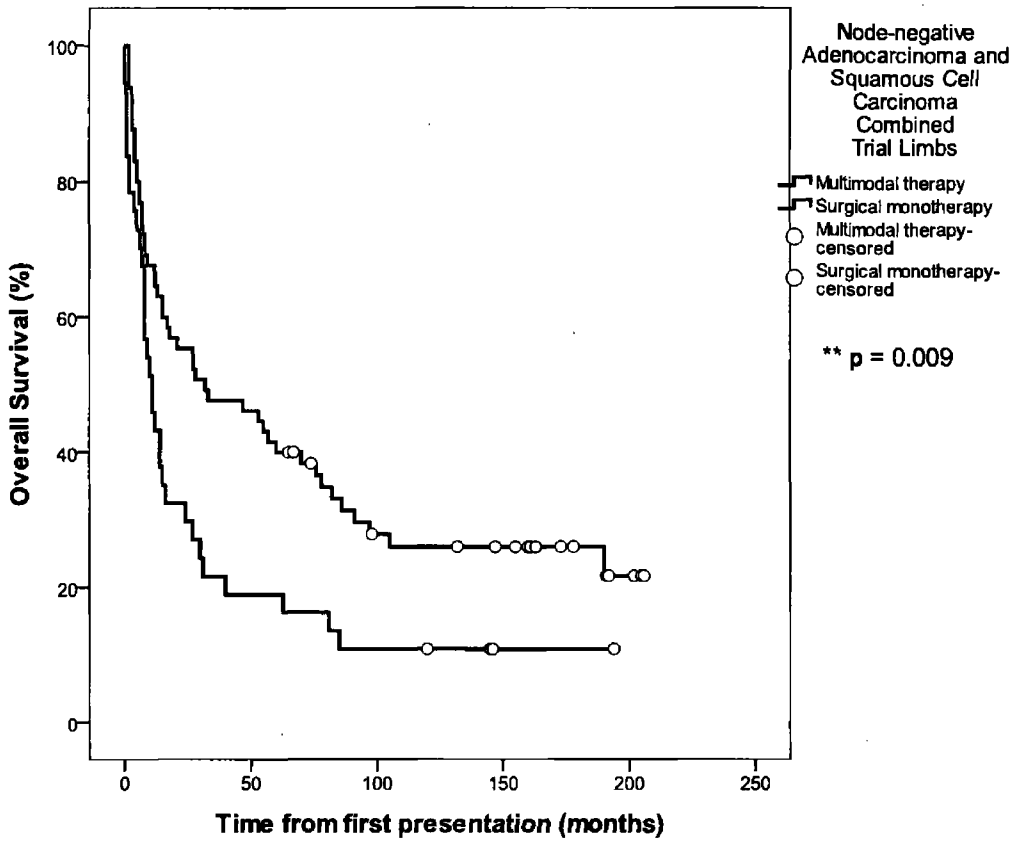


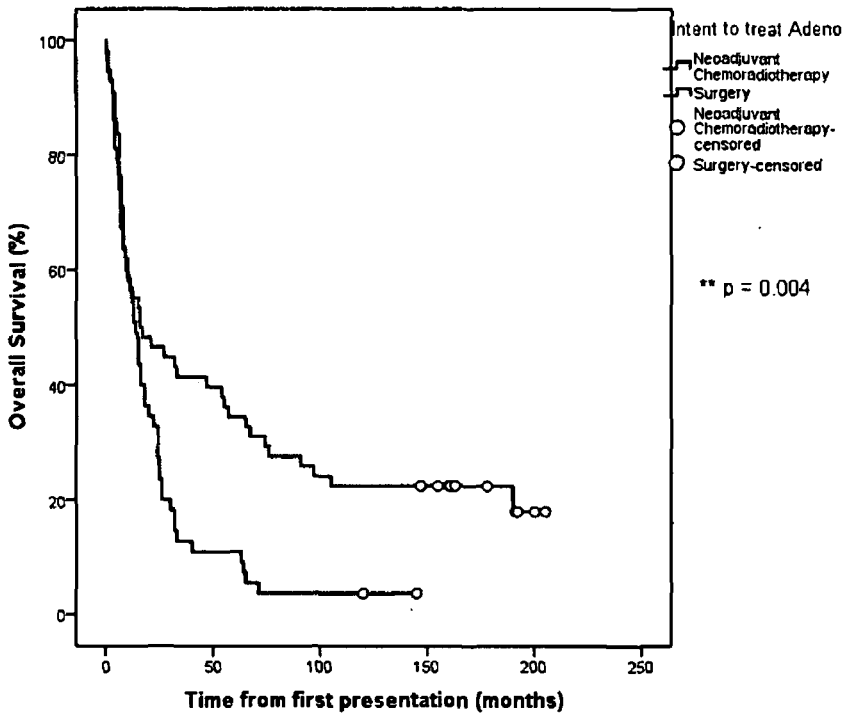
Figure 10: Kaplan–Meier Plot Of Overall Survival Of Node-Negative Patients With Adenocarcinoma And Squamous Cell Carcinoma: Multimodal Therapy Versus Surgical Monotherapy.

3.5.5.4 INTENTION TO TREAT SURVIVAL ANALYSIS

On intention to treat analysis, AC trial patients who received MMT had a statistically significant survival advantage over those who received SM (p=0.004) (**Figure 11**). In the AC MMT group, the overall three, five and ten-year survival was 41% (n=24), 34% (n=20) and 22% (n=13) respectively, compared with 13% (n=7), 11% (n=6) and 4% (n=2) in the SM group.

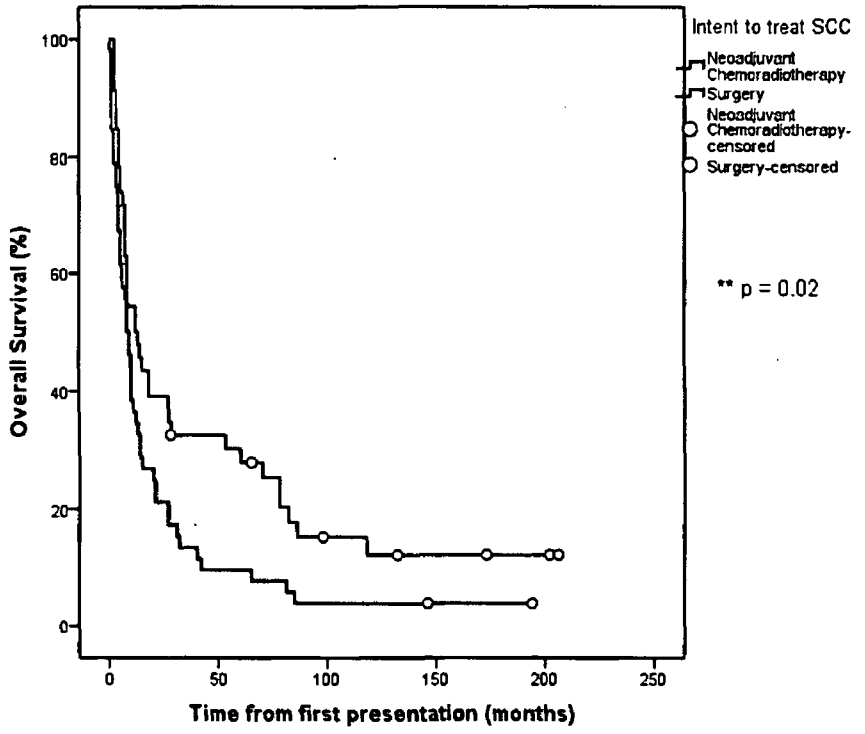
Based on intention to treat, SCC patients who received MMT also had a statistically significant survival advantage over those who received SM (p=0.02) (**Figure 12**), with three, five and ten-year survival was 30% (n=14), 28% (n=13) and 9% (n=4) respectively, compared with 13% (n=7), 10% (n=5) and 4% (n=2) in the SM group.

Combined analysis of both trials on an intention to treat basis, also revealed a statistically sigificant long-term survival advantage of MMT over SM (p<0.001) (**Figure 13**), with three, five and ten-year survival rates of 37% (n=38), 32% (n=33) and 16% (n=17) compared with 13% (n=14), 10% (n=11) and 4% (n=4) respectively.



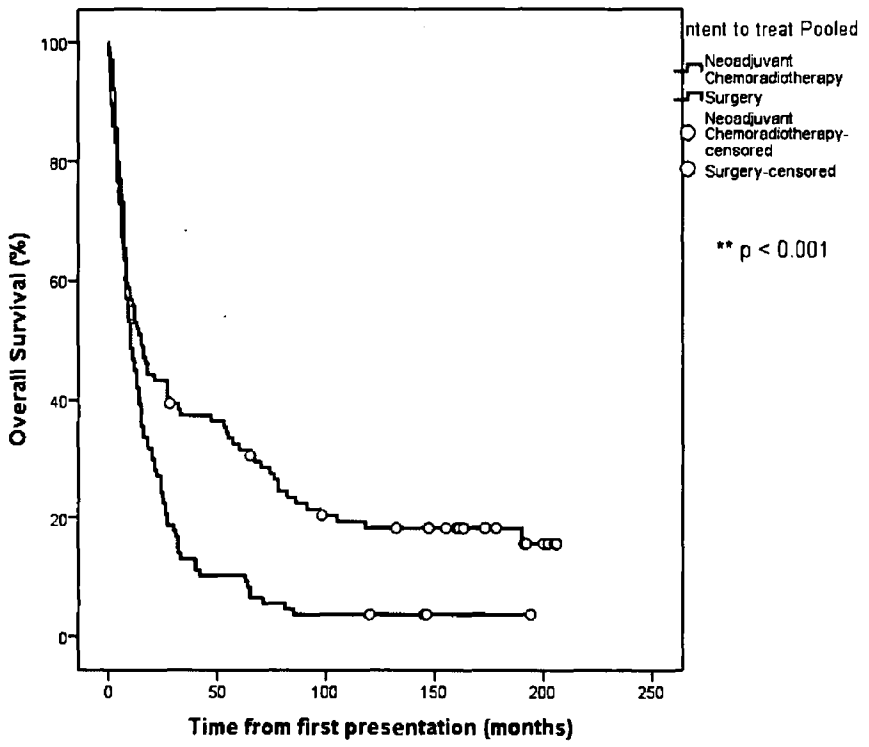
Months	0	50	100	150	200	250	5-Year Survival (%)	10-Year Survival (%)
MMT	58	23	14	11	2	0	20/58 (34%)	13/58 (22%)
SM	55	6	2	0	0	0	6/55 (11%)	2/55 (4%)

Figure 11: Kaplan–Meier Plot Of Overall Survival Based On Intention To Treat Analysis Of Patients With Adenocarcinoma: Multimodal Therapy Versus Surgical Monotherapy. The Corresponding Table Indicates Number Of Patients At Risk At Each Time Point.



Months	0	50	100	150	200	250	5-Year Survival (%)	10-Year Survival (%)
MMT	46	14	5	3	2	0	13/46 (28%)	4/46 (9%)
SM	52	5	2	1	0	0	5/52 (10%)	2/52 (4%)

Figure 12: Kaplan–Meier Plot Of Overall Survival Based On Intention To Treat Analysis Of Patients With Squamous Cell Carcinoma: Multimodal Therapy Versus Surgical Monotherapy. The Corresponding Table Indicates Number Of Patients At Risk At Each Time Point.



Months	0	50	100	150	200	250	5-Year Survival (%)	10-Year Survival (%)
MMT	104	37	19	14	4	0	33/104 (32%)	17/104 (16%)
SM	107	11	4	1	0	0	11/107 (10%)	4/107 (4%)

Figure 13: Kaplan–Meier Plot Of Overall Survival Based On Intention To Treat Analysis Of Patients With Adenocarcinoma And Squamous Cell Carcinoma (Pooled Data): Multimodal Therapy Versus Surgical Monotherapy. The Corresponding Table Indicates Number Of Patients At Risk At Each Time Point.

3.6 DISCUSSION

The main finding of this study is that the short-term survival advantage for neoadjuvant chemoradiotherapy for AC in the previously published randomised trial[182] is sustained long-term. This is reassuring and suggests that the survival benefit is due to the elimination of micrometastases rather than merely inducing dormancy[352] or destroying the majority of chemo-sensitive cells, leaving resistant clones to re-emerge, and for patients to succumb to recurrent disease[353]. Of all randomised trials reported to date the median follow-up only ranged from 2 to 8 years[123, 143, 182, 211, 213, 215, 221, 223, 224, 231, 348]. Concerns expressed by others about the short duration of follow-up of randomised trials[225, 226] may finally be laid to rest with our 17 year follow-up. The concern that pre-operative systemic therapy may simply "delay systemic relapse"[354] can no longer be considered a justification for withholding neoadjuvant therapy.

As adenocarcinoma and squamous cell carcinoma are two distinct tumours with different aetiologies, different distribution within the oesophageal lumen, and different incidence of lymph node metastases, we cannot assume that squamous cell carcinoma will have a similar response as adenocarcinoma to the 40Gy radiotherapy and 5-FU and cisplatin protocol, or that response will be sustained. It is reassuring that the short-term survival advantage previously seen in adenocarcinoma extends to oesophageal squamous cell carcinoma and is sustained in the longer term.

Our original report of a survival advantage for neoadjuvant chemoradiotherapy over surgery alone[22] has been criticised because of the poor results for surgery alone[225-230] and inadequate pre-operative staging [214, 355, 356]. Such criticism, we believe, is misplaced

as the results for surgery alone reflect recruitment criteria. The best results for surgery will be achieved in patients with earlier stage disease. But to restrict recruitment to patients with the earliest stage disease would mean abandoning the patients in most need of systemic therapy. When the original trial was designed, the authors elected pursue a liberal recruitment policy in an attempt to embrace the largest possible percentage of patients. Inclusion criteria were all medically fit patients under 76 years of age who were fit for surgery, excluding only those patients with overt metastases. As access to CT was limited, it is likely that many of these patients had early macrometastases. Access to more sophisticated staging techniques might have restricted the trial to earlier stage disease and would undoubtedly have been achieved better results for surgery alone, but there would be no additional benefit to the oesophageal cancer community. This was dramatically demonstrated in a study from the Groningen University Medical Center[140] where surgeons in academic centres appeared to provide a 5-year survival rate (49%) twice as good as surgeons in non-teaching centres (27%), but academic surgeons operated on only 10% of referred patients compared to 20% in non-teaching units. The overall impact of surgery was the same for both communities.

Most trials have had stricter recruitment criteria with earlier tumour stage[221], or younger mean age[143, 200, 223, 224]. But while restricting trials to patients with earlier disease will ensure a greater percentage survival from surgery alone, it is ultimately counterproductive as fewer patients will have systemic disease and cannot benefit from systemic therapy. Patients without macro- or micro-metastases do not require systemic therapy. Surgery will have little relevance to a disease where the majority are too old, too sick or have disease too advanced to be salvaged by surgery alone.

Restricting trials to patients with the earliest disease will require larger numbers as greater number in each limb will be cured by surgery alone, exposing trials to a risk of type 2 error.

The survival advantage identified in these two trials is consistent with the findings of three previous randomised trials. When all randomised trials[123, 143, 182, 211, 213, 215, 221, 223, 224, 231, 348], comparing neoadjuvant chemoradiotherapy and surgery with surgery alone are scrutinised, three have shown a clear survival advantage for neoadjuvant therapy[182, 215, 221] and these share certain characteristics; they are 5-fluorouracil or taxane based, in adequate dosage, with concurrent treatment radiotherapy. When these criteria have been met all trials have shown a survival advantage favouring multimodality therapy. We believe that the failure of treatments reflects the use of ineffective therapeutic agents rather than failure of concept.

There have been a number of meta-analyses[216, 217, 219, 222, 232] and even meta-analysis of meta-analyses[214] which have consistently shown a survival advantage for neoadjuvant therapy[218-220, 222]. The results of these meta-analyses are undermined by treatment protocol heterogeneity, variation of inclusion criteria, grouping of different tumour types, analysis of means rather than individual patient data and by short-term follow-up[214, 225, 226]. Meta-analysis pools effective[182, 215] with ineffective regimens[224], or indeed potentially harmful regimens[143] and pooling these data can little enhance our understanding of the sensitivity of oesophageal cancer cells to specific agents. We would not accept a meta-analysis of different antibiotics for a specific infection so it should not be acceptable in oncology. The most recent meta-analysis has shown an overall 8.7% survival advantage for chemoradiotherapy over surgery alone with equal

survival benefits in both adeno- and squamous cell carcinoma, providing additional proof of principle for the role of systemic treatment for loco-regionally advanced disease[222].

Overall 25% of patients with adenocarcinoma and 30% of those with squamous cell carcinoma had a complete pathological response. In the absence of tumour in the resection specimen, patients cannot benefit from resection but are exposed to the risks of major surgery. These patients should ideally avoid surgery. More disturbing is the finding that in these studies 13% of patients with a CPR died following an (unnecessary) resection and the rest remain exposed the morbidity and life-long negative quality of life impact of surgery. With increasing rates of CPR being reported, the issue of unnecessary oesophageal resection becomes ever more pressing. Since 25-87.5%[182, 191, 213] of patients that undergo neoadjuvant chemoradiotherapy have a complete response, depending on initial disease stage, the identification of patients who do not require resection following neoadjuvant therapy becomes ever more urgent.

Complete pathological response gave a survival advantage, especially to those with adenocarcinoma. Incomplete responders, however also benefitted as the survival advantage for incomplete responders was 17% (11/64) compared with 2.8% (3/104) for surgery alone (p=0.003) at 10 years. Our concern[182], and that of others[225, 226], that the results may not be durable has been proved groundless by this 17-year follow-up study. Patients who have achieved a complete pathological response following neoadjuvant chemoradiotherapy have an associated improved 5-year survival of up to 60%[29-31]. Recent advances have led to complete response rates from 25% in the 1990s[182] to up to 43 to 87.5%[191, 213] in more recent times. The message emerging from these trials is that

both adenocarcinoma and squamous cell carcinoma are exquisitely sensitive to chemoradiotherapy and gives the best hope of a favourable outcome.

Having identified a survival advantage for neoadjuvant chemoradiotherapy we must explore which cohort of patients needs it and those who are cured by surgery only. Clearly if patients have non-metastatic disease they can be cured by surgery alone. Unfortunately oesophageal cancer behaves aggressively in the majority. Only 5.4% of patients in one study undergoing surgery for squamous cell carcinoma had T1 tumours or earlier[141]. Even when patients with T1 tumours were subselected, the subset with submucosal tumours have a 5-year survival of less than 50%[141]. We must now recognise that all patients with disease beyond the mucosa need systemic therapy.

3.7 CONCLUSION

In conclusion, neoadjuvant chemoradiotherapy allows the best chance of long-term survival for a larger proportion of oesophageal cancer patients. There is an urgent need to optimise chemoradiotherapy regimens to increase the rate of complete pathological response and identify these patients and ultimately spare many the morbidity and mortality of surgery altogether.

CHAPTER IV: STUDY 2

COMPLETE CLINICAL RESPONSE ON ENDOSCOPIC BIOPSY FOLLOWING NEOADJUVANT CHEMORADIOTHERAPY PREDICTS OUTCOME IN OESOPHAGEAL CANCER

4.1 ABSTRACT

Background: Patients with oesophageal cancer who have a complete pathological response (CPR) to neoadjuvant chemoradiotherapy have a survival advantage over incomplete responders and patients treated with surgery alone. CPR patients cannot benefit from resection, but are exposed to all of its risks. However, CPR patients are difficult to identify with current staging techniques.

Aims. This study aimed to determine the predictive value of a negative upper-gastrointestinal endoscopy and biopsy in identifying CPR following chemoradiotherapy.

Methods. A prospectively maintained oesophageal cancer database was queried to identify patients with loco-regional disease who had undergone neoadjuvant chemoradiotherapy and who had subsequently been restaged. Patients with a complete clinical response (CCR) had negative endoscopy and biopsy and computerised tomography (CT). Luminal findings were correlated with histopathological response in the resection specimen of patients treated surgically and with long-term survival in patients treated non-operatively.

Results: Ninety patients fulfilled the selection criteria; 55 had a CCR; 31 underwent resection and 24 declined or were not offered resection but were followed-up for life. Of these, five had interval oesophagectomy following disease re-emergence and 58% survived more than 3 years and had a similar mean survival compared with those who had residual disease and immediate resection post neoadjuvant therapy (24 and 27 months respectively). Those who had a CCR and immediate resection had improved survival over those who had residual disease, with a mean survival of 53 months compared with 27 months in those with residual disease undergoing immediate

resection. CCR was 74% predictive of a CPR ($p < 0.0001$) and 80% predictive of node-negative disease ($p = 0.002$).

Conclusions: Pre-operative endoscopy with multiple tumour-bed biopsy identified with 74% accuracy a cohort of patients who had a CPR. While 26% harboured residual disease, many had systemic spread precluding cure; some were salvaged by subsequent resection but, critically, many were spared the morbidity and mortality risks of resection and survived long-term.

4.2 INTRODUCTION

While resection alone is curative for true loco-regional oesophageal cancer[138, 357], this embraces only a minority of patients[78, 120, 142, 346]. Mortality rates for resection remain up to 14%[122] and the morbidity of surgery and long-term complications continue to be significant[123-134]. Overall survival rates for surgical monotherapy remain low[182, 215], suggesting that its contribution to oesophageal cancer therapy is minimal.

The success of surgery, however, can be enhanced by the addition of effective neoadjuvant therapy[196, 198, 217, 219, 221, 222, 358], which induces tumour down-staging, increases R0 resection rates[123, 143, 217] and improves survival[182, 196, 215, 217, 219, 221, 222, 232, 359]. Depending on disease stage and the regimen employed, complete response rates between 25% and 87.5%[182, 191] have been reported, with the highest rates in patients with earliest disease[191].

The best overall and disease-specific survival rates are found amongst complete pathological responders[203, 209, 210, 212, 360-365]. These patients, however, are not readily or reliably predicted by current staging techniques and thus the role for surgery in this sub-group is not clearly defined. We hypothesised that as the disease has originated in the mucosa, it is intuitive that extensive biopsies of the mucosa at re-staging following chemoradiotherapy would provide valuable information as to the clinical response.

4.3 AIMS

The aim of this study was to correlate clinical response with histological response in the resected specimen and overall survival and outcome for patients including those treated non-operatively.

4.4 METHODS

4.4.1 PATIENTS

We queried a prospectively-accrued database to identify 90 consecutive patients who presented to Connolly Hospital with a diagnosis of primary oesophageal squamous or adenocarcinoma who had completed neoadjuvant chemoradiotherapy for loco-regional advanced disease; who had pre-and post-treatment upper-gastrointestinal endoscopy and biopsy; and who had negative dual-contrast abdomino-pelvic computerised tomography (CT) for systemic metastases. Where indicated, patients also underwent endoscopic ultrasound and or positron-emission tomography (PET).

All patients were entered on diagnosis into a prospectively-accrued oesophageal cancer database containing demographic, clinical, endoscopic, operative, pathological and follow-up data.

4.4.2 NEOADJUVANT CHEMORADIOTHERAPY

Neoadjuvant chemoradiotherapy consisted of two cycles of 5-FU and Cisplatin with 40Gy radiotherapy as previously reported[182]. In brief, this consisted of two cycles of 5-fluorouracil and cisplatin during weeks one and six, with concurrent radiotherapy (40 Gy) in 15 fractions.

Subsequently, these patients were re-staged with repeat endoscopic biopsy and CT, and PET where indicated. Patients consenting to surgery underwent *en bloc* resection within eight weeks of completion of neoadjuvant therapy by a single surgeon.

4.4.3 ENDOSCOPIC AND PATHOLOGICAL EVALUATION

All patients underwent pre- and post-treatment upper-gastrointestinal endoscopy using the same endoscopy equipment and performed by the same expert endoscopist. Pre-treatment, the site and length of the tumour were documented and multiple biopsies - at least 10 per endoscopic session - were taken for histological analysis for categorisation into subtype and differentiation. All biopsies were taken with Olympus standard oval disposable biopsy forceps (2.8mm diameter).

Post-neoadjuvant chemoradiotherapy endoscopy with multiple biopsies was repeated after four weeks as part of our restaging protocol. Endoscopic findings were categorised as either complete clinical response (CCR) or incomplete clinical response (ICR). CCR was identified when no tumour was identified on post-treatment

endoscopic evaluation, with multiple (>10) biopsies of the tumour bed (**Figures 14A and & B**). An ICR was identified when tumour was present on post-treatment endoscopy and/or in the resection specimen.

Patients selected for surgery underwent *en bloc* oesophagectomy four to eight weeks after completion of chemoradiotherapy. Resection specimens were analysed for the presence or absence of residual disease. A complete pathological response (CPR) was identified in patients undergoing resection when no tumour was identified in the resected specimen. Final histopathology reports confirmed the presence of a primary oesophageal adenocarcinoma or squamous cell carcinoma in each of the endoscopic biopsy or *en* bloc surgical specimens included in this study, and the anatomic location of the tumour was recorded. Primary tumour size was recorded as the largest diameter axis through the sectioned specimen. The total number of lymph nodes harvested and the number containing metastatic cells were recorded. Cancer staging was based on pathologic findings referenced to the sixth edition of the American Joint Committee on Cancer guidelines for oesophageal cancer[28].

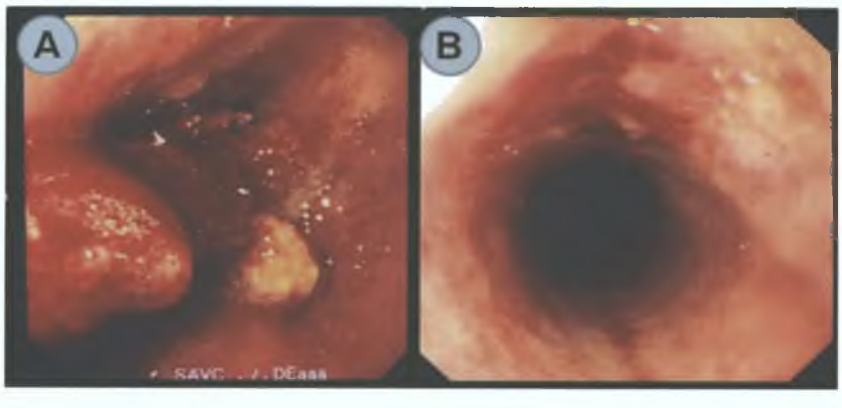


Figure 14: White-light endoscopic images of a 54 year old male with squamous cell carcinoma of the oesophagus (A) pre-treatment diagnostic upper-gastrointestinal endoscopy; this demonstrates a 1cm tumour. (B) post-neoadjuvant chemoradiotherapy. This patient had a complete luminal and complete pathological response to neoadjuvant chemoradiotherapy.

4.4.4 SURVEILLANCE

Patients opting for an observational approach or who were considered at increased risk for surgery due to age or co-morbidity were closely followed with 3-monthly endoscopy and multiple (>10) biopsies, 6-monthly CT scanning for 3 years, following which the endoscopic intervals were extended to 6-monthly to 5 years with annual follow-up thereafter and were offered interval oesophagectomy if disease re-emerged.

4.4.5 ETHICAL APPROVAL

Ethical approval was obtained for this study from the Connolly Hospital Ethics Committee.

4.4.6 STATISTICAL ANALYSIS

Overall survival was calculated from the date of recruitment until the date of death or the last recorded clinical interaction. All-cause mortality was dichotomously tabulated for extent of pathologic response, histologic variants, completion operative versus non-operative treatment and overall.

Statistical analyses were performed with PASW version 18.0 for Windows. Continuous variables were expressed as median ± standard deviation or mean ± standard error of the mean as appropriate and were compared using a two-sample t-test. Categorical variables were compared using a chi-squared test, with Fishers exact test used where appropriate. Survival probabilities for clinical, pathological, and treatment variables were estimated by the Kaplan–Meier method[350] and pairwise comparisons were made

using a log–rank test. The effect of extent of pathological response to neoadjuvant chemotherapy and 3D-conformal external-beam radiation therapy (either followed by surgical resection or surveillance), tumour histology, nodal status, size and stage on survival were examined using logistic regression, and optimal cut-offs were determined using the maximal chi-squared method. Significant univariate factors were included in a Cox proportional hazards regression model to establish independent predictors of survival. Further substratification analysis was performed where necessary using the Mantel-Haenszel test[351]. P values of less than 0.05 were considered statistically significant and the study was powered to detect a 20% difference between groups at a beta of 0.8.

4.5 RESULTS

4.5.1 PATIENT AND TUMOUR CHARACTERISTICS

4.5.1.1 PATIENT CHARACTERISTICS

Ninety patients who completed neoadjuvant chemoradiotherapy for locoregionally advanced adenocarcinoma or squamous cell carcinoma of the oesophagus fulfilled the study criteria. The mean age was 61 years (range 38-81 years). The male to female ratio was 2.5:1. Follow-up ranged between 2-144 months from date of diagnosis with a median of 38 months.

4.5.1.2 TUMOUR CHARACTERISTICS

Fifty-seven (63%) patients had adenocarcinoma (AC) and 33 (37%) patients had squamous cell carcinoma (SCC). Five tumours (6%) were well differentiated, 49 (54%) were moderately differentiated and the remaining 36 (40%) were poorly differentiated. The average tumour length, measured on initial endoscopy, was 4cm (range 1-10cm). Initial tumour length was poorly-predictive of post-treatment CCR ($p = 0.272$) and of lymph node metastasis ($p = 0.43$).

4.5.2 RESPONSE

4.5.2.1 COMPLETE CLINICAL RESPONSE

Fifty-five patients in this series (61%) had a CCR. Fifty-three percent of AC (30/57) and 76% of SCC (25/33) had a CCR. Of the 55 CCRs, 31 (56%) underwent resection while 24 (44%) declined (10/24) or were deemed unsuitable (14/24) for surgery and were placed on surveillance. Resection specimens analysed in these operative patients demonstrated an absence of lymph node metastasis in 25/31 (80%) of patients who had a pre-operative endoscopic CCR, compared with only 16/36 (44%) in those with a preoperative endoscopic ICR ($p = 0.002$, chi-square = 9.192). Five of the cohort placed on surveillance underwent interval oesophagectomy following detection of recurrence, leaving 19 that did not need or were not offered resection. All 35 patients who had an incomplete clinical response (ICR) went on to have surgical resection, and all those specimens demonstrated residual tumour.

4.5.2.2 PATHOLOGICAL RESPONSE

Thirty-one patients with a CCR underwent resection, of whom 23 (74%) had a CPR, while 8 (26%) had residual disease in the resected specimen. Overall, 26% percent of all AC (15/57) and 24% of SCC (8/33) in this series had a CPR. CCR on endoscopy was found to be highly significantly predictive of node-negative status ($p=0.002$) and of a CPR in the resection specimen ($p<0.0001$).

The post-treatment AJCC disease stage in these patients with residual disease was stage 2a in 2 patients, stage 2b in 5 patients and stage 3 in 1 patient. Six of the 8 patients were lymph node positive, with five patients having only one positive lymph node and one patient with three positive nodes.

4.5.2.3 NON-OPERATIVELY-MANAGED PATIENTS

Twenty-four patients declined or were deemed unsuitable for resection and were followed-up clinically and endoscopically until the completion of this study. Immediate surgery had not been performed due to co-morbidities or deterioration in performance status (10/24), progressive disease (4/24) or patient choice (10/24). Five of these latter 10 had an interval oesophagectomy when recurrence was detected at 3-monthly surveillance endoscopy; one at 9 months, two at 11 months and two at 12 months. The remaining 19 patients continued to be followed-up until completion of this study.

4.5.3 SURVIVAL

The mean overall survival of the 90-patient cohort was 41 months.

4.5.3.1 COMPLETE CLINICAL RESPONSE

The mean overall survival for the 55 CCR patients was 47 months (range 2-144 months); 76% were alive at 1 year (n=42), 58% at 2 years (n=32), 49% at 3 years (n=27) and 38% at 5 years (n=21). Twenty patients (36%) were alive and disease free at completion of this study. Patients with SCC and AC had a mean survival of 46 and 48 months respectively.

The mean survival of the 31 CCR patients who had immediate resection post-neoadjuvant therapy was 53 months. Twelve of these patients were alive at the conclusion of this study with a mean survival of 99 months post diagnosis (range 40-144). Eight patients died of metastatic or recurrent disease, two of broncho-pneumonia, one each of a tracheo-esophageal fistula, anastomotic leak post colonic interposition, metastatic colon cancer and upper gastro-intestinal bleed. There were four in-hospital mortalities, two of whom died of multi-organ failure following surgery, one from a pulmonary embolus and one from a cerebrovascular accident. The cause of death in one patient was unknown.

4.5.3.2 COMPLETE PATHOLOGICAL RESPONSE

The mean survival of the 23 CPR patients was 68 months (range 4-144). Of CPR patients 78% (n=18) were alive at 1 year, 74% at 2 years (n=17), 65% at 3 years (n=15) and 61% at 5 years (n=14). Patients who had a CPR to neoadjuvant chemoradiotherapy

had a statistically significant survival advantage over those patients who had residual disease (p=0.043) (**Figure 15**).

Of 23 patients with a CPR, 11 were alive at the conclusion of this study at a mean of 103 months (range 75-144). Of those who died, four patients died of metastatic or recurrent disease at 8, 22, 86 and 98 months, two from pneumonia at 7 and 81 months, one from a tracheo-oesophageal fistula at nine months, one from metastatic colon cancer at 52 months and one from unknown cause at 35 months. Three died post-operatively: one from sepsis, one from a pulmonary embolus and one from an anastomotic leak following colonic interposition.

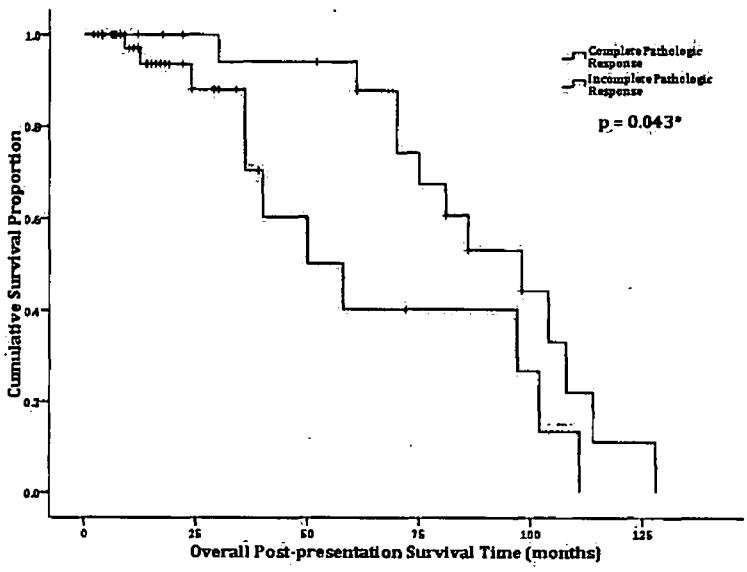


Figure 15: Kaplan-Meier Plot Comparing Overall Survival Of Patients With Complete And Incomplete Pathological Response Following Neoadjuvant Chemoradiotherapy.

4.5.3.3 INCOMPLETE RESPONSE

The mean survival for all 43 patients found to have residual disease on the resection specimen following immediate resection was 27 months (range 3-111), with 1, 2, 3 and 5-year survival of 70% (n=30), 40% (n=17), 23% (n=10) and 12% (n=5) respectively. Twenty-three (53%) died of recurrent or metastatic disease and ten were alive at completion of this study. The remainder died of pneumonia (n=4), cerebro-vascular accident (n=2), upper gastro-intestinal bleed (n=2), metastatic rectal cancer (n=1), myocardial infarction (n=1) and sepsis (n=1).

4.5.3.4 NON-OPERATIVELY MANAGED PATIENTS

The mean overall survival for 19 patients with a CCR who did not undergo immediate or interval resection was 43 months (range 6-116) with 89% of these surviving 1 year (n=17), 68% survived 2 years (n=13), 58% surviving 3 years (n=11) and 42% survived 5 years (n=8).

Of these 19 patients, seven were alive at completion of this study at 38, 39, 53, 61, 64 and two at 88 months post diagnosis. Of those who died, 5 died of metastatic disease, one of aspiration pneumonia, one of multi-organ failure, one of lung cancer and one of thoracic empyema[366] and in three the cause is unknown.

Of the 19 patients treated conservatively, 11 (58%) survived disease free for more than 3 years suggesting a complete clinical response in this cohort. This sub-group's overall mean survival was 65 months.

4.5.3.5 INTERVAL OESOPHAGECTOMY

The mean survival of patients undergoing interval oesophagectomy was 24 months post-diagnosis. One patient was still alive at completion of this study at 47 months. The remaining patients survived 10, 12, 24 and 28 months and died of metastatic or recurrent disease.

4.5.3.6 HOSPITAL MORTALITY

Overall mortality rate following surgery was 8%. The mortality rate in those who had a CPR was 9%; this was not statistically significantly different ($p = 0.834$).

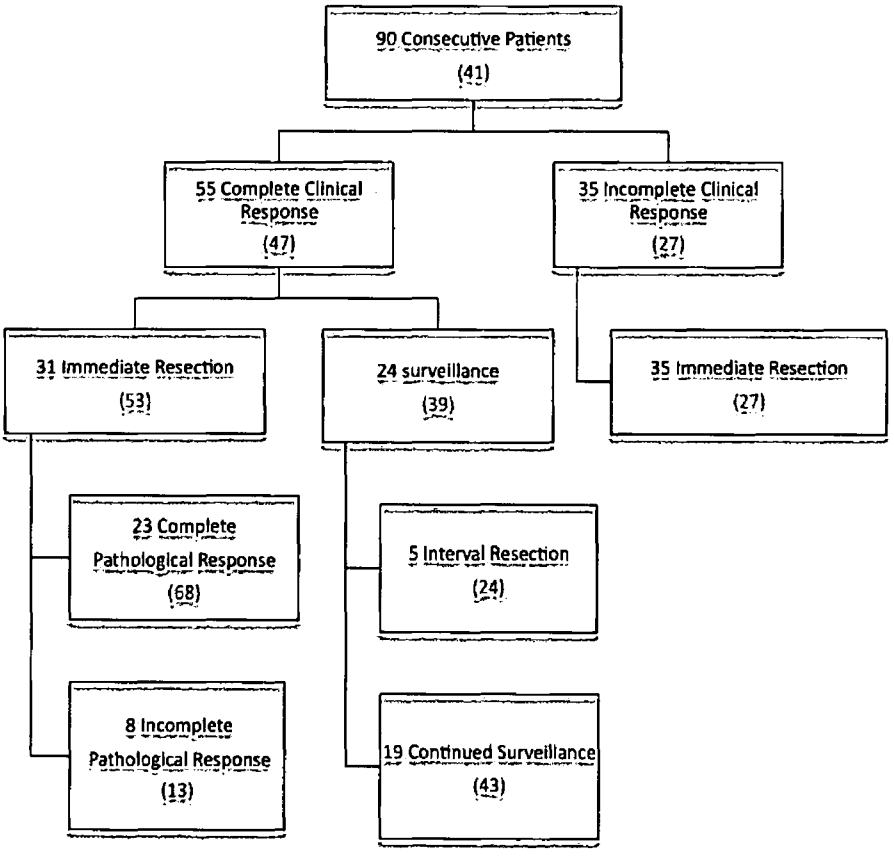


Figure 16: Flow Diagram Of Patient Distribution Following Completion Of Neoadjuvant Chemoradiotherapy. Mean Survival In Months In Parentheses.

4.6 DISCUSSION

The management of early tumours of the upper oesophagus and oropharynx has increasingly relied on definitive chemoradiotherapy, reserving surgery for patients with persistent disease. For SCC of the lower oesophagus and for AC, neoadjuvant therapy followed by surgery has remained the standard of care. Following neoadjuvant chemoradiotherapy, the identification of persistent disease in the oesophageal lumen in these latter patients at restaging presents clear, dichotomous treatment pathways. In the absence of detectable metastatic disease, resection is advised, unless precluded by co-morbidity or patient choice. A significant percentage of these patients will survive long-term following resection but the majority will succumb to metastatic disease as failure to respond is considered a negative prognotic factor[354, 367].

When no residual tumour is identified endoscopically or radiologically at restaging, the clinician is placed in a therapeutic and ethical dilemma. If resection is performed and no tumour is detected in the resected specimen, the patient has been exposed to significant mortality risk of 14%[122] and acute morbidity risks as well as the risks of chronic complications of oesophageal resection[123-133], but has gained no benefit. If no operation is performed, there may be a risk of accusation of mismanagement if the disease later re-presents at an incurable stage.

The CPR rate depends on the regimen employed and the stage of disease. Most studies report CPR rates in the order of 25 to 43%[182, 213] for loco-regionally advanced tumours. For earlier disease, a much higher CPR rate can be inferred as a recent report of

patients with T1b disease so treated had a complete response rate of 87.5% with a 4-year survival of 81%[191]. We can anticipate a greater percentage of complete responders, especially as more effective and targeted therapies are employed and extended to earlier disease stages.

The greater the CPR rate, the greater the ethical imperative to identify and avoid surgery in complete responders. A recent study of 299 patients with a CPR revealed that these patients had a 5.7% mortality rate, despite the cases being drawn from 6 centres of excellence[368]. Several studies suggest an increased surgical morbidity and mortality for patients undergoing preoperative chemoradiation[143, 149, 150] identifying a further argument against submitting all these patients to resection. We observed a 9% mortality rate in those with a CPR which was similar to the mortality rate of patients with residual disease and to overall post-operative mortality rates from large series[122]. Where resectable disease persists, a low mortality is acceptable but it is less defensible in patients with a CPR, especially when a regimen with a known high CPR rate is employed or in patients with earlier disease stage.

The ability to identify accurately which patients have responded completely to chemoradiotherapy is immensly desirable. Based chiefly on imaging, the accuracy of current cancer staging is poor since imaging techniques rely on a minimal disease burden and no current technique can reliably detect systemic microscopic disease. Thus, when untreated patients are staged as curable by surgery and undergo en bloc resection the majority will still die of their disease, succumbing to systemic micrometastases[80, 135, 138, 357]. Restaging after neoadjuvant chemoradiotherapy is even

more challenging due to the difficulty distinguishing fibrotic or necrotic or inflammatory tissue from tumour deposits, even with advanced techniques[106, 107 109].

Others have examined luminal response with varying results. Brown et al[103] reported that an endoscopically normal lumen correlated with a 50% likelihood of a CPR but neither biopsy of the lumen nor CT scanning were performed which may have reduced the accuracy of these assessments. The findings of a study from the Memorial Sloan-Kettering Cancer Center[104] were more difficult to understand as a negative endoscopy and biopsy was only 31% predictive of a CPR. In their paper, only 71% of patients were biopsied following chemoradiotherapy, and neither the number of biopsies nor the experience of the endoscopist were commented upon.

In this study, we examined the simple strategy of restaging with endoscopy, extensive biopsy and CT scanning and found that 74% of CCRs had a CPR following resection. Furthermore, 80% of the en bloc resection specimens from CCRs who underwent oesophagectomy demonstrated no microscopic nodal disease, arguing for a complete tumour field-sterilisation in response to the neoadjuvant chemoradiotherapy. Arguably a similar percentage of conservatively-managed patients had a complete response, as 19 of 24 did not re-present with locoregional disease and 58% survived long-term. The persistence of disease in 26% of patients who have a CCR is obviously a concern. This should not be equated with the loss of 26% of survivable patients, however, as a significant percentage of the patients who harbour occult disease in the oesophageal wall will also have systemic micrometastases[264], which will ultimately decide their survival. The avoidance of hospital mortality within the entire cohort who avoided surgery will further ameliorate the potentially negative effect of a conservative policy.

By offering 3-monthly interval endoscopy, interval oesophagectomy can be offered to a significant proportion of patients if the disease re-emerges with curative intent for those without systemic disease.

While we strive for greater accuracy, we are obliged to use the information from our current restaging protocols to our patients' advantage. We subscribe to the policy of engaging patients as partners in their own care. We therefore explain to our patients with a CCR that they have a 74% chance of having a CPR and therefore a 74% chance of having an unhelpful operation. While encouraging younger and fitter patients to proceed with resection, we actively encourage patients over the age of 70 and patients with significant co-morbidity to consider a "watch and wait" approach with close surveillance and interval endoscopy if necessary.

4.7 CONCLUSION

In conclusion, current neoadjuvant chemoradiotherapy protocols yield a complete response in an increasing number of patients. These patients cannot benefit from resection but are exposed to significant mortality and morbidity risks. The simple approach of endoscopy with multiple (>10) tumour bed biopsies and CT will identify with 74% accuracy a cohort of patients who will have a CPR. More accurate methods to detect a complete response are needed but the issue of what to do with complete clinical responders, especially those who are older or less fit for resection, remains controversial and in the absence of a randomised trial must be guided by common sense and patient choice.

CHAPTER V: STUDY 3

MICROMETASTASES AND LUMINAL RESPONSE TO NEOADJUVANT CHEMORADIOTHERAPY ARE PREDICTORS OF LONG-TERM SURVIVAL IN OESOPHAGEAL CANCER

5.1 ABSTRACT

Introduction: The majority of patients with oesophageal cancer die of their disease despite apparently curative resection. The addition of neoadjuvant chemoradiotherapy enhances survival. Complete responders have the best prognosis and loco-regional response is used as a surrogate marker of response and outcome but many complete responders still succumb to systemic recurrence, most likely due to persistant or resistant micrometastases.

Aims: To determine whether the addition of micrometastastic status from rib bone marrow to luminal response would more accurately predict long-term survival in oesophageal cancer.

Method: A prospectively-accrued database was used to identify a cohort of patients who had completed neoadjuvant chemoradiotherapy for oesophageal adeno- and squamous cell carcinoma, who had pre- and post-treatment endoscopy and biopsy and who had rib marrow examined for the presence of micrometastases using immunohistochemical staining with cytokeratin-18 and the alkaline phosphatase-anti-alkaline technique. Luminal response to treatment was recorded by endoscopy and biopsy. Patients were followed up until date of death or last clinical interaction.

Results: Twenty-three patients with adenocarcinoma (AC) and 20 with squamous cell carcinoma (SCC) fulfilled the selection criteria. Of 43 patients who had neoadjuvant treatment, 33% had a CPR. Twenty-seven patients had surgical resection and one had no surgery but had a post-mortem 5 years post neoadjuvant treatment. Median follow-up was 57 months (range 2-115 months). Presence of rib-marrow micrometastases predicted significantly shorter survival time in AC ($p=0.017$), but not in

SCC (p=0.47). Improved survival was predicted by CCR (p=0.009). Lymph node and marrow micrometastases negative patients with CCR were significantly more likely to be alive at study-end ($p<0.05$). Patients who had a CCR and were negative for rib micrometastatic disease were twice as likely to survive versus rib metastasis positive patients (5 year survival of 38% vs. 17%).

Conclusion: The determination of micrometastatic status improves accuracy of luminal response as a prognostic indicator. Techniques for detection of micrometastases should be standardised and evaluated in large prospective studies before incorporating micrometastatic status into pathologic staging.

5.2 INTRODUCTION

The majority of patients with oesophageal cancer die of their disease despite apparently curative resection[135, 192]. Whilst the addition of chemoradiotherapy provides a survival advantage[182, 219, 369], many complete responders still succumb to systemic recurrence[363, 370]. This may be as a result of persistent or resistant micrometastases which occur early in oesophageal cancer[119, 266, 371, 372] indicating that oesophageal cancer is a systemic rather than local-regional disease.

Neoadjuvant chemoradiotherapy targets both the local and systemic disease burden and complete pathological response (CPR) is a surrogate marker of its efficacy[209, 210, 212, 362]. We have previously shown that when a complete clinical response (CCR) is identified by negative endoscopy and biopsy, 74% of such patients have a CPR and this is predictive of long-term survival[373]. With no residual disease, these patients cannot benefit from resection, but are exposed to the same morbidity and mortality risks as those with residual disease. Over recent years, there has been a dramatic improvement in complete response rates from 43 up to 87.5% being reported[191, 213].

Because of its large blood supply and rich cellular store, bone marrow has been the focus of studies of micrometastic spread and has been found to reflect the micrometastatic disease burden[374], especially rib marrow in oesophageal cancer[119]. The detection of these tumour deposits in colon[255], gastric[258], lung[256, 374] and breast [257] cancers is indicative of poorer outcome. Micrometastases are present in the majority of patients presenting with oesophageal cancer[119, 266] but while several studies have

confirmed the prognostic implication of these occult metastatic cells on relapse-free and overall survival in oesophageal cancer[268, 375-377], others have not[263, 378]. Despite advances in technology, these micrometastases remain undetectable by current staging techniques[109].

With the introduction of new therapeutic regimes and increasing CPR rates, it is now important that we can identify those patients who cannot benefit from surgery either because they have no residual disease, or because they have incurable systemic spread and those who may benefit from adjuvant treatment.

5.3 AIMS

To determine whether the addition of micrometastastic status from rib bone marrow to luminal response to neoadjuvant chemoradiotherapy is more accurate in predicting long-term outcome in oesophageal cancer.

5.4 PATIENTS AND METHODS

We interrogated a prospectively maintained database of patients with oesophageal cancer managed at Connolly Hospital to identify a cohort of patients who had completed neoadjuvant chemoradiotherapy, had pre- and post-treatment endoscopy and biopsy and who had rib marrow examined for the presence of micrometastases as part of a previous study by Ryan et al[269]. Endoscopic findings post neoadjuvant chemoradiotherapy were extracted and correlated with rib marrow micrometatstatic status and histopathological findings of the resection specimen in those who underwent surgery. Rib marrow micrometastatic status and endoscopic response to therapy was correlated with survival. Overall survival was calculated from the date of diagnosis.

5.4.1 NEOADJUVANT CHEMORADIOTHERAPY

Neoadjuvant chemoradiotherapy consisted of two cycles of 5-FU and Cisplatin with 40Gy radiotherapy as previously reported[182]. In brief, this consisted consisted two cycles of 5-fluorouracil and cisplatin during weeks one and six, with concurrent radiotherapy (40 Gy) in 15 fractions. Those who underwent oesophagectomy had surgery on or after week 8.

5.4.2 ENDOSCOPIC AND PATHOLOGICAL EVALUATION

All patients underwent pre-and post-treatment endoscopy. Pre-treatment, the site and length of the tumour were documented and multiple biopsies - at least 10 per endoscopic session - were taken for histological analysis for categorisation into subtype and differentiation.

Post-neoadjuvant chemoradiotherapy endoscopy was repeated after four weeks as part of our restaging protocol. Endoscopic findings were categorised as either complete clinical response (CCR) or incomplete clinical response (ICR) if endoscopy, complemented by colour photography, biopsy or imaging findings identified residual disease. A CCR was identified when no tumour was identified on post-treatment endoscopic evaluation, with multiple (>10) biopsies of the tumour bed.

Patients selected for surgery underwent *en bloc* oesophagectomy four to eight weeks after completion of their chemoradiotherapy. Resection specimens were analysed for the presence or absence of residual disease in the oesophageal wall and surrounding envelope of nodes and tissue. Patients staged according to TNM guidelines as outlined by the seventh edition of the American Joint Committee on Cancer Staging[28] following histological analysis of the resection specimen. A complete pathological response (CPR) was identified in patients undergoing resection when no tumour was identified in the resected specimen.

5.4.3 DETECTION OF RIB MARROW MICROMETASTASES

The retrieval and analysis of bone marrow do not form part of this Thesis.

Bone marrow was harvested from the ribs of patients who had been diagnosed with oesophageal cancer via endoscopy and biopsy. Retrieval of a 2-3cm segment of rib removed at restaging laparoscopy at completion of neoadjuvant chemoradiotherapy or at the time of thoracotomy for oesophageal resection, as described by Ryan et al[269], was performed by Prof TN Walsh, Connolly Hospital, Blanchardstown. In order to avoid tumour micro-embolisation, the segment of rib was removed prior to surgical manipulation of the tumour and rib samples removed were not within the radiation field.

The processing and immunohistochemical staining was performed by Dr. Jacquie Kelly and Dr. Ruth Gleeson, University College, Cork. To detect micrometastases, mononuclear cells were isolated from fresh marrow and immediately stained immunohistochemically with an anti-cytokeratin-18 antibody using the APAAP technique[269]. Tumour cell viability was assessed by immunohistochemical staining of marrow cell cultures for cytokeratin-positive cells[269].

5.4.4 STATISTICAL ANALYSIS

Statistical analyses were performed using the statistical package SPSS version 15.0 for Windows (SPSS, Inc., Chicago IL). Survival probabilities for clinical, pathological, and treatment variables were estimated by the Kaplan–Meier method[350] and pairwise comparisons were made using a log–rank test. The effect of extent of pathological

response to neoadjuvant chemoradiotherapy, tumour histology, presence of micrometastases on survival were examined using logistic regression, and optimal cut-offs were determined using the maximal chi-squared method. Significant univariate factors were included in a Cox proportional hazards regression model to establish independent predictors of survival. Further substratification analysis was performed where necessary using the Mantel-Haenszel test[351]. P values of less than 0.05 were considered statistically significant.

5.5 RESULTS

5.5.1 PATIENT CHARACTERISTICS

Forty-three patients fulfilled our selection criteria. Male to female ratio was 2.6:1 and age range was 37-81 years with a mean age of 61 years. Twenty three (53%) had adenocarcinoma and 20 (47%) had squamous cell carcinoma. Six tumours (14%) were well differentiated tumours, 17 (40%) were moderately differentiated and 20 (47%) were poorly differentiated. Follow-up ranged between 2-155 months from date of diagnosis with a mean of 38 months.

Fifteen patients (4 adenocarcinoma and 11 squamous cell carcinoma) did not undergo surgical resection due to progressive disease (n=9), patient choice (n=2) and deterioration in performance status (n=4).

5.5.2 FOLLOW-UP

Follow-up ranged between 2 and 115 months from date of diagnosis with no patients lost to follow-up. The overall mean survival for the entire cohort was 38 months.

5.5.3 COMPLETE CLINICAL AND PATHOLOGICAL RESPONSE

Twenty-one patients (49%) treated with neoadjuvant chemoradiotherapy had CCR. Fourteen (67%) patients who had a CCR also had a CPR.

A CPR was achieved in fourteen patients (33%). CPR rate was 35% in adenocarcinoma (8/23) and 30% (6/20) in squamous cell carcinoma.

The 1, 2, 3 and 5 year survival of those who had a CCR were 81% (n=17), 67% (n=14), 62% (n=13) and 57% (n=12) compared with 32% (n=7), 9% (n=2), 9% (n=2) and 9% (n=2) respectively in those with a partial response on endoscopy and biopsy. Those who had a CCR had a statistically significant survival advantage (p=0.009) compared with those who had an ICR (**Figure 17**).

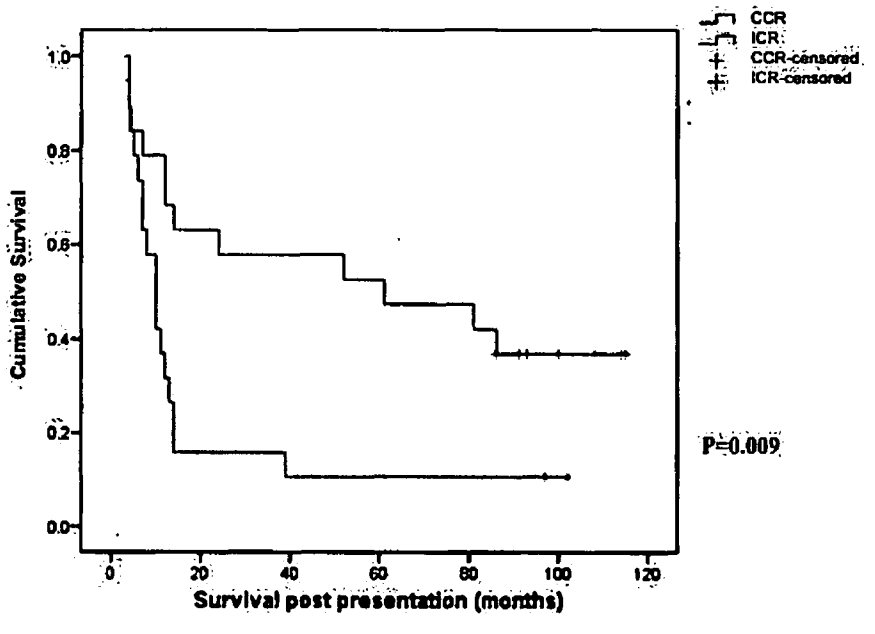


Figure 17: Kaplan–Meier Plot Of Overall Survival Of Patients With Complete Clinical Response (CCR) Versus Incomplete Clinical Response (ICR).

5.5.4 LYMPH NODE DISEASE

Sixty one percent (n=17) of patients were lymph node negative following chemoradiotherapy. Lymph node negativity significantly improved survival (p=0.006) with 75% 5 year survival for lymph node negative patients with adenocarcinoma and 80% with squamous cell carcinoma versus 20% and 0% respectively for lymph node positive patients.

5.5.5 RIB MARROW MICROMETASTASES

Rib marrow micrometastases were identified in 60% (n=26) of patients following treatment.

Patients who were negative for rib micrometastases overall had improved survival over micrometastases positive patients ($p<0.05$) with 1, 2, 3 and 5 year survival of 65% (n=11), 53% (n=9), 47% (n=8) and 41% (n=7) compared with 50% (n=13), 31% (n=8), 31% (n=8) and 23% (n=6) who had micrometastases positive disease. On subgroup analysis of tumour type, micrometastases predicted significantly shorter survival in adenocarcinoma (p=0.017) (**Figure 18**), but not in squamous cell carcinoma (p=0.47).

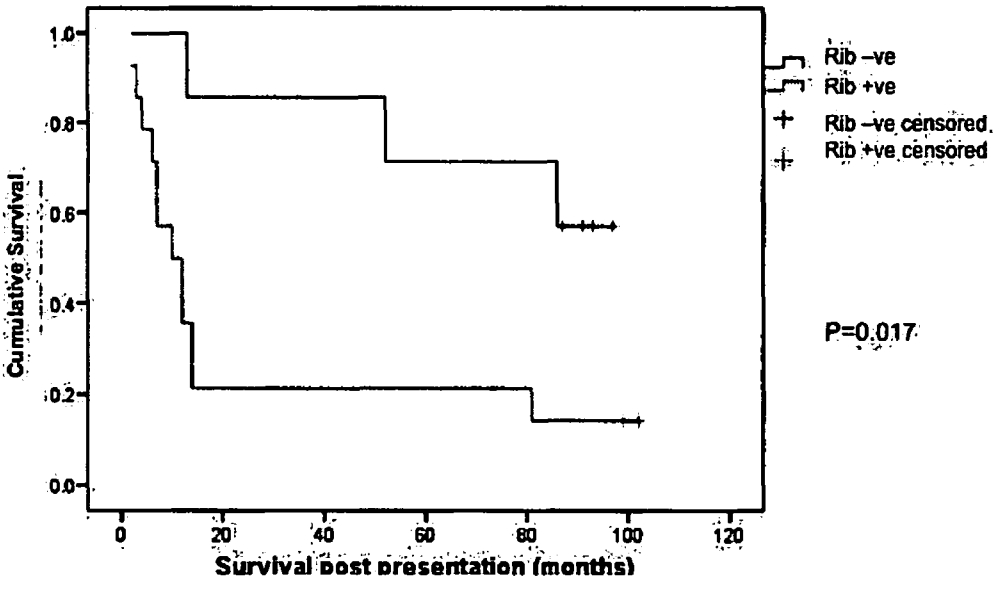


Figure 18: Kaplan–Meier Plot Of Overall Survival Of Patients With Oesophageal Adenocarcinoma With (Rib +ve) And Without (Rib -ve) Rib Micrometastases

5.5.6 COMPLETE CLINICAL RESPONSE AND RIB MICROMETASTATIC STATUS

Patients who had a CCR and were negative for rib micrometastatic disease were twice as likely to survive versus rib positive patients (5 year survival of 38% vs. 17%) but this failed to reach statistical significance (**Figure 19**).

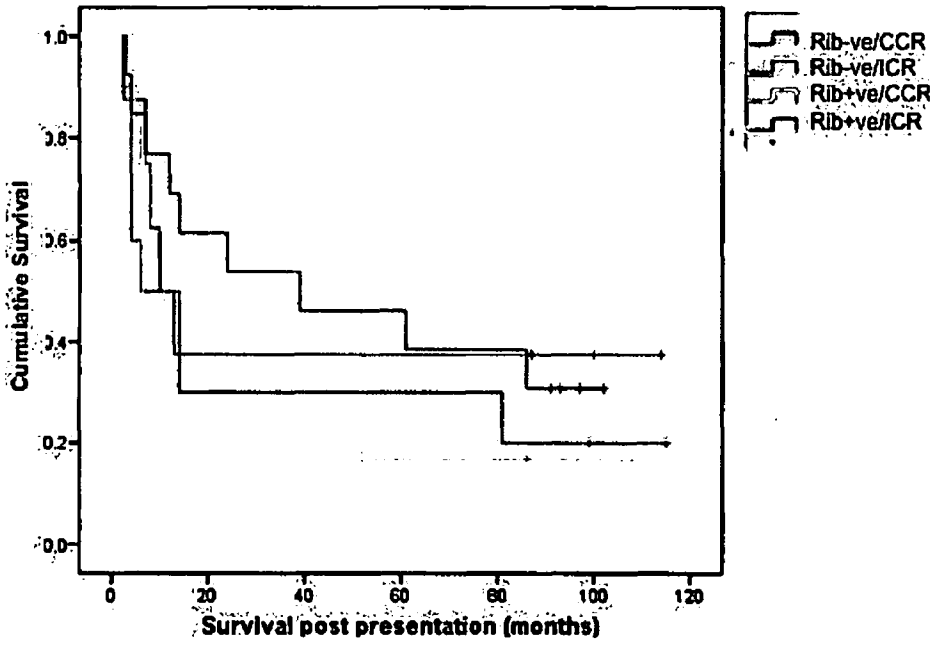


Figure 19: Kaplan–Meier Plot Of Overall Survival Of Patients With And Without Rib Micrometastases (Rib+ve And Rib-ve) And With Complete Clinical Response (CCR) Or Incomplete Clinical Response (ICR).

5.6 DISCUSSION

Survival following resection is related to disease stage in almost all published series. Patients with earliest stage disease survive longest while patients with metastases have poorest outcomes[262]. Neoadjuvant chemoradiotherapy is being increasingly employed to target both loco-regional and systemic disease. However even with the most advanced current clinical staging techniques, we are unable to differentiate between those who have had a complete response to treatment or those who have residual or micrometastatic disease[106, 107, 109]. Because of this, the majority of those with a complete clinical response to neoadjuvant therapy and those with occult metastases continue to undergo arguably unhelpful[373] and invasive surgeries associated with significant morbidity[123-134] and mortality[122]. The ability to stage and restage disease with greater accuracy would be immensly desirable and patients could be offered appropriate treatment tailored to their stage and disease burden.

This study demonstrates that down-staging to a complete loco-regional response provides best hope of long-term survival, as has been shown previously[191, 203, 209, 210, 212, 362, 379]. In a recent study and in **Chapter 4** we have shown that complete clinical response is 74% predictive of a complete pathological response[373]. It is unlikely that the accuracy of luminal response will be enhanced by imaging techniques due limitations in discriminating tumour from inflammatory or necrotic tissue by radiological means[106, 107, 109].

The presence of micrometastases is associated with the depth of penetration[258, 259] and grade[260] of the primary tumour, which are prognostic indicators[262] but the exact significance of micrometastases in oesophageal cancer is uncertain[263, 268, 375-378]. Some authors suggest that their detection merely reflects transitory shedding of cells from the primary tumour and does not indicate increased metastatic potential[264] whereas others suggest that such detection reflects biologically aggressive disease with metastatic tendency[263]. In this series, 31% of patients with micrometastases in the ribs survive 3 years, but patients with rib marrow negative for micrometastases had a survival advantage with 47% surviving 3 years.

The aim of neoadjuvant chemoradiotherapy is to reduce the tumour size and maximise local control[179], thereby allowing a higher rate of R0 resection[123, 143] as well as reducing subsequent distant failures[380]. Although the addition of chemoradiotherapy provides a survival advantage[182, 219, 369], and the best survival statistics are found amongst complete pathological responders[209, 210, 212, 360, 361] many patients with the maximum local response still succumb to systemic recurrence[363, 370]. This apparent failure to eradicate micrometastases may be explained by the fact that when given concurrently, chemotherapy enhances the effect of radiotherapy locally, including lymph node disease but systemic disease is not exposed to this synergistic effect. Additionally, because the majority of micrometastatic tumour cells may be non-proliferating[381], and thus display similar characteristics to cancer stem cells[382, 383], standard cytotoxic chemotherapy may be less effective. Like micrometastases, mounting evidence suggests that cancer stem cells are responsible for tumour resistance and re-growth, establishment of metastases and resistance to a variety of treatments[384-388]. Thus with current treatment regimes, rib micrometastases, appear to escape the full therapeutic

effect that is achieved in the locoregional domain, reflecting the quality of current chemotherapeutic agents.

The persistence of viable disseminated or micrometastatic tumour cells, some or all of which may indeed be cancer stem cells, following neoadjuvant chemoradiotherapy, including in those with an apparent complete pathological response, highlights the need for improved or additional systemic therapies. In this study, we explored the idea that by combining complete clinical response with micrometastatic status we might better identify complete treatment responders. It is clear from this, albeit small, study that information on micrometastases enhances the prognostic accuracy of luminal response. While in loco-regional incomplete-responders, rib marrow micrometastasis status less was less significant, those who had a complete loco-regional response and who had no detectable micrometastases had an improved prognosis. These patients have endured the morbidity of surgery to ascertain whether or not there was residual disease but it is questionable whether they derived any benefit. As the rate of complete pathological response increases with the introduction of new chemotherapeutic regimes, the morbidity and mortality risks that these patients are exposed to become difficult to justify. Similarly, whilst patients continue to succumb to metastatic disease in the context of good or complete loco-regional response, it is difficult to rationalise the role of surgery if their disease burden is incurable. The findings of this study suggest rib and nodal micrometastases are predictive of systemic disease and that their presence predict a poorer outcome.

5.7 CONCLUSION

The challenge remains to develop minimally invasive, affordable and reliable techniques to identify those who cannot benefit from surgery, either because of the absence of resectable disease or due to the presence of micrometastatic disease which will ultimately decide the patient's outcome. Reliable and standardised methods need to be developed and evaluated in large prospective studies before micrometastatic status is incorporated in routine clinical staging. Future therapies should target local tumour but also focus on the systemic burden as this ultimately decides the outcome.

CHAPTER VI: STUDY 4

TAILORING THERAPY FOR OESOPHAGEAL CANCER IN PATIENTS AGED 70 AND OVER

6.1 ABSTRACT

Background. Cancer is a disease of the elderly but this cohort is under-represented in randomised trials. Oesophageal cancer management in the elderly is challenging because of the morbidity and mortality risks of surgery.

Aims. To examine the outcome of a strategy of neoadjuvant chemoradiotherapy followed by surgery or surveillance, depending on response, in patients over 70.

Methods. A prospectively-accrued database identified 129 patients aged over 70 presenting with oesophageal carcinoma, of whom 66 (51%) were too advanced or unfit for curative intervention while 63 (49%) were treated with curative intent.

Results. Of 129 eligible patients, 66 (51%) received palliative measures while 63 (49%) had curative intervention: 7 had surgery alone and 56 had neoadjuvant chemoradiotherapy +/- surgery. Of the 56 patients, 33 (59%) had adenocarcinoma (AC) and 23 (42%) squamous cell carcinoma (SCC). Twenty-five (44%) had a complete clinical response (CCR) of whom 6 had immediate resection; 4 of whom (67%) had a complete pathological response (CPR); 19 patients with a CCR declined, or were unfit for surgery, and underwent surveillance; of which 3 had interval oesophagectomy; while 16 were not offered or declined resection. Eight (50%) have survived > 3 years. The mean survival was 28 months for the entire cohort; 47 months for CCRs; 61 months for patients undergoing primary resection, 29 months for those undergoing interval resection and 46 months for CCRs who did not undergo resection. In those with a CCR, surgery did not provide a survival advantage (p=0.861).

Conclusion. As one third of patients have a CPR to neoadjuvant chemoradiotherapy, resection for these, with its attendant risks in the elderly, makes little sense. Obviating

surgery yields an overall 3-year survival of 50%. With the additional option of salvage oesophagectomy for re-emergent disease, this strategy may be an attractive option for elderly patients.

6.2 INTRODUCTION

The global population is aging at a rate never observed before in human history with the older population growing at a rate considerably faster than that of the total population[389]. Within the first half of this century, the global population aged 60 years or older is projected to treble to nearly 2 billion[389]. In Europe, almost 30 per cent of the population is predicted to be aged 65 or over by 2050[389]. Cancer is a disease of the elderly[390] and the majority of gastrointestinal cancers occur in elderly patients[391]. Oesophageal cancer is no exception with median age at diagnosis of 68 years[4]. Sixty-one percent of sufferers are over the age of 65, 33% are over the age of 75 and 8% are over the age of 85[4].

Despite being one of the fastest growing and prevalent malignancies, especially in developed countries[7-10, 17, 18], the outcome of treatment of oesophageal cancer in older patients is unclear from the literature as few studies report community results[78] or results in the elderly[392, 393] and the elderly are under-represented in randomised trials[392, 393]. With this rapidly increasing incidence of oesophageal cancer and an ever-aging population[18, 389], this issue becomes evermore urgent.

As the majority of patients with oesophageal cancer have systemic disease at presentation, systemic therapy is essential. One of the advantages of providing this preoperatively is that over one third of patients will have a complete pathological response[191, 213, 215]. These patients cannot benefit from resection but are exposed to all of its risks including an risk of mortality of around 14% overall[122] and 20% in the

elderly[394-396] and a lifetime exposure to the negative quality of life impact[123-134]. We have shown that a complete clinical response (CCR) is 74% predictive of a complete pathological response (CPR) and these patients may obviate surgery[373].

6.3 AIMS

The aim of this study was to analyse the outcome of patients over the age of 70 with oesophageal cancer following treatment with chemoradiotherapy.

6.4 PATIENTS AND METHODS

6.4.1 PATIENTS

A prospectively maintained database of patients presenting to Connolly Hospital was interrogated to identify a cohort of patients over the age of 70 years who had completed chemoradiotherapy for loco-regionally advanced oesophageal adenocarcinoma and squamous cell carcinoma. Medical records were analysed for any additional data required that was not contained within the database. Patients were followed-up until death or last clinical interaction. Overall survival was calculated from the date of diagnosis.

6.4.2 CHEMORADIOTHERAPY

Neoadjuvant chemoradiotherapy consisted of two cycles of 5-FU and Cisplatin with 40Gy radiotherapy as previously reported[182]. In brief, this consisted consisted two cycles of 5-fluorouracil and cisplatin during weeks one and six, with concurrent radiotherapy (40 Gy) in 15 fractions. Patients consenting to surgery underwent resection within eight weeks of completion of neoadjuvant therapy.

6.4.3 ENDOSCOPIC AND PATHOLOGICAL EVALUATION

All patients underwent pre- and post-treatment endoscopy. Pre- and post-treatment, the site and length of the tumour were documented and multiple biopsies - at least 10 per endoscopic session - were taken for histological analysis.

Post-treatment, endoscopic findings were categorised as either CCR or incomplete clinical response if endoscopy, biopsy or imaging findings identified residual disease. A CCR was identified when no tumour was identified on post-treatment endoscopic evaluation, with multiple (>10) biopsies of the tumour bed, and a negative computerised tomography (CT). An incomplete response was identified when tumour was present on post-treatment endoscopy and/or in the resection specimen.

In patients undergoing resection, specimens were analysed for the presence or absence of residual disease. A CPR was identified when no tumour was identified in the resected specimen. Patients were staged according to TNM guidelines as outlined by the American Joint Committee on Cancer Staging[397].

6.4.4 SURVEILLANCE

Patients with a CCR opting for an observational approach or who were considered at increased risk for surgery due to age and or co-morbidity were closely followed with 3-monthly endoscopy and multiple (>10) biopsies, 6-monthly CT scanning for 3 years, following which the endoscopic intervals were extended to 6-monthly to 5 years with annual follow-up thereafter. Patients who were disease free for 3 or more years were considered complete clinical responders.

Patients known to have an incomplete response following treatment who were not offered or declined surgery were managed symptomatically and did not undergo routine investigation.

6.4.5 STATISTICAL ANALYSIS

Statistical analyses were performed with PASW version 18.0 for Windows. Continuous variables were expressed as median ± standard deviation or mean ± standard error of the mean as appropriate and were compared using a two-sample t-test. Categorical variables were compared using a chi-squared test, with Fishers exact test used where appropriate. Survival probabilities for clinical, pathological, and treatment variables were estimated by the Kaplan–Meier method[350] and pairwise comparisons were made using a log–rank test. The effect, in patients over the age of 70 years, of extent of pathological response to neoadjuvant chemotherapy and 3D-conformal external-beam radiation therapy (either followed by surgical resection or surveillance), tumour histology, nodal status, tumour length and AJCC stage on overall survival were examined using logistic regression, and optimal cut-offs were determined using the

maximal chi-squared method. Significant univariate factors were included in a Cox proportional hazards regression model to establish independent predictors of survival. Further substratification analysis was performed where necessary using the Mantel-Haenszel test[351]. P values of less than 0.05 were considered statistically significant.

6.5 RESULTS

6.5.1 PATIENT CHARACTERISTICS

Records from Connolly Hospital Oesophageal Cancer Database were extracted for a 90-month period between January 2000 and July 2007. One hundred and twenty-nine patients over the age of 70 presented during this period. Following multidisciplinary discussion, 66 patients were considered to have disease too advanced (n=41) or were too unfit for curative intent (n=25) and were treated with palliative measures. Sixty-three patients were treated with curative intent, seven of whom underwent surgery alone.

Fifty-six patients completed neoadjuvant chemoradiotherapy for locoregionally advanced AC or SCC of the oesophagus and fulfilled the selection criteria. These 56 patients formed the current study cohort.

All 56 patients were 70 years or older, 25 patients (45%) were aged 75 years or older and 10 patients (18%) were 80 years or older. The mean age was 75 years, ranging from 70 to 83 years. Thirty-five patients (62%) were male, with a male to female ratio of 1.7:1.

6.5.2 TUMOUR CHARACTERISTICS

Of the 56 patients, 33 (59%) had AC and 23 (41%) had SCC. Three tumours were well differentiated (5%), 28 were moderately differentiated (50%) and 25 were poorly differentiated (45%).

6.5.3 RESPONSE TO CHEMORADIOTHERAPY

Thirty-one of the 56 patients (55%) had residual disease on endoscopy post-chemoradiotherapy. Twenty-five (45%) had a CCR: 36% of ACs (n=12) and 54% of SCCs (n=13). Of the 25 CCRs, 6 chose immediate resection while 8 declined and 11 were deemed unsuitable for surgery and all were placed on intensive surveillance.

Three patients who initially had a CCR had re-emergence of tumour and underwent an interval oesophagectomy following detection of recurrence at 11 months in two patients and at 12 months in one patient. The remaining 16 patients continued to be followed-up clinically.

6.5.4 FOLLOW-UP

Follow-up ranged between 2 and 116 months from date of diagnosis with no patients lost to follow-up. The overall mean survival for the entire cohort was 28 months with a median of 14 +/- 1.8 months.

6.5.5 COMPLETE CLINICAL RESPONSE

Twenty-five patients had a complete clinical response. The mean overall survival of this cohort was 47 months with a median survival of 35 +/- 28.8 (95% confidence interval (CI) 0-91 months). Overall, patients with a CCR had a survival advantage over those who had an incomplete clinical response (ICR) ($p < 0.001$) (**Figure 20**).

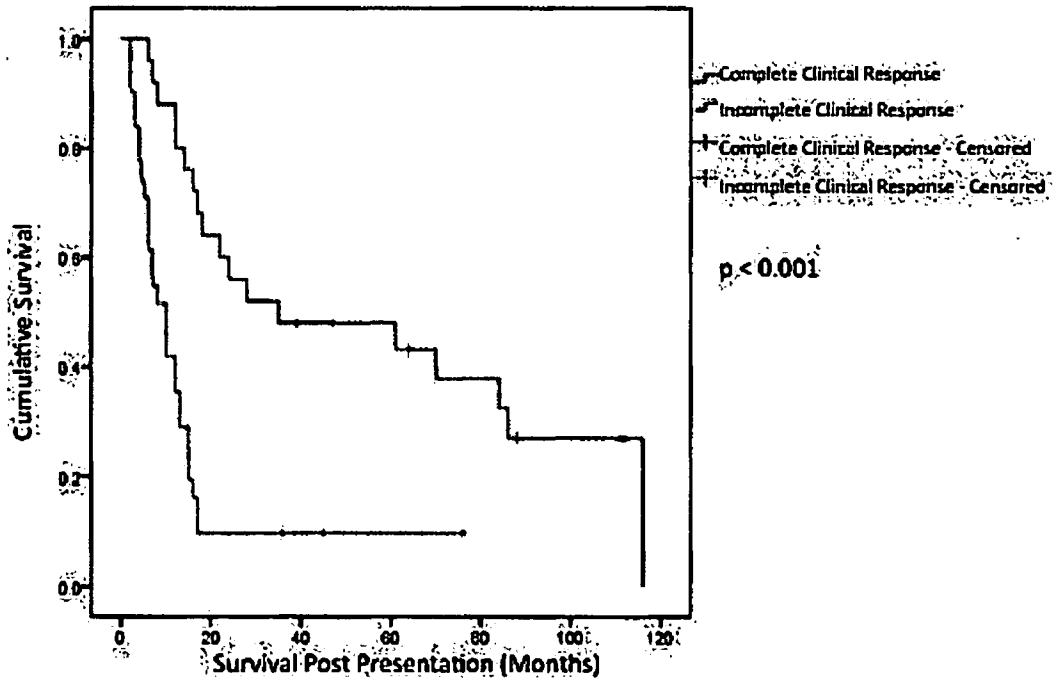


Figure 20: Kaplan–Meier Plot Of Overall Survival Of Patients With Oesophageal Cancer With Complete Clinical Response Versus Incomplete Clinical Response.

6.5.5.1 COMPLETE CLINICAL RESPONSE AND IMMEDIATE RESECTION

Of the six patients with a CCR who underwent immediate resection, four (67%) also had a complete pathological response (CPR) while one had stage 2a and one had stage 2b disease. One of these latter patients died at 12 months of recurrence and one is alive

at 111 months at completion of this study. Of the four patients with a CPR, one died at 86 months without autopsy, one died at 35 months from an unknown cause and a third died at 8 months of metastatic disease. The remaining patient was alive at 112 months at completion of this study. The mean survival of this subgroup was 61 months with a median survival of 35 +/- 10 months.

6.5.5.2 COMPLETE CLINICAL RESPONSE AND INTERVAL SURGERY

Three patients who had a CCR had an interval oesophagectomy following detection of recurrent disease on surveillance. Mean survival of these patients was 29 months. One of these patients was alive at 47 months at completion of this study, whilst two died of metastatic disease at 28 and 12 months.

6.5.5.3 COMPLETE CLINICAL RESPONSE AND NON-OPERATIVE MANAGEMENT

Sixteen patients with a CCR did not have surgery and had a mean survival 46 months (range 6-116) and a median survival of 24 +/- 36 months. The 1, 2, 3 and 5 year survival of this patient cohort was 88% (*n*=14), 56% (n=9), 50% (n=8), 44% (n=7). Seven patients died from recurrence or metastases, three died secondary to pneumonia, one from multi-organ failure and one from thoracic empyema[366]. Four patients (25%) were alive at completion of this study at 39, 64 and two at 88 months.

Patients with CCR who did not undergo surgery had a similar overall survival to those who underwent surgery (median 55.1 +/- 11 months) when compared to those undergoing oesophagectomy (median 56.4 +/- 14 months; p=0.861) (**Figure 21**).

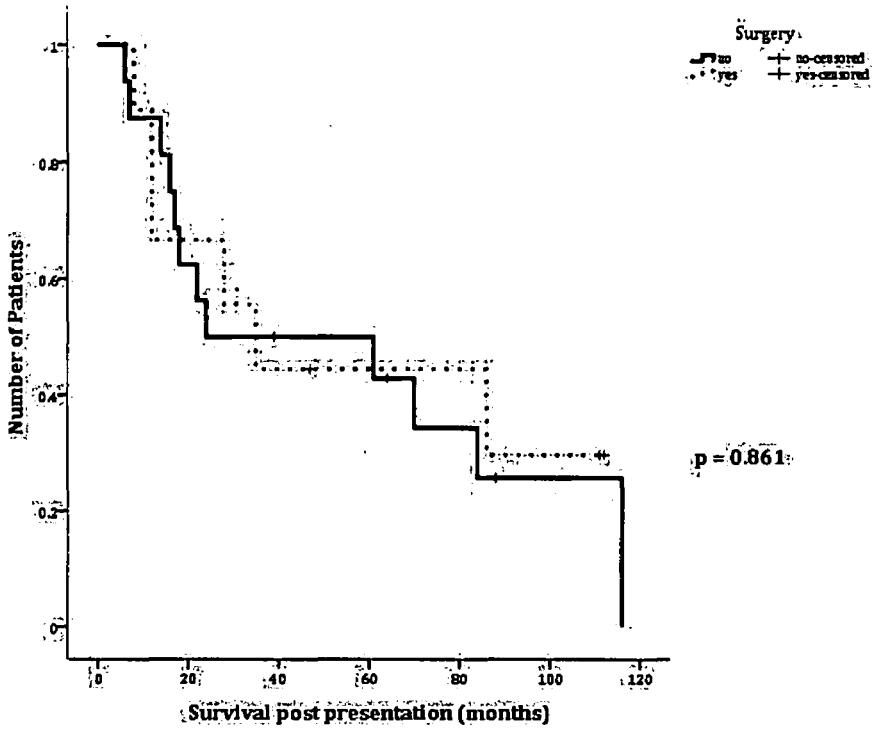


Figure 21: Kaplan–Meier Plot of Survival of Patients With Oesophageal Cancer With Complete Clinical Response To Chemoradiotherapy Managed Operatively Versus Non-Operatively.

6.5.6 INCOMPLETE CLINICAL RESPONSE

6.5.6.1 INCOMPLETE CLINICAL RESPONSE UNDERGOING RESECTION

Thirty-one patients had residual disease on endoscopy post-chemoradiotherapy. Of these, 8 underwent surgery with AJCC stage 1 in one patient, stage 2a in three patients, stage 2b in one patient and stage 3 in three patients. Fifty percent of patients had positive lymph nodes. Five of these eight died from metastases at 7, 10, 12, 16 and 17 months and three were alive at 36, 45 and 76 months at completion of this study.

6.5.6.2 INCOMPLETE RESPONSE AND NON-OPERATIVE MANAGEMENT

Twenty-three patients with an incomplete response did not undergo surgery. Of these, two died of pneumonia and two of myocardial infarctions before surgery could be considered. Five patients refused surgery due to advancing years (aged 78-81). A further five patients had progressive disease on treatment and eight had cardiorespiratory conditions with deterioration of performance status which precluded surgery. One final patient died of an upper gastrointestinal bleed at 13 months and had declined surgery due to age (aged 81). The mean survival of this cohort was 8 months (range 2-17 months) with a one-year survival of 30% (n=7) and a median survival of 6 +/- 1.1 months (95% CI = 3.6 – 8.3 months).

Of patients who had an incomplete response, those who had completion oesophagectomy had a statistically-significant overall survival advantage (median 36.2 +/- 10 months) compared with those who underwent surveillance (median 7.9 +/- 1 month, p = 0.006) (**Figure 22**).

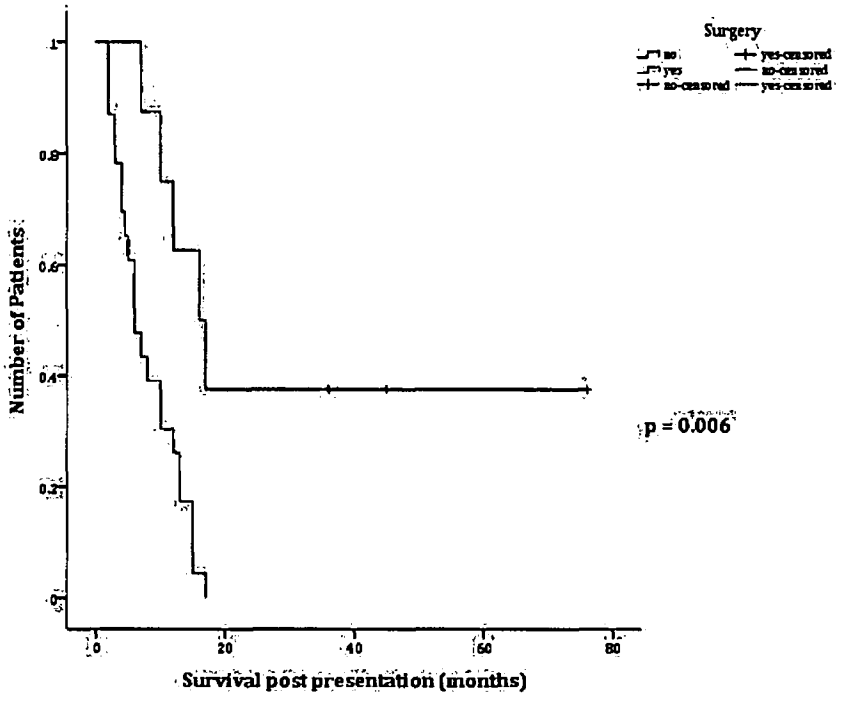


Figure 22: Kaplan–Meier Plot Of Survival Of Patients With Oesophageal Cancer With Incomplete Clinical Response To Chemoradiotherapy Managed Operatively Versus Non-Operatively.

6.5.7 LYMPH NODE STATUS

Sixty-four percent of those who underwent immediate surgery following chemoradiotherapy (9/14) were lymph node negative. Eighty-three percent of those who had a CCR and who had surgery (5/6) went on to have lymph node negative disease. Two of the three patients who had an interval oesophagectomy were also lymph node negative. Those who were lymph node negative had a mean survival of 44 months (range 8-112 months) and those with node positive disease had a mean survival of 31 months (range 7-111 months) (p=0.03).

6.5.8 HOSPITAL MORTALITY

There was one (6%) in-hospital mortality following surgery in this series. This occurred in a patient with a 50-pack year smoking history and was due to respiratory complications.

6.6 DISCUSSION

In the past, the majority of older patients diagnosed with oesophageal cancer were treated palliatively due to the combination of poor prognosis, shorter life expectancy and co-existent co-morbid conditions. With the rapidly aging global population[314] we are now more frequently encountering older patients with oesophageal carcinoma[4] for whom it is difficult to decide on best treatment, not least due to the exclusion of these patients in most randomised trials of gastrointestinal cancers[392, 393], despite the fact that the majority of cancers occur in this age cohort[391].

It has long been insisted upon that the best hope of cure for oesophageal cancer is surgery, alone or following neoadjuvant therapy. While this may hold for younger, fitter patients, it may not prove true for the elderly or for patients with significant comorbidity. While several series have shown oesophagectomy to be tolerable in elderly patients[394, 398, 399], including following chemoradiotherapy[400], there is considerable patient selection as only a small minority of elderly patients diagnosed with oesophageal cancer are referred for oesophagectomy[398, 399]. Many studies quote high co-morbidity rates of up to 86%[401-404], lower resection eligibility rates[394, 395], higher post-operative complication rates[394, 395] and mortality rates[394-396, 405] following oesophagectomy being increased up to two-fold in those over the age of 80[122]. Thus the role of surgery in patients older than 70 years is still unclear but appears appropriate only for a minority.

Several chemoradiotherapy regimes have been shown to be tolerable in the older age groups[402, 406-408] so patients should not be excluded from potentially curative treatment based on age alone[402, 403, 409, 410]. The advantages of providing neoadjuvant chemoradiotherapy include that as the majority of patients with "curable" disease have systemic micrometastases[119, 266, 371, 372] and the systemic component of the treatment will address the systemic component of the disease, enhancing survival over surgery alone[218-220]. Of greater importance is the fact that over one third of patients undergoing neoadjuvant chemoradiotherapy will have a CPR and should not require resection. Not only can they not benefit from surgery but are unnecessarily exposed to the considerable risk of mortality, severe morbidity and lifelong negative impact on quality of life[123-134]. The majority of patients treated with neoadjuvant chemoradiotherapy will have a CCR[403] with median survival of up to 35 months[402]. We have previously shown that the majority of patients with a CCR have a CPR[373]. In recent years the CPR rate from chemoradiotherapy has increased dramatically with rates of up 43% for advanced locoregional disease and up to 82.5% for early disease now being reported[191, 213].

While it is obvious that those with residual disease may benefit from surgery, the benefit to those with a CCR until now has been less clear. In this study, we have shown that patients who had chemoradiotherapy followed by surgery had no statistically significant survival advantage over those who had a CCR to chemoradiotherapy but who did not have surgery (p=0.861) with a 1, 2, 3 and 5-year survival of 83, 67, 67 and 50% compared with 88, 56, 50 and 44% respectively. Others have shown that the combination of chemotherapy and concurrent radiotherapy alone can lead to similar long-term survival to surgical monotherapy and surgery following neoadjuvant

treatment[179, 183, 411]. There is only one randomised controlled trial of 80 patients comparing definitive chemoradiotherapy alone and surgery alone[412]. This study by Chiu et al, however, did not include patients with adenocarcinoma, had a mean age of only 62 and excluded patients over the age of 75 and those with significant co-morbidity[412]. Standard oesophagectomy or chemoradiotherapy seemed to offer similar early clinical outcome and survival. Similar results were noted in a Japanese non-randomised retrospective comparison of definitive chemoradiotherapy and radical surgery in patients with resectable squamous cell carcinoma[186] with overall survival and disease-free survival rates at 3-years were 48% and 44% in the chemoradiotherapy group and 65% and 59% in the surgery group, respectively. Again patients in the 70-79 age-group were excluded. Thus chemoradiotherapy results in survival comparable with conventional surgery.

Salvage oesophagectomy may be offered those with an incomplete response to chemoradiotherapy or recurrent disease. It is accepted, however, that salvage surgery for re-emergent disease will not be as successful as immediate surgery, since tumour detected in the lumen on surveillance endoscopy may not represent early disease, but instead may represent "the tip of the iceberg" of a recurrence from without the oesophageal lumen. The patients who had salvage oesophagectomy in our study had a mean survival of 29 months and five-year survival rates of up to 25–35 per cent can be achieved in selected patients after local failure of chemoradiotherapy[246] with most series, however, reporting on younger cohorts[246].

6.7 CONCLUSION

In conclusion, we identified a number of trends in this study. Almost half of all older patients completing treatment had a CCR. Two-thirds of those with a CCR and who underwent resection had a CPR, which is similar to that previously reported for all patients[373]. Furthermore, those managed non-operatively had comparable survival to those managed with additional radical surgery. Several studies, including ours, have shown that chemoradiotherapy may provide a CPR in one third of patients obviating the need for surgery in this cohort with its attendant risks and negative impact on quality of life. The 3-year survival of 50% in this cohort compares with the best results of more selective series of younger patients and is an attractive option to both patients and clinicians. Larger-scale randomised trials inclusive of older patients which compare radical chemoradiotherapy with surgery alone in both adenocarcinoma and squamous cell carcinoma are necessary but may pose difficulties in recruitment of patients and treating clinicians alike. In their absence, it would appear reasonable to consider treating all potentially curable patients with neoadjuvant therapy and electing to observe patients achieving a complete clinical response.

CHAPTER VII: STUDY 5

THE PREDICTIVE VALUE OF MOLECULAR MARKERS P53, VEGF AND METALLOTHIONEIN IN MULTIMODALLY TREATED OESOPHAGEAL CARCINOMA.

7.1 ABSTRACT

Background. While neoadjuvant chemoradiotherapy allows improved survival over surgery alone for resectable oesophageal cancer, unresponsive patients are exposed to the negative-effects of therapy with little benefit. It would be advantageous to identify responsive patients. Because of their role in apoptosis and thus suspected involvement in treatment resistance, the molecular markers p53, metallothionein and VEGF have been examined as prognostic indicators in oesophageal cancer with variations in results largely due to study heterogeneity.

Aims. To determine whether the expression of the molecular markers p53, metallothionein and VEGF, alone or in combination can predict response and survival following neoadjuvant chemoradiotherapy in patients with oesophageal cancer.

Methods. Immunohistochemical analysis was performed on pre- and post-neoadjuvant chemoradiotherapy oesophageal tumour samples from 76 patients for expression of p53, VEGF and metallothionein and correlated with response and outcome.

Results: Pre-treatment negative expression of p53 was an independent predictor of survival ($p<0.001$). While pre-treatment tumours positive for metallothionein expression and post-treatment p53 and VEGF negativity showed a trend towards improved survival, this was not statistically significant. On combining factors, a survival advantage was identified with the association of pre-treatment tumours negative for p53 and VEGF and positive for metallothionein expression compared with all other combinations ($p<0.001$). None of the markers predicted response to treatment.

Conclusion. While these results show potential for clinical application, there is an obvious need for confirmation of these observations in a prospective study with standardised techniques in well-defined patient cohorts and should be the subject of future research. It is likely that a combination of markers will yield the most promising results.

7.2 INTRODUCTION

Neoadjuvant chemoradiotherapy provides a survival advantage over surgery alone for resectable oesophageal cancer[182, 215-222] with the greatest benefit in those with a complete response[203, 209, 210, 212, 360-365]. Those who do not respond are unlikely to derive benefit but are exposed to its side-effects and some have disease progression during treatment[182, 184, 413]. All incur considerable cost, which is a major consideration in a time of economic difficulty. The chief shortcoming of neoadjuvant chemoradiotherapy it that is still not possible to know, in advance of treatment, which patients will respond and which patients will not benefit or indeed are harmed by treatment[143].

Much effort has been expended on techniques to predict outcome and response to treatment in oesophageal cancer. Methods such as histological indices, clinical parameters, radiological imaging, and a wide range of serum and tissue markers[270] have been explored and whilst many show potential for clinical application, to date no one technique has emerged in routine clinical practice.

Response and resistance of cancer cells to chemotherapy and/or radiotherapy may be influenced by their propensity to undergo apoptosis which, when induced by chemoradiotherapy, involves various biological processes such as DNA repair, altered drug metabolism, inflammation and alteration of the cell cycle[287, 288]. The molecular markers p53, metallothionein and vascular endothelial growth factor (VEGF) all play a central role in this process and may be detected by immunohistochemical means in tumours.

Thus p53[271-286] , metallothionein[308-310, 316, 317] and VEGF[334, 336, 337] have all been studied individually or combined with other markers to a varying degree in oesophageal adeno- and squamous cell carcinoma, with promising results. To date, however, they have not been shown to be sufficiently accurate on their own, and study comparison is difficult due in part to differing techniques and tumour types and variability of results. Nor have these markers been studied together to assess prognosis and response to neoadjuvant chemoradiotherapy. Using immunohistochemistry, a process achievable in most laboratories, these three tissue markers in combination may offer valuable predictive information.

The aim of this study, therefore, was to determine whether the expression of the molecular markers p53, metallothionein and VEGF, correlates with response to treatment and survival in patients with adenocarcinoma and squamous cell carcinoma of the oesophagus who undergo neoadjuvant chemoradiotherapy.

7.3 AIM

To examine the role of p53, VEGF and metallothionein as predictive markers for response to neoadjuvant chemoradiotherapy and outcome in oesophageal cancer and to establish if their combination would prove more effective than these individual markers on their own.

7.4 PATIENTS AND METHODS

7.4.1 PATIENTS

Following Connolly Hospital ethics committee approval, 76 patients who had undergone neoadjuvant treatment for oesophageal carcinoma between January 2000 and December 2007 were identified from the prospectively-maintained Connolly Hospital Oesophageal Cancer Database. All patients had a histologically proven diagnosis of primary oesophageal adeno- or squamous cell carcinoma from biopsies obtained at oesophagoscopy. These patients had pre- or post or pre- and post-treatment pathology specimens identified as retrievable from the Connolly Hospital pathology specimen archive.

7.4.2 NEOADJUVANT CHEMORADIOTHERAPY

All patients were treated with neoadjuvant chemoradiotherapy as described previously[182]. In brief, this consisted of two cycles of 5-fluorouracil and cisplatin during weeks one and six, with concurrent radiotherapy (40 Gy) in 15 fractions. Those who underwent oesophagectomy had surgery on or after week 8.

7.4.3 PATHOLOGICAL STAGE

Following neoadjuvant treatment, those who underwent surgical resection had tumour staging as defined according to the American Joint Committee on Cancer classification[414]. A complete pathological response (CPR) was defined by the absence of residual tumour in the resected specimen and in the lymph nodes.

7.4.4 IMMUNOHISTOCHEMISTRY

Formalin-fixed paraffin-embedded tissue blocks of specimens were routinely processed for histopathological assessment of tumour type, grade and vascular invasion and staging. Patients and corresponding specimens of pre- and post-treatment biopsies and resection specimens were identified from the prospectively maintained Connolly Hospital Oesophageal Cancer Database and tissue blocks were retrieved from the Connolly Hospital pathology specimen archive. Four micron sections were cut from these blocks and mounted on Leica Microsystems "Plus" slides.

To overcome some of the limitations and variability of immunohistochemical staining, all tissue sections were processed in the same manner on a single automated staining system. This was performed with the BondMax automated staining system from Leica Microsystems (Newcastle-Upon-Tyne, UK). BondMax software 4.0 was then used to run the optimal protocol for the selected antibody marker and hence antigen to be demonstrated. Labelled slides were loaded onto the instrument and deparaffinisation was carried out using Bond™ Dewax solution (Leica Microsystems, Newcastle-Upon-Tyne, UK). Following deparaffinisation, antigen retrieval was performed with heat-induced epitope-retrieval using Bond™ Epitope Retrieval solution (Leica Microsystems,

Newcastle-Upon-Tyne, UK) for 20 minutes. Following this the primary antibody was applied (i.e. either anti-VEGF, anti-p53 or anti-metallothionein). A secondary antibody followed by a polymer containing horseradish peroxidase which bind to the primary antibody were next applied. Finally 3,3' diaminobenzinetrahydrochloride (DAB), a chromogen which produces a brown end product and which is highly insoluble in alcohol and other organic solvents, was applied. Any antigenic sites present in the tissue to which the primary antibody binds, were thus stained an intense brown colour. The reagents employed in this instance were the commercially available Monoclonal Mouse Anti-Human Antibodies p53 (DO-7) and Vascular Endothelial Growth Factor (VG1) and Monoclonal Mouse Anti-Metallothionein Clone (E9), Dako. Examples tumours with positive expression of p53, Metallothionein and VEGF are shown in **Figure 23 A, B and C.**

Once the slides were loaded, the process was allowed to continue to completion where fully stained slides were cover-slipped. Specimens of normal oesophageal mucosa were used as a positive control. The primary antibody was omitted and staining repeated as a negative control.

Analysis of the immunohistochemically stained slides was performed by a single Pathologist. Staining was considered positive for p53 when more than 10% of the cells' nuclei were strongly stained[337]. The staining pattern of metallothionein and VEGF is cytoplasmic and/or membranous and staining was considered positive when more than 10% of the tumour cells were strongly stained[279, 309, 337]. Evaluation of the immunoreactivity of specimens was performed without knowledge of the patients'

clinicopathological status or outcome. The degree of expression was then compared with a number of tumour and patient variables and correlated with outcome.

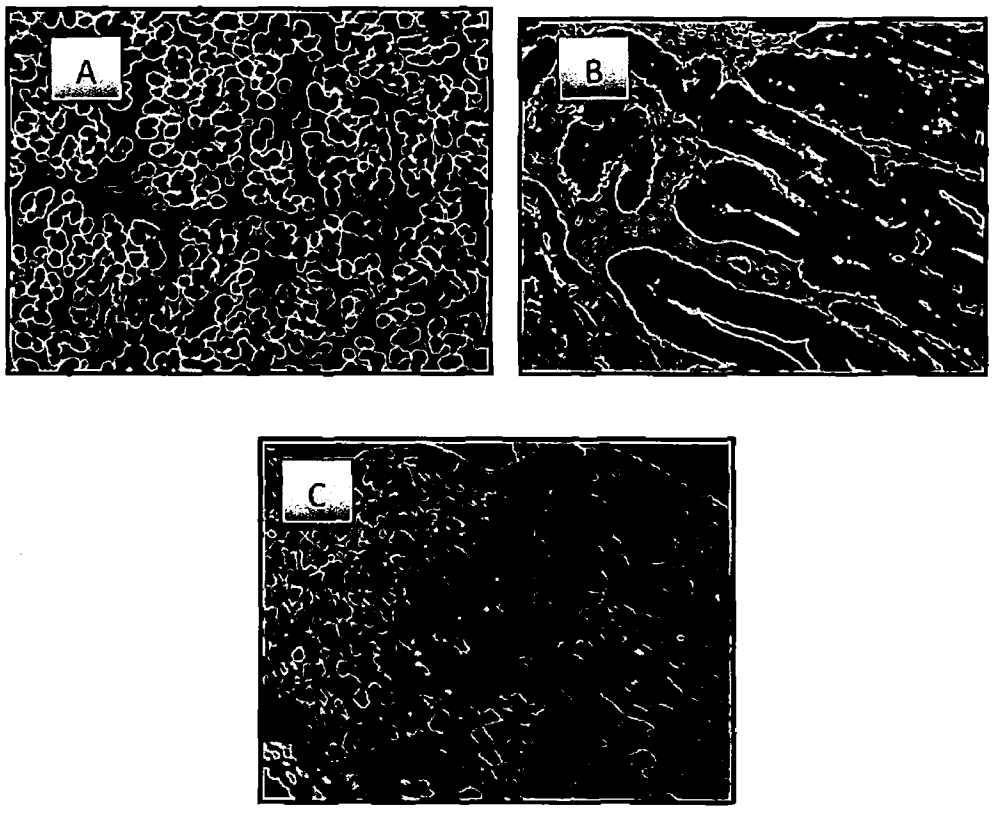


Figure 23: Examples of slides displaying high levels of expression of p53 in adenocarcinoma (A), metallothonein in adenocarcinoma (B) and VEGF in squamous cell carcinoma (C).

7.4.5 FOLLOW-UP

For the purposes of this study, all patients were followed-up until the date of death or last clinical interaction. Follow-up and cause of death, if applicable, was determined by telephone communication with their General Practitioner, review of patient records or searches in the Archives of the National Death Registry Offices, Dublin, Ireland.

7.4.6 STATISTICAL ANALYSIS

Statistical analyses were performed with PASW version 18.0 for Windows. Continuous variables were expressed as median ± standard deviation or mean ± standard error of the mean as appropriate and were compared using a two-sample t-test. Categorical and paired variables were compared using a chi-squared test. Survival probabilities for clinical, pathological, and treatment variables were estimated by the Kaplan–Meier method[350] and pairwise comparisons were made using a log–rank test. The predictive value of pre- and post-therapy oesophageal biopsy VEGF, p53 and metallothionein of the extent of pathological response to neoadjuvant chemotherapy and 3D-conformal external-beam radiation therapy (either followed by surgical resection or surveillance), tumour histology, nodal status, tumour length and AJCC stage on overall survival were examined using logistic regression, and optimal cut-offs were determined using the maximal chi-squared method. Significant univariate factors were included in a Cox proportional hazards regression model to establish independent predictors of survival. P values of less than 0.05 were considered statistically significant.

7.5 RESULTS

7.5.1 PATIENT CHARACTERISTICS

Paraffin-embedded tumour blocks from 76 patients with carcinoma of the oesophagus were studied. The mean age was 64 years (range 38-83 years). The male to female ratio was 2:1. Overall survival ranged between 0.5-111 months from date of diagnosis with a mean of 23 months.

All were treated with neoadjuvant chemoradiotherapy and all had pre-and post-treatment endoscopy and biopsy (at least 10 per session) and 37 went on to surgical resection. The remainder declined or were not offered resection due to co-morbidity, deterioration in performance status or progressive disease.

7.5.2 TUMOUR CHARACTERISTICS

Forty-nine (64%) patients had adenocarcinoma and 27 (36%) patients had squamous cell carcinoma. Five tumours (7%) were well-differentiated, 41 (54%) were moderately-differentiated and the remaining 30 (39%) were poorly-differentiated. Tumour grade was predictive of survival, with moderately- and poorly-differentiated tumours conferring a significantly-shorter median survival time (12+/-2.7 and 12.5+/-1.8 months) compared with well-differentiated tumours (61+/-40months) ($p=0.039$). The average tumour length, measured on initial endoscopy, was 5cm (range 1-12cm). Initial tumour length was poorly-predictive of survival ($p=0.325$).

7.5.3 PATHOLOGICAL RESPONSE AND PROGNOSIS

Thirty-four patients underwent resection, of whom 5 (15%) had a CPR, while 29 (85%) had residual disease in the resected specimen. Overall, 8% percent of all adenocarcinomas (2/25) and 33% of squamous cell carcinomas (3/9) had a CPR. The post-treatment AJCC disease stage in these patients with residual disease was stage 1 in 1 patient, 2a in 7 patients, stage 2b in 10 patients and stage 3 in 10 patients and stage 4 with peritoneal metastases in 1 patient 14 of the 34 patients were lymph node negative (41%).

7.5.3 DETECTION OF TUMOUR

Of 189 pre-treatment endoscopic biopsy of tumours sampled for this study, 31 did not demonstrate tumour (16%), although tumour was detected in these patients on formal pre-treatment histo-pathological assessment. One-hundred and nine post-treatment samples were analysed, 15 of which were from patients with a complete pathological response and did not demonstrate tumour. Of the remainder 94 post-treatment samples from patients with residual disease, 29 (31%) samples for this study failed to demonstrate tumour, although tumour was detected on formal histo-pathological assessment with multiple sampling.

7.5.4 P53 EXPRESSION PRE-TREATMENT

7.4.5.1 P53 EXPRESSION PRE-TREATMENT AND TUMOUR CHARACTERISTICS

A total of 59 samples from 59 patients were stained for p53; 38 adenocarcinomas and 21 squamous cell carcinomas. p53 positive staining was found in 66% (n=39) and 34% stained negative (n=20). A further 4 samples did not demonstrate tumour and were thus excluded from the analysis.

The average length of tumour in the two groups did not differ significantly with the average length in the negative and positive groups of 5.7 and 5cm respectively (range 1-12cm) (p=0.689).

In the p53 negative group, 2 tumours were well-differentiated (10%), 10 were moderately-differentiated (50%) and 8 were poorly-differentiated (40%). This compares with 3 (5%), 24 (62%) and 13 (33%) respectively in the positive group. Pre-treatment p53 status did not correlate with worse tumour grade (p=0.648).

Overall 29% of adenocarcinomas (11/38) and 43% of squamous cell carcinomas (9/21) demonstrated negative expression of p53. Fifty-five percent of negative tumours were adenocarcinomas (11/20) and 45% were squamous cell carcinomas (9/20).

7.4.5.2 P53 EXPRESSION AND RESPONSE TO NEOADJUVANT CHEMORADIOTHERAPY

Twenty patients in this group underwent resection, with the AJCC tumour stage outlined in **Table 10**. Forty-four percent of p53 negative (4/9) and 64% of p53 positive (7/11) samples had lymph node metastasis (p=0.414). Post-treatment p53 over-expression directly correlated with moderate differentiation of the tumour, while negative expression was occurred in both well-differentiated tumours and poorly-differentiated tumours (p=0.044).

7.4.5.3 P53 EXPRESSION PRE-TREATMENT AND SURVIVAL

In the negative group, the 1, 2, 3 and 5-year survival was 80% (n=16), 45% (n=9), 30% (n=6) and 20% (n=4). This compares with 44% (n=17), 13% (n=5), 3% (n=1) and 0% in the positive group. The overall mean survival for p53 negative tumours was 48 months (range 6-102 months) compared with 15 months (range 0.5-100 months) for positive tumours (p<0.001) (**Figure 24**).

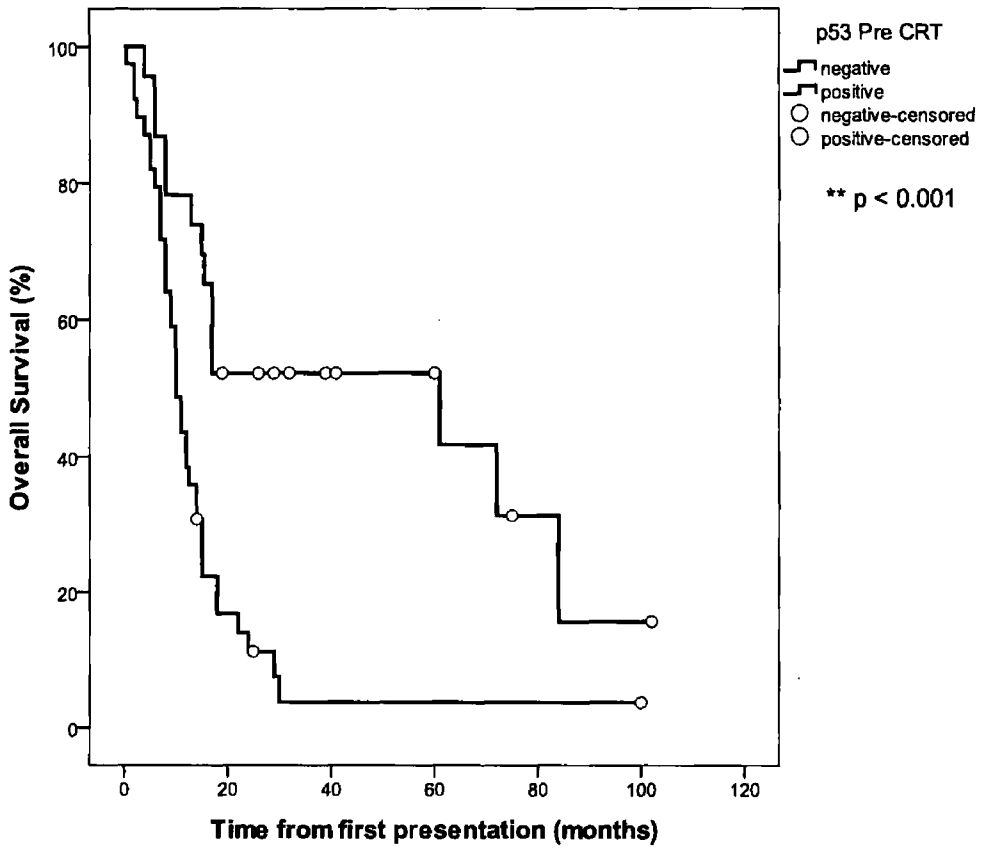


Figure 24: Kaplan-Meier Survival Analysis Comparing Survival Time For p53 Expression Pre-chemoradiotherapy.

7.4.5.4 P53 EXPRESSION POST-TREATMENT AND SURVIVAL

Thirty-six post-treatment samples were stained for p53 expression. Twenty of these did not demonstrate tumour and were excluded from analysis. Of the remainder, 13% of samples (n=2) were negative and 88% (n=14) were positive for p53 expression. There was no significant difference overall between those who were negative for p53 (mean survival of 21 months, range 6-36 months) and those positive for p53 expression post-treatment (mean survival 22 months, range 4-72) (**p**=0.235). However, whilst the Kaplan-Meier curve shows no difference in early survival with the lines overlapping at

about 20 months leading to the non-significant overall p-value, a marked divergence in long-term survival is seen, with p53 negative tumours doing better than positive ones (**Figure 25**).

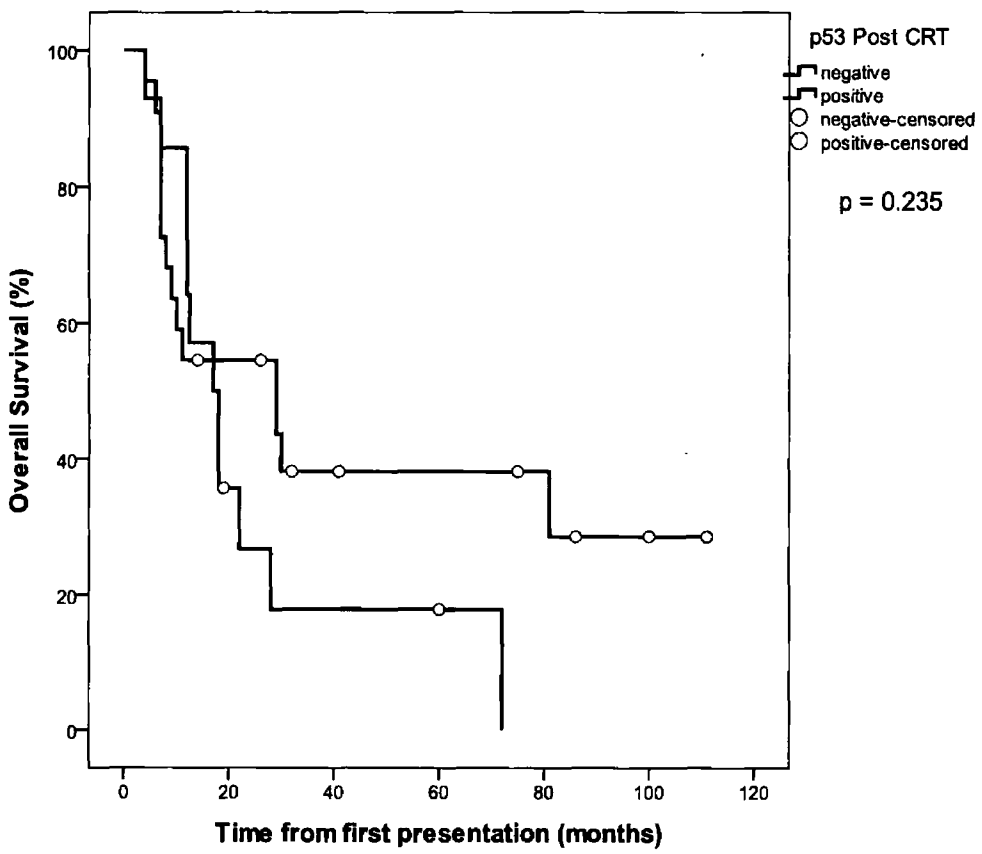


Figure 25: Kaplan-Meier Survival Analysis Comparing Survival Time For p53 Expression Post-chemoradiotherapy.

7.5.5 METALLOTHIONEIN EXPRESSION PRE-TREATMENT

7.5.6.1 METALLOTHIONEIN PRE-TREATMENT EXPRESSION AND TUMOUR CHARACTERISTICS

Metallothionein could be demonstrated in the cytoplasm, nucleus or both in normal and malignant cells but for the purpose of this study, only invasive malignancy was examined. A total of 45 samples from 45 patients were stained for metallothionein expression; 31 adenocarcinomas and 14 squamous cell carcinomas. Metallothionein positive staining was found in 64% (n=16) and 36% stained negative (n=29). A further 18 samples did not demonstrate tumour and were thus excluded from the analysis.

The average length of tumour in the two groups did not differ significantly with the average length in the negative and positive groups 5.4 of and 5cm respectively (range 1-12cm) (p=0.692).

In the negative group, no tumours were well differentiated, 12 were moderately differentiated (75%) and 4 were poorly differentiated (25%). This compares with 3 (10%), 16 (55%) and 10 (34%) in the positive group. Pre-treatment biopsy metallothionein status did not correlate with worse tumour grade (p=0.485).

Overall, 39% of adenocarcinomas (12/31) and 29% of squamous cell carcinomas (4/14) demonstrated negative expression of metallothionein. Seventy-five percent of metallothionein negative tumours were adenocarcinomas (12/16) and 25% were squamous cell carcinomas (4/16).

7.5.6.2 METALLOTHIONEIN EXPRESSION AND RESPONSE TO NEOADJUVANT CHEMORADIOTHERAPY

Thirteen patients in this group underwent resection, with the AJCC tumour stage outlined in **Table 11**. Eighty percent of metallothionein negative (4/5) and 50% of positive (4/8) samples had lymph node positive disease (p=0.279). Post-treatment metallothionein over-expression directly correlated with moderate and poor differentiation of the tumour, while expression was absent in well-differentiated tumours (p=0.023).

7.5.6.3 METALLOTHIONEIN EXPRESSION PRE-TREATMENT AND SURVIVAL

In the negative group, the 1, 2, 3 and 5-year survival was 56% (n=9), 6% (n=1), 0% and 0%. This compares with 59% (n=17), 28% (n=8), 10% (n=3) and 0% respectively in the positive group. The metallothionein negative tumours tended towards an decreased overall survival with a mean of 12 months (range 0.5-29 months) compared with 21 months (range 2-102 months) for positive tumours, but this was not found to be statistically significant (p=0.296).

7.5.6.4 METALLOTHIONEIN EXPRESSION POST-TREATMENT AND SURVIVAL

Thirty-seven post-treatment samples were stained for metallothionein expression. Eighteen of these did not demonstrate tumour and were excluded from analysis. Of the remainder, 32% of samples (n=6) were negative for metallothionein expression and 68% (n=13) were positive. The mean survival was 25 months for the negative group (range 7-111) and 24 months for the positive group (range 4-81) (p=0.213).

7.5.7 VEGF EXPRESSION PRE-TREATMENT

7.5.7.1 PRE-TREATMENT VEGF EXPRESSION AND TUMOUR CHARACTERISTICS

A total of 54 samples from 54 patients were stained for VEGF expression; 35 adenocarcinomas and 19 squamous cell carcinomas. VEGF positive staining was found in 17% (n=9) and 83% stained negative (n=45). A further 9 samples did not demonstrate tumour and were thus excluded from the analysis.

The average length of tumour in the two groups did not differ significantly with the average length in the negative and positive groups of 6.8 (range 1-12) and 5.3cm (range 2-10) respectively (p=0.949).

In the negative group, 3 tumours were well differentiated (7%), 29 were moderately differentiated (64%) and 13 were poorly differentiated (29%). This compares with 0, 4 (44%) and 5 (56%) in the positive group. Pre-treatment VEGF status did not correlate with worse tumour grade (p=0.653).

Sixty-nine percent of negative tumours were adenocarcinomas (31/45) and 31% were squamous cell carcinomas (14/45). Overall 89% of adenocarcinomas (31/35) and 36% of squamous cell carcinomas (5/14) demonstrated negative expression of VEGF.

7.5.7.2 VEGF EXPRESSION AND RESPONSE TO NEOADJUVANT CHEMORADIOTHERAPY

Twenty patients in this group underwent resection, with the AJCC tumour stage outlined in **Table 11**. Fourty-three percent of negative (6/8) and 100% of positive (3/3) samples had lymph node disease (p=0.075). Post-treatment VEGF did not correlate with worse tumour grade (p=0.608).

Table 11: AJCC Stage In Patients Undergoing Surgery Following Neoadjuvant Chemoradiotherapy Based On Expression Of p53, Metallothionein And VEGF.

	p53 Expression		Metallothionein Expression		VEGF Expression	
AJCC Stage[28]	Negative	Positive	Negative	Positive	Negative	Positive
0	1	1	0	1	2	0
1	1	0	0	0	1	0
2a	2	3	1	2	4	0
2b	3	3	2	2	3	2
3	2	4	2	3	3	1
4	0	0	0	0	0	0

7.5.7.3 VEGF EXPRESSION PRE-TREATMENT AND SURVIVAL

In the VEGF negative group, the 1, 2, 3 and 5-year survival was 58% (n=26), 22% (n=10), 9% (n=4) and 4% (n=2). This compares with 44% (n=4), 22% (n=2), 0% and 0% in the positive group. The overall mean survival for VEGF negative tumours was 19 months (range 0.5-61 months) compared with 13 months (range 2.5-29 months) for positive tumours (p=0.424).

7.5.7.4 VEGF EXPRESSION POST-TREATMENT AND SURVIVAL

Thirty-six post-treatment samples were stained for expression. Sixteen of these did not demonstrate tumour and were excluded from analysis. Of the remainder, 65% of samples (n=13) were negative for expression and 35% (n=7) were positive for expression. Those who were negative for VEGF tended towards improved survival with a mean of 43 months (range 21-65 months) versus only 24 months in the positive group (range 4-43) (p=0.19).

7.5.8 MULTIVARIATE ANALYSIS P53, METALLOTHIONEIN AND VEGF EXPRESSION

The frequency of p53, metallothionein and VEGF expression in combination (all patients, all histologies) is displayed in **Figure 26**. Separation into adenocarcinoma and squamous cell carcinoma showed similar trends, but did not achieve statistical significance, likely due to the small numbers in this series.

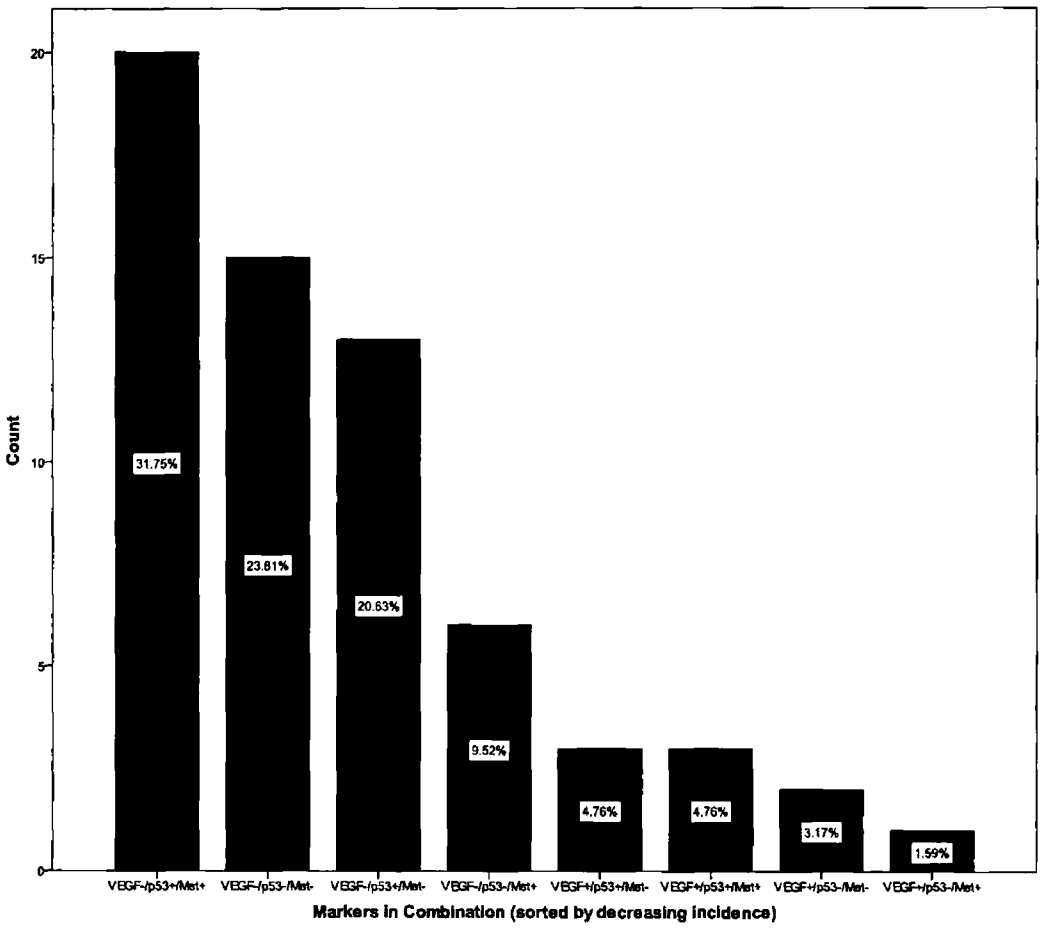


Figure 26: Frequency of p53, Metallothionein And VEGF Expression In Combination

7.5.8.1. MULTIVARIATE ANALYSIS: P53, METALLOTHIONEIN AND VEGF EXPRESSION AND SURVIVAL

Multivariate analysis of adeno- and squamous cell carcinoma combined showed that p53, VEGF and metallothionein together have prognostic significance. Tumours which were p53 and VEGF negative and metallothionein positive (p53-/VEGF-/Met+) had a strongly statistically improved outcome compared with those who were p53, VEGF and metallothionein negative (p53-/VEGF-/Met-) and all other combinations ($p<0.001$) (**Figure 27**). Mean survival for the p53-/VEGF-/Met+ was 88 +/-13 months (95%CI 62-113 months) compared with 41 +/- 9 months (95% CI 23-59 months) in the p53-/VEGF-

/Met- group. This compared with a mean survival of 20 months and 30 months for all other combinations and overall respectively.

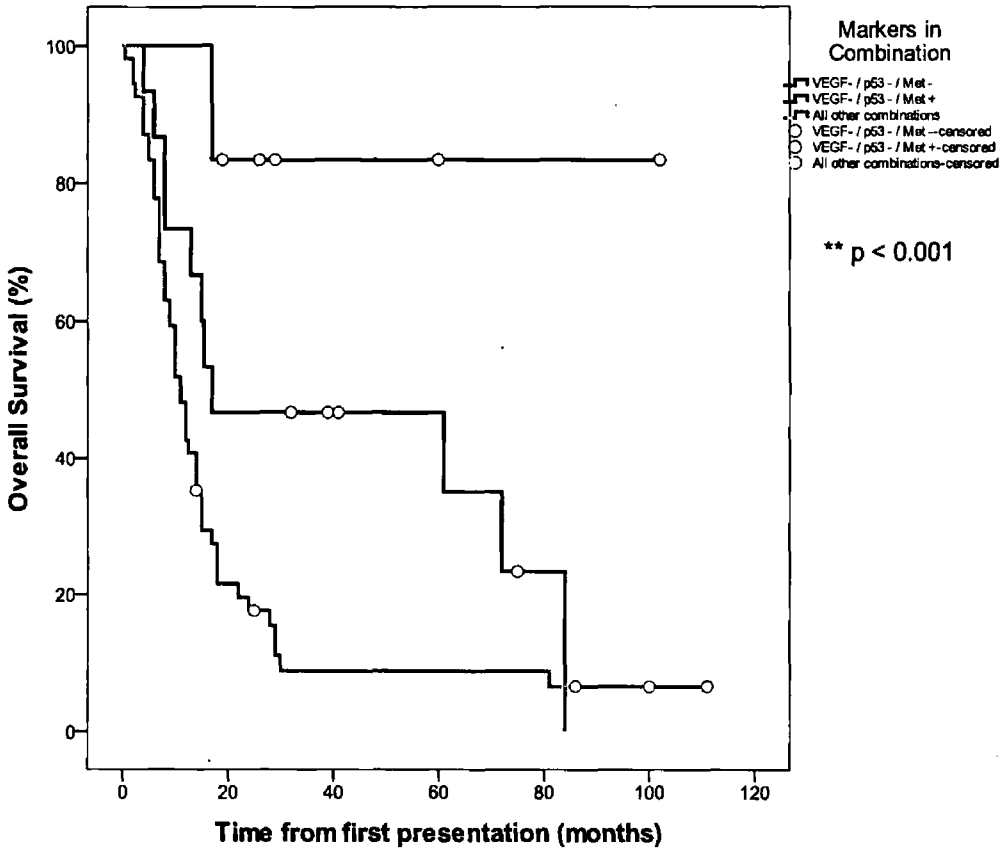


Figure 27: Kaplan-Meier Survival Analysis Comparing Survival Time For VEGF, p53 And Metallothionein In Combination Pre-Chemoradiotherapy.

7.6 DISCUSSION

Neoadjuvant chemoradiotherapy followed by surgery currently provides the best hope for cure for oesophageal cancer. A recent meta-analysis identified an 8.7% survival advantage across all randomised trials[222]. Up to 40% of patients with locally advanced disease have a complete pathological response to the most effective regimens[182, 213, 215, 221] while 87.5% of patients with early tumours[191] may have a complete response but not all regimens are equally effective and not all tumours are equally responsive. Furthermore, non-responders cannot benefit from chemoradiotherapy but continue to endure the side effects of treatment, and the disease may progress on treatment representing a lost opportunity for cure.

With the current prevalent use of neoadjuvant therapy there is an urgent need, therefore, for a marker or markers of response to treatment to guide patient management. These could offer patients both prognostic information and predict response to treatment. Patients deemed to have tumours responsive to chemoradiotherapy could be identified prospectively and be considered for neoadjuvant treatment with or without surgical intervention. Those patients predicted to respond poorly could be spared the potential morbidity, inconvenience, time and financial burden of undergoing such treatment, and may opt for alternative treatment regimes or palliative measures alone.

Because of their known role in apoptosis and thus treatment resistance, the clinical significance of p53, VEGF and metallothionein expression in pre- and post-treatment

biopsy and resection specimens was examined in patients with both oesophageal adenocarcinoma and squamous cell carcinoma. These markers have been analysed to a varying degree in the literature with wide-ranging results, but there are many limiting factors when trying to compare results such as the use of different antibodies, variation in immunohistochemical, patient and tumour characteristics, variable use of neoadjuvant therapy, variation in operative techniques and post-operative care, lack of accurate tumour staging, and the variation in study design and analytical methods. Our aim was to investigate in a single study if these markers may be utilised, alone or combined, as predictive markers for response to neoadjuvant chemoradiotherapy and outcome in oesophageal cancer.

More than 50 percent of all human tumours[291] contain a mutation or deletion of the p53 gene. Due to its many anti-cancer mechanisms such as DNA repair, genetic stabilisation, inhibition of angiogenesis and initiation of apoptosis[289], p53 is often referred to as the "guardian of the genome"[290]. A p53 abnormality is observed in over 70 percent of oesophageal cancers[272] but studies evaluating the prognostic significance of p53 expression have focused primarily in oesophageal squamous cell carcinoma[271-279] and to a lesser extent adenocarcinoma[280-283] and even fewer included both tumour types[284, 285]. The results from these studies have been conflicting[271-286]. Some have found p53 to be a good prognostic indicator for tumour invasiveness and propensity to metastasise or recur[274, 275, 286], survival[271, 273-275, 277, 278, 282, 285, 286] and response to therapy[277, 280, 283] and some have found it not to be useful[272, 276, 280, 284] unless associated with other markers[279].

Consistent with the majority of these results, our study showed that p53 negativity conferred significantly improved survival while p53 positive patients tended towards later disease stages following chemoradiotherapy. Ninety-one percent of p53 positive tumours had stage 2 or 3 disease and 64% had lymph node positivity compared with 78% and 44% respectively in the p53 negative group, but these did not achieve statistical significance. Results of post-chemoradiotherapy p53 expression status also failed to reach statistical significance, but it appeared that while there was no difference in early survival, p53 negativity was associated with a long-term survival advantage.

Squamous cell carcinomas had a higher percentage of p53 negativity compared with adenocarcinomas (43% and 29%). This may be one explanation for the fact that squamous cell carcinomas tend to be more sensitive to chemoradiotherapy and have higher complete pathological response rates.

Metallothionein is a low molecular weight, cysteine-rich protein, which has a high affinity for metal ions[298]. It is involved in many pathophysiological processes, including metal ion homoeostasis, protection against oxidative damage and cell proliferation and apoptosis[299, 300]. Its ability to inhibit apoptosis[311] and its free radical scavenging property[299] protect the cells from radiation and chemotherapeutic agents. Over-expression of metallothionein correlates significantly with a poorer prognosis in several tumour types[303-307] and metastatic tumour activity and proliferative potential[308] in oesophageal squamous cell carcinoma.

Expression of metallothionein in tumours from patients with oesophageal cancer treated with neoadjuvant chemoradiotherapy has been associated with a worse prognosis[309], but results from other studies have shown no such association[309, 310]. We found that tumours with positive expression for metallothionein pre-treatment tended towards an improved overall survival with a mean survival almost double that of negative ones, but this failed to reach statistical significance.

Metallothionein over-expression has been implicated in resistance to cisplatin in many tumour types[312-315], including oesophageal squamous cell carcinoma[309, 316, 317] and cisplatin forms the cornerstone of the most successful oesophageal cancer treatment regimens[182, 215]. While post-treatment metallothionein over-expression directly correlated with moderate and poor differentiation of tumours in our study, it did not correlate with response to treatment or outcome. While consistent with these findings reported in the literature, our results may be confounded by small numbers, combining of tumour types and the fact that almost 30% of samples evaluated did not contain tumour and therefore could not be evaluated.

Angiogenesis plays a key role in growth and metastasis of solid tumours[415, 416]. VEGF is one of the most powerful and specific inducers of neovascularisation in malignant neoplasms and plays a vital role in inhibiting tumour cell apoptosis[320]. VEGF expression has been shown to correlate with poor prognosis in many cancers[323-327] but the data in oesophageal cancer mainly refers to squamous cell carcinoma, where there is a positive correlation between VEGF expression, depth of tumour invasion and locoregional and distant metastasis[328-331, 333]. Low levels of VEGF are associated with a better long-term survival[334]. These findings, however, were not supported by our study.

Tumour microcirculation and vessel permeability are important factors in tissue oxygenation, drug delivery and radio-sensitisation of malignant cells[335]. Thus VEGF expression in oesophageal cancer has been explored as a means of predicting response to chemoradiotherapy with significantly higher levels of VEGF in pre-treatment biopsies in non-responders than in those who respond to chemoradiotherapy[336, 337]. Similarly, weak VEGF immunoreactivity in pre-treatment biopsies is associated with a higher incidence of complete tumour regression and improved long-term survival after neoadjuvant chemoradiotherapy[334]. In our study, those negative for VEGF expression post-treatment had almost double mean survival time compared to those with postitive expression, but this failed to reach significance, likely confounded by 44% of samples not demonstrating tumour.

Detection rates of p53[272, 273, 280, 284, 286], metallothionein[309, 316] and VEGF[334] postitivity in this study were similar to previous reports although VEGF positivity rates vary in the literature and our level was lower than many[330, 331, 333, 337]. This highlights the need for standardisation of techniques to allow accurate comparison across the literature. In our series, 16% pre- and 31% post-treatment samples did not identify tumour although tumour was detected on formal histo-pathological assesment pre-and post-treatment. Higher detection rates would more likely have occurred had we evaluated multiple tissue sections and thus identified tumour in more sections. These findings however underline and support our policy of taking at least 10 biopsies for histological analysis, especially post-neoadjuvant treatment where residual tumour may not be macroscopically visable at endoscopic assessment, in order to reliably assess for presence or absence of tumour.

On individual analysis, our results indicated that p53 but not metallothionein or VEGF expression pre-treatment was an independent predictor of survival. While there was a failure to find a significantly shorter disease free survival in p53, VEGF and metallothionein positive tumours post-treatment, it is possible that this may actually reflect the ability of neoadjuvant chemoradiotherapy to improve outcome. When the markers were combined, however, their predictive capacity was improved significantly with those patients with pre-treatment tumours negative for p53 and VEGF and positive for metallothionein expression having the best outcome.

7.7 CONCLUSION

The multitude of variables and heterogeneity of studies in the literature makes accurate comparison difficult. While results from our study and others show potential for clinical application of molecular markers such as p53, metallothionein, VEGF and others, there is a need to confirm these observations in a prospective study with standardised techniques in well-defined patient cohorts and should be the subject of future research. It is likely that a combination of markers will yield the most promising results.

CHAPTER VIII: GENERAL DISCUSSION AND CONCLUSIONS

Oesophageal cancer is the eighth most common cancer and sixth most common cause of death from cancer worldwide[1] but the overall prognosis remains poor, having improved minimally since the 1970s[3, 4, 5]. One of the primary reasons for the poor prognosis is the advanced stage of disease at diagnosis in most patients[80] with one third of patients having metastatic disease at presentation[78]. Explanations for this include the aggressive biological nature of this disease, resulting in rapid dissemination. Although screening programmes for patients with Barrett's oesophagus exist to detect early disease, they are costly and have yet to show any effect on survival[50, 63-67] especially since the actual progression rates to cancer are low[49-51]. In fact, most patients with Barrett's die due to causes other than oesophageal adenocarcinoma[50]. Another, and more modifiable reason, is the lack of awareness, especially among the public, of the symptoms of oesophageal cancer[81-83]. Efforts spent on Barrett's surveillance may be more appropriately placed in lifestyle modification and health education programmes and awareness campaigns to prevent disease or facilitate early recognition of symptoms by doctors and patients.

Although squamous cell carcinoma is the most prevalent histological type worldwide, adenocarcinoma is becoming the dominant histology in developed countries[7-10, 17, 18] and the patient demographics are changing along with this trend. The patients are now older[4, 13] and more obese[68-70] with signigicant co-morbidity attached to both cohorts. Thus the management of this disease is becoming evermore challenging.

Despite the dismal statistics, there is hope. We have shown that improved long-term survival rates can be achieved when chemoradiotherapy is administered and that those with a complete pathological response, or those with significant downstaging have the best outcomes (**Chapters 3 & 4**).

The chief finding of **Chapter 3** is that the short-term survival advantage for neoadjuvant chemoradiotherapy for adenocarcinoma in the previously published randomised trial[182] is sustained up to 15 years. This a valuable addition to the randomised trials reported to date, where the median follow-up only ranged from 2 to 8 years[123, 143, 182, 211, 213, 215, 221, 223, 224, 231, 348]. The findings of our long-term follow-up are reassuring and suggest that the survival benefit is due to the elimination of micrometastases rather than merely inducing dormancy[352] or allowing resistant clones to re-emerge, and for patients to succumb to recurrent disease[353].

Adenocarcinoma and squamous cell carcinoma are two different diseases in terms of their aetiology, patient population, distribution in the oesophagus and incidence of lymph node metastases. We therefore could not assume that squamous cell carcinoma would have a similar response as adenocarcinoma to the 40Gy radiotherapy and 5-FU and cisplatin protocol, or that response would be sustained. The results from **Chapter 3** has further demonstrate that the survival benefit achieved in adenocarcinoma, extends to squamous cell carcinoma, and long-term, as did the benefits of associated disease downstaging.

For now, surgery still plays a key role as complete response rates are significantly less than the ideal 100% and complete responders are not readily identifiable, but it still carries a significant mortality[122]. Indeed in our series, there was a significant post-operative mortality, especially in squamous cell carcinoma patients, which was criticised in the literature. The high post-operative mortality rate was largely due to the age and co-morbidity of the patient cohort, as squamous cell carcinoma is associated with greater cardiorespiratory risks, with both diseases being closely associated with smoking and alcohol. The low long-term survival for the surgery alone cohorts was likely to be largely related to the lack of sophistication of available pre-operative staging modalities and the wide age-range and recruitment criteria set for the trial. We believe, however, that this was ultimately to the benefit of the trial, as it demonstrated the benefits of neoadjuvant therapy in a cohort more reflective of the true disease population. To "cherry-pick" only those with the earliest of disease for inclusion into randomised trial, as so frequently occurs in the literature, does not do justice to the majority who present with advanced loco-regional disease or metastatic cancer and can have little relevance for the disease-community as a whole.

By giving neoadjuvant chemoradiotherapy in advance of surgery, a unique opportunity is presented to downstage disease, provide greater R0 resection rates, and perhaps most importantly, eradicate the systemic manifestation of the disease. In addition, these regimens can be so effective as to totally eliminate the disease calling into question the benefit of surgery in this cohort. In the two randomised trials, 25% of adenocarcinomas and 30% of squamous cell carcinomas had a complete pathological response to neoadjuvant treatment, but 12% died following an (unnecessary) resection and the rest remained exposed to the morbidity of the resection and a life-long

negative quality of life impact of surgery. In the era of ever-increasing complete pathological response rates, it is exceedingly difficult to justify the role of surgery especially in the earliest disease stages, and this issue is becoming evermore concerning.

The major limitation of neoadjuvant therapy however, is that we cannot currently reliably predict who will respond to neoadjuvant treatment and to identify those who cannot benefit from surgery, either because of the absence of viable disease or due to the presence of micrometastatic disease.

Following neoadjuvant therapy, we found that the simple approach of endoscopy with multiple tumour bed biopsies, with careful histological analysis and computerised tomography identified with 74% accuracy a cohort of patients who had a complete pathological response. Up to 31% of samples in our series analysing molecular markers did not identify tumour, although tumour was detected on formal histo-pathological assesment. These findings underscore and give strength to our policy of taking and performing histological analysis of at least 10 biopsies, especially post-neoadjuvant treatment where residual tumour may be elusive, in order to reliably assess histoligically for the presence or absence of tumour, a policy which appears not to be adopted in other institutions[103, 104].

In **Chapter 5**, we further showed that the addition of micrometastatic disease status improved accuracy of luminal response as a prognostic indicator. We highlighted the fundamental importance that assessment of response should not focus solely on loco-

regional disease response but rather to focus on the systemic burden as this ultimately decides the outcome. The locoregional response may, however, prove to be important as a surrogate marker for systemic response. While the results of this study are promising, more reliable and standardised methods need to be developed before micrometastatic status can incorporated in routine clinical staging.

A major challenge remains to develop minimally invasive, affordable and reliable techniques to identify, in advance of treatment, those who are most likely to respond; and following treatment those who cannor benefit from surgery. In **Chapter 7** we examined p53, metallothionein and VEGF as candidates for such markers. These markers have been analysed to a varying degree in the literature with wide-ranging and conflicting results[271-286, 308-310, 316, 317, 334, 336, 337] and on analysis of these studies, many limitations become apparent, such as the use of different antibodies, variation in patient, tumour and immunohistochemical characteristics, variable use of differing neoadjuvant therapies, variation in surgical techniques and post-operative care, lack of accurate tumour staging, and the variation in study design and analytical methods. In our series, while p53 positive tumours tended towards later disease stages and higher rates of lymph node positivity post-treatment, metallothionein and VEGF did not show such association and could not predict response to treatment. While there was a failure to find a significantly shorter disease free survival in p53, VEGF and metallothionein positive tumours post-treatment, it is possible that this may actually reflect the ability of neoadjuvant chemoradiotherapy to improve outcome.

While results from our study and others show potential for clinical application of molecular markers such as p53, metallothionein, VEGF and micrometastatic status,

there is a significant need to confirm these observations in a prospective study with standardised techniques in well-defined patient cohorts and should be the subject of future research. Only then may these techniques be considered to be included in pathological staging.

More accurate indicators of complete response are needed but the issue of what to do with complete clinical responders, especially those who are older or less fit for resection, remains controversial. The management of this cohort is further complicated by the underrepresentation of older patients in most randomised trials. All the elderly patients in this study were involved in the decision making about their own care. Many chose not to have surgery, while others were unfit for a radical procedure. In the past, the majority of these patients would have been palliated, but we believe they could be offered more. In **Chapter 6**, we found that almost half of all older patients completing treatment had a complete clinical response and that two-thirds of these who underwent resection had a complete pathological response. Furthermore, those managed non-operatively had comparable survival to those managed with additional radical surgery. The 3-year survival of 50% in this cohort compares with the best results of more selective series of younger patients. With these results, the approach of active observation of complete responders with the added option of salvage surgery if disease re-emerges is an attractive option to patients and clinicians alike. Larger-scale randomised trials inclusive of older patients comparing radical chemoradiotherapy and surgery alone in both adenocarcinoma and squamous cell carcinoma are necessary but are likely to face difficulties in recruiting both patients and treating clinicians.

The focus of the scientific, pharmacological and medical communities needs to adapt accordingly and be directed towards providing a cure that benefits the whole spectrum of oesophageal cancer sufferers, not just the youngest and fittest. We remain unclear as to the role that surgery will play in the future of oesophageal cancer care, but are sure that advances in neoadjuvant chemoradiotherapy will provide the best hope of cure and a positive outlook for those who are diagnosed with this formidable disease.

CHAPTER IX: DIRECTION FOR FURTHER STUDIES

Current neoadjuvant chemoradiotherapy provides a complete pathological response rate of 25-40% and has been proven to improve outcome (**Chapter 3**). While it may reduce the micrometastatic disease burden, it does not completely eradicate it providing a nidus for disease recurrence and resulting in the present high relapse rate.

Better outcomes for oesophageal cancer can be anticipated if/when new regimens of chemoradiotherapy are introduced which reliably and consistently provide high rates of complete systemic and local response response with the minimum of side effects. Toxicity must be low so they can be offered to all patients at all stages of disease reducing or obviating the necessity for formidable surgery.

Many patients still succumb to oesophageal cancer, even following a complete pathological response. The relapse pattern of oesophgeal cancer following treatment with neoadjuvant chemoradiotherapy may be explained by a number of mechanisms and thus identifies a number of potential therapeutic targets. One of the most obvious is that when given concurrently, chemotherapy enhances the effect of radiotherapy locally, including lymph node disease but systemic disease is not exposed to this synergistic effect. Another explanation may be that because the majority of micrometastatic tumour cells may be non-proliferating[381], and thus display similar characteristics to cancer stem cells[382, 383] the standard cytotoxic chemotherapies aimed at proliferating cells may be less effective allowing disease to recur.

Recently, there has been much research into the concept of the cancer stem cell since the first conclusive evidence for these cells was published in the late 1990s[417] and they have now been identified in many types of tumour[417-422]. These cells possess the same characteristics associated with normal stem cells, generating and proliferating tumours through stem cell processes of self-renewal and differentiating into multiple cell types. Mounting evidence suggests that cancer stem cells are responsible for tumour resistance and re-growth, establishment of metastases and resistance to a variety of treatments[384-388]. Several recent reports have suggested that as many as one quarter of the cancer cells within certain tumours have the properties of cancer stem cells[423, 424] but conventional chemotherapy regimens kill differentiated or differentiating cells, which form the bulk of the tumour. Thus by this mechanism, cancer stem cells could remain unaffected and result in a relapse of the disease.

The persistence of viable disseminated or micrometastatic tumour cells, some or all of which may indeed be cancer stem cells, following neoadjuvant chemoradiotherapy, including in those with an apparent complete pathological response, highlights the need for improved or additional systemic therapies. Future regimens may identify targets, such as cell surface markers, on these cells to eliminate them selectively as part of neoadjuvant treatment or allow "mopping up" of these cells with targeted adjuvant therapy, such as antibody-based therapies. Another strategy may be to find other means to sensitise systemically-circulating cells to chemotherapeutic agents to allowing a similar synergistic effect to that which radiotherapy provides loco-regionally.

Oesophageal cancer offer clears opportunities for clinical research of targeted therapies with the increasing understanding of both its clinical and biological behaviour

and response to chemoradiotherapy. It is probable that optimal therapeutic regimens will need to incorporate agents that target both cancer stem cells and non-cancer stem cells, both in the tumour and micrometastases in circulation if truly curative therapies are ever to be achieved. Elimination of these cells may achieve the ultimate goal of cancer treatment - a true complete pathological and systemic response.

To allow for the greatest impact on this disease, all patients, including older patients with both early and loco-regionally advanced oesophageal cancer should be considered for inclusion in clinical trials of targeted and tailored therapies in the search for more effective treatments, which may allow for increased rates of complete pathological response and improved outcomes. When we have developed means with which to reliably identify patients with a complete pathological response, we may eliminate the need for formidable surgery and all of its risks.

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