EFFECT OF SUPPLEMENTARY OMEGA-3 FATTY ACIDS ON THE BIOCHEMICAL, RADIOLOGICAL AND CLINICAL OUTCOME OF PATIENTS WITH METASTATIC OESOPHAGO-GASTRIC CANCER RECEIVING PALLIATIVE CHEMOTHERAPY

A Thesis Submitted for the Degree of

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by

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Abstract

Background: Omega-3 polyunsaturated fatty acids (PUFAs) have reported anti-cancer effects. Few studies have assessed oesophago-gastric cancer, either in vitro or in vivo.

Aims: To evaluate whether addition of omega-3 PUFAs (Omegaven) to palliative chemotherapy would influence the clinical and biochemical outcome of patients with oesophago-gastric cancer, and to assess the response of oesophageal adenocarcinoma cell lines to omega-3 PUFAs compared to oxaliplatin.

Methods: Participants in a phase II trial received palliative chemotherapy and Omegaven infusion. Clinical and radiological outcome were assessed. Comparison was made to historical patients who received chemotherapy alone. Serum cytokine concentrations and uptake of PUFAs in plasma and red cell membrane were assessed in trial patients. The in vitro response of cell lines to omega-3 PUFAs or oxaliplatin were evaluated.

Results: Participants who received chemotherapy and Omegaven had a higher radiological response rate than those who received chemotherapy alone ((overall response: 73% (95% CI 51 to 95) vs 43% (95% CI 25 to 61), p=0.05; partial response 73% (95% CI 51 to 95) vs 39% (95% CI 21 to 57), p=0.03)). . Grade 3/4 toxicity was observed less frequently in those who received Omegaven (thromboembolism, gastrointestinal side-effects). This translated into fewer hospital admissions. There were significant reductions in the concentrations of TNF-α ($p<0.0001$, 95% CI -0.0121 to -0.0046) & VEGF ($p=0.002$, 95% CI -0.0161 to -0.0034) following each treatment. Participants with low baseline IL-6 & TNF-α expression had a superior survival. Each infusion of Omegaven resulted in a short lived increase in plasma EPA and DHA. The serial infusions of Omegaven caused a sustained increase in the EPA content of the red cell membrane.
DHA, EPA and oxaliplatin had an anti-proliferative effect on the oesophageal cancer cell lines at all concentrations. Omegaven had a more concentration dependent anti-proliferative effect. Reduction in VEGF expression was the most consistently observed cytokine effect. The anti-proliferative effect was associated with reduction in anti-apoptotic protein and an increase in pro-apoptotic protein.

Conclusions: Infusion of omega-3 PUFAs resulted in a more favourable chemotherapy side-effect profile and an improved radiological response rate. Treatment resulted in a reduction in serum pro-inflammatory cytokines. Repeated Omegaven infusion resulted in a gradual and sustained uptake of EPA in the red cell membrane. The anti-proliferative effect of omega-3 PUFAs on oesophageal cancer was demonstrated in vitro.

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Dedication

This work is dedicated to the twenty one patients who participated in the clinical trial.

I dedicate this work to my lovely wife; Amal Bengreed for being there for me throughout my research. Without her love and support, this work would not have been made possible. I also dedicate this work to my lovely Daughter (Afnan) and Son (Abdulhadi).

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Publications and presentations derived from this work

Publications

Eltweri AM, Howells LM, Thomas AL, Dennison AR, Bowrey DJ. *Effects of Omegaven®, EPA, DHA and Oxaliplatin on oesophageal adenocarcinoma cell lines growth, cytokine and cell signal biomarkers expression.* Nutrition and Cancer: An International Journal **(*Under Review*).**

Eltweri AM, Thomas AL, Chung WY, Morgan B, Thompson J, Dennison AR, Bowrey DJ. *The effect of supplementary omega-3 fatty acids on the clinical outcome of patients with advanced oesophago-gastric adenocarcinoma receiving palliative epirubicin, oxaliplatin and capecitabine chemotherapy.* Disease of the Esophagus (***In press***)

Eltweri AM, Thomas AL, Dennison AR, Bowrey DJ. *A systematic review of the randomised phase III clinical trials employing palliative chemotherapy in the management of advanced oesophago-gastric adenocarcinoma.* Cancer research frontier 2016 May; 2(2): 184-199. doi: 10.17980/2016.184

Eltweri AM, Thomas AL, Fisk HL, Calder PC, Dennison AR, Bowrey DJ. *Erythrocyte and plasma uptake of omega-3 fatty acids from an intravenous fish oil based lipid emulsion in patients with advanced oesophago-gastric cancer.* Clin Nutr. 2016 Jun 7. pii: S0261-5614(16)30131-5. doi: 10.1016/j.clnu.2016.06.001

Eltweri AM, Thomas AL, Metcalfe M, Calder PC, Dennison AR, Bowrey DJ. *Potential applications of fish oils rich in omega-3 polyunsaturated fatty acids in management of gastrointestinal cancer.* Clin Nutr. 2016 Jan 15. pii: S0261-5614(16)00009-1. doi: 10.1016/j.clnu.2016.01.007

National and international presentations

Eltweri AM, Howells LM, Thomas AL, Dennison AR, Bowrey DJ. *Effects of EPA, DHA and Oxaliplatin on pro-inflammatory cytokine expression in two oeasophageal adenocarcinoma cell lines.* Poster presentation to ESPEN conference 2016 Copenhagen. Clinical Nutrition, Vol. 35, S70.

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Eltweri AM, Howells LM, Thomas AL, Dennison AR, Bowrey DJ. *Treatment of oesophageal cell lines with docosahexaenoic fatty acid (DHA) and oxaliplatin: Effects on proliferation, and expression of vascular endothelial growth factor.* Poster presentation to ESPEN 2016 Copenhagen. Clinical Nutrition, Vol. 35, S70.

AM Eltweri, AL Thomas, HL Fisk, A Arshad, PC Calder, M Metcalfe, AR Dennison, DJ Bowrey. *Erythrocyte and plasma uptake of omega-3 fatty acids from an intravenous fish oil based lipid emulsion in patients with advanced oesophago-gastric cancer.* Oral presentation to the Society of Academic and Research Surgeons 2016. Abstract published in BJS April 2016; 103 (S3): 6-49.

Eltweri AM, Thomas A, Dennison A, Metcalfe M, Bowrey D. *Omega-3 fatty acid infusion reduces gastrointestinal toxicity and prevents thromboembolism in patients with advanced esophagogastric cancer treated with palliative platinum based chemotherapy.* Poster presentation to the American Gastroenterological Association, Washington, US, May 2015. Abstract published in Gastroenterology 2015; 148(4):S-561

Eltweri AM, Thomas AL, Metcalfe M, Dennison AR, Bowrey DJ. *Serum triglyceride levels, safety and tolerability of maximum dose rate infusion of omega-3 rich lipid emulsion in patients with advanced oesophago-gastric cancer.* Poster presentation to the Society of Academic and Research Surgeons 2015. Abstract published in BJS 2015;102 (suppl 5):29-30.

AM Eltweri, A.L. Thomas, M. Metcalfe, A.R. Dennison, D.J. Bowrey. *Omega-3 fatty acid infusion reduces gastrointestinal toxicity and prevents thromboembolism in patients with advanced esophagogastric cancer treated with palliative platinum based chemotherapy.* Poster presentation to the British Society of Gastroenterology /

Digestive Disease Federation, London, June 2015. Abstract published in Gut 06/2015; 64 (Suppl 1):A286-A287

AM Eltweri, L.M. Howells, A.L. Thomas, A.R. Dennison, D.J. Bowrey. *Treatment of oesophageal cell lines with docosahexanoic fatty acid (DHA) and oxaliplatin: effects on proliferation, expression of vascular endothelial growth factor and IL-6.* Poster presentation to the British Society of Gastroenterology / Digestive Disease Federation, London, June 2015. Abstract published in Gut 06/2015; 64 (Suppl 1):A286-A287

Eltweri AM, Thomas AL, Al-leswas D, Isherwood J, Metcalfe M, Dennison AR, Bowrey DJ. *Serum triglyceride levels, safety and tolerability of maximum dose rate infusion of omega-3 rich lipid emulsion in patients with advanced oesophago-gastric cancer.* Poster presentation to the European Society of Parenteral and Enteral Nutrition. Abstract published in Clinical Nutrition, vol. 32, S147-S148, September 2013.

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AA	Arachidonic acid
AJCC	American joint committee on cancer
AKI	Acute kidney injury
BNF	British national formulary
BO	Barrets oesophagus
BSC	Best supportive care
Chemo	Chemotherapy
CO	Corn oil
COX-2	Cyclooxygenase 2
CTCAE	Common terminology criteria for adverse events
CT scan	Computed tomography scan
DGCA	Diffuse gastric cancer
DHA	Docosahexaenoic acid
DMH	Dimethylhydrazine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPD	Dihydropyrimidine dehydrogenase
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme linked immunosorbent assays
EN	Enteral nutrition
EORTC	European organisation of research and treatment of cancer
EPA	Eicosapentaenoic acid
EPDs	Epoxydocosapentaenoic Acid
ERK	Extracellular signal-regulated kinase
EQ-5D	Quality of life questionnaire
EUS	Endoscopic ultrasound scan
FAs	Fatty acids
FAP	Familial adenomatous polyposis
FAME	Fatty acid methyl ester
FBS	Foetal bovine serum
FCS	Foetal calve serum
FDA	Food and Drug Administration
FID	Flame ionisation detector
FO	Fish oil
FS	Functioning score
5FU	5-Fluorouracil
G	Gastric
GC	Gas chromatography
GCA	Gastric cancer
GI	Gastrointestinal
GIT	Gastrointestinal tract
HER-2	Human epidermal growth factor receptor 2

HNPCC	Hereditary non polyposis colon cancer
HPETE	5-Hydroperoxyeicosatetraenoic
ICU	Intensive care unit
ID	Identification number
Ig	Immunoglobulin
IGCA	Intestinal gastric cancer
IL	Interleukin
IV	Intravenous
K_2CO_3	Potassium carbonate
$KHCO_3$	Potassium bicarbonate
LN	Lymph node
LTB	Leukotriene-B
MDT	Multidisciplinary team meeting
MDSC	Myeloid derived suppressor cells
mg	Milligram
μM	Micro molar
M&M	Morbidity & mortality
MMP-2	Matrix metalloproteinase-2
MRI	Magnetic resonance scan
N&V	Nausea and vomiting
NEFA	Non-esterified fatty acids
NHS	National Health Service
NRES	National research ethical service
O	Oesophageal
OAC	Oesophageal adenocarcinoma
OE33	Oesophageal cell line 33
OE19	Oesophageal cell line 19
ONS	Oral nutritional supplement
OS	Overall survival
OSCC	Oesophageal squamous cell carcinoma
P	Pancreatic
PBS	Phosphate buffer saline
PC	Phosphatidyle choline
PD	Progressive disease
PFS	Progression free survival
PGE2	prostaglandin E2
PN	Parenteral nutrition
Postop	Postoperative
Preop	Preoperative
PS	Performance status
PUFAs	Polyunsaturated fatty acids
PPE	Palmar plantar erythema
PR	Partial response
QLQ C-30	Quality of life questionnaire
QoL	Quality of life
RBCs	Red blood cells

RCTs	Randomised clinical trials
RECIST	Response evaluation criteria in solid tumours
RPMI	Roswell park memorial institute (media)
SD	Stable disease
SGC-7091	Gastric cancer cell lines
SIRS	Systemic inflammatory response syndrome
SPE	Solid phase extraction
SPSS	statistical package for the social sciences
STAT3	Signal transducer activator of transcription
TP53	Tumour suppressor protein 53
TEE	Total energy expenditure
TNM	Tumour lymph node metastasis
TPN	Total parenteral nutrition
UICC	Union for International Cancer Control
UK	United Kingdom
US	United States
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor
Vs	Versus
VTE	Venous thromboembolism
WHO	World Health Organisation

Chapter One

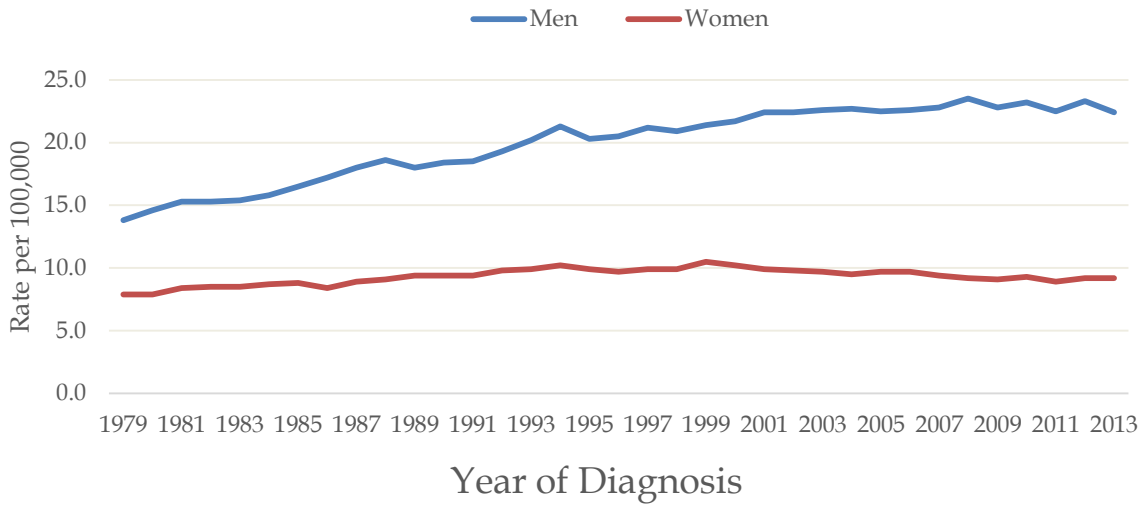
Introduction

1. Introduction

1.1 Epidemiology of Oesophageal and stomach cancers

Oesophageal and gastric cancers rank as the fourteenth and sixteenth most common cancers in the UK, with a combined reported 15,601 patients affected in 2014 [1,2]. For oesophageal cancer, the age standardised annual incidence rate is 22 per 100,000 population for men, and 9 per 100,000 populations for women in 2013 [1]. The incidence rates in Scotland (19 per 100,000) are significantly higher than in England (15 per 100,000), Wales (15 per 100,000) and Northern Ireland (15 per 100,000) in 2014 [1]. Since the 1970s there has been a sustained increase in the incidence of oesophageal adenocarcinoma in the UK, although there is evidence that this increase in incidence has now plateaued, and has indeed started to fall in women (Figure 1) [1]. The lifetime risk for the development of oesophageal cancer is around 1 in 55 for men, and 1 in 115 for women [1].

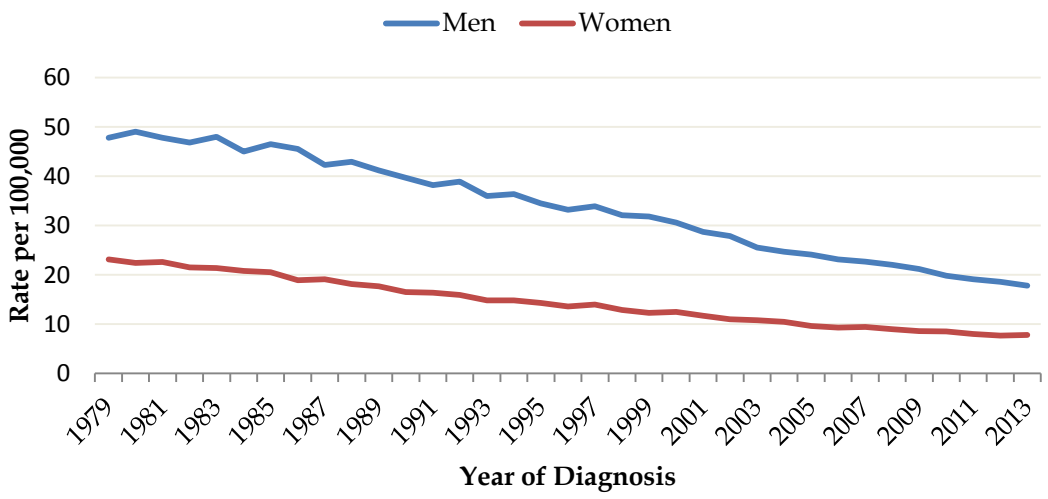
Figure 1: Oesophagus cancer incidence in UK



Cancer Research UK, http://www.cancerresearchuk.org/cancer-info/cancerstats/types/oesophagus/incidence, January 2017).

The overall age standardised annual incidence for gastric cancer is 12 per 100,000 populations [2]. As with oesophageal cancer, men are affected twice as frequently as women (men incidence: 18 per 100,000; women incidence: 8 per 100,000) [2]. Within the constituent components of the UK, the rates in England are significantly lower than the rates observed in Scotland (both sexes) and Wales (males only) [2]. Although, stomach cancer rates have been falling since the 1970s, most noticeably cancers affecting the distal stomach, cancers affecting the proximal stomach have increased in incidence, mirroring the changes in oesophageal cancer incidence (Figure 2) [2]. The lifetime risk for the development of gastric cancer is around 1 in 67 for men, and 1 in 135 for women [2].

Figure 2: Stomach cancer incidence rate in UK



Cancer Research UK, http://www.cancerresearchuk.org/cancer-info/cancerstats/types/stomach/incidence, January 2017).

Both oesophageal and gastric cancer increase in incidence with increasing age, this being most evident from age 60-64 years onwards [1,2]. The median age at diagnosis is around 70 years for oesophageal cancer and around 75 years for gastric cancer [1,2]. They are comparatively rare cancers in those aged below 50 years, with only 4% and 6% of oesophageal and gastric cancers respectively being diagnosed below that age [1,2].

There is marked geographic variation in the incidence of oesophageal and gastric cancer [3-5]. Within Europe, oesophageal cancer incidence is highest in the UK and the Netherlands, and lowest in the Balkan states. Gastric cancer incidence rates are highest in Eastern Europe and lowest in Scandinavia [3-5]. On a worldwide basis, oesophageal cancer incidence rates are highest in central Asia and lowest in Western Africa [3-5]. Gastric cancer incidence rates are highest in Russia and Ukraine, and lowest in Western Africa, India and Indonesia. There are marked social class differences, with social deprivation being linked to an increased cancer incidence [3-5].

In 2014, in UK there were 7790 deaths from oesophageal cancer and 4576 deaths from gastric cancer [1,2]. The crude one and five year survival rates for oesophageal cancer are 42% and 15% respectively [1,2]. The corresponding survival rates for gastric cancer are 42% and 19% respectively [1,2]. There is some evidence that the prognosis for younger patients with oesophageal cancer is better than that for older patients, although the reasons for this are unclear [1,2]. It

may relate to older patients being less likely to be offered radical therapy, due to the presence of concomitant comorbidities [1,2].

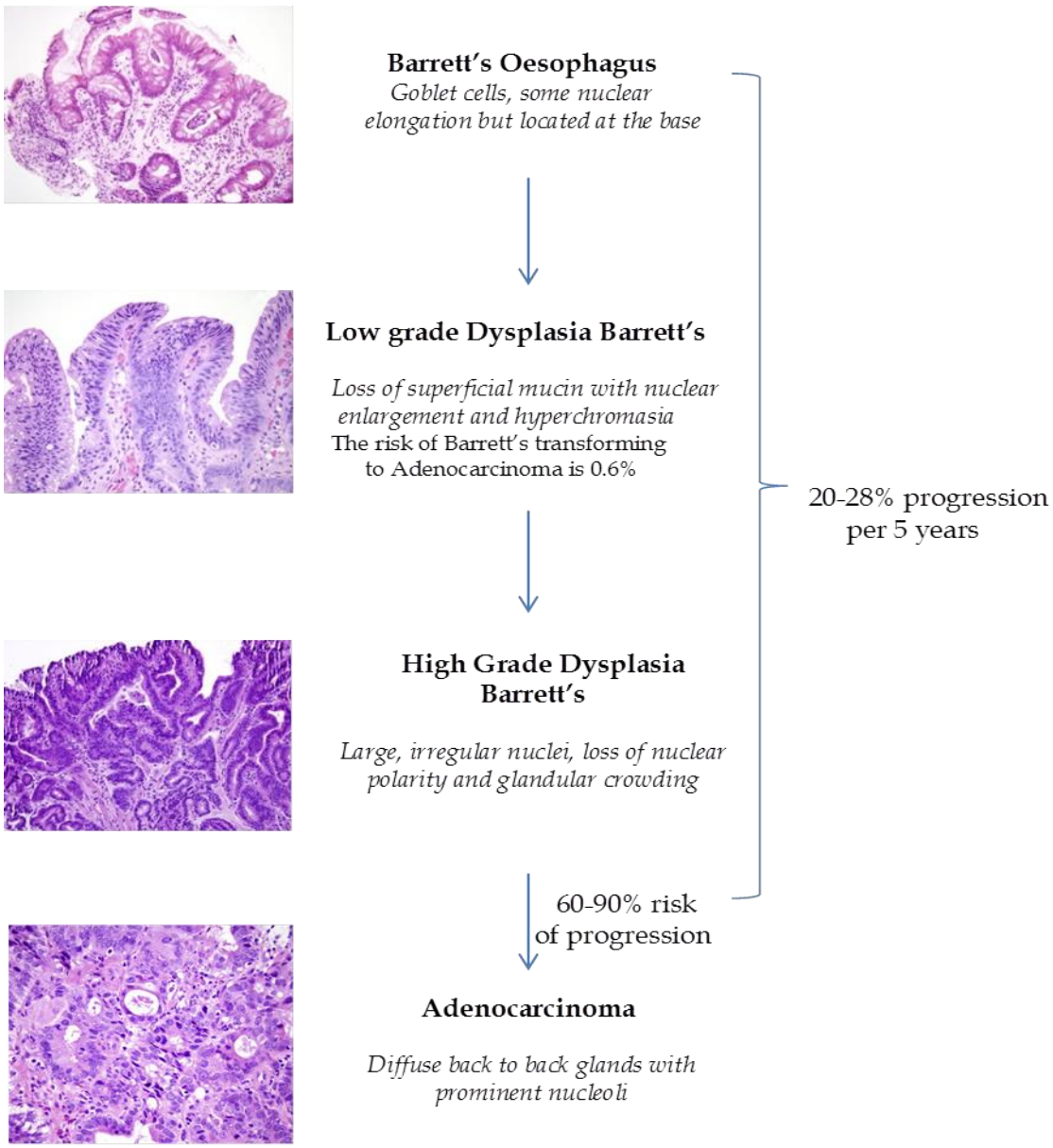
1.2 Oesophageal and gastric cancer histopathology

1.2.1 Oesophageal cancer histopathology

The two main histological subtypes of oesophageal cancer are squamous cell carcinoma and adenocarcinoma [6]. The former is considered to arise from the squamous mucosa of the oesophagus in response to similar luminal factors that result in squamous cancers at other sites, such as the oropharynx (tobacco, high consumption of alcohol) [6-8]. In contrast, adenocarcinoma is considered to arise as a result of metaplastic transformation of the squamous oesophageal mucosa to a glandular mucosa (Barrett's oesophagus). Barrett's oesophagus is considered to develop as a result of chronic gastro-oesophageal reflux disease and ensuing inflammation [6]. As with malignant transformation in the colon, Barrett's oesophagus is considered to progress through a sequence of dysplastic changes before the development of invasive cancer [6]. These cancer precursor steps, low and high grade dysplasia can be identified from endoscopic biopsies making endoscopic surveillance and management feasible (Figure 3) [6-8].

The oesophageal wall is characterised by the absence of a serosal layer. This may account for the late presentation of oesophageal cancers due to a lack of resistance to local invasion by the tumour, and because until the lumen is occluded to a significant extent, dysphagia may not be present.

Figure 3: Stages of transformation of Barrett's oesophagus to adenocarcinoma.



The images adapted and modified with permission from Voltaggio L et al [6].

1.2.2 Cancers arising around the gastro-oesophageal junction

As described in the previous Epidemiology section since the 1970s there has been a proximal shift in the location of gastric cancers, with a preponderance of cancers around the gastro-oesophageal junction [9]. To aid in the management of these cancers, the Siewert classification was introduced [10]. This defined tumours according to their epicentre in relation to the gastro-oesophageal junction. Siewert type I cancers were defined as those with their epicentre between 1 and 5 cm above the gastro-junction [10]. Siewert type II cancers were defined as those where the tumour epicentre was between 1 cm above and 2 cm below the gastroesophageal-junction. Finally, Siewert type III cancers were those where the tumour epicentre was between 2 and 5 cm below the gastro-oesophageal junction [10]. This classification was employed practically in order to assist in surgical decision making, in order to determine when a thoracic approach to resection would be required [10].

This classification, although still relevant, has been superseded by the 7th revision of the UICC staging system, with junctional cancers being dichotomised as either oesophageal or gastric [11,12].

1.2.3 Gastric cancer histopathology

The majority of gastric cancers are adenocarcinomas [13-15]. Several classification systems have been employed for gastric cancer [13-15]. The most recent classification was based on the molecular classification of gastric adenocarcinoma by the cancer genome Atlas Research Network, which divided

gastric cancer into 4 subtypes; Epstein-Barr virus positive (DNA hypermethylation), microsatellite unstable cancer (increased mutation rate), genomically stable cancer (diffuse gastric cancer and RHOA mutation) and cancer with chromosomal instability (amplification of tyrosine kinase receptors) [16]. In 1965, Lauren subdivided gastric cancer into intestinal and diffuse types, based on the histological growth pattern [17]. The World Health Organisation (WHO) categorized gastric adenocarcinoma into four subtypes; papillary, mucinous, tubular and signet ring cell adenocarcinoma [13-15]. Further classification systems include those of Ming (1977) which defined expanding and infiltrative patterns of growth [18], and that of Mulligan (1972), which recognised the morphological patterns of mucous, intestinal and pyloro-cardiac gland cell carcinoma [19].

Eighty percent of gastric cancers are considered to arise as a consequence of atrophic gastritis due to *Helicobacter pylori* infection [20,21]. Figure 4 shows the putative steps in the pathogenesis of gastric cancer and summarises the major gene and biomarker changes associated with its development [22]

Figure 4: Proposed sequences and steps in the pathogenesis both gastric cancer (GCA) subtypes; diffuse gastric cancer (DGCA) and intestinal gastric cancer (IGCA).

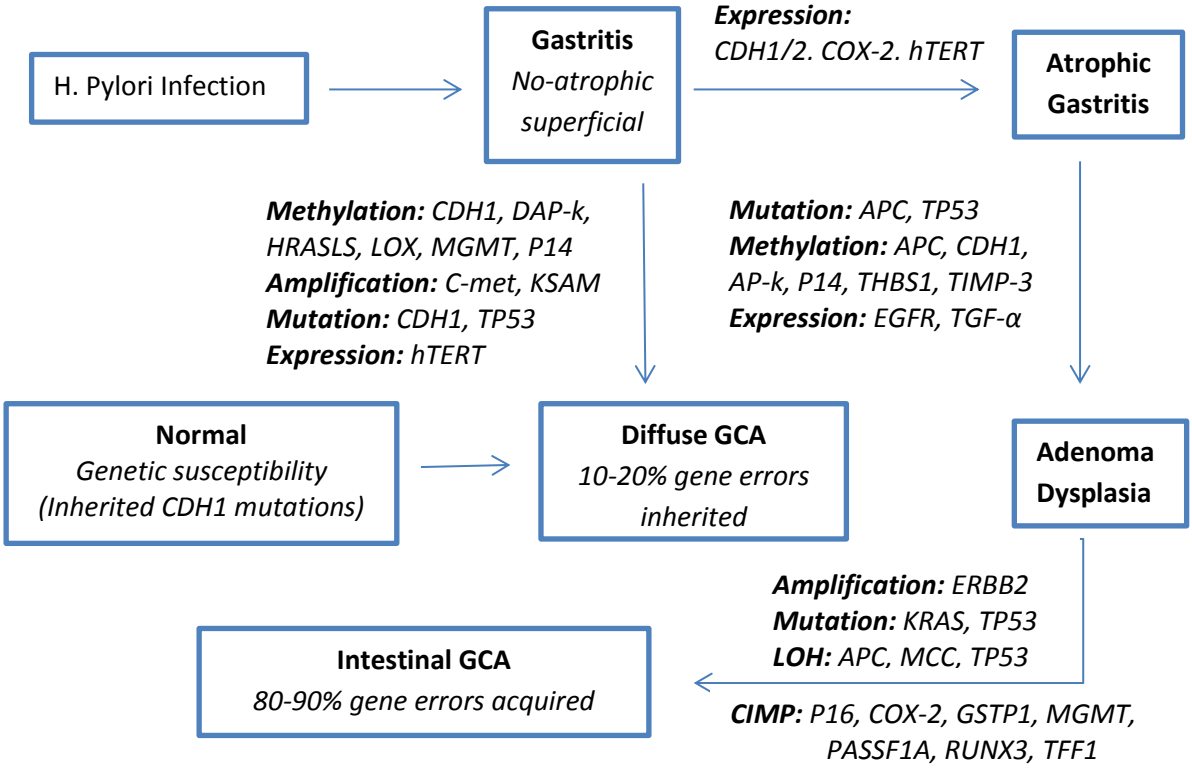


Figure modified from Vauhkonen M et al [22]

1.3 Clinical features of oesophageal and gastric cancer

The most common symptom of oesophageal or junctional cancer is dysphagia (difficulty swallowing) [23,24]. Other symptoms include odynophagia (pain at the site of the cancer on swallowing), regurgitation of undigested food and weight loss. Dysphagia occurs principally because of luminal narrowing by the cancer [23,24]. Other patients, more notably those with early disease may present with the symptoms of the underlying precursor condition for adenocarcinoma, gastro-oesophageal reflux disease or may have cancer detected as part of an endoscopic surveillance programme.

The majority of patients with gastric cancer present with non-specific symptoms such as; dyspepsia, belching, nausea, early satiety or symptoms due to occult gastrointestinal bleeding. In the advanced stages, the symptoms include haematemesis, vomiting, melaena, abdominal distension, ascites, jaundice or the presence of an abdominal mass.

The general manifestations of any malignancy such as loss of appetite, weight loss, fatigue and persistent nausea can present at any stage.

1.4 Risk factors

1.4.1 Oesophageal Squamous Cell Carcinoma (OSCC)

The principal risk factors for the development of oesophageal squamous cell carcinoma are similar to those for squamous carcinoma of the remainder of the aerodigestive tract, tobacco and cigarette smoking, and alcohol ingestion, in particular, high concentrations alcohol in the form of spirits [25-27]. Potential

mechanisms of action are through nitrosamine exposure from tobacco smoking and the alcohol metabolite, aldehyde, both of which are known carcinogens [25-27]. Other risk factors are low socioeconomic status, poor oral hygiene, achalasia and previous thoracic irradiation [24].

1.4.2 Oesophageal Adenocarcinoma (OAC)

While tobacco smoking and alcohol ingestion are implicated risk factors for the development of oesophageal adenocarcinoma, the strength of the association is not as strong as it is for oesophageal squamous cell carcinoma [28-31]. Other lifestyle and environmental risk factors reported as possibly linked to oesophageal and gastric cancer include low fruit and vegetable intake [32-34], the proposed mechanism being through the protective action of dietary antioxidants present in fruit and vegetables (e.g. vitamin E, vitamin C, selenium) [8,23,34]. As with oesophageal squamous carcinoma, prior mediastinal irradiation carries a slightly increased risk for the development of adenocarcinoma [7,35].

The most well characterised risk factors for adenocarcinoma of the oesophagus are gastro-oesophageal reflux disease and obesity [36,37]. The former likely contributes to the development of Barrett's oesophagus. For the latter, it is less clear. It may be that the two act synergistically [36,37]. Lindblad *et al.* estimated that a body mass index greater than 25 kg/m^2 was associated with a 70% increased risk for oesophageal adenocarcinoma compared to those with body mass indices in the normal range (18-25 kg/m^2) [29]. Whiteman *et al.*

reported that obesity and reflux symptoms were associated with an increased risk of oesophageal cancer independent of all other factors [36,37]

The risk of the development of oesophageal adenocarcinoma in Barrett's oesophagus although elevated, remains low, with a lifetime risk in the order of 0.6% [7,8]. Overall, oesophageal adenocarcinoma is an uncommon cause of mortality in patients with Barrett's oesophagus [7,8]. Among patients with Barrett's oesophagus, those with high grade dysplasia are at greatest risk of developing adenocarcinoma, particularly when combined with other known risk factors, such as tobacco smoking [7,8].

In contrast to its role in gastric cancer, there is evidence that *H. pylori* infection is protective against oesophageal adenocarcinoma [31,38]. The proposed mechanism of action includes a reduction in gastric acid production, thereby reducing the quantity of potential refluxate available, and a reduction in ghrelin levels causing a reduced appetite and a lower propensity for obesity [31,38].

1.5 Gastric cancer

The most well characterised risk for the development of gastric cancer is infection with *Helicobacter pylori* [39]. The microorganism was first reported to the Royal Australian College of Physicians meeting in October 1982 [39]. It is estimated to be responsible for more than 80% of intestinal and diffuse type gastric cancers subtypes [21,22] (Figure 4). The putative mechanisms include increased gastric cell proliferation and a complex interplay with host genetics causing an overexpression of pro-inflammatory cytokines such as interleukin 1

and tumour necrosis factor alpha. The end result is chronic gastritis, gastric mucosal atrophy and gastric intestinal metaplasia [40-44]. As with both subtypes of oesophageal cancer, tobacco smoking increases the risk of gastric cancer development. Lindblad M. *et al* has also reported a 50% increased risk for this cancer in patients with a body mass index greater than 25 kg/m^2 compared to patients with lower body mass indices [29].

1.6 Family history and heritable risk of oesophago-gastric cancer

A family history of oesophageal or gastric cancer in a first or second degree relative carries an increased risk for the development of cancer at those sites [45,46].

For gastric cancer, the major gene modulations and dys-regulations associated with cancer are shown in Figure 4. These include tumour protein p53 (*TP53), KRAS2,* fibroblast growth factor receptor 2 (*FGFR2)* and the mesenchymal epithelial transition factor (*MET)* [22,47]. Among the mutated genes in gastric cancer are E-cadherin type 1 (CDH-1) and human epidermal growth factor receptor 2 (HER-2) [22,47]. The condition known to be most strongly associated with the development of inherited intestinal gastric cancer is the presence of a CDH-1 [22,47]. Carriers of CDH-1 mutations should be considered for prophylactic total gastrectomy or enrolled in an endoscopic surveillance programme (Cambridge protocol) from the age of 20 years if prophylactic total gastrectomy is deferred [47,48].

Several studies have demonstrated that overexpression of HER-2 in sporadic oesophago-gastric cancer is associated with a poor prognosis and a more aggressive phenotype [49-51]. Other inherited cancer syndromes carrying an increased risk for oesophageal or gastric cancer include the Plummer-Vinson syndrome, familial adenomatous polyposis (FAP), Peutz-Jeghers syndrome (PJS) and Hereditary non polyposis colon cancer (HNPCC) [20,47,52,53].

1.7 Diagnosis and staging

The diagnosis of oesophageal or gastric cancer is made by direct tumour visualisation at endoscopy and on histological analysis of endoscopic biopsy. The tumour extent is then estimated (staged) in order to ascertain whether the cancer is amenable to radical (potentially curative) therapy [11,12]. The most widely applied staging system is the UICC/AJCC TNM staging system (7th edition) (Figure 5) and Table 1 [11,12].

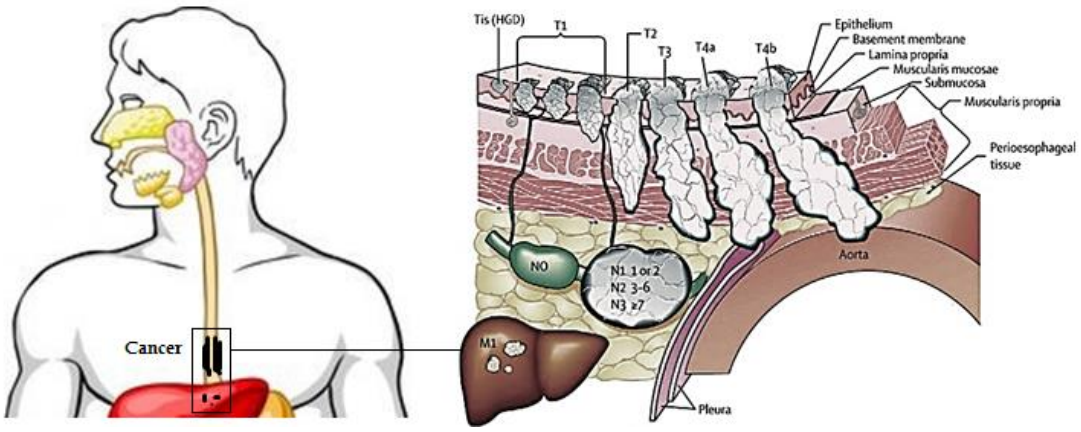
The initial staging investigation for oesophageal or gastric cancer include computed tomography scan (CT scan) of chest, abdomen and pelvis. In general terms, oesophageal cancer is assessed further with a combination of PET-CT scanning and EUS [54]. Gastric cancer is assessed further by staging laparoscopy [54]. Junctional cancers may require all three imaging modalities, where there is tumour both above and below the level of the diaphragm [54,55].

Accurate staging of oesophageal cancer defines the groups of patients for stage specific treatment and reduce the risk from inappropriate treatment. The utility of different modalities is associated with better staging of oesophageal

cancer [54]. EUS is used primarily to assess the depth of wall invasion (T stage) and regional lymph nodes (N stage) [56]. PET-CT is used primarily to exclude occult metastatic disease, not identified on CT scanning or in confirming levels of radioisotope uptake in CT-identified abnormalities [57]. The utility of laparoscopy is the detection of occult peritoneal disease, not visible on CT scanning, or in the biopsy confirmation of CT-detected peritoneal abnormalities [54,55,56,57].

Magnetic resonance imaging (MRI) has a limited role in the assessment of oesophago-gastric cancer. It use is restricted to the evaluation of CT-detected liver abnormalities and in the assessment of the spinal cord symptoms, potentially due to metastatic disease [58,59].

Figure 5: Oesophageal cancer stages illustration according to the latest TNM classification system (TNM 7.0th Edition).



Tis= Tumour in situ, HGD= high grade dysplasia. The image adapted from Pinnathur et al [24].

Table 1: The American joint committee on cancer (AJCC) histological grade and TNM staging system (7th edition) for oesophagus, gastric and oesophago-gastric adenocarcinoma

Gastric Adenocarcinoma		**Oesophageal and oesophago-gastric Adenocarcinoma**	
Tx	Tumour cannot be assessed	**Tx**	Tumour cannot be assessed
T0	No evidence of tumour	**T0**	No evidence of tumour
Tis	Intra-epithelial tumour without invasion of thelamina propria	**Tis**	High grade dysplasia
T1a	Tumour invades the lamina propria or muscularis mucosa	**T1**	Invades the lamina propria, muscularis mucosa or submucosa
T1b	Tumour invades the submucosa		
T2	Tumour invades the muscularispropria	**T2**	Invades the muscularis propria
T3	Tumour invades the subserosal connective tissue without involving the visceral peritoneum. Also includes invading the gastrocolic or gastro hepatic ligament or into the lesser or greater omentum.	**T3**	Invades the adventitia
T4a	Tumour invades the visceral peritoneum	**T4a**	Invades the pleura, pericardium and diaphragm (resectable)
T4b	Tumour invades the adjacent structures e.g spleen, pancreas and abdominal wall	**T4b**	Invades the aorta, vertebral bodies and trachea (unresectable)
Nx	Lymph nodes (LN) cannot be assessed	**Nx**	LN cannot be assessed
N0	No LN involvement	**N0**	No LN involvement
N1	1-2 LNs involved	**N1**	1-2 LNs involved
N2	3-6 LNs involved	**N2**	3-6 LNs involved
N3	≥7 LNs involved	**N3**	≥ 7 LNs involved
M0	No distant metastasis	**M0**	No distant metastasis

M1	Distant metastasis			**M1**	Distant metastasis			
Stage 0	**T category**	**N category**	**M category**	**Stage 0**	**T category**	**N category**	**M category**	**Grade**
	Tis	N0	M0		HGD	N0	M0	G1
Stage Ia	T1	N0	M0	**Stage Ia**	T1	N0	M0	G1 or 2
Stage Ib	T2 or T1	N0 or N1	M0	**Stage Ib**	T1/T2	N0	M0	G3/G1 or 2
Stage IIa	T3 or T2 or T1	N0 or N1 or N2	M0	**Stage IIa**	T2	N0	M0	G3
Stage IIb	T4a/T3/T2/ T1	N0/N1/N2/N3	M0	**Stage IIb**	T3/T1 or 2	N0/N1	M0	Any
Stage IIIa	T4a/T3/ T2	N1/N2/N3	M0	**Stage IIIa**	T1 or 2/ T3/T4a	N2/N1/N 0	M0	Any
Stage IIIb	T4b/T4a/T3	N0 or N1/N2/N3	M0	**Stage IIIb**	T3	N2	M0	Any
Stage IIIc	T4b/T4a	N2 or N3/N3	M0	**Stage IIIc**	T4a T4b/any T	N1 or 2 Any N/N3	M0	Any
Stage IV	Any T	Any N	M1	**Stage IV**	Any T	Any N	M1	Any

Grade 1= well differentiated, grade 2= moderately differentiated, grade 3= poorly differentiated, grade 4= undifferentiated. This table modified from Rice et al and Washington et al [11,12]**.**

In the UK and the United States, there is no population based screening programme for oesophageal or gastric cancer [23,60]. In some countries such as China and Japan, where the incidence of these cancers is higher, population based screening programmes are in place. The net effect is to diagnose a higher proportion of cancers at an earlier stage. In Japan, the five year survival for oesophageal cancer is approximately 90% [23,60]. The risks and benefits of rolling out such an oesophageal cancer screening programme to the UK need to be considered carefully. At the current time, there is insufficient evidence to support such a programme in Europe [23,60].

1.8 Management of oesophageal, oesophago-gastric and gastric cancer

The standard of care in the United Kingdom for all patients diagnosed with oesophageal or gastric cancer is discussion at a multidisciplinary team meeting and protocol based management. Treatment intent depends upon a combination of patient preference, disease extent (cancer stage) and patient fitness for therapy. After discussion(s) at an MDT, management will be along one of two treatment aim lines, potentially curative (radical) or palliative.

The radical treatment options include endoscopic resection or submucosal dissection (T1 oesophageal or gastric cancer), surgical resection (oesophagectomy and gastrectomy), radiotherapy / chemoradiotherapy (oesophageal cancer) [61,62,63]. The most widely applied radical therapy in the UK is surgical resection [61]. Overall, around a third of patients can expect to undergo surgical resection for oesophageal or gastric cancer [61,62,63]. Over 70% of those

undergoing surgical resection also receive preoperative (neoadjuvant) chemotherapy [64]. Eligibility criteria for surgical resection include performance status 0-1, American Society of Anaesthesiologists grade 1-3, absence of metastatic disease and patient preference [62,63].

The palliative treatment options, which can be employed to both oesophageal and gastric cancers, include endoscopic therapy, principally stenting, radiotherapy and chemotherapy [62,63]. It is of note that the National Oesophago-gastric Cancer Audit 2013/14 identified that 20% of patients in England and Wales received no active therapy at all, and were managed by best supportive care (symptom control) alone [62,63].

It is this last group of patients that form the focus of this thesis, namely those receiving palliative chemotherapy for oesophago-gastric cancer, principally because of advanced disease stage. With this in mind, the subsequent section of the Background reviews the evidence base surrounding palliative chemotherapy in advanced oesophago-gastric cancer, and the evidence base surrounding the use of omega-3 fish oils in oesophago-gastric and more generally in gastrointestinal cancer.

1.8.1 Systematic review of the randomised phase III clinical trials employing palliative chemotherapy in the management of advanced oesophago-gastric adenocarcinoma

The American Cancer Society estimate that each year over 40,000 patients are diagnosed with oesophago-gastric cancer and those more than 25,000 patients will die from these cancers [61]. Only around one third of these patients will be amenable to radical therapy, with the remaining two thirds receiving palliative oncology treatments, endoscopic therapies, principally stenting, or best supportive care only [61].

The UK National Oesophago-gastric Cancer Audit which evaluated in excess of 20,000 patients with cancer diagnosed during the time period 1st April 2011 and 31st March 2013 found that 36% of patients received curative intent treatment, 44% received palliative intent treatment, and 20% received best supportive care only. The most widely employed palliative intent treatment was chemotherapy [62,63]. Of those scheduled to receive palliative chemotherapy, just over half of the patients completed the treatment as planned (53%) [62,63]. For the remaining patients, 11% died during treatment, 18% developed progressive disease, 12% developed acute toxicity requiring discontinuation, and in 6% of patients, treatment was stopped at patient request. Older patients (aged over 80 years) and those with poorer performance status were the least likely to complete planned treatment [62,63].

The principal chemotherapy drugs employed over the last two decades have comprised fluoropyrimidine and platinum combinations, either as a two

drug regimen [65-71] or as part of a three drug regimen [72-76], with taxanes, popular in the US and anthracyclines, popular in the UK. In recent years, these have been combined with biological agents [77-83].

1.8.1.1 Palliative chemotherapy applied in this clinical trial

1.8.1.1.1 Epirubicin

Epirubicin is an anthracycline chemotherapy agent that causes DNA damage and interferes with its synthesis [84]. Because of its lower risk of cardiotoxicity compared to the other anthracyclines, epirubicin has been the most frequently used in oesophago-gastric cancer [84]. It was first used in a combination regimen Epirubicin, Cisplatin and 5-Fluorouracil for the treatment of oesophago-gastric cancer by the gastrointestinal unit at the Royal Marsden Hospital in 1991 [85].

1.8.1.1.2 Oxaliplatin

The first platinum based chemotherapeutic agent used in a clinical setting was cisplatin in the 1960s [86]. Unfortunately it has a wide range of toxicities and its clinical use was limited due to existence or development of resistance. In an attempt to overcome these resistance pathways, oxaliplatin was chemically engineered and developed in the late 1960's and approved by the United State Food and Drug Administration (FDA) in 2002 following approval in Europe in 1999 [86]. It was initially used for the treatment of metastatic colorectal cancer in United States and has been found to have a better safety profile than the other platinum based compounds [86].

1.8.1.1.3 Capecitabine

Capecitabine and 5-fluorouracil (5FU) are fluoropyrimidine chemotherapeutic agents and were first produced by Charles Heidelberger in 1957 [87,88]. 5-FU metabolites are incorporated into the DNA strands and result in cell damage at this site. 5-FU was historically administered intravenously to avoid its metabolism at the gastrointestinal tract and liver and the resulting unpredictable plasma levels [87,88]. In the last two decades, three groups of oral fluoropyrimidines have become available: 5FU prodrug, 5FU + DPD (Dihydropyrimidine dehydrogenase) inhibitors and 5FU prodrug + DPD inhibitor [87,88]. Capecitabine is a prodrug of doxifluridine (5FU prodrug) administered orally, and converted to 5FU through three enzymatic reactions: firstly, carboxylesterase to 5 deoxyfluorocytudine, then to doxifluridine by cytidine deaminase and finally to 5FU by thymidine phosphorylase mainly in the tumour tissue [87].

The following section was published in the cancer research frontiers as a literature review, the accrued evidence from published phase III randomised clinical trials (RCTs) of patients with oesophago-gastric adenocarcinoma treated with palliative chemotherapy. Included studies were those which reported on the outcome measures of interest; response rate, survival and treatment related toxicity. The rationale for restricting the inclusion criteria to phase III RCTs was on the basis that they would constitute the best available evidence.

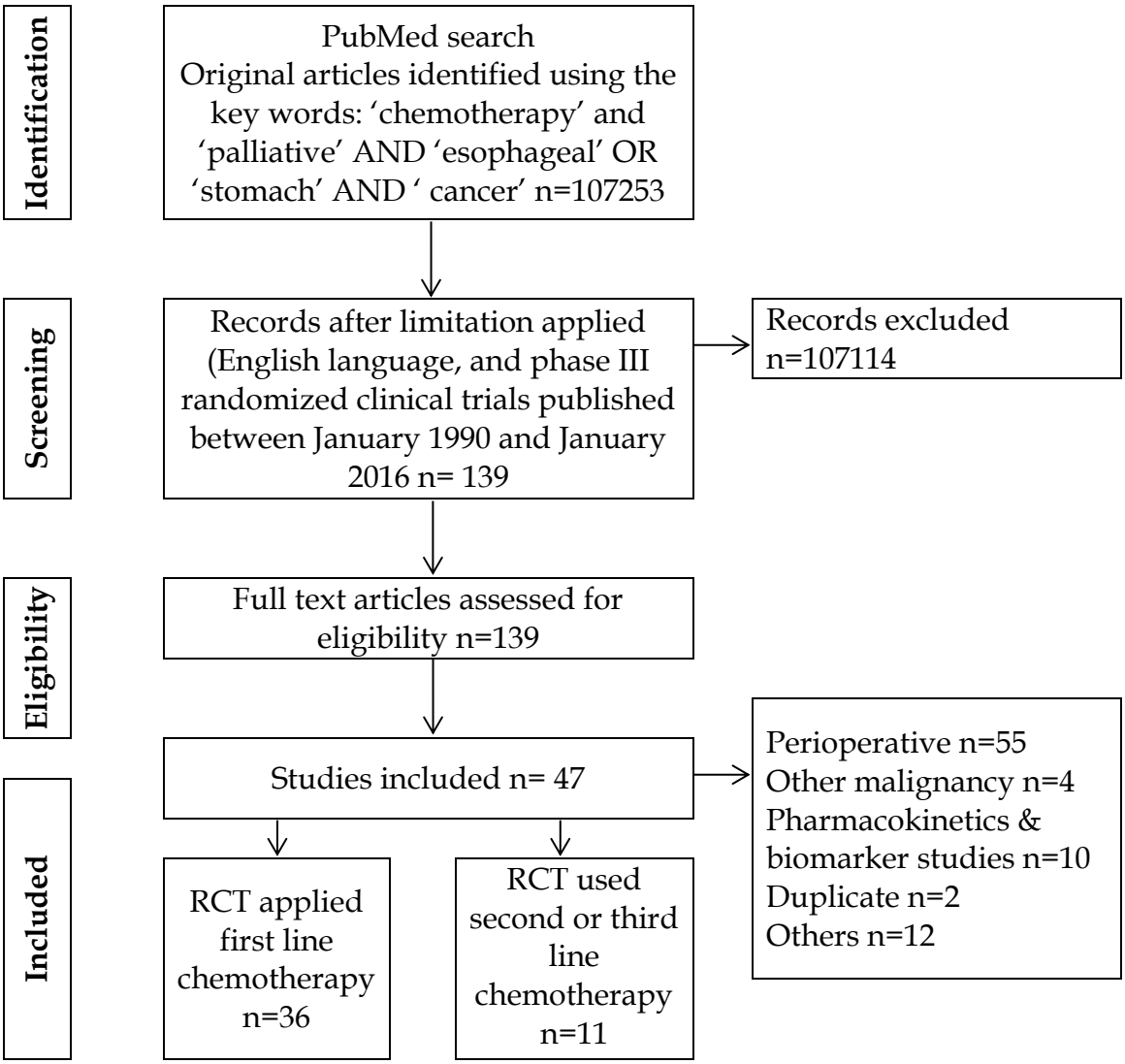
1.8.1.2 Literature search and study identification

A PubMed search of the English language literature was undertaken employing the following search words: (("drug therapy"[Subheading] OR ("drug"[All Fields] AND "therapy"[All Fields]) OR "drug therapy"[All Fields] OR "chemotherapy"[All Fields] OR "drug therapy"[MeSH Terms] OR ("drug"[All Fields] AND "therapy"[All Fields]) OR "chemotherapy"[All Fields]) AND palliative[All Fields] AND ("oesophagus"[All Fields] OR "esophagus"[MeSH Terms] OR "esophagus"[All Fields])) OR (("stomach"[MeSH Terms] OR "stomach"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields])) . Omega-3 PUFAs keywords were not included in the search keywords, as there were no phase III clinical trials in the literature investigating the effects of omega-3 PUFAs in the palliative setting for management of patients with advanced oesophago-gastric adenocarcinoma. The inclusion criteria were phase three randomised controlled trials describing patients with advanced oesophago-gastric or gastric adenocarcinoma being treated with palliative intent using either single agent or combination chemotherapy. The review included articles published in English language between January 1990 and January 2016. Exclusion criteria were combined chemotherapy and radiotherapy regimes, studies reporting exclusively on patients with squamous cell cancer or mixed study populations, where it was not possible to extract information relating to those with adenocarcinoma, and chemotherapy given in a perioperative setting, PRISMA chart details in Figure 6 and the details of this review checklist presented in appendix A. Outcome

measures of interest included the chemotherapy related toxicity, radiological response to treatment, quality of life and survival analysis. The process and inclusion of eligible papers were independently reviewed by two of the authors (AM Eltweri, DJ Bowrey).

The search strategy yielded 139 evaluable articles which were screened for inclusion. Forty seven full articles met the inclusion criteria and form the basis of this review. Thirty six of the forty seven studies reported on patients receiving first line palliative chemotherapy, and the remaining 11 studies reported on patients who received second line chemotherapy. Heterogeneity between the included studies made it impossible to perform a formal meta-analysis. The combined reports describe the outcome for a total of 14,452 patients.

Figure 6: PRISMA chart showing the search strategy



1.8.1.2.1 Summary of studies reporting on 1st line palliative chemotherapy

The clinical trials of 1st line palliative chemotherapy were summarised in Table 2. It is evident that a wide variety of drug combinations have been employed, with the majority of studies over the last decade employing two drug combinations. In those studies that compared treatment to best supportive care, overall survival for participants in the latter group was around three months. Response rates for single agent fluoropyrimidine therapy ranged from 9-39% [65,89,90,91,92,66,93,94,95], with higher values observed for S-1 compared to 5-fluorouracil. Overall survival ranged from 7.1-11.4 months [65,89,90,91,92,66,93,94,95].

Response rates for combined fluoropyrimidine / platinum combinations ranged from 20-62% [96,97,67,98,70,68,99,91,71,100,69,101,102,66,103,72,104,105,93,73,74,75,106,95] and overall survival ranged from 7.2-14.1 months [96,97,67,98,70,68,99,91,71,100,69,101,102,66,103,72,104,105,93,73,74,75,106,95]. A number of three drug combinations have been employed. Those reporting on fluoropyrimidine / platinum / anthracycline combinations (five studies) have noted response rates ranging from 15-76% [106,75,74,98,103] and overall survival ranging from 5-12 months [106,75,74,98,103]. Heterogeneous drug combinations in other studies limit meaningful conclusions. Head-to-head comparisons of regimens (where significant) indicated superiority of two agents over single agent, of capecitabine over 5-fluourouracil in combination with cisplatin, and of three drug combinations over best supportive care. Earlier studies did not indicate subsequent therapies, but it is evident from Table 2 that many of the first line trial participants in

more recently published articles went on to receive subsequent second (and third) line therapy (range 30-85%) [68,99,67].

1.8.1.2.2 Summary of studies reporting on 2nd line palliative chemotherapy

Table 3 summarises those studies reporting on second line chemotherapy [107,108,109,110,111,112,113]. Three of these compared single agent taxane or irinotecan to best supportive care [108,112,113]. Observed response rates were generally lower than for first line therapy (range 7-22%) [107,108,109,110,111,112,113], with overall survival ranging from 4.0-13.9 months [107,108,109,110,111,112,113]. By comparison, overall survival with best supportive care ranged from 2.4-5.3 months [108,112,113]. Those where significant findings were noted concluded that single agent therapy was superior to best supportive care [108,112,113].

1.8.1.2.3 Summary of studies reporting on palliative chemotherapy in combination with biological agents

Table 4 summarises those studies that employed biological agents as part of the treatment regimen [78,79,114,77,80,81,82,115,116]. With the exception of the ToGA and TyTAN studies [77,116] which enrolled only patients with proven immunohistochemical expression of the antibody target in tumour tissue, the other studies enrolled unselected participants [78,79,114,80,81,82,115].

The use of biological agents in the treatment of oesophago-gastric adenocarcinoma has been largely disappointing, with the exception of Trastuzumab (Herceptin®) [116], a human epidermal growth factor receptor 2 antibody, in patients with tumours expressing the HER-2 gene or protein. The latter was associated with impressive response rates in phase II clinical trials (up to 94%) and notably, the ability to downstage unresectable gastric cancer to potentially resectable disease [117-122]. The effects have been largely restricted to those patients whose primary cancer has demonstrated HER-2 expression [117-122]. In a phase III clinical trial, (ToGA) the use of trastuzumab plus fluoropyrimidine / platinum in patients with HER-2 positive cancers has been demonstrated to improve response rate, quality of life and survival compared to those patients treated with chemotherapy alone [116].

Therapy with the other biological agents has yielded largely negative results, with no superiority over conventional chemotherapy being demonstrated for bevacizumab (monoclonal antibody targeting VEGF) [78], cetuximab (monoclonal antibody targeting epidermal growth factor receptor) [80]

or panitumumab (monoclonal antibody targeting epidermal growth factor receptor) [81]. Ramucirumab (monoclonal antibody targeting VEGF) as a second line treatment has been shown to be superior to placebo [114], with a toxicity profile similar to conventional chemotherapy [114]. When combined with paclitaxel as second line therapy, it has been demonstrated to effect a modest improvement in survival at the expense of a higher frequency of grade 3 or 4 toxicity [79]. Lapatinib as second line therapy demonstrated a higher response rate when combined with paclitaxel compared to paclitaxel alone, but no significant improvement in survival was demonstrated [77]. Two recent studies of rilotumumab have been terminated early for safety reasons.

1.8.1.2.4 Chemotherapy related toxicities

Tables 6 and 7 summarise the reported grade 3 or 4 toxicities, non-hematological (Table 6) and haematological (Table 7). The most commonly observed toxicities were gastrointestinal adverse effects, which occurred in 0-25% with single agent fluoropyrimidine therapy, 0-26% with single agent irinotecan and 0-58% with combination therapy. Neutropenia was noted in 1-11% with single agent fluoropyrimidine therapy, 18-39% with single agent irinotecan, 15-29% with single agent taxane, and 12-82% with combination therapy.

In an attempt to reduce the risk of chemotherapy related toxicity, Cascinu S *et al.* have reported the use of glutathione in conjunction with cisplatin based chemotherapy in 50 patients with advanced gastric cancer [106]. The authors demonstrated that this reduced the risk of neurotoxicity while still

having a response rate of 76% [106]. Other authors have used leucovorin (folinic acid) in six phase III RCTs [69,73,106,123-125] as an adjunct to palliative chemotherapy especially fluoropyrimidines and methotrexate. The theory underpinning its use was that the folinic acid protects the gastrointestinal mucosa from fluoropyrimidine toxicities and minimizes the risk of bone marrow suppression caused by methotrexate and as a consequence improves quality of life [69,73,106,123-125].

Table 2: Summary of the reported phase III randomized clinical trials of 1st line palliative chemotherapy for advanced oesophago-gastric adenocarcinoma (highlighted studies are those where significant differences were identified)

Author (year)	# patients	Regimen	Response rate (%) (RECIST Criteria)	Progression free survival in months	Overall survival in months	Toxicity related mortality (%)	% receiving subsequent 2nd line treatment
Ryu *et al* (2015) [96]	625	S-1, cisplatin (SP3)	60%	5.5	14.1	NR	NR
		S-1, cisplatin (SP5)	50%	4.9	13.9	NR	NR
Ochenduszko et al (2015) [97]	56	Docetaxel, 5FU*, cisplatin	NR	6.8	11.9	NR	41%
		EOX	NR	6.4	9.5	NR	52%
Yamada *et al* (2015) [67]	685	S-1, oxaliplatin	56%	5.5	14.1	1.2%	85%
		S-1, cisplatin	52%	5.4	13.1	2.4%	84%
Guimbaud *et al* (2014) [98]	416	Epirubicin, cisplatin, capecitabine	39%	5.2	9.4	4.3%	48%
		5-FU, leucovorin, irinotecan	38%	6.7	9.7	3.4%	39%
Kim *et al* (2014) [70]	244	Capecitabine, cisplatin, placebo	29%	4.6	11.5	NR	NR
		Capecitabine, cisplatin, simvastatin	27%	5.2	11.6	NR	NR
Koizumi *et al* (2014) [65]	635	S-1, docetaxel	39%	5.3	12.5	0.6%	70%
		S-1	27%	4.2	10.8	0%	76%
Shirao *et al* (2013) [89]	237	5-FU	NR	NR	9.4	1.7%	81%
		5-FU, leucovorin, methotrexate	NR	NR	10.6	0.9%	73%
Narahara *et al* (2011) [90]	315	S-1	27%	3.6	10.5	0%	47%
		S-1, irinotecan	41%	4.5	12.8	1.2%	53%
FLAGS trial (2010) [68,99]	1053	S-1, cisplatin	29%	4.8	8.6	2.5%	30%
		5-FU, cisplatin	32%	5.5	7.9	4.9%	33%
Koizumi *et al* (2010) [91]	120	S-1	29%	4.3	9.2	0%	NR
		S-1, cisplatin	62%	5.7	12.5	0%	NR
Boku *et al* (2009) [92]	704	5-FU	9 %	2.9	10.8	0%	83%
		S-1	28 %	4.2	11.4	0.4%	74%
		Irinotecan, cisplatin	38 %	4.8	12.3	1.3%	78%
Kang *et al* (2009) [71]	316	Capecitabine, cisplatin	46%	5.6	10.5	1%	NR
		5-FU, cisplatin	32%	5.0	9.3	1%	NR

Lee *et al* (2009) [100]	174	5-FU, heptaplatin 5-FU, cisplatin	35% 36%	2.5 2.3	7.3 7.9	0% 0%	NR NR
Al-Batran *et al* (2008) [69]	220	5-FU, leucovorin, oxaliplatin 5-FU, leucovorin, cisplatin	35% 25%	5.8 3.9	10.7 8.8	NR NR	52% 59%
Dank *et al* (2008) [101,102]	333	5-FU, folinic acid, irinotecan 5-FU, cisplatin	32% 26%	5.0 4.2	9.0 8.7	0.6% 3.0%	NR NR
Koizumi *et al* (2008) [66]	298	S-1 S-1, cisplatin	31% 54%	4.0 6.0	11.0 13.0	0% 0%	75% 74%
Sadighi *et al* (2006) [103]	86	5-FU, epirubicin, cisplatin 5-FU, docetaxel, cisplatin	40% 41%	NR NR	12.0 12.0	NR NR	NR NR
Van cutsem *et al* (2006) [72,104,105]	445	5-FU, docetaxel, cisplatin 5-FU, cisplatin	37% 25%	5.6 3.7	9.2 8.6	2.7% 4.5%	32% 41%
Ohtsu *et al* (2003) [93]	280	5-FU 5-FU, cisplatin Uracil, tegafur, mitomycin	11% 34% 9%	1.9 3.9 2.4	7.1 7.3 6.0	1% 4% 1%	57% 52% 49%
Kondo et al (2000) [94]	170	Doxifluridine 5FU	NR NR	3 2.2	7.4 5.5	NR NR	NR NR
Popov *et al* (2000) [126]	120	Cisplatin, etoposide & doxorubicin (bolus) doxorubicin (infusion)	28% 20%	6 4	7 5	4.0% 3.4%	NR NR
Vanhoefer *et al* (2000) [73]	245	5-FU, leucovorin, etoposide 5-FU, cisplatin 5-FU, doxorubicin, methotrexate	9% 20% 12%	3.3 4.1 3.3	7.2 7.2 6.7	0% 2.4% 5.9%	NR NR NR
Icli *et al* (1998) [74]	131	Epirubicin, cisplatin, etoposide 5-FU, epirubicin, cisplatin	20% 15%	6 7	6 5	0% 0%	NR NR
Webb *et al* (1997) [75]	256	5-FU, epirubicin, cisplatin 5-FU, doxorubicin, methotrexate	45% 21%	7.4 3.4	8.9 5.7	0.9% 1.8%	NR NR
Cascinu *et al* (1995) [106]	50	5-FU, epirubicin, cisplatin, leucovorin & glutathione placebo	76% 52%	NR NR	14 10	NR NR	NR NR
Pyrhonen *et al* (1995) [123]	41	5-FU, epirubicin, methotrexate	29%	5.4	12.3	4.8%	NR

		Best supportive care	NA	1.7	3.1	NA	NR
Kim *et al* (1993) [95]	295	5-FU	26%	2.2	7.6	NR	NR
		5-FU, cisplatin	51%	5.4	9.2	NR	NR
		5-FU, doxorubicin, mitomycin	25%	3.0	7.3	NR	NR
Murad *et al* (1993) [124]	40	5-FU, doxorubicin, methotrexate	50%	NR	9	3.3%	NR
		Best supportive care	NA	NR	3	NA	NR

**plus Leucovorin, EOX = epirubicine, oxaliplatin, capecitabine, 5-FU = 5-fluorouracil, NA = not applicable, NR = not reported, blue highlights indicate significant difference.*

Table 3: Summary of the reported phase III randomized clinical trials of 2nd line palliative chemotherapy for advanced oesophago-gastric adenocarcinoma (highlighted studies are those where significant differences were identified)

Author (year)	# patients	Regimen	Response rate (%) (RECIST Criteria)	Progression free survival in months	Overall survival in months	Toxicity related mortality (%)
Nishikawa *et al* (2015) [107]	163	Irinotecan	15%	4.1	12.7	0%
		vs				
		Irinotecan, cisplatin	17%	4.6	13.9	0%
Ford et al (2014) [108]	168	Docetaxel	7%	3.1	5.2	0%
		vs				
		Best supportive care	NA	NR	3.6	0%
Higuchi *et al* (2014) [109]	130	Irinotecan	16%	2.8	10.1	0%
		vs				
		Irinotecan, cisplatin	22%	3.8	10.7	0%
Ohtsu et al (2013) [110]	656	Everolimus	43%	1.7	5.4	0.6%
		vs				
		Placebo	22%	1.4	4.3	0.9%
Hironaka *et al* (2013) [111]	219	Paclitaxel	21%	3.6	9.5	0%
		vs				
		Irinotecan	14%	2.3	8.4	1.8%
Kang *et al* (2012) [112]	188	Docetaxel or Irinotecan	13%	NR	5.3	NR
		vs				
		Best supportive care	NA	NR	3.8	NR
Thuss-patience *et al* (2011) [113]	40	Irinotecan	0%	2.5	4.0	0 %
		vs				
		Best supportive care	NA	NR	2.4	NA

NA = not applicable, NR = not reported, blue highlights indicate significant difference.

Table 4: Comparison of the reported phase III randomized clinical trials in patients who received palliative chemotherapy with or without biological agents

Author (year)	patients	Regimen	Response rate (%) (RECIST)	Progression free survival in months	Overall survival in months	Toxicity related mortality (%)	% receiving subsequent 2nd line therapy
AVATAR study (2015) [78]	202	Capecitabine, cisplatin, bevacizumab vs Capecitabine, cisplatin, placebo	41% 34%	6.3 6.0	10.5 11.4	4% 8%	NR
RAINBOW study (2014) [79]	665	Paclitaxel, ramucirumab vs Paclitaxel, placebo	27% 16%	4.4 2.9	9.6 7.4	12% 16%	NA
REGARD study (2014) [114]	355	Ramucirumab vs placebo	3% 3%	2.1 1.3	5.2 3.8	2% 2%	NA
TyTAN study (2014) [77]	261	Paclitaxel vs Paclitaxel, Lapatinib	9% 27%	4.4 5.5	8.9 11.0	NR NR	NA
EXPAND study (2013) [80]	904	Capecitabine, paclitaxel vs Capecitabine, paclitaxel, cetuximab	29% 30%	5.6 4.4	10.7 9.4	8% 9%	53% 53%
REAL 3 study (2013) [81]	553	Epirubicin, oxaliplatin, capecitabine vs Epirubicin, oxaliplatin, capecitabine, panitumumab	42% 46%	7.4 6.0	11.3 8.8	2% 1%	NR
AVAGAST trial (2011) [82,115]	774	Capecitabine, paclitaxel, bevacizumab vs Capecitabine, paclitaxel, placebo	46% 37%	6.7 5.3	12.1 10.1	2 % 3 %	41% 45%
ToGA study (2010) [116]	584	Capecitabine/5-FU, cisplatin, trastuzumab vs Capecitabine/5-FU, cisplatin	47 % 35 %	6.7 5.5	13.8 11.1	3% 1%	42% 45%

5-FU = 5-fluorouracil, NA = not applicable, NR = not reported, blue highlights indicate significant difference.

1.8.1.2.5 Targeted therapy additional side effects

With the exception of the REAL 3 study, the reported toxicities were similar between those treated with and without biological agents. In the REAL 3 study, the use of panitumumab was associated with an increased frequency of grade 3 and 4 toxicity, and as there was no improvement in survival, the study was terminated early. Compared to combination epirubicin, oxaliplatin and capecitabine alone, the addition of panitumumab was associated with higher frequencies of diarrhea (17%vs 11%), skin rash (11% vs 1%), mucositis (5% vs 0%) and hypomagnesaemia (5% vs 0%).

Table 5 summarises the reported toxicities of special interest to the biological drugs used and related mortalities.

Table 5: Comparison of the reported grade 3 or 4 toxicities of interest to biological drugs in those receiving and not receiving the drug

Study	Biological agent	VTE (%)	ATE (%)	HTN (%)	Bleeding (%)	GI perforation (%)	Skin reaction (%)	Heart failure (%)	Infusion related (%)
AVATAR study (2015) [78]	Bevacizumab	1%	3%	0%	4%	1%	NR	NR	NR
	Placebo	1%	4%	1%	12%	0%	NR	NR	NR
RAINBOW study (2014) [79]	Ramucirumab	2%	1%	15%	4%	1%	NR	<1%	<1%
	Placebo	3%	1%	3%	2%	0%	NR	<1%	0%
REGARD study (2014) [114]	Ramucirumab	1%	1%	8%	3%	<1%	NR	0%	0%
	Placebo	4%	0%	3%	3%	<1%	NR	0%	0%
TyTAN study (2014) [77]	Lapatinib	NR	NR	NR	NR	NR	3%	<1%	NR
	No drug	NR	NR	NR	NR	NR	0%	0%	NR
EXPAND study (2013) [80]	Cetuximab	6%	NR	NR	NR	NR	13%	<1%	3%
	No drug	3%	NR	NR	NR	NR	0%	<1%	<1%
REAL 3 study (2013) [81]	Panitumumab	11%	NR	NR	<1%	NR	11%	NR	NR
	No drug	7%	NR	NR	0%	NR	1%	NR	NR
AVAGAST study (2011) [82,115]	Bevacizumab	*6%	*1%	6%	4%	2%	NR	<1%	0%
	Placebo	*9%	*2%	<1%	4%	<1%	NR	<1%	0%
ToGA study (2010) [116]	Trastuzumab	NR	NR	NR	NR	NR	NR	<1%	6%
	No drug	NR	NR	NR	NR	NR	NR	<1%	0%

*ATE = arterial thromboembolism, GI = gastrointestinal, HTN = hypertension, VTE = venous thromboembolism, NR= not reported *grade 3-5 toxicities were reported as combined information. It was not possible to extract individual toxicity grades. Blue highlights indicate significant difference.*

Table 6: Summary of the reported non haematological grade 3 or 4 toxicities in published phase III randomized clinical trial of palliative chemotherapy in patients with advanced oesophago-gastric adenocarcinoma

	Study	Chemotherapy	Diarrhea (%)	Nausea (%)	Vomiting (%)	Lethargy/ Fatigue (%)	Infection (%)	Neuropathy (%)
Single drug regimens	**Kang *et al* 2012** [112]	Best supportive care	5%	6%	NR	27%	NR	NR
	Nishikawa *et al* 2015 [107]	Irinotecan	3%	5%	4%	4%	NR	NR
	Higuchi *et al* 2014 [109]	Irinotecan	6%	5%	0%	6%	NR	NR
	Hironaka *et al* 2013 [111]	Irinotecan	5%	5%	1%	NR	NR	0%
	Kang *et al* 2012 [112]	Irinotecan	8%	3%	NR	10%	NR	NR
	Thuss-Patience *et al* 2011 [113]	Irinotecan	26%	5%	5%	NR	16%	NR
	Koizumi *et al* 2014 [65]	S-1	5%	3%	2%	5%	NR	NR
	Narahara *et al* 2011 [90]	S-1	6%	6%	2%	7%	4%	NR
	Koizumi *et al* 2010 [91]	S-1	0%	0%	0%	NR	NR	NR
	Boku *et al* 2009 [92]	S-1	8%	6%	-	5%	6%	1%
	Koizumi *et al* 2008 [66]	S-1	3%	1%	2%	1%	1%	0%
	Shirao *et al* 2013 [89]	5-FU	1%	10%	NR	NR	6%	NR
	Boku *et al* 2009 [92]	5-FU	<1%	7%	NR	2%	4%	0%
	Ohtsu *et al* 2003 [93]	5-FU	0%	5%		NR	NR	0%
	Kim *et al* 1993 [95]	5-FU	5%	25%		NR	2%	0%
	Kondo et al 2000 [94]	5-FU	2.5%	0%		NR	NR	NR
	Kondo et al 2000 [94]	Doxifluridine	0%	0%		NR	NR	NR
	Kang *et al* 2012 [112]	Docetaxel	3%	5%	NR	26%	NR	NR
	Hironaka *et al* 2013 [111]	Paclitaxel	1%	2%	3%	NR	NR	7%
	Nishikawa *et al* 2015 [107]	Irinotecan, cisplatin	0%	4%	1%	9%	NR	NR
	Higuchi *et al* 2014 [109]	Irinotecan, cisplatin	2%	5%	0%	3%	NR	NR
	Boku *et al* 2009 [92]	Irinotecan, cisplatin	9%	21%	NR	10%	12%	1%
	Narahara *et al* 2011 [90]	Irinotecan, S-1	16%	7%	3%	6%	2%	NR
	Dank *et al* 2008 [127]	Irinotecan, 5-FU *	22%	5%	7%	7%	3%	0%
	Koizumi *et al* 2014 [65]	S-1, docetaxel	3%	6%	3%	6%	NR	NR
	Ryu *et al* 2015 [96]	S-1, cisplatin (SP3)	NR	NR	NR	NR	NR	NR

Two drug combination	**Ryu *et al* 2015** [96]	S-1, cisplatin (SP5)	NR	NR	NR	NR	NR	NR
	Yamada *et al* 2015 [67]	S-1, oxaliplatin	6%	4%	1%	6%	NR	5%
	Yamada *et al* 2015 [67]	S-1, cisplatin	7%	4%	1%	9%	NR	0%
	Ajani *et al* 2013 [68]	S-1, cisplatin	5%	7%	8%	12%	NR	<1%
	Koizumi *et al* 2010 [91]	S-1, cisplatin	0%	6%	3%	NR	NR	NR
	Koizumi *et al* 2008 [66]	S-1, cisplatin	4%	11%	4%	4%	3%	0%
	Ajani *et al* 2013 [68]	5-FU, cisplatin	4%	10%	10%	13%	NR	1%
	Lee *et al* 2009 [100]	5-FU, cisplatin	2%	29%	12%	0%	NR	NR
	Kang *et al* 2009 [71]	5-FU, cisplatin	4%	3%	8%	<1%	NR	NR
	Dank *et al* 2008 [127]	5-FU, cisplatin	7%	9%	8%	7%	5%	3%
	Al-Batran *et al* 2008 [69]	5-FU, cisplatin *	5%	9%	6%	7%	NR	2%
	Van Cutsem *et al* 2006 [72]	5-FU, cisplatin	8%	17%	17%	14%	7%	3%
	Ohtsu *et al* 2003 [93]	5-FU, cisplatin	3%	8%		NR	NR	1%
	Vanhoefer *et al* 2000 [73]	5-FU, cisplatin	6%	26%		NR	5%	1%
	Kim *et al* 1993 [95]	5-FU, cisplatin	11%	58%		NR	4%	5%
	Lee *et al* 2009 [100]	5-FU, heptaplatin	0%	8%	2%	3%	NR	NR
	Al-Batran *et al* 2008 [69]	5-FU, oxaliplatin *	6%	4%	3%	4%	NR	14%
	Kim *et al* 2014 [70]	Capecitabine, cisplatin ‡	3%	7%	3%	NR	NR	2%
	Kim *et al* 2014 [70]	Capecitabine, cisplatin †	3%	2%	3%	NR	NR	1%
	Kang *et al* 2009 [71]	Capecitabine, cisplatin	5%	2%	7%	2%	NR	NR
	Shirao *et al* 2013 [89]	5-FU, methotrexate *	10%	12%	NR	NR	8%	NR
	Vanhoefer *et al* 2000 [73]	5-FU, etoposide *	5%	7%		NR	7%	0%
Three drug combination	**Vanhoefer *et al* 2000** [73]	5-FU, doxorubicin, methotrexate	3%	8%		NR	7%	0%
	Webb *et al* 1997 [75]	5-FU, doxorubicin, methotrexate	7%	5%		NR	20%	0%
	Pyrhonen *et al* 1995 [123]	5-FU, doxorubicin, methotrexate	2%	8%		NR	NR	NR
	Murad *et al* 1993 [124]	5-FU, doxorubicin, methotrexate	NR	3%		NR	3%	0%
	Kim *et al* 1993 [95]	5-FU, doxorubicin, mitomycin	5%	38%		NR	0%	0%

Ochenduszko *et al* 2015 [97]	5-FU*, Docetaxel, cisplatin	4%	0%	0%	4%	NR	0%
Icli *et al* 1998 [74]	5-FU, epirubicin, cisplatin	1%	9%		NR	1%	0%
Webb *et al* 1997 [75]	5-FU, epirubicin, cisplatin	6%	17%		NR	8%	0%
Van Cutsem *et al* 2006 [72]	5-FU, docetaxel, cisplatin	19%	14%	14%	19%	13%	8%
Popov *et al* 2000 [126]	Etoposide, cisplatin, doxorubicin infusion	6%	5%		NR	NR	NR
Popov *et al* 2000 [126]	Etoposide, cisplatin, doxorubicin bolus	2%	8%		NR	NR	NR
Ochenduszko *et al* 2015 [97]	Epirubicin, oxaliplatin, capecitabine	3%	4%	0%	7%	NR	0%
Icli *et al* 1998 [74]	Epirubicin, cisplatin, etoposide	2%	6%		NR	2%	0%
Ohtsu *et al* 2003 [93]	Tegafur, uracil, mitomycin	0%	11%		NR	NR	0%

*5-FU = 5-fluorouracil, NR= not reported, * leucovorin was given, † simvastatin was given*

Table 7: Summary of the reported grade 3 or 4 haematological toxicities in published phase III randomized clinical trial investigating the effect of palliative chemotherapy on patients with advanced oesophago-gastric adenocarcinoma

	Study	Chemotherapy	Neutropenia (%)	Thrombocytopenia (%)	Anaemia (%)
Single drug regimens	Ford *et al* 2014 [108]	Best supportive care	NR	NR	5%
	Kang *et al* 2012 [112]	Best supportive care	2%	0%	23%
	Thuss-Patience *et al* 2011 [113]	Best supportive care	NR	NR	NR
	Nishikawa *et al* 2015 [107]	Irinotecan	28%	0%	4%
	Higuchi *et al* 2014 [109]	Irinotecan	36%	2%	18%
	Hironaka *et al* 2013 [111]	Irinotecan	39%	2%	30%
	Kang *et al* 2012 [112]	Irinotecan	18%	3%	32%
	Thuss-Patience *et al* 2011 [113]	Irinotecan	NR	NR	11%
	Kondo et al 2000 [94]	Doxifluridine	NR	NR	NR
	Ford *et al* 2014 [108]	Docetaxel	NR	NR	6%
	Kang *et al* 2012 [112]	Docetaxel	15%	2%	30%
	Hironaka *et al* 2013 [111]	Paclitaxel	29%	1%	21%
	Koizumi *et al* 2014 [65]	S-1	5%	1%	8%
	Narahara *et al* 2011 [90]	S-1	11%	4%	11%
	Koizumi *et al* 2010 [91]	S-1	4%	1%	NR
	Boku *et al* 2009 [92]	S-1	6%	NR	13%
	Koizumi *et al* 2008 [66]	S-1	11%	0%	4%
	Shirao *et al* 2013 [89]	5-FU	1%	0%	10%
	Boku *et al* 2009 [92]	5-FU	1%	NR	16%
	Kondo et al 2000 [94]	5-FU	NR	NR	NR
	Ohtsu *et al* 2003 [93]	5-FU	5%	2%	10%
	Kim *et al* 1993 [95]	5-FU	NR	0%	<1%
	Cascinu *et al* 1995 [106]	Cisplatin ‡	NR	0%	21%
	Cascinu *et al* 1995 [106]	Cisplatin ♦	NR	0%	17%
	Kim *et al* 2014 [70]	Capecitabine, cisplatin †	41%	3%	13%
	Kim *et al* 2014 [70]	Capecitabine, cisplatin ‡	41%	3%	10%
	Kang *et al* 2009 [71]	Capecitabine, cisplatin	16%	NR	NR

Two drug combination	Dank *et al* 2008 [127]	5-FU, irinotecan *	25%	2%	11%
	Ajani *et al* 2013 [68]	5-FU, cisplatin	40%	8%	19%
	Lee *et al* 2009 [100]	5-FU, cisplatin	0%	0%	0%
	Lee *et al* 2009 [100]	5-FU, heptaplatin	8%	0%	17%
	Kang *et al* 2009 [71]	5-FU, cisplatin	19%	NR	NR
	Al-Batran *et al* 2008 [69]	5-FU, cisplatin *	15%	4%	7%
	Dank *et al* 2008 [127]	5-FU, cisplatin	52%	12%	17%
	Van Cutsem *et al* 2006 [72]	5-FU, cisplatin	57%	13%	26%
	Ohtsu *et al* 2003 [93]	5-FU, cisplatin	53%	18%	25%
	Vanhoefer *et al* 2000 [73]	5-FU, cisplatin	35%	9%	NR
	Kim *et al* 1993 [95]	5-FU, cisplatin	NR	0%	<1%
	Al-Batran *et al* 2008 [69]	5-FU, oxaliplatin *	12%	5%	3%
	Yamada *et al* 2015 [67]	S-1, oxaliplatin	19%	10%	15%
	Ryu *et al* 2015 [96]	S-1, cisplatin (SP3)	39%	NR	19%
	Ryu *et al* 2015 [96]	S-1, cisplatin (SP5)	9%	NR	9%
	Yamada *et al* 2015 [67]	S-1, cisplatin	42%	10%	32%
	Ajani *et al* 2013 [68]	S-1, cisplatin	19%	5%	16%
	Koizumi *et al* 2010 [91]	S-1, cisplatin	21%	6%	NR
	Koizumi *et al* 2008 [66]	S-1, cisplatin	40%	5%	26%
	Koizumi *et al* 2014 [65]	S-1, docetaxel	29%	1%	12%
	Narahara *et al* 2011 [90]	S-1, irinotecan	27%	1%	15%
	Nishikawa *et al* 2015 [107]	Irinotecan, cisplatin	35%	1%	16%
	Higuchi *et al* 2014 [109]	Irinotecan, cisplatin	39%	0%	16%
	Boku *et al* 2009 [92]	Irinotecan, cisplatin	65%	NR	39%
	Vanhoefer *et al* 2000 [73]	5-FU, etoposide*	39%	2%	NR
	Shirao *et al* 2013 [89]	5-FU, methotrexate*	32%	2%	16%
Three drug combination	Van Cutsem *et al* 2006 [72]	5-FU, docetaxel, cisplatin	82%	8%	18%
	Webb *et al* 1997 [75]	5-FU, epirubicin, cisplatin	36%	4%	8%
	Ochenduszko et al 2015 [97]	Epirubicin, oxaliplatin, capecitabine	72%	0%	7%
	Ohtsu *et al* 2003 [93]	Uracil, tegafur, mitomycin c	38%	30%	15%
	Popov *et al* 2000 [126]	Etoposide, cisplatin, doxorubicin infusion	NR	6%	13%
	Popov *et al* 2000 [126]	Etoposide, cisplatin, doxorubicin bolus	NR	16%	19%

Ochenduszko et al 2015 [97]	5-FU*, Docetaxel, cisplatin	50%	0%	8%
Vanhoefer *et al* 2000 [73]	5-FU, doxorubicin, methotrexate	43%	5%	NR
Webb *et al* 1997 [75]	5-FU, doxorubicin, methotrexate	58%	8%	10%
Pyrhonen *et al* 1995 [123]	5-FU, doxorubicin, methotrexate	NR	2%	1%
Murad *et al* 1993 [124]	5-FU, doxorubicin, methotrexate	NR	0%	NR
Kim *et al* 1993 [95]	5-FU, doxorubicin, mitomycin c	NR	0%	1%

*5-FU = 5-fluorouracil, NR= not reported, * leucovorin was given, † simvastatin was given, ‡ placebo, ♦ glutathione was given*

1.8.1.2.6 Quality of life assessment

Quality of life (QoL) has been reported in seven phase III RCTs comparing two or more treatment regimens (biological agents employed in three) and two comparing single agent therapy with best supportive care (biological agents employed in one) [75,79,98,103,108,114,125,128,129]. The most widely used questionnaire was the EORTC QLQ-C30 questionnaire and its modules, but the reporting approach was inconsistent in all trials.

In the ToGA study [129], Satoh *et al* reported a median time to 10% deterioration in global health score of 10.2 months for patients treated with trastuzumab and chemotherapy compared to 6.4 months for those patients treated with chemotherapy alone [129]. The beneficial effect was more pronounced in those patients whose primary tumour demonstrated high levels of HER-2 protein expression [129]. In the V325 study, the authors compared the quality of life in patients receiving 5-fluorouracil and cisplatin or 5-FU, cisplatin and docetaxel [72,105]. Five percent deterioration in quality of life from baseline was observed after 4.2 months with dual therapy, and after 6.5 months, with triple therapy [72,105]. Similar findings were observed when quality of life was assessed using the EQ-5D questionnaire [72,105]. Curran *et al,* and Dank *et al* reported a 5 % deterioration of quality of life after 5.9 and 4.9 months for 5-fluorouracil, cisplatin and 5-fluorouracil, irinotecan combinations respectively [101,125].

Sadighi *et al*, compared QoL in patients who received 5-fluorouracil, cisplatin, docetaxel to that observed in patients who received 5-fluoruuracil, cisplatin, epirubicin [103]. Both groups showed improvement in their QoL measures compared to baseline scores with the exception of the domains of cognitive functioning, diarrhoea and financial aspect of the disease [103]. Those treated with the taxane containing regimen had evidence of a clinically and statistically significance improvement in global QoL (p=0.001), social functioning (p=0.03), emotional functioning (p=0.004), pain (p=0.03) and sleep difficulties (p=0.02) [103].

Ford *et al* showed statistically superior symptom control for patients who received single agent docetaxel compared to best supportive care (dysphagia, general pain, abdominal pain, nausea and vomiting and constipation) [108]. Webb *et al*, demonstrated superiority of the 5-fluorouracil, cisplatin, epirubicin regimen over 5-fluorouracil, doxorubicin, methotrexate at 24 weeks (p=0.04) [75].

In the REGARD study [114], no difference in global QoL was identified between patients treated with ramucirumab monotherapy and those treated with placebo [114]. In the RAINBOW study [79], no difference was identified in global quality of life scores between those treated with paclitaxel plus ramucirumab, and those treated with paclitaxel plus placebo [79]. Guimbaud *et al* showed no statistical significant difference in QoL between those treated with capecitabine, cisplatin, epirubicin and those treated with 5-fluorouracil, folinic acid and irinotecan [98].

1.8.1.3 Conclusions

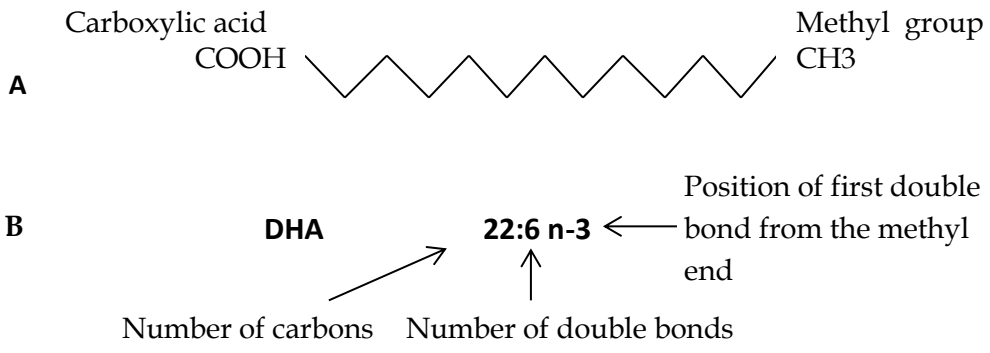
Two-thirds of patients with oesophago-gastric cancer will be treated with palliative intent, with palliative chemotherapy being the most applied therapy. Combination regimens are the most widely applied treatment worldwide, with response rates in the order of 20-62% and median overall survival in the order of 7.2-14.1 months. 30-85% of patients will go to have second or third line therapy, with reported response rates in the order of 7-22%, and median overall survival in the range 4.0-13.9 months. With the exception of trastuzumab, the effects of biological agents have been largely disappointing.

1.9 Omega-3 Fatty Acids

1.9.1 Fatty acid structure and nomenclature

Fatty acids are hydrocarbon chains with carboxylic acid group at one end and methyl group at the other end (Figure 7 A) [130]. The biological properties of FAs are determined by the length of the carbon chain, the number of double bonds, the position of the first double bond, the number and orientation of the double bonds [130]. They are classified as short chain (2-6 carbon atoms), medium chain (8-12 carbon atoms) and long chain (>12 carbon atoms). They are also subdivided into saturated, monounsaturated and polyunsaturated. Fatty acids are described by their numerical code (Figure 7B), their systematic name (Figure 7 C) and historic names [131,130].

Figure 7: Fatty acid structure and nomenclature



In plasma, the long chain fatty acids are either esterified to form more complex lipids such as phospholipids, cholesteryl ester or bound to albumin to form non-esterified fatty acids (NEFA) [132]. The phospholipids are found in only small quantities in plasma, but they are essential components of cell membranes [133,132].

1.9.2 Omega-3 polyunsaturated fatty acids

Omega-3 polyunsaturated fatty acids (PUFAs), in particular eicosapentaenoic acid (EPA, C20:5 ω-3) and docosahexaenoic acid (DHA, C22:6 ω-3,) have been reported to have multiple anti-tumour effects [134-137]. EPA and DHA are highly functional fatty acids, defined by the position of double bond closest to the methyl end of the molecule and have important roles in cell membranes [138] and as precursors of bioactive lipid mediators [139]. Despite their important roles, the human body has a very limited capacity to synthesize EPA and more especially DHA, making reliance upon dietary or supplement sources vital.

The potential anti-cancer effects of omega-3 PUFAs have been demonstrated for a number of gastrointestinal cancer types, most notably pancreatic and colon cancer in, in vitro, animal and human studies. The reported chemopreventive effects were regulation of apoptosis, inhibition of cancer growth, anti-angiogenic and anti-proliferative effects, in addition to anti-inflammatory and anti-thrombotic actions, see Figure 8 and 11.

The following section reviews the evidence base in order to determine the potential mechanism(s) of action and applications of omega-3 PUFAs in gastrointestinal cancers.

Figure 8: Inflammatory pathways that lead to cellular transformation, progression and metastasis of cancer and a suggested mode of action of omega-3 PUFAs.

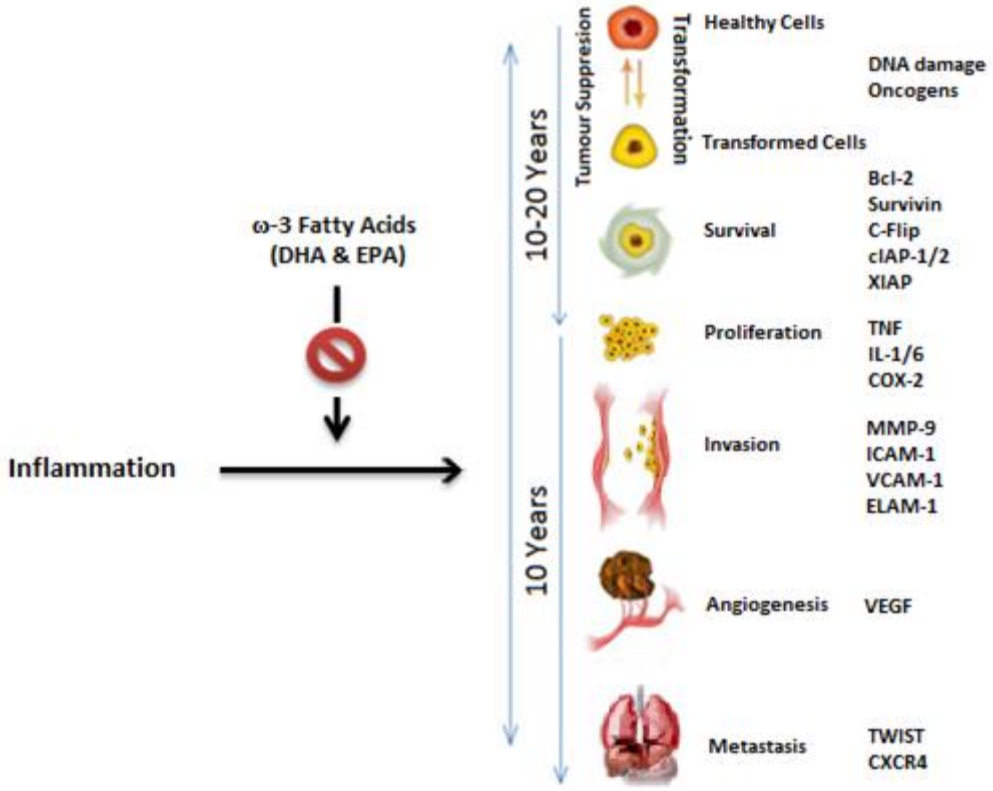


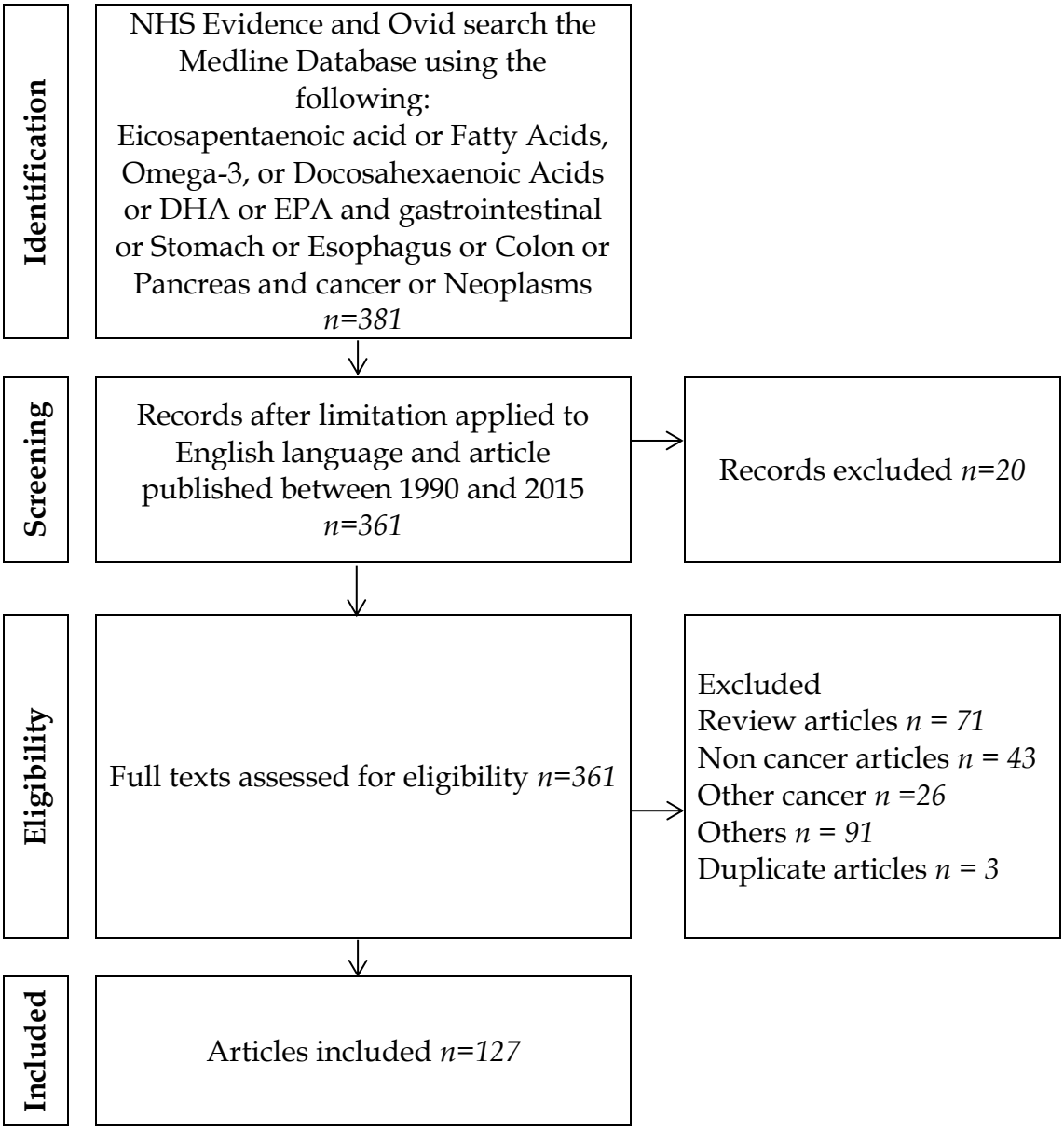
Figure modified with permission from Aggarwal BB et al [140]

1.9.2.1 Literature search strategy

A Medline search was conducted by combining the MeSH keywords "eicosapentaenoic acid", "EPA", "docosahexaenoic acid" or "DHA" with the keywords "gastrointestinal'', "stomach", "oesophagus", "colon" or "pancreas" and the keywords "cancer" or "neoplasm". Restrictions comprised English language publications during the time period January 1990 to January 2016. The abstracts of retrieved articles were reviewed for relevance and pertinent articles reviewed in full. A hand search of bibliographies of retrieved articles was performed in order to identify any additional publications, PRISMA chart presented in Figure 9.

In the subsequent text, studies have been divided into those reporting preclinical (in vitro and in vivo), and those reporting clinical evidence of EPA and DHA effects on patients with gastrointestinal cancer. All identified laboratory based and animal studies are included. Inclusion criteria for clinical studies were those that reported on patients with gastrointestinal cancer where it was possible to clearly identify the treatment effects in that group. Studies reporting on heterogeneous populations of patients that comprised gastrointestinal and non-gastrointestinal cancers were excluded if the treatment effects in those with gastrointestinal cancer were not described separately. These were largely studies assessing the effects of single agent EPA or DHA in the palliative care setting, as a potential treatment for cancer cachexia.

Figure 9: PRISMA chart showing the literature search strategy



1.9.2.1.1 Sources of omega-3 PUFAs and their absorption, metabolism and excretion by the human body

The simplest omega-3 PUFA and the metabolic precursor of EPA and DHA is α-linolenic acid (18:3 omega-3). Important dietary sources of α-linolenic acid are several vegetable oils, flaxseed and flaxseed oil and some nuts including English walnuts. EPA and DHA are found almost exclusively in seafood especially fatty fish and are present in fish oil supplements [141]. The minimum recommended daily intake of EPA and DHA is between 200 and 450 mg, depending upon the organisation making the recommendation [142]. The FDA considers that an intake up to 3 g/day is safe [143,144]. The American Heart Association recommends an intake of two servings of fatty fish e.g. cold water salmon (3.5-oz each serving) twice a week for cardiovascular prevention, 1g/day purified EPA and DHA for the treatment of cardiovascular disease, and 2 to 4 g/day for the treatment of hypertriglyceridemia.

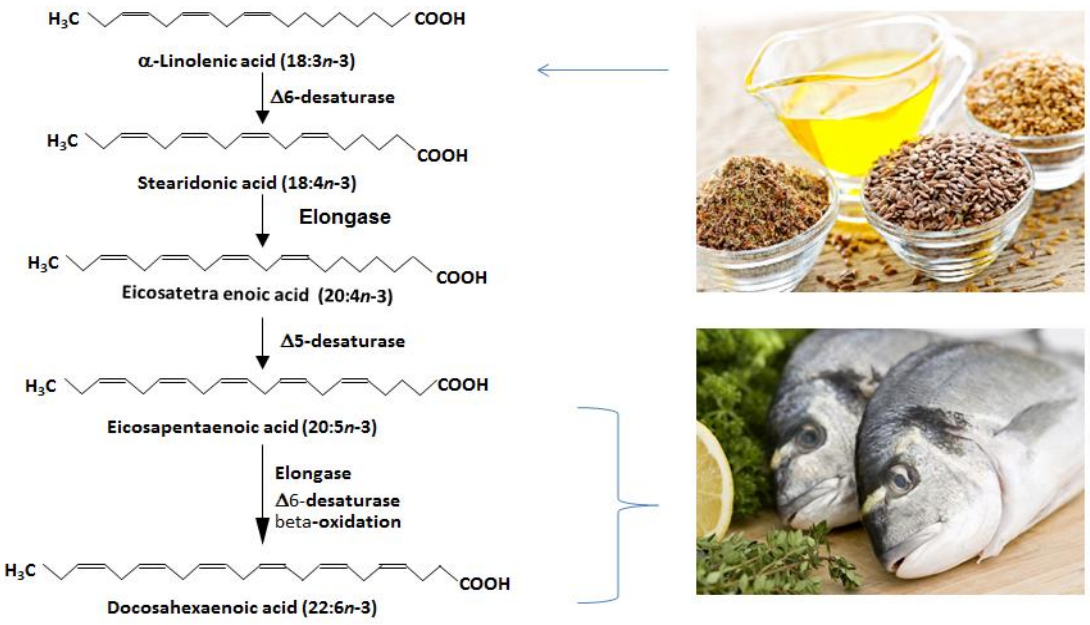
In common with most fatty acids, dietary omega-3 PUFAs are consumed complexed into triacylglycerols. These are digested in the small intestine, mainly by pancreatic lipase, and this is also the site of omega-3 PUFA absorption into enterocytes. Here fatty acids are re-esterified back into triacylglycerols which are complexed with phospholipids and apoproteins into chylomicrons. Chylomicrons are released into the lymphatic circulation [145] and later into the circulation, making the fatty acids available to cells and tissues.

EPA and DHA are incorporated into cellular membranes where their functions include the modulation of pro- and anti-inflammatory lipid mediator

(prostaglandin, leukotriene, resolvin, protectin) and cytokine production [134,146-148]. They influence different inflammatory pathways that lead to cellular transformation, progression and metastasis of cancer [140]. Thus, these properties of omega-3 PUFAs suggest that they will have important therapeutic potential in cancer management [140] (Figure 11). The beneficial anti-inflammatory effects of omega-3 fatty acids have been fairly well demonstrated [149,150]. Both the absolute amount of omega-3 PUFAs in plasma, cells and tissues as well as the ratio of omega-3 PUFAs to omega-6 PUFAs seem to be an important determinant of their beneficial effects [151,152]. This is because omega-3 PUFAs often act to oppose the action of omega-6 PUFAs. This may be especially important in inflammation and in the development and progression of cancer where mediators like prostaglandins and leukotrienes produced from omega-6 fatty acids have a key pathological role [153].

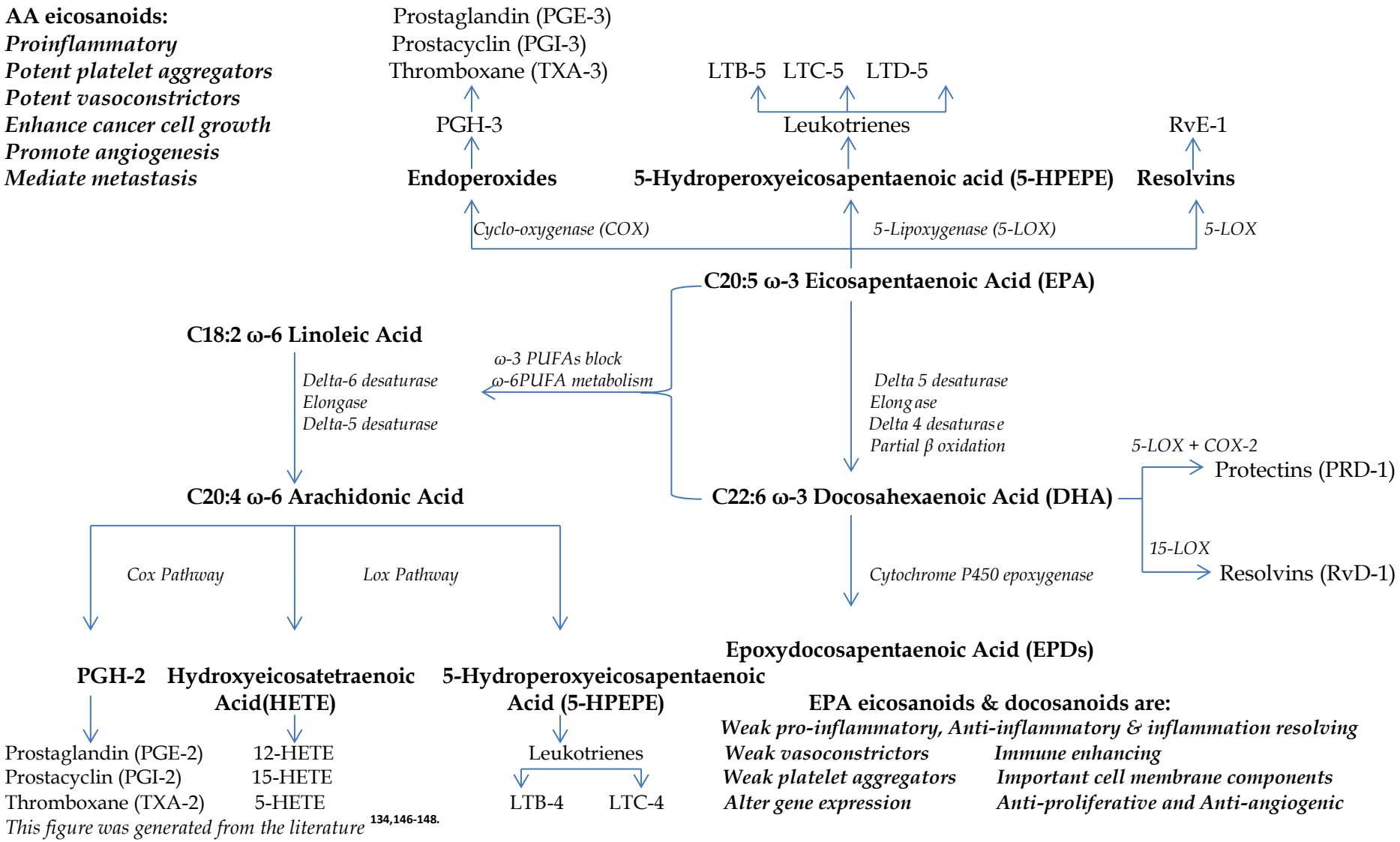
The metabolism of omega-3 PUFAs is described in Figure 10. α-Linolenic acid may be converted to EPA by elongation of the fatty acyl chain and insertion of two additional double bonds into it. EPA is further metabolised to DHA through a complex set of reactions involving chain elongation, desaturation (insertion of a double bond) and partial oxidation. After EPA and DHA are produced, or after an increase in their intake from the diet, they are incorporated into cell membranes partially replacing omgea-6 PUFAs.

Figure 10: Pathway of biosynthesis of EPA and DHA



As a consequence there will be a reduction in the generation of omega-6-derived protaglandins and leukotrienes. Some of the latter including prostaglandin E2 and leukotriene B4 have direct roles in cancer [154] and these mediators are also involved in vasoconstriction, platelet aggregation and inflammation [155]. Displacement of omega-6 by omega-3 PUFAs results in the genesis of omega-3-derived prostaglandins and leukotrienes, that have fewer inflammatory properties compared to the omega-6-derived metabolites [156] (Figure 11).

Figurse 11: Metabolism of omega-6 and omega-3 PUFAs to bioactive mediators



This figure was generated from the literature [134,146-148].

1.9.2.1.2 Pre-clinical evidence of effects of EPA and DHA on gastrointestinal cancer

1.9.2.1.2.1 Evidence from studies of human cell lines

Fifty six studies were identified reporting the effects of omega-3 PUFAs on human cell lines of relevance to gastrointestinal cancer (Tables 8-10). Of these 56 studies, 42 used colorectal cancer cell lines, six used pancreatic cancer cell lines and the remaining studies a variety of primary gastrointestinal tumour types. The concentrations of omega-3 PUFAs used in these studies were in the range 5-200 μM. Consistent findings between the studies were that omega-3 PUFAs exerted anti-proliferative and pro-apoptotic effects at the concentrations employed. The cellular mechanisms accounting for these effects included modulation of p53 dependent [157] and independent pathways, suppression of nuclear factor Kappa-light chain enhancer of activated B cells (NF-kB) [158], down regulation of Bcl-2 expression [159] and inhibition of cyclo-oxygenase-2 (Cox-2) [158]. Six in vitro studies reported an enhancement of the anti-proliferative effect of chemotherapy with omega-3 PUFAs [160-165].

1.9.2.1.2.2 Evidence from studies of animal cell lines

Three studies of animal cell lines [166-168] were identified, including two colon and one hepatoma cell line. These studies showed similar findings to the in vitro studies with human cancer cell lines [166-168]. Omega-3 PUFAs had anti-proliferative effects, with the possible mechanisms of action including suppression of Ras over-activation and modulation of the Extracellular signal

Regulated Kinases (ERK) pathway. The latter is a major determinant of cell proliferation and survival [166-168].

1.9.2.1.2.3 Evidence from studies in animal models

Table 11 summarises the in vivo animal studies exploring the effects of omega-3 PUFAs on gastrointestinal cancers; data come from a total of 33 studies. These include mouse and rat models using EPA, DHA and a combination of the two. Omega-3 PUFAs were observed to cause cellular changes consistent with the in vitro studies, with reduced cell proliferation and promotion of apoptosis. Consistent anti-cancer effects observed included reduction in tumour volume [169] and size [169] and inhibition of liver metastasis [135,170]. However, there were conflicting reports from animal models about cancer cachexia and tumour weight changes [171,172,173].

Ichihara *et al.* reported the only study to investigate the effects of parenteral omega-3 fatty acids and cancer in an animal model [174]. Further, this was the only animal study to use cancer survival as a study endpoint [174]. In this study, female mice were inoculated into the spleen with colon cancer cell lines while receiving concomitant supplementation with L-α-dimyristoylphosphatidylcholine (DMPC) and DHA [174]. The important study finding was a significantly improved median survival time for DMPC+DHA treated mice compared to their untreated counterparts [174].

Table 8: In vitro studies investigating the effect of omega-3 PUFAs in human pancreatic cell lines

Reference	Omega-3 PUFA used and concentration	Effects of omega-3 PUFAs	Possible mechanism of omega-3 PUFA effects
Fukui *et al* (2013) [169]	EPA 100 μM	Reduced cell viability Increased apoptosis	Increased intracellular reactive oxygen species accumulation Caspase-8 dependent Induced cancer autophagy
D'Eliseo *et al* (2012) [175]	DHA 12.5-100 μM	Inhibited cancer cell invasion	Decreased GrB expression
Hering *et al* (2007) [160]	EPA 100 μM EPA 100 μM + Gemcitabine	Anti-proliferative	Decreased I-kB phosphorylation and suppression of NF-kB activation
Hawkins *et al* (1998) [176]	DHA 5 μM + γ-irradiation	Increased apoptosis	Involves oxidative mechanism
Lai *et al* (1996) [177]	EPA 10-50 μM	Increased apoptosis, reduced cell number & viability	Cell cycle arrest
Falconer *et al* (1994) [178]	EPA 10-40 μM	Anti-proliferative	Increased lipid peroxidation

Caspase-8 = cysteine aspartic protease; DHA = docosahexaenoic acid; EPA = Eicosapentaenoic acid; GrB = proteinase granzyme, I-kB = inhibitor of NF-kB; μM = micromolar; NF-kB = nuclear factor kappa light chain enhancer of B cells; PUFA = polyunsaturated fatty acid

Table 9: In vitro studies investigating the effect of omega-3 PUFAs in human colorectal cell lines

Reference	Omega-3 PUFA used and concentration	Chemo	Effects of omega-3 PUFAs	Possible mechanism of omega-3 PUFA effects
Pettersen et al 2016 [179]	DHA 70 μM	-	Anti-proliferative	Induction of autophagy related gene transcription
Zhang *et al* (2015) [180]	EPA and DHA 150 μM	5FU	Anti-proliferative	Decreased PGE_2, LTB_4 and COX_2
Zhang *et al* (2014) [181]	EPA and DHA 150 μM	5FU	Increase apoptosis	Loss of mitochondrial membrane potential, generation of ROS, activation of caspase-9 & 3 Increase in Bax/Bcl2 expression
Vasudevan *et al* (2014) [182]	EPA	5FU Ox	Increase apoptosis	Decrease pAkt, normalization of β-catenin expression Decrease in pro inflammatory metabolites
Skender *et al* (2014) [183]	DHA 50 μM	-	Anti-proliferative Increase apoptosis	Enhances TRAIL induced apoptosis and Involvement of Bax dependent mitochondrial pathway
Cho *et al* (2014) [184]	DHA 50 μM	-	Increase apoptosis	Suppressing promoter methylation of the proapoptotic genes
De Carlo *et al* (2013) [161]	EPA 25 μM	5FU Ox	Anti-proliferative Enhanced effect of chemotherapy	Decreased CD133 expression Increased cytokeratin 20 and mucin 2
Granci *et al* (2013) [162]	EPA 24 μM + DHA 20.5 μM (Omegaven®)	5FU Ox IRI	Increased apoptosis Enhanced effect of chemotherapy	Involvement of Bax dependent mitochondrial pathway
Fenton *et al* (2012) [185]	DHA 50 μM + Curcumin 10 μM	-	Anti-proliferative	MAPK and MEK mediated

Fasano *et al* (2012) [186]	DHA 25 μM	-	Increased apoptosis	ERK1/2 phosphorylation ERdj5 expression, PERK phosphorylation, caspase 4 and 7 activation
Kuan *et al* (2011) [163]	EPA and DHA 0-100 μM	PTX	Increased apoptosis Enhanced effect of chemotherapy	Decreased MDR1 gene expression Increased PXR and CAR gene expression
Manda *et al* (2011) [187]	EPA (albumin complex) 30 μM ± irradiation EPA (in ethanol) 30 μM ± irradiation	-	Anti-proliferative Enhance radiosensitivity	Increased lipid peroxidation
Slagsvold *et al* (2010) [188]	DHA 70 μM	-	Cell cycle arrest (G1 & G2 phase)	DHA affects several target protein of chemotherapy and enhance cancer chemosensitivity
Rogers *et al* (2010) [189]	DHA 100 μM	-	Anti-proliferative	Inhibition of EGFR protein
Habermann *et al* (2010) [190]	DHA and EPA 50-200 μM	-	Increased apoptosis (DHA more effective than EPA)	Increased oxidative damage
Sala-Vila *et al* (2010) [164]	EPA 10-100 μM + DHA 7-70 μM	5FU	Anti-proliferative	Enhanced effect of chemotherapy
Hawcroft *et al* (2010) [191]	EPA 200 μM	-	Increased apoptosis	Alteration in PGE synthesis and EP4 receptor signalling
Dommels *et al* (2009) [192]	EPA and α-linolenic acid 300 μM	-	Inhibited gap junctional intercellular communication	Production of intercellular lipid peroxidase
Kumamoto-Yonezawa *et al* (2009) [193]	Conjugated EPA 0-100 μM	-	Anti-proliferative	Decreased polymerase expression G1 phase cell cycle arrest
Danbara *et al* (2009) [194]	EPA 56.6 μM DHA 46.8 μM Conjugated DHA 31.6 μM	-	Conjugated DHA most potent Anti-proliferative Increased apoptosis	Up-regulation of p21, down-regulation of cyclin D1/E Up-regulation of Bak and Bcl-x Down-regulation of Bcl-2

Habbel *et al* (2009) [195]	DHA 60-100 μM	-	Increased apoptosis Anti-proliferative	Down-regulation of Bcl-2, Up-regulation of p21 Suppression of arachidonic acid and of PGE-2 induced proliferation
Tang *et al* (2009) [196]	EPA 0-25 μM + Lycopene	-	Anti-proliferative	Down-regulation of the PI3K/Akt/mTOR pathway
Hossain *et al* (2008) [197]	EPA 100-150 μM DHA 100-150 μM	-	Increased apoptosis	Increased activity of caspase-3 Down-regulation of Bcl-2
Jakobsen *et al* (2008) [198]	DHA 70 μM	-	Anti-proliferative	Endoplasmic reticulum stress and disturbed calcium homeostasis
Allred *et al* (2008) [199]	EPA 100 μM	-	Anti-proliferative	Involves PPARγ1 pathway
Toit-Kohn *et al* (2008) [200]	DHA 10 μM	-	Increased apoptosis	Inhibition of Akt Ser phosphorylation and increased p38, MAPK phosphorylation
Goto *et al* (2008) [201]	DHA 30.3, 53.3 or 46.4 μM	-	Increased apoptosis	Bcl-2 regulation
Kato *et al* (2007) [202]	EPA + DHA 125 μM	-	Cell cycle arrest Anti-proliferative	p53 pathway
Calviello *et al* (2007) [203]	DHA 2.5-10 μM	-	Increased apoptosis	Inhibited β-catenin Decreased survivin
Tsuzuki *et al* (2006) [204]	DHA 10 μM	-	Increased apoptosis	Increased lipid peroxidation and P53 dependent apoptosis
Calviello *et al* (2005) [205]	DHA 2.5-10.12 μM	5FU	Increased apoptosis	Enhanced effect of chemotherapy
Narayanan *et al* (2004) [158]	DHA 5 μM ± p-XSC 2.5 μM	-	Increased apoptosis Anti-proliferative	Inhibited expression of β-catenin protein Reduced expression of NF-kB, COX-2 and iNOS
Vaculova *et al* (2004) [206]	DHA 100 μM ± TRAIL 200 ng/ml	-	Increased apoptosis	Combined treatment lead to cleavage of procaspase-3, procaspase-8 and

				PARP proteins and production of reactive oxygen species
Calviello *et al* (2004) [207]	EPA 10-30 µM or DHA 10-30 µM	-	Inhibition of VEGF	Targeting COX-2/PGE2/ERKp and HIF-1 pathways
Moonen *et al* (2004) [208]	EPA 0 - 80 µM	-	PGE_{-2} Inhibition	Compets with arachidonic acid for COX enzyme Less heterocyclic aromatic amine-DNA adduct formation
Jordan *et al* (2003) [165]	EPA 0 - 50 µM DHA 7.5 - 75 µM	5FU	Anti-proliferative Increased apoptosis	Potentiated the effect of 5FU and cell cycle arrest in S phase
Swam *et al* (2003) [209]	DHA 0-225 µM	-	Increased apoptosis Anti-proliferative	Inhibited COX-2 expression and activation
Narayanan *et al* (2003) [210]	DHA 5 µg/l	-	Increased apoptosis Anti-proliferative	Down regulation of iNOS, NF-kB, cGMP Upregulation of p21, p27
Dommels *et al* (2003) [211]	EPA 0-160 µM	-	Anti-proliferative	Inhibited COX activity
Chen *et al* (2000) [212]	DHA 150 µM	-	Increased apoptosis	Down-regulated bcl-2 expression
Jiang *et al* (1998) [213]	EPA 50 µM	-	Reduced cellular invasion	Enhances the expression of nm-23 gene
Mengeaud *et al* (1992) [214]	EPA 10-80 µg/ml	-	Anti-proliferative	Increased lipid peroxidation and membrane fluidity

Akt= protein kinase B; Bak= pro apoptotic Bcl-2 protein; Bax = a member of Bcl-2 gene family; Bcl-2= B cell lymphoma-2 (family of apoptosis regulatory protein); CAR= constitutive androstane receptor; caspase= cysteine aspartic protease; cGMP= cyclic guanosine monophosphate; chemo=chemotherapy; EGFR=epidermal growth factor receptor;EP4 receptor= prostaglandin E4 receptor; ERdj5 = endoplasmic reticulum resident protein; ERKp= phosphorylated extracellular signal regulated kinases; FU=fluorouracil ; G1/G2 phases= gap1/2 cell cycle phases; HIF-1= hypoxia inducible factor-1; iNOS= inducible nitric oxide synthase; IRI= irinotecan; µM=micromolar; MAPK= mitogen activated protein kinase; MDR1= multidrug resistance gene; MEK= mitogen activated kinase;mTOR= mammalian target of rapamycin protein; NF-kB= nuclear factor kappa light chain enhancer of B cells; nm-23 gene= tumour suppressor gene; OX= oxaliplatin; p27= cyclin dependent kinase inhibitor protein; p38= class of mitogen activated protein kinases; PGE=prostaglandin E; PI-3K= phosphatidylinositol-3 kinase; PPARγ1=peroxisome proliferator activated receptor-γ1; PTX= paclitaxel; PUFA= polyunsaturated fatty acid; PXR=pregnane X receptor; p-XSC= 1,4-phenylene bis (methylene) selenocyanate; TRAIL= TNF related apoptosis inducing ligand.

Table 10: In vitro studies investigating the effect of omega-3 PUFAs in other human cancer cell lines

Reference	Model/Cell line	Omega-3 PUFA used and concentration	Effects of omega-3 PUFAs	Possible mechanism of omega-3 PUFA effects
Zhang *et al* (2013) [134]	Umbilical vein endothelium	19,20-EDP 3μM	Inhibited angiogenesis	Inhibited VEGF inhibition Inhibited MMP-2 (weak activity) and VEGF-VEGFR2 signalling
Sun *et al* (2013) [135]	Gastric	DHA 29-80 μM	Increased apoptosis	Activated miR-15b and miR-16 Down-regulated Bcl-2
Wu *et al* (2012) [215]	Gastric	EPA 5-50 μM DHA 5-50 μM	Macrophage activated cell migration Increased apoptosis	Down-regulated MMP10 Down-regulated ERK and STAT3 pathway
Odenthal *et al* (2012) [136]	Intestinal	EPA 0.6-600 μM Curcumin 0.4-400 μM Quercetin 1-1000 μM	Anti-proliferative	Phase II detoxification enzymes seem to be trivial factor in anti-carcinogenesis effects
Zhuo *et al* (2009) [159]	Gastric	DHA 40 μg/ml 5FU μg/ml	Anti-proliferative	DHA and 5FU synergistic Down-regulated FAS, Bcl2 L12 genes Up-regulated BAX genes
Lee *et al* (2009) [137]	Gastric	DHA 50-150 μM	Anti-proliferative Increased apoptosis	ERK activation, AP-1 transactivation Increased p53, cytochrome c, BAX protein levels
Lim *et al* (2009) [216]	HCC	EPA 30 μM DHA 30-60 μM	Inhibited cancer growth Reduced cell viability	DHA inhibited COX-2, PGE-2 signalling pathways, activated GSK-3β and induced β-catenin degradation (Hep3B)
Chi *et al* (2004) [157]	Hepatoma	EPA 100 μM	Increased apoptosis	Fas mediated P53 status dependent

AP-1 = activator protein; Bax = a member of Bcl-2 gene family; β-catenin = protein regulates cell-cell adhesion and gene transcription; Bcl-2 = B cell lymphoma-2 family of apoptosis regulatory protein; Fas = TNF superfamily receptor 6; GSK-3β = glucose synthase kinase 3β; HCC = hepatocellular carcinoma; Hep3B = human hepatoma cell line

Table 11: In vivo studies investigating the effect of omega-3 PUFAs in cancer bearing animal models

Reference	Animal model	Omega-3 PUFA's used	Route	Principal findings for omega-3 PUFAs
Zou *et al* (2015) [217]	BALB/c mice injected with HCT-15 cells subcutaneously	DHA	Oral	Downregulate expression of tumour growth and metastasis related genes as COX-2, VEGF-A and MMP-1
Piazzi *et al* (2013) [218]	Five week old male C57BL/6J mice injected with AOM intraperitoneal	EPA	Oral	Reduced colorectal cancer incidence and tumour size
Fukui *et al* (2013) [169]	Athymic mice;s.c. injected with MIA-PaCa-2 cells	EPA and DHA separately	Oral	Reduced tumour volume and weight
Sun *et al* (2013) [135]	Male BALB/c mice; s.c. injected with SGC-7901 cells	DHA	IP	Reduced tumour size Inhibited metastasis
Kansal *et al* (2012) [219]	Male Wistar rats; i.p. injected with DMH	EPA and DHA together (FO)	Oral	Reduced expression of ras induced signalling pathways Anti-proliferative via inhibition of ras induced raf/MEK/Erk1/2 pathways Induced apoptosis by inhibiting ras induced Akt pathway
Cho *et al* (2012) [220]	Male Sprague-Dawley rats; injected with AOM	EPA and DHA together (FO)	Oral	Increased apoptosis by enhancing Bcl-2 promoter methylation
Hawcroft *et al* (2012) [170]	BALB/c mice; trans-splenic inoculation of MC-26 cells (mouse colon cancer)	EPA	Oral	Reduced liver tumour growth Anti-proliferative Decreased pERK1/2 signalling expression at the tumour edges
Sarotra *et al* (2011) [221]	Male Wistar rats; i.p. injected with DMH	EPA and DHA together (FO)	Oral	Chemopreventive effect mediated by decrease in cell proliferation, DNA damage, cyclin D1 and p53 expression
Ichihara *et al* (2011) [174]	Female SCID mice; intrasplenicinoculation of HCT116 cells (human colon carcinoma)	DHA	IV	Increased apoptosis Improved survival

Shah *et al* (2011) [222]	Male Sprague-Dawley rats; injected with AOM	EPA and DHA together (FO)	Oral	Down-regulated miR-19b, miR-26b and miR-203 oncogene targets
Cho *et al* (2011) [223]	Male Sprague-Dawley rats; injected with AOM	EPA and DHA together (FO)	Oral	Chemoprotective by affecting apoptosis gene expression and cell cycle regulation
Sarotra *et al* (2010) [224]	Male Wistar rats; i.p. injected with DMH	EPA and DHA together (FO)	Oral	Chemopreventive effect mediated by increased oxidative stress and apoptosis
Moreira *et al* (2009) [225]	Wistar rats; s.c. injected with DMH	EPA and DHA together (FO)	Oral	Protected against preneoplastic lesions Did not protect against colon cancer
Hong *et al* (2009) [226]	Male Sprague-Dawley rats; injected with AOM	EPA and DHA together (FO)	Oral	Increased apoptosis Down-regulated bcl-2 expression
Strouch *et al* (2011) [227]	El-kras mice	EPA and DHA together (FO)	Oral	Anti-proliferative Cell cycle arrest Increased apoptosis
Bathen *et al* (2008) [228]	Female athymic mice; s.c.injected with SW620 cells	EPA and DHA together (FO)	Oral	Decreased tumour growth
Fukunaga *et al* (2008) [229]	F334 rats; injected with DMH	EPA or DHA	Oral	Decreased proliferation Increased apoptosis
Gutt *et al* (2007) [230]	WAG/Rij rats; splenic inoculation with colonic cancer cell lines	EPA and DHA together (FO)	Oral	Reduced tumour metastasis Inhibited tumour growth
Kato *et al* (2007) [202]	Athymic mice; s.c. injected with COLO 205 (colon adenocarcinoma)	EPA and DHA together (FO) DHA	Oral	Decreased tumour growth
Heukamp *et al* (2006) [153]	Syrian golden hamsters; s.c. injected with BOP	EPA and DHA together (FO)	Oral	Reduction in macroscopically visible cancer Less liver metastasis
Calviello *et al* (2004) [207]	Athymic mice; subaxillary inoculated with HT-29 cells (colon adenocarcinoma)	EPA or DHA	Oral	Both inhibited VEGF and COX-2 expression
Davidson *et al* (2004) [231]	Male Sprague Dawley rats; s.c. injected with AOM	EPA and DHA together (FO)	Oral	Modulated balance between DNA adduct formation, apoptosis and aberrant crypt foci multiplicity
Whitehouse *et al* (2001) [172]	Female NMRI mice, s.c. injected with MAC16 tumour (murine colon carcinoma)	EPA	Oral	Decreased weight loss as a result of inhibition of ATP-dependent proteolytic pathway Inhibited cancer growth by down regulation of proteasome expression

Sauer *et al* (2000) [232]	Male Buffalo rats; inoculated with hepatoma 7288CTCs cells	EPA	Oral	Inhibition of cancer growth and anti–cachectic effect secondary to inhibitory effect on FA transport
Iwamoto *et al* (1998) [167]	Male F344 rats; injected with ACL-15 cancer cells via superior mesenteric vein	EPA	Oral	Fewer liver metastasis Decreased VCAM-1 expression
Griffini *et al* (1998) [233]	Wag-Rij rats; injected with colon cancer cells through their portal veins	EPA and DHA together (FO)	Oral	No effect on cell proliferation Enhanced colon cancer liver metastasis compared to low fat diet
Singh *et al* (1998) [234]	Male F344 rats; s.c. injected with AOM	EPA and DHA together (FO)	Oral	Inhibited farnesyl protein transferase which leads to inhibition of active ras-p21 production
Jiang *et al* (1997) [171]	Male Sprague Dawley rats; s.c. injected with AOM	EPA and DHA together (FO)	Oral	No significant difference in weight changes Blocked down regulation of colonic PKC isoenzyme
Takahashi *et al* (1997) [235]	Male F344 rats; s.c. injected with AOM	DHA	IG	Reduced the multiplicity of colon cancer
Singh *et al* (1997) [173]	Male F344 rats;s.c. injected with AOM	EPA and DHA together (FO)	Oral	No significant difference in weight changes Suppressed colon cancer by inhibition of ras-p21 protein
Ligo *et al* (1997) [236]	CDF1 mice, s.c. injected with Co 26Lu (highly metastatic colon carcinoma)	EPA or DHA	Oral	DHA had marked anti-metastatic effect, the uptake of DHA by tumour cells lead to cell membrane alteration in the tumour tissues
Suzuki *et al* (1997) [237]	CDF1 mice;s.c. injected with Co 26Lu (highly metastatic colon carcinoma)	EPA or DHA	Oral	DHA decreased activity of MMP-9
Singh *et al* (1997) [238]	Weanling male F344 rats;s.c. injected with AOM	EPA and DHA together (FO)	Oral	Anti-cancer effect via inhibiting the COX-2 expression

Akt= protein kinase B; ATP= adenosine triphosphate; AOM= azoxymethane; BALB/c=lab bred strain of house mouse; BOP= N-nitrosobis (2-oxopropyl) amine (pancreatic carcinogen); Bcl-2= B cell lymphoma-2 family of apoptosis regulatory protein, DMH= dimethylhydrazine; DMPC/DHA= L-a-dimyristoylphosphatidylcholine/ docosahexaenoic acid; DNA=deoxyribonucleic acid; IG=intragastric; IP= intraperitoneal; MEK= mitogen activated kinase; mg= milligram; MMP-9= matrix metalloprotease-9; NMRI= naval medical research institute mouse; P53=tumour suppressor phosphoprotein; pERK1/2= phosphorylated extracellular regulated signal kinase 1/2; PKC= protein kinase C; PUFA= polyunsaturated fatty acids; ras/raf/MEK/Erk1/2= a chain ofproteins in the cell that communicate a signal from receptor; s.c=subcutaneous; SCID= severe combined immunodeficiency strain of mice; SGC-7091= gastric cancer cell lines; VCAM-1= vascular cell adhesion molecules 1; VEGF= vascular endothelial growth factor

1.9.2.1.3 Evidence from clinical studies of the effects of EPA and DHA on gastrointestinal cancer (in humans)

Tables 12 and 13 summarise the clinical studies reporting the use of EPA and DHA in the curative [239,240,241,242,243,244,245,246,247,248,249,250,251,252,253,254,255,256,257,258,259,260,261,262,263,264,265,266,267,268,269,270] and palliative settings [271,272,273,274,275,276,277,278,279,280,281], respectively. In the curative setting, oral, intravenous and enteral supplementation of omega-3 PUFAs has been described in the preoperative setting, the postoperative setting or a combination of both. The studies have included heterogeneous groups of patients with different gastrointestinal cancer types [239,242,244,245,246,248,250,257,262,264,265,267,268,269] and homogenous groups of patients with colorectal [266,263,255,243], oesophageal [247,251,253,254,256,260], or gastric [240,246,249,252,258,259,261]. Table 12 indicates that omega-3 PUFAs were associated with improvement in nutritional status in three of 32 studies where reported [242,243,253]. Table 12 also indicates that the majority of studies assessed changes in haematological and immunological function [242-244,247,250,253-255,259-263,265,267,268,282] and the following observations were found: improved neutrophil count [242], decreased C-reactive protein/albumin ratio [243], favourable modulation of IL-10, less systemic inflammatory response [250,252], reduced platelet aggregation [254,260], improved immune response [255], lower complication rate [252], shorter hospital stay [261], shorter postoperative ICU stay [256], increased IL-2 [259], decreased IL-6, IL-8 and TNF-α [260], and improved liver and pancreas function [262].

Table 13 indicates that use of omega-3 fatty acids in the palliative management of gastrointestinal cancer has been restricted to colorectal and pancreatic cancer [271,273,274,276,277,278,279,280,281]. In nine of the twelve studies

[271,272,273,274,276,277,278,279,280], omega-3 PUFAs were employed as a single agent, in an analogous manner to their use in other patients with non-gastrointestinal cancer being treated for cancer cachexia. In three studies, omega-3 PUFAs were combined with palliative chemotherapy [271,272,273]. All of these studies employed oral supplementation in a relatively small number of patients, for a median of 49 days (range 12-84 days). Omega-3 PUFA use resulted in improved survival in two of the four studies where survival was reported [278,280]. Improvement in quality of life was noted in all four studies where it was evaluated [271,273,274,280].

Table 12: Clinical trials investigating the effects of omega-3 PUFAs in patients with gastrointestinal cancer in curative settings

Reference	Site of cancer	n	Surgery	Chemo	Omega-3 intervention	Days	Route	Outcome measures	Effects of omega-3 PUFAs
Ma et al 2015 [239]	GI	99	Yes	No	Omega-3 'lipoplus' TPN (EPA + DHA 8.6-17.2 g/l) postoperatively	7	IV	Inflammatory response	No significant effect
Wei et al 2014 [240]	G	48	Yes	No	10% Omegaven® (omega-3) or 20% intralipid (omega-6) enriched TPN postoperatively	6	IV	Inflammatory response Postoperative complication	Less inflammatory response (P<0.01) Less postoperative complications (P=0.03)
Nagano *et al* (2013) [241]	O	20	Yes	No	Omega-3 enriched EN for 7 days pre and 7 days postoperatively	14	Oral (pre) & gastrostomy (post)	Length of hospital stay, SIRS, Postoperative complications, Body weight (baseline, 2 weeks)	No significant effect
Bonatto *et al* (2012) [242]	GI	38	No	Adjuvant (5FU)	Fish oil capsules (2 g/day)	56	Oral	Blood neutrophils, Body weight (baseline, 8 weeks)	Improved neutrophil count (P<0.05) Improved body weight (P<0.002)
Silva *et al* (2012) [243]	CR	23	No	Neoadjuvant	Fish oil capsules (2 g/day)	63	Oral	Inflammatory markers and nutritional status	Decreased CRP/albumin ratio (P=0.005) Stabilization of body weight (P=0.01)
Cury-Boaventura *et al* (2012) [244]	GI	25	Yes	No	Fish oil infusion (0.2 g/kg body weight/day) preoperatively	3	IV	Leucocyte functions	Protected postoperative lymphocyte function (P<0.05), Upregulated anti-apoptotic genes

Ma *et al* (2012) [245]	GI	40	Yes	No	Fish oil infusion (1 - 2 g fat (15% was FO)/kg body weight/day)postop	5	IV	Length of hospital stay, Postoperative complications	No significant effect
Sultan *et al* (2012) [246]	O or G	195	Yes	No	Omega-3 enriched EN for 7 days pre and 7 days postoperatively	14	Oral	Anthropometry, Length of ICU stay, Length of hospital stay, Postoperativemorbidity and mortality	No significant effect
Miyata *et al* (2012) [247]	O	91	No	Neoadjuvant (2 cycles '7 days each' of 5-FU and Cisplatin)	Omega-3 enriched EN	17	Oral	Chemotherapy related toxicity	Enteral nutrition associated with less neutropenia (P=0.005) and leukopenia (P=0.01).
de Miranda Torrinhas *et al* (2012) [248]	GI	63	Yes	No	Fish oil infusion (0.2 g/kg/day) preoperatively	3	IV	Length of hospital stay, Length of ICU stay, Postoperative complications	No significant effect
Makay *et al* (2011) [249]	G	26	Yes	No	Fish oil infusion (0.2 g/kg/day) postoperatively	5	IV	Length of hospital stay, Postoperative complications	No significant effect
Jiang *et al* (2010) [250]	GI	206	Yes	No	Soybean oil (1 g/kg body weight/day) plus FO (0.2 g/kg body weight /day) infusion postop	7	IV	Length of hospital stay, Occurrence of SIRS, Postoperative infectious complications, Body weight	Less SIRS (P=0.03) Shorter hospital stay (P=0.04) Better immune function and less inflammatory response
Nakamura *et al* (2009) [251]	O	20	Yes	No	Omega-3 enriched EN preoperatively	7	Oral	Postoperative complications	No significant effect
Okamoto *et al*	G	60	Yes	No	Omega-3 enriched EN preoperatively	7	Oral	Postoperative complications, Body	Fewer infectious complications (P<0.05)

(2009) [252]								weight (baseline, 1 week)	Shorter duration of SIRS (P<0.05)
Ryan *et al* (2009) [253]	O	53	Yes	No	Omega-3 enriched EN for 5 days pre and 21 days postoperatively	26	Oral (pre) & jejunal (post)	Body composition, Postoperative morbidity, Body weight	Lean body mass preservation Decreased stress response
Aiko *et al* (2008) [254]	O	29	Yes	No	Omega-3 enriched EN postoperatively	7	Jejunal	Length of hospital stay, Postoperative complications	Less postoperative hyper coagulopathy (P=0.01) Reduced platelet aggregation
Liang *et al* (2008) [255]	CR	42	Yes	No	Soybean oil (1 g/kg body weight/day) plus fish oil (0.2 g/kg body weight/day) infusion postop	7	IV	Infectious complications, Length of hospital stay, Postoperative mortality, Immune and inflammatory responses	Improved immune response (P=0.03) Reduced inflammatory changes (P=0.03) No clinical effect (P=0.1)
Takeuchi *et al* (2007) [256]	O	40	Yes	No	Omega-3 enriched EN for 5 days preoperatively or14 days postop or 21 days periop	Up to 21	Oral (pre) & jejunal (post)	Length of ICU stay, Postoperative complications	Shorter length of ICU stay (P=0.04) Fewer wound infections (P=0.03) Shorter duration of SIRS (P<0.05)
Helminen *et al* (2007) [257]	GI	100	Yes	No	Omega-3 enriched EN for 5 days preoperatively and 5 days postop	10	Oral	Length of hospital stay, Postoperative complications	No significant effect
Klek *et al* (2005) [258]	G	90	Yes	No	Fish oil infusion postoperatively	7	IV	Length of hospital stay, Postop complications	No significant effect
Chen *et al* (2005) [259]	G	40	Yes	No	Omega-3 enriched EN postoperatively	7	Nasojejunal	Immune and nutrition status	Increase in IL-2 (P<0.01) Increase in IgA/G/M (P<0.05) Decrease in IL-6 and TNFα (P<0.01)

Aiko *et al* (2005) [260]	O	28	Yes	No	Omega-3 enriched EN postoperatively	7	Jejunal	Platelet aggregation, coagulation activity and inflammatory response	Reduced risk of thrombocytopenia and hypercoagulopathy (P<0.05) Decrease in IL-8 (P<0.05)
Farreras *et al* (2005) [261]	G	60	Yes	No	Omega-3 enriched EN postoperatively	7	Jejunal	Length of hospital stay, Postoperative complications	Fewer complications (P=0.01) Improved wound healing (P=0.005) Shorter hospital stay (P=0.02)
Heller *et al* (2004) [262]	GI	44	Yes	No	Soybean oil (0.8 g/kg body weight/day) plus fish oil (0.2 g/kg body weight/day) infusion postop	5	IV	Length of hospital stay, Length of ICU stay, Body weight	Improved liver (P<0.05) and pancreas function (P=0.04) No clinical effect
Braga *et al* (2002) [263]	CR	200	Yes	No	Omega-3 enriched EN for 5 days preoperatively or 13 days periop	5 or 13	Oral (pre) & jejunal (post)	Length of hospital stay, Postop complications during hospitalisation and one month after discharge	Fewer infectious complications (P<0.04) Less antibiotic therapy (P<0.005) Shorter hospital stay (P<0.0001)
Braga *et al* (2002) [264]	GI	150	Yes	No	Omega-3 enriched EN for 7 days preoperatively or 14 days periop	7 or 14	Oral (pre) & jejunal (post)	Length of hospital stay, Postoperative complications	Fewer complications (P=0.02) and shorter length of hospital stay (P=0.01) when omega-3 PUFAs given pre and postop
Wu *et al* (2001) [265]	GI	48	Yes	No	Omega-3 enriched EN postoperatively	8	Oral	Inflammatory and immune responses	Decreased TNF-α, IL-6 (P<0.05) and CRP (P<0.01)
Gee *et al* (1999) [266]	CR	49	Yes	No	Fish oil capsules (1.4 g/day EPA	12	Oral	Fatty acid composition of colonic mucosa and effect	Rapid incorporation of EPA into colonic

					and 1 g/day DHA) preoperatively			on proliferation	epithelium (P<0.001) but no effect on epithelial cytokines
Senkal *et al* (1999) [267]	GI	154	Yes	No	Omega-3 enriched EN for 5 days preoperatively and 10 days postop	15	Oral (pre) & jejunal (post)	Length of hospital stay, Postoperative complications	Fewer postoperative complications (P=0.04)
Kenler *et al* (1996) [268]	GI	35	Yes	No	Omega-3 enriched EN postoperatively	7	Jejunal	Length of hospital stay, Postoperative complications	Fewer complications (P=0.03) Improved liver and renal function
Kemen *et al* (1995) [269]	GI	42	Yes	No	Omega-3 enriched EN postoperatively	10	Jejunal	Immune response	Improved postoperative immune response (P<0.05)
Daly *et al* (1992) [270]	GI	77	Yes	No	Omega-3 enriched EN postoperatively	7	Oral	Length of hospital stay, Postoperative complications	Fewer infections Fewer wound complications (P=0.02) Shorter hospital stay (P=0.01)

Chemo= chemotherapy; CR= colorectal; CRP= C-reactive protein; DHA= docosahexaenoic acid; EN=enteral nutrition; EPA=eicosapentaenoic acid; 5FU=5 fluorouracil; FO=fish oil; G= gastric; GI= gastrointestinal; g/l=gram per litre; ICU= intensive care unit; Ig= immunoglobulin; IL=interleukin; IV= intravenous; M&M=morbidity & mortality; n = number of participants; Non-RCT= non randomised clinical trial; O= oesophageal; PN= parenteral nutrition; postop= postoperative; preop= preoperative; PUFA= polyunsaturated fatty acids; RAC= racol diet; RCT= randomised clinical trial; SIRS=systemic inflammatory response syndrome; TNF=tumour necrosis factor; TPN=total parenteral nutrition; vs=versus.

Table 13: Clinical trials investigating the effects of omega-3 PUFAs in patients with gastrointestinal cancer in palliative settings

Reference	Site of cancer	n	Chemotherapy	Omega-3 intervention			Days	Route	MOS days (range)	Outcome measure	Effects of omega-3 PUFAs
				Details	EPA (g/d)	DHA (g/d)					
Trabal *et al* (2010) [271]	CR	13	FOLFOS + Capcitabine	EPA enriched ONS	1	-	84	Oral	NR	Energy intake, QoL and weight (baseline, 12 weeks)	Tendency for weight preservation (P=0.04) Improved QoL Improved tolerability of chemotherapy
Jones *et al* (2008) [272]	O or G	54	Paclitaxel 1,100 mg/m² every 21 days	DHA infusion once every 21 days	-	NR	84	IV	262	Disease free survival, overall survival, response rate and toxicity	Less toxicity (non-haematological) DHA-paclitaxel was not superior over paclitaxel alone
Read *et al* (2007) [273]	CR	23	FOLFIRI	EPA enriched ONS for 3 wk before chemo and then for 6 wk	2.18	0.92	63	Oral	NR	Energy intake, nutritional status, QoL and (weight baseline, 3 & 9wks)	Maintained body weight (P=0.03) Improved nutritional status and QoL (P=0.03)
Moses *et al* (2004) [274]	P	24	No	EPA enriched ONS	1.1	-	56	Oral	NR	Body composition, energy expenditure energy intake, Karnofsky PS and weight (baseline, 8 weeks)	Increased TEE Improved physical activity and possibly QoL

Persson *et al* (2004) [275]	GI	24	No	Fish oil syrup (and melatonin capsules)	4.9	3.2	56	Oral	142 vs 179	Energy intake, Karnofsky PS, quality of life and weight(baseline, 4 weeks, 8 weeks)	No effect on cytokines Stable weight No effect on survival
Barber *et al* (2004) [276]	P	8	No	EPA enriched ONS	2	-	21	Oral	NR	Weight (baseline, 3 weeks)	Modulated hepatic protein synthesis (P=0.001)
Fearon *et al* (2003) [277]	P	200	No	EPA enriched ONS	2.2	0.96	60	Oral	NR	Energy intake, QoL and weight change	No effect
Wigmore *et al* (2000) [278]	P	26	No	EPA capsules: 1 g/d in 1st week 2 g/d in 2nd week 4 g/d in 3rd week 6 g/d in 4th week	Up to 6	-	12	Oral	173 (85-339)	Anthropometry, body composition, weight and WHO PS (baseline, 4 , 8, 12 weeks), survival	EPA is safe anti-cachectic agent (P<0.005) Improved survival
Barber *et al* (2001) [279]	P	22	No	EPA enriched ONS	2.2	0.96	21	Oral	NR	Metabolic response to feeding	Normalized the metabolic response (P<0.01) Increased lean body mass and weight gain (P<0.05)
Barber *et al* (1999) [280]	P	20	No	EPA enriched ONS	2.2	0.96	49	Oral	170 (90-270)	Anthropometry, appetite, body composition, energy intake and expenditure, PS and weight (baseline, 3 & 7 wks), survival	Improved weight gain (P=0.02), performance status (P=0.004) and appetite (P=0.009) at 3 weeks Significant weight gain at 7 weeks (P=0.03) Improved survival

Wigmore *et al* (1996) [281]	P	18	No	Fish oil capsules: 2 g/d for the first week, increasing by 2 g/d on a weekly basis to a max of 16 g/d	Up to 2.88	Up to 1.92	60	Oral	NR	Anthropometry and body composition analysis	Increased body weight (P<0.002)

CR = colorectal; EN=enteral nutrition; FOLFIRI= folinic acid+5-fluorouracil+irinotecan; FOLFOS=folinic acid+5-fluorouracil+oxaliplatin; G=gastric; g/d=gram per day; GI= gastrointestinal; IV= intravenous; MOS=median overall survival; n= number of patients; Non-RCT= non randomised clinical trial; NR=not reported; O= oesophageal; ONS=oral nutritional supplement; P=pancreatic;PS=performance status; RCT= randomised clinical trial; ROS=route of supplement; TEE=total energy expenditure.

Eleven randomized clinical trials evaluated the effects of parenteral omega-3 PUFAs in a total of 737 patients for a duration ranging from 3-84 days. Six of these studies investigated patients with heterogeneous gastrointestinal cancer [239,244,245,248,250,262], four included oesophageal or gastric cancer [240,249,258,272] and one included only patients with colorectal cancer [255]. These studies employed omega-3 fatty acids in the pre and or post-operative setting. The principal findings were that omega-3 PUFAs were associated with improved immune function (e.g. increase in CD3+ and CD4+ lymphocyte percentage), a protective effect on lymphocyte apoptosis and reduced inflammatory markers (e.g. increase in IL-10, decrease in IL-6 and TNF-α production) which lead to less systemic inflammatory response syndrome.

Miyata *et al.* compared the clinical effects of enteral (EN) versus parenteral (PN) omega-3 fatty acids in patients with oesophageal cancer receiving platinum based neoadjuvant chemotherapy [247]. The authors demonstrated a lower frequency of haematological toxicity in patients who received enteral compared to parenteral nutrition but there was no significant difference in tumour response rate [247].

Tumour-induced cachexia is often refractory to treatment and is associated with a poor quality of life. Cachexia is common in patients with gastrointestinal cancers causing appetite and weight loss and increasing energy expenditure. There have been two studies using chemotherapy and omega-3 PUFAs in the palliative treatment of colorectal cancer which showed improved quality of life and preservation of body weight [271,273]. The third study in

patients with oesophago-gastric cancer using DHA-paclitaxel showed no superiority over paclitaxel alone although a reduced risk of non-haematological toxicity was observed [283].

1.9.2.2 Conclusion

Omega-3 fatty acids have anti-inflammatory properties, acting mainly by blocking the production of, and the effects of, the pro-inflammatory mediators derived from omega-6 fatty acids (Figure 11). The anti-inflammatory effects of omega-3 PUFAs are important in blocking the mediators involved in the inflammatory pathway for cancer development and metastasis. There are a large number of studies reporting the anticancer and anti-cachectic effects of omega-3 PUFAs in a variety of model systems. This literature review provides evidence of the effectiveness of omega-3 PUFAs in cancer management with favourable outcome including better quality of life, less toxicity and even improved survival. There is a lack of human trials both as a phase II or phase III clinical trials in several areas, including the palliative settings for advanced oesophago-gastric cancer investigating the effects of omega-3 PUFAs supplements to the current standards of palliative chemotherapy. This thesis reports the first clinical trial to investigate the effects of intravenous omega-3 PUFAs in oesophago-gastric cancer patients looking into anticancer action including progression survival, overall survival rate, radiological response, quality of life, tolerability and safety profile in combination with palliative chemotherapy treatment.

1.10 Cancer and inflammation

Chronic inflammation has been shown in a number of clinical states to promote cancer development. The proposed mechanisms are mediated at a cellular level by the interplay between pro- and anti-inflammatory cytokines. The most well studied of the pro-inflammatory cytokines are IL-1, IL-6, and TNF-α. All have been associated with an increase in cell proliferation, disease progression and metastasis, in both in vitro and in vivo studies [284,285,286]. In colorectal cancer, the anti-inflammatory effect of omega-3 PUFAs has been shown to be mediated through inhibition of these cytokines.

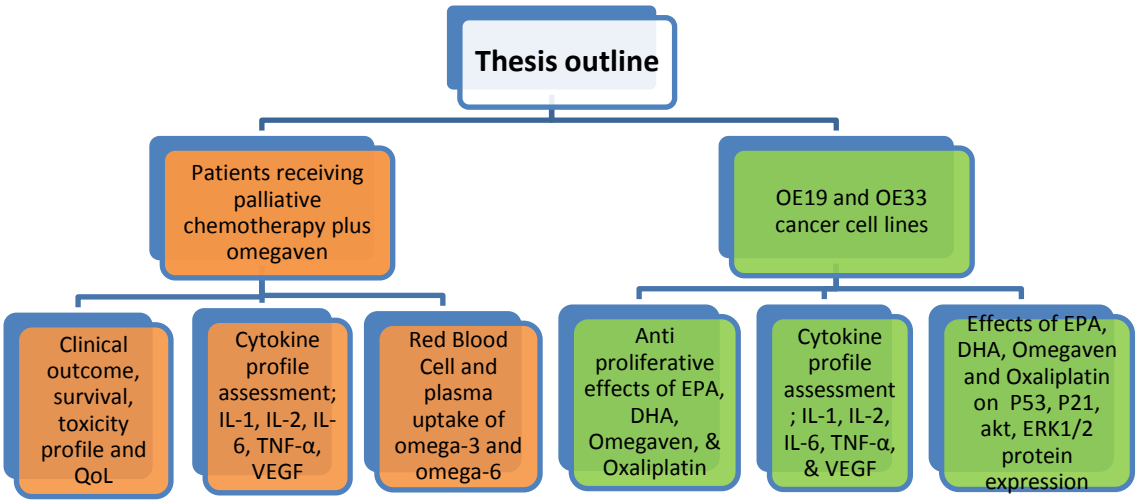
Chapter Two

Overview of the research work

2 Overview of research work

The work contained within this thesis explores the effects of omega-3 fish oils on oesophageal and gastric cancer, briefly outlined in Figure 12. Chapters 4 and 5 report on a cohort of patients receiving palliative chemotherapy, for oesophageal or gastric adenocarcinoma, in conjunction with omega-3 fatty acid infusion. Chapter 4 reports in detail the clinical effects of palliative chemotherapy plus omega-3 fatty acids intravenous supplement, compared to a historical control group of patients who received palliative chemotherapy alone. It focuses on the cellular effects of the omega-3 fatty acids, in particular the effects on the pro inflammatory cytokine pathways. Quality of life is reported for the patient cohort. In Chapter 5, the emphasis is on the red blood cell and plasma uptake of omega-3 fatty acids. Chapter 6 details translational work which focuses on the effects of EPA, DHA, Omegaven and oxaliplatin on two oesophageal cancer cell lines. Chapters 7 and 8 discuss the implications of the study findings and explore potential avenues for future work.

Figure 12: An overview of the studies in the thesis



2.1 Aims and Objectives

2.1.1 Clinical Study

2.1.1.1 Primary aim

The primary aim of the clinical study was to assess the anti-cancer activity of the combination of omega-3 fish oil with standard EOX palliative chemotherapy using as the primary endpoint of the study, progression free survival at 6 months.

2.1.1.2 Secondary aims

Secondary aims of the clinical study were to

1. To determine the toxicity profile of the combination of omega-3 fish oil with standard palliative epirubicin, oxaliplatin and capecitabine chemotherapy
2. To determine the complete and partial response rates (as per RECIST v1.1 criteria) to treatment
3. To determine overall survival (time from enrolment to death).
4. To determine the quality of life, pain ratings and the health status of patients using the EORTC QLQ-C30 questionnaire.

2.1.2 Translational work

The aims of the translational work were

1. To determine the uptake of ω-3/6 fatty acids and their ratios in the cellular membranes of plasma and red blood cells to investigate if the infusion causes changes at the cellular level.
2. To determine the effects of omega-3 PUFAs on the systemic cytokine levels, notably those known to be associated with pro- or anti-inflammatory properties (TNF-α, IL-1, IL-2, IL-6, VEGF).

To determine the effects of omega-3 PUFAs on two oesophageal cancer cell lines (OE19, OE33).

Chapter Three

Patients and Methods

3 Patients and Methods

The same patient cohort was used for the clinical trial and the associated translational work described above.

3.1 Clinical trial

3.1.1 Study design and sample size

This was a pilot and feasibility prospective, single arm phase II clinical trial, evaluating the effect of adding an intravenous (IV) infusion of omega-3 PUFAs to conventional platinum-based palliative chemotherapy in participants with advanced oesophago-gastric cancer.

As this was a pilot and feasibility study, the sample size was selected on pragmatic grounds to make an estimate of recruitment, retention and drug toxicity, while not exposing too large number of participants to the full range of experimental procedures. The power calculation was based on Simon's two stage model [287]. The expected proportion of patients with progression free survival at 6 months was estimated to be 50% (median PFS from standard care with EOX). A 20% improvement with intervention with fish oil (derived from experience in pancreatic cancer study [288]) would increase this proportion to 70%. The first stage of this clinical trial is reported in this thesis. Using these values in Simon's model and tolerating α and β error limits of 0.1 and 0.1 respectively the following study subject numbers had been derived. In the first stage up to 21 participants would need enrolment. If 11 or more of these achieved six months or more progression free survival, then the second stage of

recruitment should be completed, enrolling a further 24 participants to a total of 45. If 26 or more patients were to achieve a six months progression free survival after the second stage, this would be justification for progressing to a phase III study.

3.1.2 Patient inclusion and exclusion criteria

Eligible participants were patients with histologically confirmed gastric or oesophageal adenocarcinoma, deemed incurable as a result of standard staging investigations, after discussion at the weekly Upper Gastrointestinal Multi-Disciplinary Team meeting at the Leicester Royal Infirmary. Inoperability was determined by either the presence of locally advanced or metastatic disease.

The clinical trial recruited adult patients who provided written informed consent. Inclusion criteria for the study included: measurable disease according to RECIST 1.1 criteria on a CT scan within four weeks of study entry, a WHO performance status between 0 and 2, an estimated life expectancy greater than 12 weeks and adequate bone marrow function documented within 7 days. Women of childbearing age were required to have a negative pregnancy test (urine or serum) at the commencement of treatment.

A detailed list of the inclusion and exclusion criteria is given in Appendix A.

3.1.3 Recruitment

3.1.3.1 Intervention group

Research Ethics Committee approval for the study was granted by the NRES Committee East Midlands - Nottingham 2 in January 2012 (reference number

11/EM/0412) and the medicines and healthcare products regulation agency approval (reference number 21275/0288/001-0001) in 25th of February 2012. Recruitment opened in May 1st 2012 and closed for the current interim analysis in July 31st 2013. Eligible participants were identified at the multi-disciplinary meeting and offered a participant information sheet (Appendix E) at the first Oncology clinic visit. A minimum of 24 hours later potential participants were contacted to enquire about potential trial participation and before any trial related procedures were undertaken, the patient's written informed consent (Appendix F) was obtained. Participant follow up was carried out for a year from the date of the last treatment, disease progression or death. As this was a single arm clinical trial, the clinical outcome was compared to a historical control cohort of patients to provide an estimate of clinical efficacy.

3.1.3.2 Historical controls

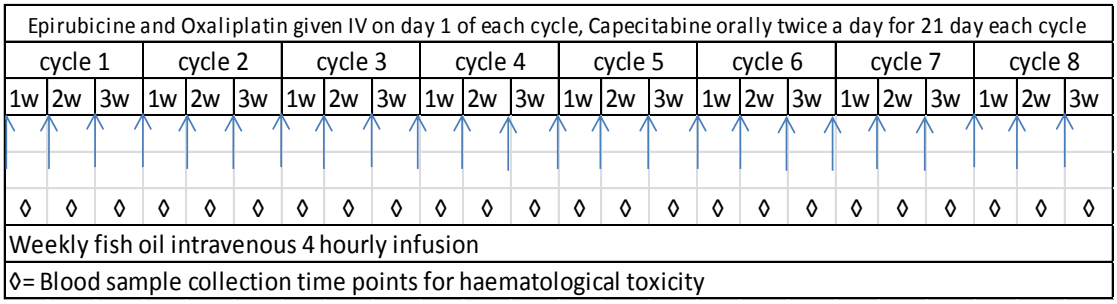
Patients who had received palliative platinum based chemotherapy for advanced oesophago-gastric adenocarcinoma between January 2010 and December 2011 were identified as a potential comparator (control) group for the purpose of comparing treatment toxicity, radiologic response where evaluable and survival. These patients were identified from the Department of Oncology clinical database and case notes reviewed in order to ascertain eligibility for inclusion in the control group. Inclusion criteria were similar to the intervention arm (EOX or ECX chemotherapy, treatment with palliative intent). Institutional Clinical Audit Standards and Effectiveness team approval was granted for this. Individual patient consent was not required as patients

were not subject to any trial intervention. Patients with squamous carcinoma were excluded from the comparator group. No attempts were made to match the two groups,

3.1.4 Intervention

Trial participants received palliative chemotherapy with intravenous Epirubicin (50 mg/m^2) and Oxaliplatin (130 mg/m^2) every 21 days, and oral capecitabine (1250 mg/m^2) daily for 21 days [289]. Treatment was capped at a body surface area of 2 m^2 to avoid toxicity and overdosing in obese patients, this was the standard practice in the UK. As part of the trial, this regimen was coupled with parenteral Omega-3 fatty acids (2 ml/Kg given over 4 hours) (Omegaven® Fresenius-Kabi). The Omega-3 fatty acid infusion was administered immediately after the chemotherapy treatment on day 1 of each cycle, and then on days 8 and 15, Figure 13. Participants started treatment within 4 weeks after baseline CT was performed.

Figure 13: Overview of timing of Omegaven® infusion and blood sample collection.



Chemotherapy (epirubicin, oxaliplatin, capecitabine) was administered in three weekly cycles, for up to eight cycles. Omegaven® was administered on a weekly basis, for up to 24 weeks

3.1.5 The clinical trial treatment and dose modification

3.1.5.1 Epirubicin, Oxaliplatin and Capecitabine

3.1.5.1.1 Dose modification

Standard dose delays and modifications of the EOX regimen were adopted as per local guidance and in line with the summary of product characteristics. These advised EOX treatment delay for up to 14 days if bone marrow recovery was delayed (absolute neutrophil count less than 1.5 x 10^9/l or platelet count less than 100 x 10^9/l on day 1 of the cycle). Treatment was also delayed for up to 14 days for any grade 2 adverse events that had not resolved to grade 0/1 on day 1 of the cycle, with the exceptions of alopecia, and nausea and vomiting when inadequate anti-emetics had been taken). Where two dose delays for the same grade 2 toxicity occurred, a 20% dose reduction in the EOX chemotherapy was applied.

If the absolute neutrophil count was less than 1.0 x 10^9/l or the platelet count was less than 50 x 10^9/l on day 1 of any cycle, a 20% dose reduction in the EOX chemotherapy was applied.

For any other grade 3 or 4 non-haematological and haematological toxicity; EOX was delayed for up to 14 days, and a 20% dose reduction of EOX was recommended when toxicity had resolved.

If further grade 3 or 4 toxicity occurred while patients received 20% dose reduction, a further 20% dose reduction was applied after an appropriate delay (as above). If toxicity recurred again, the EOX was permanently discontinued.

3.1.5.2 Omega-3 fatty acids (Omegaven®)

As part of the trial, the above chemotherapy regimen was coupled with intravenous infusion of omega-3 PUFAs as Omegaven® (Fresenius Kabi, Bad Homburg, Germany). We anticipated that many of these patients with dysphagia would find it an additional challenge, ingesting oral capsules. Hence, intravenous route was used to administer the omega-3 PUFAs (Omegaven®). The latter was infused once weekly at a rate of 2 ml/kg body weight for 4 hours (i.e., 140 ml for 4 hours in a 70 kg patient). Omegaven® is a 10% fish oil lipid emulsion containing 1.25 to 2.82 g/100 ml EPA and 1.44 to 3.09 g/100 ml DHA, to administer 0.2 ml lipid/kg body weight for 4 hours. Chemical analysis by gas chromatography revealed the EPA and DHA contents of the batch of Omegaven® used in the current study to be 2.0 and 2.3 g/100

ml, respectively. Thus, patients received 0.04 and 0.046 g EPA and DHA/kg body weight for each 4 hour infusion; in a 70 kg patient this would equate to 2.8 g EPA and 3.2 g DHA during each infusion. Omegaven® was administered via a peripheral venous line immediately after the chemotherapy treatment on day 1 of each cycle and then again on days 8 and 15 of the cycle.

3.1.5.2.1 Omegaven® dose modifications

Dose interruptions were used to manage toxicity or an elevated triglyceride level (> 3 mmol/l). No dose reductions were possible. If dose interruptions to the EOX chemotherapy occurred, treatment with Omegaven® was likewise interrupted.

3.1.5.2.2 Omegaven® infusion side effects

Patients were monitored for any signs or symptoms of Omegaven® side effects and if identified, the infusion was discontinued. Table 14 summarises the known side effect profile for Omegaven®, as reported in the latest summary of products characteristics.

Table 14: Omegaven® side effects

	Uncommon (≥1/100 to ≤1/1000)	**Very rare (≥1/10,000),**
Toxicities	Abdominal pain, nausea, vomiting, pyrexia, shivering, chills, tiredness, headache, and hypertriglyceridemia	Anaphylaxis, thrombocytopenia, haemolysis, reticulocytosis, transient increase in liver function test, rash, urticaria, hypotension, hypertension and priapism

3.1.5.2.2.1 Management of Omegaven® toxicity

Anti-emetics were administered if nausea and vomiting occurred on an as required basis. Any evidence of hypersensitivity resulted in discontinuation of therapy.

Any other treatment, which was considered necessary for the patient's safety and well-being, was administered at the discretion of the investigators and in accordance with the British National Formulary recommendations.

3.1.6 Drug storage and accountability

Omegaven® stock was stored in a secure place under appropriate storage conditions at the Leicester Royal Infirmary pharmacy located in the Windsor Building of the campus.

3.1.7 Permitted concomitant medications/procedures

Prior to receiving the chemotherapy regimen, patients were pre-medicated with anti-emetics. The employed agents included 5-HT3 antagonists (Ondansetron 4-8mg) and dexamethasone 8mg as per BNF recommendations. Diarrhoea occurring within 24 hours of administration of EOX chemotherapy was managed with loperamide. An initial dose of 4 mg was given, followed by 2 mg with each loose bowel action. Loperamide was continued for up to 5 days at a maximum daily dose of 16 mg.

3.1.8 Non-permitted concomitant medications/procedures

The interventional use of growth factors was not allowed, including erythropoietin. Any patient taking warfarin was converted to low molecular weight heparin for the duration of the study.

3.1.9 Follow up schedule

Patients who remained free of evidence of disease progression were followed up every 9-12 weeks. At each visit participants had a full clinical examination, recording tumour related signs and symptoms and an assessment of performance status, dysphagia score. Participants were asked to complete the EORTC QLQ-C30 quality of life questionnaire. Follow up continued until there was evidence of disease progression or until 12 months from last treatment had been completed. Latest participant outcome was determined from hospital records and the NHS Information Centre

Adverse events considered to be serious by the investigator or suspected to be related to the Omegaven® were followed up after the treatment discontinuation until the event had resolved or stabilised. All serious adverse events were reported to the Research and Development department as per the study protocol.

3.1.10 Trial Assessment:

Table 15 summarises the schedule of test visits and assessments.

3.1.10.1 Radiologic response assessment

EOX plus fish (intervention) group: As per the trial protocol, staging computerised tomography (CT) scans were obtained after chemotherapy cycles three, six and eight, and three months following completion of treatment (Table 15). Patients in whom chemotherapy was discontinued before completion of the third cycle were not routinely scanned.

EOX alone (Historical control) group: CT scans were performed as part of routine clinical practice after chemotherapy cycles three and six. After completion of treatment, additional CT scans were performed only if clinically indicated on symptomatic grounds.

All reports were according to the latest version of Response Evaluation Criteria in Solid Tumours (RECIST v1.1, Appendix B) and performed by single Oncology interest Radiologist.

Radiologic response was assessed in trial participants who received at least three cycles of treatment. Response from baseline was categorised as complete response, partial response, stable disease or progressive disease. Tumour evaluation by ultrasound scan or positron emission tomography (PET) scan was not employed for the assessment of radiologic response.

Overall survival was defined as the interval between enrolment into the study and death. Progression free survival was defined as the interval between trial enrolment and radiologically proven disease progression or death. In order

to provide an estimation of the treatment efficacy, the patients who included in the historical group were evaluated for radiological response.

Table 15: Trial Assessment

Planned visit/cycle	Enrolment	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Completion	Follow up	Symptomatic disease progression
Week	-4 to 0	0	3	6	9	12	15	18	21	24	36	
Informed consent	X											
Medical history	X											
Inclusion/exclusion criteria	X											
Physical examination	X	X	X	X	X	X	X	X	X			
Weight, temperature, blood pressure and pulse	X	X	X	X	X	X	X	X	X			
Electrocardiogram (patients with cardiovascular disease)	X											
Clinical chemistry	X	X	X	X	X	X	X	X	X			
Haematology	X	X	X	X	X	X	X	X	X			
Pregnancy test	X											
ECOG Performance status	X	X	X	X	X	X	X	X	X			
Radiological and Clinical Tumour Assessment (RECIST v1.1)	X				X			X		X	X	X
EOX-Omegaven® treatment cycle commences		X	X	X	X	X	X	X	X			
Adverse events review		X	X	X	X	X	X	X	X	X		
Concomitant medication	X	X	X	X	X	X	X	X	X	X		
Cytokine, fatty acid uptake blood samples	X	X	X	X	X	X	X	X	X	X		
Optional blood tests (i.e. clotting)	X	X	X	X	X	X	X	X	X			
EORTC-C30 quality of life	X	X	X	X	X	X	X	X	X	X	X	
Dysphagia score	X	X	X	X	X	X	X	X	X	X	X	

X= assessment was carried out, blood samples were collected weekly per each cycle

3.1.10.2 Blood samples and laboratory procedures

3.1.10.2.1 Routine laboratory blood samples

The following haematological and biochemical tests were performed for monitoring of treatment toxicity: full blood count, coagulation profile, urea and electrolytes, liver function test, triglyceride levels. All samples were processed by the University Hospitals of Leicester pathology laboratory. Results were collected on a weekly basis.

3.1.10.2.2 Serum pro-angiogenic, inflammatory cytokine and principles of the ELISA assay

3.1.10.2.2.1 Blood sample collection and serum separation

One 7.5 ml serum gel blood bottle was used to collect whole blood samples immediately before (baseline) and after each treatment from all patients who received at least one experimental treatment. The blood was centrifuged at 1000G for 15 minutes at 4 °C and 500 µl aliquots of serum were collected and stored at - 80 °C until analysis was performed. Multi-spot ELISA kit assay were conducted in accordance with the Meso Scale Discovery, LLC analysis protocol.

3.1.10.2.2.2 ELISA Assay

The following serum cytokine markers were assessed using ELISA: interleukin (IL)-6, IL-1, IL-2, VEGF and TNF-α.

All samples were processed in duplicate using 96 well plates (8 rows and 12 columns), the first two columns being used for calibration and the remaining

columns used for sample and control alternatively. Fifty microliters of diluted sample and calibrator (standard) were added to the 96 well plates, each plate was then sealed with adhesive plate seal and incubated at room temperature for 90 minutes, while it was agitated. The plate was irrigated three times with a minimum of 150 µL/well of wash buffer. Then 25 µL of detection antibody was added to each well, and the plate was sealed and once again incubated at room temperature for 30 minutes, while being agitated. The plates were irrigated once again with a minimum of 150 µL/well of wash buffer, 150 µL of read buffer T was added to each well and the plates were then read using the meso scale discovery reading instrument [290].

3.1.10.2.3 Polyunsaturated fatty acid uptake assessment (Gas chromatography)

3.1.10.2.3.1 Blood sample collection

Plasma was prepared from all blood samples while RBCs were prepared only from the pre-infusion blood samples. Blood was collected into EDTA blood bottles and plasma isolated by centrifugation at 1300 x g for 10 minutes. RBC membranes were isolated from the pellet by addition of serial dilutions of phosphate buffer saline (PBS) and centrifugation after each at 1300 x g for 10 minutes. All samples were stored at - 80 ˚C until analysed. Clinical outcomes will be reported separately.

3.1.10.2.3.2 The principles of GC

Gas chromatography (GC) allows separation of the fatty acids by exploiting differences in the temperature at which, each fatty acid becomes volatile. The

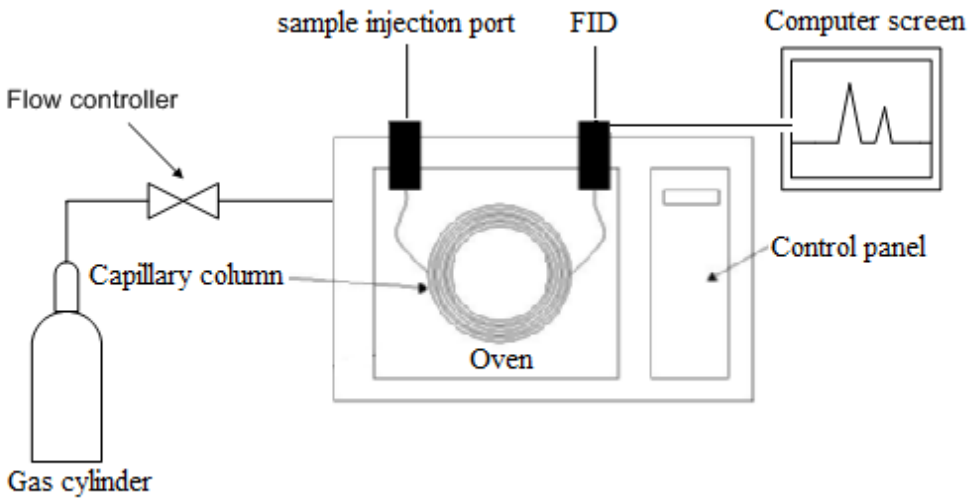
latter depends on the length of carbon chain, and the number and position of double bonds within the compound.

Increasing the length of the fatty acid carbon chain has the effect of raising the temperature at which the compound vaporises. Conversely, the greater the number of double bonds, the lower is the boiling point.

3.1.10.2.3.3 Gas chromatograph apparatus structure and mechanism of FAs analysis

It comprises a heated injection port, a fused silica capillary column located within a high temperature oven (250-300°C) and flame ionisation detector (FID) held at 250°C Figure 13.

Figure 14: Schematic diagram of gas chromatography apparatus



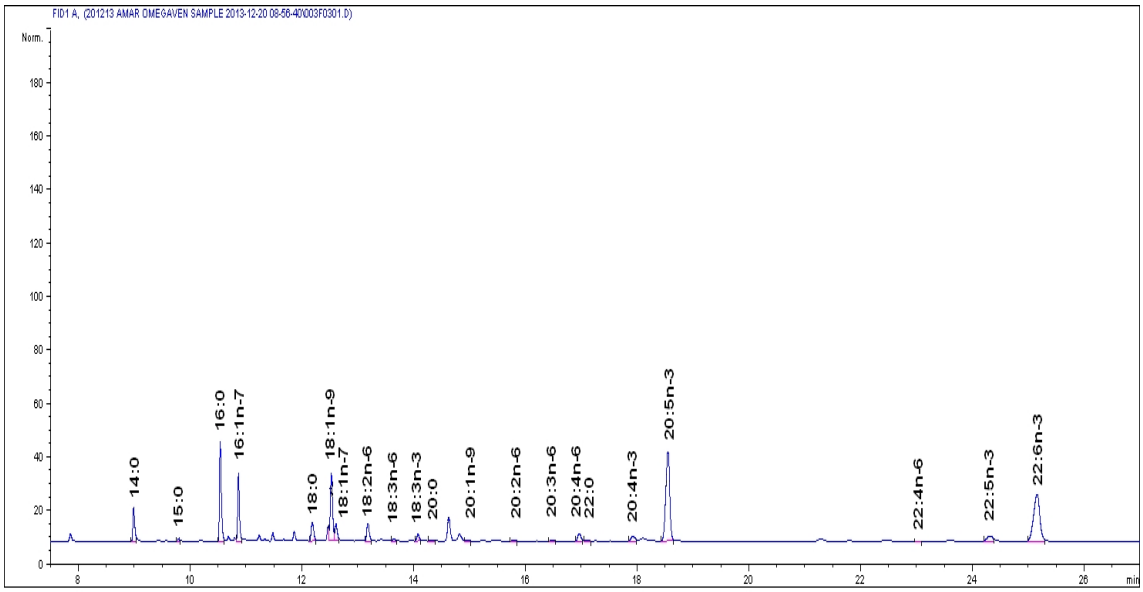
3.1.10.2.3.4 Fatty acid analysis by gas chromatography

Total lipid was extracted from plasma and RBC membranes using chloroform : methanol (2:1 vol / vol); butylated hydroxytoluene (50 mg/l) was added as an antioxidant. NEFAs and PC were isolated from the plasma lipid extract by solid phase extraction (SPE) on Bond-Elute cartridges. The lipid extract was loaded onto the SPE cartridge and triacylglycerols and cholesteryl esters were eluted with chloroform and discarded. Next, PC was eluted with chloroform : methanol (60:40, vol / vol) under vacuum suction. Finally, NEFAs were eluted with chloroform : methanol : glacial acetic acid (100:2:2, vol / vol / vol) under vacuum suction. Plasma NEFAs, plasma PC and RBC membrane lipids were dried down under nitrogen at 40°C and then redissolved in 0.5 ml of dry toluene. Then fatty acid methyl esters (FAMEs) were formed by reaction with methanol containing 2% (vol / vol) sulphuric acid and heating at 50°C for two hours. After cooling and neutralisation with potassium bicarbonate ($KHCO_{3)}$ and potassium carbonate ($K_2CO_{3)}$, FAMEs were extracted into hexane.

The following steps of FAME separation / analysis using Gas chromatography were performed by Professor P Calder and Mrs H Fisk at the Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton. FAMEs were separated and identified by gas chromatography on a Hewlett Packard 6890 gas chromatograph fitted with a BPX-70 column. The inlet temperature was 300 °C. The oven temperature was initially 115 °C and this was maintained for 2 min after injection. The oven temperature was programmed to increase to 200 °C at the rate of 10 °C/min to

hold at 200 °C for 16 min and increase to 240 °C at the rate of 60 °C/min to hold at 240 °C for 2 min. The total run time was just longer than 29 min. Helium was used as the carrier gas. FAMEs were detected by using a flame ionization detector held at a temperature of 300 °C. The instrument was controlled by, and data collected, with HPChemStation software (Hewlett-Packard). FAMEs were identified by comparison of retention times with those of authentic standards run previously. A chromatograph generated by the gas chromatography of one of the trial samples derived FAMEs (Figure 14). The following omega-3 and omega-6 PUFAs were identified - omega-3 PUFAs: α-linolenic acid, eicosatetraenoic acid, clupanodonic acid (aka docosapentaenoic acid), EPA and DHA; omega-6 PUFAs: linoleic acid, gamma linolenic acid, eicosadienoic acid, dihomo-γ-linolenic acid, adrenic acid and arachidonic acid.

Figure 15: A chromatograph generated by the gas chromatography of one of the trial samples derived FAMEs.



X axis showing time in minutes and Y axis shows the signal current in picoAmps. Each peak is corresponding to a different FAME. The relative proportions of the concentrations of an individual FAME present are equal to the relative proportions of the areas under each peak.

3.1.10.3 Patients reported outcome

3.1.10.3.1 Data collection and Interpretation of scores

All patients in the intervention group were asked to complete EORTC QLQ-C30 questionnaires (Appendix C) on a three weekly basis while Omegaven® infusion was taking place. EORTC QLQ-C30 v3 is the standard version of QLQ-C30; it is composed of multi-item scales and single item measures. These scales include five functional, three symptom related, global health status/quality of life scale and six single items. The majority of questions were answered as four point scales namely ''not at all'', ''a little'', ''quite a bit'' and ''very much''. Two

questions (Q29 & Q30) were answered on a 7 point scale ranging from 1 (very poor) to 7 (excellent).

All the raw data from EORTC QLQ-C30 scores were transformed to scores ranging from 0 to 100, a functional scale high score represents a healthy level of functioning, a high score for quality of life (QoL)/global health status represents a better QoL, but a high score for a symptom scale/item represents a high level of symptoms. For details of score calculation and transformation see Appendix D.

3.1.11 Outcome measures and statistical analysis

The primary end point was progression free survival determined up to 6 months from the day of baseline CT scan.

The secondary end points included toxicity, tolerability (side effects) and feasibility of use (number of participants requiring dose delays and/or treatment withdrawal), objective response rate (presented as a percentage of the maximum longitudinal dimensional changes using RECIST criteria version 1.1) and the overall survival (OS) defined as time from enrolment to death. Analysis was performed on the intention to treat basis. Fisher's exact and the chi square test was used to compare proportions. Survival analysis was estimated using the Kaplan-Meier method and the log-rank test. Significance was assumed at the 5% level.

3.2 Cell culture

The two oesophageal cancer cell lines used were OE19 and OE33. OE19 is a human oesophageal cancer cell line derived from a 72 year old white male patient with moderately differentiated UICC stage 3 adenocarcinoma. The OE33 cancer cell line is derived from a 73 year old white female with UICC stage 2A lower oesophageal adenocarcinoma arising in a background of known Barrett's metaplasia. These cell lines were purchased from Public Health England cell collection (The European Collection of Authenticated Cell Cultures).

3.2.1 Maintenance of cell lines

Cell lines were cultured as a monolayer at 37 °C and 5 % CO_2. Both cell lines were cultured in RPMI 1640 medium (Sigma-Aldrich, UK) supplemented with 2 mM Glutamine and 10 % foetal bovine serum (FBS).

3.2.2 Cell passaging

Cell lines were passaged no more than 15 times following resuscitation from liquid nitrogen, to reduce the risk of phenotypic alterations. Passaging was undertaken once cells had reached approximately 80 % confluence as follows: Cells were washed with 10 mL pre-warmed (37 °C) PBS once, followed by addition of 5 mL of 1X trypsin for 5 minutes at 37 °C for cell detachment. The trypsinisation process was halted following addition of an equivalent volume of RPMI media containing 10 % FBS. Cells were pelleted at 400 x g,

resuspended in fresh medium containing 10% FBS, and aliquoted appropriately into cell culture flasks as per experimental requirements.

3.2.3 Drugs and Solvents

The treatments tested were EPA, DHA, Oxaliplatin (all from Sigma-Aldrich, UK), and Omegaven® (Fresenius Kabi, Germany). EPA and DHA stocks were prepared as 50 mM stocks dissolved in DMSO and oxaliplatin was prepared as a 50 mM stock dissolved in 5 % dextrose. All treatments including the vehicle control, received equivalent volumes of DMSO or 5 % dextrose. The Omegaven® emulsion consisted of a mix of EPA and DHA with concentration ranges of 1.25 - 2.82 g of EPA and 1.44 – 3.09 g of DHA as per the Omegaven® summary of product characteristics. In order to equate this mixture to treatment concentrations using the single agents, the emulsion was diluted in RPMI medium + 10 % FCS via serial dilution to make treatments of approximately 10-50 μM of EPA and DHA. The vehicle control received medium only.

3.2.3.1 EPA, DHA and Oxaliplatin treatments

OE33 and OE19 cell lines were grown in RPMI 1640 + 2 mM Glutamine + 10 % foetal bovine serum (FBS) medium for 24 hours, then the media was removed and replaced with medium containing 0-50 μM of each treatment and the cells incubated for a further 72, 96, 120 or 144 hours. The cell culture supernatant was collected at each time point and stored at - 80 °C for cytokine analysis. The cells were then harvested and counted.

3.2.4 Cell proliferation assays

Cell proliferation was undertaken using a Z2 particle size analyser (Beckman Coulter, UK) to count raw cell numbers; this was performed in both cell lines for comparison in triplicate. OE19 & OE33 cell lines were seeded into 24 well plates at a density of 2 X 10^3 cells/well in 1 mL of RPMI 1640 medium. Cells were incubated for 24 hours, and then the media was replaced with media containing the relevant treatments. Cells were then incubated for 72, 96, 120 and 144 hours before counting; Each well was washed x1 with 1 mL PBS and 0.5 mL of 1X trypsin added to each well, which was neutralised with 0.5 mL RPMI + 10 % FBS once cells had detached. The well contents were transferred to coulter count cups containing 9 mL of isoton solution (Beckman Coulter, UK) and cells were counted using the Z2 particle analyser.

3.2.4.1 Cytokine and cell signalling biomarkers analysis

3.2.4.1.1 Cell culture supernatant collection

Cell culture media supernatants was collected at 72, 96, 120 and 144 hours after incubation with treatment as described above and stored at - 80 °C for analysis of IL-1, 2, 6, TNF-α, VEGF using an ELISA assay.

3.2.4.1.2 Cell lysate preparation

The cell lines (OE19 and OE33) were seeded at 1.5 X 10^5 cells per 75 cm flask and allowed to adhere overnight. Cells were then treated with EPA, DHA, Omegaven® or Oxaliplatin at concentrations of 0-50 μM for 72 and 96 hours.

After 72 and 96 hours, all flasks were removed from the incubator and placed on ice. Cells were gently scraped using a sterile cell scraper, media containing cells was collected and cells pelleted (400 x g, 4 °C, 3 minutes). The supernatant was discarded, and the remaining cell pellet washed with 1 mL PBS. Cells were again pelleted, the PBS carefully aspirated and 100 µL of Roche Complete Lysis M (Roche Ltd, UK) cell lysis buffer added to the cell pellet. The pellet was mixed by vigorous pipetting and the cells lysed on ice for 10 minutes. Cell debris was pelleted and the supernatant stored at - 80 °C for later analysis of total Akt, Erk1/2, P53 and P21 proteins.

3.2.4.2 Cytokine analysis using Enzyme Linked Immunosorbant Assays (ELISA)

The previously collected cell culture supernatants were defrosted at room temperature. The manufacturer's mesoscale discovery instructions for cytokine analysis were followed. In brief, this was as follows:

All reagents were brought to room temperature, and an eight point standard curve (in duplicate) was prepared spanning concentrations from 10,000 pg /mL to 2.4 pg /mL, via serial dilution. The detection antibody solution was prepared by diluting the antibody 1:50 with diluent 100 solution.

All samples were processed in duplicates using 96 well plates (8 rows and 12 columns); the first two columns used for calibrator and the remaining columns used for sample as follow:

Twenty five µL of the diluted calibrator and sample was added to the 96 well plates. The plate was sealed with adhesive plate seal and incubated at room temperature with continues vigorous shaking for 1 ½ hour to increase binding rate of the sample to the capture antibodies.

Twenty five microliter of the 1X detection antibody solution was added to each well of the MSD plate, and then the plate was sealed with an adhesive plate seal and incubated at room temperature with continuous vigorous shaking for another hour.

The plates were then washed three times with PBS + 0.05 % Tween-20.Following this, 150 µL of X2 read buffer T was added to each well of the MSD plate. The plate was analysed using the MSD reading device immediately after adding the reading buffer T.

3.2.4.3 Prototype protein ELISA assay (cell lysate)

The cell lines (OE19 and OE33) lysate was prepared as described above. The following proteins were measured; total P53, P21, Akt, and Erk1/2.

All reagents were brought to room temperature and the stock calibrator thawed on ice. All samples were processed in duplicates using 96 well plates (8 rows and 12 columns); the first two columns used for calibrator and the remaining columns used for cell lysate samples as follows:

After each of the following steps the MSD plate was washed with 150 µl/well with the Tris wash buffer 1X at least three times.

A hundred and fifty microliter of blocker A solution was added to each well, the plate was sealed with an adhesive plate seal and incubated for 1 hour at room temperature with continuous shaking.

Twenty five µL of the diluted cell lysate sample and calibrator were added to the allocated wells in the plate, and the plate was sealed again and incubated at room temperature for another hour with continuous shaking.

Then 25 µL of detection antibody solution was added to each well, and the plate sealed again and incubated at room temperature for another hour with shaking.

Finally, 150 µL of the read buffer T was added to each well and the plate was immediately placed on the MSD plate reading instrument to measure each protein concentration.

Chapter Four

The effects of supplementary omega-3 fatty acids on the clinical outcome of patients with advanced oesophago-gastric adenocarcinoma receiving palliative epirubicin, oxaliplatin and capecitabine chemotherapy

4 The effect of supplementary omega-3 fatty acids on the clinical outcome of patients with advanced oesophago-gastric adenocarcinoma receiving palliative epirubicin, oxaliplatin and capecitabine chemotherapy

4.1 Introduction

The 2013/2014 United Kingdom National Oesophago-Gastric Cancer Audit indicated that 38% of patients received potentially curative treatment, 48% received palliative treatments, and 15% received best supportive care only. Of those that received palliative treatments, palliative chemotherapy was employed in two-thirds [62,63]. In the UK, the most widely used drug combinations are epirubicin, oxaliplatin and capecitabine. Only half of these patients will complete their planned programme of treatment, because of disease progression or chemotherapy related toxicity [62,63].

Omega-3 polyunsaturated fatty acids (PUFA) are natural nutritional products derived mainly from fish and fish oil. They have strong anti-inflammatory effects, acting mainly by blocking the release of the pro-inflammatory mediators derived from omega-6 fatty acids [151]. This anti-inflammatory effect acts on and inhibits the inflammatory pathway crucial for cancer development and metastasis [140]. A number of studies have reported the favourable effects of omega-3 PUFAs in a number of cancer settings. Findings have included improved quality of life [271,273,280], reduced toxicity [272,291] and improved prognosis [278,280,281]. There is little information of their application in the palliative setting to patients with advanced oesophago-gastric cancer [292].

The cancer microenvironment is a complex setting comprised of tumour cells, fibroblasts and endothelial cells. Cytokines are the key elements controlling communication between these cells [293]. These inflammatory biomarkers are released in the cancer microenvironment and have been identified in various human cancer cells. Tumour angiogenic and pro-inflammatory cytokines include IL-1, IL-6, TNFα and VEGF. These are considered to play a major role in cancer progression, survival and metastasis [140].

4.1.1 Statistical analysis

Analysis for the clinical study was performed on the intention to treat population. Fisher's exact and the chi square test was used to compare proportions. Survival analysis was estimated using the Kaplan-Meier method, and significance was assumed at the 5% level.

In order for accurate presentation of the actual changes in serum cytokine concentrations, a statistical model was developed, that used a random coefficient model xtmixed in STATA software. This statistical model applied linear regression in which both slope and intercept were allowed to vary between each patient. The logarithms of cytokine concentrations are indicated on the y axis and the week of treatment on the x axis, each slope represents the best fit concentration for each patient. If the slope of the graph is from left to right, this indicates a negative coefficient or a reduction in cytokine concentration. In contrast, if the slope of the graph is upward from left to right, this indicates a positive coefficient or an increase in cytokine concentration.

In order to explore the potential relationship between baseline levels of pro-inflammatory biomarkers and overall survival, the cohort was subdivided into those who were high (top 50%) and low (bottom 50%) cytokine expressers based on the median concentration value of each cytokine. Kaplan-Meier curves were applied to determine if these baseline markers influenced overall survival (OS).

The quality of life was assessed using the European Organization for Research and Treatment of Cancer (EORTC QLQ-C30) questionnaire and its

modules; it is composed of both single item measure and multi-item scales. These measures include functional scales, global health status/QoL measure, the symptom scale and six single items.

As described in QLQ-C30 scoring manual, all raw QLQ-C30 scores transformed to scores ranging from 0-100, a higher scale represents a high response level; functional scale high score represents a healthy level of functioning, a high score for quality of life (QoL)/global health status represents a better QoL, but a high score for a symptom scale/item represents a high level of symptoms [294].

4.2 Results and discussion

4.2.1 Clinical outcome

4.2.1.1 Patient demographics

Fifty-six patients with advanced oesophago-gastric adenocarcinoma were identified from the weekly upper gastrointestinal cancer multi-disciplinary team meeting were screened for trial inclusion. Twenty-one patients met the inclusion criteria and agreed to participate. Sixty patients medical notes were screened for the inclusion criteria and thirty seven patients were included in the historic control group. The reasons for exclusion are indicated in Figure 16.

4.2.1.1.1 EOX plus fish oil (Intervention) Group

Twenty participants completed at least one cycle of treatment and formed the basis of this study. One participant experienced a decline in performance status after enrolment and declined any further oncology treatments. The demographic characteristics of the trial participants are indicated (Table 16). Recruitment and retention rates were 100% (21 of 21 eligible participants) and 95% (20 of 21 participants) respectively.

4.2.1.1.2 EOX alone (Historical control) Group

The case notes of 60 patients who had received palliative chemotherapy for oesophago-gastric cancer during the specified time period were reviewed. Thirty-seven patients met the inclusion criteria and they formed the control

group for comparison of radiologic and clinical outcome information only (Table 16).

Figure 16: Consort chart showing patient disposition

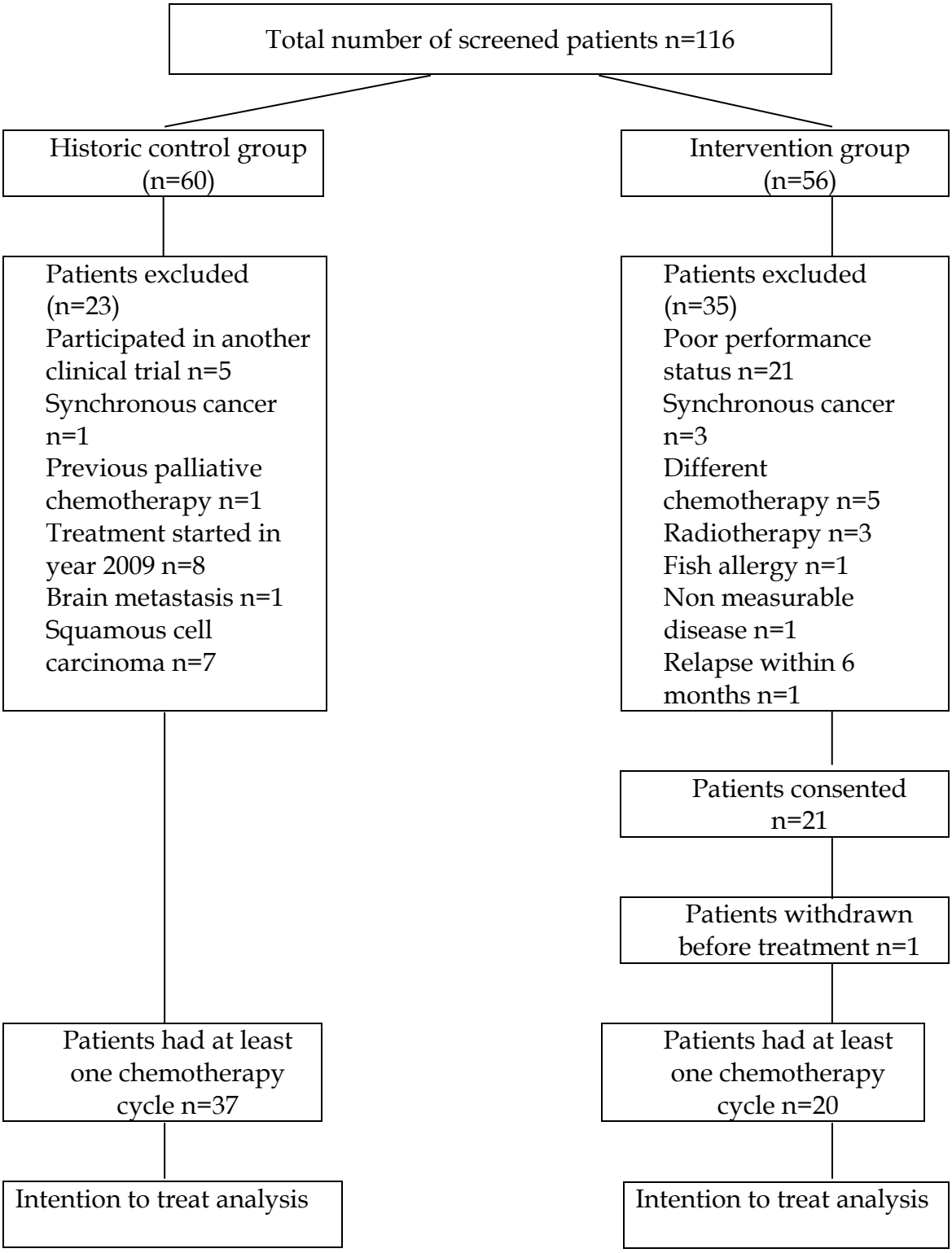


Table 16: Demographic characteristics of the EOX plus fish oil (intervention) and EOX alone (historical control) groups

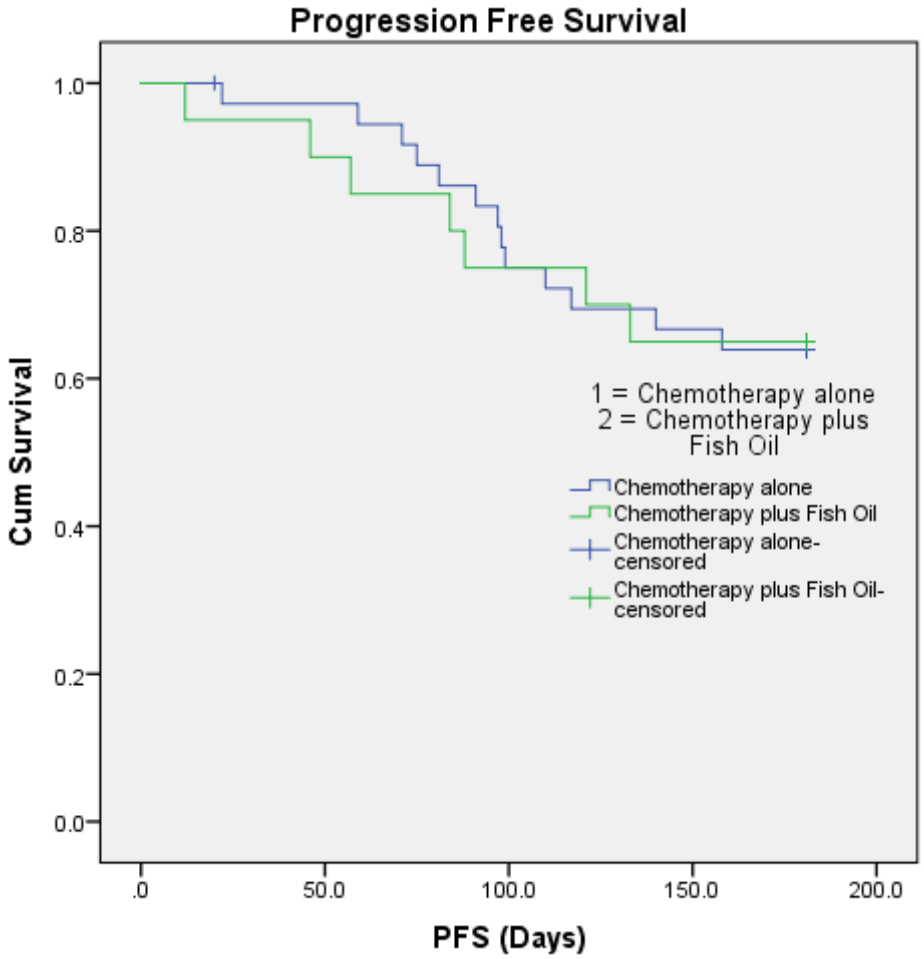
Patient characteristics			
Demographics		EOX alone n=37	EOX + Fish oil n=21
Gender	Male	26(70%)	16(76%)
	Female	11 (30%)	5(24%)
Age	(median 'years')	(66)	(67)
	Range	36-81	47-80
	>60 years	8(22%)	16(76%)
	<60 years	29(78%)	5(24%)
Performance status	0	15(40.5%)	8(38%)
	1	15(40.5%)	9(43%)
	2	7(19%)	4(19%)
Baseline Weight	Median weight in Kg (range)	70.6 (43.1-105.7)	76.5 (49.0-110.6)
Tumour site	Oesophagus	10(27%)	11(52%)
	Gastro-oesophageal junction	11(30%)	5(24%)
	Stomach	16(43%)	5(24%)
Stage	Stage 3	3(8%)	3(14%)
	Stage 4	34(92%)	18(86%)
Site of metastasis	Local or LN metastasis	8(22%)	5(24%)
	One distant organ	19(51%)	11(52%)
	Two or more organs	10(27%)	5(24%)
Number of chemotherapy cycles (median)		160 (5)	91 (6)
Number of patients completing 4 cycles (%)		23 (62%)	12 (60%)
Number of patients completing 6 cycles (%)		16 (43%)	11 (55%)

LN= lymph node

4.2.1.2 Survival analysis

Eleven of the 15 evaluable patients in the intervention group achieved at least six months progression free survival, satisfying the original trial criteria for progression to the second stage of recruitment. This interim report did not identify any statistically significant difference in either progression free (p=0.97) Figure 17, or overall Survival (p=0.69), Figure 18. These findings were similar to those previously reported by Jones *et al* in 2008, when they investigated paclitaxel plus DHA and found no survival benefits when compared to paclitaxel or docetaxel alone [272]. The intravenous use of omega-3 PUFA (Lpidim®) in advanced pancreatic cancer was previously investigated and resulted in improved survival [288] and the use of oral EPA supplements was seen to improve survival in two studies with advanced pancreatic cancer [278,280].

Figure 17: Progression free survival (6 months) of patients received palliative Chemotherapy plus Fish oil vs Chemotherapy alone (historic group), P=0.97 (Log Rank)



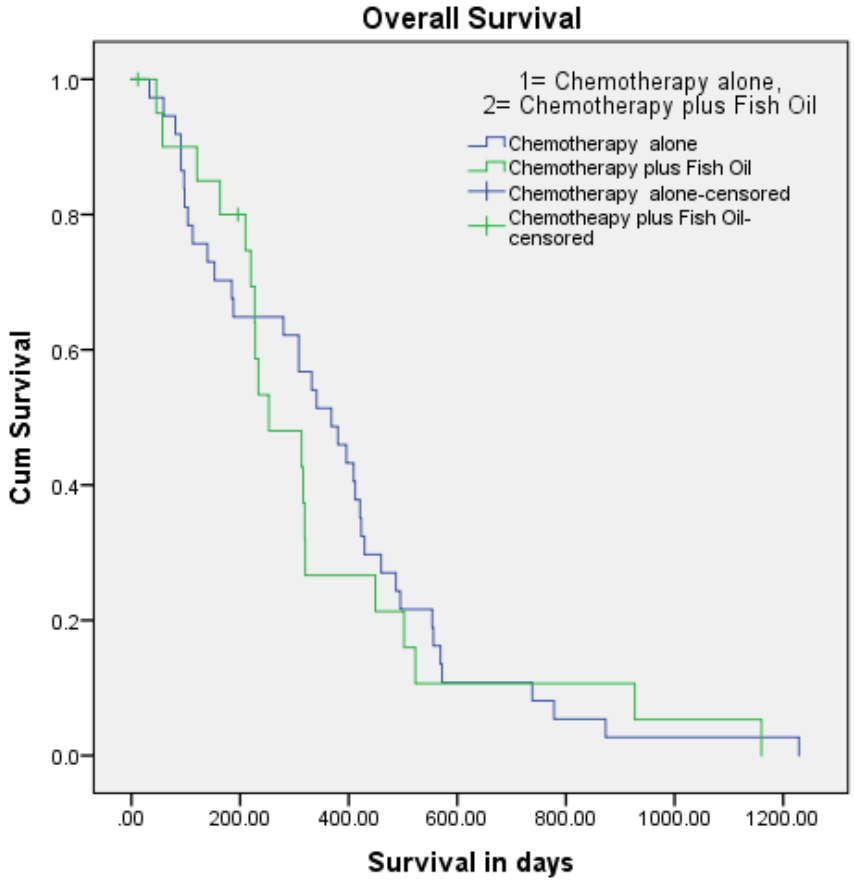
1 = Chemotherapy alone (n=37) 2 = Chemotherapy plus Fish Oil (n=20)	Mean[a] Survival			
			95% Confidence Interval	
	Estimate	Std. Error	Lower Bound	Upper Bound
Chemotherapy alone	149.472	7.750	134.283	164.661
Chemotherapy plus Fish Oil	144.700	12.224	120.741	168.659

a. Estimation is limited to the largest survival time if it is censored.

Case Processing Summary

1 = Chemotherapy alone 2 = Chemotherapy plus Fish Oil	Total N	N of Events	Censored	
			N	Percent
Chemotherapy alone	37	13	24	64.9%
Chemotherapy plus Fish Oil	20	7	13	65.0%
Overall	57	20	37	64.9%

Figure 18: Overall survival of patients received palliative Chemotherapy plus Fish oil vs Chemotherapy alone (historic group), median overall survival 253 vs 368 days, P=0.69 (Log Rank).



1= Chemotherapy only (n=37) 2= Chemotherapy plus fish oil (n=21)	Median Survival time (Days)			
			95% Confidence Interval	
	Estimate	Std. Error	Lower Bound	Upper Bound
Chemotherapy alone	368.000	53.509	263.123	472.877
Chemotherapy plus Fish Oil	253.000	60.851	133.733	372.267

Case Processing Summary

1 = Chemotherapy only (n=37) 2 = Chemotherapy plus fish oil (n=21)	Total N	N of Events	Censored	
			N	Percent
Chemotherapy alone	37	37	0	0.0%
Chemotherapy plus Fish Oil	21	19	2	9.5%

4.2.1.3 Radiological tumour response

Fifteen of the 21 patients in the intervention arm were evaluable for radiological tumour response. Among the historic control group 28 patients were evaluable for radiological tumour response. Six participants in the intervention group and 9 patients in the historical control group did not have radiological tumour response assessment, because of either clinical disease progression or discontinuation of chemotherapy prior to completion of the third cycle. There was a higher frequency of radiologic response among those treated with chemotherapy and omega-3 fatty acids ((overall response: 73% (95% CI 51 to 95) vs 43% (95% CI 25 to 61), p=0.05; partial response: 73% (95% CI 51 to 95) vs 39% (95% CI 21 to 57), p=0.03)), Table 17. There were no previous studies reported similar promising findings in the management of advanced oesophago-gastric adenocarcinoma [292]. However, this has been reported in advanced pancreatic cancer patients treated with Gemcitabine with or without omega-3 PUFA [288].

Table 17: Radiological tumour response RECIST v1.1 of the two groups

Response	EOX (n=28)	EOX plus fish oil (n=15)	*P value*
CR	*1 (4%)*	*0 (0%)*	***0.47***
PR	*11 (39%)*	*11 (73%)*	***0.03***
SD	*11 (39%)*	*3 (21%)*	***0.24***
PD	*5 (18%)*	*1 (7%)*	***0.34***
Overall response CR + PR	*12 (43%)*	*11 (73%)*	***0.05***
Disease control CR + PR + SD	***23 (82%)***	***14 (93%)***	***0.31***

CR= complete response, PR= partial response, SD= stable disease, PD= progressive disease

4.2.1.4 Adverse Events according to CTCAE v 4.03

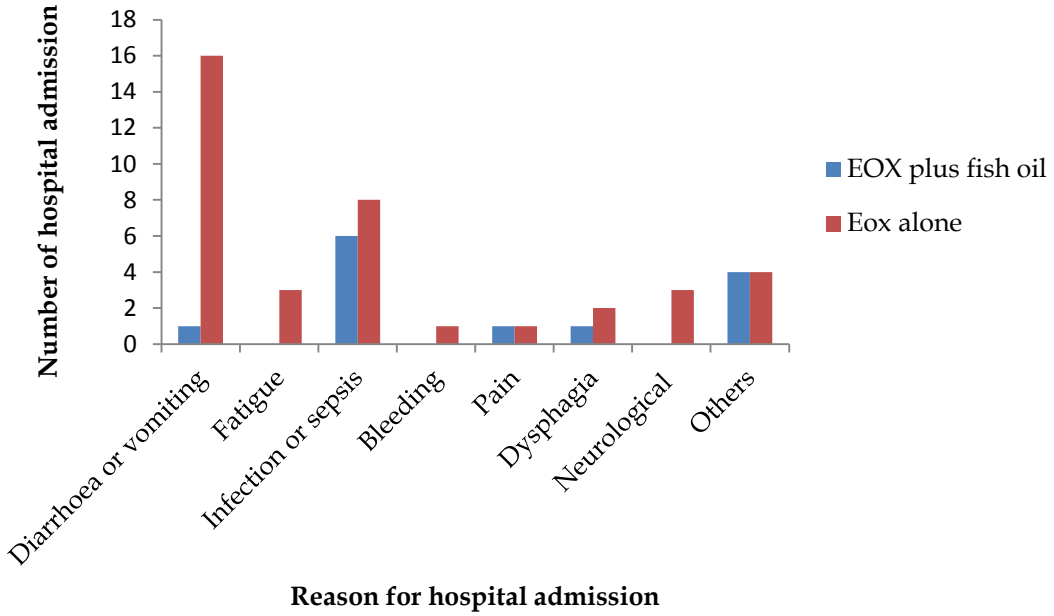
The combination of palliative EOX chemotherapy with omega-3 infusion was well tolerated by participants in the trial. The recorded grade 3 or 4 haematological and non-haematological toxicities are indicated (Table 18). There was a significant reduction in grade 3 and 4 gastrointestinal toxicity among the intervention group compared to the control group, specifically nausea and vomiting, although significant numerical reduction in diarrhoea symptoms but didn't reach statistical significance. There was also a significant reduction in the frequency of thromboembolic events among the intervention group. There has been few reports on use of omega-3 PUFA and that it was associated with less hypercoagulability and reduction in platelets aggregation in patients with advanced oesophageal cancer undergoing curative resection [254,260]. There were large number of studies investigated the use of short term oral or intravenous omega-3 PUFA with no significant effects in term of toxicity and hospital stay as highlighted by Eltweri *el al* [292].

Eleven (11/20, 55%) of the intervention group required a total of 14 hospitalisation episodes (≥ 24 hour) compared to a total of 39 hospitalisation episodes (≥ 24 hour) among 29 patients in the control group (29/37, 78%), p=0.06. The reason for reporting only those who admitted for 24 hour or more was because those in the fish oil group were admitted to the Hope unit at Leicester Royal Infirmary for 4 hour to receive Omegaven® infusion once

weekly. Similarly, in the historical control group, some patients were admitted to the oncology assessment unit.

Gastrointestinal toxicities were the main reason for hospital admissions and these were significantly less among the EOX plus omega-3 infusion group compared to EOX alone (1/14 admissions 7.1% vs 16/39 admissions 41%, p=0.01). Details of all hospital admissions among the two groups are shown in the Figure 19.

Figure 19: Toxicity related Hospital admission among both groups.



Regarding adverse events of specific interest to omega-3 infusion, no patient suffered fat overload syndrome or grade 3 or 4 hypertriglyceridaemia. The mean baseline and 7 day post treatment triglyceride levels were 1.7 mmol/l (95% CI 1.6- 1.79) and 1.66 mmol/l (95% CI 1.56-1.75) respectively. The

highest recorded triglyceride level was 4.84mmol/l. Only one patient required treatment with a statin to counteract hypertriglyceridaemia.

Dose reductions due to chemotherapy toxicity were required for 11 patients in the intervention group (55%) and for 23 patients in the control group (62%), p=0.5. Despite the incidence of haematological toxicities were higher among the fish oil group; dose reduction secondary to haematological toxicity was seen less in those received EOX plus fish oil 10 % compared to 19 % of EOX alone, p=0.4. Whereas, dose reduction secondary to non-haematological toxicity was 40 % vs 35 %, p=0.3 and for both haematological plus non haematological toxicities was 5 % vs 8 %, p=0.7 respectively. Two patients from each group required cisplatin instead of oxaliplatin chemotherapy because of oxaliplatin related peripheral neuropathy. There were no dosage modifications to the Omegaven® (omega-3 fish oil) and the only treatment delays to the administration of Omegaven® related to those associated with chemotherapy-related toxicity.

Table 18: Details of all grade 3 or 4 recorded toxicities (CTCAE v1.1) in both groups.

Grade 3 or 4 toxicity	EOX alone n (%)	EOX plus Fish oil n (%)	*P value*
	Clinical manifestations		
Diarrhoea	10/37 (27%)	2/20 (10%)	*0.18*
Nausea/vomiting	7/37 (19%)	0/20 (0%)	*0.045*
Peripheral neuropathy	5/37 (14%)	2/20 (10%)	*1.00*
Fatigue	9/37 (24%)	1/20 (5%)	*0.08*
Thromboembolism	7/37 (19%)	0/20 (0%)	*0.045*
Infection or sepsis	1/37 (3%)	4/20 (20%)	*0.75*
Constipation	1/37 (3%)	0/20 (0%)	*1.00*
PPE	1/37 (3%)	0/20 (0%)	*1.00*
	Biochemical disturbances		
Anaemia (<8)	3/37 (8%)	5/20 (25%)	*0.12*
Leucopenia (<2)	6/37 (16%)	12/20 (60%)	*<0.001*
Neutropenia (<1)	15/37 (40%)	17/20 (85%)	*0.002*
Thrombocytopenia (<50)	0/37 (0%)	2/20 (10%)	*0.12*
Thrombocytosis (>800)	3/37 (8%)	1/20 (5%)	*0.55*
Hypoalbuminaemia(<20)	1/37 (3%)	0/20 (0%)	*1.00*
AKI (creatinine >360)	0/37 (0%)	2/20 (10%)	*0.35*
High bilirubin level (>63)	0/37 (0%)	0/20 (0%)	-
High ALK (>650)	2/37 (5%)	2/20 (10%)	*0.61*
High ALT (>265)	0/37 (0%)	0/20 (0%)	-
Hypercalcaemia (>3.1)	1/37 (3%)	0/20 (0%)	*1.00*
Hyperglycaemia (>13.9)	0/3 (0%)	1/20 (5%)	*1.00*
Hypocalcaemia (<1.7)	1/37 (3%)	2/20 (10%)	*0.55*
Hyponatraemia (<130)	4/37 (11%)	1/20 (5%)	*0.64*
Hypernatraemia (>155)	0/37 (0%)	0/20 (0%)	-
Hypertriglyceridaemia (>5.7)	0/3(0%)	0/20 (0%)	-
Hypokalaemia (<3)	1/37 (3%)	2/20 (10%)	*0.28*

AKI= acute kidney injury, ALK= alkaline phosphate, ALT= alanine transaminase, PPE= palmar planta erythema, P value calculated using Fisher's exact and Chi square test

4.2.1.5 Quality of life outcome for the intervention group

EORTC QLQ-C30 quality of life questionnaires were completed by participants in the intervention group as follows: 17 participants (one week), 16 participants (three weeks), 14 participants (six weeks), 12 participants (nine weeks) and 11 participants (12 and 15 weeks). Reasons for non-completion included participant withdrawal from treatment, disease progression or death. The changes in the median QoL scores during treatment for these patients over the core domain of QLQ-C30 and its supplementary modules are shown in Tables 19 and 20.

There were improvements in the global health scores (median score 75 vs. 50), fatigue scores (median score 44 vs. 33) and the nausea and vomiting scores (median 17 vs. 8) after nine weeks of treatment compared to baseline (first week). The use of oral and intravenous omega-3 PUFA in colorectal and pancreatic cancer showed improvement in quality of life [271,273,274,280,288].

There was no significant weight gain among the fish oil group during treatment, the median weight at baseline was 70 kg compared to 67 kg after treatment.

Table 19: Changes in the QoL scores during chemotherapy treatment are presented as median score for all patients and (range).

QLQ-C30 Scales	1 wk	3 wk	6 wk	9 wk	12 wk	15 wk
	Median (range)					
Global health Score	50 (0-83)	67 (42-92)	67 (33-100)	75 (33-83)	67 (17-83)	67 (17-100)
Functional Scale Score$^{\Delta}$	69 (16-96)	81 (38-100)	80 (38-100)	70 (44-98)	73 (38-96)	78 (44-98)
Symptom Scale Score$^{\Delta}$	62 (23-100)	80 (46-97)	82 (41-97)	81 (51-95)	85 (31-100)	80 (28-95)
Physical FS$^{\Delta}$	80 (13-100)	87 (27-100)	80 (40-100)	77 (47-93)	67 (20-100)	80 (40-100)
Emotional FS$^{\Delta}$	83 (8-100)	83 (33-100)	96 (33-100)	92 (33-100)	83 (33-100)	92 (33-100)
N&V Score$^{\Delta\Delta}$	17 (0-100)	8 (0-50)	8 (0-50)	8 (0-50)	17 (0-67)	0 (0-50)
Diarrhoea Score$^{\Delta\Delta}$	3 (0-100)	0 (0-33)	0 (0-33)	0 (0-33)	0 (0-33)	0 (0-100)
Fatigue Score$^{\Delta\Delta}$	44 (0-100)	33 (0-78)	33 (0-78)	33 (0-78)	33 (0-100)	33 (0-100)

N&V= nausea and vomiting, FS=functioning score

$^{\Delta}$ *Increase in functional scores indicates improvement.*
$^{\Delta\Delta}$ *Increase in symptom scores indicates deterioration.*

Table 20: Percentage changes in global health and quality of life scores for individual patients for up to fifteen weeks of treatment compared to the first week scores (data presented as number of patients (percentage)).

Global health score	Very much improvement (≥20 % increase)	Moderate improvement (10% increase)	Stable or a little improvement (0-10% increase)	Deterioration
3 weeks	5/16 (31.2%)	5/16 (31.2%)	6/16 (37.5%)	0 (0%)
6 weeks	7/14 (50%)	4/14 (28.6%)	2/14 (14.3%)	1/14 (7.1%)
9 weeks*	6/12 (50%)	2/12 (16.7%)	1/12 (8.3%)	2/12 (16.7%)
12 weeks	3/11 (27.3%)	5/11 (45.4%)	0 (0%)	3/11 (27.3%)
15 weeks	5/11 (45.4%)	2/11 (18.2%)	1/11 (9.1%)	3/11 (27.3%)

* One patient didn't provide these details at 9 weeks

There was an improvement in functioning measures; QLQ-C30 functioning score measures include physical, role, emotional, cognitive and social functioning measures. Very much improvements was reported by at least 21% of patients after 6 weeks treatment and by nine weeks there was approximately 58% of patients had stable function when compared to first week of treatment. All details are shown in Table 21.

Table 21: Improvements in functional score measures during treatment (data presented as patients number and percentage)

Functioning Score	Very much improvement (≥20 % increase)	Moderate improvement (10% increase)	Stable or a little improvement (0-10% increase)	Deterioration
3 weeks	2/16 (12.5%)	3/16 (18.7%)	7/16 (43.7%)	4/16 (25%)
6 weeks	3/14 (21.4%)	4/14 (28.6%)	3/14 (21.4%)	4/14 (28.6%)
9 weeks	2/12 (16.7%)	1/12 (8.3%)	7/12 (58.3%)	2/12 (16.7%)
12 weeks	2/11 (18.2%)	2/11(18.2%)	4/11 (36.4%)	3/11 (27.3%)
15 weeks	2/11 (18.2%)	1/11(9.1%)	5/11 (45.4%)	3/11 (27.3%)

The details for overall symptom measures didn't show any significance, so not presented. However, when broken down by each symptom specified in the QLQ-C30 questionnaire, the number of patients and the percentage reported fatigue are shown in Table 22, diarrhoea data are shown in Table 23, nausea and vomiting data are shown in Table 24. There is approximately 50% improvement in appetite scores, data are shown in Table 28.

Table 22: Percentage changes in fatigue scores from the first week of treatment. (Data presented as patients number and percentage)

Fatigue Score	Very much deterioration (≥20 % increase)	Moderate deterioration (10% increase)	Stable or no symptoms	Improvement
3 weeks	-	4/16 (25%)	1/16 (6.2%)	11/16 (68.7%)
6 weeks	3/14 (21.4%)	-	1/14 (7.1%)	10/14 (71.4%)
9 weeks	1/12 (8.3%)	2/12 (16.7%)	3/12 (25%)	6/12 (50%)
12 weeks	1/11 (9.1%)	2/11 (18.2%)	-	8/11 (72.7%)
15 weeks	2/11 (18.2%)	1/11 (9.1%)	2/11 (18.2%)	6/11 (54.5%)

Table 23: Percentage changes in diarrhoea scores from the first week of treatment. (Data are presented as patient's number and percentage)

Diarrhoea Score	Very much deterioration (≥20 % increase)	Moderate deterioration (10% increase)	Stable or no symptoms	Improvement
3 weeks	2/16 (12.5%)	-	6/16 (37.5%)	8/16 (50%)
6 weeks	-	-	7/14 (50%)	7/14 (50%)
9 weeks*	1/12 (8.3%)	-	5/12 (41.7%)	6/12 (50%)
12 weeks	-	-	5/11 (45.4%)	6/11 (54.5%)
15 weeks	1/11 (9.1%)	-	5/11 (45.4%)	5/11 (45.4%)

Table 24: Percentage changes in nausea and vomiting scores from the first week of treatment. (Data are presented as patient's number and percentage).

N&V Score	Very much deterioration (≥20 % increase)	Moderate deterioration (10% increase)	Stable or no symptoms	Improvement
3 weeks	-	1/16 (6.2%)	4/16 (25%)	11/16 (68.7%)
6 weeks	-	-	5/14 (35.7%)	9/14 (64.2%)
9 weeks*	-	1/12 (8.3%)	2/12 (16.7%)	9/12 (75%)
12 weeks	-	2/11 (18.2%)	-	9/11 (81.8%)
15 weeks	1/11 (9.1%)	1/11(9.1%)	1/11 (9.1%)	8/11 (72.7%)

Table 25: Percentage changes in appetite scores from the first week of treatment. (Data are presented as patient's number and percentage).

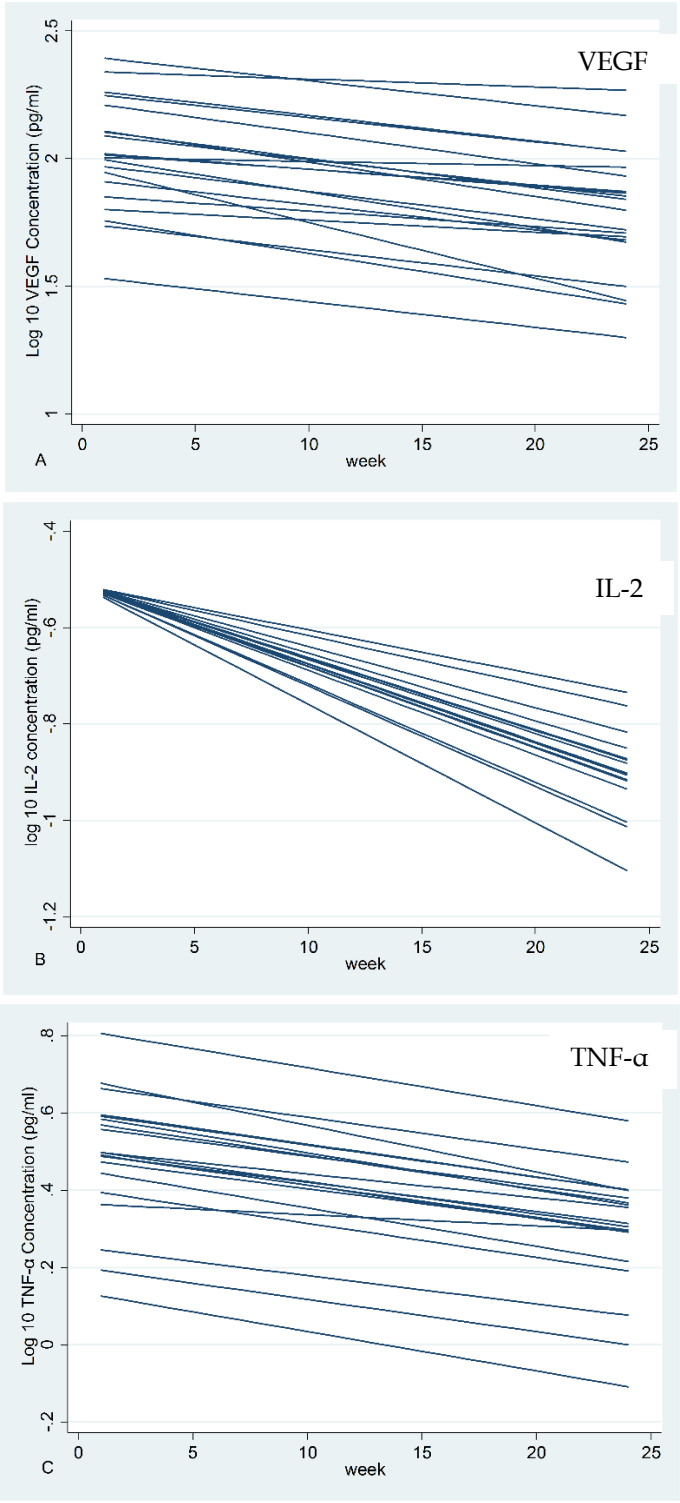
Appetite Score	Very much deterioration (≥20 % increase)	Moderate deterioration (10% increase)	No change	Improvement
3 weeks	1/15 (6.7%)	-	6/15 (40%)	8/15 (53.3%)
6 weeks	1/14 (7.1%)	-	5/14 (35.7%)	8/14 (57.1%)
9 weeks*	-	-	6/12 (50%)	6/12 (50%)
12 weeks	1/11 (9.1%)	-	5/11 (45.4%)	5/11 (45.4%)
15 weeks	1/11 (9.1%)	-	4/11 (36.4%)	6/11 (54.5%)

4.2.2 Effects of palliative EOX plus omega-3 PUFAs on cytokine expression

Immediately after infusion of omega-3 PUFAs, there was a significant reduction in the concentrations of VEGF (P= 0.002, 95% CI -0.0161 to -0.0034), TNF-α (P <0.001, 95% CI -0.0121 to -0.0046) and IL-2 (P= 0.009, 95% CI -0.0283 to -0.0039), see Figure 20. In the curative settings after short term peri operative omega-3 PUFA supplementation to be associated with reduction in inflammatroy status by reduction in TNF-α and IL-6 [265]. In our study, there were reduction in TNF-α, VEGF and IL-2 but the reduction in IL-6 didn't reach statistical significance.

The reduction in VEGF and IL-2 concentration was short lived, and was no longer evidence seven days after treatment, immediately before the subsequent scheduled treatment (VEGF: P= 0.55, 95% CI -0.0092 to 0.0172; IL-2: P=0.458, 95% CI -0.0051 to 0.1152). However, the weekly treatment resulted in a cumulative effect on TNF-α, VEGF and IL-2 expression.

Figure 20: The serial changes in log 10 cytokine concentration (pg/ml).

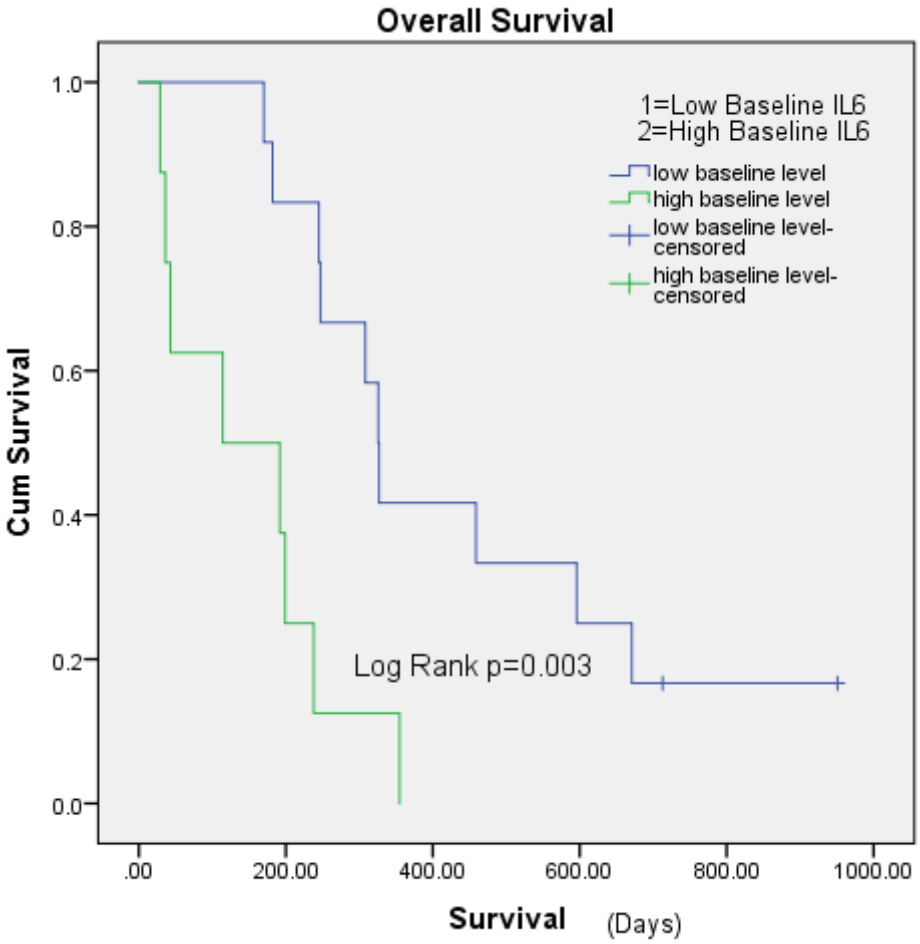


There were reductions in (A) vascular endothelial growth factor (VEGF), p=0.002, (B) interleukin-2 (IL-2) concentrations, p=0.009) and (C) tumour necrosis factor alpha (TNF-a), p<0.001 immediately post omega-3 PUFA infusion with time; each slope represents the best fit concentration for each patient. If the slope of the graph is from left to right, this indicates a negative coefficient or a reduction in cytokine concentration.

There were no significant changes in IL-1α, IL-1 β or IL-6, at any time point measured (IL-1α immediately post treatment P=0.80, 7 days post treatment P=0.25; IL-1 β immediately post treatment P=0.85, 7 days post treatment P=0.82; IL-6 immediately post treatment P=0.18 and 7 days post treatment P=0.75).

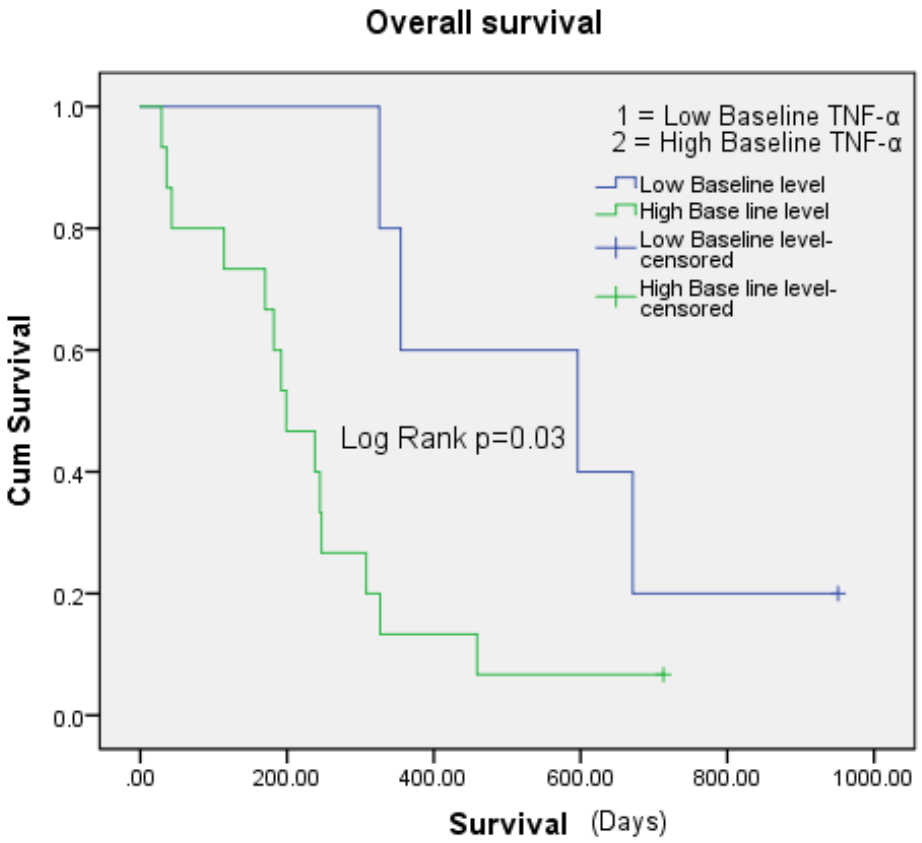
The cytokines IL-6 and TNF-α were associated with better clinical outcomes (Figures 21 and 22), while no associations were observed for the other cytokines. Specifically, patients with low baseline levels of IL-6 and TNF-α had a better overall survival compared to those who were high expressers (P=0.003 and P=0.03 respectively). Further, among those who demonstrated a radiological partial response, reduction in VEGF was the only marker that correlated with an improved overall survival (P=0.03) (Figure 23).

Figure 21: Low baseline levels of IL-6 predict better overall survival.



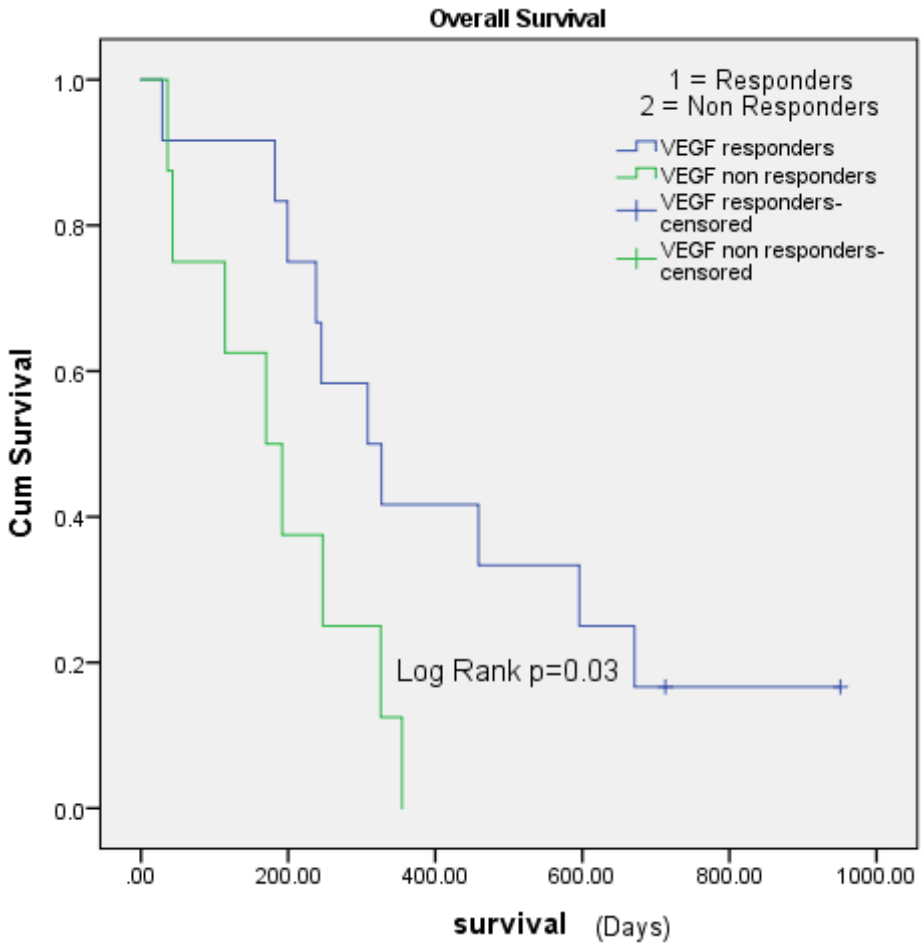
1 = Low Baseline IL6 (n=12) 2 = High Baseline IL6 (n=8)	Median Survival time (Days)			
			95% Confidence Interval	
	Estimate	Std. Error	Lower Bound	Upper Bound
Low baseline level	326.000	16.454	293.749	358.251
High baseline level	114.000	105.359	.000	320.503

Figure 22: Low levels of baseline TNF-α predict better overall survival.



Median Survival (Days)				
1 = Low Baseline TNF-α (n=5) 2 = High Baseline TNF-α (n=15)	Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Low Baseline level	596.000	264.002	78.556	1113.444
High Base line level	199.000	36.067	128.308	269.692

Figure 23: Overall survival of the patients who had confirmed radiological response to treatment and their VEGF correlation.



1 = Responders (n=12) 2 = Non Responders (n=8)	Median Survival time (Days)			
			95% Confidence Interval	
	Estimate	Std. Error	Lower Bound	Upper Bound
VEGF responders	308.000	71.014	168.812	447.188
VEGF none responders	170.000	55.154	61.898	278.102

4.3 Conclusions

In this non-randomised small study, compared to patients treated with chemotherapy alone, those treated with supplementary omega-3 fish oils infusion had reduced chemotherapy related toxicity, notably a lower frequency of gastrointestinal and thromboembolic adverse effects and this might explain the difference in hospital admission rate. Omega-3 fatty acids have the potential to ameliorate the toxicity associated with chemotherapeutic agents. We also found significant changes in serum cytokine levels in that baseline IL-6 and TNF-α were predictive biomarkers for better overall survival outcome in this clinical trial and the significant reduction in VEGF concentrations was associated with better response to the platinum based chemotherapy regimen employed in this clinical trial. There were rapid effect of treatment on cytokine expression and this effect was incremental with each treatment.

Chapter Five

Erythrocyte and plasma uptake of omega-3 fatty acids from an intravenous fish oil based lipid emulsion in patients with advanced oesophago-gastric cancer

5 Erythrocyte and plasma uptake of omega-3 fatty acids from an intravenous fish oil based lipid emulsion in patients with advanced oesophago-gastric cancer.

5.1 Introduction

As long ago as 1863 Rudolf Virchow, after noting the presence of leukocytes in cancer specimens, proposed a link between inflammation and cancer development [295-298]. Chronic inflammation leads to release of pro-inflammatory eicosanoids which are metabolites of the omega-6 polyunsaturated fatty acid (PUFA) arachidonic acid (AA). These metabolites, such as prostaglandin E_2 and leukotriene B_4, play key roles in the initiation and propagation of colorectal, prostate, breast and pancreatic cancer [296,297,299,300]. There is a substantial body of evidence supporting the anti-inflammatory and anti-cancer properties of the omega-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [130,295,301-303].

EPA and DHA are found in fish and in fish oil supplements. One of the main mechanisms of their anti-inflammatory action involves opposing the production and effects of the AA-derived eicosanoids [130]. This mechanism of action is linked to incorporation of the omega-3 PUFAs into cell membranes [131]. Because of the opposing actions of omega-6 and omega-3 PUFAs, both the content of omega-3 PUFAs and the ratio of omega-6 to omega-3 PUFAs in cell membranes are important determinants of their anti-inflammatory effects [151,152].

Omega-3 PUFAs may be administered by oral, enteral or parenteral means [271,272,304]. Carpentier *et al.* showed that intravenous (IV) infusion of a

single preparation of 80% medium-chain triacylglycerol and 20% fish oil to healthy volunteers led to an increase in EPA in platelet and white blood cell phospholipids within 60 minutes and that the observed rises remained for 48 hours [305]. Another study demonstrated incorporation of omega-3 PUFAs from IV fish oil in patients with advanced pancreatic cancer [306]. Indeed short term IV infusion of omega-3 PUFAs is more effective than oral supplementation at promoting incorporation of the bioactive omega-3 PUFAs EPA and DHA into plasma, blood cells and tissues [307]. Hence we investigated the effect of once-weekly infusions of a fish oil-based lipid emulsion (Omegaven®) for six months in patients with advanced oesophago-gastric cancer receiving palliative chemotherapy. The outcomes were appearance of EPA and DHA in two plasma lipid fractions (i.e., non-esterified fatty acids (NEFAs) and phosphatidylcholine (PC)), and in red blood cell (RBC) membranes.

5.2 Patients and methods

This section has been presented in details in both chapters 3 and 4

5.3 Statistical analysis

As data were not normally distributed, they are shown as median and interquartile range, and were log transformed prior to analysis using SPSS version 21 and significance was assumed at the 5% level.

5.4 Results and discussion

5.4.1 Patient demographics

Of the 21 patients recruited, 20 received at least one treatment and are included in the intention-to-treat analysis (Table 16). These patients comprised 16 men and 4 women diagnosed with advanced oesophago-gastric adenocarcinoma. Patients were aged 47 to 80 (median 67) years. Eighty four Omegaven® treatments were administered. As reported previously, no patient experienced grade 3 or 4 hypertriglyceridemia related to Omegaven® infusion.

5.4.2 Plasma polyunsaturated fatty acids uptake

5.4.2.1 Omega-6 and omega-3 PUFAs in plasma NEFAs and plasma PC following a single 4 hour infusion of Omegaven®

Data for the first infusion are shown in Table 26. There was a significant increase in the content of both EPA and DHA, and also of AA, in plasma NEFAs during the infusion (Table 26). Thus, there was a significant increase in total omega-3 PUFAs and a significant decrease in the ratio of omega-6 to omega-3 PUFAs in the NEFA fraction (Table 26). There was a strong trend for EPA content of plasma PC to increase during Omegaven® infusion and there was a small, but significant, decrease in the ratio of omega-6 to omega-3 PUFAs in the PC fraction (Table 26). Arshad *et al* also reported a significant increase in the contents of EPA, DHA and AA plasma NEFA [308] and Katan *et al* showed the incorporation half-life of EPA serum cholesteryl ester after oral supplement of 4.8 days [309].

5.4.2.2 Repeatability of the increase in EPA, DHA and AA in plasma NEFAs following Omegaven® infusion

The increases in EPA, DHA and AA in plasma NEFAs were examined after each of the 24 infusions with Omegaven®; the results are shown in Figure 24. It is evident that the increases in EPA of about 3.5%, in DHA of about 5% and in AA of about 0.5% seen with the first infusion (Table 26) are highly repeatable across each of the later infusion. Arshad *et al* reported no difference in omega-6 to omega-3 PUFA ratio [308], in our study it was clearly statistically significant

reduction in omega-6 to omega-3 PUFA plasma NEFA ratio. This might be because they have used different FO emulsion with different PUFAs contents.

Table 26: Omega-6 and omega-3 PUFAs in plasma NEFAs and plasma PC prior to and at the end of the first Omegaven® infusion

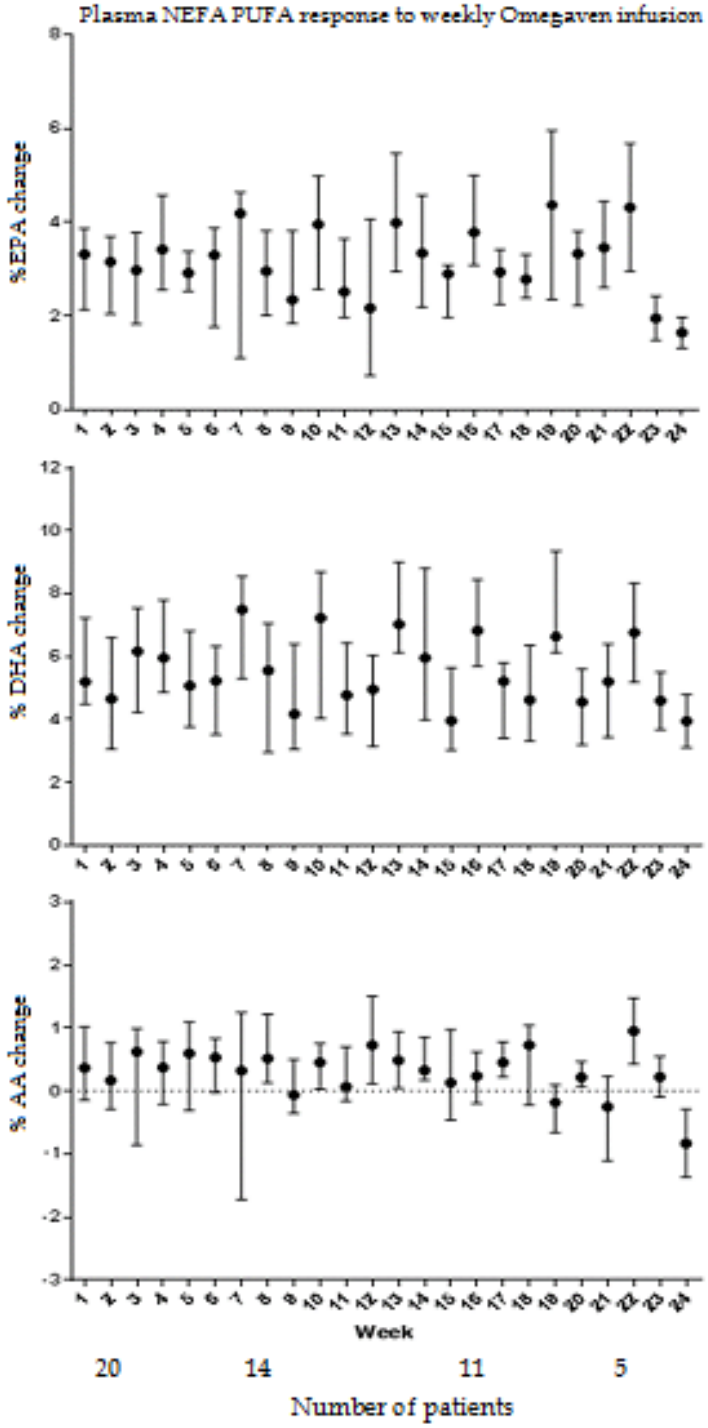
	Plasma NEFAs			Plasma PC		
Fatty acid	Pre-Omegaven® infusion	Post-Omegaven® infusion	P	Pre-Omegaven® infusion	Post-Omegaven® infusion	P
EPA	0.2 (0.1-0.6)	3.7 (2.8-4.3)	<0.001	0.9 (0.6-1.1)	0.9 (0.7-1.3)	0.067
DHA	0.9 (0.6-1.2)	6.3 (5.7-8.3)	<0.001	3.4 (2.8-3.8)	3.4 (2.8-3.7)	0.715
AA	1.2 (0.9-2.1)	1.8 (1.4-2.0)	0.035	8.6 (7.6-10.5)	8.5 (7.4-10.7)	0.315
Total omega-6 PUFAs	12.2 (11.1-13.0)	11.0 (9.7-12.6)	0.142	31.6 (30.3-34.4)	31.8 (30.1-33.2)	0.900
Total omega-3 PUFAs	2.4 (1.9-3.5)	12.6 (10.5-14.9)	<0.001	5.6 (4.9-6.3)	5.4 (5.0-6.1)	0.001
Omega-6 to Omega-3 PUFA ratio	3.5 (2.9-4.5)	1.0 (0.9-1.0)	<0.001	5.0 (4.4-5.4)	4.8 (4.5-5.1)	0.046

Data are median and interquartile range percentage of total fatty acids (n = 20 patients).
P values were calculated using paired sample t-test on log transformed data.
AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid (EPA); NEFAs = non-esterified fatty acids; PC = phosphatidylcholine; PUFA = polyunsaturated fatty acid

5.4.2.3 Omega-6 and omega-3 PUFAs in plasma NEFAs and plasma PC over the entire period of Omegaven® treatment

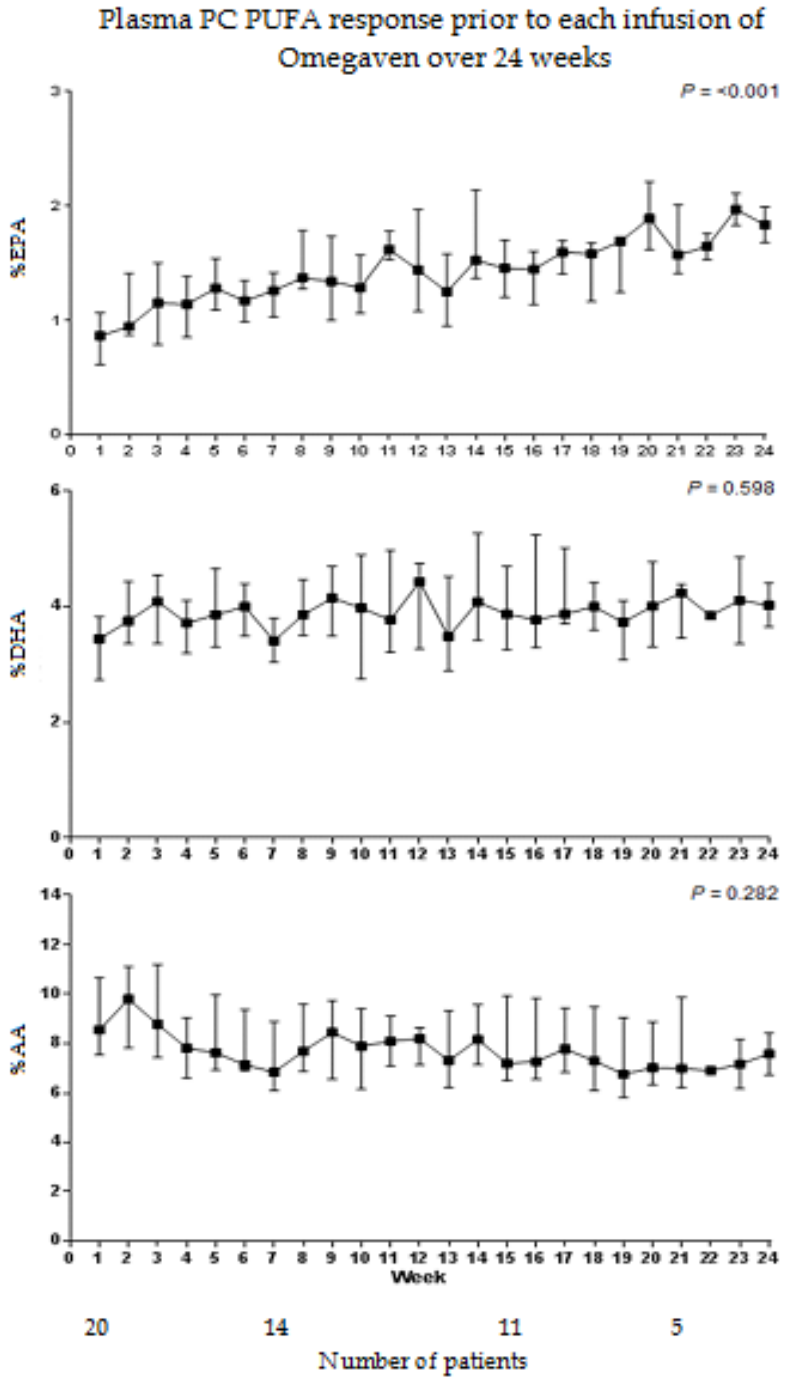
Blood samples collected prior to each Omegaven® infusion allowed the change in PUFAs in plasma NEFAs and plasma PC to be determined over the entire treatment period of 6 months. Overall there was limited effect on the fatty acids in NEFAs (Figure 24). However, the content of EPA in plasma PC increased with increasing number of infusions (i.e., with time) ($p < 0.001$), as shown in Figure 25. In contrast, there was no significant change in plasma PC DHA or AA over time (Figure 25). Consequently, the total omega-3 PUFA content of plasma PC increased and the ratio of omega-6 to omega-3 PUFAs decreased with increasing number of infusions, although this did not reach statistical significance (data not shown). Katan *et al* in 1997 reported that EPA in serum cholesteryl ester plateaued after 1 – 2 months and DHA incorporation was small and erratic and incorporation half-life was 10.3 days after oral supplement in healthy volunteers [309]. In our study, once weekly infusion resulted in gradual increase in EPA plasma PC concentration over six months period without reaching plateau state. Arshad *et al* showed no difference in any FAME proportion and omega-6 to omega-3 PUFA ratio in plasma PC [308].

Figure 24: Percentage change in EPA, DHA and AA in plasma NEFAs for each weekly infusion of Omegaven® i.e., comparison of immediate post- with pre-infusion levels for each cycle.



Data are median and interquartile range, and are for decreasing numbers of patients as the time increases.

Figure 25: Percentage of EPA, DHA and AA in plasma PC prior to each infusion of Omegaven® i.e., comparison of each baseline pre-infusion level for up to 24 weeks.



Data are median and interquartile range, and are for decreasing numbers of patients as the time increases.

5.4.3 Red Blood Cell polyunsaturated fatty acids uptake

5.4.3.1 Omega-6 and omega-3 PUFAs in RBC membranes over the entire period of Omegaven® treatment

Blood samples collected prior to each Omegaven® infusion allowed the change in PUFAs in RBC membranes to be determined over the entire treatment period of 6 months. Table 27 compares the data prior to the first and the final infusion. There was a significant increase in the content of EPA, but there were no significant changes in the content of DHA or AA in RBC membranes. Arshad *et al* red blood cell membrane uptake was similar to our findings [308]. Pittet *et al*, reported significant incorporation of EPA in platelets membrane in a dose dependent manner after single intravenous infusion [310].

Table 27: Omega-6 and omega-3 PUFAs in RBC membranes prior to the first and last infusion of Omegaven®

Fatty acid	Prior to first Omegaven® infusion	Prior to final Omegaven® infusion	*P*
EPA	0.4 (0.4 – 0.6)	0.9 (0.9 – 1.0)	0.027
DHA	3.4 (2.1 – 4.3)	2.5 (1.4 – 3.6)	0.534
AA	12.6 (8.9 – 16.4)	7.1 (3.2 – 10.9)	0.281

Data are median and interquartile range percentage of total fatty acids.

P values were calculated using paired sample t-test on log transformed data.

AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; PUFA = polyunsaturated fatty acid; RBC = red blood cell

5.5 Conclusions

A 4 hour infusion with Omegaven® enriched plasma NEFAs with EPA and DHA in patients with advanced oesophago-gastric cancer receiving palliative chemotherapy, while repeated 4 hour infusions once a week for several months enriched plasma PC and RBC membranes with EPA.

Chapter Six

Effects of EPA, DHA, Omegaven® and Oxaliplatin on oesophageal adenocarcinoma cell lines growth, cytokine expression and cell signal biomarkers expression

6 Effects of EPA, DHA, Omegaven® and Oxaliplatin on oesophageal adenocarcinoma cell lines growth, cytokine expression and cell signal biomarkers expression

6.1 Introduction

Several factors in the cancer microenvironment influence the carcinogenesis process, including the delivery of bioactive molecules, such as cytokines and growth factors that are responsible for increased cell proliferation, inhibition of apoptosis and induction of angiogenesis [286]. In many patients, oesophago-gastric cancer is considered to arise as a consequence of chronic inflammation [6]. This link is implicated for the development of gastric cancer as a result of Helicobacter pylori related chronic gastritis and atrophy [311]. For the oesophagus, the link between inflammation and cancer is strongest for adenocarcinoma as a result of chronic reflux associated inflammation [6]. In the stomach, it is established that H. pylori infection causes the induction of various pro-inflammatory cytokines such as TNF-α, Interleukin (IL) -1 and IL-6 [311].

There is an increasing interest in natural therapies with anti-inflammatory effects that exhibit anticancer benefit, including omega-3 fish oil PUFAs. The main omega-3 PUFA active products are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have anti-inflammatory effects. The human body is unable to produce these PUFA. Hence, it is dependent on external sources. The use of EPA and DHA has been extensively investigated, with proven *in vitro* anticancer effects in gastrointestinal cancers such as colorectal

and pancreatic adenocarcinoma. However, there is limited evidence in oesophageal cancer as highlighted by the author's published review summarised earlier in the Introduction section [292].

Wu *et al*, treated gastric cancer cell lines with EPA and DHA, and found that EPA and DHA inhibited macrophage activated cell migration by down regulation of the matrix metalloproteinase 10 gene, and subsequent down regulation of the ERK and STAT3 pathways [215]. Slagsvold *et al* confirmed that DHA (75 μM) had significant anticancer effects on colon cancer cell lines, through cell cycle arrest at both the G1 and G2 phases as well as through upregulation of P21 protein and downregulation of survivin and livin (inhibitors of apoptosis) [188].

We hypothesized that treatment of two oesophageal adenocarcinoma cell lines (OE33 and OE19) with EPA, DHA, Omegaven® (fish oil emulsion) and oxaliplatin would result in a reduction in levels of inflammatory cytokines, and inhibition of cell line proliferation. In this exploratory study, we evaluated the effect of the four single treatments on cell growth and expression of the following cytokines: IL-1β, IL-2, IL-6, TNF-α and VEGF in the cell culture supernatant. In addition, we also evaluated expression of the following proteins P53, P21, Akt, ERK1/2in the cell lysate.

6.2 Statistical analysis

In order to identify whether there was any significant effect of each treatment on the cell counts (proliferation) at different time points, two way ANOVA was

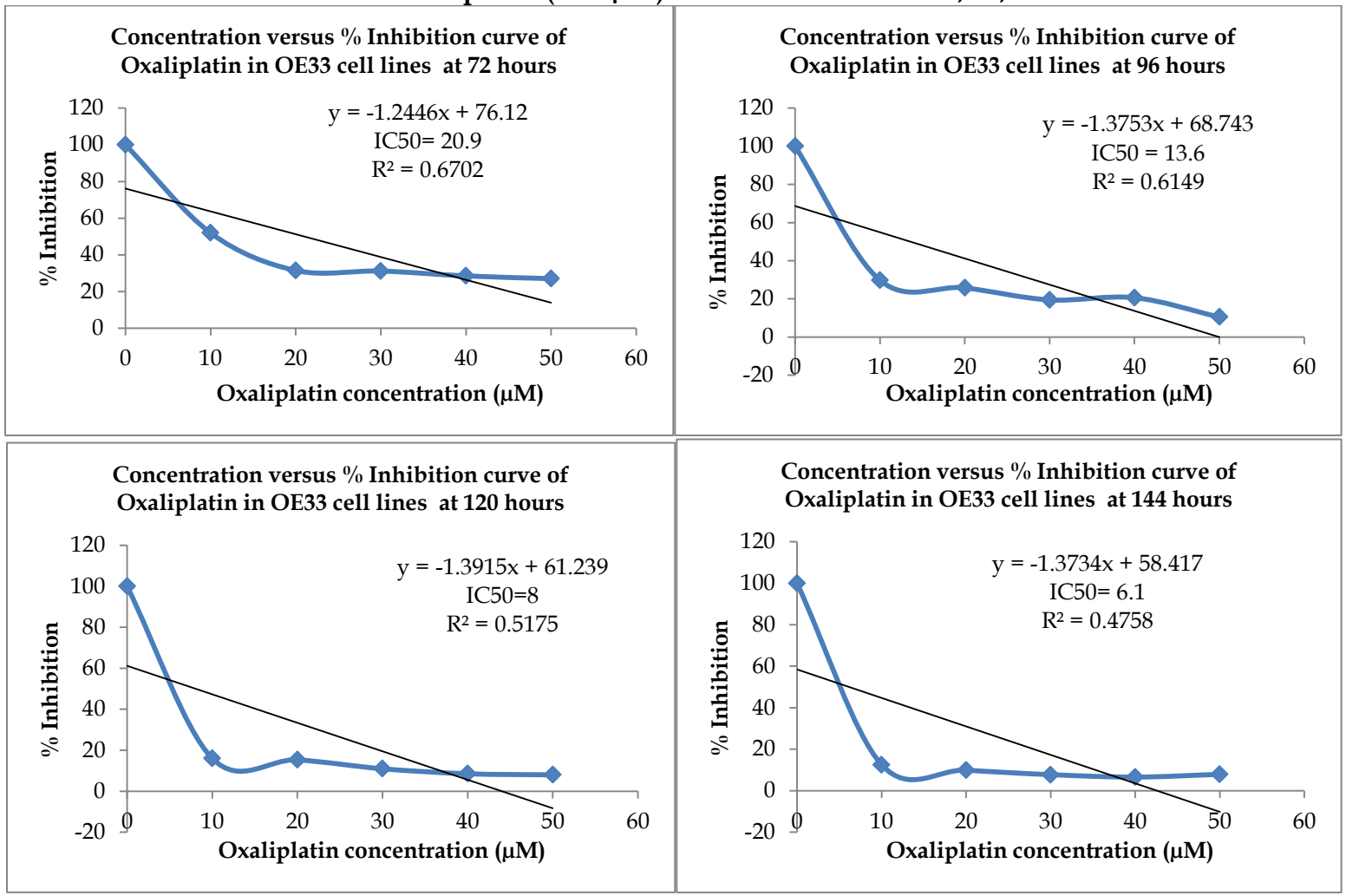
used and the two tailed t test was used to compare cell count and all cytokine expression compared to the control.

6.3 Results and discussion:

6.3.1 The half maximal inhibitory concentration (IC50) of the drugs in the experiment

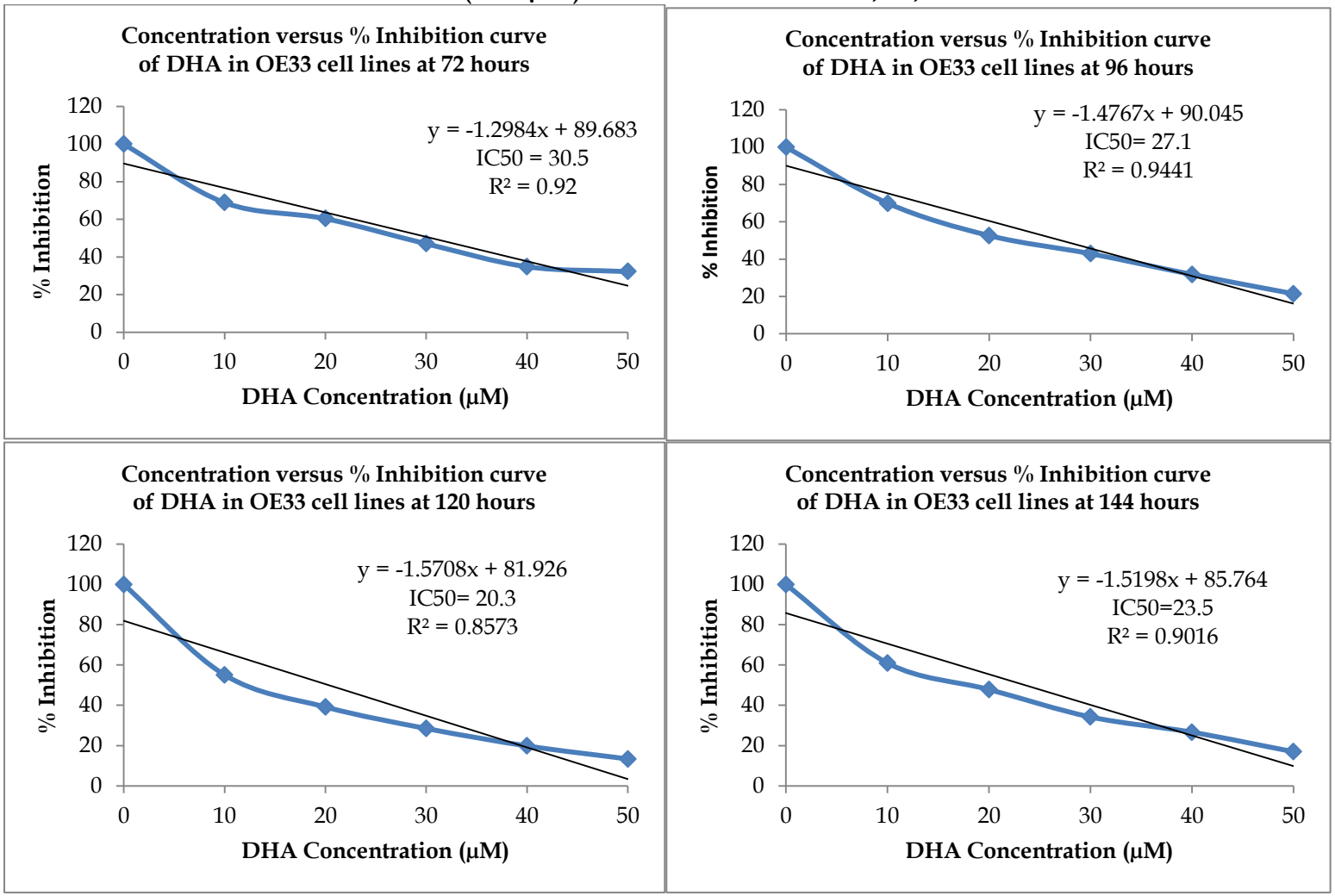
The linear line equation was used to calculate the IC50 for each drug at four time points and the data were presented in the following dose response curves Figures 26-31. Each value represents the percentage of cell number as compared to vehicle control. It was not feasible to calculate the IC50 for omegaven® due to the biphasic effects i.e. increased growth at low and high concentrations and inhibited proliferation at intermediate concentration.

Figure 26: Growth inhibition curves of Oxaliplatin (0-50 µM) in OE33 cell lines at 72, 96, 120 and 144 hours.



Linear line equation used to calculate the IC50 of Oxaliplatin at different time points
The values represent percent of cell number as compared to vehicle control

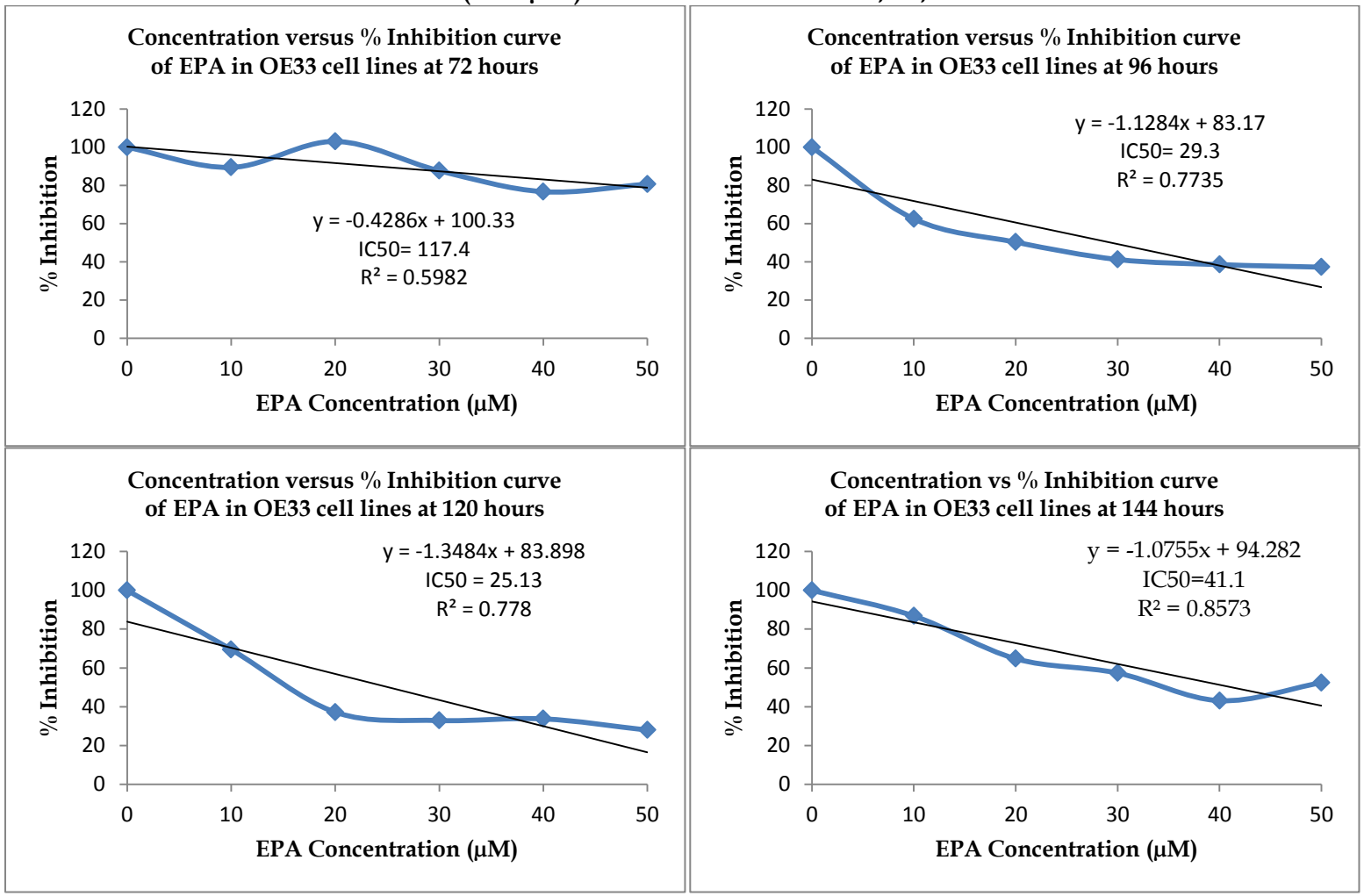
Figure 27: Growth inhibition curves of DHA (0-50 µM) in OE33 cell lines at 72, 96, 120 and 144 hours.



Linear line equation used to calculate the IC50 of DHA at different time points

The values represent percent of cell number as compared to vehicle control

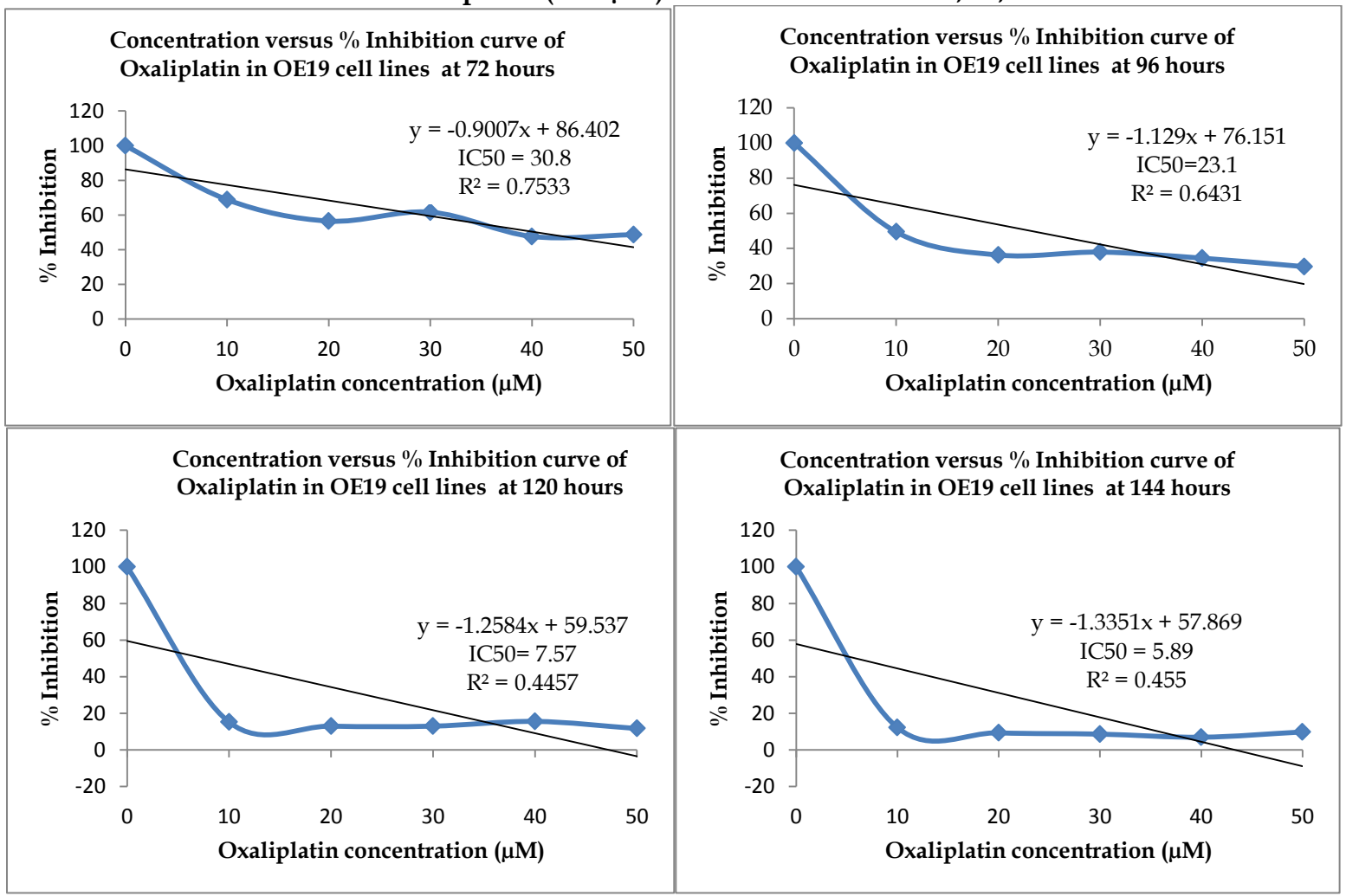
Figure 28: Growth inhibition curves of EPA (0-50 µM) in OE33 cell lines at 72, 96, 120 and 144 hours.



Linear line equation used to calculate the IC50 of EPA at different time points

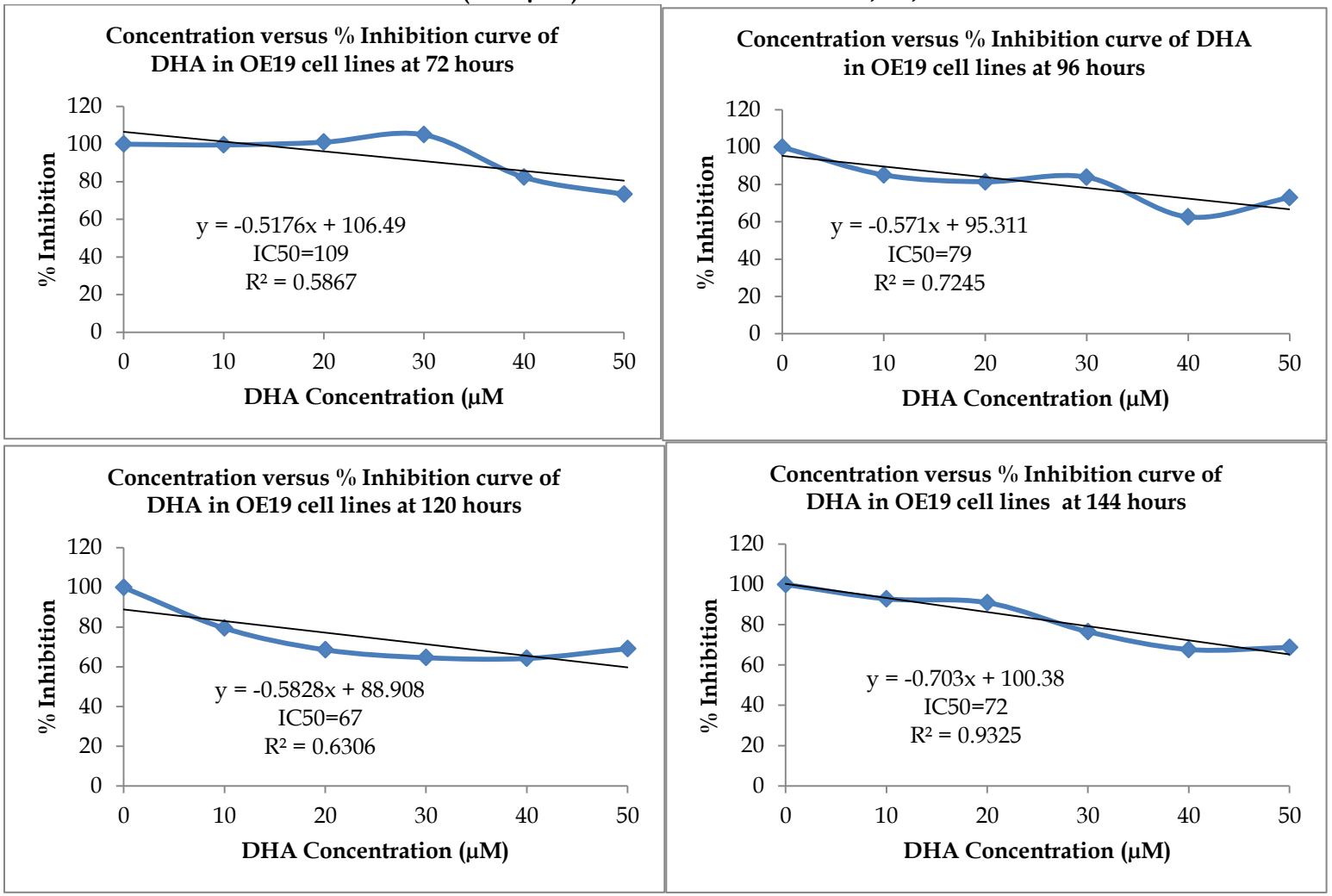
The values represent percent of cell number as compared to vehicle control

Figure 29: Growth inhibition curves of Oxaliplatin (0-50 µM) in OE19 cell lines at 72, 96, 120 and 144 hours.



Linear line equation used to calculate the IC50 of Oxaliplatin at different time points
The values represent percent of cell number as compared to vehicle control

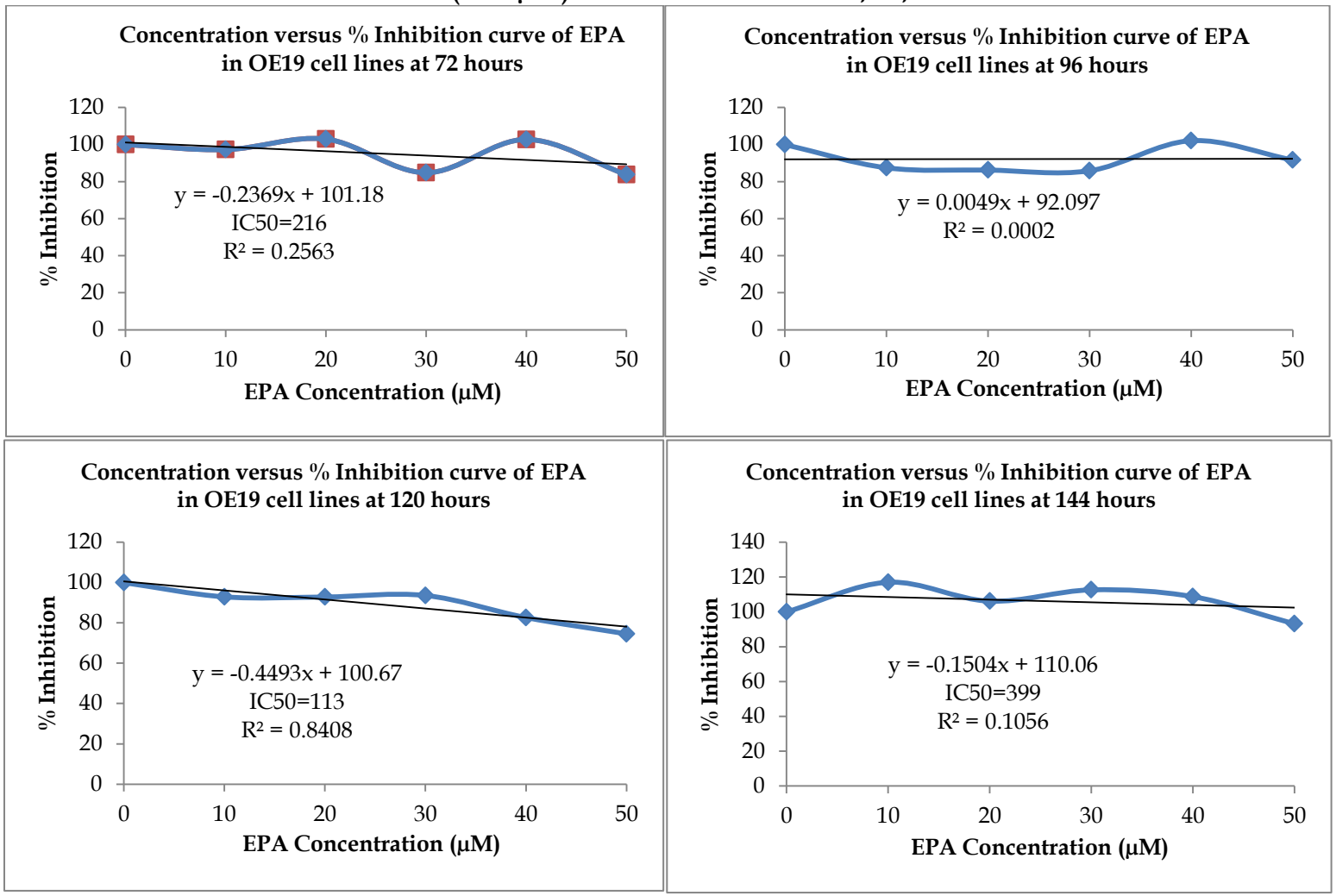
Figure 30: Growth inhibition curves of DHA (0-50 µM) in OE19 cell lines at 72, 96, 120 and 144 hours.



Linear line equation used to calculate the IC50 of DHA at different time points

The values represent percent of cell number as compared to vehicle control

Figure 31: Growth inhibition curves of EPA (0-50 µM) in OE19 cell lines at 72, 96, 120 and 144 hours.



Linear line equation used to calculate the IC50 of EPA at different time points

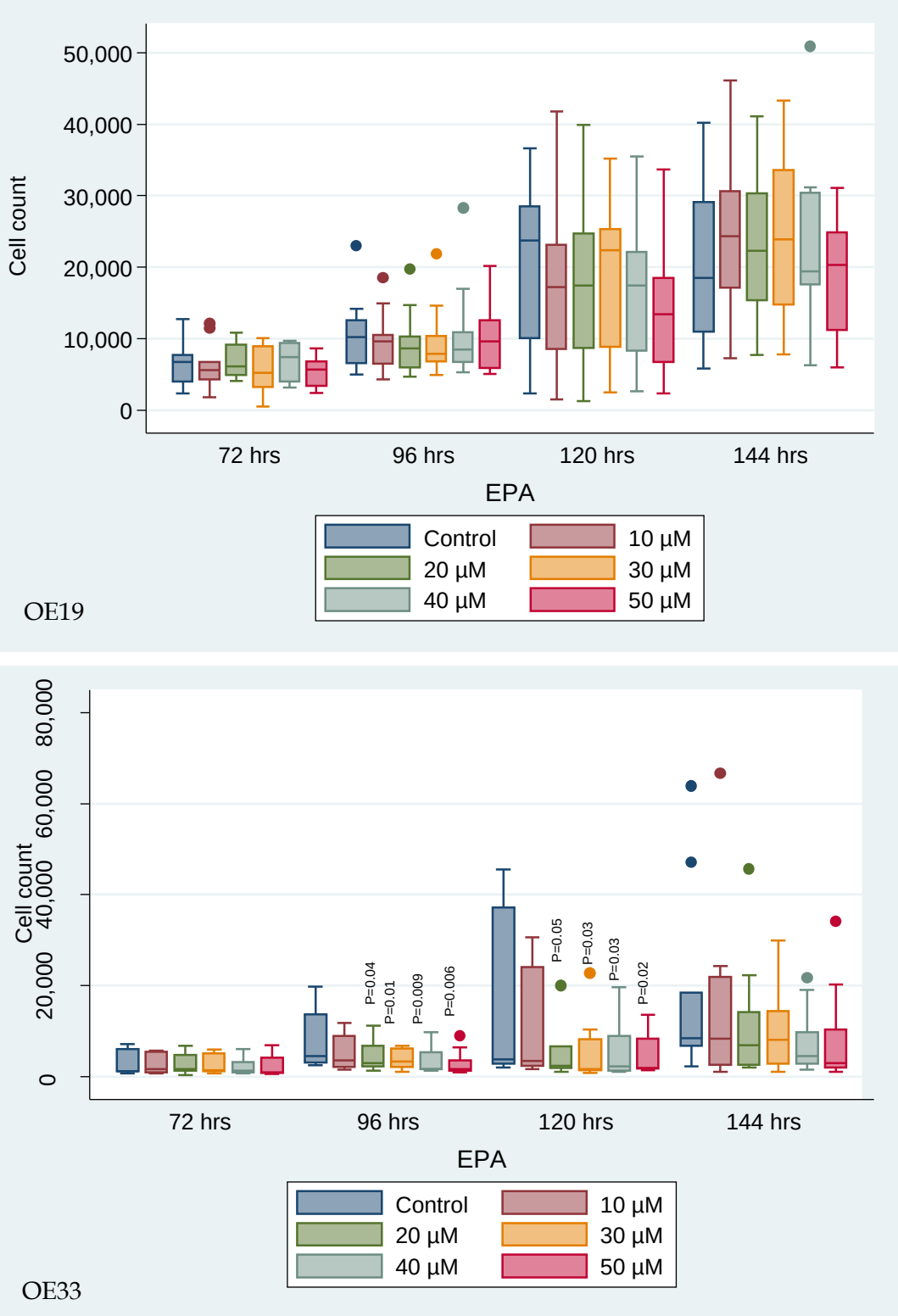
The values represent percent of cell number as compared to vehicle control

6.3.2 Effects of the four treatments on cell proliferation

6.3.2.1 Effect of Eicosapentaenoic acid (EPA) on OE19 and OE33 cells

OE33 were more sensitive to the effects of EPA than OE19 cells. For OE19 cells, although the cell numbers decreased as EPA concentration increased, the anti-proliferative effect was not statistically significant (Figure 32). For OE33 cells, growth was significantly inhibited by EPA compared to control at 96 hours (30-50 μM EPA) and 120 hours (20-50 μM), see Figure 32.

Figure 32: Effects of EPA treatment on OE19 and OE33 cell line growth at four time points

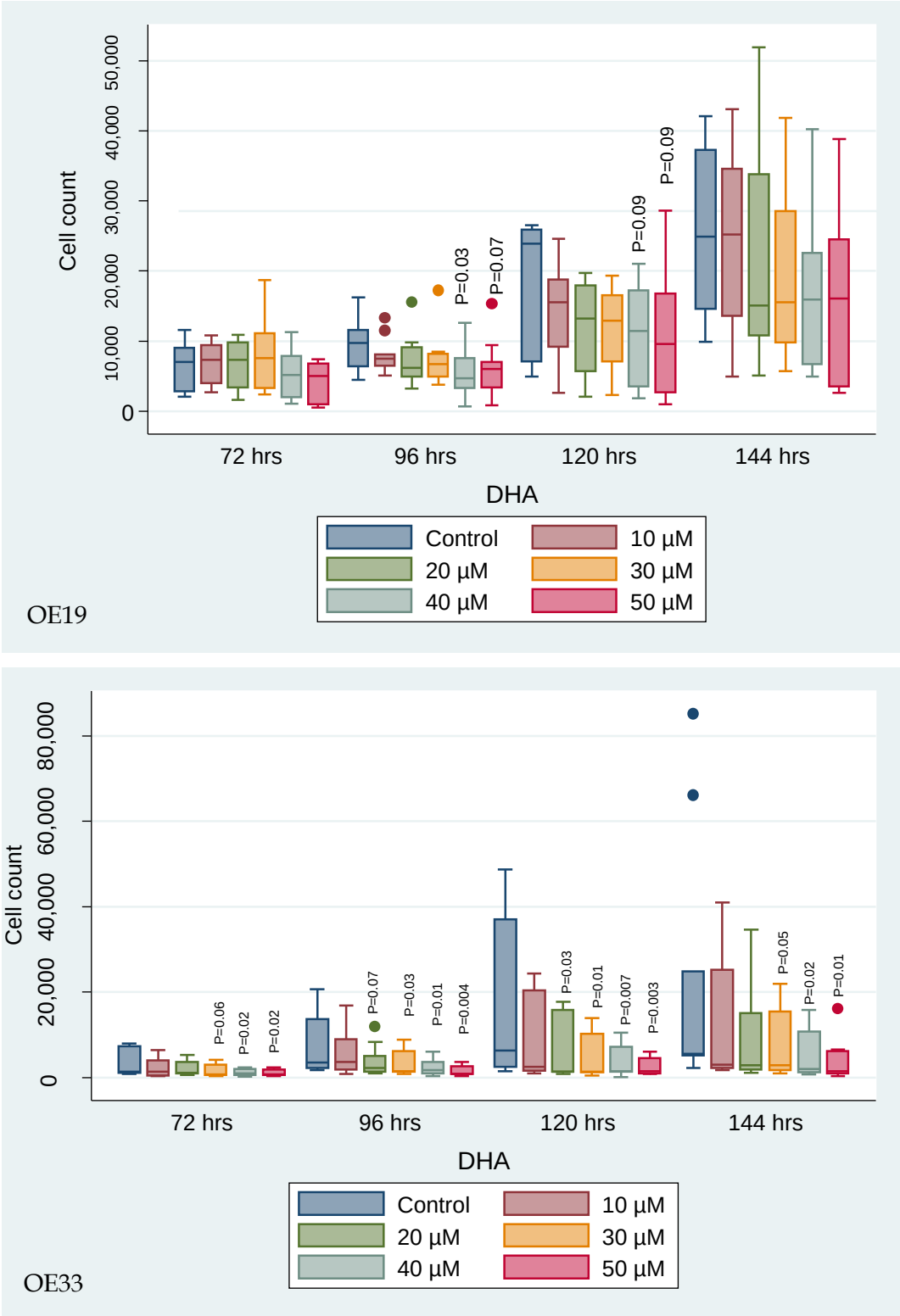


Lines represent median of the cell count and whisker bars represent interquartile range of three experiments in triplicate, dots represent outliers. Two tailed student's t test (log transformed data) was used to identify if there was any difference between each EPA concentration and the control (DMSO).

6.3.2.2 Effect of Docosahexaenoic acid (DHA) on OE19 and OE33 cells

There was a reduction in cell count with a statistical significant anti-proliferative effect when OE19 cells were treated with 40 μM of DHA for 96 hours. The effects of other concentrations exhibited a similar anti-proliferative effect but did not achieve statistical significance (Figure 33). The OE33 cell lines were more sensitive to DHA treatment across all time points: from 20 μM at 96 ($p=0.07$) and 120 hrs ($p=0.03$), and from 30 μM at 72 ($p=0.03$) and 144 hrs ($p=0.05$), see Figure 33.

Figure 33: Effects of DHA treatment on OE19 and OE33 cell growth at four time points.

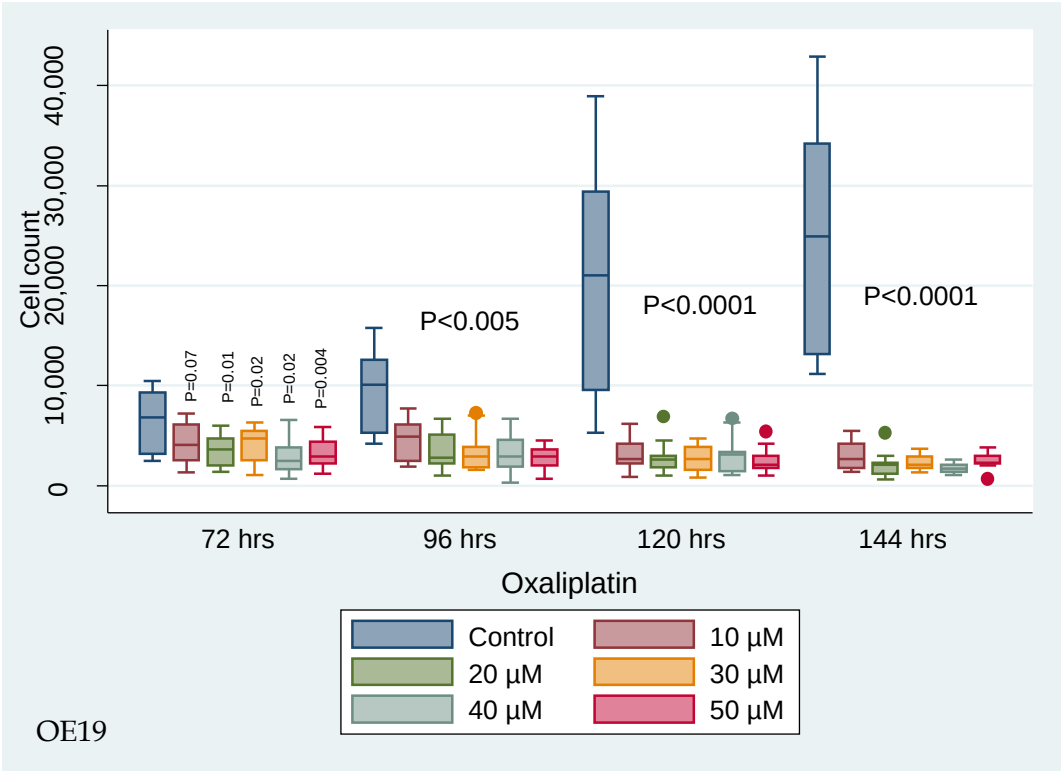


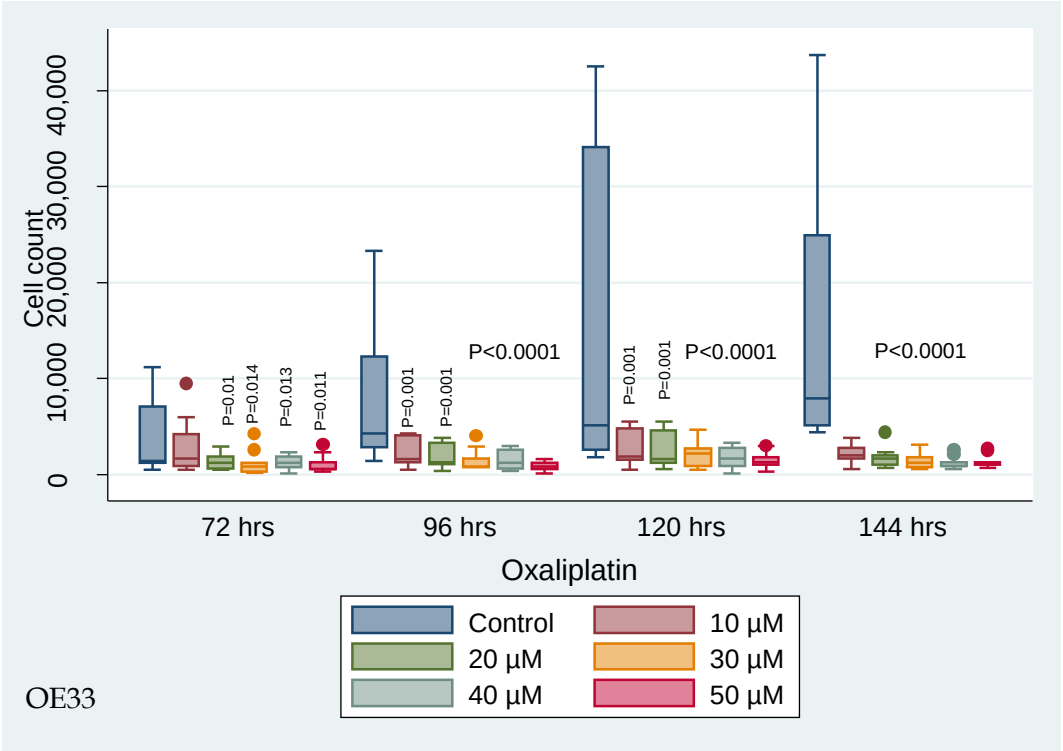
Lines represent median of the cell count and whisker bars represent interquartile range of three experiments in triplicate, dots represent outliers. Two tailed student's t test (log transformed data) was used to identify if there was any difference between each DHA concentration and the control (DMSO).

6.3.2.3 Effect of Oxaliplatin (platinum chemotherapy) on cell counts of both cell lines

There was a statistically significant decrease in cell number with increasing oxaliplatin concentration. This was evident for both cell lines at all time points from 72 hours onwards (Figure 34).

Figure 34: Effects of Oxaliplatin treatment on OE19 and OE33 cell lines growth at four time points.



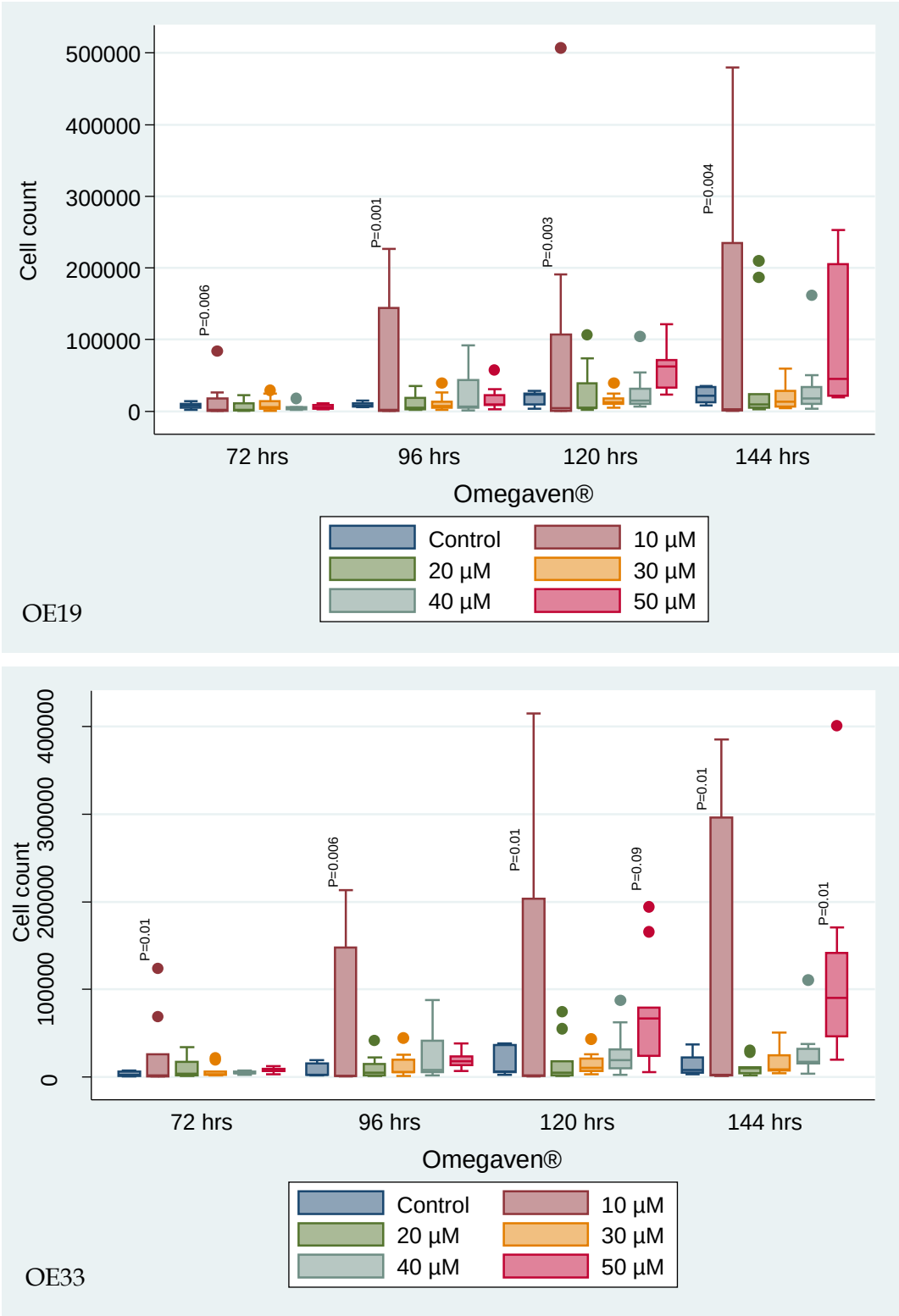


Lines represent median of the cell count and whisker bars represent interquartile range of three experiments in triplicate, dots represent outliers. Two tailed student's t test (log transformed data) was used to identify if there was any difference between each oxaliplatin concentration and the control (5% dextrose).

6.3.2.4 Effect of Omegaven® (Fish oil emulsion) on cell counts of OE19 and OE33 cells

Omegaven exerted a concentration dependent effect on cell growth for both cell lines. At both low (10 μM) and high (50 μM) concentrations of Omegaven®, there was an increase in cell number, while at intermediate concentrations (20-30 μM) of Omegaven®, there was a reduction in cell number (Figure 35).

Figure 35: Effects of Omegaven® treatment on OE19 and OE33 cell lines growth at four time points.



Lines represent median of the cell count and whisker bars represent interquartile range of three experiments in triplicate, dots represent outliers. Two tailed student's t test (log transformed data) was used to identify if there was any difference between each Omegaven® concentration and the control.

6.3.3 Cell culture supernatant cytokine assessment

The following cytokines (IL-1, 2, 6, TNF-α and VEGF) were measured using ELISA assays in the cell culture supernatant that was previously collected during cell count assessment.

6.3.3.1 Vascular Endothelial Growth Factor (VEGF) expression

The EPA treatment of OE19 cells led to significant overexpression of VEGF when the cells were treated with 20 μM EPA for 96 hours only (Figure 36). The OE33 cells showed significant overexpression of VEGF when exposed to treatment for 120 and 144 hours (Figure 36).

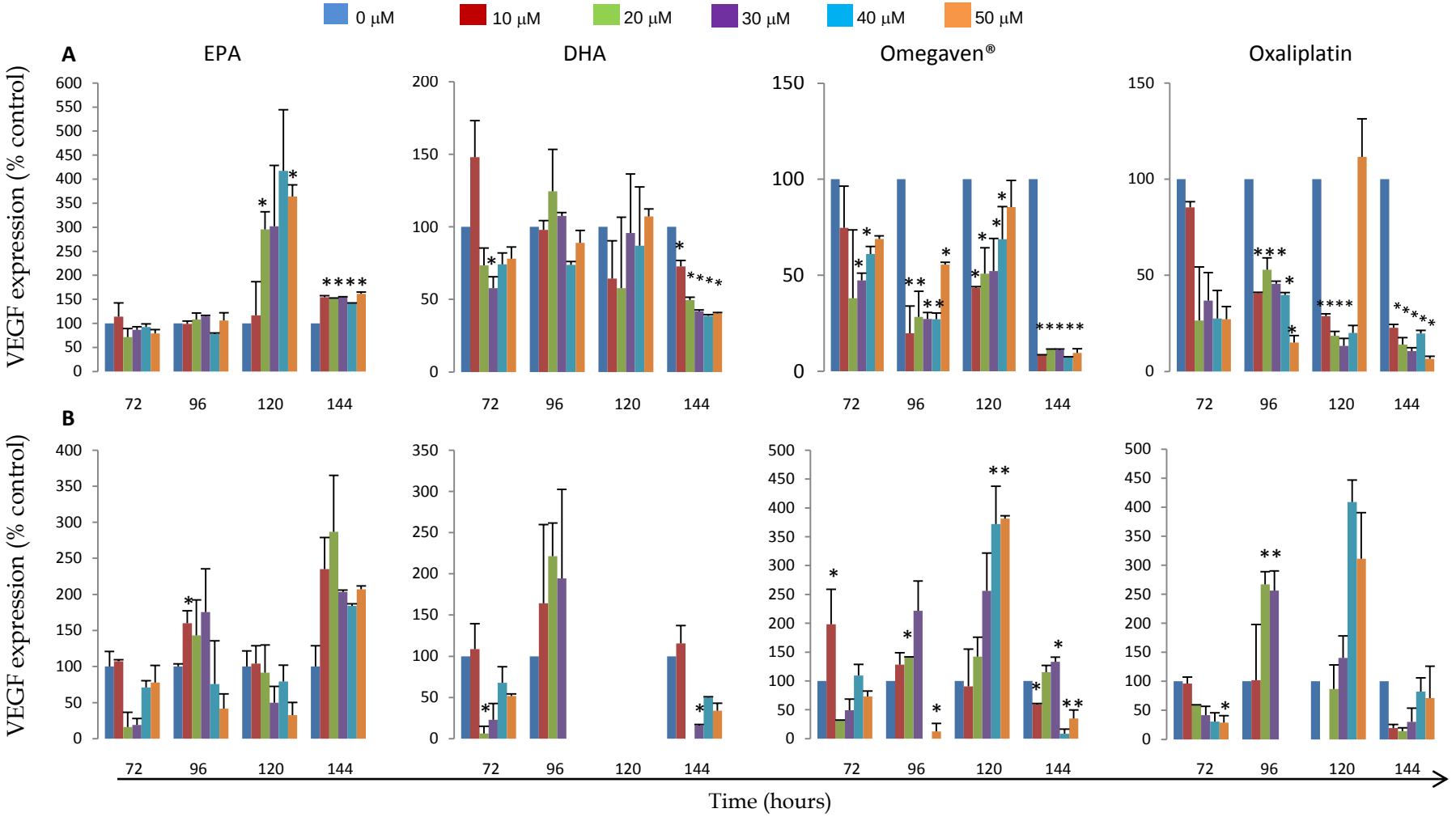
A statistically significant decrease was observed after prolonged treatment with 10-50 μM of DHA in the OE33 cells. The OE19 cells showed significant downregulation of VEGF when treated with 20 μM at 72 hours and 30 μM after 144 hours (Figure 36). The VEGF concentration was not measurable in OE19 cells after 120 hours (data not displayed)

The OE19 cells treated with Omegaven® were associated with overexpression of VEGF. Whereas, Omegaven® treatment of OE33 cell lines resulted in a significant decrease in VEGF levels across all time points (Figure 36).

The oxaliplatin treatment was associated with a significant reduction in cell counts for both cell lines and reduction of VEGF expression in OE33 cell lines at all time points and with all concentrations (Figure 36). OE19 cells were more refractory to oxaliplatin treatment than the OE33 cells, with significant

downregulation of VEGF expression only at 72 hours when treated with the highest concentration (50 μM) of oxaliplatin (Figure 36).

Figure 36: Effects of EPA, DHA, Omegaven® and oxaliplatin treatments over time on VEGF expression in (A) OE33 and (B) OE19 cells



*Analysis was made using ELISA assays in duplicate. The graphs represent the mean and standard deviation (SD) of VEGF concentration (pg/mL) expressed as a percentage of control. * indicate that the difference between the treatment and control was significant (P<0.05), using two tailed student's t test.*

6.3.3.2 TNF-α expression

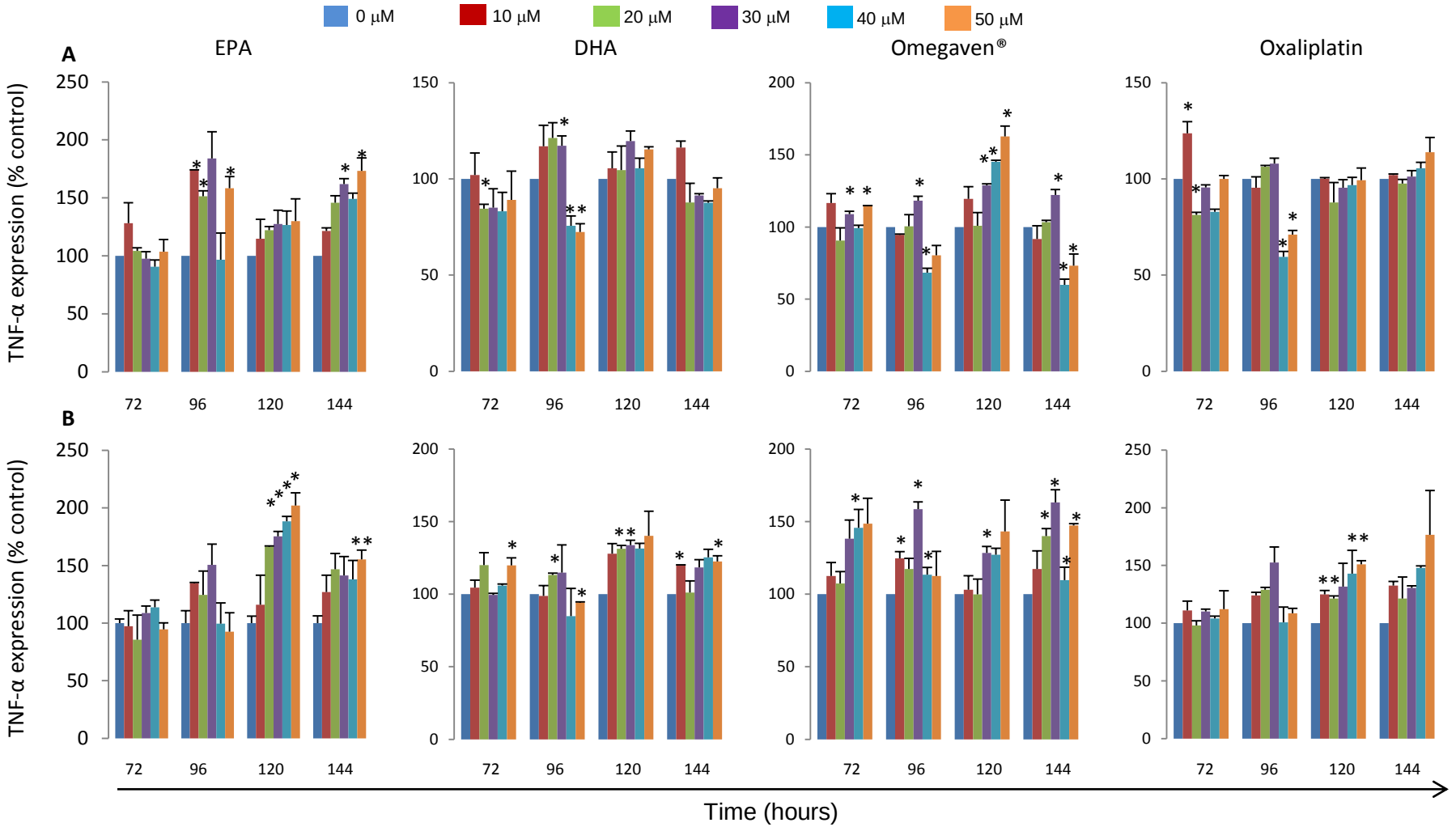
Eicosapentaenoic acid was associated with overexpression of TNF-α in both cell lines. This achieved statistical significance after prolonged exposure to treatment in OE19 cells and from 96 hours in OE33 cells (Figure 37).

The DHA treatment of the two cell lines was associated mainly with overexpression of TNF-α (Figure 37), with the exception of OE33 cells at 72 and 96 hours and OE19 cells at 96 hours when treated with the higher concentration of 40-50 μM, showed downregulation effects.

Omegaven® results in an increase in TNF-α expression as early as 72 hours in both cell lines. The exception was for OE33 cell at 96 hours and 144 hours when exposed to higher concentrations where a significant decrease was observed (Figure 37).

Despite the significant reduction in cell count and VEGF expression as presented in the previous figures, oxaliplatin did not cause a reduction in TNF-α in either cell line. The was a trend toward an upregulation of the TNF-α expression in both cells, with statistically significant changes after 120 hours of exposure to treatment in OE19 cell lines (Figure 37).

Figure 37: Effects of EPA, DHA, Omegaven® and oxaliplatin treatments over time on TNF-α expression in (A) OE33 cells and (B) OE19 cells.



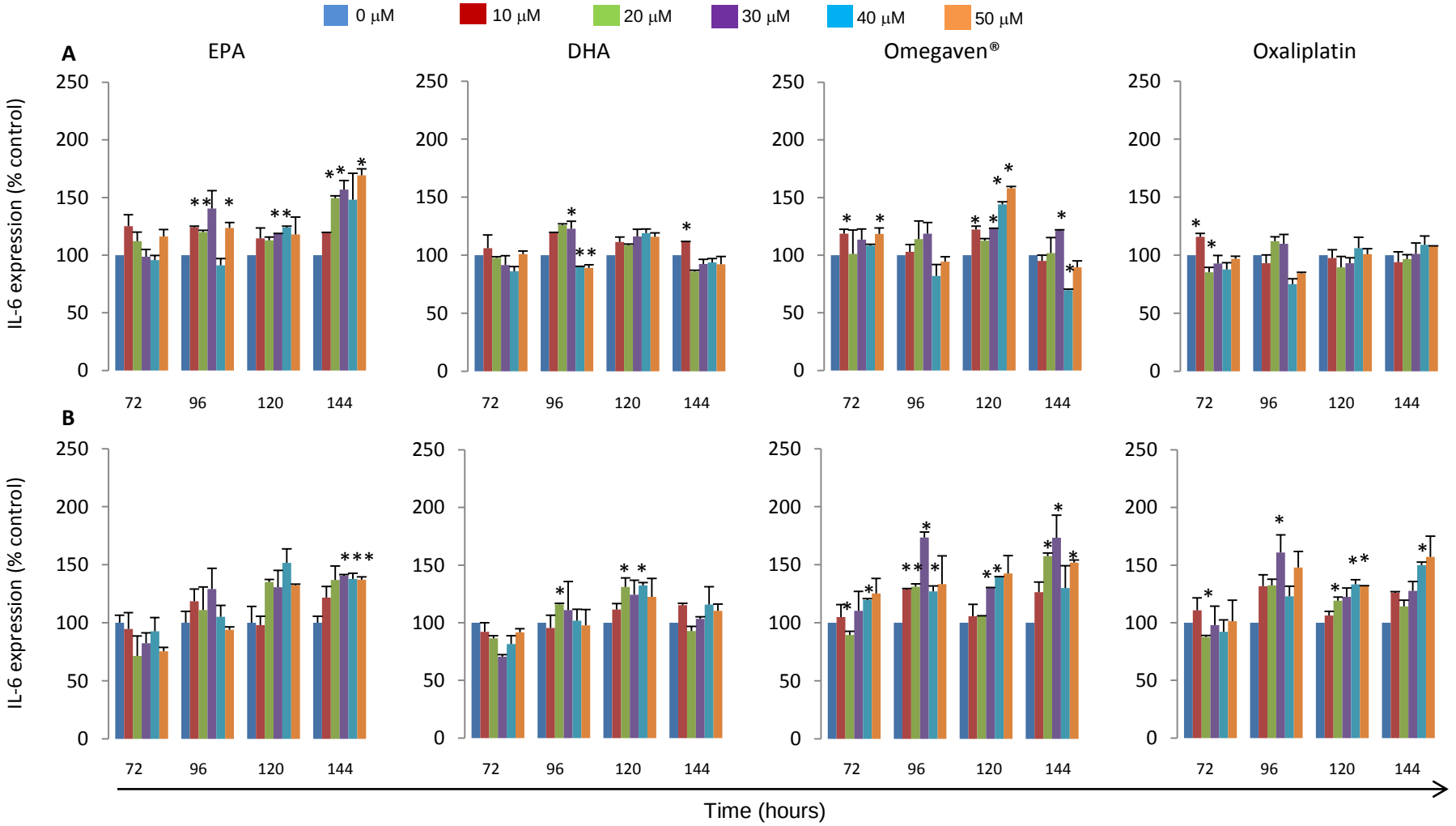
Analysis was made using ELISA assays in duplicate. The graphs represent the mean and standard deviation (SD) of TNF-α concentration (pg/mL) expressed as a percentage of control. * indicate that the difference between the treatment and control was significant ($P<0.05$), using two tailed student's t test

6.3.3.3 IL-6 expression

The EPA treatment of both cell lines was associated with an overexpression of IL-6; this effect was evident in OE33 cells as early as 96 hours and when treated with 10 μM. Although OE19 cells had a trend toward an increase in IL-6 expression, this only reached significance when exposed to treatment for a prolonged period. Kubota *et al* showed that only treating oesophageal SCC cell lines with higher concentration of EPA (≥ 300 μM) inhibits cell proliferation and suppresses IL-6 production [312], the IL-6 expression was in a similar manner as for TNF-α changes. The IL-6 changes might be stimulated by tumour necrosis factor alpha and interleukin-1 as reported by Yuzhalin *et al* in 2014 [285]. The DHA treatment elicited an increase in OE33 cells IL-6 expression and was statistically significant at 96 hours. Similarly in OE19 cells DHA increased IL-6 expression from 96 hours with 20 μM of DHA.

The Omegaven® treatment was associated with an increase in IL-6 expression by OE33 cells at 72 and 120 hours but decreased after prolonged treatment. The Omegaven® effect on OE19 cells associated with an increase in IL-6 expression at all time points. The effect of oxaliplatin on OE19 cells was associated with an increase in IL-6 expression with an increase in the concentration and time. The changes in IL-6 expression in both cell lines are presented in Figure 38.

Figure 38: Effects of EPA, DHA, Omegaven® and Oxaliplatin treatments over time on IL-6 expression in (A) OE33 cells and (B) OE19 cells



Analysis was made using ELISA assays in duplicate. The graphs represent the mean and standard deviation (SD) of IL-6 concentration (pg/mL) expressed as a percentage of control. * indicate that the difference between the treatment and control was significant ($P<0.05$), used two tailed student's t test.

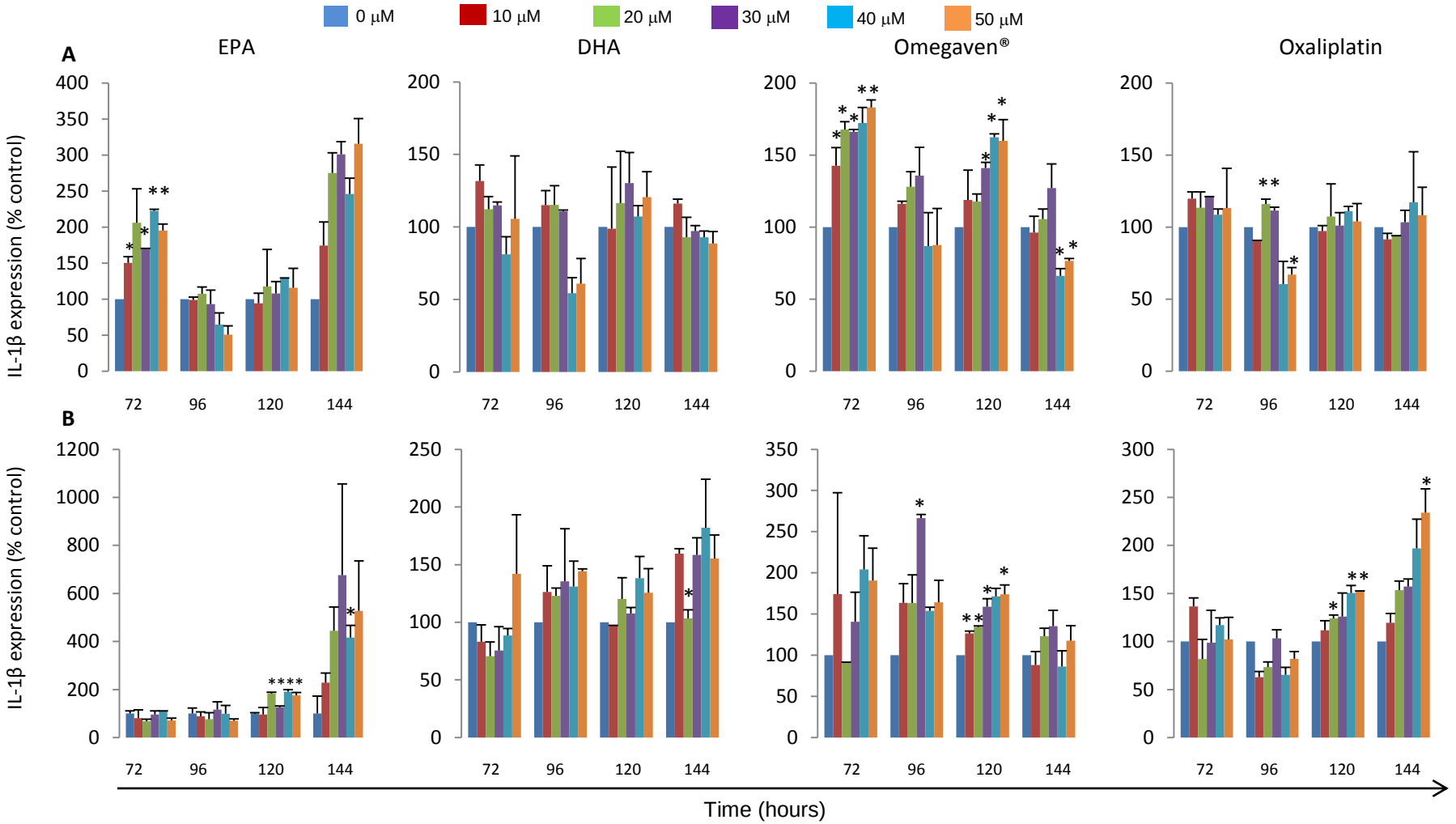
6.3.3.4 IL-1β expression

The EPA was associated with time dependent changes and a significant increase in IL-1β expression by OE33 cells at 72 hours when treated with 10-50 μM. After 120 hours of treatment with 20-50 μM EPA, OE19 cells were associated with statistically significant increase in IL-1β expression and only with 40 μM after 144 hours. The DHA effects were concentration dependent in IL-1β expression by both cell lines.

Omegaven® was associated with concentration dependent changes in both cell lines. In OE33 cell lines treated Omegaven® 10-50 μM at 72 hours and after 120 hours of 30-50 μM treatment there was an increase in expression. Higher concentrations 40-50 μM of Omegaven® after prolonged treatment of 144 hours, resulted in a decrease in IL-1β expression. In OE19 cell lines treated with Omegaven®, there was a significant increase in IL-1β expression when treated with 30 μM and 10-50 μM at 96 and 120 hours of treatment respectively.

The oxaliplatin treatment in OE33 cell lines was associated with significant concentration dependent changes at 96 hours of treatment with increase in IL-1β expression with smaller concentration (20-30 μM) and decrease in IL-1β expression with higher concentration (50 μM). In OE19 cell lines treated with oxaliplatin, there were concentration dependent changes in the first 96 hours of treatment, but then associated with significant increase in IL-1β expression at 120 hours (30-50 μM) and 144 hours (50 μM). The changes in IL-1β expression in both cells were presented in Figure 39.

Figure 39: Effects of EPA, DHA, Omegaven® and Oxaliplatin treatments over time on IL-1β expression in (A) OE33 cells and (B) OE19 cells



Analysis was made using ELISA assays in duplicate. The graphs represent the mean and standard deviation (SD) of IL-1β concentration (pg/mL) expressed as a percentage of control. * indicate that the difference between the treatment and control was significant ($P<0.05$), used two tailed student's t test.

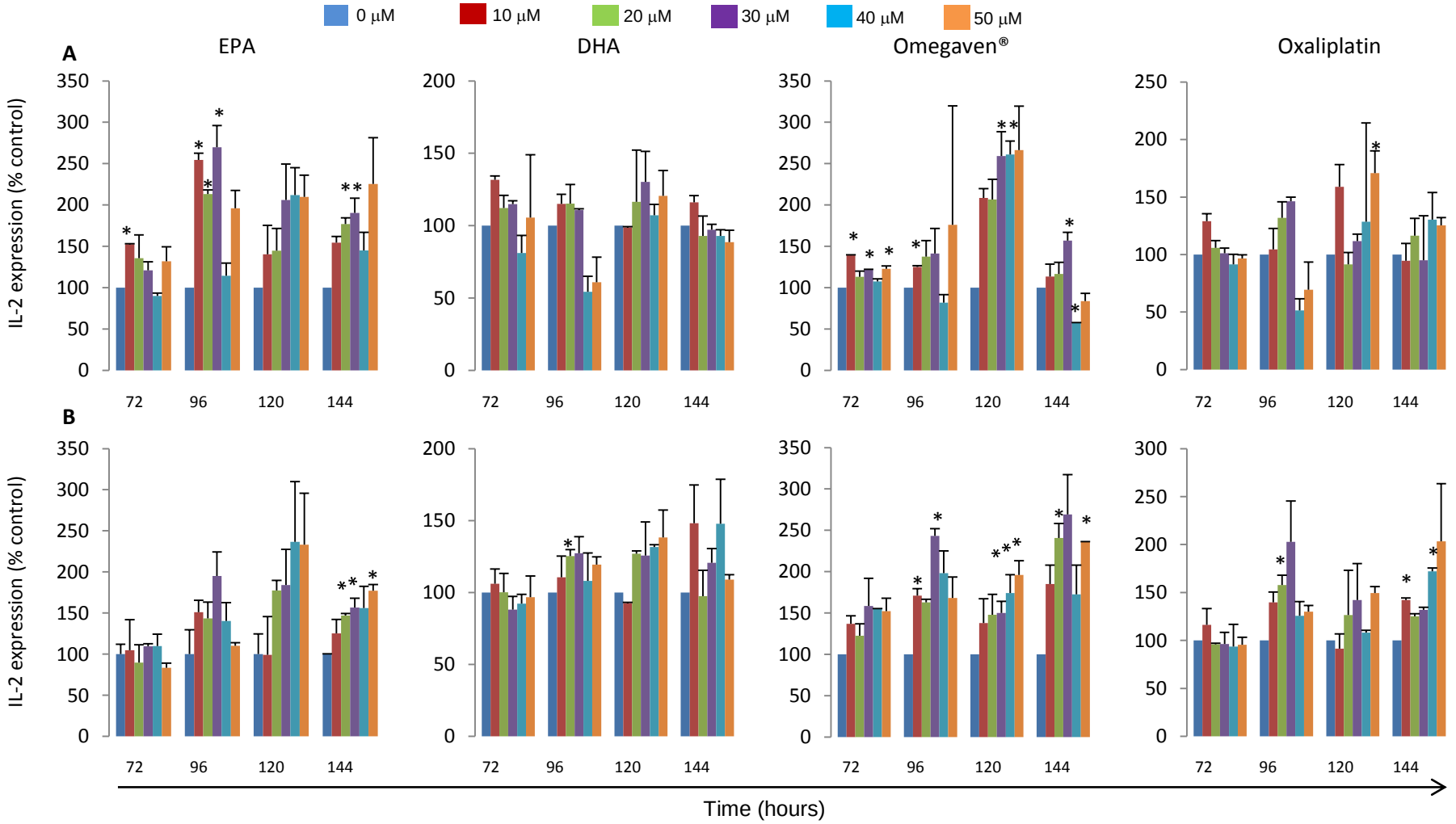
6.3.3.5 IL-2 expression

In OE33 cell lines treated with 10-30 μM of EPA at 72, 96 and 144 hours, there was significant increase in IL-2 expression. When compared to the OE19 cells treated with a similar concentration and time points, there were significant increase of IL-2 expression only after prolonged exposure to higher concentration (30-50 μM EPA). There were DHA concentration dependent changes in IL-2 expression by both cell lines (Figure 40).

Omegaven® was associated with concentration dependent changes at 96 and 144 hours after, but only increased expression of IL-2 at 72 and 120 hours with concentrations varies from 10-50 μM of Omegaven®. In OE19 cells treated with 10-50 μM Omegaven®, there was increase in IL-2 expression at 96-144 hours form treatment exposure.

There were concentration dependent changes in IL-2 expression by OE33 cells treated with oxaliplatin but only statistical significant increase at 120 hours when treated with 50 μM oxaliplatin. In OE19 cell lines had a trend toward an increase in IL-2 expression. The significant changes were evident as early as 96 hours with 20 μM concentration 10 μM at 144 hours from treatment exposure.

Figure 40: Effects of EPA, DHA, Omegaven® and oxaliplatin treatments over time on IL-2 expression in (A) OE33 cells and (B) OE19 cells.



Analysis was made using ELISA assays in duplicate. The graphs represent the mean and standard deviation (SD) of IL-2 concentration (pg/mL) expressed as a percentage of control. * indicate that the difference between the treatment and control was significant ($P<0.05$), used two tailed student's t test.

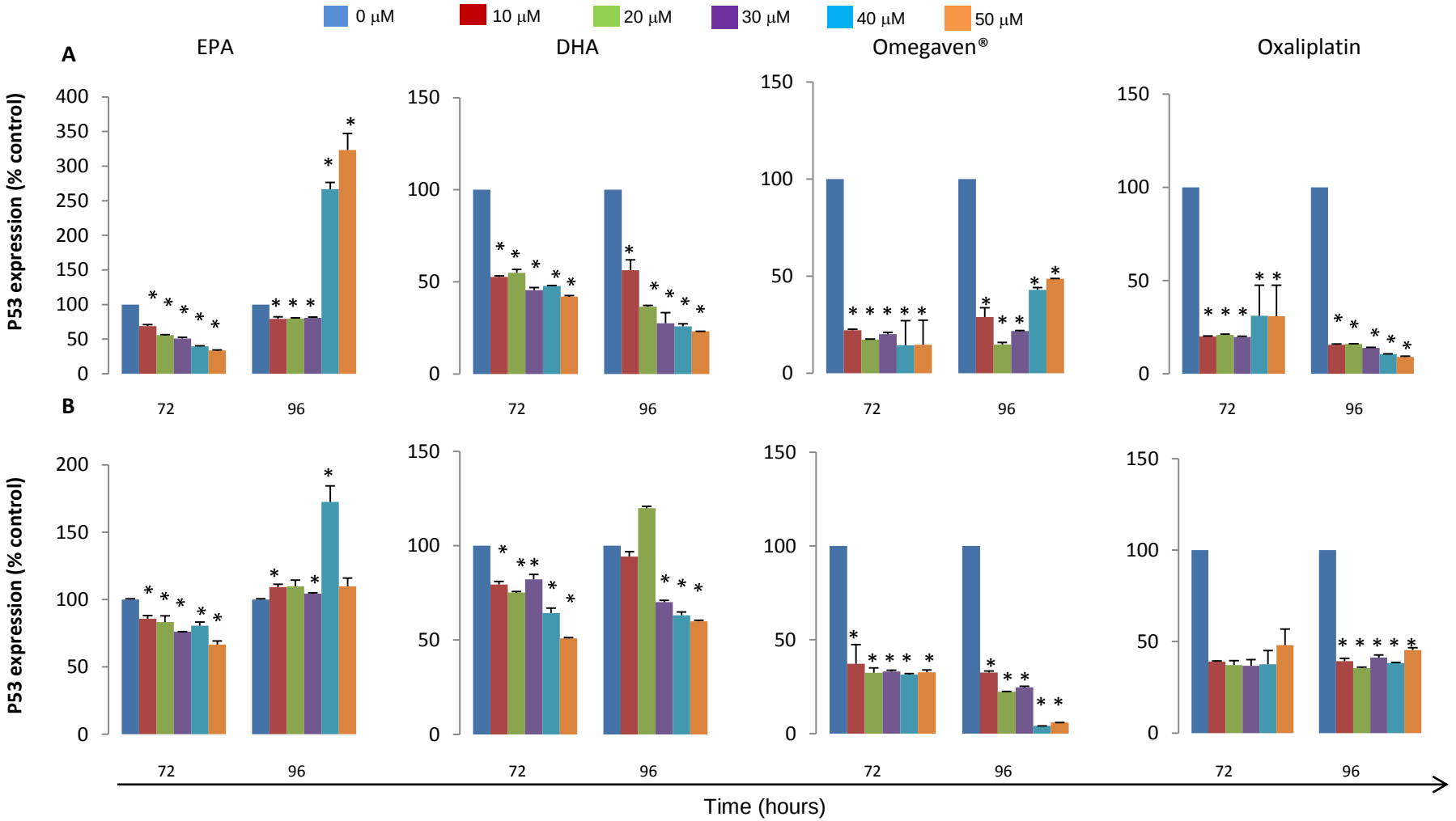
6.3.4 Cell signal biomarkers assessment

6.3.4.1 P53 protein expression

The EPA treatment had concentration and time dependent effects on OE33 cell line expression of p53 protein. At 72 hours, there was downregulation of p53 expression and at 96 hours, 10-30 μM of EPA lead to downregulation of p53 and 40-50 μM associated with upregulation of p53 protein.

The effects of 10-50 μM of DHA, Omegaven® and oxaliplatin in both cell lines, there were significant reduction in p53 protein at both 72 and 96 hours of treatment (Figure 41). Lee *et al* reported gastric cancer cell lines treated with 150 μM of DHA to be associated with increased apoptosis and p53 expression [137]. The changes in p53 protein expression in this experiment were most likely to be related to the fact that both cells were p53 mutant type.

Figure 41: **Effects of EPA, DHA, Omegaven® and Oxaliplatin treatments over time on p53 protein expression in (A) OE33 cells and (B) OE19 cells.**



Analysis was made using ELISA assays in duplicate. The graphs represent the mean and standard deviation (SD) of p53 concentration (signal) expressed as a percentage of control.* indicate that the difference between the treatment and control was significant (P<0.05), using two tailed student's t test.

6.3.4.2 P21 protein expression

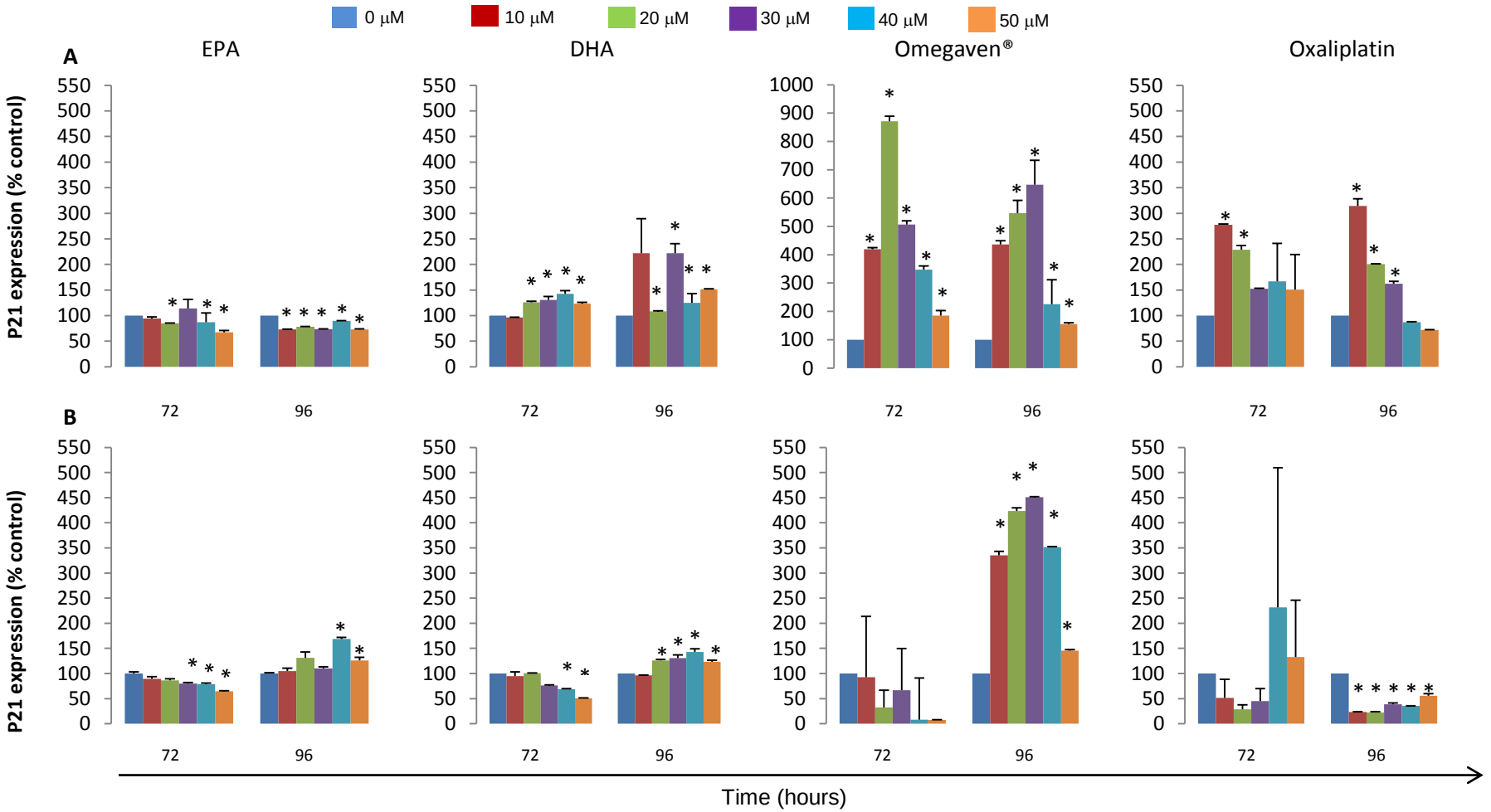
The effect of EPA on both cell lines was different. In OE33 cell lines; there were downregulation of p21 expression with all concentration and at both 72 and 96 hours. Whereas in OE19 cells, a higher concentration of EPA were associated with upregulation of p21 protein, after prolonged treatment exposure (96 hours).

In OE33 cells, 20-50 μM DHA was associated with upregulation of p21 protein at both 72 and 96 hours but only after prolonged treatment exposure of 96 hours in OE19 cells. In OE19 cells, at 72 hours 40-50 μM of DHA caused downregulation of p21 protein. The effects of Omegaven® were similar to those of DHA in both cell lines.

Oxaliplatin 10-30 μM was associated upregulation of p21 but not higher concentration in OE33 cells. In OE19 cells there was downregulation effects but statistically significant after prolonged treatment (details shown in Figure 42).

The pro apoptotic role of p21 protein had been investigated in a p53 dependent or independent manner [313,314] and the proapoptotic effect of p21 had been reported to be related to TNF-α activity [315-317]. There were no oesophageal cell line studies examining the effects of omega-3 PUFAs on either p21 or p53 protein expression [292]. In colorectal cancer cell lines, treatment with 5-100 μM of DHA lead to significant increase apoptosis and upregulation of p21 protein and Danbara *et al* reported no changes in p53 expression but upregulation of p21 protein [194,195,210].

Figure 42: Effects of EPA, DHA, Omegaven® and Oxaliplatin treatments over time on p21 protein expression in (A) OE33 cells and (B) OE19 cells



Analysis was made using ELISA assays in duplicate. The graphs represent the mean and standard deviation (SD) of p21 concentration (signal) percentage of control. * indicate that the difference between the treatment and control was significant (P<0.05). Statistical analysis was made by two tailed student's t test

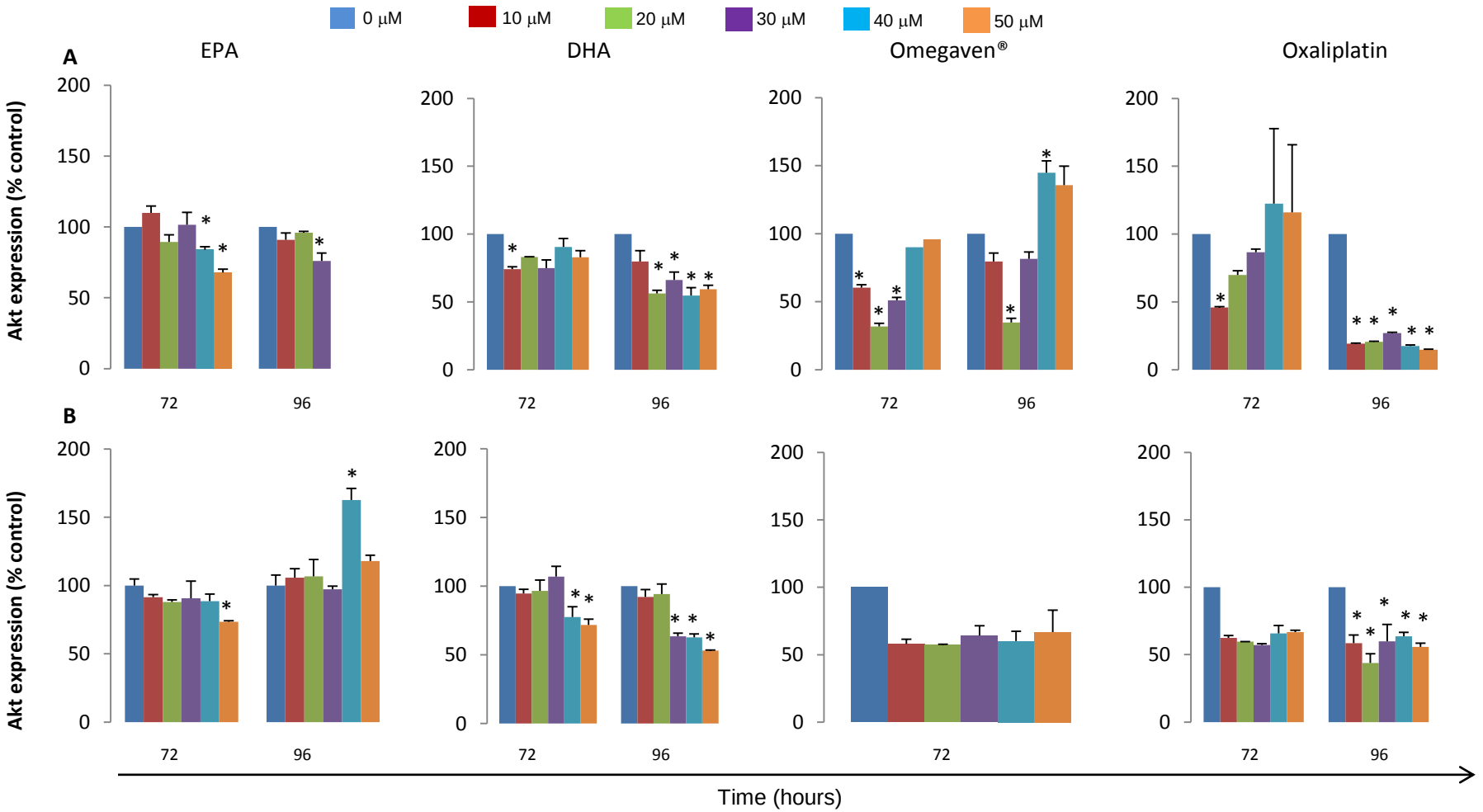
6.3.4.3 Akt expression

It has been reported that there was a positive correlation between proliferation and Akt expression in OE33 and OE19 cell lines when exposed to risk factors such as leptin and acidic media [318,319]. In our experiment, OE33 cell lines treated with EPA only higher concentrations (40-50 μM) were associated with downregulation of Akt at both 72 and 96 hours, there were similar effects on OE19 cells at 72 hours but 40-50 μM at 96 hours resulted in upregulation of Akt. The DHA treatment in both cell lines resulted in downregulation of Akt but OE33 cells (10 μM at 72 hours and 20 μM at 96 hours) more sensitive to treatment than OE19 cells (40 μM at 72hours and 30 μM at 96 hours).

Omegaven® (10-30 μM) resulted in significant downregulation of Akt in OE33 cells but not OE19 cells at 72 hours. The data from OE19 cells were not available, hence not presented. Oxaliplatin was associated with downregulation of Akt in both cell lines but the effect was statistically significant at 96 hours with 10-50 μM (Figure 43).

There were no previous studies investigated the effects of omega-3 PUFAs on oesophageal adenocarcinoma cell lines Akt expression.

Figure 43: Effects of EPA, DHA, Omegaven® and Oxaliplatin treatments over time on Akt expression in (A) OE33 cells and (B) OE 19 cells.



Analysis was made using ELISA assays in duplicate. The graphs represent the mean and standard deviation (SD) of Akt expressed as a concentration (signal) percentage of control. * indicate that the difference between the treatment and control was significant ($P<0.05$), used two tailed student's t test.

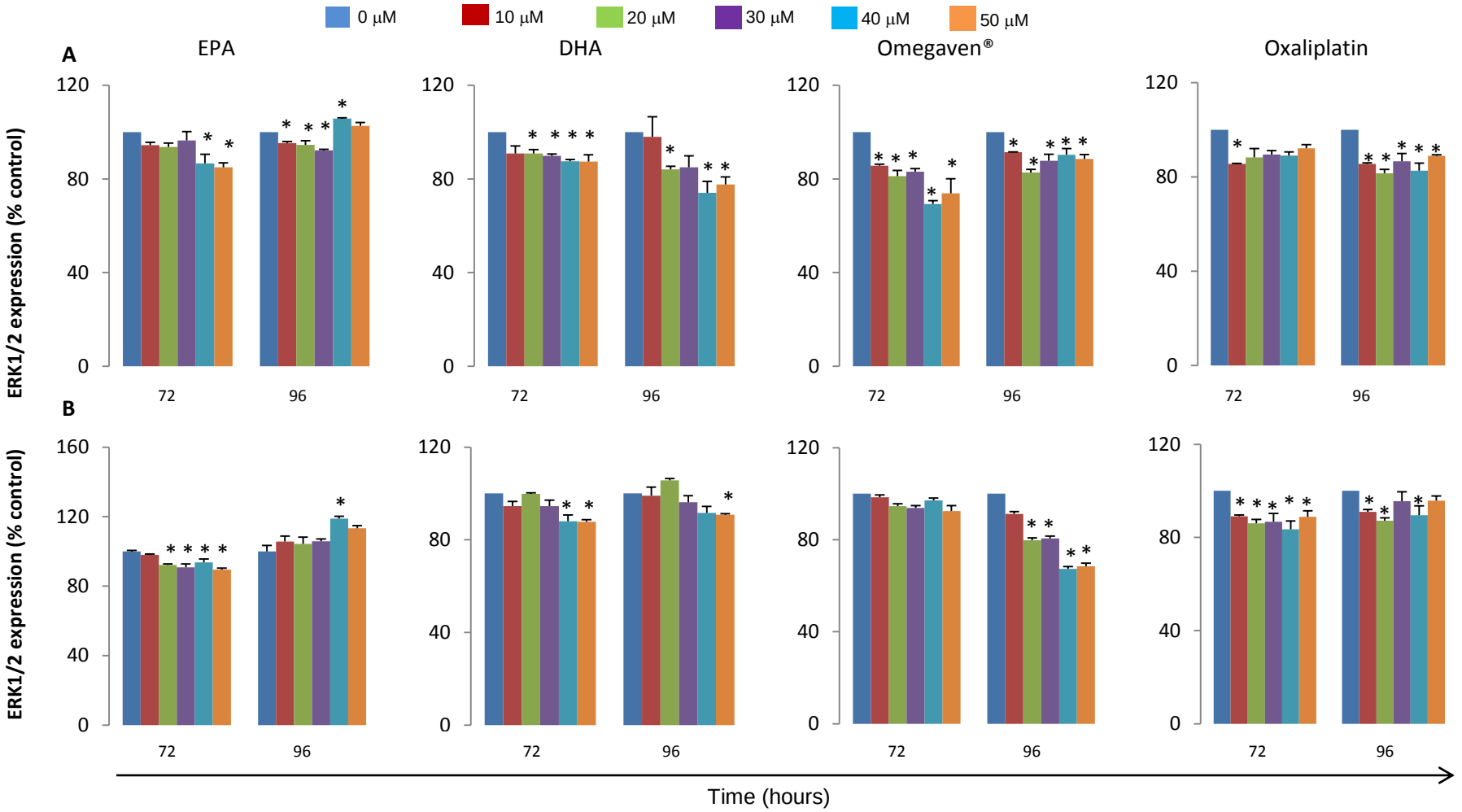
6.3.4.4 ERK1/2 expressions

It has been reported that there was a positive correlation between proliferation and ERK1/2 expression in OE33 and OE19 cell lines when exposed to certain risk factors [318,319]. There were no previous studies investigated the effects of omega-3 PUFAs on oesophageal adenocarcinoma cell lines ERK1/2 expression.

In this experiment, OE33 cells treated with 10-50 μM of DHA, Omegaven® and oxaliplatin were associated with significant downregulation of ERK1/ 2 at both 72 and 96 hours of treatment. Higher concentration of EPA (40-50 μM) resulted in downregulation of ERK1/ 2 at 72 hours and upregulation at 96 hours (Figure 44).

In OE19 cells treated with 20-50 μM of EPA at 72 hours resulted in downregulation of ERK1/2 and this effect was lost at 96 hours. Higher concentration 40-50 μM of DHA at 72 hours and 50 μM at 96 hours were associated with downregulation of ERK, Omegaven® was also associated with ERK downregulation but only after prolonged exposure (Figure 44). The downregulation effect of DHA, and Omegaven® were more prominent in OE33 treated cell lines at both time points 72 and 96 hours than in OE19 cell lines, in a similar manner to the growth inhibition presented above. Oxaliplatin was associated with downregulation of ERK1/2 in both cells.

Figure 44: Effects of EPA, DHA, Omegaven® and Oxaliplatin treatments over time on ERK1/2 expression in (A) OE33 cells and (B) OE19 cells.



Analysis was made using ELISA assays in duplicate. The graphs represent the mean and standard deviation (SD) of ERK1/2 concentration (signal) expressed as a percentage of control. * indicate that the difference between the treatment and control was significant (P<0.05), used two tailed student's t test

6.4 Conclusions

EPA, DHA, and oxaliplatin were associated with antiproliferatve effects on oesophageal adenocarcinoma cell lines. These effects were also observed with Omegaven®, but were concentration dependent, being evident at intermediate concentrations. DHA, Omegaven® and oxaliplatin were associated with a reduction in VEGF expression in both oesophageal cell lines, the effects being more evident after prolonged exposure at higher concentrations. The treatments were not associated with significant reductions in pro inflammatory cytokines in the cell lines. The treatment was associated with upregulation of the apoptosis protein p21 but not p53 protein. This treatment also resulted in a significant reduction in the total Akt and ERK1/2 in both cell lines.

Chapter Seven

General Discussion

7 Discussion

7.1. Clinical trial

7.1.1. Study design

The intention of this pilot study was to inform whether the addition of omega-3 PUFAs to the current standard of palliative chemotherapy would affect robust outcomes such as the biochemical, radiological and clinical measures in patients with metastatic oesophago-gastric cancer.

The single arm Simon's two stage model is a well recognised model for phase II clinical trials in oncology. The principal limitations of this study design are the lack of randomisation and the lack of a contemporaneous control group. For this reason, a historical group of patients who were treated with the same chemotherapy regimen during the two consecutive years prior to the start date of this study was used to provide an estimate about the clinical effectiveness of this treatment regimen. The clinical trial inclusion and exclusion criteria were used to identify the historical cohort used for comparison and to minimize the selection bias.

The dosing schedule for the omega-3 fatty acid infusion was on weekly basis. It was a challenge determining the optimum dosing regimen as the literature lacks similar studies in patients with oesophago-gastric cancer. However, this schedule was decided as a similar schedule was previously administered in our institution to patients with advanced pancreatic cancer, with the exception that gemcitabine chemotherapy was used in place of EOX[308]. The addition of the Omegaven®

meant that trial participants attended hospital on a weekly basis, compared to on a three weekly basis under normal circumstances. Despite these extra two visits per chemotherapy cycle, the recruitment rate of 21 patients during 14 month period was higher than expected, given the physical and emotional burden of cancer these patients had to deal with. We anticipated that many of these patients with dysphagia would find it an additional challenge, ingesting oral capsules. This might have reduced compliance and reduced the magnitude of any potential therapeutic effect. The differences between oral and intravenous routes were discussed in section 7.2.

7.1.2. Clinical outcome

There have been over 50 laboratory studies investigating the effects of EPA and DHA omega-3 PUFAs (fish oil) in gastrointestinal cancers, mainly of colorectal and pancreatic origin. Six studies have indicated that fish oil treatment may enhance the anti-proliferative effects of chemotherapy on treated cancer cell lines [160-165]. There have been four clinical trials examining the role of omega-3 PUFAs in the palliative treatment of advanced gastrointestinal cancers, in association with chemotherapy, two in patients with colon cancer [271,273], one in patients with pancreatic cancer [306] and one in patients with oesophago-gastric cancer [272].

In the latter study the authors administered an intravenous DHA-paclitaxel infusion every 21 days for up to four cycles (84 days). The authors identified a reduced frequency of haematological toxicity, but in other respects the clinical course of the patients who received additional DHA was similar to that of patients who received paclitaxel or docetaxel alone. In that study, the progression free and overall survivals were three and nine months respectively [272].

Arshad *et al* employed intravenous omega-3 PUFA supplements (Lipidem®) in combination with gemcitabine chemotherapy in patients with advanced pancreatic cancer [308], and reported a superior response rate and overall survival compared to historical control patients [308]. The two studies of patients with colorectal cancer that used EPA enriched oral nutritional supplements in combination with chemotherapy (folinic acid, 5FU, oxaliplatin plus capcitabine and folinic acid, 5FU plus irinotecan) reported favourable effects on body weight preservation and quality of life, but no improvement in survival [271,273].

These findings are broadly similar to the findings reported in a number of studies by the Edinburgh group of single agent oral omega-3 fish oil in advanced pancreatic cancer [278,280,281]. In those studies, single agent fish oil was associated with better weight maintenance and improved quality of life.

The current pilot phase II clinical trial has shown that the addition of weekly omega-3 PUFAs infusion (Omegaven®) to combination chemotherapy regimen (EOX) is feasible and safe [320]. The most significant findings were a reduced frequency of gastrointestinal toxicity and thromboembolic events, and a greater radiological response rate compared to historical controls who received chemotherapy alone. We did not demonstrate any survival advantage for those treated with omega-3 PUFAs.

The antithrombotic effects of omega-3 fatty acids may explain the lower frequency of thromboembolic events finding in the current study among patients receiving platinum based chemotherapy and Omegaven®. The reported biological antithrombotic actions of omega-3 PUFAs include a reduction in whole blood viscosity [321], a reduction in platelet count, a reduced sensitivity of platelets to collagen[322], inhibition of platelet aggregation and adhesion[323-325], a reduction in plasma fibrinogen levels and a prolongation of thrombin time, independent of Vitamin K[326].

We did note a higher frequency of abnormal haematologic tests in patients treated with fish oil, although it should be pointed out that this group underwent a greater level of blood sampling than historical controls. Participants in the

intervention group had full blood counts checked three times per chemotherapy cycle, while those in the control arm had their blood count checked only once per chemotherapy cycle.

The majority of previous clinical studies employed oral omega-3 fish oil supplementation. We elected to use intravenous supplementation in order to achieve better compliance and potentially higher omega-3 fatty acid levels than would be achieved with oral supplementation.

7.1.3. Effects of palliative EOX plus omega-3 PUFAs on cytokine expression

Several studies have presented the correlation of omega-3 PUFAs in reducing inflammatory biomarkers and improving immunity in cancer patients. However, no reports to date have studied the use of intravenous of omega-3 PUFAs in patients with advanced oesophago-gastric adenocarcinoma receiving palliative chemotherapy. Arshad A *et al*. have previously shown an improved overall survival for patients with low baseline expression of IL-6 and IL-8 cytokines in a cohort of patients with advanced pancreatic cancer who received palliative gemcitabine chemotherapy in combination with omega-3 PUFAs [308]. The latter study also demonstrated a significant reduction in platelets derived growth factor and fibroblast growth factor but not VEGF.

In patients with oesophagogastic cancer being treated with curative intent, Deans *et al* demonstrated that high expression of IL-1β was associated with a poorer survival[327]. El-Omar *et al* showed that IL-1β was upregulated during the course of H. *pylori* gastritis, that it played a major role in the inhibition of gastric acid secretion and as a result of associated chronic H. *pylori* gastritis with hypochlorhydria was associated with the development of gastric cancer [328]. In the current study, IL-1β changes were not correlated with survival outcome. Dvorakova *et al* have previously demonstrated that increased expression of IL-6 in patients with Barrett's oesophagus contributed to the development of apoptosis resistance and malignancy [329]. Liao *et al*, noted high expression of IL-6 cytokine to be associated with a poorer survival after gastrectomy for cancer, and that the cytokine carried greater prognostic weight than TNM staging [330]. In this study, we

demonstrated that higher expression of IL-6 was associated with a poorer prognosis.

7.2. Fatty acid uptake

7.2.1. Plasma and red blood cell membrane polyunsaturated fatty acid uptake

There is increasing interest in using fish oil (a source of the omega-3 PUFAs EPA and DHA) for cancer prevention and as a component of cancer therapy. Almost 20 years ago Caygill *et al*. reported on mortality data for breast and colorectal cancer for 24 European countries; they found an inverse correlation between colorectal and breast cancers and fish and fish oil consumption [331]. In preclinical models DHA, and to a lesser extent EPA, suppressed oestrogen-independent breast cancer [332] while in clinical settings oral DHA supplements taken during chemotherapy of breast cancer led to reduced toxicity and improved outcome of chemotherapy and chemosensitized breast tumours [64]. A combined EPA and DHA as an oral supplement increased efficacy of chemotherapy in patients with advanced non small cell lung cancer which improved the response rate and other clinical benefits[333]. The use of omega-3 PUFAs in the palliative management of gastrointestinal cancer has been investigated mainly in colorectal and pancreatic cancers [274-276,278-280,334]. In these latter studies omega-3 PUFAs were used as a single agent, but in four other studies omega-3 PUFAs were combined with palliative chemotherapy with reported improvement in chemotherapy related toxicity and preservation of lean body weight [271-273]. Fish oil supplementation resulted in improved survival in three studies [278,280,334], while improvement in quality of life was noted in four studies. [271,273,274,280]

In the studies described above [274-276,278-280,334], oral fish oil supplements (i.e., capsules) were used in patients with colorectal, breast, lung or prostate cancer, conditions in which swallowing is not compromised . However, the use of oral capsules would be more challenging for patients with advanced oesophageal cancer due to luminal obstruction and resultant dysphagia. In these patients, intravenous administration of omega-3 PUFAs in the form of fish oil could be advantageous. Not only would this circumvent problems with swallowing, but omega-3 PUFAs are reported to be more easily incorporated into plasma, blood cells and tissues when infused intravenously compared to when given orally in rats [307]. Furthermore higher doses of EPA and DHA can be given intravenously than can be consumed orally and the intravenous route assures compliance. Non-compliance has been reported to be a problem in some studies of oral supplements in cancer patients [277,335]. It was considered that repeated short infusions over a period of several months could be a strategy for supplying omega-3 PUFAs to patients with advanced oesophago-gastric cancer to increase their status of EPA and DHA.

Omegaven® is a fish oil supplement emulsified with purified egg phosphatide. The fatty acids in the fish oil are largely present in the form of triacylglycerols i.e., fatty acids esterified to glycerol; EPA and DHA contribute about 40 to 45% of the fatty acids present. Upon infusion the triacylglycerols are hydrolysed in the circulation by lipases releasing NEFAs. During the course of a single infusion we observed a marked increase in EPA and DHA in the NEFA pool, an average of 18.5- and 7-fold increases, respectively. This is consistent with the aforementioned

hydrolysis of the triacylglycerol component of the fish oil and is important because the released omega-3 NEFAs would be made available to cells and tissues where they could elicit their biological effects. These would include host cells involved in inflammation, immune and metabolic responses but also cancer cells. These cells would take the fatty acids up by general free fatty acid uptake mechanisms [133], but in addition some cells, including inflammatory macrophages, express receptors that have some specificity for omega-3 PUFAs, particularly DHA [336]. Thus, this rapid release of non-esterified EPA and DHA would act to facilitate the functional activities of these fatty acids. There was also a 50% increase in non-esterified AA during infusion. This is most likely because Omegaven® contains AA (0.1 to 0.4 g per 100 ml according to the manufacturer) which would also be freed by lipases. Patients received repeated weekly infusions of Omegaven® for up to 6 months. The appearance of EPA, DHA and AA in the NEFA pool was very similar with each infusion. To our knowledge this is the first time that fatty acid changes with such a repeated regimen of fish oil infusion have been reported.

Plasma PC, EPA, but not DHA, increased by a small amount during infusion. PC acts as a monolayer "coat" on lipoproteins and the small increase in plasma PC EPA during the 4 hour infusion would suggest recycling of the non-esterified EPA that originated in Omegaven® into PC over that period. This most likely occurs in the liver. It is not clear why DHA does not appear in plasma PC during a four hour infusion, but appearance of DHA in plasma PC takes longer than appearance

of EPA [132], probably reflecting different metabolic handling of the two omega-3 PUFAs.

Arshad *et al.* investigated short term (2 hour) infusion of an omega-3 fatty acid containing lipid emulsion in patients with advanced pancreatic cancer weekly for three consecutive weeks followed by a rest week, and assessed pre-infusion fatty acid levels for up to six months [308]. In that study, post-infusion levels were measured only for seven weeks [308]. The novelty of the current study is its use of repeated short-term infusions (a single infusion of 4 hours per week) over a long period of time (up to 6 months). This regimen resulted in increased EPA in plasma PC and in RBC membranes. These increases were progressive over time, suggesting a gradual accumulation of EPA in these pools. This demonstrates that this approach enables net accumulation of EPA in blood lipid and cell pools and this would be expected to influence cell and tissue function. Oral supply of EPA results in a time-dependent accumulation of EPA in plasma PC and in RBC membranes [132]. In the current study, DHA did not accumulate in the way that EPA did. It is not clear why this is the case, since DHA accumulates in plasma PC and RBC membranes when taken as a regular oral supplement over a period of time, although accumulation of DHA is slower than that of EPA [132]. Whatever the reason, the observation suggests that the DHA provided in each infusion is used by the body in a different way than the EPA and that it may not accumulate.

The regimen of repeated infusions of fish oil did not result in net accumulation of EPA or DHA in plasma NEFAs assessed prior to each infusion. In this state, most NEFAs would be derived from hydrolysis of triacylglycerols stored in adipose

tissue. The absence of any accumulation of EPA or DHA in plasma NEFAs would suggest that there is very limited or no storage of infused EPA and DHA in adipose tissue. It is worth noting that oral supplementation with high doses of EPA and DHA for periods as long as one year results in only very small accumulation of those fatty acids in adipose tissue [132,309].

The ratio of omega-6 to omega-3 PUFAs in the diet, in blood lipids and in cells and tissues is thought to be important in influencing metabolism and cellular processes, including proliferation of colorectal cancer cells [151]. In the current study, the changes in the fatty acid content of plasma NEFAs during infusion and in plasma PC following repeated infusions resulted in a lowered ratio of omega-6 to omega-3 PUFAs. This would likely be of functional significance.

7.3. Effects of treatment on two oesophageal cell lines

It has been reported in four previous studies on gastric adenocarcinoma cell lines that omega-3 PUFAs mainly EPA and DHA, present in fish oil to be associated with anti-proliferative effects [135,137,159,215], but the literature lacks evidence on oesophageal adenocarcinoma cell lines [292]. We have shown that EPA, DHA, and oxalipatin have anti-proliferative effects on two oesophageal adenocarcinoma cell lines. There was a more pronounced dose response with DHA and oxaliplatin than EPA. Oxaliplatin as a single treatment or coupled with EPA has been previously investigated in colorectal cancer cell lines[161,182]. These studies demonstrated that the addition of omega-3 PUFAs enhanced the oxaliplatin cytotoxic effects and reduced cell growth[161,182]. In this study, oxaliplatin was used as a single treatment only and was associated with significant reduction in cell growth from as low as 10 μM. The combination of oxaliplatin and omega-3 PUFAs was not explored in the current series of experiments, but this remains the potential next avenue of research.

This study differs from previous studies investigated omega-3 PUFAs in that we used EPA and DHA as a single treatment and in combination in the form of a complex fatty acids emulsion (Omegaven® lipid emulsion) in oesophageal adenocarcinoma cell lines for the first time.

At low concentrations (10 μM) of Omegaven®, treatment resulted in a significant increase in cell number for both OE19 and OE33 cell lines, at intermediate concentrations of 20-30 μM, there was reduction in cell count. This has been

previously investigated in colorectal cancer cells and the findings were similar to our study in that higher concentrations of Omegaven® emulsion (0.72-1.44 ml/l equivalent to 50-100 μM of EPA) [165].

Granci *et al*, investigated the effects of Omegaven® (EPA and DHA equivalent of 24 μM and 20.5 μM respectively) in combination with oxaliplatin in colorectal cancer HT-29 cells, this combination was associated with further significant reduction cell viability when compared to oxaliplatin alone [162]. This concentration dependent effect had also been reported previously in breast cancer cell proliferation using another dietary agent (Genistein) and the authors reported increased cell proliferation at smaller concentrations (1 μM) and proliferation was decreased with higher genistein concentration (25 μM) [337].

Omegaven® emulsion has various other substances such as preservatives and stabilisers present, which might theoretically have altered the growth characteristics of the oesophageal cancer cell lines. In addition, it was very challenging to calculate accurate equivalent concentrations of EPA and DHA, as each 100 mL bottle of Omegaven® merely specified a concentration range; 1.25 - 2.82 g of EPA and 1.44 – 3.09 g of DHA according to the summary of product characteristics of Omegaven®. Hence, these findings should be interpreted cautiously.

In colonic cancer cells, both EPA and DHA (10-30 μM) were associated with inhibition of VEGF expression [338]. *In vivo*, mice injected with colorectal cancer cells and fed EPA and or DHA were associated with a reduction in tumour size and reduced expression of VEGF [338]. Higher baseline serum VEGF levels have been shown to be associated with poor survival [339], and promotion of metastasis [340].

In the current series of experiments, the most profound effects on cytokine production were on VEGF. The treatment of OE33 cells with DHA, Omegaven® and oxaliplatin in this experiment, showed significant decreases in VEGF expression after prolonged treatment of 144 hours (Figure 36). The changes in OE19 cells were less marked than those seen on OE33 cells. Although both cell lines are derived from tumour tissue from patients with oesophageal adenocarcinoma, the OE33 cell lines arose in the setting of extensive Barrett's oesophagus. These phenotypic differences may explain some of the differences in cytokine expression under exogenous influences.

The effects of treatment on TNF-α was examined and we found that smaller concentration of DHA (30 μM), Omegaven® (30 μM) and oxaliplatin (10 μM) resulted in TNF-α upregulation and that 40-50 μM resulted in downregulation of TNF-α in OE33 cells. This sort of bi-phasic low dose vs high dose effect appears to be a common finding in response to a variety of dietary agents and has previously been reported by Lavigne *et al* in 2008, while investigating the effects of isoflavones (Genistein) in breast cancer cell lines [337]. In our experiment, the EPA treatment was associated with a significant increase in TNF-α expression as early as 96 hours of treatment in OE33 cells and 120 hours in OE19 cells. DHA,

Omegaven® and oxaliplatin were associated with only upregulation of TNF-α in OE19 cells.

Interleukin-1, IL-6 and TNF-α are cytokines that serves as a growth factors for oesophageal and gastric cancers and they are regulated by NF- kB [140], the concentration dependent changes in cytokine expression and the reduction in cell growth found in this experiment might be through downregulation of the NF- kB protein pro inflammatory pathway but this was not investigated in this experiment. The suppression of NF- kB protein expression by colorectal cell lines (CaCo-2) treated with higher dose of DHA [158] and pancreatic cells treated with 100 μM of EPA was reported [160].

In the current studies, IL-2 expression was increased when OE33 and OE19 cells were treated with EPA, DHA, Omegaven® and oxaliplatin. There have been no previous studies using gastrointestinal cancer cell lines investigated the effects of omega-3 fatty acids on IL-2 expression[292]. However, in a clinical study by Chen *et al*, patients receiving enteral omega-3 PUFAs immunonutrition feeding had a significant increase in serum IL-2 concentration, suggesting that omega-3 PUFAs enriched immunonutrition restores immunity [259].

The novelty of this study was that we investigated the effects of EPA, and DHA as single agents and used Omegaven® for the first time in oesophageal adenocarcinoma cell lines. The assessment of various pro inflammatory cytokines and cell signal biomarkers forms the initial evidence of the effects of omega-3 PUFAs treatment in oesophageal adenocarcinoma cell lines to allow for future

research in identifying the potential mechanism of action in cell growth inhibition. Nevertheless, there are limitations to the current studies. All markers were assessed using ELISA but were not confirmed by Western blot analysis. Further, the current studies measured total protein concentrations. It would have been more sophisticated to assess the effects of treatment on protein phosphorylation for AKT and ERK1/2.

In light of the *in vitro* findings and the improved response rate to chemotherapy plus intravenous fish oil reported in our clinical trial, it is suggested that the possible anti-cancer mechanism of omega-3 PUFA might be mediated through inhibition of ERK and Akt protein signalling pathways. However, this requires confirmation in further studies that study the effects of treatment on protein phosphorylation rather than the total expression.

Chapter Eight

Conclusion and future directions

8 Conclusion and future directions

The study demonstrated no survival benefit. The principal findings from this pilot and feasibility study were that intravenous supplementation with Omegaven® (Fish oil) through a peripheral line was feasible, well tolerated and associated with a favourable safety/toxicity profile. The addition of Omegaven® to palliative chemotherapy for patients with oesophago-gastric cancer resulted in a lower frequency of thromboembolic and gastrointestinal adverse effects, an improved radiological response rate and a cytokine profile favouring an anti-inflammatory state.

The *in vitro* experiments using EPA, DHA and oxaliplatin as a single treatment demonstrated the anti-proliferative effects of these agents on two oesophageal adenocarcinoma cell lines. Omegaven® was associated with a biphasic reponse, causing increased cell proliferation at low concentrations and decreased cell proliferation at higher concentrations. This type of response is not uncommonly observed with dietary agents, and has been previously shown with use of isoflavons in breast cancer cell lines [337].

The most profound effect on cytokine expression was on VEGF. DHA, Omegaven® and oxaliplatin were all associated with a reduction in VEGF expression in both cell lines, the effects being more noticeable after prolonged exposure at higher concentrations.

The novelty of the current study is its use of repeated short-term infusions of nutritional supplement during palliative chemotherapy (a single infusion of

Omegaven® 4 hours per week) over a long period of time (up to 6 months) and the assessment of the cytokine biomarkers over the entire treatment period. Further, the assessment of the fatty acid composition of two plasma lipid pools and one cellular (RBC) pool is novel. A control group not receiving intravenous fish oil was not necessary in the current study because in the absence of an exogenous supply of EPA and DHA, their concentrations in plasma NEFAs, plasma PC and RBC membranes do not change, as shown by previous long term oral supplementation studies [132,309] and in short term intravenous infusion studies [341,342]

There are limitations to the current study, not least the small number of participants and the use of historical patients as a control group. Nonetheless, with a recruitment rate of 37% and a retention rate of 95%, it seems feasible that a larger scale study can be planned. Further, 60% of those recruited completed four cycles of treatment. The original intention had been to perform an interim analysis after the first phase of recruitment, and proceed with the second stage of recruitment up to 45 participants. As safety and tolerability of the regimen have been proven in the current study, it may be more appropriate to move directly to a randomised controlled trial to see of the findings of the current study are replicated in a multi-centre study.

Future work to elucidate the mechanism of the interaction of the trial treatment regimen on myeloid derived suppressor cell accumulation is warranted. This can be done by assessing the change in the number of cells with treatment using fluorescence-activated cell sorting analysis. In brief, myeloid derived suppressor

cell are driven by the pro-inflammatory mediators such as prostaglandins, vascular endothelial growth factor, and Interleukin-6. All of these biomarkers are produced in the cancer microenvironment by the host stromal cells. Activating the myeloid derived cells allows the pro-inflammatory mediators to inhibit the tumour immunity [343,344]. Hence, it will be interesting to know the effect of this treatment regimen on the myeloid derived suppressor cell accumulation and activation.

In term of *in vitro* experiments, the current experiments assessed only the effects of single agents on two oesophageal adenocarcinoma cell lines. To take this further, combination treatment with omega-3 PUFAs and oxaliplatin should be studied. Further, developing an oxaliplatin resistant oesophageal cell line model may yield clues as to future therapies. This would involve repeat treatment of the oesophageal cancer cell lines with low concentrations of oxaliplatin until the cell lines become refractory to treatment.

Appendix A

Section/topic	#	Check list item	Reported on page
TITLE	**1**	Title 1 Identify the report as a systematic review, meta-analysis, or both.	**45**
ABSTRACT			
Structured summary	**2**	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	**NA**
INTRODUCTION			
Rationale	**3**	Describe the rationale for the review in the context of what is already known.	**47**
Objectives	**4**	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	**47**
METHODS			
Protocol and registration	**5**	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	**Not existing**
Eligibility criteria	**6**	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	**47, 48 & 50**
Information sources	**7**	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	**48**
Search	**8**	Present full electronic search	**48 and 50**

		strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	**9**	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	**49**
Data collection process	**10**	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	**NA**
Data items	**11**	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	**NA**
Risk of bias in individual studies	**12**	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	**NA**
Summary measures	**13**	State the principal summary measures (e.g., risk ratio, difference in means).	**NA**
Synthesis of results	**14**	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis.	**NA**
Risk of bias across studies	**15**	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	**NA**
Additional analyses	**16**	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	**NA**
RESULTS			
Study selection	**17**	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for	**50 PRISMA chart**

		exclusions at each stage, ideally with a flow diagram.	
Study characteristics	**18**	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	**51-70**
Risk of bias within studies	**19**	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	**NA**
Results of individual studies	**20**	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	**NA**
Synthesis of results	**21**	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	**NA**
Risk of bias across studies	**22**	Present results of any assessment of risk of bias across studies (see Item 15).	**NA**
Additional analysis	**23**	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	**NA**
DISCUSSION			
Summary of evidence	**24**	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	**51-70**
Limitations	**25**	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	**NA**
Conclusions	**26**	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	**71**
FUNDING			
Funding	**27**	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders	**NA**

		for the systematic review.	

From: Moher D, et al, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. doi:10.1371/journal.pmed1000097

Appendix B

Inclusion criteria

Patients meeting all of the following criteria may be included in the trial:

1. Patients with histologically confirmed gastric or oesophageal carcinoma (irrespective of subtype), deemed incurable as a result of standard staging investigations.
2. Measurable disease according to RECIST 1.1 criteria on CT within 4 weeks of study entry
3. WHO Performance status 0-2
4. Aged >18 years
5. Able to give informed written consent
6. Life expectancy >12 weeks
7. Adequate hepatic and renal function documented within 7 days prior to treatment (estimated GFR>50ml/min, serum bilirubin <1.5x ULN; ALT or AST <2.5x ULN; ALP <3x ULN (in the absence of liver metastases). If liver metastases are present, serum transaminases <5x ULN are permitted.)
8. Adequate bone marrow function documented within 7 days (Haemoglobin ≥9g/dL (can have transfusion or growth factors, Platelets ≥100,000cells/mm3, Neutrophil count ≥1500cells/mm^3)
9. No known hyperlipidaemic state
10. Women of childbearing age must have a negative pregnancy test (urine or serum) at commencement of treatment

11. Willingness to comply with scheduled visits, treatment, laboratory test, and other aspects of the trial

Exclusion criteria

If any of the following criteria apply, patients cannot be included in the trial:

1. Prior radical treatment within 6 months of relapse
2. Prior treatment with any systemic chemotherapy for metastatic disease
3. Prior adjuvant radio- or chemotherapy within 4 weeks of starting the study
4. Patients with locally advanced disease deemed suitable for radical chemo-radiotherapy
5. Known hyperlipidaemic state
6. Hypersensitivity to fish- or egg protein or to any of the active substances or constituents in the lipid emulsion
7. Patients with known coagulation disorders
8. Any general contra-indications to infusion therapy – pulmonary oedema, hyperhydration, decompensated cardiac insufficiency
9. Any unstable medical conditions – uncontrolled diabetes mellitus, acute myocardial infarction, stroke, embolic disease, metabolic acidosis, sepsis, pancreatitis
10. Known HIV or hepatitis B or C carrier
11. Dementia or significantly altered mental status that would prohibit the understanding or rendering of informed consent and compliance with requirements of the protocol

12. History of malignancy other than gastric or oesophageal cancer, with the exception of curative treatment for skin cancer (other than melanoma) or *in situ* breast or cervical carcinoma, or those treated with curative intent for any other cancer with no evidence of disease for 5 years
13. Major surgical procedure or significant traumatic injury within 4 weeks of treatment
14. Cerebral metastases
15. History of interstitial lung disease (e.g., pneumonitis or pulmonary fibrosis) or evidence of interstitial lung disease on baseline chest CT scan
16. Known peripheral neuropathy >Grade 1 (absence of deep tendon reflexes as the soleneurological abnormality does not render the patient ineligible).
17. Lack of physical integrity of the upper gastro-intestinal tract, malabsorption syndrome, or inability to take oral medication (administration of capecitabine by naso-gastric or jejunostomy feeding tube is permitted).

Appendix C

Response Evaluation Criteria in Solid Tumours v1.1

Measurable disease: measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter (LD) to be recorded) with the least possible size of 10 mm by CT scan (the CT scan slice thickness should be not greater than 5 mm).

Non-measurable disease: Small lesions (longest diameter<10mm or pathological Lymph node ≥10 to ≤ 15mm short axis). Truly non-measurable lesions consisting of; Leptomeningeal disease, ascites, pleural or pericardial effusion, lymphatic involvement of lung or skin, breast inflammatory disease, abdominal masses and or abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques.

Target lesions: when more than one measurable lesion is present at the baseline, all lesions up to a maximum of 5 lesions in total (a maximum of two lesions per organ) representative of all organs involved. Target lesions should be selected on the basis of their size (lesions with the longest diameter 'LD'), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. Occasionally, the largest lesion does not lend itself to reproducible repeated measurements, in which circumstances the next largest lesion which can be measured reproducibly should be selected.

Non-Target Lesions: Measurable lesions numbering greater than 5 (or 2 in any one organ). All non-measurable lesions (or sites of disease) plus pathological

lymph nodes ≥10 to ≤ 15mm short axis) should be identified as non-target lesions and should be recorded at the baseline. Measurements are not required and these lesions should be followed as present, absent or unequivocal progression.

Response Criteria:

All patients will have their best response on study classified as outlined below:

Target lesions response evaluation:

Complete Response (CR):

Disappearance of all target lesions, any pathological lymph nodes must have reduction in short axis to <10 mm (whether target or non-target). Non target lesions must also disappear and there must be no new lesions.

Partial response:

At least a 30% decrease in the sum of LD of target lesions, taking as reference the baseline sum LD.

Stable disease: steady state of disease and it is defined as neither sufficient reduction to qualify for PR nor sufficient increase to qualify for PD. Taking as reference the smallest sum diameter while on study.

Progressive disease: at least 20% rise in the sum of diameters of target lesions; taking as reference the smallest sum on study (includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also show an absolute increase of least 5mm. (the appearance of one or more new lesions also considered as progression)

Evaluation of non-target lesions:

Complete response: disappearance of all non-target lesions and normalization of tumour markers level. All lymph glands must be < 10mm in short axis (non-pathological).

Non complete response/ non progression disease: persistence of one or more non-target lesions and /or maintenance of tumour marker level above the normal limits.

Progressive disease: unequivocal progression of existing non-target lesions (the appearance of one or more new lesions is also considered as progression).

Appendix D

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:

Your birthdate (Day, Month, Year):

Today's date (Day, Month, Year):

Not all=1 A little=2 Quite a bit=3 Very much=4

1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase? 1 2 3 4

2. Do you have any trouble taking a long walk? 1 2 3 4

3. Do you have any trouble taking a short walk outside of the house? 1 2 3 4

4. Do you need to stay in bed or a chair during the day? 1 2 3 4

5. Do you need help with eating, dressing, washing yourself or using the toilet? 1 2 3 4

During the past week:

6. Were you limited in doing either your work or other daily ctivities? 1 2 3 4

7. Were you limited in pursuing your hobbies or other leisure time activities? 1 2 3 4

8. Were you short of breath? 1 2 3 4

9. Have you had pain? 1 2 3 4

10. Did you need to rest? 1 2 3 4

11. Have you had trouble sleeping? 1 2 3 4

12. Have you felt weak? 1 2 3 4

13. Have you lacked appetite? 1 2 3 4

14. Have you felt nauseated? 1 2 3 4

15. Have you vomited? 1 2 3 4

16. Have you been constipated? 1 2 3 4

During the past week:

17. Have you had diarrhea? 1 2 3 4

18. Were you tired? 1 2 3 4

19. Did pain interfere with your daily activities? 1 2 3 4

20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television? 1 2 3 4

21. Did you feel tense? 1 2 3 4

22. Did you worry? 1 2 3 4

23. Did you feel irritable? 1 2 3 4

24. Did you feel depressed? 1 2 3 4

25. Have you had difficulty remembering things? 1 2 3 4

26. Has your physical condition or medical treatment interfered with your family life? 1 2 3 4

27. Has your physical condition or medical treatment interfered with your social activities? 1 2 3 4

28. Has your physical condition or medical treatment caused you financial difficulties? 1 2 3 4

For the following questions please circle the number between 1 and 7 that best applies to you

Very poor=1 Excellent=7

29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7

Appendix E

As described in QLQ-C30 scoring manual, all raw QLQ-C30 scores transformed to scores ranging from 0-100, a higher scale represents a high response level; functional scale high score represents a healthy level of functioning, a high score for quality of life (QoL)/global health status represents a better QoL, but a high score for a symptom scale/item represents a high level of symptoms.

The principle for scoring these scales was the same in all cases;

1. Calculate the raw score (RS); estimate of the mean of the component items of a scale.
2. Use a linear transformation to standardise the raw score, so that score range from 0-100.

If items $I_1, I_2, I_3, I_4, \ldots I_n$ were included in a scale the scoring procedure was as follow;

Raw Score (RS) = $(I_1+I_2+\ldots+I_n)/n$

Apply the linear transformation 0-100 to obtain the score (S);

Functional scale: S = {1-(RS-1)/range} X100

Symptom scales/items: S = {(RS-1)/range} X100

Global health status/QoL: S = {(RS-1)/range} X100

Range = the difference between the maximum possible value and the minimum possible value of RS. Most items are scored 1 to 4 giving range of 3, except for the items contributing to the global health status / QoL which are 7 point questions with a range = 6 [294].

Appendix F

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Short Title: EOX & Omegaven in Oesophago-gastric Cancer Patients

Phase II trial of epirubicin, oxaliplatin and capecitabine (EOX) chemotherapy combined with Omega-3 fish oil infusion in patients with oesophago-gastric carcinoma

Patient Information Sheet

Please read this information carefully and feel free to ask any questions or to request further information

Investigators: Mr Amar Eltweri (Research Fellow), Dr Anne Thomas (Consultant Oncologist), Mr David Bowrey (Consultant Surgeon)

Date: 11/12/2011

REC number: 11/EM/0412

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you

would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The purpose of this trial is to look into the potential benefits of giving fish oil supplements in addition to your chemotherapy. The fish oil is rich in omega-3 fatty acids which have been shown to have an anti-cancer effect. The fish oil is given in an intravenous form, through a plastic tube (a drip) placed in a vein in your arm.

Why have I been chosen?

You have been chosen because you are being considering and are considered chemotherapy for cancer of the oesophagus or stomach. We wish to investigate the beneficial effects of fish oils on oesophageal and gastric cancer.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

Are there any exclusion criteria that mean I would not be able to take part in the trial?

In general terms, if you are suitable to receive the commonly used chemotherapy drugs against cancers of the oesophagus or stomach, then you would be suitable to participate in the study.

What will happen to me if I take part?

You are being considered or are considering treatment with chemotherapy for oesophageal or stomach cancer. The drugs involved are called epirubicin, oxaliplatin and capecitabine; if you had any intolerance to the oxaliplatin then cisplatin will be used instead. These are currently the best drugs available for the type of cancer you have. If you have agreed to have treatment with these drugs, you may be suitable to receive omega-3 fish oils in addition.

If you agree to take part in this trial, you will begin with having a blood test and being examined by a doctor to check that it would be safe for you to enter the trial – this would happen to any patient prior to starting chemotherapy. Assuming everything is fine, you will commence your treatment with epirubicin, oxaliplatin & capecitabine.

To receive your epirubicin, oxaliplatin & capecitabine treatment, you will need to attend the department of Oncology at Leicester Royal Infirmary once every three weeks, for six months. Each time you attend for the epirubicin, oxaliplatin & capecitabine, you will need to have a small plastic tube inserted into one of the veins in your arms (a drip) in order to give the drug. The epirubicin, oxaliplatin will be given via a drip over two hours & the capecitabine is given in tablet form in two daily doses. This is what happens normally.

If you agree to enter the study, you will be required to attend the hospital once every week for four hours to have omega-3 fish oil containing liquid as a drip into your arm. You will be informed about the date and the time once you agree to participate in the study. During this time you will be able to eat and drink what ever you wish. You will have a blood sample taken before the epirubicin, oxaliplatin & capecitabine /fish oil treatment at each visit. The blood samples will consist of 4 small bottles (a total of about 4 teaspoons) to analyse the fat levels in your body and also your kidney and liver function. This would normally happen if you were only being treated with epirubicin, oxaliplatin & capecitabine. Separate blood samples will be taken for analysis at a later date to assess the effect of the fish oil on your body. The collected blood and tissue samples may be stored for future research purposes.

During the epirubicin, oxaliplatin & capecitabine /fish oil treatment, you will regularly be seen by a doctor. Should you experience any side effects or abnormalities in the blood tests, this may require the treatment to be altered or stopped – again, this would happen during normal treatment.

During your treatment, you will be given a questionnaire to fill in to find out how you have been feeling during the previous weeks, what your appetite and energy levels are like, etc. The answers to these questionnaires will be kept confidential, and stored with your case notes.

During your treatment, you will be required to have regular CT scans. You will have one initial scan before any treatment is started. If there is a delay in you

starting the trial treatment, this scan would need to be repeated. In summary, you may require one additional scan that you would not normally have. After 3 cycles of epirubicin, oxaliplatin & capecitabine /fish oil treatment (9 weeks), you will undergo another CT (CAT scan), similar to one you had before being offered chemotherapy. This will be used to assess the size and spread of your cancer. If the extent of the cancer is either the same or less than before, and you have not had any major problems with the treatment, then you will be offered to continue with epirubicin, oxaliplatin & capecitabine /fish oil treatment. If the cancer has grown, then you will exit this trial and your Oncologist will discuss alternative treatments with you. Nevertheless, you will have further two CT scans, if you finished your six months chemotherapy treatment.

Following your last treatment with epirubicin, oxaliplatin & capecitabine / fish oil, we would like to see you in the oncology clinic to find out how you have been getting on, even if you have started alternative treatment. At this time you will have another CT scan and blood tests, and we will also ask you to fill out a final questionnaire. Throughout the trial, we will keep you informed of any new information about fish oils.

What do I have to do?

If after you have read this information and discussed the trial with one of our investigators, you wish to take part, then we will ask you to sign a consent form saying that you understand the potential benefits and risks of the trial. We will then take some blood tests and examine you to ensure that it is safe for you to

enter the trial. If it is, then we will organise for you to attend for the first treatment with epirubicin, oxaliplatin & capecitabine and fish oils at the Leicester Royal Infirmary and the trial will start. The rest will be down to us.

What is being tested?

The substance that is being tested is omega-3 fatty acids. These are fats found in fish oils. Our bodies cannot make these fats; therefore we must gain them from our diet. They are found in preparations like cod liver oil tablets.

What are the side effects of any treatment received when taking part?

Your Oncologist (cancer doctor) will already have discussed the potential side effects of epirubicin, oxaliplatin & capecitabine with you, prior to you agreeing to treatment. There are very few additional side effects from the omega-3 fish oils. Omega-3 fish oils have been shown to be a safe nutritional supplement, which is why preparations like cod liver oil are available in the supermarket. The small risks in this trial are that you may find that you are allergic to the omega-3 fish oils. It also can have effects on other salt and sugar levels in your blood, which is why you will have blood tests before and after treatment. This can happen when you receive the chemotherapy drugs on their own. It is also important to monitor the fat levels in your blood to ensure that they do not rise too high, as this may affect some of the organs in your body including your liver. It may also make you feel a bit nauseated, may reduce your appetite and may affect your blood pressure. There is an extremely small chance of it causing breathing problems or priapism (painful erection) in males. All of these side effects are very uncommon.

Omega-3 fish oils have been given via a drip to many other patients in Leicester without any major problems.

What are the possible disadvantages and risks of taking part?

The potential risks of this trial are virtually identical to those that would occur if you received standard chemotherapy and did not participate in this trial. The additional potential side effects of omega-3 fish oil listed above are very uncommon. The insertion of the tube into your arm to give the fish oil carries very small risks, notably infection at the site of the cannula (drip) and bleeding (bruising). In addition there will be the inconvenience of needing to stay at the hospital for four hours, compared to the standard two hours. We will reimburse any parking charges that you incur because of this.

What are the possible benefits of taking part?

Omega-3 fatty acids are components of the membranes surrounding the cells in the body and are important in controlling production of certain chemicals called cytokines. These cause inflammation (similar to when you bang your arm or get bitten by an insect and it becomes red, warm, painful and swollen). Omega-3 fish oils have been shown to have beneficial anti-inflammatory effects. They have also been shown to have other benefits in patients undergoing surgery, including reducing infection rates, reducing the need for antibiotics, reducing the need for re-operation, and reducing the risk of blood clots in the leg and lung (deep vein thrombosis DVT and pulmonary embolism). They have also been shown to

reduce stay in hospital after operation by up to 7 days and to reduce the death rate following major surgery.

Omega-3 fatty acids have been shown to have beneficial effects against cancer cells. In laboratory and animal experiments, omega-3 fatty acids have been shown to stop the growth of oesophago-gastric cancer cells. Omega-3 fish oils have also been shown to reduce the spread of oesophago-gastric cancer in animal experiments. Recently, omega-3 fish oils have been shown to make oesophago-gastric cancer cells more responsive to treatment with epirubicin, oxaliplatin & capecitabine; however these results have not been confirmed in humans. In addition, many clinical trials have shown that taking omega-3 fatty acids improves appetite, reduces tiredness, and improves quality of life in patients with advanced oesophago-gastric cancer. However, this is an exploratory study and therefore there may be no direct benefits.

Will I receive any financial benefit for taking part?

You will not receive any financial benefit for taking part in the study. However, because the study will involve longer hospital visits than standard treatment, we will reimburse any parking expenses that you incur.

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make

arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

What happens when the research study stops?

Treatment with the epirubicin, oxaliplatin & capecitabine and fish oil will continue until one of several reasons for stopping treatment. The first is that the cancer of the oesophagus and/or stomach is no longer controlled by the treatment and the cancer has grown. This is the reason why you will have CT scans (CAT scans) every 8 weeks. The second reason is that the side effects of the epirubicin, oxaliplatin & capecitabine become intolerable to you. The third reason is that you change you mind about being in the trial.

After the trial, you will proceed with standard clinical management of your condition, and discuss with your Oncologist (cancer doctor) as to what further treatment might be beneficial.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect

of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital/surgery will have your name and address removed so that you cannot be recognised from it.

We would like to ask if you would consent to us NHS Information Centre flagging

We would like to notify your own GP of your participation in this trial. This may be important for them to know should they alter any medication that you are on. We ask for your permission to do this.

What will happen to the results of the research study?

The results of the study will be fed back to you and the other participants. Should the treatment be beneficial, the results may be shown to other patients in your condition and treatment be offered to them. The results may also be reported in medical journal and at conference presentations to educate other medical professionals. All information will be strictly confidential.

Who is organising and funding the research?

The Departments of Surgery (Mr Eltweri, Mr Bowrey, Mr Sutton, Mr Williams, Mr Metcalfe, Mr Dennison) and Oncology (Dr Thomas) at the University Hospitals of

Leicester are organising this study with assistance from Fresenius Kabi, the pharmaceutical company who manufacture the fish oil compound.

Who has reviewed the study?

All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee (East Midlands Nottingham 2 Research Ethics Committee) before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

Contact for Further Information

If you would like any further information, please feel free to contact us. The contact details are given below.

Finally, thank you very much for reading this information. After you have read this information, if you would like to participate in the project, we will ask you to sign a consent form. We will give you a copy of the signed consent form and this information sheet to keep.

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Appendix G

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REC number : 11/EM/0412
01162585631
Patient Identification number for this trial:

CONSENT FORM

Phase II trial of palliative epirubicin, oxaliplatin and capecitabine (EOX) chemotherapy combined with Omega-3 fish oil infusion in patients with oesophago-gastric carcinoma

Investigators: Mr Amar Eltweri (Research fellow), Dr Anne Thomas (Consultant Oncologist), Mr David Bowrey (Consultant Surgeon)

Please initial each box

1. I confirm that I have read and understand the patient information sheet dated 11/12/2011 version 1.6 for the above study. I have had the opportunity to consider the information, ask questions and have had these questions answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree for my blood and tissue samples to be stored and used for future research ☐
4. I understand that relevant sections of my medical notes and/or data may be looked at by responsible individuals from the study team, Research Ethics Committee, NHS Trust or form regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
5. I agree to my NHS number being checked through information held by the NHS and the General Register Office.
6. I agree to my GP being informed about my participation in the study ☐
7. I agree to take part in the above study. ☐

Name of Patient (BLOCK LETTERS) Date Signature

I confirm that I have explained the nature of the study, as detailed in the Participant Information leaflet, in terms which in my judgement are suited to the understanding of the patient

Name of Person taking consent (if different from researcher) Date Signature

Researcher (BLOCK LETTERS) Date Signature

When completed: 1 for patient; 1 for researcher; 1 to be kept with hospital notes

References:

1. Cancer Research UK. Cancer statistics, http://Www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/oesophageal-cancer/incidence#heading-zero, January 2017. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/oesophageal-cancer/incidence#heading-Zero. Updated 2016. Accessed January/21, 2017.

2. Cancer Research UK. Cancer statistics, http://Www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/stomach-cancer/incidence#heading-two, January 2017. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/stomach-cancer/incidence#heading-Two. Updated 2016. Accessed January/21, 2017.

3. Parkin DM. International variation. *Oncogene*. 2004;23(38):6329-6340.

4. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;55(2):74-108.

5. Eslick GD. Epidemiology of esophageal cancer. *Gastroenterol Clin North Am*. 2009;38(1):17-25, vii.

6. Voltaggio LM, EA. Histopathology of barrett esophagus. . 2011;17(2):41-49.

7. Lepage C, Drouillard A, Jouve JL, Faivre J. Epidemiology and risk factors for oesophageal adenocarcinoma. *Dig Liver Dis*. 2013;45(8):625-629.

8. Reid BJ, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: Time for a new synthesis. *Nat Rev Cancer*. 2010;10(2):87-101.

9. Crew KD, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol*. 2006;12(3):354-362.

10. Siewert JR, Stein HJ. Classification of adenocarcinoma of the oesophagogastric junction. *Br J Surg*. 1998;85(11):1457-1459.

11. Rice TW, Blackstone EH, Rusch VW. 7th edition of the AJCC cancer staging manual: Esophagus and esophagogastric junction. *Ann Surg Oncol*. 2010;17(7):1721-1724.

12. Washington K. 7th edition of the AJCC cancer staging manual: Stomach. *Ann Surg Oncol*. 2010;17(12):3077-3079.

13. Werner M, Becker KF, Keller G, Hofler H. Gastric adenocarcinoma: Pathomorphology and molecular pathology. *J Cancer Res Clin Oncol*. 2001;127(4):207-216.

14. Dicken BJ, Bigam DL, Cass C, Mackey JR, Joy AA, Hamilton SM. Gastric adenocarcinoma: Review and considerations for future directions. *Ann Surg*. 2005;241(1):27-39.

15. Jass JR, Sobin LH, Watanabe H. The world health organization's histologic classification of gastrointestinal tumors. A commentary on the second edition. *Cancer*. 1990;66(10):2162-2167.

16. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014;513(7517):202-209.

17. LAUREN P. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. an attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand*. 1965;64:31-49.

18. Ming SC. Gastric carcinoma. A pathobiological classification. *Cancer*. 1977;39(6):2475-2485.

19. Mulligan RM. Histogenesis and biologic behavior of gastric carcinoma. *Pathol Annu*. 1972;7:349-415.

20. Lochhead P, El-Omar EM. Gastric cancer. *Br Med Bull*. 2008;85:87-100.

21. Sipponen P. Gastric cancer: Pathogenesis, risks, and prevention. . 2002;37(13):39-44.

22. Vauhkonen M, Vauhkonen H, Sipponen P. Pathology and molecular biology of gastric cancer. *Best Pract Res Clin Gastroenterol*. 2006;20(4):651-674.

23. Glenn TF. Esophageal cancer. facts, figures, and screening. *Gastroenterol Nurs*. 2001;24(6):271-3; quiz 274-5.

24. Pennathur A, Gibson MK, Jobe BA, Luketich JD. Oesophageal carcinoma. *Lancet*. 2013;381(9864):400-412.

25. Yang SJ, Wang HY, Li XQ, et al. Genetic polymorphisms of ADH2 and ALDH2 association with esophageal cancer risk in southwest china. *World J Gastroenterol*. 2007;13(43):5760-5764.

26. Gammon MD, Schoenberg JB, Ahsan H, et al. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst*. 1997;89(17):1277-1284.

27. Vaughan TL, Davis S, Kristal A, Thomas DB. Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia: Adenocarcinoma versus squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*. 1995;4(2):85-92.

28. Engeland A, Tretli S, Bjorge T. Height and body mass index in relation to esophageal cancer; 23-year follow-up of two million norwegian men and women. *Cancer Causes Control*. 2004;15(8):837-843.

29. Lindblad M, Rodriguez LA, Lagergren J. Body mass, tobacco and alcohol and risk of esophageal, gastric cardia, and gastric non-cardia adenocarcinoma among men and women in a nested case-control study. *Cancer Causes Control*. 2005;16(3):285-294.

30. Steevens J, Schouten LJ, Goldbohm RA, van den Brandt PA. Alcohol consumption, cigarette smoking and risk of subtypes of oesophageal and gastric cancer: A prospective cohort study. *Gut*. 2010;59(1):39-48.

31. Zhuo X, Zhang Y, Wang Y, Zhuo W, Zhu Y, Zhang X. Helicobacter pylori infection and oesophageal cancer risk: Association studies via evidence-based meta-analyses. *Clin Oncol (R Coll Radiol)*. 2008;20(10):757-762.

32. Freedman ND, Park Y, Subar AF, et al. Fruit and vegetable intake and esophageal cancer in a large prospective cohort study. *Int J Cancer*. 2007;121(12):2753-2760.

33. Gonzalez CA, Pera G, Agudo A, et al. Fruit and vegetable intake and the risk of stomach and oesophagus adenocarcinoma in the european prospective investigation into cancer and nutrition (EPIC-EURGAST). *Int J Cancer*. 2006;118(10):2559-2566.

34. Kubo A, Corley DA, Jensen CD, Kaur R. Dietary factors and the risks of oesophageal adenocarcinoma and barrett's oesophagus. *Nutr Res Rev*. 2010;23(2):230-246.

35. Ahsan H, Neugut AI. Radiation therapy for breast cancer and increased risk for esophageal carcinoma. *Ann Intern Med*. 1998;128(2):114-117.

36. Pandeya N, Webb PM, Sadeghi S, Green AC, Whiteman DC, Australian Cancer Study. Gastro-oesophageal reflux symptoms and the risks of oesophageal cancer: Are the effects modified by smoking, NSAIDs or acid suppressants? *Gut*. 2010;59(1):31-38.

37. Whiteman DC, Sadeghi S, Pandeya N, et al. Combined effects of obesity, acid reflux and smoking on the risk of adenocarcinomas of the oesophagus. *Gut*. 2008;57(2):173-180.

38. Islami F, Kamangar F. Helicobacter pylori and esophageal cancer risk: A meta-analysis. *Cancer Prev Res (Phila)*. 2008;1(5):329-338.

39. Buckley MJ, O'Morain CA. Helicobacter biology--discovery. *Br Med Bull*. 1998;54(1):7-16.

40. Osman MA, Bloom GS, Tagoe EA. Helicobacter pylori-induced alteration of epithelial cell signaling and polarity: A possible mechanism of gastric carcinoma etiology and disparity. *Cytoskeleton (Hoboken)*. 2013;70(7):349-359.

41. Bolin TD, Hunt RH, Korman MG, Lambert JR, Lee A, Talley NJ. Helicobacter pylori and gastric neoplasia: Evolving concepts. *Med J Aust*. 1995;163(5):253-255.

42. Eslick GD, Lim LL, Byles JE, Xia HH, Talley NJ. Association of helicobacter pylori infection with gastric carcinoma: A meta-analysis. *Am J Gastroenterol*. 1999;94(9):2373-2379.

43. Talley NJ, Zinsmeister AR, Weaver A, et al. Gastric adenocarcinoma and helicobacter pylori infection. *J Natl Cancer Inst*. 1991;83(23):1734-1739.

44. Forman D. Helicobacter pylori infection and cancer. *Br Med Bull*. 1998;54(1):71-78.

45. Turati F, Edefonti V, Bosetti C, et al. Family history of cancer and the risk of cancer: A network of case-control studies. *Ann Oncol*. 2013.

46. Yu J, Fu B, Zhao Q. Family history of malignant neoplasm and its relation with clinicopathologic features of gastric cancer patients. *World J Surg Oncol*. 2013;11(1):201.

47. Lynch HT, Grady W, Suriano G, Huntsman D. Gastric cancer: New genetic developments. *J Surg Oncol*. 2005;90(3):114-33; discussion 133.

48. Kluijt I, Sijmons RH, Hoogerbrugge N, et al. Familial gastric cancer: Guidelines for diagnosis, treatment and periodic surveillance. *Fam Cancer*. 2012;11(3):363-369.

49. Prins MJ, Ruurda JP, van Diest PJ, van Hillegersberg R, Ten Kate FJ. The significance of the HER-2 status in esophageal adenocarcinoma for survival: An immunohistochemical and an in situ hybridization study. *Ann Oncol*. 2013;24(5):1290-1297.

50. Tanner M, Hollmen M, Junttila TT, et al. Amplification of HER-2 in gastric carcinoma: Association with topoisomerase IIalpha gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab. *Ann Oncol*. 2005;16(2):273-278.

51. Gravalos C, Jimeno A. HER2 in gastric cancer: A new prognostic factor and a novel therapeutic target. *Ann Oncol*. 2008;19(9):1523-1529.

52. Lao-Sirieix P, Caldas C, Fitzgerald RC. Genetic predisposition to gastro-oesophageal cancer. *Curr Opin Genet Dev*. 2010;20(3):210-217.

53. Anderson SR, Sinacori JT. Plummer-vinson syndrome heralded by postcricoid carcinoma. *Am J Otolaryngol*. 2007;28(1):22-24.

54. Fox MP, van Berkel V. Management of gastroesophageal junction tumors. *Surg Clin North Am*. 2012;92(5):1199-1212.

55. Plukker JT, van Westreenen HL. Staging in oesophageal cancer. *Best Pract Res Clin Gastroenterol*. 2006;20(5):877-891.

56. Rampado S, Bocus P, Battaglia G, Ruol A, Portale G, Ancona E. Endoscopic ultrasound: Accuracy in staging superficial carcinomas of the esophagus. *Ann Thorac Surg*. 2008;85(1):251-256.

57. Thurau K, Palmes D, Franzius C, et al. Impact of PET-CT on primary staging and response control on multimodal treatment of esophageal cancer. *World J Surg*. 2011;35(3):608-616.

58. Pultrum BB, van der Jagt EJ, van Westreenen HL, et al. Detection of lymph node metastases with ultrasmall superparamagnetic iron oxide (USPIO)-enhanced

magnetic resonance imaging in oesophageal cancer: A feasibility study. *Cancer Imaging*. 2009;9:19-28.

59. Kantarci M, Polat P, Alper F, et al. Comparison of CT and MRI for the diagnosis recurrent esophageal carcinoma after operation. *Dis Esophagus*. 2004;17(1):32-37.

60. Lao-Sirieix P, Fitzgerald RC. Screening for oesophageal cancer. *Nat Rev Clin Oncol*. 2012;9(5):278-287.

61. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA: A Cancer Journal for Clinicians*. 2016;66(1):7-30.

62. Chadwick G, Groene O, Cromwell D et al. The national oesophago-gastric cancer audit. an audit of the care received by people with oesophago-gastric cancer in england and wales. 2013 annual report. in: NHS information centre; 2013. .

63. Chadwick G, Taylor A, Groene O et al. The national oesophago-gastric cancer audit. an audit of the care received by people with oesophago-gastric cancer in england and wales. 2014 annual report. in: Nhs information centre; 2014. .

64. Bougnoux P, Hajjaji N, Ferrasson MN, Giraudeau B, Couet C, Le Floch O. Improving outcome of chemotherapy of metastatic breast cancer by docosahexaenoic acid: A phase II trial. *Br J Cancer*. 2009;101(12):1978-1985.

65. Koizumi W, Kim YH, Fujii M, et al. Addition of docetaxel to S-1 without platinum prolongs survival of patients with advanced gastric cancer: A randomized study (START). *J Cancer Res Clin Oncol*. 2014;140(2):319-328.

66. Koizumi W, Narahara H, Hara T, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): A phase III trial. *Lancet Oncol*. 2008;9(3):215-221.

67. Yamada Y, Higuchi K, Nishikawa K, et al. Phase III study comparing oxaliplatin plus S-1 with cisplatin plus S-1 in chemotherapy-naive patients with advanced gastric cancer. *Ann Oncol*. 2015;26(1):141-148.

68. Ajani JA, Buyse M, Lichinitser M, et al. Combination of cisplatin/S-1 in the treatment of patients with advanced gastric or gastroesophageal adenocarcinoma: Results of noninferiority and safety analyses compared with cisplatin/5-

fluorouracil in the first-line advanced gastric cancer study. *Eur J Cancer*. 2013;49(17):3616-3624.

69. Al-Batran SE, Hartmann JT, Probst S, et al. Phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil, leucovorin plus either oxaliplatin or cisplatin: A study of the arbeitsgemeinschaft internistische onkologie. *J Clin Oncol*. 2008;26(9):1435-1442.

70. Kim ST, Kang JH, Lee J, et al. Simvastatin plus capecitabine-cisplatin versus placebo plus capecitabine-cisplatin in patients with previously untreated advanced gastric cancer: A double-blind randomised phase 3 study. *Eur J Cancer*. 2014;50(16):2822-2830.

71. Kang YK, Kang WK, Shin DB, et al. Capecitabine/cisplatin versus 5-fluorouracil/cisplatin as first-line therapy in patients with advanced gastric cancer: A randomised phase III noninferiority trial. *Ann Oncol*. 2009;20(4):666-673.

72. Van Cutsem E, Moiseyenko VM, Tjulandin S, et al. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: A report of the V325 study group. *J Clin Oncol*. 2006;24(31):4991-4997.

73. Vanhoefer U, Rougier P, Wilke H, et al. Final results of a randomized phase III trial of sequential high-dose methotrexate, fluorouracil, and doxorubicin versus etoposide, leucovorin, and fluorouracil versus infusional fluorouracil and cisplatin in advanced gastric cancer: A trial of the european organization for research and treatment of cancer gastrointestinal tract cancer cooperative group. *J Clin Oncol*. 2000;18(14):2648-2657.

74. Icli F, Celik I, Aykan F, et al. A randomized phase III trial of etoposide, epirubicin, and cisplatin versus 5-fluorouracil, epirubicin, and cisplatin in the treatment of patients with advanced gastric carcinoma. turkish oncology group. *Cancer*. 1998;83(12):2475-2480.

75. Webb A, Cunningham D, Scarffe JH, et al. Randomized trial comparing epirubicin, cisplatin, and fluorouracil versus fluorouracil, doxorubicin, and methotrexate in advanced esophagogastric cancer. *J Clin Oncol*. 1997;15(1):261-267.

76. Kim NK, Park YS, Heo DS, et al. A phase III randomized study of 5-fluorouracil and cisplatin versus 5-fluorouracil, doxorubicin, and mitomycin C versus 5-fluorouracil alone in the treatment of advanced gastric cancer. *Cancer*. 1993;71(12):3813-3818.

77. Satoh T, Xu RH, Chung HC, et al. Lapatinib plus paclitaxel versus paclitaxel alone in the second-line treatment of HER2-amplified advanced gastric cancer in asian populations: TyTAN--a randomized, phase III study. *J Clin Oncol*. 2014;32(19):2039-2049.

78. Shen L, Li J, Xu J, et al. Bevacizumab plus capecitabine and cisplatin in chinese patients with inoperable locally advanced or metastatic gastric or gastroesophageal junction cancer: Randomized, double-blind, phase III study (AVATAR study). *Gastric Cancer*. 2015;18(1):168-176.

79. Wilke H, Muro K, Van Cutsem E, et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): A double-blind, randomised phase 3 trial. *Lancet Oncol*. 2014;15(11):1224-1235.

80. Lordick F, Kang YK, Chung HC, et al. Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): A randomised, open-label phase 3 trial. *Lancet Oncol*. 2013;14(6):490-499.

81. Waddell T, Chau I, Cunningham D, et al. Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): A randomised, open-label phase 3 trial. *Lancet Oncol*. 2013;14(6):481-489.

82. Ohtsu A, Shah MA, Van Cutsem E, et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: A randomized, double-blind, placebo-controlled phase III study. *J Clin Oncol*. 2011;29(30):3968-3976.

83. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): A phase 3, open-label, randomised controlled trial. *Lancet*. 2010;376(9742):687-697.

84. Bonfante V, Bonadonna G, Villani F, Martini A. Preliminary clinical experience with 4-epidoxorubicin in advanced human neoplasia. *Recent Results Cancer Res*. 1980;74:192-199.

85. Findlay M, Cunningham D, Norman A, et al. A phase II study in advanced gastro-esophageal cancer using epirubicin and cisplatin in combination with continuous infusion 5-fluorouracil (ECF). *Ann Oncol*. 1994;5(7):609-616.

86. Graham J, Mushin M, Kirkpatrick P. Oxaliplatin. *Nat Rev Drug Discov*. 2004;3(1):11-12.

87. Lamont EB, Schilsky RL. The oral fluoropyrimidines in cancer chemotherapy. *Clin Cancer Res*. 1999;5(9):2289-2296.

88. Heidelberger C, Chaudhuri NK, Danneberg P, et al. Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature*. 1957;179(4561):663-666.

89. Shirao K, Boku N, Yamada Y, et al. Randomized phase III study of 5-fluorouracil continuous infusion vs. sequential methotrexate and 5-fluorouracil therapy in far advanced gastric cancer with peritoneal metastasis (JCOG0106). *Jpn J Clin Oncol*. 2013;43(10):972-980.

90. Narahara H, Iishi H, Imamura H, et al. Randomized phase III study comparing the efficacy and safety of irinotecan plus S-1 with S-1 alone as first-line treatment for advanced gastric cancer (study GC0301/TOP-002). *Gastric Cancer*. 2011;14(1):72-80.

91. Koizumi W, Tanabe S, Azuma M, et al. Impacts of fluorouracil-metabolizing enzymes on the outcomes of patients treated with S-1 alone or S-1 plus cisplatin for first-line treatment of advanced gastric cancer. *Int J Cancer*. 2010;126(1):162-170.

92. Boku N, Yamamoto S, Fukuda H, et al. Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: A randomised phase 3 study. *Lancet Oncol*. 2009;10(11):1063-1069.

93. Ohtsu A, Shimada Y, Shirao K, et al. Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: The japan clinical oncology group study (JCOG9205). *J Clin Oncol*. 2003;21(1):54-59.

94. Kondo K, Sakamoto J, Nakazato H, et al. A phase III randomized study comparing doxifluridine and 5-fluorouracil as supportive chemotherapy in advanced and recurrent gastric cancer. *Oncol Rep*. 2000;7(3):485-490.

95. Kim NK, Park YS, Heo DS, et al. A phase III randomized study of 5-fluorouracil and cisplatin versus 5-fluorouracil, doxorubicin, and mitomycin C versus 5-fluorouracil alone in the treatment of advanced gastric cancer. *Cancer*. 1993;71(12):3813-3818.

96. Ryu MH, Baba E, Lee KH, et al. Comparison of two different S-1 plus cisplatin dosing schedules as first-line chemotherapy for metastatic and/or recurrent gastric cancer: A multicenter, randomized phase III trial (SOS). *Ann Oncol*. 2015;26(10):2097-2101.

97. Ochenduszko S, Puskulluoglu M, Konopka K, et al. Comparison of efficacy and safety of first-line palliative chemotherapy with EOX and mDCF regimens in patients with locally advanced inoperable or metastatic HER2-negative gastric or gastroesophageal junction adenocarcinoma: A randomized phase 3 trial. *Med Oncol*. 2015;32(10):242-015-0687-7. Epub 2015 Sep 9.

98. Guimbaud R, Louvet C, Ries P, et al. Prospective, randomized, multicenter, phase III study of fluorouracil, leucovorin, and irinotecan versus epirubicin, cisplatin, and capecitabine in advanced gastric adenocarcinoma: A french intergroup (federation francophone de cancerologie digestive, federation nationale des centres de lutte contre le cancer, and groupe cooperateur multidisciplinaire en oncologie) study. *J Clin Oncol*. 2014;32(31):3520-3526.

99. Ajani JA, Rodriguez W, Bodoky G, et al. Multicenter phase III comparison of cisplatin/S-1 with cisplatin/infusional fluorouracil in advanced gastric or gastroesophageal adenocarcinoma study: The FLAGS trial. *J Clin Oncol*. 2010;28(9):1547-1553.

100. Lee KH, Hyun MS, Kim HK, et al. Randomized, multicenter, phase III trial of heptaplatin 1-hour infusion and 5-fluorouracil combination chemotherapy comparing with cisplatin and 5-fluorouracil combination chemotherapy in patients with advanced gastric cancer. *Cancer Res Treat*. 2009;41(1):12-18.

101. Dank M, Zaluski J, Barone C, et al. Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5-fluorouracil in chemotherapy naive patients with advanced adenocarcinoma of the stomach or esophagogastric junction. *Ann Oncol*. 2008;19(8):1450-1457.

102. Curran D, Pozzo C, Zaluski J, et al. Quality of life of palliative chemotherapy naive patients with advanced adenocarcinoma of the stomach or esophagogastric junction treated with irinotecan combined with 5-fluorouracil and folinic acid: Results of a randomised phase III trial. *Qual Life Res*. 2009;18(7):853-861.

103. Sadighi S, Mohagheghi MA, Montazeri A, Sadighi Z. Quality of life in patients with advanced gastric cancer: A randomized trial comparing docetaxel,

cisplatin, 5-FU (TCF) with epirubicin, cisplatin, 5-FU (ECF). *BMC Cancer*. 2006;6:274.

104. Ajani JA, Moiseyenko VM, Tjulandin S, et al. Clinical benefit with docetaxel plus fluorouracil and cisplatin compared with cisplatin and fluorouracil in a phase III trial of advanced gastric or gastroesophageal cancer adenocarcinoma: The V-325 study group. *J Clin Oncol*. 2007;25(22):3205-3209.

105. Ajani JA, Moiseyenko VM, Tjulandin S, et al. Quality of life with docetaxel plus cisplatin and fluorouracil compared with cisplatin and fluorouracil from a phase III trial for advanced gastric or gastroesophageal adenocarcinoma: The V-325 study group. *J Clin Oncol*. 2007;25(22):3210-3216.

106. Cascinu S, Cordella L, Del Ferro E, Fronzoni M, Catalano G. Neuroprotective effect of reduced glutathione on cisplatin-based chemotherapy in advanced gastric cancer: A randomized double-blind placebo-controlled trial. *J Clin Oncol*. 1995;13(1):26-32.

107. Nishikawa K, Fujitani K, Inagaki H, et al. Randomised phase III trial of second-line irinotecan plus cisplatin versus irinotecan alone in patients with advanced gastric cancer refractory to S-1 monotherapy: TRICS trial. *Eur J Cancer*. 2015;51(7):808-816.

108. Ford HE, Marshall A, Bridgewater JA, et al. Docetaxel versus active symptom control for refractory oesophagogastric adenocarcinoma (COUGAR-02): An open-label, phase 3 randomised controlled trial. *Lancet Oncol*. 2014;15(1):78-86.

109. Higuchi K, Tanabe S, Shimada K, et al. Biweekly irinotecan plus cisplatin versus irinotecan alone as second-line treatment for advanced gastric cancer: A randomised phase III trial (TCOG GI-0801/BIRIP trial). *Eur J Cancer*. 2014;50(8):1437-1445.

110. Ohtsu A, Ajani JA, Bai YX, et al. Everolimus for previously treated advanced gastric cancer: Results of the randomized, double-blind, phase III GRANITE-1 study. *J Clin Oncol*. 2013;31(31):3935-3943.

111. Hironaka S, Ueda S, Yasui H, et al. Randomized, open-label, phase III study comparing irinotecan with paclitaxel in patients with advanced gastric cancer without severe peritoneal metastasis after failure of prior combination chemotherapy using fluoropyrimidine plus platinum: WJOG 4007 trial. *J Clin Oncol*. 2013;31(35):4438-4444.

112. Kang JH, Lee SI, Lim do H, et al. Salvage chemotherapy for pretreated gastric cancer: A randomized phase III trial comparing chemotherapy plus best supportive care with best supportive care alone. *J Clin Oncol*. 2012;30(13):1513-1518.

113. Thuss-Patience PC, Kretzschmar A, Bichev D, et al. Survival advantage for irinotecan versus best supportive care as second-line chemotherapy in gastric cancer--a randomised phase III study of the arbeitsgemeinschaft internistische onkologie (AIO). *Eur J Cancer*. 2011;47(15):2306-2314.

114. Fuchs CS, Tomasek J, Yong CJ, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): An international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet*. 2014;383(9911):31-39.

115. Van Cutsem E, de Haas S, Kang YK, et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: A biomarker evaluation from the AVAGAST randomized phase III trial. *J Clin Oncol*. 2012;30(17):2119-2127.

116. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): A phase 3, open-label, randomised controlled trial. *Lancet*. 2010;376(9742):687-697.

117. Chua C, Tan IB, Yamada Y, et al. Phase II study of trastuzumab in combination with S-1 and cisplatin in the first-line treatment of human epidermal growth factor receptor HER2-positive advanced gastric cancer. *Cancer Chemother Pharmacol*. 2015;76(2):397-408.

118. Kurokawa Y, Sugimoto N, Miwa H, et al. Phase II study of trastuzumab in combination with S-1 plus cisplatin in HER2-positive gastric cancer (HERBIS-1). *Br J Cancer*. 2014;110(5):1163-1168.

119. Gravalos C, Gomez-Martin C, Rivera F, et al. Phase II study of trastuzumab and cisplatin as first-line therapy in patients with HER2-positive advanced gastric or gastroesophageal junction cancer. *Clin Transl Oncol*. 2011;13(3):179-184.

120. Sawaki A, Ohashi Y, Omuro Y, et al. Efficacy of trastuzumab in japanese patients with HER2-positive advanced gastric or gastroesophageal junction cancer: A subgroup analysis of the trastuzumab for gastric cancer (ToGA) study. *Gastric Cancer*. 2012;15(3):313-322.

121. Bekaii-Saab TS, Roda JM, Guenterberg KD, et al. A phase I trial of paclitaxel and trastuzumab in combination with interleukin-12 in patients with HER2/neu-expressing malignancies. *Mol Cancer Ther*. 2009;8(11):2983-2991.

122. Ryu MH, Yoo C, Kim JG, et al. Multicenter phase II study of trastuzumab in combination with capecitabine and oxaliplatin for advanced gastric cancer. *Eur J Cancer*. 2015;51(4):482-488.

123. Pyrhonen S, Kuitunen T, Nyandoto P, Kouri M. Randomised comparison of fluorouracil, epidoxorubicin and methotrexate (FEMTX) plus supportive care with supportive care alone in patients with non-resectable gastric cancer. *Br J Cancer*. 1995;71(3):587-591.

124. Murad AM, Santiago FF, Petroianu A, Rocha PR, Rodrigues MA, Rausch M. Modified therapy with 5-fluorouracil, doxorubicin, and methotrexate in advanced gastric cancer. *Cancer*. 1993;72(1):37-41.

125. Curran D, Pozzo C, Zaluski J, et al. Quality of life of palliative chemotherapy naive patients with advanced adenocarcinoma of the stomach or esophagogastric junction treated with irinotecan combined with 5-fluorouracil and folinic acid: Results of a randomised phase III trial. *Qual Life Res*. 2009;18(7):853-861.

126. Popov I, Jelic S, Radulovic S, Radosavljevic D, Nikolic-Tomasevic Z. Eight-hour infusion versus bolus injection of doxorubicin in the EAP regimen in patients with advanced gastric cancer: A prospective randomised trial. *Ann Oncol*. 2000;11(3):343-348.

127. Dank M, Zaluski J, Barone C, et al. Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5-fluorouracil in chemotherapy naive patients with advanced adenocarcinoma of the stomach or esophagogastric junction. *Ann Oncol*. 2008;19(8):1450-1457.

128. Ajani JA, Moiseyenko VM, Tjulandin S, et al. Quality of life with docetaxel plus cisplatin and fluorouracil compared with cisplatin and fluorouracil from a phase III trial for advanced gastric or gastroesophageal adenocarcinoma: The V-325 study group. *J Clin Oncol*. 2007;25(22):3210-3216.

129. Satoh T, Bang YJ, Gotovkin EA, et al. Quality of life in the trastuzumab for gastric cancer trial. *Oncologist*. 2014;19(7):712-719.

130. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim Biophys Acta*. 2015;1851(4):469-484.

131. Calder PC. The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins Leukot Essent Fatty Acids*. 2008;79(3-5):101-108.

132. Browning LM, Walker CG, Mander AP, et al. Incorporation of eicosapentaenoic and docosahexaenoic acids into lipid pools when given as supplements providing doses equivalent to typical intakes of oily fish. *Am J Clin Nutr*. 2012;96(4):748-758.

133. Glatz JF, Luiken JJ, Bonen A. Membrane fatty acid transporters as regulators of lipid metabolism: Implications for metabolic disease. *Physiol Rev*. 2010;90(1):367-417.

134. Zhang G, Panigrahy D, Mahakian LM, et al. Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. *Proc Natl Acad Sci U S A*. 2013;110(16):6530-6535.

135. Sun H, Meng X, Han J, et al. Anti-cancer activity of DHA on gastric cancer--an in vitro and in vivo study. *Tumour Biol*. 2013;34(6):3791-3800.

136. Odenthal J, van Heumen BW, Roelofs HM, et al. The influence of curcumin, quercetin, and eicosapentaenoic acid on the expression of phase II detoxification enzymes in the intestinal cell lines HT-29, caco-2, HuTu 80, and LT97. *Nutr Cancer*. 2012;64(6):856-863.

137. Lee SE, Lim JW, Kim H. Activator protein-1 mediates docosahexaenoic acid-induced apoptosis of human gastric cancer cells. *Ann N Y Acad Sci*. 2009;1171:163-169.

138. Valentine RC, Valentine DL. Omega-3 fatty acids in cellular membranes: A unified concept. *Prog Lipid Res*. 2004;43(5):383-402.

139. Calder PC. Omega-3 fatty acids and inflammatory processes. *Nutrients*. 2010;2(3):355-374.

140. Aggarwal BB, Gehlot P. Inflammation and cancer: How friendly is the relationship for cancer patients? *Curr Opin Pharmacol*. 2009;9(4):351-369.

141. Kris-Etherton PM, Harris WS, Appel LJ, American Heart Association. Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*. 2002;106(21):2747-2757.

142. Agostoni C, Bresson J, Fairweather-Tait S, Flynn A, Golly I, Korhonen H, Lagiou P, Løvik M, Marchelli R, Martin A, Moseley B, Neuhäuser-Berthold M, Przyrembel H, Salminen S, Sanz Y, Strain S, Strobel S, Tetens I, Tomé D, Loveren H, Verhagen H. Sceintific opinion on the tolerable upper intake level of eicospentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *European Food Safety Authority (EFSA), Parma, Italy*. 2012;10(7).

143. U.S. Food and Drug Administration Centre for Food Safety and applied Nutrition. Agency response letter. GRAS notice no. GRN 000105. october 15, 2002. http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt000343-041-appx-E-Ref-29-GRAS-vol268.pdf. Accessed March 29, 2004.

144. Department of Health and Human Services, US Food and Drug Administration. Substances affirmed as generally recognized as safe: Menhaden oil. *federal register*. june 5, 1997. vol. 62, no. 108: Pp 30751–30757. 21 CFR part 184 [docket no. 86G-0289]. available at: Http://Frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1997_register&docid=fr05jn97-5. Accessed june 2014.

145. Food and Nutrition Board, Institute of Medicine of the National Academies. Dietary fats: Total fat and fatty acids. In: *Dietary reference intakes for energy, carbohydrate,fiber, fat, fatty acids,cholesterol, protein, and amino acids*. The national Academies Press, Washington D.C.; 2005:422-541.

146. Gomez-Candela C, Villarino Sanz M, Horrisberger A, Loria Kohen V, Bermejo LM, Zamora Aunon P. Efficacy evaluation of an oral powder supplement enriched with eicosapentaenoic acid in cancer patients. *Nutr Hosp*. 2011;26(6):1385-1393.

147. Serhan CN, Hong S, Gronert K, et al. Resolvins: A family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med*. 2002;196(8):1025-1037.

148. Balvers MG, Verhoeckx KC, Bijlsma S, et al. Fish oil and inflammatory status alter the n-3 to n-6 balance of the endocannabinoid and oxylipin metabolomes in mouse plasma and tissues. *Metabolomics*. 2012;8(6):1130-1147.

149. Mayer K, Gokorsch S, Fegbeutel C, et al. Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. *Am J Respir Crit Care Med*. 2003;167(10):1321-1328.

150. Weiss G, Meyer F, Matthies B, Pross M, Koenig W, Lippert H. Immunomodulation by perioperative administration of n-3 fatty acids. *Br J Nutr*. 2002;87 Suppl 1:S89-94.

151. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother*. 2002;56(8):365-379.

152. Pizato N, Bonatto S, Yamazaki RK, et al. Ratio of n6 to n-3 fatty acids in the diet affects tumor growth and cachexia in walker 256 tumor-bearing rats. *Nutr Cancer*. 2005;53(2):194-201.

153. Heukamp I, Kilian M, Gregor JI, et al. Impact of polyunsaturated fatty acids on hepato-pancreatic prostaglandin and leukotriene concentration in ductal pancreatic cancer -- is there a correlation to tumour growth and liver metastasis? *Prostaglandins Leukot Essent Fatty Acids*. 2006;74(4):223-233.

154. Tong WG, Ding XZ, Talamonti MS, Bell RH, Adrian TE. LTB4 stimulates growth of human pancreatic cancer cells via MAPK and PI-3 kinase pathways. *Biochem Biophys Res Commun*. 2005;335(3):949-956.

155. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated fatty acids. *J Nutr Metab*. 2012;2012:539426.

156. Simopoulos AP. Omega-3 fatty acids and cancer. . Indoor Built Environ 2003;12:405-412.

157. Chi TY, Chen GG, Lai PB. Eicosapentaenoic acid induces fas-mediated apoptosis through a p53-dependent pathway in hepatoma cells. *Cancer J*. 2004;10(3):190-200.

158. Narayanan BA, Narayanan NK, Desai D, Pittman B, Reddy BS. Effects of a combination of docosahexaenoic acid and 1,4-phenylene bis(methylene) selenocyanate on cyclooxygenase 2, inducible nitric oxide synthase and beta-catenin pathways in colon cancer cells. *Carcinogenesis*. 2004;25(12):2443-2449.

159. Zhuo Z, Zhang L, Mu Q, et al. The effect of combination treatment with docosahexaenoic acid and 5-fluorouracil on the mRNA expression of apoptosis-related genes, including the novel gene BCL2L12, in gastric cancer cells. *In Vitro Cell Dev Biol Anim*. 2009;45(1-2):69-74.

160. Hering J, Garrean S, Dekoj TR, et al. Inhibition of proliferation by omega-3 fatty acids in chemoresistant pancreatic cancer cells. *Ann Surg Oncol*. 2007;14(12):3620-3628.

161. De Carlo F, Witte TR, Hardman WE, Claudio PP. Omega-3 eicosapentaenoic acid decreases CD133 colon cancer stem-like cell marker expression while increasing sensitivity to chemotherapy. *PLoS One*. 2013;8(7):e69760.

162. Granci V, Cai F, Lecumberri E, Clerc A, Dupertuis YM, Pichard C. Colon cancer cell chemosensitisation by fish oil emulsion involves apoptotic mitochondria pathway. *Br J Nutr*. 2013;109(7):1188-1195.

163. Kuan CY, Walker TH, Luo PG, Chen CF. Long-chain polyunsaturated fatty acids promote paclitaxel cytotoxicity via inhibition of the MDR1 gene in the human colon cancer caco-2 cell line. *J Am Coll Nutr*. 2011;30(4):265-273.

164. Sala-Vila A, Folkes J, Calder PC. The effect of three lipid emulsions differing in fatty acid composition on growth, apoptosis and cell cycle arrest in the HT-29 colorectal cancer cell line. *Clin Nutr*. 2010;29(4):519-524.

165. Jordan A, Stein J. Effect of an omega-3 fatty acid containing lipid emulsion alone and in combination with 5-fluorouracil (5-FU) on growth of the colon cancer cell line caco-2. *Eur J Nutr*. 2003;42(6):324-331.

166. Fox JC, Hay RV. Eicosapentaenoic acid inhibits cell growth and triacylglycerol secretion in McA-RH7777 rat hepatoma cultures. *Biochem J*. 1992;286 (Pt 1)(Pt 1):305-312.

167. Iwamoto S, Senzaki H, Kiyozuka Y, et al. Effects of fatty acids on liver metastasis of ACL-15 rat colon cancer cells. *Nutr Cancer*. 1998;31(2):143-150.

168. Collett ED, Davidson LA, Fan YY, Lupton JR, Chapkin RS. N-6 and n-3 polyunsaturated fatty acids differentially modulate oncogenic ras activation in colonocytes. *Am J Physiol Cell Physiol*. 2001;280(5):C1066-75.

169. Fukui M, Kang KS, Okada K, Zhu BT. EPA, an omega-3 fatty acid, induces apoptosis in human pancreatic cancer cells: Role of ROS accumulation, caspase-8 activation, and autophagy induction. *J Cell Biochem*. 2013;114(1):192-203.

170. Hawcroft G, Volpato M, Marston G, et al. The omega-3 polyunsaturated fatty acid eicosapentaenoic acid inhibits mouse MC-26 colorectal cancer cell liver

metastasis via inhibition of PGE2-dependent cell motility. *Br J Pharmacol*. 2012;166(5):1724-1737.

171. Jiang YH, Lupton JR, Chapkin RS. Dietary fish oil blocks carcinogen-induced down-regulation of colonic protein kinase C isozymes. *Carcinogenesis*. 1997;18(2):351-357.

172. Whitehouse AS, Smith HJ, Drake JL, Tisdale MJ. Mechanism of attenuation of skeletal muscle protein catabolism in cancer cachexia by eicosapentaenoic acid. *Cancer Res*. 2001;61(9):3604-3609.

173. Singh J, Hamid R, Reddy BS. Dietary fat and colon cancer: Modulating effect of types and amount of dietary fat on ras-p21 function during promotion and progression stages of colon cancer. *Cancer Res*. 1997;57(2):253-258.

174. Ichihara H, Zako K, Komizu Y, Goto K, Ueoka R. Therapeutic effects of hybrid liposomes composed of phosphatidylcholine and docosahexaenoic acid on the hepatic metastasis of colon carcinoma along with apoptosis in vivo. *Biol Pharm Bull*. 2011;34(6):901-905.

175. D'Eliseo D, Manzi L, Merendino N, Velotti F. Docosahexaenoic acid inhibits invasion of human RT112 urinary bladder and PT45 pancreatic carcinoma cells via down-modulation of granzyme B expression. *J Nutr Biochem*. 2012;23(5):452-457.

176. Hawkins RA, Sangster K, Arends MJ. Apoptotic death of pancreatic cancer cells induced by polyunsaturated fatty acids varies with double bond number and involves an oxidative mechanism. *J Pathol*. 1998;185(1):61-70.

177. Lai PB, Ross JA, Fearon KC, Anderson JD, Carter DC. Cell cycle arrest and induction of apoptosis in pancreatic cancer cells exposed to eicosapentaenoic acid in vitro. *Br J Cancer*. 1996;74(9):1375-1383.

178. Falconer JS, Ross JA, Fearon KC, Hawkins RA, O'Riordain MG, Carter DC. Effect of eicosapentaenoic acid and other fatty acids on the growth in vitro of human pancreatic cancer cell lines. *Br J Cancer*. 1994;69(5):826-832.

179. Pettersen K, Monsen VT, Hakvag Pettersen CH, et al. DHA-induced stress response in human colon cancer cells - focus on oxidative stress and autophagy. *Free Radic Biol Med*. 2016;90:158-172.

180. Zhang C, Yu H, Ni X, Shen S, Das UN. Growth inhibitory effect of polyunsaturated fatty acids (PUFAs) on colon cancer cells via their growth

inhibitory metabolites and fatty acid composition changes. *PLoS One*. 2015;10(4):e0123256.

181. Zhang C, Yu H, Shen Y, Ni X, Shen S, Das UN. Polyunsaturated fatty acids trigger apoptosis of colon cancer cells through a mitochondrial pathway. *Arch Med Sci*. 2015;11(5):1081-1094.

182. Vasudevan A, Yu Y, Banerjee S, et al. Omega-3 fatty acid is a potential preventive agent for recurrent colon cancer. *Cancer Prev Res (Phila)*. 2014;7(11):1138-1148.

183. Skender B, Hofmanova J, Slavik J, et al. DHA-mediated enhancement of TRAIL-induced apoptosis in colon cancer cells is associated with engagement of mitochondria and specific alterations in sphingolipid metabolism. *Biochim Biophys Acta*. 2014;1841(9):1308-1317.

184. Cho Y, Turner ND, Davidson LA, Chapkin RS, Carroll RJ, Lupton JR. Colon cancer cell apoptosis is induced by combined exposure to the n-3 fatty acid docosahexaenoic acid and butyrate through promoter methylation. *Exp Biol Med (Maywood)*. 2014;239(3):302-310.

185. Fenton JI, McCaskey SJ. Curcumin and docosahexaenoic acid block insulin-induced colon carcinoma cell proliferation. *Prostaglandins Leukot Essent Fatty Acids*. 2013;88(3):219-226.

186. Fasano E, Serini S, Piccioni E, et al. DHA induces apoptosis by altering the expression and cellular location of GRP78 in colon cancer cell lines. *Biochim Biophys Acta*. 2012;1822(11):1762-1772.

187. Manda K, Kriesen S, Hildebrandt G, Fietkau R, Klautke G. Omega-3 fatty acid supplementation in cancer therapy : Does eicosapentanoic acid influence the radiosensitivity of tumor cells? *Strahlenther Onkol*. 2011;187(2):127-134.

188. Slagsvold JE, Pettersen CH, Storvold GL, Follestad T, Krokan HE, Schonberg SA. DHA alters expression of target proteins of cancer therapy in chemotherapy resistant SW620 colon cancer cells. *Nutr Cancer*. 2010;62(5):611-621.

189. Rogers KR, Kikawa KD, Mouradian M, et al. Docosahexaenoic acid alters epidermal growth factor receptor-related signaling by disrupting its lipid raft association. *Carcinogenesis*. 2010;31(9):1523-1530.

190. Habermann N, Schon A, Lund EK, Glei M. Fish fatty acids alter markers of apoptosis in colorectal adenoma and adenocarcinoma cell lines but fish consumption has no impact on apoptosis-induction ex vivo. *Apoptosis*. 2010;15(5):621-630.

191. Hawcroft G, Loadman PM, Belluzzi A, Hull MA. Effect of eicosapentaenoic acid on E-type prostaglandin synthesis and EP4 receptor signaling in human colorectal cancer cells. *Neoplasia*. 2010;12(8):618-627.

192. Dommels YE, Alink GM, Linssen JP, van Ommen B. Effects of n-6 and n-3 polyunsaturated fatty acids on gap junctional intercellular communication during spontaneous differentiation of the human colon adenocarcinoma cell line caco-2. *Nutr Cancer*. 2002;42(1):125-130.

193. Kumamoto-Yonezawa Y, Sasaki R, Ota Y, et al. Cell cycle arrest triggered by conjugated eicosapentaenoic acid occurs through several mechanisms including G1 checkpoint activation by induced RPA and ATR expression. *Biochim Biophys Acta*. 2009;1790(5):339-346.

194. Danbara N, Yuri T, Tsujita-Kyutoku M, et al. Conjugated docosahexaenoic acid is a potent inducer of cell cycle arrest and apoptosis and inhibits growth of colo 201 human colon cancer cells. *Nutr Cancer*. 2004;50(1):71-79.

195. Habbel P, Weylandt KH, Lichopoj K, et al. Docosahexaenoic acid suppresses arachidonic acid-induced proliferation of LS-174T human colon carcinoma cells. *World J Gastroenterol*. 2009;15(9):1079-1084.

196. Tang FY, Cho HJ, Pai MH, Chen YH. Concomitant supplementation of lycopene and eicosapentaenoic acid inhibits the proliferation of human colon cancer cells. *J Nutr Biochem*. 2009;20(6):426-434.

197. Hossain Z, Hosokawa M, Takahashi K. Growth inhibition and induction of apoptosis of colon cancer cell lines by applying marine phospholipid. *Nutr Cancer*. 2009;61(1):123-130.

198. Jakobsen CH, Storvold GL, Bremseth H, et al. DHA induces ER stress and growth arrest in human colon cancer cells: Associations with cholesterol and calcium homeostasis. *J Lipid Res*. 2008;49(10):2089-2100.

199. Allred CD, Talbert DR, Southard RC, Wang X, Kilgore MW. PPARgamma1 as a molecular target of eicosapentaenoic acid in human colon cancer (HT-29) cells. *J Nutr*. 2008;138(2):250-256.

200. Toit-Kohn JL, Louw L, Engelbrecht AM. Docosahexaenoic acid induces apoptosis in colorectal carcinoma cells by modulating the PI3 kinase and p38 MAPK pathways. *J Nutr Biochem*. 2009;20(2):106-114.

201. Goto K, Tanaka Y, Matsumoto Y, Ueoka R. Induction of apoptosis of human tumor cells by hybrid liposomes including docosahexaenoic acid. *Bioorg Med Chem Lett*. 2008;18(6):1880-1883.

202. Kato T, Kolenic N, Pardini RS. Docosahexaenoic acid (DHA), a primary tumor suppressive omega-3 fatty acid, inhibits growth of colorectal cancer independent of p53 mutational status. *Nutr Cancer*. 2007;58(2):178-187.

203. Calviello G, Resci F, Serini S, et al. Docosahexaenoic acid induces proteasome-dependent degradation of beta-catenin, down-regulation of survivin and apoptosis in human colorectal cancer cells not expressing COX-2. *Carcinogenesis*. 2007;28(6):1202-1209.

204. Tsuzuki T, Kambe T, Shibata A, Kawakami Y, Nakagawa K, Miyazawa T. Conjugated EPA activates mutant p53 via lipid peroxidation and induces p53-dependent apoptosis in DLD-1 colorectal adenocarcinoma human cells. *Biochim Biophys Acta*. 2007;1771(1):20-30.

205. Calviello G, Di Nicuolo F, Serini S, et al. Docosahexaenoic acid enhances the susceptibility of human colorectal cancer cells to 5-fluorouracil. *Cancer Chemother Pharmacol*. 2005;55(1):12-20.

206. Vaculova A, Hofmanova J, Andera L, Kozubik A. TRAIL and docosahexaenoic acid cooperate to induce HT-29 colon cancer cell death. *Cancer Lett*. 2005;229(1):43-48.

207. Calviello G, Di Nicuolo F, Gragnoli S, et al. N-3 PUFAs reduce VEGF expression in human colon cancer cells modulating the COX-2/PGE2 induced ERK-1 and -2 and HIF-1alpha induction pathway. *Carcinogenesis*. 2004;25(12):2303-2310.

208. Moonen HJ, Dommels YE, van Zwam M, et al. Effects of polyunsaturated fatty acids on prostaglandin synthesis and cyclooxygenase-mediated DNA adduct formation by heterocyclic aromatic amines in human adenocarcinoma colon cells. *Mol Carcinog*. 2004;40(3):180-188.

209. Swamy MV, Cooma I, Patlolla JM, Simi B, Reddy BS, Rao CV. Modulation of cyclooxygenase-2 activities by the combined action of celecoxib and

decosahexaenoic acid: Novel strategies for colon cancer prevention and treatment. *Mol Cancer Ther*. 2004;3(2):215-221.

210. Narayanan BA, Narayanan NK, Simi B, Reddy BS. Modulation of inducible nitric oxide synthase and related proinflammatory genes by the omega-3 fatty acid docosahexaenoic acid in human colon cancer cells. *Cancer Res*. 2003;63(5):972-979.

211. Dommels YE, Haring MM, Keestra NG, Alink GM, van Bladeren PJ, van Ommen B. The role of cyclooxygenase in n-6 and n-3 polyunsaturated fatty acid mediated effects on cell proliferation, PGE(2) synthesis and cytotoxicity in human colorectal carcinoma cell lines. *Carcinogenesis*. 2003;24(3):385-392.

212. Chen ZY, Istfan NW. Docosahexaenoic acid is a potent inducer of apoptosis in HT-29 colon cancer cells. *Prostaglandins Leukot Essent Fatty Acids*. 2000;63(5):301-308.

213. Jiang WG, Hiscox S, Bryce RP, Horrobin DF, Mansel RE. The effects of n-6 polyunsaturated fatty acids on the expression of nm-23 in human cancer cells. *Br J Cancer*. 1998;77(5):731-738.

214. Mengeaud V, Nano JL, Fournel S, Rampal P. Effects of eicosapentaenoic acid, gamma-linolenic acid and prostaglandin E1 on three human colon carcinoma cell lines. *Prostaglandins Leukot Essent Fatty Acids*. 1992;47(4):313-319.

215. Wu MH, Tsai YT, Hua KT, Chang KC, Kuo ML, Lin MT. Eicosapentaenoic acid and docosahexaenoic acid inhibit macrophage-induced gastric cancer cell migration by attenuating the expression of matrix metalloproteinase 10. *J Nutr Biochem*. 2012;23(11):1434-1439.

216. Lim K, Han C, Dai Y, Shen M, Wu T. Omega-3 polyunsaturated fatty acids inhibit hepatocellular carcinoma cell growth through blocking beta-catenin and cyclooxygenase-2. *Mol Cancer Ther*. 2009;8(11):3046-3055.

217. Zou S, Meng X, Meng Y, et al. Microarray analysis of anti-cancer effects of docosahexaenoic acid on human colon cancer model in nude mice. *Int J Clin Exp Med*. 2015;8(4):5075-5084.

218. Piazzi G, D'Argenio G, Prossomariti A, et al. Eicosapentaenoic acid free fatty acid prevents and suppresses colonic neoplasia in colitis-associated colorectal cancer acting on notch signaling and gut microbiota. *Int J Cancer*. 2014;135(9):2004-2013.

219. Kansal S, Negi AK, Bhatnagar A, Agnihotri N. Ras signaling pathway in the chemopreventive action of different ratios of fish oil and corn oil in experimentally induced colon carcinogenesis. *Nutr Cancer*. 2012;64(4):559-568.

220. Cho Y, Turner ND, Davidson LA, Chapkin RS, Carroll RJ, Lupton JR. A chemoprotective fish oil/pectin diet enhances apoptosis via bcl-2 promoter methylation in rat azoxymethane-induced carcinomas. *Exp Biol Med (Maywood)*. 2012;237(12):1387-1393.

221. Sarotra P, Kansal S, Sandhir R, Agnihotri N. Chemopreventive effect of different ratios of fish oil and corn oil on prognostic markers, DNA damage and cell cycle in colon carcinogenesis. *Eur J Cancer Prev*. 2012;21(2):147-154.

222. Shah MS, Schwartz SL, Zhao C, et al. Integrated microRNA and mRNA expression profiling in a rat colon carcinogenesis model: Effect of a chemo-protective diet. *Physiol Genomics*. 2011;43(10):640-654.

223. Cho Y, Kim H, Turner ND, et al. A chemoprotective fish oil- and pectin-containing diet temporally alters gene expression profiles in exfoliated rat colonocytes throughout oncogenesis. *J Nutr*. 2011;141(6):1029-1035.

224. Sarotra P, Sharma G, Kansal S, et al. Chemopreventive effect of different ratios of fish oil and corn oil in experimental colon carcinogenesis. *Lipids*. 2010;45(9):785-798.

225. Moreira AP, Sabarense CM, Dias CM, et al. Fish oil ingestion reduces the number of aberrant crypt foci and adenoma in 1,2-dimethylhydrazine-induced colon cancer in rats. *Braz J Med Biol Res*. 2009;42(12):1167-1172.

226. Hong MY, Chapkin RS, Davidson LA, et al. Fish oil enhances targeted apoptosis during colon tumor initiation in part by downregulating bcl-2. *Nutr Cancer*. 2003;46(1):44-51.

227. Strouch MJ, Ding Y, Salabat MR, et al. A high omega-3 fatty acid diet mitigates murine pancreatic precancer development. *J Surg Res*. 2011;165(1):75-81.

228. Bathen TF, Holmgren K, Lundemo AG, et al. Omega-3 fatty acids suppress growth of SW620 human colon cancer xenografts in nude mice. *Anticancer Res*. 2008;28(6A):3717-3723.

229. Fukunaga K, Hossain Z, Takahashi K. Marine phosphatidylcholine suppresses 1,2-dimethylhydrazine-induced colon carcinogenesis in rats by inducing apoptosis. *Nutr Res*. 2008;28(9):635-640.

230. Gutt CN, Brinkmann L, Mehrabi A, et al. Dietary omega-3-polyunsaturated fatty acids prevent the development of metastases of colon carcinoma in rat liver. *Eur J Nutr*. 2007;46(5):279-285.

231. Davidson LA, Nguyen DV, Hokanson RM, et al. Chemopreventive n-3 polyunsaturated fatty acids reprogram genetic signatures during colon cancer initiation and progression in the rat. *Cancer Res*. 2004;64(18):6797-6804.

232. Sauer LA, Dauchy RT, Blask DE. Mechanism for the antitumor and anticachectic effects of n-3 fatty acids. *Cancer Res*. 2000;60(18):5289-5295.

233. Griffini P, Fehres O, Klieverik L, et al. Dietary omega-3 polyunsaturated fatty acids promote colon carcinoma metastasis in rat liver. *Cancer Res*. 1998;58(15):3312-3319.

234. Singh J, Hamid R, Reddy BS. Dietary fish oil inhibits the expression of farnesyl protein transferase and colon tumor development in rodents. *Carcinogenesis*. 1998;19(6):985-989.

235. Takahashi M, Fukutake M, Isoi T, et al. Suppression of azoxymethane-induced rat colon carcinoma development by a fish oil component, docosahexaenoic acid (DHA). *Carcinogenesis*. 1997;18(7):1337-1342.

236. Iigo M, Nakagawa T, Ishikawa C, et al. Inhibitory effects of docosahexaenoic acid on colon carcinoma 26 metastasis to the lung. *Br J Cancer*. 1997;75(5):650-655.

237. Suzuki I, Iigo M, Ishikawa C, et al. Inhibitory effects of oleic and docosahexaenoic acids on lung metastasis by colon-carcinoma-26 cells are associated with reduced matrix metalloproteinase-2 and -9 activities. *Int J Cancer*. 1997;73(4):607-612.

238. Singh J, Hamid R, Reddy BS. Dietary fat and colon cancer: Modulation of cyclooxygenase-2 by types and amount of dietary fat during the postinitiation stage of colon carcinogenesis. *Cancer Res*. 1997;57(16):3465-3470.

239. Ma CJ, Wu JM, Tsai HL, et al. Prospective double-blind randomized study on the efficacy and safety of an n-3 fatty acid enriched intravenous fat emulsion in postsurgical gastric and colorectal cancer patients. *Nutr J*. 2015;14:9-2891-14-9.

240. Wei Z, Wang W, Chen J, Yang D, Yan R, Cai Q. A prospective, randomized, controlled study of omega-3 fish oil fat emulsion-based parenteral nutrition for patients following surgical resection of gastric tumors. *Nutr J*. 2014;13:25-2891-13-25.

241. Nagano T, Fujita H, Tanaka T, et al. Randomized controlled trial comparing antioxidant-enriched enteral nutrition with immune-enhancing enteral nutrition after esophagectomy for cancer: A pilot study. *Surg Today*. 2013;43(11):1240-1249.

242. Bonatto SJ, Oliveira HH, Nunes EA, et al. Fish oil supplementation improves neutrophil function during cancer chemotherapy. *Lipids*. 2012;47(4):383-389.

243. Silva Jde A, Trindade EB, Fabre ME, et al. Fish oil supplement alters markers of inflammatory and nutritional status in colorectal cancer patients. *Nutr Cancer*. 2012;64(2):267-273.

244. Cury-Boaventura MF, Torrinhas RS, de Godoy AB, Curi R, Waitzberg DL. Human leukocyte death after a preoperative infusion of medium/long-chain triglyceride and fish oil parenteral emulsions: A randomized study in gastrointestinal cancer patients. *JPEN J Parenter Enteral Nutr*. 2012;36(6):677-684.

245. Ma CJ, Sun LC, Chen FM, et al. A double-blind randomized study comparing the efficacy and safety of a composite vs a conventional intravenous fat emulsion in postsurgical gastrointestinal tumor patients. *Nutr Clin Pract*. 2012;27(3):410-415.

246. Sultan J, Griffin SM, Di Franco F, et al. Randomized clinical trial of omega-3 fatty acid-supplemented enteral nutrition versus standard enteral nutrition in patients undergoing oesophagogastric cancer surgery. *Br J Surg*. 2012;99(3):346-355.

247. Miyata H, Yano M, Yasuda T, et al. Randomized study of clinical effect of enteral nutrition support during neoadjuvant chemotherapy on chemotherapy-related toxicity in patients with esophageal cancer. *Clin Nutr*. 2012;31(3):330-336.

248. de Miranda Torrinhas RS, Santana R, Garcia T, et al. Parenteral fish oil as a pharmacological agent to modulate post-operative immune response: A randomized, double-blind, and controlled clinical trial in patients with gastrointestinal cancer. *Clin Nutr*. 2013;32(4):503-510.

249. Makay O, Kaya T, Firat O, et al. Omega-3 fatty acids have no impact on serum lactate levels after major gastric cancer surgery. *JPEN J Parenter Enteral Nutr*. 2011;35(4):488-492.

250. Jiang ZM, Wilmore DW, Wang XR, et al. Randomized clinical trial of intravenous soybean oil alone versus soybean oil plus fish oil emulsion after gastrointestinal cancer surgery. *Br J Surg*. 2010;97(6):804-809.

251. Nakamura M, Iwahashi M, Takifuji K, et al. Optimal dose of preoperative enteral immunonutrition for patients with esophageal cancer. *Surg Today*. 2009;39(10):855-860.

252. Okamoto Y, Okano K, Izuishi K, Usuki H, Wakabayashi H, Suzuki Y. Attenuation of the systemic inflammatory response and infectious complications after gastrectomy with preoperative oral arginine and omega-3 fatty acids supplemented immunonutrition. *World J Surg*. 2009;33(9):1815-1821.

253. Ryan AM, Reynolds JV, Healy L, et al. Enteral nutrition enriched with eicosapentaenoic acid (EPA) preserves lean body mass following esophageal cancer surgery: Results of a double-blinded randomized controlled trial. *Ann Surg*. 2009;249(3):355-363.

254. Aiko S, Yoshizumi Y, Ishizuka T, et al. Enteral immuno-enhanced diets with arginine are safe and beneficial for patients early after esophageal cancer surgery. *Dis Esophagus*. 2008;21(7):619-627.

255. Liang B, Wang S, Ye YJ, et al. Impact of postoperative omega-3 fatty acid-supplemented parenteral nutrition on clinical outcomes and immunomodulations in colorectal cancer patients. *World J Gastroenterol*. 2008;14(15):2434-2439.

256. Takeuchi H, Ikeuchi S, Kawaguchi Y, et al. Clinical significance of perioperative immunonutrition for patients with esophageal cancer. *World J Surg*. 2007;31(11):2160-2167.

257. Helminen H, Raitanen M, Kellosalo J. Immunonutrition in elective gastrointestinal surgery patients. *Scand J Surg*. 2007;96(1):46-50.

258. Klek S, Kulig J, Szczepanik AM, Jedrys J, Kolodziejczyk P. The clinical value of parenteral immunonutrition in surgical patients. *Acta Chir Belg*. 2005;105(2):175-179.

259. Chen DW, Wei Fei Z, Zhang YC, Ou JM, Xu J. Role of enteral immunonutrition in patients with gastric carcinoma undergoing major surgery. *Asian J Surg*. 2005;28(2):121-124.

260. Aiko S, Yoshizumi Y, Tsuwano S, Shimanouchi M, Sugiura Y, Maehara T. The effects of immediate enteral feeding with a formula containing high levels of omega-3 fatty acids in patients after surgery for esophageal cancer. *JPEN J Parenter Enteral Nutr*. 2005;29(3):141-147.

261. Farreras N, Artigas V, Cardona D, Rius X, Trias M, Gonzalez JA. Effect of early postoperative enteral immunonutrition on wound healing in patients undergoing surgery for gastric cancer. *Clin Nutr*. 2005;24(1):55-65.

262. Heller AR, Rossel T, Gottschlich B, et al. Omega-3 fatty acids improve liver and pancreas function in postoperative cancer patients. *Int J Cancer*. 2004;111(4):611-616.

263. Braga M, Gianotti L, Vignali A, Carlo VD. Preoperative oral arginine and n-3 fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. *Surgery*. 2002;132(5):805-814.

264. Braga M, Gianotti L, Nespoli L, Radaelli G, Di Carlo V. Nutritional approach in malnourished surgical patients: A prospective randomized study. *Arch Surg*. 2002;137(2):174-180.

265. Wu GH, Zhang YW, Wu ZH. Modulation of postoperative immune and inflammatory response by immune-enhancing enteral diet in gastrointestinal cancer patients. *World J Gastroenterol*. 2001;7(3):357-362.

266. Gee JM, Watson M, Matthew JA, et al. Consumption of fish oil leads to prompt incorporation of eicosapentaenoic acid into colonic mucosa of patients prior to surgery for colorectal cancer, but has no detectable effect on epithelial cytokinetics. *J Nutr*. 1999;129(10):1862-1865.

267. Senkal M, Zumtobel V, Bauer KH, et al. Outcome and cost-effectiveness of perioperative enteral immunonutrition in patients undergoing elective upper gastrointestinal tract surgery: A prospective randomized study. *Arch Surg*. 1999;134(12):1309-1316.

268. Kenler AS, Swails WS, Driscoll DF, et al. Early enteral feeding in postsurgical cancer patients. fish oil structured lipid-based polymeric formula versus a standard polymeric formula. *Ann Surg*. 1996;223(3):316-333.

269. Kemen M, Senkal M, Homann HH, et al. Early postoperative enteral nutrition with arginine-omega-3 fatty acids and ribonucleic acid-supplemented diet versus

placebo in cancer patients: An immunologic evaluation of impact. *Crit Care Med*. 1995;23(4):652-659.

270. Daly JM, Lieberman MD, Goldfine J, et al. Enteral nutrition with supplemental arginine, RNA, and omega-3 fatty acids in patients after operation: Immunologic, metabolic, and clinical outcome. *Surgery*. 1992;112(1):56-67.

271. Trabal J, Leyes P, Forga M, Maurel J. Potential usefulness of an EPA-enriched nutritional supplement on chemotherapy tolerability in cancer patients without overt malnutrition. *Nutr Hosp*. 2010;25(5):736-740.

272. Jones RJ, Hawkins RE, Eatock MM, et al. A phase II open-label study of DHA-paclitaxel (taxoprexin) by 2-h intravenous infusion in previously untreated patients with locally advanced or metastatic gastric or oesophageal adenocarcinoma. *Cancer Chemother Pharmacol*. 2008;61(3):435-441.

273. Read JA, Beale PJ, Volker DH, Smith N, Childs A, Clarke SJ. Nutrition intervention using an eicosapentaenoic acid (EPA)-containing supplement in patients with advanced colorectal cancer. effects on nutritional and inflammatory status: A phase II trial. *Support Care Cancer*. 2007;15(3):301-307.

274. Moses AW, Slater C, Preston T, Barber MD, Fearon KC. Reduced total energy expenditure and physical activity in cachectic patients with pancreatic cancer can be modulated by an energy and protein dense oral supplement enriched with n-3 fatty acids. *Br J Cancer*. 2004;90(5):996-1002.

275. Persson C, Glimelius B, Ronnelid J, Nygren P. Impact of fish oil and melatonin on cachexia in patients with advanced gastrointestinal cancer: A randomized pilot study. *Nutrition*. 2005;21(2):170-178.

276. Barber MD, Preston T, McMillan DC, Slater C, Ross JA, Fearon KC. Modulation of the liver export protein synthetic response to feeding by an n-3 fatty-acid-enriched nutritional supplement is associated with anabolism in cachectic cancer patients. *Clin Sci (Lond)*. 2004;106(4):359-364.

277. Fearon KC, Von Meyenfeldt MF, Moses AG, et al. Effect of a protein and energy dense N-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: A randomised double blind trial. *Gut*. 2003;52(10):1479-1486.

278. Wigmore SJ, Barber MD, Ross JA, Tisdale MJ, Fearon KC. Effect of oral eicosapentaenoic acid on weight loss in patients with pancreatic cancer. *Nutr Cancer*. 2000;36(2):177-184.

279. Barber MD, McMillan DC, Preston T, Ross JA, Fearon KC. Metabolic response to feeding in weight-losing pancreatic cancer patients and its modulation by a fish-oil-enriched nutritional supplement. *Clin Sci (Lond)*. 2000;98(4):389-399.

280. Barber MD, Ross JA, Voss AC, Tisdale MJ, Fearon KC. The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. *Br J Cancer*. 1999;81(1):80-86.

281. Wigmore SJ, Ross JA, Falconer JS, et al. The effect of polyunsaturated fatty acids on the progress of cachexia in patients with pancreatic cancer. *Nutrition*. 1996;12(1 Suppl):S27-30.

282. Kemen M, Senkal M, Homann HH, et al. Early postoperative enteral nutrition with arginine-omega-3 fatty acids and ribonucleic acid-supplemented diet versus placebo in cancer patients: An immunologic evaluation of impact. *Crit Care Med*. 1995;23(4):652-659.

283. Jones RJ, Hawkins RE, Eatock MM, et al. A phase II open-label study of DHA-paclitaxel (taxoprexin) by 2-h intravenous infusion in previously untreated patients with locally advanced or metastatic gastric or oesophageal adenocarcinoma. *Cancer Chemother Pharmacol*. 2008;61(3):435-441.

284. Ohira T, Nishio K, Ohe Y, et al. Improvement by eicosanoids in cancer cachexia induced by LLC-IL6 transplantation. *J Cancer Res Clin Oncol*. 1996;122(12):711-715.

285. Yuzhalin AE. *Interleukins in cancer biology: Their heterogeneous role.* Amsterdam; Boston: Elsevier/AP, Academic Press is an imprint of Elsevier; 2014.

286. Inacio Pinto N, Carnier J, Oyama LM, et al. Cancer as a proinflammatory environment: Metastasis and cachexia. *Mediators Inflamm*. 2015;2015:791060.

287. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials*. 1989;10(1):1-10.

288. Arshad A, Isherwood J, Mann C, et al. Intravenous omega-3 fatty acids plus gemcitabine: Potential to improve response and quality of life in advanced pancreatic cancer. *JPEN J Parenter Enteral Nutr*. 2015.

289. Cunningham D, Starling N, Rao S, et al. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med*. 2008;358(1):36-46.

290. Lee JW, Devanarayan V, Barrett YC, et al. Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm Res*. 2006;23(2):312-328.

291. Minami Y, Miyata H, Doki Y, et al. Omega-3 fatty acid-containing diet (racol) reduces toxicity of chemoradiation therapy for patients with esophageal cancer. *Gan To Kagaku Ryoho*. 2008;35(3):437-440.

292. Eltweri AM, Thomas AL, Metcalfe M, Calder PC, Dennison AR, Bowrey DJ. Potential applications of fish oils rich in omega-3 polyunsaturated fatty acids in the management of gastrointestinal cancer. *Clin Nutr*. 2016.

293. Wilson J, Balkwill F. The role of cytokines in the epithelial cancer microenvironment. *Semin Cancer Biol*. 2002;12(2):113-120.

294. Fayers PM, Aaronson NK, Bjordal K, Groenvold M, Curran D, Bottomley A, eds.
On behalf of the EORTC quality of life group. the EORTC QLQ-C30 scoring manual (3rd edition).
. 3rd edition ed. Brussels: European Organisation for Research and Treatment of Cancer; 2001.

295. Philpott M, Ferguson LR. Immunonutrition and cancer. *Mutat Res*. 2004;551(1-2):29-42.

296. Waldner MJ, Foersch S, Neurath MF. Interleukin-6--a key regulator of colorectal cancer development. *Int J Biol Sci*. 2012;8(9):1248-1253.

297. Dethlefsen C, Hojfeldt G, Hojman P. The role of intratumoral and systemic IL-6 in breast cancer. *Breast Cancer Res Treat*. 2013;138(3):657-664.

298. Mantovani A. Cancer: Inflaming metastasis. *Nature*. 2009;457(7225):36-37.

299. Kornman KS. Interleukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. *Am J Clin Nutr*. 2006;83(2):475S-483S.

300. Wang D, Dubois RN. Eicosanoids and cancer. *Nat Rev Cancer*. 2010;10(3):181-193.

301. Calder PC. Very long chain omega-3 (n-3) fatty acids and human health. *European Journal of Lipid Science and Technology*. 2014;116:1280-1300.

302. Merendino N, Costantini L, Manzi L, Molinari R, D'Eliseo D, Velotti F. Dietary omega -3 polyunsaturated fatty acid DHA: A potential adjuvant in the treatment of cancer. *Biomed Res Int*. 2013;2013:310186.

303. Vaughan VC, Hassing MR, Lewandowski PA. Marine polyunsaturated fatty acids and cancer therapy. *Br J Cancer*. 2013;108(3):486-492.

304. Chen R, Cai JL, Zhou B, Jiang AF. Effect of immune-enhanced enteral diet on postoperative immunological function in patients with colorectal cancer. *Zhonghua Wei Chang Wai Ke Za Zhi*. 2005;8(4):328-330.

305. Carpentier YA, Hacquebard M, Portois L, Dupont IE, Deckelbaum RJ, Malaisse WJ. Rapid cellular enrichment of eicosapentaenoate after a single intravenous injection of a novel medium-chain triacylglycerol:Fish-oil emulsion in humans. *Am J Clin Nutr*. 2010;91(4):875-882.

306. Arshad A, Chung WY, Isherwood J, et al. Cellular and plasma uptake of parenteral omega-3 rich lipid emulsion fatty acids in patients with advanced pancreatic cancer. *Clin Nutr*. 2014;33(5):895-899.

307. Barros KV, Carvalho PO, Cassulino AP, et al. Fatty acids in plasma, white and red blood cells, and tissues after oral or intravenous administration of fish oil in rats. *Clin Nutr*. 2013;32(6):993-998.

308. Arshad A, Chung WY, Isherwood J, et al. Cellular and plasma uptake of parenteral omega-3 rich lipid emulsion fatty acids in patients with advanced pancreatic cancer. *Clin Nutr*. 2014;33(5):895-899.

309. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: An 18-month controlled study. *J Lipid Res*. 1997;38(10):2012-2022.

310. Pittet YK, Berger MM, Pluess TT, et al. Blunting the response to endotoxin in healthy subjects: Effects of various doses of intravenous fish oil. *Intensive Care Med*. 2010;36(2):289-295.

311. Lochhead P, El-Omar EM. Helicobacter pylori infection and gastric cancer. *Best Practice & Research Clinical Gastroenterology*. 2007;21(2):281-297.

312. Kubota H, Matsumoto H, Higashida M, et al. Eicosapentaenoic acid modifies cytokine activity and inhibits cell proliferation in an oesophageal cancer cell line. *Anticancer Res*. 2013;33(10):4319-4324.

313. Kondo S, Barna BP, Kondo Y, et al. WAF1/CIP1 increases the susceptibility of p53 non-functional malignant glioma cells to cisplatin-induced apoptosis. *Oncogene*. 1996;13(6):1279-1285.

314. Lincet H, Poulain L, Remy JS, et al. The p21(cip1/waf1) cyclin-dependent kinase inhibitor enhances the cytotoxic effect of cisplatin in human ovarian carcinoma cells. *Cancer Lett*. 2000;161(1):17-26.

315. Ryazantseva NV, Novitskii VV, Zhukova OB, et al. Role of NF-kB, p53, and p21 in the regulation of TNF-alpha mediated apoptosis of lymphocytes. *Bull Exp Biol Med*. 2010;149(1):50-53.

316. Lee RH, Yoon N, Reneau JC, Prockop DJ. Preactivation of human MSCs with TNF-alpha enhances tumor-suppressive activity. *Cell Stem Cell*. 2012;11(6):825-835.

317. Piccolo MT, Crispi S. The dual role played by p21 may influence the apoptotic or anti-apoptotic fate in cancer. . 2012;1(2):189-202.

318. Beales IL, Ogunwobi OO. Leptin synergistically enhances the anti-apoptotic and growth-promoting effects of acid in OE33 oesophageal adenocarcinoma cells in culture. *Mol Cell Endocrinol*. 2007;274(1-2):60-68.

319. Beales IL, Ogunwobi O, Cameron E, El-Amin K, Mutungi G, Wilkinson M. Activation of akt is increased in the dysplasia-carcinoma sequence in barrett's oesophagus and contributes to increased proliferation and inhibition of apoptosis: A histopathological and functional study. *BMC Cancer*. 2007;7:97.

320. SARS abstracts. *Br J Surg*. 2015;102(s5):3-52.

321. Woodcock BE, Smith E, Lambert WH, et al. Beneficial effect of fish oil on blood viscosity in peripheral vascular disease. *Br Med J (Clin Res Ed)*. 1984;288(6417):592-594.

322. Larson MK, Shearer GC, Ashmore JH, et al. Omega-3 fatty acids modulate collagen signaling in human platelets. *Prostaglandins Leukot Essent Fatty Acids*. 2011;84(3-4):93-98.

323. Li XL, Steiner M. Fish oil: A potent inhibitor of platelet adhesiveness. *Blood*. 1990;76(5):938-945.

324. Dona M, Fredman G, Schwab JM, et al. Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. *Blood*. 2008;112(3):848-855.

325. Chen P, Vericel E, Lagarde M, Guichardant M. Poxytrins, a class of oxygenated products from polyunsaturated fatty acids, potently inhibit blood platelet aggregation. *FASEB J*. 2011;25(1):382-388.

326. Vanschoonbeek K, Feijge MA, Paquay M, et al. Variable hypocoagulant effect of fish oil intake in humans: Modulation of fibrinogen level and thrombin generation. *Arterioscler Thromb Vasc Biol*. 2004;24(9):1734-1740.

327. Deans DA, Wigmore SJ, Gilmour H, Paterson-Brown S, Ross JA, Fearon KC. Elevated tumour interleukin-1beta is associated with systemic inflammation: A marker of reduced survival in gastro-oesophageal cancer. *Br J Cancer*. 2006;95(11):1568-1575.

328. El-Omar EM, Carrington M, Chow WH, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*. 2000;404(6776):398-402.

329. Dvorakova K, Payne CM, Ramsey L, et al. Increased expression and secretion of interleukin-6 in patients with barrett's esophagus. *Clin Cancer Res*. 2004;10(6):2020-2028.

330. Liao WC, Lin JT, Wu CY, et al. Serum interleukin-6 level but not genotype predicts survival after resection in stages II and III gastric carcinoma. *Clin Cancer Res*. 2008;14(2):428-434.

331. Caygill CP, Charlett A, Hill MJ. Fat, fish, fish oil and cancer. *Br J Cancer*. 1996;74(1):159-164.

332. Rose DP, Connolly JM. Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacol Ther*. 1999;83(3):217-244.

333. Murphy RA, Mourtzakis M, Chu QS, Baracos VE, Reiman T, Mazurak VC. Supplementation with fish oil increases first-line chemotherapy efficacy in patients with advanced nonsmall cell lung cancer. *Cancer*. 2011;117(16):3774-3780.

334. Gogos CA, Ginopoulos P, Salsa B, Apostolidou E, Zoumbos NC, Kalfarentzos F. Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy: A randomized control trial. *Cancer*. 1998;82(2):395-402.

335. Fearon KC, Barber MD, Moses AG, et al. Double-blind, placebo-controlled, randomized study of eicosapentaenoic acid diester in patients with cancer cachexia. *J Clin Oncol*. 2006;24(21):3401-3407.

336. Oh DY, Talukdar S, Bae EJ, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell*. 2010;142(5):687-698.

337. Lavigne JA, Takahashi Y, Chandramouli GV, et al. Concentration-dependent effects of genistein on global gene expression in MCF-7 breast cancer cells: An oligo microarray study. *Breast Cancer Res Treat*. 2008;110(1):85-98.

338. Calviello G, Di Nicuolo F, Gragnoli S, et al. N-3 PUFAs reduce VEGF expression in human colon cancer cells modulating the COX-2/PGE2 induced ERK-1 and -2 and HIF-1alpha induction pathway. *Carcinogenesis*. 2004;25(12):2303-2310.

339. Cheng JC, Graber MS, Hsu FM, et al. High serum levels of vascular endothelial growth factor-A and transforming growth factor-beta1 before neoadjuvant chemoradiotherapy predict poor outcomes in patients with esophageal squamous cell carcinoma receiving combined modality therapy. *Ann Surg Oncol*. 2014;21(7):2361-2368.

340. Shih CH, Ozawa S, Ando N, Ueda M, Kitajima M. Vascular endothelial growth factor expression predicts outcome and lymph node metastasis in squamous cell carcinoma of the esophagus. *Clin Cancer Res*. 2000;6(3):1161-1168.

341. Barbosa VM, Miles EA, Calhau C, Lafuente E, Calder PC. Effects of a fish oil containing lipid emulsion on plasma phospholipid fatty acids, inflammatory markers, and clinical outcomes in septic patients: A randomized, controlled clinical trial. *Crit Care*. 2010;14(1):R5.

342. Al-Taan O, Stephenson JA, Spencer L, et al. Changes in plasma and erythrocyte omega-6 and omega-3 fatty acids in response to intravenous supply of omega-3 fatty acids in patients with hepatic colorectal metastases. *Lipids Health Dis*. 2013;12:64-511X-12-64.

343. Ostrand-Rosenberg S. Cancer and complement. *Nat Biotechnol*. 2008;26(12):1348-1349.

344. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: Linking inflammation and cancer. *J Immunol*. 2009;182(8):4499-4506.