PHASE II TRIAL OF THE EFFECT OF GEMCITABINE WITH INTRAVENOUS OMEGA-3 FISH OIL INFUSION IN PATIENTS WITH UNRESECTABLE PANCREATIC ADENOCARCINOMA

Thesis submitted for the degree of Doctor of Medicine at the University of Leicester

By

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Abstract

Phase II trial of the effect of gemcitabine with intravenous omega-3 fish oil infusion in patients with unresectable pancreatic adenocarcinoma

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Introduction

Advanced Pancreatic Cancer (APC) has an appalling prognosis characterised by rapidly declining quality of life mediated by circulating pro-inflammatory cytokines and growth factors (CAF). Omega-3 fatty acids (n-3FAs) are proven to have anti-neoplastic and anti-inflammatory effects. Oral trials of n-3FAs in patients with advanced cancer have shown mixed results due in part to poor bioavailability and compliance with these preparations.

Methods

A phase II single arm trial was carried out using gemcitabine and intravenous n-3FAs in patients with APC. Primary outcome measure was overall radiological response rate (ORR) with secondary outcome measures of Overall (OS) and Progression-Free Survival (PFS), quality of life using validated questionnaires, Clinical Benefit Response (CBR) rates, adverse events, changes in CAF, complement, uptake of n-3FAs into cell membranes and plasma.

Results

Twenty nine patients were recruited: 21 were evaluable for ORR which was 3/21 (14.3%). Median OS=4.8 months, median PFS=3.5 months. Improvements and percentage of patients experiencing it in QOL outcomes of at least 10% over baseline was seen in the following domains: Global health- 57%, Summated QOL- 43%, Pain scores- 57%. CBR rates were 38%. PDGF, TRAIL and FGF concentrations reduced significantly with treatment over time. Low baseline IL-6 and IL-8 were correlated with improved OS. PDGF responders showed a tendency towards improved OS and FGF responders a significantly improved PFS. Restoration of hypoactive Mannose Binding Lectin complement activity was associated with improved time to progression. Proportions of n-3FA fractions in cell membranes increased significantly with time.

Conclusions

Intravenous n-3FAs plus gemcitabine may improve quality of life and provide clinical benefit response in patients with APC. These changes may be mediated by manipulation of CAFs and complement pathways. The independent effect of n-3FAs over gemcitabine warrants further investigation in randomised trials.

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Peer reviewed publications

1**. Arshad A**, Al-Leswas D, Al-Taan O, Stephenson J, Metcalfe M, Steward WP and Dennison AR. Pooled Survival and Response Data From Phase III Randomized Controlled Trials for Gemcitabine-based Regimes in the Treatment of Advanced Pancreatic Cancer. Am J Clin Oncol 2011 Mar [Epub ahead of print]

2**. Arshad A**, Al-Leswas D, Stephenson J, Metcalfe M and Dennison AR. Potential applications of fish oils rich in n-3 fatty acids in the palliative treatment of advanced pancreatic cancer. Br J Nutr 2011;106(6):795-800.

3. **Arshad A,** Chung WY, Steward WP, Metcalfe MS and Dennison AR. Reduction in circulating pro-angiogenic and pro-inflammatory factors is related to improved outcome in patients with advanced pancreatic cancer treated with gemcitabine and intravenous omega-3 fish oil. HPB 2013;15(6):428-432.

4. **Arshad A**, Chung WY, Isherwood J, Steward WP, Metcalfe AR and Dennison AR. Restoration of Mannose-Binding Lectin Complement Activity Is Associated With Improved Outcome in Patients With Advanced Pancreatic Cancer Treated With Gemcitabine and Intravenous ω-3 Fish Oil. J Parenter Enteral Nutr 2013 Feb[Epub ahead of print]

International presentations to learned societies

1. **Arshad A**, Hall TC, Bilku D, Al-Leswas D, Stephenson JA, Pollard C, Mann C, Metcalfe MS, Steward WP, Dennison AR. An Omega-3 rich lipid infusion can help prevent tumour-related weight loss in patients with advanced pancreatic cancer. *Poster presentation*, European Society for Parenteral and Enteral Nutrition, Gothenburg, September 2011

2. **Arshad A**, Al Leswas D, Hall TC, Bilku D, Mann C, Morgan B, Metcalfe MS, Steward WP, Dennison AR An Omega-3 rich lipid infusion in combination with gemcitabine can improve quality of life in patients with advanced pancreatic cancer. *Poster presentation*, European Society for Parenteral and Enteral Nutrition, Gothenburg, September 2011

3. **Arshad A**, Bilku D, Hall TC, Al-Leswas D, Metcalfe MS, Steward WP, Dennison AR. Serum triglyceride levels, safety and tolerability of high-dose-rate infusion of omega-3 rich lipid emulsion in patients with advanced pancreatic cancer. *Poster presentation*, European Society for Parenteral and Enteral Nutrition, Gothenburg, September 2011

4**. Arshad A**, Hall T, Al-Leswas D, Bilku D, Cairns V, Mann C, Morgan B, Metcalfe M, Steward WP, Dennison AR. Phase II Trial of Gemcitabine and an Omega-3 Rich Lipid Infusion in Advanced Pancreatic Cancer. *Poster presentation*, ECCO/ESMO/ESSO Multidisciplinary Cancer Conference, Stockholm, September 2011

5. **Arshad A,,**Chung WY, Al-Leswas D,Hall T, Bilku D, Pollard C, Metcalfe M, Steward WP, Dennison AR. Restoration of Activity in Mannose-binding Lectin Complement Pathway in Patients with Advanced Pancreatic Cancer Treated with Intravenous Omega-3 Rich Lipid Infusion and Gemcitabine is Associated with Improved Outcome. *Poster presentation*, ECCO/ESMO/ESSO Multidisciplinary Cancer Conference, Stockholm, September 2011

6. **Arshad A,** Hall T, Chung WY, Al-Leswas D, Pollard C, Steward WP, Metcalfe M and Dennison AR. Reduction in circulating pro-angiogenic and pro-inflammatory factors is related to improved outcome in patients with advanced pancreatic cancer treated with gemcitabine and intravenous omega-3 fish oil : *best oral presentation session,* International Hepato-Pancreatico-Biliary Association World Congress, Paris, July 2012

7. **Arshad A**, Al-Leswas D, Hall TC, Chung WY, Mann C, Pollard C, Steward WP, Metcalfe MS and Dennison AR. Phase II trial of gemcitabine plus intravenous omega-3 fish oil in advanced pancreatic cancer : *poster presentation*, , International Hepato-Pancreatico-Biliary Association World Congress, Paris, July 2012

8. **Arshad A**, Al-Leswas D, Bilku D, Chung WY, Pollard C, Steward WP, Metcalfe MS and Dennison AR Intravenous omega-3 fish oil plus gemcitabine ameliorates tumour cachexia and stabilises quality of life and pain scores in patients with advanced pancreatic cancer: *poster presentation*, International Hepato-Pancreatico-Biliary Association World Congress, Paris, July 2012

9. **Arshad A**, Chung WY, Hall TC, Al-Leswas D, Pollard C, Steward WP, Metcalfe MS and Dennison AR. Baseline Interleukin 6 and 8 predict clinical outcome in patients with advanced pancreatic cancer treated with gemcitabine and intravenous omega-3 fish oil: *poster presentation*, International Hepato-Pancreatico-Biliary Association World Congress, Paris, July 2012

10. **Arshad A**, Chung WY, Hall TC, Al-Leswas D, Pollard C, Steward WP, Metcalfe MS and Dennison AR. Expression of circulating pro-angiogenic and pro-inflammatory factors is reduced in patients with advanced pancreatic cancer treated with gemcitabine and an intravenous omega-3 rich lipid emulsion : *poster presentation*, International Hepato-Pancreatico-Biliary Association World Congress, Paris, July 2012

11. **Arshad A**, Chung WY, Bilku D, Al-Leswas D, Pollard C, Steward WP, Metcalfe MS and Dennison AR. Restoration of Mannose-binding lectin pathway activity is related to improved outcome in patients with advanced pancreatic cancer treated with gemcitabine and intravenous omega-3 fish oil : *poster presentation*, International Hepato-Pancreatico-Biliary Association World Congress, Paris, July 2012

12 **Arshad A**, Chung WY, Mann CD, Steward WP, Metcalfe MS and Dennison AR. Quality of life improvement in patients with advanced pancreatic cancer treated with intravenous omega-3 fish oil and gemcitabine. *Accepted for virtual poster discussion session*, European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life conference, Brussels, October 2012

Abbreviations used

ALA Alpha linolenic acid
BSA Body surface area
CAF Circulating pro-angiogenic and pro-inflammatory factors
CCD Charged coupled devices
CE Cholesterol ester
CT Computed tomography
DHA Docosahexaenoic acid
DNA Deoxyribonucleic acid
DPA Docosapentaenoic acid
ECOG Eastern cooperative oncology group
EGF Epidermal growth factor
EGFR Epidermal growth factor receptor
ELISA Enzyme-linked immunosorbent assay
EMA European Medicines Agency
EPA Eicosapentaenoic acid
FAME Fatty acid methyl ester
FDA Food and Drug Administration
FGF Fibroblast growth factor
FOLFIRINOX Folinic Acid, 5-Fluorouracil, Irinotecan, Oxaliplatin
HGF Hepatocyte growth factor
HPB Hepatopancreaticobiliary
HRP Horse radish peroxidase
HRQOL Health related quality of life
IFN-γ Interferon gamma
IL-1β Interleukin-1 beta
IL-6 Interleukin-6
IL-8 Interleukin-8
LA Linoleic acid
MBL Mannose binding lectin
MDSC Myeloid derived suppressor cells
MDT Multidisciplinary team
NEFA Non-esterified fatty acids
N-3FA Omega-3 fatty acid
N-3FAs Omega-3 fatty acids
N-6FA Omega-6 fatty acid
PC Phosphatidylcholine
PDGF Platelet derived growth factor
PE Phosphatidylethanolamine
QoL Quality of life
RANKL Receptor activator of nuclear factor kappa beta ligand

RECIST Response evaluation criteria in solid tumours
TAG Triacylglycerol
TNF-α Tumour necrosis factor alpha
TRAIL TNF-α related apoptosis inducing ligand
ULN Upper limit of normal
VEGF Vascular endothelial growth factor

Contents

1 INTRODUCTION

1.1 Incidence and epidemiology of pancreatic adenocarcinoma

  Pancreatic cancer is responsible for more than 7900 deaths from cancer each year in the UK making it the fifth most common cause of death from all cancer sites and the third most common from gastrointestinal sources. It is the eleventh most common cancer in the UK, with over 8300 new cases per annum. This represents 9 per 100,000 of the UK population. The male:female ratio is roughly equal, although the incidence in men has fallen slightly in the last 30 years, the female incidence has remained unchanged (figure 1.1).   One year survival rates are in the region of 14% overall, with rates of 25% in the under 50s. Five year survival rates are 2-3% (Coupland et al. 2012). This makes the prognosis one of the worst amongst all cancers. Risk factors for the development of pancreatic cancer include a strong association with cigarette smoking, chronic pancreatitis, diabetes, obesity and family history, especially in a first degree relative. (Bansal, Sonnenberg 1995, Berrington de Gonzales, Sweetland & Spencer 2003, Doll et al. 1994, Ekbom 1994, Malka 2002, Permuth-Wey, Egan 2008, Stevens, Roddam & Beral 2007)
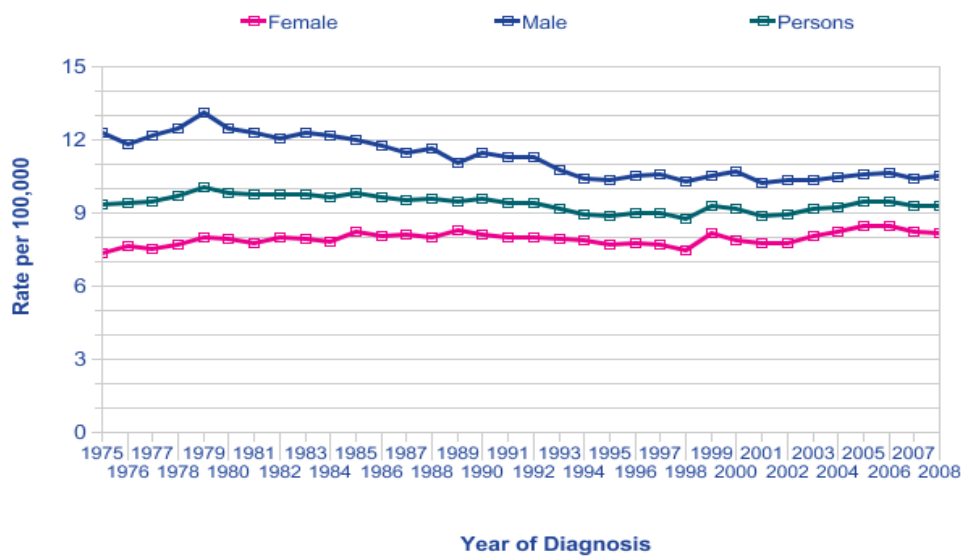
Figure 1.1 Age-standardised incidence rates of pancreatic cancer for Great Britain, 1975-2008 (pan-European data from the cancer-research UK database).

1.2 Current treatments for resectable pancreatic adenocarcinoma

Of all the patients presenting with pancreatic tumours approximately 95% will prove to have adenocarcinoma on histology, the remainder being neuro-endocrine tumours. The head of the pancreas is the most common site of tumour, with approximately 70% arising in this region. However due to their often late presentation they are only rarely amenable to surgical resection with resection rates of approximately 10% in most centres (Witkowski et al. 2013). Surgical resection usually involves pancreatico-duodenectomy (Whipple's procedure) if the tumour is localised to the head of the pancreas, and distal pancreatectomy with splenectomy or total pancreatectomy if the tumour is in the tail or body of the pancreas respectively. These resections are usually followed up with adjuvant chemotherapy depending on

the loco-regional nodal involvement. Postoperative 5-year survival is in the region of 20-40% depending on the lymph node involvement status (Bachmann et al. 2006).

1.3 Current treatments for non-resectable pancreatic adenocarcinoma

1.3.1 Symptomatic treatment

All patients with a diagnosis of pancreatic cancer require symptomatic treatment in the first instance. This includes treatment for obstructive jaundice due to encroachment and compression of the distal extra-hepatic common bile duct by the tumour, treated by endoscopic biliary stenting. Patients with gastric outlet obstruction may require surgical gastrojejunostomy. Systemic malaise and pain must be adequately controlled with medication.

1.3.2 Disease modifying treatment

A minority of patients will be fit enough, with an ECOG performance status of 0 or 1, to undergo treatment with palliative chemotherapy with the aim of extending survival and quality of life. The current standard of care is intravenous gemcitabine therapy on a weekly basis which confers a survival benefit over bolus 5-fluoro-uracil (5FU) (Burris et al. 1997). Response rates for gemcitabine are modest: typically 12-18% will exhibit radiological partial response (a decrease in sum of target lesion diameters by 30% or more), with a median overall survival of 6.2 months in recent pooled cohort of reported phase III trials (Arshad et al. 2011a). Combination

chemotherapies such as capecitabine or oxaliplatin with gemcitabine have not shown

clear survival advantages and hence their utility is questionable (Lee et al. 2009b,

Cunningham et al. 2009). A recent study showed a significant survival benefit

(Median OS = 11.1 vs 6.8 months OR 0.57 CI 0.45-0.73 p<0.001) for a

FOLFIRINOX (5-FU, folinic acid, irinotecan and oxaliplatin) regimen over single-

agent gemcitabine but at the expense of significantly increased side effects such as

neutropaenia, febrile neutropaenia, diarrhoea and sensory neuropathy and hence this

regimen has not been widely adopted to date (Conroy et al. 2011). Certainly

FOLFIRINOX can only be administered to fit patients because of its toxicity, which

is the minority of patients with advanced pancreatic cancer. Its utility consequently

occupies a small niche in the group of patient who will receive palliative

chemotherapy.

1.3.3 Gemcitabine structure and mechanism of action

Gemcitabine (2′,2′-difluoro-2′-deoxycytidine) is structurally similar to the

nucleoside cytidine. The two hydrogen atoms attached to the 2'carbon position are

substituted by fluorine atoms (figure 1.2).
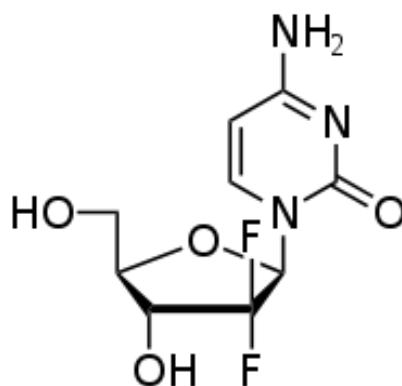
Figure 1.2. Chemical structure of Gemcitabine.

Gemcitabine is hydrophilic and cannot cross the cell membrane without use of a specialised transport system. Two distinct families of nucleoside transporter exist: sodium dependent and independent or equilibrative. Gemcitabine transport in humans is mostly by the human equilibrative nucleoside transporters 1 and 2 (hENT1 and 2) (Andersson et al. 2009).

Once inside the cell, gemcitabine undergoes phosphorylation by deoxycitadine kinase (dCK) and to a far lesser extent thymidine kinase 2 (TK2), to produce gemcitabine monophosphate (Ohhashi et al. 2008). It is then further phosphorylated by less specific enzymes to the most important and active metabolite gemcitabine triphosphate. This is incorporated into the C sites of the forming DNA strand catalysed by DNA polymerases, where one more nucleotide can be added before polymerisation is terminated, resulting in DNA damage (Andersson et al. 2009). This then leads to apoptosis (programmed cell death).

1.4 Pooled survival and response data from Phase III randomized controlled trials for gemcitabine-based regimens in the treatment of advanced pancreatic cancer

Single agent gemcitabine is currently the first line treatment of choice for patients with advanced pancreatic cancer since demonstrating significant superiority in median and progression free survival over single agent 5-fluorouracil (Burris et al. 1997). Thirteen years later, median survival times remain around 6 months with little in the way of meaningful improvement, and so investigations into novel chemotherapeutic and biological agents which can potentiate the effects of gemcitabine are ongoing. A recent encouraging trial showing significantly improved survival benefit of the

combination of FOLFIRINOX chemotherapy over single-agent gemcitabine has not been translated into widespread clinical use due to the significantly higher incidence of adverse effects such as febrile neutropaenia in the FOLFIRINOX arm (Conroy et al. 2011). Most trials have compared gemcitabine alone or with placebo versus gemcitabine combined with an investigational agent, although a handful have directly compared single investigational agents with gemcitabine. Many trials of novel compounds which have shown statistically superior action to single agent gemcitabine in small Phase II trials have shown no statistical benefit when investigated in randomized Phase III trials with much larger numbers of patients (Xiong et al. 2004, Philip et al. 2010). It is important when designing single-arm phase II trials that an accurate and contemporaneous view of median and progression free survival times for single agent gemcitabine can be obtained. Finding accurate historical control data is difficult and may be misleading when applied to a single hospital department.

A recent systematic literature review was carried out in order to evaluate the pooled results of all published randomized phase III trials comparing single agent gemcitabine with other agents either alone or in combination (Arshad et al. 2011a). Data including number of patients, investigational product, median, progression free and 1 year survival and radiological response rates was collected. The most common Grade 3 and 4 toxicities reported were detailed by study and arm: these were namely neutropaenia, thrombocytopaenia, anaemia, nausea/vomiting (pooled data for both where given separately) and diarrhoea. Twenty one randomized phase III trials were identified (table 1.1) from 1997 to 2010. All had single agent once-weekly gemcitabine as their control group. Seventeen trials investigated combination therapies and four single agent novel therapies against gemcitabine. Two studies comparing 5-Fluorouracil alone with gemcitabine and BAY 12-9566 (a selective

matrix metalloproteinase inhibitor) with gemcitabine showed significantly better median and progression free survivals for single agent gemcitabine (Burris et al. 1997, Moore et al. 2007). One non-gemcitabine containing chemotherapy regimen (5-fluorouracil, leucovorin, epirubicin and carboplatin) showed statistically superior median and progression free survival over single agent gemcitabine (Cantore et al. 2004). It should be noted that this regimen was administered intra-arterially directly into the coeliac axis three times with a three week interval between treatments. In several separate trials, the doublet combinations of cisplatin, 5-fluorouracil, oxaliplatin, cisplatin, epirubicin and capecitabine each individually along with gemcitabine showed statistically prolonged progression free survival but without improved overall survival (Colucci et al. 2002, Berlin et al. 2002, Louvet et al. 2005, Reni et al. 2005, Cunningham et al. 2009). The only agent so far to show improved overall and progression free survival in Phase III as a combination therapy with gemcitabine is erlotinib (Moore et al. 2007). This benefit although statistically significant was an improvement in 10 days of median overall survival and 6 days in progression free survival. In addition 36% of the patients receiving erlotinib had at least a grade 2 skin rash, the most frequent and serious adverse effect associated with it.

All phase III trials had comparable cohorts of disease stage and performance status. In total, a pool of 3171 patients were investigated in the single-agent gemcitabine arm. The pooled median survival was 6.15 (range 3.6-9.1) months. Pooled 1 year survival was 22% and progression free survival 3.3 (range 1.9-4.2) months. In general response rates were reported by best response for each patient on evaluation of CT images. Overall objective radiological response rate, which includes both complete and partial response on RECIST evaluation ranged from 4.4% to 17.3%

(mean=8.3%) for the gemcitabine control arms and 0% to 38.5% for the combination therapy arms. An objective response was seen in 264/3171 patients in the gemcitabine control groups giving an overall response rate of 8.3% for the trials. Complete response with single agent gemcitabine was never seen in more than 1% of patients. Specific stable disease rates were reported by only eleven of the studies and ranged from 18.6-56% for the gemcitabine arm and 11.0-60.2% for the combination arms. Significantly improved objective response rates were seen in trials adding Cisplatin, Irinotecan, Oxaliplatin, Cisplatin, Epirubicin and 5-fluorouracil and Capecitabine to Gemcitabine, but none of these studies demonstrated improved median survival times (Colucci et al. 2002, Rocha Lima et al. 2004, Louvet et al. 2005, Reni et al. 2005, Cunningham et al. 2009).

1.4.1 Toxicities and Adverse Events

The most common haematological toxicities for all trials were neutropaenia, thrombocytopaenia and anaemia. Nausea and/or vomiting and diarrhoea are the most common non-haematological adverse events. Neutropaenia is particularly common for gemcitabine-based regimens with rates of 8.3% to 32% of patients in the gemcitabine arm having grade 3 or 4 toxicities. Of note, no combination therapy was able to significantly reduce haematological or non-haematological toxicities experienced with gemcitabine (table 1.2).

Combination therapies with gemcitabine have shown disappointing activity in Phase III trials and investigation must continue into novel agents which can prolong median and progression free survival with an acceptable toxicity profile. Some therapies such as single-agent 5-fluorouracil have in-fact showed significantly worse

survival times when compared with single-agent gemcitabine and have strengthened the evidence for its current use as first line chemotherapy for advanced pancreatic cancer. Although a handful of trials investigating combination therapies have improved objective response rates, no agent has achieved improvement in all three of the most common outcome measures evaluated which are median survival, progression free survival and objective response rates. This analysis of a large number of patients indicates that the current median survival for patients with advanced pancreatic cancer treated with single-agent gemcitabine is just over 6 months with progression free survival of just over 3 months. Objective response rates for gemcitabine are between 4.4% and 17.3% (mean=8.3%). These are the benchmarks upon which the design of single-armed phase II trials involving gemcitabine-based combination regimens should be based and their results evaluated.

Author	Gem Pts	Comp Pts	Gem OS	Comp OS	P	GEM 1yr	Comp 1yr	Gem PFS	Comp PFS	P	GEM ORR	Comp ORR
Burris	63	63	5.65	4.41	0.025	18%	2%	2.33	0.92	0.0002	5.6%	0
Colucci	54	53	4.6	7	0.43	11%	11.3%	2	5	0.048	7.6%	26.4%
Berlin	162	160	5.4	6.7	0.09	<20%	<20%	2.2	3.4	0.022	5.6%	6.9%
Wang	20	22	9.1	7.2	NS	31.3%	11.1%	NS	NS	NS	6.3%	11%
Bramhall	119	120	5.5	5.5	0.95	17%	18%	3.2	3.1	0.68	16%	11%
Smith	25	30	3.6	5.1	NS	NS	NS	1.9	2.3	NS	8%	3%
Moore	139	138	6.59	3.74	<0.001	25%	10%	3.5	1.68	<0.001	5%	1%
Van Cutsem	347	341	6.1	6.4	0.75	24%	49%	3.6	3.7	0.72	8%	6%
Rocha-Lima	169	173	6.6	6.3	0.789	22%	21%	3	3.5	0.352	4.4%	16.1%
Cantore	67	71	5.8	7.9	0.036	21%	35%	4.2	5.4	0.013	5.9%	14%
Louvet	156	157	7.1	9	0.13	27.8%	34.7%	3.7	5.8	0.04	17.3%	26.8%
Reni	46	51	NS	NS	NS	21.3%	38.5%	3.3	5.4	0.003	8.5%	38.5%
Oettle	282	283	6.3	6.2	0.847	13.8%	17.3%	3.3	3.9	0.1109	7.1%	14.8%
Strathopoulos	70	60	6.5	6.4	0.97	21.8%	24.3%	2.9	2.8	0.795	10%	15%
Heinemann	95	95	6	7.5	0.15	24.7%	25.3%	3.1	5.3	0.053	8.2%	10.2%
Abou Alfa	174	175	6.2	6.7	0.52	21%	23%	3.8	3.6	0.22	4.6%	6.3%
Moore	284	285	5.91	6.24	0.038	17%	23%	3.55	3.75	0.004	8.6%	8%
Herrmann	159	160	7.2	8.4	0.234	30%	32%	3.9	4.3	0.103	7.8%	10%
Poplin	275	272	4.9	5.7	0.22	16%	21%	2.6	2.7	0.1	6%	9%
Cunningham	266	267	6.2	7.1	0.08	22%	24.3%	3.8	5.3	0.004	12%	19.1%
Colucci	199	201	8.3	7.2	0.38	32%	28.4%	3.9	3.8	0.8	10.1%	12.9%
Totals	**3171**	**3177**	**6.15**	**6.55**		**22%**	**23%**	**3.3**	**3.725**		**264/3171 (8.3%)**	

Table 1.1. Clinical results of Phase III trials of Gemcitabine based regimens for

advanced pancreatic cancer. Total number of patients along with mean values for each

column are shown.

Key : OS=Median Overall Survival (months) Gem=Gemcitabine arm
Comp=Comparator arm Pts=Number of Patients PFS=Progression Free Survival
(months). ORR=Overall radiological response rate

Author	Neut Gem	Neut Comp	Anaem Gem	Anaem Comp	Plt Gem	Plt Comp	N/V Gem	N/V Comp	Diarr Gem	Diarr Comp
Burris	25.9	4.9	9.7	0	9.7	1.6	12.7	4.8	1.6	4.8
Colucci	9.0	18.0	4.0	6.0	2.0	2.0	2.0	2.0	0.0	4.0
Berlin	16.0	29.0	10.0	10.0	11.0	19.0	19.0	15.0	4.0	10.0
Bramhall	NS	NS	7.0	3.0	NS	NS	NS	NS	NS	NS
Smith	4.0	16.7	4.0	0.0	1.0	13.3	0.0	10.0	4.0	6.7
Moore	12.0	1.4	10.7	10.9	8.6	0.0	8.6	10.9	2.2	1.4
Van Cutsem	30.0	40.0	16.0	20.0	12.0	15.0	17.0	14.0	3.0	4.0
Rocha-Lima	32.0	37.6	13.0	16.2	14.2	19.7	18.4	30.7	1.8	18.5
Cantore	7.5	19.7	2.9	14.0	1.4	25.3	4.4	NS	2.9	NS
Louvet	27.6	20.4	10.3	6.4	3.2	14.0	9.0	19.1	1.3	5.7
Reni	12.2	19.5	4.4	3.6	1.8	28.6	7.9	5.9	0.0	0.5
Oettle	12.8	45.1	2.9	13.9	6.2	17.9	6.6	6.6	0.7	2.9
Strathopoulos	15.7	26.7	4.3	5.0	0.0	5.0	4.3	3.4	2.9	3.3
Heinemann	8.3	10.0	10.6	13.3	10.6	4.4	5.9	22.2	4.7	3.3
Abou Alfa	14.0	30.0	8.0	5.0	4.0	15.0	6.0	9.0	0.0	1.0
Moore	27.0	24.0	NS	NS	11.0	10.0	NS	NS	2.0	6.0
Herrmann	19.0	23.0	6.0	8.0	8.0	4.0	8.0	11.0	2.0	5.0
Poplin	16.0	12.0	10.0	5.0	13.0	11.0	7.0	16.0	3.0	6.0
Cunningham	22.0	35.0	6.0	4.0	6.0	11.0	12.0	13.0	4.0	5.0
Colucci	14.0	25.0	1.0	5.0	5.0	16.0	2.0	6.0	2.0	0.5

Table 1.2. Grade 3+4 toxicities for Phase III trials of Gemcitabine based regimens for advanced pancreatic cancer. Gem = Patients in single-agent gemcitabine arm. Comp = Patients in comparator arm. Neut = Percentage of patients with neutropaenia. Anaem = Percentage of patients with anaemia. Plt = Percentage of patients with thrombocytopaenia. N/V = Percentage of patients with nausea or vomiting. Diarr = Percentage of patients with diarrhoea.

1.5 Novel biological combination therapies for advanced pancreatic adenocarcinoma

The palliation of patients with locally advanced and metastatic pancreatic carcinoma has seen little in the way of development over the past ten years. The priority for patients with unresectable disease is extending survival times and improving quality of life. The need for better therapeutic options to use alone or in combination with existing chemotherapy regimens drives the investigation and development of novel therapeutic targets. Unfortunately promising results from early-phase studies have so far failed to translate into improved survival except for the Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitor erlotinib, which is the only novel biological agent to demonstrate an improved median overall survival in a phase III randomised controlled trial when added to a standard chemotherapy regimen (Moore et al. 2007). There have been many attempts at other therapeutic strategies which have not yielded any benefit in survival or objective response. A recent systematic literature review was undertaken in order to assess the clinical effectiveness of novel biological agents to date using pubmed/medline and the American Society for Clinical Oncology abstract database.

1.5.1 Phase I trials

Matuzumab

Matuzumab is a humanised monoclonal antibody against the epidermal growth factor receptor with a longer half-life than lapatinib. Murine models utilising human pancreatic cancer cell lines have shown long-lasting anti-tumour effects particularly

in combination with gemcitabine (Kleespies et al. 2008). In a Phase I trial,
Matuzumab combined with gemcitabine in patients with advanced pancreatic cancer,
reported Partial Response (PR) or Stable Disease (SD) in eight out of 12 evaluated
patients (66.7%), with three patients showing PR among six evaluated in the group
receiving 800 mg weekly. It also inhibited phosphorylated EGFR and affected
receptor-dependent signalling and transduction even in the 400mg weekly (lowest
dose) group (Graeven et al. 2006). Phase II development planning is in process.

Imatinib

Imatinib is a protein tyrosine kinase inhibitor of Platelet Derived Growth Factor
(PDGF) receptor that was developed initially for its selective action against the BCR-
ABL fusion protein expressed in nearly all patients with chronic myeloid leukaemia.
In animal models with experimental pancreatic cancer, the combination of
gemcitabine and imatinib induced tumour regression of >70% compared to 36% in
those given imatinib alone when compared to controls and also inhibited metastases
to the liver. There was also decreased expression of activated PDGFR alpha and beta,
reduced angiogenesis and increased apoptosis (Hwang et al. 2003). A phase I trial
randomising patients to receive either gemcitabine chemotherapy or Imatinib showed
no response in either group and no benefit in survival or quality of life with Imatinib
(Chen et al. 2006). A phase II trial of Imatinib mono-therapy in patients with
metastatic pancreatic cancer showed no significant benefit (Gharibo et al. 2008).

1.5.2 Phase II trials

Lapatinib

Lapatinib is a tyrosine kinase inhibitor against ErbB-1 and ErbB-2 which can be commonly expressed in pancreaticobiliary cancers. In a Phase I trial of lapatinib and gemcitabine, and lapatinib with the combination of gemcitabine and oxaliplatin, 25 patients were enrolled of which 18 had pancreatic cancer and 7 biliary cancer. The maximum tolerated dose with weekly gemcitabine was 1500mg per day and with gemcitabine and oxaliplatin 1000mg/day. Mean survival was 11 months with a 1 year survival of 48%. A phase II trial was terminated after 29 patients due to futility analysis indicating four months median survival and with the single agent lapatinib group, all patients progressing by 1 month. Lapatinib is not effective in combination with gemcitabine or isolation in pancreatic cancer and is unlikely to be investigated further (Safran et al. 2009).

Masitinib

Masitinib is a tyrosine kinase inhibitor targeting c-Kit, PDGFR, FGFR3 and the FAK pathway and can enhance the anti-proliferative effect of gemcitabine in human pancreatic cancer cell lines. A single-armed phase II study of 22 patients with advanced pancreatic cancer gave patients daily oral masitinib and standard weekly gemcitabine. Median time to progression was 6.4 months with median OS 7.1 months and a disease control rate (PR+CR+SD) of 73% was demonstrated (Hammel et al. 2009).

Sorafenib

Sorafenib has inhibitory effects on Raf-1 kinase and VEGFR-2 and is thus a potentially potent anti-angiogenesis agent. A phase I trial enrolled a total of 42 patients (with 23 in an extended advanced pancreatic cancer arm) to receive sorafenib in combination with gemcitabine and demonstrated that 13 patients (56.5%) achieved disease control. There was good tolerability and no drug interaction between the two agents (Siu et al. 2006). A Phase II trial of sorafenib plus gemcitabine in advanced pancreatic cancer enrolled 17 patients. There were no objective responses and three patients (23%) had SD with no demonstrable benefit in survival. Their conclusion was that Sorafenib in combination with gemcitabine is inactive in advanced pancreatic cancer patients (Wallace, Locker & Nattam 2007).

Axitinib

Axitinib is a potent inhibitor of VEGFRs. In a Phase II trial, 103 patients with locally advanced or metastatic pancreatic cancer were randomised in a 2:1 ratio to receive gemcitabine with axitinib or gemcitabine alone. The main objective of the trial was to determine the Overall Survival (OS). Median OS was longer in the combination group than in the gemcitabine only group (6.9 vs 5.6 months). The hazard ratio for survival with the combination group compared to the gemcitabine only group was 0.71, and the safety profiles in the two groups were similar (Spano et al. 2008).

S-1

S-1 is an oral analogue of 5-fluorouracil which was the established intravenous bolus chemotherapy treatment for metastatic pancreatic cancer before gemcitabine. Experimental murine models have been employed to investigate the optimal

combination chemotherapy to use with S-1 and have found that gemcitabine provides the best results and seems to act synergistically (Yoshizawa et al. 2009). The mechanism of this synergistic action appears to be the up-regulation of the human equilabrative nucleoside transporter 1 by S-1. It is therefore suggested that S-1 be given prior to gemcitabine in any combination regimen (Nakahira et al. 2008). Phase I trials showed good tolerability and promising anti-tumour activity of S-1 in combination with gemcitabine in human subjects (Ueno et al. 2005, Nakamura et al. 2005). A multicentre phase II study investigated gemcitabine and S-1 in combination in 38 patients with a best response of PR achieved in 11 (32%). Their median OS was 8.4 months with median Progression Free Survival (PFS) 5.4 months. This combination was well tolerated (Oh et al. 2010). A further Phase II study enrolled 32 patients to receive S-1 and gemcitabine combination therapy and showed best response of PR in 14 (44%) with SD in 8 (25%) and Progressive Disease (PD) in 8 (25%). Median PFS was 4.9 months and median OS was 7.9 months again with acceptable tolerance (Lee et al. 2009a). In another Phase II study, S-1 in combination with gemcitabine was given to 55 patients with advanced pancreatic cancer. No Complete Response (CR) was observed, PR was achieved in 24 patients, resulting in an overall response rate of 44% and 26 patients (48%) had SD. The median PFS was 5.9 months and the median OS was 10.1 months with a 1-year survival of 33%. The major grade 3-4 toxicities were neutropaenia, leucopoenia, thrombocytopenia, anorexia, rash, nausea and fatigue. This combination produced an encouraging response rate and survival associated with an acceptable toxicity. A randomised Phase III trial is currently recruiting (Ueno, Okusaka & Furuse 2007). There have also been limited quality trials using irinotecan in combination with S-1 with PFS of 4.9 months and OS 11.3 months in 16 patients although some had undergone previous treatment

with gemcitabine (Shitara et al. 2008). Finally a phase II trial of S-1 as mono-therapy in gemcitabine-refractory metastatic pancreatic cancer has shown marginal anti-tumour clinical benefit with an acceptable toxicity profile (Morizane et al. 2009).

Celecoxib

Celecoxib is a selective COX-2 inhibitor widely used as an analgesic agent. Pre-clinical studies in human pancreatic tumour cell lines suggest an involvement of COX-2 derived bioactive molecules in tumour-dependent angiogenesis and provide the rationale for inhibition of the COX pathway as an effective therapeutic approach (Wu et al. 2005, Gregor et al. 2005). In a Phase II trial of gemcitabine plus celecoxib (Celebrex®), 42 patients enrolled with advanced pancreatic adenocarcinoma (26 metastatic and 16 with locally advanced disease), four patients (9%) achieved a PR and 26 (62%) had SD, gaining a total disease control in 30(71%) patients. Neither grade 4 neutropaenia nor grade 3-4 thrombocytopenia was observed. Grade 3 neutropaenia was detected in 19% of patients. Median survival was 9.1 months (Ferrari et al. 2006). A more recent phase II trial of the same combination in 25 patients revealed grade 3 neutropaenia in 32%. There was no significant improvement in measured clinical outcomes compared to historical controls: 4 (17%) patients had PR and 7 (35%) had SD (Dragovich et al. 2008).

1.5.3 Phase III trials

Bevacizumab

Bevacizumab (Avastin ®) is a recombinant humanized monoclonal antibody to VEGF, which is already used for treatment of certain forms of metastatic colon, lung and breast cancer. A multicenter single-armed Phase II trial using bevacizumab in combination with gemcitabine in previously untreated patients with advanced

pancreatic cancer showed promising results. Fifty-two patients were enrolled out of which total of 11 patients (21%) had confirmed PR, and 24 (46%) had SD. The 6-month survival rate was 77% with a median survival of 8.8 months. Median PFS was 5.4 months and these data were historically superior to single-agent gemcitabine (Kindler et al. 2005). Another phase II trial enrolled 50 patients treating them in a single arm group with gemcitabine, capecitabine and bevacizumab. They showed a radiological (PR+CR) response rate of 22% with PFS of 5.8 months and overall survival of 9.8 months (Javle et al. 2009). The third phase II trial enrolled 52 patients to receive gemcitabine, cisplatin and bevacizumab. They showed 10 (19.2%) had an unconfirmed response and 30 (57.7%) had stable disease. In this cohort, 20/35 patients with elevated baseline CA19-9 levels had a greater than 50% decline in these levels during treatment. Unfortunately in this study group there were some serious complications including gastrointestinal bleeding, thromboembolic events, bowel perforation, cardiac events and hypertension (Ko et al. 2008). The only Phase III trial to date reported that the addition of bevacizumab to gemcitabine in patients with advanced pancreatic cancer, compared with gemcitabine plus placebo, resulted in no improvement in any efficacy results. These were median OS: 5.8/5.9 months, median progression free survival 3.8/2.9 months, with overall response rates of 13%/10% (Kindler et al. 2010).

In a multicenter Phase III study of gemcitabine and erlotinib with or without bevacizumab in 607 patients with metastatic pancreatic cancer, PFS was significantly improved in the bevacizumab group. However the trial did not show an improvement in OS which was the primary outcome measure. No new safety events were observed with the addition of bevacizumab in this trial (Van Cutsem et al. 2009).

Cetuximab

Cetuximab is a monoclonal antibody which acts as an EGF receptor blocker. It inhibits EGF receptor autophosphorylation *in vitro* and *in vivo*, and potentiates the cytotoxic effects of gemcitabine on pancreatic cancer cell lines (Bruns et al. 2000). In a phase II study of 41 patients with advanced pancreatic cancer who had immunohistochemical evidence of EGFR expression, the combination of gemcitabine and cetuximab resulted in PR in 5(12.2%) and SD in 26(63.4%). The median time to progression was 3.8 months, OS was 7.1 months, and the 1-year survival rate was 31.7% (Xiong et al. 2004).Another Phase II trial enrolled 64 patients in a single arm study to receive cetuximab, gemcitabine and oxaliplatin. Their overall response rate was 33% (2% CR + 31% PR), 31% SD and 36% PD, with a median OS of 7.0 months. There was no increase in response or survival with this combination although it was well tolerated (Kullmann et al. 2009). A further Phase II trial randomised 84 patients to receive either cetuximab, gemcitabine and cisplatin OR gemcitabine and cisplatin. They showed no significant difference in objective response, disease control, overall survival or median survival (Cascinu et al. 2008). The only Phase III trial to date showed that patients with locally advanced or metastatic pancreatic cancer had no statistically significant improvement in survival when the combination of gemcitabine and cetuximab was given compared with gemcitabine alone, with a median survival of 6.4 and 5.9 months, respectively. There was a trend toward improvement in PFS that was not statistically significant: 3.5 and 3.0 months, respectively (Philip et al. 2010).

Erlotinib

Erlotinib is another EGF receptor blocker that also acts by inhibiting the receptor autophosphorylation. Preclinical studies in mice demonstrated that gemcitabine-induced apoptosis was augmented when given in combination with Erlotinib (Ng et al. 2002). In a double blind randomised controlled Phase III trial, 569 patients were assigned to receive gemcitabine plus erlotinib or placebo. Overall survival was significantly prolonged for the erlotinib/gemcitabine arm with a median survival of 6.24 months vs 5.91 months (P=0.038). One year survival was also increased (23% vs 17% P=0.023). PFS was also significantly improved with an estimated HR of 0.77 (P=0.004). Overall objective response rates were not significantly different. This trial was the first to show statistically improved survival for any agent added to gemcitabine chemotherapy and led to the approval of erlotinib by the US FDA in locally advanced and metastatic pancreatic cancer (Moore et al. 2007). Contrasting with the FDA however the European Medicines Agency (EMA) only approved erlotinib in the metastatic setting partly due to the potentially serious side-effects with cutaneous reactions particularly common. The phase III trial of bevacizumab with erlotinib and gemcitabine has been discussed previously, both arms of this trial received gemcitabine and erlotinib (Van Cutsem et al. 2009). A phase III crossover trial of gemcitabine plus erlotinib (GE) followed by capecitabine or capecitabine plus erlotinib(CE) followed by gemcitabine showed that the GE arm had significantly prolonged time to first line treatment failure but overall survivals and time to second line treatment failure were similar (Boeck et al. 2010). A single-armed phase II trial of the combination of gemcitabine, capecitabine, erlotinib and bevacizumab in 44 patients showed PFS and OS of 8.5 and 12.8 months respectively (Watkins et al. 2010). Further combinations of gemcitabine and bevacizumab with either cetuximab

or erlotinib in a randomised phase II trial were investigated in 139 patients. Although results were superior to historical controls of single-agent Gemcitabine, the combinations were not considered sufficiently active to warrant phase III evaluation (Kindler et al. 2008).

Glufosfamide

Glufosfamide is glucose linked to isophosphoramide mustard, the active metabolite of ifosfamide. Cancer cells use glucose at a higher rate than healthy cells, which may lead to preferential metabolic targeting by glufosfamide and delivery of the cytotoxic agent to the cancer cells. A Phase II study evaluated the safety and efficacy of glufosfamide in combination with gemcitabine in patients with locally advanced and metastatic pancreatic cancer. A total of 29 patients were enrolled to receive 4500mg/m^3 of intravenous glufosfamide at the start of a monthly chemotherapy cycle with gemcitabine once a week for the first three weeks only. Five of 28 (18%; 95% CI: 6-37%) had a PR (duration >1.0-5.8 months), and one unconfirmed PR; 11 of 28 patients (39%) had SD (median duration 5.3 months), median PFS was 3.7 months and the median OS was 6-months. One year survival was 32%. This combination may benefit patients with pancreatic cancer, although haematological and renal toxicity was seen in 79% and 37% of patients respectively and were pronounced. It was suggested that alternative dosing regimens should be explored (Chiorean et al. 2010). A recently published Phase III trial randomised 303 patients with metastatic pancreatic cancer previously treated with gemcitabine to either glufosfamide mono-therapy or best supportive care. They found an non-statistically significant improvement in median OS from 84 days for the gemcitabine group to 105 days for the glufosfamide group (Ciuleanu et al. 2009).

1.5.4 Immunotherapy

There has been recent interest in novel immunotherapeutic options such as vaccine therapy for advanced pancreatic cancer. Strategies including combined dendritic cell vaccination and parenteral injection of activated lymphocytes or lymphokine-activated killer lymphocytes have resulted in a median survival of 9 months in patients with advanced pancreatic cancer (Nakamura et al. 2009). Personalised peptide vaccination using reacting peptides pre-vaccinated into patients with pancreatic cancer also showed promising results in early human trials with 85% of patients showing clinical response with reduction in tumour size or levels of tumour markers (Yanagimoto et al. 2007). Dendritic cell-based immunisation causing activation of tumour-specific cytotoxic T-cell lymphocytes in conjunction with gemcitabine and oral S-1 has shown promising results with radiological response rates of 69.2% (Okamoto et al. 2009). Telomerase is expressed in 90% of pancreatic cancers and immunogenic telomerase peptides have been isolated and are now the subject of randomised clinical trials. Telomerase is considered vital for tumour immortalisation and growth. GV1001 is a peptide vaccine which binds multiple HLA class II molecules and harbours class I epitopes. It may therefore elicit combined CD4/CD8 T-cell responses resulting in cell-mediated cytotoxiticy of cancer cells (Kyte 2009). It is given as a subcutaneous injection in conjunction with granulocyte-macrophage stimulating factor (GM-CSF). Phase I/II trials demonstrated that GV1001 was safe to use and generated an immune response, particularly in an intermediate dose group (Bernhardt et al. 2006). A Phase III multi-centre trial of gemcitabine mono-therapy versus GV1001 plus GM-CSF plus sequential gemcitabine therapy enrolled 365 patients in total but was terminated prematurely due to a preliminary analysis showing no survival benefit (Buanes et al. 2009).

Novel approaches to the treatment of locally advanced and metastatic pancreatic cancer have arisen out of necessity due to the poor performance of currently available therapies. Pre-clinical studies have identified biological targets on the molecular level which have in some cases gone on to useful clinical studies. Unfortunately the vast majority of these trials have shown no statistical benefit in clinical response or survival over historical results of single-agent gemcitabine chemotherapy (table 1.3). To date only one large phase III trial has provided clinicians with an agent of proven statistical benefit with regards to survival, and thus the search for effective agents which can provide results in terms of objective response, disease stabilisation and prolonged survival in combination with a tolerable side-effect profile must continue.

Agent	Author	Type	Patients	OS	PFS	ORR
Bevacizumab	Kindler[2005]	2S	52	8.8	5.4	21.0%
Bevacizumab+Capecitabine	Javle	2S	50	9.8	5.8	22.0%
Bevacizumab+Cisplatin	Ko	2S	52	8.2	6.6*	19.2%
Bevacizumab+Cetuximab	Kindler[2008]	2R	68	7.8	5.0	23.0%
Bevacizumab+Erlotinib	Kindler[2008]	2R	71	7.2	5.1	18.0%
Bevacizumab	Kindler[2010]	3R	535 T	5.7	4.8	13.1%
Bevacizumab+Erlotinib	Van Cutsem	3R	607 T	7.1	4.6	13.5%
Cetuximab	Xiong	2S	41	7.1	3.8	12.2%
Cetuximab+Oxaliplatin	Kullman	2S	64	7.0	3.9*	33.0%
Cetuximab+Cisplatin	Cascinu	2R	84 T	7.5	3.4	17.5%
Cetuximab	Philip	3R	745 T	6.3	3.4	12.0%
Erlotinib	Moore	3R	569 T	6.2	3.8	8.6%
Erlotinib+Capecitabine	Boeck	3R	299 T	6.9	2.4	5.0%

Table 1.3. Investigative efficacy history of novel agents explored in phase III in combination with gemcitabine. Type : 2S= Single-arm phase II trial, 2R= Randomized phase II trial, 3= Randomised phase III trial. T=Total number of patients in randomised trials, OS=Median Overall Survival (months) PFS=Progression Free Survival (months), *= Time to progression. ORR=Objective response rate.

1.6 Omega-3 fatty acids and fish oil

Omega-3 fatty acids (n-3FAs) are a family of unsaturated fatty acids that have in common a first carbon-carbon double bond as the third carbon-carbon bond from the terminal methyl end of the carbon chain. Important n-3 polyunsaturated fatty acids involved in human nutrition are α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids have 3,5 or 6 double bonds in a carbon chain of 18,20 or 22 carbon atoms respectively (figure 1.3). The capacity of human metabolism to derive EPA and DHA by the desaturation of α-linolenic acid is negligible. Furthermore, this synthesis of longer chain, n-3FAs from linolenic acid is competitively slowed by omega-6 analogues (figure 1.4).
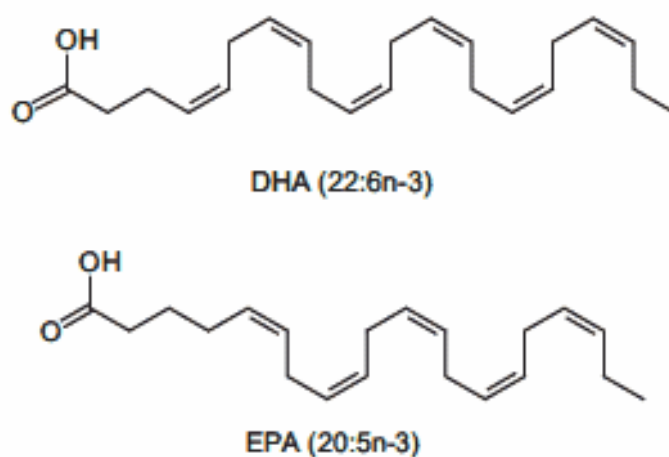
Figure 1.3. Chemical structure of DHA and EPA.

Omega-6	Enzymes	Omega-3
Linoleic Acid (LA) 18:2		α-Linolenic acid (ALA) 18:3
▽	6-Desaturase	▽
Gamma-linoleic acid (GLA) 18:3		Stearidonic acid (SRA) 18:4
▽	Elongase	▽
Dihomo-gamma-linolenic acid (DGLA) 20:3		Eicosatetraenoic acid (ETA) 20:4
▽	5-Desaturase	▽
Arachidonic acid (ARA) 20:4		Eicosapentaenoic acid (EPA) 20:5
▽	Elongase	▽
Adrenic acid (DTA) 22:4		Docosapentanoic acid (DPA) 22:5
▽	3 steps	▽
Docosapentanoic acid (DPA) 22:5		Decosahexaenoic acid (DHA) 22:6

Figure 1.4. Pathway of in vivo conversion of LA and ALA to other polyunsaturated fatty acids.

Therefore their concentration in tissues is enhanced when they are directly ingested or when competing amounts of omega-6 fatty acids are relatively small. N-3FAs are synthesised in abundance by algae and plankton. By consuming these small organisms, fish build up large concentrations of n-3FAs in their tissues and become the main dietary source of essential n-3FAs in humans. In particular cold-water oily fish such as mackerel, salmon, herring, anchovies and sardines provide relatively large amounts of n-3FAs compared to other fish.

1.6.1 Anti-inflammatory actions of n-3FAs

N-3FAs can exert profound anti-inflammatory effects both in vitro and in vivo (Schmocker et al. 2007, Sierra et al. 2006). These are mediated mainly by the

immunomodulating effects of the metabolised products of the eicasanoids EPA and DHA which are preferentially incorporated into the cell wall lipid bilayer. EPA and DHA metabolites produced by the action of cyclo-oxygenase 2 (COX-2) such as leukotrienes and thromboxanes are far less pro-inflammatory than the metabolites of arachadonic acid derivates from an omega-6 substrates (figure 1.5). DHA metabolism in particular has been closely studied, and while the exact mechanisms of its anti-inflammatory properties are poorly understood, several clues have been suggested. DHA is highly susceptible to peroxidation which results in a production of bioactive lipid species such as the compound cyclopentenone neuroprostanes, which are highly reactive and similar in structure to anti-inflammatory prostaglandins. However synthetic cyclopentenone neuroprostanes suppress lipopolysaccharide-induced expression of inducible nitric-oxide synthase and COX-2 in macrophages as well as Nuclear Factor Kappa Beta (NFKB) activation (Musiek et al. 2008). EPA and DHA also competitively inhibit the formation of the omega-6 fatty acid derived pro-inflammatory mediators such as LTB4 and prostaglandin E2 (PGE2). N-3FAs can also form potent anti-inflammatory mediators such as resolvins and protectins; these mediators attenuate neutrophil migration and enhance removal (Serhan, Chiang 2008). These actions together suppress the production of pro-inflammatory cytokines and enzymes (Kang, Weylandt 2008, Singer et al. 2008). N-3FAs also significantly lower nitric oxide (NO) production in lipopolysaccharide stimulated macrophages in vitro through altered inducible nitric oxide synthatase (NOS) protein expression (Aldridge et al. 2008). This mechanism of NOS inhibition is independent of COX-2 derived PGE2 (Razzak et al. 2008). Oral EPA and DHA consumption results in downregulation of gene expression from peripheral blood mononuclear cells involved in pro-inflammatory pathways such as nuclear transcription factor kappaB (NFKB)

(Bouwens et al. 2009). Diets with higher omega 3/omega 6 ratios are have also been shown to increase the concentration of EPA and DHA in erythrocyte cell membranes and reduce the production of pro-inflammatory mediators such as PGE1, LTB4, IL-1, IL-6 and granulocyte colony-stimulating factor (GCSF) from stimulated neutrophils (Schubert et al. 2007, Weaver et al. 2009, Vedin et al. 2008). In rats with experimentally induced pancreatitis who have endogenously increased tissue levels of n-3FAs, lung neutrophil infiltration and circulating plasma IL-6 levels were shown to be significantly reduced (Weylandt et al. 2008).
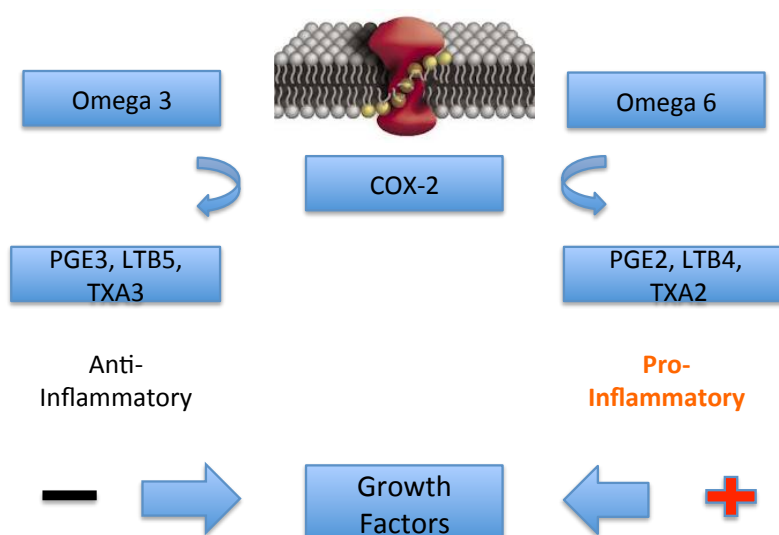
Figure 1.5. Mechanism of anti-inflammatory action of n-3FAs. Cell-membrane n-3FA is converted to Prostaglandin E3 (PGE3), Leukotriene B5 (LTB5) and Thromboxane A3 (TXA3) series which are far less inflammatory than their Omega-6 family lipid metabolite counterparts : Prostagladin E2 (PGE2), Leukotriene B4 (LTB4) and Thromboxane A2 (TXA2) series. These metabolites then have an inhibitory (Omega-3 stream) or activating downstream effect on growth factors.

It has been shown that septic patients given a total parenteral nutrition (TPN) regimen rich in n-3FAs have higher levels of LTB5 and enhanced neutrophil function (Morlion et al. 1996, Mayer et al. 2003b, Mayer et al. 2003a). This may also be translated into shorter intensive care unit stays and lower rates of severe infection in postoperative surgical patients given omega-3 fish oil emulsions (Weiss et al. 2002). Patients with severe acute pancreatitis given TPN rich in n-3FAs have fewer respiratory and renal complications (Wang et al. 2008). The optimum ratio of omega-3 to omega-6 fatty acids in lipid emulsion to achieve maximal anti-inflammatory effects while minimising immunosuppression is not yet known, but is probably in the region of 1:2 to 1:4 (Grimm et al. 1994, Grimm, Kraus 2001).

1.7 Potential applications of omega-3 fish oils in the palliative treatment of advanced pancreatic cancer

Patients with advanced pancreatic cancer commonly experience profound tumour-related cachexia, which is mediated by highly potent pro-inflammatory cytokines such as TNFα, IL-1,6 and 8. Novel therapies which may alleviate this distressing symptom as well as potentially provide enhanced anti-cancer activity are of particular interest. N-3FAs may provide a multi-facetted approach to therapy which through anti-inflammatory action could potentially alleviate tumour cachexia, and through anti-angiogenic action could potentially effect stabilisation of growth or regression of tumour cells. As previously described, anti-angiogenic strategies have been widely investigated in advanced pancreatic cancer. Their failure to date may be in part due to the fact that only a single molecule or receptor is targeted (such as VEGF or EGF). By contrast, the attraction of using agents which target the relevant mediator cascades at

source and therefore attenuate a whole host of downstream metabolites lies in the potential to overcome the problem of redundancies built in to the inflammatory systems overwhelming the effect of putative therapeutic intervention.

1.7.1 Laboratory studies of omega-3 in pancreatic cancer models

Both DHA and EHA have been shown to have beneficial effects on pancreatic adenocarcinoma cell lines in vitro. They inhibit growth of human pancreatic adenocarcinoma cell lines in a dose dependant manner (Falconer, Ross & Fearon 1994, Lai et al. 1996, Ravichandran, Cooper & Johnson 2000). They also induce apoptosis of the same cells also in a dose dependant manner (Lai et al. 1996, Merendino et al. 2003, Merendino et al. 2005, Shitara et al. 2008, Zhang et al. 2007). They have been shown to inhibit proliferation in gemcitabine-resistant cell lines irrespective of the level of gemcitabine resistance (Hering et al. 2007). There are various mechanisms postulated for this action including induction of apoptosis, cell cycle arrest, intracellular glutathionine depletion, downregulation of cyclin E, and inhibition of NFκB expression (Merendino et al. 2005, Dekoj et al. 2007). In rat models given azaserine to induce neoplastic pancreatic lesions, a diet with a high omega-3 to omega-6 ratio of fatty acids decreased the development of pre-neoplastic atypical acinar cell nodules (O'Connor et al. 1989). A different model using N-nitrosobis-2-oxypropylamine to induce ductal pancreatic adenocarcinoma in rats found that a group fed with a diet rich in omega-3 only had significantly lower incidence of macroscopic tumours and liver metastases compared to the groups fed on a diet rich in omega- 6 alone, or omega 3,6 and 9 together (Heukamp et al. 2006, Gregor et al. 2006). More recently, the incidence, frequency and proliferative index

of pre-neoplastic pancreatic lesions in an experimental rat model was shown to be reduced in the cohort fed on a high omega-3 fat diet (Strouch et al. 2011). Omega-6 has been shown to stimulate the development of pancreatic carcinoma in xenograft models through the increased production of COX-2 generated prostaglandin E2, whereas in the same model omega 3 was shown to reduce development of pancreatic carcinoma through reversal of the prostaglandin E2:E3 ratio (Funahashi et al. 2008).

Rapidly growing tumours require new blood vessel formation or angiogenesis in order to initiate and sustain proliferation. Angiogenesis is dependent on many different growth factors, in particular vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Omega-3 fatty acids suppress VEGF-stimulated cell proliferation, migration and tube formation during angiogenesis (Yang, Morita & Murota 1998, Tsuzuki et al. 2007a, Tsui, Murota & Morita 2003). Omega-3 fatty acids inhibit the production of PDGF-like protein from vascular endothelial cells and inhibit vascular smooth muscle proliferation by interfering with the PDGF signalling pathway (Terano, Shiina & Tamura 1996). In addition, angiogenesis is critically dependent upon the production of nitric oxide and the action of cyclo-oxygenase-2 (COX-2). N-3FAs inhibit nitric oxide production and nitric oxide synthase in vitro as well as in animal models (Boutard et al. 1994, Jeyarajah et al. 1999, Ohata et al. 1997). Several recent studies have shown that n-3FAs combined with COX-2 inhibitors inhibit growth in experimental cancer cell lines and xenograft models (Naravanan, Naravanan & Reddy 2005, Reddy et al. 2005).

Furthermore, n-3FAs have been shown to potentiate the effects of gemcitabine chemotherapy on human cancer cell lines. Postulated mechanisms for this action include up-regulation of cytotoxic transporters and initiation of oxidative stress processes. These studies are summarised in table 1.4.

Study (Year)	Model used	Parameters Measured + Outcome
Falconer (1996)	PC cell lines + different fatty acids	Reduction in cell numbers, viability and proliferation with EPA.
Lai (1996)	PC cell lines + EPA	Decrease in cell count and viability mediated by cell cycle arrest and apoptosis with EPA
Ravichandran (2000)	PC cell lines/mouse Xenograft + EPA + GLA	Growth inhibition in cell lines with EPA+GLA but no effect in xenograft model.
Merendino (2003, 2005)	PC cell lines + Butyric acid, DHA or ALA	Reduced cell growth and induction of apoptosis, probably by glutathione depletion, with DHA.
Shirota (2005)	PC cell lines + EPA	Dose-dependent inhibition of proliferation + induction of apoptosis with EPA
Zhang (2007)	PC cell lines +EPA or DHA	Growth and proliferation inhibition with induction of apoptosis with EPA or DHA.
Hering (2007)	PC cell lines + n-3FA / n-6FA +- gemcitabine	Inhibition of proliferation in n-3FA group regardless of gemcitabine. Inhibition of NF-kB activation and restoration of apoptosis in gemcitabine resistant cells with n-3FA.
Dekoj (2007)	PC cell lines + n-3FA or n-6FA	Cell cycle arrest and induction of apoptosis with n-3FA.
O'Connor (1989)	Rats treated with azaserine to induce preneoplastic pancreatic lesions + different n3:n6 FA ratios	Decreased development of pre-neoplastic lesions with increased n3-FA component.
Heukamp (2006)	Hamsters treated with BOP to induce PC + n-6FA rich diet followed by n-3 or n-3,-6 and -9FA rich diet.	Decreased incidence of liver metastases in n-3FA group.
Gregor (2006)		Decreased incidence of macroscopically visible pancreatic tumours in n-3FA, although microscopic appearances are no different to other fatty acid groups.
Strouch (2009)	EL-Kras mice + n-3FA rich or standard diet PC cell lines + DHA	Incidence, frequency and proliferation index of pancreatic precancer reduced with n-3FA rich diet. Decrease in cell line proliferation and induction of apoptosis with DHA.
Funahashi (2008)	PC cell lines + n-3FA or n-6FA. Mice injected with PC cells + n-3 or n-6FA rich diet.	Decreased cell growth with n-3FA. Decrease in growth in mouse model with n-3FA rich diet.
Yang (1998)	Bovine endothelial cell lines treated with VEGF + n-3 or n-6FA	Suppression of VEGF-induced proliferation with EPA.
Tsuzuki (2007a)	Human umbilical vein cells treated with VEGF + EPA	Inhibition of VEGF-stimulated migration and tube formation. Reduction in MMP2 and MMP9.
Tsui (2003)	Bovine endothelial cell lines treated with VEGF + EPA or Docosapentaenoic acid (DPA) or DHA.	Inhibition of VEGF-stimulated migration and tube formation and VEGF-receptor 2 expression strongest with DPA compared with DHA or EPA.
Fox (1988)	Vascular endothelial cells + EPA	Inhibition of PDGF production with EPA.

Table 1.4. Preclinical studies using omega-3 in neoplastic and proliferative cell lines and xenograft models.

PC=Pancreatic cancer, GLA=Gamma-linolenic acid, NF-kB=Nuclear factor kappa beta, BOP= N-nitrosobis(2-oxopropyl)amine, VEGF=Vascular endothelial growth factor, MMP=Matrix metalloproteinase, COX=Cyclo-oxygenase.

Terano (1996)	Vascular smooth muscle cells + EPA or DHA	Inhibition of vascular smooth muscle proliferation through inhibition of PDGF signal transduction with EPA greater than DHA.
Boutard (1994)	Macrophages isolated from rats fed n-3FA rich or deficient diets.	Nitric Oxide production in response to Lipopolysaccharide reduced by n-3FA rich diet.
Jeyarajah (1999)	Mouse macrophages (treated with IFN-gamma or TNF-a) + DHA	DHA inhibitory to Nitric Oxide production at all doses of IFN-gamma or TNF-alpha.
Ohata (1997)	Mouse macrophage cell line treated with lipopolysaccharide + n-3, n-6, n-9FA or stearic acid.	Inhibition of Nitric Oxide production with n-3FA in contrast to no inhibition with any other FA regimen.
Narayanan (2005)	Prostate cancer cell lines + DHA +- Celexoxib (COX-2 inhibitor)	Inhibition of growth and induction of apoptosis greatest with low dose combination of DHA + Celecoxib.
Reddy (2005)	Colon cancer rat model + Celecoxib + n-3FA rich or deficient diet.	Inhibition of tumour incidence in multiplicity in n-3FA rich diet with low dose celecoxib.

Table 1.4. Continued.

1.7.2 Human studies into effects on tumour-related cachexia and quality of life

It has been suggested for 20 years that n-3FAs may be useful in the alleviation of tumour-related cachexia (Tisdale, Dhesi 1990). In particular, most studies have been performed on patients with pancreatic and upper gastrointestinal tract cancers, although there is some data showing benefit in patients with other solid cancers (Jatoi et al. 2004). Barber showed that patients with pancreatic cancer given approximately 2g of EPA and 1g of DHA for 7 weeks showed significant weight gain and improvement in functional status and appetite, in both one single and two double armed non-randomised studies comprising 72 patients and 12 controls (Barber et al. 1999b, Barber et al. 1999a, Barber et al. 2000). It was also shown that high doses (up to 18g) of EPA were well tolerated but with greater side effects such as pain, steatorrhoea and nausea (Barber, Fearon 2001, Burns et al. 1999). Burns went on to

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show 66% weight stabilisation and 17% weight gain in the 22 patients he enrolled in a single armed study with the best quality of life scores in the patients with weight gain (Burns et al. 1999). Wigmore showed significant weight gain, with a mean of 0.3kg/month in pancreatic cancer patients given fish oil for 3 months as well as stabilisation of resting energy expenditure by indirect calorimetry (Wigmore et al. 1996). They went on to examine an escalating dose of EPA from 1g/day for 4 weeks to 6g/day for 12 weeks. This study showed a weight gain of 0.5kg at 1 month which remained stable at 12 weeks (Wigmore et al. 2000). The best quality and largest study in pancreatic cancer patients to date is from Fearon who randomised 200 patients to receive 2.2g EPA per day or placebo. They noted weight and lean tissue gain in the EPA group as well as improved Quality of Life scores (Fearon et al. 2003). Bruera however noted no difference in weight, functional status or well being in their randomised controlled trial comprising 60 patients given either DHA and EPA or olive oil, although it should be noted that this group had tumours of diverse anatomical origin (Bruera et al. 2003). Kenler studied 35 patients with surgically operated upper gastrointestinal malignancies and noted a significant reduction in gastrointestinal complications of distension, diarrhoea and nausea with a significant decrease in the need for TPN, and improvement in liver and renal function in the EPA/DHA group (Kenler et al. 1996). Moses found a significant increase in total resting energy expenditure and physical activity level in the patients to whom they gave EPA for 8 weeks (Moses et al. 2004). In summary, there does seem to be at least some evidence to show a beneficial relationship of omega-3 fish oils in the alleviation of tumour related cachexia and improving QoL scores. There is limited evidence on the optimal dose: these studies all used oral supplementation, and although most showing benefit used a dose greater than 1.5g/day, with some showing

improved results in the 1.5-4.0g/day range (Colomer et al. 2007). In addition many of these studies reported problems with patient compliance in taking the oral omega-3 preparations, mainly due to the large number of tablets or volume of liquid that was required to achieve the desired dose. These studies are summarised in table 1.5.

1.7.3 Clinical applications of parenteral omega-3 fish oils

Many studies have reported beneficial immunomodulatory and nutritional effect of omega-3 containing lipid emulsions as part of total parenteral nutrition (Calder et al. 2010). So far, few have examined the use of n-3FA emulsions independently in the treatment of inflammatory conditions and there are no published case series or controlled trials of intravenous n-3FA preparations in the adjuvant treatment of cancers. However, animal models as discussed previously using n-3FA preparations do support the potential utility of omega-3 emulsions in the adjuvant treatment of human pancreatic adenocarcinoma. Notwithstanding the potential direct tumour effects and potential response for patients undergoing chemotherapy for unresectable pancreatic adenocarcinoma there is a reasonable body of evidence that quality of life scores and tumour cachexia may be improved.

High strength oral preparations are available, as previously mentioned, with EPA purity of up to 95% containing up to 18g of EPA in 100 mL of emulsion (Barber, Fearon 2001). However data concerning the oral bioavailability of EPA and DHA is limited and there is no published data comparing oral and intravenous bioavailability (Dyerberg et al. 2010). Intravenous preparations containing 10g of omega-3 triglycerides per 500mL are commercially available.

The safety of high-dose n-3FA parenteral emulsions is well established when it has been used as a component of total parenteral nutrition, but further studies would be required to establish its tolerability and efficacy as a combination therapy in conjunction with gemcitabine chemotherapy for the treatment of advanced pancreatic cancer.

The effective palliative treatment of patients with advanced pancreatic cancer has undergone very little advancement in terms of improving overall survival since gemcitabine chemotherapy was first introduced 16 years ago (Casper et al. 1994). Novel agents which can prolong survival, improve quality of life and alleviate cachexia in patients with advanced pancreatic cancer are currently unavailable. Putative adjuvant therapies including parenteral omega-3 emulsions have the potential to address all of these outcome targets and have the additional benefit of proven safety and tolerability albeit in a different study population. The marginal benefits on tumour cachexia and quality of life shown in trials using oral omega-3 supplementation may warrant further investigation with parenteral preparations as compliance and maintenance of optimal dosing should be easier to achieve. Clinical trials to investigate omega-3 emulsions in combination with gemcitabine in patients with advanced pancreatic cancer are clearly warranted. Even if there is no demonstrated anti-neoplastic activity, an improvement in cachexia and quality of life could result in n-3FA emulsions becoming part of standard care in this challenging patient group.

Study (Year)	EPA /DHA (g) per day	Duration of Treatment (Weeks)	Number of patients in EPA/DHA group and diagnosis	Outcome
Barber (1999)	2.2 / 0.9	7	20 (PC)	Significant wt gain, PS and appetite at 3 weeks and wt gain at 7 weeks.
Barber (1999)	2.0 / 1.0	3	18 (PC+ supplement) + 18 (PC-supplement) + 6 (Healthy Controls)	Weight gain in treatment group with stabilisation of acute phase protein response.
Barber (2000)	2.2 / 0.96	3	16 (PC) + 6 (Healthy Controls)	Increase in body weight of PC patients and normalization of metabolic response
Barber (2001)	18 EPA	4	5 (PC)	Can be tolerated by PC patients.
Burns (1999)	Up to 7.9/5.2	4 (median)	22 (Mixed cancers)	High dose well tolerated.
Wigmore (1996)	2.16/1.44 (median)	12	18 (PC)	Weight gain and reduction in acute phase protein response.
Wigmore (2000)	5.7 EPA	12	26 (PC)	Well tolerated + stabilised weight.
Fearon (2003)	1.54 EPA	8	95 (PC+supplement) 105 (PC+control)	Weight gain + improved QOL if taken at intended dose (2.2g/day)
Bruera (2003)	1.8 / 1.2 (median)	2	Mixed Cancer : 30 (treatment) + 30 (placebo)	No significant difference in any parameter
Kenler (1996)	3.27 / 1.48 (median)	1	17 (treatment)+18 (control -surgery patients)	Reduction in infections and complications in fish oil group.
Moses (2004)	2.09 EPA (median)	8	9 (PC+treatment) + 15 (PC+control)	Increase in physical activity may be related to QoL.

Table 1.5. Clinical studies in patients with pancreatic cancer treated with omega-3 rich oral preparations. PC=Pancreatic Cancer, PS=Performance status, QoL=Quality of life.

1.8 Quality of life in advanced pancreatic cancer

Quality of life (QoL) is extremely important to advanced pancreatic cancer (APC)

patients and is significantly reduced when using validated questionnaires compared to

normal individuals (Müller-Nordhorn et al. 2006). Self-reported quality of life

outcomes by patients rather than an attempt at objective assessment by health

professionals, offer a more accurate measurement of quality of life, as there can often

be discordance between patients and health professionals (Fitzsimmons et al. 1999a).

Measurement of quality of life can be difficult, but several tools have been developed

to aid the investigator in its evaluation. These include the EORTC QLQ-C30 and its

pancreatic cancer supplementary module the PAN-26(Fitzsimmons et al. 1999b).

QOL measures are probably under-reported in clinical trials, and their significance in

comparison with traditional endpoints such as overall survival, progression free

survival or objective tumour response rates is often deemed to be minor. Weight

stabilisation is a very important component of stabilisation or improvement in quality

of life, and is also correlated with improved survival in some studies (Davidson et al.

2004, Gupta, Lis & Grutsch 2006). Interpretation of QoL questionnaire scores can be

difficult. An increase with respect to baseline of 10% is probably clinically

significant, whereas an increase of 20% is probably highly clinically significant

(Osoba et al. 1988). It is fifteen years since the first study to demonstrate improved

quality of life in patients with advanced pancreatic cancer undergoing palliative

gemcitabine chemotherapy compared to the current standard of care at the time, 5-

Fluorouracil chemotherapy (Burris et al. 1997).In this study, the primary efficacy

measure was clinical benefit response (CBR), defined by improvement in any of

Karnofsky performance status, pain scores or weight gain sustained for greater than 4

weeks without decline in either of the other parameters. It was shown that 23.8% of

gemcitabine treated patients versus 4.8% of 5-FU treated patients experienced CBR. This was an intial benchmark figure by which subsequent single-arm clinical trials evaluating quality of life compared their results to.

Phase III randomised clinical trials incorporating quality of life measures as one of the outcome measures are scarce. Van Cutsem examined gemcitabine with the farnesyltransferase inhibitor tipifarnib or placebo in patients with APC (Van Cutsem et al. 2004). Quality of life was assessed using performance status and the FACTPa questionnaire. Differences between the two groups were not significant. Rocha-Lima examined gemcitabine with irinotecan or placebo in APC patients (Rocha Lima et al. 2004). FACT-Hep questionnaires, bodyweight and performance status were used as measures of QoL. No differences in quality of life were demonstrated. Heinemann et al examined gemcitabine with or without cisplatin in patients with APC (Heinemann et al. 2006). The measure used was that according to Spitzer (Spitzer et al. 1981). This is an out of date and very superficial measurement tool for QoL which only utilised five questions and was never validated. No difference in QoL between the two arms was demonstrated. Bernhard et al used the definition of CBR used by Burris along with a linear analogue scale quality of life questionnaire examining seven different variables to evaluate differences in quality of life between patients undergoing gemcitabine plus capecitabine versus gemcitabine alone chemotherapy for APC (Bernhard et al. 2008). There was no significant difference in CBR or QoL between these two groups in this study (20% versus 19%). Cunningham et al examined the same treatment combination but used the EORTC QLQ-C30 questionnaire to assess QOL; again there were no differences in QoL between the two arms (Cunningham et al. 2009). Moinpour examined pain and emotional well-being in patients treated with gemcitabine with or without cetuximab for APC (Moinpour et al. 2010). There were

no significant differences between the treatment arms. Bonnetain examined time until deterioration in QoL scores, using the EORTC QLQ C30 questionnaire in patients undergoing treatment with 5FU, folinic acid, cisplatin and gemcitabine either in that order or in reverse order, but showed no difference in time to QoL deterioration between the two arms (Bonnetain et al. 2010). Conroy in their landmark study of the first chemotherapeutic regimen (oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) to show improved overall survival over a gemcitabine-based regimen examined quality of life using QLQ-C30 (Conroy et al. 2011). While they did not show any significant differences between the arms in terms of change in quality of life scores during treatment, they noted that at the end of 6 months treatment, 31% of the FOLFIRINOX group had a definite decline in quality of life scores compared to 66% in the gemcitabine group. Loehrer used the FACT-Hep questionnaire to examine quality of life differences between patients undergoing radiotherapy in addition to single-agent gemcitabine chemotherapy for locally advanced pancreatic cancer and noted no significant differences between the two groups (Loehrer et al. 2011). Romanus used the Euroqol EQ5-D instrument to assess QoL in APC patients treated with gemcitabine plus bevacizumab or placebo (Romanus et al. 2012). They specifically examined the relationship between QoL and objective response rate to chemotherapy and failed to show any relationship concluding that in this group, chemotherapy did not improve health-related QoL even in those who had a response to their treatment. Colucci used Burris's definition of CBR in combination with the EORTC QLQ-C30 and PAN-26 questionnaires to assess QoL in APC patients treated with gemcitabine with or without cisplatin (Colucci et al. 2010).There was no significant difference in CBR (23%, gemcitabine, 15.1%, gemcitabine plus cisplatin) or global health QoL scores (mean increase of 6.2% in global health over baseline)

between the two groups although subgroup analysis of the QoL questionnaires did reveal statistically significant differences in social functioning and limitation in planning, favouring the single agent gemcitabine group and in hepatic symptoms favouring the gemcitabine plus cisplatin group. Reni compared QoL in patients having treatment with cisplatin, epirubicin, 5-fluorouracil and gemcitabine combination chemotherapy versus gemcitabine alone (Reni et al. 2006). They used the EORTC QLQC-30 and PAN26 questionnaires as well and noted significant improvement as defined by a score improvement of greater than 10% in several domains, favouring the combination chemotherapy. In particular a 10% increase in global health scores was recorded in 55% of the experimental group compared with 29% of the gemcitabine group. They also noted that patients experiencing partial response on CT evaluation of tumour dimensions also had improved quality of life in many domains over those with stable disease.

Tumour cachexia is a major component of declining QoL in APC patients and is thought to be driven by pro-inflammatory cytokines such as Interleukin 1-Beta, 6,8 and tumour necrosis factor alpha (Fearon et al. 2013). Compounds which can reduce the production of these cytokines are therefore of interest in potentially tackling tumour cachexia and in turn improving QoL.

Clinical trials of oral omega-3 rich lipid preparations have demonstrated improved quality of life and amelioration of tumour cachexia in patients with a range of malignancies. These preparations have proven anti-inflammatory and anti-neoplastic effects both in vitro and in vivo (Arshad et al. 2011b). When presented to cells, omega-3 is preferentially incorporated into the cell membrane over the omega-6 lipid family: the action of cyclo-oxygenase 2 on these omega-3 related membrane lipids creates downstream metabolites such as leukotriene B5 and prostaglandin E3 which

are far less pro-inflammatory than their omega-6 related metabolites. It is these properties that are of interest in cancer therapy and the potential action of these compounds in improving quality of life. More dramatic results for oral trials of omega-3 rich compounds were potentially limited by the limited bioavailability and tolerability of high dose oral lipids.

1.9 Pro-inflammatory cytokines and pro-angiogenic growth factors in pancreatic cancer

There is a clear association between angiogenesis and the development of most human solid tumours, evidenced by data showing increased serum concentrations of circulating pro-angiogenic factors (CAF) such as VEGF, PDGF, TGF-B, EGF and FGF in these patients (Ugurel et al. 2001, Kwon et al. 2010, Tsushima et al. 2001, Rahbari et al. 2011a). VEGF inhibition as an anti-angiogenic strategy for treatment of solid tumours gained particular interest due to overexpression and its correlation with poor outcome (Poon, Fan & Wong 2001, Poon et al. 2003). This was reinforced by improved outcome versus standard treatment in late phase randomized clinical trials using agents which target receptors for these factors, such as bevacizumab (VEGF-a, colorectal and lung), cetuximab (EGF, colorectal, head and neck cancer) and erlotinib (EGF, lung cancer). However, when applied to pancreatic cancer in randomized clinical trials, most of these strategies have proven to have no clinical benefit (Philip et al. 2010, Kindler et al. 2010). Only erlotinib was shown to have an overall survival benefit, and although statistically significant this has not translated into widespread use as the clinical difference was marginal at best (10 days overall survival improvement over gemcitabine alone) (Moore et al. 2007). This may be due to the

fact that pancreatic cancers are not highly vascular tumours, and that they usually

have a dense stromal reaction around the tumour which may protect neoplastic cells

from targeted agents. Studies examining changes in pro-angiogenic cytokines and

growth factors in APC patients have shown significantly increased expression of

PDGF, VEGF and EGF compared to healthy controls (Rahbari et al. 2011b). High

concentrations of VEGF have been shown to be related to poor outcome in studies of

patients with APC (Seo et al. 2000, Niedergethmann et al. 2002, Chang et al. 2008).

The role of PDGF in neoplasia is less clear. PDGF-BB stimulation may enhance

invasiveness in pre-clinical cell line models (Singh et al. 2007). There may be a

synergistic role for PDGF and VEGF in tumourigenesis, with PDGF blockade

potentiating the anti-neoplastic action of VEGF blockade in cell lines (Shen et al.

2007). PDGF expression is correlated with poor clinical outcome in gastric cancer

and osteosarcoma patients (Katano et al. 1998, Sulzbacher et al. 2003).

Omega-3 fatty acids (n-3FAs) have been shown in pre-clinical experiments and

clinical trials to be able to modulate CAF and therefore have anti-angiogenic potential

(Spencer et al. 2009, Arshad et al. 2011b). They are rapidly incorporated into cell

membrane phospholipid bilayers by competition with omega-6 fatty acids. Cyclo-

oxygenase-2 acting on n-3FAs produces metabolites which are far less pro-

inflammatory and pro-angiogenic than their n-6FA related counterparts. These

metabolites downregulate transcription of pro-angiogenic growth factors. N-3FAs

have been shown to reduce expression of PDGF both in vivo and in randomized

clinical trials using healthy volunteers (Baumann et al. 1999).

1.10 The complement cascade and pancreatic cancer

The complement system is a component of human immunity consisting of over 25 proteins and protein fragments which when activated have four basic functions: opsonisation, chemotaxis, cell lysis and clumping of antigen-bearing agents. There are three distinct pathways by which complement can be activated: the alternative pathway (AP), classical pathway (CP) and mannose binding lectin pathway (MBL).

The association of complement with cancer is well established. The link is certainly more complex than the early simplistic view that chronic inflammation associated with complement activation was responsible for neoplastic proliferation (figure 1.6). In particular, the recent discovery of the association of myeloid derived suppressor cells (MDSC) with complement activation and the subsequent blockade of anti-tumour immunity is potentially crucial and may explain some of the associations of complement activity with clinical outcomes (Markiewski et al. 2008).

Figure 1.6. Potential mechanisms of action of omega-6 related metabolites to activate MDSC and complement pathways and block anti-tumour immunity (Ostrand-Rosenberg 2008- reproduced with permission).

The MBL pathway in particular has a proven strong link with carcinogenesis: the early assumption that MBL-related proteins in particular might have anti-carcinogenic properties is probably over-simplistic (Nakagawa et al. 2003). MBL activity has been shown to be increased in patients with patients with colorectal cancer and paediatric solid malignancy patients compared to healthy volunteers (Fisch et al. 2011, Ytting et al. 2004). High concentrations of MBL associated serine protease-2 (MASP-2), a component of the MBL pathway, can be found in paediatric patients with haematological malignancies, CNS malignancies and are associated with poor survival and recurrence amongst colorectal cancer patients(Fisch et al. 2011, Ytting et al. 2005, Ytting et al. 2008). High cellular expression of MASP-2 can also be found in oesophageal and ovarian cancer patients' epithelial cells (Verma et al. 2006, Swierzko et al. 2007). Conversely in paediatric patients with haematological

malignancy, especially lymphoma, higher MASP-2 serum concentrations are significantly associated with improved outcome in terms of event free survival (Zehnder et al. 2009). The mechanisms at work to explain the relationships observed between cancer patients outcomes and MBL and MASP-2 concentrations are unclear.

There is little reported in the literature on the association of complement with pancreatic cancer specifically. It has been suggested that plasma concentration of soluble iC3b, which is generated after binding of autoantibodies to tumour cells and subsequent inactivation of the complement cascade could be correlated with radiological recurrence or development of pancreatic cancer (Märten et al. 2010). One of the possible mechanisms of explaining this effect is that iC3b released from cells undergoing apoptosis binds to dendritic cells preventing their maturity which in turn induces immunological tolerance (Schmidt et al. 2006). Certainly there is evidence on mass-spectrometry of advanced pancreatic cancer patients sera that concentrations of complement 3C are elevated compared to healthy volunteers sera (Hanas et al. 2008).

Production of pro-inflammatory cytokines such as interleukin 1-β, 6,8 and TNF-alpha may activate complement pathways and MDSC directly which may block anti-tumour immunity. However there is currently no known mechanism by which omega-3 fatty acids may affect the MBL pathway directly other than by manipulating pro-inflammatory cytokines responsible for its activation.

1.11 Omega-3 fatty acid uptake into cells and plasma

Studies of uptake of n-3FAs have almost exclusively used gas chromatography to assess changes in percentage composition and ratios of omega 3 out of the total fatty

acids in membrane phospholipids of plasma, white blood cells (WBC) and red blood cells (RBC).

1.11.1 Principles of Gas Chromatography

Gas chromatography (GC) is a well-established technique for analysis of lipids which are either volatile or can be made volatile by modification of chemical groups. The major limitation of GC is that complex lipids cannot be studied intact and so potentially important information regarding the combinations of fatty acids in these molecules is lost. However, provided the data are interpreted carefully, this technique is sufficient for most nutritional studies.

The main principle underlying the separation of fatty acids by GC is that they differ in the temperature at which they become volatile. This depends upon carbon chain length, number and position of double bonds. Increasing chain length increases the temperature at which fatty acids enter the vapour phase. In contrast, the greater the number of double bonds, the lower the boiling point. In addition to the effects of temperature, differences in the interaction between fatty acids and the lining of the GC column are also used to separate fatty acids. Although some GC columns are capable of separating underivatised fatty acids, this requires high temperatures and separation of very long chain fatty acids is often poor. Typically, a methyl group is added to the carboxylic end of the fatty acid to form a fatty acid methyl ester (FAME) by reaction with a methyl donor such as methanol, in the presence of a catalyst, for example sulphuric acid, sodium methoxide or boron trifluoride. This lowers the boiling point that, in turn, allows separations of a wide range of fatty acid at moderate temperatures.

The GC apparatus consists of a heated injection port, a fused silica capillary column located within a high efficiency oven and a detector (figure 1.7). FAMEs injected into the injection port of the gas chromatographer are rapidly heated to $250 - 300°C$ and so become volatile. The FAMEs are carried into the capillary column by a stream of helium (or hydrogen in some applications). The column is held at a lower temperature than the injection port, and FAMEs rapidly condense on the column lining. Very volatile substances, such as the hexane used to dissolve the FAMEs prior to injection, do not condense. The column is then heated and FAMEs dissociate from the column lining as their boiling point is reached. After entering the vapour phase FAMEs then transiently interact with the column lining. The strength of this interaction is determined by chemical properties of the FAME including the number of double bonds. The longer the column, the more interactions occur and the greater the resolution of FAMEs. Different column linings will cause differences in the order in which fatty acids elute.

The end of the column is located within the flame ionisation detector, held at about $250°C$. The hydrogen flame causes combustion of the FAMEs and thus generates an ion current proportional to the amount of FAME in the sample.

The resulting chromatogram contains a series of peaks, each corresponding to a FAME. The area under each peak is proportional to the mass of the FAME injected onto the column (figure 1.8).
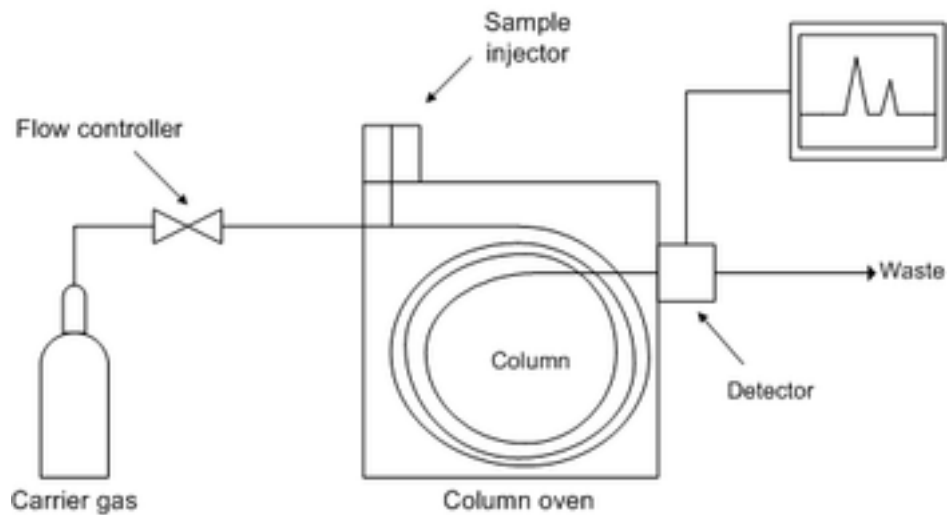
Figure 1.7. Schematic diagram of gas chromatography apparatus.
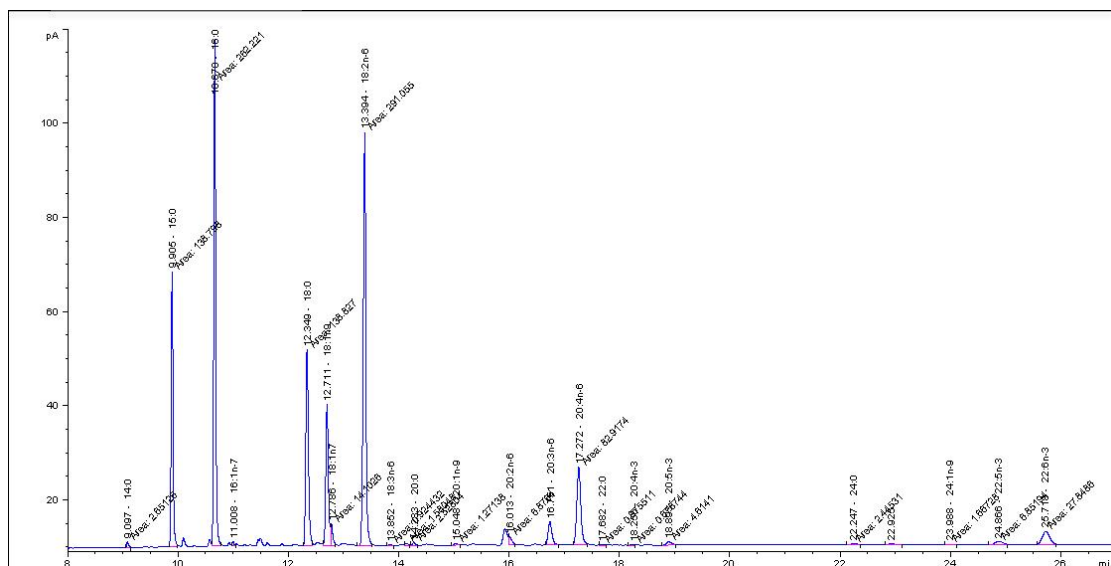
Figure 1.8. Typical chromatogram produced by gas chromatography of plasma derived FAMEs. X axis = time (minutes). Y axis = signal current (picoAmps). Peaks on the x axis correspond to individual FAMEs, the relative proportions of the concentration of each FAME present are equal to the relative proportions of the areas under each peak.

1.11.2 Uptake of oral n-3FA rich preparations

Faber gave 12 healthy subjects 2.4g of EPA and 1.2g of DHA orally each day for 1 week to investigate short term uptake of omega-3 fatty acids into plasma, RBC and

WBC phospholipids (Faber et al. 2011). After 1 day of nutritional supplementation, the percentage of EPA of total fatty acids in phospholipids of WBC increased from 0.5% at baseline to 1.3% ($P < 0.001$). After 7 days, the percentage of EPA rose to 2.8% ($P < 0.001$, compared with baseline). No effect was observed on the percentage of DHA in phospholipids of WBC after 7 days of nutritional supplementation, whereas the percentage of docosapentaenoic acid (DPA) increased within 7 days ($P < 0.001$). The percentage of arachidonic acid (AA) was reduced from day 2 onwards ($P < 0.001$). After 1 day of nutritional supplementation, the percentage of n-3 fatty acids increased from 5.6% at baseline to 6.8% ($P < 0.001$) and at day 7, to 9.4% ($P < 0.001$). Correspondingly, the percentage of n-6 fatty acids in phospholipids of WBC decreased after 7 days ($P < 0.001$), as did the ratio of n-6/n3 fatty acids. In RBC, the percentage of EPA increased after 1 day of nutritional supplementation and increased further to 7 days ($P < 0.001$). Furthermore, in RBC, there was an increase in the percentage of DHA. DPA showed a small but significant increase after 7 days of nutritional intervention ($P < 0.001$). The n-6 fatty acid AA demonstrated a reduction at day 2 ($P = 0.001$), although at days 4 and 7, no significant differences could be detected. The percentage of n-3 fatty acids increased and the percentage of n-6 fatty acids and the ratio of n-6/n-3 fatty acids decreased within 1 day of nutritional intervention ($P \leq 0.009$). Rapid incorporation of EPA in particular even 12 hours after a fish-oil rich meal has been demonstrated (Gibney, Daly 1994). Other studies of oral fish oil administration in healthy volunteers have shown similar results with the ability to increase both plasma phospholipid and erythrocyte cell membrane EPA, DHA and DPA content over several weeks (Vidgren et al. 1997). However, EPA concentrations may plateau after 2 weeks of oral administration (Gibney, Hunter 1993).

1.11.3 Uptake of parenteral n-3FA rich preparations

Pittet studied the effect on platelet phospholipid and membrane incorporation of a bolus infusion of 3 different doses of n-3FA rich fish oil in groups of healthy volunteers (Pittet et al. 2010). In the plasma phospholipids, the percentages of EPA, DHA, and DPA all increased from baseline at all time points between 2 and 24 hours later ($P < 0.001$). AA demonstrated a significant reduction only at day 7 ($P < 0.001$). The percentage of n-3FA increased and the percentage of n-6 fatty acids and the ratio of n-6/n-3FA decreased within 1 day of nutritional intervention (all $P < 0.001$). They observed that a single intravenous infusion of fish oil significantly increased EPA content in cell membranes. There was a direct relation between the dose of fish oil and the platelet membrane incorporation. They suggested a much quicker incorporation of n-3FA into phospholipids using a parenteral rather than an enteral route. Simoens gave 8 healthy volunteers a 5 hour a day infusion over 4 days of 50:50%, LCT and MCT (n-6FA rich) lipid emulsion and then after a 6 week break, a 5 hour a day, 4 day infusion of a 50:40:10% MCT:LCT:Fish oil (n-3FA rich) lipid emulsion to investigate uptake in cells (Simoens et al. 2008). The infusion of the n-3FA rich emulsion resulted in a rapid (within hours) and substantial incorporation of EPA in membrane phospholipids of platelets and leukocytes. A marked enrichment (2–3-fold) was already observed after the first infusion and remained largely present on day 2 after more than 16 hours of lipid-free interval. The EPA content was increased 7-fold in platelets and more than 2-fold in leukocytes after 4 consecutive infusions of the n-3FA rich emulsion. In contrast, the DHA content was not raised in blood cell membrane phospholipids, even after 4 consecutive infusions. Although the DHA concentration in the n-3FA rich emulsion (13%) was half that of EPA (26%), one would expect a proportional DHA enrichment in platelet and white blood cell

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phospholipids if the uptake and cellular metabolism of both fatty acid groups were similar.   The effect of an n-3FA rich versus standard MCT/LCT emulsion over a 5-day infusion was examined by Senkal in 40 patients undergoing colorectal surgery requiring parenteral nutrition (Senkal et al. 2007). The n-3FA rich group had significantly increased levels of EPA in erythrocyte cell membranes expressed as a percentage of fatty acid methyl esters (FAME) using gas chromatography (2.0% versus 0.8% on day 6 and 2.0% versus 0.5% on day 10) than the standard MCT/LCT group. EPA in serum phospholipids showed similar significant increases in the n-3FA group (7.0% versus 1.3% on day 6 and 3.6% versus 1.0% on day 10). DHA levels in serum phospholipids also showed significant increases over the MCT/LCT group (11.8% versus 8.4% on day 6 and 11.2% versus 8.5% on day 10).

2 Aims

To investigate the combination of gemcitabine and intravenous n-3FA rich lipid emulsion in patients with locally advanced and metastatic pancreatic cancer. To assess radiological response rates, survival, quality of life and adverse event outcomes. To assess changes in CAF and complement activity and correlate these with clinical outcomes. To assess n-3FA uptake into plasma and cell membranes with treatment.

The primary aim of investigation is to elucidate if n-3FAs can synergistically improve the action of gemcitabine to stabilise tumour growth, and hence improve survival and by its whole host of other postulated mechanisms outlined previously, attenuate the pro-inflammatory cascade to the extent that it improve quality of life. The clinical outcomes must be related to biochemical changes in terms of reduction in measured cytokine expression. Finally proof of principle that n-3FAs are incorporated into cell membranes over the course of treatment to effect these changes is crucial. The study is important as no agent investigated to date has so far proved effective in these measures.

2.1 Hypothesis

The null hypothesis is that the combination of gemcitabine and intravenous n-3FA rich lipid emulsion will have no effect on response rates, survival and quality of life over that seen with single-agent gemcitabine. These changes will not be associated with reduction in CAF or modulation of complement pathways. Uptake of n-3FAs into plasma phospholipids and cell membranes will not occur and be no different from baseline n-3FA levels in these mediums.

3 Methods

3.1 Study design

 The study was conceived as a phase II, single arm, single-centre study of gemcitabine plus parenteral omega-3 in patients with chemotherapy-naïve advanced pancreatic cancer. Patients had radiological tumour assessments performed every 8 weeks as they would have done with standard single-agent gemcitabine.

 The power calculation was based on Simon's two-stage design to test the null hypothesis that the response rate would be <10% versus >25% (Simon 1989) and twenty-one patients were planned to be enrolled in the first stage. If two or fewer patients have an objective response, the trial would be terminated for lack of efficacy. Otherwise, an additional 29 patients would be enrolled, to a total of 50 patients. This design would yield at least a 0.90 probability of a positive result if the true response rate is at least 25% and at least 0.90 probability of a negative result if the true response rate is at most 10%. The first stage of recruitment is reported in this thesis.

 A single-arm phase II design was employed to give a reasonable number of patients who could be recruited and evaluated over a 2 year period as a preliminary study to answer the clinical questions posed prior to embarking on larger randomised trials.

3.1.1 Primary outcome measure

 Objective response rate (both partial and complete response) on contrast-enhanced CT scan of the abdomen assessed by RECIST v1.1 criteria by an independent

experienced radiologist. This was a well-recognised endpoint for single-arm phase II trials, widely used in other trials.

3.1.2 Secondary outcome measures

- Overall survival, progression free survival, and duration of response.

- Safety and tolerability of gemcitabine plus parenteral n-3FAs.

- Health-related quality of life, pain ratings, and health status of patients as measured by the European Organisation for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) questionnaire, and Brief pain inventory short form (BPI-sf)

- Uptake analysis by measuring omega-3/6 fatty acid levels and ratios in plasma phospholipids and cellular membranes of erythrocytes.

- Changes in CAF with time and treatment outcome

- Changes in complement activity with time and treatment outcome

3.1.3 Patient Inclusion Criteria

Patients with histologically confirmed pancreatic adenocarcinoma, in whom the disease is assessed as unresectable by the HPB MDT in Leicester, either due to metastatic or locally advanced disease, and deemed suitable to receive gemcitabine chemotherapy were eligible for the study. Essentially the only criteria barring inclusion into the study, where the patient would have been suitable for gemcitabine chemotherapy were related to prior chemotherapy, prior malignant disease hyperlipidaemia and adverse reaction to Lipidem components. This was to ensure

minimum bias in interpreting clinical results and obviously to ensure safety of the patients undergoing trial treatment. The inclusion criteria were as follows:

- Aged >18 years

- Able to give informed written consent

- ECOG performance status of 0 or 1 (Appendix 1)

- Life expectancy >12 weeks

- Adequate hepatic and renal function documented within 14 days prior to treatment

    - AST and ALT ≤2.5x upper limit of normal (ULN), unless liver metastases present, in which case ≤5.0xULN

    - Total bilirubin ≤1.5xULN

    - Serum creatinine ≤1.5xULN or calculated creatinine clearance ≥60mL/min

    - Urinary protein <1+ by urine dipstick. If ≥1+, then 24-hour urine collection was done and could then only be enrolled if urine protein was <2g/24hours

- Adequate bone marrow function

    - Haemoglobin ≥9g/dL (could have transfusion or growth factors)

    - Platelets ≥100,000cells/mm^3

    - Neutrophil count ≥1500cells/mm^3

- No significant hyperlipidaemia

- No severe blood coagulation disorders (anticoagulants were allowed)

- Women of childbearing age had a negative pregnancy test (urine or serum) at commencement of treatment

- All patients were willing to comply with scheduled visits, treatment, laboratory test, and other aspects of the trial

3.1.4 Patient exclusion criteria

Patients were excluded from this trial if they had:

- Prior treatment with any systemic chemotherapy for metastatic disease

- Prior adjuvant radio- or chemotherapy within 4 weeks of starting the study

- Previous treatment with gemcitabine

- Hypersensitivity to fish-, egg-, or soy protein, or to any of the active substances or constituents in the lipid emulsion

- Any general contra-indications to infusion therapy – pulmonary oedema, hyper hydration, decompensated cardiac insufficiency

- Any unstable medical conditions – uncontrolled diabetes mellitus, acute myocardial infarction, stroke, embolic disease, metabolic acidosis, sepsis, pancreatitis

- Known HIV or AIDS

- Dementia or significantly altered mental status that would prohibit the understanding or rendering of informed consent and compliance with requirements of the protocol

- History of malignancy other than pancreatic 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

- Major surgical procedure or significant traumatic injury within 4 weeks of treatment

- Female patients were either surgically sterilised or postmenopausal or agree to use two adequate contraception measures during the period of therapy which would be continued for 6 months after the last dose of gemcitabine. Male patients were either surgically sterilised or agree to use adequate contraception for the same period.

- Patients deemed unsuitable for gemcitabine chemotherapy

- Patients taking oral fish oil supplements were not excluded

3.2 Trial treatments

3.2.1 Administration

- Study treatment was administered in cycles of 4-week duration

- Individual dose adjustments of gemcitabine were made on the basis of the adverse events observed as per current clinical practice

- Treatment was continued until progression of disease, unmanageable adverse events, death, and withdrawal of patient consent or completion of six cycles.

3.2.2 Gemcitabine

- Treatment consisted of gemcitabine ($1000mg/m^2$) administered as a 30-minute intravenous infusion once weekly for 3 weeks, followed by one week of rest from gemcitabine treatment. Preparation and administration precautions were contained in the product labelling for gemcitabine and are as per standard clinical practice.

- All gemcitabine dosing was determined by the patient's BSA as calculated from actual weight and height. Dose banding was permitted according to standard hospital practice.

- Prefilled bags and dose banding were allowed as per local practice.

- Gemcitabine reductions below dose level 3 was not allowed.

- If gemcitabine was withheld for 4 weeks, then it was discontinued.

- Standard dose-modifications for gemcitabine were used.

3.2.3 Gemcitabine dose modification for haematological toxicity

Gemcitabine doses were modified for myelosuppression based on blood counts taken within 1 day before the planned dosing day as per current clinical practice. Gemcitabine doses held on Day 8 or 15 of a cycle were not be made up at a later date. If gemcitabine was held, the Lipidem was continued. If gemcitabine was discontinued, the Lipidem was also discontinued.

3.2.4 Gemcitabine dose modification for febrile neutropenia

If febrile neutropenia (defined as 1 reading of oral temperature >38.5ºC or 3 readings of oral temperature >38ºC in a 24-hour period concomitant with Absolute Neutrophil Count<500/mm^3) developed in a given cycle, dose reductions were allowed. Colony stimulating factors could be started at the discretion of the investigator until resolution of febrile neutropenia or septic episode. Growth factors were not allowed to be substituted in lieu of dose reduction.

Gemcitabine and Lipidem dosing were held during febrile neutropenia. Doses missed on Days 8 or 15 of therapy were not made up. After neutropenia resolved, gemcitabine was resumed at one lower dose than the previous cycle administered. This gemcitabine dose (or lower) was used for all subsequent cycles. Lipidem was resumed as before, when neutropenia is resolved.

3.2.5 Gemcitabine dose modification for toxicity

Dose modifications were allowed for hepatic toxicity at anytime during treatment as indicated in the protocol.

For grade 3 and 4 toxicities, gemcitabine treatment was withheld until the toxicity resolved to grade 1 or less, then reinstituted (if medically appropriate) at one lower dose level. Gemcitabine doses that were held on day 8 or 15 were not be made up at a later date. If gemcitabine treatment was withheld for longer than 4 weeks, gemcitabine and Lipidem were discontinued.

One patient, G05, had gemcitabine held for two weeks while undergoing surgical treatment for gastric outlet obstruction, but Lipidem was continued throughout this time as this was not a treatment toxicity. Doses reduced for drug-related toxicity were not re-escalated.

3.2.6 Lipidem

- Lipidem (200mg/mL) was supplied by BBraun, Melsungen.
- Lipidem was infused via a peripheral line or Peripherally inserted central catheter (PICC line)

- The patients were allowed to eat and drink normally between infusions

- The starting dose of Lipidem infusion was 500mL of Lipidem 200mg/mL over 4 hours (25g of triglyceride/hour)

- This dose was reduced in the interests of patient safety at the discretion of the investigator if the full dose was poorly tolerated.

- Infusions were administered immediately following gemcitabine chemotherapy on days 1,8 and 15 of each cycle with a rest week in keeping with the gemcitabine dosing regimen.

3.2.7 Monitoring during Lipidem infusion

Patients were carefully monitored for any signs or symptoms or anaphylactic reaction. If present the infusion was immediately interrupted. The patient had hourly observations taken (temperature, pulse rate, blood pressure). The patients were also carefully monitored for signs of fat overload syndrome.

Potential risks of parenteral fish-oil infusion were:

- Anaphylactic/hypersensitivity reactions (1/1000-1/10000)

- Metabolic acidosis (risk reduced by concomitant infusion of carbohydrates and amino acids)

- Hypoglycaemia and other metabolic disturbances associated with TPN

- Fat overload syndrome

- Hyper- and hypotension (1/1000-1/10000)

- Dyspnoea (1/1000-1/10000)

- Lack of appetite (1/100-1/1000)

- Nausea and vomiting (1/100-1/1000)

- Priapism (<1/10000)

3.2.8 Fat Overload Syndrome

This is caused by impaired capacity to eliminate triglycerides which may be caused by an overdose. There are various causes such as genetic predisposition, renal impairment and sepsis.

It is characterised by hyperlipidaemia, fever, fat infiltration, hepatomegaly with or without jaundice, splenomegaly, anaemia, leukopenia, thrombocytopenia, coagulopathy, haemolysis and reticulocytosis, abnormal LFTs and coma. The symptoms are usually reversible is the infusion of fat emulsion is discontinued.

3.2.9 Drug storage and drug accountability

The hospital clinical trials pharmacist ensured that all study drugs were stored in a secure area, under recommended storage conditions and in accordance with applicable regulatory requirements.

To ensure adequate records, all Lipidem was accounted for in the case report form and drug accountability inventory forms.

3.2.10 Concomitant medications and therapies

Palliative and supportive care for disease-related symptoms, including pain management was offered to all patients on this trial as per standard clinical practice. Patients who developed obstructive jaundice were offered endoscopic biliary stenting within 48 hours. Patients who developed gastric outflow obstruction were offered expedient surgical gastroenterostomy if thought to be clinically appropriate. Low dose oral steroids (defined as less than 5mg per day of prednisolone or equivalent) or topical or inhaled steroids at any dose were allowed to be taken during the study. No other chemotherapy, hormonal therapy, radiotherapy, or experimental anticancer medications were permitted while the patient is on study. Any disease progression requiring other forms of specific anticancer therapy was cause for discontinuation from study treatment.

3.3 Trial procedures

3.3.1 Screening

All patients being considered for the study and eligible for screening signed an informed consent form for the study prior to any study specific procedures. The required screening assessments and laboratory tests are summarised in the schedule of tests and procedures (table 6). Following completion of the pre-treatment assessments and confirmation of eligibility, patients were registered.

A complete physical examination was performed including the assessment of all body systems, the measurement of body weight, height, ECG, pulse, and assessment

of ECOG performance status (see Appendix 1). Body surface area (BSA) was determined for gemcitabine dosing. If a patient had ≥10% change in weight, BSA was recalculated. All examinations were performed by qualified health care professionals. Findings of all physical examinations were recorded in the source documents, and any change from baseline considered by the investigator to be clinically significant was recorded as an adverse event in the case report form.

3.3.2 Trial period

Patients received treatment with gemcitabine and Lipidem as outpatients. . Additional assessments were performed as necessary to evaluate specific adverse events until they resolved to baseline or CTCAE Grade ≤1.

3.3.3 Follow-up visit

The primary reason for a patient's discontinuation of the study medication was clearly documented on the case report form. A final safety assessment was done, where possible no sooner than 28 days after the last dose of gemcitabine or Lipidem (whichever is later). If a patient had died or was too unwell to attend clinic at this time, this was recorded.

Adverse events that are serious, suspected to be related to Lipidem, or considered significant by the investigator, were followed after the time of therapy discontinuation until the event of its sequelae resolved or stabilised at a level acceptable to the investigator. Each serious adverse event was reported to the hospital Research and Development department.

3.3.4 Subject Withdrawal

Subjects could withdraw from the trial at any time at their own request, or could be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioural, or administrative reasons.

Observation	Screening Day -14 to 0	Day 1 of each cycle	Days 8 and 15 of each cycle (predose)	Day 1, 8 and 15 each cycle (postdose)	Follow-up 28 days after last dose
Informed consent	X				
Medical history	X				
Physical examination	X	X			X
Weight, temperature, blood pressure, pulse	X	X	X		X
ECOG performance status (Appendix 1)	X	X			X
12-lead Electrocardiogram	X				X
Serum lipids, haematology, liver function, renal function, glucose, coagulation screen	X	X	X	X	X
Urinalysis (and pregnancy test for female patients)	Day -3 to 0	X			X
Tumour assessment	Day -28 to 0	Every 8 weeks			X
Serum CA19.9		Every 8 weeks			X
Health-related quality of life questionnaire (QLQ-C30; QLQ-PAN26) and health status (EQ-5D)	X	X	X		X
Cytokine, complement and uptake blood samples		X	X	X	
Gemcitabine treatment		X	X		
Lipidem infusion		X	X		
Survival	Continuously monitored				

Table 3.1. Schedule of tests and procedures. X=assessment or procedure carried out at that time. If blank then was not carried out.

3.4 Trial assessments

3.4.1 Efficacy assessments

All baseline radiological tumour assessments were performed as close as possible to but not more than 28 days before start of trial. CT of the chest, abdomen and pelvis were always performed at baseline, all subsequent assessments and at follow-up assessment. At baseline, tumour lesions were categorised as target or non-target. All patients were evaluated for response according to RECIST v1.1.

The same method and technique were used to characterise each identified and reported lesion at baseline and during the study treatment period. Tumour evaluation by positron emission tomography (PET) scan or ultrasound was not allowed to substitute CT scans.

Radiological tumour assessments were performed at screening, every 8 weeks during the study and whenever disease progression was suspected.

All patients were continuously followed up until death. Survival duration was defined as the interval between first study treatment and death. Progression free survival duration was defined as the interval between first study treatment and first occurring event of radiologically demonstrated progression or death. Kaplan-Meir survival curves for progression free-survival and overall survival were constructed using Graphpad software.

3.4.2 Historical controls

In order to provide an estimate of efficacy, a historical cohort of patients undergoing single-agent gemcitabine chemotherapy for advanced pancreatic cancer was evaluated. These were all consecutive patients with evaluable disease by RECIST criteria attending for single-agent gemcitabine chemotherapy in the 12 months prior to study commencement. Their baseline characteristics and outcome data can be found in chapter 4. Statistical analyses comparing study data with historical data was performed using Graphpad software.

3.5 Assessment of response by CT imaging using RECIST criteria (Response Evaluation Criteria In Solid Tumours)

At baseline, tumour lesions were characterised as measurable or non-measurable (defined below)

3.5.1 Eligibility

- Only patients with measurable disease at baseline were included.

- Measurable disease: the presence of at least one measurable lesion.

- Measurable lesions: lesions that can be accurately measured in at least one dimension with longest diameter ≥ 20 mm using conventional techniques or ≥ 10 mm with spiral CT scan.

- Non-measurable lesions: all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, ascites, pleural/pericardial effusion, lymphangitis cutis/pulmonitis, cystic lesions, and also abdominal masses that were not confirmed and followed by imaging techniques.

- All measurements were taken and recorded in metric notation using electronic measurement. All baseline evaluations were performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of treatment.

- The same method of assessment and the same technique was used to characterize each identified and reported lesion at baseline and during follow-up.

- Clinical lesions were only considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes).

3.5.2 Methods of measurement

- CT was used to assess measurable disease as this is the best currently available and reproducible method to measure target lesions selected for response assessment. Spiral CT was performed using a 5 mm contiguous reconstruction algorithm.

- All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs were identified as target lesions and recorded and measured at baseline.

- Target lesions were selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements.

- A sum of the longest diameter (LD) for all target lesions was calculated and reported as the baseline sum LD. The baseline sum LD was used as reference by which to characterize the objective tumour response.

- All other lesions (or sites of disease) were identified as non-target lesions and were also recorded at baseline. Measurement of these lesions was not required, but the presence or absence of each was noted throughout follow-up.

The following RECIST criteria were the primary method utilised in this study for the assessment and reporting of tumour response data.

* Complete Response (CR): Disappearance of all target lesions and no appearance of new lesions. Each had to be documented on two separate occasions separated by at least 4 weeks. Disappearance of all non-target lesions and normalization of tumour marker level.

* Partial Response (PR): At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD.

* Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

* Stable Disease (SD):   Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started. Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.

3.5.3 Evaluation of best overall response

The best overall response was the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started- table 3.2). In general, the patient's best response assignment depended on the achievement of both measurement and confirmation criteria.

Determination of Best Overall Response			
Target lesions	**Non-Target lesions**	**New Lesions**	**Overall response**
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Table 3.2. Determination of best overall response by RECIST criteria. CR= Complete Response, PR= Partial Response, SD= Stable Disease, PD= Progressive Disease.

3.5.4 Reporting of results

All patients included in the study were assessed for response to treatment. Each patient was assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 8) unknown (not assessable, insufficient data).

3.6 Blood samples

3.6.1 Routine laboratory blood samples

The following haematological and biochemical tests were performed as described in Table 3.1: Full blood count and coagulation screen, urea, creatinine, sodium (Na^+),

potassium (K^+), bicarbonate (HCO_3^-), alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, bilirubin, cholesterol, triglycerides and glucose.

CA19.9 was measured in all patients at baseline and every 8 weeks.

3.6.2 Pharmacokinetic, cytokine evaluation and complement blood samples

Blood samples for pharmacokinetic analysis of omega-3/6 fatty acid components in cellular membranes of blood erythrocytes, changes in serum levels of circulating pro-inflammatory and pro-angiogenic factors and components of the complement pathways were taken at time points as set out in table 3.1. These samples were collected immediately prior to and after treatment each week and transferred on crushed ice to a laboratory for processing as soon as possible after venepuncture and in any case within 30 minutes. One serum gel and one heparinised blood collection tube were used to collect whole blood, and these centrifuged at 1000G for 15 minutes at 4°C in order to produce 4 250uL aliquots of serum and plasma respectively. This was stored at -80°C until analysis. Erythrocyte cell membrane pellets for uptake analysis were produced by cell lysis using serial dilutions of phosphate buffered saline with centrifugation at 10,000 RPM for 10 minutes in between. The final pellet was then suspended in 10mmol PBS and frozen using liquid nitrogen before storage at -80°C until analysis.

3.7 Cytokine quantification by multiplex array

Whole blood was taken from the patients as described above and transferred to a serum gel tube which was centrifuged within 30 minutes at 1000G for 15 minutes at 4°C. The serum was transferred to eppendorf tubes and stored at -80°C. At the time

of analysis, the serum was thawed and subject to cytokine concentration quantification using a chemiluminescant multiplex ELISA array (Aushon biosystems) in the following manner (Moody et al 2001).

The following pro-inflammatory and pro-angiogenic cytokines and growth factors were evaluated in the multiplex array: IL-1 beta, TNF-alpha, IL-6, IL-8, IFNγ, VEGF-a, VEGF-c, VEGF-d, TRAIL, RANKL, PDGF, HGF, FGF, EGF.

The serum was thawed on ice and pipetted into the plate in duplicate. The serum samples were either run neat, in a 1:2 dilution or a 1:4 dilution depending on the expected concentration of factors to be detected and the dynamic range of the array. The extent of dilution required for each plate was assessed by the author using trial plates, before deciding upon the correct dilution to bring sample concentrations into the capable dynamic range of array detection.   The standards supplied with the kit representing known concentrations of each analyte were made up in duplicate and the appropriate dilutions transferred to the plate. Once all the wells were filled with standard (first 16 wells) or serum samples (next 80 wells), the plate was gently agitated for 1 hour using an automated plate shaker. The plate was then thoroughly washed manually using the Aushon custom wash, and biotinylated antibody added to each well. This was then agitated again for 30 minutes and washed manually 3 times. Streptavadin-HRP conjugate was then added to each well and the plate agitated for 30 minutes and washed 3 times. Finally a luminal-based substrate was added and the plate read within 2 minutes in the custom-built cooled CCD camera image detector. The Aushon Searchlight software was used to capture and analyse the image to provide a concentration in each well of each analyte compared to the standards. The concentrations were then entered into an excel spreadsheet to provide data on changes with treatment and time for each patient. The changes over time in the logarithms of

the concentrations of each factor were modelled using a random co-efficients model fitted using xtmixed in STATA software (Rabe-Hasketh, Skrondal 2005). This model fits a linear regression in which both the intercept and the slope are allowed to vary randomly between individuals. Kaplan-Meir survival curves were constructed using Graphpad software to analyse overall and progression-free survival relationships with baseline cytokine concentrations and response in cytokines with treatment.

3.8 Complement quantification by ELISA

Stored serum was collected, stored and then thawed at time of analysis as described above. Serum was diluted 1/101 with custom diluent and left at room temperature for 15 minutes. 100μL per well of positive control, negative control, blank (diluent) and diluted patients serum was pipetted into the 96-well plate. This was then incubated at 60-70 minutes at 37°C. After serum incubation, the wells were emptied and washed 3 times with 300μL of custom washing solution, filling and emptying the wells each time. 100μL of conjugate was added to each well and the plate incubated for 30 minutes at room temperature. The plates were washed 3 times and 100μL of substrate solution added to each well. The plate was incubated for 30 minutes at room temperature and the absorbance read at 405nm on a microplate reader (Roos et al. 2003, Fredikson et al. 1993). The results were calculated by subtracting the absorbance of the blank (diluent) from the negative control, positive control and the samples. These were used in a semi-quantitative way to calculate percentage complement activity as follows: (Sample-NC)/(PC-NC)x100. Changes in complement activity over time, restoration of complement activity to 100% from a low baseline

and its relationship to survival and progression-free survival outcomes were calculated using Graphpad software to construct Kaplan-Meir survival curves.

3.9 Gas chromatography for lipid uptake quantification

The author assisted with preparation and analysis of some samples for lipid uptake quantification, and performed statistical analysis of all sample data. All other samples were prepared and analysed by other research staff in the laboratory of nutritional immunology, Southampton University in the following manner. Total lipid extract was prepared from plasma. Plasma was thawed at room temperature and then mixed by vortexing and centrifuged at 13,000 RPM for 5 minutes to remove denatured protein. Following this, 100μL of plasma was added to 700uL of NaCl and transferred to a screw-cap glass tube. To this tube, 5 mL of chloroform:methanol (2:1 mix) was added containing BHT (50mg/l) antioxidant. NaCl 1mL was added and mixed thoroughly by vortexing. The tube was then centrifuged at 2000G for 10 minutes at room temperature. The lower phase was collected by aspiration with a Pasteur pipette and transferred to a screw-cap glass tube which was then dried under nitrogen at 40°C (Folch et al. 1957)

Total lipid extract was prepared form erythrocyte cell membrane pellets in exactly the same way as for plasma described above. Lipid classes were separated and purified using solid phase extraction. The SPE tank was connected to the vacuum pump and aminopropylsilica SPE cartridges placed on the on the tank. A screw-capped tube was placed under each cartridge to collect waste (Triacylgylerol (TAG) and Cholesterol Esters (CE) fractions). 2mL chloroform was added using a dispenser to each cartridge and allowed to drip through under gravity. The total lipid extract prepared earlier was dissolved in 1.0 mL dry chloroform, and vortex mixed. This

sample was then applied to the relevant column with a Pasteur pipette and allowed to drip through under gravity. When no drips fell, the remaining liquid was removed by vacuum. The column was washed with 2 x 1.0 mL dry chloroform under vacuum. These washes were discarded and new screw-capped glass tubes labeled PC placed under the cartridges. Phosphatidylcholine (PC) lipid classes were eluted by addition of 2.0 mL dry chloroform:methanol (60:40, v/v) under vacuum until dry. The tubes now containing PC were removed and dried under nitrogen at 40°C. To elute Phosphatidylethanolamine (PE) lipid classes, new glass tubes were placed under the same cartridges and the columns washed with 2.0 mL dry methanol under vacuum. This was then replaced with another screw capped glass tube, and NEFA eluted by adding 2.0 mL chloroform: methanol:glacial acetic acid (100 : 2 : 2, v/v/v) under vacuum until dry. This fraction was also dried under nitrogen at 40°C (Yao et al. 1985).

Preparation of fatty acid methyl esters was performed by adding 0.5mL of dry toluene to the purified lipid classes and vortex mixing. Methanol containing sulphuric acid was added drop wise, and then mixed by inversion. This mix was heated at 50°C for 2 hours and then allowed to cool. Neutralising solution and dry hexane was added and then vortex mixed. This was then centrifuged at 1000 RPM for 2 mins at room temperature. The upper phase containing the FAMEs was collected and transferred to a round bottom glass tube (Iverson, Sheppard 1975). This was dried under nitrogen at 40°C. 75μL of dry hexane was added and vortexed and then transferred to a GC autosampler vial before being injected into the gas chromatographer (Hewlett Packard 6890).

Chromatograms produced by the GC software (Agilent Chemstation) included peaks which corresponded to individual FAMEs. These were identified by analysing known

standards of FAME prior to experimental analysis, and thus the lipid structure corresponding to each position of each peak on the x axis of the chromatograph in the experimental output could be accurately identified. The area under each peak was quantified by accurate integration of peak areas, which was performed automatically by the chemstation software, and then each one checked manually to ensure the correct baseline and peaks had been analysed by the software. This area was proportional to the concentration of that particular FAME, and the relative areas under each peak equal to the relative proportions of each FAME present in the sample (Christie 1989). Analysis of the differences of FAME proportions for pre-treatment versus post-treatment samples each week were analysed for the first two cycles using Wilcoxon matched-pairs signed rank test. Analysis of changes in weekly pre-treatment FAME proportions over the entire treatment course for each patient were analysed using the same statistical model described previously as was used for the cytokine analysis.

3.10 Patient reported outcomes

 Patient-reported outcomes of health-related quality of life (HRQoL), pancreatic cancer specific symptoms, pain and health status were measured using the EORTC QLQ-C30/QLQ-PAN26, and Brief Pain Inventory-Short Form (BPI-sf) (Appendix 2) completed each treatment week.

 The EORTC QLQ-C30 consists of 30 questions that assess aspects of patient functioning (physical, emotional, role, cognitive, and social), symptoms (e.g. nausea and vomiting, pain, fatigue), and global health status (Aaronson et al. 1993). The QLQ-PAN26 consists of 26 questions relating to disease symptoms, treatment side effects, and emotional issues specific to pancreatic cancer. These include questions

on altered bowel habit, pain, dietary changes, disease and treatment-related

symptoms, and issues related to emotional and social well-being. The PAN26 is

developed for use in conjunction with the QLQ-C30 and not as a stand-alone

measure. The majority of questions are answered on the 4-point Likert scale ranging

from 1 'Not at All' to 4 'Very Much', with two questions being answered on a 7-point

scale, ranging from 1 'Very Poor' to 7 'Excellent'. The questionnaire takes

approximately 15-20 minutes to complete, has excellent psychometric properties and

has been translated into many languages.

The BPI-sf is an 11-item self-report questionnaire that is designed to assess the

severity of pain and the impact of pain on daily functions. The BFI-sf has

demonstrated excellent reliability and validity and has been translated into several

languages. At the core of the BFI-sf are 4 questions that assess pain intensity (worst,

least, average, right now) and 7 questions that assess the impact of pain on daily

function (general activity, mood, walking ability, normal work, relations with other

people, sleep, enjoyment of life). Each question is answered on a scale ranging from

0 'no pain' to 10 'pain as bad as you can imagine'. There is no summary score for all

the items in the questionnaire. The 4 pain intensity questions can be totalled to derive

an index of pain intensity and the 7 function questions can be totalled to derive an

index for pain interference. The measure can also be scored by item, with lower

scores being indicative of less pain or pain interference.

Patient-reported outcome assessments were performed at baseline (prior to first

cycle of therapy) and thereafter during each treatment

3.10.1 Quality of life questionnaire scoring

   Scoring of questionnaires was carried out following guidelines published by the European Organisation for Research and Treatment of Cancer (Fayers et al. 2001). The QLQ-C30 is composed of both multi-item scales and single-item measures. These include five functional scales, three symptom scales, a global health status / QoL scale, and six single items. Each of the multi-item scales includes a different set of items - no item occurs in more than one scale. All of the scales and single-item measures range in score from 0 to 100. A high scale score represents a higher response level. Thus a high score for a functional scale represents a high / healthy level of functioning; a high score for the global health status / QoL represents a high QoL, but a high score for a symptom scale / item represents a high level of symptomatology / problems.

The principle for scoring these scales is the same in all cases:

1. Estimate the average of the items that contribute to the scale; this is the raw score.

2. Use a linear transformation to standardise the raw score, so that scores range from 0 to 100; a higher score represents a higher ("better") level of functioning, or a higher ("worse") level of symptoms.

Technical Summary

In practical terms, if items I1 , I2, ... In are included in a scale, the procedure is as follows:

Raw score: Calculate the raw score

RawScore = RS = (I1+I2+... In)/n

Linear transformation: Apply the linear transformation to 0-100 to obtain the score ,S

Functional scales: $S = (1-((RS-1)/range)))\times100$

Symptom scales / items: $S = ((RS -1)/ range)\times100$

Global health status / QoL: $S =((RS -1)/range)\times100$

Range is the difference between the maximum possible value of RS and the minimum possible value. The QLQ-C30 has been designed so that all items in any scale take the same range of values. Therefore, the range of RS equals the range of the item values. Most items are scored 1 to 4, giving range = 3. The exceptions are the items contributing to the global health status / QoL, which are 7-point questions with range = 6, and the initial yes/no items on the earlier versions of the QLQ-C30 which have range = 1.

3.10.2 Interpretation of scores

As described previously, the raw QLQ-C30 scores can be transformed to scores ranging from 0 to 100. The use of these transformed scores has several advantages, but transformed scores may be difficult to interpret. Also, there are no grounds for regarding, say, an emotional function score of 60 as being equally good or bad as scores of 60 on the other functioning scales. However, there are a number of ways to ease the interpretation of QLQ-C30 results.

Changes in scores over time and differences between groups may be more difficult to interpret than absolute scores. The fact that a change is statistically significant does

not necessarily imply that it also has clinical significance. Lydick and Epstein (1993) reviewed the different approaches used to define the 'Minimal Clinically Important Difference' and grouped these into anchor-based and distribution-based interpretations.

Anchor-based interpretations compare the changes seen in QoL scores ('anchored') against other clinical changes or results. Examples of such approaches used with the QLQ-C30 include the Subjective Significance Questionnaire (SSQ) (Osoba et al., 1998). The SSQ asks patients about perceived changes in physical, emotional, and social functioning and in global QL, using a 7-point scale ranging from 'much worse' over 'no change' to 'much better'. Patients filled in the QLQ-C30 at two occasions. At the second completion they also filled in the SSQ. Patients who reported 'a little' change for better or worse on a particular scale (function or symptom) had QLQ-C30 changes about 5 to 10.

Those reporting 'moderate' change had changed about 10 to 20, and 'very much' change corresponded to a change greater than 20, hence the notion that a 10% increase in QLQ-C30 main domains such as global health is clinically significant whereas a 20% change is highly clinically significant.

3.10.3 Brief pain inventory scoring

Each of the 11 question items where 10 was a score indicating worse pain or functioning were added together to reach a score out of 110. The question assessing relief from symptoms of pain where 10 indicated complete relief was also scored. The scores were then subject to linear transformation in the same manner described

above as for the quality of life questionnaires in order to produce a score out of 100: where 100 was the best possible score reflecting least pain or best function and 0 was the worst.

3.11 Statistical methods

Response rate comparisons with historical controls were analysed using fisher's exact test. Overall and progression-free survival data was analysed with Kaplan-Meir curves with Gehan-Breslow-Wilcoxon test used. This was utilised in preference to the log-rank test because it gives more weight to early events which was typical of the disease pattern expected in these patient cohorts. Changes in cytokines were analysed using a statistical model in STATA software in which multiple logistic regression analysis lines were fitted which allowed random variation of slope and intercept between individuals. This was used as it was the best model which allowed for missing data, which was prevalent in the study due to heterogeneity in treatment course length as well as some patients missing treatments due to investigator or patient choice. Clinical outcomes were correlated with cytokine and complement changes using survival curves analysed with a log-rank test. In this situation the events contributing to the curves were expected to be spread out more evenly across the treatment course for both groups.

4 Clinical results

4.1 Recruitment

34 patients were screened from whom 29 patients were enrolled of which 27 patients had at least one trial treatment. The patients were predominantly male and late-middle aged with a high proportion of metastatic disease (table 4.1.1). Twenty-one patients were evaluable for response on RECIST CT. Eighty-four cycles of treatment were administered with 233 individual patient treatments in total. Median number of cycles of treatment administered per patient was 3.5 (table 4.1.2).

Sex- Male : Female	17:12
Median age (range)	66 years (40-73 years)
Locally advanced : metastatic	8:21
ECOG performance status 0:1	13:16
Raised Baseline CA19-9 (%)	25 (86%)

Table 4.1.1. Baseline characteristics of study group patients.

Total number of treatment cycles / total treatments	84 / 233
Median cycles per patient (range)	3.5 (0-6)

Table 4.1.2. Number of treatment cycles, total number of treatments and median cycles per patient.

Mean Lipidem dose administered was 73.6g, with mean baseline serum triglycerides of 1.4mmol/litre and mean triglycerides in serum isolated from blood taken within 10 minutes of infusion termination 12.2 mmol/litre (table 4.1.3).

Mean Lipidem dose administered (95% C.I.)	73.6g (65.8-81.4g)
Mean baseline serum triglycerides (95% C.I.)	1.4 mmol/litre (1.2-1.7)
Mean post infusion serum triglycerides	12.2 mmol/litre (10.1-14.2)

Table 4.1.3. Lipidem dose, baseline (pre-infusion) and post-infusion triglyceride data.

Dose reductions of Lipidem from the planned dose of 100g took place in all patients at some point during their treatment due to poor tolerability of the full dose or adverse events. The commonest reason for dose reduction was grade II nausea or belching followed by grade II-III chills (as described in section 3.8).

There was a clear linear relationship between immediate post-infusion serum triglyceride level , taken within 10 minutes of infusion termination and the lipidem dose by bodyweight in mL/kg (figure 4.1).
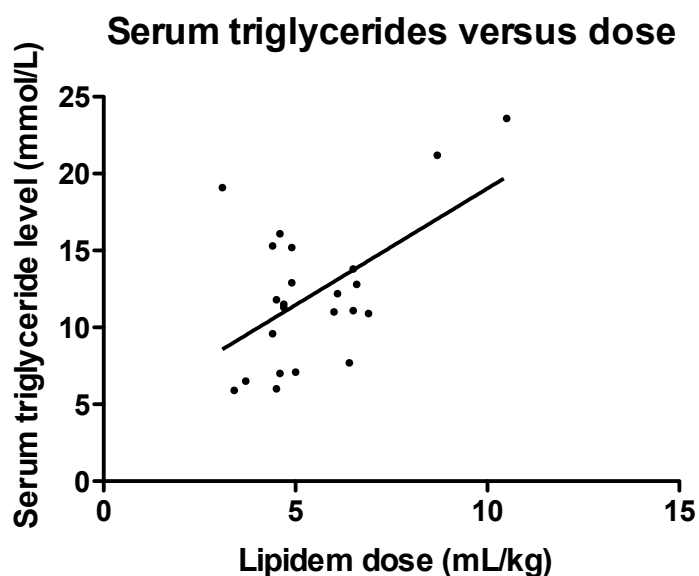

Figure 4.1. Relationship between serum triglyceride concentration and lipidem dose administered with bodyweight with best fit line (R^2=0.3, F=8.837, p=0.007).

4.2 Historical control cohort

As this was a single-arm trial, in order to provide an estimate of clinical efficacy, a historical control cohort was identified and analysed. This consisted of 26 consecutive patients recruited in the 12 months before the trial who had undergone palliative chemotherapy treatment for locally advanced or metastatic pancreatic cancer using single-agent gemcitabine at University Hospitals of Leicester. This cohort were more homogenous than the trial cohort, with a slight predisposition towards younger and female patients. A particular difference to the trial cohort was the equal proportion of locally advanced and metastatic disease (table 4.2.1). Of the patients identified, 17 were evaluable for response using RECIST criteria

Sex- Male : Female	12:14
Median age (range)	59 (39-75)
Locally advanced : metastatic	13:13

Table 4.2. Baseline characteristics of historical control cohort.

4.3 Radiological tumour response

The following tables illustrate best tumour response by maximum longitudinal dimension assessment using RECIST criteria.

Complete response was not observed in any patient. Partial response was seen in 3/21, stable disease in 13/21 and progressive disease in 5/21 patients. Disease control rate, which was a combination of complete response, partial response and stable disease was 16/21 patients (table 4.3).

	Proportion	Percent
Complete response (CR)	0/21	0
Partial response (PR)	3/21	14.3
Stable disease (SD)	13/21	61.9
Progressive disease (PD)	5/21	23.8
Disease control (CR+PR+SD)	16/21	85.7

Table 4.3. Experimental group overall response rates (gemcitabine plus Lipidem).

Baseline target lesion dimensions for both the primary pancreatic lesion (this was not measurable due to difficulties in recognising inflammatory and neoplastic components in one patient- G25) and any metastatic lesions, along with baseline serum CA19-9 levels (normal range 0-37) are shown in table 4.4.

Patient	Pancreas	Liver mets	Lung mets	Other	Total	CA19-9 (U/mL)
G01	4.0	1.5		1.5	7.0	906
G02	5.7	5.1		3.5	14.3	1612
G03	4.4	1.2			5.6	>10000
G04	4.0	9.7			13.7	X
G05	3.2	4.6	4.3		12.1	<3
G06	6.6				6.6	3411
G07	2.4				2.4	>10000
G08	3.1	4.2			7.3	>10000
G09	4.2	1.7		2.9	8.8	>10000
G10	2.5				2.5	1169
G11	3.6	6.5			10.1	68534
G12	3.0		5.9		8.9	773
G13	4.8	6.4	1.3		12.5	130701
G14	3.7			2.6	6.3	>99999
G15	3.5				3.5	1410
G16	2.4	4.2			6.6	37
G17	3.4	7.7			11.1	>99999
G18	3.9			1.7	6.5	26644
G19	7.5			1.4	8.9	14
G20	2.9				2.9	36
G21	3.3				3.3	2357
G22	5.0	5.6		2.6	13.2	>100000
G23	4.0				4.0	513
G24	4.7	2.2			6.9	9707
G25	X	6.8		2.6	9.4	>100000
G26	5.5	4.6		4.3	14.4	9349
G27	3.8			1.1	4.9	7195
G28	4.0				4.0	1696
G29	2.1			3.4	5.5	270

Table 4.4. Baseline target lesion dimensions (cm) and CA19-9 levels. U=Units.

Dimensions of all individual target lesions as well as total dimensions along with

CA19-9 levels after 2 cycles (table 4.5), 4 cycles (table 4.6) and 6 cycles (table 4.7) of

treatment are shown below for each patient (pt). Percentage change in dimensions of

liver metastases (LM) and total change along with response assigned by RECIST

criteria are also shown.

Pt	Pancreas	RECIST Liver mets	ONE Lung mets	Other	Total	CA19-9 (U/mL)	LM change (%)	Total change (%)	Response
G01	4.0	1.1		1.3	6.4	416	**-26.7**	**-8.6**	**SD**
G02	4.6	3.3		2.5	10.4	825	**-35.3**	**-27.3**	**PR**
G03	4.0	0.8			4.8	7659	**-33.4**	**-14.3**	**SD**
G05	2.9	3.1	3.7		9.7	8	**-32.6**	**-19.8**	**SD**
G06	6.0				6.0	494		**-9.1**	**SD**
G07	2.4				2.4	5210		**0.0**	**SD**
G08	3.7	7.7			11.4	120324	**+83.3**	**+56.2**	**PD**
G10	2.5				2.5	820		**0.0**	**SD**
G11	3.2	5.4			8.6	5118	**-16.9**	**-14.9**	**SD**
G12	2.8		5.9		8.7	246		**-2.2**	**SD**
G13	4.6	5.1	1.3		11.0	9190	**-20.3**	**-13.4**	**SD**
G15	3.8				3.8	581		**8.6**	**SD**
G17	3.9	10.1			14.0	>40000	**31.2**	**26.1**	**PD**
G18	3.4	3.4		2.1	8.9	28236		**36.9**	**PD**
G19	7.3			1.2	8.5	7		**-4.5**	**SD**
G20	2.5				2.5	18		**-13.8**	**SD**
G21	3.1				3.1	1109		**-4.8**	**SD**
G24	2.5	2.0			4.5	7778	**-9.1**	**-34.8**	**PD**
G25	X	5.2		6.1	11.3	>40000	**-23.6**	**20.2**	**PD**
G26	4.7	3.6		3.2	11.5	754	**-21.8**	**-20.1**	**SD**
G29	2.1			3.4	5.5	320		**0.0**	**SD**

Table 4.5. Response dimensions (cm) and CA19-9 by RECIST criteria after two cycles.

Pt	Pancreas	RECIST Liver mets	TWO Lung mets	Other	Total	CA19-9 (U/mL)	LM change (%)	Total change (%)	Response
G01	4.0	0.8		1.3	6.1	569	**-46.7**	**-12.9**	**SD**
G03	3.6	1.1			4.7	4365	**-8.4**	**-16.1**	**SD**
G05	2.9	2.8	4.0		9.7	21	**-39.1**	**-19.8**	**SD**
G06	5.8				5.8	2153		**-12.1**	**SD**
G07	2.7				2.7	4000		**+12.5**	**SD**
G10	3.0				3.0	2410		**+20.0**	**PD**
G11	2.6	4.0			6.6	787	**-38.5**	**-34.7**	**PR**
G12	3.2		8.9		12.1	151		**+36.0**	**SD**
G13	4.4	3.5	1.2		9.1	3629	**-45.3**	**-27.2**	**SD**
G15	2.8				2.8	576		**-20.0**	**SD**
G20	2.7				2.7	24		**-6.9**	**SD**
G21	2.8				2.8	525		**-15.2**	**SD**
G26	5.0	3.3		3.3	11.6	2670	**-28.3**	**-19.4**	**SD**
G29	2.2			3.4	5.6	X		**-9.1**	**SD**

Table 4.6. Response dimensions (cm) and CA19-9 by RECIST criteria after four cycles.

Pt	Pancreas	RECIST Liver mets	THREE Lung mets	Other	Total	CA19-9 (U/mL)	LM change (%)	Overall change (%)	Response
G01	5.1	4.3		1.6	11.0	6466	**+287**	**+57**	PD
G03	3.9	1.1			5.0	2950	**-8.4**	**-11.8**	PD
G05	2.6	2.8	3.9		9.3	12	**-39.1**	**-23.2**	PD
G06	5.3				5.3	6178		**-19.7**	PD
G07	3.8				3.8	10947		**+58.3**	PD
G11	2.3	3.2			5.5	X	**-50.8**	**-45.5**	PR
G12	3.2		8.9		12.1	233		**+36.0**	PD
G13	4.5	3.0	1.2		8.7	4161	**-53.2**	**-30.4**	PR
G15	3.2				3.2	703		**-8.6**	SD
G20	2.9				2.9	44		**0**	SD
G21	2.7				2.7	485		**-18.2**	SD

Table 4.7. Response dimensions (cm) and CA19-9 by RECIST criteria after six cycles.

Response rates in the historical control cohort are shown below (table 4.8). There
was no complete response observed. Partial response was observed in 2/17, stable
disease in 4/17, with the majority (11/17) experiencing progressive disease as their
best response. Disease control rate was 6/17.

	Proportion	Percent
Complete response (CR)	0/17	0
Partial response (PR)	2/17	11.8
Stable disease (SD)	4/17	23.5
Progressive disease (PD)	11/17	64.7
Disease control (CR+PR+SD)	6/17	35.3

Table 4.8. Historical control (single-agent gemcitabine) group overall response rates.
The proportions and percent of the cohort experiencing each category of response by
RECIST criteria as described previously are shown.

The disease control rate in the experimental group was significantly better than the
historical control group (16/21 versus 6/17 p=0.02- figure 4.2)
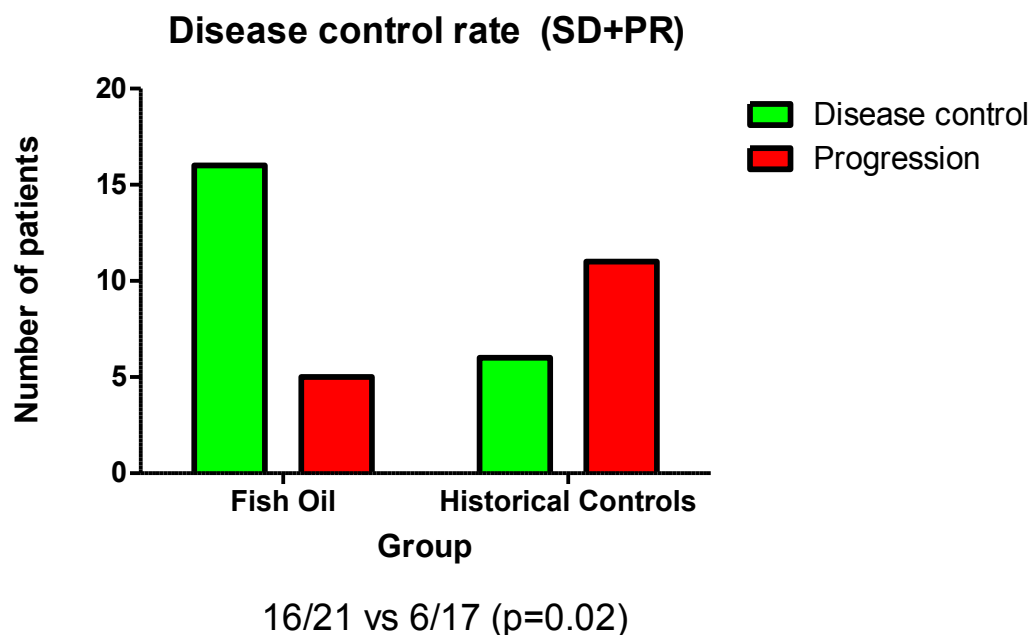
Figure 4.2. Comparative analysis of response rates using fisher's exact test. Disease
control rate (sum of stable disease and partial response rates) of the Fish Oil group is
significantly superior to the historical control group.

Proportions of patients experiencing disease control is perhaps best demonstrated and understood by waterfall plots below. Each bar represents a single patient, with their best overall response of all dimensions (figure 4.3) and liver metastases response (figure 4.4) shown relative to the baseline measurements. Note that one patient- G24, experienced partial response by size criteria in target lesions (34.8% reduction), but developed new target lesions and hence although depicted on the waterfall plot as a responder was classified as progressive disease for the purposes of the trial outcomes.
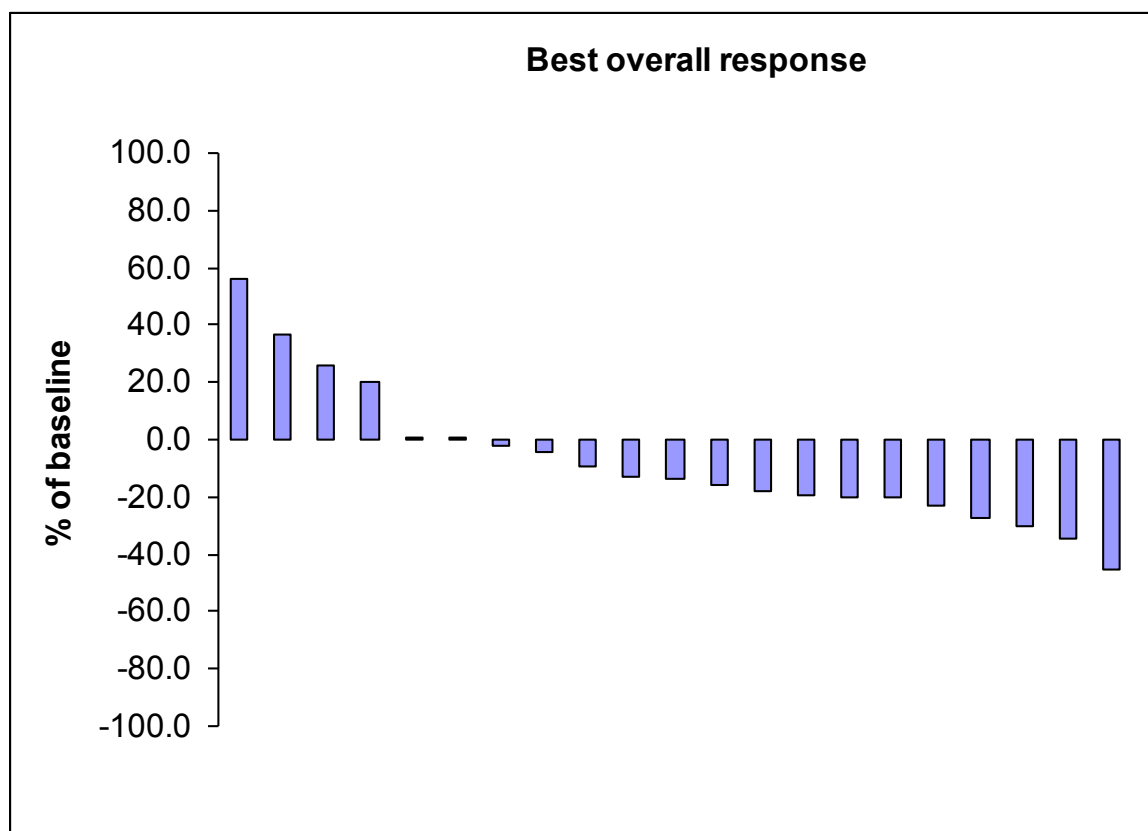
Figure 4.3. Waterfall plot of best overall response for experimental group.
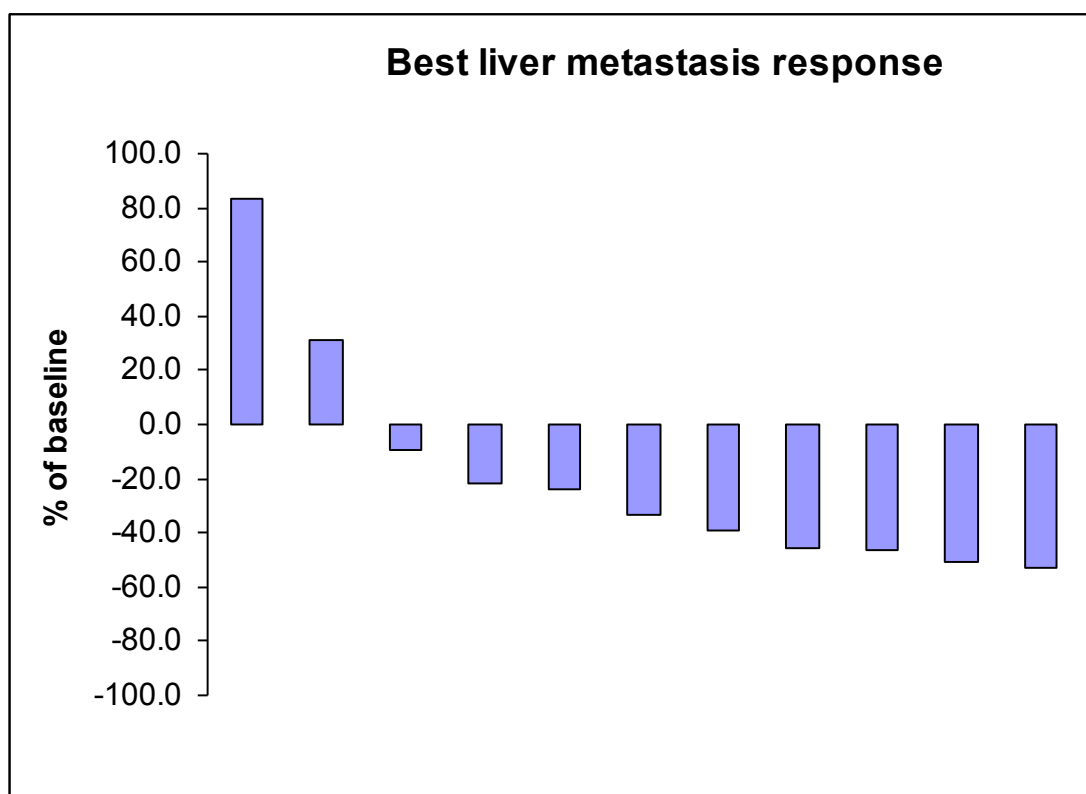
Figure 4.4. Waterfall plot of best liver metastasis response.

4.3.1 Outcome in relation to primary outcome measure

The outcome for stage one of Simon's two stage model in order to proceed to the second stage of recruitment was therefore achieved (3 partial responses out of 21 evaluable patients)

4.4 Survival analysis

Overall survival and progression-free survival for each patient as defined previously are shown in the table below.

Patient	OS (months)	PFS (months)
G01	7.0	5.3
G02	2.0	2
G03	10.9	5.7
G04	3.2	3.2
G05	7.0	5.2
G06	11.2	5.3
G07	7.4	5.3
G08	4.5	1.9
G09	2.0	2.0
G10	4.3	3.5
G11	10.3	9.0
G12	7.7	5.5
G13	13.1	7.5
G14	0.5	0.5
G15	8.2	8.2
G17	6.0	3.4
G18	2.4	1.5
G19	2.7	2.7
G20	18.9	10.6
G21	7.6	7.0
G22	1.0	1.0
G23	1.6	1.6
G24	4.4	1.5
G25	2.0	1.8
G26	4.8	4.8
G27	2.9	2.9
G29	8.9	8.9

Table 4.9. Overall survival (OS) and progression-free survival (PFS) by patient from first treatment date.

Kaplan-Meir survival curves constructed for both OS and PFS for the experimental group and the historical control group demonstrated no difference in OS (figure 4.5) but a significantly improved PFS in the experimental group (figure 4.6). The Gehan – Breslow-Wilcoxon test was used to analyse the survival curves rather than the log-

rank test as it gives more weight to earlier events, which was to be expected in this
group of patients.

Overall Survival

Median OS = 4.8 vs 4.15 months (p=0.56)

p= 0.56 (Gehan-Breslow-Wilcoxon test)
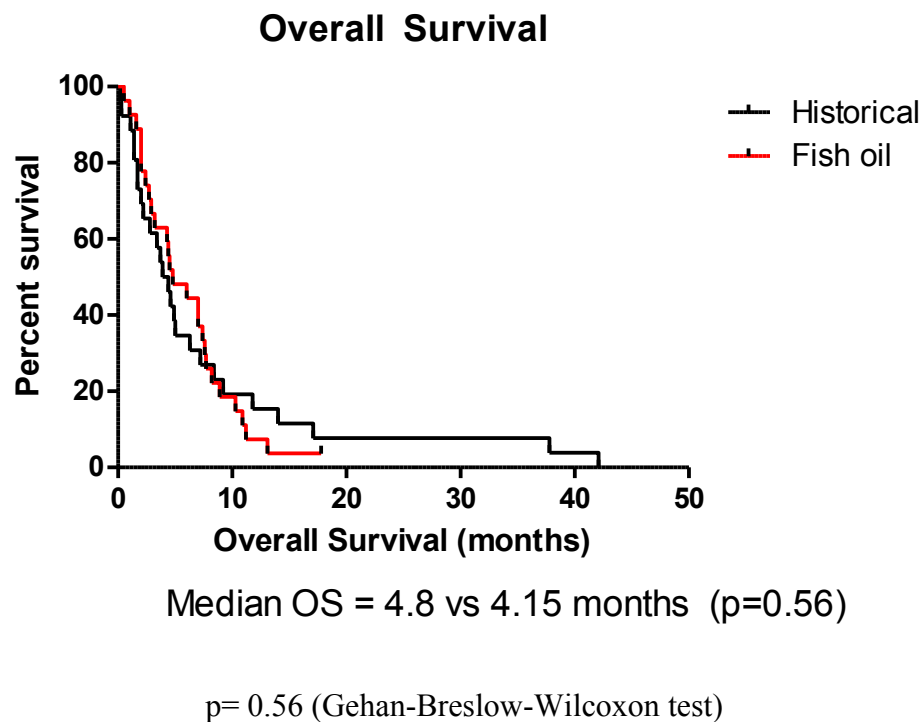
Figure 4.5. Overall survival of experimental versus historical cohorts.

Progression-free survival

Median PFS = 3.5 vs 2.35 months (p=0.02)

p=0.02 (Gehan-Breslow-Wilcoxon test)
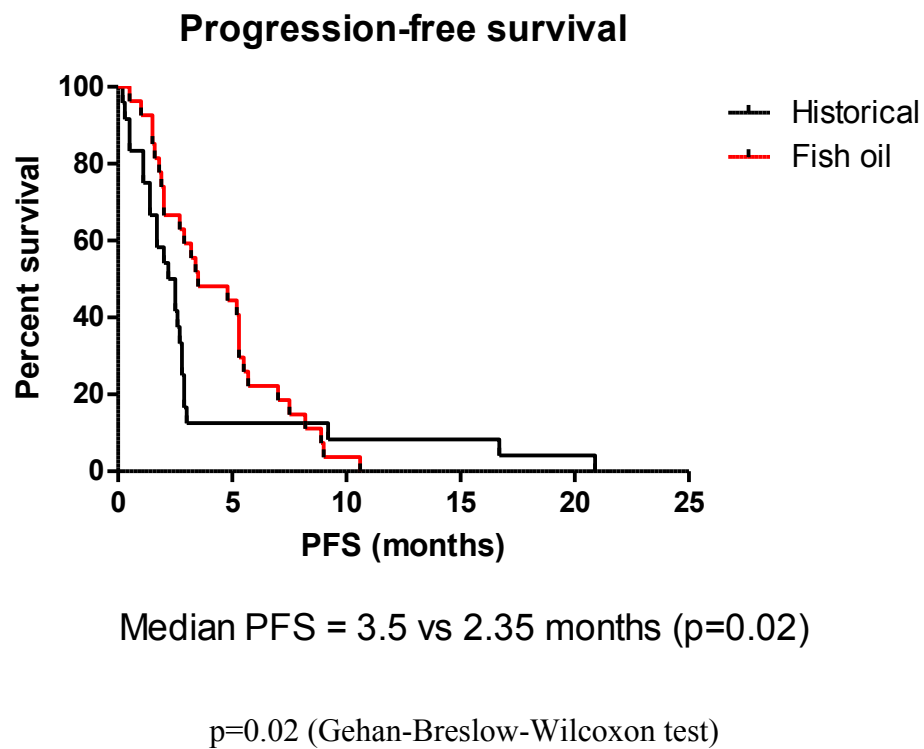
Figure 4.6. Progression-free survival of experimental versus historical cohorts.

4.5 Serum CA19-9 antigen response

Serum CA19-9 antigen concentration was raised in 31/34 patients at baseline screening investigations. The median concentration was 1511 units/mL with a range of <3 to 130,701 units/mL (table 4.10). The normal range for CA19-9 is 0-37 units/mL.

Proportion (percent) raised	31/34 (91%)
Median (range)	1511 (<3 – 130,701) (Units/mL)

Table 4.10. Baseline CA19-9 values (all patients screened).

Of the 21 patients assessable for CA19-9 response, having completed a minimum of 2 cycles, 10/21 (47.6%) had >50% decrease in CA19-9. In a similar fashion to response dimension changes, best CA19-9 response relative to baseline for each patient is shown in a waterfall plot below (figure 4.7)
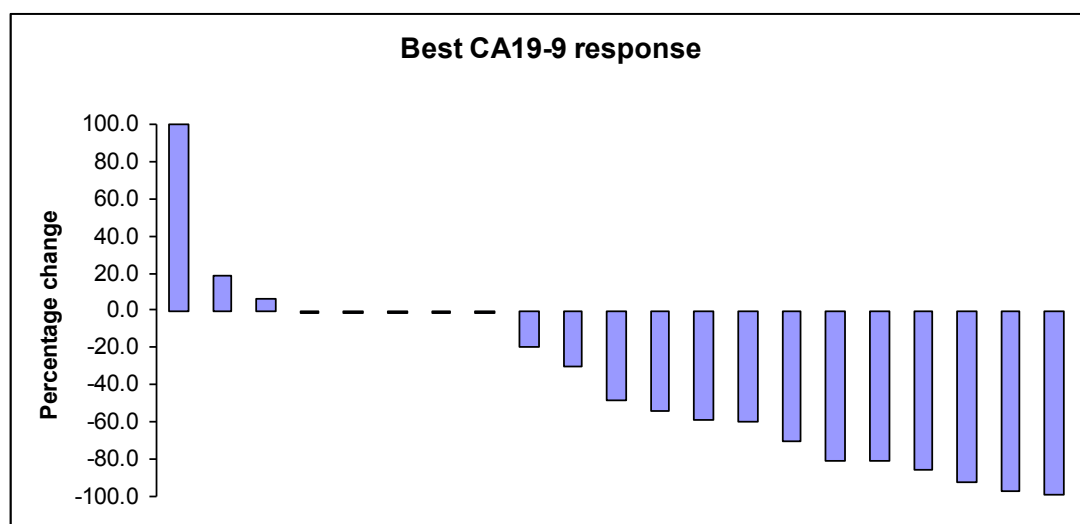
Figure 4.7. Waterfall plot of best CA19-9 response.

4.6 Patient-reported outcomes: Quality of life and brief pain inventory assessments

Twenty-one patients were assessable for QoL response over at least a 4 week period, having completed at least 4 trial treatments. All scores when transformed from questionnaires according to EORTC guidelines had a maximum of 100 and a minimum of 0. Baseline scores for these patients over the core domains in the QLQ-C30, PAN-26 and BPI questionnaires are shown below in table 4.6.1. Median score for global health was 50.0; for functional and symptom scores in the QLQ-C30 questionnaire: 71.1 and 66.7 respectively. For the supplementary disease specific PAN-26 module, functional and symptom median baseline scores were 55.6 and 60.8 respectively. Median support scores, detailing questions relating to medical and holistic support were 100 at baseline. Median BPI score at baseline was 68.3.

	Median (range)
Global health	50.0 (16.7-91.7)
QLQ-C30 Functional Scores	71.1 (20-95.6)
QLQ-C30 Symptom Scores	66.7 (24.2-90.9)
PAN-26 Disease specific functional scores	55.6 (16.7-100)
PAN-26 Disease specific symptom scores	60.8 (21.6-92.2)
PAN-26 Support scores	100 (0-100)
BPI Scores	68.3 (27.5-100)

Table 4.11. Baseline scores for quality of life.

All scores were calculated at baseline: only changes over a greater than 4 week period were considered.

As has been previously described, of particular interest was the number of patients experiencing stable, 10% increase or 20% increase in scores over and above the baseline values for at least a 4 week period. In the global health domain these proportions were 14/21, 12/21 and 10/21 respectively. The BPI score proportions for stable, 10% increase or 20% increase were 16/21, 12/21 and 6/21 respectively (table 4.6.2).

	Stable or better	10% increase	20% increase
Global health score	14 (67%)	12 (57%)	10 (48%)
Summated QoL score	18 (86%)	9 (43%)	6 (29%)
BPI score	16 (76%)	12 (57%)	6 (29%)

Table 4.12. Percentage changes in quality of life scores from baseline.

When broken down by domain for both the core (QLQ-C30) and supplementary module (PAN-26) questionnaires in a similar fashion to the baseline scores in table 4.6.1, the numbers of patients with a >10% improvement in scores over baseline were also identified (table 4.6.3)

HRQOL outcome	Number (percentage) with >10 point improvement over baseline
QLQ-C30 Functional Scores	7 (33.3)
QLQ-C30 Symptom Scores	13 (61.9)
PAN-26 Disease specific Functional Scores	13 (61.9)
PAN-26 Disease specific Symptom Scores	14 (66.7)
PAN-26 Support Scores	6 (28.6)

Table 4.13. Improvement in different HRQOL outcome measures. Note 15 (71.4%) patients had support scores of 100% at baseline.

Waterfall plots to depict best change in global health scores (figure 4.8), summated QoL scores (figure 4.9) and BPI scores (figure 4.10) for each patient were also constructed to give a pictorial representation of responders versus non-responders for these domains. Again changes recorded were only those lasting for at least 4 weeks. As before each bar represents a patient, and peak values above baseline were thus recorded and charted. If there was no improvement in score over baseline lasting for at least 4 weeks, then the trough value was charted so as to give a true representation of QoL score changes.
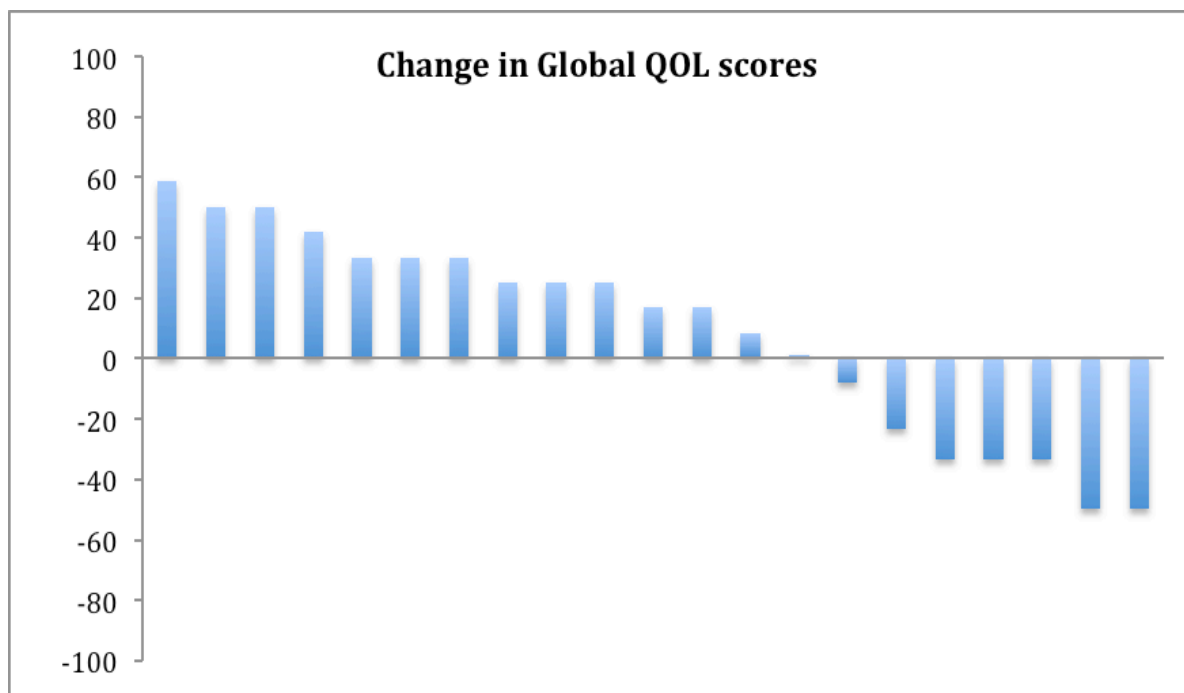
Figure 4.8. Waterfall plot of best percentage change in Global Quality of Life scores from baseline.
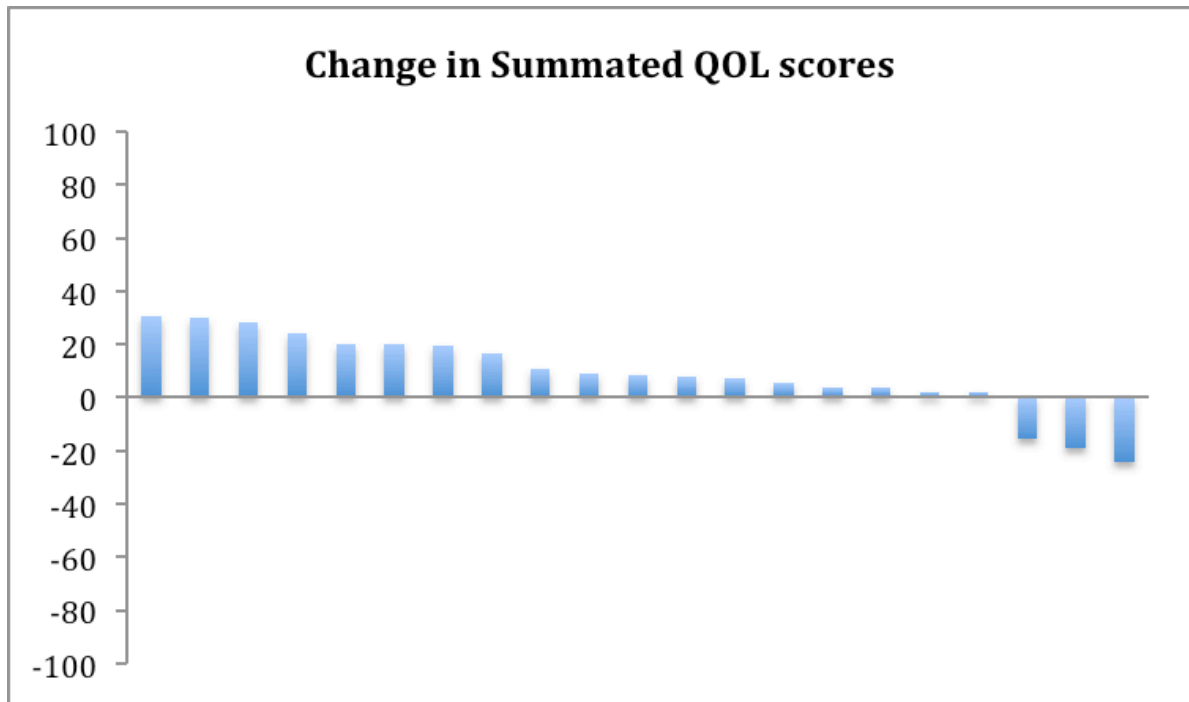
Figure 4.9. Waterfall plot of best percentage change in summated Quality of Life

scores from baseline.
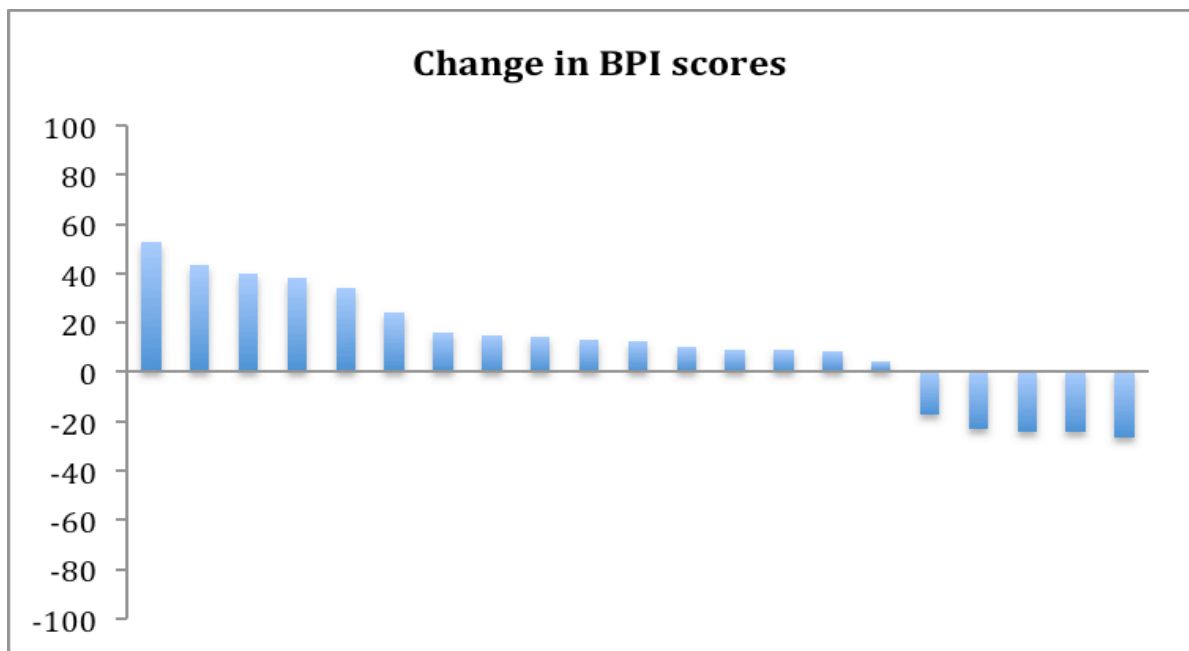
Figure 4.10. Waterfall plot of best percentage change in Brief Pain Inventory (BPI)
scores.

4.7 Clinical benefit response and weight changes

Clinical benefit response (CBR) was defined by stable or increased global health score without decrease in weight or brief pain inventory scores over at least 4 consecutive weeks. CBR by these criteria was seen in 8/21 patients (38%). Median duration of CBR was 20.5 weeks. Weight stabilisation or improvement was seen in 14/21 (66.7%) of patients.

Changes in bodyweight with treatment were depicted on a waterfall plot. In a similar fashion to the QoL data described above, peak bodyweight above baseline at a timepoint at least 4 weeks after initiation of treatment is recorded for each patient. If there was no improvement in bodyweight over the treatment course lasting for at least 4 weeks, then the trough weight was plotted
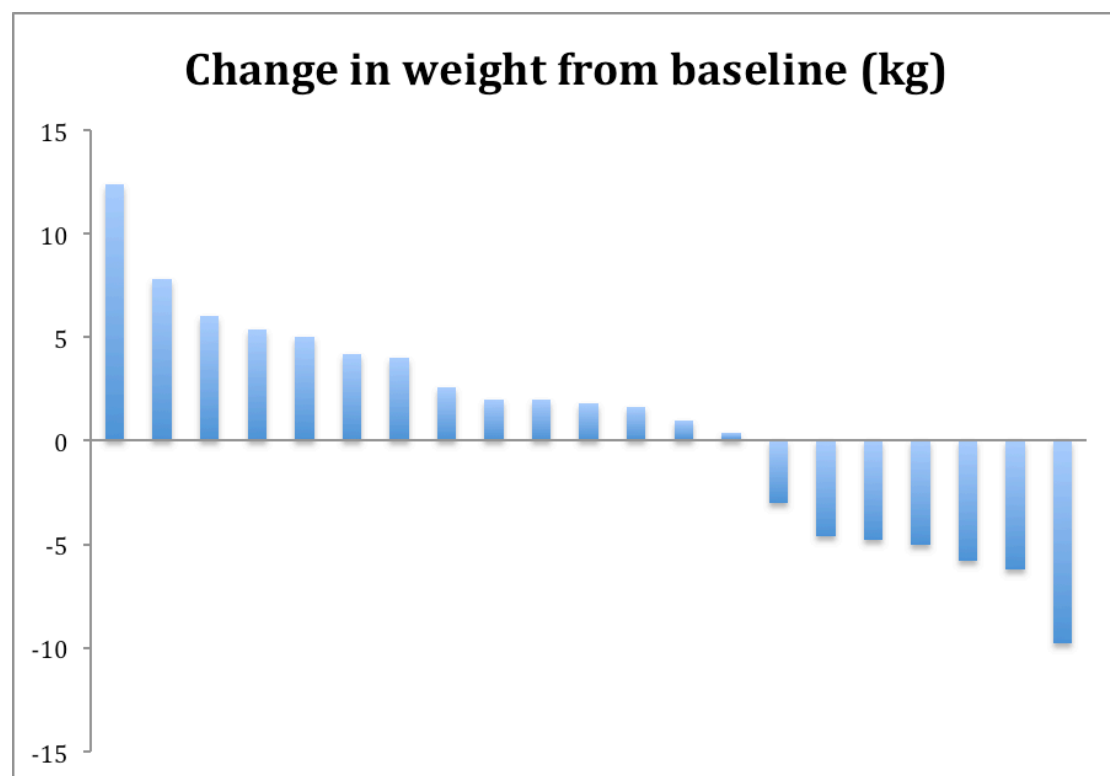
Figure 4.10. Changes in weekly bodyweight. Each bar represents a patient. Peak weight gain is recorded above and trough loss below the baseline.

4.8 Adverse Events (CTCAE criteria)

The experimental combination of gemcitabine and Lipidem was relatively well tolerated in general, although not at the planned therapeutic dose of 100g. Dose reductions from this Lipidem dose planned in the trial protocol had to be carried out for all patients at some point due to either grade 1 nausea and vomiting, chills or bloating. Highest grade of commonly experienced adverse events by CTCAE v4.0 criteria for each patient are shown in table 4.7 and figure 4.11. There were no other grade 2 or higher adverse events experienced.

Patient	Platelets	Neutrophils	Nausea / Vomiting	Chills	Diarrhoea
G1	0	1	1	1	1
G2	0	1	1	0	0
G3	2	2	1	1	0
G4	0	0	1	0	0
G5	0	1	3	0	1
G6	3	2	1	3	0
G7	0	2	1	1	1
G8	3	2	3	3	0
G9	0	1	1	2	0
G10	0	1	1	0	1
G11	0	1	2	3	1
G12	1	2	2	2	1
G13	1	1	1	1	0
G14	0	0	0	0	0
G15	1	2	1	1	0
G17	0	0	2	0	0
G18	1	1	1	2	1
G19	2	2	0	0	0
G20	1	2	0	1	0
G21	0	3	2	2	0
G22	0	3	0	0	2
G23	0	1	0	0	0
G24	0	1	2	0	0
G25	0	0	1	1	1
G26	2	1	0	0	0
G27	0	0	0	2	2
G29	1	4	1	0	0

Table 4.14. Highest CTCAE V4.0 grade of commonly experienced adverse events for each patient who underwent at least one trial treatment.
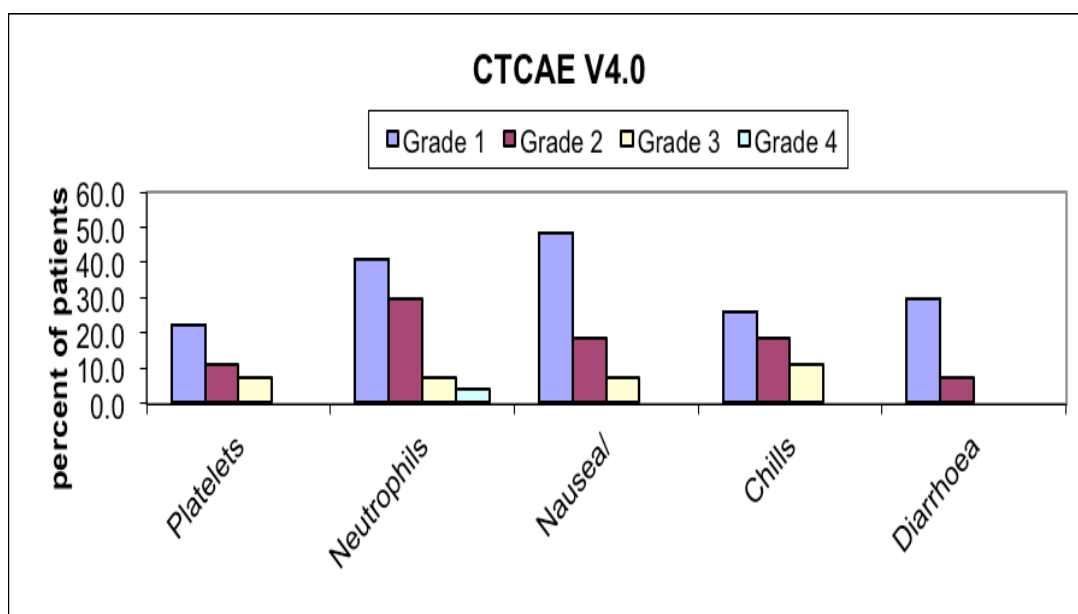
Figure 4.11. Common adverse events for the experimental group.

The proportions of patients experiencing common grade 3-5 adverse events in gemcitabine-containing regimens were identified in the experimental group and historical control cohort. These adverse events were thrombocytopaenia, neutropaenia and nausea/vomiting. This was then graphically charted along with the published data on all phase III trials (Arshad et al 2011a). As can be seen, the proportions of patients experiencing these adverse events in each of the three groups were broadly comparable (figure 4.12).
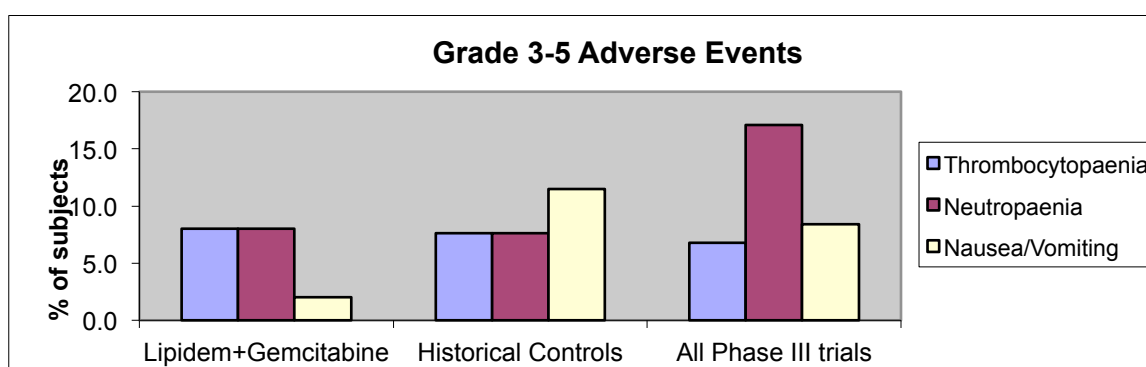
Figure 4.12. Grade 3-5 adverse events compared to historical controls and existing pooled phase III trial data for comparison.

5 Laboratory results

5.1 Changes in serum CAF concentrations with treatment

Changes in serum platelet-derived growth factor (PDGF) concentrations with time were evaluated using multiplex cytokine ELISA array as described previously. These data were plotted with mean values for all patients in each treatment week along with standard error of the mean bars. It can be clearly seen there is a reduction in PDGF concentration during each cycle, with the effect lost during the rest week (figure 5.1).
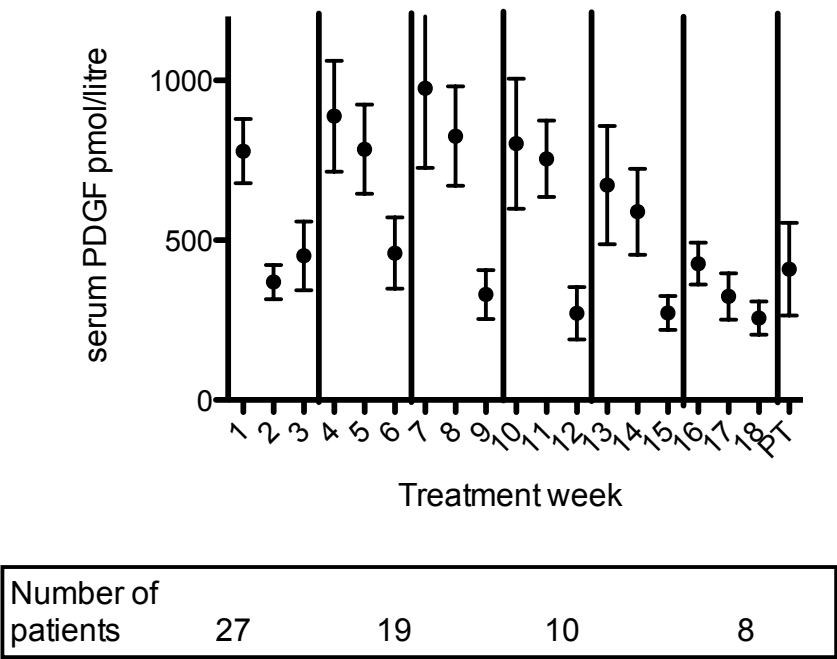

Figure 5.1. Changes in mean serum PDGF concentrations for all patients with each treatment timepoint. Standard error of the mean (SEM) bars are shown. Vertical lines after every third week indicate the rest week, and thus subdivide the data by treatment cycle. PT=post-treatment sample. Number of patients at each timepoint are shown to illustrate attrition.

In order to more accurately model the actual changes in serum cytokine concentrations, a statistical model was created which used mixed-effects logistic regression allowing the slope and intercept to vary between individuals. The

logarithms of the concentrations are shown on the 'y' axis, with treatment week on

the 'x' axis. These models produced a graphical output in which the actual or

projected concentration best-fit individual lines represent an individual patient. If

sloping downwards from left to right, this indicates a negative co-efficient, or a

reduction in concentration, whereas if sloping upwards from left to right this is a

positive co-efficient or an increase in concentration. These models were used to

evaluate PDGF concentration changes over all cycles (figure 5.2) and changes within

each PDGF cycle (figures 5.3-5.8), all of which demonstrated a significant reduction

in PDGF concentration with time.
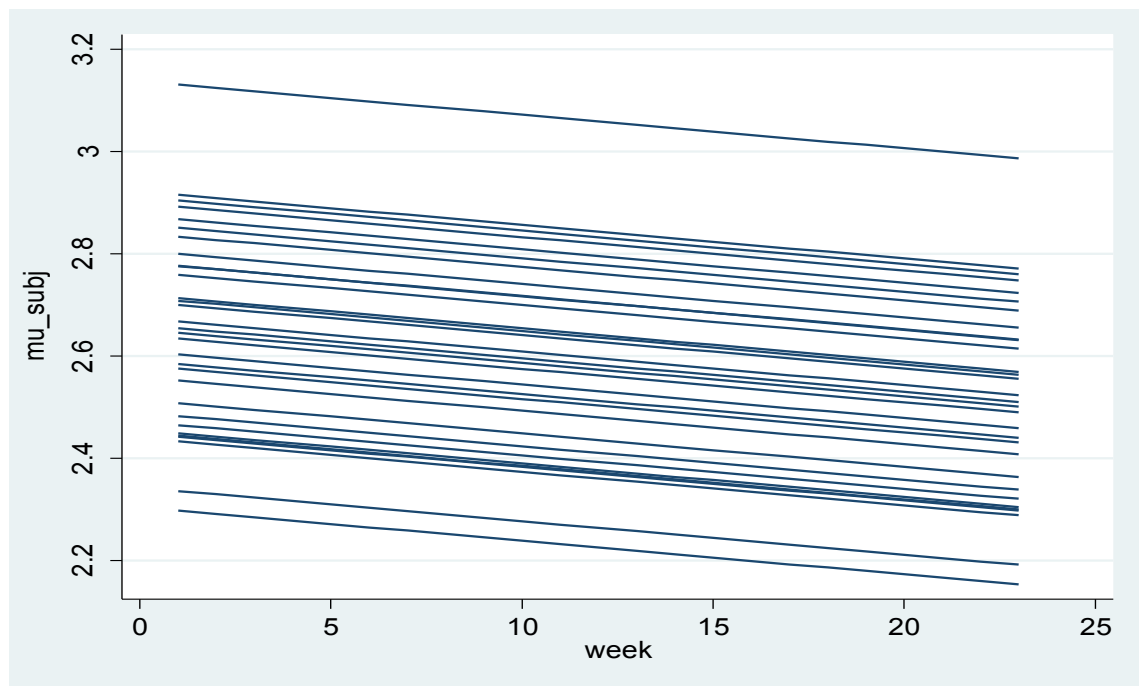
Figure 5.2. Statistical model output showing reduction in PDGF for all cycles with time in treated patients: p=0.05. Logarithms of concentrations (mu_subj) are shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.
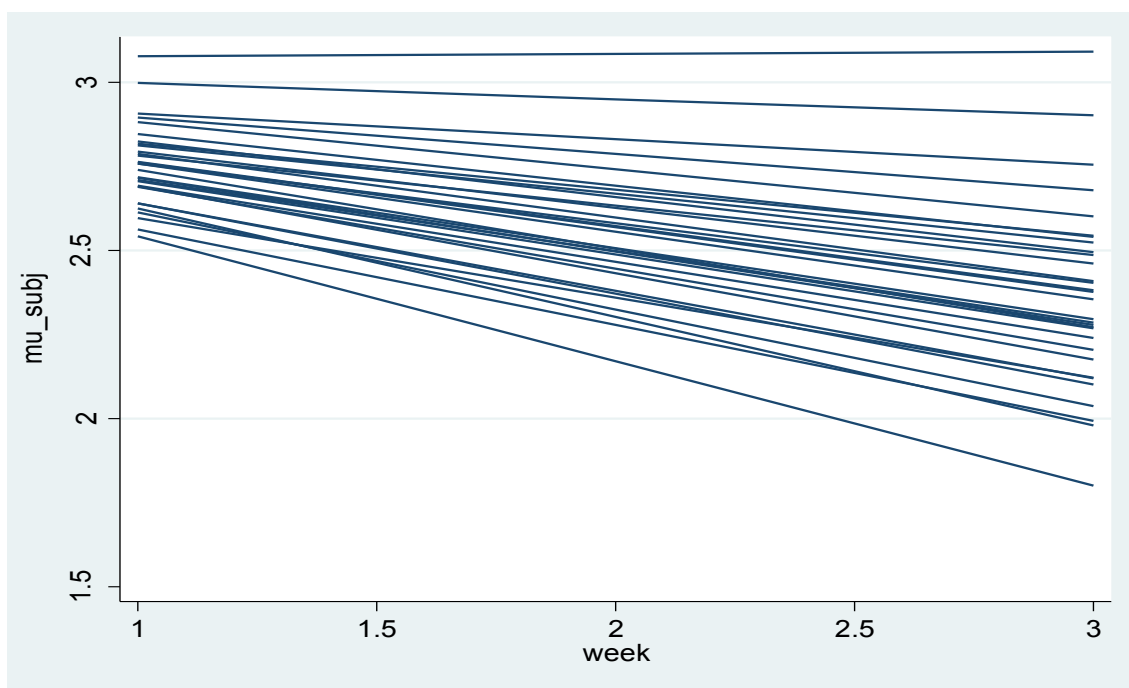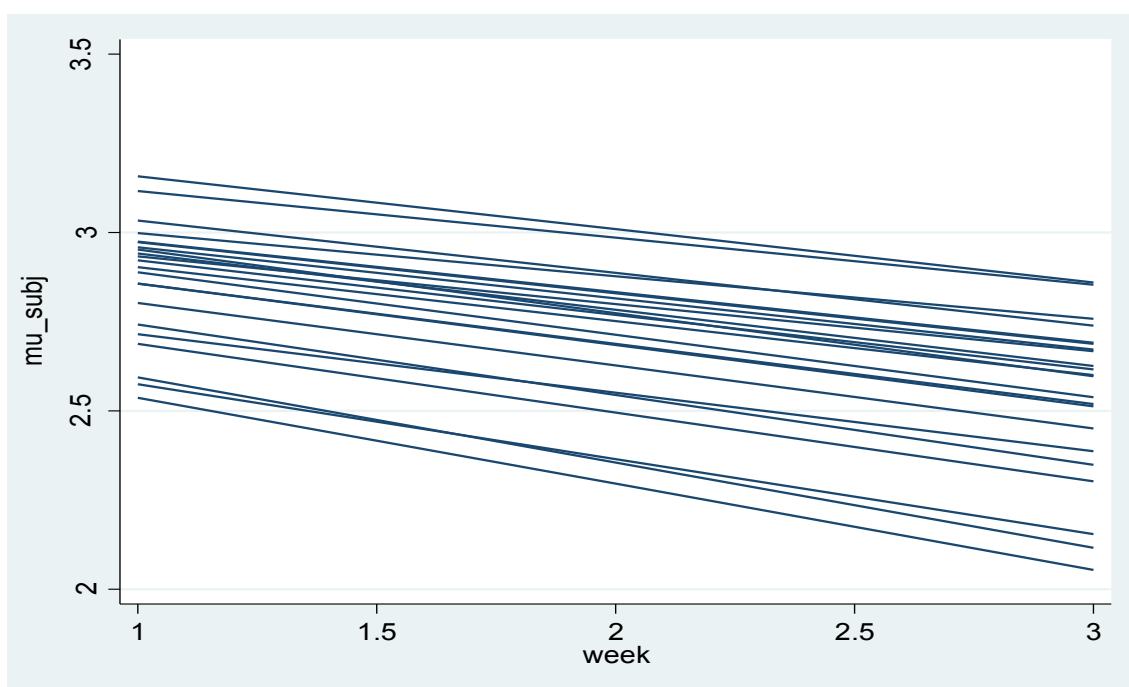
Figure 5.3. Statistical model output showing reduction in PDGF for cycle 1 with time in treated patients: p<0.001. Logarithms of concentrations (mu_subj) are shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.

Figure 5.4. Statistical model output showing reduction in PDGF for cycle 2 with time in treated patients: p<0.001. Logarithms of concentrations (mu_subj) are shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.
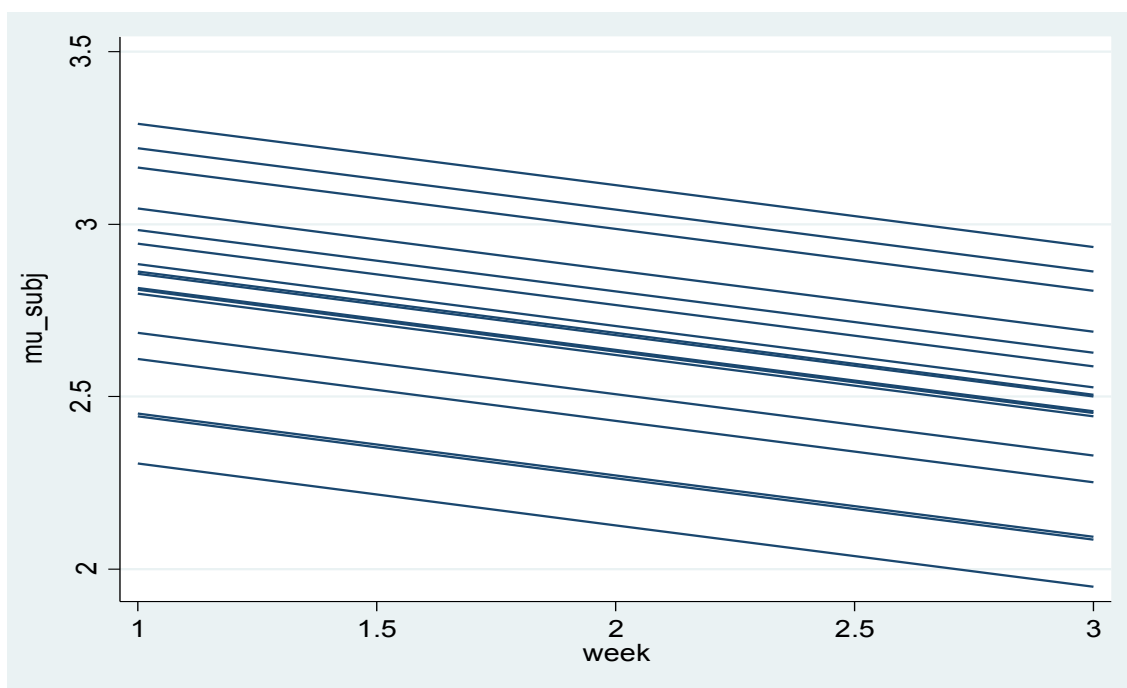
Figure 5.5. Statistical model output showing reduction in PDGF for cycle 3 with time in treated patients: p<0.001. Logarithms of concentrations (mu_subj) are shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.
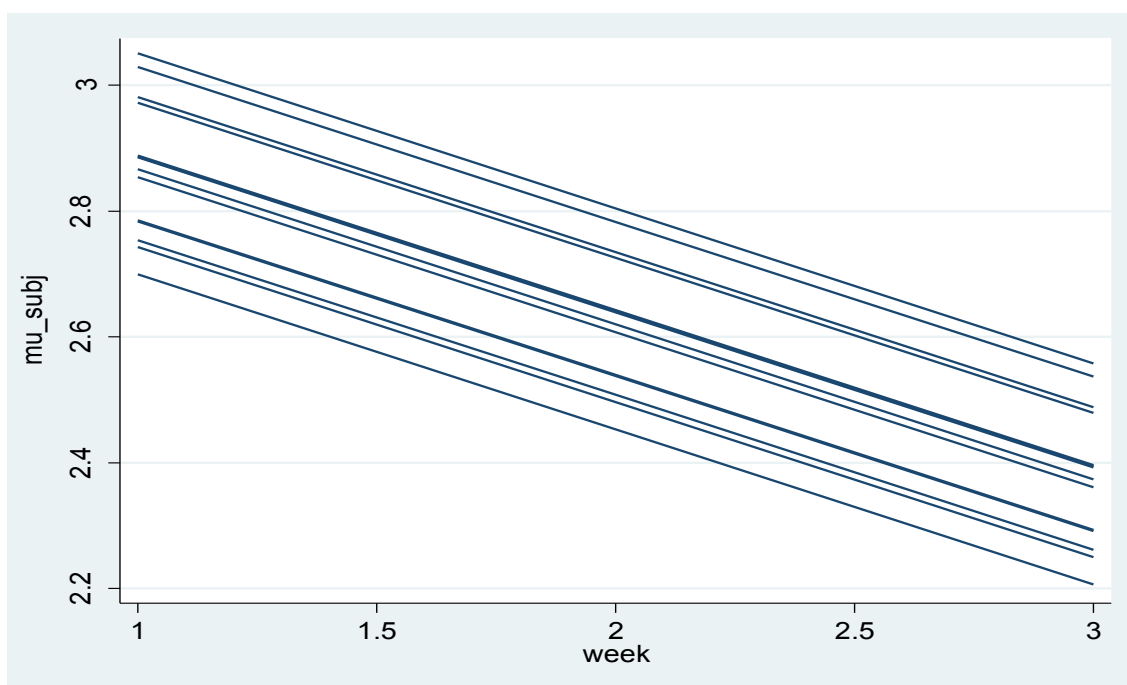

Figure 5.6. Statistical model output showing reduction in PDGF for cycle 4 with time in treated patients: p<0.001. Logarithms of concentrations (mu_subj) are shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.

Figure 5.7. Statistical model output showing reduction in PDGF for cycle 5 with time in treated patients: p<0.001. Logarithms of concentrations (mu_subj) are shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.
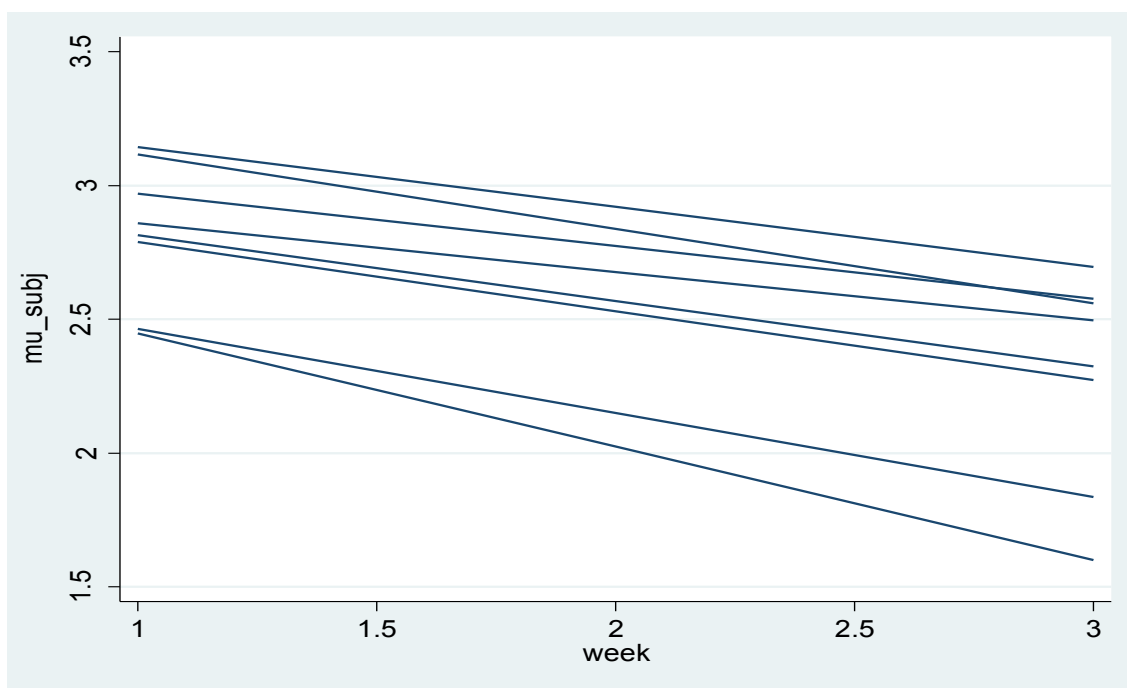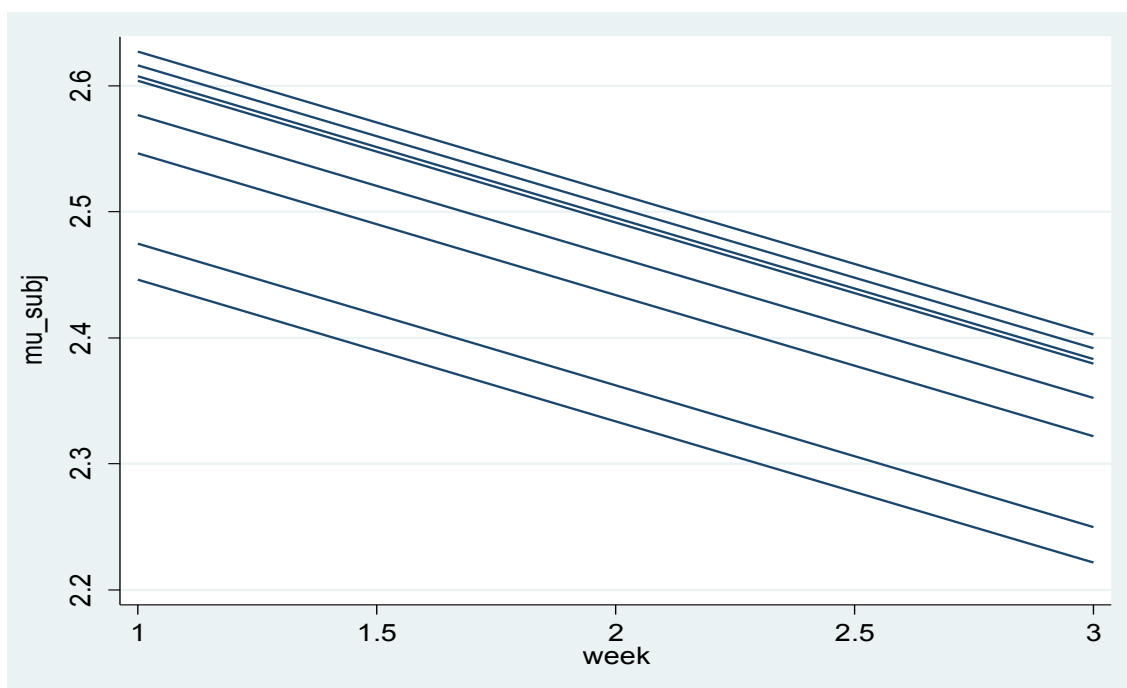

Figure 5.8. Statistical model output showing tendency to reduction in PDGF for cycle 6 with time in treated patients: p=0.06. Logarithms of concentrations (mu_subj) are shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.

In order to assess whether the PDGF reductions were in any way related to a
reduction in platelet count with treatment, as might be expected from any cytotoxic
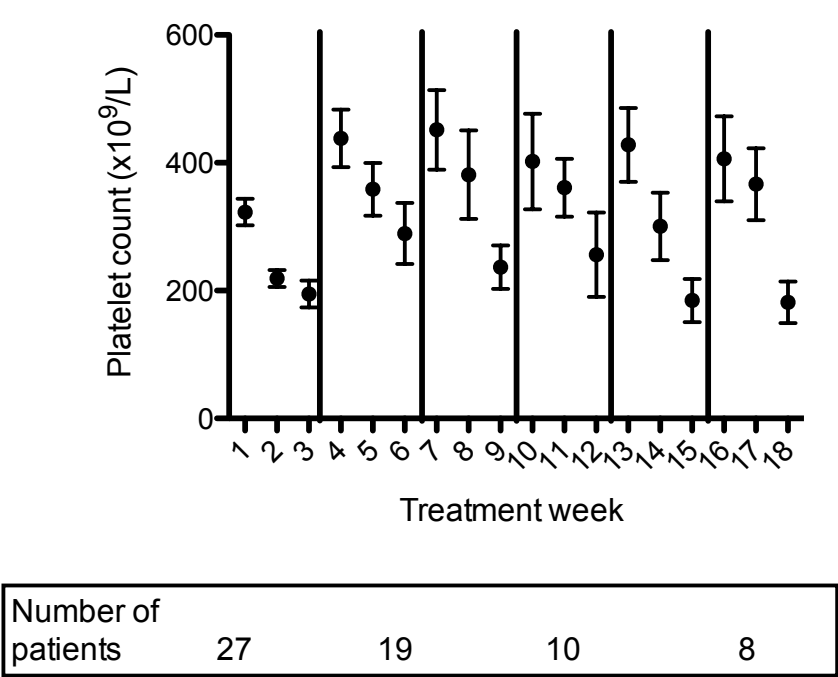regimen, separate analyses were carried out for this.

Figure 5.9. Changes in mean platelet count for all patients with each treatment
timepoint. Standard error of the mean (SEM) bars are shown. Vertical lines after
every third week indicate the rest week, and thus subdivide the data by treatment
cycle. Number of patients at each timepoint are shown to illustrate attrition.

There was no change in platelet count when the same statistical model was used to
analyse changes over the entire treatment course (p=0.6). However when analysed on
an intra-cycle basis, as for PDGF above, platelet count reduced significantly for every
cycle except cycle 4 (table 5.1). This may indicate that the reduction in PDGF
observed was related, at least in part, to a reduction in platelet count.

Cycle analysed	Reduction / Increase	p
All cycles	No significant change	0.6
Cycle 1	Reduction	<0.001
Cycle 2	Reduction	0.001
Cycle 3	Reduction	0.002
Cycle 4	No significant Change	0.088
Cycle 5	Reduction	<0.001
Cycle 6	Reduction	<0.001

Table 5.1. Statistical model analysis of change in platelet count across entire treatment course and on an intra-cycle basis.

Fibroblast growth factor (FGF) concentrations were also plotted in a similar way to that described above (figure 5.10).
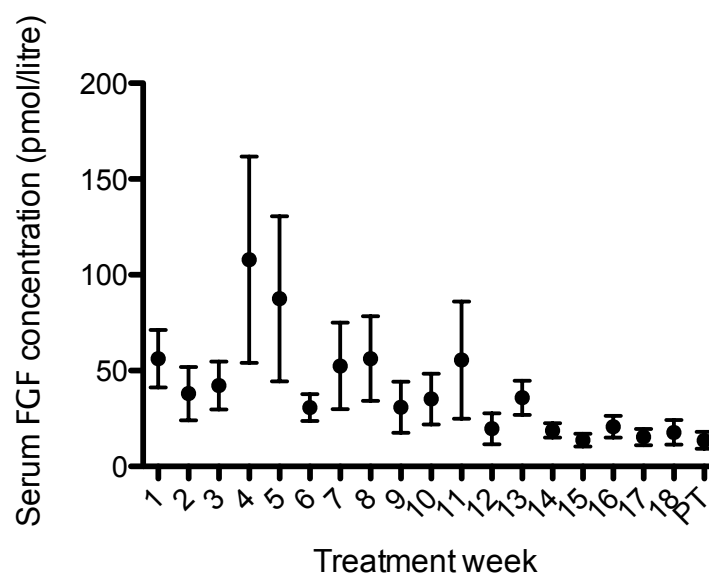
Number of patients	27	19	10	8

Figure 5.10. Changes in mean serum FGF concentration with treatment. SEM error bars are also shown. PT=post treatment sample. Number of patients at each timepoint are shown to illustrate attrition.

The same mixed-effects model was used to accurately identify changes in FGF

concentration with time. Again a negative co-efficient is demonstrated showing a

significant reduction in FGF concentration with time (figure 5.11).

Figure 5.11. Statistical model output showing reduction in serum FGF for all cycles
with time: p=0.03. Logarithms of concentrations (mu_subj) are shown on the y axis,
with time on the x axis. Each line represents a patient, with the best fit output from the
model shown.

Serum tumour-necrosis factor alpha apoptosis inducing ligand (TRAIL)

concentration was plotted in a similar way to that described above (figure 5.12).

Number of patients	27	19	10	8

Figure 5.12. Changes in mean serum TRAIL concentration with treatment. SEM error bars are shown. PT=post-treatment sample. Number of patients at each timepoint are shown to illustrate attrition.

The statistical model graphical output shown depicts a significant decrease in serum

TRAIL concentrations across the treatment course (figure 5.13)
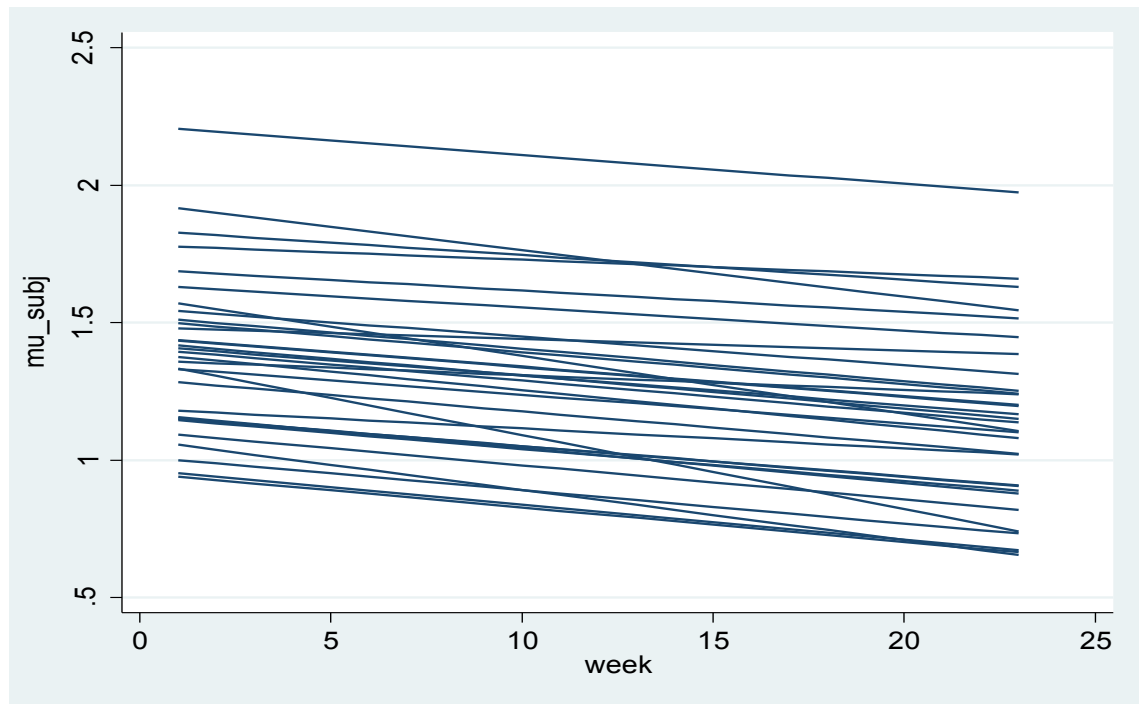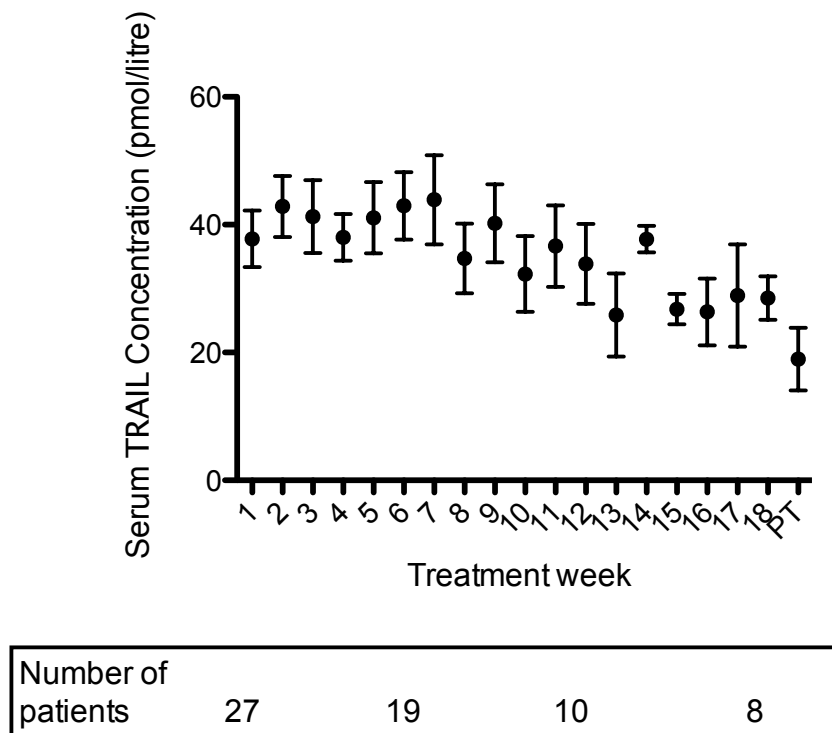
Figure 5.13. Statistical model output showing reduction in serum TRAIL for all cycles with time: p=0.009. Logarithms of concentrations (mu_subj) are shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.

Serum vascular endothelial growth factor-d (VEGF-d) concentrations were plotted in

a similar way to that described above (figure 5.14).

Number of patients	27	19	10	8

Figure 5.14. Changes in mean serum VEGFd concentration with time. SEM error bars are shown. PT=post-treatment sample. Number of patients at each timepoint are shown to illustrate attrition.

As was perhaps expected by the gross depiction of data above in figure 5.14, the statistical model graphical output shown depicts a significant increase in serum VEGF-d concentrations across the treatment course (figure 5.15)
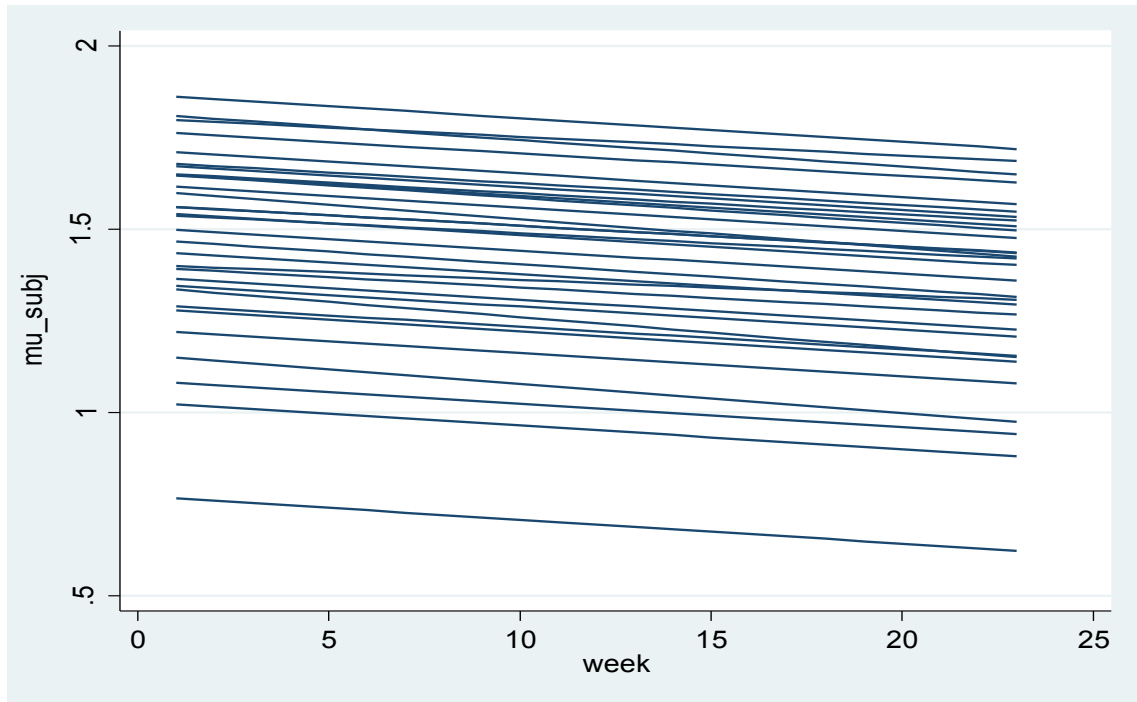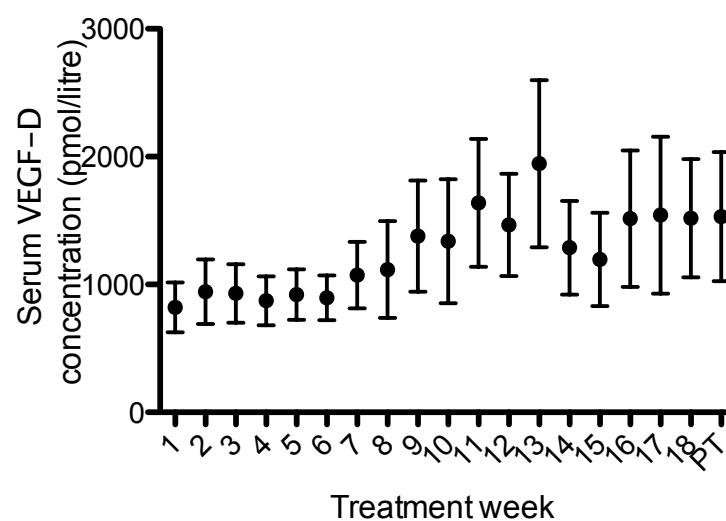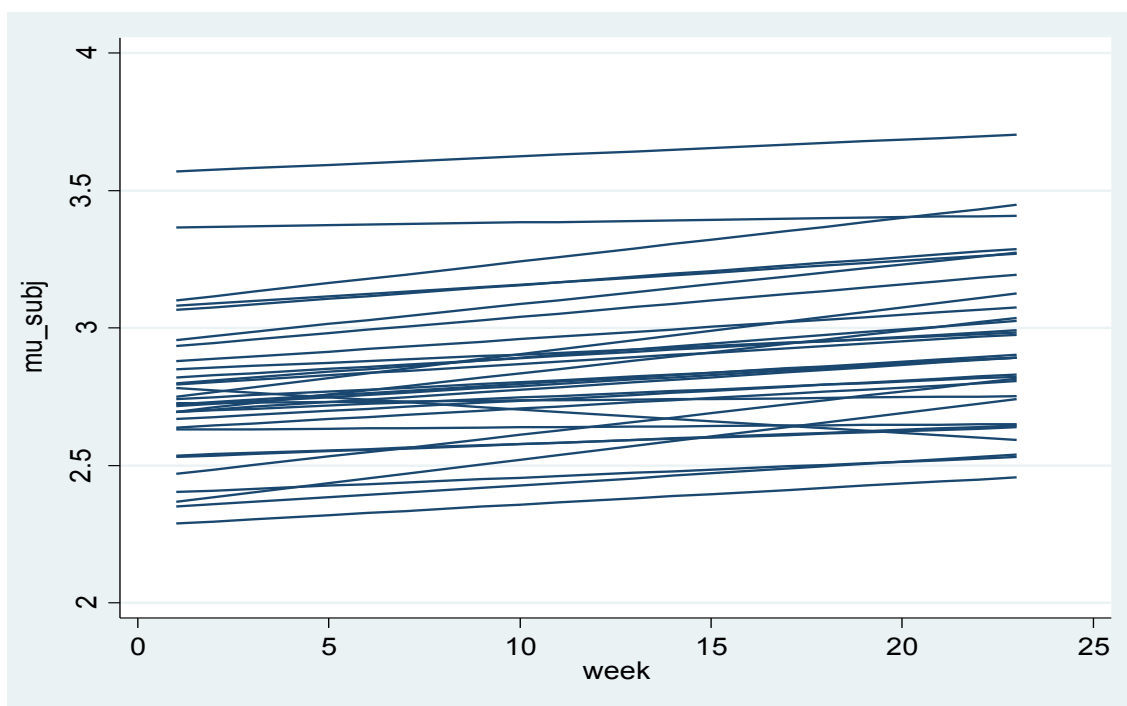
Figure 5.15. Statistical model output showing increase in serum VEGFd for all cycles with time: p=0.01. Logarithms of concentrations (mu_subj) are shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.

For all other serum CAF concentrations examined there was no change with the treatment course across the cohort as a whole. The non-significant changes are described in table 5.2.

CAF	p
IFNγ	0.13
IL1-β	0.74
IL6	0.06
IL8	0.82
TNFα	0.57
VEGFc	0.49
EGF	0.78
RANKL	0.65
HGF	0.58
VEGFa	0.92

Table 5.2. Non-significant change in serum cytokine concentrations with time.

5.2 Kaplan-Meir curves of high versus low baseline (defined by the median value) CAF concentrations correlated with overall survival

Baseline serum CAF concentrations were evaluated for each patient, and two groups created around the median value: a "high" group and a "low" group. Kaplan meir curves were then drawn, with log-rank analysis to determine differences between overall survival between these two groups for each CAF. The curves and outcomes from log-rank analysis for IL6 (figure 5.16) and IL8 (figure 5.17) are shown below.
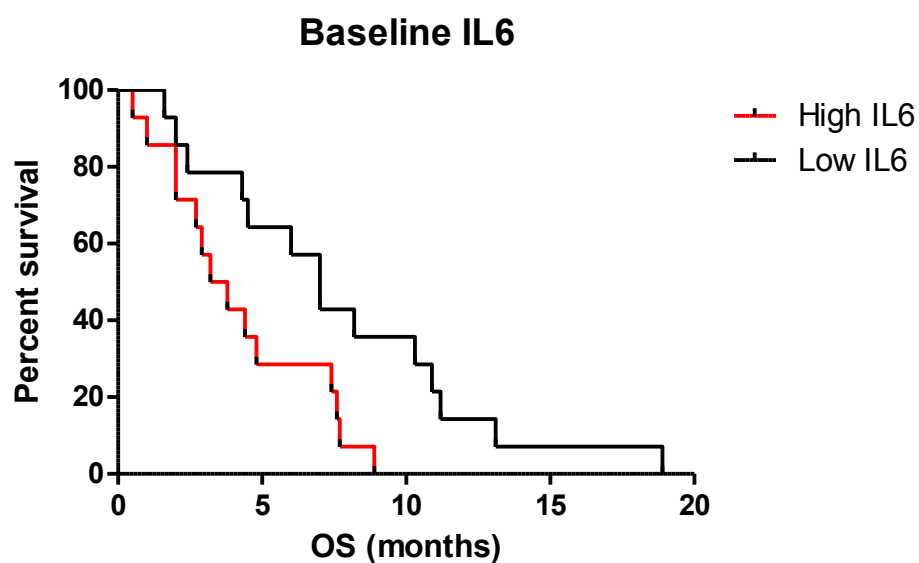

Figure 5.16. Median OS= 3.5 months (High IL6) versus 7.0 months (Low IL6): Log rank p=0.02.

Figure 5.17. Median OS= 3.5 months (High IL8) versus 7.3 months (Low IL8): Log rank p=0.05.

Non-significant relationships between baseline CAF concentration and OS are shown

in table 5.3.

CAF	High – OS (months)	Low – OS (months)	p (log rank)
IFNγ	4.35	5.4	0.64
IL1-β	4.45	5.4	0.91
TNFα	4.35	5.4	0.82
EGF	7.2	4.35	0.21
RANKL	7.0	3.35	0.83
TRAIL	5.75	4.6	0.67
PDGF	3.75	5.4	0.83
HGF	7.2	4.45	0.51
FGF	5.4	4.45	0.53
VEGFa	7.0	4.3	0.40
VEGFd	5.9	4.45	0.53

Table 5.3. Non-significant relationships between high and low baseline CAF concentration and OS.

5.3 Kaplan-Meir curves of high versus low baseline CAF concentrations correlated with progression-free survival

Baseline serum CAF concentrations were evaluated for each patient, and two groups

created around the median value:a "high" group and a "low" group. Kaplan meir

curves were then drawn, with log-rank analysis to determine differences in

progression-free survival between these two groups for each CAF. The curves and

outcomes from log-rank analysis for IL6 (figure 5.18) and IL8 (figure 5.19).

Baseline IL6

Figure 5.18. Median PFS=2.8 months (High IL6) versus 5.3 months (Low IL6): Log rank p=0.06.
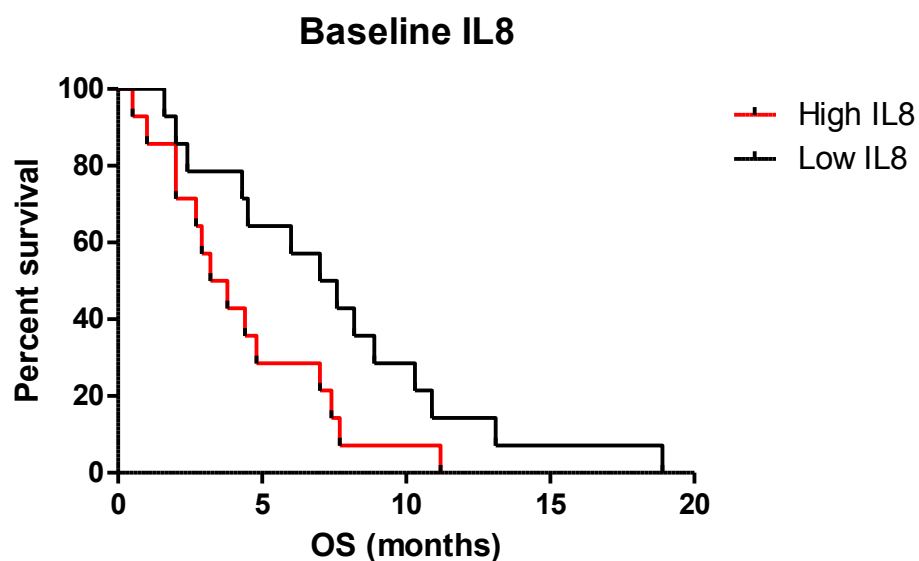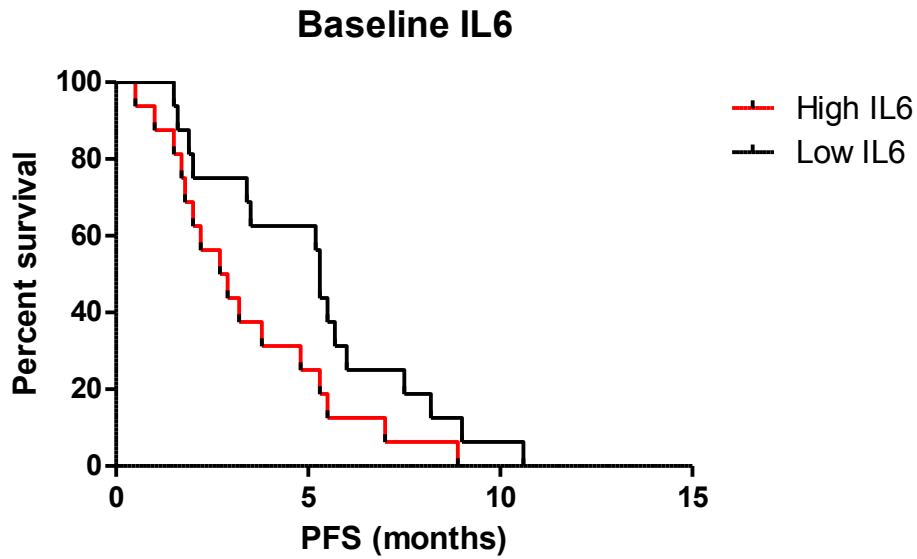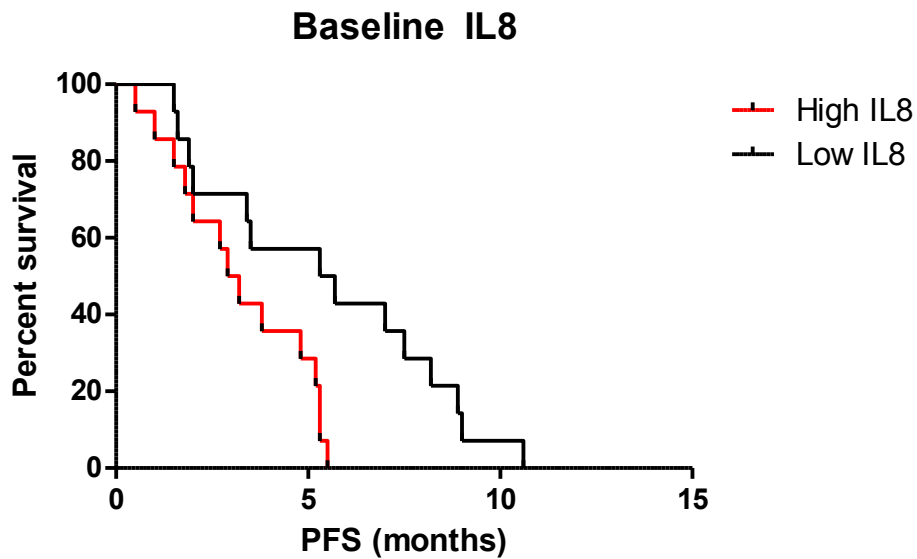
Baseline IL8

Figure 5.19. Median PFS=3.05 months (High IL8) versus 5.5 months (Low IL8): Log rank p=0.01.

Non-significant relationships between baseline CAF concentration and PFS are shown in table 5.4.

CAF	High - PFS (months)	Low - PFS (months)	p (log rank)
IFNγ	3.65	4.1	0.43
IL1-β	3.65	4.1	0.42
TNFα	3.65	4.1	0.47
EGF	5.3	3.45	0.26
RANKL	5.3	3.15	0.68
TRAIL	4.2	3.65	0.71
PDGF	3.35	4.3	0.67
HGF	5.25	3.45	0.13
FGF	4.3	2.95	0.12
VEGFd	5.05	3.45	0.61
VEGFa	5.25	2.3	0.29

Table 5.4: Non-significant relationships between high and low baseline CAF concentration and PFS.

5.4 Kaplan Meir curves of patients experiencing reduction in CAF during treatment and their correlation with overall survival

Responders were classified as those patients who had a 30% reduction or greater relative to baseline in serum cytokine concentration at any point during their treatment. Intra-cycle responders were those who had a 30% reduction during any cycle with baseline being the time point immediately before first treatment in that cycle. The chosen figure of 30% was recognised as being fairly arbitrary but is reasonable given that there are no prior published studies examining a relationship between magnitude of reduction of serum cytokine concentration from baseline and clinical outcome of any sort.

A Kaplan-meir curve was drawn to examine the difference in overall survival between PDGF intra-cycle responders and non-responders:i.e. those who experienced a 30% or greater reduction in PDGF during any one of their cycles of treatment

compared to baseline at the start of that cycle. There was a non-significant tendency

towards longer OS in PDGF responders versus non-responders (figure 5.20).
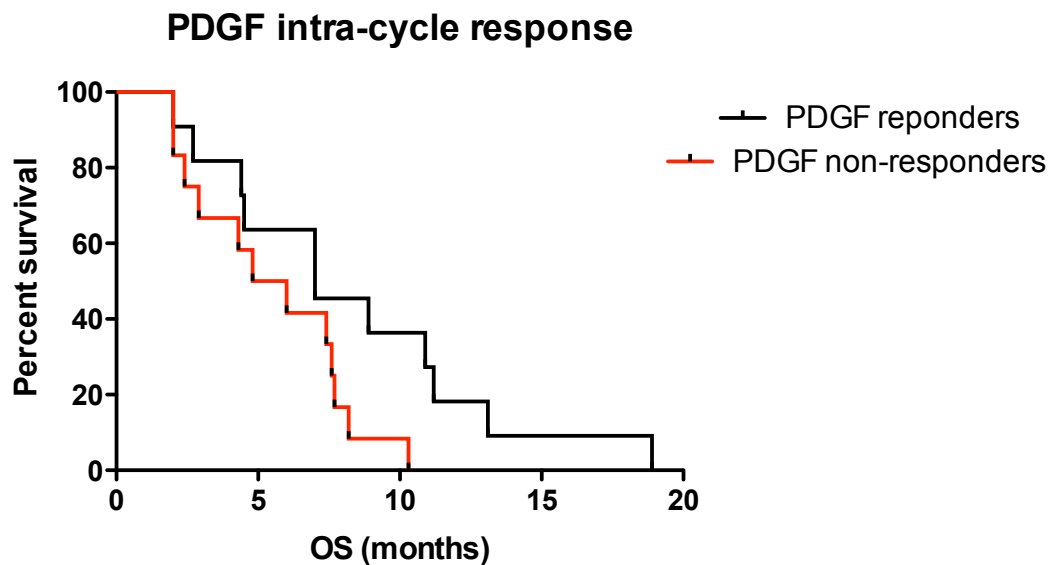
Figure 5.20. Median OS=7.0 months (PDGF intra-cycle responders) versus 5.4 months (PDGF intra-cycle non-responders): Log rank p=0.06.

Non-significant relationships between CAF responder status and OS are shown in

table 5.5.

CAF	Responder – OS (months)	Non Responder – OS (months)	P (log rank)
IFNγ	5.3	7.0	0.72
IL1-β	7.4	4.8	0.51
IL6	7.0	6.5	0.63
IL8	7.0	4.45	0.57
TNFα	7.2	4.5	0.41
VEGFc	5.2	7.0	0.84
EGF	7.0	6.0	0.58
RANKL	7.4	4.55	0.94
TRAIL	7.0	6.0	0.27
VEGFa	7.0	4.3	0.25
HGF	7.4	5.3	0.11
FGF	7.0	4.4	0.34
VEGFd	7.4	4.4	0.31

Table 5.5. Non-significant relationships between CAF responders and OS.

5.5 Kaplan Meir curves of patients experiencing reduction in CAFs during treatment and their correlation with progression-free survival

 The same criteria for CAF reduction were applied to examine the relationship

between CAF responder status and PFS.

   Kaplan-meir curves were drawn to examine the difference in PFS between

responders and non-responders for TNFα (figure 5.21), HGF (figure 5.22) and FGF

(figure 5.23). There was a non-significant tendency towards improved PFS in TNFα

and HGF responders versus non responders (p=0.06 and 0.09 respectively). There was

significantly improved PFS in FGF responders versus non responders (p=0.001).

Figure 5.21. Median PFS=5.3 months (TNFα responders) versus 2.7 months (TNFα non-responders): Log rank p=0.06.

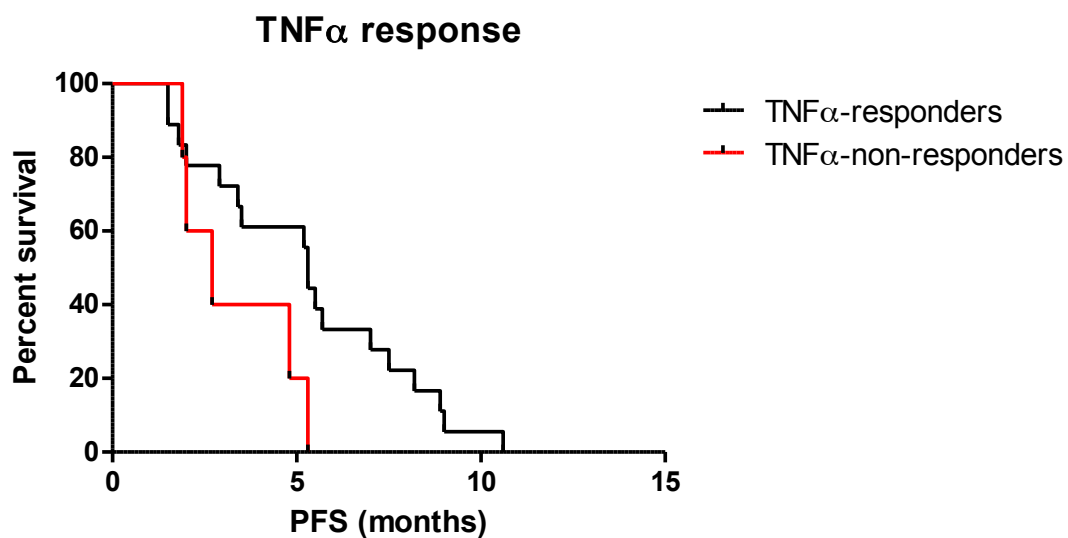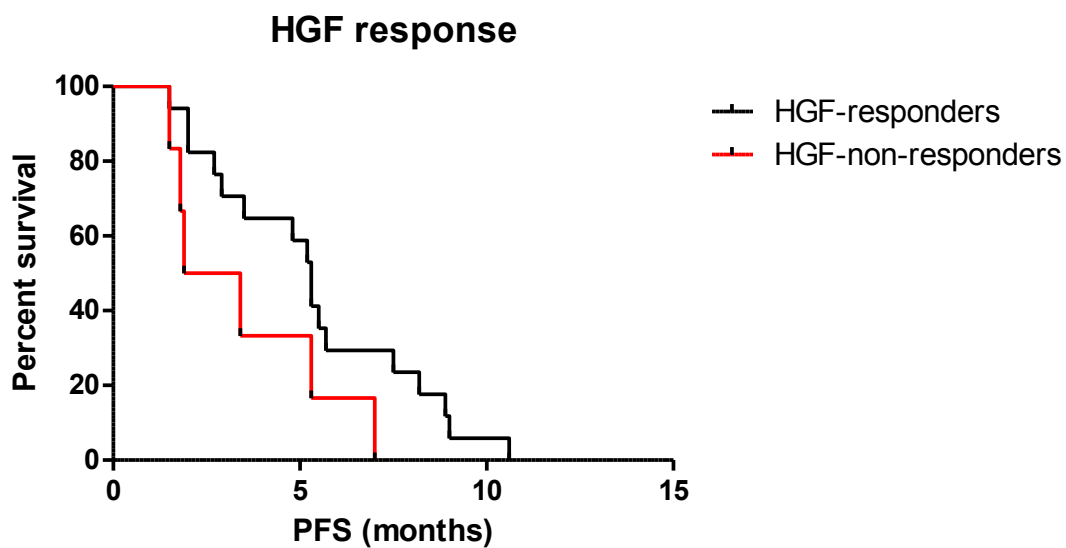Figure 5.22. Median PFS=5.3 months (HGF responders) versus 2.7 months (HGF non-responders): Log rank p=0.09.
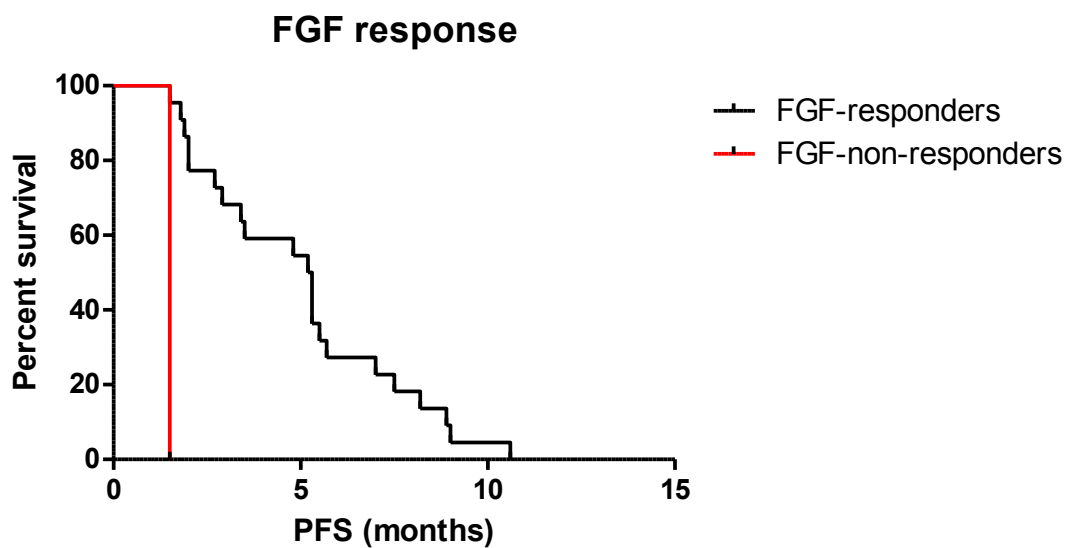
Figure 5.23. Median PFS=5.3 months (FGF responders) versus 1.5 months (FGF non-responders): Log rank p=0.001.

Non-significant relationships between CAF responder status and PFS are shown in table 5.6.

CAF	Responder – PFS (months)	Non Responder – PFS (months)	p (log rank)
IFNγ	4.2	5.2	0.37
IL1-β	5.3	3.9	0.34
IL6	5.3	4.4	0.90
IL8	5.3	3.2	0.62
PDGF	5.3	4.2	0.55
VEGFc	3.05	5.3	0.70
EGF	5.25	3.5	0.94
RANKL	5.3	3.5	0.71
TRAIL	5.25	3.5	0.84
VEGFa	5.25	3.5	0.53
VEGFd	5.3	3.2	0.23

Table 5.6. Non-significant relationships between CAF responders and PFS.

5.6 The relationship between CAF responders and QoL scores

CAF responders as defined previously were correlated with QoL scores for those patients experiencing a 10% or 20% increase in global health (GH), quality of life (QoL) or brief pain inventory (BPI) scores and Clinical benefit Response (CBR) rates from baseline lasting at least 4 weeks from first treatment. A two by two contingency table was created for each CAF across the 6 QoL measures described above. The correlations were then analysed using Fisher's exact test, with the Bonferroni correction for multiple testing applied. There was no correlation between any CAF change and QoL outcome measure (table 5.7).

CAF Responders	10% GH	20% GH	10% QOL	20% QOL	10% BPI	20% BPI	CBR
IFNγ	1.000	1.000	1.000	1.000	0.63	1.000	1.000
IL-1β	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IL-6	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IL-8	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TNFα	1.000	1.000	1.000	1.000	0.63	1.000	1.000
VEGF-c	1.000	1.000	1.000	1.000	1.000	1.000	1.000
EGF	1.000	1.000	1.000	1.000	1.000	1.000	1.000
RANKL	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TRAIL	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PDGF	1.000	1.000	1.000	1.000	1.000	1.000	1.000
HGF	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FGF	1.000	1.000	1.000	1.000	1.000	1.000	1.000
VEGF-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
VEGF-d	1.000	1.000	1.000	1.000	1.000	1.000	0.252

Table 5.7. CAF responders correlated with patient reported outcomes. Numbers are p values (all are non-significant). Key : 10% = an in increase in 10% over baseline scores. 20%= increase in 20% over baseline scores. GH= Global Health. QoL= Remainder of quality of life scores. BPI= Brief pain inventory scores. CBR= Clinical benefit response.

5.7 Baseline complement activity correlated with clinical outcome

Complement activity was assessable in 23 patients who completed at least 2 cycles of treatment. Complement activity for alternative and classical pathways was at least 100% of positive control in all patients at baseline. Baseline MBL complement pathway complement activity was low (<70% of positive control) in 10/23 patients (43.5%). Two groups were created according to baseline MBL complement activity: low activity or high activity. Kaplan-Meir curves were drawn to examine the relationship between baseline MBL complement activity, OS and time to progression (TTP) , with log rank analyses to evaluate these differences. There was no difference in OS or TTP between patients with high or low baseline MBL activity (figure 5.24 and 5.25).

Overall survival by baseline MBL activity
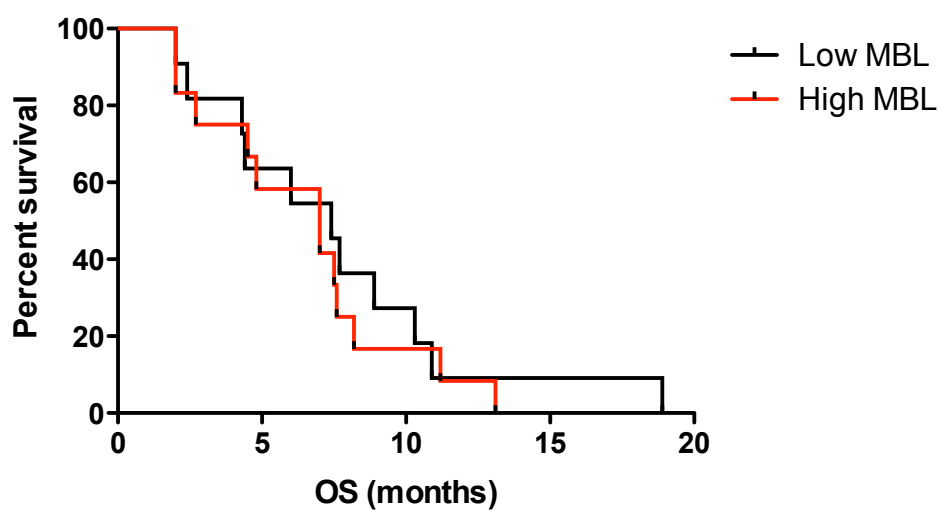
Figure 5.24. Overall survival by baseline MBL activity. Median OS = 7.0 versus 7.4 months: Log rank p=0.63.

Time To Progression by baseline MBL activity
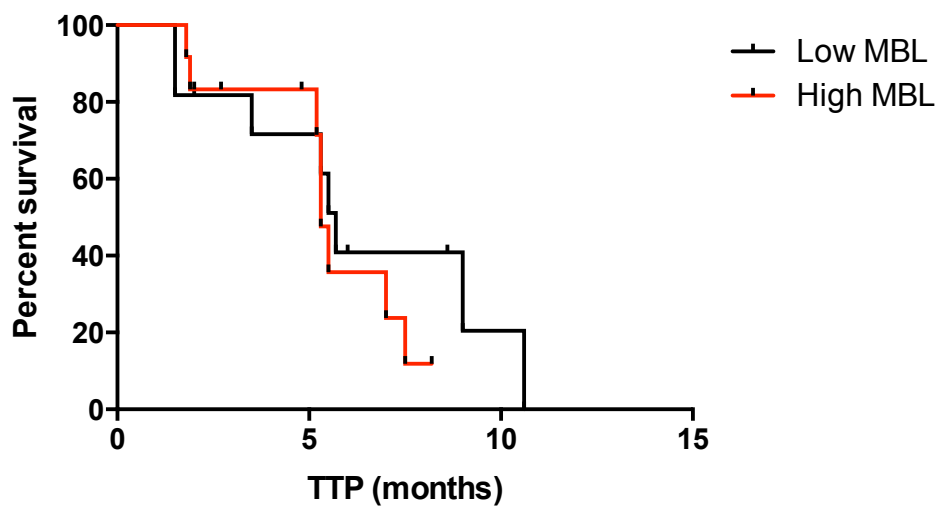
Figure 5.25. TTP by baseline MBL activity. Median TTP= 5.7 versus 5.3 months: Log rank p=0.44.

5.8 MBL pathway response correlated with clinical outcome

A further two groups were created out of the patients with low MBL baseline activity: those who had restoration of MBL activity to >70% of positive control and those who did not. Kaplan-Meir curves were drawn to examine the relationship between restored or unrestored MBL patients , OS and time to progression (TTP) , with log rank analyses to evaluate these differences. There was a tendency to improved OS in restored MBL patients (p=0.07- figure 5.26). There was a significant improvement in time to progression in restored versus unrestored MBL patients (p=0.03- figure 5.27).

Overall survival for restored vs unrestored MBL activity
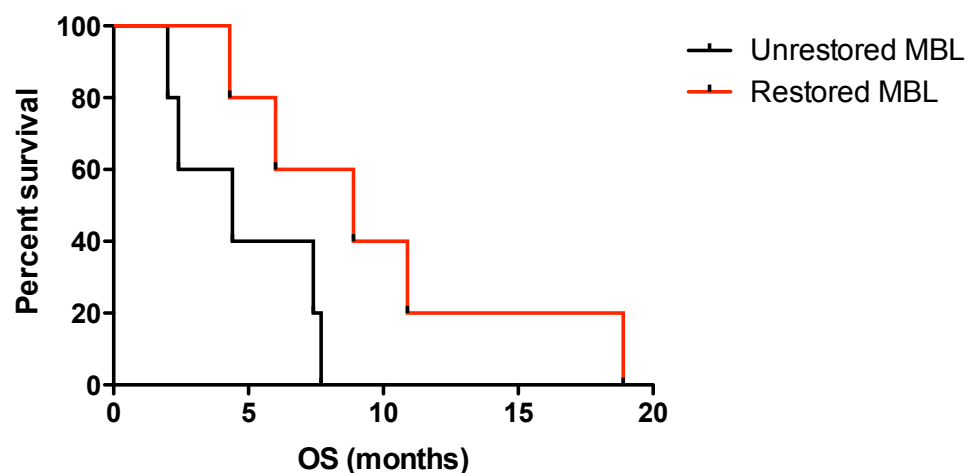
Figure 5.26. Overall survival by MBL restoration. Median OS = 8.9 versus 4.4 months: Log-rank p=0.07.

MBL restoration and Time To Progression
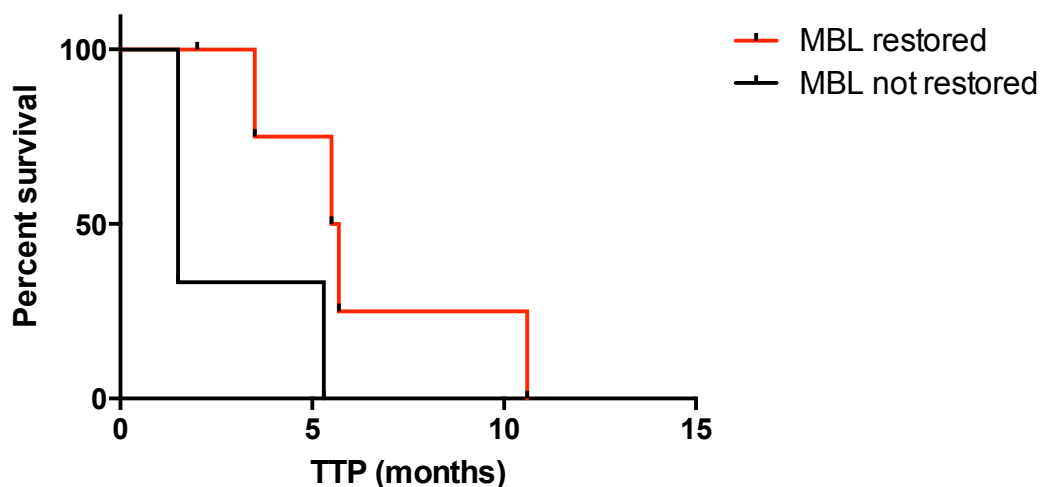
Figure 5.27. Time To Progression by MBL restoration. Median TTP = 5.6 versus 1.5 months: Log-rank p=0.04.

5.9 Uptake of n-3FAs into plasma phospholipids and erythrocyte cell membranes

5.9.1 Analysis of pre-treatment versus post-treatment FAME proportions for plasma NEFA

Chromatograms produced by gas chromatography analysis of plasma NEFA samples as described previously were analysed and proportions of each fatty acid out of the total were evaluated (Figure 5.28). Proportions in plasma NEFA taken immediately prior to treatment each week was compared to that taken within 10 minutes of infusion termination to evaluate changes in EPA (figure 5.29), DHA (figure 5.30) and all n-6 fatty acids (figure 5.31) with treatment. There were significant increases in proportions of all three of these groups in the post-treatment compared to the pre-treatment samples.
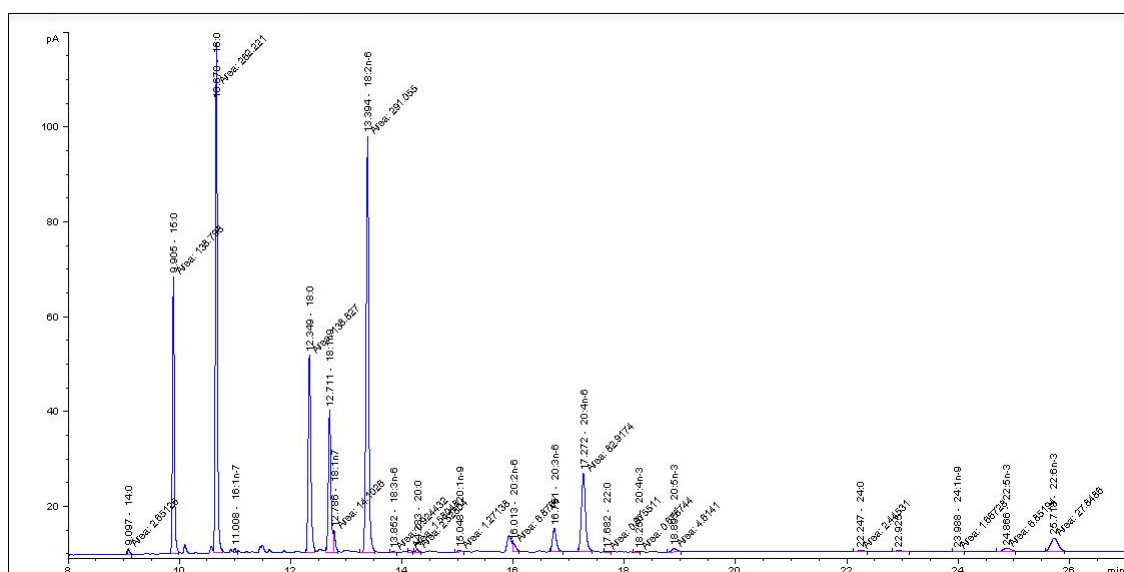
Figure 5.28. Chromatogram from plasma NEFA FAME analysis. As described previously, each peak is identified by known position of known standards along the x axis (time in minutes), and relative areas under each peak corresponding to position on y axis- signal in picoAmps (pA) is identical to relative proportions of FAMEs in the sample.
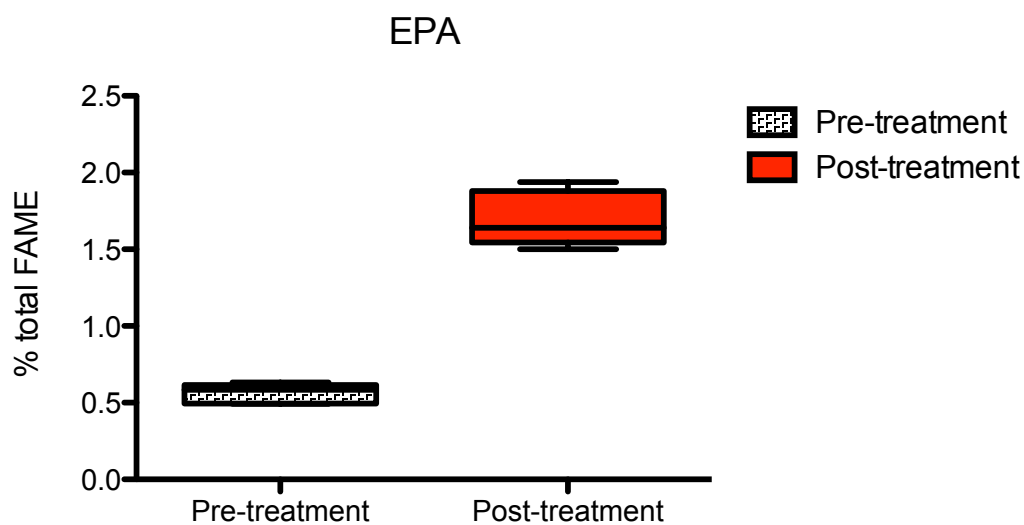
Figure 5.29. Box plot of pre-treatment versus post-treatment EPA in plasma NEFA: p=0.031. Whiskers are minimum to maximum values.

DHA
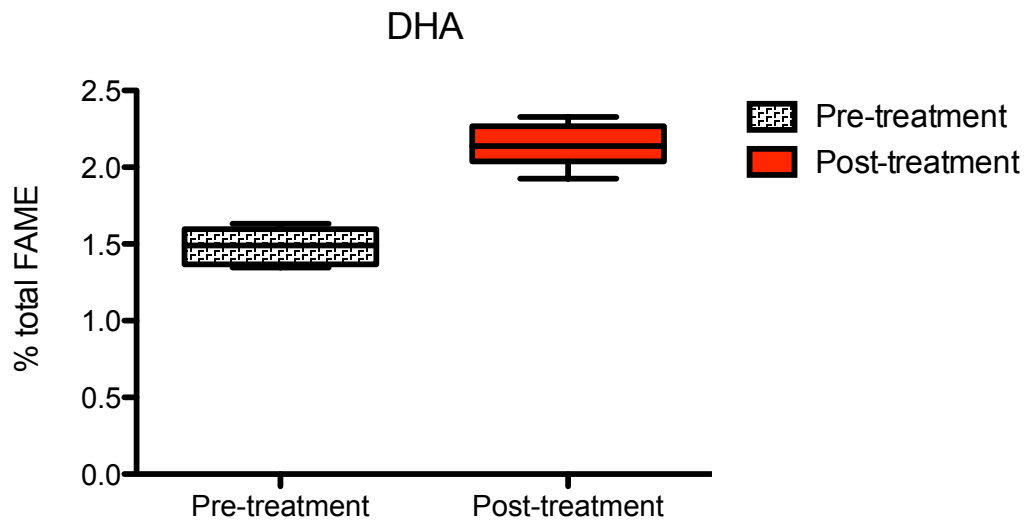
Figure 5.30. Box plot of pre-treatment versus post-treatment DHA in plasma NEFA: p=0.031. Whiskers are minimum to maximum values.

All n-6 fatty acids

Figure 5.31. Box plot of pre-treatment versus post-treatment n-6 fatty acids in plasma NEFA: p=0.031. Whiskers are minimum to maximum values.

There was no difference in n-6:n-3 FAME ratio for pre-treatment versus post-treatment samples in plasma NEFA. There was no difference in any FAME proportion or the n6:n3 FAME ratio for pre-treatment versus post treatment samples in plasma PC.

5.9.2 Analysis of pre-treatment versus post-treatment FAME proportions in erythrocyte cell membranes

Chromatograms produced by gas chromatography analysis of erythrocyte cell membrane (ECM) samples as described previously were analysed and proportions of each fatty acid out of the total were evaluated. Proportions in ECM taken immediately prior to treatment each week were compared to that taken within 10 minutes of infusion termination to evaluate changes in EPA (figure 5.32), DHA (figure 5.33) and all n-6 fatty acids (figure 5.34) with treatment. EPA concentrations in ECM showed a significant increase in the post-treatment versus pre-treatment samples whereas concentrations of DHA and all n-6 fatty acids showed a significant decrease.
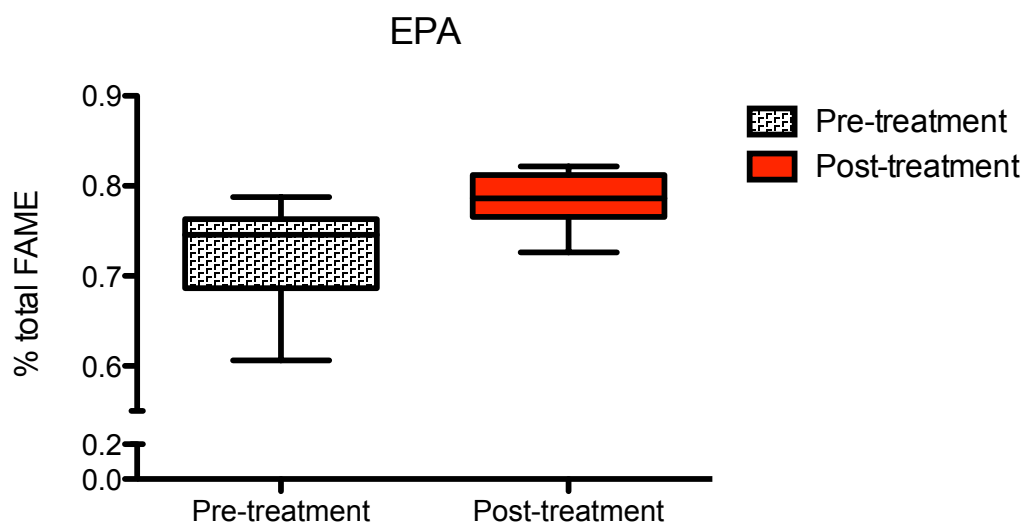
Figure 5.32. Box plot of pre-treatment versus post-treatment EPA in erythrocyte cell membranes: p=0.031. Whiskers are minimum to maximum values.

DHA
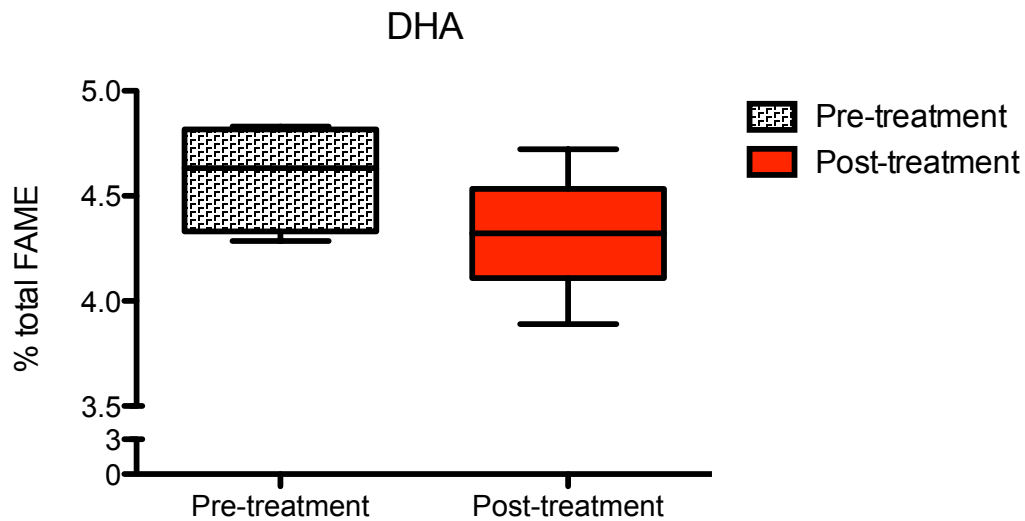
Figure 5.33. Box plot of pre-treatment versus post-treatment DHA in erythrocyte cell membranes: p=0.031. Whiskers are minimum to maximum values.

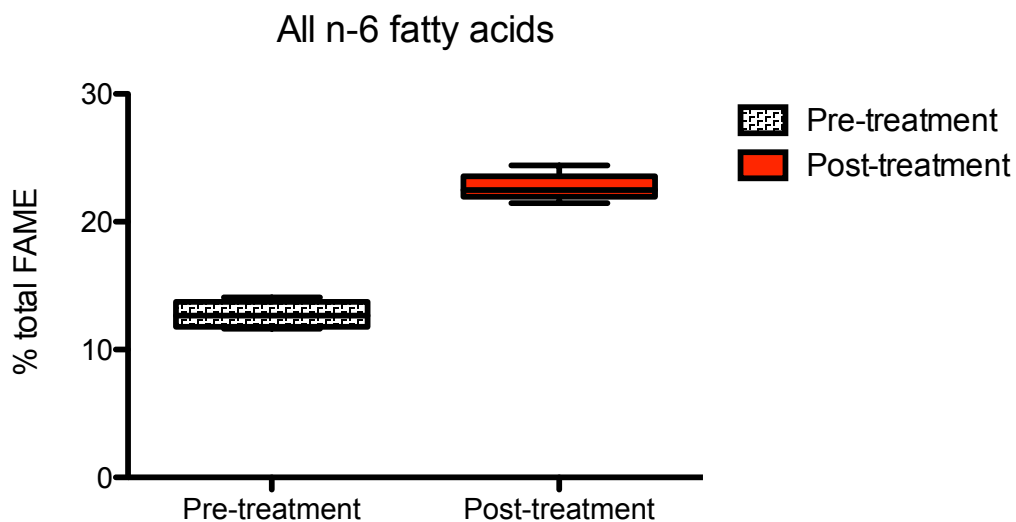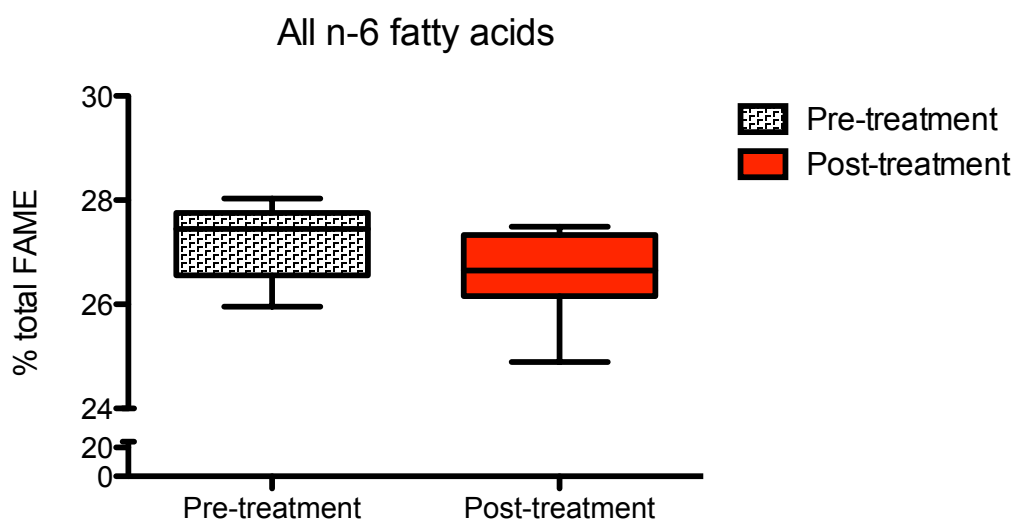## All n-6 fatty acids

Figure 5.34. Box plot of pre-treatment versus post-treatment n-6 fatty acids in erythrocyte cell membranes: p=0.031. Whiskers are minimum to maximum values.

There was no difference in n-6:n3 FAME ratio for pre-treatment versus post-treatment samples in erythrocyte cell membranes. There was no difference in any

FAME proportion or the n6:n3 FAME ratio for pre-treatment versus post treatment samples in plasma PC.

5.9.3 Analysis of changes in weekly pre-treatment FAME proportions in plasma and erythrocyte cell membranes with time

The same statistical model which was used to evaluate cytokine changes with time, namely a mixed multiple logistic regression model allowing for random effects, has been described previously and was used to evaluate changes in FAME proportions in plasma and ECM over the treatment course. In addition the total proportion of n-6 FAMEs was divided by the total proportion of n-3 FAMEs identified for each timepoint, and a ratio calculated to allow evaluation of changes in this ratio with time using the model.

There was no statistically significant change with time in any FAME proportions or the n6:n3 FAME ratio in pre-treatment plasma NEFA or PC samples.

In ECM there was a significant increase in EPA (figure 5.35) and DHA (5.36) with treatment course. There was a significant decrease in the n6:n3 ratio of FAME proportions over time (figure 5.37).
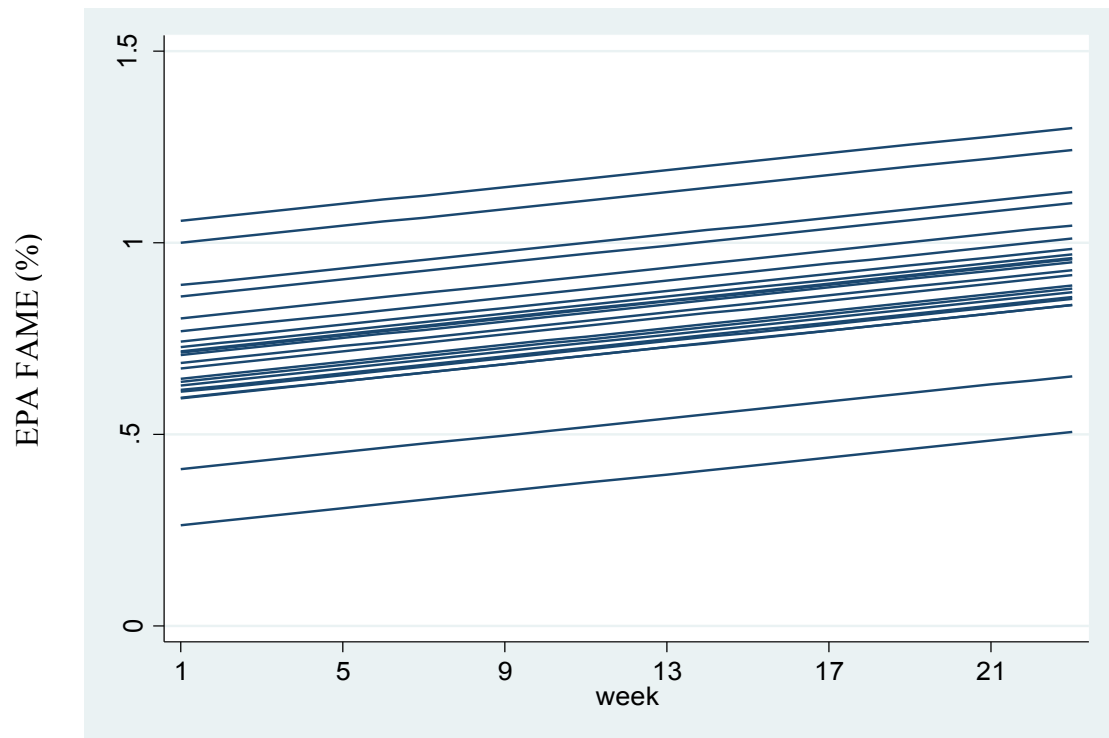
Figure 5.35. Statistical model output showing increase in erythrocyte cell membrane EPA FAME proportions with time: p=0.005. Proportion of FAME is shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.
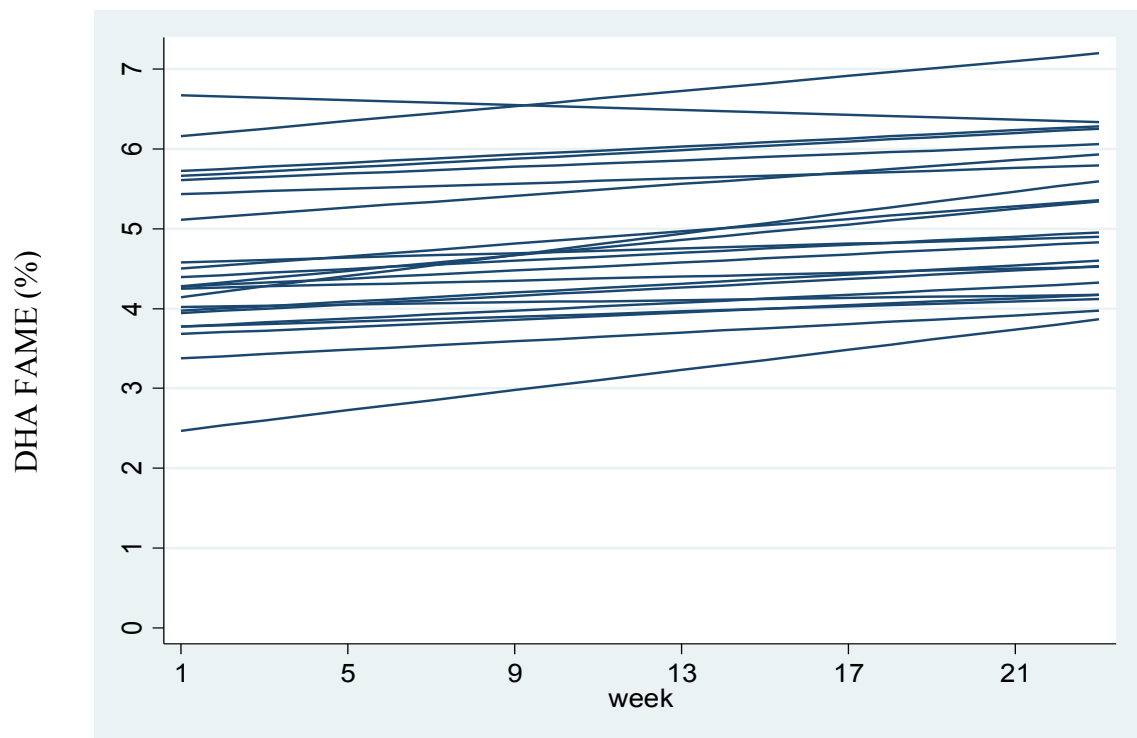
Figure 5.36. Statistical model output showing increase in erythrocyte cell membrane DHA FAME proportions with time: p<0.001. Proportion of FAME is shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.
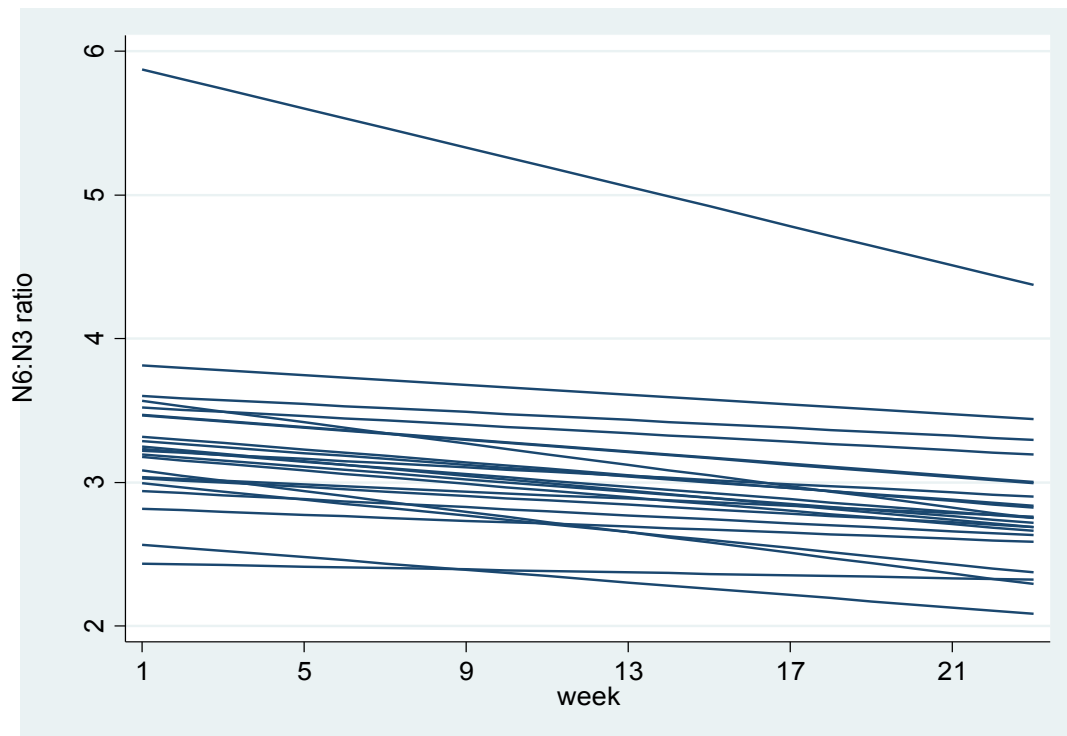
Figure 5.37. Statistical model output showing decrease in erythrocyte cell membrane n-6:n-3 ratio of FAME proportions with time: $p<0.001$. Ratio of n6:n3 is shown on the y axis, with time on the x axis. Each line represents a patient, with the best fit output from the model shown.

6 Discussion

6.1 Trial design

This trial was designed to answer the question of whether further study was indicated in the form of larger randomised trials using the investigational combination therapy of lipidem and gemcitabine in advanced pancreatic cancer. To this end a single arm-phase II design was chosen primarily to ensure that numbers of patients needed to complete recruitment and answer this question were realistic within the constraints of a two-year recruitment window. This was based on estimated recruitment into the trial of 10-15 patients per annum. The single-arm phase II design is well recognised in clinical oncology trials, but of course does not lend itself well to comparative analysis as there is no control group. Perhaps the most obvious criticism of the design therefore is the lack of randomisation with a control arm. This is important as without a control arm to which we may compare investigational data generated from the trial, investigators are limited to searching published literature to assess the effectiveness of trial treatments with potentially heterogenous groups used as the comparators. A historical control cohort was selected for analysis to try to provide an estimate of clinical efficacy of the investigational treatment. To eliminate bias, similar selection criteria for inclusion were applied in terms of lesions being evaluable on CT. This cohort had a significantly lower proportion of metastatic disease than the experimental group and so this could have affected the results in terms of a worse clinical outcome in the experimental group, which actually was not realised.

The trial was designed to add an investigational product: Lipidem, to the established standard of care in this patient cohort: single-agent gemcitabine. Lipidem was given

at the same treatment sitting as gemcitabine, on a weekly basis for three weeks followed by a rest week as this was the schedule that was thought to cause least disruption to this medically and physically frail patient cohort. However, this dosing schedule is open to criticism as this was the only consideration for it, and there were no detailed pharmacokinetic studies carried out prior to planning the study and writing the protocol. Lipidem had originally been designed for use as an additive in total parenteral nutrition, and all pharmacokinetic data to date had been obtained based on a much more prolonged duration of infusion (Simoens et al. 2008). It could be argued that the proposed biological effect of administering n-3FAs intravenously for such a short time with a long period in between each infusion would not lead to persistent biological change in the patient and that a more sustained intravenous administration, perhaps over a few days as an inpatient, would have been preferable. Whilst this may be true, it is certain that the both the initial recruitment rate would have been lower and the subsequent trial attrition rate higher should we have adapted this strategy. The possibility of adding oral n-3FAs to the schedule between intravenous administration dates could also have been studied, as this might have led to slower washout, more sustained uptake, and greater biological effect, especially during the rest week from chemotherapy. All patients who were already taking oral n-3FA supplementation before trial enrolment decided to stop taking these supplements once they were receiving parenteral n-3FA treatment, but there was no instruction or compulsion from the investigators for them to do this, nor were their decisions based on any known pharmacokinetic data.

 The recruitment rate for the trial of 29 patients from 34 screened was exceptionally high for a clinical trial in this challenging patient cohort. This is probably due to the perceived familiarity and safety of the product as well as the relatively convenient

dosing schedule with little over and above the standard interventions the patients would have received anyway. It should be noted that patients were re-imbursed for parking charges incurred while receiving trial therapy which would not have occurred should they have not been enrolled in the trial: however this was not thought to have played a significant role in their acceptance of enrolment. However this rate of recruitment bodes well for accrual into late phase large randomised controlled trials.

6.2 Clinical aspects

6.2.1 Response data

The primary outcome measure from the trial was response rate on CT, and the overall partial responses seen in 3/21 evaluable patients (14.3% of evaluable patients) were broadly comparable with other published clinical trial data (Arshad et al. 2011a) The disease stabilisation rate of 16/21 (85.7%) however is higher than expected, and when compared with our own historical control cohort is significantly higher. As can be seen by the waterfall plot, there was a majority of patients who had either static tumour dimensions or minor response not fulfilling RECIST partial response criteria out of the trial cohort. Response from serum CA19-9 data also compared very favourably with published trial data with the experimental cohort showing 10/21 (47.6%) of patients with raised CA19-9 experiencing a decrease of at least 50% in concentration. The realisation of 3 partial responses in the first 21 evaluable patients means that the first stage criteria of the study design was achieved and that recruitment could continue on to a total of 50 patients. This response rate is therefore considered favourable according to the study design, which was based on previous

published clinical trial data in patients with advanced pancreatic cancer treated with gemcitabine. It is debatable whether overall response rate on RECIST criteria is actually a good surrogate marker for clinical effectiveness in practice: it certainly has failed to show good correlations with overall survival in phase II trials which have been taken into phase III (Dhani et al. 2009). In fact there are multiple clinical trials using new regimens in advanced pancreatic cancer showing improved response rate in phase II which have failed to demonstrate overall survival benefit in randomised phase III trials (Kindler et al. 2010, Colucci et al. 2010, Cunningham et al. 2009). The reason that response rate is in widespread use in single arm phase II trials is that it is one of the only true objective measures, other than overall survival which is minimally open to bias. Overall survival is not a good outcome measure to use in small phase II trials because to show statistically significant differences of small amounts, very large sample sizes are required, such as in the erlotinib + gemcitabine trial (Moore et al. 2007). The costs of conducting trials of this magnitude both financially and in terms of staff and patient resources are very high, particularly if the investigational arm shows no benefit over standard care.

The high disease stabilisation rate is interesting in this study: of course it is not known whether this represents tumour stasis or a tumouricidal effect with lack of growth, or simply if the CT appearances of most tumours represent surrounding stroma and inflammatory tissue rather than active neoplastic tissue. Peri-tumour stroma is of particular importance in pancreatic cancer for several reasons. Firstly it may represent inflammation secondary to pancreatitis which in turn is caused by obstruction of the pancreatic duct by the tumour. This inflammatory tissue then causes release of pro-inflammatory cytokines and growth factors which may not only contribute to tumour growth, but also tumour-related cachexia. In addition it has been

postulated that the stroma may prevent adequate access to the tumour cells by chemotherapeutic agents by acting as a barrier between the blood and the cells (Feig et al. 2012). Finally, there is increasing evidence of tumour-stromal cross-talk in terms of cellular messengers released on both sides which may contribute to tumour resistance and growth in the presence of contemporary chemotherapeutic agents (Erkan et al. 2012). The reduction in growth factors and cytokines, which could be in part be due to by omega-3 fatty acids, could contribute to disease stabilisation by limiting new angiogenesis. It is not possible to comment with any certainty on whether this disease stabilisation on CT evaluation could be translated into improved progression free survival data, as although the trial data was promising, it was not powered for this outcome measure.

6.2.2 Survival data

Median overall survival for the cohort was broadly comparable to existing published trial data at 4.8 months and not significantly different to the historical control cohort (Arshad et al. 2011a). Median progression-free survival was slightly better than published trial data at 3.5 months and significantly superior to the historical control cohort. This is probably reflected by the high rate of disease stabilisation in the trial cohort. The trial was not powered to detect changes in overall or progression free survival as previously discussed. Nearly all late-phase randomised trials in clinical oncology are powered for a primary outcome of overall survival, with a select few using a primary outcome of progression-free survival. Progression-free survival is usually seen as an inferior endpoint to overall survival primarily because the outcomes are more open to variation. Overall survival is a very definite time period

starting on first treatment and ending on patient death. Progression free survival time period endpoints can be manipulated: one might suggest that timings of CT scans to look for progression are not always rigidly followed: a clinician might be less inclined to perform extra CT scans to look for progression if that was the primary outcome measure: whether trial withdrawal due to clinical progression in the absence of radiologically demonstrated progression counts as an event under progression free survival is also open to debate. In terms of power calculations used during the design of clinical trials, suggested differences that these studies are seeking to detect are usually in the order of 1-2 months resulting in a sample size of 500-600 patients across two arms. This is obviously not suitable for a small exploratory early phase trial such as this. The historical control data is certainly open to criticism. Firstly, our historical controls were simply taken as the unselected 26 patients who had undergone single-agent gemcitabine treatment in the calendar year prior to trial enrolment. The two cohorts not matched for baseline characteristics: in fact the historical cohort has a much lower proportion of metastatic disease than the trial cohort and thus should have superior outcomes. This was not seen, with the historical cohort overall survival being inferior to that expected from single-agent gemcitabine arms of large scale phase III clinical trials. Comparisons with historical control groups are always difficult due to inherent variations in patient selection and biases towards trial treatments, and these are acknowledged (Tannock 1992). This is one reason why the historical control group has not been more extensively compared to the trial cohort and only used as a guide to which the survival data can be gauged in an institutional context.

6.2.3 Quality of life outcomes and bodyweight changes

Rates of global health increase from baseline were high compared to existing trial data. An increase of 10% and 20% in scores compared to baseline were seen in 57% and 48% respectively over a period of at least 4 weeks. The only other study examining defined changes in global health is that by Reni, which showed 29% of patients in the single-agent gemcitabine group experienced a 10% or greater increase (Reni et al. 2006). Clinical benefit response (CBR) rates were also higher than existing trial data with 38% experiencing this, compared to 23.8% in Burris landmark trial and 20% for the Bernhard trial and 23% for the Colucci trial (Burris et al. 1997, Bernhard et al. 2008, Colucci et al. 2010). Some investigators criticise CBR as an outcome measure for cancer clinical trials: it was generated during the Burris trial when the more established survival outcomes had shown no difference between gemcitabine and 5-FU in pancreatic cancer patients, but the investigators had recognised improvements in certain other measures such as Karnofsky performance status, bodyweight and pain scores. Of course all of these three measures are open to wide variation in scoring and therefore criticism. Karnofsky performance status in particular is a physician assessment of health and is open to variation between assessing individuals and is highly subjective. Bodyweight in the Burris trial was not measured as lean body mass, but total bodyweight, which does not allow for fluid retention either as ascites or within interstitial compartments such as the legs. The high CBR rates demonstrated in the Lipidem and gemcitabine trial described in this thesis were obtained using not identical but similar criteria in the definitions. These outcomes may also be related to improved disease stabilisation by the trial regimen. The disease specific (PAN26) and non-disease specific (QLQC-30) changes in quality

of life are difficult to interpret in the absence of other published clinical trial data examining these domains, but appear to be encouraging.

Weight stabilisation or improvement was seen in 14/21 (66.7%) of patients. However it should be noted that total bodyweight was used, rather than lean body mass, the results of which do not account for fluid retention as a cause of weight gain as described above. Change in lean body mass (LBM), and even activity related outcome measures such as daily step counts are much more recognised clinical outcome measures in the context of tracking tumour-related cachexia in the modern era (Fearon et al. 2013). Future trials of n-3FAs in cancer settings would need to be designed with LBM as an outcome measure rather than total bodyweight changes as well as potentially utilising other activity related outcomes.

Overall the quality of life data seems to indicate positive activity of the trial regimen in improving these outcome measures and is certainly worthy of further investigation in randomised clinical trials not withstanding any effect on survival or response rates.

6.2.4 Adverse events and safety profile

The trial regimen had a good safety profile compared to the known expected events using gemcitabine alone in large scale randomised phase III trials (Arshad et al. 2011a). In fact the rates of grade 4-5 neutropaenia were lower both in the trial regimen and the historical cohort than in the pooled data from randomised clinical trials. In comparison to other novel biological agents which may be associated with debilitating side effects (such as erlotinib and grade 3-4 skin rashes), the side effect profile was excellent (Moore et al. 2007). Other adverse events were broadly comparable with the exception of a higher rate of grade 1-2 belching, nausea and

chills in the trial group. This is probably related to a high intravenous triglyceride load in a short period of time, but none of these adverse events developed into grade 3 or worse. Although these adverse events were perhaps deemed of minor importance to the investigators by CTCAE criteria, they were of major importance to the patients and resulted in dose reductions in all patients from the target treatment dose: either due to patient request, which had to be honoured, or the investigator's decision. Once it had been established that dose reductions were inevitable, a lowest treatment dose was established (250mL/50g) of Lipidem below which lack of tolerability would result in withdrawal from treatment by the investigator, but this was not necessary for any patient. One criticism of the planned dose in the trial is that it was not based on any detailed pharmacokinetic data and a phase I/II design might have led to a more evidence-based and therefore potentially successful design for a larger randomised trial.

6.3 Laboratory

6.3.1 Circulating cytokine and growth factor analysis

The hypothesis that intravenous n-3FA administration and uptake would reduce certain circulating cytokines and growth factors by the intrinsic action of COX-2 on the n-3FA component of the cell membrane lipid bilayer was supported in particular by the highly significant reduction in PDGF when analysed on a per-cycle basis. The fact that this PDGF reduction effect was lost during the rest week was also interesting, and could give rise to further questions when designing large-scale clinical trials such as if further n-3FAs should be given during the rest week and if this should be intravenously delivered or a given as a high-dose oral alternative. There is evidence

in both preclinical and clinical trials that n-3FAs may reduce production of PDGF. (Fox et al. 1988, Terano, Shiina & Tamura 1996, Kaminski et al. 1993).

When platelet count was analysed for all patients across the treatment course, there was no significant change. However, platelet count also reduced highly significantly when analysed on a per-cycle basis, with this effect lost during the rest week. PDGF is synthesised mainly by megakaryocytes, but also by macrophages, endothelial cells, fibroblasts, glial cells, astrocytes, myoblasts, smooth muscle cells and some tumour cells (Antoniades 1991). It is stored within platelets, and released after their activation (Ross et al. 1974). Therefore it could be argued that the reduction in PDGF during each cycle was in fact related to the reduction in platelet count, although the reduction in PDGF across the entire treatment course was probably unrelated to change in platelet count.

Reductions in FGF and TRAIL were also demonstrated with treatment across the cohort. FGF is known to be over-expressed in pancreatic cancer cells, and there is evidence that FGF inhibitors may have activity against mouse models of pancreatic neuroendocrine cancer (Wagner et al. 1998, Allen et al. 2011). However FGF inhibition as a target in pancreatic cancer is relatively novel, and there are no published clinical trials using this strategy to date. Pre-clinical data suggests that n-3FAs have no effect on FGF production, but this is limited to two small studies and further work is required to examine this relationship (Yang et al. 1998, Murota, Onodera & Morita 2000). TRAIL is a member of the TNFα family which induces apoptosis by activating NFKB, which as mentioned previously can be down-regulated by n-3FA administration (Wiley et al. 1995, Musiek et al. 2008, Bouwens et al. 2009). There are limited reports linking the effects of DHA and EPA on TRAIL-mediated apoptosis in tumour cells in laboratory experiments (Vaculova et al. 2005, Tsuzuki et

al. 2007b). However the mechanism by which n-3FAs might cause a reduction in TRAIL across the treatment course is unclear or what effect this could have had on tumour cell apoptosis in the absence of a control group. The apparent increase in VEGF-d cannot be readily explained, particularly as there was no change in VEGF-a, or c. There are several pre-clinical studies and one small trial in healthy human subjects using oral n-3FAs which show that n-3FAs can reduce production of VEGF as a whole (Yang et al. 1998, Tsuzuki et al. 2007a, Tsui et al. 2003, Ambring et al. 2006). However changes in the concentrations of individual sub-types of VEGF were not analysed by these studies. Certainly VEGF-d has been shown to play a pivotal role in lymphangiogenesis and lymphatic metastasis in models of pancreatic cancer (Von Marschall et al. 2005). It may also the most potent initiator of angiogenesis and lymphangiogenesis of the VEGF family (Rissanen et al. 2003). It may be possible that VEGF-d production escaped control by the trial treatment, while the production of the other VEGF family growth factors was stabilised, as these did not show an increase in concentration over the treatment course. Whether these effects are caused by gemcitabine or intravenous n-3FAs, or are independent of the treatment given is of course unknown and open to criticism. The effects could only be properly assessed in a randomised controlled trial.

The correlations of baseline IL-6 and IL-8 with overall and progression free survival are certainly interesting. It can be argued that this effect is independent of n-3FA administration and that raised serum IL-6 and IL-8 concentrations at baseline are simply a surrogate biomarker for poor outcome regardless of any treatment. Indeed high pre-chemotherapy IL-6 concentrations have been shown to be correlated with reduced overall survival in patients with APC receiving single-agent gemcitabine (Mitsunaga et al. 2013). However the putative link between markers of severe

inflammation and neoplastic progression and ultimately death is related to the basic hypothesis and rationale of the trial, and the differences could be explained by those patients having hyper-active inflammation being too far advanced to have benefit from the trial regimen.

Correlations of reductions in serum CAF concentration with overall and progression free survival revealed a tendency to improved overall survival with PDGF responders (those showing a >30% decrease in serum PDGF concentration during treatment). As mentioned previously, this figure of 30% is recognised as arbitrary, but was deemed reasonable as there was no previous published data correlating reduction in cytokine concentrations over treatment courses with clinical outcomes in cancer patients.

In conjunction with the demonstrated reduction in PDGF across the cohort this is perhaps the most striking result from the cytokine work. FGF responders also had improved outcome compared to non-responders. However as there were only 2 non-responders, the data is difficult to interpret in terms of the Kaplan-Meir survival curves. However the statistical analyses allow for this and demonstrated highly significant differences.

Correlations of CAF responders with QoL and CBR outcomes revealed no significant results when the Bonferroni correction for multiple testing was applied.

6.3.2 Complement analysis

Baseline complement activity analysis revealed full activity across all patients in the alternative and classical pathways. Attention was focussed on the mannose binding lectin (MBL) pathway as there were some patients who had hypoactivity in this at baseline. As for the CAF responders, the definition of hypoactivity in the MBL

pathway was fairly arbitrary, with a cutoff of 70% of positive control, but in the absence of any published data on the relationship of MBL activity and clinical response in pancreatic cancer this was deemed reasonable. There were no correlations between baseline MBL activity and either overall or progression free survival in the trial cohort. However, when analysing MBL "responders", or those who had hypoactive MBL activity at baseline, which was then restored to positive control levels, these responders had a significantly improved time to progression and a tendency to improved overall survival over non-responders. It is probable that hypoactivity of complement actually represents consumption of the factors detected by the assay, and in fact when the complement assay activity is restored with treatment, this represents reduction in consumption of complement and actually attenuated biological activity. The mechanisms of action of the clinical correlations with complement activity are not clear but several hypotheses are possible. Complement including the MBL pathway, along with inflammatory cytokines, growth factors and prostaglandins may all act to activate myeloid derived suppressor cells (MDSC), which may in turn block anti-tumour immunity by the host (Ostrand-Rosenberg 2008). There are no prior published studies examining the relationship between complement and n-3FAs. Once again it is not possible to say if it whether the gemcitabine or n-3FA component or the combination of the two in the regimen is responsible for the action, but this would be addressed in a large scale randomised trial.

6.3.3 Uptake of n-3FAs into erythrocytes and plasma phospholipids

In plasma NEFA samples, EPA, DHA and all n-6 FAME proportions showed a significant increase for post-treatment compared to pre-treatment samples. This may have represented physiological uptake or spill-over from adipose tissue, but as NEFA are essentially free fatty acids, it may also simply represent un-cleared fish oil infusion as it is likely that fatty acids present in the infusion are in this form in any case, and the samples were taken within 20 minutes of stopping the infusion.

The data for erythrocyte cell membrane FAME proportion changes immediately post treatment is interesting. This shows a statistically significant increase in EPA, which must represent rapid uptake of EPA from the infusion into cellular membranes, certainly within hours of the infusion start. However, DHA showed a significant decrease which is difficult to explain. Certainly the comparative proportions of EPA and DHA present in Lipidem are not clear in the manufacturers summary of product characteristics: it simply states the total EPA+DHA. It is possible that some batches of the product contain negligible concentrations of DHA and much higher concentrations of EPA. Furthermore, it is possible in these patients that aelongation and desaturation of EPA to produce DHA from the infused product had not taken place by the time the sample was taken. Finally, the postulated higher concentrations of EPA may have undergone uptake into cells competitively against DHA. In a similar fashion, the explanation for a statistically significant reduction in n-6 FAME proportions post-treatment versus pre-treatment is unclear. One explanation may be that n-6 uptake into cell membranes was competitively reduced by the presence of relatively high n-3FA concentrations and that the increase in n-3FA proportions in the cell membrane leads to a relative decrease in the n-6FA proportions.

The increase in EPA proportions out of total red cell membrane lipid in the post-treatment samples compared to pre-treatment was only of the order of 1.0%. Over the entire treatment course, the increase in pre-treatment weekly EPA proportions, although significant, was of the order of 0.5% or less. The increase in DHA proportions was of the order of 1.0% over the treatment course. The proportion of EPA and DHA resulting from uptake of oral n-3FA preparations in order to provide biological effects has been well studied. In cancer patients, improved immunological function was demonstrated using relatively modest oral doses of n-3FAs (0.3g EPA and 0.4g DHA daily) correlated with an increase in EPA concentration of 0.42% and DHA of 0.25% from baseline in neutrophil cell membranes (Bonatto et al 2012). Improved immune function in healthy volunteers had been shown with oral n-3FA supplementation with increase in EPA concentration of 2.3% over baseline (Faber et al 2011).

There was no change in pre-treatment plasma NEFA or PC FAME proportions over the treatment course. However, erythrocyte cell membrane EPA and DHA FAME proportions showed a significant increase over the treatment course of up to 24 weeks. This is the longest period of time for which intravenous n-3FA supplementation has ever been given to a cohort of patients in a clinical trial, and certainly generates novel pharmacokinetic data. Indeed there did not seem to be the plateau in n-3FA proportions over the initial weeks which have been described previously by other studies (Gibney, Hunter 1993). The fact that pre-treatment proportions increased each week indicates that the weekly schedule of high dose intravenous infusion of n-3FA was sufficient to have an effect on uptake even though there were at least 6 days between infusions, with 13 days after each cycle. Interestingly, the n-6:n-3 ratio also showed a significant decrease with time,

indicating competitive inhibition of n-6FA uptake by n-3FAs, which is in keeping with the aim of the study therapeutic intervention and the original hypothesis. It is therefore proposed that the increased uptake of n-3FAs observed in cells may be responsible for the effects observed in both clinical and laboratory outcomes previously described.

6.4 Cost Analysis

One of the features lending n-3FAs to further study in this setting is the relatively low cost of treatment compared to existing novel agents. For example a six- cycle course of Lipidem as used in this study, allowing for full dosage each occasion and no missing treatments would cost £684. The cost of gemcitabine for the same treatment course in an average sized patient is £4,500. The only novel agent approved for use by the FDA in the USA, Erlotinib would cost an additional £7,944 for the same treatment course.

6.5 Further suggested work

Phase II trial completion to enrol 50 patients is underway, with the protocol for a randomised phase II trial leading in to a randomised phase III trial drafted, to be activated pending full results. This is based on survival endpoints as the primary outcome measure, with response rates and HRQOL outcomes as the secondary measures. Work to further elucidate the mechanisms of interaction of the trial regimen with MDSCs in particular is underway using an automated cell sorter to assess for changes in number of MDSCs with treatment. Correlations of tumour

sarcopenia on CT imaging with clinical outcome and cytokine changes in the trial cohort are also planned.

Novel endpoints for future phase II trials in cancer patients using n-3FA based therapy in particular can and probably should include items such as change in lean muscle mass and change in markers of activity such as step count using a portable pedometer (Gallagher et al. 2012). These are hard endpoints with clearly defined values which are easily assessable and for change in lean muscle mass in particular, much less prone to bias than traditional endpoints such as progression free survival (Dhani et al. 2009).

In terms of pre-clinical studies, work to further elucidate the mechanisms of anti-neoplastic and anti-inflammatory action of n-3FA related metabolites, in particular in combination with current chemotherapeutic regimens is indicated. The interaction of n-3FAs with complement, in particular MBL-pathway components requires further study. Novel models which can better represent pancreatic cancer in human subjects, in particular allowing for stromal reaction around tumour cells have been developed to accurately assess planned therapeutic intervention in the laboratory setting, moving away from simple cell line incubation with these agents both in vitro and in vivo.

Should the novel FOLFIRINOX regimen be adopted widely in favour of gemcitabine in patients with adequate performance status, this regimen should also be studied as an arm of a controlled trial in addition to gemcitabine (Conroy et al. 2011). However it is anticipated that FOLFIRINOX patients may occupy a different niche bearing in mind the worse toxicity profile compared to gemcitabine and the prevalence and severity of cancer cachexia leading to impaired performance status typically seen in this group of patients.

Appendix 1 : ECOG performance status

GRADE	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature
2	Ambulatory and capable of all self care but unable to carry out any work. Up and about more than 50% of waking hours
3	Capable of only limited self care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry out any self care. Totally confined to bed or chair
5	Dead

Appendix 2 : EORTC QLQ-C30 and PAN-26 questionnaires

PATIENT ID

DATE OF COMPLETING THIS QUESTIONNAIRE : ___ / ___ / _____

WEEK / CYCLE ____ /____

EORTC QLQ-C30/QLQ-PAN26

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.

		Not at All	A Little	Quite a Bit	Very Much
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 <u>long</u> 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 activities?	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

		Not at All	A Little	Quite a Bit	Very Much
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
17.	Have you had diarrhoea?	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 you social activities?	1	2	3	4
28.	Has you physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions circle the number between 1 and 7 that best applies.

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

    1   2   3   4   5   6   7

    Very Poor   Excellent

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

    1   2   3   4   5   6   7

    Very Poor   Excellent

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems <u>during the past week</u>. Please answer by circling the number that best applies to you.

		Not at All	A Little	Quite a Bit	Very Much
31.	Have you had abdominal discomfort?	1	2	3	4
32.	Did you have a bloated feeling in your abdomen?	1	2	3	4
33.	Have you had back pain?	1	2	3	4
34.	Did you have pain during the night?	1	2	3	4
35.	Were you uncomfortable in certain positions (e.g. lying down)?	1	2	3	4
36.	Were you restricted in the types of food you can eat as a result of your disease or treatment?	1	2	3	4
37.	Were you restricted in the amounts of food you could eat as a result of your disease or treatment?	1	2	3	4
38.	Did food and drink taste different from usual?	1	2	3	4
39.	Have you had indigestion?	1	2	3	4
40.	Were you bothered by gas (flatulence)?	1	2	3	4
41.	Have you worried about you weight being too low?	1	2	3	4
42.	Did your arms and legs feel weak?	1	2	3	4
43.	Did you have a dry mouth?	1	2	3	4
44.	Have you had itching?	1	2	3	4
45.	To what extent was your skin yellow?	1	2	3	4
46.	Did you have frequent bowel movements?	1	2	3	4

Please go on to the next page

		Not at All	A Little	Quite a Bit	Very Much
47.	Did you feel a sudden urge to have a bowel movement?	1	2	3	4
48.	Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
49.	Have you been dissatisfied with your body?	1	2	3	4
50.	To what extent have you been troubled by side-effects from your treatment?	1	2	3	4
51.	Have you been worried about what your health might be like in the future?	1	2	3	4
52.	Were you limited in planning activities in advance (e.g. meeting friends)?	1	2	3	4
53.	Have you received adequate support from your health care professionals?	1	2	3	4
54.	Has the information given about your physical condition and treatment been adequate?	1	2	3	4
55.	Have you felt less sexual enjoyment?	1	2	3	4

Appendix 3 : Brief Pain Inventory questionnaire

PATIENT ID _____

DATE OF COMPLETING THIS QUESTIONNAIRE : ___ / ___ / _____

WEEK / CYCLE ___ / ___

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains and toothaches). Have you had pain other than these every-day kinds of pain today ?
    YES / NO

2. Please rate your pain by circling the one number that best describes your pain at its **worst** in the last 24 hours

    0   1   2   3   4   5   6   7   8   9   10
    No pain

    Pain as bad as you can imagine

3. Please rate your pain by circling the one number that best describes your pain at its **least** in the **last 24 hours**

    0   1   2   3   4   5   6   7   8   9   10
    No pain

    Pain as bad as you can imagine

4. Please rate your pain by circling the one number that best describes your pain on **average**

    0   1   2   3   4   5   6   7   8   9   10
    No pain

    Pain as bad as you can imagine

5. Please rate your pain by circling the one number that tells how much pain you have **right now**

0   1   2   3   4   5   6   7   8   9   10
No pain

Pain as bad as
you can
imagine

6. What treatments or medications are you receiving for your pain?

_____

7. **In the last 24 hours**, how much relief have pain treatments or medications provided ? Please circle the **one percentage** that shows how much relief you have received.

0%   10%   20%   30%   40%   50%   60%   70%   80%   90%   100%
   No relief
Complete

Relief

8. Circle the one number that describes how, **during the past 24 hours**, pain has interfered with your :

A. General Activity

0   1   2   3   4   5   6   7   8   9   10

No Interference   Completely
                                                   Interferes

B. Mood

0   1   2   3   4   5   6   7   8   9   10

No Interference   Completely
                                                   Interferes

C. Walking ability

0   1   2   3   4   5   6   7   8   9   10

No Interference   Completely
                                                   Interferes

D. Normal Work (includes both work outside the home and housework)

| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

No Interference   Completely
                          Interferes

E. Relations with other people

| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

No Interference   Completely
                          Interferes

F. Sleep

| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

No Interference   Completely
                          Interferes

G. Enjoyment of life

| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

No Interference   Completely
                          Interferes

Appendix 4 : Lipidem composition data

1000 mL emulsion contains:

Medium-chain triglycerides	100.0 g
Soya-bean oil, refined	80.0 g
Omega-3-acid triglycerides	20.0 g

Content of essential fatty acids per litre:

Linoleic acid (omega-6)	38.4 - 46.4 g
Alpha-linolenic acid (omega-3)	4.0 - 8.8 g
Eicosapentaenoic acid and docosahexaenoic acid (omega-3)	8.6 - 17.2 g

200 mg/mL (20%) correspond to total content of triglycerides.

Total energy per litre	7900 kJ ≅ 1910 kcal
	6.5 - 8.5

Excipients:
1000 mL emulsion contains 2.6 mmol sodium (as sodium hydroxide and oleate)

Pharmaceutical form

Emulsion for infusion
White, homogeneous emulsion.

Osmolality	approximately 410 mOsm/kg
Titration (to pH 7.4)	less than 0.5 mmol/l NaOH or HCl
pH	6.5 - 8.5

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