Translating curcumin into clinical practice for treatment of metastatic colorectal cancer: the CUFOX trial

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ABSTRACT

Translating curcumin into clinical practice for treatment of metastatic colorectal cancer: the CUFOX trial - Chinenye Oluchi Obiageri Iwuji

Palliative treatment of metastatic colorectal cancer provides an overall median survival rate of approximately 21 months, with response rates of less than 60%. Curcumin is a low molecular weight polyphenol derived from the spice turmeric that inhibits carcinogenesis *in vitro* and *in vivo* via multi-targeted mechanisms. A clinical study was established investigating the safety and feasibility of administering curcumin with standard oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer and in parallel biomarker analysis was conducted to identify potential biomarkers of efficacy and toxicity.

Methods: Phase I was a dose escalation phase using the traditional escalation rule (3+3+3) design to establish the maximum target dose of curcumin. Phase IIa was an open-labelled, two-armed, randomised controlled feasibility trial. Patients received standard oxaliplatin and 5-FU chemotherapy with or without the maximum target dose of curcumin established in Phase I. Biomarker studies were conducted involving measurement of miR-122, curcumin/curcuminoids and DNA platination in patient plasma samples, and proteomic analysis of treated explant media from patient-derived colorectal liver metastasis.

Results: Phase I dose escalation was successfully completed with thirteen patients receiving curcumin plus standard oxaliplatin-based chemotherapy up to the target dose of 2 grams daily with no significant issues identified with toxicity or feasibility. Eighteen patients had been recruited into Phase IIa at the time of this report with no notable safety concerns. Changes in miRNA and curcumin/curcuminoid levels were successfully measured in patient plasma samples. Explant culture analysis showed proteins involved in apoptosis, angiogenesis and inflammation/immune response were selectively upregulated following treatment with CUFOX.

Conclusion: Addition of curcumin to standard FOLFOX chemotherapy up to a dose of 2 grams daily has shown good tolerability, feasibility and no safety concerns across Phase I and IIa of this study. Potential biomarkers for future investigation have been identified.

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LIST OF ABBREVIATIONS

5-FU	5-Fluorouracil
5-FUTP	5-fluorouridine triphosphate
8-oxo-dG	8-oxo-7,8-dihydro-2'-deoxyguanosine
ACTN	α-actinin
ADH	Alcohol dehydrogenase
AJCC	American Joint Committee on Cancer
AP-1	Angiopoietin-1
AP-2	Angiopoietin-2
APC	Adenomatous polyposis coli
bDMC	Bisdemethoxycurcumin
bFGF	Basic fibroblast growth factor
BMI	Body mass index
BSA	Bovine serum albumin
CDC25	Cell division cycle 25
CDF	Cancer drugs fund
CEA	Carcinoembryonic Antigen
CID	Collision induced dissociation
CRC	Colorectal cancer
CST	Curcumin and standard treatment
CTC AE	Common Terminology Criteria for Adverse Events
COX	Cyclo-oxygenase
DAVID	Database for annotation, visualization and integrated discovery
DCC	Deleted in colorectal cancer
DLT	Dose-limiting toxicity
DMC	Demethoxycurcumin
DMEM	Dulbecco's modified eagle's medium
DPD	Dihydropyrimidine dehydrogenase
DTT	DL-Dithiothreitol
ERK	Extracellular-signal-regulated kinases
ESI	Electrospray ionisation

F-dUMP	5-5-fluoro-2'-deoxyuridine-5'-O-monophosphate
FAP	Familial adenomatous polyposis
FCS	Foetal calf serum
FLN	Filamin
FOBT	Faecal occult blood test
FPP	Ficoll-Paque PLUS
GERCOR	Groupe Cooperateur Multidisciplinaire en Oncologie
GFP	[Glu1]-Fibrinopeptide
GST	Glutathione *S*-transferase
HNPCC	Hereditary non-polyposis colorectal cancer
HSP	Heat shock protein
IAA	Iodoacetamide
IMS	Ion mobility separator
iNOS	Inducible nitric oxide synthase
IP_3R	Inositol 1,4,5-triphosphate receptor
JNK	c-Jun N-terminal
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIF1B	Isoform 3 of kinesin-like protein KIF1B
KIR3DL1	Killer cell immunoglobulin-like receptor 3DL1
KRAS	Kirsten rat sarcoma 2 viral oncogene homologue
M_1dG	Pyrimidopurinone adduct of deoxyguanosine
MAPK	Mitogen-activated protein kinases
MAPKAPK	Mitogen-activated protein kinase-activated protein kinase
mCRC	Metastatic colorectal cancer
MEK1	Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Kinase 1
MEKK1	Mitogen-activated protein kinase kinase kinase 1
MMP1	Metalloproteinase inhibitor 1
MOSAIC	Multicentre International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer
MTD	Maximum target dose
NCI	National Cancer Institute

NGAL	Neutrophil gelatinase-associated lipocalin precursor
NO	Nitric oxide
NF-κB	Nuclear factor kappa B
NSAIDS	Non-steroidal anti-inflammatory agents
PBL	Peripheral blood lymphocytes
PBS	Phosphate buffered saline
PFS	Progression-free survival
PG	Prostaglandin
PGE_2	Prostaglandin E_2
PIH1D1	Protein interacting with Hsp90 1 domain-containing protein 1
PKG	Protein kinase G
PKM2	Pyruvate kinase type M2
PLGS	Protein Lynx Global Server
PP1	Protein phosphatase 1
POLR3D	DNA-directed RNA polymerase III subunit RPC4
RECIST	Response Evaluation Criteria in Solid Tumours
RPAP	RNA polymerase II-associated protein
ROS	Reactive oxygen species
RR	Response rates
SAPK	Stress-activated protein kinases
SEK1	Stress-activated protein kinase/extracellular-signal regulated kinase 1
SMC3	Structural maintenance of chromosomes 3
ST	Standard treatment
TIC	Tumour initiating cells
TS	Thymidylate synthase
UHL	University Hospitals of Leicester
UPLC	Ultraperformance Liquid Chromatography
UTP	Uridine triphosphate
UK	United Kingdom

1. Introduction

1.1. **Epidemiology**

Colorectal cancer (CRC) is the fourth most common form of cancer in the UK after breast, lung and prostate cancer (excluding non-melanoma skin cancer) with over 40,000 new cases diagnosed each year[1]. The lifetime risk of developing CRC has increased for both men and women in the UK[2] leading to increased attention on the management of this disease. Management of colorectal cancer has significantly improved over the past 40 years, however current standard treatment of metastatic colorectal cancer using FOLFOX chemotherapy with or without biological agents still only has a response rates of less than 60% (47% - 54%)[3,4]. Furthermore, 50% of patients treated with curative intent will develop recurrent disease[5]. Efforts to improve outcomes for CRC are aimed not only at improving patient care in terms of optimising management and patient quality of life, but also in encouraging research in areas such as chemoprevention, early detection, tailor-made treatments and improving survival.

Factors including age and economic development contribute to colorectal cancer incidence. Developed countries lead in incidence with 60% of CRC cases being diagnosed in the West[6], whilst 86% of diagnoses are made in patients over age 60[2]. The distribution between the genders is equal until age 50, after which the rates become higher in men. In comparison to 1975, the lifetime risk has increased from 3.5% to 6.9% in men, and from 3.9% to 5.4% in women[2].

In 2012, there were 16,187 deaths from CRC in the UK[7]. It is the second most common cause of cancer deaths in the UK[7]. Fortunately this mortality rate has been consistently decreasing over the last 40 years across sexes and age groups. The 5-year survival rate for colon cancer in men has almost doubled since the 1970s from 22% to 50%, and in women from 23% to 51%. The same improvement is seen in rectal cancers (men: 25% to 51%; women: 27% to 55%). This improvement in outcome has been attributed to multiple factors including earlier detection, improved diagnostic techniques, more public awareness and better treatment. The bowel cancer screening program initially started in England in 2006 and has now been implemented throughout the UK. In England, this screening program has been shown to reduce the risk of dying from bowel cancer by 25%[2].

Another determining factor in a patient's prognosis is the presence of metastatic disease. The 5-year survival for patients diagnosed with early stage disease (Dukes A disease - not invading the bowel wall) is 93% compared to 7% in patients diagnosed with widespread metastases (Dukes D stage)[2]. Twenty-five per cent of patients diagnosed with CRC present with metastatic disease[8]. The liver is the primary site for disease metastasis with almost 50% of patients having liver metastases either at primary diagnosis or with disease recurrence[9].

1.2. **Aetiology and Risk Factors**

The causes of CRC appear to be a combination of inherited susceptibility and environmental factors, ranging from diet and body habitus to family history.

1.2.1. Lifestyle

More than half of the cases of CRC diagnosed in the UK are thought to be linked to diet and environmental factors[10]. A higher incidence is particularly linked to the western diet, where populations also tend to have greater proportions of obesity and lower levels of exercise [11]. Diets that are high in fibre reduce the risk of CRC. Research also suggests that garlic, milk and calcium probably have a protective effect, whilst there is convincing evidence that red and processed meat increase the risk of CRC[12]. There is limited evidence that vegetables, fruit and dietary vitamin D may also reduce the risk of bowel cancer.

The risk of CRC is greater in overweight and obese individuals, particularly men. Overweight men (body mass index/BMI 25 – 29.9kg/m^2) have a 23% greater risk of colon cancer compared to healthy men (BMI < 25). The risk increases further to 53% in obese men (BMI ≥ 30 kg/m^2)[13]. Physical activity, on the other hand, has been shown to lower the risk of colon cancer by up to 24%[14]. Both cigarette smoking and alcohol intake have also been linked to an increased risk of CRC. Data for the UK in 2010 suggests that 11% of bowel cancers were linked to alcohol and 8% to cigarette smoking[15,16].

1.2.2. Family history

Family history contributes to 25% of CRCs[17]. This is either by inherited syndromes (e.g. familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer) or by familial colorectal cancers where families have an increased incidence of CRC but no identifiable hereditary syndrome.

Approximately 5% of CRCs are associated with the genetic predisposition syndromes: familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC; also called Lynch syndrome)[18]. FAP is an autosomal dominant inherited disorder caused by a mutation in the adenomatous polyposis coli (APC) gene. This results in numerous adenomatous polyps developing in the colon, particularly the epithelium of the large bowel. Initially these polyps are benign, but then undergo malignant transformation into colon cancer. If left untreated, almost all FAP patients develop CRC by age 40 years[19]. Nonetheless, only 1% of CRCs are caused by this syndrome[20].

Hereditary nonpolyposis colorectal cancer causes an estimated 1-4% of CRCs. This autosomal dominant inherited condition is caused by inherited mutations affecting DNA mismatch repair. The lifetime risk for HNPCC patients developing colon cancer is almost 85% (13), with approximately 91% of men and 69% of women being affected by age 70[21]. HNPCC also increases the lifetime risk of developing endometrial, gastric, biliary tract, urinary tract, and ovarian cancers[22].

Twenty per cent of CRCs are due to hereditary factors not associated with genetic syndromes i.e. familial colorectal cancer[18]. Having a first-degree relative with CRC doubles the risk of CRC and increases the risk of colorectal adenomas by 70%[23,24]. This risk is further increased if the relative is younger than 45 years old or if more than one first-degree relative is affected.

1.2.3. Medical history

Ulcerative colitis and Crohn's disease are both forms of inflammatory bowel disease that increase the risk of CRC. In ulcerative colitis patients, this risk increases from 2% after 10 years of colitis to 8% after 20 years and 18% after 30 years[25]. Crohn's disease increases the risk of CRC to almost 3 times that of the general population[26].

Other medical conditions linked to an increased risk of CRC include type II diabetes mellitus, human papillomavirus (HPV) infection and previous radiation exposure[27-29].

1.3. **Pathogenesis**

Tumorigenesis of colorectal carcinomas is the result of an accumulation of genetic alterations leading to the transformation of normal colonic epithelium into a colon adenocarcinoma. The two pathways thought to direct this process are the adenomatous

polyposis coli (APC) pathway (adenoma-carcinoma sequence) and the DNA mismatch pathway.

Mutation and inactivation of the tumour suppressor APC gene occurs in the APC pathway, accounting for 80% of colorectal carcinomas. The subsequent chromosomal instability produces a stepwise progression from epithelial proliferation to malignant carcinoma via the accumulation of mutations in tumour suppressor genes and oncogenes (Fig 1.1).

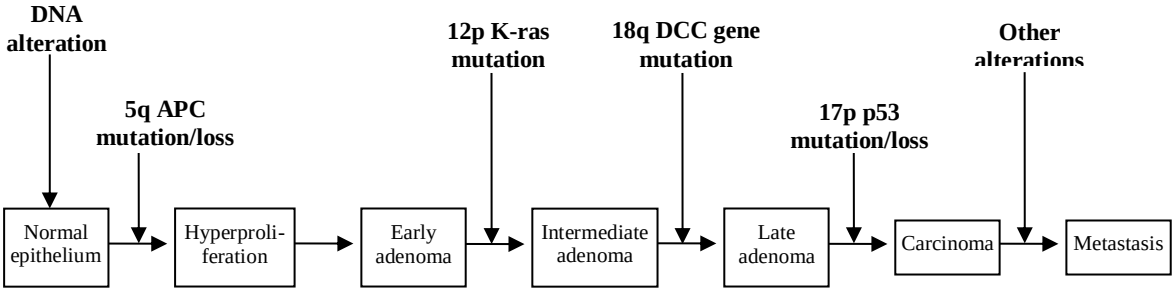


Figure 1.1 Multistep model of carcinogenesis (adapted from Fearon[30]**).** APC – adenomatous polyposis coli; K-ras - V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; DCC – deleted in colorectal cancer gene

The genes identified as playing key roles in this process include:

APC gene mutation/loss (chromosome 5q21): the APC gene is responsible for degradation of β-catenin[31]; APC loss or mutation usually occurs early in the adenoma-carcinoma sequence. This event results in accumulation of β-catenin, which stimulates cell hyperproliferation.

K-ras[2] gene mutation (chromosome 12p12): mutation of this oncogene stimulates growth and inhibits apoptosis via the MAP (mitogen-activated protein) kinase pathway. The K-ras mutation is present in 35% - 45% of colorectal cancers[32].

DCC[3] gene mutation (chromosome 18q): encodes a transmembrane protein involved in cell growth and apoptosis. This gene is deleted in >70% of colorectal carcinomas[33].

p53 gene deletion (chromosome 17p): p53 tumour suppressor plays a key role in conserving genomic stability via regulation of the cell cycle and induction of apoptosis. Its deletion occurs late in the carcinogenesis pathway and is seen in 75% of colorectal carcinomas[33].

[2] Kirsten rat sarcoma 2 viral oncogene homologue
[3] Deleted in colorectal cancer

Although the genetic alterations tend to occur at characteristic points in the pathway, variations in this pattern have been seen. The accumulation of at least four to five of these mutations is thought to be the key factor for the development of malignancy, rather than the actual sequence in which the mutations occur.

In the DNA mismatch pathway mutations occur in DNA mismatch repair genes (MMR genes e.g. MSH2, MSH6, MLH1, PMS1, PMS2[4]) leading to tumour development and progression. MSH2 and MLH1 are the predominant MMR gene mutations identified in colorectal cancer. The resultant microsatellite instability produces an accumulation of mutations in genes associated with regulation of proliferation and apoptosis such as the BAX, type II TGF-β receptor and insulin-like growth factor receptor II genes. Colorectal cancers occurring secondary to this pathway (~15%) have a better prognosis compared to the APC pathway(34). Trials have also shown that MMR status can be used as a predictor of response to chemotherapy given after primary surgery in the curative setting (adjuvant chemotherapy). The aim of adjuvant chemotherapy is to reduce the risk of cancer recurrence. Patients with MMR-proficient tumours gain a significant survival benefit with adjuvant chemotherapy, whereas patients with MMR-deficient tumours experience no benefit(34-36). Therefore adjuvant chemotherapy is not recommended for MMR-deficient tumours, particularly in stage II disease.

1.4. **Staging**

The staging of colorectal cancer is vital in determining the treatment and prognosis of patients. There are two main forms of staging colorectal cancer: the American Joint Committee on Cancer (AJCC) or TNM staging, and the Dukes classification system.

The TNM staging system is the most commonly used, where T represents tumour invasion of the bowel wall ($T_1 – T_4$), N represents lymph node involvement ($N_0 – N_2$), and M represents disease metastases ($M_0 – M_1$)(37). The Dukes classification system essentially uses the same parameters and ranges from Dukes A (localized disease) to Dukes D (metastatic disease)(38). The TNM or Dukes classification can be used to define the stage group of the disease. Stages for colorectal cancer range from Stage I to IV (Table 1.1).

[4] MMR – mismatch repair; MSH2 - MutS protein homolog 2; MSH6 - MutS protein homolog 6; MLH1 - MutL homolog 1; PMS1 - postmeiotic segregation increased 1; PMS2 - postmeiotic segregation increased 2; BAX - BCL2-Associated X Protein; TGF-β – Transforming growth factor beta

Table 1.1 – Staging of colorectal cancer (Adapted from [39])

Stage	T	N	M	Dukes
0 **(Carcinoma in situ or intramucosal carcinoma)** The tumour does not extend beyond the colorectal mucosa.	Tis	N0	M0	–
I The tumour invades through the muscularis mucosa into the submucosa (T1) or into the muscularis propria (T2). There is no lymph node involvement (N0) or distant metastasis (M0).	T1	N0	M0	A
	T2	N0	M0	A
IIA The tumour extends through the muscularis propria into the serosa (or visceral peritoneum) (T3) but does not invade surrounding organs. There is no lymph node involvement (N0) or distant metastasis (M0).	T3	N0	M0	B
IIB The tumour has penetrated through the serosa but does not involve surrounding tissues or organs (T4a). There is no lymph node involvement (N0) or distant metastasis (M0).	T4a	N0	M0	B
IIC The tumour has penetrated through the bowel wall and is attached to or has grown into nearby tissues or organs (T4b). There is no lymph node involvement (N0) or distant metastasis (M0).	T4b	N0	M0	B

Stage	T	N	M	Dukes
IIIA T1 – T2 disease with metastasis to 1 to 3 regional lymph nodes (N1), or T1 disease with metastasis in 4 to 6 regional lymph nodes (N2a). There are no distant metastases.	T1–T2	N1	M0	C
	T1	N2a	M0	C
IIIB T3 – T4a disease with metastasis to 1 to 3 regional lymph nodes (N1), or T2 – T3 disease with metastasis to 4 to 6 regional lymph nodes (N2a), or T1 – 2 disease with metastasis to 7 or more regional lymph nodes (N2b). There are no distant metastases.	T3–T4a	N1	M0	C
	T2–T3	N2a	M0	C
	T1–T2	N2b	M0	C
IIIC T4a disease with metastasis to 4 to 6 regional lymph nodes, or T3 – T4a disease with metastasis to 7 or more regional lymph nodes, or T4b disease with any lymph node metastasis. There are no distant metastases.	T4a	N2a	M0	C
	T3–T4a	N2b	M0	C
	T4b	N1–N2	M0	C
IVA Any T, any N with metastasis to 1 distant organ or site e.g. liver, lung, ovary or non-regional lymph node (M1a).	Any T	Any N	M1a	–
IVB Any T, any N with metastasis to more than one distant organ or the peritoneum (M1b).	Any T	Any N	M1b	–

1.5. **Treatment of localized colorectal cancer**

Surgery is the definitive treatment for localized colorectal cancer (Stages I – III) and the only curative option. For a curative resection, the entire primary tumour must be excised as well as its lymphatic drainage and a surrounding margin of normal tissue. Total mesorectal excision is recommended for rectal tumours where patients are able to tolerate radical surgery. This reduces the local recurrence rate and improves survival[40,41]. Preoperative chemoradiotherapy or short course radiotherapy is also recommended for rectal tumours. Preoperative chemoradiotherapy is useful for downsizing locally advanced tumours and is also more beneficial and less toxic than post-operative chemoradiotherapy[42]. Colonic tumours may be resected by open or laparoscopic colectomy with similar survivals.

The prognosis following surgical resection alone is generally good in early stage disease. The 5-year survival is 85% - 95% for stage I disease, 60 – 80% for stage II disease and 30 – 60% for stage III disease[43]. Adjuvant chemotherapy is required following surgery in stage III and 'high-risk'[5] stage II disease. Results from the MOSAIC (Multicentre International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer) trial showed that 5-FU/leucovorin with oxaliplatin (FOLFOX chemotherapy regimen, discussed below) significantly increases disease-free survival, reduces the risk of disease recurrence, and in stage III disease, it also improves overall survival[44]. Capecitabine with oxaliplatin or as a single agent may also be used as adjuvant treatment. Trials have not shown any benefit for the use of irinotecan as adjuvant treatment[45-47].

1.6. **Treatment of metastatic disease**

1.6.1. Curative treatment of metastatic disease

Twenty-five per cent of patients diagnosed with colorectal cancer present with metastatic disease[38]. The liver is the primary site for disease metastasis with almost 50% of patients having liver metastases either at primary diagnosis or with disease recurrence[20]. Surgical resection, where possible, is the only potentially curative option in locally recurrent disease or metastatic disease localised to the liver- or lung-only. Only 10% - 15% of patients with liver metastases are considered suitable for curative

[5] High-risk stage II disease is defined as having one of the following characteristics: less than 12 lymph nodes sampled, poorly differentiated tumour, vascular/lymphatic/perineural invasion, obstruction or perforation at presentation and pT4 stage.

resection of liver metastases[48]. This is dependent on the number of lesions, intrahepatic locations of lesions, vascular involvement, absent or limited extrahepatic disease and potential functional hepatic reserve. The post-operative mortality is relatively low at 2.8%, with a 5-year survival rate of approximately 30%[49]. The 5-year survival for patients following resection of lung metastases is 25% to 35%[50].

Perioperative chemotherapy with FOLFOX has been shown to improve progression free survival by 7 - 8% at 3 years in patients with resectable liver metastases[51]. Adjuvant FOLFOX should also be considered in cases where no pre-operative chemotherapy was administered. Liver metastases that are initially unresectable may become resectable after downsizing with neoadjuvant treatment. Resection rates are improved in up to 40% of patients with initially unresectable metastases following treatment with FOLFOX or FOLFIRI chemotherapy[50]. Addition of the targeted agent cetuximab (see Section 1.6.6) has been shown to further improve response rates and R0 resection rates for hepatic metastases in mCRC[3,52].

The majority of patients, however, are not suitable for surgery and receive palliative treatment using multi-agent chemotherapy with or without biological agents. Currently, the drugs licensed for first line treatment of metastatic colorectal cancer are 5-fluorouracil, capecitabine, raltitrexed, irinotecan, oxaliplatin, bevacizumab, cetuximab and panitumumab.

1.6.2. 5-Fluorouracil, capecitabine and ralitrexed

5-Fluorouracil (5-FU) is a cytotoxic fluoropyrimidine used in the treatment of colorectal cancer. 5-FU produces its cytotoxic effect via the actions of its three metabolites: 1) 5-5-fluoro-2'-deoxyuridine-5'-O-monophosphate (F-dUMP), 2) fluorouridine triphosphate (FUTP), and 3) fluorodeoxyuridine triphosphate (F-dUTP). F-dUMP inhibits DNA synthesis by targeting the enzyme thymidylate synthase. Thymidylate synthase (TS) metabolises the conversion of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (dTMP) for thymidine production during DNA synthesis. F-dUMP irreversibly inhibits this enzyme and results in thymidine depletion[53]. 5-FUTP mimics the RNA nucleotide uridine triphosphate (UTP). During nucleic acid replication 5-FUTP becomes incorporated instead of UTP and inhibits the synthesis of ribosomal and messenger RNA[54]. F-dUTP similarly becomes incorporated into

cellular DNA during DNA synthesis[55]. Together these mechanisms act to inhibit replication of malignant cells (Fig 1.2).

Bolus 5-FU was used for 20 years (~1975 – 1995) as the first chemotherapeutic agent to improve survival in metastatic colorectal cancer (mCRC). However, partial responses were seen in less than 18% of patients and complete responses in fewer than 8%[56]. Median survival was less than 10 months[53]. The addition of folinic acid (leucovorin) to 5-FU stabilises the binding of 5-FU to TS and improves response rates to 20-25%. Tumour response rates were also significantly improved by giving infusional (22%) as opposed to bolus 5-FU (14%)[57].

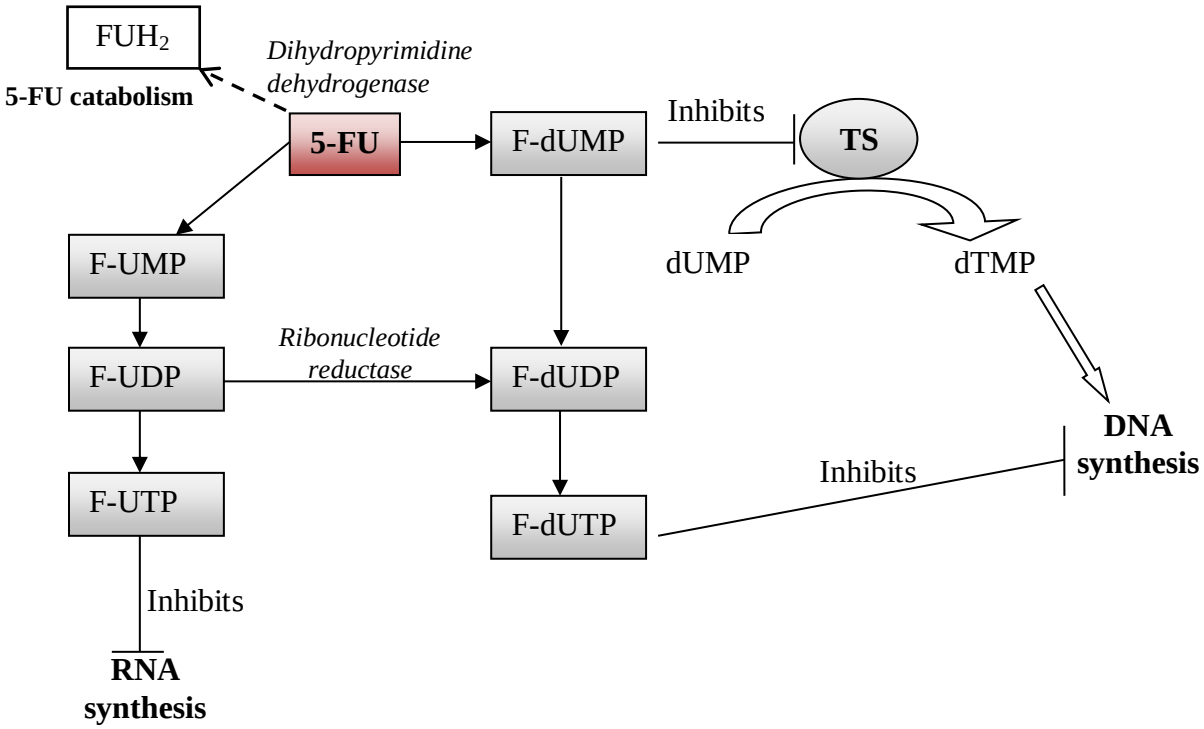


Figure 1.2 5-Fluorouracil Mechanism of Action (Adapted from [58])

5-FU - 5-fluorouracil
TS - Thymidylate synthase
dUMP - deoxyuridine monophosphate
dTMP - thymidine monophosphate
F-dUMP - 5-5-fluoro-2'-deoxyuridine-5'-O-monophosphate
F-dUDP - fluorodeoxyuridine diphosphate
F-dUTP - fluorodeoxyuridine triphosphate
F-UH_2 - dihydro-5-fluorouracil
F-UMP - fluorouridine monophosphate
F-UDP - fluororuridine diphosphate
F-UTP - fluorouridine triphosphate

Capecitabine is an oral fluoropyrimidine that is also used in the treatment of mCRC. This prodrug is converted to its active metabolite by the enzyme thymidine phosphorylase, which is found in higher concentrations in tumours and the liver

compared to normal tissue. Phase III trials show that although capecitabine has a higher response rate compared to 5-FU/leucovorin (26% vs. 17%), there is no significant difference in progression free survival or overall survival[59].

The main dose-limiting toxicities of 5-FU are myelosuppression and mucositis. More rarely, 5-FU has been associated with dermatitis, conjunctivitis, ataxia and cardiotoxicity[54]. Diarrhoea, nausea, vomiting and hand-foot syndrome are seen more frequently with Capecitabine, but less myelosuppression and stomatitis compared with 5-FU[60].

Dihydropyrimidine dehydrogenase (DPD) is a pyrimidine catabolic enzyme encoded by the *DYPD*[6] gene. It catalyses the initial and rate-limiting step in the catabolism of 5-FU (See Fig 1.2). Over 80% of 5-FU and capecitabine standard doses are deactivated by DPD[61]. DPD deficiency is associated with severe toxicity after administration of 5-FU, increasing the drug half-life and resulting in excess 5-FU accumulation. True DPD deficiency is a rare occurrence estimated to affect approximately 0.5% of caucasions, whereas 3% to 5% have variations in the *DPYD* gene causing partial deficiency[62]. The primary toxicity associated with this deficiency is grade IV neutropenia[7] [63]. Furthermore, onset of toxicity occurs significantly earlier in patients with reduced DPD activity compared to those with normal activity[64]. The IVS14+1G>A mutation is the most commonly reported *DPYD* variant and has been detected in 24% to 28% of patients experiencing severe 5-FU toxicity[63]. Therefore DPD deficiency is an important pharmacogenetic syndrome affecting the use of 5-FU in the treatment of patients with colorectal cancer.

Raltitrexed, like 5-FU, is an inhibitor of thymidylate synthase (TS), and acts by inhibiting the binding of TS to its folate cofactor[65]. Treatment with ralitrexed compared to 5-FU produced no significant difference in overall survival, progression-free survival or response rates[66-68], however it is associated with a reduced cardiotoxicity risk compared to 5-FU[69]. Ralitrexed is therefore licensed as an alternative to 5-FU in treatment of metastatic colorectal cancer where 5-FU is not tolerated, or in patients at risk of cardiotoxicity[38].

[6] *DYPD* gene – dihydropyrimidine dehydrogenase gene
[7] Neutrophils < 500/mm^3

1.6.3. Irinotecan

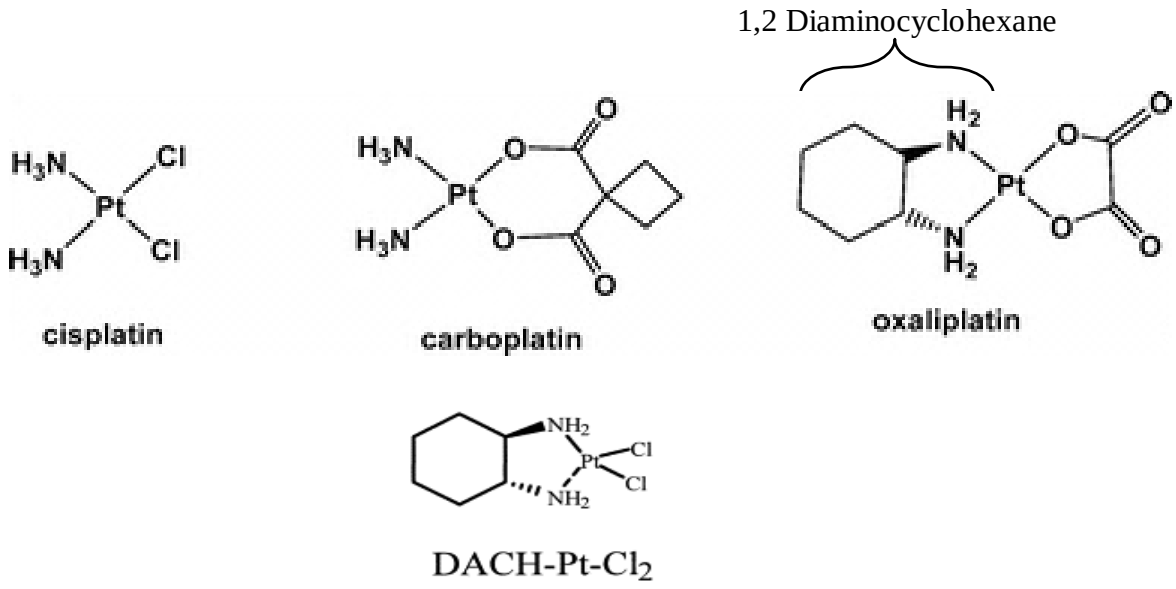
In the 1990s, irinotecan emerged as the second chemotherapeutic agent shown to improve patient survival in mCRC. Irinotecan (7-ethyl-10-[4-(l-piperidino)-l-piperidino]carbonyloxy- camptothecin, CPT-11, Camptosar) is a semi-synthetic derivative of the topoisomerase I inhibitor camptothecin. It binds to and stabilises DNA/topoisomerase adducts, which prevents DNA unwinding during replication and causes single-strand breaks. This results in the inhibition of DNA replication and transcription.

Clinical trials showed that single agent irinotecan produces similar responses to 5-FU/leucovorin when used as 1st line treatment in mCRC[70]. Irinotecan also significantly improved survival compared to 5-FU and best supportive care as 2nd line treatment in patients who had progressed following previous treatment with 5-FU[71,72]. The main toxicities were neutropenia, diarrhoea, fatigue, nausea and vomiting. The addition of irinotecan to 5-FU/leucovorin significantly improves response rate (39% vs. 21%), progression-free survival (7.0 vs. 4.3 months) and overall survival (median: 14.8 vs. 12.6 months)[70]. Although this combination resulted in more Grade 3 (severe) diarrhoea, incidence of Grade 4 (life-threatening) diarrhoea was similar to that seen with 5-FU/leucovorin. Severe mucositis, neutropenia and neutropenic fever were less common with irinotecan plus 5-FU/leucovorin.

Although the FOLFIRI regimen (irinotecan dose 180mg/m^2 with infusional 5-FU) is licensed as 1st and 2nd line treatment for mCRC, NICE guidelines recommend the use of irinotecan as a single agent (350mg/m^2) or in combination with 5-FU for 2nd line treatment of mCRC[38]. Irinotecan may also be given in combination with Capecitabine (XELIRI regimen – irinotecan 250mg/m^2 on Day 1 with capecitabine 1000mg/m^2 twice daily).

1.6.4. Oxaliplatin

Oxaliplatin (oxalato(trans-L-1,2-diaminocyclohexane)platinum) (See Fig 1.3) is a third-generation DNA-alkylating platinum derivative that is the only platinum compound to have shown clinical activity in colorectal cancer. It differs from cisplatin and carboplatin by possessing the 1,2 diaminocyclohexane-containing carrier ligand.



DACH-Pt-Cl_2

Figure 1.3 Chemical structures of cisplatin, carboplatin, oxaliplatin and reactive form of oxaliplatin, DACH-$PtCl_2$ (Adapted from [73] and [74]). DACH-$PtCl_2$ - Dichloro(1,2-diaminocyclohexane)platinum(II)

1.6.4.1. Pharmacology

Oxaliplatin undergoes non-enzymatic transformation in plasma to produce reactive compounds, including the dichloro(DACH)-platinum complex[75]. The dichloro-(DACH)platinum compounds enter the cell nucleus where they bind a nitrogen atom (N7) of guanine, or less preferentially, adenine to form DNA monofunctional adducts. These subsequently form diadducts by reacting with a second nucleophile to form crosslinks[74]. Oxaliplatin primarily forms DNA intra-strand crosslinks between two nucleobases of a single DNA strand. It also induces DNA inter-strand and DNA-protein crosslinks, but these appear to be less significant in terms of cytotoxic effects[76]. Formation of DNA cross-links inhibit DNA replication and transcription, leading to induction of the apoptotic pathway via caspase 3 activation, Bax translocation and cytochrome C release[77].

Although DNA adducts are formed more rapidly with cisplatin, oxaliplatin has been shown to be a more potent cytotoxic agent compared to both cisplatin and carboplatin. This is thought to be because the DACH-platinum adducts are more bulky leading to conformational distortions that make their structure distinct from that of cisplatin and carboplatin adducts and more effective in preventing DNA synthesis. The mismatch

repair pathway (MMR) is a key mechanism for cellular sensitivity to cisplatin and carboplatin. This system is responsible for recognising base-to-base mismatches caused by cisplatin and carboplatin DNA adducts and activates downstream signalling pathways resulting in apoptosis[76]. In colorectal cancer, there is failure of the MMR system leading to genetic instability and resistance to carboplatin and cisplatin[76,78]. However, the MMR complex has a poor affinity for oxaliplatin adducts due to their altered conformation[79]. Therefore, the deficiency of the MMR pathway does not affect the function of oxaliplatin and enables it to be effective in colorectal cancer[80].

Oxaliplatin exerts its antitumour activity by undergoing rapid nonenzymatic biotransformation to release free platinum species into the plasma. The unbound platinum is eliminated via a triphasic process consisting of two short phases with half-lives of 0.43 hours ($t_{1/2}\alpha$) and 16.8 hours ($t_{1/2}\beta$), and long terminal phase with a half-life of 391 hours ($t_{1/2}\gamma$)[81]. Oxaliplatin reaches its steady-state concentration during the first cycle of treatment. There is no significant accumulation of platinum in blood cells or plasma ultrafiltrate. Inter- and intra-patient variability in platinum exposure is moderate to low. Oxaliplatin binds rapidly and irreversibly to plasma proteins and erythrocytes. Clearance of platinum from the plasma is via covalent binding to tissues and renal excretion. The platinum is eliminated predominantly by renal clearance, with 54% of platinum being excreted in the urine. Renal clearance correlates with the glomerular filtration rate and platinum clearance is reduced in patients with moderate renal impairment. Nonetheless, there is no marked increase in drug toxicity[82].

1.6.4.2. Therapeutic effects

As a single agent, oxaliplatin has shown response rates of 20% as 1st line treatment and 10% as 2nd line treatment in patients who have previously been treated with 5-FU[83]. Clinical trials showed addition of oxaliplatin to 5-FU/folinic acid regimens resulted in more than double the response rates compared to 5-FU/folinic acid alone in 1st line treatment, from 16 – 22% up to 48.3 – 53%[81]. Response rates were also improved to 45% in 2nd line treatment[84]. Randomised controlled trials comparing 5-FU/leucovorin to 5-FU/leucovorin with oxaliplatin showed progression free survival improved by over 2 months (6.1 to 8.7 months) with the addition of oxaliplatin[83]. However, there appears to be no significant improvement in median overall survival.

The FOLFOX4 regimen consists of oxaliplatin (85mg/m^2) with infusional 5-FU/leucovorin (5-FU 400mg/m^2 bolus then 600mg/m^2 infusion, leucovorin 200mg/m^2). This regimen was compared with IFL (irinotecan 125mg/m^2, 5-FU 500 mg/m^2 bolus and leucovorin 20mg/m^2) as 1st line treatment for patients with mCRC. Results showed the FOLFOX4 regimen improved response rates (31% to 45%), progression free survival (6.9 months to 8.7months), and overall survival (15.0 months to 19.5 months) compared to IFL[(85)]. However, it is important to acknowledge that IFL is a bolus regimen as opposed to the infusional regimen of FOLFIRI that is known to be optimal. Subsequently two studies compared FOLFOX to FOLFIRI as 1st line treatment in mCRC[(4,86)]. Both these studies found that there was no significant difference in response rates, progression-free survival and overall survival between the two regimens. The GERCOR (Groupe Cooperateur Multidisciplinaire en Oncologie) study allowed cross-over between the two arms upon progression on first-line therapy. Both treatment sequences showed prolonged survival and similar efficacy[(4)]. The main difference between treatment arms was in toxicity. The main dose limiting toxicity with oxaliplatin is peripheral sensory neuropathy. Severe neutropenia was also more common with FOLFOX. However, in comparison with irinotecan-based treatments, FOLFOX showed lower rates of severe nausea, vomiting, diarrhoea, febrile neutropenia and dehydration[(85)]. The NICE guidelines recommend the use of FOLFOX as 1st line treatment for metastatic colorectal cancer[(38)].

UHL[8] currently uses the FOLFOX6 chemotherapy regimen. This differs from FOLFOX 4 in that higher doses of oxaliplatin (85 – 100 mg/m^2) may be given and therefore the leucovorin dose is increased from 200 mg/m^2 to 400 mg/m^2. The 5-FU infusional dose also differs at 2400 to 3000 mg/m^2 infused over 46 hours in FOLFOX6, compared to 600 mg/m^2 for 22 hours on two consecutive days[(20)]. Studies comparing these two regimens in patients with chemotherapy-refractory disease showed FOLFOX6 to have a slightly higher response rate of 27% compared to 19% in 5-FU refractory disease, and 15% compared to 9.9% in FOLFIRI-refractory disease[(4,87)]. The toxicity profiles are similar for the two regimens[(88)]. The main advantage appears to be the convenience of the FOLFOX6 regimen. This allows outpatient treatment with only one hospital visit every two weeks compared to the two hospital visits required for FOLFOX4[(89)].

[8] University Hospitals of Leicester NHS trust

Phase III trials substituting capecitabine (XELOX) for 5-FU as 1st line treatment of mCRC have shown no significant difference in response rate, progression-free survival or overall survival between the two regimens[90,91]. Therefore XELOX can be considered a suitable substitute for FOLFOX.

1.6.4.3. Toxicity

The grade 3 or 4 toxicities that occur with oxaliplatin monotherapy include neurotoxicity, nausea, vomiting, diarrhoea, mucositis, thrombocytopenia and neutropenia[81]. The severity of oxaliplatin-related myelotoxicity is dose-related, occurring at 85 – 135 mg/m^2. Myelosuppression is more common with oxaliplatin compared to cisplatin, however, despite Grade 3 – 4 neutropenia occurring frequently, there is only a 4% incidence of neutropenic sepsis[4]. The gastrointestinal side-effects are usually of mild or moderate severity. Alopecia is rare, whilst nephrotoxicity and ototoxicity are not associated with oxaliplatin.

Neurotoxicity tends to be the principal dose-limiting toxicity with this treatment. It may present as an acute, early-onset peripheral neuropathy characterized by cold-induced paraesthesia, dysethesia, or allodynia affecting the extremities, lip and oropharyngolaryngeal area. It can begin during the oxaliplatin infusion or up to 2 days afterwards, then resolves within a few hours or days. The symptoms frequently reoccur with subsequent oxaliplatin doses and may be associated with jaw tightness, cramps, fasciculations and other forms of muscular hypersensitivity[92]. This acute oxaliplatin-induced neuropathy occurs in up to 85% of patients[81]. It is thought to be caused by the oxaliplatin metabolite, oxalate, altering voltage-gated sodium channels by causing changes in current and chelating calcium and magnesium[93]. Chronic oxaliplatin-induced neuropathy occurs in 10 – 15% of patients, usually developing when the total cumulative dose of oxaliplatin reaches approximately 800mg/m^2. This is thought to be due to platinum compounds accumulating in the dorsal root ganglia, resulting in neuronal atrophy and mitochondrial dysfunction[94]. It usually manifests as a sensory peripheral neuropathy affecting the extremities and causing a reduction in distal sensations, sensory ataxia, deficit in fine sensory-motor coordination and proprioception. The symptoms persist between cycles and frequently accumulate to the extent that patients may become limited in the ability to perform activities of daily living. On cessation of oxaliplatin, over 80% of patients will recover from symptoms

within 3 – 4 months, and less than 5% of patients are left with irreversible symptoms[75,81]. Over 60% of patients require dose reduction or cessation of oxaliplatin prior to completing 12 cycles of chemotherapy due to chronic oxaliplatin-induced peripheral neuropathy[95].

The two main approaches that have been studied in the prevention or treatment of oxaliplatin-induced peripheral neuropathy are intermittent oxaliplatin dosing and administering neuromodulatory agents. Intermittent oxaliplatin dosing consisting of the 'stop-and-go' strategy was investigated in a number of trials including OPTIMOX1, OPTIMOX2, CONcePT, and MACRO. Overall, these trials showed that as long as maintenance treatment is provided, stop-and-go oxaliplatin treatment shows potential as a means of reducing neurotoxicity, however the optimal regimen and timing for oxaliplatin reintroduction is yet to be determined[90,96-98]. The neuromodulatory agents investigated have included calcium/magnesium infusions, carbamazepine, and xaliproden. These have all failed to produce consistent significant improvements in peripheral neuropathy[90,95,99,100]. Smaller studies investigating glutathione, glutamine, and N-acetylcysteine have all shown benefits in reducing neurotoxicity, however larger trials will be required to confirm these result.

1.6.4.4. DNA platination

Clinical studies with platinum-based cytotoxic agents have shown that drug-DNA adduct levels in surrogate tissues are associated with tumour response and toxicity[101-103]. Although drug-DNA adduct measurements are two- to five-times higher in tumour biopsies[104], measurement of platinum-DNA adduct levels in peripheral blood cells remains more predictive of tumour response to platinum-based therapy than previous treatment with platinum-based drugs, disease stage, histological type and tumour grading[101]. DNA-platinum adduct levels have shown a significant correlation with prolonged disease-free survival, and treatment outcome in patients with head-and-neck carcinoma and non-small cell cancer, showing potential as a biomarker for patient response to platinum-based treatment[105,106]. For germ cell tumours, however, high DNA-adduct levels did not predict a favourable treatment outcome[107]. This discrepancy has been credited to issues including method of adduct measurement, concurrent medication and tumour type[104].

In relation to oxaliplatin, a study measuring oxaliplatin-DNA adducts in peripheral blood lymphocytes across a variety of malignancies demonstrated significantly higher levels of adducts after 24 and 48 hours in patients who responsed to treaments compared to non-responders[108]. Similarly, the oxaliplatin-DNA adduct levels have been reported as a good indicator for patients' exposure to oxaliplatin[109].

1.6.5. Bevacizumab

Bevacizumab is a humanized monoclonal antibody that targets vascular endothelial growth factor (VEGF), which is the main angiogenic factor in CRC. VEGF is an anti-apoptotic, pro-angiogenic cytokine that is associated with increased CRC metastasis, increased incidence of disease recurrence and poor prognosis[100]. Bevacizumab prevents VEGF from binding with receptors on vascular endothelial cells. This results in inhibition of angiogenesis, regression of VEGF-dependent vessels and normalization of blood vessel structure, thus improving delivery of chemotherapeutic agents to the tumour[110].

Clinical trials have shown that addition of bevacizumab to standard chemotherapy significantly improves outcomes in the treatment of mCRC. A randomized phase III trial comparing IFL to IFL plus bevacizumab as 1st line treatment for colorectal cancer showed significant improvements in median survival (15.6 months vs. 20.3 months), progression-free survival (6.2 months versus 10.6 months) and response rate (34.8% vs. 44.8%)[111]. Bevacizumab added to oxaliplatin-based chemotherapy (XELOX or FOLFOX4) as 1st line treatment of mCRC resulted in significantly improved progression-free survival (9.4 vs. 8.0 months). However, the increase in median overall survival was not statistically significant (19.9 vs. 21.3 months)[3]. As 2nd line treatment, bevacizumab and FOLFOX4 significantly increased median survival by 2 months (10.8 to 12.9 months), and progression-free survival by almost 3 months (4.7 vs. 7.3 months). The overall response rate was 22.7% for bevacizumab and FOLFOX compared to 8.6% for FOLFOX only[112].

Bevacizumab has also been shown to significantly improve response rates in colorectal cancer with liver metastases[113]. The addition of bevacizumab to chemotherapy regimens significantly reduced the percentage of pathologically viable tumour cells from 45.3% in regimens consisting of 5FU+Oxaliplatin only to 32.9% with bevacizumab ($p = 0.02$). Measurement of tumour viability is an important factor to

consider in assessing responses to chemotherapy and biological agents such as bevacizumab. Radiological methods alone are often unable to distinguish between areas of viable tumour and areas of fibrotic replacement caused by effective treatment. The use of bevacizumab in the treatment of malignant gliomas has particularly highlighted this, due to the phenomenon of pseudoprogression. Pseudoprogression is a non-tumoural increase in the size of a lesion that can be mistaken for disease progression. Although pseudoprogression has been reported primarily with bevacizumab use in malignant gliomas[114] and not in colorectal cancer, it is nonetheless essential that careful review of both pathological and clinical responses should be taken into consideration when evaluating treatment efficacy.

The adverse effects associated with bevacizumab commonly include hypertension (Grade 3), proteinuria, delayed wound healing, and less frequently, gastrointestinal perforation, increased incidence of arterial thromboembolic events and cardiac ischaemia[115].

Bevacizumab in combination with 5-FU/FA based chemotherapy is currently licensed in the UK as treatment for mCRC.

1.6.6. Cetuximab and panitumumab

Cetuximab and panitumumab target the epidermal growth factor receptor (EGFR) signalling pathway. This pathway regulates cell differentiation, proliferation, migration, angiogenesis and apoptosis but is deregulated in malignant cells. Up-regulation of EGFR expression occurs in 60% to 80% of colorectal cancers and is related to more aggressive disease and poor prognosis[116,117].

Cetuximab is a chimeric IgG1 monoclonal antibody to EGFR. Its high affinity for the epidermal growth factor receptor enables it to competitively bind and inhibit the ligand-induced phosphorylation of EGFR[118]. This binding also stimulates antibody-dependent cell-mediated cytotoxicity. Cetuximab affects the signaling pathway at the surface of the cell membrane, therefore its efficacy can be disrupted by mutations that promote activation of the pathway downstream of the EGFR. In particular, the mutation status of the K-ras has been shown to significantly impact treatment outcomes with cetuximab.

K-ras is a member of the *ras (*rat sarcoma) proto-oncogene family, which consists of small (21 kDa) GTP-binding proteins. K-ras is located in the internal plasma membrane. It has GTPase activity that enables it to convert GTP to GDP when activated. Once the conversion to GDP has occurred, K-ras returns to an inactive state. Mutations within the K-ras gene make the protein resistant to inactivation via the action of regulatory GTP-ase activating proteins. This constitutive activation renders EGFR inhibitors ineffective[119]. The K-ras mutation is found in 30% - 60% of colorectal adenocarcinomas[120]. At least 90% of the K-ras mutations are found in codons 12 and 13 of exon 2 of the K-ras gene[121]. Initially it was thought that the absence of this mutation in codons 12 and 13 of exon 2 was sufficient for positive responses to cetuximab treatment (See Fig 1.4). However, it has now been established that the presence of other RAS mutations (K-ras exons 3/4 and N-ras exons 1/2/3/4) can also predict resistance to anti-EGFR monoclonal antibodies[122,123]. Therefore evidence suggests that extended RAS testing should be undertaken to indentify patients who are most likely to benefit from treatment with these agents.

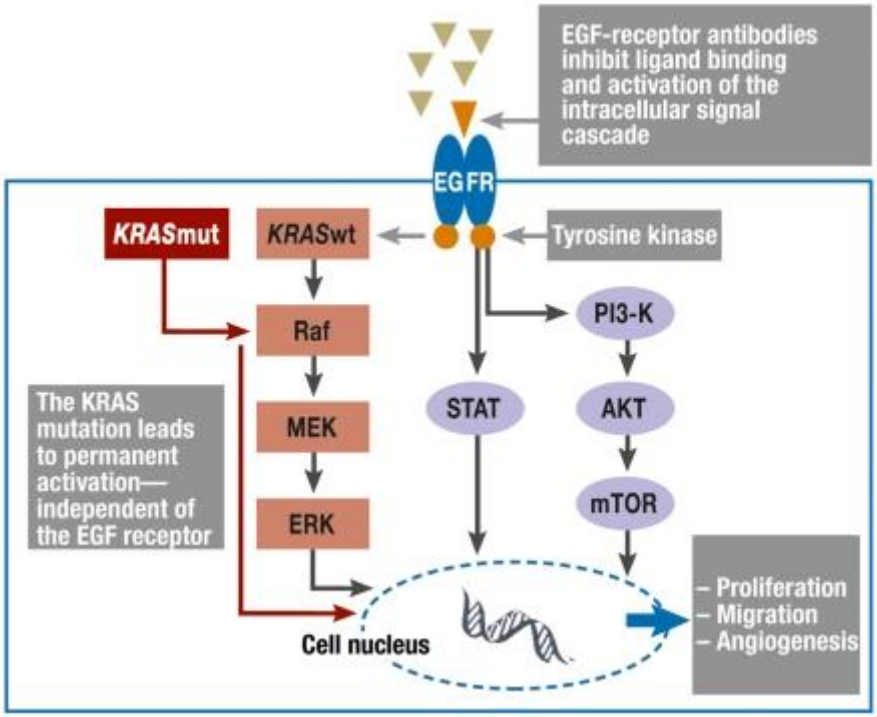


Figure 1.4 K-ras and EGFR pathway (from [121]). K-ras - V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; EGFR – epithelial growth factor receptor.

The CRYSTAL study was a phase III study investigating the benefit of adding cetuximab to FOLFIRI as 1st line treatment for mCRC. Results showed that for patients with K-ras and RAS wild-type, the addition of cetuximab improved response rate, progression-free survival and R0 resection rate in patients with mCRC compared to FOLFIRI alone[124,125]. The OPUS trial similarly showed that the addition of cetuximab to FOLFOX as 1st line treatment for patients with mCRC significantly improved progression-free survival and response rates in patients with K-ras wild-type tumours[126]. Subsequent reanalysis of these results with extended RAS testing showed that patients with mutation in K-ras and N-ras exons 2 – 4, gained no benefit from treatment with cetuximab and may suffer detrimental effects[127]. Unfortunately, these results were not confirmed by the COIN (Addition of Cetuximab to Oxaliplatin-based First-line Combination Chemotherapy for Treatment of Advanced Colorectal Cancer) trial, a phase III trial that investigated the addition of cetuximab to oxaliplatin-based combination chemotherapy as 1st line treatment for mCRC. Although the addition of cetuximab improved the response rate, there was no improvement in progression-free survival or overall survival, even in K-ras wild-type patients[128]. These results mean that cetuximab plus oxaliplatin-based chemotherapy is not recommended as 1st line palliative treatment for mCRC.

The addition of cetuximab to both oxaliplatin-based and irinotecan-based combination therapy improves the resection of liver metastases, including R0 resections[129]. Single agent cetuximab has also a 12% response rate in patients with disease refractory to fluoropyrimidines, irinotecan and oxaliplatin[130]. In combination with irinotecan-based chemotherapy, cetuximab improves response rates, progression-free survival and overall survival as 3rd line treatment of mCRC[131].

NICE guidelines recommend the use of cetuximab for 1st line treatment in mCRC only in cases where all the following criteria are fulfilled[132]:

- The primary tumour has been resected or is potentially operable,
- Metastatic disease is confined to the liver and is unresectable
- The primary tumour can be resected and the liver metastases become resectable after treatment with cetuximab.

Panitumumab is a recombinant, fully human IgG2 monoclonal antibody against EGFR. Phase III trials confirm that panitumumab improves progression-free survival compared to best supportive care in chemotherapy refractory mCRC[133]. The PRIME study (Randomized phase III trial of panitumumab with infusional fluorouracil, leucovorin and oxaliplatin (FOLFOX4) versus FOLFOX alone) initially showed that as 1st line treatment for mCRC, panitumumab improved progression-free survival compared to FOLFOX in patients with wild-type *K-ras*, but no significant difference in overall survival was found[134]. Further retrospective extended RAS analysis demonstrated a significant improvement in both progression-free survival and overall survival in the subgroup of patients who had no RAS mutations[122]. As 2nd line treatment, panitumumab with FOLFIRI improved progression-free survival but not overall survival in *K-ras* wild-type patients with mCRC refractory to fluoropyrimidine treatment[135].

Panitumumab is currently licensed as monotherapy for treatment of EGFR-expressing mCRC with K-ras wild-type after failure of treatment with fluoropyrimidine, irinotecan and oxaliplatin. However, NICE guidelines do not recommend the use of Panitumumab in this setting.

The K-ras gene has played a substantial role as a biomarker for responses to cetuximab and panitumumab. Not only do patients with wild-type K-ras significantly benefit from treatment with cetuximab and panitumumab in contrast to those with the K-ras mutation, the K-ras mutation resulted in no improvement and occasionally worse outcomes. Results from the OPUS study showed that treatment of K-ras mutant tumours with FOLFOX-cetuximab significantly reduced response rates (34% vs. 53%; odds ratio 0.459, $p = 0.0290$) and progression-free survival compared to patients who received FOLFOX alone (5.5 months vs. 8.6 months, hazard ratio 1.720, $p = 0.0192$)[126]. Therefore K-ras mutational status is a highly predictive selection criterion for treatment of mCRC with EGFR inhibitors.

1.6.7. Cancer drugs fund

The Cancer Drugs Fund (CDF) was set up by the government in October 2010 to improve access to cancer drugs that were not routinely available on the NHS. These drugs were generally agents that were either awaiting appraisal, or had been appraised by NICE but not approved for routine NHS use because they did not meet the required

clinical and/or cost-effectiveness criteria. It was initially commissioned to continue until March 2014, but was subsequently extended until March 2016. By 2014/15, 1 in 5 cancer patients starting new treatment were using drugs supported by the CDF **(Ref Report – Investigation into the Cancer Drugs Fund, National Audit Office, Department of Health and NHS England, Session 2015 – 2016, 17 September 2015)**.

Patients received access to drugs such as Bevacizumab and Cetuximab via the CDF. From April 2013 – March 2015, 19% of patients using the fund were provided access to Bevacizumab. Patients at University Hospitals of Leicester (UHL) receiving first-line treatment for metastatic colorectal cancer were able to access Bevacizumab via the CDF from the onset of the CUFOX study. Cetuximab plus FOLFIRI as first line treatment for metastatic colorectal cancer became available at UHL after September 2012. From this point, FOLFIRI plus Cetuximab became the recommended first-line treatment for patients found to have K-ras wild-type mCRC.

1.7. **Curcumin**

1.7.1. Background

Curcumin is derived from turmeric (*Curcuma longa*), a rhizomatous plant found in southern and south eastern tropical Asia[136]. This perennial herb is a member of the ginger family and has a wide range of functions in Asian communities. The average Indian diet is thought to contain a daily intake of 2 to 2.5 grams of turmeric. Based on the curcumin content of 3 – 5% in the dry rhizome, this is the equivalent of approximately 60 to 100 mg of curcumin daily[137]. Turmeric is also used as a dietary pigment, a preservative, and in the textile and pharmaceutical industries. Its use in Asian medicine dates back to at least the second millennium BC. Ancient Hindu texts, including the Ayurveda, have described its various uses[138]. Its medicinal properties include treatment for wounds, inflammation, biliary disorders, anorexia, cardiovascular disease, hepatic disorders, rheumatism, sinusitis and tumours.

Curcuminoids are phenolic compounds derived from turmeric by ethanol extraction. Curcumin [1,7-bis-(4-hydroxy-3methoxyphenyl)-1,6-heptadiene-3,5-dione] is a low molecular weight polyphenol and is the most active constituent of turmeric[136]. Vogel and Pellatier first described the structure of curcumin as $C_{21}H_{20}O_6$ in 1815[139]. In 1910

Lampe *et al* identified it as a diferuloylmethane, and this was confirmed by chemical synthesis in 1913[140]. It is a bright yellow crystalline powder that is responsible for the colour of turmeric and curry powder. Curcumin is used internationally as a colouring agent in various foods (e.g. cheese, mustards, spices, yoghurts, soups and cereals) and carries the food additive number E100. It is also available in an encapsulated form as a health food supplement.

Over the past few decades, there have been numerous studies showing the benefit of curcumin as an antioxidant, anti-inflammatory, and cancer chemopreventive agent. Research also indicates that curcumin may have potential as a chemotherapeutic agent. The characteristics of curcumin and the research conducted so far will be discussed in more detail.

1.7.2. Physical and chemical properties

Extracted curcumin powder contains approximately 75% curcumin (molecular weight/MW 368.87), 10% - 20% demethoxycurcumin (DMC; MW 338) and less than 5% bisdemethoxycurcumin (bDMC; MW 308)[141]. BDMC is thought to convert to DMC which then becomes curcumin[142] (See Fig 1.5). Curcumin is highly photosensitive and has a melting point of 183°C.

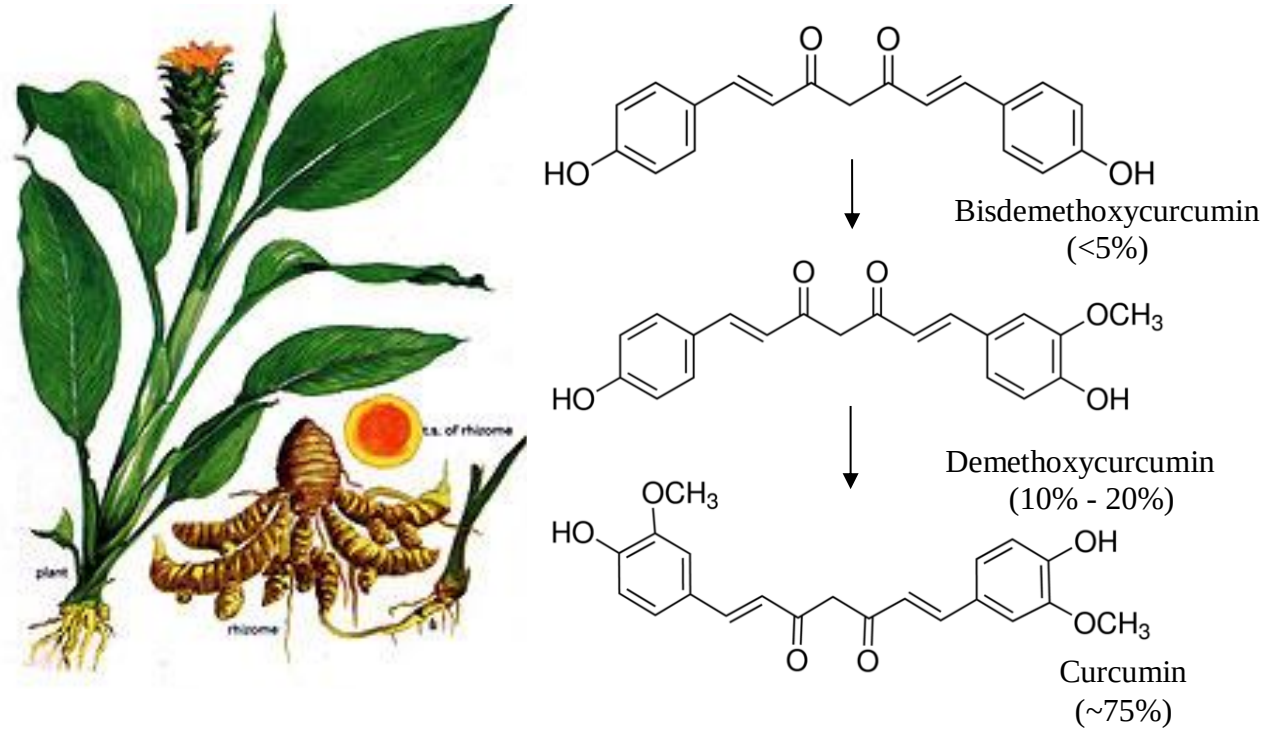


Figure 1.5 ***Curcuma longa*** **and curcuminoids** (Adapted from [136])

Curcumin is a lipophilic molecule that is soluble in acetone, ethanol and dimethylsulfoxide, but relatively insoluble in water. It consists of two aromatic rings linked by two unsaturated carbonyl groups, with hydrogen-bonding of the central OH group providing the stability for the molecule. Curcumin has the biochemical properties to act as a free-radical scavenger and anti-oxidant as mechanisms of preventing cell damage. Under alkaline conditions curcumin is very unstable and degrades in less than 30 minutes to *trans*-6-(4'hydroxy-3'methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, feruloylmethane and vanillin[143]. The rate of decomposition is significantly slower under acidic conditions, with less than 20% of the total curcumin being degraded at 1 hour[144]. This accounts for curcumin's stability in the gastrointestinal tract where the pH is 1 – 6. Curcumin degradation appears to be completely inhibited in the presence of foetal calf serum or human blood, or by adding antioxidants (e.g. ascorbic acid, *N*-acetylcysteine or glutathione). In cell culture medium containing 10% foetal calf serum or in human blood, less than 20% of the total curcumin is degraded in 1 hour compared to 90% within 30 minutes in serum-free medium[144]. Curcumin is also photosensitive and should be protected from light to avoid degradation.

1.7.3. Anti-inflammatory effects

Curcumin has been shown to have potent anti-inflammatory effects, primarily due to its inhibition of COX-2 expression and the resultant reduction in prostaglandin synthesis. COX-2 expression can be induced by cytokines, growth factors, hormones, oncogenes and tumour promoters. Overexpression of COX-2 promotes tumour growth by inhibiting apoptosis and disrupting cell adhesion properties[145], and is observed in a variety of tumours including colon, rectum, breast, head and neck, lung, pancreas, stomach and prostate[146]. Studies have shown that inhibition or genetic knock-out of COX-2 in mice with a germline knock-out mutation of the APC tumour suppressor gene, and in rats exposed to azoxymethane (colon carcinogen), significantly inhibited the development of colonic tumours[147].

Curcumin has been shown to inhibit COX-2 gene expression in oral and colon epithelial cells *in vitro*[148]. At a concentration of 20 μM, curcumin significantly reduced chemically induced PGE2 production in colon cells to pre-induction levels[149]. Curcumin's inhibition of COX-2 is thought to be at the transcriptional level, as opposed to inhibiting the enzyme's catalytic activity. COX-2 induction is mediated by the

transcription factor nuclear factor kappa B (NF-κB). NF-κB release and resultant nuclear translocation occurs following the phosphorylation of IκB (inhibitor of kappa B) by NF-κB-inducing kinase (NIK) and IκB kinases (IKK[9]α and β). Curcumin, like aspirin, acts by preventing IκB phosphorylation by the NIK/IKK signalling pathway and inhibits NF-κB release[(150)]. Therefore the use of curcumin should reinstate cellular apoptosis. This was confirmed by research showing that the use of curcumin in rats exposed to azoxymethane increased the percentage of apoptotic cells and decreased tumour incidence by 50%[(151)].

1.7.4. Pro-apoptotic effects

Curcumin has a stimulatory effect on both the intrinsic and extrinsic apoptotic pathways. The intrinsic (mitochondrial) pathway involves p53 regulation of the pro-apoptotic protein Bax. This protein inhibits the anti-apoptotic protein Bcl-2 and enables release of cytochrome c. Cytochrome c enables the initiation of the caspase cascade that leads to cell death. Curcumin upregulates p53 expression by increasing Bax expression and thus stimulates the release of cytochrome c[(152)]. It also inhibits the expression of Bcl-2 in breast cancer cell lines[(153)]. Apoptosis was increased in breast cancer cell lines treated with curcumin, associated with an increase in p53, raised p53 DNA binding and Bax expression[(154)]. The extrinsic (death receptor) pathway is triggered by "death activators" (e.g. TNF[10]-α and Fas ligand) binding to and activating to their cell surface receptors. The activated receptors stimulate the caspase cascade via activation of caspase 8. A melanoma study showed that curcumin promotes aggregation of Fas receptors and increases caspase 8 levels, which stimulates apoptosis[(155)]. Curcumin has been shown to stimulate caspase-3-induced apoptosis in hepatic cancer cell lines[(156)].

1.7.5. Effects on angiogenesis and metastasis

Curcumin has demonstrated anti-angiogenic activity *in vitro* and *in vivo* by inhibiting pro-angiogenic growth factors, enzymes and inflammatory mediators associated with neovascularisation including basic fibroblast growth factor (bFGF), VEGF, angiopoetin-1 and 2 (AP-1 and AP-2), COX-2, matrix metalloproteinase-9 (MMP-9) and NF-κB[(157)]. Hepatocellular carcinoma cells treated with curcumin showed decreased tumour neocapillary density compared to untreated cells, with reduced levels

[9] IKK – Iκβ kinase
[10] Tumour necrosis factor

of angiogenic biomarkers COX-2 and VEGF[158]. There is also evidence that curcumin affects intracellular adhesion molecules (e.g. β-catenin, E-cadherin and APC) that are involved in tumour growth and metastasis[159].

The effects of curcumin on NF-κB could also be significant as NF-κB over-expression is associated with drug-resistance. A number of chemotherapy agents (e.g. paclitaxel, doxorubicin, oxaliplatin) interact with this pathway, therefore its inhibition by curcumin could result in enhanced cytotoxic effects[160].

1.7.6. Curcumin and cancer stem cells

According to the cancer stem cell hypothesis, tumour initiating cells (TIC) are a subpopulation of highly tumourigenic cells with stem cell-like phenotypes that are responsible for tumour formation. These cells have the capacity for self-renewal, differentiation, invasion and metastasis[161]. They are thought to have a higher degree of innate or acquired chemo-resistance and failure to eradicate these cells results in disease recurrence. Resistance to cisplatin and paclitaxel in breast cancer[162] and gemcitabine in pancreatic cancer have been attributed to TICs[163]. Curcumin inhibits the Notch, hedgehog and *Wnt* signalling pathways that are associated with TICs[164,165]. *Wnt* signalling has been significantly reduced after curcumin administration in both normal and malignant breast stem cells without causing toxicity in differentiated cells[166]. Chemoresistant (FOLFOX-surviving) HCT-116 or HT-29 colon cancer cells also responded with marked decreases in TICs after treatment with curcumin either as a single agent or in combination with FOLFOX[167]. Therefore curcumin could have a useful influence in preventing chemoresistance.

1.7.7. Antioxidant activity

The chemical structure of curcumin provides it with antioxidant activity. It is able to inhibit lipid peroxidation and neutralize lipid free radicals[168]. *In vitro* and *in vivo* data in rats demonstrate curcumin's ability to scavenge reactive oxygen species (ROS) produced by macrophages[169]. Inducible nitric oxide synthetase (iNOS) is an enzyme induced in macrophages in response to oxidative stress. It generates large volumes of nitric oxide (NO) that can react with superoxide radicals and produce toxic peroxynitrite. Curcumin reduces the activation of ROS by downregulating iNOS activity in macrophages[170].

1.7.8. Preclinical studies

1.7.8.1. Preclinical pharmacokinetics

Preclinical and clinical studies show curcumin to have limited systemic bioavailability[138,171,171]. Studies of oral curcumin administered to rats demonstrated that the vast majority was excreted in faeces with 60 - 66% of the given dose being absorbed and approximately one-third not being systemically metabolised[172]. Both intravenous and intraperitoneal doses were excreted in bile with over 50% of the intravenous dose excreted within 5 hours[173]. Preclinical work with isolated human hepatocytes or liver or gut microsomes and further studies of oral administration of curcumin in rats suggests very rapid metabolism of curcumin, which occurs in a matter of minutes. Plasma levels of curcumin in the rat studies were low, with higher levels of curcumin metabolites[149]. Traces of curcumin are also found at low concentrations in the rat liver and kidneys, suggesting there is some uptake in organs distal to the intestine[172]. Therefore it appears that curcumin has low systemic bioavailability, with oral doses being metabolised in the intestine while absorbed curcumin is excreted in the bile following rapid first-pass metabolism[141].

The chemotherapeutic potential of curcumin has been demonstrated in a number of *in vivo* studies performed using murine models (see Table 1.2).

Table 1.2 - Examples of pre-clinical studies reporting mechanisms of action and efficacy of curcumin in cancer. 8-oxo-dG - 8-Oxo-2'-deoxyguanosine; AP-1 – Activator protein 1; APC – adenomatous polyposis coli; COX-2 – cyclooxygenase-2; IBD – Inflammatory bowel disease; M_1G - 3-(2-deoxy-β-di-erythro-pentafuranosyl)-pyr[1,2-α]-purin-10(3*H*)one; MAPK - Mitogen-Activated Protein Kinases; NF-κβ - Nuclear factor kappa B; NO – Nitric oxide; SLJ/J - Swiss Jim Lambert J; TRAMP - Transgenic Adenocarcinoma of the Mouse Prostate; TNF-α – Tumour necrosis factor α

Cancer	Model	Curcumin dose	Observations	Ref
Colon cancer	Male Rat	0.6% curcumin + celecoxib (≈0.4g/kg)	Augments inhibitory effect of celecoxib on tumour growth	(174)
Colon Adenoma	APC Min Mouse N = 4 per group (4 vs 14 wks curcumin)	2% (≈1.2g/kg) 4 vs 14 weeks	Lifetime curcumin reduced COX-2 expression, 8-oxo-dG and M_1G Short-term feeding did not affect total adenoma number or COX-2 expression, but decreased M_1G	(175)
Prostate	Female TRAMP mice N = 9 controls vs 12 cancer	2% (≈1.2g/kg) 10-16 weeks	Decreased tumour formation Increased apoptosis Decreased proliferation	(176)
Squamous cell carcinoma	Female nude mouse xenograft N = 12 curcumin vs 5 controls	50-250 μmol Topical paste 3 weeks	Xenograft tumour growth inhibition by curcumin paste	(177)
Breast cancer	Female Foxn1$^{nu/nu}$ mice, N = 8 controls and 8 curcumin	0.65 curcumin for 6 weeks	Inhibition of tumour growth and angiogenesis. Downregulation of NF-κB regulated expression of cyclin D1, PECAM-1, and p65.	(178)
Pancreatic cancer	Nude mice, Sprague Dawley, N = 5 per group (curcumin vs liposomes vs saline)	40 mg/kg IV, 3 times/week	Reduction in tumour size and angiogenesis attributed to inhibition of NF-κB mechanisms. No toxicity.	(179)
Lung cancer	CD-1 female nude mice, N = 4 per group (curcumin for 3 vs 9 days vs control)	50mg/2.5ml/kg intraperitoneal injections,	Dose dependent suppression of Stat3 phosphorylation and reduced cell proliferation.	(180)
Cervical cancer	Female Swiss albino mice. N = 12 per group (curcumin vs paclitaxel vs curcumin+paclitaxel)	Curcumin 25 mg/kg i.p. Paclitaxel 10 mg/kg	Curcumin enhances tumour anti-proliferative effect of paclitaxel by downregulation of Akt/AP-1/MAPK and NFkB.	(181)
Glioma	U87-derived xenografts female nude mice.	30, 60, 120 mg/kg i.p. daily for 30 days	Anti-proliferative effect on tumour xenografts. Decreased endothelial cell migration and tube formation, Decreased matrix metalloprotease activity.	(182)

Dietary effects of curcumin have also been demonstrated in Min/+ mouse models of human familial APC where it has been shown to inhibit the development of adenomas[171]. The rate of adenoma formation was reduced by 39% and 40%

respectively with diets containing 0.2% and 0.5% curcumin respectively. This indicates chemopreventive potential for human inherited colorectal cancer. Data from this study suggests that a daily dose of 1.6 g of oral curcumin would be required for a response in humans. Lifetime administration of curcumin to APC $^{Min+}$ mice downregulates COX-2 transcription by 66% and reduces levels of DNA adducts, the pyrimidopurinone adduct of deoxyguanosine (M_1dG) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) by 39% and 24% respectively[175].

1.7.8.2. Curcumin and chemotherapy

In vitro and *in vivo* studies have shown that curcumin is able to enhance the cytotoxic effects of chemotherapy and inhibit chemoresistance[183-185]. One such study, conducted in Leicester, compared the antiproliferative effects of oxaliplatin and curcumin, as single agents and in combination, on colorectal cancer cell lines[183]. The combination of oxaliplatin and curcumin produced increased efficacy compared to either of the single agents in both p53wt (HCT116 – p53 wild type) and p53mutant (HT29) cell lines. Both single agents induced apoptosis, but there was a 16-fold increase in p53 induction with agents combined. Likewise, in HCT116 p53wt and $p53^{-/-}$ cell lines, addition of curcumin enhanced the cytotoxic effects of oxaliplatin in overcoming chemoresistance, irrespective of p53 status, both *in vitro* and *in vivo*, without altering oxaliplatin's ability to form DNA-platinum adducts[185]. Therefore the addition of curcumin to oxaliplatin may constitute a multitargeted approach, where curcumin and oxaliplatin operate using independent mechanisms of action. The p53 wild type cell lines were more sensitive, suggesting that p53 upregulation does have some influence on response. Translation of the combination to an *in vivo* setting resulted in a decreased tumour volume compared to control, oxaliplatin and curcumin alone, accompanied by decreased immunoreactivity in the pro-proliferation markers Ki-67 and Notch-1 and a 4.4-fold increase in the apoptosis marker caspase-3. These heightened effects have been associated with curcumin inhibiting EGFR and IGF-1R (insulin-like growth factor 1 (IGF-1) receptor) signalling pathways[184].

1.7.8.3. Curcumin and neuropathy

In vitro and *in vivo* studies suggest that curcumin use may be beneficial in alleviating peripheral neuropathy[186,187]. Oral curcumin was shown to reduce the neuropathy

phenotype in the Trembler-J mouse model for human neuropathy[187]. Further studies in diabetic mice also showed that chronic treatment with curcumin had an antinociceptive effect, with significantly reduced thermal hyperalgesia and hot-plate latencies[188]. These results indicate that curcumin may potentially alleviate dose-related peripheral sensory neuropathy, which is one of the dose-limiting toxicities experienced with oxaliplatin chemotherapy.

1.7.9. Clinical studies

1.7.9.1. Bioavailability of curcumin

Clinical studies have investigated the pharmacokinetics of curcumin in humans. A phase I study was performed in Leicester where doses of 0.45 g to 3.6 g daily of curcumin were administered to 15 patients with advanced colorectal cancer refractory to standard chemotherapy[189]. The 3.6 g daily dose resulted in detectable levels of curcumin and its conjugates in plasma and urine samples. The lower doses did not produce significant detection of curcumin or its metabolites in the urine samples. This suggests that curcumin is not preferentially excreted by the kidneys. Renal function appears to have no effect on curcumin metabolism, whilst there is evidence to suggest that curcumin could improve renal function[190]. Other trials have shown only trace levels of curcumin in serum following oral doses of less than 2 g daily. Oral administration of 4 g and 8 g of curcumin daily have produced serum levels of 0.51 μM and 1.77 μM respectively[191]. Oral doses of up to 12 g daily have been given to patients, resulting in peak plasma concentrations at 1 to 2 hours after loading, and trough levels at 12 hours[191].

Further studies administered doses of 0.45 g to 3.6 g oral curcumin daily for 7 days to patients prior to surgery for colorectal cancer or liver metastasis secondary to colorectal cancer[192,193]. The colorectal patients who received 3.6 g daily showed concentrations of 12.7 ± 5.7 nmol/g in normal and 7.7 ± 1.8 nmol/g in malignant colorectal tissues[192]. There were metabolites (curcumin sulphate and curcumin glucuronide) found in the intestinal tissue and trace levels of curcumin in peripheral circulation. Topical levels were detected on the mucosa for several days after ingestion, which is noteworthy because the therapeutic effects of curcumin might involve both topical and systemic activity[177]. The patients with liver metastases showed low nanomolar levels of

curcumin and its conjugates in peripheral blood samples and no parent compound in the liver tissue. Only trace levels of curcuminoids were detected in the liver[193]. Therefore 3.6 g daily of oral curcumin results in sufficient levels in colorectal tissue to produce a pharmacological effect, however there is negligible distribution in tissues distant to the gut. Evidence from a clinical trial on patients with pancreatic cancer does suggest that despite its poor bioavailability and limited evidence of its presence at the target site, curcumin may still exert a clinical effect on organs distal to the intestine[194]. Nonetheless the question remains as to what dose of curcumin is required to produce a pharmacologically and clinically efficacious level of curcumin in tissues outside the gastrointestinal tract.

Table 1.3 - Summary of completed phase I and II clinical trials reporting pharmacodynamics and pharmacokinetics of curcumin C3-complex (n=10) and other similar curcumin formulations (n=20). 5-HETE - 5-hydroxy-eicosatetraenoic acid; BAX - BCL2-Associated X Protein; BCL2 - B-cell lymphoma 2; CRC – colorectal cancer; ESR – erythrocyte sedimentation rate; GST - glutathione S-transferase; HDL – high density lipoprotein cholesterol; IL-1β – interleukin 1β; IL-4 – interleukin 4; M_1G - 3-(2-deoxy-**β**-di-erythro-pentafuranosyl)-pyr[1,2-α]-purin-10(3*H*)one; PD – Pharmacodynamics; PGE – Prostaglandin E; PK – Pharmacokinetics; SUSAR - Suspected unexpected serious adverse reaction; VEGF - Vascular endothelial growth factor

Year	Country	Trial design, study group and size	Curcumin regimen	Observations	Ref
1992	India	Phase I N=10 Healthy volunteers	500 mg /day one week	Raises HDL, lowers overall cholesterol 11% and peroxidises 33%. Tolerated.	(195)
2001	UK	Phase I N=15 Colorectal cancer Pharmacokinetic Pharmacodynamic	Up to 4 g/day curcuma extract 4 months	Well tolerated. No toxicity. No curcumin or metabolites found in blood or urine but in faeces. 440 mg caused maximal (59%) reduction in GST activity. Some efficacy noted after 2-4 months of treatment.	(196)
2001	Taiwan	Phase I N=25 Various pre-malignancies Tolerability Pharmacokinetics	0.5-12 g/day 3 months	Tolerated up to 8 g when drug became too bulky. No toxicity up to this dose. Not found in urine. Plasma peak 1-2 hours. Plasma peaks; 4 g curcumin/0.51 μmol, 6 g/6.33 μmol, 8 g /1.77 μmol. No SUSARs reported.	(191)
2004	UK	Phase I N=12 Colorectal liver metastases Pharmacokinetic Pharmacodynamic	450-3600 mg/day 1 week C3-complex	Doses of curcumin required to furnish hepatic levels sufficient to exert pharmacological activity are probably not feasible in humans. No parent compound in liver. Curcuminoids in liver only at 3.6 g. No change in DNA adducts. Not found in urine. Trace in serum at highest dose, Increase in M_1G adduct levels post-treatment perhaps due to surgery.	(193)
2004	UK	Phase I N=15 Healthy volunteers Biomarker study	0.45 – 3.6 g/day 4 months C3-complex	Low systemic bioavailability. Recommend 3.6 g minimum. No toxicity, mild diarrhoea. 3.6 g inhibits PGE2 in leucocytes. M_1G and GST not affected. 3.6 g generates detectable parent compound and conjugates in plasma and urine (expressed as adducts/107 nucleotides).	(189)
2005	USA	Phase I N=5 Inflammatory bowel disease	360 mg 4x /day 2 months	Tolerated. Reduced symptoms. Reduced inflammatory markers. No PK/PD data. No toxicities reported.	(197)

Year	Country	Trial design, study group and size	Curcumin regimen	Observations	Ref
2005	UK	Phase I N=12 Colorectal cancer Pharmacokinetic Pharmacodynamic	450-3600 mg/day 1 week C3-complex	COX-2 not reduced in CRC tumour. Decreases M_1G. Dose up to 3.6 g safe and is probably minimum required for efficacy. Negligible distribution outside the gut; 12.5 nmol/g in normal mucosa, 7.7 nmol/g in malignant tissue.	(192)
2005	India	Phase I N=20 Tropical pancreatitis Efficacy	500 mg/day + 5 mg piperine 6 weeks	Reduced inflammation (ESR) Increase glutathione synthesis. No change in pain. 100% compliance, no adverse events.	(198)
2006	Japan	Phase II N=43 with control arm Ulcerative colitis	2 g per day with mesalazine 6 months	Safe medication for maintaining remission in patients with quiescent ulcerative colitis	(199)
2006	USA	Phase I N=24 Healthy volunteers Dose escalation	500-12000 mg/day 14 days C3-complex	30% (7 of 24 subjects) experienced minimal toxicity. Tolerance of curcumin in high single oral doses appears to be excellent. 7 grade I adverse events. No curcumin in serum in doses up to 8000 mg. Traces found in serum at doses above 10000 mg /50 ng/mL and 12000 mg/57.6 ng/mL in 2 patients.	(200)
2007	USA	Phase II N=25 Pancreatic cancer Feasibility	8 g/day, initially 8 weeks then until progression C3-complex	Feasibility and tolerability demonstrated. No treatment-related toxic effects. Possible efficacy despite low bioavailability. Peak curcumin 41 ng/mL. Plasma peaks of curcumin released from conjugate forms ranged from 0 to 125 ng/mL at 2 hours.	(194)
2008	USA	Phase I N=12 Healthy volunteers PK and PD	6 pts 10 g/day 6 pts 12 g/day 72hr assay C3-complex	Only 1 patient had detectable free curcumin. Peak serum 35 μg/ml. t1/2 3 hr and 6 hr for 10 g and 12 g respectively. Tmax 10 hr. No adverse events above grade I were reported.	(201)
2010	Israel	Phase II N=17 Pancreatic cancer Feasibility	8 g/day + gemcitabine 4 weeks C3-complex	Feasible, Safe, possible efficacy. 8 g is a large dose to administer and can cause abdominal pains. Dose reduction to 4 g favourable. 30% stopped due to abdominal pains. Increase in serum cytokines. No PK data.	(202)
2011	USA	Phase IIa Colon cancer screening N = 41	2 or 4 g for 30 days C3-complex	25 of 41 participants (61%) had grade-1 and -2 toxicity, primarily gastrointestinal disturbances. Decrease aberrant crypt foci number, PGE2 concentrations did not change significantly. Curcumin also did not reduce 5-HETE concentrations	(203)
2011	China	Phase I Colon cancer N = 63, with controls	360 mg TDS 10 – 30 days	Up-regulation of p53. Weight gain in patients taking curcumin. Increase in BAX, decrease in BCl-2. No toxicities reported.	(204)

Year	Country	Trial design, study group and size	Curcumin regimen	Observations	Ref
2011	Iran	Phase II Type 2 diabetic nephropathy N = 20 + 20 controls	500 mg turmeric 3 x daily (66.3 mg curcumin), 2 months	No adverse events	(205)
2012	Iran	Phase II Sulfur mustard-induced pruritis N = 46 + N = 50 controls	1 g curcumin daily for 4 weeks + 5 mg Bioperine	Increased antioxidant enzyme activity, alleviates pruritic symptoms and improves quality of life scores. 6 participants did not complete study due to gastrointestinal side effects.	(206)
2012	USA	Pilot study Rheumatoid arthritis N=15 per group	500 mg curcumin twice daily +/- diclofenac	Curcumin provided significant improvement in patients with active rheumatoid arthritis and was not associated with any adverse events. Curcumin safe and well tolerated	(207)
2012	Australia	Phase II Monoclonal gammopathy of undetermined significance (MGUS), N= 19 Multiple myeloma, N=17	4 g curcumin daily for 3 months followed by 3 month placebo cross-over. Optional 8 g extension study for further 3 months.	Free light chain ratio decreased in both patient groups suggestive of better prognosis. 1 patient withdrew at 4 g dose. No safety issues reported.	(208)
2012	UK	Pilot study Colon cancer N=28	2.35 g curcuminoids daily for 14 days prior to surgical resection. C3-complex	Measurement of curcumin in plasma and colorectal tissues. All biopsies saw levels of 18.85+/-6.8 µg/g tissue. 13 adverse events (grades 1-2) attributable to curcumin in 6 patients. Abdominal pain, bloating, diarrhoea, dyspepsia, flatulence, nausea, vomiting.	(209)
2013	USA	Phase II Breast cancer – radiation induced dermatitis N=15 + N=15 controls	2 g curcumin, 3 x daily.	Significantly fewer curcumin-treated patients exhibited moist desquamation. No adverse events reported.	(210)
2013	USA	Phase II Castration resistant prostate cancer. Combination with docetaxel. N=30	6 g/day. 7 days per docetaxel cycle beginning 4 days prior and ending 2 days post.	4 complete PSA responses and 13 partial responses observed. No adverse events were attributable to curcuminoids.	(211)

Year	Country	Trial design, study group and size	Curcumin regimen	Observations	Ref
2014	Brazil	Phase II Gingival plaque reduction in combination with photodynamic therapy, vs chlorhexidine varnish. N = 45	Curcumin solution at 1.5 mg/mL + photodynamic therapy. 1 x weekly for 4 weeks.	Decrease in gingival bleeding index, but no improvement in plaque formation compared to standard care. No adverse events reported	(212)
2015		Randomised, double blind, placebo-controlled study investigating the potential of peripheral biomarkers to predict treatment response and antidepressant mechanisms of change. N = 50	Curcumin taken for 8 weeks at 500 mg twice daily	Curcumin influences markers associated with ant-depressant mechanisms of action, No adverse events reported	(213)
2015	Iran	Phase II open labelled trial. Compared curcumin/piperine with standard anti-depressant medication. N = 111	100 mg curcumin (C3 complex) + 10 mg piperine, daily for 6 weeks	Curcumin/piperine co-administration significantly improves treatment efficacy in alleviation of symptoms of depression. No adverse events reported.	(214)
2014		Randomised, double-blind, placebo-controlled study, assessing efficacy of curcumin in major depressive disorder. N = 56	500 mg curcumin twice daily for 8 weeks.	Evidence for efficacy observed 4 – 8 weeks following end of intervention. No adverse events reported.	(215)
2014	Iran	Randomised, double blind, placebo-controlled study, investigating curcumin for knee osteoarthritis. N = 40	500 mg curcumin + 5 mg bioperine 3 x daily for 6 weeks.	Curcuminoids showed significant nociceptive effect in osteoarthritis. No significant adverse effects were reported. AEs reported were of a mild gastrointestinal nature, and were not significantly increased in frequency compared to placebo control.	(216)
2014	Iran	Randomised, double blind, placebo-controlled study, investigating curcumin for effects on serum cytokines in obese individuals. N = 37	2 x 500 mg curcumin + 5 mg bioperine for 30 days, 2 week wash-out and then cross-over to placebo or intervention accordingly.	Curcumin decreased circulating IL-1β, IL-4 and VEGF. Seven participants withdrew due to adverse events consisting of: constipation, bloating and increased frequency of urination.	(217)

Year	Country	Trial design, study group and size	Curcumin regimen	Observations	Ref
2014	Thailand	Double blind randomised controlled trial assessing efficacy of curcuma extra vs ibuprofen in pain relief for knee osteoarthritis. N = 367	1.2 g ibuprofen vs 1.5 g curcuma extracts daily for 4 weeks.	Curcuma extracts were as efficacious at pain reduction and functional improvement as ibuprofen. Dyspepsia and nausea rates were higher in the ibuprofen group, whereas the occurrence of loose stools was higher in the curcuma group.	(218)
2015	Italy	Phase III, single-dose, randomized, double-blind, placebo-controlled clinical trial in patients with chronic plaque psoriasis, using Meriva. N = 63	Topical methylprednisolone aceponate 0.1% plus 2 g per day of Meriva (2 tablets of 500 mg, twice daily) (arm 1) or topical methylprednisolone aceponate 0.1% ointment plus matching placebo	Curcumin downregulated T-cell mediated inflammation. One adverse event of diarrhoea was reported.	(219)

1.7.9.2. Curcumin dosing for pharmacological effect

Dosing of curcumin remains a challenge due to its poor absorption, rapid metabolism and low systemic bioavailability. Clinical studies have required oral doses ranging from 4 g to 8 g daily to achieve serum levels necessary for efficacy. *In vivo* studies suggest 1.6 g oral curcumin daily might be sufficient to exert a biological effect in humans, producing a colonic mucosal concentration of 0.1 μmol/g[174]. Conversion of pre-clinical data to human dosing is complex. Trials giving 2 g oral curcumin daily detected trace levels of curcumin in human serum, whilst curcuminoids were detected in hepatic tissue after a 1 week course of 3.6 g curcumin daily[193]. Thus it appears that a minimum dose of 2 – 4 g is required for a pharmacological effect, particularly in organs distal to the gut[193]. Compliance rates at this dose range are excellent[189,193]. There is the risk that higher doses, with the possibility of larger capsule size and number, would reduce compliance. Despite concerns about increased adverse effects, trials have shown tolerance to, and feasibility of oral doses of 8 g for three months[191], 4 g for four months[189,193] and 5 g for 5 months[220]. Optimal peak plasma levels would be achieved if curcumin was ingested as a single daily dose. If, however, it becomes evident that the topical effect is more beneficial against luminal disease, divided doses may prove equally effective[221].

1.7.9.3. Clinical trials with curcumin

Curcumin has shown promising results in phase I trials investigating its anticancer effects. Curcumin was used as topical treatment in patients with oral cancers and leukoplakia. Ten per cent of the 62 participants showed a reduction in lesion size, whilst 70% of patients noted drying of their lesions[222]. In colorectal cancer, two phase I studies investigated the effect of oral curcumin given in doses of 0.36 g to 1.80 g daily, and 0.45 g to 3.6 g daily for 4 months in patients with advanced disease[189,223]. In the first trial, 5 out of the 15 participants demonstrated radiologically stable disease for 2-4 months of treatment, and a significant decrease in carcinoembryonic antigen (CEA) was noted in another patient[223]. Two participants in the second trial experienced radiologically stable disease for up to 4 months[189].

Curcumin has been combined with docetaxel chemotherapy in a phase I trial of patients with advanced and metastatic breast cancer[224]. The maximum tolerated dose in this

study was 8 g oral curcumin daily. Fourteen patients were recruited with eight having disease measurable according to RECIST criteria. Five of these patients demonstrated a partial response to treatment and three showed stable disease, with no reports of disease progression. One of these patients, who had initially been classed as having inoperable disease, had no residual tumour after 6 cycles of treatment and subsequently underwent a mammectomy.

A number of phase II trials have investigated the use of curcumin in patients with pancreatic cancer[194,202,225]. A study enrolling 21 patients with gemcitabine-resistant pancreatic cancer administered 8 g oral curcumin daily in combination with gemcitabine-based chemotherapy[225]. The phase I arm of this study established 8 g as the recommended dose for phase II investigations. No dose-limiting toxicities were observed and the median compliance rate was 100%. A second phase II study recruited 17 patients with advanced pancreatic cancer, again using 8 g oral curcumin daily in combination with gemcitabine[202]. Of the 11 evaluable patients, one (9%) had a partial response, 4 (36%) had stable disease, and 6 (55%) experienced disease progression. Median time to progression was 2 and a half months (range 1 – 12 months) with a median overall survival of 5 months (range 1 – 24 months). This study reported low compliance rates with the 8 g dose when taken with gemcitabine. Curcumin has also shown biological activity when used as a single agent in patients with advanced pancreatic cancer[194]. Despite showing poor oral bioavailability, a curcumin dose of 8 g daily resulted in one of the 21 patients evaluated having stable disease for over 18 months. A second patient experienced 73% tumour regression, however this response was short-lived, lasting only 1 month. No toxicities were reported in this study.

1.7.9.4. Toxicity

Preclinical studies of curcumin have revealed no significant toxicity. *In vivo* studies were conducted in rats where doses of up to 5 g/kg were administered orally and produced no apparent toxic effects[226]. Almost 40 clinical trials recruiting over 800 participants have been conducted using curcumin with little discernible toxicity (Table 1.4)[221]. Side-effects tend to be gastrointestinal and dose-related, with symptoms improving after dose reduction. These typically include loose stools, abdominal bloating, reflux and abdominal discomfort. No toxicities were reported in a trial where up to 8 g daily of oral curcumin was administered to patients with pre-invasive

malignant or high risk pre-malignant conditions for 3 months[191]. Similarly, minimal side-effects were reported in patients with advanced colorectal cancer given doses of up to 3.6 g oral curcumin daily for up to 4 months[189]. The two adverse events reported were of diarrhoea (US National Cancer Institute (NCI) grades 1 or 2) in one patient consuming 0.45 g daily after one month of treatment, and a second patient taking 3.6 g daily after 4 months. Nausea (NCI toxicity grade 2) was reported in one patient taking 0.9 g curcumin daily, however this resolved spontaneously with the patient continuing treatment. Elevated serum alkaline phosphatase levels were noted in four patients (2 patients – NCI grade 1, 2 patients – NCI grade 2), which may be related to curcumin. Three patients also experienced a 150% rise in serum lactate dehydrogenase compared to pre-treatment values. However, it remains unclear whether these abnormal blood results were due to curcumin toxicity or disease progression. No effect on biochemical and haematological parameters has been attributed to curcumin.

The main limiting factor with curcumin dose escalation is the volume of capsules required to deliver doses greater than 8 g orally. One trial studying the safety of curcumin in healthy volunteers administered single oral doses of curcumin escalating from 0.5 g to 12 g[200]. Adverse effects (NCI grade 1), including diarrhoea, headache, rash and yellowish stool, were experienced in 7 of the 24 participants. The maximum tolerated dose of curcumin was not established because participants were unable to tolerate the volume of tablets required for doses greater than 12 g.

Table 1.4 - Summary of clinical studies reporting chemotherapy used in combination with curcumin C3 complex (n=3) and similar oral curcumin formulations (case report), including toxicities observed.

Year	Cancer	Country	Study size	Dose of curcumin	Agent in Combination	Observations	Ref
2008	Pancreas	Israel	Phase II n=17	8 g daily 4 weeks C3-complex	Gemcitabine	Feasible, safe, possible efficacy. 8 g is a lot to take and gives abdominal pains. Reduces to 4 g.	(202)
2009	Colon with liver metastasis	Germany	Case report n=1	5 g daily 5 months	FOLFOX, interrupted at 3rd cycle	50% reduction in tumour size. Curcumin tolerated.	(220)
2010	Breast	France	Phase I n=14	500 mg-8g for 1 week, 3 weekly C3-complex	Docetaxel	Feasible, safe, possible efficacy. Most patients observed biological and or clinical improvements. Possibly dose limiting toxicity due to curcumin, 2 patients with grade III diarrhoea.	(224)
2011	Pancreas	Japan	Phase I/II N=21	8 g daily C3-complex	Gemcitabine	8 g per day accepted as a tolerable dose of curcumin. No patients withdrew from the study. No toxicities attributable to curcumin. Early survival data – no significant difference.	(225)
2013	Prostate	USA	Phase II N=30	6g daily	Docetaxel	4 complete PSA responses and 13 partial responses observed. No adverse events were attributable to curcuminoids	(211)
2015	Bowel	UK	Phase I dose escalation N = 12	0.5 – 2 g daily	FOLFOX	Eleven of twelve participants (91.7%) showed stable disease or partial response to treatment after 6 cycles of chemotherapy. The most common curcumin-induced side effects were constipation (25.0%), dry mouth (16.7%) and flatulence (16.7%). All adverse events reported were grade 2 or less.	(227)

1.8. **Biomarkers**

A biomarker is a biological characteristic that may be molecular, anatomical, physiological or biochemical in nature, which is objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacological responses to therapeutic interventions. The roles of biomarkers include:

- Detecting a specific disease as early as possible – diagnostic biomarker (PSA – prostate cancer)
- Assessing the risk of developing a disease – susceptibility/risk biomarker (BRCA 1 – breast cancer)
- Predict evolution of a disease (indolent vs. aggressive) – prognostic biomarker (HER2 – breast cancer)
- Predict response or toxicity to a particular therapy – predictive biomarker (e.g. Dihydropyrimidine dehydrogenase (*DPD*) deficiency and fluoropyrimidines in gastrointestinal cancer)

The potential benefit of biomarkers has been highlighted by examples such as the discovery of the role of human epidermal growth factor receptor (HER)-2/*neu* oncogene as a predictive and prognostic biomarker in breast cancer, and EGFR-status for treatment of non-small cell lung cancer. In colorectal cancer *K-ras* mutational status has played a vital role as a predictive biomarker for response to treatment with cetuximab and panitumumab (See Section 1.6.6). Similarly, decreased DPD activity detected in peripheral blood mononuclear cells can act as a predictive biomarker for toxicity to 5-FU chemotherapy (See Section 1.6.2).

Nonetheless, there remains a paucity of known or validated biomarkers for detection, treatment, follow-up and targeted therapy in cancer. Linking biomarker studies to drug development could be a key step in identifying patient populations that would benefit the most from new treatments and possibly also identify gauges for monitoring patient compliance.

1.8.1. Proteomics

A proteome is the entire complement of proteins expressed by a genome, cell, tissue or organism at a given time and under defined conditions. This set of proteins will vary with time and particular circumstances such as the environmental stresses encountered

by the organism. Proteomics is a large-scale characterization of a specific proteome, including assessment of variations in protein content and abundance as a means of characterizing cellular processes. This information can then be utilized in the identification of biomarkers of disease, prognosis and therapeutic effects of medication. Validating biomarkers of efficacy can be problematic in the absence of target tissues to use as comparators. In cancer studies, proteomics provides a means of interrogating a variety of biospecimens for cancer-related proteins, providing a substitute for biopsy tissue that is rarely available. These studies enable comparison of protein levels under variable conditions as a means to identify biomarkers for particular cellular processes involved in cancer development, progression and therapeutic intervention.

The discovery of a biomarker for therapeutic efficacy does not necessarily result in a successful clinical outcome [228]. One reason for this the inability of the laboratory model to mimic the biological processes that take place within the patient. In particular, there is increasing appreciation of the impact of the tumour microenvironment on cellular functions including proliferation, differentiation and morphology [229,230]. Using tissue from patients to build *ex vivo* models can provide a setting for drug analysis that is more akin to the clinical disease [227,231,232].

Proteomics studies have been conducted investigating biomarkers for therapeutic intervention in colorectal cancer. One such study analysed tissue from primary colorectal tumours and liver metastases from 17 patients[233]. Because obtaining biological material from metastatic lesions is often less feasible, this study aimed to evaluate whether proteomic analysis of biological material from the primary tumour could be used to predict the outcome in liver metastatases. This study identified 5768 discrete proteins, 5 of which predicted histopathological response to fluorouracil-based chemotherapy regimens. In particular, immunohistochemistry was used to validate the flavin adenine dinucleotide (FAD) binding protein, NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1). Expression of this protein in the primary tumour is predictive of response to neoadjuvant chemotherapy in liver metastases, whilst inhibition of NQO1 at genetic and functional levels improves chemosensitivity. Another study explored the alterations in protein secretion secondary to stimulation and inhibition of the EGFR pathway in colorectal cancer cells to identify a biomarker of cetuximab therapy[234]. This study used 3D spheroids from colorectal cancer cells to generate secretomes with a drug-sensitivity profile that matched patients with colorectal

cancer. Based on their findings, they concluded that sensitivity to cetuximab and inhibition of the EGFR pathway was associated with over-secretion of phosphorylated-EGFR (pEGFR). This was validated by comparison with plasma samples from patients. Thus measurement of circulating pEGFR levels could potentially serve as a biomarker for tumour response to cetuximab.

Overall, results from these studies show that proteomic sequencing in colorectal cancer is a feasible method for detecting biomarkers of therapeutic response. Furthermore, the use of patient samples in analysis could offer a more clinically relevant predictive model.

1.8.2. MicroRNA

MicroRNAs (miRNAs) are small non-protein-coding RNA molecules (containing approximately 22 nucleotides) that regulate gene expression and play an important role in oncogenesis. miRNAs have a variety of roles in processes including development, differentiation, cell proliferation, apoptosis and stress responses[(235)]. Studies have shown that miRNA expression is distinctly altered in colorectal cancer[(236)], with miRNAs playing a part in all stages of colorectal cancer initiation, progression and metastases. miRNAs are able to demonstrate either oncogenic and tumour suppressive functions depending on the environment in which they are expressed (See Table 1.5).

Table 1.5 - Oncogenic and tumour suppressor miRNAs in colon cancer and their target genes (adapted from [236])

miRNA	Oncogene vs tumour suppressor	miRNA targets
miR-17-92 cluster	Oncogene	*E2F1*
miR-21	Oncogene	*PDCD4, PTEN, RECK, NFIB, TPM1, SPRY2,RHOB, TIMP3, maspin, CDC25a, TIAM1, MSH2*
miR-95	Oncogene	*SNX1*
miR-135a/b	Oncogene	*APC*
miR-155	Oncogene	*MLH1, MSH2, MSH6*
miR-499	Oncogene	*FOXO4, PDCD4*
let-7	Tumour suppressor	*K-RAS*
miR-29	Tumour suppressor	*MMP2, DNMT3A/B*
miR-30a	Tumour suppressor	*DTL*
miR-34a	Tumour suppressor	*FRA1, SIRT1, MYC, BCL2*
miR-101	Tumour suppressor	*COX2*
miR-137	Tumour suppressor	*CDC42*
miR-143	Tumour suppressor	*K-RAS, DNMT3A, ERK5*
miR-145	Tumour suppressor	*IRS-1, c-Myc, YES1, STAT1, OCT4, SOX2, KLF4, FLI1*
miR-342	Tumour suppressor	*DNMT1*
miR-365	Tumour suppressor	*CCND1, BCL-2*
miR-451	Tumour suppressor	*MIF*
miR-675	Tumour suppressor	*RB*

A number of studies have investigated the use of miRNAs as potential biomarkers for diagnosis, prognosis and disease response in colorectal cancer. Circulating miRNA levels can be detected in blood serum or plasma to identify patients with colorectal cancer[237]. miR-92, for example, has higher expression in colorectal cancer patients compared to healthy patients and can distinguish between these groups with 65-70% specificity and 82-89% sensitivity[237],[238]. Furthermore, circulating miR-92 levels decrease following surgical resection of colorectal tumours. Thus monitoring miR-92 levels can be used in conjunction with markers such as CEA to augment screening accuracy or monitor for disease recurrence. Similarly miR-141 and miR-21 have been considered as markers of prognosis in colorectal cancer. miR-141 is elevated in metastatic colorectal cancer and associated with poor prognosis[239]. miR-21 is an oncogenic miRNA that is over-expressed in several malignancies[236]. Over-expression of this miRNA has been linked with worse survival and therapeutic outcomes, regardless of the disease stage[240]. In particular, elevated miR-21 expression has been

shown to induce resistance to 5-FU in cancer cell lines by inhibiting the DNA mismatch repair gene MutS homolog 2 (MSH2)[241]. In this context, miR-21 can also be used to identify microsatellite instability in colorectal cancer patients. Equally, miRNA expression patterns may prove useful in classifying other phenotypic subgroups (K-ras mutation, and TP53 status) that influence therapeutic response.

1.8.2.1. miRNA-122

miR-122 (miRNA-122) is a microRNA expressed abundantly and almost exclusively in hepatocytes. It constitutes up to 70% of the hepatic miRNA population[242]. miR-122 has been investigated for its potential as a biomarker for liver damage in human and murine studies, particularly with alcohol-,viral- and chemical-induced (e.g. acetaminophen and D-galactosamine) liver injury [243,244),(245]. Currently, liver injury or disease is most commonly assessed using 'liver function tests'. The most specific marker of hepatocellular injury in these tests is alanine aminotransferase (ALT) which is a cytoplasmic enzyme most commonly found in hepatocytes. In previous studies, ALT measurements have been used as a standard for comparison to establish miR-122's feasibility for use as a biomarker of liver damage [243,244]. miR-122 levels have been shown to significantly correlate with ALT levels in alcohol-, viral-, and chemical-induced acute liver injury[243),(245]. However significant increases in miR-122 were identified earlier and at lower doses of drugs (e.g. acetaminophen) compared with ALT[243]. Moreover miR-122 changes showed greater specificity for liver injury compared to ALT, correlated with liver histologic staging and may be detectable before the onset of microscopically detectable changes in liver cells[245]. These findings suggest that miR-122 would be at least an equally reliable biomarker and possibly supplement information provided by standard liver function tests in monitoring potential liver toxicity related to curcumin use.

As an oncological biomarker, the majority of research into miR-122 has been conducted in the setting of hepatocellular carcinoma (HCC). It is thought to play a tumour-suppressive role in this cancer and has been shown to be frequently down-regulated in HCC and HCC cell lines [246]. miR-122 has also been shown to play a key role in pathways intimately associated with carcinogenic progression (e.g. ADAM10, SRF, and the PI3K/Akt/mTOR/p70S6K) and thus affect non-hepatic cancers [247]. More recently, it has become apparent that miR-122 may also play in important role in colorectal cancer, particularly in the context of chemoresistance. miR-122 expression is

significantly downregulated in 5-FU resistant cell lines[248], corroborating its role as a tumour suppressor gene. Conversely, overexpression of miR-122 significantly increased 5-FU-induced cell cytotoxicity in both 5-FU-sensitive and -resistant cell lines. The mechanism of action for this is thought to be miR-122's direct inhibition of pyruvate kinase type M2 (PKM2). PKM2 is an isoenzyme of pyruvate kinase that is involved in aerobic glycolysis and tumour cell growth[248]. PKM2 overexpression has been demonstrated in colon cancer cells and is associated with advanced stage and metastatic disease[249].

1.9. **Study objectives**

Pre-clinical and early clinical trials suggest that curcumin has potential to be a beneficial and well-tolerated adjunct to standard chemotherapy in the treatment of metastatic colorectal cancer. Thus far, no trials have been conducted to confirm that addition of curcumin to standard chemotherapy in patients with metastatic colorectal cancer is safe and well-tolerated. Likewise, data to identify potential biomarkers of efficacy and toxicity is limited, but nonetheless essential to predict and measure treatment outcomes. This trial aims to address these issues and provide a foundation for future trials to confirm whether addition of curcumin to standard chemotherapy results in improved patient outcomes.

1.9.1 Hypothesis

The combination of oral curcumin with first-line Oxaliplatin-based chemotherapy will be a safe and tolerable regimen for long-term administration to patients with metastatic colorectal cancer.

1.9.1. Primary objective

- To establish the safety, tolerability and feasibility of administering oral curcumin at increasing doses escalating to 4 capsules (2 g) during standard FOLFOX chemotherapy for palliation of colorectal liver metastases.

1.9.2. Secondary objectives

- To collect blood samples for future translational work including identification of putative biomarkers of efficacy and toxicity.
- To observe any changes to the neuropathic side-effects of chemotherapy.
- To observe potential for efficacy in terms of disease response and survival.

2. Methods

2.1. **Clinical methods**

2.1.1. Ethical approval

This trial was conducted following ethical approval from the Nottingham Research Ethics Committee (REC) 1 (REC reference number 11/EM/0263 – 25/08/11) and University Hospitals of Leicester NHS Trust (UHL) Research and Development (R&D) approval (25/10/11). It was sponsored by the University of Leicester (UNOLE225). The trial consists of a phase I dose escalation study followed by an open-labelled phase IIa two arm-randomised controlled trial.

2.1.2. Study population

The target population were patients presenting with a histological diagnosis of metastatic colorectal cancer who were suitable for palliative FOLFOX-based chemotherapy. The main inclusion criteria also required patients to have disease measurable by RECIST 1.1 (Response Evaluation Criteria in Solid Tumours version 1.1), be aged above 18 years with the ability to give informed consent, an ECOG (Eastern Cooperative Oncology Group) performance status of at least 1 and a life expectancy estimated as greater than 12 weeks. Patients were identified via oncology multidisciplinary team meetings and specialist referrals, and then reviewed in oncology outpatient clinics. The trial was discussed with eligible patients who were also given information sheets regarding the trial. Written consent was obtained from participants prior to enrolment into the trial.

The phase I study uses the traditional escalation rule (TER or 3+3+3) design, where patients are recruited in tiers of 3 participants. This requires the recruitment of a minimum of 9 and a maximum of 18 patients. The phase IIa study aims to recruit 33 participants in total – with 22 participants randomised to the FOLFOX and curcumin arm, and 11 participants in the FOLFOX only arm. As this is the first study administering this regimen in humans, there is no data available upon which to base power calculations. The sample size is comparable to that used in similar studies and will provide adequate data with which to power future studies, within a realistic time frame.

2.1.3. Phase I

Recruitment for the phase I study began in February 2012. The first tier of participants were recruited to receive 500 mg (1 capsule) of oral curcumin (C3-complex – 500 mg capsules) daily. The three participants started their curcumin doses 7 days before their scheduled chemotherapy. Their chemotherapy treatment was subsequently commenced once no dose-limiting toxicities (DLTs) were recorded during the preceding 7 days of curcumin. The FOLFOX-based chemotherapy consists of 2-weekly cycles of chemotherapy given to a maximum of 12 cycles or until withdrawal from the trial. (Where CAPOX chemotherapy is used, this consists of eight 3-weekly cycles of treatment.) Once the first tier of patients had no DLTs at 7 days after completing two cycles of chemotherapy, a further 3 patients could be recruited to the next escalation dose of 1 g (2 capsules) daily.

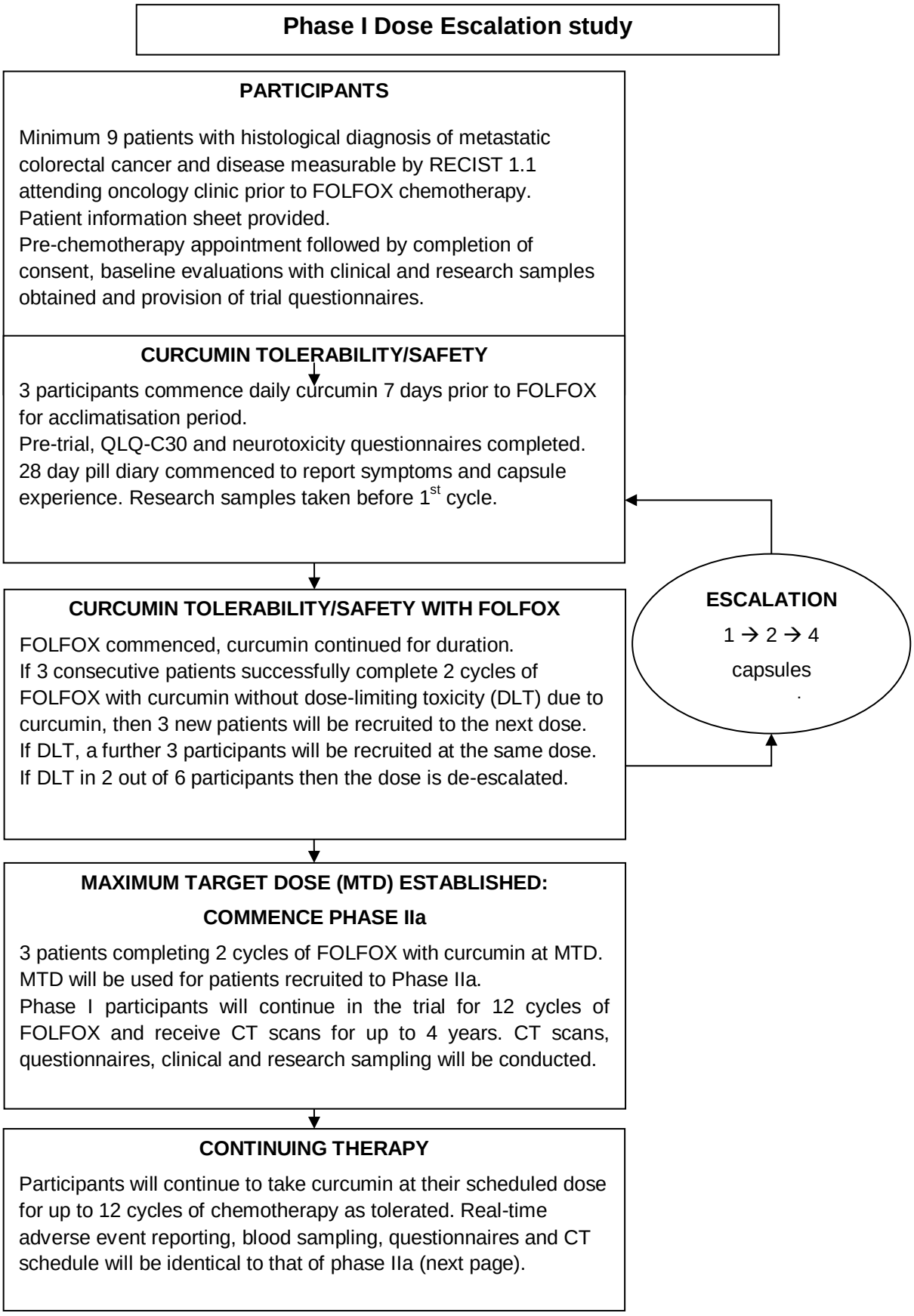
The same procedure was observed prior to escalation to 2 g (4 capsules) daily. In the event of DLTs occurring at a given dose, another 3 patients were recruited at that dose. If 2 of 6 participants suffer DLTs at a given dose, de-escalation occurred. The phase IIa study began recruitment after 3 participants from phase I had reached 7 days after completion of two cycles of FOLFOX at the maximum target dose (MTD).

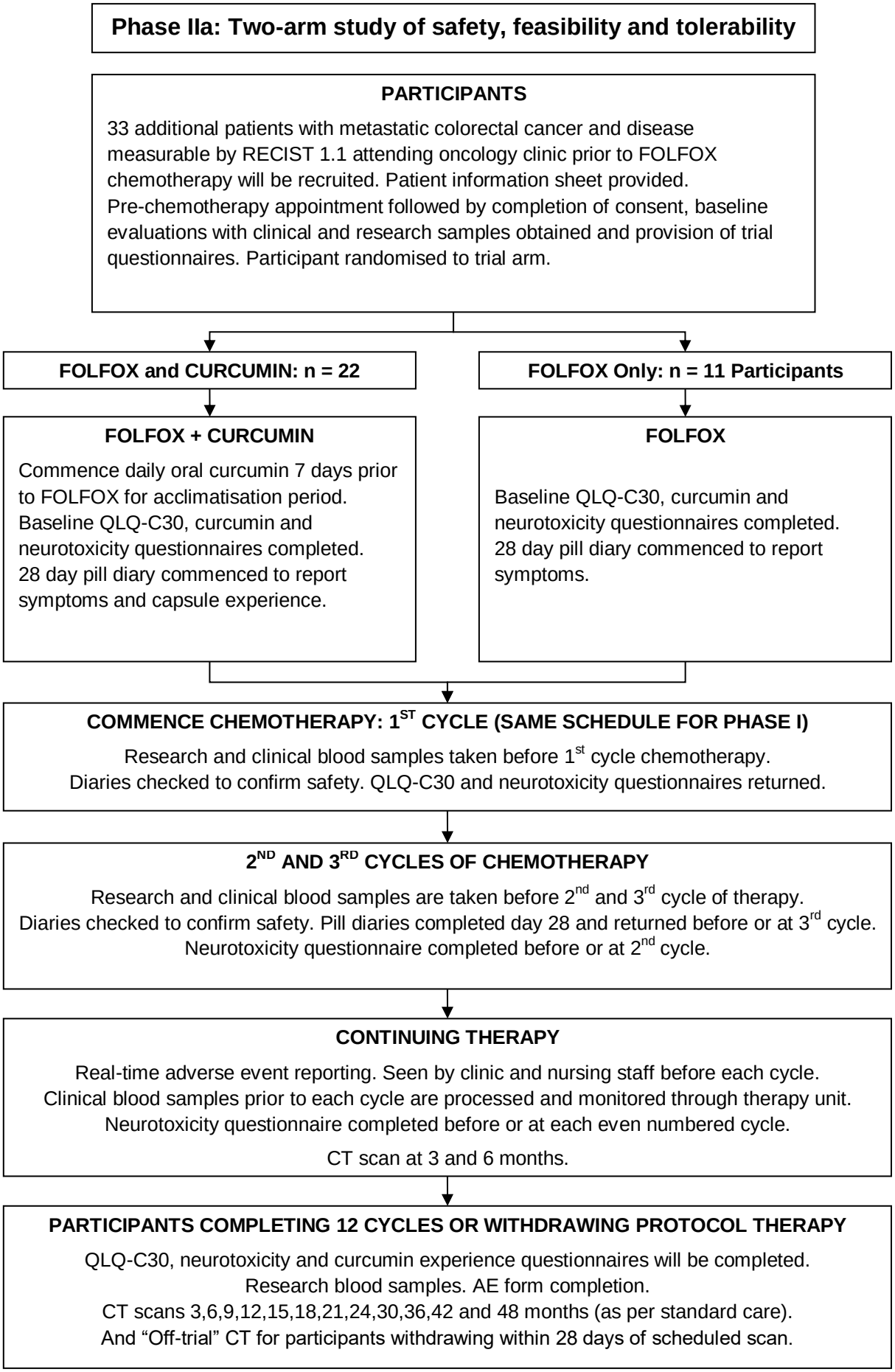
2.1.4. Phase IIa

Participants in the phase IIa randomised controlled trial were randomised to receive either Oxaliplatin-based chemotherapy and the MPD of daily oral curcumin (22 participants) as per the finding of phase I, or standard treatment only with no curcumin (11 participants). For the randomisatin process, an electronic random number generator was used to assign consecutive numbers from 1 to 33 to either treatment group 1, combination therapy or group 2, chemotherapy only. These numbers corresponded to patients' trial identity numbers. Each trial ID number and its treatment group was placed in a sealed envelope. Both the researcher and the patient were blinded at the point of recruitment. Once the consent form had been signed, the randomisation envelope was opened to reveal which treatment group the patient had been allocated to.

Patients in the FOLFOX and curcumin arm began taking curcumin 7 days before chemotherapy, and then commenced their first cycle of chemotherapy providing there were no DLTs.

2.1.5. Trial schema




Phase IIa: Two-arm study of safety, feasibility and tolerability
PARTICIPANTS
33 additional patients with metastatic colorectal cancer and disease measurable by RECIST 1.1 attending oncology clinic prior to FOLFOX chemotherapy will be recruited. Patient information sheet provided. Pre-chemotherapy appointment followed by completion of consent, baseline evaluations with clinical and research samples obtained and provision of trial questionnaires. Participant randomised to trial arm.
FOLFOX and CURCUMIN: n = 22
FOLFOX Only: n = 11 Participants
FOLFOX + CURCUMIN
Commence daily oral curcumin 7 days prior to FOLFOX for acclimatisation period. Baseline QLQ-C30, curcumin and neurotoxicity questionnaires completed. 28 day pill diary commenced to report symptoms and capsule experience.
FOLFOX
Baseline QLQ-C30, curcumin and neurotoxicity questionnaires completed. 28 day pill diary commenced to report symptoms.
COMMENCE CHEMOTHERAPY: 1ST CYCLE (SAME SCHEDULE FOR PHASE I)
Research and clinical blood samples taken before 1st cycle chemotherapy.
Diaries checked to confirm safety. QLQ-C30 and neurotoxicity questionnaires returned.
2ND AND 3RD CYCLES OF CHEMOTHERAPY
Research and clinical blood samples are taken before 2nd and 3rd cycle of therapy.
Diaries checked to confirm safety. Pill diaries completed day 28 and returned before or at 3rd cycle.
Neurotoxicity questionnaire completed before or at 2nd cycle.
CONTINUING THERAPY
Real-time adverse event reporting. Seen by clinic and nursing staff before each cycle.
Clinical blood samples prior to each cycle are processed and monitored through therapy unit.
Neurotoxicity questionnaire completed before or at each even numbered cycle.
CT scan at 3 and 6 months.
PARTICIPANTS COMPLETING 12 CYCLES OR WITHDRAWING PROTOCOL THERAPY
QLQ-C30, neurotoxicity and curcumin experience questionnaires will be completed.
Research blood samples. AE form completion.
CT scans 3,6,9,12,15,18,21,24,30,36,42 and 48 months (as per standard care).
And “Off-trial” CT for participants withdrawing within 28 days of scheduled scan.

2.1.6. Baseline evaluation

The baseline evaluation for each participant included a complete physical examination, measurement of body weight, height, baseline observations (temperature, blood pressure, heart rate), 12-lead electrocardiogram and assessment of ECOG performance status. Urine samples were also obtained from patients due to start on Bevacizumab to rule-out proteinuria and for pregnancy testing in pre-menopausal females. The standard pre-chemotherapy blood samples (full blood count, urea and electrolytes, liver function tests) were obtained from patients at their screening visit and prior to each cycle of chemotherapy. CEA was measured at baseline and will be repeated on a 12-weekly basis (Table 2.1).

2.1.7. Duration of treatment

A full course of standard chemotherapy for metastatic colorectal cancer consists of 24 weeks (6 months) of treatment. With FOLFOX chemotherapy, this consisted of twelve 2-weekly cycles of chemotherapy. For patients receiving CAPOX, this would require eight 3-weekly cycles of treatment, however none of the study patients at the point of writing this thesis required CAPOX chemotherapy. Curcumin was taken over the duration of treatment until cessation of chemotherapy. In the event of postponement of chemotherapy treatment, patients continued to take their curcumin capsules inbetween cycles.

The criteria for cessation of treatment prior to completion of the full 24 weeks of chemotherapy were:

- A serious, non-resolving adverse event or any intercurrent illness that compromised the patients ability to tolerate further chemotherapy
- The patient requested withdrawal from the study
- The investigator determined that withdrawal from the study was in the best interest of the patient
- Disease progression was recorded whilst on active treatment, indicating that an alternative form of treatment was required.

Disease progression was determined using RECIST 1.1 criteria to assess the size of target lesions. Progressive disease was defined as an increase of at least 20% in the sum of diameters of the target lesions, with that sum demonstrating an absolute increase of at

least 5 mm. Similarly the appearance of one or more new lesions on the CT scan signified progression. Finally, patients who showed clinical deterioration consistent with known symptoms and signs of their disease, in the absence of radiological evidence or alternative pathology as a causative factor, were classed as demonstrating clinical disease progression.

Table 2.1 Schedule of Tests and Procedures

Observation	Screening Pre-curcumin	Week 1/ curcumin loading	Week 3/ Before 2nd cycle	Week 5/ Before 3rd cycle	Week 24/ After Cycle 12 or withdrawal
Informed consent[1]	X				
EORTC QLQ-C30	X				X
Curcumin questionnaire	X				X
Medical history	X				
Physical examination	X				
Weight, temperature, blood pressure, pulse	X		X	X	X
ECOG performance status	X				X
12-lead Electrocardiogram	X				
Haematology, liver function, renal function[2]	X	Performed as routine prior to each cycle of FOLFOX			
Urine sample (pregnancy test for female patients)	X				
Tumour assessment[3]	Day -28 to 0	3 monthly CT scan to 24 months. 6 monthly CT 24 to 48 months when appropriate			
Serum CEA[2]	X	3 monthly CEA until CT scans end			
Symptom diary	X	Daily for first 4 weeks			
Neurotoxicity questionnaire	X	Questionnaire every 2 cycles, after cycle 12 or withdrawal			
Blood samples for curcumin, platinum and biomarker analysis	X	X	X	X	X[4]
FOLFOX treatment[5]		Up to 12 cycles, at 2 week intervals			
Survival		Continuously monitored once protocol therapy has ended[6]			

1. Can be obtained at any point prior to start of trial.
2. Patients on FOLFOX chemotherapy will routinely have these blood tests done prior to each cycle.
3. Baseline assessment of disease (CT/MRI of chest, abdomen and pelvis) must be done within 28 days and should be repeated every six cycles (12 weeks) during FOLFOX and then 3 monthly to 24 months and 6 monthly to 48 months.
4. Research samples after final cycle should be as close to 14 days as possible, but more than 7 and can be arranged to coincide with the next clinic appointment.
5. FOLFOX will be up to 12 cycles (approximately 24 weeks). Central line is placed prior to chemotherapy by trained staff.
6. Direct patient involvement in the trial will cease after curcumin has completed. Patient episodes following this will be confined to follow-up CT scans. Patients will remain in the standard care pathway.

2.1.8. Monitoring of toxicity, quality of life and compliance

On commencing the study, patients were issued with a 28-day pill diary to record their compliance with taking curcumin and record any symptoms or significant experiences. Neurotoxicity was monitored by participants completing neurotoxicity questionnaires at baseline and then at every even numbered cycle. Participants were also given quality of life (EORTC QLQ-C30) and curcumin questionnaires for completion at baseline and after 12 cycles of chemotherapy or withdrawal from the study. Baseline CT scans were performed within 4 weeks of recruitment and repeated 3-monthly, as per standard care.

The EORTC quality of life questionnaire is a 30-item questionnaire designed to assess the quality of life of cancer patients. It uses both multi-item scales and single-item measures to assess factors including physical functioning, social functioning, symptoms such as pain, and fatigue, as well as overall quality of life. The questionnaire content consists of five functional scales, three symptom scales, a global health status/quality of life scale and six single items. Each scale or single-item measure has a score ranging from 0 to 100, with the higher scores representing a higher response level. The scores for each of the scales was determined by firstly calculating the average of the items (I) contributing to the scale, known as the raw score. Linear transformation was then used to standardise the raw score, giving scores ranging from 0 to 100. Therefore the score (S) for each scale was obtained as follows:

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

Linear transformation to obtain score (S):

Functional scales: $S = \left\{1 - \frac{(RS - 1)}{\text{range}}\right\} \times 100$

Symptom scales/items: $S = \{(RS - 1)/ \text{range}^{11}\} \times 100$

Global health status/QoL: $S = \{(RS - 1)/ \text{range}\} \times 100$

With the functional and global health status scoring, a higher score represents a better level of functioning or quality of life. With the symptom scale, a higher score signifies worse symptoms.

Patients were reviewed in oncology outpatient clinics prior to each cycle of chemotherapy. Any reported toxicities were assessed using the National Cancer

[11] Range is the difference between the maximum and minimum possible values of RS.

Institute Common Terminology Criteria for Adverse Events 4.0 (NCI CTC AE 4.0). Patients were issued with new supplies of curcumin every 3rd cycle. At this point, any curcumin tablets remaining from their previous prescription were returned to pharmacy and counted.

2.1.9. Blood collection

Peripheral blood samples were obtained from patients either by venepuncture or via central venous access using aseptic techniques. Samples for standard pre-chemotherapy blood tests were taken using an EDTA tube for full blood count and a brown-topped tube for biochemistry and tumour markers. In addition to this, a further three blood samples were obtained. Two 7.5 mL samples (15 mL total) were collected in lithium-heparin tubes for curcumin/curcuminoid levels and biomarker studies. One 9 mL sample was collected in a serum Z tube for analysis of circulating miRNA.

Standard blood tests were taken at the screening visit (prior to commencing curcumin) and then prior to each cycle of chemotherapy. The standard pre-chemotherapy samples were sent to the Pathology Department at Leicester Royal Infirmary for analysis. Trial blood samples were taken from participants at the following time points:

- Screening visit (Baseline)
- Immediately before their 1st cycle of chemotherapy, after 1 week of taking curcumin only (Curcumin)
- Immediately before their 2nd cycle of chemotherapy, having completed at least 3 weeks of curcumin and 2 weeks after their 1st cycle of chemotherapy (Post #1)
- Immediately before their 3rd cycle of chemotherapy, having completed at least 5 weeks of curcumin and 2 weeks after their 2nd cycle of chemotherapy (Post #2)
- At their end of trial visit. This was arranged to be more than 7 days post-treatment and as close to 14 days as possible. Patients were instructed to stop taking their curcumin on the day their 5-FU pumps were disconnected (i.e. 2 days after commencing cycle) (End of Tx).

The trial blood samples were stored immediately on ice, in a covered ice-box for transport to the laboratory. These samples were processed within one hour of collection.

2.1.10. Trial outcome measures

Primary outcome measures:

- Phase I: Completion of dose escalation in 3 consecutive participants at 4 capsules or MTD of curcumin in combination with FOLFOX without adverse effects attributable to curcumin one week after two cycles of chemotherapy.
- Phase II: Completion of 12 cycles of chemotherapy (including dose reductions) or withdrawal from therapy by the target population.
- Suspected unexpected serious adverse reaction or serious adverse drug reaction attributable to curcumin
- Disease progression on CT scan

Secondary outcome measures:

- Improvement in progression free survival (PFS), objective response rate (ORR) and overall survival (OS) from enrolment to death as assessed by RECIST 1.1
- Improvement in neurotoxicity scores
- Analysis of blood for biomarker studies
- Analysis of blood for curcumin/curcuminoid levels

Curcumin will be discontinued when chemotherapy is withdrawn or completed.

2.2. **Laboratory methods**

2.2.1. Materials

Chemicals	**Supplier**
Acetic acid	Fisher Scientific
Acetone (HPLC grade)	Fisher Scientific
Acetonitrile	Fisher Scientific
Ammonium bicarbonate	Sigma
Applied Biosystems Taqman miRNA Reverse Trascription Kit	Thermo Scientific
Curcumin C3-complex	Sabinsa
Deoxycholic acid	Sigma
Dimethyl sulfoxide	Sigma
DL-Dithiothreitol (DTT, ultragrade)	Sigma
Ethanol 96 – 100%	Fisher Scientific
Ficoll Paque PLUS	GE Healthcare
Foetal calf serum	GE Healthcare
Formic acid	Fisher Scientific
HPLC gradient grade water	Fisher Scientific
Iodoacetamide (IAA, ultragrade)	Sigma
Methanol (HPLC grade)	Fisher Scientific
Millicell cell culture inserts	Fisher Scientific
miRNeasy mini kit	QIAGEN
Nitric acid, Aristar	Merck
Nuclease-free water, Ambion	Thermo Scientific
Ortho-phosphoric acid	Fisher Scientific
Oxaliplatin (Eloxatin)	Sanofi-Aventis synthelab
Pierce BCA protein assay kit	Thermo Scientific
Phosphate buffer solution tablets	Oxoid
Protein assay reagent	BioRad
ProteoMiner sequential elution kit	BioRad
QIAamp DNA blood MIDI kit	QIAGEN
QIAzol lysis reagent	QIAGEN
RPMI 1640 Media	GE Healthcare

Sequencing grade modified trypsin	Promega
Trypsin	Promega
Water (HPLC grade)	Fisher Scientific

2.2.2. Addresses of Suppliers

- BioRad, Hertfordshire, UK
- Fisher Scientific, Loughborough, UK
- GE Healthcare, Buckingham, UK
- Gibco-BRL (Invitrogen life technologies), Paisley, UK
- Merck Group, Darmstadt, Germany
- Nova Laboratories Ltd, Leicester, UK
- Oxoid, Hampshire, UK
- Promega, Southampton, UK
- QIAGEN, West Sussex, UK
- Sabinsa, Langen, Germany
- Sanofi-Aventis Synthelab, Surrey, UK
- Sarstedt, Numbrecht, Germany
- Sigma-Aldrich, Dorset, UK
- Thermo Scientific, Loughborough, UK
- Waters, Elstree, UK

2.2.3. Plasma separation

One 7.5 mL lithium-heparin sample was used for plasma separation. On arrival at the laboratory, samples were centrifuged as soon as possible at 3300 x g at 4°C for 10 minutes. This formed a superficial layer of plasma that was immediately aliquoted into 500 μL aliquots. The samples were stored in the freezer at -80°C until future use.

2.2.4. Peripheral blood lymphocyte extraction

One 7.5 mL lithium heparin sample was used for peripheral blood lymphocyte (PBL) extraction. PBL extraction was performed using density centrifuging with Ficoll-PaqueTM PLUS (FPP). This procedure was carried out in a class I hood at room temperature, using methods based on the manufacturer's instructions.

In brief, the anticoagulant-treated blood was mixed with an equal volume of RPMI 1640 media. 3.7 mL of the 1:1 blood/RPMI mixture was carefully layered upon 3 mL of FFP in 15 mL falcon tubes. The samples were centrifuged at 381 x g for 40 minutes at 18°C using a centrifuge with a swing-out rotor. Differential migration resulted in a layer of lymphocytes between the FPP and plasma that was then aspirated off with a Pasteur pipette. The aspirate was washed and centrifuged twice in RPMI 1640 media. The cells were suspended in 1 mL of RPMI/10% DMSO/20% FCS (DMSO – dimethylsulphoxide; FCS - foetal calf serum). The lymphocyte count was obtained using a haemocytometer. The sample was then stored at -80°C for future DNA extraction.

2.2.5. DNA analysis

2.2.5.1. DNA extraction

The platinum concentration in DNA extracted from patients' lymphocytes (PBLs) was measured to establish potential variance in DNA platination following curcumin treatment. This was to ensure that the addition of curcumin did not affect the efficacy of oxaliplatin in forming DNA platinum adducts. Genomic DNA extraction from the PBLs was performed using QIAamp DNA blood MIDI extraction kits (QIAGEN), as per the manufacturer's instructions.

The PBLs, previously frozen in 1 mL RPMI/10% DMSO/20% FCS at -80°C, were equilibrated to room temperature and centrifuged. The resultant pellet was resuspended in 1 mL of phosphate buffered saline (PBS) (pH 7.2). The sample was mixed with

QIAGEN Protease and Buffer AL then incubated at 70°C for cell lysis. The sample was centrifuged with ethanol and then washed twice using buffer solutions provided in the QIAamp MIDI kit. The sample was transferred onto the QIAamp Midi column provided and placed in a 15 mL centrifuge tube then centrifuged at 1850 x g for 3 minutes. Having discarded the filtrate, the sample was centrifuged with Buffer AW1 at 4500 x g for 3 minutes, then Buffer AW2 at 4500 x g for 15 minutes.

After discarding the filtrate, 200 µL Buffer AE was added to the QIAamp Midi column, then incubated at room temperature for 5 minutes. The column was centrifuged at 4500 x g for 2 minutes. To maximise DNA yield, a further 600 µL Buffer AE was added to the column, incubated and then centrifuged as previously. DNA concentrations were obtained using the Nanodrop spectrophotometer (Thermo Fisher Scientific Inc, Hertfordshire, United Kingdom). The samples were stored at -80°C until final analysis of DNA platination.

2.2.5.2. DNA platinum content analysis

All the Phase I samples were reconstituted to a final DNA concentration of 10 µg/mL in 500 µL 3% Aristar nitric acid (Merck, Darmstad, Germany) diluted with Buffer AE. DNA was also extracted from healthy volunteers in order to estimate what baseline platinum levels would be. I transported these samples to the Department of Earth Sciences, University of Durham and carried out DNA platinum analysis (under supervision of Dr C. Ottley). Platinum levels were measured on a Thermo Scientific X-Series 2 ICP-MS using a standard cross-flow nebulizer and Scott-type double-pass spray chamber. Samples were administered using a peristaltic pump into a standard cross-flow nebulizer fitted to the double-pass spray chamber. The nebulizer gas flow rates were between 0.8 to 1 L/min and optimized to maintain production of cerium oxide (CeO^+) equal to or less than 3% of the total Ce^+ signal. Because Ce^+ is prone to oxide formation, it was used for measurement of oxide formation for monitoring elemental interference.

Platinum standards of 100, 500, 1000 and 2000 ppt were prepared from a 1000 ppm stock solution. These standards were run pre- and post-samples to rule out instrumental drift. Similarly, the 500 ppt standard was re-run at intervals between samples to check for instrumental drift. As a result of alteration in the standard measurements during the

experiment, the standard curve plotted at the end of all measurements was used to determine platinum concentrations. Three platinum isotopes were monitored – ^{194}Pt (32.97% abundance), ^{195}Pt (33.83% abundance) and ^{196}Pt (25.24% abundance). Monitoring the differences between the levels of these isotopes provided a check for possible isobaric errors or interference. The concentrations measured for each platinum (Pt) isotope correlated with expected values based on abundance (within analytical error), therefore potential interferences were deemed negligible. The values of the three platinum isotopes were averaged to calculate the samples' platinum concentration. Where values for procedural blanks were significant, these were subtracted from platinum concentrations to correct the value. Hafnium forms oxide species (^{178}Hf^{16}O and ^{179}Hf^{16}O) which cause isobaric interference when measuring platinum levels. Measured hafnium oxide levels in the DNA solutions were too low to significantly impact on Pt measurements and therefore it was not necessary to correct for hafnium oxide.

The platinum measurements obtained were used to calculate the number of platinum atoms per nucleotide using the following formulae:

$$Pt\ per\ nucleotide = \frac{Number\ of\ Pt\ atoms\ per\ litre}{Number\ of\ nucleotides\ per\ litre}$$

$$No\ of\ Pt\ atoms\ per\ litre = \left(\frac{Pt\ \times 0.000001}{195.078}\right) \times Avogadros\ constant$$[12]

$$No\ of\ nucleotides\ per\ litre = \left(\frac{DNA\ concentration \times 0.001}{307.61}\right) \times Avogadros\ constant$$

2.2.5.3. Statistical analysis – DNA platination

Statistical analysis of data was conducted using IBM SPSS Statistics 22. The difference in platinum per nucleotide readings between treatments, tiers and the interaction between the treatment and tiers was analysed using a mixed design analysis of variance (mixed design ANOVA, with Bonferroni's post hoc test) model.

[12] Where Pt = platinum, Avogadro's constant = $6.02214129 \times 10^{23}$ mol^{-1}, 195.078 = atomic mass of platinum

2.2.6. Analysis of circulating tumour miRNA

Circulating tumour miRNA profiling was performed to assess the use of miRNA as a potential biomarker. A 9 mL blood sample was collected in a serum Z tube. The sample was left to clot at room temperature for 20 – 30 minutes then centrifuged at 2600 x g for 20 minutes. The separated upper serum layer was then aliquoted in 1 mL volumes into cryovial tubes and stored at -80˚C. I transported these serum samples to the Department of Pharmacology and Therapeutics, University of Liverpool where I analysed them for miRNA content (under supervision of Dr P. Starkey-Lewis laboratory).

2.2.6.1. miRNA extraction

For miRNA extraction, 40 µL of each serum sample was made up to 200 µL with nuclease free water. The samples were vortexed with 700 µL Qiazol reagent (3 x 5 seconds) and incubated at room temperature for nucleo-protein disruption. After adding 140 µL chloroform, the samples were shaken vigorously for 15 seconds then centrifuged at 12,000 x g for 15 minutes at 4˚C. The resultant supernatant was loaded into the Qiacube system (robotic workstation) for automated completion of miRNA extraction and purification. The eluted samples were frozen overnight at -80˚C.

2.2.6.2. miRNA reverse transcription

The samples were thawed on ice prior to use the following day. miRNA reverse transcription was performed (Applied Biosystems Taqman miRNA Reverse Trascription Kit) using an miRNA-122 primer and let-7d as the control primer. Each 15 µL reverse transcription reaction consisted of 12.25 µL of master mix, 0.75 µL of primer and 2 µL of eluted sample. Sufficient master-mix was prepared for the required number of reactions plus 10% to account for pipetting. The master-mix components were prepared as follows:

Component	Master-Mix volume per 15 µL (µL)
Nuclease-free water	9.41
100 mM dNTPs (with dTTP)	0.15
10X Reverse transcription buffer	1.50
RNase Inhibitor, 20 U/µL	0.19
MultiScribe™ Reverse Transcriptase, 50 U/µL[13]	1.00
Total volume	**13.00**

A separate master-mix was prepared for the control samples as follows:

Component	Master-Mix volume per 15 µL (µL)
Nuclease-free water	12.16
100 mM dNTPs (with dTTP)	0.15
10X Reverse transcription buffer	1.5
RNase Inhibitor, 20 U/µL	0.19
MultiScribe™ Reverse Transcriptase, 50 U/µL[5]	1.00
Total volume	**15.00**

A 96-well plate was loaded with 2 µL of each sample in duplicate (one for miRNA-122, one for let-7d) then 13 µL of master-mix was added for a final volume of 15 µL. For the controls, 15 µL of master-mix was loaded onto 3 wells. The plate was sealed and centrifuged for a few seconds to mix the samples and pull them to the bottom of the plates. After a 5 minute incubation on ice, the samples were loaded into the GeneAmp PCR system 9700 for reverse transcription cycles. The reverse transcription cycles were programmed as follows:

Step type	Time (minutes)	Temperature (˚C)
HOLD	30	16
HOLD	30	42
HOLD	5	85
HOLD	∞	4

2.2.6.3. miRNA PCR reactions

For the subsequent PCR reactions, the PCR reaction mix was prepared under dimly-lit conditions because the primers were light sensitive. The PCR reaction mix was prepared to be sufficient for each sample to be run in duplicate plus a 10% excess for volume loss from pipetting. The reverse transcription primer was diluted to 1:15 of the

[13] Add all components to master-mix except reverse transcriptase. Load samples into wells, then add reverse transcriptase to master-mix just before it is pipetted into the wells. This is to prevent the reaction beginning too early.

final qPCR reaction. Two PCR reaction mixes were made using the following components, where one used the miRNA-122 and the other the let-7d control primer:

Component	Volume per 20 µL reaction (µL)
Nuclease-free water	7.67
TaqMan®Universal PCR MasterMix II (2x)	10.00
TaqMan® Small RNA Assay (20x) (Primer)	1.00
Total volume	**18.67**

A 384-well plate was loaded with 1.33 µL of each reverse transcription sample in duplicate. The master-mix (18.67 µL) was added to each well, making a total volume of 20 µL per well. The samples were sealed with polypropylene and wrapped for light-protection then centrifuged at 500 x g for 5 seconds. The plate was loaded onto the PCR instrument (ViiA^{TM} Real-Time PCR System, 384-well) and run as below:

Step	**Enzyme activation**	**PCR**	
	HOLD	**Cycle (per sample)**	
		Denature	**Anneal/extend**
Temp (˚C)	95	95	60
Time	10 minutes	15 seconds	60 seconds

2.2.6.4. miRNA calculations versus ALT

The PCR results were given in terms of cycle threshold values (C_T). This represented the number of cycles required for the fluorescent signal to cross the threshold. C_T levels are inversely proportional to miRNa levels. C_T values are recorded on a log2 scale and therefore the following formula was used to transform the data:

2^ - C_T (target)/ 2^- C_T (normalizer) = normalized miRNA

(Where C_T (target) = mean miRNA-122; C_T(normalizer) = mean let-7d; ^ = to the power of)

The normalized miRNA is also called delta C_T. Each delta C_T value was divided by the value at baseline to calculate the fold change in miRNA ($C_{Tx}/C_{Tbaseline}$ = C_T fold-change), also called delta delta C_T.

Patients had blood tests measuring alanine transaminase (ALT) levels prior to every cycle of chemotherapy, as an indicator of liver function. The fold changes in ALT were

calculated by dividing each value by the ALT at baseline ($ALT_x/ALT_{baseline}$ = ALT fold-change).

The changes in miRNA were compared to the ALT changes to determine whether there was any correlation between these two biomarkers.

2.2.6.5. Statistical analysis – miRNA results

No statistical calculations were conducted on the miRNA results due to the limited number of samples analysed.

2.2.7. Analysis of plasma curcumin and curcuminoids

Levels of circulating curcumin and curcuminoids were measured as an indicator of compliance and to facilitate correlation between dosage and effect. This has previously been undertaken in a pilot study (Pilot study of curcumin to determine colonic tissue levels in patients undergoing colorectal endoscopy or colorectal cancer resection - UHL10364; EudraCT Number 2007-001971-13) and a method was established. For our study, liquid phase extraction was used to measure the levels of curcumin and curcumin metabolites in patients' plasma samples.

Patients' plasma samples were defrosted to room temperature (see Methods 2.2.3). Acetone was combined with 0.25M formic acid at a ratio of 9:1. Two hundred microliters of plasma was added to 400 µL of the acetone:formic acid then vortexed[14]. After incubation for 30 minutes at -20˚C the samples were again vortexed, then centrifuged at 13000 x g for 20 minutes at 4˚C. The supernatant was extracted and stored in the fridge. Another 400 µL acetone:formic acid added to re-extract the precipitate. The sample was again incubated at -20˚C for 30 minutes then centrifuged at 13000 x g for 20 minutes at 4˚C. The two supernatants were combined then dried down in a speed vac at 45˚C. The residue was re-suspended in 100 µL of 50:50 ammonium acetate:acetonitrile, vortexed then centrifuged at 13000 x g for three minutes.

The resulting sample was analysed for curcumin and its metabolites by high-performance liquid chromatography (HPLC), using the Alliance HPLC system with UV visible detector and an Atlantic dC18 3 µM reverse phase column at 15˚C. The mobile phases comprised of 10 mM ammonium acetate, pH 4.5 (A), and acetonitrile (B). These solvents were programmed to begin at 95% A, then progress to 55% A at 20

[14] These samples were reanalysed (credit J.Mahale) using 100 µL of plasma as per the validated methodology.

minutes and 5% A at 33 minutes, with a flow rate of 1.0 mL/min. Curcumin and its metabolites were detected at 426 nm using a Waters 2487 dual λ absorbance detector.

Curcumin, curcumin glucuronide and sulphate stocks were prepared to the following concentrations 0.2, 0.5, 1, 5, 10, 20, 50 and 100 µg/mL in DMSO to a final volume of 1 mL. The stock samples were used to run 'extracted standards' by adding 2 µL of each stock to 100 µL of plasma from healthy volunteers. The curcumin was extracted using the same method described above for patient samples. 'Unextracted standards' were also prepared by adding 2 µL of the stock samples to 100 µL of 50:50 ammonium acetate (10 mM, pH 4.5):acetonitrile, vortexing and centrifuging at 13000 x g for 3 minutes. Fifty microliters of the extracted and unextracted standards were injected into the HPLC and the results were used to assess the linearity of the method, estimate retention times for curcumin and its metabolites and plot standard calibration curves. Three sets of extracted standards were run against one set of unextracted standard.

The limit of quantification (LoQ) was defined as the lowest standard concentration that could be determined with an accuracy and precision of greater than 20%. Once extracted, each set of patient samples was run on the HPLC alongside curcumin standards that included concentrations from the lower (LLoQ) to the upper limit of quantification (ULoQ). Quantitation was performed using the resultant standard curve, without an internal standard.

2.2.8. Conversion of curcumin metabolites to parent curcuminoids[15]

β-glucuronidase was dissolved in 0.1 M phosphate buffer (pH 6.8) to produce 1800 units of β-glucuronidase per 100 µL buffer. An aliquot of 100 µL from each plasma sample was combined with 100 µL β-glucuronidase and vortexed for optimal mixing. The samples were incubated at 37˚C for 3.5 hours, then extraction of curcuminoids was carried out as described above.

2.2.9. Biomarker studies – Proteomic analysis of explant cultures

2.2.9.1. Explant culture

Tissue samples of colorectal liver metastases from patients undergoing surgical resection of their disease at University Hospitals of Leicester NHS Trust hospitals had

[15] Credit J.Mahale

been obtained for an excess tissue study at University of Leicester[16]. For this study, 9 segments of tumour (1mm x 1mm x 1mm) were incubated for 15 hours (minimum) on inserts at 37˚C, 5% CO_2 in 6-well plates containing 1.5 mL of media (DMEM[17] 1% FCS antibiotic/antimycotic). Half the media was then replaced with a 0.75 mL treatment of one of the following: DMSO (control), 5 µM curcumin, 2 µM oxaliplatin + 5 µM 5-FU, or 5 µM curcumin + 2 µM oxaliplatin + 5 µM 5-FU, then incubated for a further 24 hours prior to formalin fixation/paraffin embedding. The explant media from these samples was extracted and stored at -20˚C.

2.2.9.2. Desalting of explant samples

The explant samples were desalted/cleaned in preparation for enzymatic digestion and mass spectrometry. For this process, 300 µL of 50 mM ammonium bicarbonate was pipetted into an Amicon Ultra-0.5 filter device and centrifuged at 11,000 rpm for ten minutes at 4˚C. The filtrate liquid was discarded then 500 µL of explant sample was loaded onto the filter device. The sample was centrifuged at 11,000 rpm for twenty minutes at 4˚C then the filtrate was discarded. This was repeated with the remainder of the explant sample, using 500 µL aliquots until the entire sample had been loaded. The filter was washed x 3 with 450 µL of 50 mM ammonium bicarbonate. The Amicon Ultra-0.5 filter was then placed upside-down in a clean collection tube and centrifuged at 1000 rpm for one minute at 4˚C to transfer the remaining concentrated sample into the tube. Any residual proteins were washed into the collection tube by repeatedly pipetting 100 µL of ammonium bicarbonate along the sides of the filter device. The filter was again centrifuged upside-down at 1000 rpm for 1 minute at 4˚C. Forty µL of the ultrafiltrate was used for protein determination, and the remainder of the sample stored at -20˚C until use.

2.2.9.3. Protein assay

A protein assay was performed to determine the protein concentration of each sample prior to tryptic digestion. A stock of 1 mg/mL bovine serum albumin (BSA) was prepared by combining equal volumes of albumin with deionised water. This was diluted to produce 100 µL volumes of standards at concentration of 0.2 to 1.0 mg/mL. A 96-well plate was loaded with 10 µL of each explant sample and 10 µL of each BSA standard (in triplicate). To all these samples, 200 µL Bicinchoninic Acid (BCA)

[16] Leicestershire, Northamptonshire and Rutland ethics committee – REC reference 09/H0402/45
[17] Dulbecco's Modified Eagle's Medium (DMEM)

working solution was added, and the samples incubated at 30˚C for 30 minutes before measuring absorbance at a wavelength of 595 nm using the FLUOstar Optima Microplate reader (BMG Labtech, Bucks, UK). Protein concentrations were calculated by comparing the sample readings to the standard curve values (y = mx + c), then 100 µg protein was extracted from each sample for tryptic digestion.

2.2.9.4. Tryptic digest

Tryptic digestion was used to reduce the explant proteins into their peptide constituents prior to analysis by mass spectrometry. A mass of 100 µg protein from each sample was initially heated at 80˚C for 15 minutes. They were then chemically reduced by heating with 100 mM DL-dithiothreitol (DTT) (5 µL per 100 µg protein) at 60˚C for 15 minutes. Following this, the samples were alkylated by incubating them in the dark for 30 minutes with 200 mM iodoacetamide (IAA) (5 µL per 100 µg protein). The resulting solution was digested overnight with trypsin (1 µg trypsin per 40 µg protein). The digestion process was arrested by adding 2 µL formic acid, then the samples were centrifuged at 13000 rpm for 1 minute to remove insoluble material. The supernatant was extracted and concentrated using a SpeedVac (90 min) before 3 hours of freeze drying in preparation for mass spectrometer analysis.

2.2.9.5. Nano Ultra Performance Liquid Chromatography (NanoUPLC)

The samples were reconstituted in 10µL of 0.1% formic acid/3% acetonitrile. Then 5µL of this sample was combined with 5µL of alcohol dehydrogenase (ADH – 50fM/µL) as an internal standard. The samples were analysed using a Waters NanoAcquity UPLC system. Two µL of each sample was loaded onto a Waters Symmetry C18 trap column (180 µm x 20 mm, 5 µm) to desalt and chromatographically focus the peptides prior to elution onto a HSS T3 C_{18} 75 µm x 150 mm, 1.7 µm analytical column. For elution, HPLC-grade water with 0.1% formic acid was used as Solvent A and Solvent B was Acetonitrile with 0.1% formic acid. The flow rate was set to 0.3 µL/min. The gradient began after a 3 minute (5 µl/min) trapping stage on the trap column. The gradient used was as follows: 0 min – 1%B, 90 min – 40%B, 92 min – 85%B. The gradient was held at 85% B at 93 minutes, then returned to starting conditions at 95 min to equilibrate. The total run time was 110 min.

2.2.9.6. Mass spectrometry

The Nano Acquity UPLC system was coupled to a Waters Synapt G2 HDMS for analysis of individual samples. The instrument was operated in positive ion nanoelectrospray ionisation mode. The capillary voltage was set at 3.4 kV, cone voltage of 30 V and temperature 80˚C. Picotip emitters (10 μm internal diameter) were used for the nanostage probe, directing flow from the analytical column to the source. A helium gas flow of 180 mL/min and ion mobility separator (IMS) nitrogen gas flow of 90 mL/min with a pressure of 2.5 mbar was used. The IM wave velocity was set to 600 m/s and the wave height at 40 V throughout each run. For ion mobility separation, nitrogen was used for drift gas with an incorporated helium cell for ion stream focusing. During $HDMS^E$ acquisition low collision induced dissociation (CID) energy, 2 V was applied across the transfer ion guide. For high CID energy acquisition, a ramp of 27 to 50 V was applied. Argon was used as the CID gas. Lockspray maintained mass accuracy throughout the chromatographic run, using [Glu1]-Fibrinopeptide (GFP) with 785.8427 m/z. Data was assimilated in $HDMS^\varepsilon$ mode from m/z 100 – 1950 with a scan speed 0.9s/scan using MassLynx 4.1.

2.2.9.7. Data analysis

The raw data was processed using Protein Lynx Global Server (PLGS) 3.0. This version of PLGS uses the drift time of ion mobility-separated peptides to optimise the specificity of alignment/association for the precursor and product ions. Data were extracted, aligned and searched against the Uniprot human proteomic database, version 2013-11, appended with the alcohol dehydrogenase (*S.cerevisiae)* sequence. The ion accounting algorithm used has been outlined previously[250,251]. Data was further processed using Microsoft Excel 2010, GraphPad Prism 6, IdentityE and Scaffold (Scaffold_4.3.4, Proteome Software).

2.2.9.8. Protein identification

Differential protein expression was analysed using Progenesis QI software. The optimal threshold was set to 100-10-750. The raw data was aligned using the QC1 run to allow for composite comparison between the different groups. Fixed modification of carbamidomethylation and variable modifications consisting of deamidation N, deamidation Q, oxidation M, Phosphoryl STY were selected for protein identification.

A stringent FDR of less than 1% was chosen. For peptide matching 2 or more fragments were required. 5 fragments were required for protein identification and 2 peptides required for protein identification. For protein quantification Hi-3 was used. Triplicate injections of the same sample were analysed for all patients.

Functional analysis of the resultant proteins was conducted using DAVID (the database for annotation, visualization and integrated discovery) Bioinformatics Resources 6.7 [252,253], which co-ordinated with the KEGG (Kyoto Encyclopedia of Genes and Genomes) database to generate maps of functional pathways[254,255].

3. Phase I

3.1. Introduction

The primary objective of the CUFOX study was to establish the safety, tolerability and feasibility of administering oral curcumin (up to 2 g daily) during standard oxaliplatin-based chemotherapy as palliative treatment for patients with metastatic colorectal cancer. Phase I used a 3+3+3 dose escalation design to determine the maximum target dose of curcumin that could be tolerated in combination with standard chemotherapy. Once established, this dose was used in the phase IIa randomised controlled trial comparing patients being treated with curcumin at the maximum target dose in combination with standard treatment, versus patients being treated with standard treatment only.

The tools used to assess safety, tolerability and feasibility in this trial included clinical assessment and patient feedback via questionnaires. The Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 is the primary system used for the documentation of adverse events in clinical trials and was employed in this trial for the clinician's documentation of adverse events. Patients recorded their symptoms and compliance using a 28-day pill diary and the EORTC QLQ-C30[18] questionnaire. Discrepancies in symptom reporting between patients and clinicians have been shown to occur primarily when grading subjective symptoms such as fatigue and dyspnoea(256). Therefore collating data from both the clinician and patient viewpoints will ensure a more comprehensive account of events.

Alongside the traditional clinical trial outcome measures of progression-free and overall survival, quality of life assessment is now recognised as an important outcome measure in the treatment of cancer patients. For patients with metastatic colorectal cancer, their quality of life is already impacted by symptoms from disease and in turn this affects which treatment modalities they can be offered. Patients with stage IV colorectal cancer tend to experience a negative trend in quality of life over the duration of their treatment(257). It is therefore imperative to minimise further detrimental effects from treatment toxicity on the quality of life of these patients.

[18] European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire. C30 is a validated 30-item cancer specific questionnaire.

3.1. **Phase I Clinical Results**

3.1.1. Recruitment

Twenty-five patients were screened between February 2012 and March 2013 for recruitment into phase I of the clinical trial. Eleven patients failed screening as they did not fulfil the inclusion criteria for the trial and one patient declined. Reasons for failing screening included poor performance status (4 patients), bone metastases (1 patient), not being suitable for chemotherapy due to comorbidities (1 patient), liver disease operable without chemotherapy (1 patient), unable to give consent (1 patient), peptic ulcer disease (2 patients), and no liver metastases (1 patient). The remaining thirteen eligible patients opted to be recruited into the trial (Table 3.1). Therefore there was a 92.8% recruitment rate amongst eligible patients, and overall recruitment was 37.5%. Eight men (61.5%) and five women (38.5%) were recruited with a median age of 65 years old (range 41 to 76 years old). All the participants were of white British ethnicity and had lived in Britain all their lives. The three participants recruited onto tier 1 all completed 2 cycles of chemotherapy without experiencing any curcumin-related dose-limiting toxicity and thus fulfilled the criteria for recruitment to begin on the second tier. Likewise, dose-escalation onto the third tier began after the 3 patients from tier 2 completed 2 cycles of chemotherapy without curcumin-related dose-limiting toxicities. One patient from the initial three recruited into tier 3 experienced a curcumin-related dose-limiting toxicity (see Section 3.2.4 – Toxicity) and therefore a further three patients (6 in total) were entered into and completed tier 3 of Phase I at a dose of 2 grams daily.

Table 3.1 Screening and recruitment of patients into Tiers 1, 2 and 3 for Phase 1

Tier	N^{o} screened	N^{o} failed screening	N^{o} recruited	N^{o} declined	Overall recruitment rate (%)	Recruitment rate - eligible (%)
1 (500mg)	8	5	3	0	37.5	100.0
2 (1 gram)	4	0	4	0	100.0	100.0
3 (2 grams)	13	6	6	1	53.8	85.7
Total	25	11	13	1	56.0	92.9

3.1.2. Treatment

The three participants recruited into the first tier were all treated with 5-FU/oxaliplatin/ folinic acid (FOLFOX) chemotherapy and Bevacizumab, with the addition of 500 mg (1 capsule) oral curcumin daily (See Table 3.2). Two of these participants completed 12 cycles of chemotherapy. Participant CUFOX1 003 did not require any alteration to chemotherapy or Bevacizumab treatment. However, participant CUFOX1 001 had the oxaliplatin dose initially reduced by 25% for cycle 9 and then completely omitted for the last three cycles due to chronic peripheral neuropathy. Both of these patients tolerated the full curcumin dose throughout their treatment. Treatment was stopped for participant CUFOX1 002 after 7 cycles due to chemotherapy side-effects. A total of 47 doses of curcumin were omitted for this participant, primarily due to curcumin being stopped during hospital admissions.

One participant in tier 2 (CUFOX1 004) was treated with FOLFOX[19] only whilst all the other participants on this tier received FOLFOX and Bevacizumab[20] as well as a daily dose of 1 gram curcumin (Table 3.2). One participant from tier 2 (CUFOX1 005) was withdrawn from the trial after suffering a myocardial infarction following one cycle of chemotherapy (See Section 3.2.4 – Toxicity). Although curcumin use was not thought to have been a contributing factor, this patient was withdrawn from the trial because chemotherapy was terminated, and the patient replaced with another participant. Participant CUFOX1 004 completed the full twelve cycles of chemotherapy. However, only 75% of the oxaliplatin dose was given for cycle 12 due to symptoms of chronic peripheral neuropathy. One participant (CUFOX1 006) stopped treatment after 9 cycles due to toxicity and therefore curcumin was also stopped. Although participant CUFOX1 007 was tolerating treatment well with no dose limiting toxicities, they chose to withdraw from the trial after 6 cycles of treatment due to the trial's impact on their lifestyle. This participant continued on standard treatment without trial follow-up. The curcumin dose for participant CUFOX1 004 was temporarily halved (from 1 gram to 500 mg) with cycle 5 of chemotherapy due to symptoms of abdominal pain and flatulence. However this dose reduction failed to improve the patient's symptoms so the full dose of curcumin was reinstated with the symptoms subsequently being

[19] 5-FU (fluorouracil) + oxaliplatin + folinic acid

[20] Bevacizumab contraindications include: Tumour invading blood vessels, surgical procedure within past 28 days, uncontrolled hypertension, significant cardiovascular/cerebrovascular disease, inherited bleeding diathesis or coagulopathy, non-healing wounds.

controlled with ancillary treatment. All the other participants on this tier tolerated the full dose of curcumin throughout their treatment.

In tier 3, all participants were treated with FOLFOX and bevacizumab, except CUFOX1 010 who received FOLFOX chemotherapy only. Patient CUFOX1 008 experienced significant peripheral neuropathy resulting in dose reductions and eventual cessation of oxaliplatin. This patient also had bevacizumab stopped after 8 cycles of treatment after being diagnosed with pulmonary emboli. Patient CUFOX1 011 was diagnosed with venous stasis and therefore bevacizumab was omitted from cycle two. CUFOX1 010, 012 and 013 all completed 12 cycles of treatment. The treatment doses for both 5-FU and oxaliplatin were reduced to 75% from cycle 2 for patient CUFOX1 010, and from cycle 8 for CUFOX1 012. (Details of altered oxaliplatin doses will be discussed in Chapter 3.2.5 Peripheral Neuropathy.)

Overall 50% of patients completed 12 cycles of chemotherapy and curcumin. That included both of the patients treated with FOLFOX chemotherapy and 40% of the patients treated with FOLFOX and Bevacizumab.

Table 3.2 Summary of curcumin doses and standard treatments allocated to patients, cycles completed and compliance rates. Compliance rates documented as per patient feedback. Curc – Curcumin; g – grams; Tx – treatment; F+B – 5-FU (fluorouracil)/oxaliplatin/folinic acid (FOLFOX) + Bevacizumab; F – 5-FU (fluorouracil)/oxaliplatin/folinic acid (FOLFOX)

Study ID CUFOX1	Curc dose (g)	Tx	N° cycles	Tx Outcome	Curc compliance	Reason for non-compliance
001	0.5	F+B	12	Completed	100%	-
002	0.5	F+B	7	Stopped	52.0%	Hospital admission: nausea, diarrhoea, infection, dehydration
003	0.5	F+B	12	Completed	98.8%	Forgot to take curcumin
004	1.0	F	12	Completed	100%	-
006	1.0	F+B	9	Stopped	100%	-
007	1.0	F+B	6	Withdrew	100%	-
008	2.0	F+B	10	Stopped	97.1%	Hospital admission: pulmonary embolism
009	1.0[21]	F+B	10	Stopped	83.0%	Dose limiting toxicity
010	2.0	F	12	Completed	96.9%	Dry throat caused difficulty swallowing capsules
011	2.0	F+B	4	Died	96.4%	Hospital admission: DVT, 1 day diarrhoea - resolved spontaneously so curcumin restarted
012	2.0	F+B	12	Completed	99.4%	Forgot to take one dose
013	2.0	F+B	12	Completed	100%	-

3.1.3. Curcumin compliance

Curcumin compliance is described in Table 3.2. The average compliance rate across all participants was 93.8%. Comparing the different tiers, the mean compliance rates were 83.6% for tier 1, 100% for tier 2 ($p^{22} = 0.27$) and 95.5% for tier 3 ($p = 0.13$). Patient CUFOX1 002 had the lowest compliance rate at 52.0% after missing 47 doses due to his hospital admissions. CUFOX1 009 missed 23 out of 140 curcumin doses (83.0% compliance), primarily due to suffering dose-limiting toxicity, and was dose-reduced from 2 grams to 1 gram of curcumin daily after three cycles of chemotherapy. No further issues of compliance were reported on this lower dose. CUFOX1 010's initial

[21] Started on 2grams daily, reduced to 1 gram after 3 cycles due to dose limiting toxicity.
[22] Tier 1 is used as baseline. P values are from comparison of tiers 2 and 3 versus tier 1.

difficulty swallowing capsules was resolved by spreading each dose over 30 minutes. A 100% compliance rate was achieved by five patients (41.7%).

3.1.4. Toxicity

Five of twelve participants (41.7%) did not experience any curcumin-induced adverse events during the one week of curcumin-only treatment (Fig 3.1). The most common curcumin-induced side effects were constipation (25.0% of patients), dry mouth (16.7%) and flatulence (16.7%). All adverse events reported were grade 2 or less and therefore did not prevent patients from progressing onto chemotherapy.

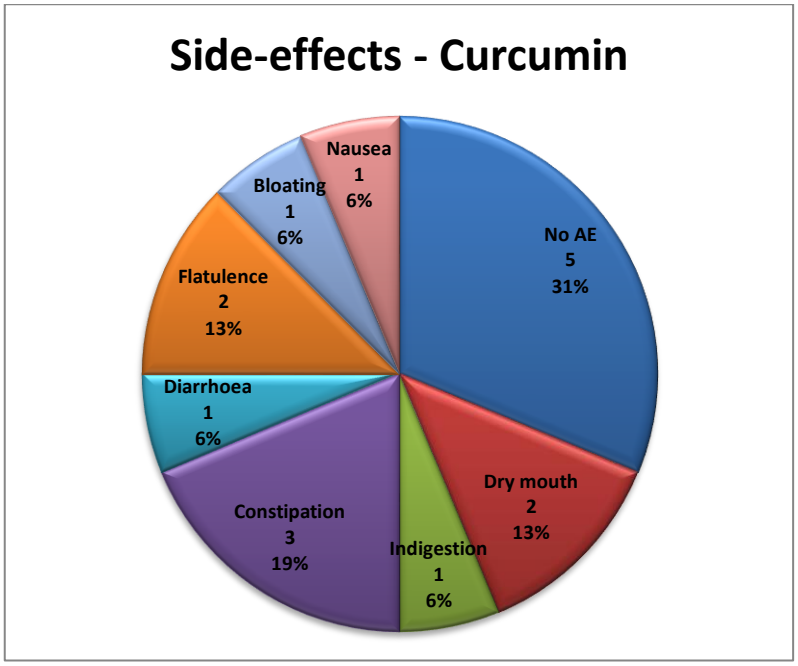


Figure 3.1 Adverse events (AE) reported by patients whilst treated with curcumin only (pre-chemotherapy). Number of patients affected (Group total = 13).

On commencing chemotherapy, the most commonly reported side effect was peripheral neuropathy, which was noted in eleven patients (91.7%). Other commonly reported toxicities were fatigue (83.3%), diarrhoea (66.7%) and oral mucositis (58.3%). Side effects that could possibly be attributed to curcumin are shown in Table 3.3. Despite the addition of chemotherapy, the majority of adverse events (90.0%) were classed as grade 1 or 2, while 10.0% were grade 3. Overall, three patients described grade 3 toxicities that could have been attributed to curcumin. Grade 3 diarrhoea was deemed a dose-limiting toxicity for one patient, therefore their curcumin dose was maintained at 1 gram daily. After another seven cycles of treatment, this patient was again admitted

with grade 3 diarrhoea and at this point all treatment was stopped. Other grade 3 toxicities that were not thought to have been curcumin-related included fatigue, hyponatraemia, neutropenia, thromboembolic events, urinary tract infections and weight loss. No grade 4 event was recorded. One grade 5 event occurred where a patient (CUFOX1 011) was admitted with a severe chest infection and subsequently died. This event was not thought to be due to curcumin use.

Table 3.3 Treatment side-effects which may possibly be attributable to curcumin Grade 3 adverse events in **bold**.

Adverse event	N° patients	% patients	Grade pre-chemotherapy	Grade with chemotherapy	Grade 3 N° patients	Grade 3 % events
Abdominal pain	4	33.3%	1,2	**1,2,3**	1	16.7%
Acute kidney injury	1	8.3%	-	**1,3**	1	33.3%
Anorexia	4	33.3%	-	**2,3**	3	75.0%
Bloating	1	8.3%	2	-	-	-
Constipation	5	41.7%	1	1,2	-	-
Diarrhoea	8	66.7%	1	**1,2,3**	2	30.8%
Dry mouth	3	25.0%	1	1	-	-
Dyspepsia	4	33.3%	1	2	-	-
Flatulence	3	25.0%	1	2	-	-
Oral mucositis	7	58.3%	-	**1,2,3**	2	16.7%
Nausea	4	33.3%	1	**1,2,3**	1	16.7%
Rash	2	16.7%	-	1,2	-	-
Vomiting	2	16.7%	-	1,2	-	-
Weight loss	4	33.3%	-	**1,2,3**	1	20.0%

There were eleven serious adverse events (SAEs) reported in phase I, which resulted either in hospital admission or were potentially life-threatening (Fig 3.2). The only serious adverse event where curcumin was considered to have contributed is diarrhoea, despite this also being a known side-effect of chemotherapy. Acute kidney injury secondary to diarrhoea and dehydration was reported in the same patient on two occasions. These episodes were reported as suspected unexpected serious adverse events.

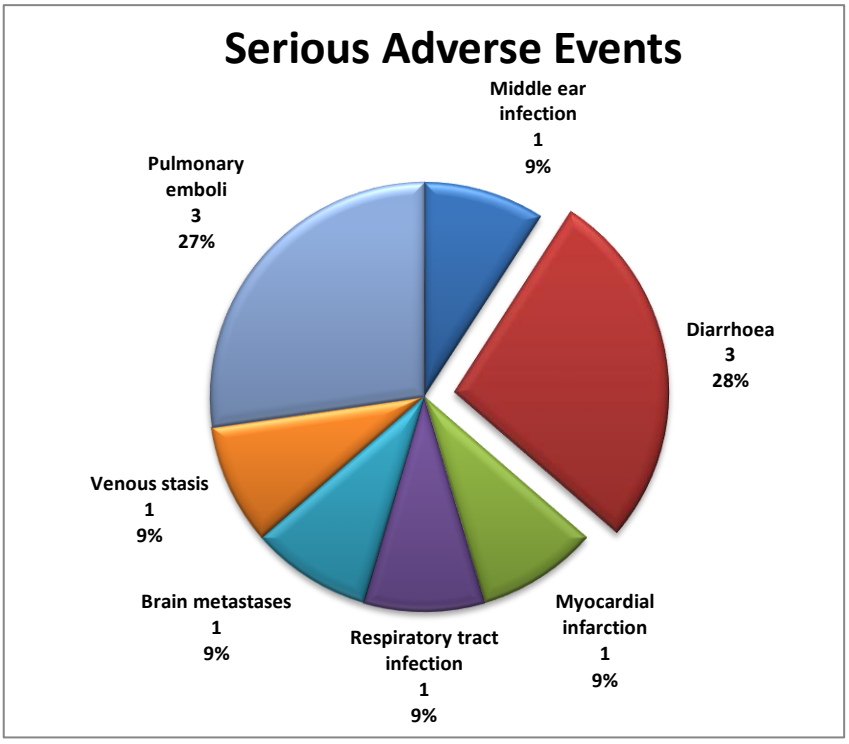


Figure 3.2 Serious adverse events experienced by patients during treatment with curcumin plus standard chemotherapy. Diarrhoea (reported in 3 patients) was the only serious adverse event attributed to curcumin. (11 serious adverse events reported in group of 13 patients)

3.1.5. Peripheral neuropathy

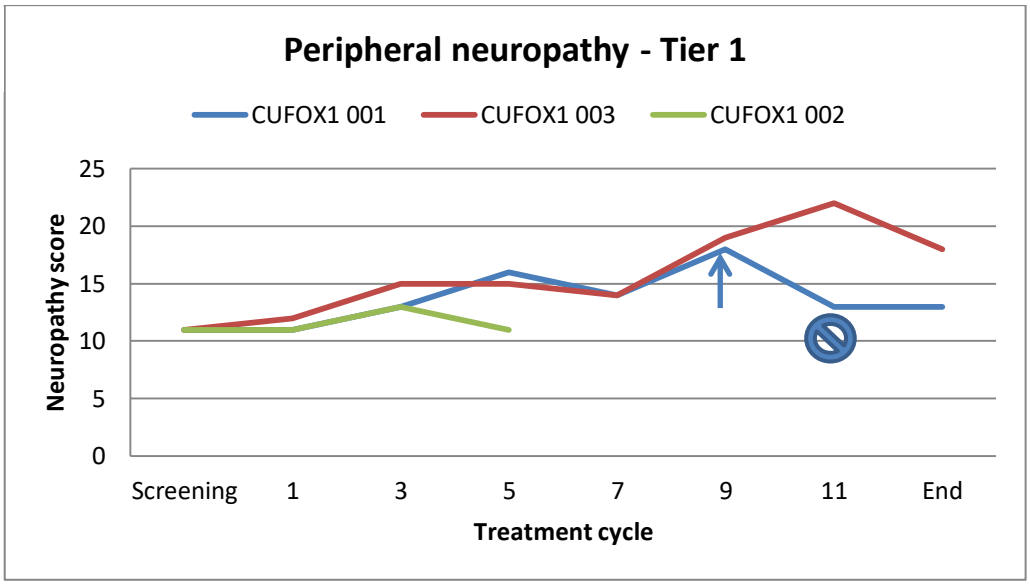
All the patients reported some degree of sensory peripheral neuropathy in the neurotoxicity questionnaires (Table 3.4, Fig 3.3). For the majority of participants, symptoms consisted of short-lived episodes of cold-induced neuropathy which resolved between treatment cycles. The trend showed a worsening of peripheral neuropathy with continued cycles of chemotherapy. Six participants (50%) required oxaliplatin dose reductions after displaying symptoms of chronic peripheral neuropathy. Three patients (25%) (CUFOX1 008, 010, and 012) eventually required a 50% dose reduction in their oxaliplatin dose. Oxaliplatin was completely stopped after 9 cycles of treatment in one participant (CUFOX1 001), following which there was an improvement in symptoms.

Table 3.4 Peripheral neuropathy experienced by patients over course of chemotherapy. Increasing score represents worsening peripheral neuropathy. Minimum score = 11; Maximum score = 29 (of potential 44). CUFOX1 011 died after cycle 3 (not curcumin-related), therefore no 'End' score.

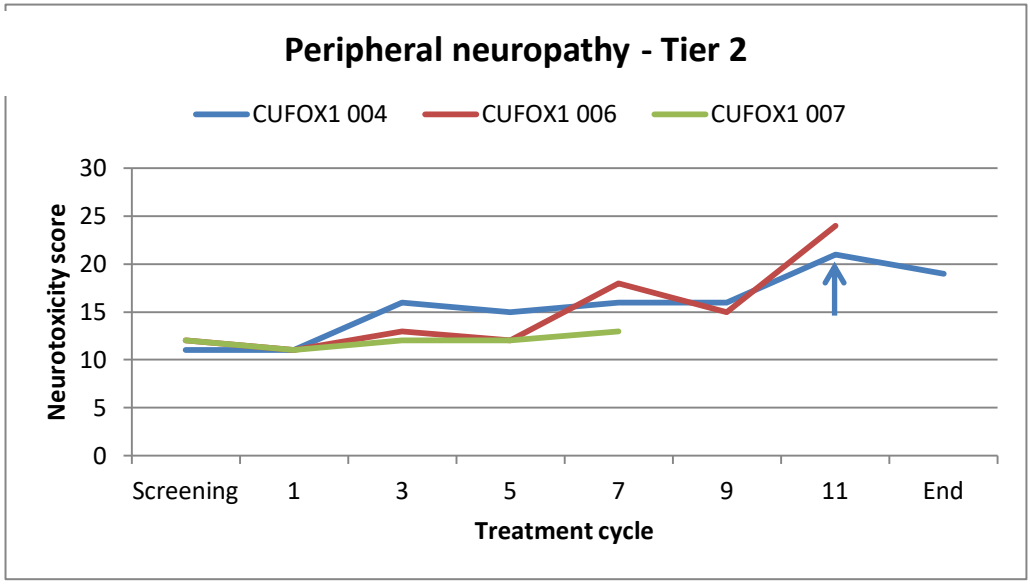
Study ID	Treatment cycles							
	Screening	1	3	5	7	9	11	End
CUFOX1 001	11	11	13	16	14	18	13	13
CUFOX1 002	11	11	13	11	-	-	-	11
CUFOX1 003	11	12	15	15	14	19	22	18
CUFOX1 004	11	11	16	15	16	16	21	19
CUFOX1 006	12	11	13	12	18	15	-	24
CUFOX1 007	12	11	12	12	-	-	-	13
CUFOX1 008	13	15	20	25	25	29	-	29
CUFOX1 009	13	15	17	18	21	20	-	21
CUFOX1 010	11	15	14	15	19	19	24	24
CUFOX1 011	11	12	18	-	-	-	-	-
CUFOX1 012	11	11	11	21	15	18	21	18
CUFOX1 013	11	13	15	15	14	13	19	16

Oxaliplatin 75% - ; Oxaliplatin 50% - ; Oxaliplatin stopped -

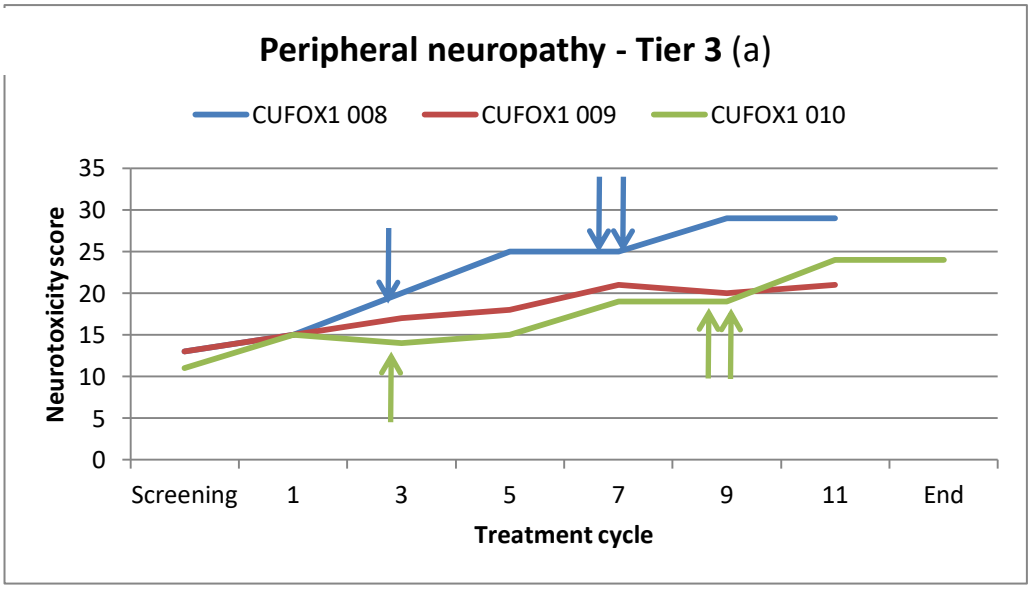
A


Peripheral neuropathy - Tier 1
CUFOX1 001
CUFOX1 003
CUFOX1 002
Neuropathy score
25
20
15
10
5
0
Screening
1
3
5
7
9
11
End
Treatment cycle

B


Peripheral neuropathy - Tier 2
CUFOX1 004
CUFOX1 006
CUFOX1 007
Neurotoxicity score
30
25
20
15
10
5
0
Screening
1
3
5
7
9
11
End
Treatment cycle

C



D

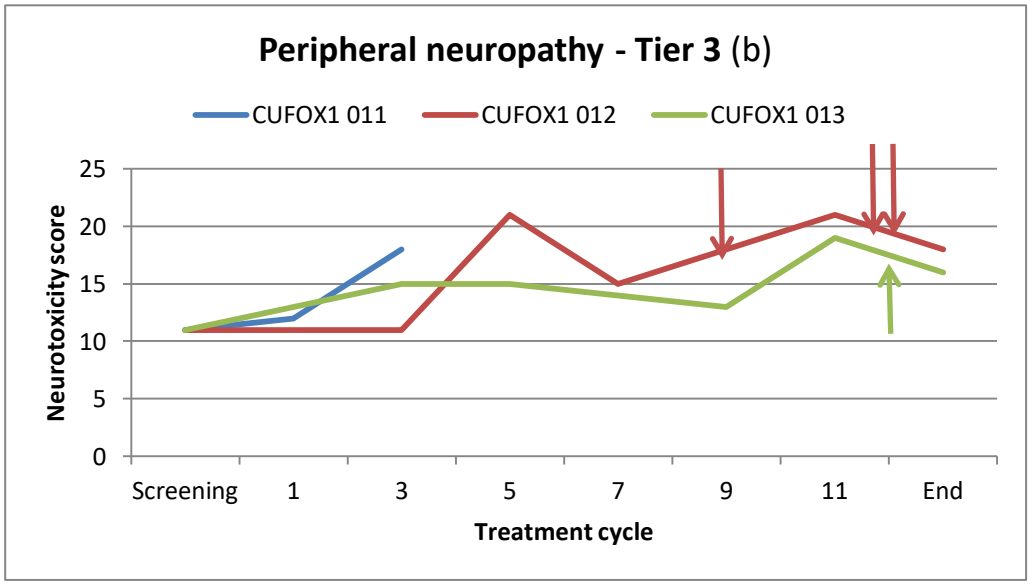


Figure 3.3 Peripheral neuropathy scores over duration of treatment for patients in Tiers 1 (A), 2 (B) and 3 (C, D); Higher score represents worsening neuropathy.
↑- Oxaliplatin reduced 75%; ↑↑ - Oxaliplatin reduced 50%; ⊘ - Oxaliplatin stopped

3.1.6. Treatment outcomes

Eleven of the twelve participants (91.7%) demonstrated either a partial response (PR) or stable disease (SD) after 6 cycles of treatment (Table 3.5, Fig 3.4). A reduction in target lesions of at least 30% (partial response) was recorded in more than half the patients (RR[23] = 58.3%). The best response recorded was 75.8% in patient CUFOX1 007. One participant (CUFOX1 011) was admitted to hospital after four cycles of

[23] RR = response rate

treatment and although the CT scan showed reduction in target lesions, the increase in non-target lesions depicted progressive disease.

At the end of treatment, eight of the remaining ten participants (80%) showed continued disease control. 5 patients (50%) achieved a partial response, while 3 patients (30%) showed stable disease. CUFOX1 010 (Tier 3) had initially shown a good partial response of 45.1% in his scan, but then presented to hospital three weeks later and a CT scan showed brain metastases[24]. CUFOX1 012 (Tier 3) showed a partial response (40.7%) in target lesions but increases of over 30% in non-target lesions confirmed disease progression. Therefore, overall the response rate for phase I patients whilst on treatment was 50.0%.

Table 3.5 Treatment outcomes according to RECIST 1.1 criteria after 6 cycles of chemotherapy, at end of treatment and 3 months post-treatment. PFS – progression-free survival. Partial response ≥30% reduction in target lesion diameter. Progression ≥ 20% increase in target lesion diameter. Stable – inadequate changes to class as partial response or progression. Non-target lesions reported as incomplete or complete response.

		Outcomes[25]			**PFS (wks)**
	Lesions	**6 cycles**	**Tx end**	**3-m post-tx**	
C1 001	Target	40.0% reduction	40.0% reduction	18.2% reduction	
	Non-target	Incomplete	Incomplete	Increase in size	
	Overall response	Partial	Partial	Progression	**39**
C1 002	Target	46.5% reduction	48.8% reduction	2.4% increase from baseline	
	Non-target	Incomplete	Incomplete	Increase in size	
	Overall response	Partial	Partial	Progression	**34**
C1 003	Target	38.8% reduction	55.1% reduction	32.7% reduction, increase from last scan	
	Non-target	Unchanged	Incomplete	Increase in size	
	Overall response	Partial	Partial	Progression	**34**
C1 004	Target	24.6% reduction	27.5% reduction	37.7% reduction	
	Non-target	Incomplete	Incomplete	Incomplete	
	Overall response	Stable	Stable	Partial	**51**

[24] Routine CT scans assessing disease response do not include CT head.
[25] Outcomes compared to measurements at baseline.

	Outcomes[26]				PFS (wks)
	Lesions	**6 cycles**	**Tx end**	**3-m post-tx**	
C1 006	Target	20.4% reduction	23.3% reduction	1.0% increase from baseline	
	Non-target	Incomplete	Incomplete	Increase in size	
	Overall response	Stable	Stable	Progression	**35**
C1 007	Target	75.8% reduction	-	-	
	Non-target	Complete response			
	Overall response	Partial			
C1 008	Target	15.9% reduction	15.5% reduction	2.2% increase from baseline	
	Non-target	Incomplete	Incomplete	Increase in size; new peritoneal disease	
	Overall response	Stable	Stable	Progression	**36**
C1 009	Target	46.7% reduction	46.7% reduction	41.7% reduction, increase from last scan	
	Non-target	Unchanged/ Incomplete	Incomplete	Increase in size	
	Overall response	Partial	Partial	Progression	**37**
C1 010	Target	34.5% reduction	45.1% reduction	-	
	Non-target	Incomplete	Incomplete/Brain metastases[27]		
	Overall response	Partial	Partial/ Progression		**29**
C1 011	Target	22.3% reduction	-	-	
	Non-target	Increased adenopathy			
	Overall response	Progression			**9**
C1 012	Target	33.5% reduction	40.7% reduction	-	
	Non-target	Incomplete	Increased lung lesions&new lung lesion		
	Overall response	Partial	Progression		**26**
C1 013	Target	28.1% reduction	33.3% reduction	35.4% reduction	
	Non-target	Incomplete	Incomplete/ Complete response in 1˚ tumour	Incomplete/ Complete response in 1˚ tumour	
	Overall response	Stable	Partial	Partial	NA

[26] Outcomes compared to measurements at baseline.
[27] End of treatment scan (26/3/13) showed partial response; CT brain (17/4/13) showed brain metastases - Progression

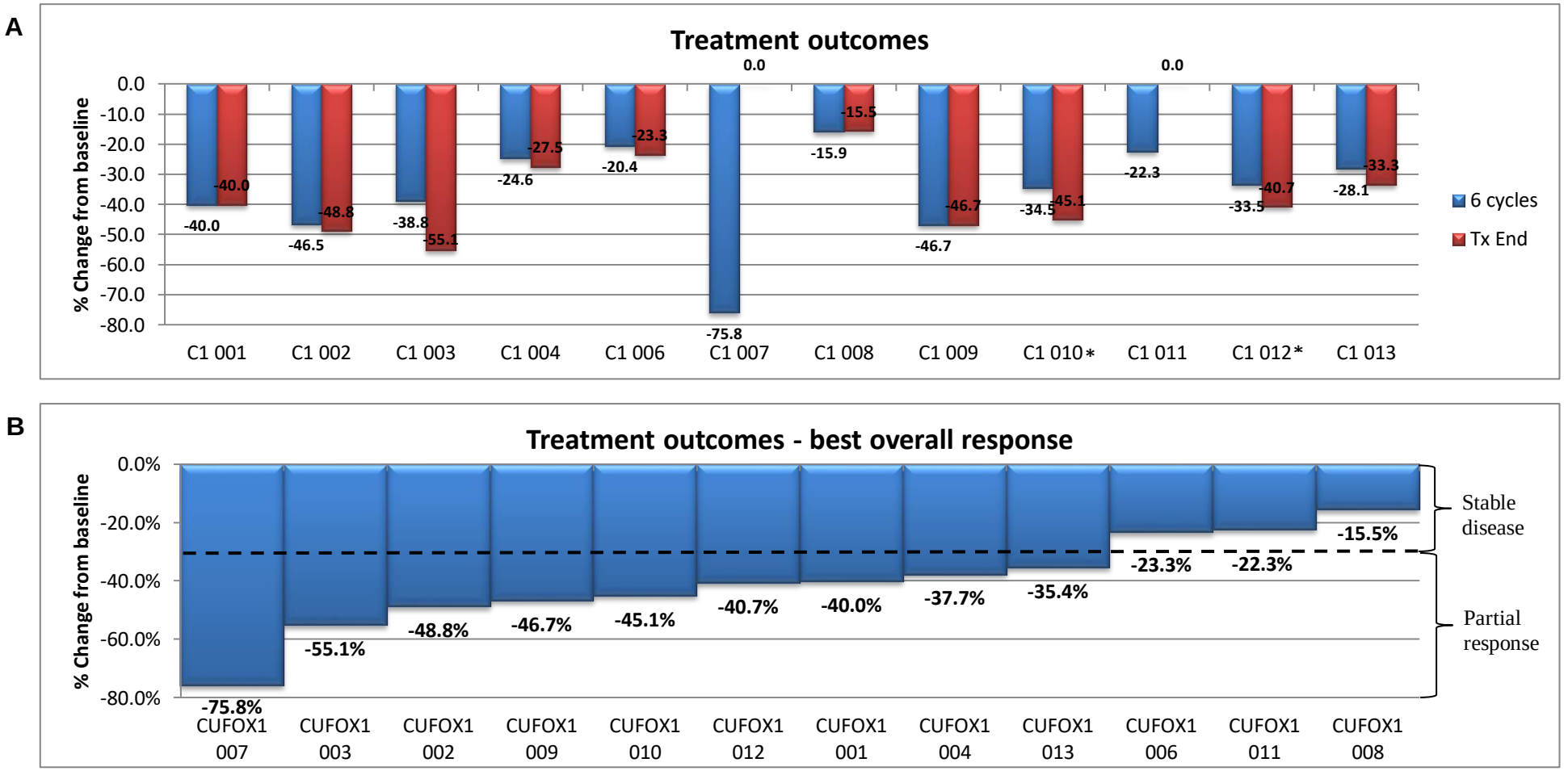
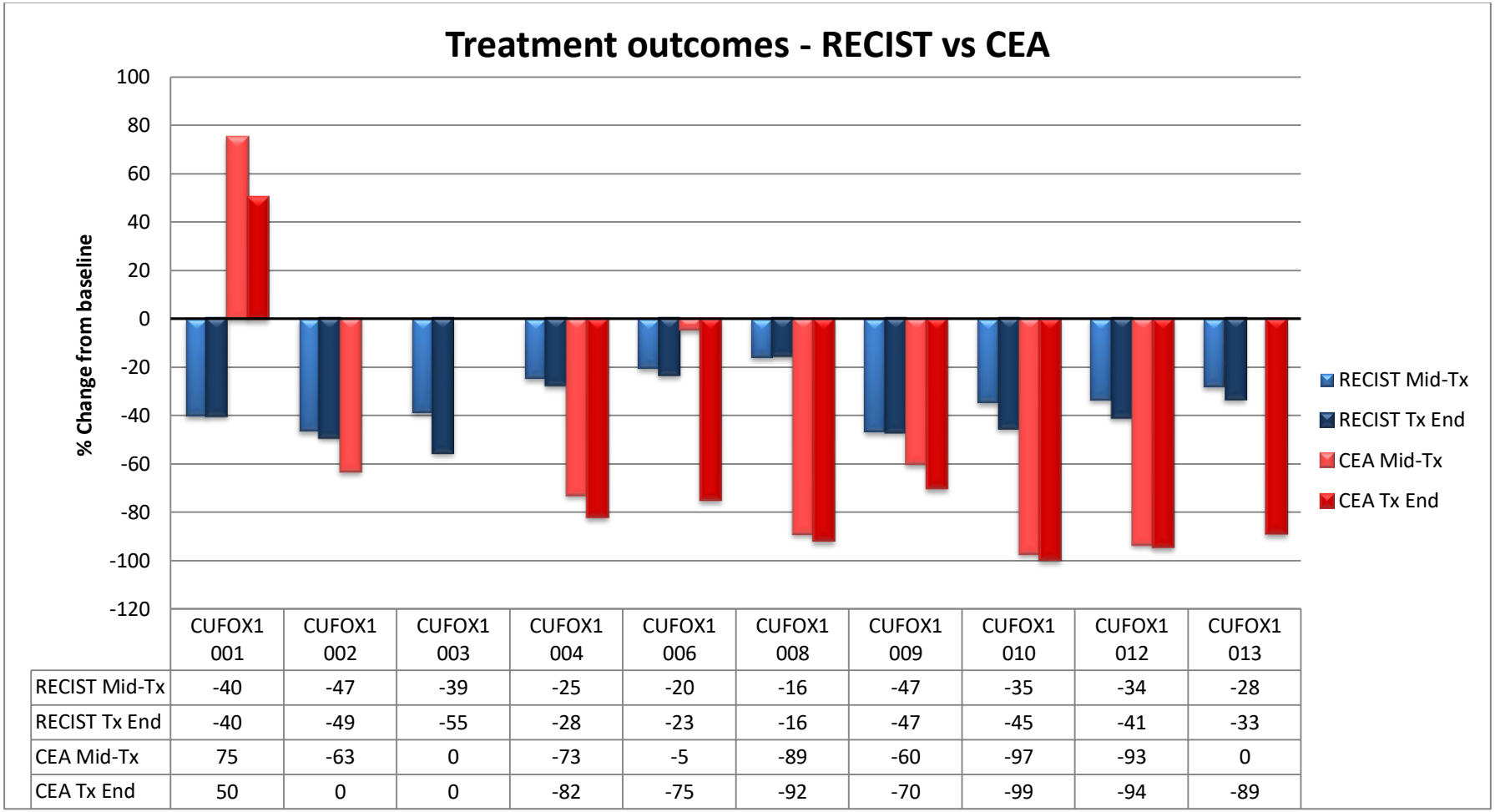


Figure 3.4 A – Change in tumour size at mid-point and end of treatment scans compared to baseline. *Patients showing disease progression by the end of treatment (in some cases reduction measured in target lesions, but progression due to new lesions). **B – Waterfall plot of best responses to treatment.** Best overall response – best response across all time points for each patient. Partial response ≥30% reduction in target lesion diameter. Progression ≥ 20% increase in target lesion diameter. Stable – inadequate changes to class as partial response or progression.

The median progression-free survival[28] was 8.5 months (95% CI, 6.3 to 9.8 months). Disease progression was reported in six participants (50.0%) at 3 months post-treatment. Two patients (CUFOX1 004 and CUFOX1 013) showed progression approximately 6 months post-treatment. These patients were withdrawn from trial follow-up and referred for alternative chemotherapy or palliative treatments.

Patients' CEA levels were measured at baseline and compared to CEA readings after 6 cycles of treatment, then at the end of treatment. The changes in CEA readings were compared to tumour responses measured by RECIST criteria (Fig 3.5). CEA readings were not obtained after cycle 6 for patients CUFOX1 007, CUFOX1 011 and CUFOX1 013. This was because CUFOX1 011 did not complete 6 cycles of treatment. With the other two patients this tumour marker was omitted in error when the blood tests were taken. CEA readings were also not obtained at the end of treatment for patients CUFOX1 002 and CUFOX1 011 because these patients were too unwell by the end of their treatment to come in specifically for trial blood tests. In one case (CUFOX1 003) the CEA reading was <2 (lowest possible measurement) at baseline and remained at this level for the duration of their treatment. The number of samples obtained was not sufficient to calculate a statistical correlation between changes in tumour size measured using RECIST criteria versus changes in CEA readings. Even so, the outcomes recorded did not suggest there was a clear association between these two measurements.

[28] Progression-free survival measured as the time from screening to clinical/radiological progression or death from any cause.



	CUFOX1 001	CUFOX1 002	CUFOX1 003	CUFOX1 004	CUFOX1 006	CUFOX1 008	CUFOX1 009	CUFOX1 010	CUFOX1 012	CUFOX1 013
RECIST Mid-Tx	-40	-47	-39	-25	-20	-16	-47	-35	-34	-28
RECIST Tx End	-40	-49	-55	-28	-23	-16	-47	-45	-41	-33
CEA Mid-Tx	75	-63	0	-73	-5	-89	-60	-97	-93	0
CEA Tx End	50	0	0	-82	-75	-92	-70	-99	-94	-89

Figure 3.5 Change in tumour size measured at mid-point and end of treatment scans compared to baseline versus changes in CEA measurements for the same timepoints. Patients CUFOX1 007 and CUFOX1 011 excluded because mid-treatment and end of treatment measurements were not available for these patients.

3.1.7. Quality of Life

The quality of life questionnaires were used to determine whether adding curcumin altered patients' quality of life during treatment. For phase I in particular, the emphasis was on whether increased curcumin doses were associated with a reduction of the detrimental effect of chemotherapy on quality of life (Table 3.6, Fig 3.6). The quality of life questionnaires measured effects on: a) functionality; b) symptoms; c) overall subjective well-being i.e. global health score. The majority of patients noted an improvement (increased score) in their functional and global health scores. However, most patients reported higher symptom scores following treatment. The patients who completed 12 cycles of treatment all reported improved functionality and global health scores, as well as their symptoms improving or remaining stable.

Table 3.6 Comparison of quality of life scores before and after treatment. Increase in functional score represents improved physically functionality. Increase in global health score represents improved overall perceived wellbeing. Increase in symptom score represents worsening symptoms. Patients in grey completed 12 cycles of chemotherapy.

	Functional		Symptoms		Global	
	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx
C1 001	71.1	88.9	10.3	10.3	66.7	83.3
C1 002	84.4	53.3	23.1	30.8	66.7	41.7
C1 003	68.9	86.7	7.7	20.5	58.3	66.7
C1 004	64.4	75.6	23.1	23.1	50.0	66.7
C1 006	97.8	75.6	2.6	18.0	100.0	58.3
C1 007	100.0	95.6	0.0	2.6	83.3	100.0
C1 008	84.4	55.6	28.2	41.0	75.0	58.3
C1 009	93.3	78.6	5.1	18.0	75.0	66.7
C1 010	73.3	93.3	23.1	10.3	33.3	83.3
C1 012	71.1	92.9	10.3	7.7	66.7	83.3
C1 013	68.9	95.6	35.9	7.7	33.3	66.7

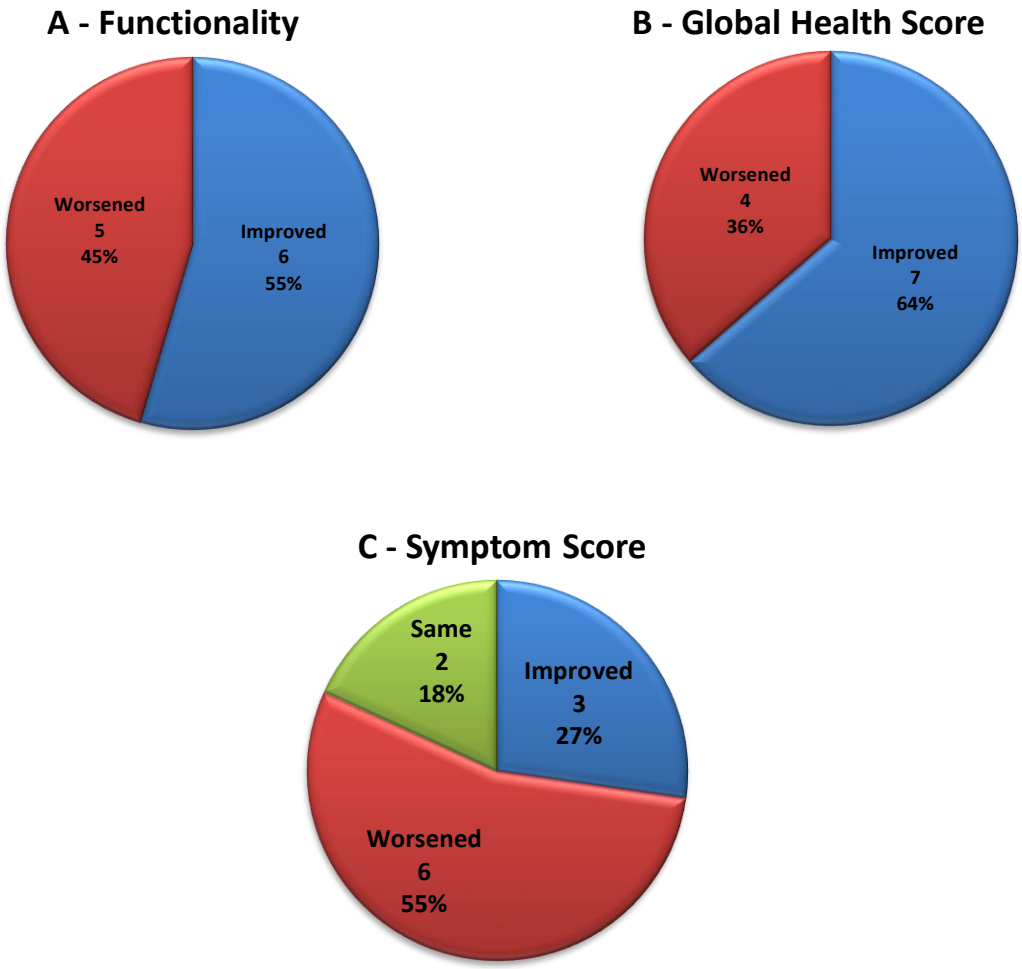


Figure 3.6 Results from EORTC quality of life questionnaires. A – Functional score, assessing physical functionality; B – Global health score, assessing overall perceived well-being; C – Symptom score, assessing degree of symptoms experienced. (Group total = 11 patients)

3.1.8. Curcumin questionnaire

The curcumin questionnaires were designed to establish patients' knowledge, preconceptions and concerns regarding curcumin prior to commencing on the trial, then reassess their attitudes at the end of treatment. Not many significant concerns were raised by participants regarding curcumin use prior to treatment. Five reported some concern ('a little' or 'quite a bit') about the large size of the capsules (Table 3.7). However following treatment, only two participants had 'a little' difficulty taking the capsules, whilst one participant reported 'quite a bit' of difficulty with taking capsules this size. Three participants (one from each tier) were 'a little' worried about the number of capsules they would be taking. Almost all the patients (91.7%) were 'not at

all' concerned about the once daily dose frequency. By the end of treatment, the majority of patients (72.7%) reported no difficulties at all with the number, size or frequency of their curcumin doses. The number of capsules did cause 'a little' bit of difficulty for one patient in tier one (1 capsule) and two patients in tier 3 (4 capsules).

The potential side-effects of treatment were a minor concern for 41.7% of patients (Table 3.7). However, by the end of treatment, 81.8% of patients had no concerns regarding the side-effects from curcumin. All participants had experienced side-effects to some degree, but only 36.4% attributed these toxicities to curcumin, whilst all participants believed chemotherapy caused some side-effects.

The main side-effects associated with curcumin use from previous studies include yellow stools, flatulence, bloating and abdominal pain. At the end of the study, complaints of yellow stools had increased by 29.6% (Table 3.7). There were also more reports of flatulence (6.0%) and bloating (3.8%). On the contrary, abdominal pain improved with a 40.9% decrease in this symptom.

Prior to treatment, participants were asked what measures they anticipated would improve compliance in taking curcumin. The suggestions made were: smaller capsules (8.3%) and fewer side-effects (16.7%). The remainder of participants either were not sure (16.7%), or gave no suggestions (58.3%). By the end of treatment, none of the participants believed that side-effects had a negative impact on curcumin compliance (Fig 3.7). One patient taking four capsules on tier 3 suggested that taking fewer capsules would be beneficial. Reducing the size of the capsules was suggested by two participants. However, most patients (72.7%) stated that they did not feel anything was required to improve their willingness to take curcumin. The same percentage of patients reported that they would be willing to continue taking curcumin beyond the six months of treatment, and indefinitely (Fig 3.8). One patient (Tier 3) did state that 6 months of taking curcumin would be the maximum they could tolerate.

Table 3.7 Comparison of patients' experience of curcumin from curcumin questionnaire pre- and post-treatment

	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx
	Not at all		A little		Quite a bit		Very Much	
Nº of capsules difficult	75.0%	72.7%	25.0%	27.3%	0.0%	0.0%	0.0%	0.0%
Size of capsules difficult	58.3%	72.7%	25.0%	18.2%	16.7%	9.1%	0.0%	0.0%
Frequency of capsules difficult	91.7%	72.7%	8.3%	18.2%	0.0%	9.1%	0.0%	0.0%
Concerned about side-effects	58.3%	81.8%	41.7%	9.1%	0.0%	9.1%	0.0%	0.0%
Experienced side-effects	-	0.0%	-	45.5%	-	36.4%	-	18.2%
Side-effects due to chemotherapy	-	0.0%	-	27.3%	-	27.3%	-	45.5%
Side-effects due to curcumin	-	54.5%	-	27.3%	-	9.1%	-	0.0%
Worried if missed dose	25.0%	54.5%	58.3%	27.3%	0.0%	9.1%	16.7%	0.0%
Treatment gave peace of mind	25.0%	63.6%	33.3%	18.2%	33.3%	9.1%	0.0%	9.1%
Inconvenient with other medication	83.3%	72.7%	8.3%	27.3%	0.0%	0.0%	0.0%	0.0%
Affects other medication	66.7%	72.7%	8.3%	18.2%	0.0%	0.0%	0.0%	0.0%
6 months too long	83.3%	81.8%	16.7%	18.2%	0.0%	0.0%	0.0%	0.0%
Yellow stools	75.0%	45.5%	25.0%	9.1%	0.0%	18.2%	0.0%	27.3%
Flatulence	33.3%	27.3%	41.7%	63.6%	8.3%	9.1%	16.7%	0.0%
Bloating	58.3%	54.5%	25.0%	36.4%	0.0%	9.1%	16.7%	0.0%
Abdominal pain	50.0%	90.9%	25.0%	9.1%	16.7%	0.0%	8.3%	0.0%

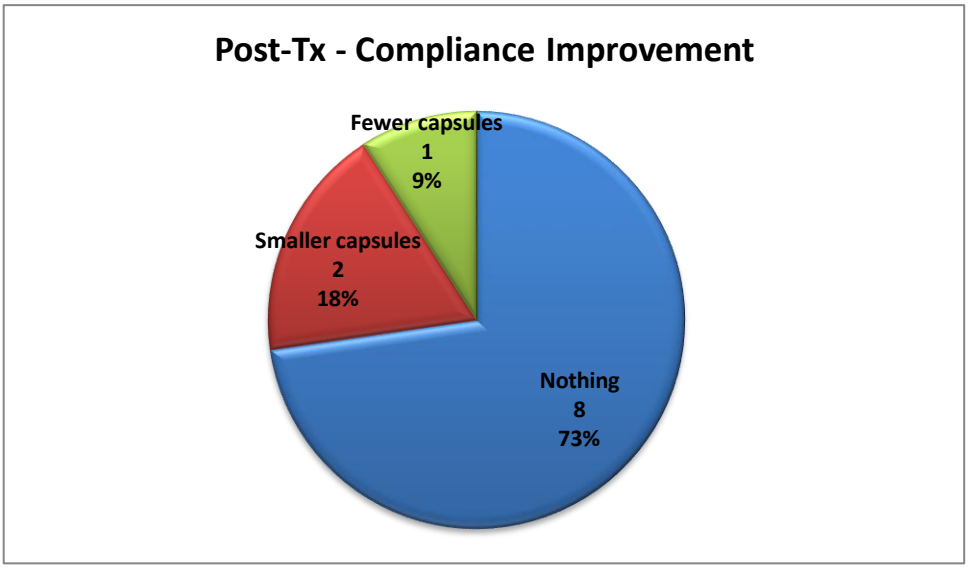


Figure 3.7 Patient suggestions of measures to improve compliance from curcumin questionnaire; S/E – Side-effects, Tx – treatment (Group total = 11 patients)

Compared to the start of treatment, there was an increase of 33.3% in patients' awareness of curcumin (Fig 3.8). None of the participants had previously used curcumin as a health supplement and only one was aware of using curcumin or turmeric regularly in their diet. By the end of treatment, seven of the eleven participants (63.6%) indicated that they would consider using curcumin as a health supplement and regularly in their diet.

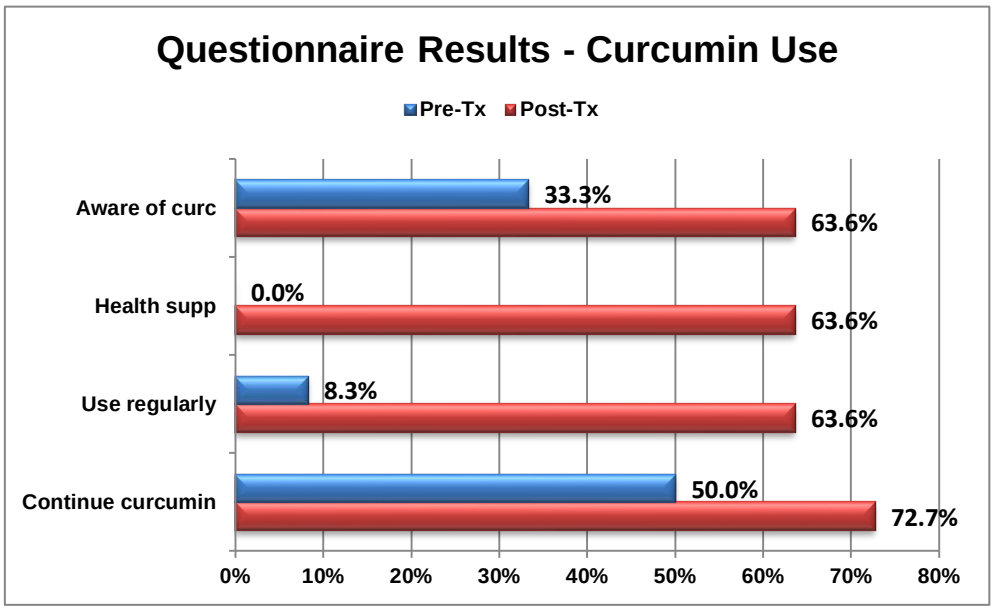


Figure 3.8 Comparison of patients' attitude to curcumin from pre- and post-treatment curcumin questionnaires; Questionnaires assessed patients' awareness of benefits of curcumin, whether patients would consider using curcumin as a health supplement, use of turmeric regularly in diet, and whether patients would have been willing to continue curcumin beyond the 6-month trial period; Tx – treatment.

3.2. **Discussion - Phase I**

3.2.1. Recruitment

The clinical protocol had estimated a recruitment rate of two patients per month, with approximately 2.5 months required for recruitment and completion of two cycles of chemotherapy. Recruitment for the first two tiers was completed much more quickly than expected with seven patients completing the dose escalation period in less than 5 months. Recruitment for tier 3 was more protracted, requiring 10 months for recruitment and two cycles of chemotherapy to be completed. This appears to be primarily due to a reduced number of patients being referred and meeting the inclusion criteria over that time period. For tiers 1 and 2, 12 patients were screened in just over 4 months (approx. 3 patients/month) compared to the 13 patients screened in 10 months of recruiting for tier 3 (<2 patients/month).

The overall recruitment rate[29] reported for cancer clinical trials in the UK is 18% to 21% of all incident cancer cases (between 2012 – 2013), with 76% of studies meeting over 90% of the planned recruitment target during the planned window[(258)]. In our trial, 56.0% of patients who were reviewed and received information about entering the trial (verbal ± patient information sheets) were recruited. Only one participant (4.5%) for tier 3 declined entry into the study. Therefore, the larger curcumin dose of 2 grams daily, did not appear to substantially deter eligible patients from entering the trial.

The overall recruitment rates were, however, reduced by the number of patients who failed initial screening (44.0%). This was high in comparison to previous cancer clinical trials, where typically 28% of patients failed to meet initial screening criteria [(259)]. As a result, the inclusion and exclusion criteria for this trial were reviewed by the principal investigator, and trial committee members prior to commencing recruitment for phase II. The exclusion of patients with peptic ulcer disease was reassessed. Two patients had been excluded from the study due to this criterion that were otherwise fit to have FOLFOX chemotherapy. Initially, there had been concerns that curcumin's anti-inflammatory COX-2 inhibition might cause gastrointestinal toxicities such as peptic ulcers and gastrointestinal bleeding, as seen with aspirin. However, it is known that aspirin inhibits both COX-1 and COX-2, and these adverse effects are attributed to COX-1 inhibition only. There is no significant evidence to suggest that curcumin use

[29] Percentage of patients diagnosed with cancer who entered clinical trials.

exacerbates peptic ulcer disease. Likewise, there are no substantial data confirming that the presence of peptic ulcer disease affects curcumin absorption. In view of the above, the presence of peptic ulcer disease was removed from the exclusion criteria for the phase IIa study.

Initial estimations predicted that phase I would be completed in 15 months. Despite the slower recruitment rate in tier 3, all phase I patients completed dose escalation within 15 months, and recruitment for phase IIa began in April 2013.

3.2.2. Curcumin compliance

Compliance is a key issue that affects the efficacy of any drug therapy. The compliance rate of 93.8% in our study was encouraging, being comparable to those in other clinical trials of oral antineoplastic agents such as Tamoxifen, which showed compliance rates of 72% to 96% (203). Similarly, a phase I/II study investigating the addition of curcumin to gemcitabine for patients with pancreatic cancer reported an excellent median compliance rate of 100% (Range 79 – 100%, 21 patients)[(225)]. For tier 1, the two cases of missed doses (CUFOX1 002 and CUFOX1 003) were due to patients forgetting their doses or being admitted to hospital (not curcumin related) as opposed to any adverse effects or difficulty swallowing curcumin. All patients in tier 2 recorded 100% compliance for the duration of their treatment. For three of the 5 patients, doses were missed as a result of hospital admission or because they forgot to take their capsules. In one case this was related to the dose limiting toxicity and in the other due to difficulty swallowing 4 capsules at once. Once the doses of these patients were adjusted accordingly, no further compliance issues were reported. No significant trend was noted when comparing compliance rates to increases in curcumin doses.

Cancer patients are generally expected to be highly motivated due to their awareness of the gravity of their illness. Yet despite this and the many benefits of oral antineoplastic agents, there are significant concerns regarding patient adherence to treatment regimes. This issue was demonstrated in a study investigating adherence to oral tamoxifen in women being treated for breast cancer. Over the 2-year follow-up period, 17% of women stopped taking their tamoxifen, with 68% of these patients doing so within the first year[(260)]. Potential reasons for non-adherence to treatment are complex and multifactorial. However, key strategies to improve compliance require informational and behavioural interventions [(261)]. Informational intervention was employed by

providing patients with clear information about potential benefits and risks of their treatment in the form of verbal discussion during their screening appointment and written patient information sheets for the CUFOX trial. Behavioural measures that may have contributed to better compliance included keeping the curcumin dosing to a once daily regime over a 6 month period. The number of daily doses has been reported to be inversely proportional to compliance rates, thus a once daily dose is likely to improve adherence to treatment and likewise the shorter duration of treatment (204). Giving patients the option of administering their doses over 15 to 30 minutes if required also may have improved patient co-operation by tailoring the regime to better suit the individual. Equally, the perceived low toxicity of the medication may also have influenced patients' compliance.

3.2.3. Toxicity

The patients generally tolerated their week of curcumin (pre-chemotherapy) with minimal adverse events. The events reported were primarily gastrointestinal and consistent with those reported in previous curcumin trials. The side-effects reported were all mild (Grade 1 or 2), either resolving spontaneously or after medication. Once chemotherapy was added, the range of toxicities broadened and in some cases severity increased. Diarrhoea was the only serious adverse event where curcumin was likely to have contributed. Causality was established based on whether stopping and subsequently reintroducing curcumin affected this symptom. The effects of standard treatment were similarly assessed. Diarrhoea is commonly seen in patients treated with the combination of FOLFOX and Bevacizumab (11.1%) [(262)]. With FOLFOX alone grade 3-4 diarrhoea was reported in 10.0% of patients[(4)]. Therefore it is difficult to determine how much each of the treatment agents contributed to the grade 3 diarrhoea seen in two trial patients. In the case of the dose-limiting diarrhoea experienced by one patient in tier 3, this could be attributed primarily to curcumin because increasing the curcumin dose exacerbated this side-effect whereas re-starting chemotherapy did not. This participant had already had loose stools, controlled with Loperamide, prior to starting on treatment but no significant worsening of bowel habit had been reported during curcumin pre-dosing. No other patients in tier 3 experienced grade 3 diarrhoea.

One of the patients stopped treatment after seven cycles of chemotherapy following a series of hospital admissions. Reasons for admission primarily involved increased stoma output plus a background of poor oral intake of both nutrition and fluids leading

to acute renal impairment. In this case, the increased stoma output could not be attributed to curcumin because the patient's diarrhoea remained settled when taking curcumin in the absence of chemotherapy. It is therefore more likely that these events were primarily due to standard treatment.

Fatigue and anorexia were the key factors in deciding to stop treatment for the other patients who failed to complete twelve cycles of chemotherapy. Again, fatigue is not uncommon in patients being treated with FOLFOX and Bevacizumab (9.0%)[(262)]. In our study 60% of patients completed twelve cycles of chemotherapy. In the NSABP C-08[30] trial, the rate was higher at 77 – 85%[(262)]. However it is important to note that the NSABP C-08 patients all had stage II to III disease, thereby having less advanced disease than patients recruited into the CUFOX study. Cardiac side-effects of myocardial infarction caused one patient to be withdrawn. These are not side-effects that have previously been reported with curcumin. Likewise the other serious adverse events of infection and pulmonary emboli are unlikely to be due to curcumin use. It will be important to monitor side-effects in phase IIa to confirm that there is no link between curcumin and these side-effects.

3.2.4. Peripheral neuropathy

The results of curcumin use on peripheral neuropathy are currently inconclusive, particularly in view of the small cohort of patients who have completed treatment. On the one hand, all participants experienced at least acute peripheral neuropathy with an accumulation of symptoms as treatment progressed, as is the known pattern with oxaliplatin treatment. With 50% of patients requiring either a dose reduction or cessation of oxaliplatin, this is still a lower rate of treatment alteration than reported in other studies (~60%) (78). Although these results appear encouraging, outcomes from Phase IIa of this trial may shed more light on whether the level of peripheral neuropathy is reduced in participants taking curcumin compared to standard treatment alone.

3.2.5. Treatment response

The treatment response rates for this study were encouraging with 50.0% of patients achieving either a partial response or stable disease. This is comparable to the response rates seen with standard treatment (FOLFOX RR = 45% - 54% [(263)]; FOLFOX + Bevacizumab (RR = 47% [(3)]) in previous clinical trials in metastatic colorectal cancer,

[30] National Surgical Adjuvant Breast and Bowel Project C-08

which have shown response rates of 49% with FOLFOX chemotherapy and 47% with the addition of Bevacizumab [3,81]. The median progression-free survival for FOLFOX and Bevacizumab is 9.4 months, and 8.0 months for FOLFOX only[3]. Therefore the progression-free survival of 8.5 months reported in phase I of this trial is comparable. Overall, the addition of curcumin to standard treatment appears to maintain similar progression-free survival compared to standard treatment alone. Further evaluation of this will be required by directly comparing these parameters in phase IIa.

3.2.6. Quality of life and curcumin questionnaires

Over half the trial participants (55%) reported deterioration in their symptoms by the end of their treatment. Nonetheless, the majority of patients (72.7%) reported that they would be willing to continue curcumin for as long as it was necessary. This evidently reflects the perception by all the participants that most of their side-effects were due to chemotherapy. In addition to this, patients noted improvement in functionality and quality of life (global health scores), with little or no difficulty in taking the curcumin. Overall, it appears that the minimal perceived adverse effects of adding curcumin to standard treatment resulted in patients reporting favourable opinions on the tolerability and feasibility of this treatment.

3.3. **Conclusion – Phase I**

The addition of curcumin to standard oxaliplatin-based treatment has shown good tolerability, feasibility and no significant safety concerns with doses of up to 2 grams of curcumin daily. Patients have shown excellent compliance and given positive feedback regarding the long-term use of curcumin. Alterations and cessation of oxaliplatin secondary to peripheral neuropathy is currently slightly lower than those reported in previous studies. However more data is required before the effects of curcumin on peripheral neuropathy can be defined. Response rates and progression-free survival is comparable to those observed for standard treatment alone.

4. Phase IIa

4.1. **Introduction**

Phase IIa of this study aimed to further investigate the safety, tolerability and feasibility of the 2 gram daily maximum target dose of curcumin established in Phase I of the CUFOX study. As an open-labelled, two-armed, randomised controlled feasibility trial, comparisons could be made between experiences and outcomes for patients having standard treatment alone, versus those having standard treatment and curcumin. As curcumin has not previously been administered to humans with this regimen of chemotherapy, no data exists to estimate efficacy or to use as a basis for power calculations. Thus data from this study will also be useful in the powering of future studies.

As in phase I, questionnaires were used to collect information regarding each patient's perceptions and experience before, during and after the study. Comparing the results between the two study arms would provide greater understanding of whether addition of curcumin to standard treatment raises new safety concerns.

4.2. **Phase IIa Clinical Results**

4.2.1. Recruitment

Screening for Phase IIa began in April 2013 and continues to date. From April 2013 until January 2015, 31 patients were screened and 18 patients recruited onto the CUFOX trial. Eleven of the 15 patients who were not recruited had failed screening, whilst 2 patients declined entry. Patients failed to meet the eligibility criteria based on poor performance status (4 patients), inability to give consent (1), not for Oxaliplatin-based chemotherapy (3), and co-existing medical conditions (3). The recruitment rate was approximately 0.86 patients per month (one patient every 5 weeks) with 86% of eligible patients and 55% of screened patients entering the study.

Following randomisation, 13 patients received curcumin with standard treatment (CST) and five patients received standard treatment (ST) alone. The demographics across the two groups were similar in terms of age, sex and ethnicity (see Table 4.1). However, all the patients recruited to standard treatment alone had extrahepatic disease (4 – lung, 1 – adrenal), whilst 4 patients treated with curcumin showed no extrahepatic metastases.

Table 4.1 Demographics of patients recruited onto Phase IIa

	Curcumin	No Curcumin
N^{o} of participants	13	5
Male	9 (69%)	3 (60%)
Female	4 (31%)	2 (40%)
Age range	54 – 77 yrs	58 – 78 yrs
Median age	67	71
Ethnicity	All white British	All white British
Extra-hepatic metastases	9[31] (69%)	5 (100%)

4.2.2. Treatment

In the CST arm, 8 patients (61.5%) were treated with FOLFOX plus Bevacizumab, and 5 patients (38.5%) with FOLFOX only as standard treatment. All patients on ST arm received FOLFOX + Bevacizumab.

Three patients on the ST arm completed less than 6 cycles of treatment (Fig 4.1). One of these patients was withdrawn from the study after completing only one cycle of chemotherapy, having been found retrospectively to be ineligible for the trial. This patient's CT scan had initially been reported as showing liver metastases. However, on subsequent review, there was uncertainty as to whether these liver lesions represented metastatic disease or liver abscesses. In the absence of confirmed liver metastases, or any other measurable disease, this patient's (CUFOX2A 003) treatment was suspended and they were withdrawn from the CUFOX trial. A second patient developed bowel obstruction after 2 cycles of chemotherapy. He/she underwent surgery to treat the obstruction, but then died of complications following the procedure. The third patient suffered significant toxicity with their treatment and only tolerated 3 cycles of FOLFOX and Bevacizumab.

[31] Extra-hepatic metastases in curcumin arm: 3 – lung, 4 – peritoneal, 1 – lung and peritoneal, 1 – lymphadenopathy; non-curcumin arm: 4 – lung, 1 - adrenal

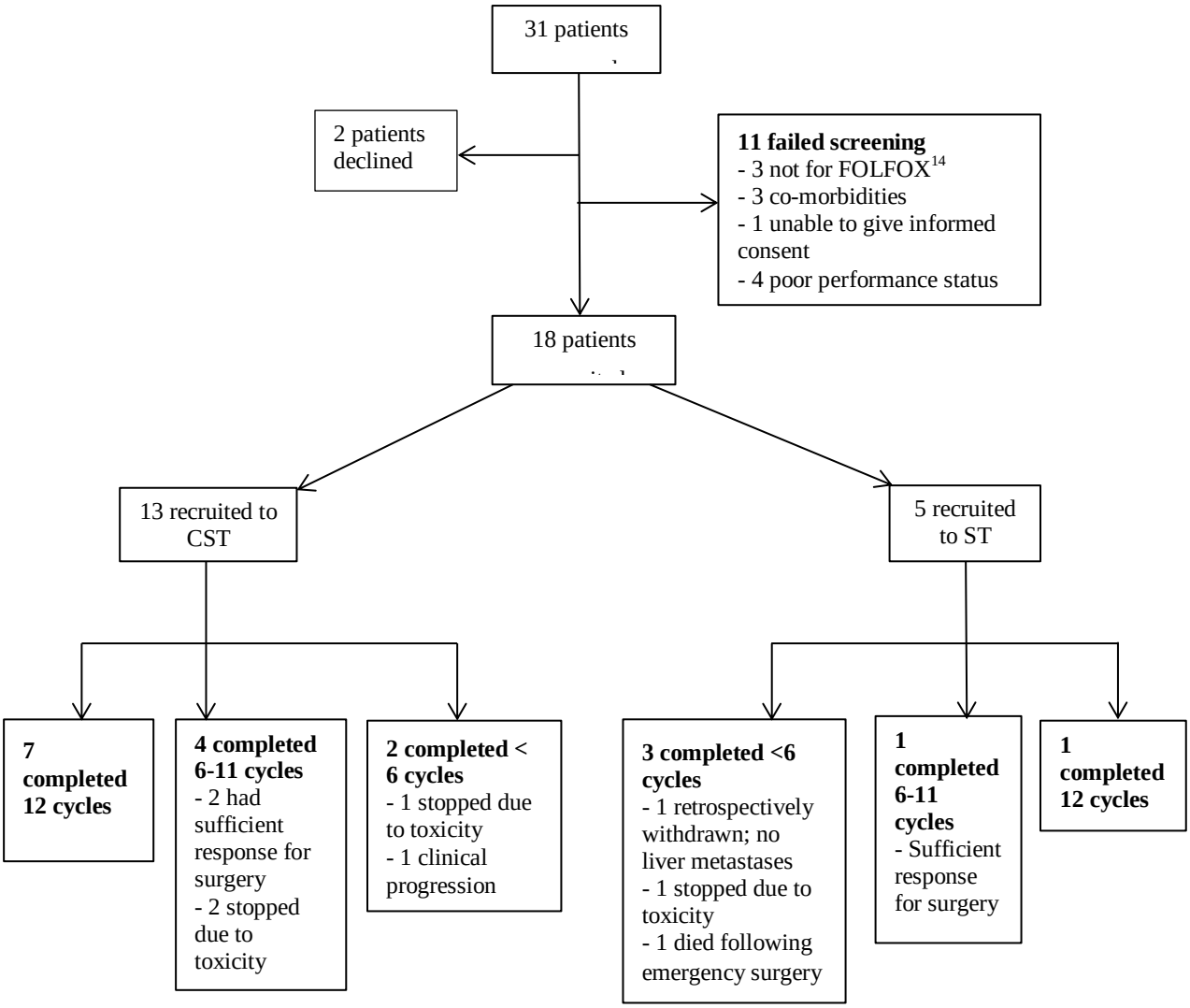


Figure 4.1 Summary of patient recruitment, randomisation and treatment in Phase IIa[32]

There were 2 patients in the CST arm who completed less than 6 cycles of treatment. Treatment was stopped on one of these patients due to continued symptomatic deterioration. This patient also stopped taking curcumin after his second cycle of chemotherapy due to its side-effects (nausea and belching). Another patient was admitted to hospital with treatment-induced toxicity after one cycle of chemotherapy. As a result, this patient's chemotherapy was stopped and she was withdrawn from the CUFOX study as per protocol. Two other patients in this arm stopped treatment after 8 and 10 cycles of chemotherapy due to toxicity.

The interim CT scan (after 6 cycles) for 2 CST and 1 ST patient showed treatment responses that were sufficient for these patients to proceed to surgical resection of their

[32] FOLFOX – 5-FU (fluorouracil)/oxaliplatin/folinic acid

disease. Therefore chemotherapy was stopped before these patients completed 12 cycles of treatment.

The target 12 cycles of chemotherapy was completed by 7 patients in the CST arm; 4 of whom received FOLFOX + Bevacizumab, and 3 received FOLFOX as standard treatment. One patient in the ST arm completed 12 cycles of chemotherapy.

4.2.3. Compliance

Compliance was recorded primarily based on patient reports. The average compliance rate was 94.1%[33]. Over half the patients (53.8%) reported a 100% compliance rate. Reasons for missing doses included hospital admissions (33.3%), curcumin side-effects (16.7%), feeling unwell (16.7%), forgot (16.7%), and being on holiday (16.7%).

4.2.4. Toxicity

All 17 patients in Phase IIa of this study experienced adverse events. The majority of these adverse events were Grade 1 or 2 (91.3%) (See Fig 4.2). Grade 3 events were reported in 9 of the 17 patients (52.9%); overall these accounted for 7.9% of all documented adverse events. One patient experienced a grade 4, and subsequently grade 5 event, accounting for 0.8% of all adverse events. The toxicities experienced by the most patients were fatigue/lethargy (94.1%), sensory neuropathy (82.4%), dyspepsia (52.9%) and nausea (52.9%).

[33] There was an episode of delayed curcumin supply that resulted in 2 patients missing curcumin doses (CUFOX2A 012 – 2 doses, CUFOX2A 016 – 5 doses). However this was not recorded as non-compliance because the patients were not at fault.

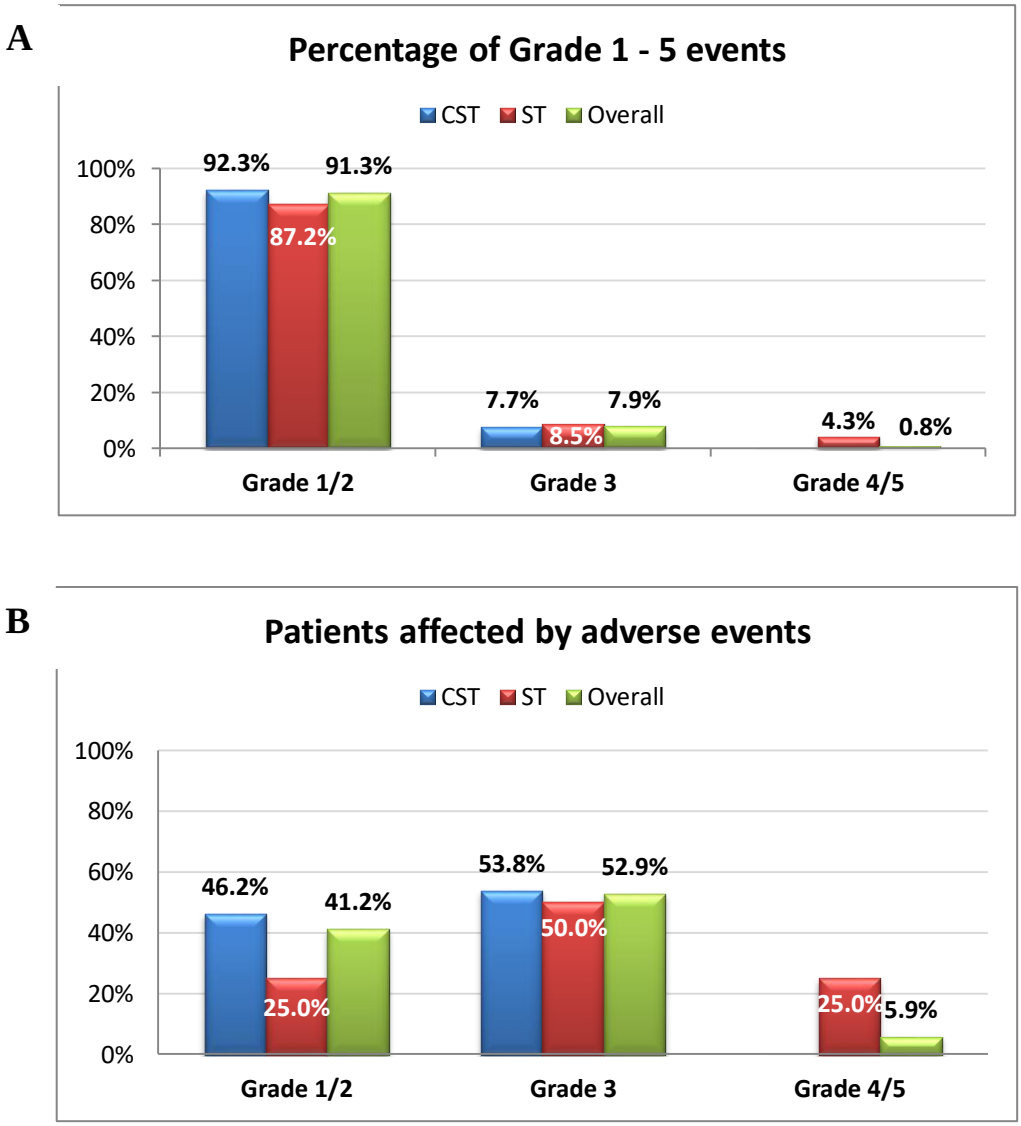


Figure 4.2 A - Percentage of Grade 1-2, 3 and 4-5 adverse events reported; B - Percentage of patients affected by Grade 1-2, 3 and 4-5 adverse events in the curcumin and standard treatment (CST) group, standard treatment (ST) group, and overall

In the CST group, grade 1/2 adverse events were experienced by 6 patients (46.2%) compared to 7 patients (53.8%) who experienced grade 3 adverse events (see Fig 4.2). The majority of the adverse events overall were grade 1 or 2 (92.3%), with grade 3 events making up 7.7% (See Fig 4.2). No grade 4 or 5 adverse events were reported in this group. Approximately half of the patients (7 patients, 53.8%) did not experience any adverse events prior to commencing chemotherapy (see Fig 4.3). For the remaining 6 patients, the toxicities reported included gastrointestinal symptoms (e.g. abdominal pain, diarrhoea, constipation, and flatulence), fatigue, peripheral oedema and dry mouth. All these were reported as grade 1 toxicity, except for diarrhoea which was grade 2.

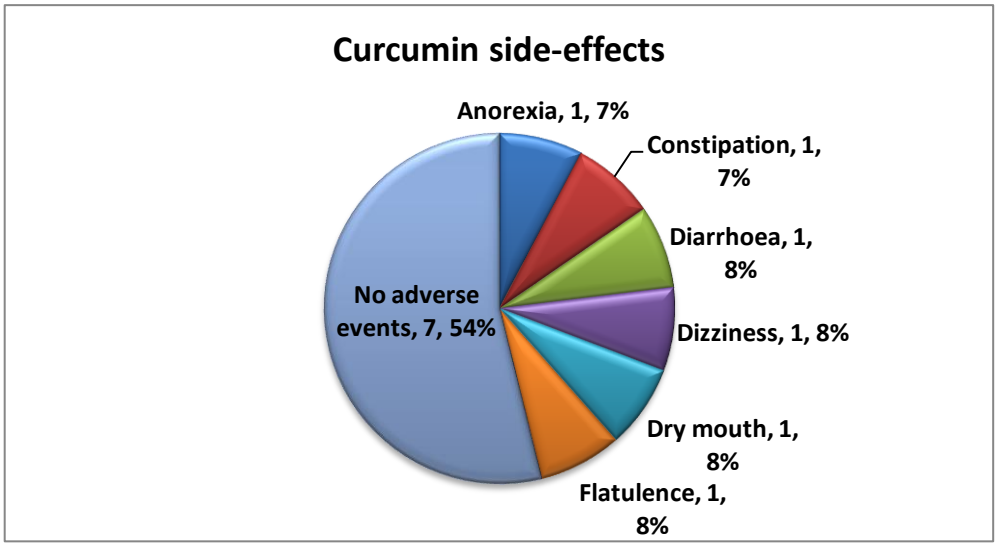


Figure 4.3 Side-effects reported by patients whilst treated with curcumin only and the number of patients affected (pre-chemotherapy). (Group total = 13 patients)

On combining curcumin with chemotherapy, the toxicities experienced by most patients were fatigue/lethargy (100%), sensory neuropathy (92.3%), dyspepsia (53.8%), oral mucositis (53.8%), and thrombocytopaenia (53.8%). Nausea, vomiting, and diarrhoea were also frequently reported (all 46.2%). The adverse events that were thought to be possibly or likely related to the use of curcumin are detailed in Table 4.2. On average, these adverse events were primarily grade 1/2 (93.4%).

Table 4.2 Adverse events possibly or likely related to curcumin use, and the percentage of patients experiencing grade 1 – 5 events. Shaded cells represent grade 3 or greater toxicity.

Curcumin-related AEs	% Gd 1/2	% Gd 3	% Gd 4/5	% patients
Abdominal bloating	100.0%	0.0%	0.0%	5.9%
Abdominal pain	80.0%	20.0%	0.0%	29.4%
Acute kidney injury	100.0%	0.0%	0.0%	5.9%
Anorexia	85.7%	14.3%	0.0%	41.2%
Constipation	100.0%	0.0%	0.0%	29.4%
Diarrhoea	87.5%	12.5%	0.0%	47.1%
Dizziness	100.0%	0.0%	0.0%	17.6%
Dry mouth	100.0%	0.0%	0.0%	11.8%
Dyspepsia	100.0%	0.0%	0.0%	52.9%
Flatulence	100.0%	0.0%	0.0%	11.8%
Nausea	88.9%	11.1%	0.0%	52.9%
Oral Mucositis	100.0%	0.0%	0.0%	47.1%
Vomiting	85.7%	14.3%	0.0%	41.2%
Weight loss	80.0%	20.0%	0.0%	29.4%
MEAN	**93.4%**	**6.6%**	**0.0%**	**34.5%**

Of the four patients in the ST group, one patient experienced only grade 1/2 toxicities, two patients reported grade 3 toxicities (50.0%), and one patient reported grade 4/5 toxicities (see Table 4.3). For these patients, the most common toxicities were fatigue/lethargy (75.0%) and nausea (75.0%).

Table 4.3 Grade 3 or greater adverse events reported in the standard treatment group

	N° of events (%)	**N° of patients (%)**
Abdominal pain[34]	1 (20%)	1 (25%)
Anorexia	1 (20%)	1 (25%)
Hyperkalaemia	1 (20%)	1 (25%)
Surgical procedure[3]	1 (20%)	1 (25%)
Sepsis[3,35]	1 (20%)	1 (25%)

Serious adverse events were reported in 8 patients overall (47.1%): 7 patients in the CST arm and 1 in the ST arm. All the SAEs in the CST group occurred after chemotherapy had commenced. The SAEs most frequently reported in the CST group were diarrhoea (15.4%), and febrile neutropenia (15.4%) (See Fig 4.4). Curcumin was thought to possibly contribute to the diarrhoea experienced by these patients. Acute kidney injury was recognised as an SAE that can develop in severe cases of diarrhoea and hence could be linked to curcumin use. None of the other SAEs were thought to be due to curcumin use. One patient in the ST group reported SAEs of colonic obstruction then developed a wound infection and subsequently died.

[34] All these events were reported in the same patient over a single episode.
[35] Grade 5 sepsis resulting in patient's death.

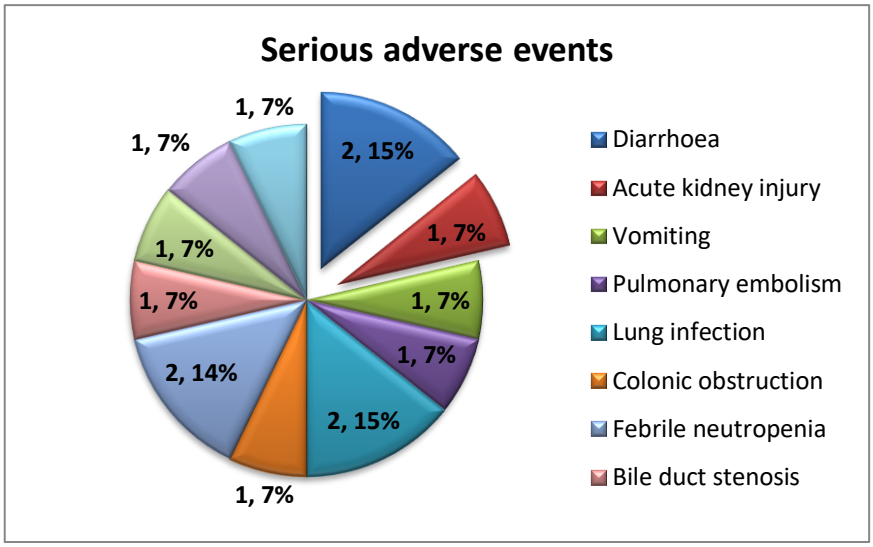


Figure 4.4 Serious adverse events reported by patients in the curcumin + standard treatment (CST) group. (Group total = 13 patients). Curcumin possibly contributed to diarrhoea and acute kidney injury. Diarrhoea, lung infection and febrile neutropenia were the most commonly occurring serious adverse events (15% of all serious adverse events).

4.2.5. Peripheral neuropathy

Participants in both the CST and ST arms all experienced sensory peripheral neuropathy. The peripheral neuropathy scores recorded in the ST group were significantly higher than those in the CST group over the course of treatment ($p = 0.0002$), where a higher score represented worsening neuropathy (See Fig 4.5).

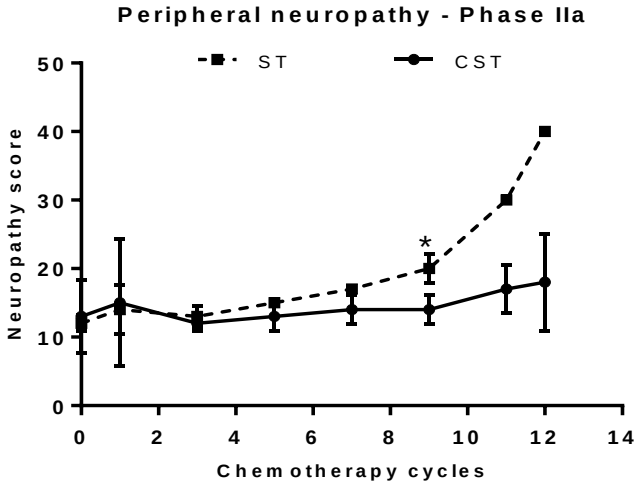


Figure 4.5 Comparison of peripheral neuropathy scores between curcumin + standard treatment group (CST) vs standard treatment (ST) only group over 12 cycles of chemotherapy. Neurotoxicity measured at baseline and cycles 1,3,5,7,9,11, and 12. Statistically significant difference between values analysed using t-tests where $p < 0.05$ represented by asterisk (*). T-tests comparisons could not be performed for cycles 7, 11 and 12 due to insufficient patient numbers from ST group – only one patient completed 12 cycles.

The full oxaliplatin dose was maintained for 23.1% (3 patients) of the CST patients for the duration of their treatment. In this group, symptoms of chronic peripheral neuropathy resulted in an oxaliplatin dose reduction (20 – 25%) in 23.1% (3 patients) and early cessation of oxaliplatin in over half of the patients (53.8%) (See Fig 4.6). In the ST arm, the oxaliplatin dose was reduced for two of the four patients (50.0%). The remaining two patients (50.0%) tolerated the full oxaliplatin dose for the duration of their treatment, however CUFOX2A 006 completed only 2 cycles of chemotherapy[36].

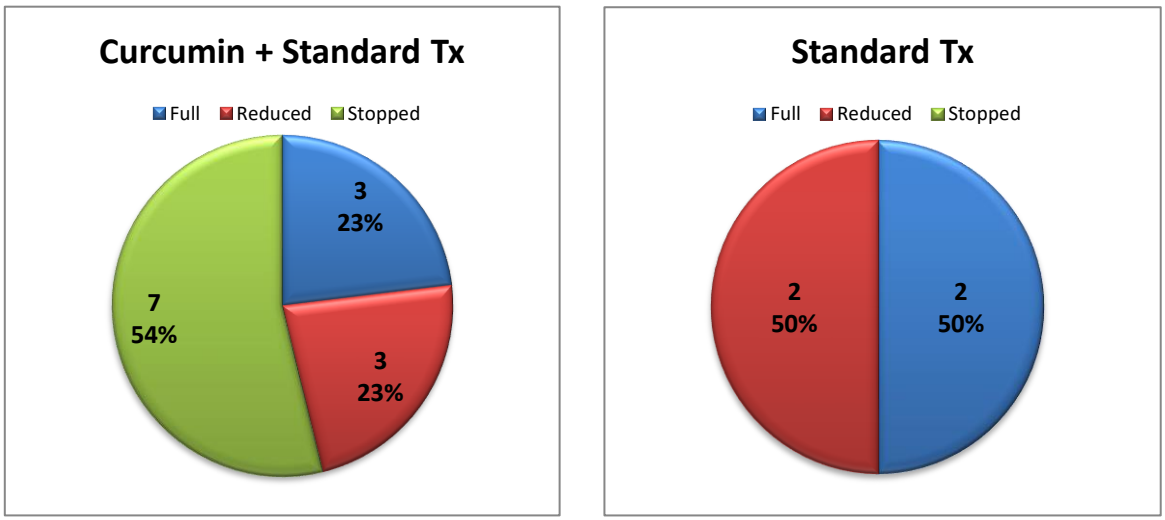


Figure 4.6 Comparison of oxaliplatin dose alterations over treatment in curcumin + standard treatment group vs standard treatment only. (Group total Curcumin + Standard = 13 patients; Group total Standard = 4 patients).

4.2.6. Treatment outcomes

By the end of treatment in the CST arm, 7 patients (53.8%) had achieved a partial response[37], 1 patient (7.7%) maintained stable disease, and 4 patients (30.8%) showed disease progression (See Fig 4.7). The one patient who stopped treatment after one cycle of chemotherapy did not have a follow-up CT scan. Sufficient disease reduction was achieved in 3 patients (23.1%) for them to proceed to surgical resection of their lesions. These responses were observed at the mid-treatment (post-6 cycles) CT scans for two patients, and after 12 cycles of chemotherapy for the last patient.

[36] CUFOX2A 006 died after 2 cycles of chemotherapy (See Section 3.5.4 - Toxicity)
[37] Partial response = at least 30% reduction in the sum of the diameters of target lesions; Progressive disease = at least 20% increase in the sum of the diameters of target lesions; Stable disease = neither sufficient shrinkage to qualify as partial response or sufficient increase to qualify as progressive disease

The ST arm (See Fig 4.7) had one participant that showed a partial response (25.0%) to treatment in his mid-treatment scan and underwent surgical resection of his disease. A second patient obtained a partial response after 6 cycles but was found to have disease progression (25.0%) after completing 12 cycles of treatment. One patient showed stable disease (25.0%), however this was after only 3 cycles of treatment, following which toxicity necessitated cessation of chemotherapy on the trial. The final patient died after 2 cycles of chemotherapy and did not have a follow-up CT scan.

CEA measurements were again obtained at baseline, after 6 cycles of treatment and at the end of treatment for Phase IIa patients. Two of these patients (CUFOX2A 005, CUFOX2 006) only had baseline CEA measurements taken as they tolerated less than 6 cycles of chemotherapy and then were too unwell for blood tests at the time of treatment cessation. For two patients (CUFOX2A 001, CUFOX2A 017) only one further CEA reading was obtained following baseline as a result of errors in blood requests. CUFOX2A 012 maintained a CEA of <2 throughout treatment. As in Phase I, no obvious correlation could be demonstrated between tumour response via RECIST criteria and CEA readings.

For this interim data analysis, progression-free survival (PFS[38]) was compared between the two arms using data available up to April 1st 2015 to give a median 7-month follow-up[39] (See Fig 4.9). Patients who underwent surgery post-trial were not included in analysis of PFS. The median PFS was significantly longer in the CST arm compared to ST, at 7.6 months vs 5.5 months respectively (HR = 0.06 (95% CI 0.006 – 0.6); log-rank p = 0.02). All the patients recruited onto the ST arm had either demonstrated disease progression or died during this follow-up period. Data was censored at the given date for 3 patients in the CST arm who thus far show no evidence of disease progression.

[38] Progression-free survival measured as the time from screening to clinical/radiological progression or death from any cause.
[39] Follow-up measured as time from screening until time of progression, death, or 1/4/15.

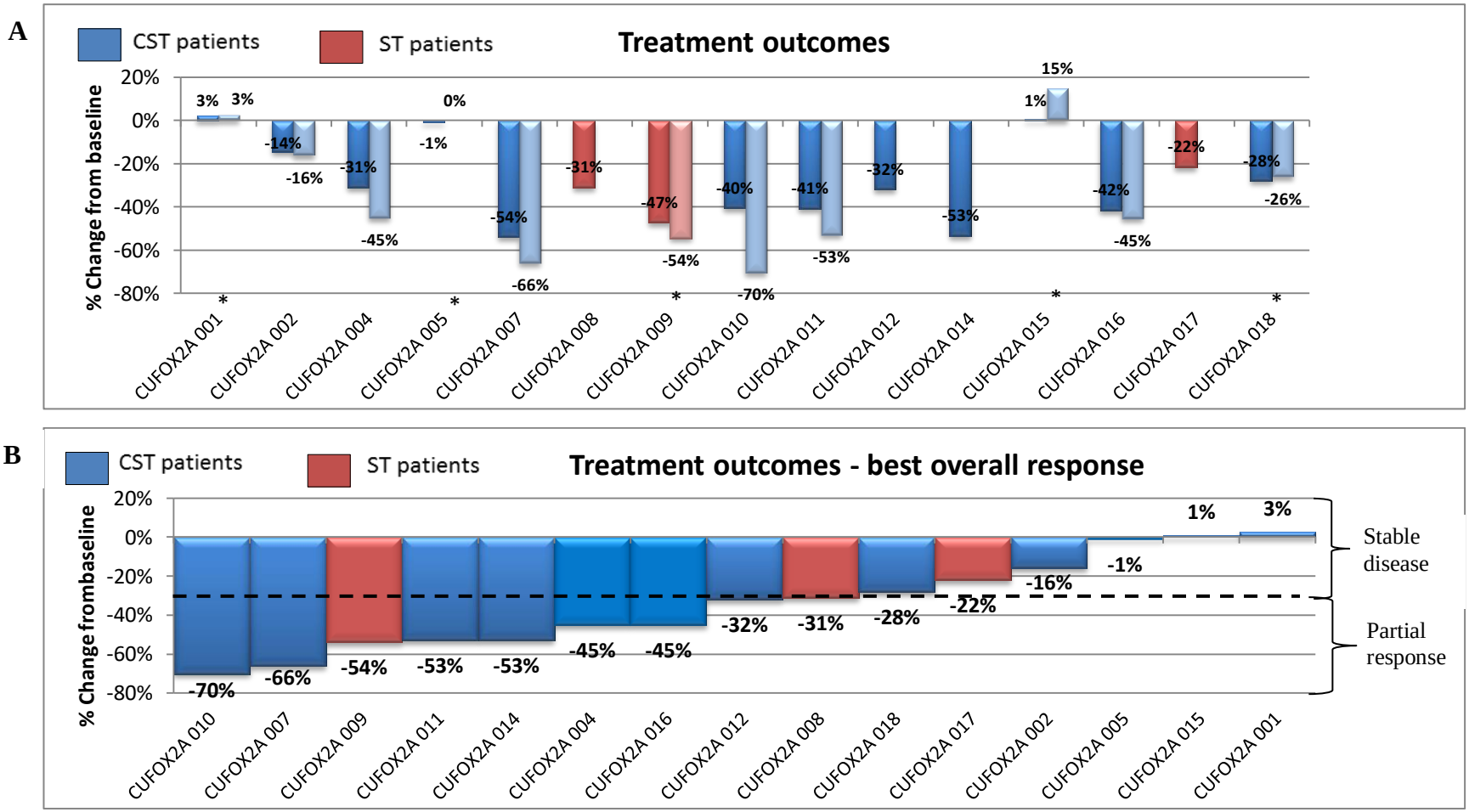
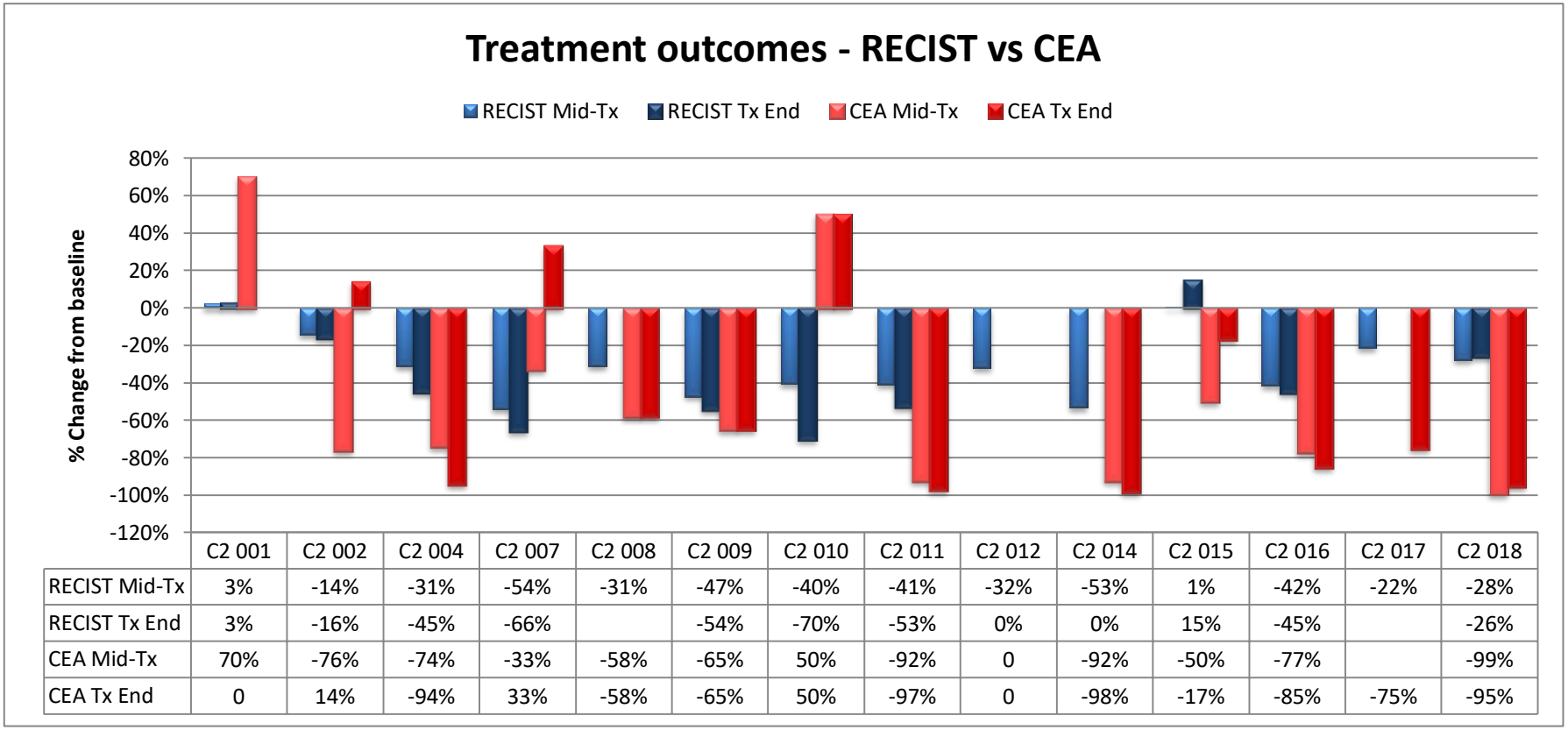


Figure 4.7 A - Change in tumour size at mid-point (dark bars) and end of treatment scans (light bars) compared to baseline for all trial patients. CST – patients having curcumin + standard treatment. ST – patients having standard treatment alone. *Patients showing disease progression by the end of treatment (in some cases reduction measured in target lesions, but progression due to new lesions). **B – Waterfall plot of best responses to treatment.** Best overall response – best response across all time points for each patient. Partial response - ≥30% reduction in target lesion diameter. Progression - ≥ 20% increase in target lesion diameter. Stable – inadequate changes to class as partial response or progression.



	C2 001	C2 002	C2 004	C2 007	C2 008	C2 009	C2 010	C2 011	C2 012	C2 014	C2 015	C2 016	C2 017	C2 018
RECIST Mid-Tx	3%	-14%	-31%	-54%	-31%	-47%	-40%	-41%	-32%	-53%	1%	-42%	-22%	-28%
RECIST Tx End	3%	-16%	-45%	-66%		-54%	-70%	-53%	0%	0%	15%	-45%		-26%
CEA Mid-Tx	70%	-76%	-74%	-33%	-58%	-65%	50%	-92%	0	-92%	-50%	-77%		-99%
CEA Tx End	0	14%	-94%	33%	-58%	-65%	50%	-97%	0	-98%	-17%	-85%	-75%	-95%

Figure 4.8 Change in tumour size measured at mid-point and end of treatment scans compared to baseline versus changes in CEA measurements for the same timepoints. Patients excluded because mid-treatment and end of treatment RECIST data or CEA readings were not available.

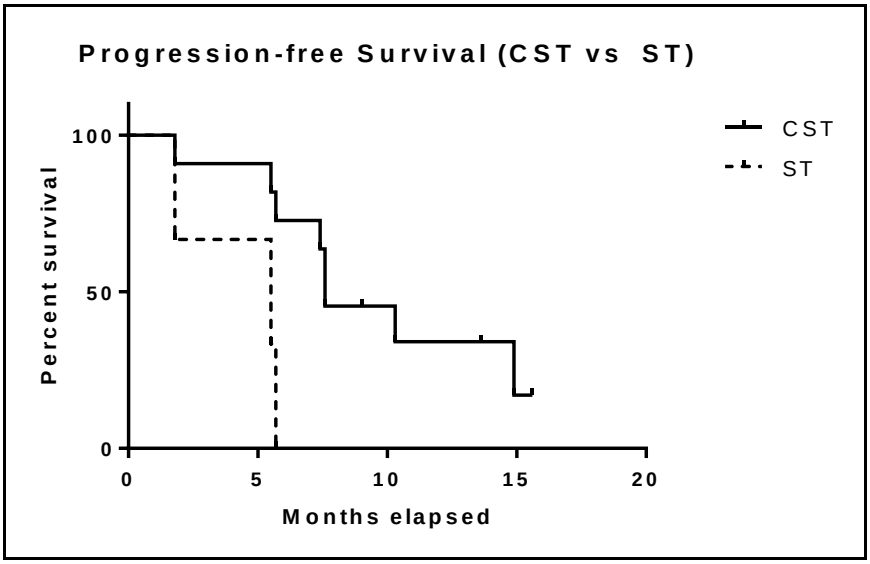


Figure 4.9 Comparison of the progression-free survival for patients in curcumin + standard treatment (CST) group versus standard treatment alone (ST). Data was sensored at time of report for three patients in CST arm who had not yet progressed. An improvement of 2.1 months was reported in the CST arm with a hazard ratio of 0.06, p = 0.02.

4.2.7. Quality of life

The EORTC QLQ-C30 quality of life questionnaire was used to measure the impact of treatment on patients' quality of life. These questionnaires enabled comparison between the CST and ST groups in 3 areas: 1) functionality; b) symptoms; c) overall subjective well-being, measured as the global health score. In the CST arm, 41.7% of patients reported an improvement in functionality, 50% deteriorated, while 8.3% noticed no change. Three of the 5 patients (60.0%) with improved functionality displayed an increase of over 10 points in their functionality. By comparison, in the ST arm there was a noted improvement (>10 points) in 33.3% of patients and deterioration in 66.7%. Overall comparison of pre- versus post-treatment scores, demonstrated an average decrease in functionality in both groups (See Fig 4.10). Although this deterioration was more marked in the ST group, the difference was not statistically significant (Functional score[40] change CST = -5.7; ST = -19.3, p = 0.47).

Symptom scoring showed that 66.7% of patients in the ST group experienced a deterioration in symptoms compared to 33.3% in the CST group. Symptoms improved in 41.7% of patients in the CST group versus 33.3% in the ST group. Again, the overall

[40] Higher score = improved function

difference in pre- versus post-treatment scores for both groups reflected a worsening of symptoms, with a non-significantly greater score (i.e. worse symptoms) in the ST arm (Symptoms score[41] change CST = 1.2; ST = 3.2, p = 0.77).

Equal proportions of patients in the CST arm reported improvement in their global health score (41.7%) as those reporting a deterioration. The score was static in 16.7% of patients in this group. In the ST arm, 66.7% of patients reported a worsening and 33.3% an improvement in global health. The mean global health score deteriorated to a greater extent in the ST group compared to the CST group (Global health score change CST = -2.8; ST = -11.1, p = 0.84).

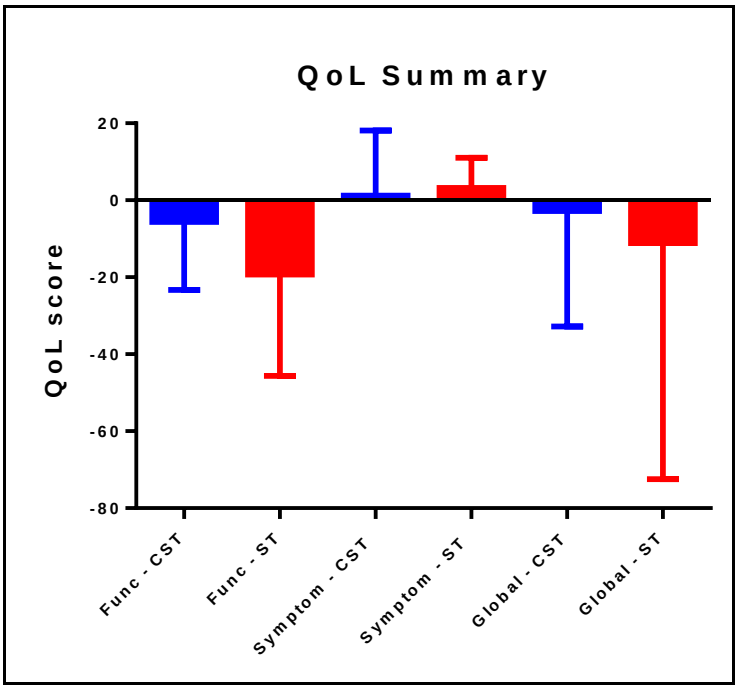


Figure 4.10 Graph comparing the changes in quality of life scores in the curcumin plus standard treatment (CST) group versus standard treatment (ST) alone; Increase in functional score (Func) represents improved physical functionality. Increase in global health (Global) score represents improved overall perceived wellbeing. Increase in symptom score represents worsening symptoms.

4.2.8. Curcumin questionnaire

As with Phase I, the Phase IIa patients completed questionnaires to examine their views on taking curcumin. However, the post-treatment curcumin questionnaires were only completed by the patients in the CST arm.

[41] Higher score = worsening symptoms

Most participants (70.6%) had not heard of curcumin prior to starting the trial and none used it as a health supplement. Three patients (17.6%) reported using turmeric regularly in their diet, however they did not clarify how frequently (See Table 4.4, Fig 4.11). On completion of treatment, there was an increase of 57.4% in patients who would consider using curcumin in their diet and 58.3% as a health supplement (see Fig 4.11).

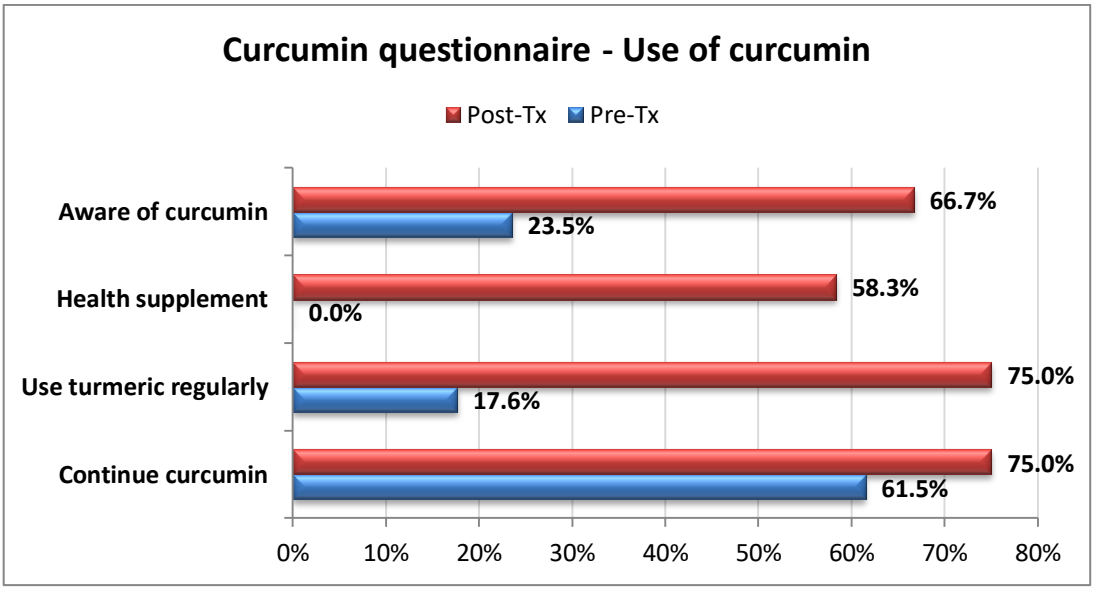


Figure 4.11 Comparison of patients' attitude to curcumin from pre- and post-treatment curcumin questionnaires; Questionnaires assessed patients' awareness of benefits of curcumin, whether patients would consider using curcumin as a health supplement, use of turmeric regularly in diet, and whether patients would have been willing to continue curcumin beyond the 6-month trial period; Tx – treatment.

Some patients had expressed concerns about the potential side-effects of curcumin (35.3% - 'a little') and the effect curcumin would have on their other medication (29.4% - 'a little' or 'quite a bit') in their pre-treatment questionnaires. On completion of treatment, 91.6% of patients reported that they had experienced side-effects. All of these patients attributed these side-effects to chemotherapy whilst one patient also attributed curcumin ('a little'). Nonetheless, none of the participants reported concerns about the side-effects of curcumin by the end of their treatment. The proportion of patients concerned about the interaction of curcumin with their medication also reduced from 29.4% to 16.3%.

Table 4.4 Comparison of patients' experience of curcumin from curcumin questionnaire pre- and post-treatment

	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx
	Not at all		A little		Quite a bit		Very much	
Capsules difficult to take (N^o)	82.4 %	91.7%	5.9%	0.0%	5.9%	8.3%	5.9%	0.0%
Capsules difficult to take (Size)	76.5 %	91.7%	11.8 %	0.0%	5.9%	8.3%	0.0%	0.0%
Capsules difficult to take (frequency)	88.2 %	91.7%	0.0%	0.0%	0.0%	8.3%	0.0%	0.0%
Concerned about side-effects	58.8 %	100%	35.3 %	0.0%	0.0%	0.0%	0.0%	0.0%
Experienced side-effects	-	8.3%	-	50.0%	-	33.3%	-	8.3%
Side-effects due to chemo	-	8.3%	-	25.0%	-	33.3%	-	33.3%
Side-effects due to curcumin	-	83.3%	-	8.3%	-	0.0%	-	0.0%
Worried if missed dose	23.5 %	50.0%	23.5 %	25.0%	5.9%	16.7%	29.4 %	0.0%
Treatment gave peace of mind	17.6 %	25.0%	17.6 %	41.7%	23.5 %	16.7%	17.6 %	16.7%
Inconvenient with other meds	82.4 %	83.3%	0.0%	8.3%	0.0%	0.0%	0.0%	0.0%
Affects other medication	52.9 %	66.7%	11.8 %	8.3%	17.6 %	8.3%	0.0%	0.0%
6 months too long	64.7 %	100%	11.8 %	0.0%	0.0%	0.0%	0.0%	0.0%
Yellow stools	58.8 %	58.3%	35.3 %	33.3%	0.0%	8.3%	0.0%	0.0%
Flatulence	23.5 %	41.7%	64.7 %	33.3%	5.9%	16.7%	5.9%	8.3%
Bloating	41.2 %	83.3%	52.9 %	8.3%	0.0%	0.0%	5.9%	8.3%
Abdominal pain	35.3 %	100%	41.2 %	0.0%	17.6 %	0.0%	5.9%	0.0%

In terms of gastrointestinal symptoms, levels of flatulence (76.5% to 58.3%) and abdominal bloating (58.8% to 16.6%) were reduced by the end of treatment, and abdominal pain was resolved (64.7% to 0%) (see Table 4.4). Yellow stools, however, increased from 35.3% to 41.6%.

With the exception of one patient, participants reported no problems with the number, size or frequency of the curcumin dose. Suggestions to improve compliance included smaller capsules, fewer capsules or splitting the dosage from once to twice daily (see Fig 4.12). However, the larger contingent (83.3%) did not think anything was required to improve compliance. Curcumin side-effects were not reported as a potential problem for treatment compliance.

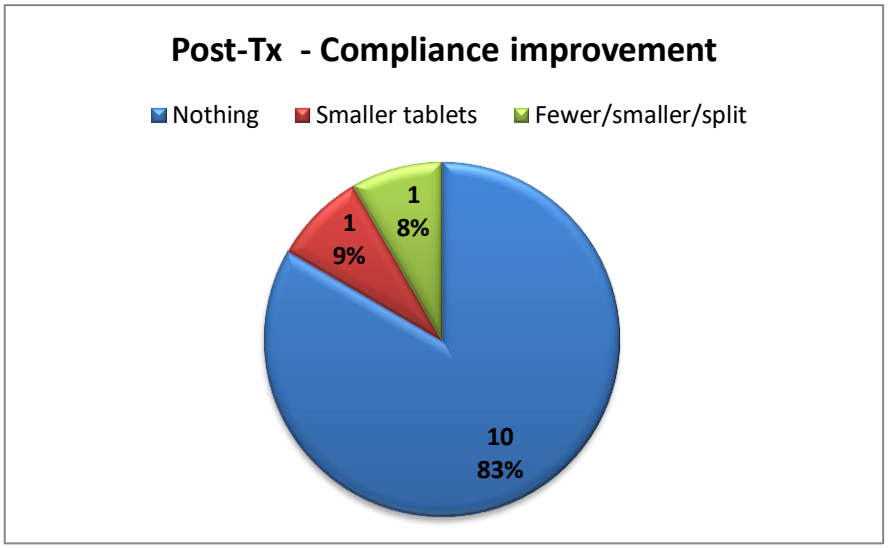


Figure 4.12 Patient suggestions of measures to improve compliance from curcumin questionnaire; S/E – Side-effects, Tx – treatment (Group total = 12 patients)

All the participants stated that they had no problems at all with taking curcumin for 6 months. Eight participants (66.7%) would have been willing to continue taking curcumin indefinitely, whilst 2 participants (16.7%) reported 6 months as the maximum duration they would tolerate. One patient recommended 12 months as the maximum duration for taking curcumin.

4.3. **Discussion – Phase IIa**

4.3.1. Recruitment

As seen towards the end of Phase I, recruitment was slower than expected in Phase IIa of this study. With the estimated recruitment rate of 2 patients per month, approximately 40 – 42 patients would have been the projected number of patients recruited over the 21 month period of Phase IIa recruitment so far. However, this study's recruitment rate (Phase IIa – 0.86 patients/month) is in keeping with the average of 0.83 patients/month/site reported for clinical trials in patients with advanced metastatic colorectal cancer [(264)]. A number of factors could have contributed to the decrease in trial recruitment from Phase I tier 2 to Phase I tier 3 and Phase IIa. A decrease in clinical trial recruitment was reported by NCRI for 2013/2014 where the percentage of cancer patients recruited relative to incidence fell to 9.5% compared to 30% in 2011/2012[(265)]. For this study in particular, the low recruitment rate is more likely to be a reflection of the reduction in the number of patients presenting with

metastatic colorectal cancer. Only 33 patients with metastatic colorectal cancer were screened during this time. Again the overall recruitment rate of 55% remains high compared to those reported for UK cancer clinical trials during this time period.

The introduction of the National Bowel Cancer Screening Programme in 2006 was designed to enable diagnosis of bowel cancer at an earlier stage, which would in turn improve treatment options and mortality for patients (see Fig 4.13). A study comparing patients diagnosed with colorectal cancer through screening compared to the 2-week suspected colorectal cancer referral guidelines, reported that the proportion of patients diagnosed at an earlier stage (T1/T2 or Duke A) was significantly higher in the screened versus the symptomatic group ($p < 0.001$) [266].

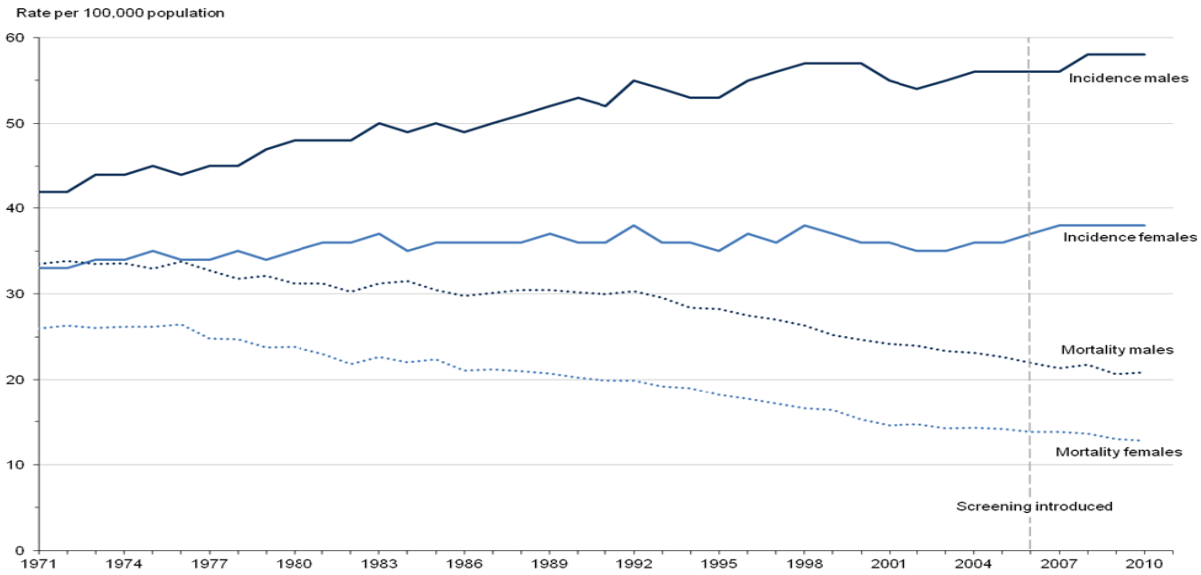


Figure 4.13[267] **Bowel cancer incidence and mortality rates, by sex, England, 1971 – 2010;** Age-standardized rates per 100,000 population, standardized using the European Standard Population

Similarly, improvements in surgical techniques and chemotherapeutic agents have resulted in more opportunities for resection of liver metastases[268]. Therefore the number of patients referred for chemotherapy with unresectable liver metastases could be declining. Another factor affecting trial recruitment would be the availability of FOLFIRI plus Cetuximab as first-line treatment for patients with *K-ras* wild-type colorectal cancer. Access to this chemotherapy regimen via the cancer drugs fund, made FOLFIRI + Cetuximab the preferred chemotherapy regimen for patients meeting the eligibility criteria, resulting in fewer patients being screened for the CUFOX trial.

Overall, the percentage of eligible patients who consented to entry into the trial in Phase IIa was similar to that seen in Phase I (Phase I: 85.7 – 100%, Phase IIa – 86.0%). Despite amendments in the inclusion/exclusion criteria at the end of Phase I, the proportion of patients who failed screening in Phase IIa remained similar to that seen in Phase I (45.5%). The eligibility criteria have therefore been reviewed again by the trials committee. A substantial amendment has been submitted allowing patients with non-hepatic metastases to be recruited in the trial. Furthermore, FOLFOX + Cetuximab has recently been added to the East Midlands guidelines for the treatment of patients with *K-ras* wild-type metastatic colorectal cancer. Therefore it has been recommended that this chemotherapy regimen be accommodated in the CUFOX protocol.

4.3.2. Treatment

Patients were randomised in a 2:1 ratio to curcumin and standard treatment versus standard treatment alone. Being a pilot study, there was no previous data available to provide a basis for accurate power calculations. Nonetheless, the recruitment numbers and ratios were based on information from similar phase I studies in other tumour sites and advice from the University of Leicester Clinical Trials Unit. The initial recruitment of 13 CST versus 5 ST patients would have provided reasonable data for comparing the two groups. However, unforeseeable complications resulted in only 2 of the 5 patients in the ST group receiving more than 6 cycles of chemotherapy. Data from this arm was therefore limited, and larger recruitment figures will be required to substantiate these initial findings.

4.3.3. Toxicity

As with phase I, no significant safety concerns were raised by the combination of curcumin with standard chemotherapy. The adverse events from the curcumin-only period remained low grade. Of the more commonly reported adverse events, curcumin is likely to have contributed to oral mucositis and dyspepsia. Dry mouth is a likely precursor for oral mucositis and has featured as a pre-chemotherapy toxicity in both phase I and IIa. Dyspepsia was reported pre-chemotherapy in phase I and again frequently experienced by patients in the CST arm.

With the addition of chemotherapy, a greater proportion of serious adverse events was reported in the CST group. Diarrhoea remains the primary contentious issue and associated acute kidney injury remain the primary problems for patients in this group.

Grade 3-4 diarrhoea was reported by two patients (15.4%) in the CST arm which is a higher percentage than the 10% - 11.1% reported for patients treated with FOLFOX ± Bevacizumab[4,262]. Neither of these patients had been experiencing diarrhoea at baseline. Conversely, the ST group reported no episodes of grade 3 or more diarrhoea. Therefore monitoring for diarrhoea and subsequent acute kidney injury in the remainder of this cohort will be essential.

The most significant toxicity reported was in the ST arm where a patient died following wound dehiscence and sepsis after developing bowel obstruction. This patient had been receiving FOLFOX and Bevacizumab as standard treatment. Bevacizumab is known to contribute to a number of post-surgical complications including impaired wound healing, wound dehiscence, surgical site bleeding, ecchymosis and wound infection[269]. For this reason it is recommended that patients wait at least 6 to 8 weeks after cessation of bevacizumab to have surgery or at least 28 days before postoperative initiation[269]. In this case the surgical procedure was performed as an emergency less than 28 days after Bevacizumab, which is likely to have been a significant factor in this event. Either way, the patient had not been taking curcumin during treatment, thus there can be no link between the trial drug and this patient's death.

Overall, these results provide no significant safety concerns for the addition of curcumin to standard chemotherapy in this patient group.

4.3.4. Compliance and curcumin questionnaire

The reported Phase IIa compliance to taking curcumin was again satisfactory at 94.1%. However it is important to note that these results are based on patient reports. Unfortunately, monitoring of the number of capsules returned was not conducted accurately. Patients were required to return any empty pill bottles or unused tablet to the trials team each time a new batch of capsules was dispensed and at the end of the trial. The number of capsules remaining would be counted by the trials team as a means of verifying compliance. This return of pill bottles and capsules proved to be erratic with some patients never returning any medication. Accounting for medication was further complicated when patients were admitted to hospital, where the trial medication was often withheld and sometimes misplaced. There were also two occasions were the supply of curcumin to the pharmacy was delayed. This resulted in alterations in the dispensing schedule and subsequent difficulty in tallying number of capsules returned

with a pre-determined value. It was therefore not possible to validate patient-reported compliance using pill counts. Discrepancies in treatment compliance could significantly affect reported outcomes of clinical and pharmacological data. It is therefore imperative that a more reliable method of measuring compliance be adopted for future studies to ensure that patient reports can be endorsed. The reference standard for monitoring patient compliance would be the use of electronic monitoring devices. However the additional cost of adopting this technique would need to be considered.

Having said that, the high compliance rates reported do correlate with the feedback from the end of trial curcumin questionnaires where the majority of patients did not think anything was required to improve compliance. Concerns raised prior to starting treatment regarding potential curcumin side-effects and its interaction with other medication, all reduced by the end of treatment. In fact, patients reported a reduction in some of the gastrointestinal symptoms associated with curcumin (e.g. flatulence, abdominal discomfort).

As previously discussed, dose frequency and perceived toxicity can be significant barriers to patient compliance. As with phase I, the phase IIa curcumin questionnaires show that the majority of patients did not encounter these problems with their curcumin capsules. As a result, over 70% of the CST patients would have been willing to continue taking curcumin beyond the 6-month trial period.

4.3.5. Peripheral neuropathy

A significant difference was reported in the peripheral neuropathy scores between the CST and ST arms. However, the neurotoxicity score only reached significance when comparing the results after completion of 12 cycles of treatment. Thus these results were based on 3 CST patients versus one ST patient. Data from such a small number can only be viewed as encouraging but preliminary.

In phase I, the proportion of patients needing oxaliplatin dose reduction or cessation, was lower than expected at 50.0%. Conversely, 76.9% of phase IIa patients (CST arm) required either dose reductions or cessation of treatment. Over the two phases, 63.5% of patients have required alteration in their oxaliplatin dose, a similar proportion to that reported in previous studies (62.5%)[95]. Only 50% (2 patients) in the ST arm reported any peripheral neuropathy, but it should be noted that the other two patients both completed less than 3 cycles of treatment. One of the two patients in this arm who had

no oxaliplatin dose reductions only completed 2 cycles of chemotherapy in total. It is essential that any distinction in peripheral neuropathy scores between the two trial groups is classified over the remainder of the trial. Oxaliplatin-induced peripheral neuropathy remains a stumbling block in the treatment of colorectal cancer. There is still no recommended agent for the prevention or treatment of this toxicity due to a lack of high quality and consistent evidence[270]. Chronic neurotoxicity is estimated to affect up to 18% of patients treated with oxaliplatin [84] and the detrimental impact of this toxicity has been reported, not only in terms of affecting treatment administration, but also on patients' subsequent quality of life. Severe peripheral neuropathy has been associated with depression[271], decreased social activity and impaired quality of life[272]. The method of screening patients in this study was via patient questionnaires and clinical consultation. Discrepancies between patient perception and clinical assessment are a known problem in the evaluation of chemotherapy-induced peripheral neuropathy[273]. This could affect clinical judgement of how and when to change treatment, as well as the interpretation of trial data. Efforts are ongoing to reduce the impact of neuropathy following treatment of colorectal cancer.

4.3.6. Quality of life

The post-treatment quality of life questionnaires were undertaken within 14 days of the end of chemotherapy. It is therefore not surprising an overall deterioration in all three quality of life outcome parameters (function, symptoms and global health) was recorded for both groups compared to baseline. Within this short time frame most of the acute chemotherapy effects are unlikely to have resolved. It is therefore difficult to extrapolate this data and predict the impact of these treatments on patients' longer term quality of life.

Comparing the quality of life scores between the two groups, the CST group showed a higher proportion of patients with improved outcomes for all three factors measured. With such a small cohort, and particularly when comparing results from a group of 12 patients to one of 3 patients, no substantial conclusions can be reached. However, if this remains the pattern as the cohort expands, it would substantiate the current suggestion that quality of life was improved by the addition of curcumin to standard treatment. For survivors of colorectal cancer, who are disease-free up to 3 years post-diagnosis, quality of life has been shown to be comparable to that of the general population in terms of physical functioning and overall quality of life.[274]. Already over

40% of the patients in the CST arm reported improvements in functionality and their global health score by the end of treatment. This has the potential to improve further if disease control can be sustained post-treatment.

4.3.7. Treatment outcomes

In phase I, 58.3% of patients showed a partial response after treatment with curcumin (doses 500 mg, 1 g and 2 g daily) combined with FOLFOX ± Bevacizumab. Similarly, 53.8% of patients in the CST arm of phase IIa demonstrated a partial response to their treatment. These outcomes are similar to those previously reported for FOLFOX (RR = 45% - 54% [263]) and FOLFOX + Bevacizumab (RR = 47% [3]). Overall, over 60% of CST patients (61.5%) demonstrated disease control with either a partial response or stable disease.

One patient in the ST arm demonstrated a partial response to treatment, suggesting a 25.0% response rate. Stable disease was demonstrated in one patient, however this was after only 3 cycles of chemotherapy following which all treatment was stopped due to this patients' clinical deterioration. Analysis of data such as response rates in this small cohort of only 4 patients has to be interpreted with caution.

Surgical resection of initially unresectable disease was undertaken in 23.1% of patients in the CST group. This is a higher proportion than reported with standard treatment where systemic chemotherapy in patients with previously unresectable disease rendered 13% to 16% of patients resectable[275-277]. Surgical resection is the only option for long term survival in patients with liver metastases. Despite advances in surgical technique, 80% of these patients will be classed as having unresectable disease at the time of presentation[278]. Yet for those whose disease is adequately downgraded to proceed to surgery, survival rates become significantly better than patients receiving chemotherapy only and similar to those seen in patients with initially resectable disease[275,279]. The 5-year survival for patients treated with chemotherapy only ranges from 0% to 8% [275,279]. In patients with resectable liver metastases, this improves to 40% to 50%[276]. Yet following rescue surgery for patients with previously unresectable disease, studies have reported 5-year survivals of 33% to 42.6%, and 10-year survivals of 16% to 27% [280,281]. These studies also reported that 16% to 23% of patients were considered to

have been cured of their disease[42]. Therefore the suggestion that surgical resection rates could be improved by addition of curcumin to standard chemotherapy is of considerable potential importance. This data will need to be validated on completion of this study by further trials with larger cohorts. Appropriate analysis of the progression-free survival data cannot be undertaken at this time as some patients in the CST arm continue to show no evidence of disease recurrence.

4.4. **Conclusion – Phase IIa**

The addition of curcumin at a dose of 2 grams daily to standard oxaliplatin-based chemotherapy continues to exhibit good tolerability with no significant safety concerns. Interim analysis shows no significant decline in quality of life for patients taking curcumin compared to those on standard treatment alone. Excellent compliance rates were achieved with patients reporting no concerns with the curcumin regimen and a willingness to continue treatment beyond 6 months. Data acquired on completion of phase IIa will be used to validate these initial results and assess effects on peripheral neuropathy, disease response and survival.

[42] Cure was defined as a disease-free interval of 5 years or more after the last hepatectomy or last resection of extrahepatic metastases. Furthermore, patients had to be free of disease at last follow-up. Patients who died of not curatively resected metastases or disease recurrence were defined "noncured".

5. Biomarkers

The following chapter describes results for three biomarker strategies that were outlined within the CUFOX clinical protocol, which set out to assess potential toxicity of the combined therapy, effect of curcumin on DNA platination and curcuminoid levels in patient plasma.

5.1. **MicroRNA**

5.1.1. Introduction

miR-122 has been established as a biomarker for various forms of liver damage[243-245]. Currently, liver toxicity is routinely measured using liver function tests, with alanine aminotransferase (ALT) in particular considered the most specific marker of liver toxicity. As well as showing significant correlation with ALT levels, miR-122 expression alters earlier and with greater specificity to herald liver damage[245]. As well as a biomarker of liver toxicity, recent studies have also indicated the role of miR-122 in enhancing the cytotoxicity of 5-FU in colorectal cancer[248].

In this study we measured changes in miR-122 readings at set time points for each patient, assessing how changes in miR-122 corresponded to ALT readings as a possible biomarker of liver toxicity following treatment with curcumin and chemotherapy. Increased miR-122 levels, with a corresponding rise in ALT levels, could indicate increased hepatocellular injury. We also investigated whether miR-122 expression related to treatment response.

5.1.2. MiRNA results

Serum samples were collected from patients CUFOX1 008, 009, 010, 011, 012 and 013 (n=6) for miRNA analysis. All these patients were in Tier 3 and had been taking 2 grams of curcumin daily. Samples were collected at the five designated time points[43] for all patients except CUFOX1 011, who died before completing treatment and therefore underwent only four sample collections.

[43] Time points: 1 – Baseline; 2 – After 1 week of curcumin only; 3 – After 1 cycle of chemotherapy; 4 – After 2 cycles of chemotherapy; 5 – At the end of treatment.

All but one patient (CUFOX1 013) showed a decrease in miR-122 between baseline and commencing chemotherapy, following consumption of daily curcumin for 1 week (See Figures 5.1 & Table 5.1). After one cycle of chemotherapy, four patients (CUFOX1 008, 009, 010, 012) showed increases in miR-122.

No formal statistics calculations were conducted due to the limited number of miRNA samples, therefore the statistical significance of these results could not be defined. Nonetheless, there was a noteable increase in the mean miR-122 fold change at the end of treatment compared to baseline (End of treatment fold change - 2.51; see Table 5.1). Overall, there appeared to be an increase in the miR-122 fold change measurements progressing over the cycles of treatments, however it was not possible to calculate whether this was a significant trend.

Table 5.1 Summarised data for miR-122 readings over treatment time points. Mean miR-122 results at treatment time points compared to readings at baseline.

Time point	Mean miR-122 fold change	Mean change from baseline
Baseline	1.00	-
Pre-cycle 1	0.68	-0.32
Pre-cycle 2	0.98	-0.02
Pre-cycle 3	1.37	0.37
End of Tx	2.51	1.51

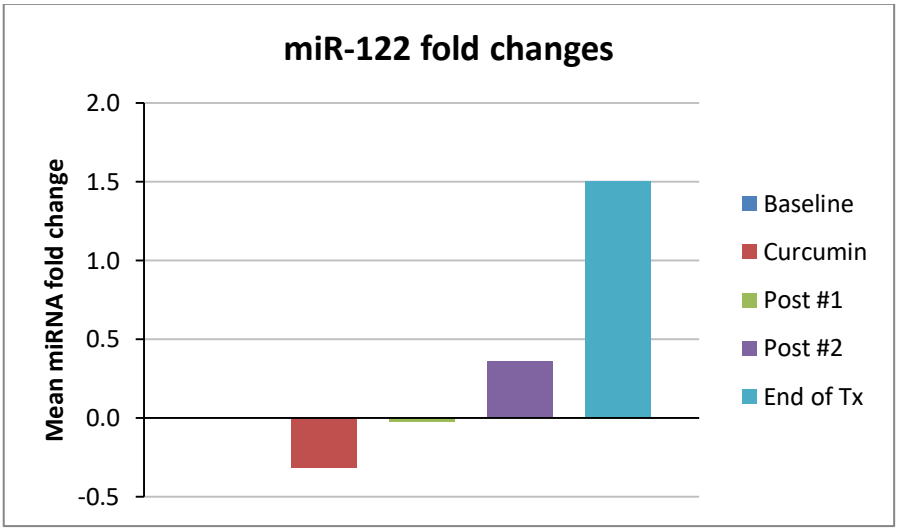


Figure 5.1 – Mean miR-122 fold changes over treatment time points. Mean miR-122 fold change compared to baseline. Curcumin – After 1 week curcumin only, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – end of treatment.

There was no obvious pattern observed in the changes between the mean ALT measurements for any treatment time points compared to baseline (See Table 5.2 and Figure 5.2). Similarly, no trend was observed across the changes in ALT over the duration of treatment.

Table 5.2 – Summarised data for ALT readings over treatment time points. Mean ALT results at treatment time points compared to readings at baseline.

Time point	Mean ALT fold change	Mean change from baseline
Baseline	1.00	-
Pre-cycle 1	1.01	0.008
Pre-cycle 2	0.96	-0.045
Pre-cycle 3	1.03	0.032
End of Tx	1.47	0.472

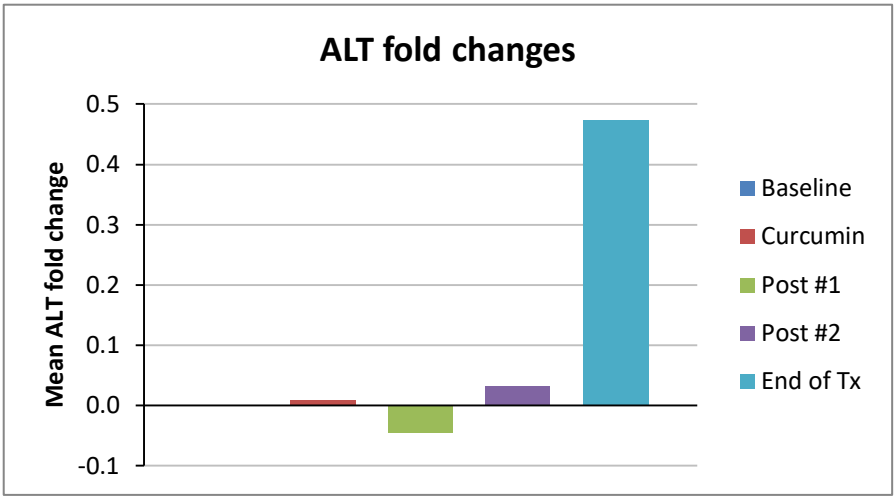


Figure 5.2 – Mean ALT fold changes over treatment time points. Mean ALT fold change compared to baseline. Curcumin – After 1 week curcumin only, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – end of treatment.

Although statistical significance could not be established, the miR-122 and ALT fold changes did appear to follow a similar trend over the treatment time points (See Fig 5.3). The graphs for two patients in particular showed very similar fold changes in miRNA compared to ALT (CUFOX1 009 and CUFOX1 013), then to a lesser extend with CUFOX1 008 and CUFOX1 010 (See Appendix 8.10, Fig A.2).

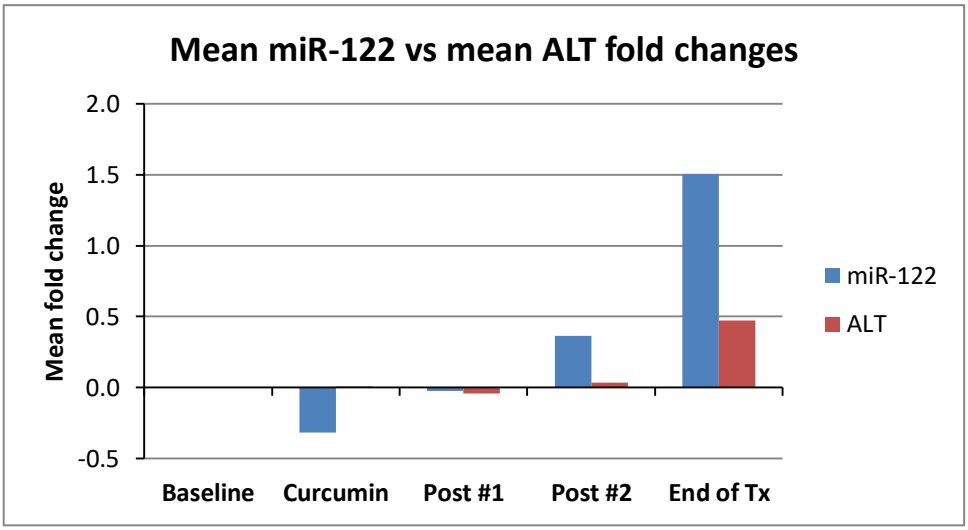


Figure 5.3 – Mean miR-122 fold changes compared to mean ALT fold changes over treatment time points. Curcumin – After 1 week curcumin only, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – end of treatment.

miR-122 expression was compared with treatment response to assess whether increased expression related to enhanced cytotoxicity. In comparing miR-122 fold change to best response to treatment, no correlation was demonstrated (See Fig 5.4).

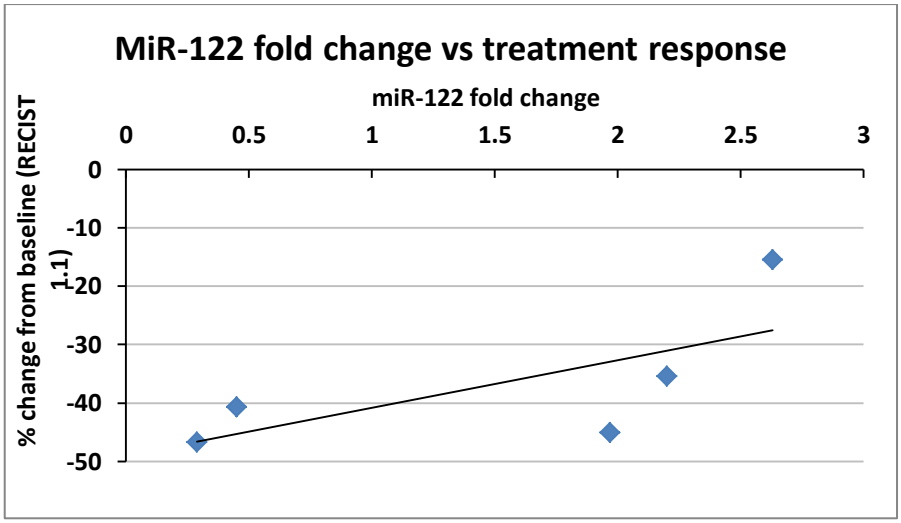


Figure 5.4 – Correlation between mean miR-122 fold changes compared to best treatment responses. Response measured as percentage change compared to baseline according to RECIST 1.1 criteria.

5.1.3. Discussion

miR-122 is important in the maintenance of liver homeostasis, with key roles in cholesterol metabolism and hepatocyte gene expression[282]. Serum miR-122 levels have been shown to be significantly raised in patients with acute liver injury, and correlated with peak alanine aminotransferase (ALT) levels[243],[245]. However, miR-122 is thought to display greater sensitivity to tissue injury than ALT because it is detected earlier, at lower drug concentrations and, unlike ALT, it does not increase with aetiologies such as muscle injury, polymyositis and extreme exercise. Furthermore, the liver-specific nature of miR-122 enables differentiation between hepatic and non-hepatic increases in serum ALT. A similar trend was noted across treatment time points between miR-122 and ALT fold changes, although the statistical significance of this could not be established. Further assessment of this relationship will need to be undertaken in phase IIa. If a correlation can be established between these two distinct biomarkers, then ALT could act as a reference point for validating the miR-122 results obtained. The combination of 5-FU and folic acid has been associated with hepatic steatosis and alterations in serum alkaline phosphatase, transaminases, and bilirubin levels [283],[284]. Oxaliplatin-based chemotherapy has also been linked to hepatotoxicity, particularly hepatic sinusoidal obstruction syndrome following oxaliplatin-based treatment [285]. Monitoring of miR-122 levels in conjunction with ALT readings could be a useful tool to determine whether addition of curcumin to FOLFOX chemotherapy adds to potential liver toxicity.

Our results showed a marked increase in miR-122 levels at the end of treatment compared to baseline. Although the ALT readings did not show as clear a trend over treatment time points, ALT fold changes recorded at the end of treatment were generally higher than baseline. It is therefore possible that data from this study corroborates miR-122's ability to detect liver toxicity earlier than ALT, as shown in previous studies. To this end miR-122 could act as a useful biomarker in determining early treatment-induced toxicity. These are useful preliminary results that can be analysed in more detail with the larger patient population in Phase IIa.

Pre-clinical studies have suggested miR-122 may have a role as an oncological biomarker, but there are no clinical studies to corroborate this as yet. MiR-122 has generally been considered a liver-specific microRNA, however it has now also been shown to modify expression of pro-carcinogenic proteins such as cyclin G, Bcl-W and

ADAM17 (*a d*isintegrin *a*nd *m*etalloproteinase 17), thus its tumour suppressor activity is likely to extend beyond hepatocellular cancer. To illustrate this, down-regulation of miR-122 has been reported in gastrointestinal, breast and pancreatic cancers [247,286,287]. For colorectal cancer particularly, loss of function of the APC tumour suppressor gene contributed to miR-122 down-regulation via the APC/β-catenin signalling pathway, thus stimulating proliferation of cancer cells. Conversely, introduction of an miR-122 mimic inhibited cell growth[287]. Furthermore, rat models of colorectal cancer showed miR-122 suppression in healthy tissue may be related to carcinogenesis elsewhere in the colon [288]. miR-122 also plays a part in cytotoxicity by re-sensitizing 5-FU resistant cells via its inhibition of pyruvate kinase M2 (PKM2)[248]. PKM2 is an isoform of pyruvate kinase that is essential for rapid growth of cancer cells [289]. No definite relationship was established between miR-122 fold-change and changes in tumour size in this study. This would need to be assessed further with the larger cohort in phase IIa,. Firstly it will be key to determine whether changes in miR-122 levels correlate most significantly with changes in ALT and consequently indicate hepatotoxicity. Alternatively if raised miR-122 levels correspond with decreased tumour size, this may suggest miR-122 affecting tumour sensitivity to cytotoxic agents. Another potential assessment of miR-122 as a biomarker could include a panel of miRNAs that would be analysed alongside miR-122. This panel could include, for example, miR-21 and miR-34, which are known to play a role in colorectal cancer and can be affected by curcumin administration. MiR-21 is a microRNA that promotes invasion and metastasis of CRC by targeting mRNAs of tumour suppressor genes e.g. *PDCD4* (programmed cell death 4), *CCL21* (chemokine (C-C motif) ligand 20) and *Cdc25A* (cell division cycle 25 homolog A). Curcumin has been shown to inhibit miR-21 activity by stabilizing the expression of *PDCD4* in colorectal cell lines and restoring the PTEN-Akt pathway in chemoresistant cell lines, thereby preventing miR-21-induced cell proliferation, invasion and metastases. The miR-34 family (miR-34a, b and c) regulates cell cycle progression, apoptosis and inhibits cancer stem-like cells. MiR-34 expression is controlled by p53 and is markedly reduced in colorectal cancer. Difluorinated curcumin (CDF) is a curcumin analogue that has been shown to restore expression of the miR-34 family and induce apoptosis in chemo-resistant cell lines [290]. Examples of other microRNAs that could be included in an analysis panel are listed in Table 5.3.

Table 5.3 – miRNAs in colorectal cancer [291]

miRNA	Function in colorectal cancer
miR-497	Down-regulates insulin-like growth factor 1 receptor gene (*IGF1-R*). Results in inhibition of cell survival, proliferation, invasion and increased sensitivity to apoptosis, e.g. secondary to treatment with chemotherapeutic agents cisplatin and 5-FU[292].
miR-126	Regulates phosphatidylinositol-3-kinase (PI3-K) signalling pathway by targeting regulatory subunit, p85beta. Down-regulated in primary colorectal cancer CRC). Upregulation inhibits growth of CRC cells *in vitro*[293].
miR-143	Targets metastasis-associated in colon cancer-1 (*MACC1*), a gene that promotes tumour metastasis via the HGF/MET signalling pathway. Cell growth, migration and invasion in CRC cells are inhibited by introducing miR-143, and vice versa. miR-143 levels are reduced in CRC tumour samples[294].
miR-103 miR-107	Targets mRNAs of death-associated protein kinase 1 (DAPK) and Krüppel-like factor 4 (KLF4) causing increased cell motility, cell-matrix adhesion, decreased cell-cell adhesion and decreased epithelial marker expression. Upregulation associated with local invasion and liver metastasis in mouse models. [295].

Curcumin has already been shown to increase treatment sensitivity in oxaliplatin-resistant cell lines [183,185]. Studies have also shown that curcumin is able to attenuate liver injury induced by toxins such as ethanol, thioacetamide, iron overdose, carbon tetrachloride (CCl4) intoxication and cholestatis[296]. In the case of CCl4 toxicity, curcumin is able to reverse cirrhosis to some degree via its activity on hepatic stellate cells (HSCs)[297]. HSCs are activated by liver injury and transdifferentiate into highly proliferative and motile myofibroblasts, resulting in liver fibrosis. HSCs are also known to be significant components of the premetastastic liver microenvironment[298]. They stimulate the production of extracellular proteins that contribute to tumour initiation and progression[299]. Curcumin inhibits the proliferation and activation of HSCs, and induces the apoptosis of activated HSCs [297,300]. It would be interesting to determine whether curcumin's activity in preventing liver injury can be related to miRNA activity and treatment outcomes in our patient samples.

Overall the data from this pilot study shows that measurements of miRNA from our patient samples is feasible and shows promise as a biomarker to assess potential treatment-related liver toxicity. More investigation is required to examine miR-122's role as a biomarker of efficacy. Further analysis of Phase IIa samples will be required to substantiate these results.

5.2. **DNA platination**

5.2.1. Introduction

This study assessed the impact of curcumin on platinum-DNA adduct formation in white blood cells as an indication of treatment efficacy. Oxaliplatin exerts its cytotoxic activity by forming mono- or bifunctional adducts with DNA[79,301]. The resulting DNA damage disrupts cellular processes and activates apoptotic pathways leading to cell death. The level of DNA platination in the target tissue is thought to reflect the level of efficacy of oxaliplatin. Pre-clinical studies have shown that curcumin enhanced the cytotoxicity of oxaliplatin in vitro in oxaliplatin-resistant cell lines, and also improved efficacy in vivo without affecting oxaliplatin's mode of action or significantly affecting accumulation of oxaliplatin DNA adducts in tumour tissue [185] (See Section 1.7.8.2). In this study, we aimed to measure oxaliplatin-DNA adduct levels as a biomarker to determine whether addition of curcumin to standard oxaliplatin-based chemotherapy affects oxaliplatin's efficacy in the clinical setting.

Obtaining tumour tissue samples for the duration of this clinical trial was not practical. However a significant correlation has been observed between the level of platinum-DNA adduct formation in lymphocytes from patient blood samples and tumour response [101]. Therefore measurement of DNA platination in white blood cells or lymphocytes has been deemed an appropriate surrogate for monitoring oxaliplatin activity[82]. Oxaliplatin generally has a lower rate of platinum-DNA adduct formation compared to cisplatin[302] and therefore it was essential that a sensitive and validated method of measurement was employed. Having proved sensitive and effective as a means of measuring platinum-DNA adduct levels after oxaliplatin treatment in preclinical studies and clinical trials[109,185], ICP-MS was adopted as the method of choice in this study.

5.2.2. Results

The healthy volunteer DNA samples yielded a mean platinum concentration of 3.48 (± 2.46) Pt:10^6 nucleotides. DNA concentrations were too low for analysis from 3 of the participants in tier 3.Therefore from that cohort only samples from three (CUFOX1 008, CUFOX1 009, CUFOX1 010) instead of six patients were analysed for DNA platination (See Table 5.4).

Table 5.4 Mean platinum results (Pt per 10^6 nucleotides) for each tier of patients at designated time points

	Tier 1 (Pt:10^6 nucleotides)		Tier 2 (Pt:10^6 nucleotides)		Tier 3 (Pt:10^6 nucleotides)	
	Mean	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
Baseline	4.44	2.87	4.51	1.91	5.16	2.55
Curcumin	5.10	3.39	9.14	3.33	7.41	6.60
Post #1	4.69	1.12	47.30	67.58	3.78	2.09
Post #2	4.86	2.59	5.16	0.87	7.76	7.50
Tx End	3.32	0.28	8.71	10.05	2.67	2.42

The median values ranged from 2.81 to 11.13 Pt:10^6 nucleotides. The highest mean platinum concentration recorded was 47.30 Pt:10^6 nucleotides for Tier 2 after one cycle of chemotherapy. The lowest was 2.67 Pt:10^6 nucleotides at the end of treatment for patients in Tier 3. The DNA platination measurements suggested that patients in Tier 1 generally had the lowest platination levels (See Fig 5.5) across the treatment time points. However the variation between platinum readings between the three tiers was not statistically significant ($F(2.00, 6.00) = 1.30$, $p = 0.340$). Likewise, no significant difference or trend was noted across treatment time points ($F(1.07, 6.44) = 1.013$, $p = 0.358$) and no correlation was found between treatment time points and the curcumin dosage ($F(2.15, 6.44)$, $p = 0.379$).

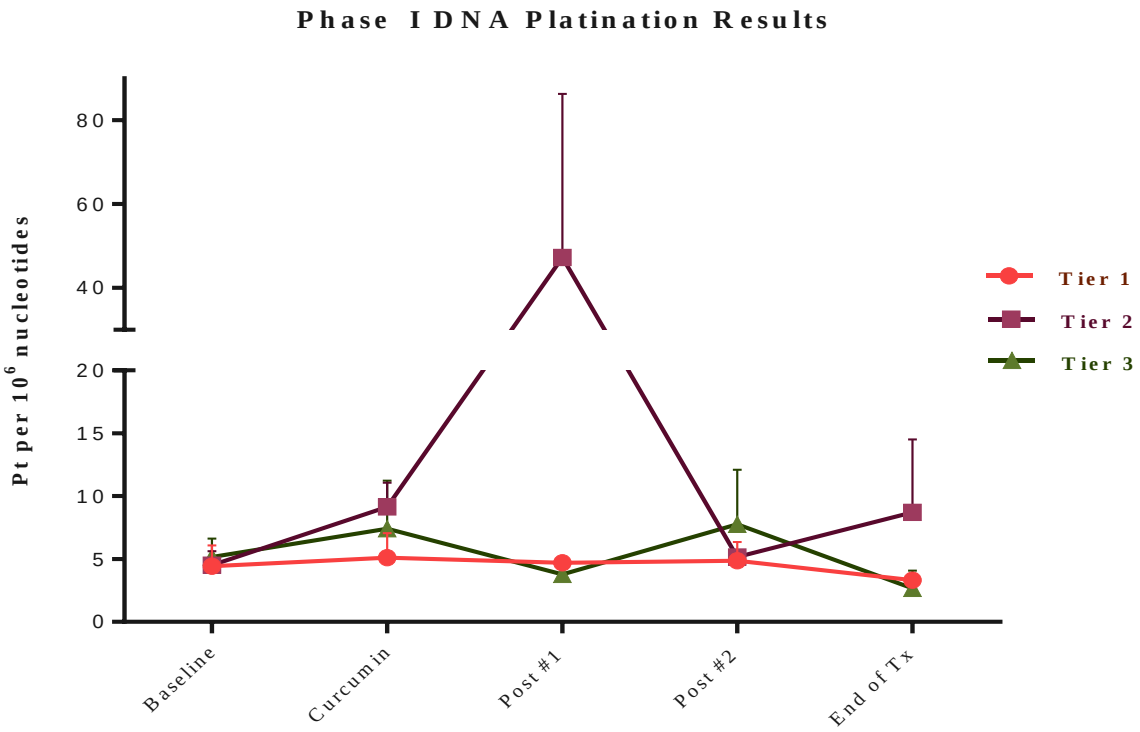


Figure 5.5 Mean phase I DNA platination results for each curcumin dose tier at designated time points. Platinum atoms per 10^6 nucleotides calculated for patients receiving curcumin doses of 500 mg (Tier 1), 1 gram (Tier 2) and 2 grams (Tier 3) daily. Results show the mean platinum readings and standard error bars at baseline, after 1 week of curcumin only, after cycle 1, then cycle 2 of chemotherapy, and at the end of treatment. Error bars represent ± Standard Error.

5.2.3. Discussion

The DNA analysis from the Phase I samples recorded baseline platinum concentrations, with no significant changes after patients commenced chemotherapy. The only notable increase in platinum readings was seen in tier 2, after cycle 1 chemotherapy. This was because patient CUFOX1 006 showed a mean platinum reading of 125.26 Pt:10^6 nucleotides, which raised the average for that tier to 47.30 Pt:10^6 nucleotides (SD 67.58, St error of mean 39.02). Nonetheless no statistically significant difference was calculated. After cycle 2 chemotherapy, this reading dropped to 5.16 Pt:10^6 nucleotides (SD 5.16, St error of mean 0.50). It is therefore most likely that this unusually high result was in error and possibly due to sample contamination.

Platinum-DNA adduct formation in white blood cells of patients treated with oxaliplatin has been previously investigated, primarily at oxaliplatin doses of 130mg/m^2 (FOLFOX dose 85mg/m^2). The median maximum platinum-nucleotide ratio (A_{max}) recorded was 4.43 Pt atoms per 10^6 nucleotides, recorded at either 4 or 24 hours from the onset of the infusion[82]. In this study, the baseline values ranged from 4.44 to 5.16 Pt:10^6 nucleotides, which are comparable to A_{max} levels seen in patients within 48 hours of their infusions. Although these baseline readings are higher than those seen in other studies, they are consistent with those recorded in our healthy volunteer samples. This might be due to a high sensitivity of the icp-ms, or perhaps the reagents used for this procedure contained slightly higher ambient platinum.

Subsequently these platinum levels did not significantly increase even after treatment with oxaliplatin. In interpreting these results, the first consideration was whether an error occurred during the DNA extraction or nitrification process, which affected the dissociation of platinum from DNA strands. If the DNA strands were not sufficiently digested by the addition of nitric acid, the platinum ions would remain bound to the DNA strands, preventing accurate detection by mass spectrometry. To investigate this further, our method of DNA preparation was compared to an alternative validated protocol for DNA extraction and digestion with nitric acid. No significant difference in platinum concentration was noted between these two methods. Therefore the low platinum readings recorded in this study were not due to our method of DNA preparation.

The second possible explanation involves the uptake of platinum into peripheral lymphocytes of patients. In order to fit in with patients' standard of care pathway, blood samples for lymphocyte extractions were taken just prior to the patients' next cycle of chemotherapy, therefore two weeks had elapsed since oxaliplatin was administered. Previous investigations into the pharmacokinetics of oxaliplatin has shown that removal of platinum adducts is rapid. In some cases, no platinum is detected at 24 hours post-treatment, and by 5 days after treatment few patients have any recordable levels[82]. Thus it is more likely that the two-week interval after treatment enabled the platinum to be cleared back to baseline levels before the next set of blood samples were obtained. As a result of this, there were no remarkable differences in platinum concentrations taken before or after treatment with oxaliplatin.

The final important question is whether the addition of curcumin to standard treatment did, in fact, impact oxaliplatin's efficacy in forming platinum adducts. Pre-clinical trials investigating the addition of curcumin to oxaliplatin showed no evidence of curcumin affecting DNA platination[185]. Likewise, in terms of overall clinical response, there is no evidence of a reduced response rate or progression-free survival in the trial patients compared to those expected with standard treatment alone. The phase I results obtained offer no means of clarifying this query. However, it does tentatively suggest that increasing doses of curcumin in combination with oxaliplatin do not contribute to off-target effects (i.e. altering ability of DNA-platinum adduct formation) that may exacerbate platinum-related toxicities. In phase IIa of this study, the platinum readings will be compared between patients who received curcumin versus those who had standard treatment only.

5.3. **Curcumin and curcuminoids**

5.3.1. Introduction

Curcumin is known to have poor bioavailability due to its poor absorption, rapid metabolism and efficient systemic elimination. Nonetheless, curcumin has exhibited therapeutic activity despite low serum concentrations. As a result, studies continue to aim to evaluate the dosage and plasma concentrations of curcumin required to elicit a pharmacological effect. Curcumin glucuronides and sulfates are the predominant metabolites of curcumin, reaching their peak concentrations one hour after curcumin administration. The activity of glucuronides and sulfates compared to curcumin is unclear. However, these metabolites have been shown to be less effective at inhibiting COX-2 expression, a known mechanism of action for curcumin [(149)].

In this trial the maximum target dose was designed to be the dose that would exert a pharmacological response, without increasing adverse effects and which would also maintain good patient compliance. Healthy volunteers have tolerated curcumin doses of up to 12 g per day with minimal side effects [(189)], however in combination with chemotherapy doses of greater than 4 g daily have been associated with side-effects (e.g. intractable abdominal pain and bloating) leading to dose reduction [(202)]. Excellent compliance rates (92%) have been recorded with doses of up to 2.35 mg daily taken as 5 daily capsules [(209)] but this study also showed that patients would be reluctant to take higher doses if this required a greater size or number of capsules. Considering that patients were required to take curcumin for the duration of their chemotherapy, which could last up to six months, and would also be contending with chemotherapy-induced side effects such as nausea and altered bowel habit, a target dose of 2 g daily was least likely to exacerbate toxicities and expected to optimise compliance. As the first phase I study in patients undergoing standard oxaliplatin-based chemotherapy treatment for metastatic colorectal cancer, dose escalation was required to ensure the safety and tolerability for these patients up to the target dose. Therefore, the aims for this part of the study were 3-fold: to assess participant's continued compliance; to analyse any potential association between curcumin dosage and plasma levels of curcumin/curcuminoids; to assess whether plasma levels had any association with patient outcomes.

5.3.2. Results

The curcumin, curcumin glucuronide and curcumin sulfate standards ranging from 40 ng/ML to 10000 ng/mL were plotted as per Figure 5.6 below.

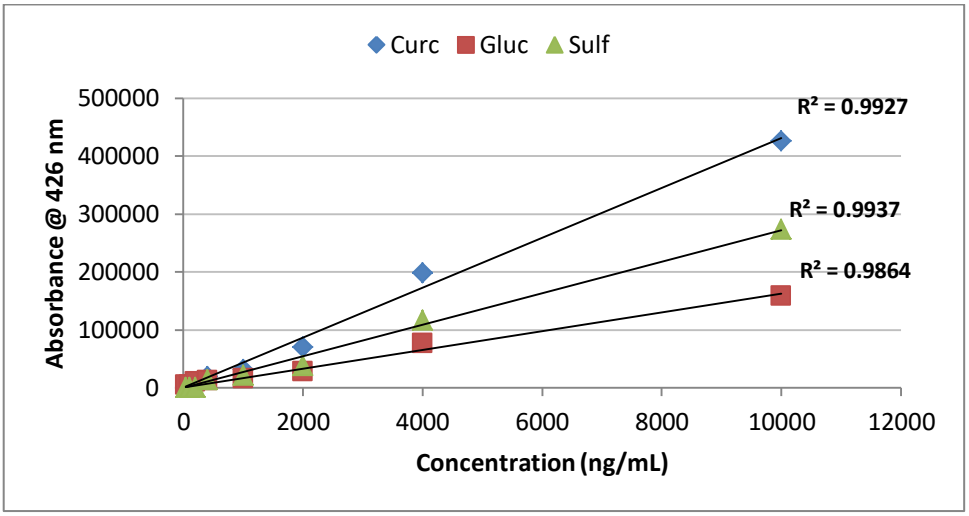


Figure 5.6 Standard curves for curcumin, curcumin glucuronide and curcumin sulfate. Curc – curcumin, Gluc – curcumin glucuronide, Sulf – curcumin sulfate (Calculated on MicroSoft Excel)

The retention time for curcumin was approximately 23.4 minutes, preceded by bisdemethoxycurcumin (bDMC) at 23.0 minutes and DMC at 23.2 minutes. Retention times for curcumin glucuronide and curcumin sulfate were at approximately 15.6 minutes and 19.4 minutes respectively (see Fig 5.7). The limit of detection (based on a signal to noise ratio of 1:3) was established as 25 ng/mL (0.005 – 0.007 nM) for curcumin, curcumin glucuronide, and for curcumin sulfate.

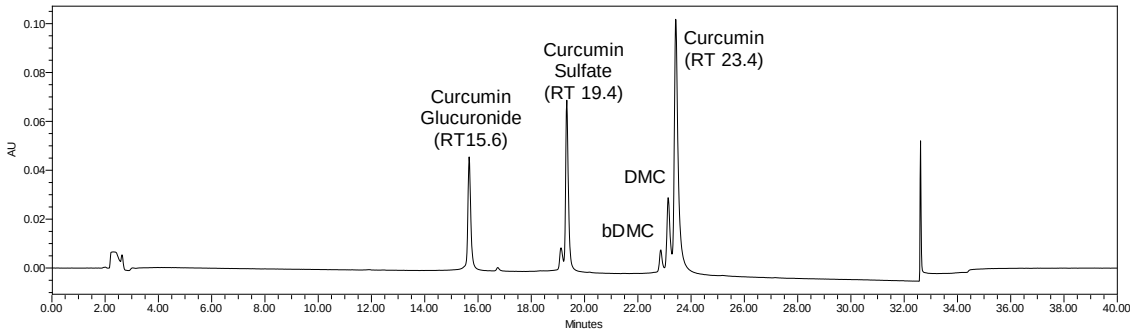


Figure 5.7 – Standards HPLC chromatogram showing retention times of 23.4 minutes for curcumin, 19.4 minutes for curcumin sulfate and 15.6 minutes for curcumin glucuronide following spiking of human plasma with 100 µg/mL of each and subsequent liquid phase extraction for plasma extraction of curcumin, bisdemethoxycurcumin (bDMC), demethoxycurcumin (DMC), curcumin glucuronide and curcumin sulfate. RT – retention time. AU = absorbance units.

In tier 1, the mean time interval between patients' taking their curcumin and having trial blood samples taken was 5 hours and 37 minutes. For patients in tiers 2 and 3, the time interval was 12 hours and 18 minutes, and 4 hours and 48 minutes respectively. The average number of days between the last curcumin dose and the end of trial blood samples were 17 days for tier 1, 12 days for tier 2 and 13 days for tier 3.

The curcumin concentrations measured from plasma samples for patients were converted from ng/mL to nanomoles. On initial analysis, the curcumin concentrations obtained from plasma samples ranged from 0.13 nM to 0.72 nM in Tier 1, 0.01 nM to 0.30 nM in Tier 2 and 0.11 nM to 0.31 nM in Tier 3. The lowest mean curcumin level was recorded at baseline (Curcumin concentration = 0.065 nM), while the highest was after the second cycle of chemotherapy (Curcumin concentration = 0.160 nM) (See Fig 5.8). No significant difference was demonstrated between the curcumin levels at different time points in any of the tiers (Tier 1: $p = 0.655$, Tier 2: $p = 0.995$, Tier 3: $p = 0.307$, All tiers: $p = 0.625$). Similarly, no significant difference was reported in any tier when comparing each of the time points to baseline (see Table 5.5 and Fig 5.8).

Table 5.5 Curcumin, curcumin glucuronide, and curcumin sulfate concentrations measured for patients in tiers 1, 2 and 3 at all time points. P-values represent comparison of treatment time point versus baseline. Curcumin – curcumin only, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – End of treatment.

	Curcumin (nM)			Curcumin glucuronide (nM)			Curcumin sulfate (nM)		
Time point	**Mean**	**Std Dev**	**P value**	**Mean**	**Std Dev**	**P value**	**Mean**	**Std Dev**	**P value**
TIER 1									
Baseline	0.116	0.201	-	0	0	-	-	-	-
Curcumin	0.271	0.228	0.429	0	0	-	-	-	-
Post #1	0.251	0.228	0.486	0.010	0.018	0.423	-	-	-
Post #2	0.412	0.361	0.299	0.005	0.008	0.423	-	-	-
End of Tx	0.175	0.159	0.710	0	0	-	-	-	-
TIER 2									
Baseline	0.067	0.117	-	0.005	0.009	-	-	-	-
Curcumin	0.078	0.128	0.925	0.006	0.010	0.968	-	-	-
Post #1	0.073	0.075	0.947	0.016	0.014	0.359	-	-	-
Post #2	0.103	0.178	0.789	0.006	0.010	0.968	-	-	-
End of Tx	0.062	0.107	0.954	0	0	0.423	-	-	-
TIER 3									
Baseline	0.039	0.028	-	0.237	0.293	-	0.238	0.429	-
Curcumin	0.034	0.038	0.808	0.276	0.281	0.819	0.125	0.194	0.577
Post #1	0.129	0.123	0.136	0.167	0.217	0.649	0.073	0.085	0.395
Post #2	0.062	0.092	0.577	0.115	0.155	0.394	0.054	0.076	0.347
End of Tx	0.087	0.102	0.351	0.063	0.072	0.212	0.056	0.061	0.349
ALL TIERS									
Baseline	0.065	0.106	-	0.120	0.232	-	0.238	0.429	-
Curcumin	0.104	0.153	0.480	0.139	0.237	0.840	0.125	0.194	0.577
Post #1	0.145	0.149	0.144	0.090	0.167	0.721	0.073	0.085	0.395
Post #2	0.160	0.238	0.228	0.060	0.119	0.438	0.054	0.076	0.347
End of Tx	0.104	0.117	0.410	0.029	0.056	0.211	0.056	0.061	0.349

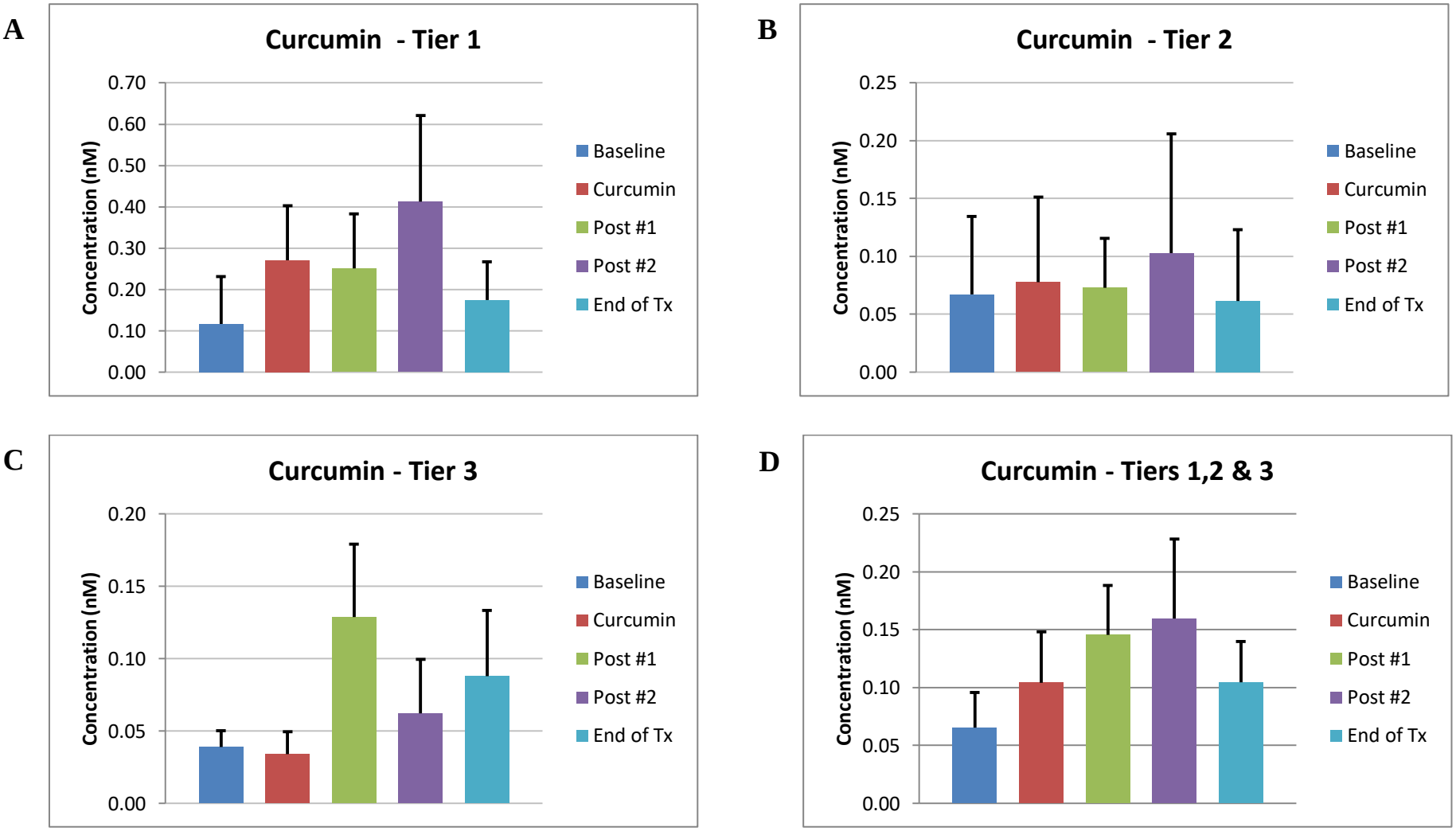


Figure 5.8 Mean plasma curcumin concentrations over treatment time points, following standard liquid phase extraction. Curcumin – After 1 week curcumin only, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – end of treatment. Error bars represent ± Standard error. (Calculated using Microsoft Excel).

Curcumin glucuronide was detected in 2 samples from the same patient (CUFOX1 003) in Tier 1 at levels of 0.03 nM and 0.14 nM post cycles 1 and 2 chemotherapy respectively. In tier 2, the curcumin glucuronide concentrations measured from 0.017 nM to 0.026 nM, whilst in tier 3 the range was from 0.013 nM to 0.719 nM. The lowest mean curcumin glucuronide levels were reported at the end of treatment (Curcumin glucuronide concentration = 0.029 nM), whilst the highest were seen after treatment with one week of curcumin only (Curcumin glucuronide concentration = 0.139 nM). Again, no significant difference was demonstrated between curcumin glucuronide levels at different time points (Tier 1: $p = 0.545$, Tier 2: $p = 0.454$, Tier 3: $p = 0.510$, All tiers: $p = 0.577$), nor when each time point was compared to baseline (See Table 5.5 and Fig 5.9).

Curcumin sulfate was not detected in any samples from tiers 1 and 2. However in tier 3, it was identified in samples from 4 of the 5 patients at concentrations of 0.009 nM to 1.09 nM. The lowest mean curcumin sulfate levels were recorded after cycle 2 of chemotherapy (Curcumin sulfate concentration = 0.054 nM), whilst the highest levels were demonstrated at baseline (Curcumin sulfate concentration = 0.238 nM). No significant difference was calculated between the curcumin sulfate concentrations at different time points ($p = 0.592$), nor when comparing each time point to baseline (See Table 5.5 and Fig 5.10).

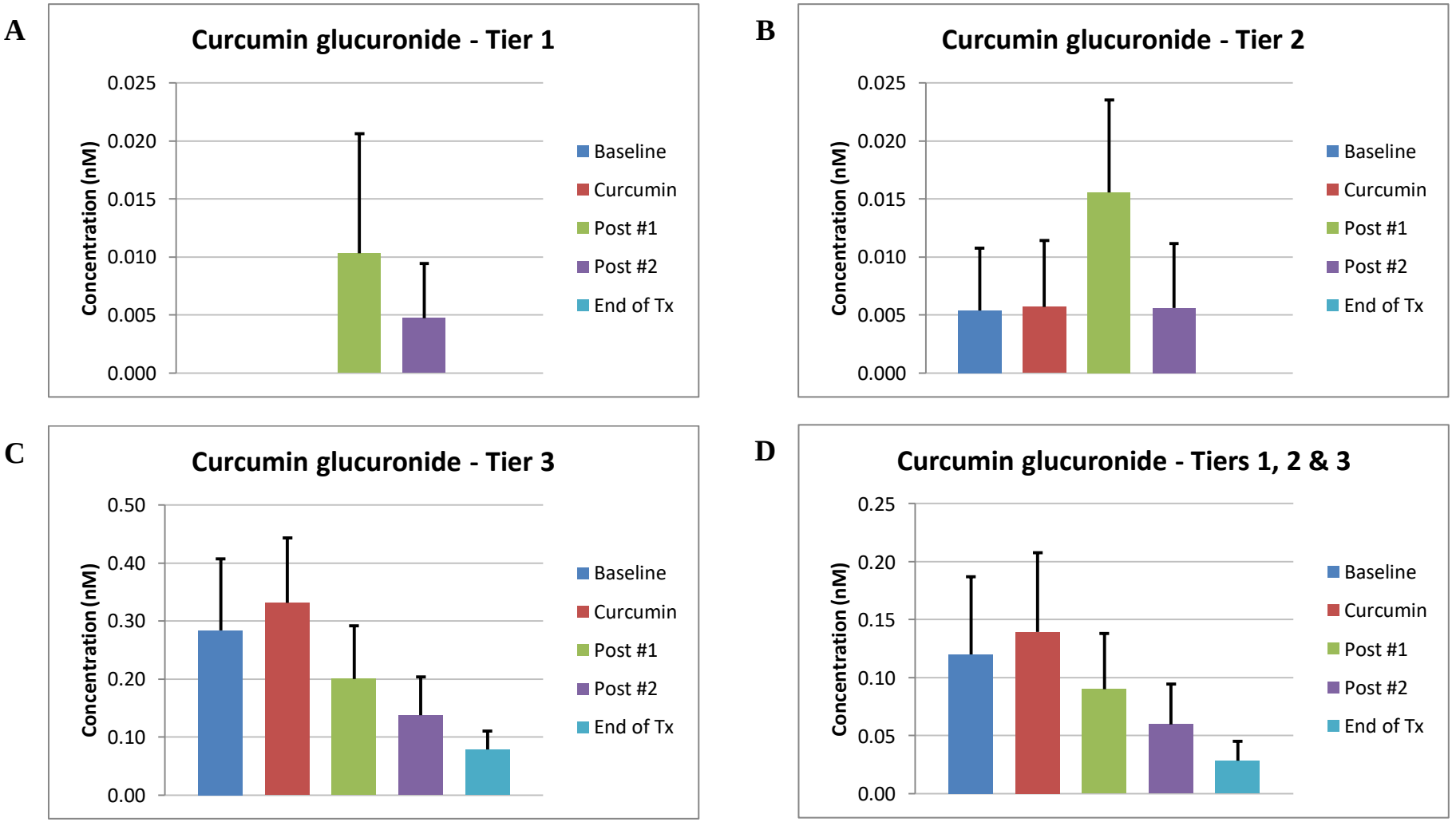


Figure 5.9 Mean plasma curcumin glucuronide concentrations over treatment time points, following standard liquid phase extraction. Curcumin – After 1 week curcumin only, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – end of treatment. Error bars represent ± Standard error. (Calculated using MicroSoft Excel).

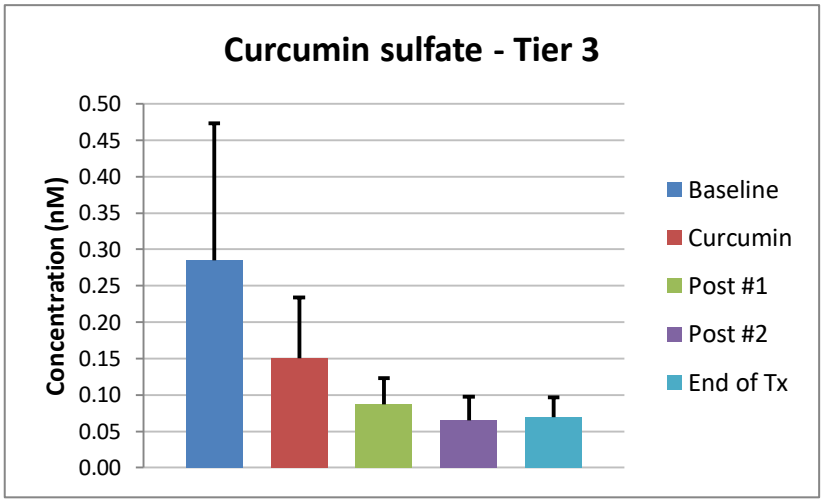


Figure 5.10 Mean curcumin sulfate concentrations over treatment time points for Tier 3, following standard liquid phase extraction. Curcumin – After 1 week curcumin only, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – end of treatment. Error bars represent ± Standard error. (Calculated using MicroSoft Excel)

Due to the unexpected appearance of curcuminoids in the baseline samples, the samples were re-extracted in order to assess the validity of this result. In this case, measurement of levels of curcuminoids in the native plasma was undertaken first, and followed by conversion of curcumin metabolites to parent curcuminoids. Levels of curcumin produced following this enzymatic conversion were also measured (See Fig 5.11). Results from this second measurement of curcumin/curcuminoids (credit J. Mahale) produced levels of curcumin and its metabolites below the level of detection from all native plasma samples. However, following enzymatic conversion back to parent curcuminoids, curcumin was detected in samples from four patients in Tier 3 (See Table 5.6 & Fig 5.12).

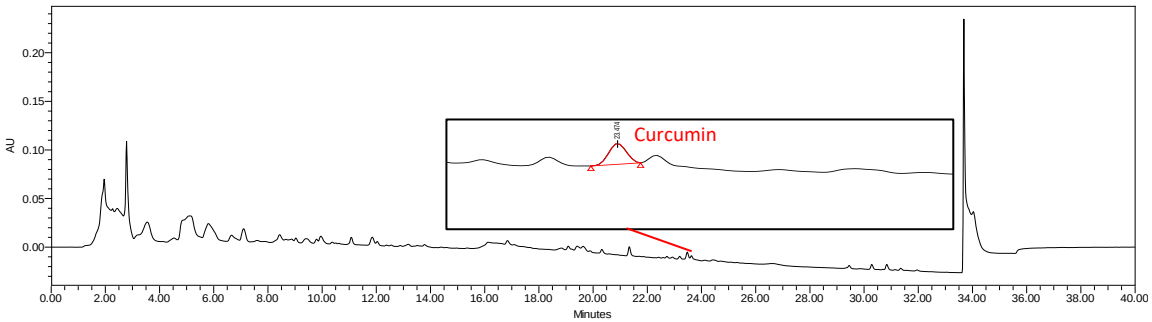


Figure 5.11 Representative chromatograph of enzymatically converted plasma showing curcumin peak

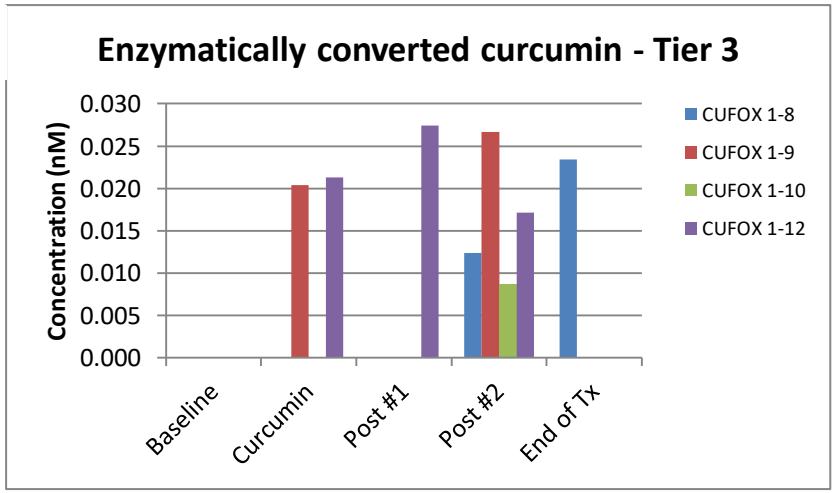
Following the re-evaluation of plasma, curcumin was identified most consistently in plasma samples taken after the second cycle of chemotherapy. Curcumin concentrations ranged from 0.009 nM to 0.027 nM. None of the samples taken at baseline had detectable levels of curcumin. The highest mean curcumin levels following enzymatic conversion were recorded after cycle 2 of chemotherapy (Curcumin concentration = 0.011 nM). This was the only value calculated as significantly greater than baseline curcumin levels ($p^{44} = 0.05$). No significant difference was seen between any of the other time points compared to baseline, nor when comparing concentrations across all the time points ($p = 0.403$) (See Fig 5.13).

Table 5.6 Curcumin concentrations from enzymatically converted plasma. Curcumin –curcumin only, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – End of treatment

Patient	Treatment	Concentration (nM)
CUFOX 1-8	Baseline	0
	Curcumin	0
	Post #1	0
	Post #2	0.012
	End of Tx	0.023
CUFOX 1-9	Baseline	0
	Curcumin	0.020
	Post #1	0
	Post #2	0.027
	End of Tx	0
CUFOX 1-10	Baseline	0
	Curcumin	0
	Post #1	0
	Post #2	0.009
	End of Tx	0
CUFOX 1-12	Baseline	0
	Curcumin	0.021
	Post #1	0.027
	Post #2	0.017
	End of Tx	0

[44] $P \leq 0.05$ = statistically significant

A



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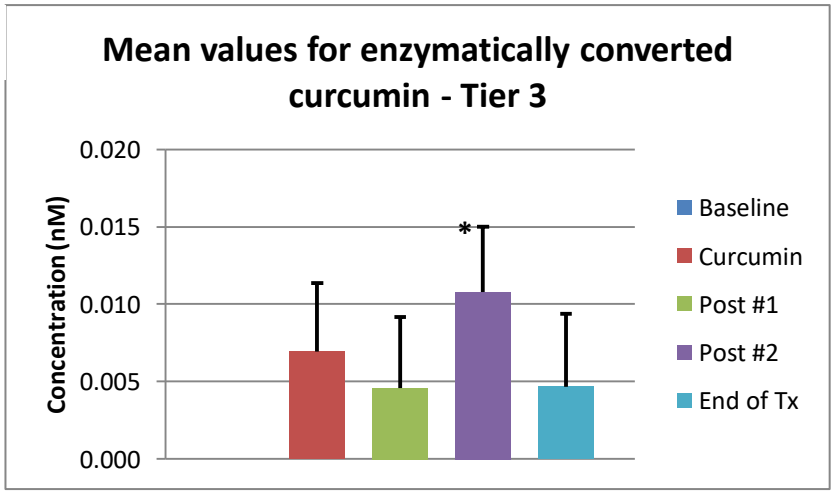

Figure 5.12 A - Concentrations of curcumin measured for patients in Tier 3 following enzymatic conversion. B - Mean curcumin concentrations and standard deviation from enzymatically converted plasma samples from tier 3 patients. Mean concentration +/- standard error. Curcumin – After 1 week curcumin only, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – end of treatment. Error bars represent ± Standard error. *Represents statistical significance ($p \leq 0.05$). (Calculated using GraphPad Prism unpaired t-test with Welch's correction).

The tier 3 plasma curcumin concentrations were compared to the concentrations obtained after enzymatic conversion. There was no significant correlation between these two values (r^{45} = 0.020, p = 0.487).

In order to assess the effects of curcumin on treatment response, the highest levels of curcumin and enzymatically converted curcumin were compare to the best treatment response for each patient (See Fig 5.13). No significant correlation was found between best treatment response and patient curcumin levels (R^2 = 0.0004, p = 0.969), nor between best treatment response and enzymatically converted curcumin levels (R^2 = 0.0447, p = 0.688).

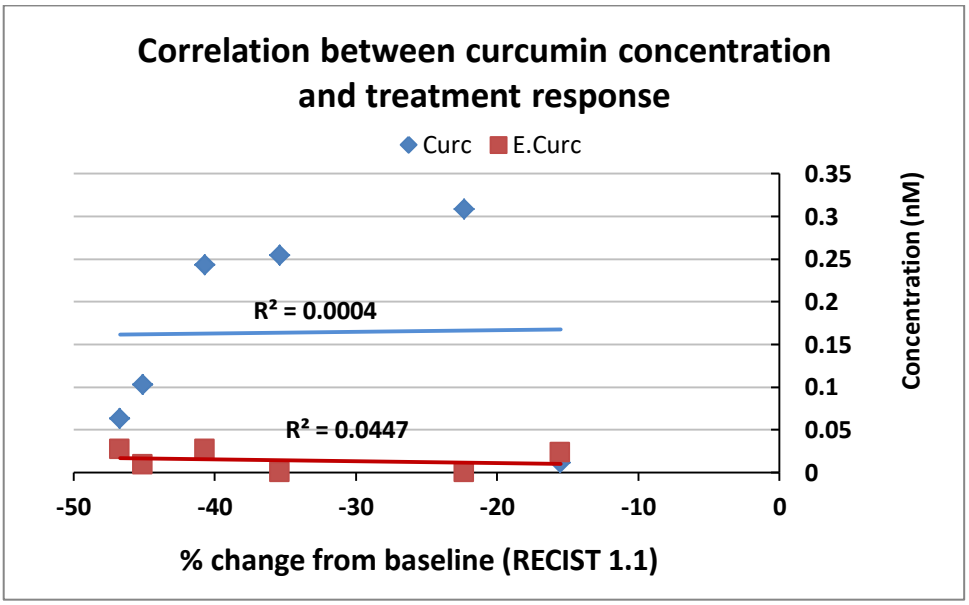

Figure 5.13 Scatter plot to illustrate correlation between best treatment responses (RECIST 1.1) versus highest curcumin level and highest enzymatically converted curcumin level. Best treatment response is measured as the percentage change in the sum of target lesions compared to baseline. Curc = Concentration of curcumin extracted from patient plasma. E.Curc = Concentration of enzymatically converted curcumin measured in patient plasma. R^2 = 0.0 means there is no correlation between the two values. R^2 = 1.0 means there is a strong correlation between the two values. R^2 calculated using Microsoft Excel.

[45] P ≤ 0.05 is statistically significant.

5.3.3. Discussion

Two measurements of curcumin/curcuminoids were carried out on patient plasma samples for all tiers. For the first analysis curcumin and its metabolites were extracted from patient plasma samples taken at the designated time points and the resultant concentration of curcumin/curcuminoids recorded. A previous clinical study with colorectal cancer patients where up to 2.2 g daily of curcumin were ingested over 29 days resulted in neither curcumin nor its metabolites being detected [(196)]. Doses of 4 g or 8 g daily, however, did produce serum levels of 0.51 µM and 1.77 µM respectively. Based on this the dosage of curcumin (0.5 g to 2 g) used in this study was expected to produce minimal or no detectable curcumin. However, curcumin, curcumin glucuronide and curcumin sulfate were detected in samples from across all tiers in this study. Although concentrations were all in the nanomolar range, our results showed recordable levels of curcumin at baseline (before curcumin dosing began) and in plasma from patients taking only 0.5 - 1 g of curcumin. Doses of 10 g and 12 g of curcumin have produced no detectable free curcumin at time points up to 72 hours post-dose in previous studies [(201)]. Therefore the levels of curcuminoids detected in our study samples raised the suspicion of sample contamination or operator error during sample processing. Another possibility would be that these patients, intentionally or accidentally, already had a greater than expected curcumin intake. Of note is the point that the two patients who demonstrated this (CUFOX1 009, CUFOX1 013) had both stated in their pre-trial questionnaires that they were already aware of the benefits of curcumin. However their questionnaires did not testify to a known regular health supplement or dietary intake of curcumin.

In order to clarify our initial results a second analysis was undertaken, measuring the levels of extracted curcuminoids, as well as levels of curcumin produced following enzymatic back-conversion. In this case the extracted curcumin and its metabolites were all below the limit of detection. Back-conversion produced detectable curcumin levels in some tier 3 samples (2 grams daily). Notably, none of the baseline samples (pre-curcumin dosing) produced detectable curcumin even after back-conversion. These results are more in keeping with expectations based on previous studies. It is therefore most likely that some low level contamination occurred during the first analysis of plasma samples, resulting in the unexpected levels of curcumin particularly at baseline and in tiers 1 and 2. Thus the method of enzymatic conversion to parent

curcumin could prove helpful as a means of verifying preliminary measurements of curcumin and its metabolites.

Plasma concentrations from oral curcumin dosing have been shown to peak at 1 to 2 hours after loading and trough at 12 hours [191]. The time intervals between patients taking the curcumin capsules and trial blood tests ranged from 1 hour 10 minutes to 23 hours 55 minutes, with tier 3 patients having the shortest mean time interval of 4 hours 48 minutes. The majority of patients' blood samples were taken after the peak of two hours but before the trough of 12 hours. Unfortunately, however, there was insufficient data to draw any conclusions regarding the impact of these time intervals on plasma curcuminoid levels. The symptom and capsule diaries recorded data for 4 weeks and therefore did not include the time of the curcumin dose prior to the fourth blood test (post-cycle 2 chemotherapy). The symptom diaries were not always accurately or fully completed. This was complicated further by hospital admissions, which disrupted both the diary-keeping and curcumin administration. Nonetheless, there is the overall reduction seen in curcuminoid levels in the end-of-treatment bloods that could reflect the fact that patients had generally stopped taking their curcumin capsules for at least 10 days before this blood test. In fact, with this time interval, the levels of curcumin detected in our patient samples are unexpected as curcumin should have been completely cleared. Nonetheless, curcumin and curcuminoids from end-of-treatment samples were consistently detected, both on initial analysis and subsequent enzymatic conversion, albeit at quantities bordering the level of detection. To optimise data in future studies, a greater emphasis should be placed on co-ordinating the time of patients' dosing with the time of trial blood taking. Ideally trial blood samples would be taken throughout the duration of the patients' treatment, which would provide more robust data for analysis.

Because the dosage required for curcumin to produce a pharmacological effect remains uncertain, it is difficult to assess how the levels measured in this study may have impacted upon patient outcomes. At 3.6 g daily, curcumin concentrations of 7.7 ± 1.8 nmol/g were measured in malignant colorectal tissue. Based on effects on M(1)G and COX-2 protein, this was deemed as having displayed pharmacological activity in the colorectum [192]. It is possible that despite the generally low levels of curcuminoids detected, these may have been sufficient to produce a pharmacological response, particularly in the tier 3 patients where these compounds were detected more

consistently. As a phase I study, there were a relatively small number of samples analysed and compared. To this end it is not surprising that it was difficult to reach statistically significant outcomes. Similarly, no significant correlations were established between curcumin levels and treatment response. This could be further assessed in the phase II stage of this study where patients' response to standard chemotherapy alone versus chemotherapy plus curcumin, can be evaluated alongside the levels of curcuminoids detected in patient plasma.

In summary, the results from analysis of levels of curcumin and its metabolites corroborates the association between curcumin dosage and subsequent plasma levels of curcumin and its metabolites. This can therefore be useful as a biomarker for patient compliance at these higher doses. This will be investigated further with samples from phase IIa of this study.

6. Proteomics

6.1. **Introduction**

Predicting the efficacy of cancer therapies in the clinical setting remains a significant hurdle in drug development. Identifying biomarkers of efficacy is key to overcoming this hurdle. Potential methods for characterising biomarkers include genomic, metobonomic and transcriptomic analysis. However, proteomic analysis has produced most of the biomarkers currently in use, particularly because a large proportion of current drug targets are proteins[303]. As well as discovering these proteins, it is essential that these biomarkers translate from preclinical models to clinical outcomes.

In the absence of target tissues for biomarker studies, proteomic analysis was conducted on explant media samples from patient-derived colorectal liver metastases treated with curcumin, oxaliplatin+5-FU chemotherapy and a combination of oxaliplatin+5-FU+curcumin (CUFOX). Mass spectrometry and proteomic analysis were used to evaluate the proteins expressed following these treatments. These proteins were mapped onto their functional biological pathways so comparisons could be made between the pathways and expression of their associated protein constituents, with standard treatment in the presence and absence of curcumin. Ultimately the aim was to identify biomarkers particularly associated with triple therapy (i.e. curcumin + oxaliplatin + 5-FU), therefore analysis was focussed on the biological pathways and proteins associated with this treatment group. Outcomes from these analyses could indicate which proteins are key players in curcumin's mechanism of action, and thus could potentially serve as biomarkers of efficacy and compliance.

6.2. **Results**

6.2.1. Proteins and peptides

Explant media from 6 different tissue samples was obtained. Each tissue sample was divided into 4 treatment groups of: DMSO (control), 5 µM curcumin, 2 µM oxaliplatin + 5 µM 5-FU, or 5 µM curcumin + 2 µM oxaliplatin + 5 µM 5-FU. A total of 34719 peptides and 2891 proteins were identified across all samples following HPLC mass spectrometry analysis (See Methods Section 2.2.8). Over 2000 proteins (2002) were altered following treatment with curcumin, oxaliplatin + 5-FU or curcumin + oxaliplatin

+ 5-FU and 1670 of these proteins were only identified in the treatment group (see Table 6.1, Fig 6.1).

Table 6.1 Proteins and peptides identified by HPLC in explant media samples (N = 6). DMSO = control; Ox + 5-FU = treated with oxaliplatin and 5-fluorouracil; Curcumin = treated with curcumin only; CUFOX = treated with curcumin, oxaliplatin and 5-fluorouracil. Unique proteins = proteins identified exclusively in that treatment group; Total proteins = proteins identified in that group +/- overlapping with other treatments.

	DMSO	Ox + 5-FU	Curcumin	CUFOX
Peptides	10920	14087	16703	14392
Unique proteins	475	428	538	498
Total proteins	1221	1230	1377	1316

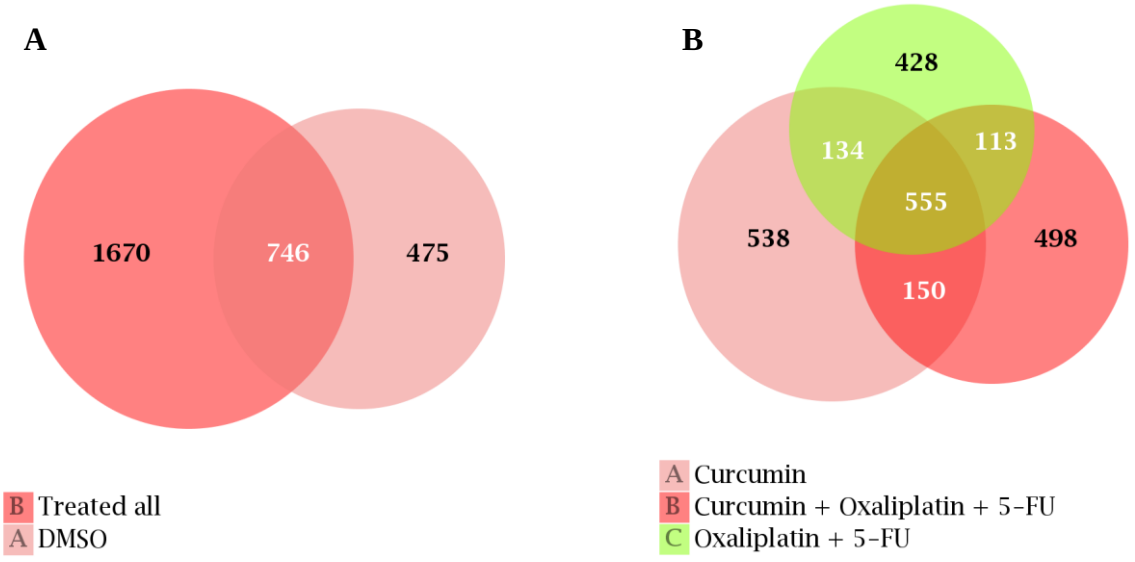

Figure 6.1 A - Venn diagram showing number of proteins significantly altered in DMSO control (A) versus curcumin, oxaliplatin+5-FU, and curcumin+oxaliplatin+5-FU groups combined (B). B – Venn diagrams showing number of proteins significantly altered following treatment with curcumin (A), curcumin + oxaliplatin + 5-FU (B), and oxaliplatin + 5-FU (C). Diagrams generated using ProteinCentre Professional Edition, Software Version 3.14.10006, from analysis of explant media samples (N=6).

Combination treatment with curcumin+oxaliplatin+5-FU produced significant alterations in 226 proteins compared to DMSO. Of these proteins, 218 (96.5%) were increased whilst 8 (3.5%) proteins were decreased compared to DMSO. Treatment with curcumin alone altered a greater number of proteins - 524 proteins compared to control. From this total, 470 (89.7%) were upregulated following treatment, whereas 54 (10.3%) were downregulated. Conversely, with FOLFOX treatment only 98 proteins were

significantly changed, with only 7 proteins (7.1%) upregulated, whilst 91 proteins (92.9%) were significantly higher in the control group (See Fig 6.2).

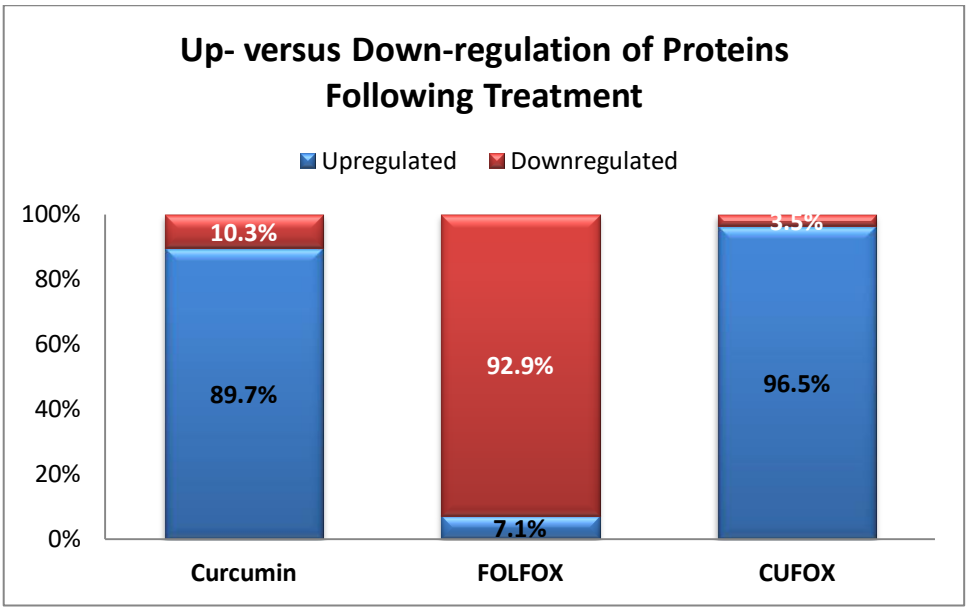

Figure 6.2 – Comparison of the percentage of proteins upregulated or downregulated with curcumin, oxaliplatin+5-FU or curcumin+oxaliplatin+5-FU compared to control (DMSO). Percentages represent the proportion of the total number of proteins altered by that treatment.

6.2.2. Curcumin + Oxaliplatin + 5-FU (CUFOX)

In seeking to isolate potential biomarkers for triple treatment, the list of proteins was further refined to identify proteins that were significantly altered following treatment with curcumin+oxaliplatin+5-FU, but were not significantly altered in the other treatment groups (ANOVA, $p < 0.05$). Based on this criteria, 51 proteins were found to be significantly upregulated following treatment with CUFOX compared to control, whilst 2 proteins were significantly downregulated (See Tables 6.2 and 6.3). The proteins significantly altered following CUFOX treatment (curcumin + oxaliplatin + 5-FU) are distributed throughout the cell complex, however they are most frequently located in the cytoplasm (48.9%), membrane (42.8%) and nucleus (38.7%) of cells.

Detailed pathway analysis was not possible for this relatively small number of proteins, nonetheless the function of these proteins and their involvement in biological pathways were investigated (See Tables 6.2 and 6.3).

Table 6.2 Proteins significantly and uniquely upregulated following treatment with curcumin+oxaliplatin+5-FU (CUFOX) compared to DMSO ($p < 0.05$).

Protein	Function	Pathways	Ref
4-aminobutyrate aminotransferase, mitochondrial	Insulin secretion	Insulin pathway	(304)
Acidic leucine-rich nuclear phosphoprotein 32 family member A	Implicated in a number of cellular processes, including proliferation, differentiation, caspase-dependent and caspase-independent apoptosis, suppression of transformation	Apoptosis	(305-308)
Alpha-2-HS-glycoprotein	Promotes endocytosis, possesses opsonic properties and influences the mineral phase of bone	Phosphorylation, insulin receptor signalling, inflammation	(309)
Alpha-protein kinase 2	Kinase that recognizes phosphorylation sites in which the surrounding peptides have an alpha-helical conformation	Phosphorylation	(310)
Adaptor protein complex 2 (AP-2) complex subunit beta	Component of the adaptor protein complex 2 (AP-2). Regulates protein transport and involved in clathrin-dependent endocytosis.	EGFR regulation, protein transport, endocytosis, apoptosis	(311-313)
Bile salt-activated lipase	Catalyses fat and vitamin absorption.	Lipid metabolism	(314)
Calcium-binding protein 1	Modulates calcium-dependent activity of inositol 1,4,5-triphosphate receptors (ITPRs). Inhibits agonist-induced intracellular calcium signalling.	Calcium signalling.	
Chromobox protein homolog 3	Seems to be involved in transcriptional silencing in heterochromatin-like complexes.	Negative regulation of transcription	(315)
Cleavage stimulation factor subunit 3	One of the multiple factors required for polyadenylation and 3'-end cleavage of mammalian pre-mRNAs.		(316)
Cytochrome P450 4F12	Catalyses leukotriene B4 omega-hydroxylation and arachidonic acid omega-hydroxylation but with an activity much lower than that of CYP4F2.	Drug metabolism	(317,318)
Dipeptidyl peptidase 3	Cleaves and degrades the opioid peptide encephalin	Inflammation, oxidative stress, protein catabolism	(319)
DNA-directed RNA polymerase III subunit RPC4	DNA-dependent RNA polymerase catalyses the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates	Positive regulation of immune response	(320)

Protein	Function	Pathways	Ref
Dynein heavy chain 17, axonemal	Force generating protein of respiratory cilia.	Microtubule based movement, migration	(321)
Dystonin	Cytoskeletal linker protein. Acts as an integrator of intermediate filaments, actin and microtubule cytoskeleton networks.	Cell adhesion, cell cycle arrest, migration	(322)
Elongation factor 1-beta	EF-1-beta and EF-1-delta stimulate the exchange of GDP bound to EF-1-alpha to GTP	Protein biosynthesis	(323)
Glucose 1,6-bisphosphate synthase	Glucose 1,6-bisphosphate synthesis	Glucose metabolism	(324,325)
Heat shock protein 105 kDa	Inhibits HSPA8/HSC70 ATPase and chaperone activities, positive regulation of MHC class I biosynthetic process, positive regulation of NK T cell activation	Overexpressed in cancer, apoptosis	(326)
Hypoxanthine-guanine phosphoribosyltransferase	Converts guanine to guanosine monophosphate, and hypoxanthine to inosine monophosphate.	Purine biosynthesis	(327)
Ig kappa chain V-III region GOL	Antigen binding	Immune response, endocytosis	(328)
Inter-alpha-trypsin inhibitor heavy chain H2	Endopeptidase inhibitor activity, involved in extracellular matrix stabilization and in prevention of tumour metastasis.	Regulation of endopeptidase activity	(329)
Isoform 1 of Four and a half LIM domains protein 1	Ion channel binding	Involved in development of myopathy	(330)
Isoform 2 of Centrosomal protein of 164 kDa	Plays a role in microtubule organization	Microtubule organization, migration, apoptosis	(331-333)
Isoform 2 of Nucleoside diphosphate kinase A	Major role in the synthesis of nucleoside triphosphates other than ATP, Involved in cell proliferation, differentiation and development, signal transduction, G protein-coupled receptor endocytosis, and gene expression	Cell proliferation, signal transduction, G protein-coupled receptor endocytosis	(334-336)
Isoform 2 of Protein SOGA1	Regulates autophagy by playing a role in the reduction of glucose production	Regulation of gluconeogenesis, apoptosis	(337)
Isoform 2 of SH2 domain-containing protein 3C	Eph receptor-binding protein which may be a positive regulator of TCR signalling	EGF-dependent cell migration	(338)
Isoform 3 of Calumenin	Involved in regulation of vitamin K-dependent carboxylation of multiple N-terminal glutamate residues	Platelet degranulation	(339)

Protein	Function	Pathways	Ref
Isoform 3 of Heterogeneous nuclear ribonucleoprotein D0	Acts as a transcriptional regulator. Promotes transcription repression.	Regulation of transcription	(340-342)
Isoform 3 of Kinesin-like protein KIF1B	Motor for anterograde transport of mitochondria	Apoptosis, migration, intracellular transport	(343)
Isoform 3 of Plasminogen activator inhibitor 1 RNA-binding protein	May play a role in the regulation of mRNA stability	Apoptosis	(344)
Isoform 4 of Ankyrin repeat and KH domain-containing protein 1	May play a role as a scaffolding protein	Anti-apoptosis	(345)
Isoform A1 of CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase	Involved in protein glycosylation	Involved in mental retardation and encephalopathy	(346)
Keratin, type II cytoskeletal 7	Blocks interferon-dependent interphase and stimulates DNA synthesis in cells	DNA synthesis	(347)
Killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1)	Regulation of cell death via immune response, natural killer cell mediated cytotoxicity, signal transduction. Inhibits the activity of NK cells thus preventing cell lysis. Involved in HIF-1 (hypoxia inducible factor 1) pathway	Apoptosis, immune response	(348)
Metalloproteinase inhibitor 1	Metalloproteinase inhibitor	Migration, angiogenesis	(349-351)
Nesprin-2	Forms network between organelles and the actin cytoskeleton to maintain the subcellular spatial organization. Facilitates centrosome migration during early ciliogenesis.	Migration	(352-354)
Neuropilin and tolloid-like protein 2	Accessory subunit of neuronal kainate-sensitive glutamate receptors	Regulates glutamate receptor activity	(355,356)
Neutral alpha-glucosidase AB	Oligosaccharide precursor of immature glycoproteins	Post translational protein modification	(357-359)
Neutrophil gelatinase-associated lipocalin	Iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity and renal development.	Apoptosis	(360)

Protein	Function	Pathways	Ref
Peroxiredoxin-4	Probably involved in redox regulation of the cell. Regulates the activation of NF-kappa-B in the cytosol by a modulation of I-kappa-B-alpha phosphorylation.	Transcription regulation, oxidation, apoptosis	(361)
Pikachurin	Promotes matrix assembly and cell adhesiveness	Migration	(362)
Proliferation-associated protein 2G4	May play a role in a ERBB3-regulated signal transduction pathway. Implicated in growth inhibition and the induction of differentiation of human cancer cells	Proliferation, negative regulator of apoptosis, cell cycle	(363,364)
Putative heat shock protein HSP 90-beta-3	Putative molecular chaperone, may promote maturation, structural maintenance and regulation of specific target proteins	Stress response, protein regulation	(365)
Putative inactive carboxylesterase 4	Carboxylesterase hydrolase activity	Regulation of carboxylesterase	(366)
Sentrin-specific protease 7	Deconjugates SUMO2 and SUMO3 from targeted proteins	SUMO protein metabolism, Wnt signalling pathway,	(367)
Short transient receptor potential channel 1	Thought to form a receptor-activated non-selective calcium permeant cation channel. Involved in JNK activation via its interaction with TRAF2.	Calcium transport and metabolism;	(368)
Speedy protein E3	Regulation of protein kinase activity	Protein kinase regulation	(369)
T-complex protein 1 subunit eta	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis.	Protein folding, toxin transport	(370)
Transforming growth factor-beta-induced protein ig-h3	Binds to type I, II, and IV collagens. This adhesion protein may play an important role in cell-collagen interactions.	Angiogenesis, cell adhesion, proliferation, apoptosis	(371-373)
Type II inositol 3,4-bisphosphate 4-phosphatase	Catalyses the hydrolysis phosphatidylinositol 3,4-bisphosphate, inositol 1,3,4-trisphosphate and inositol 1,4-bisphosphate.	Protein kinase B regulation	(374)
Tyrosine-protein kinase Tec	Involved in human growth factor-induced ERK signalling pathway. Regulatory role in development, function and differentiation of T-cells.	Immune regulation, tyrosine kinase signalling pathway, protein phosphorylation	(375-377)
Ubiquitin carboxyl-terminal hydrolase 48	Involved in the processing of poly-ubiquitin precursors. Interacts with RELA and TRAF2 to regulate NF-kappa-B activation by TNF receptor superfamily.	Transcription regulation, immune response, apoptosis	(378)

Table 6.3 Proteins significantly and uniquely downregulated following treatment with curcumin+oxaliplatin+5-FU (CUFOX) compared to DMSO (control) ($p < 0.05$).

Protein	Function	Pathways	Ref
Isoform 3 of PIH1 domain-containing protein 1	Involved in the assembly of C/D box small nucleolar ribonucleoprotein (snoRNP) particles. Inhibits apoptosis.	Apoptosis, epithelial cell differentiation	(379,380)
Probable G-protein coupled receptor 150	G protein coupled receptor activity	G protein signalling pathway	(381)

The proteins that are altered in the CUFOX treatment group affect pathways which are associated with the regulation of apoptosis (15 proteins - 29.4%), migration (8 proteins - 15.7%) and inflammation/immune responses (7 proteins – 13.7%) (See Fig 6.3 and Tables 6.4, 6.5 and 6.6). Pathways involving changes to ≤ 2 proteins are not named in the figure below, but include signal transduction, insulin metabolism, transport, calcium transport and metabolism, oxidation, cell cycle, glucose metabolism, stress response, cell adhesion and endocytosis.

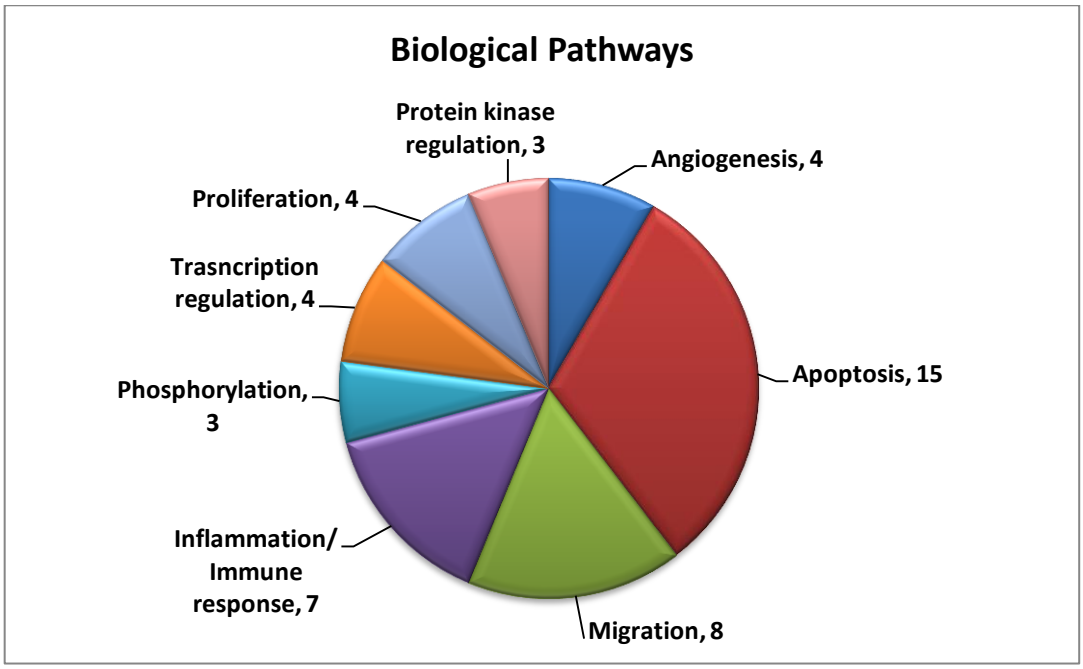

Figure 6.3 – Biological pathways involving proteins upregulated exclusively following treatment with curcumin+oxaliplatin+5-FU. Numbers represent number of proteins involved in each pathway. Pathways involving ≤2 upregulated proteins are not included.

Table 6.4 Proteins upregulated exclusively in CUFOX: roles in apoptosis and cancer.

Protein	Involvement in Apoptosis/Cancer	Ref
DNA-directed RNA polymerase III subunit RPC4	Regulates apoptosis via induction of NF-kappa-B.	(320)
Heat shock protein 105 kDa	Inhibits apoptosis via suppression of p38 MAPK signalling pathway	(382)
Isoform 2 of centrosomal protein of 164 kDa (CEP164)	Critical role in regulating G2/M checkpoint and nuclear divisions.	(332)
Isoform 2 of Protein SOGA1	Regulates insulin-receptor signalling pathway, important in cell death and proliferation. Possible stimulation of apoptosis via inhibition of PI3K.	(337,383)
Isoform 3 of Kinesin-like protein KIF1B	Induces neuronal apoptosis. Loss-of-function results in neuroblastomas, pheochromocytomas and medulloblastoma. Downregulation of KIF1B correlates with poor prognosis in hepatocellular carcinoma. Acts as tumour suppressor.	(343,384)
Isoform 3 of Plasminogen activator inhibitor 1 RNA-binding protein (PAI)	Conflicting data for role in apoptosis: both pro- and anti-apoptotic effects. Binds to caspases to mediate cellular apoptosis. Inhibits apoptosis in prostate cancer cell lines. Also shown to induce apoptosis and inhibit tumour cell proliferation in prostate cancer cell lines by stimulating endothelial apoptosis in tumour vasculature.	(385,386)
Isoform 4 of Ankyrin repeat and KH domain-containing protein 1	Overexpressed in and promotes growth of acute lymphoblastic leukemia. Involved in anti-apoptotic activity via regulation of caspases.	(345,387)
Killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1)	Generates an inhibitory signal on natural killer cells and prevents cell lysis. Presence of KIR 3DL1 associated with recurrent diseases in mCRC.	(388,389)
Metalloproteinase inhibitor 1	Functions as a growth factor mediating cell differentiation, migration and cell death and activates cellular signalling cascades via CD63 and β1 integrin (ITGB1). Inhibits apoptosis, promotes tumour invasion and metastases.	(350,390-392)

Protein	Involvement in Apoptosis/Cancer	Ref
Neutrophil gelatinase-associated lipocalin (NGAL)	Involved in apoptosis due to interleukin-3 (IL3) deprivation: iron-free form induces expression of the proapoptotic protein BCL2L11/BIM, promoting apoptosis. Over-expression related to higher rates of colorectal metastases. Inhibits metastases in CRC cell lines; inhibits invasion, metastases and angiogenesis in pancreatic cancer.	(360,393-395)
Peroxiredoxin-4	Involved in apoptosis via regulation of NF-kappa-B activation via modulation of I-kappa-B-alpha phosphorylation. Over-expressed in CRC, associated with reduced survival and may play a role in cell proliferation, infiltration and lymph node metastasis of CRC. Induces proliferation in breast cancer cell lines. Contributes to survival of lung carcinoma cells *in vivo* and associated with high-grade squamous cell lung carcinoma.	(361,396-398)
Proliferation-associated protein 2G4	2 isoforms, p48 and p42, which differentially mediate cell survival and differentiation. p48 suppresses apoptosis via MAPK cascade and AKT activity. p42 promotes cell differentiation. Overexpressed in colorectal cancer and cancer stem cell side populations. Suppresses proliferation and induces differentiation of ERBB3-positive breast, and bladder cancer cell lines. Inhibits growth and metastasis of salivary adenoid cystic carcinoma cells.	(363,399-401)
Transforming growth factor-beta-induced protein ig-h3	Associated with high-grade human colon cancers. Over-expression increased metastatic potential and poor prognosis in colorectal cancer. Promotes cell survival and proliferation by activating the FAK/AKT/AKT1S1/PRS6/EIF4EBP pathway.	(402,403)
Type II inositol 3,4-bisphosphate 4-phosphatase	Involved in regulation of PI3K apoptosis pathway.	(404)
Ubiquitin carboxyl-terminal hydrolase 48	Inhibits cell proliferation, causes cell cycle arrest in G2/M phase and promotes apoptosis via the intrinsic caspase-dependent pathway. Stabilizes p53 through the ubiquitination pathway in CRC, HCC and other digestive cancers.	(405)

Table 6.5 Proteins upregulated exclusively in CUFOX: roles in migration and cancer.

Protein	Involvement in Migration/Cancer	Ref
AP-2 complex subunit beta isoform b	Down-regulation of AP-2 causes enhanced tumour growth and inhibits chemotherapy-induced apoptosis, migration and invasion. Promotes clathrin lattice assembly and endocytosis.	(311,312,406)
Dynein heavy chain 17, axonemal	ATPase activity and involved in microtubule-dependent transport processes. Role in interkinetic nuclear migration in neuronal cells	(321,407)
Dystonin	Regulates stability of microtubule network.	(408)
Isoform 3 of Kinesin-like protein KIF1B	Involved in transporting membranous organelles, protein complexes and mRNAs in a mictotubule- and ATP-dependent manner. Transports tumour suppressor proteins.	(409-411)
Metalloproteinase inhibitor 1	Regulate cell migration, tumour invasion and metastases. Identified as potential biomarker in lung adenocarcinoma.	(349)
Nesprin-2	Belongs to family of nuclear envelope proteins stimulated in response to DNA damage; suggested tumour suppressor activity. Regulates endothelial cell shape and migration. Mediates centrosome migration and is essential for ciliogenesis via remodelling of actin cytoskeleton.	(352,412,413)
Neutrophil gelatinase-associated lipocalin precursor	Decreased expression in mCRC. Suppresses CRC cell invasion and inhibits liver metastases. Inhibits adhesion/invasion by suppressing FAK activation in pancreatic cancer cells.	(394,395)
Nucleoside diphosphate kinase A isoform a	Regulates c-myc expression – c-myc inhibition reduces cell migration, invasion and proliferation. Inhibits migration in gastric lymphoma, hepatocellular carcinoma, and oral squamous cell carcinoma. Inhibits metastases in cancer e.g. lung cancer. Role in CRC prognosis is equivocal.	(414-418)

Table 6.6 Proteins upregulated exclusively in CUFOX: roles in immune response and cancer.

Protein	Involvement in Immune Response/Cancer	Ref
Alpha-2-HS-glycoprotein	Acts as negative acute phase reactant. AHSG deficiency is associated with inflammation.	(419,420)
Dipeptidyl peptidase 3	Regulates encephalin and inflammatory mechanisms in peripheral tissues. Increased activity associated with endometrial, ovarian and squamous cell lung cancers.	(421-423)
DNA-directed RNA polymerase III subunit RPC4	Stimulates type I interferons through the RIG-I pathway.	(320,424)
Ig kappa chain V-III	Immune marker predicting metastasis-free survival and response to chemotherapy in breast, lung and colon cancer.	(425)
Killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1)	Regulates the activation thresholds of NK cells and some T cells; KIR blockade enhances tumour cell killing	(426)
Neutrophil gelatinase-associated lipocalin precursor	Involved in innate immunity. Acute phase protein, chronic inflammation and cancer cause increase in circulating NGAL levels. Potential biomarker in ovarian ca.	(360,427,428)
Tyrosine-protein kinase Tec	Key role in T-cell signalling and activation. Regulate NKT cell maturation, cytokine production, and survival	(429,430)

Of the 15 upregulated proteins involved with apoptosis, 3 are known to have pro-apoptotic activity (isoform 2 of protein SOGA1, isoform 3 of kinesin-like protein KIF1B, and ubiquitin carboxyl-terminal hydrolase 48), whilst 7 other proteins appear to inhibit apoptosis (heat shock protein 105 kDa, isoform 4 of Ankyrin repeat and KH domain-containing protein 1, killer cell immunoglobulin-like receptor 3DL1, metalloproteinase inhibitor 1, peroxiredoxin-4, proliferation-associated protein 2G4 and transforming growth factor-beta-induced protein ig-h3). Although the remaining proteins are known to contribute to apoptotic activity, their roles are unclear either due to limited information, or data showing they may have both pro- and anti-apoptotic functions (See Table 6.4).

The group of proteins involved in migration included 5 proteins (dynein heavy chain 17, isoform 3 of kinesin-like protein KIF1B, neutrophil gelatinase-associated lipocalin, dystonin and AP-2 complex subunit beta isoform b) that stimulated migration and 3

proteins that inhibited migration (metalloproteinase inhibitor 1, nesprin-2 and nucleoside diphosphate kinase A isoform a) (See Table 6.5).

The 7 upregulated proteins with known roles in immune response were alpha-2-HS-glycoprotein, dipeptidyl peptidase 3, DNA-directed RNA polymerase III subunit RPC4, Ig kappa chain V-III, killer cell immunoglobuline-like receptor 3DL1, neutrophil geltainase-associated lipocalin precursor and tyrosine-protein kinase Tec (See Table 6.6).

6.2.3. Pathway analysis

A larger protein group was required in order to conduct more detailed analysis of the biological pathways involved in the mechanism of action of CUFOX. For this assessment, the total number of proteins significantly altered following treatment with CUFOX was analysed (DAVID and KEGG pathway analysis), including proteins not exclusively altered by CUFOX.

Functional analysis identified 23 pathways with the proteins upregulated following treatment with CUFOX, compared with 38 pathways with curcumin and 6 with FOLFOX (See Table 6.7). The pathways unique to treatment with CUFOX included the adherens junction, MAPK (mitogen-activated protein kinase) signalling, VEGF (vascular endothelial growth factor) signalling, purine metabolism and dilated cardiomyopathy pathways (See Figs 6.4, 6.5, & 6.6). The oocyte meiosis pathway was altered following treatment with curcumin and oxaliplatin+5-FU, but not CUFOX (See Fig 6.7).

Table 6.7 Comparison of pathways associated with proteins upregulated following treatment (DAVID/KEGG pathway analysis). Pathway analysis of proteins with significant change ($p<0.05$) compared to DMSO control following triplicate analysis of individual explant media samples (N = 6). Explant media samples treated with: CUFOX – curcumin+oxaliplatin+5-FU; FOLFOX – oxaliplatin+5-FU; Curcumin. Blue highlight – pathways upregulated following all 3 treatments; Pink highlight – pathways upregulated with CUFOX and Curcumin; Green highlight – pathways upregulated with Curcumin and FOLFOX. Yellow highlight – pathways upregulated with CUFOX only.

CUFOX Pathways	Curcumin Pathways	FOLFOX Pathways
Focal adhesion	Focal adhesion	Focal adhesion
Insulin signalling pathway	Insulin signalling pathway	Insulin signalling pathway
Regulation of actin cytoskeleton	Regulation of actin cytoskeleton	Regulation of actin cytoskeleton
Antigen processing & presentation	Antigen processing & presentation	Oocyte meiosis
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	
Complement & coagulation cascades	Complement & coagulation cascades	
Gap junction	Gap junction	
Glutathione metabolism	Glutathione metabolism	
Glycolysis / Gluconeogenesis	Glycolysis / Gluconeogenesis	
Hypertrophic cardiomyopathy (HCM)	Hypertrophic cardiomyopathy (HCM)	
Leukocyte transendothelial migration	Leukocyte transendothelial migration	
Pathogenic Escherichia coli infection	Pathogenic Escherichia coli infection	
Pentose phosphate pathway	Pentose phosphate pathway	
Ribosome	Ribosome	
Spliceosome	Spliceosome	
Systemic lupus erythematosus	Systemic lupus erythematosus	
Tight junction	Tight junction	
Viral myocarditis	Viral myocarditis	
Adherens junction	Oocyte meiosis	
Dilated cardiomyopathy		
MAPK signalling pathway		
Purine metabolism		
VEGF signalling pathway		

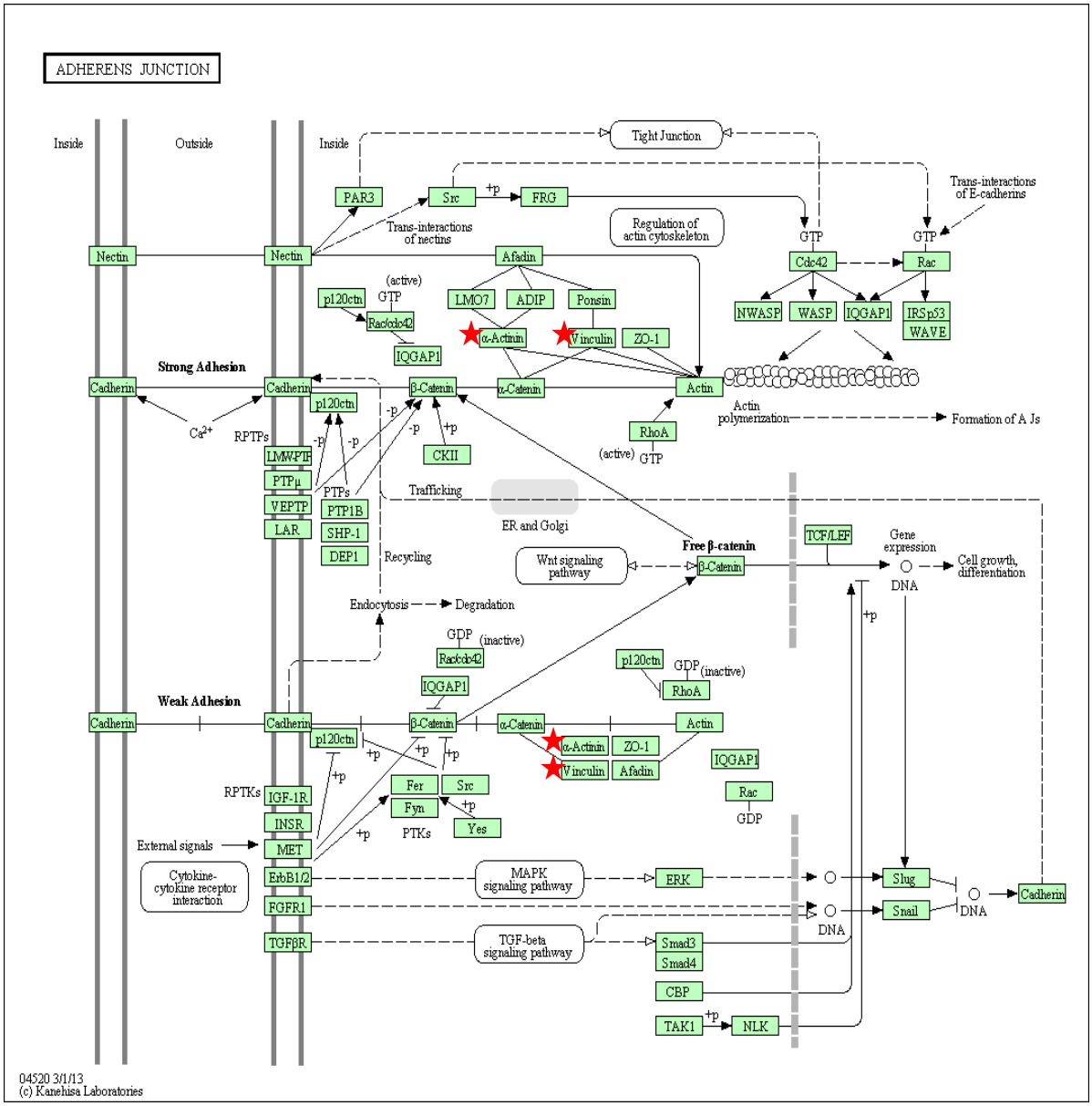

Figure 6.4 – Adherens junction signalling pathway. Red stars highlight proteins significantly upregulated ($p < 0.05$ compared to DMSO) following treatment with curcumin+oxaliplatin+5-FU: actinin α1, α2 & α4, and vinculin.

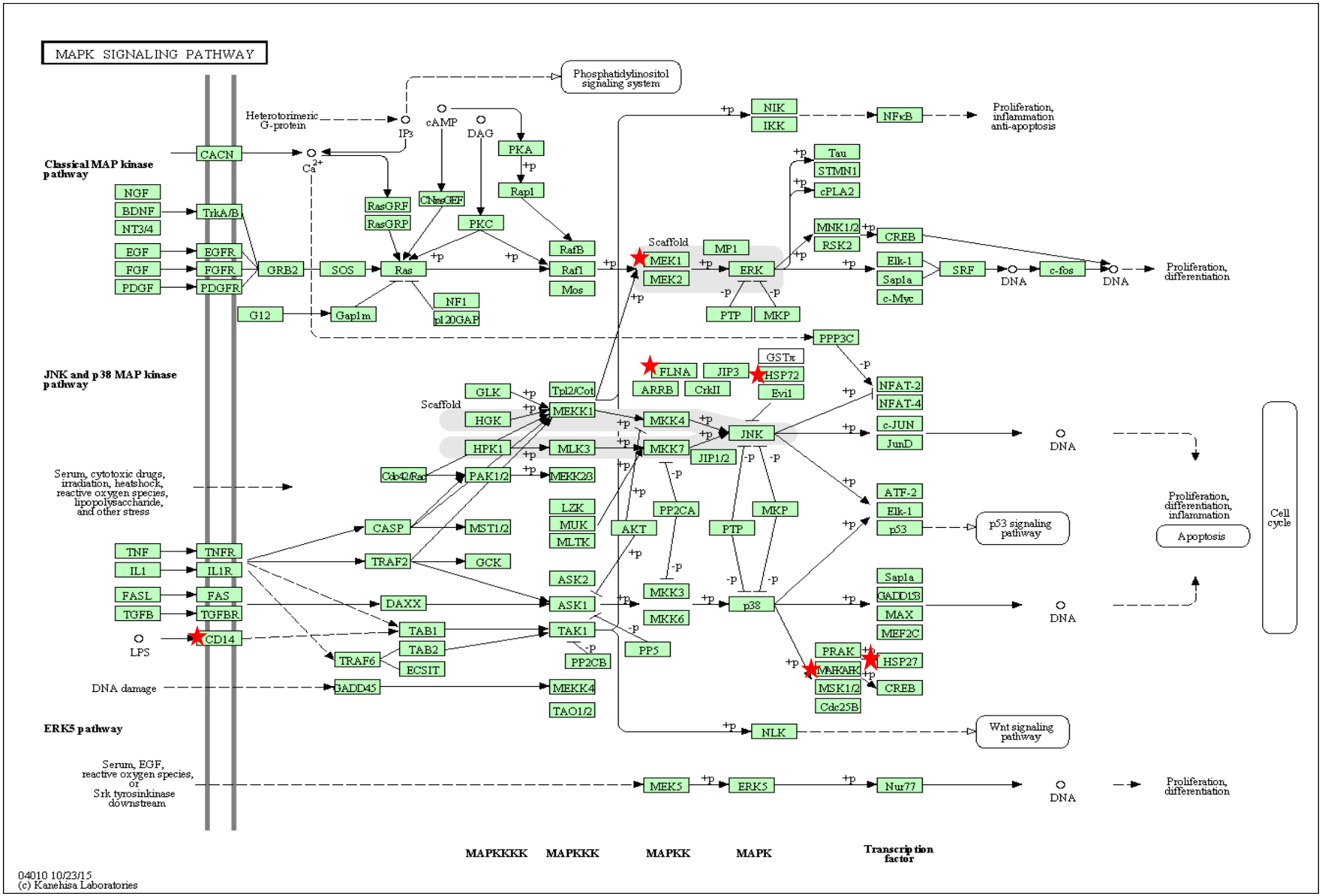

Figure 6.5 – MAPK (mitogen-activated protein kinase) signalling pathway. Red stars highlight proteins significantly upregulated ($p < 0.05$ compared with DMSO control) following treatment with curcumin+oxaliplatin+5-FU: CD14 molecule (CD14), filamin B (FLN), heat shock protein (HSP), mitogen-activated protein kinase-activated protein kinase (MAPKAPK), and mitogen-activated protein kinase kinase (MEK).

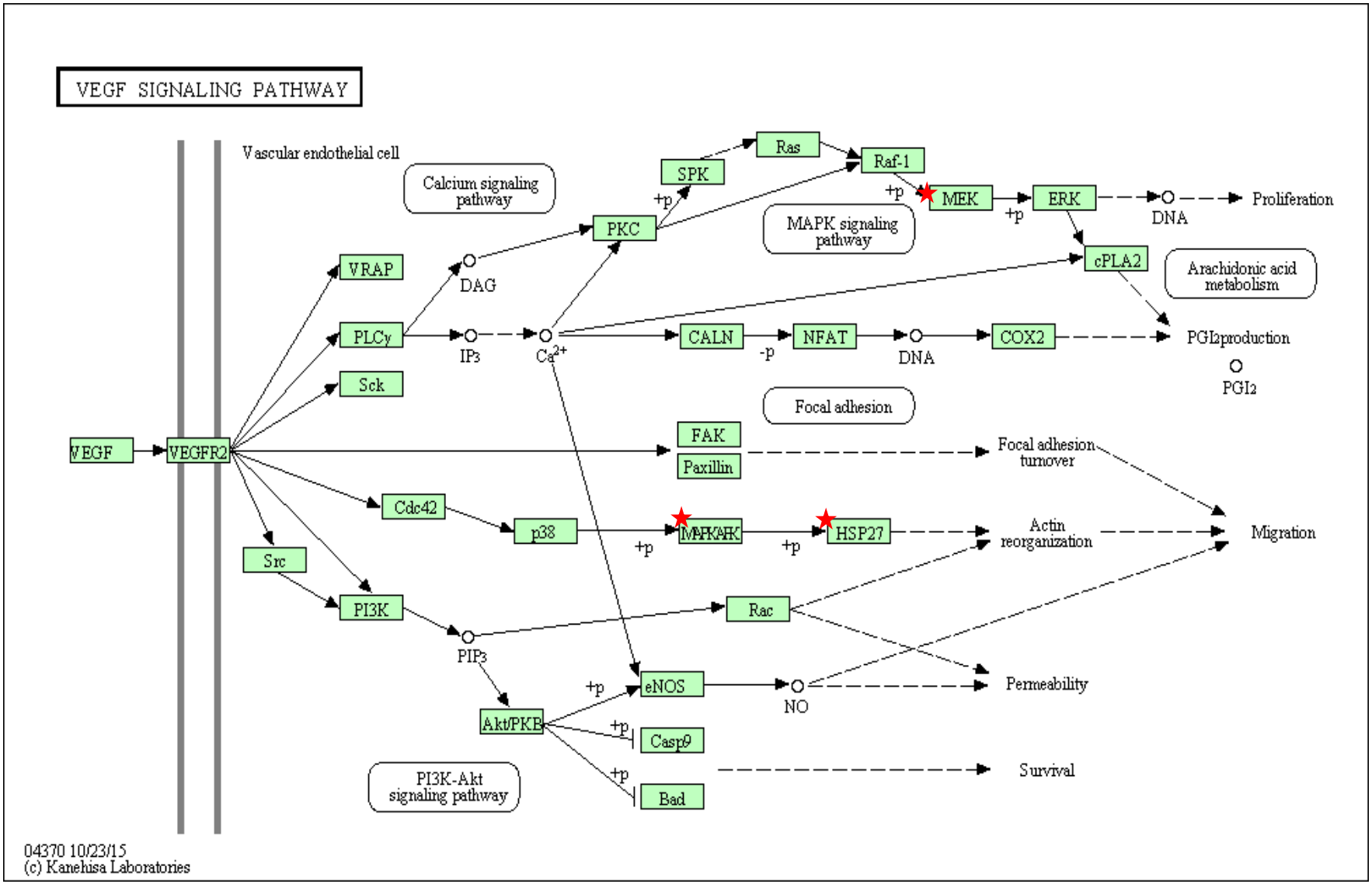

Figure 6.6 – VEGF (vascular endothelial growth factor) signalling pathway. Red stars highlight proteins significantly upregulated ($p < 0.05$ compared with DMSO control) following treatment with curcumin+oxaliplatin+5-FU: heat shock protein (HSP), mitogen-activated protein kinase-activated protein kinase (MAPKAPK), and mitogen-activated protein kinase kinase (MEK).

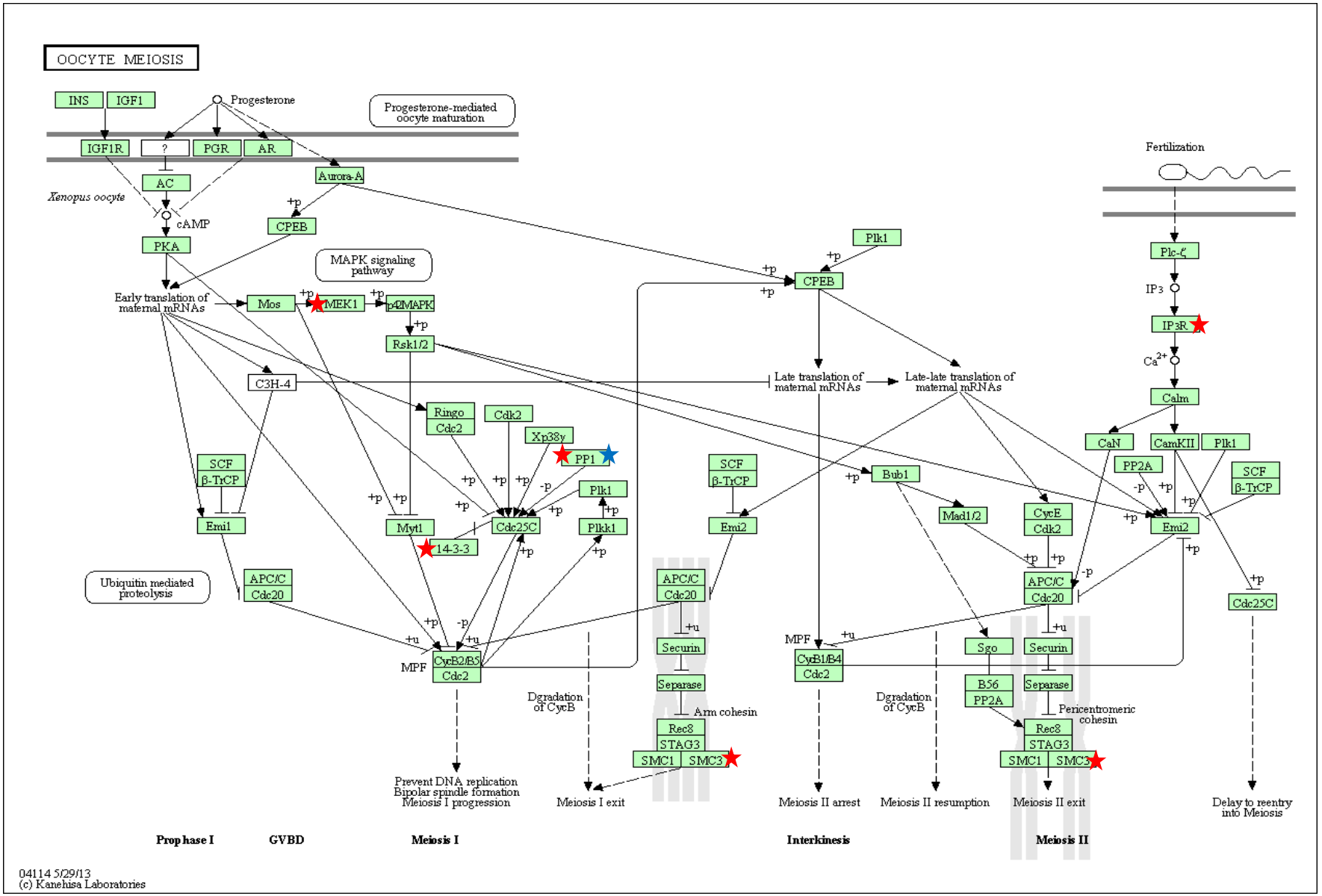

Figure 6.7 – Oocyte meiosis pathway. +p – phosphorylation; -p – dephosphorylation. Red stars – proteins significantly upregulated with curcumin, blue star – proteins significantly upregulated with oxaliplatin+5-FU: inositol 1,4,5-triphosphate receptor (IP_3R), mitogen activated protein kinase kinase 1 (MEK1), protein phosphatase 1 (PP1), 14-3-3 protein epsilon, structural maintenance of chromosomes 3 (SMC3)

6.2.4. Potential biomarkers

Proteins for further investigation as potential biomarkers were chosen from the group of 51 proteins uniquely altered following treatment with curcumin. A literature search (via PubMed) was conducted to select proteins that are known to be involved in more than one of the biological functions of apoptosis, migration and immune response, and also associated with at least one of the key signalling pathways related to CUFOX treatment only (ie MAPK, VEGF or adherens junction signalling pathway).

As a result, 5 proteins were isolated for further evaluation as potential biomarkers of efficacy: neutrophil gelatinase-associated lipocalin precursor (NGAL), metalloproteinase inhibitor 1 (MMP1), isoform 3 of kinesin-like protein KIF1B (KIF1B), killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1) and DNA-directed RNA polymerase III subunit RPC4 (POLR3D) (See Tables 6.4, 6.5 and 6.6).

6.3. **Discussion**

The focus for this study was to identify the proteins significantly altered following treatment with CUFOX in particular and determine whether they may have a potential role as biomarkers of efficacy. A substantial number of proteins were found to be significantly altered following treatment with curcumin, FOLFOX and CUFOX. The largest proportion of upregulated proteins was seen in the explant media samples treated with CUFOX. Further in depth analysis identified 51 proteins uniquely altered following treatment with CUFOX. These proteins were assigned to their functional pathways and the pathways that featured were those involved in apoptosis migration and inflammation or the immune response. The computer programme (KEGG pathway) used to map proteins to their pathways was limited in its ability to correlate the majority of proteins to a pathway. Initial analysis of all the proteins altered by CUFOX was conducted using this program, and was able to highlight some interesting biological pathways related uniquely to treatment with CUFOX. However once the 51 unique CUFOX proteins were isolated, more in depth exploration was required to identify each protein's function.

The 3 major functional pathways identified were involved in apoptosis, migration and inflammation/immune response. Apoptosis, or programmed cell death, plays an important role in both physiological and pathological processes. Loss of balance between cell division and apoptosis is one of the steps in the pathogenesis of cancer.

Likewise abnormalities in apoptotic signalling affect tumour responses to cancer treatments. Our results show that the greater proportion of proteins involved in apoptosis and uniquely altered by CUFOX were inhibitors of apoptosis. These proteins exerted their effects via a variety of mechanisms including effects on the MAPK pathway, regulation of caspases, cell cycle progression, inhibition of NF-kappa-B and natural killer cells or modulation of p53.

Metastasis of adenocarcinomas relies on the ability of the cancer cell to migrate and invade adjacent tissues with subsequent spread into the blood and lymphatic vessels. This cell motility is dependent on chemotaxis where gene expression from the microenvironment can initiate tumour invasion. The cellular translocation takes place as a result of the motility cycle involving the protrusion and retraction of the cell's actin-based cytoskeleton[(431)-(432)]. The CUFOX proteins shown to be involved in motility had roles in regulating cell motility, stabilising components of the actin skeleton and remodelling of the actin skeleton. Such proteins could be potential targets for chemotherapeutic agents, particularly with the aim of preventing disease metastases.

The altering of immune-related proteins following treatment with CUFOX could also be significant in that both the innate and adaptive immune systems are known to play a regulatory role in the development of cancer. Growing evidence and recent drug developments have confirmed that stimulating an appropriate immune response can produce substantial results in halting and reversing tumour progression. However, some tumour-related forms of inflammation have been known to promote cancer proliferation, invasion and metastases[(433)].

Interestingly the biological pathways identified as uniquely altered by the CUFOX proteins using the KEGG mapping program, reinforced the functions featured above. The mitogen-activated protein kinases (MAPK) signalling pathway, adherens junction pathway, and the VEGF signalling pathway are involved in the regulation of apoptosis, cell migration, and cancer proliferation. These pathways their roles in cancer are discussed in more detail below.

6.3.1. Upregulated pathways

Adherens junction

Proteins upregulated following treatment with CUFOX were shown to be involved in the adherens junctions pathway, namely α-actinin-1, α-actinin-2, α-actinin-4 and

vinculin. This pathway is important in regulating cell-cell adhesion, and cell migration. Alpha-actinins (ACTN) are cytoskeletal actin-binding proteins that crosslink actin filaments in migrating cells [434]. The four isoforms of ACTN are classified as either muscle-type (ACTN 2 and ACTN 3) or non-muscle type (ACTN 1 and ACTN 4) [435]. The muscle-type isoforms are exclusively expressed in muscle tissues and facilitate interactions between actin filaments and the Z-disk. The non-muscle isoforms are located along actin filaments and at adhesion junctions, where they facilitate the cell adhesion and migration[436]. ACTN1 and ACTN4 appear to play contrasting roles in focal adhesion formation. Focal adhesions are large dynamic protein structures that connect the cytoskeleton of the cell with the extracellular matrix. They act as traction sites whereby the strength of the adhesion and the dynamics of the focal adhesion enable cell detachment and motility[437,438]. ACTN1 promotes the formation of stable focal adhesions, whereas ACTN4 promotes the formation of immature focal adhesion with rapid turnover, leading to increased cell motility and cancer invasion[434]. Overexpression of ACTN4 is associated with a poor prognosis in several cancers, including colorectal, breast, lung, pancreatic, ovarian and bladder[434]. In colorectal cancer particularly, suppression of ACTN4 resulted in reduced migration and proliferation of cancer cells, whist overexpression stimulated lymph node metastases in immunodeficient mice[439].

Vinculin is a cytoskeletal protein that regulates cell-cell and cell-matrix junctions, playing regulatory role in focal adhesion formation, cell proliferation and regulation of the actin cytoskeleton[440,441]. Vinculin has shown tumour suppressor activity by inhibiting cell invasion and metastases[442]. Downregulation of vinculin is associated with the development of squamous carcinoma, rhabdomyosarcoma and breast cancer. *In vitro* and *in vivo* studies in colorectal cancer show vinculin inhibits invasion, migration and metastasis. It is significantly downregulated in metastatic colorectal cell lines and metastatic tissues[440]. Vinculin is activated by binding to binding partners, one of which is ACTN. The interaction of vinculin with ACTN exposes its binding sites and enables its localisation to focal adhesions[440,441].

The three isoforms of ACTN expressed following treatment with CUFOX have contrasting individual roles in the setting of colorectal cancer. However, in the context of the adherens junction signalling pathway, it is possible that these ACTNs are acting primarily to activate vinculin in its role as a tumour suppressor. This will have to be

investigated further by measuring the levels of ACTNs and vinculin in patient plasma samples. With these clinical trial samples, evaluation should confirm whether ACTNs and vinculin are solely isolated in patients treated with CUFOX and not with FOLFOX, as predicted by explant samples. Furthermore, an assessment of whether there is a significant correlation between the presence of one or both of these proteins and treatment outcomes will have to be performed. Vinculin has already been identified as a potential biomarker for prognosis in colorectal, pancreatic and prostate cancers[440,443,444]. If vinculin and ACTNs are confirmed as being uniquely expressed following patient treatment with CUFOX and a robust association with treatment response is confirmed, these proteins could be considered potential biomarkers for efficacy following treatment with CUFOX.

MAPK signalling pathway

The MAPK signalling pathway is a cell-proliferation signalling pathway between the cell surface and the nucleus[445]. This pathway is known to influence critical cellular processes involved in the development and progression of cancer including growth, proliferation, differentiation, migration, angiogenesis and apoptosis[446]. The 3 main subfamilies of MAPK are the extracellular-signal-regulated kinases (ERK MAPK, Ras/Raf1/MEK/ERK), the stress-activated protein kinases (SAPK) or c-Jun N-terminal (JNK), and MAPK14 (also called p38-α).

The ERK MAPK (extracellular signal-regulated kinase mitogen-activated protein kinases) signalling pathway is dysregulated in approximately one-third of human cancers and particularly associated with cell proliferation[446]. Overexpression of the ERK MAPK pathway is involved in the development and progression of colorectal cancer [447]. Activation of the ERK MAPK pathway is triggered following phosphorylation by MEK (MAPK/ERK 1 and 2). This in turn activates multiple targets including kinases, phosphatases, transcription factors and cytoskeletal proteins [448]. Thus, depending on the cell type involved, this pathway influences cellular proliferation, differentiation, survival, migration, angiogenesis and chromatin remodelling [448,449]. MEK1 was one of the proteins upregulated following treatment with CUFOX. This protein kinase is usually activated by stimuli including growth factors, cytokine, membrane depolarisation and calcium influx [450]. Because of its role in promoting the ERK MAPK pathway, MEK inhibitors have been investigated as

potential anti-cancer agents and are currently being assessed in the clinical trial setting [446]. However studies investigating the role of MEK signalling in chemoresistance have produced contradictory results[451]. In some cases (e.g. non-Hodgkins lymphoma, pancreatic and prostate cancer cells) activation of MEK resulted in chemoresistance increased anti-apoptotic activity, and inhibition of MEK activity increased tumour sensitivity to chemotherapeutic agents [452-454]. Whereas other studies have shown that MEK activity is essential for cancer cell apoptosis following treatment with the chemotherapy agent cisplatin [455,456]. In these studies, the use of chemical inhibitors of the MEK/ERK signalling pathway resulted in inhibition of cisplatin-induced apoptosis, whilst activators of the pathway increased cell sensitivity to cisplatin[456]. In colorectal cancer specifically, sensitivity to oxaliplatin was inhibited following activation of the MEK/ERK pathway with subsequent inhibition of apoptosis [457].

HSP27 was upregulated following treatment with CUFOX as was heat shock protein 72 (HSP72). Both of these proteins are associated with poor prognosis across various tumour types. Heat shock protein 27 (HSP27) is phosphorylated by the p38 MAPK in response to heat shock, cell stresses and pro-inflammatory stimuli [458-460]. The p38/MAPK14 pathway is also involved in controlling cellular proliferation and differentiation, but may also have some anti-apoptotic activity[461]. This pathway works in conjunction with other signalling pathways including the JNK, and ERK MAPK pathways to mediate the balance between cell survival and cell death. Therefore p38 MAPKs have been implicated in opposing roles in tumour development, both enabling cell survival and promoting apoptosis, depending on the circumstances[462]. The p38-HSP27 interaction is shown to promote drug resistance to cisplatin and anti-angiogenic agents in colorectal cancer stem cells [463,464]. However there are some cancer types, namely endometrial adenocarcinomas and oesophageal cancer where HSP27 expression improves prognosis[465]. HSP72 exerts its adverse prognostic effect by inhibition of the p53 pathway. This protein regulates the expression of MEK1/2 and inhibits stimulation of the ERK MAPK pathway[466].

The c-Jun NH_2-terminal kinase (JNK) pathway is activated by the PKG/MEKK1/SEK1/JNK[46] cascade in response to stress and cytokine stimulation[467].

[46] PKG/MEKK1/SEK1/JNK - protein kinase G (PKG)/Mitogen-activated protein kinase kinase kinase 1 (MEKK1)/stress-activated protein kinase/extracellular-signal regulated kinase 1 (SEK1) and c-Jun kinase (JNK)

This pathway regulates cell proliferation and induces apoptosis [468]. Filamin B (FLNB) was upregulated following treatment with CUFOX, whilst filamin A (FLNA) expression was reduced. Filamins are large non-muscle actin-binding proteins consisting of 3 isoforms: filamin A, B and C [469,470]. Filamin B is reported to act as a molecular scaffold in the JNK signalling pathway and in doing so stimulates interferon-induced apoptosis[471]. FLNB regulates the expression of metalloproteinases and vascular endothelial growth factor (VEGF), which are involved in tumour growth, metastasis and angiogenesis. Therefore, inhibition of FLNB has been shown to stimulate tumour growth and metastasis in human cancer cells via stimulation of the ERK MAPK pathway [472]. Conversely, FLNA does not bind to JNK, but is stimulated by stress conditions to promote cell survival [473,474]. Overexpression of this protein is associated with increased aggression in breast, prostate, pancreatic and hepatocellular cancers [475-478]. Inhibition of FLNA significantly reduces lung, splenic and systemic tumour metastasis in nude mice, suggesting that FLNA promotes tumour progression [479]. Overall this suggests that FLNB upregulation by CUFOX stimulates the JNK pathway to increase apoptosis, whilst downregulating FLNB and thereby inhibiting ERK MAPK-induced tumour growth and progression.

Overall it is not yet clear the impact that upregulation of the MAPK signalling pathway due to CUFOX treatment will have on treatment outcomes and patient prognosis. The proteins expressed in this pathway have shown variable and even conflicting roles in cancer treatment outcomes. This pathway is established as a key component in tumourigenesis and cancer progression, therefore its upregulation is noteworthy. However further analysis in the clinical setting is required to validate its significance in this study.

VEGF pathway

Vascular endothelial growth factor (VEGF) is a potent stimulator of tumour angiogenesis. Angiogenesis is known to be an essential process in the onset and progression of malignancy. Activation of the VEGF signalling pathway by binding of VEGF to vascular endothelial growth factor receptors (VEGFR-1 and VEGFR-2) results in the stimulation of proteins and pathways involved in the proliferation and migration of endothelial cells [480,481]. Consequently there is promotion of cell survival and increased vascular permeability. This is effect is via activation of the PI3K

(phosphatidylinositol 3 kinase), FAK (focal adhesion kinase), ERK MAPK and p38 MAPK pathways. The VEGF pathway proteins upregulated following treatment with CUFOX were MEK, mitogen-activated protein kinase-activated protein kinase (MAPKAPK), and HSP27, which are associated with the ERK MAPK and p38 MAPK pathways in particular. ERK MAPK activation, stimulated by MEK phosphorylation, promotes cell proliferation. Triggering of the p38 MAPK pathway promotes activation of MAPKAPK and HSP27 phosphorylation. This in turn stimulates actin organization, with recruitment of vinculin to focal adhesions, and mediates cell migration[482].

Due to the varying roles of these proteins and pathways, it is difficult to predict the mechanism of action and outcomes of CUFOX treatment in metastatic colorectal cancer. The addition of curcumin to standard chemotherapy clearly has an impact on key biological pathways in cancer development and these pathways are interlinked in their effect on tumour growth and progression. Further investigation into the representation of these pathways in patient samples and association with treatment outcomes is required.

6.3.2. Downregulated pathways

Treatment with curcumin+oxaliplatin+5-FU (CUFOX) resulted in the loss of proteins from the oocyte meiosis pathway. This was primarily due to the loss of protein phosphatase 1 (PP1) that was upregulated following treatment with curcumin and oxaliplatin+5-FU (FOLFOX), but not identified in the proteins upregulated following treatment with CUFOX. Protein phosphatase 1 is a serine/threonine phosphatase that regulates a range of cellular processes including cell-cycle progression, protein synthesis, transcription and neuronal signaling[483]. PP1 regulates cell-cycle progression via its effect on the phosphatase CDC25 (cell division cycle 25). CDC25 phosphatases are key regulatory proteins of the cell cycles and particularly regulate checkpoint pathways in response to DNA damage. Regulation of entry into mitosis requires the PP1 dephosphorylating of CDC25B and CDC25C. This activates cyclin-dependent kinase 1 (CDK1) which promotes further binding of CDC25 to PP1 and together this three proteins promote mitosis. CDC25 overexpression, particularly the CDC25A and CDC25B isoforms, have been reported in a number of cancers including colorectal, breast, lung and ovarian cancers[484]. CDC25B overexpression in colorectal cancer has been linked to poor prognosis and disease progression[485]. Reports of CDC25C overexpression in cancer are more limited, with this isoform generally showing a lower

rate of overexpression compared to CDC25A and CDC25B. Nonetheless overexpression of CDC25C has also been reported in some colorectal tumours[485]. In view of the stimulatory role PP1 plays in expression of CDC25, the loss of this protein following treatment with CUFOX may indicate increased treatment efficacy with this combination compared to curcumin or standard FOLFOX alone. Studies in cervical cancer have reported protein phosphatase 1 inhibitor 5 (IPP5) as a tumour suppressor that inhibits expression of PP1 and prevents progression through the G2/M checkpoint during the cell cycle[486,487]. Similarly, treatment with radiotherapy produced significant upregulation of the protein phosphatase 1 regulatory subunit 7. It is possible that, as in cervical cancer, knock down of PP1 by combination CUFOX treatment could signify improved therapeutic response.

Another protein downregulated following treatment with CUFOX was PIH1D1, isoform 3 (protein interacting with Hsp90 1 domain-containing protein 1). PIH1D1 has been reported to have anti-apoptotic effects and inhibit doxorubicin-induced cell death[380]. It forms a complex with RNA polymerase II-associated protein (RPAP3) and Monad proteins, pontin and reptin, which inhibits the assembly of apoptosis-related proteins. This cell protective function has distinguished PIHID1 as a potential target for cancer therapy[488].

Biomarker analysis of patient samples in the CUFOX study could measure the levels of PP1 and PIH1D1 and correlate the presence or absence of these proteins against treatment and tumour responses. This data could confirm whether these proteins are downregulated in samples from patients treated with CUFOX compared to standard chemotherapy alone. If this downregulation also corresponds to improved patient outcome, this could be the next step in validating these proteins as potential biomarkers for patient compliance and treatment efficacy.

6.3.3. Proteins for further investigation as potential biomarkers

The information obtained about the important pathways involved in the mechanism of action of CUFOX was used to wean down the list of proteins and select those that showed potential for further investigation as biomarkers of efficacy. NGAL is involved in all 3 functions of apoptosis, migration and immune response and its activity has been associated with the MAPK, adherens junction and VEGF signalling pathways[395,447,489-491]. MMP1 regulates cell migration and apoptosis, and is itself regulated via the MAPK signalling pathway[349,350,350,390,391,492]. It also plays a role in the VEGF and adherens

junction pathways[493,494]. KIF1B acts as a tumour suppressor by inducing apoptosis and regulates migration via its actions on the adherens junction[343,343,384,384,409-411,495]. KIR3DL1 controls the activation of the immune response and prevents apoptosis via stimulation of the MAPK pathway[388,389,426,496]. POLR3D has apoptotic and immune functions and is linked with the MAPK signalling pathway[320,424,497].

In addition, investigation of proteins altered following CUFOX treatment and highlighted as strategic proteins in functional pathways, could also warrant additional exploration as potential biomarkers of efficacy. This would be true of the following proteins: α-actinins, vinculin, MEK1, heat shock proteins 27 and 72 and filamin B. These proteins were all only significantly upregulated following treatment with curcumin or CUFOX.

The proteomic analysis conducted thus far is a starting point with relatively subjective criteria for the identification of potential biomarkers. Nonetheless, it provides useful data for further exploration, particularly for the patient samples obtained in the CUFOX trial.

6.3.4. Application of proteomics results in the clinical setting

Reviewing the balance of functions of the proteins in these pathways, it remains difficult to predict how treatment with CUFOX will affect carcinogenic processes in patients. This will depend on how accurately the changes in the secretome will correlate to changes seen in patients during treatment. Numerical fold changes observed in the laboratory setting do not necessarily predict biologically significant changes.

Oncology is one of the medical fields that has seen some success in identifying molecular tests that can predict treatment outcomes. Examples of this include human epidermal growth factor receptor 2 (HER2) expression in breast cancer, BRAF mutations in melanoma and K-ras status in colorectal cancer. However, in medicine as a whole, the majority of claims for novel biomarker candidates have not been successfully validated in clinical studies, often due to an inability to equate preclinical findings with human disease [303]. For this reason the running of biomarker studies alongside drug development trials has gained popularity.

Having identified potential biomarkers from our analysis of explant media samples, the next step will involve comparing whether a similar cohort of proteins is identified in our trial patient samples. Proteomic analysis of patient plasma samples will be conducted. The proteins identified will be compared to the predicted outcomes from our explant samples in terms of proteins and pathways identified at baseline, following treatment with curcumin only (pre-chemotherapy), and after treatment with standard chemotherapy versus chemotherapy plus curcumin. Additional investigations will examine how these proteins and pathways may be altered over treatment time points and how they relate to patient treatment outcomes.

6.3.5. Summary

Proteomic analysis of explant media samples treated with curcumin, FOLFOX and CUFOX revealed a large number of proteins that were altered with treatment. Treatment with CUFOX particularly altered the expression of proteins associated with pathways affecting apoptosis, migration and immune/inflammatory responses. Particular proteins that showed potential as biomarkers of efficacy included NGAL, MMP-1, POLR3D, KIR3DL1, KIF1B, and possibly vinculin, α-actinins, PP1 and PIH1D1. These outcomes will need to be compared to proteomics results from trial patients' plasma samples to gain further insight into their implication in the clinical setting.

7. General Discussion

7.1. **Conclusion**

Despite significant improvements in the management of colorectal cancer over the past 40 years, this malignancy remains the second most common cause of cancer deaths in the UK, accounting for 10% of all cancer deaths[7,498]. Disease metastasis is present in 25% of patients at diagnosis and reduces their 5-year survival to 7%[2,8]. Treatment for metastatic colorectal cancer with oxaliplatin-based regimens (e.g. FOLFOX) is hampered by toxicities, particularly peripheral neuropathy, resulting in delays, dose reductions and early cessation of treatment in up to 60% of patients[95]. Subsequently approximately 54% of patients will achieve a response to this treatment and progression-free survival is approximately 8 months[4]. Pre-clinical studies and early clinical trials have highlighted curcumin as a potentially beneficial adjunct to chemotherapy due to its multi-targeted cancer chemopreventive and co-therapeutic properties, associated with comparatively low toxicity[185,202,221,224,225].

Results from the phase I dose escalation established the dose of 2 grams of curcumin daily as safe and well-tolerated when added to standard treatment for this patient group. This enabled progression into phase IIa of this trial comparing outcomes for patients treated with standard chemotherapy alone versus those treated with standard treatment plus 2 g curcumin daily. At the time of this report 18 patients had been recruited onto phase IIa of this study with no safety concerns reported. Throughout phase I and thus far in phase IIa, excellent compliance rates have been sustained, with patients reporting a willingness to continue curcumin for longer than the 6 months of the trial. Data thus far is not sufficient to determine the effect of curcumin on chemotherapy-induced peripheral neuropathy. Based on the phase I results, addition of curcumin to standard chemotherapy maintained response rates and progression-free survival at the same level as observed with standard treatment only.

Analysis of miR-122 expression, DNA platination and curcumin/curcuminoid plasma concentrations was conducted to examine their utility in identifying any potentially unfavourable effects of adding curcumin to standard treatment. Results from phase I plasma samples showed that miR-122 could be measured in patient samples and used in conjuction with ALT levels to examine treatment-induced hepatotoxicity. The DNA platination results were not conclusive due to the lack of a significant increase in

platination levels in treated samples compared to baseline. Even so, response rates and patient reported outcomes showed no evidence that the addition of curcumin to standard chemotherapy affected the ability of oxaliplatin to form DNA adducts (and thus its efficacy) nor had a detrimental effect in terms of platinum-induced toxicity. Measurement of curcumin/curcuminoid levels showed detectable curcumin levels in patients receiving 2g daily curcumin, despite blood draws being undertaken after the predicted Tmax. Therefore this could serve as a measure for patient compliance, particularly with the use of enzymatic back-conversion to corroborate results.

Patient-derived liver metastases were treated with curcumin, oxaliplatin+5-FU (FOLFOX) and curcumin+oxaliplatin+5-FU (CUFOX) and proteomic analysis was conducted on the resultant explant media samples. The aim was to provide an ex-vivo assay that maintained tumour architecture from primary patient samples, thus potentially providing a more relevant model by which to examine the biological effects of CUFOX treatment on tumour tissues. Altered expression of an extensive range of proteins and biological pathways was achieved. Pathways involved in apoptosis, migration and immune/inflammatory responses were predominantly altered following treatment with curcumin. Of note was the upregulation of proteins involved in the adherens junction, MAPK and VEGF pathways, all of which are known to play prominent roles in the development or control of cancer. The proteins singled out as potentially worthy for further investigation included NGAL, MMP-1, POLR3D, KIR3DL1, KIF1B, and possibly vinculin, α-actinins, PP1 and PIH1D1.

7.2. **Phase IIa**

Since the onset of this report, the number of patients recruited onto phase IIa of this trial has increased from 18 to 26. The expectation is that this larger cohort will provide more conclusive results when addressing the effects of curcumin on peripheral neuropathy, treatment efficacy and toxicity. Thus far, not only have patients reported a high compliance rate with this treatment, they also showed a willingness to continue curcumin for longer than the 6 months of this trial. The fact that these patients attributed almost all their side-effects to chemotherapy and not curcumin no doubt has a significant bearing on this positive outlook. Results thus far corroborate the low toxicity profile associated with curcumin. However, the head-to-head comparison of toxicities from phase IIa will need to be completed to verify that chemotherapy is indeed primarily responsible for the bulk of side-effects experienced. Similarly, it is

essential to confirm that curcumin did not actually have detrimental impact on patient outcomes. Therefore head-to-head comparison of peripheral neuropathy, treatment response and progression-free survival rates between patients having standard treatment alone versus combination treatment will provide stronger evidence for this. As this was a pilot study, we were unable to conduct power calculations to ensure statistically significant outcomes with our recruitment numbers. Nonetheless, our results will provide a platform for power calculations in future studies to validate any potentially significant results.

miR-122 analysis was only performed for 6 phase I patients due to a delay in obtaining ethical approval for this line of work. Therefore, repeating these measurements in the larger phase IIa cohort is essential to ensure reproducibility. Hopefully the larger sample size will also provide clarity on the relationship between miR-122 and ALT levels. Contrasting miR-122 levels between patients treated with standard chemotherapy only versus curcumin plus standard treatment in conjunction with liver toxicity and treatment outcomes could establish whether miR-122 would serve best as a biomarker of toxicity or potentially be associated with curcumin's effect on treatment outcomes.

Investigation of DNA platination with phase IIa samples will be essential in determining the effect of curcumin on the ability of oxaliplatin to form DNA adducts in the clinical setting. Direct comparison of the DNA platination levels achieved with standard chemotherapy versus combination treatment can clarify whether the lack of a substantial increase in DNA platination following treatment with oxaliplatin in phase I was secondary the time lapse between treatment and sample analysis, enabling clearance of platinum from the plasma before readings can be obtained. These time points were established to align with the standard care pathway and minimize patient visits. In hindsight, the time frames may not have been suitable. This can be a point for investigation in future studies. Alternatively it could point to curcumin altering oxaliplatin's mechanism of action.

All the patients taking curcumin in phase IIa of this trial were given the dose of 2 g daily. Enzymatic back-conversion to the parent compound produced detectable curcumin/curcuminoid levels at this dosage in phase I patients. These measurements will have to be repeated to determine reproducibility. Furthermore comparisons will

need to be conducted between readings for patients treated with and without curcumin. Our analysis of phase I plasma samples was limited by the sensitivity of our method of detection. Experiments conducted by the Cancer Studies group here at the University of Leicester (credit J.Mahale) have presented mass spectrometry as a more sensitive modality for the measurement of curcumin/curcuminoid levels in plasma samples (not yet published). This method enabled the detection of picomolar concentrations of curcumin in human plasma samples following the oral ingestion of up to 100 mg of curcumin. Detection of curcumin/curcuminoids to this degree of sensitivity will be invaluable in the analysis of our patient plasma samples. Continued measurement of curcumin/curcuminoid concentrations in phase IIa samples, with the possible addition of mass spectrometry analysis, could validate the feasibility of measuring curcumin/curcuminoids as biomarkers of patient compliance.

7.3. **Proteomics**

Results from proteomic analysis have highlighted proteins and pathways that may be involved in the mechanism of action of combination treatment exclusively. These results need to be further validated by secondary techniques such as western blotting or enzyme linked immunosorbent assay (ELISA). Proteomic analysis is yet to be conducted on samples from phase I and phase IIa of this trial. The phase I analysis will be significant in distinguishing whether proteins and pathways altered in explant media are consistently altered in patient plasma samples and whether these alterations are dose dependent. Phase IIa analysis will confirm whether these alterations are indeed exclusively associated with CUFOX. A number of the altered proteins and pathways had ambiguous functions in cancer, whereby they may promote carcinogenic processes under certain circumstances, but inhibit cancer growth in others. Therefore it will be essential to not only identify these proteins, but also establish their role in terms of treatment outcomes. Overall, these results should provide a suitable foundation for identification of robust biomarkers of treatment efficiency for CUFOX in patients with metastatic colorectal cancer.

7.4. **Future work**

A number of factors can influence each patient's outcome following treatment with curcumin plus standard chemotherapy. Previous preclinical and early clinical studies have provided snapshots linking curcumin ingestion and bioavailability with pharmacological effect, however the oral dosing required for a pharmacological effect

remains uncertain. Results from the CUFOX trial should enable a more comprehensive picture of curcumin and its biological effects. Our measurements of curcumin/curcuminoids thus far produced detectable levels of parent curcumin in only a third of all the samples. Yet previous studies suggest that these trace levels of curcumin or its metabolites may be sufficient to exert a response[194]. Previously, levels of M(1)G adducts, COX-2 protein and prostaglandin E(2) have been used as biomarkers to determine the pharmacological effects of curcumin [192,193,223]. The biomarker analysis conducted in explant media samples now provides an additional range of potential biomarkers of efficacy. Ideally, alterations in the levels of these proteins and their related pathways would be compared to plasma curcumin/curcuminoid concentrations to establish the plasma curcumin/curcuminoid concentrations required to produce this biological change. However, this would require blood draws at T_{max} for optimal correlation between plasma levels and dose, and therefore may not be practical within this clinical study. The final step would then be to correlate the plasma curcumin/curcuminoid concentrations and protein biomarkers to patient outcomes. This trial targeted a treatment dose of 2 grams daily to optimize patient compliance and minimize toxicity from combination treatment. However, this dosage might need to be reconsidered if higher plasma curcumin plasma concentrations are required to elicit a pharmacological response, or conversely if lower doses of curcumin are sufficient for treatment efficacy.

Validation of any potentially significant results from this trial would require expansion with a considerably larger cohort of patients. For this reason it is essential that the safety profile of this combination treatment is robust. In order to ensure clinically relevant results, future phase II and phase III trials should include treatment regimens involving irinotecan, cetuximab, and possibly panitumumab, in addition to oxaliplatin, 5-FU, and capecitabine. Future clinical trials should also consider increasing the time points for trial blood samples so that more comprehensive analysis for outcome measures such as DNA platination and biomarker studies can be achieved. In view of the favourable indications from this trials patient cohort, the benefit of continuing single-agent curcumin as maintenance treatment could also be explored. If shown to be beneficial, curcumin's minimal side-effects and relatively low cost would make it ideal for this setting.

Overall, these initial results from the CUFOX trial have shown the safety and feasibility of translating promising preclinical results for curcumin as a chemopreventive agent, into clinical application in the treatment of metastatic colorectal cancer.

8. Appendix

8.1. **Trial synopsis**

Study title	A phase I/IIa study combining curcumin (Curcumin C3 Complex, Sabinsa) with standard care FOLFOX chemotherapy in patients with inoperable colorectal cancer.
Short title	FOLFOX plus curcumin in patients with inoperable colorectal cancer.
Abbreviated title	CUFOX
EudraCT No	2011-002289-19
ISRCTN or other No	To be confirmed after REC submission
Funder	Bowel Disease Research Foundation Hope Foundation Royal College of Surgeons, England With support from NIHR, ECMC and Cancer Research UK (CRUK)
Chief Investigator	Prof Anne Thomas
Sponsor	University of Leicester
Sponsor No	UNOLE0225
Study period	7 years
Phase	I/IIa
Number of arms	Single arm phase 1, Two-arm phase 2
Design	Phase I Dose Escalation Study Phase IIa Two arm (2:1) randomised study
Number of participants	Phase I, n = minimum 9, maximum 18 Phase IIa, n = 33 Total, n = 42 (maximum 51)
Objectives **Primary** To establish the safety, tolerability and feasibility of administering oral curcumin at	

increasing doses escalating to 4 capsules (2 g) during standard FOLFOX-chemotherapy for palliation of colorectal metastases. **Secondary** To collect blood samples for future translational work including identification of putative disease biomarkers. To observe any changes to the neuropathic side-effects of chemotherapy. To observe potential for efficacy in terms of disease response and survival.
Main inclusion criteria Histological or cytological diagnosis of metastatic colorectal cancer Measurable disease by Response Evaluation Criteria in Solid Tumours version 1.1 (RECIST 1.1) (Appendix 1) Adequate haematological, hepatic and renal function Age ≥ 18 years Eastern Cooperative Oncology Group (ECOG) Performance status 0 or 1 (Appendix 2) Patients must have recovered from effects of any recent major surgery Willing to use contraception if applicable Informed consent Life expectancy estimated to be more than 12 weeks
Main exclusion criteria Contraindications to FOLFOX chemotherapy: Peripheral neuropathy NCI CTC >1 (Appendix 3), Liver failure, uncontrolled coronary heart disease, myocardial infarction within the previous 6 months. Unwilling or unable to comply with the study protocol. Patients who are pregnant or lactating or contemplating pregnancy. Patients or their partners who become pregnant during the study will be referred to the appropriate experts. Undergone chemotherapy (other than adjuvant for CRC) or participating in another drug study. Previous cancer <5 years (other than colorectal, basal cell carcinoma, in-situ cervical cancer).

Major surgery within 4 weeks of starting the study Co-existing active infection or serious concurrent medical condition Significant cardiovascular disease Bone metastases Known brain or leptomeningeal metastases Surgery or hospital admissions for symptomatic intra-abdominal adhesions
Treatments Curcumin will be administered for 7 days prior to the commencement of chemotherapy and continued until the 12th cycle or patient withdrawal from the trial. **FOLFOX will be given to all patients as per routine care** Oxaliplatin 85 mg/m^2 administered in 250 ml glucose 5% as 2-hour IV infusion. Folinic acid 350 mg administered as a two hour infusion on day 1; followed by a loading dose of 5- Fluorouracil (5-FU) (400 mg/m^2) IV bolus administered on day 1, then 5-FU (2400 mg/m2) administered by ambulatory pump for a period of 46 hours every two weeks. Modifications or additions (e.g. Bevacizumab) will be permitted as clinically required and as per local practice. **Curcumin** **Phase I Dose escalation study (Traditional escalation response (TER) model)** Participants will receive 1, 2 or 4 capsules of daily oral curcumin according to the escalation level to which they are recruited until chemotherapy ceases. If at 7 days after completing 2 cycles of FOLFOX, 3 consecutive participants starting at 1 capsule per day have not experienced dose-limiting toxicity (DLT) then a further 3 patients will be recruited and commenced on the next dose of 2 daily capsules. This process will be repeated for doses of 2 and then 4 daily capsules. Recruitment will begin for phase IIa after 3 participants have reached 1 week after completion of 2 cycles of FOLFOX whilst receiving the target dose of daily oral curcumin. If a DLT occurs at a particular dose then a further 3 patients will be recruited at that dose. De-escalation will occur in the event of DLT in 2 of 6

patients and phase IIa will continue at a revised maximum target dose (MTD).

Phase IIa FOLFOX and curcumin combination group

Participants will receive the MTD of daily oral curcumin until chemotherapy ceases. MTD dictated by the findings of phase I. Chemotherapy will be given and monitored as per the standard care pathway.

Phase IIa FOLFOX only group

Will receive standard care FOLFOX chemotherapy and no curcumin.

Trial assessments following consent

Baseline

Medical history

Clinical examination

Computerised Axial Tomography (CT) scan of chest, abdomen and pelvis to evaluate measurable disease by RECIST 1.1

Haematology, serum biochemistry

ECOG performance status

Pregnancy test (females of child bearing potential only)

Baseline toxicity including neurotoxicity (NTX) score (Appendix 4)

Pre-trial curcumin experience questionnaire

European Organisation for research and treatment of cancer quality of life questionnaire form C30 (EORTC QLQ-C30)(Appendix 5)

Prior to or at each cycle of chemotherapy, as per routine care

Haematology and serum biochemistry

Evaluation of toxicities using NCI CTC AE 4.0 and real-time adverse event (AE) reporting

Documentation of dose delays and/or reduction

And in addition to this:

Blood sampling for biomarker analysis and compliance will be taken at baseline,

and end of weeks 1 and 3, 5 and at the final cycle of chemotherapy
Neurotoxicity questionnaire (NTX) at before/at second cycle of chemotherapy
Daily pill diary for the first 4 weeks
Pre-chemotherapy checklist each cycle.
EORTC QLQ-C30 and post-trial curcumin experience questionnaire

3 monthly to 24 months and then 6 monthly for up to 48 months
CT scan of chest, abdomen and pelvis to evaluate disease response by RECIST

Endpoints

Safety
Safety Review of toxicities will be on going.

Primary
Completion of 2 cycles of chemotherapy in dose-escalation phase by 3 consecutive patients at MTD.
Time to completion of chemotherapy or withdrawal from therapy of all participants.
Tolerability and feasibility of using this curcumin regimen with FOLFOX chemotherapy.

Secondary
Improvement in progression free survival (PFS), objective response rate (ORR) and overall survival (OS) from enrolment to death as assessed by RECIST 1.1.
Improvement in neurotoxicity scores.
Analysis of blood for biomarker studies.
Analysis of blood for curcumin/curcuminoid levels

8.2. **Neurotoxicity questionnaire**

University of Leicester

UNOLE0225 CUFOX
Neurotoxicity Questionnaire

University Hospitals of Leicester NHS

Patient Unique ID_____ **Date**_____

The previous cycle of chemotherapy _____

	Questions about feelings in your hands and feet (see key at bottom)	**1** ☺	**2**	**3**	**4** ☹
1	Do you have any difficulty in buttoning buttons?				
2	Do you have any stiffness or tightness in your hands?				
3	Do you have any stiffness or tightness in your feet?				
4	Do you feel clumsy?				
5	Do you feel any discomfort in your hands?				
6	Do you feel any discomfort in your feet?				
7	When holding an object in your hand(s), are you able to feel this shape?				
8	Do you have tingling in your hands?				
9	Do you have tingling in your feet?				
10	Do you have numbness in your hands?				
11	Do you have numbness in your feet?				

1 = "not at all"; 2 = "a little"; 3 = "quite a bit", 4 = "very much".

Please complete at the start of the study, 2 weeks after your last cycle of chemotherapy and before or at every second cycle. Please ask a trial investigator for help if you require it.

Neurotoxicity Questionnaire Version 1, 22/05/2011
Sponsor Number UNOLE0225
Sponsor: University of Leicester, College of Medicine, Maurice Shock Building, University Road, PO Box 138, Leicester, LE1 9HN

8.3. **EORTC QLQ-C30 (Quality of life) questionnaire**

EORTC QLQ-C30 (version 3)

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

Today's date (Day, Month, Year): 31

		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 long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:		Not at All	A Little	Quite a Bit	Very Much
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
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

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

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

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 overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

8.4. **Pre-trial questionnaire**

University of Leicester **UNOLE0225 CUFOX** University Hospitals of Leicester NHS NHS Trust

PRE-TRIAL QUESTIONNAIRE

Patient Unique ID: **Initials:** **Date:**

Age:

How many years have you lived in the UK: ☐ **All my life** ☐ **Other, please state**

Gender: ☐ Male ☐ Female
(on birth certificate)

Marital Status: ☐ Married ☐ Single
☐ Separated ☐ Living with Partner
☐ Divorced ☐ Widowed
☐ Prefer not to say

Ethnic Origin: Which one of the following groups do you feel most adequately describes your ethnic origin?

☐ White British	☐ Asian or Asian British – Bangladeshi
☐ White Irish	☐ Other Asian Background
☐ Other White Background	☐ Black or Black British – Caribbean
☐ Mixed –White and Black Caribbean	☐ Black or Black British – African
☐ Mixed –White and Black African	☐ Other Black Background
☐ Mixed –White and Asian	☐ Chinese
☐ Other –Mixed Background	☐ Other Ethnic Background
☐ Asian or Asian British - Indian	☐ Unknown
☐ Asian or Asian British - Pakistani	☐ Prefer not to say

Religious beliefs: To which of the following religions, bodies or belief systems, if any, do you belong or affiliate with?

☐ Christian (including Church of England, Catholic, Protestant and all other Christian denominations)	☐ Muslim
☐ Buddhist	☐ Sikh
☐ Hindu	☐ I have no religious beliefs
☐ Jewish	☐ Other (please state)
	☐ Prefer not to say

Employment:

☐ Full time	☐ Retired	☐ Other (please state)
☐ Part time	☐ Student	☐ Prefer not to say
☐ Unemployed	☐ Disabled or retired due to ill health	

Highest level of education:

☐ GCSE/O Level	☐ Bachelor or equivalent (NVQ4)	☐ No formal qualifications
☐ Vocational/NVQ 1 or 2	☐ Masters/MD/PhD	☐ Still studying
☐ A-Level or equivalent (NVQ3)	☐ Other	

1

Regarding your diet and curcumin:	**No**	**Yes**	**Not Sure**
Before this study, had you heard of curcumin?	0	1	2
Before this study, had you ever used curcumin as a health supplement?	0	1	2
Do you regularly, use turmeric or curcumin in your diet?	0	1	2
If so please specify:	DAILY	WEEKLY	MONTHLY

Regarding curcumin, pills and your own (if any) medications:	**Not at all**	**A little**	**Quite a bit**	**Very Much**
Do you think that taking one dose of 1 / 2 / 4 pills will be difficult? (circle your dose)	1	2	3	4
Do you think the size of the pills will make them difficult to swallow?	1	2	3	4
Do you think the daily frequency of taking the pills is too much?	1	2	3	4
Do you worry about the pills having serious side-effects?	1	2	3	4
Would you be worried if you skipped a dose of curcumin?	1	2	3	4
Do you think curcumin will give you peace of mind?	1	2	3	4
Do you think curcumin pills will be inconvenient to take with your other medications?	1	2	3	4
Do you think curcumin will affect how your other medication works?	1	2	3	4

Do you think it would be difficult taking curcumin for 6 months?	1	2	3	4
How long do you think you could take curcumin for:	_____			
What, if anything, would make it easier to take for longer:	_____			

Regarding your bowels and abdomen, during the last week:	**Not at all**	**A little**	**Quite a bit**	**Very Much**
Did you have pale or yellow stools?	1	2	3	4
Did you suffer from excess flatulence (wind)?	1	2	3	4
Did you suffer from bloating?	1	2	3	4
Did you suffer from abdominal pain?	1	2	3	4

8.5. **Post-trial questionnaire**

University of Leicester

University Hospitals of Leicester NHS
NHS Trust

UNOLE0225 CUFOX
POST-TRIAL QUESTIONNAIRE

Patient Unique ID: **Initials:** **Date:**

Regarding your diet and curcumin:	**No**	**Yes**	**Not Sure**
Are you aware of any potential benefits of curcumin?	0	1	2
Would you ever use curcumin as a health supplement?	0	1	2
Would you regularly, use turmeric or curcumin in your diet?	0	1	2
If so please specify:	DAILY	WEEKLY	MONTHLY

Regarding curcumin, pills and your own (if any) medications:	**Not at all**	**A little**	**Quite a bit**	**Very Much**
Did you think that taking one dose of 1 / 2 / 4 pills was difficult? (circle your dose)	1	2	3	4
Did you think the size of the pills made them difficult to swallow?	1	2	3	4
Did you think the daily frequency of taking the pills was too much?	1	2	3	4
Did you think the pills had serious side-effects?	1	2	3	4
Did you suffer any side-effects during your chemotherapy?	1	2	3	4
Did you think the side-effects were due to the Chemotherapy?	1	2	3	4
Or the Curcumin?	1	2	3	4
Were you worried if you skipped a dose of curcumin?	1	2	3	4
Do you think taking curcumin provided peace of mind?	1	2	3	4

	Not at all	A little	Quite a bit	Very Much
Did you think curcumin pills were inconvenient to take with your other medications?	1	2	3	4
Did you think curcumin affected how your other medication works?	1	2	3	4
Would it be difficult taking curcumin for 6 months?	1	2	3	4
How long do you think you could take curcumin for:	_____			
What, if anything, would make it easier to take for longer:	_____			

Regarding your bowels and abdomen, during the last week:	**Not at all**	**A little**	**Quite a bit**	**Very Much**
Did you have pale or yellow stools?	1	2	3	4
Did you suffer from excess flatulence (wind)?	1	2	3	4
Did you suffer from bloating?	1	2	3	4
Did you suffer from abdominal pain?	1	2	3	4

Thank you very much for your participation in this study.

8.6. **Patient Information Sheet – Phase I**

University of Leicester

University Hospitals of Leicester NHS
NHS Trust

Department of Oncology
2nd floor Osborne Building
Leicester Royal Infirmary
LE1 5WW
0116 2587597

Study title: Clinical trial investigating curcumin given with standard FOLFOX chemotherapy to patients with advanced bowel cancer

Principle Investigator: Prof William Steward, Consultant Oncologist, Head of Cancer Studies

Patient Unique ID: **Initial:** **Date:**

An invitation to be part of the trial

You are invited to take part in a research study. Participation is voluntary. This study provides an addition to your normal care. Before you decide to take part it is important for you to understand why this research is being done and what it involves. Please take time to read this carefully and discuss it with others if you wish. Please ask us if anything is unclear or if you would like more information.

What is the purpose of the study?

The purpose of this study is to investigate the potential benefits of a naturally occurring plant extract, called **curcumin**, when it is combined with a type of chemotherapy called **FOLFOX** which is usually given to patients with advanced bowel cancer for up to 6 months.

Why have I been chosen?

You have been chosen because unfortunately you suffer from bowel cancer and as part of your care you will receive FOLFOX chemotherapy to try to shrink this. We are hoping to observe the effects in about 45 patients.

What is FOLFOX chemotherapy?

FOLFOX is usually the first choice of chemotherapy offered to patients with bowel cancer. It is a combination of 3 common chemotherapy medicines given together (fluorouracil, folinic acid and oxaliplatin) and injected into a vein every 2 weeks for up to 6 months. If you feel any side-effects from the chemotherapy, the dose or type of medicines can be altered.

What is Curcumin?

Curcumin is a natural substance found in turmeric, part of the Ginger family. It is a spice common in Asian food and used to make many biscuits, cheese and cereals. It is yellow food colouring (E100) and may have beneficial properties as a medicine.

Why add Curcumin to chemotherapy?

How well and how long any type of chemotherapy works for will be different for each patient, even after several cycles. We know from laboratory studies that curcumin can shrink tumours. It may also improve how chemotherapy works against tumours that are resistant to treatment. In this study we aim to apply these findings to see if patients can safely take curcumin while receiving chemotherapy and if there are any potential benefits from doing so.

In addition to this, FOLFOX can produce side-effects such as unusual or painful feelings in the hands and feet, called peripheral sensory neuropathy. Sometimes it is necessary to change the chemotherapy medicines to try and improve the side-effects but this may also effect how well therapy works or lead to other side-effects. We would like to investigate if curcumin can improve side-effects experienced.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you decide to take part you will be given this document to keep and asked to sign a consent form. If you decide to take part you are free to withdraw at any time and without giving a reason. A decision to not participate will not affect the standard of care you receive.

What do I have to do?

If after you have read this information and discussed it with an investigator you wish to take part, then we will ask you to sign the consent form accompanying this leaflet. We will take some blood tests and examine you to check it is safe for you to start the trial. Assuming there are no reasons preventing you taking part in the study, we will organise for you to start treatment on curcumin tablets and then after a week attend again to start FOLFOX at Leicester Royal Infirmary.

You can take your curcumin capsules at any time of day but ideally together and roughly at the same time of day with meals. You can continue to take your normal medicines. Taking curcumin does not restrict you from any of your normal activities.

What will happen to me if I take part?

You will receive the normal chemotherapy course of up to 12 cycles lasting about 6 months. During this time it is usual to attend for appointments and blood tests every two weeks. During and after chemotherapy, you would also have a series of CT ("CAT") body scans every 3 months for up to 2 years. You may need scans for up to 4 years.

In addition to your chemotherapy, we would like you to take curcumin capsules by mouth, daily for as long as you can manage while you are having your course of chemotherapy, which can last for up to 6 months. We would also ask you to complete a short questionnaire at the start and end of your course of curcumin to let us know your thoughts and experiences from taking it.

To help monitor any side-effects you experience we would ask you to fill in a simple daily diary for the first 28 days. We would also like to monitor any changes in sensation you may feel in your hands and feet from the chemotherapy and would like you to complete a brief

symptom questionnaire to help with this. At the start and end of your chemotherapy we would like you to complete a questionnaire to let us know if your quality of life has been affected in anyway during this study.

When you come to the department we will ask you to let us take some blood samples. Most extra samples required for the study will be performed as part of or at the same time as your routine tests and are taken at 5 of your visits. These consist of 4 small bottles (a total of about 4 teaspoons) to help with our study as well as measure your blood count, kidney and liver function as part of your regular health check while on chemotherapy.

Is curcumin safe?

Laboratory studies have not revealed any adverse effects of curcumin when it is used as a chemotherapy agent. Curcumin has been used in 18 clinical trials taken by over 350 patients. Two trials have given curcumin to patients taking chemotherapy. Curcumin has been given in doses of up to 16 grams (g) per day but because of the number of tablets required to give this amount and the bowel symptoms some patients feel at this dose, a better tolerated dose is probably less than half of this (< 8 g or 2 heaped teaspoons). The maximum dose to be used in this study is 2 g and we will continue to monitor trial participants for any problems that may arise.

Although curcumin has been given to patients on chemotherapy before it has not been given to patients receiving this type of chemotherapy. Curcumin will firstly be given at a very low dose and increased step-wise across 3 groups of patients.

The first group will receive a small dose of one capsule a day (half a gram). This is of a similar magnitude to what is found in a curry rich diet. If this dose is tolerated safely a second group will then receive 2 capsules a day (1 gram). If the second group can safely tolerate 2 capsules a day, a third group will then receive 4 capsules a day. Curcumin is not spicy hot and it comes in a capsule to swallow shown in the photograph (actual size). Your trial doctor will explain to you the dose you need to take if you wish to take part.

Are there any side-effects from curcumin?

Most patients who have had curcumin do not report any side-effects. We know from our recent study in Leicester about 1 in 4 patients may feel some kind of mild bowel disturbance. Side-effects can include bloating, increased bowel movements, sometimes diarrhoea or abdominal pain and particularly at higher doses. Several studies have used up to 4 g per day and for up to 4 months without severe side-effects. This study will use a lower dose of no more than 2 g a day (1 level teaspoon).

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

You will not be able to take part if you meet any of the following criteria:

- If you have had any previous chemotherapy for cancer in the last 5 years except bowel cancer.
- If you have had a cancer other than bowel cancer. Exceptions to this are skin cancer (other than melanoma) or early breast or cervical cancer, or if you have had treatment to cure any other cancer and have remained in remission for at least 5 years
- If you have an allergy to turmeric
- If you have any medical conditions that would mean treatment with chemotherapy might be harmful – including uncontrolled heart failure, recent heart attack or stroke, liver disease, kidney disease
- If you have HIV or AIDS
- If you have had a major operation or injury within 4 weeks of treatment
- If you are female, you must have undergone a surgical sterilisation, be post-menopause, or agree to use two adequate forms of contraception at all times during treatment and for at least 6 months after the last dose of FOLFOX. If you are male and your partner has childbearing potential, you must have been surgically sterilised or agree to use adequate contraception.
- If you are breast feeding
- You have peptic ulcers or colitis proven on endoscopy, recurrent pain from abdominal adhesions or chronic diarrhoea.

In addition, if either you or your doctor should decide that you are not suitable to receive chemotherapy, then you would also not be able to enter the trial

Am I safe to take part?

If you agree to take part in this trial, you will have blood tests and an examination by a doctor to check that it is safe for you to receive chemotherapy. Assuming everything is fine, you will commence your treatment. During the trial, you will regularly be seen by a doctor or nurse. Should you experience any side effects or abnormalities in the blood tests, this may require the treatment to be altered or stopped. This happens to all patients on chemotherapy.

What are the extra requirements of being in the trial?

There are very few extra requirements in addition to the standard care pathway other than those mentioned already. If you agree to enter the study you will start taking oral curcumin one week before starting FOLFOX. This will require up to 4 capsules taken with food once a day. This will continue for the duration of your chemotherapy. During this time you will be able to eat and drink as you would normally and take your normal medications.

What are the additional blood tests for?

We will measure the level of curcumin in your blood. It is broken down by the body very quickly and it is useful to know if chemotherapy alters this. We will also perform experiments to look at proteins in the blood that may help detect or measure disease and the response to treatments. We also intend to measure the amount of platinum from the chemotherapy that

attaches to DNA in cells (we are NOT looking at your genes or doing genetic research). Developing these experiments is important for future research and we request your permission to store your blood samples for analysis in future studies looking at proteins in your blood. This is optional.

Are there any other problems I might encounter and what do I do about them?

Before you agree to treatment your Oncologist (cancer doctor) will discuss possible side effects of FOLFOX with you. Many patients experience peripheral sensory neuropathy requiring alteration to the chemotherapy. FOLFOX can sometimes cause problems to the heart, kidneys and liver which is why we monitor everybody closely with regular check-ups.

Curcumin has been used in many trials and at higher doses without significant drug related toxicity. It has so far been shown to be safe. Some patients have had bowel disturbances (bloating and loose stool) at higher doses which is one of our reasons for choosing a lower dose. We will monitor any side-effects by the diary that you fill in. You should record symptoms in the diary or report them to an investigator or nurse if you have concerns.

What are the possible disadvantages and risks of taking part?

The potential risks of this trial are virtually identical to those that would occur should you not participate in this trial and were taking FOLFOX. Potential side-effects of curcumin have been explained and are mostly mild bowel disturbances. In addition, there will be the possible inconvenience of taking curcumin capsules every day for up to 6 months, provide extra blood samples, fill in questionnaires and a drug diary.

What are the possible benefits of taking part?

This is an exploratory study and therefore there may be no direct benefits. The information we obtain is important for future therapies and study design.

Curcumin has been shown to have beneficial effects against cancer cells in laboratory experiments. Recently, curcumin has made a small difference in other forms of cancer (pancreas) when combined with chemotherapy. Some experiments suggest curcumin has potential to improve peripheral sensory neuropathy. We will investigate if the neuropathy symptoms from FOLFOX alter if curcumin is used with it.

Will I receive any financial benefit for taking part?

You will not receive any financial benefit for taking part in the study. However, if you are required to make extra hospital visits than you would on standard treatment, we will reimburse any additional parking/travel expenses that you incur.

What if new information becomes available?

Sometimes during the course of a study, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. They will explain the reasons and arrange for your care to continue.

What happens when the research study stops?

Treatment with the FOLFOX and curcumin will continue until one of several reasons for stopping treatment:

1. Cancer is no longer controlled by the treatment and has grown. This is the reason why you will have CT scans (CAT scans) every 3 months.
2. Side effects of the FOLFOX become intolerable to you.
3. Side effects of the curcumin become intolerable to you.
4. You change you mind about being in the trial.
5. The course of chemotherapy finishes.

When you end the course we will also ask you to fill out a final questionnaire and have a final blood test. Your care will then continue as it would for any other patient. We would still like to carry out CT scans after the course has ended until it is necessary to stop.

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

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital/surgery will have your name and address removed so that you cannot be recognised from it. We would like to notify your own GP of your participation in this trial. This may be important for them to know should they alter any medication that you are on. We ask for your permission to do this.

What will happen to the results of the research study?

The results of the study will available to you and other participants. Should the treatment be beneficial, the results may be shown to other patients with your condition and treatment be offered to them. Findings may be reported to the media, in medical journals and at conferences to inform medical professionals and the public. Personal information is strictly confidential.

Who is organising and funding the research?

The Department of Cancer Studies, University of Leicester is running the study at the department of Oncology, University Hospitals of Leicester with funding supported by Cancer Research UK, Hope Foundation, Bowel Disease Research Foundation and the Royal College of Surgeons.

Who has reviewed the study?

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

Contact for Further Information

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

Finally, thank you very much for taking the time to read this information. If you would like to take part, we will ask you to sign a consent form, provide a copy of the signed consent form and this information sheet to keep.

Project co-ordinators Dr Lynne Howells and Glen Irving
5th Floor Robert Kilpatrick Clinical Sciences Building
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Consultant Oncologist and Head of Cancer Studies
Department of Oncology
2nd floor Osborne Building
Leicester Royal Infirmary
LE1 5WW
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8.7. **Patient Information Sheet – Phase II**

University of Leicester

University Hospitals of Leicester NHS
NHS Trust

Department of Oncology
2nd floor Osborne Building
Leicester Royal Infirmary
LE1 5WW
0116 2587597

Study title: Clinical trial investigating curcumin given with standard FOLFOX chemotherapy to patients with advanced bowel cancer

Principle Investigator: Prof William Steward, Consultant Oncologist, Head of Cancer Studies

Patient Unique ID: **Initial:** **Date:**

An invitation to be part of the trial

You are invited to take part in a research study. Participation is voluntary. This study provides an addition to your normal care. Before you decide to take part it is important for you to understand why this research is being done and what it involves. Please take time to read this carefully and discuss it with others if you wish. Please ask us if anything is unclear or if you would like more information.

What is the purpose of the study?

The purpose of this study is to investigate the potential benefits of a naturally occurring plant extract, called **curcumin**, when it is combined with a type of chemotherapy called **FOLFOX** which is usually given to patients with advanced bowel cancer for up to 6 months.

Why have I been chosen?

You have been chosen because unfortunately you suffer from bowel cancer and as part of your care you will receive FOLFOX chemotherapy to try to shrink this. We are hoping to observe the effects in about 45 patients.

What is FOLFOX chemotherapy?

FOLFOX is usually the first choice of chemotherapy offered to patients with bowel cancer. It is a combination of 3 common chemotherapy medicines given together (fluorouracil, folinic acid and oxaliplatin) and injected into a vein every 2 weeks for up to 6 months. If you feel any side-effects from the chemotherapy, the dose or type of medicines can be altered.

What is Curcumin?

Curcumin is a natural substance found in turmeric, part of the Ginger family. It is a spice common in Asian food and used to make many biscuits, cheese and cereals. It is yellow food colouring (E100) and may have beneficial properties as a medicine.

Why add Curcumin to chemotherapy?

How well and how long any type of chemotherapy works for will be different for each patient, even after several cycles. We know from laboratory studies that curcumin can shrink tumours. It may also improve how chemotherapy works against tumours that are resistant to treatment. In this study we aim to apply these findings to see if patients can safely take curcumin while receiving chemotherapy and if there are any potential benefits from doing so.

In addition to this, FOLFOX can produce side-effects such as unusual or painful feelings in the hands and feet, called peripheral sensory neuropathy. Sometimes it is necessary to change the chemotherapy medicines to try and improve the side-effects but this may also effect how well therapy works or lead to other side-effects. We would like to investigate if curcumin can improve side-effects experienced.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you decide to take part you will be given this document to keep and asked to sign a consent form. If you decide to take part you are free to withdraw at any time and without giving a reason. A decision to not participate will not affect the standard of care you receive.

What do I have to do?

If after you have read this information and discussed it with an investigator you wish to take part, then we will ask you to sign the consent form accompanying this leaflet. We will take some blood tests and examine you to check it is safe for you to start the trial. Assuming there are no reasons preventing you taking part in the study, we will organise for you to start treatment and attend again for FOLFOX chemotherapy at Leicester Royal Infirmary.

What will happen to me if I take part?

You will receive the normal chemotherapy course of up to 12 cycles lasting about 6 months. During this time it is usual to attend for appointments and blood tests every two weeks. During and after chemotherapy, you would also have a series of CT ("CAT") body scans every 3 months for up to 2 years. You may need scans for up to 4 years.

Sometimes because we do not know which way of treating patients is best, we need to make comparisons. People will be put into 1 of 2 groups and then compared. The groups are selected by a computer which has no information about the individual – i.e. by chance. Patients in both groups will receive standard chemotherapy, but only one group will receive curcumin. If you agree to take part, you will have a 2 in 3 chance of being allocated to the group taking curcumin. You will only be told which group you are in once you have consented to take part. This is the normal way of doing things to help make the quality of the trial design as good as possible.

<u>If you are allocated to take curcumin with your chemotherapy (two thirds of patients)</u>
In addition to your chemotherapy, we would like you to take [1/2/4*] curcumin capsules by mouth, daily for as long as you can manage while you are having your course of chemotherapy, which can last for up to 6 months. We would also ask you to complete a short

questionnaire at the start and end of your course of curcumin to let us know your thoughts and experiences from taking it. [* precise dose will be decided from phase I]

You can take your curcumin capsules at any time of day but ideally together and roughly at the same time of day with meals. You can continue to take your normal medicines. Taking curcumin does not restrict you from any of your normal activities.

Whether or not you are taking curcumin with your chemotherapy (all patients)
To help monitor any side-effects you experience we would ask you to fill in a simple daily diary for the first 28 days. We would also like to monitor any changes in sensation you may feel in your hands and feet from the chemotherapy and would like you to complete a brief symptom questionnaire to help with this. At the start and end of your chemotherapy we would like you to complete a questionnaire to let us know if your quality of life has been affected in anyway during this study.

When you come to the department we will ask you to let us take some blood samples. Most extra samples required for the study will be performed as part of or at the same time as your routine tests and are taken at 5 of your visits. These consist of 4 small bottles (a total of about 4 teaspoons) to help with our study as well as measure your blood count, kidney and liver function as part of your regular health check while on chemotherapy.

Is curcumin safe?

Laboratory studies have not revealed any adverse effects of curcumin when it is used as a chemotherapy agent. Curcumin has been used in 18 clinical trials taken by over 350 patients. Two trials have given curcumin to patients taking chemotherapy. Curcumin has been given in doses of up to 16 grams (g) per day but because of the number of tablets required to give this amount and the bowel symptoms some patients feel at this dose, a better tolerated dose is probably less than half of this (< 8 g or 2 heaped teaspoons). The dose to be used in this study is [*500 mg/ 1 g/ 2 g] or [1/2/4 capsules*] and we will continue to monitor trial participants for any problems that may arise. [* precise dose will be decided from phase I]

Although curcumin has been given to patients on chemotherapy before it has not been given to patients receiving this type of chemotherapy. In the 1st stage of this study, we have already given curcumin at a very low dose to one group of patients receiving FOLFOX. We have then gradually increased the dose step-wise across further groups of patients. Completing this earlier study has helped us to decide what will most likely be a safe and well tolerated dose to take for other patients.

The dose is of a similar order of magnitude to what is found in a curry rich diet. Curcumin is not spicy hot and it comes in a capsule to swallow shown in the photograph (actual size).

Are there any side-effects from curcumin?

Most patients who have had curcumin do not report any side-effects. We know from our recent study in Leicester about 1 in 4 patients may feel some kind of mild bowel disturbance. Side-effects can include bloating, increased bowel movements, sometimes diarrhoea or abdominal pain and particularly at higher doses. Several studies have used up to 4 g per day and for up to 4 months without severe side-effects. This study will use a lower dose of no more than 2 g a day (1 level teaspoon).

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

You will not be able to take part if you meet any of the following criteria:

- If you have had any previous chemotherapy for cancer in the last 5 years except bowel cancer.
- If you have had a cancer other than bowel cancer. Exceptions to this are skin cancer (other than melanoma) or early breast or cervical cancer, or if you have had treatment to cure any other cancer and have remained in remission for at least 5 years
- If you have an allergy to turmeric
- If you have any medical conditions that would mean treatment with chemotherapy might be harmful – including uncontrolled heart failure, recent heart attack or stroke, liver disease, kidney disease
- If you have HIV or AIDS
- If you have had a major operation or injury within 4 weeks of treatment
- If you are female, you must have undergone a surgical sterilisation, be post-menopause, or agree to use two adequate forms of contraception at all times during treatment and for at least 6 months after the last dose of FOLFOX. If you are male and your partner has childbearing potential, you must have been surgically sterilised or agree to use adequate contraception.
- If you are breast feeding
- You have peptic ulcers or colitis proven on endoscopy, recurrent pain from abdominal adhesions or chronic diarrhoea.

In addition, if either you or your doctor should decide that you are not suitable to receive chemotherapy, then you would also not be able to enter the trial

Am I safe to take part?

If you agree to take part in this trial, you will have blood tests and an examination by a doctor to check that it is safe for you to receive chemotherapy. Assuming everything is fine, you will commence your treatment. During the trial, you will regularly be seen by a doctor or nurse. Should you experience any side effects or abnormalities in the blood tests, this may require the treatment to be altered or stopped. This happens to all patients on chemotherapy.

What are the extra requirements of being in the trial?

There are very few extra requirements in addition to the standard care pathway other than those mentioned already. If you agree to enter the study you will start taking oral curcumin one week before starting FOLFOX. This will require 1/2/4* capsules taken with food once a

day. This will continue for the duration of your chemotherapy. During this time you will be able to eat and drink as you would normally and take your normal medications. [* precise dose will be decided from phase I]

What are the additional blood tests for?

We will measure the level of curcumin in your blood. It is broken down by the body very quickly and it is useful to know if chemotherapy alters this. We will also perform experiments to look at proteins in the blood that may help detect or measure disease and the response to treatments. We also intend to measure the amount of platinum from the chemotherapy that attaches to DNA in cells (we are NOT looking at your genes or doing genetic research). Developing these experiments is important for future research and we request your permission to store your blood samples for analysis in future studies looking at proteins in your blood. This is optional.

Are there any other problems I might encounter and what do I do about them?

Before you agree to treatment your Oncologist (cancer doctor) will discuss possible side effects of FOLFOX with you. Many patients experience peripheral sensory neuropathy requiring alteration to the chemotherapy. FOLFOX can sometimes cause problems to the heart, kidneys and liver which is why we monitor everybody closely with regular check-ups.

Curcumin has been used in many trials and at higher doses without significant drug related toxicity. It has so far been shown to be safe. Some patients have had bowel disturbances (bloating and loose stool) at higher doses which is one of our reasons for choosing a lower dose. We will monitor any side-effects by the diary that you fill in. You should record symptoms in the diary or report them to an investigator or nurse if you have concerns.

What are the possible disadvantages and risks of taking part?

The potential risks of this trial are virtually identical to those that would occur should you not participate in this trial and were taking FOLFOX. Potential side-effects of curcumin have been explained and are mostly mild bowel disturbances. In addition, there will be the possible inconvenience of taking curcumin capsules every day for up to 6 months, provide extra blood samples, fill in questionnaires and a drug diary.

What are the possible benefits of taking part?

This is an exploratory study and therefore there may be no direct benefits. The information we obtain is important for future therapies and study design.

Curcumin has been shown to have beneficial effects against cancer cells in laboratory experiments. Recently, curcumin has made a small difference in other forms of cancer (pancreas) when combined with chemotherapy. Some experiments suggest curcumin has potential to improve peripheral sensory neuropathy. We will investigate if the neuropathy symptoms from FOLFOX alter if curcumin is used with it.

Will I receive any financial benefit for taking part?

You will not receive any financial benefit for taking part in the study. However, if you are required to make extra hospital visits than you would on standard treatment, we will reimburse any additional parking/travel expenses that you incur.

What if new information becomes available?

Sometimes during the course of a study, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. They will explain the reasons and arrange for your care to continue.

What happens when the research study stops?

Treatment with the FOLFOX and curcumin will continue until one of several reasons for stopping treatment:

1. Cancer is no longer controlled by the treatment and has grown. This is the reason why you will have CT scans (CAT scans) every 3 months.
2. Side effects of the FOLFOX become intolerable to you.
3. Side effects of the curcumin become intolerable to you.
4. You change you mind about being in the trial.
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Contact for Further Information

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Finally, thank you very much for taking the time to read this information. If you would like to take part, we will ask you to sign a consent form, provide a copy of the signed consent form and this information sheet to keep.

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Prof William Steward
Consultant Oncologist and Head of Cancer Studies
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2nd floor Osborne Building
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LE1 5WW
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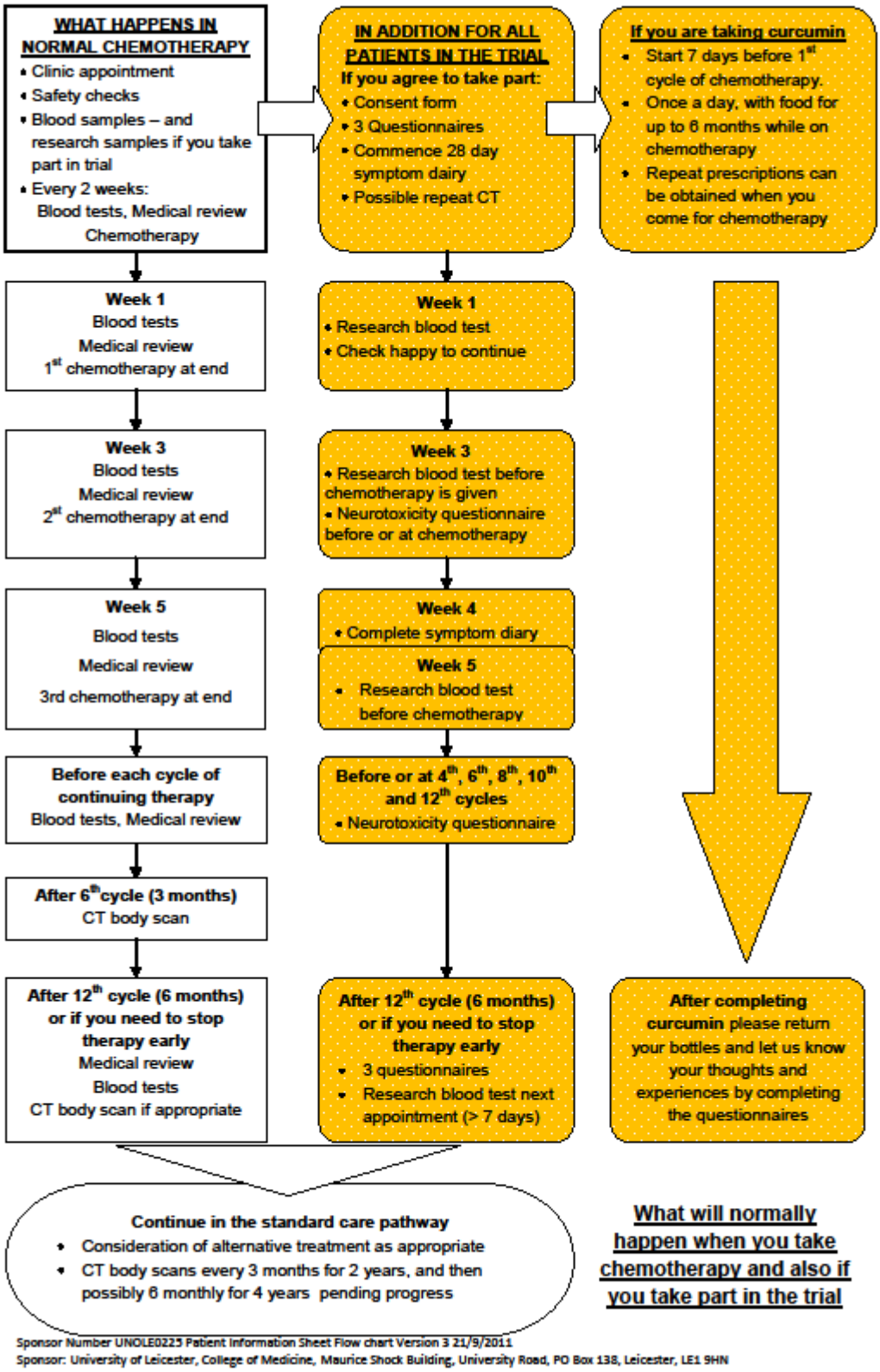
8.8. **Patient information flowchart**



8.9. **Phase IIa adverse events**

Table A.1 Adverse events experienced by patients in Phase IIa

Adverse Events	N° - Grade				Precentage (%) - Grade			
	1/2	3	4/5	Total	1/2	3	4/5	Total
Abdominal bloating	1	0	0	1	100%	0%	0%	0%
Abdominal pain	4	1	0	5	80%	20%	0%	2%
Acute kidney injury	2	0	0	2	100%	0%	0%	1%
Agitation	1	0	0	1	100%	0%	0%	0%
Allergic reaction	1	0	0	1	100%	0%	0%	0%
Alopecia	3	0	0	3	100%	0%	0%	1%
Altered vision	3	0	0	3	100%	0%	0%	1%
Anaemia	1	0	0	1	100%	0%	0%	0%
Anorexia	6	1	0	7	86%	14%	0%	3%
Chest infection	3	0	0	3	100%	0%	0%	1%
Colonic obstruction	0	1	1	2	0%	50%	50%	1%
Constipation	5	0	0	5	100%	0%	0%	2%
Cough	3	0	0	3	100%	0%	0%	1%
Diarrhoea	10	1	0	11	91%	9%	0%	5%
Dizziness	5	0	0	5	100%	0%	0%	2%
Dry mouth	2	0	0	2	100%	0%	0%	1%
Dry skin	2	0	0	2	100%	0%	0%	1%
Dysgeusia	7	0	0	7	100%	0%	0%	3%
Dyspepsia	9	0	0	9	100%	0%	0%	4%
Dyspnoea	2	0	0	2	100%	0%	0%	1%
Epistaxis	5	0	0	5	100%	0%	0%	2%
Fatigue/Lethargy	26	1	0	27	96%	4%	0%	11%
Fracture	1	0	0	1	100%	0%	0%	0%
Flatulence	2	0	0	2	100%	0%	0%	1%
Headache	3	0	0	3	100%	0%	0%	1%
Hiccups	1	0	0	1	100%	0%	0%	0%
Hyperkalaemia	2	1	0	3	67%	33%	0%	1%
Hyperpigmentation	1	0	0	1	100%	0%	0%	0%
Hypertension	4	0	0	4	100%	0%	0%	2%
Hypokalaemia	1	1	0	2	50%	50%	0%	1%
Infection (non-neutropenic)	6	0	0	6	100%	0%	0%	3%
Insomnia	2	0	0	2	100%	0%	0%	1%
Laryngitis	2	0	0	2	100%	0%	0%	1%
Musculoskeletal (other)	1	0	0	1	100%	0%	0%	0%
Nasal congestion/Corysal	3	0	0	3	100%	0%	0%	1%

No of AEs	Nº - Grade				Precentage (%) - Grade			
	1/2	3	4/5	Total	1/2	3	4/5	Total
Nausea	10	1	0	11	91%	9%	0%	5%
Neutropenia	2	1	0	3	67%	33%	0%	1%
Neutropenic sepsis	0	1	0	1	0%	100%	0%	0%
Oral Mucositis	9	0	0	9	100%	0%	0%	4%
Pain (not abdominal)	7	0	0	7	100%	0%	0%	3%
Peripheral oedema	2	0	0	2	100%	0%	0%	1%
Proteinuria	1	0	0	1	100%	0%	0%	0%
Pruritis	1	0	0	1	100%	0%	0%	0%
Pyrexia	3	0	0	3	100%	0%	0%	1%
Rash	1	0	0	1	100%	0%	0%	0%
Raised ALP	0	1	0	1	0%	100%	0%	0%
Sensory neuropathy	21	1	0	22	95%	5%	0%	9%
Sepsis (not neutropenic)	0	0	1	1	0%	0%	100%	0%
Surgical procedure (other)	0	1	0	1	0%	100%	0%	0%
Syncope	2	0	0	2	100%	0%	0%	1%
Thirst	1	0	0	1	100%	0%	0%	0%
Thrombocytopenia	11	1	0	12	92%	8%	0%	5%
Thromboembolic event	0	1	0	1	0%	100%	0%	0%
Tremor	1	0	0	1	100%	0%	0%	0%
Urinary frequency	2	0	0	2	100%	0%	0%	1%
UTI	5	0	0	5	100%	0%	0%	2%
Voice alteration	1	0	0	1	100%	0%	0%	0%
Vomiting	7	1	0	8	88%	13%	0%	3%
Weight loss	4	1	0	5	80%	20%	0%	2%

8.10. **Graphs of patient miRNA measurements**

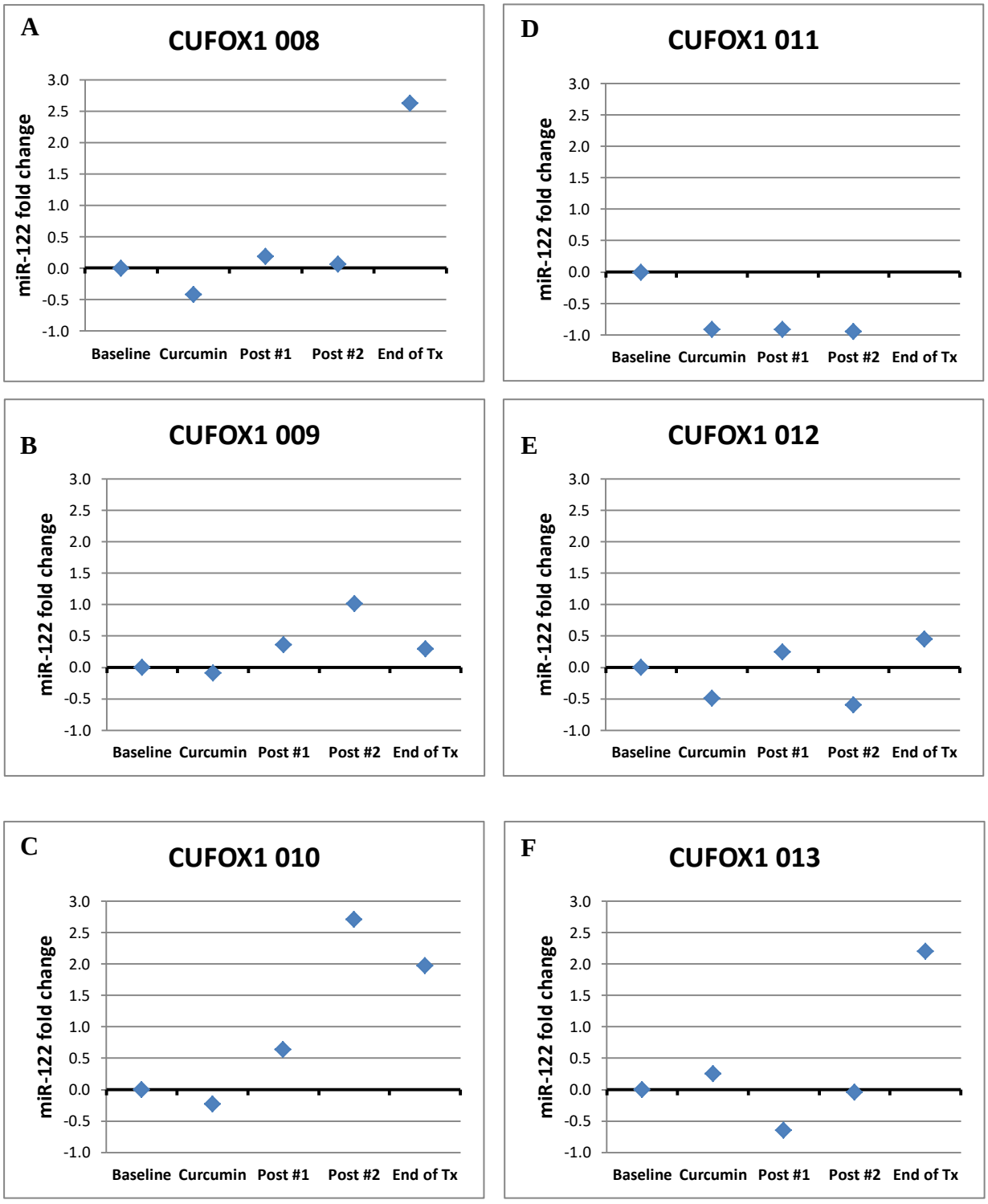


Figure A.1 – miR-122 fold-changes over treatment time points. Results for patients CUFOX2A 008 (**A**), CUFOX2A 009 (**B**), CUFOX2A 010 (**C**), CUFOX2A 011 (**D**), CUFOX2A 012 (**E**), and CUFOX2A 013 (**F**). Fold changes in miR-122 readings plotted against time points: Baseline, Curcumin - after 1 week of curcumin only, Post #1 - after cycle 1 chemotherapy, Post #2 - after cycle 2 chemotherapy, End of Tx – end of treatment.

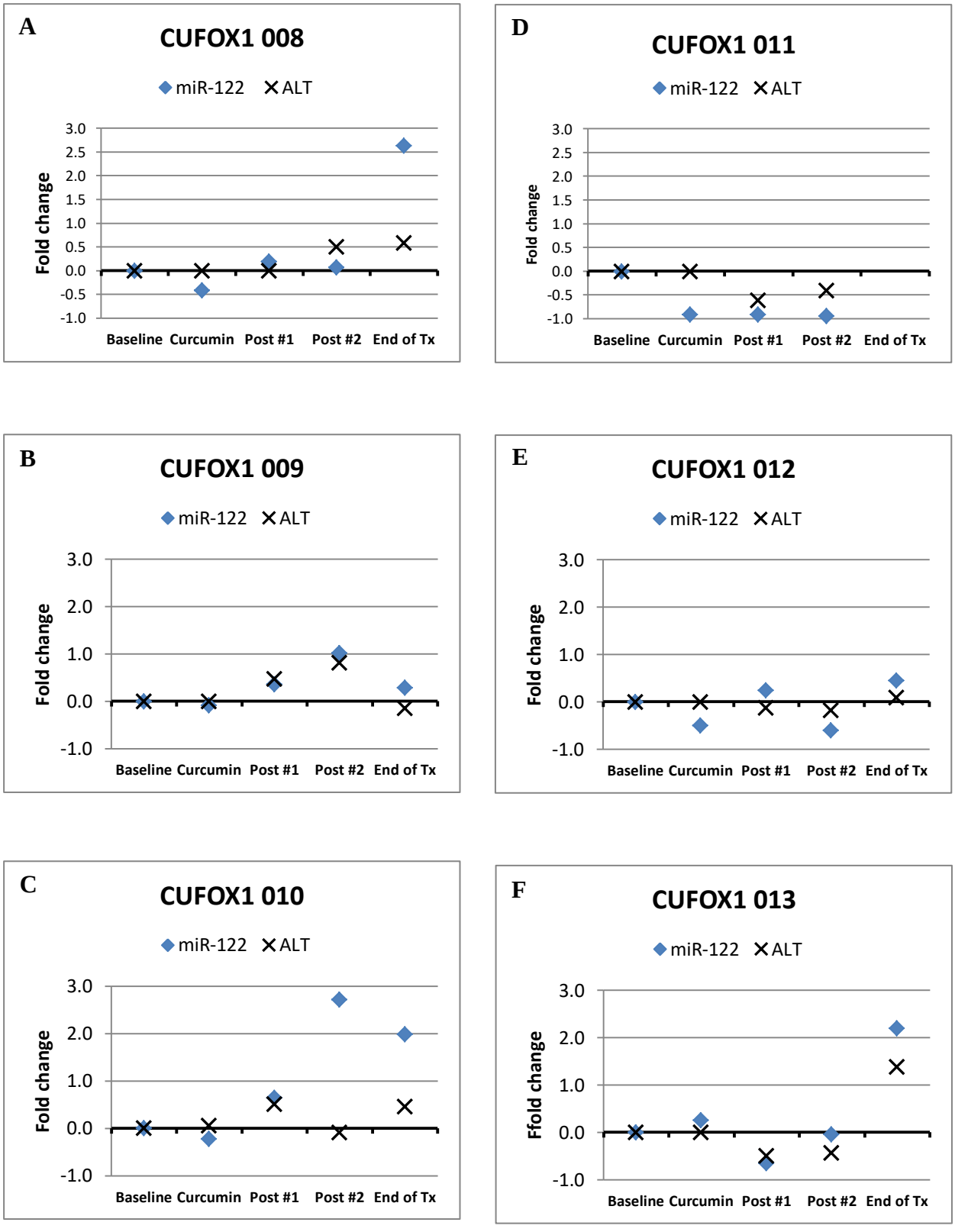


Figure A.2 – Comparison of miR-122 versus ALT fold-changes over treatment time points. Results for patients CUFOX2A 008 (**A**), CUFOX2A 009 (**B**), CUFOX2A 010 (**C**), CUFOX2A 011 (**D**), CUFOX2A 012 (**E**), and CUFOX2A 013 (**F**). Fold changes in miR-122 and ALT readings plotted against time points: Baseline, Curcumin - after 1 week of curcumin, Post #1 – after cycle 1 chemotherapy, Post #2 – after cycle 2 chemotherapy, End of Tx – End

Table A.2 Phase I DNA platination results

		Platinum concentrations (ppt)			Std Dev			%RSD		
		194Pt	195Pt	196Pt	194Pt	195Pt	196Pt	194Pt	195Pt	196Pt
Sample	1-1	48.55	48.99	48.54	3.78	5.31	3.48	7.78	10.84	7.16
Sample	1-2	55.05	57.06	56.41	6.27	4.27	6.26	11.39	7.49	11.10
Sample	1-3	23.85	25.12	24.02	2.82	0.64	0.91	11.81	2.56	3.79
Sample	1-4	25.78	26.82	26.42	0.73	0.77	2.21	2.81	2.86	8.36
Sample	1-5	19.49	19.01	20.49	0.41	0.31	1.21	2.08	1.64	5.93
Sample	2-1	21.35	22.40	22.16	1.24	2.45	0.55	5.80	10.93	2.49
Sample	2-2	26.72	25.64	26.83	1.66	0.85	0.14	6.23	3.32	0.51
Sample	2-3	37.25	37.82	38.05	4.86	6.37	5.41	13.06	16.84	14.21
Sample	2-4	48.40	47.84	51.10	9.20	6.97	6.49	19.00	14.57	12.69
Sample	2-5	22.34	23.08	23.90	2.52	1.78	3.06	11.27	7.73	12.81
Sample	3-1	13.86	13.16	14.56	0.94	0.91	0.06	6.77	6.94	0.38
Sample	3-2	14.63	15.01	13.91	0.11	0.25	0.08	0.73	1.66	0.60
Sample	3-3	26.17	27.71	27.30	1.96	3.78	2.40	7.50	13.63	8.80
Sample	3-4	17.54	16.90	16.99	0.34	0.48	2.90	1.95	2.82	17.05
Sample	3-5	19.34	20.07	21.87	11.07	11.68	11.89	57.25	58.20	54.37
Sample	4-1	14.39	15.30	16.10	2.37	2.03	2.62	16.45	13.29	16.29
Sample	4-2	58.05	58.20	57.93	1.09	0.33	2.07	1.87	0.57	3.57
Sample	4-3	33.87	35.25	35.61	8.62	9.50	7.92	25.45	26.95	22.24
Sample	4-4	32.93	33.86	33.73	3.37	0.93	2.40	10.24	2.76	7.12
Sample	4-5	22.18	23.22	24.12	1.17	0.58	3.09	5.29	2.50	12.82
Sample	6-1	30.70	30.91	33.38	1.28	2.20	0.86	4.17	7.12	2.56
Sample	6-2	35.59	36.91	37.87	6.53	5.08	3.45	18.35	13.77	9.11
Sample	6-3	795.00	795.60	792.80	29.80	35.58	51.70	3.75	4.47	6.52
Sample	6-4	38.12	37.03	38.24	4.91	4.21	5.13	12.87	11.37	13.42
Sample	6-5	128.60	128.50	128.70	19.55	23.97	16.94	15.20	18.65	13.16
Sample	7-1	39.04	38.53	39.24	5.44	4.75	4.55	13.93	12.33	11.60
Sample	7-2	78.92	77.51	80.64	8.24	7.55	7.92	10.44	9.75	9.82
Sample	7-3	69.73	69.92	72.21	1.22	1.06	3.84	1.74	1.51	5.31
Sample	7-4	25.56	27.16	27.69	3.09	3.78	4.50	12.11	13.91	16.25
Sample	7-5	13.12	14.45	14.36	1.00	1.41	0.61	7.64	9.76	4.23
Sample	8-1	20.11	20.07	20.35	0.73	1.81	0.25	3.61	8.99	1.22
Sample	8-2	93.23	95.26	95.74	8.94	2.28	2.79	9.59	2.40	2.91
Sample	8-3	38.83	38.72	40.06	6.12	3.69	3.95	15.77	9.54	9.85
Sample	8-4	26.60	26.17	27.12	1.75	0.23	0.99	6.58	0.87	3.66
Sample	8-5	30.29	33.63	31.24	0.06	0.98	0.30	0.21	2.90	0.97
Sample	9-1	50.27	50.79	52.00	5.40	4.42	1.35	10.74	8.70	2.60
Sample	9-2	29.10	29.37	29.56	7.77	6.74	8.14	26.69	22.96	27.54
Sample	9-3	15.04	15.02	14.77	0.85	0.52	0.64	5.67	3.45	4.30
Sample	9-4	16.46	16.72	18.38	0.85	0.93	1.49	5.19	5.58	8.11
Sample	9-5	17.79	16.94	19.04	0.09	0.83	0.06	0.48	4.90	0.29

		Platinum concentrations (ppt)			Std Dev			%RSD		
		194Pt	**195Pt**	**196Pt**	**194Pt**	**195Pt**	**196Pt**	**194Pt**	**195Pt**	**196Pt**
Sample	**10-1**	26.58	27.57	26.65	1.47	1.12	2.70	5.54	4.06	10.15
Sample	**10-2**	16.61	16.45	17.52	2.35	1.54	2.26	14.13	9.34	12.91
Sample	**10-3**	16.87	18.12	18.57	0.41	0.66	0.61	2.40	3.66	3.27
Sample	**10-4**	104.90	103.10	103.70	8.22	4.57	8.14	7.83	4.43	7.85
Sample	**10-5**	1.03	1.00	1.29	0.11	0.10	0.33	10.40	10.40	25.71
Sample	**12-4**	73.65	75.04	72.90	2.75	0.62	0.99	3.74	0.83	1.36
Sample	**13-2**	40.19	40.58	40.58	8.43	6.70	11.37	20.97	16.51	28.01

8.11. **Proteomics**

Table A.3 Proteins upregulated following treatment with CUFOX

1	Ig kappa chain V-I region Roy OS=Homo sapiens PE=1 SV=1
2	Receptor-interacting serine/threonine-protein kinase 3 OS=Homo sapiens GN=RIPK3 PE=1 SV=2
3	Isoform 5 of Cytosolic acyl coenzyme A thioester hydrolase OS=Homo sapiens GN=ACOT7
4	60S acidic ribosomal protein P0-like OS=Homo sapiens GN=RPLP0P6 PE=5 SV=1
5	14-3-3 protein gamma OS=Homo sapiens GN=YWHAG PE=1 SV=2
6	Desmoglein-2 OS=Homo sapiens GN=DSG2 PE=1 SV=2
7	Tyrosine-protein kinase Tec OS=Homo sapiens GN=TEC PE=1 SV=2
8	Mucolipin-2 OS=Homo sapiens GN=MCOLN2 PE=2 SV=2
9	Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4
10	Xaa-Pro dipeptidase OS=Homo sapiens GN=PEPD PE=1 SV=3
11	Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=1 SV=4
12	Inositol 1,4,5-trisphosphate receptor type 1 OS=Homo sapiens GN=ITPR1 PE=1 SV=3
13	Uncharacterized protein C6orf203 OS=Homo sapiens GN=C6orf203 PE=1 SV=1
14	Isoform 3 of Kin of IRRE-like protein 1 OS=Homo sapiens GN=KIRREL
15	Docking protein 2 OS=Homo sapiens GN=DOK2 PE=1 SV=2
16	X-ray repair cross-complementing protein 5 OS=Homo sapiens GN=XRCC5 PE=1 SV=3
17	Dipeptidyl peptidase 3 OS=Homo sapiens GN=DPP3 PE=1 SV=2
18	E3 ubiquitin-protein ligase RAD18 OS=Homo sapiens GN=RAD18 PE=1 SV=2
19	Sentrin-specific protease 7 OS=Homo sapiens GN=SENP7 PE=1 SV=4
20	Cadherin-17 OS=Homo sapiens GN=CDH17 PE=1 SV=3
21	Protein Red OS=Homo sapiens GN=IK PE=1 SV=3
22	Hypoxanthine-guanine phosphoribosyltransferase OS=Homo sapiens GN=HPRT1 PE=1 SV=2
23	Inter-alpha-trypsin inhibitor heavy chain H3 OS=Homo sapiens GN=ITIH3 PE=1 SV=2
24	Peroxiredoxin-4 OS=Homo sapiens GN=PRDX4 PE=1 SV=1
25	Polypyrimidine tract-binding protein 1 OS=Homo sapiens GN=PTBP1 PE=1 SV=1
26	Ribonuclease UK114 OS=Homo sapiens GN=HRSP12 PE=1 SV=1
27	Tau-tubulin kinase 1 OS=Homo sapiens GN=TTBK1 PE=1 SV=2
28	Centromere protein J OS=Homo sapiens GN=CENPJ PE=1 SV=2
29	Histone H3.1t OS=Homo sapiens GN=HIST3H3 PE=1 SV=3
30	Chromobox protein homolog 3 OS=Homo sapiens GN=CBX3 PE=1 SV=4
31	Protein S100-A9 OS=Homo sapiens GN=S100A9 PE=1 SV=1
32	Killer cell immunoglobulin-like receptor 3DL1 OS=Homo sapiens GN=KIR3DL1 PE=1 SV=1
33	DNA-directed RNA polymerase III subunit RPC4 OS=Homo sapiens GN=POLR3D PE=1 SV=2
34	Vitamin D-binding protein OS=Homo sapiens GN=GC PE=1 SV=1
35	F-actin-capping protein subunit alpha-1 OS=Homo sapiens GN=CAPZA1 PE=1 SV=3
36	Heterogeneous nuclear ribonucleoprotein A1-like 2 OS=Homo sapiens GN=HNRNPA1L2 PE=2 SV=2
37	Isoform 2 of Girdin OS=Homo sapiens GN=CCDC88A
38	Latent-transforming growth factor beta-binding protein 4 OS=Homo sapiens GN=LTBP4 PE=1 SV=2
39	4-aminobutyrate aminotransferase, mitochondrial OS=Homo sapiens GN=ABAT PE=1 SV=3
40	Isoform ASF-3 of Serine/arginine-rich splicing factor 1 OS=Homo sapiens GN=SRSF1

41	AF4/FMR2 family member 4 OS=Homo sapiens GN=AFF4 PE=1 SV=1
42	Neutrophil gelatinase-associated lipocalin OS=Homo sapiens GN=LCN2 PE=1 SV=2
43	Heat shock 70 kDa protein 6 OS=Homo sapiens GN=HSPA6 PE=1 SV=2
44	Isoform 2 of Centrosomal protein of 164 kDa OS=Homo sapiens GN=CEP164
45	Ig kappa chain V-III region GOL OS=Homo sapiens PE=1 SV=1
46	Septin-7 OS=Homo sapiens GN=SEPT7 PE=1 SV=2
47	Isoform 2 of Sialate O-acetylesterase OS=Homo sapiens GN=SIAE
48	Isoform 5 of Otoferlin OS=Homo sapiens GN=OTOF
49	Protein disulfide-isomerase A4 OS=Homo sapiens GN=PDIA4 PE=1 SV=2
50	Lactotransferrin OS=Homo sapiens GN=LTF PE=1 SV=6
51	Cullin-associated NEDD8-dissociated protein 1 OS=Homo sapiens GN=CAND1 PE=1 SV=2
52	Coiled-coil domain-containing protein 17 OS=Homo sapiens GN=CCDC17 PE=2 SV=2
53	Keratin, type II cytoskeletal 4 OS=Homo sapiens GN=KRT4 PE=1 SV=4
54	AP-2 complex subunit beta OS=Homo sapiens GN=AP2B1 PE=1 SV=1
55	Keratin, type I cytoskeletal 17 OS=Homo sapiens GN=KRT17 PE=1 SV=2
56	Bifunctional epoxide hydrolase 2 OS=Homo sapiens GN=EPHX2 PE=1 SV=2
57	Heterogeneous nuclear ribonucleoprotein C-like 1 OS=Homo sapiens GN=HNRNPCL1 PE=1 SV=1
58	Isoform 4 of Liprin-alpha-2 OS=Homo sapiens GN=PPFIA2
59	Deoxycytidylate deaminase OS=Homo sapiens GN=DCTD PE=1 SV=2
60	Phosphoglycerate kinase 1 OS=Homo sapiens GN=PGK1 PE=1 SV=3
61	Proteasome subunit beta type-4 OS=Homo sapiens GN=PSMB4 PE=1 SV=4
62	Fibrinogen beta chain OS=Homo sapiens GN=FGB PE=1 SV=2
63	6-phosphogluconate dehydrogenase, decarboxylating OS=Homo sapiens GN=PGD PE=1 SV=3
64	AMP deaminase 3 OS=Homo sapiens GN=AMPD3 PE=1 SV=1
65	Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6
66	Cleavage stimulation factor subunit 3 OS=Homo sapiens GN=CSTF3 PE=1 SV=1
67	Isoform 2 of Septin-11 OS=Homo sapiens GN=SEPT11
68	Isoform 3 of Calumenin OS=Homo sapiens GN=CALU
69	Exonuclease 3'-5' domain-containing protein 1 OS=Homo sapiens GN=EXD1 PE=2 SV=4
70	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
71	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2
72	Isoform 4 of Ankyrin repeat and KH domain-containing protein 1 OS=Homo sapiens GN=ANKHD1
73	Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens GN=IDH2 PE=1 SV=2
74	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens GN=ITIH2 PE=1 SV=2
75	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3
76	Isoform 1 of Four and a half LIM domains protein 1 OS=Homo sapiens GN=FHL1
77	Isoform 1c of Oxysterol-binding protein-related protein 3 OS=Homo sapiens GN=OSBPL3
78	Myosin-6 OS=Homo sapiens GN=MYH6 PE=1 SV=5
79	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1
80	Adenylyl cyclase-associated protein 2 OS=Homo sapiens GN=CAP2 PE=1 SV=1
81	Epididymal secretory protein E1 OS=Homo sapiens GN=NPC2 PE=1 SV=1
82	mRNA turnover protein 4 homolog OS=Homo sapiens GN=MRTO4 PE=1 SV=2
83	Nucleophosmin OS=Homo sapiens GN=NPM1 PE=1 SV=2
84	Interleukin-13 receptor subunit alpha-1 OS=Homo sapiens GN=IL13RA1 PE=1 SV=1

85	Short transient receptor potential channel 1 OS=Homo sapiens GN=TRPC1 PE=1 SV=1
86	Inorganic pyrophosphatase 2, mitochondrial OS=Homo sapiens GN=PPA2 PE=1 SV=2
87	MAP kinase-activated protein kinase 3 OS=Homo sapiens GN=MAPKAPK3 PE=1 SV=1
88	Dynein heavy chain 17, axonemal OS=Homo sapiens GN=DNAH17 PE=1 SV=2
89	Leukocyte elastase inhibitor OS=Homo sapiens GN=SERPINB1 PE=1 SV=1
90	Brain acid soluble protein 1 OS=Homo sapiens GN=BASP1 PE=1 SV=2
91	Adenylyl cyclase-associated protein 1 OS=Homo sapiens GN=CAP1 PE=1 SV=5
92	Ubiquitin carboxyl-terminal hydrolase 5 OS=Homo sapiens GN=USP5 PE=1 SV=2
93	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4
94	Acidic leucine-rich nuclear phosphoprotein 32 family member A OS=Homo sapiens GN=ANP32A PE=1 SV=1
95	UPF0686 protein C11orf1 OS=Homo sapiens GN=C11orf1 PE=2 SV=1
96	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1
97	Keratin, type II cytoskeletal 7 OS=Homo sapiens GN=KRT7 PE=1 SV=5
98	Isoform 2 of Ubiquitin carboxyl-terminal hydrolase 45 OS=Homo sapiens GN=USP45
99	INO80 complex subunit C OS=Homo sapiens GN=INO80C PE=1 SV=1
100	Coiled-coil domain-containing protein 88B OS=Homo sapiens GN=CCDC88B PE=1 SV=1
101	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4
102	Actin-related protein 3 OS=Homo sapiens GN=ACTR3 PE=1 SV=3
103	Zinc finger protein Rlf OS=Homo sapiens GN=RLF PE=1 SV=2
104	Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3
105	Isoform 8 of YY1-associated protein 1 OS=Homo sapiens GN=YY1AP1
106	Glutathione peroxidase 3 OS=Homo sapiens GN=GPX3 PE=1 SV=2
107	Isoform 4 of Clusterin OS=Homo sapiens GN=CLU
108	Ig kappa chain V-II region Cum OS=Homo sapiens PE=1 SV=1
109	LIM/homeobox protein Lhx5 OS=Homo sapiens GN=LHX5 PE=2 SV=1
110	Alpha-actinin-1 OS=Homo sapiens GN=ACTN1 PE=1 SV=2
111	Complement factor B OS=Homo sapiens GN=CFB PE=1 SV=2
112	Teneurin-3 OS=Homo sapiens GN=TENM3 PE=2 SV=3
113	Dystonin OS=Homo sapiens GN=DST PE=1 SV=4
114	60S ribosomal protein L30 OS=Homo sapiens GN=RPL30 PE=1 SV=2
115	Vinculin OS=Homo sapiens GN=VCL PE=1 SV=4
116	Glucose 1,6-bisphosphate synthase OS=Homo sapiens GN=PGM2L1 PE=1 SV=3
117	Alpha-actinin-2 OS=Homo sapiens GN=ACTN2 PE=1 SV=1
118	Isoform 2 of La-related protein 1B OS=Homo sapiens GN=LARP1B
119	Gelsolin OS=Homo sapiens GN=GSN PE=1 SV=1
120	Isoform 3 of Heterogeneous nuclear ribonucleoprotein D0 OS=Homo sapiens GN=HNRNPD
121	Ubiquitin carboxyl-terminal hydrolase 48 OS=Homo sapiens GN=USP48 PE=1 SV=1
122	C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens GN=MTHFD1 PE=1 SV=3
123	Plexin-A3 OS=Homo sapiens GN=PLXNA3 PE=1 SV=2
124	Speedy protein E3 OS=Homo sapiens GN=SPDYE3 PE=2 SV=2
125	Barrier-to-autointegration factor OS=Homo sapiens GN=BANF1 PE=1 SV=1
126	Uncharacterized protein KIAA1614 OS=Homo sapiens GN=KIAA1614 PE=2 SV=3
127	Heterogeneous nuclear ribonucleoprotein D-like OS=Homo sapiens GN=HNRNPDL PE=1 SV=3
128	Fructose-1,6-bisphosphatase 1 OS=Homo sapiens GN=FBP1 PE=1 SV=5

129	Isoform 4 of Bromodomain adjacent to zinc finger domain protein 2B OS=Homo sapiens GN=BAZ2B
130	Neuropilin and tolloid-like protein 2 OS=Homo sapiens GN=NETO2 PE=1 SV=1
131	Putative beta-actin-like protein 3 OS=Homo sapiens GN=POTEKP PE=5 SV=1
132	Leucine-rich repeat and calponin homology domain-containing protein 4 OS=Homo sapiens GN=LRCH4 PE=1 SV=2
133	Baculoviral IAP repeat-containing protein 1 OS=Homo sapiens GN=NAIP PE=1 SV=3
134	Neurofilament light polypeptide OS=Homo sapiens GN=NEFL PE=1 SV=3
135	Histone H2B type 1-O OS=Homo sapiens GN=HIST1H2BO PE=1 SV=3
136	Pantetheinase OS=Homo sapiens GN=VNN1 PE=1 SV=2
137	Isoform A1 of CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase OS=Homo sapiens GN=ST3GAL3
138	Transforming growth factor-beta-induced protein ig-h3 OS=Homo sapiens GN=TGFBI PE=1 SV=1
139	Metalloproteinase inhibitor 1 OS=Homo sapiens GN=TIMP1 PE=1 SV=1
140	Protein shisa-7 OS=Homo sapiens GN=SHISA7 PE=2 SV=3
141	Filamin-B OS=Homo sapiens GN=FLNB PE=1 SV=2
142	Pikachurin OS=Homo sapiens GN=EGFLAM PE=1 SV=2
143	Alpha-protein kinase 2 OS=Homo sapiens GN=ALPK2 PE=2 SV=3
144	Glutathione synthetase OS=Homo sapiens GN=GSS PE=1 SV=1
145	Eukaryotic translation initiation factor 4E OS=Homo sapiens GN=EIF4E PE=1 SV=2
146	Ribonuclease inhibitor OS=Homo sapiens GN=RNH1 PE=1 SV=2
147	Isoform 2 of SH2 domain-containing protein 3C OS=Homo sapiens GN=SH2D3C
148	Gamma-enolase OS=Homo sapiens GN=ENO2 PE=1 SV=3
149	Peroxiredoxin-5, mitochondrial OS=Homo sapiens GN=PRDX5 PE=1 SV=4
150	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2
151	Endoplasmic reticulum resident protein 44 OS=Homo sapiens GN=ERP44 PE=1 SV=1
152	Proliferation-associated protein 2G4 OS=Homo sapiens GN=PA2G4 PE=1 SV=3
153	HERV-MER_4q12 provirus ancestral Env polyprotein
154	Protein SOGA2 OS=Homo sapiens GN=SOGA2 PE=1 SV=5
155	Cystatin-C OS=Homo sapiens GN=CST3 PE=1 SV=1
156	Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5
157	Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1
158	Putative inactive carboxylesterase 4 OS=Homo sapiens GN=CES1P1 PE=5 SV=2
159	Isoform 2 of Carbonic anhydrase 12 OS=Homo sapiens GN=CA12
160	Bile salt-activated lipase OS=Homo sapiens GN=CEL PE=1 SV=3
161	Heat shock protein 105 kDa OS=Homo sapiens GN=HSPH1 PE=1 SV=1
162	Scaffold attachment factor B1 OS=Homo sapiens GN=SAFB PE=1 SV=4
163	Isoform 3 of Plasminogen activator inhibitor 1 RNA-binding protein OS=Homo sapiens GN=SERBP1
164	Myb-related protein B OS=Homo sapiens GN=MYBL2 PE=1 SV=1
165	Isoform 3 of Kinesin-like protein KIF1B OS=Homo sapiens GN=KIF1B
166	Dual specificity mitogen-activated protein kinase kinase 1
167	Lamin-B2 OS=Homo sapiens GN=LMNB2 PE=1 SV=3
168	Protein NLRC5 OS=Homo sapiens GN=NLRC5 PE=1 SV=3
169	Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2
170	E3 ubiquitin-protein ligase UBR4 OS=Homo sapiens GN=UBR4 PE=1 SV=1

171	Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1
172	Profilin-1 OS=Homo sapiens GN=PFN1 PE=1 SV=2
173	Putative heat shock protein HSP 90-beta-3 OS=Homo sapiens GN=HSP90AB3P PE=5 SV=1
174	Cytosol aminopeptidase OS=Homo sapiens GN=LAP3 PE=1 SV=3
175	Isoform 2 of Nucleoside diphosphate kinase A OS=Homo sapiens GN=NME1
176	Cytochrome P450 4F12 OS=Homo sapiens GN=CYP4F12 PE=1 SV=2
177	Monocyte differentiation antigen CD14 OS=Homo sapiens GN=CD14 PE=1 SV=2
178	Glial fibrillary acidic protein OS=Homo sapiens GN=GFAP PE=1 SV=1
179	T-complex protein 1 subunit eta OS=Homo sapiens GN=CCT7 PE=1 SV=2
180	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1
181	Type II inositol 3,4-bisphosphate 4-phosphatase OS=Homo sapiens GN=INPP4B PE=2 SV=4
182	Intermediate filament family orphan 1 OS=Homo sapiens GN=IFFO1 PE=2 SV=2
183	Nesprin-2 OS=Homo sapiens GN=SYNE2 PE=1 SV=3
184	Isoform 2 of Suppressor of IKBKE 1 OS=Homo sapiens GN=SIKE1
185	Spectrin beta chain, non-erythrocytic 4 OS=Homo sapiens GN=SPTBN4 PE=1 SV=2
186	Small nuclear ribonucleoprotein-associated proteins B and B'
187	Isoform LAMP-2B of Lysosome-associated membrane glycoprotein 2 OS=Homo sapiens GN=LAMP2
188	Histone H2B type 1-D OS=Homo sapiens GN=HIST1H2BD PE=1 SV=2
189	WD repeat-containing protein 87 OS=Homo sapiens GN=WDR87 PE=1 SV=3
190	Keratin, type II cytoskeletal 5 OS=Homo sapiens GN=KRT5 PE=1 SV=3
191	Collagen alpha-1(XVIII) chain OS=Homo sapiens GN=COL18A1 PE=1 SV=5
192	Keratin, type II cytoskeletal 80 OS=Homo sapiens GN=KRT80 PE=1 SV=2
193	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3
194	Elongation factor 1-gamma OS=Homo sapiens GN=EEF1G PE=1 SV=3
195	Thyroglobulin OS=Homo sapiens GN=TG PE=1 SV=5
196	Isoform 2 of Zinc finger and SCAN domain-containing protein 20 OS=Homo sapiens GN=ZSCAN20
197	60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1
198	Beta-2-microglobulin OS=Homo sapiens GN=B2M PE=1 SV=1
199	Carcinoembryonic antigen-related cell adhesion molecule 1
200	Tetratricopeptide repeat protein 21B OS=Homo sapiens GN=TTC21B PE=1 SV=2
201	Keratin, type I cytoskeletal 20 OS=Homo sapiens GN=KRT20 PE=1 SV=1
202	Keratin, type II cytoskeletal 8 OS=Homo sapiens GN=KRT8 PE=1 SV=7
203	Multifunctional protein ADE2 OS=Homo sapiens GN=PAICS PE=1 SV=3
204	Complement C2 OS=Homo sapiens GN=C2 PE=1 SV=2
205	Ankyrin repeat and sterile alpha motif domain-containing protein 1B
206	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6
207	40S ribosomal protein S15a OS=Homo sapiens GN=RPS15A PE=1 SV=2
208	Serine/threonine-protein kinase OSR1 OS=Homo sapiens GN=OXSR1 PE=1 SV=1
209	Isoform 2 of Protein SOGA1 OS=Homo sapiens GN=SOGA1
210	Calcium-binding protein 1 OS=Homo sapiens GN=CABP1 PE=1 SV=5
211	Heparan sulfate glucosamine 3-O-sulfotransferase 4 OS=Homo sapiens GN=HS3ST4 PE=2 SV=3
212	Complement component receptor 1-like protein OS=Homo sapiens GN=CR1L PE=1 SV=3
213	Junctional adhesion molecule A OS=Homo sapiens GN=F11R PE=1 SV=1

214	Laminin subunit alpha-2 OS=Homo sapiens GN=LAMA2 PE=1 SV=4
215	Elongation factor 1-beta OS=Homo sapiens GN=EEF1B2 PE=1 SV=3
216	Eukaryotic translation initiation factor 4H OS=Homo sapiens GN=EIF4H PE=1 SV=5
217	Spindle assembly abnormal protein 6 homolog OS=Homo sapiens GN=SASS6 PE=1 SV=1
218	Isoform 2 of Solute carrier family 22 member 7 OS=Homo sapiens GN=SLC22A7

Table A.4 Proteins downregulated following treatment with CUFOX

1	Probable G-protein coupled receptor 150 OS=Homo sapiens GN=GPR150 PE=2 SV=1
2	Isoform 3 of PIH1 domain-containing protein 1 OS=Homo sapiens GN=PIH1D1
3	Complement factor H-related protein 3 OS=Homo sapiens GN=CFHR3 PE=1 SV=2
4	Late cornified envelope protein 1D OS=Homo sapiens GN=LCE1D PE=2 SV=1
5	Peptidyl-prolyl cis-trans isomerase FKBP4 OS=Homo sapiens GN=FKBP4 PE=1 SV=3
6	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4
7	Azurocidin OS=Homo sapiens GN=AZU1 PE=1 SV=3
8	Zinc finger protein 571 OS=Homo sapiens GN=ZNF571 PE=2 SV=3

Table A.5 Proteins upregulated following treatment with FOLFOX

1	Rab9 effector protein with kelch motifs OS=Homo sapiens GN=RABEPK PE=1 SV=1
2	Laminin subunit beta-2 OS=Homo sapiens GN=LAMB2 PE=1 SV=2
3	5,6-dihydroxyindole-2-carboxylic acid oxidase OS=Homo sapiens GN=TYRP1 PE=1 SV=2
4	Isoform 2 of Serine/threonine-protein phosphatase PP1-alpha catalytic subunit OS=Homo sapiens GN=PPP1CA
5	Isoform 4 of E3 ubiquitin-protein ligase UBR3 OS=Homo sapiens GN=UBR3
6	Microsomal triglyceride transfer protein large subunit OS=Homo sapiens GN=MTTP PE=1 SV=1
7	MKL/myocardin-like protein 2 OS=Homo sapiens GN=MKL2 PE=1 SV=3

Table A.6 Proteins downregulated following treatment with FOLFOX

1	ETS translocation variant 3 OS=Homo sapiens GN=ETV3 PE=1 SV=2
2	Glycerol-3-phosphate dehydrogenase, mitochondrial OS=Homo sapiens GN=GPD2 PE=1 SV=3
3	Isoform Del-1790 of Myosin light chain kinase, smooth muscle OS=Homo sapiens GN=MYLK
4	Histone H2A type 1-A OS=Homo sapiens GN=HIST1H2AA PE=1 SV=3
5	Isoform ASF-3 of Serine/arginine-rich splicing factor 1 OS=Homo sapiens GN=SRSF1
6	Isoform 4 of Minor histocompatibility antigen H13 OS=Homo sapiens GN=HM13
7	Dedicator of cytokinesis protein 5 OS=Homo sapiens GN=DOCK5 PE=1 SV=3
8	Methionine aminopeptidase 2 OS=Homo sapiens GN=METAP2 PE=1 SV=1
9	Bifunctional ATP-dependent dihydroxyacetone kinase/FAD-AMP lyase (cyclizing) OS=Homo sapiens GN=DAK PE=1 SV=2

10	Protein transport protein Sec23A OS=Homo sapiens GN=SEC23A PE=1 SV=2
11	Nuclear-interacting partner of ALK OS=Homo sapiens GN=ZC3HC1 PE=1 SV=1
12	Protein transport protein Sec23B OS=Homo sapiens GN=SEC23B PE=1 SV=2
13	Calmodulin OS=Homo sapiens GN=CALM1 PE=1 SV=2
14	Isoform 2 of Collagen triple helix repeat-containing protein 1 OS=Homo sapiens GN=CTHRC1
15	Keratin, type II cytoskeletal 72 OS=Homo sapiens GN=KRT72 PE=1 SV=2
16	Band 4.1-like protein 3 OS=Homo sapiens GN=EPB41L3 PE=1 SV=2
17	Zinc finger and BTB domain-containing protein 2 OS=Homo sapiens GN=ZBTB2 PE=1 SV=1
18	Complement factor H-related protein 3 OS=Homo sapiens GN=CFHR3 PE=1 SV=2
19	Isoform 4 of Cdc42-interacting protein 4 OS=Homo sapiens GN=TRIP10
20	Isoform 2 of Granulins OS=Homo sapiens GN=GRN
21	Proteasome subunit alpha type-7 OS=Homo sapiens GN=PSMA7 PE=1 SV=1
22	WD repeat- and FYVE domain-containing protein 4 OS=Homo sapiens GN=WDFY4 PE=1 SV=3
23	Myb/SANT-like DNA-binding domain-containing protein 1 OS=Homo sapiens GN=MSANTD1 PE=2 SV=2
24	Tenascin OS=Homo sapiens GN=TNC PE=1 SV=3
25	Histone H2A type 3 OS=Homo sapiens GN=HIST3H2A PE=1 SV=3
26	Peptidyl-prolyl cis-trans isomerase FKBP4 OS=Homo sapiens GN=FKBP4 PE=1 SV=3
27	Alpha-N-acetylglucosaminidase OS=Homo sapiens GN=NAGLU PE=1 SV=2
28	Histone H1oo OS=Homo sapiens GN=H1FOO PE=2 SV=1
29	Nuclear transport factor 2 OS=Homo sapiens GN=NUTF2 PE=1 SV=1
30	Heterogeneous nuclear ribonucleoprotein A/B OS=Homo sapiens GN=HNRNPAB PE=1 SV=2
31	Ankyrin repeat and MYND domain-containing protein 1 OS=Homo sapiens GN=ANKMY1 PE=2 SV=2
32	Malignant T-cell-amplified sequence 1 OS=Homo sapiens GN=MCTS1 PE=1 SV=1
33	Glutamate dehydrogenase 1, mitochondrial OS=Homo sapiens GN=GLUD1 PE=1 SV=2
34	CMT1A duplicated region transcript 1 protein OS=Homo sapiens GN=CDRT1 PE=2 SV=3
35	Proteasome subunit alpha type-4 OS=Homo sapiens GN=PSMA4 PE=1 SV=1
36	Activating transcription factor 7-interacting protein 1 OS=Homo sapiens GN=ATF7IP PE=1 SV=3
37	Isoform Short of Galectin-9 OS=Homo sapiens GN=LGALS9
38	Zinc finger protein 571 OS=Homo sapiens GN=ZNF571 PE=2 SV=3
39	Zinc finger CCCH-type antiviral protein 1-like OS=Homo sapiens GN=ZC3HAV1L PE=1 SV=2
40	Isoform 3 of WD repeat-containing protein 48 OS=Homo sapiens GN=WDR48
41	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4
42	Ubiquitin-like domain-containing CTD phosphatase 1 OS=Homo sapiens GN=UBLCP1 PE=1 SV=2
43	TELO2-interacting protein 2 OS=Homo sapiens GN=TTI2 PE=1 SV=1
44	Keratin, type I cytoskeletal 16 OS=Homo sapiens GN=KRT16 PE=1 SV=4
45	Histone H1.3 OS=Homo sapiens GN=HIST1H1D PE=1 SV=2
46	Isoform 2 of Alcohol dehydrogenase class 4 mu/sigma chain OS=Homo sapiens GN=ADH7
47	Isoform 2 of Uncharacterized protein C2orf61 OS=Homo sapiens GN=C2orf61
48	Endoplasmic reticulum resident protein 29 OS=Homo sapiens GN=ERP29 PE=1 SV=4

49	Histone H2A deubiquitinase MYSM1 OS=Homo sapiens GN=MYSM1 PE=1 SV=1
50	Isoform 2 of Eukaryotic translation initiation factor 6 OS=Homo sapiens GN=EIF6
51	NLR family CARD domain-containing protein 4 OS=Homo sapiens GN=NLRC4 PE=1 SV=2
52	A-kinase anchor protein 13 OS=Homo sapiens GN=AKAP13 PE=1 SV=2
53	Cytotoxic and regulatory T-cell molecule OS=Homo sapiens GN=CRTAM PE=1 SV=2
54	Isoform 2 of Heterogeneous nuclear ribonucleoprotein A/B OS=Homo sapiens GN=HNRNPAB
55	IQ motif and SEC7 domain-containing protein 2 OS=Homo sapiens GN=IQSEC2 PE=1 SV=1
56	Keratin, type II cytoskeletal 1b OS=Homo sapiens GN=KRT77 PE=2 SV=3
57	Coiled-coil domain-containing protein 33 OS=Homo sapiens GN=CCDC33 PE=1 SV=3
58	Tubulin beta-6 chain OS=Homo sapiens GN=TUBB6 PE=1 SV=1
59	Basigin OS=Homo sapiens GN=BSG PE=1 SV=2
60	Neuritin OS=Homo sapiens GN=NRN1 PE=1 SV=1
61	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2
62	Coiled-coil domain-containing protein 144A OS=Homo sapiens GN=CCDC144A PE=1 SV=1
63	Rho GTPase-activating protein 32 OS=Homo sapiens GN=ARHGAP32 PE=1 SV=1
64	Heat shock-related 70 kDa protein 2 OS=Homo sapiens GN=HSPA2 PE=1 SV=1
65	Isoform 4 of Cadherin-13 OS=Homo sapiens GN=CDH13
66	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1
67	UPF0415 protein C7orf25 OS=Homo sapiens GN=C7orf25 PE=1 SV=1
68	Guanine nucleotide-binding protein subunit beta-2-like 1 OS=Homo sapiens GN=GNB2L1 PE=1 SV=3
69	Isoform 2 of Sulfotransferase family cytosolic 2B member 1 OS=Homo sapiens GN=SULT2B1
70	Ribosome-binding protein 1 OS=Homo sapiens GN=RRBP1 PE=1 SV=4
71	Isoform 3 of Cancer-associated gene 1 protein OS=Homo sapiens GN=CAGE1
72	Isoform 2 of Proteasome subunit beta type-8 OS=Homo sapiens GN=PSMB8
73	Glucosamine-6-phosphate isomerase 1 OS=Homo sapiens GN=GNPDA1 PE=1 SV=1
74	High mobility group protein B1 OS=Homo sapiens GN=HMGB1 PE=1 SV=3
75	Metabotropic glutamate receptor 8 OS=Homo sapiens GN=GRM8 PE=2 SV=2
76	Beta-actin-like protein 2 OS=Homo sapiens GN=ACTBL2 PE=1 SV=2
77	Plasminogen OS=Homo sapiens GN=PLG PE=1 SV=2
78	Talin-1 OS=Homo sapiens GN=TLN1 PE=1 SV=3
79	Actin-related protein 3B OS=Homo sapiens GN=ACTR3B PE=2 SV=1
80	BAH and coiled-coil domain-containing protein 1 OS=Homo sapiens GN=BAHCC1 PE=2 SV=3
81	Protein SOX-15 OS=Homo sapiens GN=SOX15 PE=1 SV=1
82	Zinc finger and SCAN domain-containing protein 9 OS=Homo sapiens GN=ZSCAN9 PE=2 SV=1
83	Ig heavy chain V-III region TIL OS=Homo sapiens PE=1 SV=1
84	Collagen alpha-1(IV) chain OS=Homo sapiens GN=COL4A1 PE=1 SV=3
85	Azurocidin OS=Homo sapiens GN=AZU1 PE=1 SV=3
86	WD repeat-containing protein 65 OS=Homo sapiens GN=WDR65 PE=1 SV=3
87	Leucine-rich repeat-containing protein 8B OS=Homo sapiens GN=LRRC8B PE=2 SV=2
88	S-formylglutathione hydrolase OS=Homo sapiens GN=ESD PE=1 SV=2
89	HLA class I histocompatibility antigen, A-24 alpha chain

90	Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2
91	Tetranectin OS=Homo sapiens GN=CLEC3B PE=1 SV=3

Table A.7 Proteins upregulated following treatment with curcumin

1	Adenylyl cyclase-associated protein 2
2	Ig kappa chain V-I region Roy
3	Fructose-1,6-bisphosphatase 1
4	Tau-tubulin kinase 1
5	Four and a half LIM domains protein 3 OS=Homo sapiens GN=FHL3 PE=1 SV=4
6	Polypyrimidine tract-binding protein 1 OS=Homo sapiens GN=PTBP1 PE=1 SV=1
7	Dynein light chain 2, cytoplasmic OS=Homo sapiens GN=DYNLL2 PE=1 SV=1
8	Dixin OS=Homo sapiens GN=DIXDC1 PE=1 SV=2
9	Isoform 2 of Girdin OS=Homo sapiens GN=CCDC88A
10	X-ray repair cross-complementing protein 5 OS=Homo sapiens GN=XRCC5 PE=1 SV=3
11	Septin-7 OS=Homo sapiens GN=SEPT7 PE=1 SV=2
12	Centromere protein J OS=Homo sapiens GN=CENPJ PE=1 SV=2
13	Phosphoglycerate kinase 1 OS=Homo sapiens GN=PGK1 PE=1 SV=3
14	Calsyntenin-2 OS=Homo sapiens GN=CLSTN2 PE=1 SV=2
15	Isoform 5 of Cytosolic acyl coenzyme A thioester hydrolase OS=Homo sapiens GN=ACOT7
16	Rab9 effector protein with kelch motifs OS=Homo sapiens GN=RABEPK PE=1 SV=1
17	Kelch repeat and BTB domain-containing protein 8 OS=Homo sapiens GN=KBTBD8 PE=2 SV=2
18	Transforming growth factor beta regulator 1 OS=Homo sapiens GN=TBRG1 PE=1 SV=1
19	Mucolipin-2 OS=Homo sapiens GN=MCOLN2 PE=2 SV=2
20	Fructose-bisphosphate aldolase B OS=Homo sapiens GN=ALDOB PE=1 SV=2
21	Isoform 5 of Otoferlin OS=Homo sapiens GN=OTOF
22	Gelsolin OS=Homo sapiens GN=GSN PE=1 SV=1
23	Brain acid soluble protein 1 OS=Homo sapiens GN=BASP1 PE=1 SV=2
24	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2
25	Pancreatic lipase-related protein 2 OS=Homo sapiens GN=PNLIPRP2 PE=1 SV=1
26	Epididymal secretory protein E1 OS=Homo sapiens GN=NPC2 PE=1 SV=1
27	Complement C2 OS=Homo sapiens GN=C2 PE=1 SV=2
28	Protein unc-80 homolog OS=Homo sapiens GN=UNC80 PE=2 SV=2
29	mRNA turnover protein 4 homolog OS=Homo sapiens GN=MRTO4 PE=1 SV=2
30	Hemoglobin subunit gamma-2 OS=Homo sapiens GN=HBG2 PE=1 SV=2
31	Isoform 1c of Oxysterol-binding protein-related protein 3 OS=Homo sapiens GN=OSBPL3
32	Isoform 2 of Sorcin OS=Homo sapiens GN=SRI
33	Exonuclease 3'-5' domain-containing protein 1 OS=Homo sapiens GN=EXD1 PE=2 SV=4
34	Small nuclear ribonucleoprotein-associated proteins B and B' OS=Homo sapiens GN=SNRPB PE=1 SV=2
35	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3
36	Inositol 1,4,5-trisphosphate receptor type 1 OS=Homo sapiens GN=ITPR1 PE=1 SV=3

37	Hemoglobin subunit beta OS=Homo sapiens GN=HBB PE=1 SV=2
38	Fibrinogen beta chain OS=Homo sapiens GN=FGB PE=1 SV=2
39	Midkine OS=Homo sapiens GN=MDK PE=1 SV=1
40	Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3
41	Protein shisa-7 OS=Homo sapiens GN=SHISA7 PE=2 SV=3
42	INO80 complex subunit C OS=Homo sapiens GN=INO80C PE=1 SV=1
43	Isoform 4 of Liprin-alpha-2 OS=Homo sapiens GN=PPFIA2
44	Profilin-1 OS=Homo sapiens GN=PFN1 PE=1 SV=2
45	Apoptosis-inducing factor 1, mitochondrial OS=Homo sapiens GN=AIFM1 PE=1 SV=1
46	Actin-related protein 3 OS=Homo sapiens GN=ACTR3 PE=1 SV=3
47	Beta-soluble NSF attachment protein OS=Homo sapiens GN=NAPB PE=1 SV=2
48	Protein NLRC5 OS=Homo sapiens GN=NLRC5 PE=1 SV=3
49	Histone H2B type 1-O OS=Homo sapiens GN=HIST1H2BO PE=1 SV=3
50	Isoform C1 of Heterogeneous nuclear ribonucleoproteins C1/C2 OS=Homo sapiens GN=HNRNPC
51	Lactotransferrin OS=Homo sapiens GN=LTF PE=1 SV=6
52	Heat shock 70 kDa protein 6 OS=Homo sapiens GN=HSPA6 PE=1 SV=2
53	Isoform 2 of Nascent polypeptide-associated complex subunit alpha OS=Homo sapiens GN=NACA
54	Keratin, type II cuticular Hb4 OS=Homo sapiens GN=KRT84 PE=2 SV=2
55	Histone H2B type 1-D OS=Homo sapiens GN=HIST1H2BD PE=1 SV=2
56	Collagen alpha-3(VI) chain OS=Homo sapiens GN=COL6A3 PE=1 SV=5
57	Ubiquitin-conjugating enzyme E2 N OS=Homo sapiens GN=UBE2N PE=1 SV=1
58	Enhancer of rudimentary homolog OS=Homo sapiens GN=ERH PE=1 SV=1
59	Cadherin-17 OS=Homo sapiens GN=CDH17 PE=1 SV=3
60	Alpha-actinin-2 OS=Homo sapiens GN=ACTN2 PE=1 SV=1
61	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1
62	Peroxiredoxin-5, mitochondrial OS=Homo sapiens GN=PRDX5 PE=1 SV=4
63	Proteasome subunit beta type-1 OS=Homo sapiens GN=PSMB1 PE=1 SV=2
64	Ras-associated and pleckstrin homology domains-containing protein 1 OS=Homo sapiens GN=RAPH1 PE=1 SV=3
65	Copine-9 OS=Homo sapiens GN=CPNE9 PE=1 SV=3
66	Protein disulfide-isomerase A4 OS=Homo sapiens GN=PDIA4 PE=1 SV=2
67	T-complex protein 1 subunit alpha OS=Homo sapiens GN=TCP1 PE=1 SV=1
68	Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5
69	Cullin-associated NEDD8-dissociated protein 1 OS=Homo sapiens GN=CAND1 PE=1 SV=2
70	Barrier-to-autointegration factor OS=Homo sapiens GN=BANF1 PE=1 SV=1
71	Disks large homolog 2 OS=Homo sapiens GN=DLG2 PE=1 SV=3
72	Eukaryotic translation initiation factor 6 OS=Homo sapiens GN=EIF6 PE=1 SV=1
73	Receptor-interacting serine/threonine-protein kinase 3 OS=Homo sapiens GN=RIPK3 PE=1 SV=2
74	Keratin, type I cytoskeletal 17 OS=Homo sapiens GN=KRT17 PE=1 SV=2
75	High mobility group protein B2 OS=Homo sapiens GN=HMGB2 PE=1 SV=2
76	Neurofilament light polypeptide OS=Homo sapiens GN=NEFL PE=1 SV=3
77	Laminin subunit alpha-2 OS=Homo sapiens GN=LAMA2 PE=1 SV=4
78	Proteasome subunit beta type-3 OS=Homo sapiens GN=PSMB3 PE=1 SV=2

79	Inorganic pyrophosphatase OS=Homo sapiens GN=PPA1 PE=1 SV=2
80	Uncharacterized protein C6orf203 OS=Homo sapiens GN=C6orf203 PE=1 SV=1
81	Junctional adhesion molecule A OS=Homo sapiens GN=F11R PE=1 SV=1
82	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
83	Protein S100-A10 OS=Homo sapiens GN=S100A10 PE=1 SV=2
84	AMP deaminase 3 OS=Homo sapiens GN=AMPD3 PE=1 SV=1
85	Disks large homolog 3 OS=Homo sapiens GN=DLG3 PE=1 SV=2
86	DNA helicase MCM9 OS=Homo sapiens GN=MCM9 PE=1 SV=4
87	Microsomal triglyceride transfer protein large subunit OS=Homo sapiens GN=MTTP PE=1 SV=1
88	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2
89	Nck-associated protein 1-like OS=Homo sapiens GN=NCKAP1L PE=1 SV=3
90	Isoform 2 of Phosphatidate phosphatase LPIN3 OS=Homo sapiens GN=LPIN3
91	Haptoglobin-related protein OS=Homo sapiens GN=HPR PE=1 SV=2
92	GTP:AMP phosphotransferase AK3, mitochondrial OS=Homo sapiens GN=AK3 PE=1 SV=4
93	Pantetheinase OS=Homo sapiens GN=VNN1 PE=1 SV=2
94	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1
95	Arginase-1 OS=Homo sapiens GN=ARG1 PE=1 SV=2
96	UPF0686 protein C11orf1 OS=Homo sapiens GN=C11orf1 PE=2 SV=1
97	Rho GTPase-activating protein 5 OS=Homo sapiens GN=ARHGAP5 PE=1 SV=2
98	14-3-3 protein sigma OS=Homo sapiens GN=SFN PE=1 SV=1
99	Sterile alpha motif domain-containing protein 15 OS=Homo sapiens GN=SAMD15 PE=2 SV=1
100	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2
101	Peroxisomal multifunctional enzyme type 2 OS=Homo sapiens GN=HSD17B4 PE=1 SV=3
102	Histone-lysine N-methyltransferase EZH1 OS=Homo sapiens GN=EZH1 PE=1 SV=2
103	Intermediate filament family orphan 1 OS=Homo sapiens GN=IFFO1 PE=2 SV=2
104	MKL/myocardin-like protein 2 OS=Homo sapiens GN=MKL2 PE=1 SV=3
105	Phosphatidylethanolamine-binding protein 1 OS=Homo sapiens GN=PEBP1 PE=1 SV=3
106	Isoform 2 of NAD(P)H-hydrate epimerase OS=Homo sapiens GN=APOA1BP
107	POTE ankyrin domain family member I OS=Homo sapiens GN=POTEI PE=3 SV=1
108	Protein Red OS=Homo sapiens GN=IK PE=1 SV=3
109	Protein Shroom3 OS=Homo sapiens GN=SHROOM3 PE=1 SV=2
110	Docking protein 2 OS=Homo sapiens GN=DOK2 PE=1 SV=2
111	Fibrinogen gamma chain OS=Homo sapiens GN=FGG PE=1 SV=3
112	Vacuolar protein sorting-associated protein 13D OS=Homo sapiens GN=VPS13D PE=1 SV=1
113	Myosin-15 OS=Homo sapiens GN=MYH15 PE=1 SV=5
114	Keratin, type I cytoskeletal 15 OS=Homo sapiens GN=KRT15 PE=1 SV=3
115	Aspartate aminotransferase, cytoplasmic OS=Homo sapiens GN=GOT1 PE=1 SV=3
116	UMP-CMP kinase OS=Homo sapiens GN=CMPK1 PE=1 SV=3
117	Zinc finger protein Rlf OS=Homo sapiens GN=RLF PE=1 SV=2
118	Keratin, type II cytoskeletal 71 OS=Homo sapiens GN=KRT71 PE=1 SV=3
119	Isoform 2 of Serine/threonine-protein phosphatase PP1-alpha catalytic subunit

	OS=Homo sapiens GN=PPP1CA
120	UTP--glucose-1-phosphate uridylyltransferase OS=Homo sapiens GN=UGP2 PE=1 SV=5
121	Isoform 2 of UDP-glucose 6-dehydrogenase OS=Homo sapiens GN=UGDH
122	Inter-alpha-trypsin inhibitor heavy chain H3 OS=Homo sapiens GN=ITIH3 PE=1 SV=2
123	Isoform 4 of Sex hormone-binding globulin OS=Homo sapiens GN=SHBG
124	AF4/FMR2 family member 4 OS=Homo sapiens GN=AFF4 PE=1 SV=1
125	Keratin, type II cytoskeletal 4 OS=Homo sapiens GN=KRT4 PE=1 SV=4
126	Glutathione peroxidase 3 OS=Homo sapiens GN=GPX3 PE=1 SV=2
127	Heme-binding protein 2 OS=Homo sapiens GN=HEBP2 PE=1 SV=1
128	Dual specificity mitogen-activated protein kinase kinase 1 OS=Homo sapiens GN=MAP2K1 PE=1 SV=2
129	Eukaryotic translation initiation factor 4H OS=Homo sapiens GN=EIF4H PE=1 SV=5
130	Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4
131	Moesin OS=Homo sapiens GN=MSN PE=1 SV=3
132	Tetratricopeptide repeat protein 28 OS=Homo sapiens GN=TTC28 PE=1 SV=4
133	Serine/threonine-protein kinase haspin OS=Homo sapiens GN=GSG2 PE=1 SV=3
134	Major vault protein OS=Homo sapiens GN=MVP PE=1 SV=4
135	Histone H3.3 OS=Homo sapiens GN=H3F3A PE=1 SV=2
136	40S ribosomal protein S15a OS=Homo sapiens GN=RPS15A PE=1 SV=2
137	Hemoglobin subunit alpha OS=Homo sapiens GN=HBA1 PE=1 SV=2
138	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1
139	Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5 OS=Homo sapiens GN=MACF1 PE=1 SV=4
140	Lamin-B1 OS=Homo sapiens GN=LMNB1 PE=1 SV=2
141	Inorganic pyrophosphatase 2, mitochondrial OS=Homo sapiens GN=PPA2 PE=1 SV=2
142	Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=1 SV=4
143	Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1
144	DNA-(apurinic or apyrimidinic site) lyase OS=Homo sapiens GN=APEX1 PE=1 SV=2
145	ELAV-like protein 1 OS=Homo sapiens GN=ELAVL1 PE=1 SV=2
146	E3 ubiquitin-protein ligase UBR4 OS=Homo sapiens GN=UBR4 PE=1 SV=1
147	Nuclease-sensitive element-binding protein 1 OS=Homo sapiens GN=YBX1 PE=1 SV=3
148	Isoform 2 of Apoptosis-inducing factor 2 OS=Homo sapiens GN=AIFM2
149	WD repeat-containing protein 87 OS=Homo sapiens GN=WDR87 PE=1 SV=3
150	Keratin, type II cytoskeletal 8 OS=Homo sapiens GN=KRT8 PE=1 SV=7
151	Protein DPCD OS=Homo sapiens GN=DPCD PE=1 SV=2
152	Ig kappa chain V-II region Cum OS=Homo sapiens PE=1 SV=1
153	Periostin OS=Homo sapiens GN=POSTN PE=1 SV=2
154	T-complex protein 1 subunit theta OS=Homo sapiens GN=CCT8 PE=1 SV=4
155	Lamin-B2 OS=Homo sapiens GN=LMNB2 PE=1 SV=3
156	3-hydroxyisobutyrate dehydrogenase, mitochondrial OS=Homo sapiens GN=HIBADH PE=1 SV=2
157	Isoform 3 of Kin of IRRE-like protein 1 OS=Homo sapiens GN=KIRREL
158	40S ribosomal protein S4, X isoform OS=Homo sapiens GN=RPS4X PE=1 SV=2
159	Spectrin alpha chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTAN1 PE=1 SV=3
160	Xaa-Pro dipeptidase OS=Homo sapiens GN=PEPD PE=1 SV=3

161	Protein FAM228B OS=Homo sapiens GN=FAM228B PE=2 SV=1
162	Alpha-actinin-1 OS=Homo sapiens GN=ACTN1 PE=1 SV=2
163	Alpha-2-antiplasmin OS=Homo sapiens GN=SERPINF2 PE=1 SV=3
164	Ankyrin repeat and sterile alpha motif domain-containing protein 1B OS=Homo sapiens GN=ANKS1B PE=1 SV=2
165	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=2
166	60S acidic ribosomal protein P0-like OS=Homo sapiens GN=RPLP0P6 PE=5 SV=1
167	Thioredoxin domain-containing protein 12 OS=Homo sapiens GN=TXNDC12 PE=1 SV=1
168	Mis18-binding protein 1 OS=Homo sapiens GN=MIS18BP1 PE=1 SV=1
169	Scaffold attachment factor B1 OS=Homo sapiens GN=SAFB PE=1 SV=4
170	POTE ankyrin domain family member F OS=Homo sapiens GN=POTEF PE=1 SV=2
171	Endoplasmic reticulum resident protein 44 OS=Homo sapiens GN=ERP44 PE=1 SV=1
172	Serine/threonine-protein kinase OSR1 OS=Homo sapiens GN=OXSR1 PE=1 SV=1
173	Protein S100-A9 OS=Homo sapiens GN=S100A9 PE=1 SV=1
174	14-3-3 protein epsilon OS=Homo sapiens GN=YWHAE PE=1 SV=1
175	Histone H3.1t OS=Homo sapiens GN=HIST3H3 PE=1 SV=3
176	Putative heat shock protein HSP 90-alpha A2 OS=Homo sapiens GN=HSP90AA2 PE=1 SV=2
177	HERV-MER_4q12 provirus ancestral Env polyprotein OS=Homo sapiens GN=ERVMER34-1 PE=2 SV=1
178	GTP-binding nuclear protein Ran OS=Homo sapiens GN=RAN PE=1 SV=3
179	Isoform 2 of Zinc finger and SCAN domain-containing protein 20 OS=Homo sapiens GN=ZSCAN20
180	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3
181	Keratin, type I cytoskeletal 20 OS=Homo sapiens GN=KRT20 PE=1 SV=1
182	Desmoglein-2 OS=Homo sapiens GN=DSG2 PE=1 SV=2
183	Monocyte differentiation antigen CD14 OS=Homo sapiens GN=CD14 PE=1 SV=2
184	Zinc finger protein 536 OS=Homo sapiens GN=ZNF536 PE=1 SV=3
185	Cystatin-C OS=Homo sapiens GN=CST3 PE=1 SV=1
186	Isoform 2 of La-related protein 1B OS=Homo sapiens GN=LARP1B
187	X-ray repair cross-complementing protein 6 OS=Homo sapiens GN=XRCC6 PE=1 SV=2
188	10 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPE1 PE=1 SV=2
189	Acyl-coenzyme A synthetase ACSM2A, mitochondrial OS=Homo sapiens GN=ACSM2A PE=1 SV=2
190	Keratin, type II cytoskeletal 5 OS=Homo sapiens GN=KRT5 PE=1 SV=3
191	6-phosphogluconolactonase OS=Homo sapiens GN=PGLS PE=1 SV=2
192	Laminin subunit beta-1 OS=Homo sapiens GN=LAMB1 PE=1 SV=2
193	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3
194	DNA topoisomerase 1 OS=Homo sapiens GN=TOP1 PE=1 SV=2
195	Glutathione synthetase OS=Homo sapiens GN=GSS PE=1 SV=1
196	Fructose-bisphosphate aldolase C OS=Homo sapiens GN=ALDOC PE=1 SV=2
197	Carcinoembryonic antigen-related cell adhesion molecule 1 OS=Homo sapiens GN=CEACAM1 PE=1 SV=2
198	Tetratricopeptide repeat protein 21B OS=Homo sapiens GN=TTC21B PE=1 SV=2
199	Keratin, type I cytoskeletal 18 OS=Homo sapiens GN=KRT18 PE=1 SV=2
200	Deoxycytidylate deaminase OS=Homo sapiens GN=DCTD PE=1 SV=2

201	Peptidyl-prolyl cis-trans isomerase A-like 4A/B/C OS=Homo sapiens GN=PPIAL4A PE=2 SV=1
202	C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens GN=MTHFD1 PE=1 SV=3
203	Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2
204	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4
205	Tissue alpha-L-fucosidase OS=Homo sapiens GN=FUCA1 PE=1 SV=4
206	Poly(rC)-binding protein 3 OS=Homo sapiens GN=PCBP3 PE=2 SV=2
207	Glucose-6-phosphate isomerase OS=Homo sapiens GN=GPI PE=1 SV=4
208	Cytochrome c OS=Homo sapiens GN=CYCS PE=1 SV=2
209	Multifunctional protein ADE2 OS=Homo sapiens GN=PAICS PE=1 SV=3
210	Caspase-6 OS=Homo sapiens GN=CASP6 PE=1 SV=2
211	Uncharacterized protein KIAA1614 OS=Homo sapiens GN=KIAA1614 PE=2 SV=3
212	RNA polymerase II-associated factor 1 homolog OS=Homo sapiens GN=PAF1 PE=1 SV=2
213	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2
214	Alcohol dehydrogenase 1B OS=Homo sapiens GN=ADH1B PE=1 SV=2
215	L-lactate dehydrogenase A-like 6A OS=Homo sapiens GN=LDHAL6A PE=2 SV=1
216	Dynein heavy chain 14, axonemal OS=Homo sapiens GN=DNAH14 PE=2 SV=3
217	Nuclear migration protein nudC OS=Homo sapiens GN=NUDC PE=1 SV=1
218	Apolipoprotein E OS=Homo sapiens GN=APOE PE=1 SV=1
219	C4b-binding protein alpha chain OS=Homo sapiens GN=C4BPA PE=1 SV=2
220	Small nuclear ribonucleoprotein Sm D3 OS=Homo sapiens GN=SNRPD3 PE=1 SV=1
221	Transgelin OS=Homo sapiens GN=TAGLN PE=1 SV=4
222	Triosephosphate isomerase OS=Homo sapiens GN=TPI1 PE=1 SV=3
223	Thioredoxin OS=Homo sapiens GN=TXN PE=1 SV=3
224	Centrosomal protein of 120 kDa OS=Homo sapiens GN=CEP120 PE=2 SV=2
225	Heterogeneous nuclear ribonucleoprotein D-like OS=Homo sapiens GN=HNRNPDL PE=1 SV=3
226	Isoform 2 of Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA
227	Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2
228	Heterogeneous nuclear ribonucleoprotein A1-like 2 OS=Homo sapiens GN=HNRNPA1L2 PE=2 SV=2
229	Acetyl-CoA acetyltransferase, mitochondrial OS=Homo sapiens GN=ACAT1 PE=1 SV=1
230	60S acidic ribosomal protein P2 OS=Homo sapiens GN=RPLP2 PE=1 SV=1
231	Protein S100-A11 OS=Homo sapiens GN=S100A11 PE=1 SV=2
232	Beta-2-microglobulin OS=Homo sapiens GN=B2M PE=1 SV=1
233	Malate dehydrogenase, mitochondrial OS=Homo sapiens GN=MDH2 PE=1 SV=3
234	Cytosolic non-specific dipeptidase OS=Homo sapiens GN=CNDP2 PE=1 SV=2
235	Protein SOGA2 OS=Homo sapiens GN=SOGA2 PE=1 SV=5
236	Vitamin D-binding protein OS=Homo sapiens GN=GC PE=1 SV=1
237	dCTP pyrophosphatase 1 OS=Homo sapiens GN=DCTPP1 PE=1 SV=1
238	Desmin OS=Homo sapiens GN=DES PE=1 SV=3
239	Myb-related protein B OS=Homo sapiens GN=MYBL2 PE=1 SV=1
240	Vinculin OS=Homo sapiens GN=VCL PE=1 SV=4
241	Teneurin-3 OS=Homo sapiens GN=TENM3 PE=2 SV=3
242	Tuftelin OS=Homo sapiens GN=TUFT1 PE=2 SV=1

243	Neutrophil cytosol factor 2 OS=Homo sapiens GN=NCF2 PE=1 SV=2
244	Ubiquitin carboxyl-terminal hydrolase 5 OS=Homo sapiens GN=USP5 PE=1 SV=2
245	Centromere-associated protein E OS=Homo sapiens GN=CENPE PE=1 SV=2
246	Creatine kinase U-type, mitochondrial OS=Homo sapiens GN=CKMT1A PE=1 SV=1
247	Isoform 3 of Ubiquitin carboxyl-terminal hydrolase 46 OS=Homo sapiens GN=USP46
248	Sodium- and chloride-dependent GABA transporter 2 OS=Homo sapiens GN=SLC6A13 PE=1 SV=3
249	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2
250	Keratin, type I cuticular Ha5 OS=Homo sapiens GN=KRT35 PE=2 SV=5
251	Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3
252	Elongation factor 1-gamma OS=Homo sapiens GN=EEF1G PE=1 SV=3
253	ADP-sugar pyrophosphatase OS=Homo sapiens GN=NUDT5 PE=1 SV=1
254	Protein Jumonji OS=Homo sapiens GN=JARID2 PE=1 SV=2
255	Leucine-rich repeat and calponin homology domain-containing protein 4 OS=Homo sapiens GN=LRCH4 PE=1 SV=2
256	Isoform 3 of Contactin-4 OS=Homo sapiens GN=CNTN4
257	Cytosol aminopeptidase OS=Homo sapiens GN=LAP3 PE=1 SV=3
258	Plastin-2 OS=Homo sapiens GN=LCP1 PE=1 SV=6
259	Isoform 2 of Solute carrier family 22 member 7 OS=Homo sapiens GN=SLC22A7
260	Insulin-like growth factor-binding protein 7 OS=Homo sapiens GN=IGFBP7 PE=1 SV=1
261	Transcription termination factor 2 OS=Homo sapiens GN=TTF2 PE=1 SV=2
262	Testis-expressed sequence 11 protein OS=Homo sapiens GN=TEX11 PE=1 SV=3
263	Chromodomain-helicase-DNA-binding protein 7 OS=Homo sapiens GN=CHD7 PE=1 SV=3
264	Rho GTPase-activating protein 12 OS=Homo sapiens GN=ARHGAP12 PE=1 SV=1
265	Isoform 2 of Niban-like protein 1 OS=Homo sapiens GN=FAM129B
266	Actin-binding LIM protein 1 OS=Homo sapiens GN=ABLIM1 PE=1 SV=3
267	Collagen alpha-1(IX) chain OS=Homo sapiens GN=COL9A1 PE=1 SV=3
268	Thioredoxin reductase 1, cytoplasmic OS=Homo sapiens GN=TXNRD1 PE=1 SV=3
269	Protocadherin Fat 3 OS=Homo sapiens GN=FAT3 PE=2 SV=2
270	T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3
271	Bifunctional epoxide hydrolase 2 OS=Homo sapiens GN=EPHX2 PE=1 SV=2
272	Pigment epithelium-derived factor OS=Homo sapiens GN=SERPINF1 PE=1 SV=4
273	Keratin, type II cytoskeletal 80 OS=Homo sapiens GN=KRT80 PE=1 SV=2
274	Coiled-coil domain-containing protein 162 OS=Homo sapiens GN=CCDC162P PE=2 SV=3
275	5'(3')-deoxyribonucleotidase, cytosolic type OS=Homo sapiens GN=NT5C PE=1 SV=2
276	Adenylyl cyclase-associated protein 1 OS=Homo sapiens GN=CAP1 PE=1 SV=5
277	Histone H3.1 OS=Homo sapiens GN=HIST1H3A PE=1 SV=2
278	Glutathione reductase, mitochondrial OS=Homo sapiens GN=GSR PE=1 SV=2
279	Spectrin beta chain, non-erythrocytic 4 OS=Homo sapiens GN=SPTBN4 PE=1 SV=2
280	Ras-related protein Rab-24 OS=Homo sapiens GN=RAB24 PE=1 SV=1
281	Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6
282	Protein disulfide-isomerase A6 OS=Homo sapiens GN=PDIA6 PE=1 SV=1
283	Gamma-glutamylcyclotransferase OS=Homo sapiens GN=GGCT PE=1 SV=1
284	Heparan sulfate glucosamine 3-O-sulfotransferase 4 OS=Homo sapiens GN=HS3ST4 PE=2 SV=3

285	Alcohol dehydrogenase 4 OS=Homo sapiens GN=ADH4 PE=1 SV=5
286	Proteasome subunit beta type-6 OS=Homo sapiens GN=PSMB6 PE=1 SV=4
287	Eukaryotic translation initiation factor 5A-1 OS=Homo sapiens GN=EIF5A PE=1 SV=2
288	Isoform 3 of Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK
289	60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1
290	Isoform 2 of Abl interactor 1 OS=Homo sapiens GN=ABI1
291	Isoform 3 of Melanocyte protein PMEL OS=Homo sapiens GN=PMEL
292	Isoform 8 of YY1-associated protein 1 OS=Homo sapiens GN=YY1AP1
293	Isoform 2 of Carbonic anhydrase 12 OS=Homo sapiens GN=CA12
294	Fatty acid-binding protein, epidermal OS=Homo sapiens GN=FABP5 PE=1 SV=3
295	Palladin OS=Homo sapiens GN=PALLD PE=1 SV=3
296	Neurobeachin OS=Homo sapiens GN=NBEA PE=1 SV=3
297	Protein FAM13A OS=Homo sapiens GN=FAM13A PE=1 SV=2
298	Eukaryotic translation initiation factor 4E OS=Homo sapiens GN=EIF4E PE=1 SV=2
299	Eukaryotic translation initiation factor 3 subunit B OS=Homo sapiens GN=EIF3B PE=1 SV=3
300	Alpha-1-acid glycoprotein 2 OS=Homo sapiens GN=ORM2 PE=1 SV=2
301	Myosin-6 OS=Homo sapiens GN=MYH6 PE=1 SV=5
302	Isoform 4 of E3 ubiquitin-protein ligase UBR3 OS=Homo sapiens GN=UBR3
303	Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3
304	Cytohesin-interacting protein OS=Homo sapiens GN=CYTIP PE=1 SV=2
305	Ezrin OS=Homo sapiens GN=EZR PE=1 SV=4
306	Isoform 2 of Tubulin polyglutamylase TTLL5 OS=Homo sapiens GN=TTLL5
307	Stabilin-1 OS=Homo sapiens GN=STAB1 PE=1 SV=3
308	Heterogeneous nuclear ribonucleoprotein C-like 1 OS=Homo sapiens GN=HNRNPCL1 PE=1 SV=1
309	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2 OS=Homo sapiens GN=DDAH2 PE=1 SV=1
310	Four and a half LIM domains protein 2 OS=Homo sapiens GN=FHL2 PE=1 SV=3
311	Isoform 4 of Clusterin OS=Homo sapiens GN=CLU
312	Galectin-1 OS=Homo sapiens GN=LGALS1 PE=1 SV=2
313	Coiled-coil domain-containing protein 88B OS=Homo sapiens GN=CCDC88B PE=1 SV=1
314	UV excision repair protein RAD23 homolog B OS=Homo sapiens GN=RAD23B PE=1 SV=1
315	Creatine kinase B-type OS=Homo sapiens GN=CKB PE=1 SV=1
316	Putative heat shock protein HSP 90-alpha A5 OS=Homo sapiens GN=HSP90AA5P PE=1 SV=1
317	Transmembrane protein 87A OS=Homo sapiens GN=TMEM87A PE=1 SV=3
318	Interleukin-13 receptor subunit alpha-1 OS=Homo sapiens GN=IL13RA1 PE=1 SV=1
319	14-3-3 protein beta/alpha OS=Homo sapiens GN=YWHAB PE=1 SV=3
320	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1
321	Neurofilament heavy polypeptide OS=Homo sapiens GN=NEFH PE=1 SV=4
322	Calcium-binding protein 39-like OS=Homo sapiens GN=CAB39L PE=1 SV=3
323	Isoform 2 of Sialate O-acetylesterase OS=Homo sapiens GN=SIAE
324	Isoform 2 of Actin-binding protein anillin OS=Homo sapiens GN=ANLN
325	Hematopoietically-expressed homeobox protein HHEX OS=Homo sapiens GN=HHEX

	PE=1 SV=1
326	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6
327	Lupus La protein OS=Homo sapiens GN=SSB PE=1 SV=2
328	Glycogen phosphorylase, brain form OS=Homo sapiens GN=PYGB PE=1 SV=5
329	Nck-associated protein 1 OS=Homo sapiens GN=NCKAP1 PE=1 SV=1
330	N-acylneuraminate cytidylyltransferase OS=Homo sapiens GN=CMAS PE=1 SV=2
331	Structural maintenance of chromosomes protein 3 OS=Homo sapiens GN=SMC3 PE=1 SV=2
332	Probable G-protein coupled receptor 132 OS=Homo sapiens GN=GPR132 PE=2 SV=1
333	Acyl-coenzyme A thioesterase 2, mitochondrial OS=Homo sapiens GN=ACOT2 PE=1 SV=6
334	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2
335	LIM/homeobox protein Lhx5 OS=Homo sapiens GN=LHX5 PE=2 SV=1
336	Carbonyl reductase [NADPH] 3 OS=Homo sapiens GN=CBR3 PE=1 SV=3
337	Isoform 3 of Malate dehydrogenase, cytoplasmic OS=Homo sapiens GN=MDH1
338	Malate dehydrogenase, cytoplasmic OS=Homo sapiens GN=MDH1 PE=1 SV=4
339	Isoform 2 of Septin-11 OS=Homo sapiens GN=SEPT11
340	Quinone oxidoreductase OS=Homo sapiens GN=CRYZ PE=1 SV=1
341	1-phosphatidylinositol 3-phosphate 5-kinase OS=Homo sapiens GN=PIKFYVE PE=1 SV=3
342	Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3
343	Keratin, type II cytoskeletal 75 OS=Homo sapiens GN=KRT75 PE=1 SV=2
344	Nidogen-1 OS=Homo sapiens GN=NID1 PE=1 SV=3
345	6-phosphogluconate dehydrogenase, decarboxylating OS=Homo sapiens GN=PGD PE=1 SV=3
346	Complement factor B OS=Homo sapiens GN=CFB PE=1 SV=2
347	Keratin, type I cuticular Ha2 OS=Homo sapiens GN=KRT32 PE=1 SV=3
348	High mobility group protein B3 OS=Homo sapiens GN=HMGB3 PE=1 SV=4
349	POTE ankyrin domain family member E OS=Homo sapiens GN=POTEE PE=1 SV=3
350	Isoform 3 of Cytochrome b5 OS=Homo sapiens GN=CYB5A
351	Cytochrome b5 OS=Homo sapiens GN=CYB5A PE=1 SV=2
352	Peroxidasin homolog OS=Homo sapiens GN=PXDN PE=1 SV=2
353	Nucleophosmin OS=Homo sapiens GN=NPM1 PE=1 SV=2
354	Isoform 2 of Glucosidase 2 subunit beta OS=Homo sapiens GN=PRKCSH
355	Histone H2A type 2-B OS=Homo sapiens GN=HIST2H2AB PE=1 SV=3
356	Isoform 2 of 6-phosphofructokinase, liver type OS=Homo sapiens GN=PFKL
357	Gamma-enolase OS=Homo sapiens GN=ENO2 PE=1 SV=3
358	Peroxiredoxin-6 OS=Homo sapiens GN=PRDX6 PE=1 SV=3
359	Isoform 3 of Protein unc-79 homolog OS=Homo sapiens GN=UNC79
360	Baculoviral IAP repeat-containing protein 1 OS=Homo sapiens GN=NAIP PE=1 SV=3
361	F-actin-capping protein subunit alpha-2 OS=Homo sapiens GN=CAPZA2 PE=1 SV=3
362	Lipopolysaccharide-responsive and beige-like anchor protein OS=Homo sapiens GN=LRBA PE=1 SV=4
363	Isoform 3 of SH3 and multiple ankyrin repeat domains protein 2 OS=Homo sapiens GN=SHANK2
364	Centrosomal protein of 128 kDa OS=Homo sapiens GN=CEP128 PE=1 SV=2

365	Isoform 3 of Apoptosis-associated speck-like protein containing a CARD OS=Homo sapiens GN=PYCARD
366	Fanconi anemia group M protein OS=Homo sapiens GN=FANCM PE=1 SV=2
367	Alpha-parvin OS=Homo sapiens GN=PARVA PE=1 SV=1
368	Latent-transforming growth factor beta-binding protein 4 OS=Homo sapiens GN=LTBP4 PE=1 SV=2
369	Laminin subunit beta-2 OS=Homo sapiens GN=LAMB2 PE=1 SV=2
370	Ribonuclease UK114 OS=Homo sapiens GN=HRSP12 PE=1 SV=1
371	Testis-expressed sequence 10 protein OS=Homo sapiens GN=TEX10 PE=1 SV=2
372	Isoform 2 of Metallophosphoesterase 1 OS=Homo sapiens GN=MPPE1
373	Far upstream element-binding protein 1 OS=Homo sapiens GN=FUBP1 PE=1 SV=3
374	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2
375	Beta-lactamase-like protein 2 OS=Homo sapiens GN=LACTB2 PE=1 SV=2
376	Phenylalanine--tRNA ligase beta subunit OS=Homo sapiens GN=FARSB PE=1 SV=3
377	Isoform LAMP-2B of Lysosome-associated membrane glycoprotein 2 OS=Homo sapiens GN=LAMP2
378	Isoform 2 of Suppressor of IKBKE 1 OS=Homo sapiens GN=SIKE1
379	UHRF1-binding protein 1 OS=Homo sapiens GN=UHRF1BP1 PE=1 SV=1
380	WD repeat-containing protein 13 OS=Homo sapiens GN=WDR13 PE=1 SV=2
381	PDZ and LIM domain protein 7 OS=Homo sapiens GN=PDLIM7 PE=1 SV=1
382	Stress-induced-phosphoprotein 1 OS=Homo sapiens GN=STIP1 PE=1 SV=1
383	E3 SUMO-protein ligase RanBP2 OS=Homo sapiens GN=RANBP2 PE=1 SV=2
384	Isoform 4 of Ubiquitin conjugation factor E4 B OS=Homo sapiens GN=UBE4B
385	Filamin-B OS=Homo sapiens GN=FLNB PE=1 SV=2
386	MAP kinase-activated protein kinase 3 OS=Homo sapiens GN=MAPKAPK3 PE=1 SV=1
387	Phosphoglycerate kinase 2 OS=Homo sapiens GN=PGK2 PE=1 SV=3
388	Glutathione S-transferase omega-1 OS=Homo sapiens GN=GSTO1 PE=1 SV=2
389	Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4
390	Death-inducer obliterator 1 OS=Homo sapiens GN=DIDO1 PE=1 SV=5
391	Putative beta-actin-like protein 3 OS=Homo sapiens GN=POTEKP PE=5 SV=1
392	Cofilin-1 OS=Homo sapiens GN=CFL1 PE=1 SV=3
393	Nostrin OS=Homo sapiens GN=NOSTRIN PE=1 SV=2
394	Collagen alpha-1(XVIII) chain OS=Homo sapiens GN=COL18A1 PE=1 SV=5
395	Keratin, type II cytoskeletal 73 OS=Homo sapiens GN=KRT73 PE=1 SV=1
396	14-3-3 protein gamma OS=Homo sapiens GN=YWHAG PE=1 SV=2
397	Isoform 3 of Coatomer subunit epsilon OS=Homo sapiens GN=COPE
398	Cyclin-D1-binding protein 1 OS=Homo sapiens GN=CCNDBP1 PE=1 SV=2
399	Mitochondrial import inner membrane translocase subunit Tim13 OS=Homo sapiens GN=TIMM13 PE=1 SV=1
400	Isoform Short of Macrosialin OS=Homo sapiens GN=CD68
401	ES1 protein homolog, mitochondrial OS=Homo sapiens GN=C21orf33 PE=1 SV=3
402	Serpin B8 OS=Homo sapiens GN=SERPINB8 PE=1 SV=2
403	Ig heavy chain V-II region ARH-77 OS=Homo sapiens PE=4 SV=1
404	F-actin-capping protein subunit alpha-1 OS=Homo sapiens GN=CAPZA1 PE=1 SV=3
405	Myosin light chain 6B OS=Homo sapiens GN=MYL6B PE=1 SV=1
406	Ig heavy chain V-III region TEI OS=Homo sapiens PE=1 SV=1

407	Tubby-related protein 1 OS=Homo sapiens GN=TULP1 PE=1 SV=3
408	Protein dpy-30 homolog OS=Homo sapiens GN=DPY30 PE=1 SV=1
409	Calcium-binding and coiled-coil domain-containing protein 2 OS=Homo sapiens GN=CALCOCO2 PE=1 SV=1
410	Aspartate aminotransferase, mitochondrial OS=Homo sapiens GN=GOT2 PE=1 SV=3
411	Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1
412	Uncharacterized protein C3orf67 OS=Homo sapiens GN=C3orf67 PE=2 SV=2
413	FYVE and coiled-coil domain-containing protein 1 OS=Homo sapiens GN=FYCO1 PE=1 SV=3
414	E3 UFM1-protein ligase 1 OS=Homo sapiens GN=UFL1 PE=1 SV=2
415	40S ribosomal protein S16 OS=Homo sapiens GN=RPS16 PE=1 SV=2
416	Proactivator polypeptide OS=Homo sapiens GN=PSAP PE=1 SV=2
417	Elongation factor 1-delta OS=Homo sapiens GN=EEF1D PE=1 SV=5
418	Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens GN=IDH2 PE=1 SV=2
419	Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1
420	Immunoglobulin superfamily member 11 OS=Homo sapiens GN=IGSF11 PE=2 SV=3
421	Protein fantom OS=Homo sapiens GN=RPGRIP1L PE=1 SV=2
422	E3 ubiquitin-protein ligase RAD18 OS=Homo sapiens GN=RAD18 PE=1 SV=2
423	Isoform 2 of Transketolase OS=Homo sapiens GN=TKT
424	Protocadherin-15 OS=Homo sapiens GN=PCDH15 PE=1 SV=2
425	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4
426	Rab GDP dissociation inhibitor alpha OS=Homo sapiens GN=GDI1 PE=1 SV=2
427	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial OS=Homo sapiens GN=NDUFV2 PE=1 SV=2
428	Ras-related protein Rab-1A OS=Homo sapiens GN=RAB1A PE=1 SV=3
429	Centrosomal protein of 290 kDa OS=Homo sapiens GN=CEP290 PE=1 SV=2
430	Leukocyte elastase inhibitor OS=Homo sapiens GN=SERPINB1 PE=1 SV=1
431	Fibronectin OS=Homo sapiens GN=FN1 PE=1 SV=4
432	Plexin-A3 OS=Homo sapiens GN=PLXNA3 PE=1 SV=2
433	Complement C4-B OS=Homo sapiens GN=C4B PE=1 SV=2
434	Complement component receptor 1-like protein OS=Homo sapiens GN=CR1L PE=1 SV=3
435	Transaldolase OS=Homo sapiens GN=TALDO1 PE=1 SV=2
436	Proteasome subunit alpha type-2 OS=Homo sapiens GN=PSMA2 PE=1 SV=2
437	Protein FAM53C OS=Homo sapiens GN=FAM53C PE=1 SV=1
438	Isoform 2 of Kinesin-like protein KIF17 OS=Homo sapiens GN=KIF17
439	60S ribosomal protein L30 OS=Homo sapiens GN=RPL30 PE=1 SV=2
440	Ribonuclease inhibitor OS=Homo sapiens GN=RNH1 PE=1 SV=2
441	L-xylulose reductase OS=Homo sapiens GN=DCXR PE=1 SV=2
442	Potassium-transporting ATPase subunit beta OS=Homo sapiens GN=ATP4B PE=1 SV=1
443	Putative FAM120A opposite strand protein OS=Homo sapiens GN=FAM120AOS PE=5 SV=1
444	Beta-hexosaminidase subunit beta OS=Homo sapiens GN=HEXB PE=1 SV=3
445	Phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger 2 protein OS=Homo sapiens GN=PREX2 PE=2 SV=1
446	Keratin, type I cytoskeletal 13 OS=Homo sapiens GN=KRT13 PE=1 SV=4

447	T-complex protein 1 subunit beta OS=Homo sapiens GN=CCT2 PE=1 SV=4
448	Ras-related protein Rap-1b-like protein OS=Homo sapiens PE=2 SV=1
449	Activated RNA polymerase II transcriptional coactivator p15 OS=Homo sapiens GN=SUB1 PE=1 SV=3
450	Isoform 4 of Bromodomain adjacent to zinc finger domain protein 2B OS=Homo sapiens GN=BAZ2B
451	Isocitrate dehydrogenase [NADP] cytoplasmic OS=Homo sapiens GN=IDH1 PE=1 SV=2
452	ELMO domain-containing protein 2 OS=Homo sapiens GN=ELMOD2 PE=1 SV=1
453	Coiled-coil domain-containing protein 17 OS=Homo sapiens GN=CCDC17 PE=2 SV=2
454	Isoform 2 of Ester hydrolase C11orf54 OS=Homo sapiens GN=C11orf54
455	Alcohol dehydrogenase 1A OS=Homo sapiens GN=ADH1A PE=1 SV=2
456	Olfactomedin-4 OS=Homo sapiens GN=OLFM4 PE=1 SV=1
457	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2
458	Coronin-1A OS=Homo sapiens GN=CORO1A PE=1 SV=4
459	Chromodomain-helicase-DNA-binding protein 2 OS=Homo sapiens GN=CHD2 PE=1 SV=2
460	Fibulin-1 OS=Homo sapiens GN=FBLN1 PE=1 SV=4
461	Interleukin enhancer-binding factor 2 OS=Homo sapiens GN=ILF2 PE=1 SV=2
462	14-3-3 protein theta OS=Homo sapiens GN=YWHAQ PE=1 SV=1
463	Eukaryotic initiation factor 4A-I OS=Homo sapiens GN=EIF4A1 PE=1 SV=1
464	Chromodomain-helicase-DNA-binding protein 3 OS=Homo sapiens GN=CHD3 PE=1 SV=3
465	Bcl-2-like protein 13 OS=Homo sapiens GN=BCL2L13 PE=1 SV=1
466	Isoform 2 of Ubiquitin carboxyl-terminal hydrolase 45 OS=Homo sapiens GN=USP45
467	Thyroglobulin OS=Homo sapiens GN=TG PE=1 SV=5
468	Putative heat shock protein HSP 90-beta 2 OS=Homo sapiens GN=HSP90AB2P PE=1 SV=2
469	Glial fibrillary acidic protein OS=Homo sapiens GN=GFAP PE=1 SV=1
470	Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=3

Table A.8 Proteins downregulated following treatment with curcumin

1	Kinesin-like protein KIF3B OS=Homo sapiens GN=KIF3B PE=1 SV=1
2	Ribosome-binding protein 1 OS=Homo sapiens GN=RRBP1 PE=1 SV=4
3	Macrophage-capping protein OS=Homo sapiens GN=CAPG PE=1 SV=2
4	5,6-dihydroxyindole-2-carboxylic acid oxidase OS=Homo sapiens GN=TYRP1 PE=1 SV=2
5	Cytotoxic and regulatory T-cell molecule OS=Homo sapiens GN=CRTAM PE=1 SV=2
6	IQ motif and SEC7 domain-containing protein 2 OS=Homo sapiens GN=IQSEC2 PE=1 SV=1
7	Zinc finger CCCH-type antiviral protein 1-like OS=Homo sapiens GN=ZC3HAV1L PE=1 SV=2
8	Tenascin OS=Homo sapiens GN=TNC PE=1 SV=3
9	ETS translocation variant 3 OS=Homo sapiens GN=ETV3 PE=1 SV=2
10	A-kinase anchor protein 13 OS=Homo sapiens GN=AKAP13 PE=1 SV=2
11	Complement factor H-related protein 3 OS=Homo sapiens GN=CFHR3 PE=1 SV=2
12	Apoptotic protease-activating factor 1 OS=Homo sapiens GN=APAF1 PE=1 SV=2
13	Late cornified envelope protein 1D OS=Homo sapiens GN=LCE1D PE=2 SV=1

14	Alpha-N-acetylglucosaminidase OS=Homo sapiens GN=NAGLU PE=1 SV=2
15	Keratin, type I cytoskeletal 16 OS=Homo sapiens GN=KRT16 PE=1 SV=4
16	Serine/threonine-protein phosphatase 2A activator OS=Homo sapiens GN=PPP2R4 PE=1 SV=3
17	Isoform 2 of Proteasome subunit beta type-8 OS=Homo sapiens GN=PSMB8
18	Inhibin beta E chain OS=Homo sapiens GN=INHBE PE=1 SV=1
19	WD repeat- and FYVE domain-containing protein 4 OS=Homo sapiens GN=WDFY4 PE=1 SV=3
20	Endoplasmic reticulum resident protein 29 OS=Homo sapiens GN=ERP29 PE=1 SV=4
21	Genetic suppressor element 1 OS=Homo sapiens GN=GSE1 PE=1 SV=3
22	Peptidyl-prolyl cis-trans isomerase FKBP2 OS=Homo sapiens GN=FKBP2 PE=1 SV=2
23	Proteasome subunit alpha type-4 OS=Homo sapiens GN=PSMA4 PE=1 SV=1
24	Mitogen-activated protein kinase kinase kinase MLK4 OS=Homo sapiens GN=MLK4 PE=1 SV=1
25	WD repeat-containing protein 65 OS=Homo sapiens GN=WDR65 PE=1 SV=3
26	Isoform 2 of Alcohol dehydrogenase class 4 mu/sigma chain OS=Homo sapiens GN=ADH7
27	Isoform 2 of Collagen triple helix repeat-containing protein 1 OS=Homo sapiens GN=CTHRC1
28	Apolipoprotein C-III OS=Homo sapiens GN=APOC3 PE=1 SV=1
29	Histone H2A type 3 OS=Homo sapiens GN=HIST3H2A PE=1 SV=3
30	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4
31	Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 1 OS=Homo sapiens GN=AGAP1 PE=1 SV=4
32	Glutamate dehydrogenase 1, mitochondrial OS=Homo sapiens GN=GLUD1 PE=1 SV=2
33	Isoform 4 of Minor histocompatibility antigen H13 OS=Homo sapiens GN=HM13
34	T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4
35	Isoform 2 of Eukaryotic translation initiation factor 6 OS=Homo sapiens GN=EIF6
36	Alpha-fetoprotein OS=Homo sapiens GN=AFP PE=1 SV=1
37	14-3-3 protein zeta/delta OS=Homo sapiens GN=YWHAZ PE=1 SV=1
38	Transmembrane protease serine 6 OS=Homo sapiens GN=TMPRSS6 PE=1 SV=3
39	Tubulin alpha-3C/D chain OS=Homo sapiens GN=TUBA3C PE=1 SV=3
40	Ribose-5-phosphate isomerase OS=Homo sapiens GN=RPIA PE=1 SV=3
41	Isoform 4 of Cadherin-13 OS=Homo sapiens GN=CDH13
42	Golgin subfamily A member 8-like protein 2 OS=Homo sapiens PE=2 SV=2
43	Histone H2A deubiquitinase MYSM1 OS=Homo sapiens GN=MYSM1 PE=1 SV=1
44	Rab3 GTPase-activating protein non-catalytic subunit OS=Homo sapiens GN=RAB3GAP2 PE=1 SV=1
45	Calpain-3 OS=Homo sapiens GN=CAPN3 PE=1 SV=2
46	Guanine nucleotide-binding protein subunit beta-2-like 1 OS=Homo sapiens GN=GNB2L1 PE=1 SV=3
47	Pre-mRNA 3'-end-processing factor FIP1 OS=Homo sapiens GN=FIP1L1 PE=1 SV=1
48	Isoform 2 of Heterogeneous nuclear ribonucleoprotein A/B OS=Homo sapiens GN=HNRNPAB
49	Phospholipid transfer protein OS=Homo sapiens GN=PLTP PE=1 SV=1
50	Neuritin OS=Homo sapiens GN=NRN1 PE=1 SV=1
51	Azurocidin OS=Homo sapiens GN=AZU1 PE=1 SV=3

52	Leucine-rich repeat-containing protein 66 OS=Homo sapiens GN=LRRC66 PE=2 SV=1
53	Interferon-related developmental regulator 1 OS=Homo sapiens GN=IFRD1 PE=1 SV=4
54	Transmembrane protein 200C OS=Homo sapiens GN=TMEM200C PE=2 SV=2

9. Bibliography

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