
# FATIGUE MEASUREMENT IN RENAL CANCER PATIENTS AND

# PRECLINICAL EVALUATION OF COMBINATION THERAPY IN RCC

**By**

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*This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.*



***Signed:***

***Date:***

**Dedication**

***This thesis is dedicated to my parents who believed that I could do it and supported me throughout my life.***

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# Table of Contents

# List of Figures

# List of Tables

## Abbreviations

| | |
|---|---|
| **AA** | Amyloid A |
| **ACTH** | Adrenocorticotropic hormone |
| **AJCC** | American Joint Committee on Cancer |
| **ANOVA** | Analysis of Variance |
| **ATP** | Adenosine triphosphate |
| **BFI** | Brief Fatigue Inventory |
| **BFS** | Bidimensional Fatigue Scale |
| **BHD** | Birt-Hog-Dube |
| **BMI** | Body Mass Index |
| **CD** | Crohn's Disease |
| **CI** | Chief Investigator |
| **CNS** | Central Nervous System |
| **CRF** | Cancer-Related Fatigue |
| **CRH** | Corticotropin-Releasing Hormone |
| **CSF** | Colony-Stimulating Factor |
| **CT** | Computerised Tomography |
| **CYP3A4** | Cytochrome P450 3A4 |
| **DFS** | Disease-Free Survival |
| **DMSO** | Dimethyl Sulfoxide |
| **ECOG** | Eastern Cooperative Oncology Group |
| **EGFR** | Epidermal Growth Factor Receptor |
| **EORTC-QLQ** | European Organisation for Research and Treatment of Cancer Questionnaire |
| **EPO** | Erythropoietin |
| **FACIT-F** | Functional Assessment Chronic Illness Therapy - Fatigue |
| **FACT-G** | Functional Assessment of Cancer Therapy - General |
| **FCS** | Foetal Calf Serum |
| **FQ** | Fatigue Questionnaire |
| **FSKI-15** | Functional Assessment of Cancer Therapy - Kidney Symptom Index-15 |
| **GABA** | Gamma-Aminobutyric Acid |
| **GBM** | Glioblastoma Multiform |
| **GDS** | Geriatric Depression Scale |
| **GIST** | Gastrointestinal Stromal Tumour |
| **GM-CSF** | Granulocyte-Macrophage-Colony Stimulating Factor |
| **HAMD** | Hamilton Depression Rating Scale |
| **HLRCC** | Hereditary Leiomyomatosis Renal Cell Carcinoma |
| **HPA** | Hypothalamic–Pituitary–Adrenal |

| | |
|---|---|
| **HPRC** | Hereditary Papillary Renal Cancer |
| **HUVECs** | Human Umbilical-Vein Endothelial Cells |
| **IBD** | Inflammatory Bowel Disease |
| **IL-2** | Interleukine-2 |
| **INF-α** | Interferon-Alpha |
| **IR** | Ionising Radiation |
| **IRAS** | Integrated Research Application System |
| **KPS** | Karnofsky Performance Status |
| **LDH** | Lactate dehydrogenase |
| **M.Wt** | Molecular Weight |
| **MCS** | Macmillan Cancer Support |
| **MDASI** | M. D. Anderson Symptom Inventory |
| **MDSC** | Myeloid-Derived Suppressor Cells |
| **MEM** | Minimum Essential Medium |
| **MEM NEAA** | Minimum Essential Medium Non-Essential Amino Acids |
| **MFSI** | Multidimensional Fatigue Symptom Inventory |
| **MHRA** | Medicine Health Regulatory Agency |
| **MOS-SF-36** | Medical Outcomes Study-Short Form |
| **mRCC** | Metastatic Renal Cell Carcinoma |
| **MRI** | Magnetic Resonance Imaging |
| **MSKCC** | Memorial Sloan-Kettering Cancer Centre |
| **mTOR** | Mammalian Target of Rapamycin |
| **NCCN** | National Comprehensive Cancer Network |
| **NCI-CTCAE** | Common Toxicity Criteria Adverse Events |
| **NF-kB** | Nuclear Factor–kappa B |
| **NHS** | National Health System |
| **NICE** | National Institute for Health and Care Excellence |
| **NSAIDs** | Non-Steroidal Anti-Inflammatory Drugs |
| **PAC** | Chemotherapy Paclitaxel |
| **PDGF** | Platelet-derived Growth Factor |
| **PE** | Plating Efficiency |
| **PET** | Positron Emission Tomography |
| **PFS** | Progression-Free Survival |
| **PI-3-K** | Phosphoinositide 3-Kinase |
| **PIS** | Patient Information Sheet |
| **PsA** | Psoriatic Arthritis |
| **PTEN** | Phosphatase and Tensin Homolog |
| **RBCs** | Red Blood Cells |
| **RCC** | Renal Cell Carcinoma |

| | |
|---|---|
| **ROS** | Reactive Oxygen Species |
| **RPF** | Revised Piper Fatigue |
| **RR** | Relative Risk |
| **SBRT** | Stereotactic Body Radiation Therapy |
| **SCID** | Structured Clinical Interview for Depression |
| **SD** | Standard Deviation |
| **SF** | Survival Fraction |
| **SSI** | Site-Specific Forms |
| **TCC** | Transitional Cell Cancer |
| **TKI** | Tyrosine Kinase Inhibitor |
| **TNM** | Tumour Node Metastasis |
| **TSC** | Tuberous Sclerosis Complex |
| **TSH** | Thyroid-Stimulating Hormone |
| **UC** | Ulcerative Colitis |
| **UICC** | Union for Cancer Control |
| **ULN** | Upper Limit of Normal |
| **VAFS** | Visual Analogue Fatigue Scale |
| **VEGF** | Vascular Endothelial Growth Factor |
| **VHL** | Von-Hippel-Lindau |

## Summary

In the United Kingdom, although only 3% of all new cancer diagnoses among men and even less for women are of kidney cancer, reports have also shown that the incidence of kidney cancer has increased not only in the United Kingdom, but also worldwide. In fact, in the United Kingdom, kidney cancer mortality rates have increased by 71% since the early 1970s. In response, the treatment and management of kidney cancer depends on whether the disease is clinically localised or metastasised at initial diagnosis. In metastatic patients, molecularly targeted therapies, including those using tyrosine kinase inhibitors (TKI) sunitinib and pazopanib, have largely replaced therapies with immunotherapy agents, which are less efficacious and more toxic than TKI. However, fatigue remains a commonly reported side effect of kidney cancer treatment involving TKI, which might make them intolerable. Accordingly, accurately measuring fatigue and identifying possible reasons for its onset and increase are vital to its early management.

In chapter 2, we measured fatigue in kidney cancer patients received sunitinib or pazopanib by using the Functional Assessment Chronic Illness Therapy for Fatigue (FACIT-F) tool. We also evaluated cancer symptoms and the impact of sunitinib and pazopanib on the quality of life of patients according to core items on the M. D. Anderson Symptom Inventory in four consecutive cycles. Among the 65 recruited patients receiving treatment at Beatson West of Scotland Cancer Centre in Glasgow, who participated in the study, 47 completed all four treatment cycles. Our results showed that the mean fatigue score range, based on the FACIT-F tool, in participants was 29.5–31.8, which considered diagnostic fatigue of < 34 based on the cutoff point of FACIT-F. Furthermore, we found that the severity of fatigue score increased when cancer symptoms increased in those patients.

Such results encouraged us to investigate a combination therapy proposed in the literature to minimise the incidence of side effects with a combination of reduced dose of TKI, sunitinib or pazopanib, and radiotherapy. In chapter 3, we report a preclinical laboratory study that we conducted to examine the efficacy of sunitinib and pazopanib in killing renal cancer cells in vitro when used as single agents, as well as their potential use in combination studies. Our results demonstrated that combination therapy was superior to monotherapy and that both sunitinib and pazopanib as single agents and in combination therapy with radiotherapy could induce apoptosis in both renal cell lines, 786-O and ACHN. Furthermore, we report our investigation into the cytotoxic effects of disulfiram, an anti-alcoholism drug not previously interrogated, in renal cell cancer alone and in combination with radiotherapy. Disulfiram demonstrated a cytotoxic effect, but not in a dose-dependent manner. Our results additionally demonstrated that copper could enhance the cytotoxicity of disulfiram in renal cell lines only with a low dose of disulfiram ≤ 10 μM. By contrast, radiotherapy combined with disulfiram ± copper did not decrease cell viability, and disulfiram alone could induce apoptosis in renal cell lines.

In sum, our results reveal that fatigue is a significant issue for most kidney cancer patients receiving sunitinib or pazopanib. TKI agents could improve the radiosensitivity of the renal cancer cell line and constitute an interesting option for managing kidney cancer in the hopes of discovering a novel combination regimen with the same efficacy and with less toxicity. In short, disulfiram exhibits potential anticancer properties in renal cancer cell lines.

# Chapter 1: Introduction to Renal Cancer

## 1.1 Anatomy of the renal system

Kidneys are regulatory organs that maintain the volume and composition of body fluids by filtering the blood and selectively secreting or reabsorbing filtered solutes. The kidneys are located behind the peritoneum, situated on the posterior wall of the abdomen on each side of the vertebral column, around the level of the twelfth rib. The right kidney is slightly lower in the abdomen than the left due to the presence of the liver pushing the right kidney downwards (1). The kidneys receive blood directly from the aorta via the renal arteries, filter it and any unabsorbed fluids and solutes then form urine. Urine contains a variety of waste materials excreted from the kidneys and passes via the fibromuscular ureters and into the bladder. The bladder can hold 700-1000 ml of urine without inducing high-pressure damage to the renal system because the bladder muscle is capable of distending. When urine is voided, the urethral sphincter at the base of the bladder relaxes and then the detrusor contracts. At that point, the urine is cleared via the urethra (1).

On sectioning (Figure 1), the kidney has a pale outer region - the cortex - and a darker inner region - the medulla. The medulla is divided into 8-18 conical regions, called the renal pyramids. The base of each pyramid starts at the corticomedullary border, and the apex ends in the renal papilla, which merges to form the renal pelvis and then on to form the ureter. In humans, the renal pelvis is divided into two or three spaces - the major calyces - which in turn divide into further minor calyces (1).

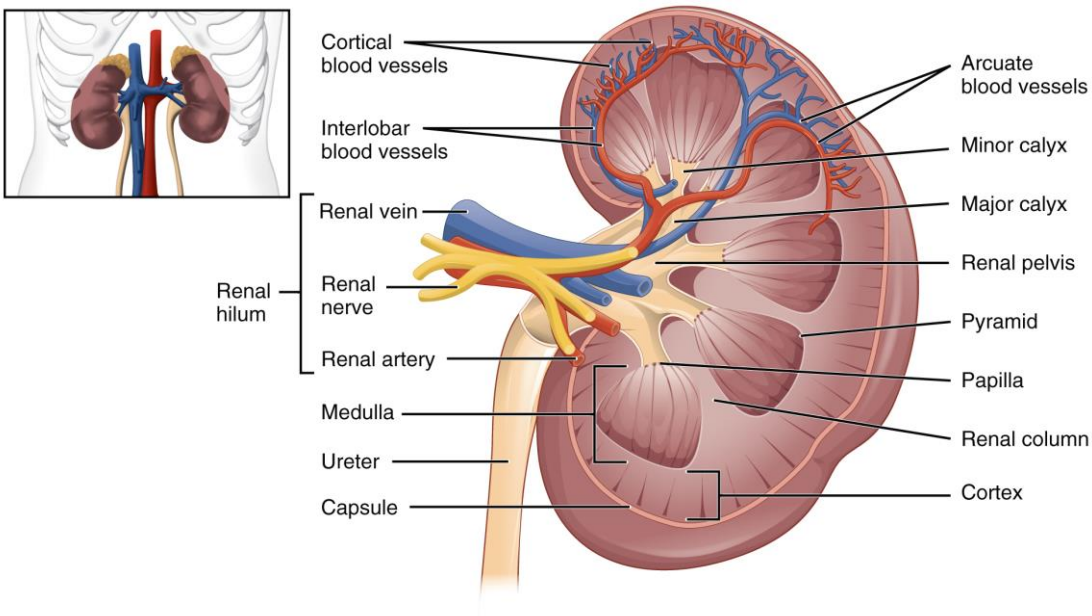


**Figure 1: Internal anatomy of the kidney. Reproduced from Betts *et al*. (1).**

The walls of the calyces, pelvis, and ureters are lined with smooth muscle that can contract to force urine towards the bladder by peristalsis. The cortex and the medulla are made up of nephrons; these are the functional units of the kidney, and each kidney contains about 1.3 million nephrons. The kidneys produce three hormones: calcitriol (1,25-dihydroxy vitamin $D_3$), which promotes intestinal absorption of dietary calcium, erythropoietin (EPO), which stimulates bone marrow to produce red blood cells (RBCs), and renin, which regulates blood pressure (1).

## 1.2 Renal cancer

Cancer is a disease caused by abnormal growth of cells, which tend to proliferate in an uncontrolled way and, in some cases, to metastasise (2). In the UK, kidney cancer accounts for almost 3% of adult malignancies with more than 11,000 new cases diagnosed per year and around 4,000 deaths reported annually (2). Renal cell

carcinoma (RCC), which originates within the renal cortex, is responsible for 85 to 90% of all primary renal neoplasms. Transitional cell carcinomas of the renal pelvis are the next most common (approximately 8%) type of renal cancer. Other parenchymal epithelial tumours, such as oncocytomas, collecting duct tumours, and renal sarcomas, occur infrequently (2). Nephroblastoma or Wilms' tumour is a kidney cancer common in children (5 to 6% of all primary renal tumours), while renal medullary carcinoma is a rare form of renal cancer, seen in individuals with sickle cell disease (2). The five-year survival for patients diagnosed with early-stage RCC is 57% but only 10% for patients with RCC who present with advanced or metastatic disease (3). Additionally, local recurrence or distant metastasis develops in up to 40% of patients treated for non-metastatic tumours (M0) (3).

## 1.3 Epidemiology of renal cancer

Only three percent of all new cancer diagnoses among men in the UK are kidney cancer, and the percentage is less for women. Thus, kidney cancer is considered an uncommon cancer. However, the incidence of kidney cancer has been increasing in the UK as well as globally. This increase may be related to the wider application of diagnostic imaging techniques that lead to the incidental detection of asymptomatic kidney cancer. In the UK, kidney cancer is considered the seventh most common cancer. In 2013, adult new cases of kidney cancer numbered 11,873 in the UK (4). In males, it is the fifth most common cancer (4% of all cancer in males), with 7,455 (63%) new cases diagnosed in 2013 whilst it is tenth in females (3% of all female cancer cases), with 4,418 (37%) of new cases diagnosed in 2013 (4). Thus, the male

to female ratio is approximately 17:10 in the UK. The crude incidence rate shows that there are 23.6 new cases of kidney cancer for every 100,000 males in the UK and 13.6 for every 100,000 females annually. The number of new cases that have been diagnosed in England, Scotland, Wales and North Ireland in 2013 is shown in Table 1 (4).

**Table 1: Number of new cases, crude and age-standardised (AS) incidence rates per 100,000 population (4).**

| Country | New Cases | Crude Rate[1] | AS Rate[2] |
|---|---|---|---|
| **Scotland** | | | |
| Men | 655 | 25.3 | 29.0 |
| Women | 405 | 14.8 | 14.9 |
| Total | 1,060 | 19.9 | 21.1 |
| **England** | | | |
| Men | 6,254 | 23.6 | 28.1 |
| Women | 3,646 | 13.3 | 13.9 |
| Total | 9,900 | 18.4 | 20.4 |
| **Wales** | | | |
| Men | 357 | 23.6 | 25.9 |
| Women | 232 | 14.8 | 14.1 |
| Total | 589 | 19.1 | 19.3 |
| **North Ireland** | | | |
| Men | 189 | 21.1 | 27.6 |
| Women | 135 | 14.5 | 16.2 |
| Total | 324 | 17.7 | 21.3 |
| **UK** | | | |
| Men | 7,455 | 23.6 | 28.0 |
| Women | 4,418 | 13.6 | 14.1 |
| Total | 11,873 | 18.5 | 20.4 |

[1] is calculated by dividing the total number of cases in a given time period by the total number of persons in the population. [2] Directly AS Rate (ASR).

## 1.4 Incidence rate by age

The incidence rate for kidney cancer has a directly proportional relationship to age (2). The number of diagnosed cases is greater in elderly patients than younger people, and rates of incidence show that kidney cancer diagnoses increase sharply after 45 years of age. Approximately half of kidney cancer cases (50%) are in patients over the age of 70, and the highest incidence by age ranges between 80 and 84 years old for men and between 75 and 79 years old for women (Figure 2) (4).

In contrast, kidney cancer in children is very rare, with only 85 cases diagnosed in the UK each year (4). Most of these cases are in children under five years old and are considered to be cases of Wilm's tumour, the most common paediatric kidney cancer (4). Von Hippel-Lindau disease, which is a hereditary kidney cancer, has also appeared rarely in paediatric cases (4). Figure 2 shows the average number of new cases of kidney cancer per year and age-specific incidence rates per 100,000 population in the United Kingdom.

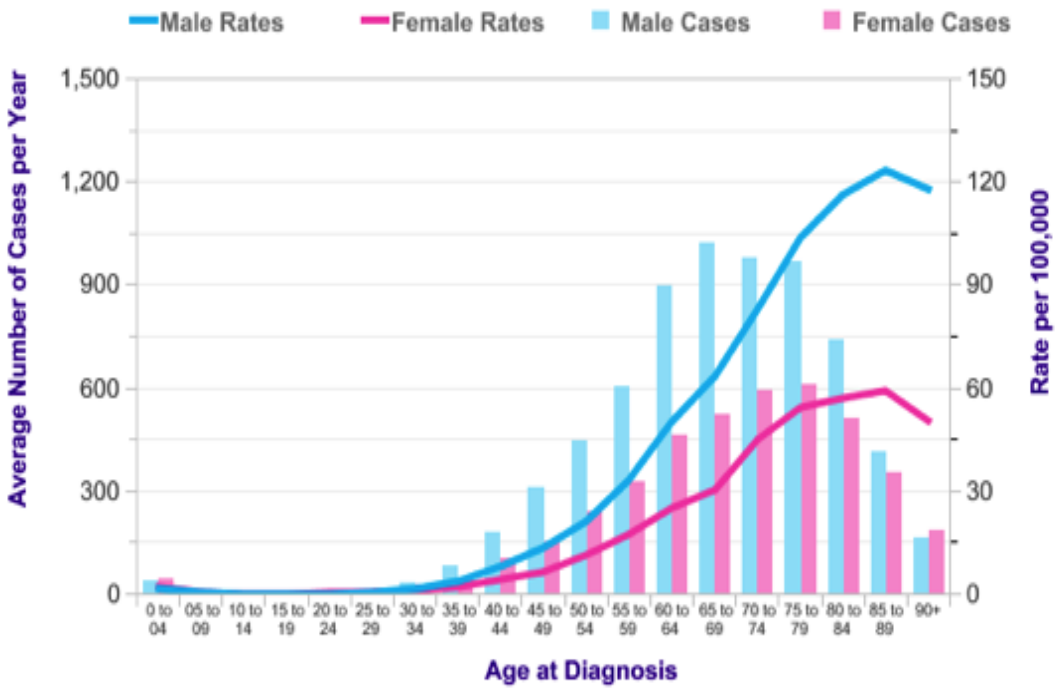


**Figure 2: Kidney cancer, average number of new cases per year and age-specific incidence rates per 100,000 population, UK, 2011-2013. Reproduced from Cancer Research UK (4).**

## 1.5 Trend incidence over time

The incidence of kidney cancer in the United Kingdom has been increasing since 1975, when there were 7.1 cases per 100,000 men; by 2013, this had increased to 28.0 cases per 100,000 men. In women, there were 3.2 cases in 1975 and 14.0 cases per 100,000 women in 2013. Most of this increase was observed in patients over the age of 60. Figure 3 shows trend incidence over time in the UK from 1979 to 2013 (4). This increase is likely due to the implementation of new imaging techniques including ultrasound, magnetic resonance imaging (MRI), and

computerised tomography (CT) scans that have led to the incidental detection of asymptomatic kidney cancer. However, the rise is probably also attributable to the changing prevalence of risk factors such as smoking and obesity.

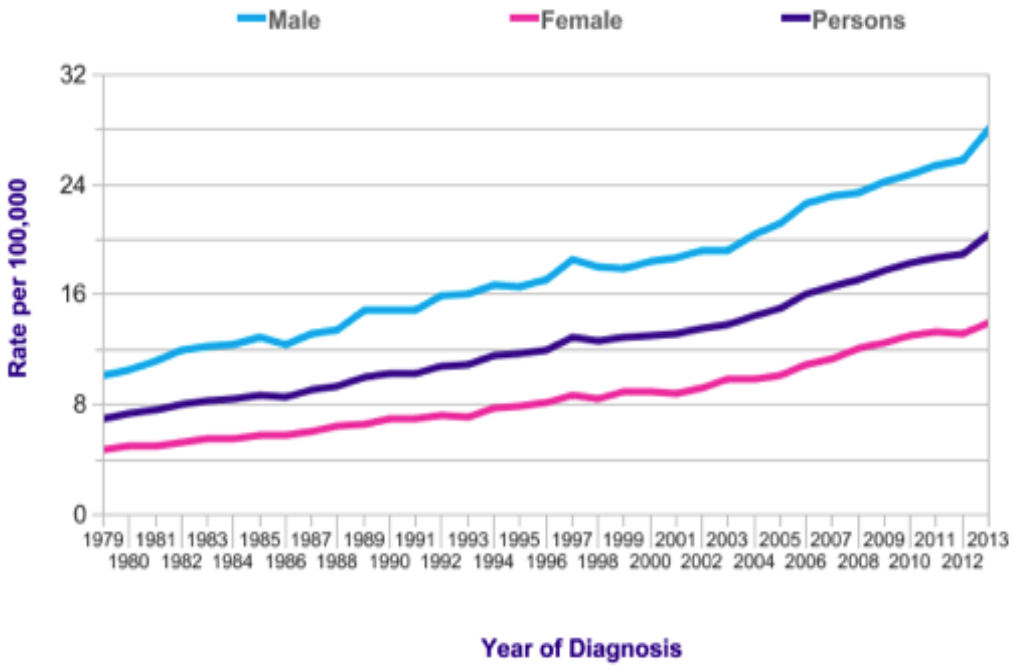


**Figure 3: European age-standardised incidence rates per 100,000 population, by sex of kidney cancer in the UK. Reproduced from Cancer Research UK (4).**

## 1.6 Incidence rate in Europe and worldwide

Kidney cancer is considered the seventh most common cancer in Europe, with more than 115,000 new cases diagnosed in 2012 (3% of the total cancer cases diagnosed in Europe). The Czech Republic has the highest age-standardised incidence rates for kidney cancer for both men and women in Europe; the lowest rates for women are in Cyprus and, for men, in Macedonia (5). Worldwide, kidney cancer is estimated to be the 13th most common cancer, with around 338,000 new cases diagnosed in 2012 (2% of total cancer cases worldwide). In the United States, the incidence rates are the highest with 57,500 cases, while the lowest rates are in Turkey with 3,600 cases (5). The differences among countries may reflect the varying prevalence of risk factors, and diagnostic methods.

## 1.7 Incidence rate in the UK by ethnicity

Table 2 shows the age standardisation rates for kidney cancer disease among different ethnicities (6). From this table we can identify that white males have the highest incidence rates of kidney cancer, while Asian females have the lowest incidence rates (6).

**Table 2: Incidence rate of kidney cancer in the UK based on ethnicity (6).**

| Ethnicity | Incidence rates per 100,000 | |
|---|---|---|
| | Male | Female |
| **White** | 11.2 to 11.8 | 5.7 to 6.0 |
| **Asian** | 5.3 to 9.2 | 1.9 to 3.8 |
| **Black** | 5.9 to 10.8 | 3.0 to 6.0 |

## 1.8 Prevalence

Prevalence refers to the number of people who have previously received a diagnosis of cancer and who are still alive at a given time point (Table 3) (7). Table 3 shows the burden of kidney cancer, information which can help to inform health care service for the future.

**Table 3: Kidney cancer prevalence in the UK, December 2006 (7).**

| Gender | 1-year prevalence | 5-year prevalence | 10-year prevalence |
|---|---|---|---|
| **Male** | 3,186 | 10,771 | 16,468 |
| **Female** | 1,894 | 6,466 | 10,035 |
| **Persons** | 5,080 | 17,237 | 26,503 |

## 1.9 Risk factors

As previously mentioned, several risk factors have been well established for renal cell carcinoma, including tobacco use, obesity, hypertension, acquisition of cystic disease, occupational exposure to chemical carcinogens, analgesics, and genetic factors (8). However, the complexity of these associations and their mechanisms have yet to be fully elucidated. Other risk factors, such as reproductive and hormonal factors, occupational exposures, and dietary habits have also been implicated, but the evidence remains inconclusive (8).

### 1.9.1 Established risk factors

Cigarette smoking is associated with an increased risk of developing RCC and incidence is proportional to the extent of exposure. Smoking possibly contributes to RCC development in around one-third of all cases. In addition, an increased use of

cigarettes has been observed to be related to a more advanced disease at diagnosis (9).

Hypertension predisposes the body to RCC development, which seems to be independent of anti-hypertensive medications or obesity (10). The independent contribution of antihypertensive medications and obesity has been difficult to clarify due to their close correlation with hypertension. The underlying biological explanations linking hypertension to RCC remain unclear (10).

A prospective analysis of over 300,000 participants in the National Institutes of Health and the American Association for Retired Persons (NIH-AARP) Diet and Health Study proved that excessive body weight is a risk factor for RCC in both men and women and concluded that the relative risk (RR) of RCC increased progressively with baseline body mass index (BMI) (11).

In addition, a clinical study by Brennan et al. suggested that the risk of developing RCC from acquired cystic disease is estimated to be 30 times greater in dialysis patients with acquired polycystic disease of the kidney compared with the general population (12).

Some occupations and industrial exposures have also been linked to RCC, but generally RCC is not considered an occupational disease. Occupational exposure to toxic compounds, such as cadmium, asbestos, and petroleum by-products, has however been associated with an increased risk of RCC (8). The prolonged ingestion of analgesic combinations, particularly compounds containing phenacetin (now discontinued but of which acetaminophen is a major metabolite) and aspirin, can

lead to chronic renal failure (8). Epidemiological studies have proven that an increased use of aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), and acetaminophen lead to an increased risk of RCC (8).

Furthermore, the risk of recurrence of metachronous renal cell carcinoma is increased in patients who have been treated for one renal cancer. This increased risk is most apparent in younger patients with a first diagnosis of RCC, suggesting that early-onset renal cancer has a genetic component (16). Factors that favour a hereditary contribution in patients without a clear genetic disease include first-degree relatives with a tumour, onset before the age of 40, and bilateral or multifocal disease. Other individuals with a clear genetic contribution have abnormalities on chromosome 3, and additional genetic abnormalities have been identified in other families: Von-Hippel–Lindau (VHL), hereditary papillary renal cancer (HPRC), Birt-Hog-Dube (BHD), hereditary leiomyomatosis and renal cell carcinoma (HLRCC), and tuberous sclerosis (8).

### 1.9.2 Other factors that modify the risk

A history of diabetes mellitus, and those using insulin for glycaemic control, has been linked with a modest increase in the risk of RCC in some studies, but not in others (13). This may be mediated through an increased incidence of hypertension. Other clinical factors that may increase the risk of developing RCC include dietary factors such as the intake of nitrites from processed meat sources, reproductive factors such as a high number of pregnancies, and prior radiation therapy (8).

## 1.10 Pathology

The most common type of kidney cancer in adults is renal cell carcinoma (RCC); 85 to 90% of kidney cancer patients are diagnosed with RCC. In renal cell carcinoma the cancerous cells line the smallest tubes inside the nephrons. Other types of kidney cancers are known as transitional cell cancer (TCC) and Wilms' tumour. TCC is less common and represents only 7 to 8% of patients diagnosed with kidney cancer. The treatment of this type of cancer is similar to the treatment of bladder cancer. Wilms' tumour mainly affects children (8).

Previously, lesions smaller than 3 cm were thought to represent benign adenomas, but now it is clear that even small tumours frequently represent carcinomas. The method of distinguishing between a malignant and a benign tumour based upon size alone is no longer in use. Instead, basic histological criteria are used to discriminate between a malignant or benign growth. Thus, all solid renal masses require resection or biopsy for accurate diagnosis (14).

RCC was previously classified by cell type and growth pattern. This classification has recently changed to more accurately reflect the morphology, growth patterns, cell of origin, histochemical, and molecular basis of the different types of adenocarcinomas. Several distinct subtypes of RCC have been identified, including:

1. Clear cell, which is represented in 75 to 85% of tumours;
2. Papillary, which appear in 12 to 14% of tumours;
3. Chromophobe, which is observed in 4 to 6% of tumours;

4. Oncocytic, which represent around 2 to 4% of tumours;

5. Collecting duct (Bellini's duct), which is observed very rarely (1%).

Less than 5% of RCCs are considered unclassified. These unclassified tumours had a worse prognosis compared with clear cell cancers, although the outcome was not different after adjusting for adverse clinical pathological features such as stage and grade (15). Other tumour types that have been reported to arise in the kidney include soft tissue sarcomas (e.g. leiomyosarcoma, liposarcoma), lymphomas and carcinoids (15).

### 1.10.1 Clear cell carcinomas

The most common type of RCC tumour are clear cell carcinomas, which typically have a deletion of chromosome 3p and arise from the proximal tubule (17). Macroscopically, they may be solid or, less commonly, cystic.

### 1.10.2 Papillary carcinomas

The second most common subtype of renal cell carcinoma is papillary renal cell carcinoma, also known as chromophilic renal cell carcinoma because the cell takes up certain dyes and looks pink coloured under the microscope. Papillary renal cell carcinoma forms in the lining of the kidney's tubules, which are very small tubes that filter waste products from the blood and produce urine (16).

### 1.10.3 Chromophobe carcinomas

Histologically, chromophobe carcinomas are composed of sheets of cells that are darker than clear cell carcinoma. They lack the abundant lipid and glycogen that is characteristic of most RCCs and originate from the intercalated cells of the collecting system (16).

### 1.10.4 Oncocytomas

Oncocytomas account for approximately 2 to 4% of all renal tumours. Like chromophobe carcinomas, oncocytomas appear to originate from the intercalated cells of the collecting ducts. While sporadic oncocytomas are usually unilateral and single, multiple and bilateral oncocytomas occur in patients with genetic syndromes such as tuberous sclerosis complex (TSC) and Birt-Hogg-Dube syndrome (16).

### 1.10.5 Collecting duct tumours

Although collecting duct (Bellini's duct) tumours are very rare, they tend to occur in younger patients and are frequently aggressive. They commonly present with massive haematuria (16).

## 1.11 Clinical manifestations

Most RCC symptoms do not appear until the disease is advanced. Patients with a localised disease can present with a wide array of symptoms and/or laboratory abnormalities, or they may be diagnosed incidentally. A review of 309 consecutive patients with RCC represented the most common presenting symptoms as

haematuria, abdominal mass, pain, and weight loss (18). Nowadays, there is an increased frequency of incidental diagnosis due to developing radiological procedures. This may contribute to better outcomes in RCC (18). In a series of 701 patients, those who were diagnosed incidentally had a significantly better disease-specific survival rate of five years (76%, versus 44% in those with symptoms). Multivariate analysis showed that the difference was due to the earlier stage and lower histological grade at the time of diagnosis (19).

## 1.12 Signs and symptoms

### 1.12.1 Classic signs and symptoms

The classic symptoms and signs for RCC are haematuria, flank pain, and a palpable abdominal renal mass, occurring in most kidney cancer patients. Haematuria is observed only with tumour invasion of the collecting system and is observed in around 40% of patients. When the haematuria is severe, the bleeding can cause clots and "colicky" discomfort. An abdominal or flank mass is more commonly palpated in thin adults and is associated with lower pole tumours. The mass is generally firm, non-tender, homogeneous, and moves with respiration. Scrotal varicoceles, the majority of which are left-sided, are observed in as many as 11% of men with RCC. This finding should always arouse suspicion for a kidney tumour that has obstructed the gonadal vein where it enters the renal vein. Inferior vena cava involvement can produce various clinical manifestations, including ascites, lower extremity oedema, hepatic dysfunction, and pulmonary emboli (16).

Among patients with disseminated disease, signs or symptoms may be due to a metastatic tumour; the most common sites of the spread include the lungs, bones, lymph nodes, liver, and brain. In this setting, the diagnosis is often made either by finding a renal mass on abdominal computed tomography or by biopsy of an accessible metastasis (16).

### 1.12.2 Paraneoplastic symptoms

Many patients with RCC present with or subsequently develop systemic symptoms or paraneoplastic symptoms. In some instances, these may be due to ectopic production of various hormones (e.g. erythropoietin, parathyroid hormone-related protein, gonadotropins, renin, glucagon and insulin).

Anaemia, which can precede the diagnosis of RCC by several months, has been reported in around 29% of patients with advanced disease (20). The anaemia is often disproportionately severe, can be either normocytic or microcytic, and is frequently associated with typical iron profiles of those observed with anaemia of chronic disease.

Hepatic dysfunction is an uncommon occurrence in patients with RCC; it is called Stauffer's syndrome when it occurs in the absence of liver metastases. When hepatic dysfunction is present, it is frequently associated with fever, weight loss, fatigue, and a poor prognosis. The dysfunction may result from the tumour production of cytokines, such as granulocyte macrophage-colony stimulating factor (GM-CSF) and possibly interleukin-6 (21). Fever, which occurs in up to 20% of

patients, is usually intermittent and is frequently accompanied by night sweats, anorexia, weight loss, and fatigue (20). Hypercalcaemia happens in around 15% of patients with RCC. Also, Erythrocytosis presents in 1 to 5% of patients with RCC and appears to be due to the constitutive production of erythropoietin (22).

Secondary amyloid A (AA) amyloidosis reflects a chronic inflammatory response as the amyloid fibrils are composed of fragments of the acute phase reactant serum amyloid A protein. This appears in 3 to 5% of patients (23). Thrombocytosis is very rare in patients with RCC, but its presence is associated with a poor prognosis. The underlying mechanism is not established but could be related to IL-6 production by the tumour. Polymyalgia rheumatica has been reported with RCC. Polymyalgia rheumatic is an inflammatory disorder that causes muscle pain and stiffness in different parts of the body, especially affecting the neck, shoulder or arms.

## 1.13 Diagnostic evaluation

### 1.13.1 Radiographic testing

Patients with unexplained haematuria, or other symptoms, signs or findings suggestive of a possible RCC, must undergo radiographic evaluation for the presence of a renal mass. The usual first test is an abdominal ultrasound or computer tomography. Incidental diagnosis is becoming more common as a result of the frequent use of ultrasonography and/or abdominal CT for the evaluation of an unrelated problem (16).

### 1.13.2 Magnetic resonance imaging (MRI)

MRI may be used for RCC diagnosis when CT and ultrasonography are non-diagnostic and/or radiographic contrast cannot be received because of an allergy or renal failure. MRI may be particularly valuable if a tumour is present; it can help identify the presence of local invasion of the collecting system or inferior vena cava. Although the diagnosis of RCC is occasionally established by a biopsy, this is used in most cases to obtain tissue for histology and treatment. Other procedures for assessing distant metastases that may be used are bone scan, CT of the chest, MRI, and positron emission tomography (PET). A bone scan is indicated only in patients with bone pain and/or elevated serum alkaline phosphatase. A CT of the chest is indicated to evaluate for evidence of pulmonary or mediastinal lymph node metastases. MRI scanning with gadolinium is superior to CT for the evaluation of the right atrium and inferior vena cava when tumour involvement is suspected. PET scanning has high sensitivity and specificity for the primary lesion. Although a PET scan might be sensitive for the detection of bone metastases, it is highly expensive and is therefore of limited use for routine staging (24).

## 1.14 Stages and grades

Primarily abdominal CT determines the extent of local and regional involvement, which is extremely accurate in staging RCC. The Tumour Node Metastasis (TNM) staging system is used for staging all histologic variants of renal carcinoma. This system is supported by both the American Joint Committee on Cancer (AJCC) and the International Union for Cancer Control (UICC) and was revised in 2010 (25). Kidney cancer stage can be reported using the TNM system (Table 4) or stage number (Table 5).

**Table 4: TNM stages of renal cell carcinoma. Modified from Cancer Research UK (25).**

| **Primary tumour (T)** | |
|---|---|
| **T0** | No evidence of primary tumour |
| **T1** | Tumour ≤ 7 cm in greatest dimension, limited to the kidney |
| **T2** | Tumour >7 cm in greatest dimension, limited to the kidney |
| **T3** | Tumour extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota's fascia |
| **T4** | Tumour invades beyond Gerota's fascia (including contiguous extension into the ipsilateral adrenal gland). |
| **Regional lymph nodes (N)** | |
| **NX** | Regional lymph nodes cannot be assessed |
| **N0** | No regional lymph node metastasis |
| **N1** | Metastasis in regional lymph node(s) |
| **Distant metastasis (M)** | |
| **M0** | No distant metastasis |
| **M1** | Distant metastasis |

**Table 5: Stage numbers of renal cell carcinoma. Modified from Cancer Research UK (25).**

| **Anatomic stage/prognostic groups** | | | |
|---|---|---|---|
| **Stage I** | T1 | N0 | M0 |
| **Stage II** | T2 | N0 | M0 |
| **Stage III** | T1 or T2<br>T3 | N1<br>N0 or N1 | M0<br>M0 |
| **Stage IV** | T4<br>Any T | Any N<br>Any N | M0<br>M1 |

**Grades**

In general, doctors grade cancer to indicate how quickly or slowly a cancer is likely to grow and spread. Therefore, we could say that the grade affects the prognosis not the treatment. The grading of RCC is based on the microscopic morphology of neoplasm with hematoxylin and eosin (H and E) staining. The most popularly used system for grading is the nuclear grading system described in 1982 by Fuhrman et al. (26). The pathology report usually uses the "Fuhrman Grade". A grade correlates with stages of the disease; later stages tend to correspond to higher grade (grade 4). Table 6 expresses the Fuhrman Grade (26).

**Table 6: Fuhrman grades of renal cell carcinoma (26).**

| **Grade** | **Nuclear** | | **Nuclear quality** |
|---|---|---|---|
| | **Nuclear size** | **Shape** | |
| **Grade 1** | 10µm | Small, round and uniform | Absent to inconspicuous |
| **Grade 2** | 15µm | Slightly irregular nuclear membrane | Evident at high power (x400 magnification) |
| **Grade 3** | 20µm | Obviously irregular membrane | Prominent, large at lower power (x100 magnification) |
| **Grade 4** | > 20µm | Irregular nuclear membrane, pleomorphic, multi-lobed with clumped chromatin | Heavy chromatin clump |

## 1.15 Prognosis of disease

The TNM staging system is the most consistent factor that correlates with prognosis in patients with RCC.

**Stage I/II** — Patients with Stage I RCC have a five-year survival rate of over 90% (25). The survival rate may be slightly lower in patients with stage II disease, with reported five-year survival rates ranging from 75 to 95%. Patients with stage I or II RCC that invades the urinary collecting system appear to have a significantly worse prognosis. The 10-year survival rates for patients with T1 or T2 primary lesions that invade the urinary collecting system are 43 and 41%, respectively (25).

**Stage III** — The five-year survival rate for patients with stage III RCC who undergo nephrectomy ranges from 59 to 70%. In addition, invasion of the urine collecting system also appears to be a prognostic factor in patients with stage III RCC (25).

**Stage IV** — The median survival rate for patients with stage IV disease is 16 to 20 months, and the five-year survival rate is less than 10% in patients with distant metastases (27).

In addition, there is increasing evidence that local and systemic inflammatory responses play a significant role in the progression of various solid tumours, such as lung, prostate, bladder and renal. Modified Glasgow Prognosis Survival (mGPS) offers prognostic information in hrenal cancer patients. mGPS was structured by measuring preoperative C- reactive protein and albumin concentrations. A cohort study was conducted with 79 localised renal cancer patients who had undergone potential curative resection (28). The study concluded that mGPS is an independent predictor of poor cancer-specific survival in localised renal cancer patients undergoing potential curative resection. Table 7 below illustrates that mGPS criteria. Also, the prognostic role of tumour necrosis is well studied in various tumours like lung and colorectal. However, there are still some conflicting results and it is not approved as an independent prognostic factor in renal cancer patients.

**Table 7: modified Glasgow Prognostic Score (28).**

| mGPS | Score |
|---|---|
| C-RP ≤10mg/l and albumin ≥35g/l | 0 |
| C-RP >10mg/l | 1 |
| C-RP >10mg/l and albumin <35g/l | 2 |

mGPS: modified Glasgow Prognostic Score. C-RP: C- Reactive Protein.

## 1.16 Screening

Screening of asymptomatic individuals is not recommended in the general population. Individuals with high risk for the development of RCC should undergo periodic monitoring with abdominal ultrasonography, CT, or magnetic resonance imaging to detect the disease early. Candidates for screening include patients with any of the following conditions: an inherited disease associated with an increased incidence of RCC (including Von Hippel-Lindau syndrome and tuberous sclerosis), end-stage renal disease, younger patient who have been on dialysis for three to five years or more, family history of RCC, or prior kidney irradiation or tumour (16).

## 1.17 Management of renal cell carcinoma

### 1.17.1 Introduction

The therapeutic process to manage renal cell carcinoma is guided by the probability of cure, which is directly related to the stage or degree of tumour metastases. More than half of patients with early stage renal cell carcinoma are cured. In contrast, the clinical outcome for stage IV disease is poor. Reviewing all available treatment options and the associated benefits and risks with the patient is recommended for management of the renal mass. This review should include oncologic issues, renal functional issues, and potential complications. The treatment options for renal cell cancer are as follows: surgery, radiation therapy, immunotherapy, molecular targeted therapy or mammalian target of rapamycin agents (27).

As discussed, the management of RCC depends on whether or not the disease is clinically localised or metastasised at initial diagnosis. When patients are diagnosed with localised disease, surgical resection can be curative (27). Unfortunately, many RCCs are clinically silent. Thus, the diagnosis is frequently not made until the disease is either locally advanced or metastatic. The prognosis for long-term, disease-free survival for patients with locally advanced or metastatic RCC is generally poor. RCC can be classified as localised disease, which includes stage I, II, and III RCC and advanced disease that includes the tumour invading beyond Gerota's fascia or extending into the ipsilateral adrenal gland (T4) and metastatic disease (M1). Either of these findings constitutes stage IV RCC (27).

### 1.17.2 Treatment of localised RCC

#### 1.17.2.1 Surgery

Surgery is curative in the majority of patients without metastatic renal cell carcinoma and is, therefore, the preferred treatment for patients with stages I, and II of the disease. Treatment can involve either a radical nephrectomy or a variety of renal-sparing approaches (partial nephrectomy or ablative techniques) in carefully selected patients, depending upon the extent of the disease (28).

Elderly patients and those with significant comorbidities may not be suitable candidates for surgical resection. Therefore, nonsurgical procedures such as cryoablation and radiofrequency ablation may be useful. Cryotherapy kills cancer cells by freezing them and is usually used if the tumour is less than 4 cm in diameter

and the patient cannot have surgery. The medical term for cryotherapy procedures is percutaneous cryotherapy for renal cancer. Radiofrequency ablation is an electrical energy method that kills cancer cells by heating the tumour. This is mainly used in situations similar to cryotherapy or if the patients have more than one small tumour in both kidneys. Most small tumours grow slowly and do not become symptomatic or metastasise. In addition, almost 40% of tumours less than 1 cm in diameter may be benign. In this setting, observation with routine re-evaluation is recommended (27).

#### 1.17.2.2 Adjuvant therapy

Adjuvant therapy is used with either immunotherapy or molecularly targeted agents after surgery and has not been shown to improve the outcomes for localised RCC (27).

#### 1.17.2.3 Immunotherapy

The ability of immunotherapy to induce objective tumour responses in some patients with advanced RCC has led to the evaluation of various immunotherapeutic strategies as adjuvant therapy following surgical resection. An overview by Atkins et al. stated that multiple randomised control trials have not demonstrated a survival benefit from interleiukin-2 or interferon-alpha agents when used as adjuvant therapy after surgery in renal cancer patients (27).

#### 1.17.2.4 Molecularly targeted therapy

The ASSURE clinical trial (adjuvant sunitinib or sorafenib versus placebo in patients with resected renal cell carcinoma) is currently examining the role of molecularly targeted therapy (such as sunitinib or sorafenib) as adjuvant therapy in renal cancer patients (30), comparing sunitinib and sorafenib to placebos in patients with resected intermediate or high-risk RCC. However, initial results of the ASSURE study failed to extend disease-free survival (DFS) in patients with locally advanced kidney cancer who are at surgical resection RCC stage (30).

### 1.17.3 Treatment of advanced RCC

The majority of patients with stage IV RCC have unresectable tumours. Generally, patients with metastatic RCC should receive medical therapy, such as molecular target therapy, immunotherapy, chemotherapy or hormonal therapy**.** However, for a subset of patients in whom the tumour directly involves the ipsilateral adrenal gland, a radical nephrectomy, which includes adrenalectomy, is potentially curative (27).

Molecularly targeted therapy has largely replaced the use of immunotherapy for patients with advanced RCC. However, immunotherapy with interleukine-2 (IL-2) or interferon-alpha (INF-α) remains an option for select patients. In summary, treatment can be stratified by whether or not patients were treated for advanced RCC.

The National Institute for Health and Care Excellence (NICE) reported that, for patients with Eastern Cooperative Oncology Group (ECOG) performance status 0-1 (Karnofsky Performance Status (KPS) ≥ 80%) and good organ function, targeted agents, Tyrosine Kinase Inhibitor (TKI), sunitinib and pazopanib, are approved for treatment in patients with metastatic or advanced RCC. Clinical trials data have shown that sunitinib and pazopanib prolong progression-free survival when compared with INF-α (sunitinib) or placebo (pazopanib), and should, therefore, be offered to newly diagnosed patients (31, 32). The clinical management guidelines for metastatic renal cell carcinoma adapted in the West of Scotland Beatson Cancer Centre, in which we undertook our trial, are shown in Figure 4.

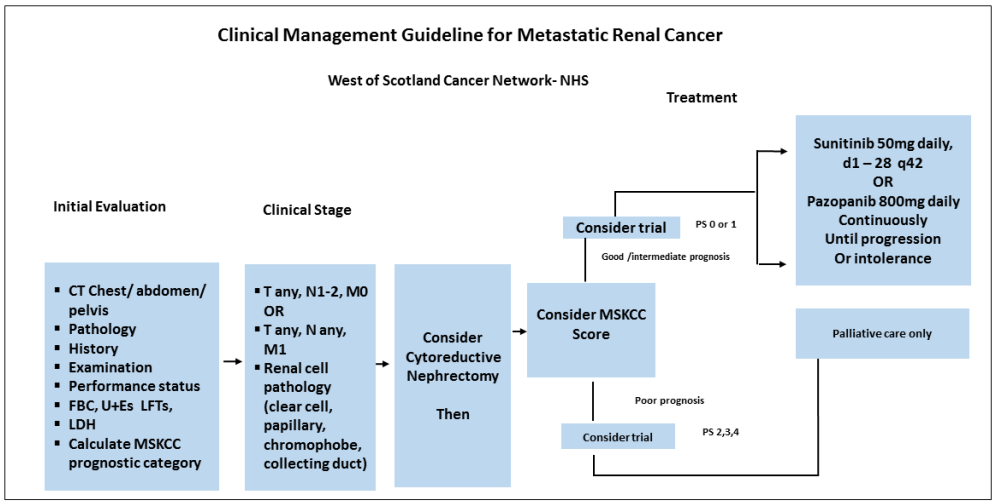


**Figure 4: Clinical management guideline for metastatic renal cell carcinoma.**

Reproduced from the Clinical Management Guideline of Metastatic Renal Cell Carcinoma (mRCC) (33).

For patients whose disease progresses after treatment with a TKI, sunitinib or pazopanib, other drugs such as the monoclonal antibody (bevacizumab) or the use of different TKI agents (axitinib) are considered as a reasonable option (27). Due to the absence of prospective trials, a decision to use either agent is based on individual patient considerations, such as tumour response to the prior VEGF pathway inhibitor (34). For patients who are not candidates for molecular targeting agents, immunotherapies including high dose IL-2 or INF-α is considered. Beyond these choices, additional options including surgery and radiation therapy may be used in carefully selected patients. Chemotherapy and hormonal therapy have limited use in clear cell carcinoma (27).

#### 1.17.3.1 Molecularly targeted therapy

##### 1.17.3.1.1 Vascular endothelial growth factor (VEGF) pathway

Two different approaches play a clinical role in blocking the VEGF pathway: first, small molecule tyrosine kinase inhibitor (TKI) (i.e. sunitinib, pazopanib, axitinib and sorafenib) block the intracellular domain of the VEGF receptor, and, secondly, a monoclonal antibody (bevacizumab) binds circulating VEGF and prevents it from activating the VEGF receptor (27). Randomised clinical trials have established the role of the VEGF receptor inhibitors for the initial management of advanced RCC and for the treatment of patients with disease progression after cytokine therapy, which will be discussed in section 1.18.

The anti-VEGF monoclonal antibody, bevacizumab, is considered to be an alternative treatment for patients who are not able to receive TKI medications

because of intolerable side effects or disease progress. Bevacizumab has potential activity in both untreated patients and those who have failed cytokine therapy. The activity of bevacizumab in treatment-naïve patients was demonstrated in a phase III trial in combination with IFN-α compared with IFN-α alone, but results left it unclear to what extent the concomitant IFN-α administration contributed to the activity of the combination (35). The trial enrolled 732 metastatic renal cell carcinoma patients who were not treated previously and concluded that overall survival for the bevacizumab plus IFN-α arm was 18.3 months compared with 17.4 months with IFN-α given as a monotherapy (35).

#### 1.17.3.1.2 Mammalian target of rapamycin (mTOR) pathway

Mammalian target of rapamycin is a protein that helps control several cell functions, including cell division and survival, and which binds to rapamycin and other drugs (27). The mTOR pathway is downstream of the phosphoinositide 3-kinase and Akt pathways, which are regulated by the phosphatase and tensin homolog (PTEN) tumour suppressor gene. The importance of the physiological function of PTEN is illustrated by its frequent disruption in cancer. By suppressing the phosphoinositide-3-kinase (PI-3-K)–AKT–mammalian target of the rapamycin pathway through its lipid phosphatase activity, PTEN controls a plethora of cellular processes including survival, proliferation, energy metabolism and cellular architecture. Therefore, inhibition of the mTOR pathway has the potential to limit tumour progression at multiple levels (27).

The mTOR targeting agents such as everolimus, which is given orally, and temsirolimus, which is administered intravenously, are inhibitors of the mammalian target of rapamycin, a component of an intracellular signalling pathway that regulates cellular metabolism, growth, proliferation, and angiogenesis. These agents bind to an intracellular protein, FKBP-12, forming a complex that inhibits the mTOR serine-threonine kinase. Temsirolimus and everolimus are alternatives to VEGF receptor inhibition (36).

#### 1.17.3.2 Immunotherapy

Before the development of active, molecularly targeted agents, immunotherapy with either IL-2 or INF-$\alpha$ represented the primary choices for the treatment of patients with metastatic RCC.

##### 1.17.3.2.1 Interleukin-2 (IL-2)

High-dose bolus IL-2 can activate an immune response against RCC that results in tumour regression in a minority of patients. Although treatment is associated with severe toxicity, responses often persist for many years, even in the absence of additional therapy. The majority of complete responders remain free of relapse long term. High-dose IL-2 remains an important option for carefully selected patients who are able to tolerate the toxicity associated with this approach and who have access to this treatment, because of its ability to induce durable long-term remission in approximately 10% of patients (27).

#### 1.17.3.2.2 Interferon alpha (INF-α)

The activity of monotherapy with INF-α in metastatic RCC has been extensively evaluated. Using a variety of preparations, doses, and schedules, the overall response rate may be as high as 15% (27). The median time to response is about four months, and most responses are partial and rarely persist beyond one year. A meta-analysis by Coppin et al. based on four studies that included a total of 644 patients reported that treatment with IFN-α was associated with an average median improvement in survival of almost four months (29). Molecularly targeted agents that have demonstrated improved efficacy compared with INF- α without additional toxicity have largely replaced the use of INF-α (29).

### 1.17.3.3 Other treatments

Chemotherapy and hormonal therapy have limited use in renal cell carcinoma. Additional options, such as radiation therapy, may be used in carefully selected patients.

#### 1.17.3.3.1 Chemotherapy

Chemotherapy does not have an established role in the management of patients with advanced or metastatic RCC, in contrast to other malignancies. A review of early studies reported that fluorinated pyrimidines were the most active agents, but the objective response rate was only 5 to 10% (37). Subsequent studies with capecitabine and a formulation of the 5-fluorouracil prodrug tegafur, have also shown evidence of at least some activity, as have studies with gemcitabine (37).

Recently reported phase II studies using combinations of gemcitabine plus capecitabine and bevacizumab have reported response rates of 8 to 24% (38). At least one study has suggested that this combination may be more effective when given in combination with sorafenib (39). Additional clinical trials will be required to determine whether or not this approach may have a role in patients who are no longer responsive to molecularly targeted therapies (39).

**1.17.3.3.2 Hormonal agents**

Progestational agents have been extensively evaluated in patients with advanced RCC, but do not appear to have antitumor activity. Medroxyprogesterone is the most widely studied of these. Despite occasional reports of responses, a review of medroxyprogesterone treatment concluded that RCC is neither hormone-dependent nor hormone-responsive, although some patients with severe anorexia may derive symptomatic benefit from hormonal therapy. There is no evidence that other hormonal agents (e.g. androgens, anti-oestrogens, or combinations of hormones and chemotherapy) are any more effective (27).

**1.17.3.3.3 Radiation therapy (RT)**

Despite the characterisation of RCC as a radioresistant tumour, conventional and stereotactic radiation therapy are frequently useful to treat a single or a limited number of metastases. In these settings, the utility of RT is similar to that in other metastases from other tumour types. Examples of situations where RT is useful include painful bone metastases, brain metastases, and painful recurrences in the renal bed. Radiation therapy has also been used as an adjuvant following

nephrectomy, but the role of RT in this setting remains unproven and is generally discouraged (27)**.** More details about the role of radiotherapy in renal cancer will discuss in Chapter 3, section 3.1.1.

## 1.18 Clinical use of the TKI agents sunitinib and pazopanib

### 1.18.1 Efficacy and adverse events

#### 1.18.1.1 Sunitinib

Sunitinib is a small molecule TKI with multiple targets on growth receptors. Sunitinib is a potent inhibitor of the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors. This imparts sunitinib with anti-tumour and anti-angiogenic actions. Sunitinib is a tyrosine kinase inhibitor (TKI) that targets multiple receptors, including vascular endothelial growth factor receptor-1, 2 and 3, platelet-derived growth factor receptor α and β, stem cell growth factor, Fms-like tyrosine kinase receptor 3 (FLT-3), neurotropic factor receptor and colony-stimulating factor (CSF) (40).

The most common dosing cycle of sunitinib in clinical practice is 50 mg once daily for four weeks, followed by a two-week interval before another dose cycle. This should be repeated until disease progression or any unacceptable adverse effect appears. For patients intolerant to sunitinib, the dose should be decreased in steps of 12.5 mg, regimens that Pfizer produces in hard capsule form (Sutent® 12.5 mg, 25 mg, 37.5 mg and 50 mg). If a patient misses a daily dose, then no additional dose should be taken. Sunitinib can be ingested on either an empty or full stomach (41).

Before initiating a cycle of sunitinib, and regularly during the cycle, patients' thyroid function, full blood count, urea and electrolyte, liver function, calcium, lactate dehydrogenase, magnesium, and blood pressure should be examined and electrocardiography and urinalysis performed. Blood pressure should be less than 160/100 mmHg before sunitinib is administered. Sunitinib should be prescribed to patients diagnosed with advanced renal cell carcinoma, with an ECOG performance status of 0 or 1, and adequate hepatic, renal and cardiac function (41).

Sunitinib is an orally administered drug that undergoes the first-pass metabolism. Sunitinib's half-life and that of its active metabolite range from 40 to 60 hours and 80 to 110 hours, respectively. Sunitinib is metabolised by the cytochrome P-450 3A (CYP3A4). Strong inducers and inhibitors of CYP3A4 can influence sunitinib metabolism and resultant concentration in the body. Therefore, the avoidance of sunitinib (interrupt treatment) or dose adjustments of sunitinib are required with particular medications, such as inducers (e.g. rifampicin) and inhibitors (e.g. ketoconazole) (40).

#### 1.18.1.1.1 Clinical efficacy of sunitinib

A randomised clinical trial demonstrated the clinical efficacy of sunitinib in 750 recruited advanced kidney cancer patients who had not yet received any treatment (31). These patients were randomly assigned to receive 50 mg of sunitinib orally once a day for four weeks followed by a two-week break or to an alternate arm of two weeks of an IFN-$\alpha$ subcutaneous treatment three times a week. The study showed that the progression-free survival time (11 v 5 months, respectively) and

median overall survival of the sunitinib group was better than those of the IFN-α group (26.4 v. 21.8 months, respectively; 29). The sunitinib group was associated with greater objective response rates than the IFN- α group. Eleven sunitinib patients achieved a complete response, whereas only four patients treated with IFN-α group therapy achieved a complete response. The median duration of treatment was 11 months for the sunitinib group and only four months in the INF-α group. Fifty-two patients took sunitinib (compared with six patients in the INF-α group) and continued until the time of completing the study. Thus, those taking sunitinib expressed a better quality of life than the patients in the INF-α group. The sunitinib group experienced less severe symptoms of a lack of energy, bone pain, fatigue, weight loss, breathlessness, fever, and coughing (all $p < 0.01$). Patients receiving sunitinib showed higher Functional Assessment of Cancer Therapy-Kidney Symptom Index-15 item (FSKI-15) and 9 item for Disease Related Symptom (FSKI-DRS) scores than did patients receiving INF-α. Higher scores achieved by these tools means less severity of cancer symptoms. The Functional Assessment of Cancer Therapy-General (FACT-G) tool was also implemented and recorded that the sunitinib patients had a better quality of life, demonstrated by a higher FACT-G score, than patients using INF-α. The FACT-G tool consists of 28 items, divided into four main sections which are used to examine well-being, physical, social, emotional and functional items. It is widely used for assessing health-related quality of life for cancer patients (31).

In March 2009, the National Institute for Health and Care Excellence recommended sunitinib as a first-line treatment option for patients with advanced and/or metastatic renal cell carcinoma who are suitable for immunotherapy and have an ECOG performance status of 0 or 1 (42).

#### 1.18.1.1.2 Adverse events of sunitinib

Researchers in 2013 performed a randomised, open-label, phase III trial of pazopanib versus sunitinib in the first-line treatment of patients with metastatic renal cell carcinoma (34). This trial examined the side effects of both agents and reported that the agents have similar side effects but at a different incidence. Fatigue (both of grades 3 and 4) was the most common adverse reaction of sunitinib and was observed in 63% of the patients. Diarrhoea presented in 57%, nausea in 46% and hypertension in 41% of the patients. In addition, hand-foot syndrome, taste alteration, and thrombocytopenia have been observed in 50%, 36%, and 34% of the patients, respectively. There were no reports of grade 4 fatigue in any of the trials.

In another clinical trial, a decrease in heart ejection fraction was recorded; however, in wider clinical use no adverse cardiac events have been reported (43). An additional clinical study reported that sunitinib caused laboratory abnormalities (white blood cells or haemoglobin) of neutropenia and anaemia in 43% and 28% of metastatic renal cancer patients respectively (40). Neutropenia occurring in patients treated with sunitinib was not associated with an infection or fever. Hypothyroidism was also observed in 4% of patients receiving sunitinib (40). However, it was

reported in 13% of patients receiving sunitinib in another trial (43). Therefore, thyroid levels should be assessed for renal cancer patients before initiating therapy with a TKI agent.

In 2013, a clinical trial examined the safety and activity of sunitinib in 68 elderly (≥ 70 years old) renal cell carcinoma patients (44). Forty-seven of the patients underwent dose reduction because of toxicity, 22 of the patients underwent dose reduction before starting the medication because those patients received medications known to inhibit cytochrome p450, which is known to lead to increased sunitinib concentration, 12 patients underwent dose reduction after the first cycle, and 13 patients underwent dose reduction after subsequent cycles. The reasons for dose reduction included haematological side effects, cardiac events, fatigue or neutropenia. In addition, 10 of the patients had to stop treatment after the first cycle because of progressive disease or toxicity, and one patient had to stop because of severe fatigue. Therefore, half of the enrolled participants in this study underwent dose reduction because of various adverse effects, and in light of that, even if sunitinib has a good efficacy in renal cancer patients, it may still be accompanied by intolerable adverse effects.

Some further clinical trials demonstrated that a significant correlation exists between TKI medications and thyroid function. One study examined 66 renal cancer patients receiving sunitinib and concluded that 56 (85%) of them developed one or more abnormalities in their thyroid function test, and 47 out of 56 (84%) of those patients had signs and symptoms of hypothyroidism (45). Seventeen patients

underwent thyroid replacement therapy, and symptoms disappeared in nine of them (45). Fatigue was one of the most common side effects recorded with those patients; therefore, monitoring of TFT is warranted for patients receiving any TKI agent.

#### 1.18.1.2 Pazopanib

Pazopanib hydrochloride (Votrient®) is a multiple TKI. Pazopanib targets VEGF and PDGF receptors. Furthermore, pazopanib is an oral medication and undergoes first-pass metabolism. Pazopanib's mean half-life is 30.9 hours. Pazopanib, like sunitinib, is metabolised by the CYP3A4 enzyme. The dosing and frequency of pazopanib are 800 mg once daily. The dose may be reduced to 200 mg according to drug tolerability in order to manage adverse drug reactions. In February 2011, NICE guidelines recommended pazopanib as a first-line treatment option for people with advanced renal cell carcinoma who have not received prior cytokine therapy and have an ECOG performance status of 0 or 1 (46).

Available in 200 and 400 mg tablets, and if necessary, dose reductions should be made in steps of 200 mg. Pazopanib should be taken 1–2 h before or after eating, swallowed with water, and not crushed or broken when ingested. If any dose is missed or vomiting occurs after ingestion, no additional dose should be taken. Pazopanib should be taken once daily until disease progression or any undesirable side effects appear (46).

Significant drug interactions with pazopanib include those with strong inhibitors of cytochrome P450 3A4 (CYP3A4), including ketoconazole, voriconazole, and

clarithromycin, which increase the concentration of pazopanib in the blood. CYP3A4 is an enzyme primarily found in the liver and intestine that oxidises small, foreign organic molecules (i.e. xenobiotics) such as medications and toxins for removal from the body. Therefore, inhibitors of the enzyme, such as grapefruit juice, increase the concentration of the medication and should be avoided. Medications which strongly induce CYP3A4 reduce pazopanib plasma concentrations (46).

Before initiating a cycle of pazopanib and regularly during treatment, patients' thyroid function, full blood count, urea and electrolyte, liver function, calcium, lactate dehydrogenase, magnesium, and blood pressure should be examined, and electrocardiography and urinalysis performed. Blood pressure should be well controlled before pazopanib is prescribed (46).

#### 1.18.1.2.1 Clinical efficacy of pazopanib

In 2010, researchers conducted a randomised, double-blind, placebo-controlled phase III study to evaluate the efficacy and safety of pazopanib (47). Of 435 patients with advanced kidney cancer, 290 patients were randomly assigned to pazopanib and 145 were randomly assigned to the placebo. The primary outcome of the trial was progression-free survival (PFS), which is defined as the time interval between the date of the randomisation process and the date of disease progression or death. The secondary end points include overall survival, which is defined as the time interval between the date of random assignment and date of death, tumour response rate, and safety. Only 148 patients in the pazopanib arm and 98 patients in the placebo arm continued at the time of the PFS analysis. The PFS was

significantly increased with pazopanib treatment compared with the placebo group in the overall study population. Median PFS was 9.2 months for pazopanib group compare with 4.2 months for the placebo group. The response rate for patients receiving pazopanib in the overall study population was 30% with a median duration of response of 58.7 weeks compared with only 3% in the placebo group. With regard to quality of life, there was no evidence of a significant difference between pazopanib and placebo patients.

#### 1.18.1.2.2 Adverse events of pazopanib

The most common side effects that the pazopanib patients reported in a COMPARZ trial (randomised trial comparing the efficacy and safety of pazopanib and sunitinib as first-line therapy in metastatic renal cell carcinoma) are diarrhoea, fatigue, and hypertension at incidence rates of 63%, 55%, and 46%, respectively. The COMPARZ trial concluded that 55% of the patients who received pazopanib suffered from fatigue, while 63% of the patients who received sunitinib suffered from fatigue (34). Moreover, the incidence of fatigue reported by a PISCES trial (a double-blind cross-over study evaluating patient preference for pazopanib or sunitinib in metastatic renal cell carcinoma) is similar at 29% for pazopanib and 30% for sunitinib (48).

While taking pazopanib, some patients have experienced palmar-plantar erythrodysesthesia (i.e. hand-foot syndrome) skin changes which led to limited activity in daily living and pain that required dose reduction by 200 mg. Stomatitis is another side effect of pazopanib that involves ulcers in the mouth; in the case of severe ulceration, the drug cycle should be discontinued until ulceration recedes,

then resumed with a 200 mg dose reduction. Hypothyroidism also frequently occurs in patients taking pazopanib, for whom a levothyroxine supplement is recommended to retain thyroid-stimulating hormone (TSH) at levels less than 5 mU/L (46).

Cases of hepatic impairment have been reported in less than 1% of patients who received pazopanib. A patient with mild hepatic impairment should be treated with 800 mg once a day with no change of dose. However, a dose reduction to 200 mg once daily is recommended for moderate hepatic function. For severe hepatic impairment, pazopanib should not be used. Alanine transaminase and hair colour change have been reported with pazopanib patients (31% and 30% respectively) more so than for sunitinib patients (18% and 10% respectively) (34).

For hypertensive patients who are receiving pazopanib, the PISCES trial recommended to monitor their blood pressure in the first week of starting treatment and frequently thereafter to ensure blood pressure control. Anti-hypertensive agents and dose modification can, however, manage hypertension. If blood pressure persists above 140/90 mmHg, then the agent should be discontinued (48). Only 13% of participants in the PISCES trial needed to undergo dose reductions or discontinuations for pazopanib due to side effects compared with 20% of the patients in the sunitinib group (48).

A pazopanib versus sunitinib patient preference study in treating naïve locally advanced or metastatic renal cell carcinoma recommended that both agents do not require dose adjustments in patients with creatinine clearance above 30 ml/min,

and should be used with caution with patients who have a creatinine clearance of less than 30 ml/min (48).

### 1.18.2 Patient and physician preference

The PISCES trial demonstrated that more patients preferred pazopanib to sunitinib (70% vs. 22%; $p < 0.001$), with the remaining 8% of patients expressing no preference for either treatment (48). There is also a high level of concordance between patients and physicians, because 61% of physicians prefer pazopanib over sunitinib (22% preference) in the PISCES trial. To compare the influence of fatigue on patient choice in this study, one question in this study asked the patients, "Please indicate which factors had an influence on your choice of treatment". Forty-seven out of 80 (59%) patients preferred pazopanib because fatigue had less of an impact on their lives. Only 12 out 25 (48%) patients preferred sunitinib for the same reason in PISCES trial (48).

The COMPARZ trial reported that the differentiated safety profile of pazopanib included a lower incidence of fatigue, hand-foot syndrome, thrombocytopenia, and changes in taste compared with sunitinib (34). Patients also had a higher tolerance for pazopanib, which was indicated by their better quality of life scores (34).

### 1.18.3 Tyrosine kinase inhibitors induced fatigue

Different pathways can be attributed to why TKI agents cause fatigue. However, researchers have confirmed that endocrine disorders and anaemia are the most common mechanisms by which TKI agents induce fatigue (49).

#### 1.18.3.1 Fatigue as a consequence of TKI-induced endocrine disorders

##### 1.18.3.1.1 Adrenal dysfunction

Adrenal dysfunction might progress in patients who receive TKI agents. Symptoms of adrenal dysfunction are nonspecific and variable, including fatigue, muscle and joint pain, muscle strength, weight loss, and abdominal complaints (e.g. nausea and vomiting). However, a clinical study reviewed endocrine-related side effects of TKI agents and reported that adrenal insufficiency was rare in patients treated clinically with TKI (55).

The US Food and Drug Administration approved sunitinib with a summary caution mentioning that no clear clinical dysfunction had been recognised in patients who had taken the drug (51). However, since subclinical toxicity may be unmasked by physiological stress, it is recommended to monitor adrenal dysfunction in patients with stressors such as surgery, trauma, or severe infection. For those patients, adrenal deficiency should be suspected and treated by appropriate steroid replacement therapy (51).

#### 1.18.3.1.2 Thyroid alteration

Thyroid dysfunctions are common in patients who receive any TKI agent with an incidence of 20–80% reported (50). Probable mechanisms include iodine uptake failure, destructive thyroiditis, and the direct inhibition of thyroid peroxidase activity. The clinical manifestation of thyroid alteration, specifically hypothyroidism includes fatigue, hair loss, and dry skin (49).

Hypothyroidism is most commonly observed (52). Due to the high incidence of hypothyroidism, patients who receive TKI should have TSH and free thyroxine monitored at baseline and before every drug cycle; some sources recommend monitoring at baseline and every four weeks for four months, then every two to three months. Hypothyroidism is easily controlled by L- thyroxine supplementation (49).

#### 1.18.3.1.3 Metabolic alterations

Bone and related metabolic abnormalities, including vitamin D deficiency and hypophosphatemia, may accompany renal cancer as a direct effect of TKI agents' use and induce fatigue due to a nonspecific inhibition of kinases expressed by osteoclasts and osteoblasts and a reduction in intestinal vitamin D absorption. Vitamin D deficiency is a well-recognised cause of fatigue and myopathy, and it is recommended to assess bone density and vitamin D levels in patients who receive TKI agents at baseline, as well as during treatment, and to correct lower levels of vitamin D with appropriate supplements if needed (53).

#### 1.18.3.2 Fatigue as a consequence of TKI-induced anaemia

Anaemia ranks among the most common abnormalities induced by TKI agents and is a contributing factor to fatigue in cancer patients (49). Various studies have shown a high incidence of anaemia in patients who receive TKI agents, and phase III trials for sunitinib and pazopanib showed an incidence of anaemia with sunitinib in 4% of patients examined, and with pazopanib in 3% of patients (31, 47). The COMPARZ trial measured the side effects of sunitinib and pazopanib and found anaemia to be among the most common side effects, with 7% prevalence with sunitinib and 1% with pazopanib (34). Obvious clinical manifestations in patients suffering from anaemia are fatigue, decreased capacity for activity, and exhaustion and usually treated by iron supplementation or blood transfusion in severe iron deficiency cases (34).

## 1.19 Introduction to the next chapter

Typically described as an overwhelming, all-embracing feeling of exhaustion, tiredness, weariness, and malaise that cannot be relieved by rest or sleep, fatigue is a common side effect in cancer patients. Cancer-related fatigue (CRF) represents a constant feeling of fatigue, yet of unpredictable capacity, meaning that patients who feel healthy might suddenly feel fatigued for no apparent reason (54). Symptoms of fatigue in cancer patients might come from cancer itself, as a side effect of anticancer treatment, or as part of a confounding factor such as age, by which elderly patients are more likely to suffer comorbidities and psychological disorders.

Fatigue in cancer patients is a multidimensional condition that involves a physical component (i.e. decreased capacity to perform normal daily activities), an emotional component (i.e. depression and upset mood that affect social relationships), and a cognitive component (i.e. loss of concentration and confusion). Cancer patients who suffer from fatigue might exhibit one or more of those aspects (54). Sunitinib and pazopanib, first-line treatments for renal cancer patients, are sometimes not tolerated by the patient due to the occurrence of different side effects, including fatigue. The onset of moderate or severe fatigue can have serious implications for treatment that require incremental dose reductions until symptoms have resolved. These reductions may consequently affect outcomes in patients treated with TKI given the highly negative influence of fatigue on the daily lives of cancer patients. Therefore, accurately measuring fatigue and identifying possible reasons for the onset and increase of fatigue is vital to its early management. In fact, the early detection and management of fatigue will help to increase the tolerability of TKI agents and support cancer survivors living lives that are as healthy and of high a quality as possible.

In the next chapter, we describe how two questionnaires were administered to explore and measure the incidence and severity of fatigue in renal cancer patients at the West of Scotland Beatson Cancer Centre taking sunitinib or pazopanib, chiefly in order to examine the correlation between clinical laboratory variables and general cancer symptoms in patients who scored high for fatigue and the impact of sunitinib and pazopanib on their quality of life.

# Chapter 2:

# Measuring the incidence and severity of fatigue in renal cancer patients receiving sunitinib or pazopanib

## 2.1 Introduction to Cancer-Related Fatigue

Worldwide, more than 40% of the population will be diagnosed with a cancer during their lifetimes. Despite such a high incidence, the mortality rates of prevalent types of cancer have decreased significantly during the past three decades (55). For example, in 2008, more than two million people in the United Kingdom were estimated to have survived cancer following their diagnosis with the disease (56). In the United Kingdom, 50% of patients diagnosed with cancer survive for at least 10 years (4). According to the Cancer in Scotland report from Health Services Scotland, roughly 352,000 new cases of cancer emerged in the UK in 2013, including approximately 31,000 new cases in Scotland (56). There has also been an increasing trend of survival since 2006, when more than 200,000 cancer patients in the United Kingdom were alive a year after their diagnosis and around 1.13 million were alive up to 10 years after (56).

Due to both improvements in cancer survival rates and the increased number of new cases occurring, the number of cancer patients is expected to double by 2050. Such growth prompts the major challenges of identifying and managing treatment-related complications, of improving patients' quality of life, and of enhancing the overall functioning of patients receiving long-term treatment or follow-up care (55).

Of the most common and debilitating symptoms that all cancer patients experience, fatigue, its severity and impact on quality of life, has been of great interest in recent years (57). The National Comprehensive Cancer Network (NCCN) has defined cancer-related fatigue as "a distressing, persistent, subjective sense of physical,

emotional, and/or cognitive tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual functioning" (58). CRF has also been defined as "a persistent, subjective sense of tiredness related to cancer and cancer treatment that interferes with usual functioning" (59).

Experienced by most cancer patients at some point during treatment, CRF continues to manifest in some patients for months or even years after the completion of primary management. CRF is distressing, has a highly negative impact on patients' quality of life, and interferes with the performance of daily activities. However, healthcare practitioners infrequently measure CRF, which suggests its mismanagement during and after treatment (55).

Unlike normal fatigue experienced by healthy individuals, rest or sleep cannot relieve CRF. Cancer-related fatigue occurs both as a consequence of cancer and as a side effect of cancer treatment. In that sense, CRF is usually used as an umbrella term to describe various sensations of reduced capacity at mental, physical, social, and emotional levels (60).

Fatigue is highly complex and overlaps biological processes involving and affecting various body systems. Its pathophysiology is thus not fully understood and there is no specific mechanism in cancer patients who suffer from fatigue; it depends on patient conditions, such as cancer type or patients' comorbidities. Although there have been several attempts to identify the mechanism of fatigue specifically in cancer patients, a predominant mechanism has not been identified (59, 61).

### 2.1.1 Incidence rates

Incidence rates of CRF have been reported in clinical trials to be up to 70 to 80% among cancer patients (50). The variations in patients' experiences of fatigue are due to the type of cancer, its treatment and importantly also due to the method of fatigue assessment (50). For example: CRF has been reported for 37% to 78% of patients with lung cancer, 28% to 91% of patients with breast cancer and only 15% of patients with prostate carcinoma (50, 62, 63). Another clinical study reported that fatigue was the most common symptom reported by breast cancer patients with 84% of patients reporting CRF compared with 75% reporting pain and less than 30% of patients reporting nausea (51). In general, fatigue has been reported as a side effect in patients with all types of cancer and following all forms of cancer treatments other than surgery, including radiation, chemotherapy, and hormonal and biological therapies. Therefore, the National Comprehensive Cancer Network recommends that cancer patients should be screened, assessed and managed for fatigue, based on clinical practice guideline (58).

In terms of presentation of CRF, the severity of CRF among patients receiving chemotherapy was reported to reach its peak within four or five days after completion of treatment and fatigue decreased gradually over time (64). In contrast, with radiotherapy, the severity of CRF increased gradually over the course of treatment (65). More specifically, fatigue is one of the most common adverse reactions seen in patients treated with tyrosine kinase inhibitors such as sunitinib and pazopanib, which are used in the treatment of renal cancer. Previous studies

have reported that fatigue is one of the common side effects of sunitinib and pazopanib, experienced by 63% and 55% patients, respectively (48).

### 2.1.2 Confounding factors of CRF

Since CRF is a multifaceted, personal, physiological, and psychological condition influenced by cancer treatment, it is difficult to define the nature of CRF. Fatigue among cancer patients differs from patient to patient based on individual factors such as anticancer treatment received, comorbid medical conditions, and age. Many studies have examined contributing factors of CRF that might cause or exaggerate fatigue in cancer patients and these can be categorised as age-related, physiological, or psychological factors.

#### 2.1.2.1 Age-related factors

It has been hypothesised that cancer-related fatigue might be more common with advancing age. For example, a clinical study examined cancer patients with different tumours during outpatient treatment with chemotherapy or pamidronate at the H. Lee Moffitt Cancer Centre in the United States (66). They examined 76 varieties of cancer in patients that were over 60 years of age at the time of the study. Fatigue was assessed using a fatigue symptom inventory (FSI), which is a self-reporting measurement tool, designed to assess the severity, frequency, and daily pattern of fatigue as well as its perceived interference with quality of life. The study reported that 72% of these patients reported fatigue at the time of assessment. A further finding was that 52% of patients reported that they rated average fatigue as grade

4; the National Cancer Institute - Common Toxicity Criteria Adverse Events (NCI-CTCAE) define grade 4 fatigue as when the patient is bed-bound or has a severe disability. Furthermore, another clinical study by Brunello et al. examined the safety and activity of sunitinib in 68 elderly patients (≥ 70 years) with metastatic renal cell carcinoma. The study assessed adverse events in those patients using a comprehensive geriatric assessment (CGA) and observed that fatigue was the most common adverse event in 55 of 68 patients (80.9%) (44).

#### 2.1.2.2 Physiological factors

Several physiological factors are thought to contribute to fatigue; the most common include anaemia, cachexia, metastatic tumours, malnutrition and hypothyroidism (59). The NCCN classified anaemia as one of the treatable factors that may contribute to cancer-related fatigue. When anaemia is associated with cancer, it may be caused by multi-factorial manifestations of cancer such as haemodialysis, bleeding, nutritional deficiencies and bone marrow infiltration. Each of these factors might contribute to the development of anaemia in cancer patients. Furthermore, inflammatory cytokines inhibit erythropoiesis, leading to decreased production of erythrocytes, a condition commonly manifesting in cases of fatigue and anaemia. Many studies have reported a strong relationship between anaemia and fatigue in cancer patients. Haemoglobin function is altered in cancer patients, often in response to neoplastic disease and cancer treatments, which change the membrane transport characteristics of erythrocytes through changes in potassium

and chloride levels and decreases red blood cells, leading to less oxygen transportation and consequently results in contribution to fatigue (59).

Other factors have also been shown to contribute to fatigue. For example, cachexia, or wasting disease, involves the loss of muscle mass and adipose tissue which may lead to fatigue, anorexia and weight loss. In cancer patients, cachexia is one of the primary contributing factors in the development of fatigue (59).

The third factor in cancer-related fatigue is the presence of metastatic disease. Stone et al. (68) and Given et al. (69) both analysed patients with different types of tumours, and demonstrated that the number and duration of metastatic tumours have a direct relationship to the degree of fatigue experienced by cancer patients (67 - 69). The first study compared groups of patients with metastatic breast, prostate or non-small lung cancer receiving palliative care with groups of patients recently diagnosed with breast, lung, or prostate cancer that had fewer metastatic events. The authors found that the first group of patients with metastatic disease experienced a higher degree of fatigue than the second group with minimal metastatic spreading of their tumour (68). The second study reported a high incidence of fatigue among patients diagnosed with late-stage cancer compared with those diagnosed in the early stages of the disease (69).

Further factors that affect cancer patients and can also induce fatigue are malnutrition resulting from vomiting, loss of appetite, or hyper-metabolism and hypothyroidism (70). Hypothyroidism, which often manifests itself with symptoms of fatigue, is one of the most common side effects of treatment with TKI agents,

specifically sunitinib and pazopanib (34). These two drugs contribute to hypothyroidism by inhibiting the activity of the enzyme thyroid peroxidase. This enzyme adds iodine to a protein called thyroglobulin, a critical step in the synthesis of thyroid hormones. Therefore, the inhibition caused by TKI leads to a decrease in thyroid hormones synthesis. For this reason, it is recommended that patients who receive TKI agents should be monitored for thyroid function before the instigation of therapy and every two to three months during the treatment; in cases of hypothyroidism, physicians should consider thyroid hormone replacement therapy (71).

#### 2.1.2.3 Psychological factors

Many clinical studies examined the link between CRF and increased levels of depression, anxiety, and mood disturbance and found a significant and direct correlation between CRF and such psychological features (72). These three psychological symptoms may affect the patient's ability to accomplish daily activities, which can lead to a negative impact on patient treatment outcomes by reducing survival times. For example, a clinical survey was designed to assess psychological impact of fatigue on the lives of cancer patients at the United States. It examined 379 cancer patients with a prior history of chemotherapy using 25-minute telephone interviews in which the patients were asked a series of questions about fatigue and its impact on their quality of life. Of their participants, 74% of patients reported feelings of isolation and 72% felt depressed (63).

In recognition of the psychological problems faced by cancer patients, a study undertaken by Tchekmedyian et al. examined 250 lung cancer patients suffering from anaemia (72). They used two assessment tools: The Brief Symptom Inventory (BSI) depression and anxiety scale and the Functional Assessment Chronic Illness Therapy - Fatigue (FACIT-F). They then compared changes in patients' BSI scores with changes in their FACIT-F scores and found that the scores had a directly proportional relationship. Therefore, better fatigue management may help reduce the incidence of psychological disorders in cancer patients.

Major depression is the most common psychiatric disorder in cancer patients and the prevalence of depression in cancer patients has been found to range from 6% to 40% (73). The variation in reports of the percentage of cancer patients suffering from depression between studies likely arises from the wide variety of different depression measurement tools used in various studies. For example, the study by Ciaramella and Poli measured the prevalence of depression in different types of cancers using two scales: The Structured Clinical Interview for Depression (SCID) and the Hamilton Depression Rating Scale (HAMD) (73). Using both of these tests, they found that the prevalence of depression among cancer patients was 49% SCID and 29% when measured using the HAMD scale. Furthermore, they concluded that the prevalence of depression bears no significant relationship with age, sex, or the site of the cancer. Nevertheless, they did note that unsurprisingly patients with metastatic cancer had significantly higher rates of depression ($p < 0.01$) than patients with non-metastatic cancer.

A clinical study demonstrated a significant and positive correlation between depressive symptoms and fatigue severity ($p < 0.01$) (66). This study measured fatigue with the FSI tool and depression with the Geriatric Depression Scale (GDS). They found that fatigue and depression could co-exist in cancer patients even if they are not caused by the same factors.

One of the complexities of examining the real rates of depression or of fatigue in cancer patients, are that most of the symptoms of depression resemble the symptoms of cancer as well as the symptoms of fatigue and include low energy, weight loss, and sleep disturbances which are somatic symptoms. However, another clinical study reported in 1984 suggested that depression prevalence dropped from 42 to 24% when all somatic symptoms were excluded as diagnosis criteria, and by considering non-somatic symptoms such as depressed mood, suicidal ideas, helplessness or hopelessness as criteria for depression assessment (74).

### 2.1.3 Measurement diagnostic

It is difficult to make a standard tool to measure fatigue because, until now, there has been no standard definition of cancer-related fatigue. Furthermore, individual patients' experiences of fatigue are subjective and occur during different stages of the treatment or the disease (75, 76).

### 2.1.4 Grade of fatigue

The National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) grades fatigue on a scale from 1 to 4 (version 4, 2010) (77). The CTCAE created this scale according to a functional assessment of patients that uses either the Karnofsky performance status scale or the Eastern Cooperative Oncology Group scale, described in Table 8 (75).

**Table 8: ECOG and Karnofsky performance status scales corresponding to the grade of fatigue. Reproduced from Larkin et al. (75).**

| Status | Karnofsky | Fatigue Grade | ECOG |
|---|---|---|---|
| **Normal** | **100** | **0** | Fully active |
| ***Able to carry on normal activity** | **90** | **1** | *Restricted in physically strenuous activity but able to carry out work |
| ***Normal activity with effort** | **80** | | |
| ***Unable to carry on normal activity or to do active work.** | **70** | **2** | *Ambulatory but unable to carry out any work activities. |
| ***Requires occasional assistance** | **60** | | *Ambulatory for more 50% of waking hours |
| ***Required considerable assistance and frequent medical care** | **50** | **3** | *Capable of only limited self-care |
| ***Required special care and assistance** | **40** | | *Confined to bed or chair for more than 50% of waking hours |
| ***Severely disabled** | **30** | **4** | *Completely disabled |
| *** Very sick** | **20** | | *Cannot carry on any self-care |
| *** Moribund** | **10** | | *Totally confined to bed or chair |
| ***Dead** | **0** | | *Dead |

### 2.1.5 Assessment of cancer-related fatigue

Fatigue assessment tools can be divided into three techniques. The first technique applies tools that assess fatigue as a part of the quality of life measures, so it requires a quality of life questionnaire such as the European Organisation for

Research and Treatment of Cancer Quality of Life Questionnaire (EORTC-QLQ) (77). The second category comprises of unidimensional tools, such as the Brief Fatigue Inventory (BFI) tool and the last category is multidimensional tools.

The first category (quality of life questionnaire) is not widely used in clinical trials to measure CRF, because the questionnaire is very long and does not focus solely on fatigue assessment. Conversely, the second category consists of unidimensional tools which are commonly used for assessing fatigue in clinical trials because they are simple, easy to complete, and typically consider only one feature of fatigue, which is the physical aspect. The third category for assessing CRF is multidimensional tools, which can examine physical, emotional, and mental aspects of fatigue simultaneously, but are more complex to administer, and the benefits of measuring additional aspects of fatigue are not clear from a clinical assessment perspective. Therefore, they are not commonly used in clinical research, compared with unidimensional tools (78).

Any tool that one wishes to use for assessing CRF must have two characteristics. Firstly, the tool should be simple and brief enough to be completed by cancer patients who are experiencing fatigue and, secondly, the tool must be designed specifically for CRF (79). Many types of tools have been used to measure fatigue in cancer patients. Therefore, selecting the appropriate tool is challenging but several questions can be considered to help the selection of the best tools for a clinical trial. Researchers should first determine which tool can measure whether patients have experienced fatigue and whether they are healthy. The Revised Piper Fatigue (RPF)

Scale is the only scale that can measure patients experiencing fatigue at the point of the survey; the assessment questions' point is "now", so this tool is valid in patients experiencing fatigue at the time of assessment. In contrast, FSI, BFI and FACIT-F tools can be used with individuals who may or may not be experiencing fatigue at the time of sampling (80).

Researchers need additionally to consider the time frame that the measurement tool will cover. For example the FSI and FACIT-F measure fatigue over the previous week while the BFI assesses fatigue over the past 24 hours. Therefore, the time frames of the tools must correspond to the period that the researchers wish to assess.

The third issue that researchers should consider is the strength of the evidence for the measure's reliability, validity, and quality of the methods used to derive the measure's format. Another related issue to be considered is the type of cancer, and the anticancer treatment since some measurements are validated for only one type of cancer. For example, the Multidimensional Fatigue Symptom Inventory (MFSI) has only been used in breast cancer patients who have undertaken chemotherapy. This feature could be advantageous for assessing fatigue in breast cancer patients or disadvantageous for measuring fatigue in other patient populations. The last issue to consider is the correlation between the target measurement (severity of fatigue or impact of fatigue on mental, cognitive, or physical health) and the questions. The tools that have been most commonly used in clinical trials for

assessing fatigue in cancer patients will be discussed below based on their dimensional properties.

#### 2.1.5.1 Unidimensional tools

Unidimensional tools come in two types: one is a single item and the other is a multi-item unidimensional tool. The single-item tools focus on detecting the presence or absence of fatigue. Some of these tools focus on the severity of fatigue and its influence on a patient's life (72). The simple numeric scale of a single-item tool typically ranges from 0 to 10. Patients are usually asked, "How would you rate your fatigue over the past 7 days?" A score of zero indicates no fatigue; a score of 1-3 indicates mild fatigue that does not require clinical management; and 4-6 and 7-10 indicate moderate and severe fatigue, respectively, which requires clinical management. A review article overviewed and critiqued measures commonly used to assess CRF and highly recommended utilisation of the single question assessment tool due to it being the simplest tool currently available (80). The Visual Analogue Fatigue Scale (VAFS) is another example of a single-item tool (72). The VAFS is advisable for cancer patients and is suitable for healthy individuals, and thus it enables researchers to compare fatigued with healthy individuals.

An example of a multi-item (unidimensional) tools that have been used for patients with cancer is the BFI, which is a measure of the severity of fatigue over the previous 24 hours. The BFI also assesses the impact of fatigue and its impact on daily life. The BFI tool was developed by a clinical researcher in 1999 and has been used since as a screening tool for fatigue in many clinical trials (81). It has nine

items, on a scale from 0 to 10, which assess the severity of fatigue in the last 24 hours. It is easy to complete, but its ability to assess the severity of the fatigue is limited because the cut-off between severity levels is unclear.

FACIT-F is also an example of a multi-item unidimensional tool and is considered to be the most commonly used scale in clinical trials to measure fatigue in cancer patients. A systematic review that examined tools that have been used in clinical studies and research into cancer-related fatigue assessment concluded that FACIT-F, EORTC QLQ C30, and the Fatigue Questionnaire (FQ) have been the most commonly used scales (78). Data for the FACIT-F were collected from more than 5000 patients, and it is the largest volume of data that has been assessed for all the examined tools. The FACIT-F tool also has more advantages than other tools because it has a validated, clinically significant score change and it includes consideration of the social impact of fatigue (78).

FACIT-F is a unidimensional tool that is a subscale of the FACIT-general multidimensional tool (28 items). FACIT-F is 13-item fatigues subscale with a 5-point scale of 0-4, focusing on the presence or absence of fatigue symptoms, the intensity of the fatigue, the effective aspects of fatigue, and the perceived interference with daily functioning. FACIT-F outcomes result in a score from 0 to 52 in which a resultant high score indicates a low level of fatigue. FACIT-F is widely used in clinical trials for fatigue measurement in cancer patients. It has also been used to measure fatigue among a variety of cancer types and following various treatments. In every study that used FACIT-F as a tool to measure fatigue, the majority of patients

reported some degree of fatigue regardless of the type of cancer or the treatment modality (80).

Recent clinical trials have used FACIT-F as the fatigue measurement with advanced kidney cancer patients. For example, the PISCES study interrogated the patients' preference for pazopanib or sunitinib in the treatment of advanced kidney cancer (48). PISCES assessed the fatigue level of health-related quality of life via the FACIT-F scale and reported that the primary reason that patients preferred pazopanib was that fatigue during treatment with this drug had less impact on their life.

A further clinical study evaluated FACIT-general, anaemia, and fatigue items with 60 cancer patients who had cancers of various sites and reported that the internal consistency of FACIT-F is strong with a coefficient alpha range of 0.93-0.95 (82). Moreover, the test-retest reliability for FACIT-F items was stable with a score of r = 0.90, which means high reliability of the FACIT-F tool. This study also reported that FACIT-F could be useful for measuring one's quality of life during treatment if the surveyor adds more focus to the problems of fatigue. The authors recommended that the fatigue subscale might also stand alone as a very brief but reliable and valid measure of fatigue. In addition, they reported the initial and test-retest administration results for the FACIT-F independently, and noted the tool's ability to be used as an independent, brief, multidimensional measure of fatigue.

Other examples of unidimensional tools are the Rotterdam Symptom Checklist, Medical Outcomes Study 36-item Short-Form Health Survey, and M.D. Anderson

Symptom Inventory. These tools are of limited use for measuring fatigue, because they are not developed specifically to measure CRF (80).

#### 2.1.5.2 Multidimensional tools

The fatigue that patients receiving anticancer treatment experience is variable and has many facets and may affect somatic, cognitive, and emotional functions. For this reason, several researchers have developed and validated multidimensional tools of fatigue for use with cancer patients. Multidimensional tools take longer to complete than unidimensional tools but provide more quantitative and qualitative details in the assessment of fatigue.

One such assessment tool, the Multidimensional Fatigue Symptom Inventory-Short Form has been validated for use with cancer patients. MFSI-SF evaluates the general, physical, emotional, mental, and energetic qualities of cancer patients using 30 items. Another study in 2004 evaluated this tool by applying it to 304 cancer patients following their fourth cycle of chemotherapy (83). They compared the MFSI-SF with several other measures of psychosocial functioning, including the Medical Outcomes Study-Short Form (MOS-SF-36) and the FSI. Their study approved and supported MFSI-SF as a valuable tool for the multidimensional assessment of CRF, and they concluded that this tool is significantly reliable and sensitive to differences in fatigue between cancer patients and non-cancer controls. Moreover, the MFSI-SF has a unique advantage: it is not disease-specific nor does it assume the presence of fatigue, thus increasing its clinical research utility. The main advantage that the MFSI-SF tool has over other multidimensional tools is its ability

to provide information about the patient's overall level of fatigue. It also asks patients about the fatigue that they experience across five important domains in which fatigue may be observed such as behavioural, cognitive, somatic, and affective. The main disadvantage is that MFSI has been used only with breast cancer patients undertaking chemotherapy treatment.

The Fatigue Symptom Inventory developed by Hann et al. is another multidimensional tool that measures fatigue and considers temporal variation in fatigue (84). It has 14 items on a scale of 0 to 10 that measure the frequency, severity, and duration of the fatigue as well as its interference with daily activity over the previous seven days. Hann and his research group also evaluated the reliability and convergent validity of the FSI with breast cancer patients, and reported that the α coefficient (a coefficient of internal consistency) for all items ranges from 0.93 to 0.95, which is above the acceptable range of 0.70-0.80 with the exception of the item that asked about a patient's ability to bath and dress, which had an alpha-coefficient of 0.60 (84). The main disadvantage of the FSI is that its test-retest reliability is weak, which means that the same person might give different results for the same question even under the same conditions. Another disadvantage of the FSI is that most of the clinical studies that used the FSI tool had recruited only breast cancer patients who were undergoing chemotherapy or radiotherapy (84).

In our study, we used a multi-item unidimensional tool, the FACIT-F tool, to measure fatigue in renal cancer patients. The FACIT-F tool has more advantages

than other tools because it is valid, easy to read and answer and has high reliability (r = 0.90). Furthermore, two review articles reported that the FACIT-F tool has more advantages than other tools because it has a validated, clinically significant score change and it includes consideration of the social impact of fatigue (78, 82). Another advantage is that recent clinical trial, the PISCES trial, have used FACIT-F as the fatigue measurement with advanced kidney cancer patients, which are the same target patients as our research (34, 48).

### 2.1.6 Impact of cancer-related fatigue on patient quality of life

Various studies on cancer patients have revealed a significant negative correlation between CRF and daily life, regardless of the type of cancer diagnosed and anticancer treatment. A clinical survey undertaken by Curt et al. in 2000 examined the impact of CRF on 406 cancer patients diagnosed with various types of cancer and receiving chemotherapy and/or radiotherapy by contacting their carers for 25 minute telephone interviews involving 50 questions about the patients' current conditions, medical history, and frequency of experiencing fatigue (64). They found that 91% of the patients' fatigue, reported by carers, prevented them from living normal lives and nearly 88% expressed that fatigue changed their daily routine.

Another study examined 64 patients with a range of cancers diagnosed at baseline after three cycles of chemotherapy treatment in terms of the relationship between fatigue score and physical and emotional activity using FACIT-F and motor and cognitive functions of the short form of the Medical Outcomes Study (36-item) (85). They calculated a positive spearman correlation coefficient between fatigue score and function of participants in the motor aspect at baseline 0.35 ($p < 0.01$) and after three cycles 0.30 ($p < 0.05$). The cognitive aspect was 0.45 ($p < 0.01$) at baseline and 0.42 ($p < 0.01$) after three cycles. These results demonstrated the fatigue symptom had a significant impact on the quality of cancer patient's life in both physical and psychological aspects.

A further study examined 60 women diagnosed with uterine cancer who were receiving radiotherapy during and after the completion of treatment by using a self-report assessment (86). They showed a significant negative correlation between fatigue and multiple domains of quality of life, including physical, social, cognitive, and emotional functioning (all $p < 0.001$, except social functioning, $p < 0.002$).

### 2.1.7 Management of cancer-related fatigue

Cancer-related fatigue should be screened for in all patients of all ages and at all stages of cancer, at both their initial and regular visits during and after treatment. CRF should also be assessed and managed according to clinical practice guidelines. The algorithm for CRF assessment is recommended by NCCN, 2016 (87), as shown in the algorithm in Figure 5.

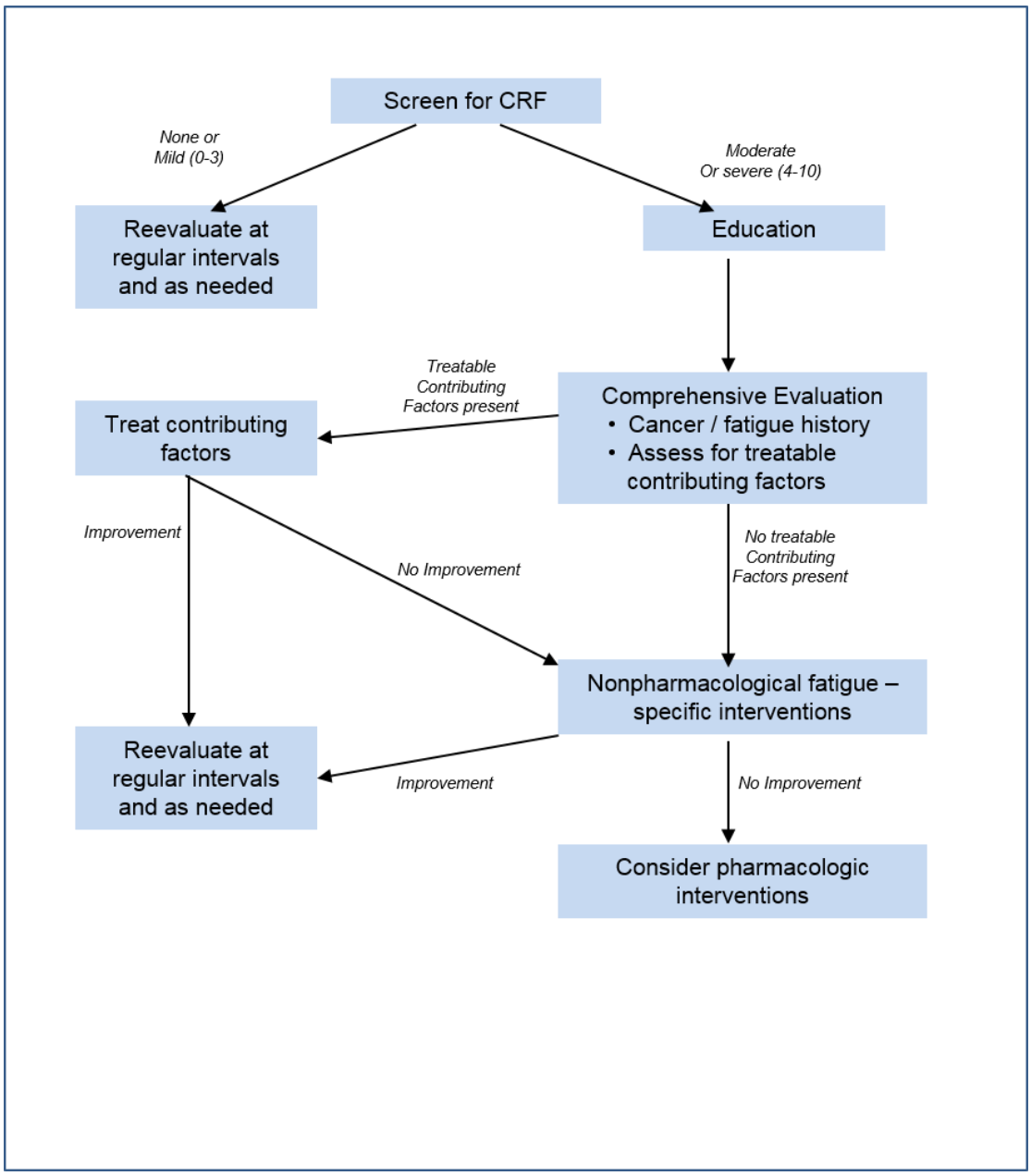


**Figure 5: Assessment of CRF by NCCN guidelines. Reproduced from National Comprehensive Cancer Network (87).**

The NCCN has established guidelines for the standard of management of CRF, and it recommends that fatigue be systemically screened for and assessed in all patients with the appropriate tools. When recognised, fatigue should be treated according to clinical practice guidelines. The treatment of fatigue should be initiated from the

start of cancer therapy and continued after the completion of cancer therapy (87). The NCCN recommends four types of intervention for fatigue, which have been abbreviated in Table 9 below.

**Table 9: NCCN (2016) guidelines for management of CRF for patients on active treatment.**

| Patient/Family education/ counselling | General strategies for management of fatigue | Non-pharmacological intervention | Pharmacological intervention |
|---|---|---|---|
| - Fatigue information during and after treatment<br>- The treatment related fatigue is not an indicator of disease progression. | - Self-monitoring of fatigue levels<br>- Energy conservation<br>- Use distraction (e.g. reading, music or games) | - Activity enhancement: consider starting and maintaining exercise, yoga, physical and/or occupational therapy<br>- Physically based therapies like massage<br>- Psychosocial interventions<br>- Nutrition consultation<br>- Cognitive behavioural therapy for sleep | - Psycho-stimulant (Methylphenidate) after excluding other causes of fatigue<br>- Treat for anaemia, pain and emotional distress<br>- Treatment of sleep disturbance, optimise nutritional deficiency. |

Reproduced from National Comprehensive Cancer Network (87).

The first intervention is educating and counselling cancer patients and their families, which include providing patients with consultation on the physical symptoms that they are likely to experience during treatment. If they receive this education before they begin their anticancer medications, then the patients will be expecting the symptoms and will feel reassured that the fatigue is not a sign of disease progression (75, 87).

The second intervention is general strategies, which include self-monitoring of fatigue levels, energy conservation and distraction. The aim of this intervention is to teach the patient how to include time for rest and inactivity into their daily lives so that they can maintain their energy levels (75).

The third strategy is non-pharmacological interventions that include activity enhancement, physically based therapy, psychological interventions, and cognitive behavioural therapy for sleep and nutrition consultation. Activity enhancement combines intensive exercise with physical training and therapy. Patients who exercise during and after treatments have demonstrated reduced fatigue, improved quality of life, greater functional capacity, and less emotional distress. Continuous exercise can increase the patient's functional capacity and can decrease the effort that the patient needs to exert to perform daily activities. No specific form of exercise has been found to be more effective than others, so the type of exercise will depend on the patient's preference (75, 87).

Psychological intervention can enhance patient morale by giving the patient a sense of control over the symptoms. For example, cognitive behavioural therapy for sleep is a kind of non-pharmacological therapy for fatigue in cancer patients. Sleep disturbance (insomnia or hypersomnia) is a common feature of CRF. Sleep hygiene programs have been approved that benefit daytime functioning, sleep parameters, and fatigue (57). A meta-analysis of 56 studies showed that behavioural intervention and exercise significantly improve the fatigue of cancer patients (88). However, one large randomised trial of 219 breast cancer patients reported no benefit of sleep therapy on fatigue (89).

The latest strategy is pharmacological intervention in which agents that relieve cancer related fatigue and other conditions such as depression, anaemia and/or sleep disturbance. Psychostimulants or central nervous stimulants such as modafinil

and methylphenidate have the greatest potential to alleviate CRF (75). Traditional central nervous system (CNS) stimulants are infrequently prescribed to cancer patients because of the high frequency of side effects (90). On the other hand, modafinil is a CNS stimulant but is not chemically related to traditional amphetamine-class CNS drugs. Modafinil has a selective site of action in the brain and is believed to work within the hypothalamus to block the outflow of Gamma-AminoButyric Acid (GABA). Because of this selective action, modafinil has fewer side effects and is more tolerated than the traditional CNS stimulants. Modafinil has no addictive potential, has a low abuse potential, and leads to less anxiety. The other advantage of modafinil is that it has no effect on heart rate or blood pressure. Nevertheless, the most common side effects of modafinil are nausea, anxiety, headache, and dizziness. These adverse effects can be controlled by dose adjustment, and their degree of severity is usually mild to moderate. Severe symptoms have been recorded but are rare. A pilot study showed that modafinil had a significant positive effect on fatigue relief in advanced lung cancer patients (90). A randomised phase III clinical trial examined the effects of modafinil on CRF among 631 cancer patients who received chemotherapy, and showed that modafinil only had measurable effects in patients who present with severe fatigue with no usefulness in patients with mild or moderate fatigue (91).

Methylphenidate, which is a CNS stimulant, might also be used to manage fatigue in cancer patients. There are two small open label studies that have suggested that methylphenidate improves CRF. However, methylphenidate is not as popular as modafinil, because it frequently causes side effects. Despite its side effects,

methylphenidate has been shown to treat anxiety, depression, loss of appetite, pain, nausea, and drowsiness in cancer patients (88). A phase II trial reported that dexmethylphenidate significantly reduced patients' fatigue, but it caused more side effects in the treatment group than it did in the placebo group (92). It should be noted that both modafinil and methylphenidate are not presently licenced in the UK for the treatment of cancer-related fatigue.

Many cancer patients develop anaemia as an outcome of their cancer and the treatments. Fortunately, anaemia is a reversible cause of CRF. Anaemia may be caused by deficiencies in iron, B12 or folate, or as a consequence of haemolysis. In some situations, it may be helpful for patients to have a blood transfusion (93). Another treatment for anaemia is erythropoietin (EPO). EPO is a hormone produced by the kidneys that stimulates red blood cell production. A number of studies have proven that EPO may increase levels of haemoglobin in the body and improve people's quality of life. On the other hand, some research has found that EPO may also increase the chance of some types of cancer coming back after treatment (93). However, the benefits of EPO may outweigh the risks for some people. EPO is licenced by NICE guidelines for people with cancer and it is recommended that EPO (with iron injections) should only be given to people with anaemia related to their cancer treatment if they cannot have blood transfusions or women with ovarian cancer who have had platinum-based chemotherapy, such as carboplatin or cisplatin (93).

In conclusion, practitioners and researchers should rule out a number of potential causes before starting CRF management. For example, fatigue may be caused or exacerbated by something as simple as dehydration. Adequate fluid and nutritional intake should be ensured. Regular laboratory and clinical evaluations should be done to eliminate other possible causes of fatigue, such as hypothyroidism, anaemia, or depression.

Because fatigue is considered a highly common side effect in cancer patients, symptoms of fatigue might come from anticancer treatment or other confounding factors such as age, as previously discussed. Fatigue symptoms are reported as a common side effect in cancer patients receiving a first-line treatment such as sunitinib or pazopanib for metastatic renal cell carcinoma, as discussed in Chapter 1. Renal cancer patients receiving sunitinib or pazopanib sometimes do not tolerate these treatments due to the occurrence of fatigue, and the onset of moderate or severe fatigue can have serious implications for treatment, requiring incremental dose reductions until symptom have resolved. These reductions may consequently affect outcomes in patients treated with TKI, sunitinib or pazopanib, given the highly negative influence of fatigue on the daily lives of cancer patients. Therefore, accurately measuring fatigue and identifying possible reasons for the onset and increase of fatigue is vital to its early management. The early detection and management of fatigue will help to increase the tolerability of TKI agents, sunitinib or pazopanib, and support cancer survivors living lives that are as healthy and of high a quality as possible.

In conclusion, in this research, we used a multi-item unidimensional fatigue measurement (FACIT-F tool) to explore and measure the incidence and severity of fatigue in renal cancer patients at the West of Scotland Beatson Cancer Centre taking sunitinib or pazopanib. We examined the correlation between possible clinical and laboratory confounding factors and fatigue score. We also examined the influence of TKI agents, sunitinib and pazopanib, on renal cancer patient's quality of life, as well as the correlation between common cancer symptoms, measured by the M.D. Anderson Symptom Inventory, and fatigue score.

### 2.1.8 Description of the problem

Cancer patients may experience cancer-related fatigue before, during and even after receiving anti-cancer treatment. Around 40% of cancer patients reported fatigue at diagnosis, and most patients experience fatigue at some point while receiving cancer therapy, when the reported rates are 80 to 90% for patients being treated with chemotherapy or radiotherapy, respectively. For renal cancer management, chemotherapy and radiotherapy are not usually used, but the rate of fatigue is still high with cytokine therapies (IL-2 and INF-α), traditionally the most recommended anticancer therapy for renal cancer (34). In the last few years, a new group of medications called tyrosine kinase inhibitors have been launched and two of these, sunitinib and pazopanib, are now considered the first-line treatment for metastatic renal cancer patients (34). Fatigue is reported as one of the most common adverse reactions for this group, but at a lower rate (around 60%) compared with cytokine therapy, chemotherapy and radiotherapy (34).

Specifically, fatigue is one of the most common adverse reactions to TKI drugs (sunitinib and pazopanib) used to treat renal cancer patients. Use of TKI drugs in renal cancer patients has recently increased. All studies to date that have focused on the efficacy and safety of these drugs have identified fatigue as the most common adverse effect. Therefore, the aim of this research is to accurately assess the nature of fatigue in metastatic renal cancer receiving sunitinib or pazopanib, measure the differentiation (if any) between numbers of treatment cycles, and compare the two agents based on fatigue score.

Fatigue might influence by many factors that may negatively impact on the incidence or severity of fatigue. Therefore, this study also examines these confounding factors and determines whether there is a correlation between individual patient's fatigue scores and these factors. Cancer symptoms like depression, sleep disturbance and lack of appetite might also aggravate the fatigue score. Finally, this study evaluates the impact of pazopanib or sunitinib on the quality of life of renal cancer patients. Therefore, in this study, two main issues have been evaluated and answered. The first issue is the incidence and severity of outcomes (fatigue) on a specific group of patients (renal cancer patients receiving pazopanib or sunitinib) and which variables (clinical and laboratory) may affect the fatigue score in a group of patients. The second issue is a measurement of the cancer symptoms that correlate with fatigue score and the impact of these two agents on quality of life. During the treatment phase, the patients underwent regular assessments for safety, quality of life and disease assessment without any intervention.

## 2.2 Methodology

### 2.2.1 Format for the protocol

The structure of a research protocol is generally written to the following form: research project title, investigator's details, project summary, background for the proposed research, project description (research questions, rationales, objectives, methodology, data management and statistical analysis), ethical consideration and references (94).

### 2.2.2 Research questions

**Primary research question:** What is the incidence and severity of fatigue in renal cancer patients receiving pazopanib or sunitinib during routine clinical practice?

**Secondary research question:**

- What are the significant confounding factors among the clinical and laboratory variables of patients experiencing fatigue during treatment with pazopanib or sunitinib, and do they correlate with the incidence and severity of fatigue?
- What is the correlation between a patient's cancer symptoms and incidence of fatigue?
- What is the impact of pazopanib or sunitinib on the quality of life of a renal cancer patient?

### 2.2.3 Aims and objectives

**Primary aim:** Measure the incidence and severity of fatigue in renal cancer patients receiving pazopanib or sunitinib by using the Functional Assessment Chronic Illness Therapy – Fatigue (FACIT-F) questionnaire.

**Primary objective:** Measure the incidence and severity of fatigue in renal cancer patients receiving pazopanib or sunitinib.

**Secondary objectives:**

- Evaluate the impact of clinical and laboratory variables (sections 2.2.7.1 and 2.2.7.2) on the incidence and severity of fatigue.
- Evaluate the correlation between cancer symptoms (sensory symptoms as defined and measured using M.D. Anderson Symptoms Inventory tool which includes: sadness, pain, fatigue, nausea, disturbed sleep, upset, shortness of breath, difficulty remembering, lack of appetite, drowsiness, dry mouth, vomiting and numbness) and of fatigue measurements in renal cancer patients receiving pazopanib or sunitinib.
- Evaluate the influence of pazopanib or sunitinib on renal cancer patients' lifestyles (reactive symptoms as defined and measured using M.D. Anderson Symptoms Inventory).

### 2.2.4 Study design

The structure of this study is an observational prospective cohort study.

### 2.2.5 Setting

The research was carried out at the renal clinic at the Beatson West of Scotland Cancer Centre, NHS Greater Glasgow and Clyde.

### 2.2.6 Subjects/Patients

Patients who are diagnosed with renal cancer and receiving the TKI drugs sunitinib or pazopanib.

### 2.2.7 Clinical and laboratory variables

#### 2.2.7.1 Clinical variables

1. Duration of treatment on a TKI at inclusion into study (≤ 4 treatment cycles versus > 4 treatment cycles).
2. Sex (male versus female).
3. Age (< 60 years versus ≥ 60 years).
4. World Health Organisation (WHO) Performance Status (0 or 1 versus ≥ 2).
5. Interval between cancer diagnosis and initiation of sunitinib/pazopanib therapy (< 12 months versus ≥ 12 months).
6. History of nephrectomy (No versus Yes).
7. History of cytokine therapy: INF-α or interleukin-2 (No versus Yes).

8. Did the participant have comorbidities? (No versus Yes) If yes, record co-morbidities.
9. Tumour stage (I, II versus III, IV).
10. Histology (Clear cell RCC versus non–clear cell RCC).
11. Does the participant have metastatic cancer? (No versus Yes) If yes, record site.
12. Did the participant receive thyroid replacement therapy? At baseline and each cycle (No versus Yes).
13. Did the participant receive any concurrent medication(s)? (No versus Yes) If yes, record medication(s).
14. Have there been any changes to the medication since the last treatment cycle (yes/no)? Record if yes.
15. Have there been any changes to the co-morbidities since the last treatment cycle (yes/no)? Record if yes.

#### 2.2.7.2 Laboratory variables

1. Haemoglobin levels: Lower Limit Normal (LLN): male: 130 g/L, female: 115 g/L (≥ LLN versus < LLN g/L) at baseline and at each cycle (record if no reading is taken for that cycle and when last reading taken).
2. Corrected calcium levels at baseline (≤ 2.5 mmol/L versus > 2.5 mmol/L).
3. Lactate dehydrogenase (LDH) levels at baseline (≤ 360 U/L versus > 360 U/L).

4. Thyroid function test (TFT); Thyroid stimulating hormone (TSH > 5 mU/L versus ≤ 5 mU/L) at baseline and at each cycle (record if no reading is taken for that cycle and when last reading taken).
5. Neutrophil count at baseline: Upper Limit of Normal (ULN): 7.5 x $10^9$/L (≤ ULN versus > ULN).
6. Platelet count at baseline: Upper Limit of Normal (ULN): 400 x $10^9$ /L (≤ ULN versus > ULN).

All variables designed in collection form and used with each participant at baseline cycles and some items for next three consecutive cycles. The collection form can be found in Appendix 1.

#### 2.2.7.3 Rationale for selecting these variables

The clinical and laboratory variables used in the collection form can be divided into the two following categories: independent prognostic factors (Motzer and Heng criteria), and patient baseline characteristics.

Seven clinical and laboratory variables reported in the collection form were related to independent prognostic factors in metastatic renal cancer disease. Renal cell carcinoma is a heterogeneous disease with widely varying clinical outcomes. Therefore, establishing independent prognostic factors for overall survival is required for the purposes of clinical trial design, for selecting patients for appropriate therapy, and for evaluating patients' condition and patient counselling to design future clinical trials. The most widely used prognostic factor is from the Memorial Sloan-Kettering Cancer Centre (MSKCC), which examined 463 patients

with metastatic renal cell carcinoma treated with interferon alpha (INF-α) to investigate which factors might influence overall survival. The MSKCC system stratified factors into the following three groups: a favourable-risk group (with no prognostic factors), an intermediate-risk group (one or two prognostic factors) and a poor-risk group (three or more prognostic factors). These factors are now called the Motzer criteria, named after the oncologist who authored this study. The Motzer study identified five prognostic factors (95). These criteria must be met for all patients who are diagnosed with renal cancer in the West of Scotland Beatson Cancer Centre as baseline patient details. The five Motzer criteria are:

- Serum haemoglobin less than the lower limit of normal (LLN),
- Corrected calcium greater than the upper limit of normal (ULN),
- Serum lactate dehydrogenase (LDH) greater than 1.5 times the ULN,
- Karnofsky performance status less than 80% (which qualifies as WHO performance status 0 or 1), and
- Interval time from diagnosis to start of treatment of less than one year.

These Motzer prognostic criteria were derived from patients treated by immunotherapy medication. Therefore, Heng and his colleagues examined independent prognostic factors in patients with metastatic renal cell carcinoma treated by VEGF- targeted medications to clarify whether or not they are the same prognostic factors previously identified (96).

The Heng study examined 645 patients receiving treatment with sorafenib, sunitinib or bevacizumab and concluded that four of the previous five criteria are independent prognostic factors – haemoglobin levels, corrected calcium, Karnofsky performance status, and time from diagnosis to treatment (96). As well as these four criteria, neutrophils and platelets levels are also called Heng criteria. Therefore, this study measured all of the Motzer and Heng criteria at baseline and some criteria after every treatment cycle to examine its relation to fatigue score results. Haemoglobin levels (lower limit normal [LLN]: male: 130 g/L, female: 115 g/L ≥ LLN vs. < LLN g/L [after every cycle]), lactate dehydrogenase (LDH) levels at baseline (≤ 360 U/L vs. > 360 U/L), corrected serum calcium levels at baseline (≤ 2.5 mmol/L vs. >2.5 mmol/L), World Health Organisation (WHO) Performance Status (0 or 1 vs. ≥ 2), the time interval between cancer diagnosis and initiation of systemic treatment (or recurrence after nephrectomy) (< 12 months vs. ≥ 12 months), neutrophils and platelets count greater than upper limit of normal (ULN) (≤ ULN versus > ULN).

The clinical and laboratory variables included the patient baseline characteristics, as well as the clinical and pathological characteristics of these patients. The baseline characteristics of each patient included gender (male vs. female), age (< 60 years vs. ≥ 60 years), tumour stage (I, II vs. III, IV) and histology of the disease (clear cell RCC vs. non-clear cell RCC). Cancer metastasis (yes vs. no [if yes, circle site: brain, liver lung, central nervous system, bone or other…]), history of nephrectomy (yes vs. no), history of cytokine therapy (INF-α (INF-α) or interleukin-2 (IL-2) (yes vs. no)) were also recorded. In addition, participant co-morbidities (yes vs. no [if yes, record every

cycle]), concurrent medications (yes vs. no [if yes, record every cycle]), type of TKI (sunitinib vs. pazopanib), initiation or maintenance dose, duration of TKI treatment at inclusion in study (≤ 4 treatment cycles vs. > 4 treatment cycles) were recorded. Finally, thyroid function test (TFT), thyroid stimulating hormone (TSH > 5 mU/L vs. ≤ 5 mU/L [after every cycle]) and whether the participant received thyroid replacement therapy (yes vs. no after every cycle) were recorded. All these variables have been examined for their possible relationship with fatigue score.

### 2.2.8 Recruitment method

At the period of recruiting the participants, staff from the clinical care team approached patients in the renal clinic at the Beatson West of Scotland Cancer Centre, and verbally introduced the study's aims and objectives and provided each potential recruit with a participant information sheet before asking for his/her consent within a reasonable time frame (a minimum of 24 hrs). When a patient agreed to participate, he/she received a consent form requesting his/her signature.

Participants completed two questionnaires whilst attending a routine clinical appointment at the renal clinic at the Beatson West of Scotland Cancer Centre. These questionnaires were the 13-item FACIT-F and M. D. Anderson Symptom Inventory (MDASI) core items. In all, the study recruited participants for four cycles of questionnaires starting on the day they join the study.

### 2.2.9 Inclusion criteria

- Adult patients (age ≥ 18 years).
- Diagnosed with renal cancer (any length of disease duration).
- Receiving or planning to receive pazopanib or sunitinib at any dose as part of their standard care package.
- Patients must have given written informed consent and are willing to complete a questionnaire for four consecutive treatment cycles at the point of recruitment.
- Able to understand English and complete the questionnaires.

### 2.2.10 Exclusion criteria

An exclusion criterion was any renal cancer patients who were not treated at the Beatson West of Scotland Cancer Centre.

### 2.2.11 Data

The data were collected using standard tools:

#### 2.2.11.1 Functional Assessment Chronic Illness Therapy-Fatigue (Appendix 2)

The Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) is a multi-dimensional tool that is a subscale of the FACT-general (28 items). FACT-F is a 13-item fatigue subscale with a 5-point scale of 0-4 that focuses on the presence or absence of fatigue symptoms, the severity of the fatigue, the effective aspects of

fatigue, and the perceived interference with daily functioning. The FACT-F is scored from 0 to 52, with a high score indicating a low level of fatigue (97). The FACIT-F tool has been previously validated among cancer patients, as described in section 2.1.7.1

**2.2.11.2 The M.D. Anderson Symptom Inventory core items (Appendix 3)**

The M. D. Anderson Symptom Inventory (MDASI) is a multi-symptom patient reported outcome measure for clinical and research use. The MDASI includes items that report the "sensory" dimension of symptoms (intensity, or severity) and the "reactive" dimension of symptoms (interference with daily function). Severity is assessed for the 13 core MDASI symptom items (pain, fatigue, nausea, disturbed sleep, distress (emotional), shortness of breath, lack of appetite, drowsiness, dry mouth, sadness, vomiting, difficulty remembering, and numbness or tingling) and for the six interference items (general activity, mood, walking ability, normal work, relations with other people, and enjoyment of life). The MDASI has several advantages over other symptom-assessment scales in that it applies broadly to different cancer types and treatments, includes items related to symptom interference with daily life, and is easy for patients to complete (98).

There are two-recall periods in the MDASI tool: past week and past 24 hours. These recall periods give the research team an option to choose the most applicable timeframe. This study used past week to be compatible with the other tool, the FACIT-F scale.

Test-retest reliability reflects the stability of scores over time. The MDASI test-retest has been examined in several studies and shows that MDASI has a strong result averaging between 0.83 and 0.96. Internal consistency reflects whether the items in a domain are inter-correlated. The internal consistency demonstrated by Cronbach coefficient alphas should be > 0.7 to reflect strong consistency. The MDASI demonstrated high internal consistency, with 0.85 for general symptom severity items and 0.82 for the interference items (99).

Therefore, this tool, based on the M. D. Anderson Symptom Inventory, was chosen to measure the correlation between fatigue score and cancer symptoms in renal cancer patients because it is one of the most common cancer symptom measurement tools used in clinical studies; it contains the most high prevalence symptoms among cancer patients; the recall period of time of one week for the MDASI tool is the same as FACIT-F; it can measure interference of symptoms with exposure and measure impact of treatment on quality of life for patients at the same time; it is easy to complete and does not require a lot of time; and finally, it demonstrates strong reliability and validity (97, 99).

#### 2.2.11.3 Data entry

After patients agreed to participate in our research and signed the consent form (Appendix 4), the researcher filled the collection form (Appendix 1) from the patient electronic file at the clinic. Then, participants filled the first FACIT-F tool (fatigue measurement tool) and M.D. Anderson Inventory tool (cancer symptom measurement tool). The participants, again, filled in these two questionnaires for each of further consecutive cycles as long as they kept receiving sunitinib or pazopanib based on their treatment plan. Additional progress information was added to the collection forms based on a patient cycle. All data from collection forms and questionnaires were transferred from hardcopies to the SPSS program by the researcher for further statistical analysis.

#### 2.2.11.4 Validation

I attended all the renal clinics (Monday morning and Thursday afternoon) at Beatson West of Scotland Cancer Centre from January 2015 to February 2016. Therefore, I recruited 65 renal cancer patients receiving sunitinib or pazopanib at that time. The number 65 represented more than 90% of all patients registered in this clinic who met our criteria. Our method did not include measuring fatigue in the normal population in order to compare with fatigue in renal cancer patients, and that is because there was evidence of fatigue score in the general population in the literature review.

**2.2.11.5 Statistical analysis**

- One-way repeated measures ANOVA (within subject) compared the mean fatigue score (FACIT-F tool) of each cycle of treatment with the previous cycle. The differentiation was considered significant when the p value < 0.05.

- As mentioned in sections 2.2.7.1 and 2.2.7.2, we divided each clinical variable and laboratory variable for each TKI drug into two levels and statistically analysed their scores using the methodology described above. We examined the effect of those variables on the results of fatigue scores using independent t-tests, which compares the means of each variable and mean fatigue scores.

- Correlation coefficients measured the degree of correlation between cancer symptoms (sensory symptoms in M.D. Anderson Symptoms Inventory) and fatigue measurement in renal cancer patients receiving TKI. The correlation was considered significant when the p value < 0.05.

- The interference of TKI medication on the quality of life in renal cancer patients was measured using split plot ANOVA (between subject) tests. Six quality of life items were divided into two categories: activity-related items (work, activity and walking) and mood-related items (relationships with people, enjoyment and mood) and we examined the influence of medication on each category. In every statistical test that is used in the study, we considered p < 0.05 to be a statically significant value.

- All the statistical considerations were reviewed and validated by the statistical consultant, Dr. Stephen Corson (Department of Mathematics and Statistics at Strathclyde University).

Along with Dr. Corson, we calculated the sample size test for the research to reach a number of high statistical powers. We found that the number that should have been recruited was very high (302 patients), to reach the highest statistical power score. The number of patients eligible in the renal clinic at Beatson West of Scotland Cancer Centre and receiving sunitinib or pazopanib was 70 (which is much less than the suggested 302), and there was also limited time available for PhD research. Therefore, we decided to continue to analyse the data, which represent the Scottish population, and it was normally distributed after being examined with the normality test. Our data are normally distributed after being examined using the Kolmogorov-Smirnov normality test. The result of this test was a non-significant difference between the data ($p = 0.2$).

### 2.2.12 Intervention

There was no active intervention in this study.

### 2.2.13 Patient withdrawal/discontinuation

Participants who did not complete four consecutive cycles of the questionnaires still had their data used in analysis unless consent for this was withdrawn.

### 2.2.14 Ethical Considerations

#### 2.2.14.1 Ethical conduct of the study

Ethical approval was sought from the National Health Institute (NHI) Health Research Authority (NRES Committee London - South East) before patients were entered into this clinical trial. Patients were only allowed to enter the study once they had provided written informed consent. The chief investigator (CI) was responsible for updating the Ethics Committee of any new information related to the study. Approval was received on 07-01-2016 with REC reference: 14/LO/2135. Details are recorded in Appendix 5.

#### 2.2.14.2 Approval by ethics review committees

For all studies on human participants or involving human biological materials, the research protocol should be approved by the local institution's ethics committee or the national committee. For that purpose, in the United Kingdom, the Integrated Research Application System (IRAS) is an online tool that enables researchers to create a core data set and then to create the forms needed for applications to R&D, National Health System (NHS) Ethics Committees, the Medicine Health Regulatory Agency (MHRA), and a number of other national bodies. IRAS contains, at the start, project filter questions so that system will provide the researcher appropriate questions for the research and the identity of the appropriate committees. IRAS then contains different questions about the project. The headings for IRAS questions are: administrative details, overview of the research, purpose and design

of the research, risks and ethical issue (research participants / procedures, risks and benefits / recruitment and informed consent / confidentiality / storage and use of data after the end of the study / incentives and payments / notification of other professionals / publication and dissemination), scientific and statistical review, management of the research, details of the research sponsor, insurance/indemnity to meet potential legal liabilities, overview of research site (create a Specific Site Information form for the research site) and finally, declarations. The IRAS application also includes site-specific forms (SSI) that should be filled for each site in the study. The SSI form for this research was filled for Beatson West of Scotland Cancer Centre and accompanied with the REC form. The IRAS application form for this research was created on 19-03-2014 with project name (measure the incidence/severity of fatigue in renal cancer patients). This form was submitted on 21-11-2014, and the following documents were uploaded with the application form: protocol form, collection form, patient information sheet, consent form, current C.V. for chief investigator, academic supervisor, principle investigator at research site and research, covering letter on headed paper (covering page) and validated questionnaire (FACIT-Fatigue & MDASI core items questionnaires). The research sponsor, the University of Strathclyde, provided two letters to cover professional indemnity and employers' liability. All these documents were submitted to the NHS Greater Glasgow and Clyde R&D management office on 21-11-2014. The IRAS's application and documents were reviewed before submission by Mrs. Helen Baigrie, Contract Manager, Research and Knowledge Exchange Services at the University of Strathclyde.

The project went through IRAS and was subsequently granted permission together with institutional approval. Actions and documentation relating to the protocol timeline, patient information sheet (PIS), protocol of the research, no harm to individuals-anonymity, confidentiality, dissemination and funding can be found in Appendixes 6, 7 and 8.

### 2.2.15 Research group

The research protocol form, patient information sheet, consent form and collection form were shared with my supervisor, Professor Alex Mullen, my co-supervisor Dr. Marie Boyd, Professor Rob Jones (Professor of Clinical Cancer Research) and senior clinical pharmacists Mary Maclean and Jennifer Laskey before being submitted as a final draft and starting the study.

## 2.3 Results

A total of 70 eligible patients were enrolled in the renal clinic at the West of Scotland Beatson Cancer Centre in Glasgow, United Kingdom. Sixty-five patients agreed to join the study by signing the consent form; 47 participants completed four consecutive cycles. Eighteen participants did not complete all four of the cycles for a variety of reasons. Figure 6 shows the consort diagram of the inclusion and dropped-off participants.

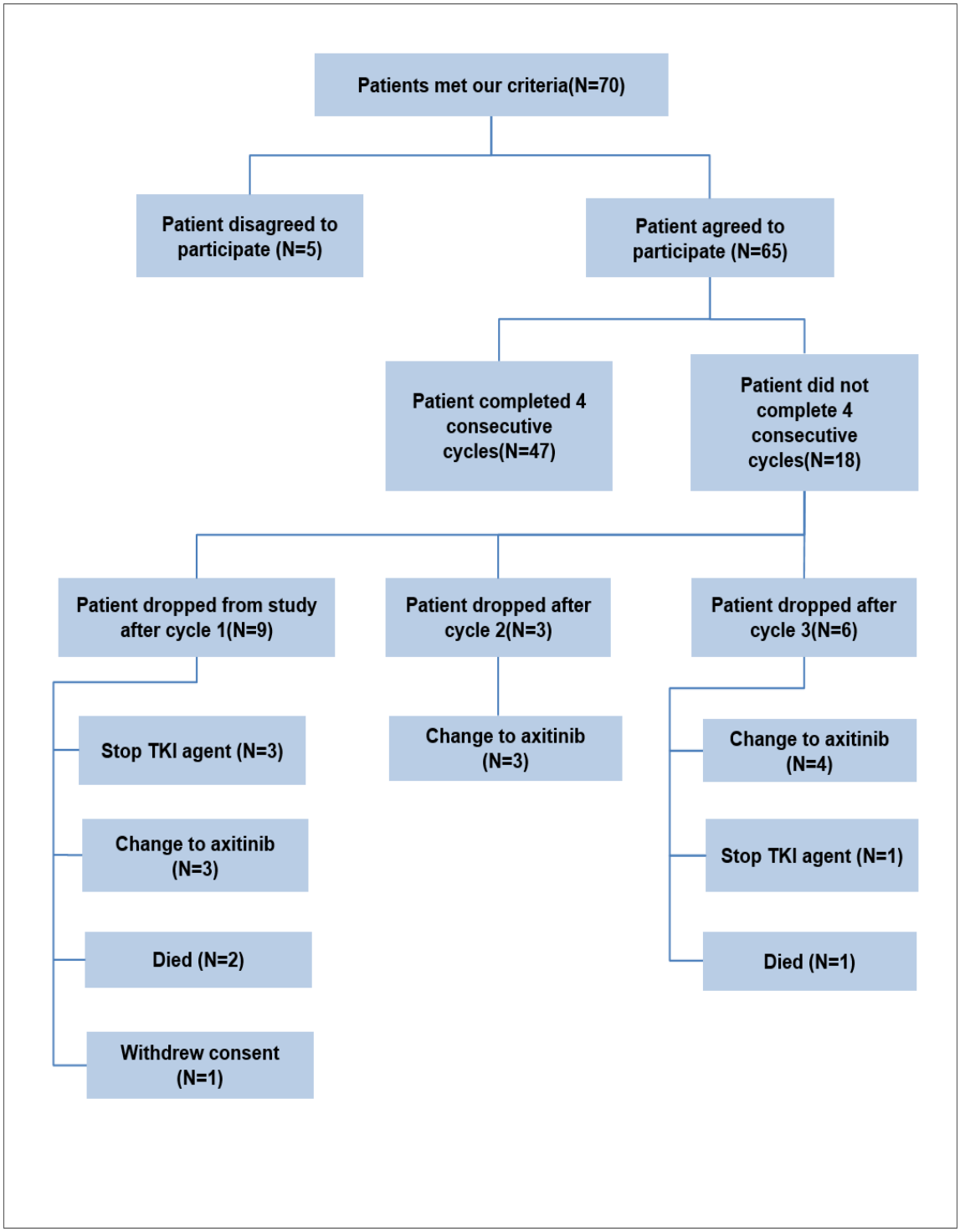


**Figure 6: Consort diagram. Number of participants who met the inclusion criteria.**

### 2.3.1 Demographic, clinical and laboratory variables data

Table 10 shows the demographic (gender and age), Motzer and Heng criteria, clinical and laboratory variables data for all participants (65 patients) recruited in our research. All the items have been divided into two categories and collected using the collection form at baseline and from each four cycles. The table also shows histology of renal cancer, tumour stage, number of metastatic organs, history of nephrectomy, history of cytokine therapy, and duration of treatment on a TKI agent at inclusion into study. Data on the thyroid function test, does the participant received thyroid replacement therapy, did the participants have concurrent medications, and did the participant have medical history and dose of TKI agents, sunitinib and pazopanib are also shown.

**Table 10: The demographical, clinical and laboratory characteristics of sunitinib and pazopanib treated patients.**

| Items | | Total Sample (N = 65) | | Sunitinib (N = 23) | | Pazopanib (N = 42) | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| **Gender** | Male | 44 | 68 | 17 | 26.2 | 27 | 41.5 |
| | Female | 21 | 32 | 6 | 9.2 | 15 | 23.1 |
| **Age** | < 60 Years | 23 | 35 | 9 | 13.8 | 14 | 21.5 |
| | ≥ 60 Years | 42 | 65 | 14 | 21.5 | 28 | 43.1 |
| **Motzer Criteria [95]** | | | | | | | |
| **Haemoglobin level [95,96] Lower limit of normal: male130g/L, femal:115g/L.** | ≥ LLN g/L | 38 | 58 | 8 | 12.3 | 30 | 46.2 |
| | < LLN g/L | 27 | 42 | 15 | 23.1 | 12 | 18.5 |
| **Lactate dehydrogenase level [95]** | ≤ 360 U/L | 61 | 94 | 21 | 32.3 | 40 | 61.5 |
| | > 360 U/L | 4 | 6 | 2 | 3.1 | 2 | 3.1 |
| **Corrected serum calcium level [95, 96]** | ≤ 2.5 mmol/L | 62 | 95 | 23 | 35.4 | 39 | 60.0 |
| | > 2.5 mmol/L | 3 | 5 | 0 | 0 | 3 | 4.6 |
| **WHO performance status [95, 96]** | ≤ 2 | 62 | 95 | 23 | 35.4 | 39 | 60.0 |
| | > 2 | 3 | 5 | 0 | 0 | 3 | 4.6 |
| **Interval between cancer diagnosis and initiation of systemic treatment [95, 96]** | ≥ 12 Months | 18 | 28 | 9 | 13.8 | 9 | 13.8 |
| | < 12 Months | 47 | 72 | 14 | 21.5 | 33 | 50.8 |
| **Heng Criteria [96]** | | | | | | | |
| **Neutrophil count [96] (Upper Limit of Normal = 7.5 x$10^9$/L)** | ≤ ULN | 63 | 97 | 23 | 35.4 | 40 | 61.5 |
| | > ULN | 2 | 3 | 0 | 0.0 | 2 | 3.1 |
| **Platelet count [96] (Upper Limit of Normal = 400 x$10^9$/L)** | ≤ ULN | 64 | 99 | 23 | 35.4 | 41 | 63.1 |
| | > ULN | 1 | 2 | 0 | 0.0 | 1 | 1.5 |
| **Tumour stage** | III, IV | 65 | 100 | 23 | 35.4 | 42 | 64.6 |
| **Histology** | Clear cell RCC | 48 | 74 | 9 | 13.8 | 39 | 60.0 |
| | Non-clear cell RCC | 17 | 26 | 14 | 21.5 | 3 | 4.6 |
| **Does the participant have metastatic cancer?** | Brain | 8 | 12 | 2 | 3.1 | 6 | 9.2 |
| | Liver | 5 | 8 | 2 | 3.1 | 3 | 4.6 |
| | Lung | 36 | 55 | 13 | 20.0 | 23 | 35.4 |
| | Lymph node | 3 | 5 | 2 | 3.1 | 1 | 1.5 |
| | Bone | 8 | 12 | 2 | 3.1 | 6 | 9.2 |
| | Others | 5 | 8 | 2 | 3.1 | 3 | 4.6 |

| Items | | Total Sample (N = 65) | | Sunitinib (N = 23) | | Pazopanib (N = 42) | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| **History of nephrectomy** | No | 25 | 39 | 7 | 10.8 | 18 | 27.7 |
| | Yes | 40 | 62 | 16 | 24.6 | 24 | 36.9 |
| **History of cytokine therapy: Interferon - alpha or interleukin -2 (IL-2)** | No | 60 | 92 | 20 | 30.8 | 40 | 61.5 |
| | Yes | 5 | 8 | 3 | 4.6 | 2 | 3.1 |
| | Yes | 5 | 8 | 3 | 4.6 | 2 | 3.1 |
| **Duration of treatment on a TKI at inclusion into study** | ≤ 4 treatment cycle | 19 | 29 | 5 | 7.7 | 14 | 21.5 |
| | > 4 treatment cycle | 46 | 71 | 18 | 27.7 | 28 | 43.1 |
| **Thyroid Function Test (TFT)** | TSH > 5 mU/L | 9 | 14 | 6 | 9.2 | 3 | 4.6 |
| | TSH ≤ 5 mU/L | 56 | 86 | 17 | 26.2 | 39 | 60.0 |
| **Did the participants receive thyroid replacement therapy?** | No | 45 | 69 | 9 | 13.8 | 36 | 55.4 |
| | Yes | 20 | 31 | 14 | 21.5 | 6 | 9.2 |
| **Did the participants have co-morbidities?** | No | 9 | 14 | 1 | 1.5 | 8 | 12.3 |
| | Yes | 56 | 86 | 22 | 33.8 | 34 | 52.3 |
| **Did the participants receive any concurrent medication?** | No | 3 | 5 | 1 | 1.5 | 2 | 3.1 |
| | Yes | 62 | 95 | 22 | 33.8 | 40 | 61.5 |
| **Thyroid Function Test (TFT) (cycle 2)** | TSH > 5 mU/L | 8 | 14 | 5 | 8.9 | 3 | 5.4 |
| | TSH ≤ 5 mU/L | 48 | 86 | 15 | 26.8 | 33 | 58.9 |
| **Did the participants receive thyroid replacement therapy (cycle 2)?** | No | 38 | 68 | 8 | 14.3 | 30 | 53.6 |
| | Yes | 18 | 32 | 12 | 21.4 | 6 | 10.7 |
| **Any changes to the co-morbidities since last treatment (cycle 2)?** | No | 52 | 93 | 17 | 30.4 | 35 | 62.5 |
| | Yes | 4 | 7 | 3 | 5.4 | 1 | 1.8 |
| **Any changes to the medication since last treatment cycle (cycle 2)** | No | 47 | 84 | 17 | 30.4 | 30 | 53.6 |
| | Yes | 9 | 16 | 3 | 5.4 | 6 | 10.7 |
| **Haemoglobin Lower Limit Normal (LLN) (cycle 2)** | ≥ 130 g/L | 39 | 70 | 10 | 17.9 | 29 | 51.8 |
| | < 130 g/L | 17 | 30 | 10 | 17.9 | 7 | 12.5 |
| **Thyroid Function Test (TFT) (cycle 3)** | TSH > 5 mU/L | 9 | 17 | 5 | 9.4 | 4 | 7.5 |
| | TSH ≤ 5 mU/L | 44 | 83 | 15 | 28.3 | 29 | 54.7 |

| Items | | Total Sample (N = 65) | | Sunitinib (N = 23) | | Pazopanib (N = 42) | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| **Did the participants receive thyroid replacement therapy (cycle 3)?** | No | 36 | 68 | 8 | 15.1 | 28 | 52.8 |
| | Yes | 17 | 32 | 12 | 22.6 | 5 | 9.4 |
| **Any changes to the co-morbidities since last treatment cycle (cycle 3)?** | No | 49 | 92 | 19 | 35.8 | 30 | 56.6 |
| | Yes | 4 | 8 | 1 | 1.9 | 3 | 5.7 |
| **Any changes to the medication since last treatment cycle (cycle 3)?** | No | 41 | 77 | 15 | 28.3 | 26 | 49.1 |
| | Yes | 12 | 23 | 5 | 9.4 | 7 | 13.2 |
| **Haemoglobin Lower Limit Normal (LLN) (cycle 3)** | ≥ 130 g/L | 36 | 68 | 10 | 18.9 | 26 | 49.1 |
| | < 130 g/L | 17 | 32 | 10 | 18.9 | 7 | 13.2 |
| **Did the participants receive thyroid replacement therapy (cycle 3)?** | No | 36 | 68 | 8 | 15.1 | 28 | 52.8 |
| | Yes | 17 | 32 | 12 | 22.6 | 5 | 9.4 |
| **Any changes to the co-morbidities since last treatment cycle (cycle 3)?** | No | 49 | 92 | 19 | 35.8 | 30 | 56.6 |
| | Yes | 4 | 8 | 1 | 1.9 | 3 | 5.7 |
| **Any changes to the medication since last treatment cycle (cycle 3)?** | No | 41 | 77 | 15 | 28.3 | 26 | 49.1 |
| | Yes | 12 | 23 | 5 | 9.4 | 7 | 13.2 |
| **Haemoglobin Lower Limit Normal (LLN) (cycle 3)** | ≥ 130 g/L | 36 | 68 | 10 | 18.9 | 26 | 49.1 |
| | < 130 g/L | 17 | 32 | 10 | 18.9 | 7 | 13.2 |
| **Thyroid Function Test (TFT) (cycle 4)** | TSH > 5 mU/L | 9 | 19 | 5 | 10.6 | 4 | 8.5 |
| | TSH ≤ 5 mU/L | 38 | 81 | 13 | 27.7 | 25 | 53.2 |
| **Did the participants receive thyroid replacement therapy (cycle 4)?** | No | 31 | 66 | 8 | 17.0 | 23 | 48.9 |
| | Yes | 16 | 34 | 10 | 21.3 | 6 | 12.8 |
| **Any changes to the co-morbidities since last treatment (cycle 4)?** | No | 45 | 96 | 16 | 34.0 | 29 | 61.7 |
| | Yes | 2 | 4 | 2 | 4.3 | 0 | 0 |
| **Any changes to the medication since last treatment (cycle 4)?** | No | 37 | 79 | 12 | 25.5 | 25 | 53.2 |
| | Yes | 10 | 21 | 6 | 12.8 | 4 | 8.5 |
| **Haemoglobin Lower Limit Normal (LLN) (cycle 4)** | ≥ 130 g/L | 29 | 62 | 9 | 19.1 | 20 | 42.6 |
| | < 130 g/L | 18 | 38 | 9 | 19.1 | 9 | 19.1 |
| **Motzer Score** | Mean ± SD | | | 1.29 (0.84) | | | |
| **Heng Score** | Mean ± SD | | | 1.28 (0.82) | | | |
| **Dosage in Mg** | 25 | 4 | 17.4 | 4 | 17.4 | 0 | 0.0 |
| | 37.5 | 8 | 34.8 | 8 | 34.8 | 0 | 0.0 |
| | 50 | 11 | 47.8 | 11 | 47.8 | 0 | 0.0 |
| | 400 | 3 | 7.1 | 0 | 0 | 3 | 7.1 |
| | 600 | 14 | 33.3 | 0 | 0 | 14 | 33.3 |
| | 800 | 25 | 59.6 | 0 | 0 | 25 | 59.6 |

N: number of patients. SD: Standard Deviation.

As shown in Table 10, of the total 65 participants, 68% of the patients (n = 44) were males and 32% (n = 21) were females. The majority of participants (65%; n = 42)

were equal to or above 60 years of age and 35% (n = 23) were less than 60 years of age. From collected data, 42% (n = 27) of the participants showed haemoglobin levels of less than 130 g/L and 94% (n = 61) to have lactate dehydrogenase level of less than 360 U/L. Ninety-five percent of patients (n = 62) had corrected serum calcium levels of less than 2.5 mmol/L and 95% (n = 62) of patients the number had WHO performance status levels of less than 2. Sixty-three participants (97%) had a neutrophil count of less than the upper limit of normal whereas 99% (n = 64) of the participants' platelet counts was less than the upper limit of normal. Collected data showed that the majority of participants (71%; n = 46) had more than four treatment cycles of TKI at inclusion into the study. Collected data showed that the majority of participants (86%; n = 56) also had comorbidities and 95% of all participants (n = 62) received concurrent medication.

As shown in Table 10, the mean ± SD of the Motzer and Heng scores for all participants were 1.29 ± 0.84 and 1.28 ± 0.82 respectively. The Motzer scores of patients within the range between 1 and 2 out of 5 placed participants in the intermediate risk group (median survival rate for cancer patients is 10 months). Meanwhile, the Heng scores showed that patients scoring within the range between 1 and 2 out of 6, placed participants in the intermediate prognosis group (median survival rate for cancer patients is 22.5 months).

The recommended dose of pazopanib as described in the manufacturer's marketing authorisation for the treatment of RCC is 800 mg once daily. An 800 mg dose of pazopanib was taken by 59.6% (n = 25) of participants being treated with

pazopanib, a 600 mg dose by 33.3% (n = 14) and a 400 mg dose by 7.1% (n = 3). The recommended dose of sunitinib as described in the manufacturer's marketing authorisation for the treatment of RCC is 50 mg. A 50 mg dose of sunitinib was taken by 47.8% (n = 11) of participants being treated with sunitinib, a 37.5 mg dose by 34.8% (n = 8) and a 25 mg dose by 17.4% (n = 4).

### 2.3.2 Measurement of the incidence and severity of fatigue using the FACIT-F questionnaire

The primary objective of this research is measuring the incidence and severity of fatigue in renal cancer patients receiving sunitinib or pazopanib by using the FACIT-F tool. Based on the agreed research method, a one-way repeated measure ANOVA test is the valid statistical test for our research to measure the fatigue score in four different cycles. There were 47 participants who completed four consecutive cycles as described previously in Figure 6. Table 11 is a descriptive result of one-way ANOVA tests using SPSS, showing the mean fatigue score (standard deviation) for four different cycles and number of patients. Table 11 shows the mean and standard deviation of fatigue score of participants from cycle 1 to cycle 4.

**Table 11: Descriptive results of mean fatigue score and standard deviation in cycles 1, 2, 3 and 4 for 47 patients who completed four consecutive cycles.**

| Cycles | Mean fatigue score | SD | Number of patients |
|---|---|---|---|
| **Cycle 1** | 30.8 | 12.7 | 47 |
| **Cycle 2** | 31.8 | 13 | 47 |
| **Cycle 3** | 30.7 | 13.9 | 47 |
| **Cycle 4** | 29.5 | 13.9 | 47 |

In order to compare the incidence and severity of fatigue between four different cycles, we used a one-way ANOVA test based on the agreed method to test the null hypothesis, that there is no mean difference in fatigue scores between four cycles. Mauchly's test was used, to test the assumptions of the ANOVA were met, which is the formal and commonly used statistical test suitable for this form of investigation. Following application of Mauchly's test, if $p < 0.05$ the null hypothesis could be rejected and it could be assumed that the variance of the difference is equal. As shown in Table 12, the p-value was found to be less than 0.05 ($p = 0.022$), suggesting that the null hypothesis could be rejected. However, to confirm that the null hypothesis could be rejected, it was recommended by the research statistician consultant that one of two conservative tests (within subject), Greenhouse-Geisser or Huynh-Feldt tests, should be conducted. Table 13 shows that both conservative tests resulted in a p value of more than 0.05, which means the null hypothesis can be accepted; i.e. there was no statistically significant difference between four cycles in mean fatigue score for renal cancer patients receiving sunitinib or pazopanib.

**Table 12: Mauchly's test.**

| Within-Subjects Effect | Mauchly's | df | Sig. | Epsilon | |
|---|---|---|---|---|---|
| | | | | Greenhouse-Geisser | Huynh-Feldt |
| **Fatigue** | .745 | 5 | .022 | .850 | .904 |

df: degrees of freedom. Sig.: significance

**Table 13: Results of the conservative test.**

| Within-Subject Cycles | p-value |
|---|---|
| **Greenhouse-Geisser** | 0.307 |
| **Huynh-Feldt** | 0.308 |

### 2.3.2.1 Mean fatigue score through treatment time (year)

The patients recruited in our research were at different years of receiving TKI agents, sunitinib or pazopanib. Therefore, we decided to investigate the number of patients in each year and the mean fatigue score. We calculated the mean fatigue score for the patients in each year for both group of medications, sunitinib and pazopanib. Figure 7 shows the mean fatigue score of patients at the time of receiving agents, sunitinib or pazopanib, in years, 1, 2, 3 and 4 of treatment.

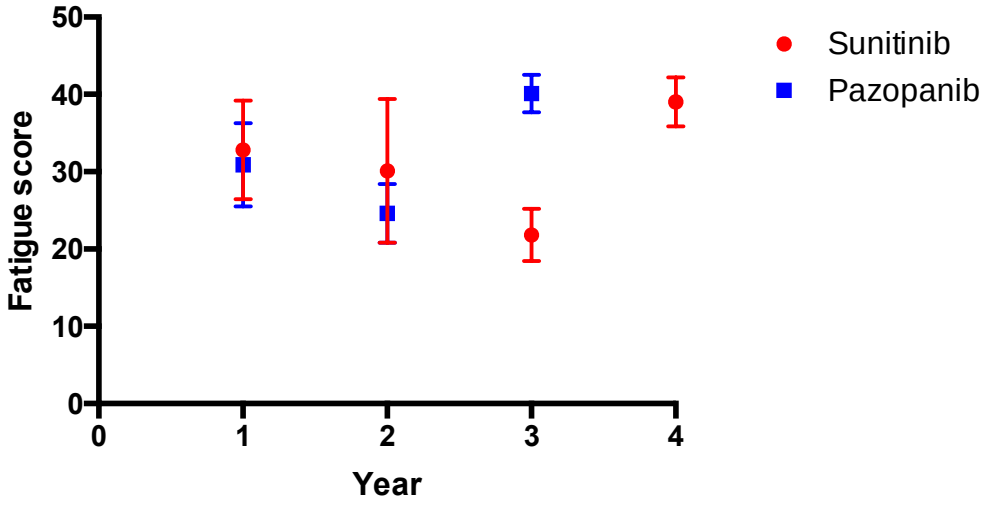


**Figure 7: Mean fatigue score (±SD) for groups taking sunitinib and pazopanib, based on year recruited to the study. The figure was created using Prism Software.**

Table 14 shows descriptive results for fatigue score through treatment time of receiving sunitinib or pazopanib. Our data showed that there were 26 patients recruited in the study at year 1 of receiving TKI agents (eight patients receiving sunitinib and 18 patients receiving pazopanib). There were ten patients in year 2 (two patients receiving sunitinib and eight patients receiving pazopanib). In year 3,

there were seven patients (four patients receiving sunitinib and three patients receiving pazopanib), and in year 4, there were only four patients receiving sunitinib. Therefore, we concluded that most of the recruited participants in our research were concentrated in years 1 and 2. Our results also demonstrated that sunitinib was received by patients for up to four years, but pazopanib was only received for up to three years. This might be related to the fact that pazopanib was approved in the market after sunitinib.

**Table 14: Mean fatigue score with SD as assessed by the FACIT-F tool for patients receiving sunitinib and pazopanib grouped by how many years they had been taking the agents when they were recruited to the study. (See also Figure 7).**

| Drug | Year 1<br>Mean ±SD<br>(Number of patients) | Year 2<br>Mean ±SD<br>(Number of patients) | Year 3<br>Mean ±SD<br>(Number of patients) | Year 4<br>Mean ±SD<br>(Number of patients) |
|---|---|---|---|---|
| **Sunitinib** | 32.8 ± 6.4<br>(8) | 30.1 ± 9.3<br>(2) | 21.8 ± 3.4<br>(4) | 39 ± 3.2<br>(4) |
| **Pazopanib** | 30.9 ± 5.4<br>(18) | 24.6 ± 3.8<br>(8) | 40.1 ± 2.4<br>(3) | No patients |

#### 2.3.2.2 Comparison of mean fatigue scores between sunitinib and pazopanib through four cycles

From the literature review, the researcher identified that most of the previous studies that compared sunitinib and pazopanib measured the fatigue score in week four in each cycle for both agents. However, our research measured the fatigue score at the end of each cycle, at week six. Therefore, we conducted this comparison between sunitinib and pazopanib medications, the results of which are shown in Table 15.

**Table 15: Descriptive statistics of mean fatigue score with standard deviation for sunitinib and pazopanib groups of patients during cycles 1-4.**

| Cycles | Drug | Mean fatigue score | SD | Number of patients |
|---|---|---|---|---|
| **Cycle 1** | **Sunitinib** | 33.1 | 13.6 | 18 |
| | **Pazopanib** | 29.2 | 12 | 29 |
| | **Total** | 30.7 | 12.7 | 47 |
| **Cycle 2** | **Sunitinib** | 31.2 | 14 | 18 |
| | **Pazopanib** | 32.1 | 12.6 | 29 |
| | **Total** | 31.7 | 13 | 47 |
| **Cycle 3** | **Sunitinib** | 30.9 | 15.7 | 18 |
| | **Pazopanib** | 30.5 | 12.9 | 29 |
| | **Total** | 30.6 | 13.9 | 47 |
| **Cycle 4** | **Sunitinib** | 30.4 | 15.4 | 18 |
| | **Pazopanib** | 28.8 | 13 | 29 |
| | **Total** | 29.4 | 13.9 | 47 |

Table 15 shows the descriptive statistics including the mean fatigue score and standard deviation for sunitinib and pazopanib groups and the total number of patients in each cycle. A mean fatigue score is considered to be the dependent variable and TKI drugs are the independent variable with two levels, sunitinib and

pazopanib. Thus, the mean fatigue score of cycle 1 to cycle 4 will be measured on two occasions of TKI drug administration of both pazopanib and sunitinib by using a split plot ANOVA between subjects, which was discussed and agreed with the research statistician consultant (100). As shown in Table 16, the p-value achieved following application of this statistical test is more than 0.05 (p = 0.735). Therefore, there is no statistically significant difference in measured fatigue score between TKI agents, sunitinib and pazopanib, in any given cycle.

**Table 16: Split-plot ANOVA test (between subject: sunitinib and pazopanib groups) in fatigue score.**

| Source (Between subjects) | df | Sig. |
|---|---|---|
| **Intercept** | 1 | 0.000 |
| **Type of TKI** | 1 | 0.735 |
| **Error** | 45 | |

df: degree of freedom. Sig.: significance.

### 2.3.3 Evaluation of the impact of clinical and laboratory variables on the incidence of fatigue

One of the secondary objectives in our research is evaluating the impact of clinical and laboratory variables (sections 2.2.7.1 and 2.2.7.2) on the incidence and severity of fatigue. An independent sample t-test was conducted to determine if there was any statistically significant impact on the clinical and laboratory variables of the patients (which were collected from the collection form) on the mean fatigue score (as determined by the FACIT-F tool). The statistical test, independent t-test, was conducted after agreement from the statistician consultant. Table 17 below shows the results after running the statistical test using SPSS software.

**Table 17: t-test comparing clinical and laboratory variables collected at baseline and each cycle of all recruited participants during treatment with sunitinib or pazopanib.**

| Variable | Category | N | Mean fatigue score (SD) | p- value |
|---|---|---|---|---|
| **Sex** | Male | 44 | 30.5 (10.8) | 0.266 |
| | Female | 21 | 26.4 (14.8) | |
| **Age** | < 60 | 23 | 27.5 (10.4) | 0.419 |
| | ≥ 60 | 42 | 30.1 (13.2) | |
| **Haemoglobin levels: Lower Limit Normal (LLN): male: 130 g/L, female: 115 g/L** | ≥ LLN | 38 | 30.4 (12.5) | 0.373 |
| | < LLN | 27 | 27.6 (11.9) | |
| **History of nephrectomy** | No | 25 | 27.9 (12.1) | 0.510 |
| | Yes | 40 | 30 (12.5) | |
| **WHO performance status** | 0 or 1 | 62 | 29.8 (11.9) | 0.083 |
| | ≥ 2 | 3 | 17.3 (16.3) | |
| **Histology** | Clear cell | 48 | 30.2 (11.8) | 0.291 |
| | Non-clear cell | 17 | 26.5 (13.4) | |
| **History of cytokine therapy: INF-alpha or IL-2?** | No | 60 | 29.6 (12.5) | 0.385 |
| | Yes | 5 | 24.6 (9.1) | |
| **Type of TKI** | Sunitinib | 22 | 29 (14.4) | 0.934 |
| | Pazopanib | 43 | 29.3 (11.2) | |
| **Thyroid Function Test (TFT); Thyroid Stimulating Hormone** | > 5 | 9 | 31.4 (17.5) | 0.561 |
| | ≤ 5 | 56 | 28.9 (11.4) | |
| **Did the participant receive thyroid replacement therapy?** | No | 45 | 29.2 (11) | 0.983 |
| | Yes | 20 | 29.2 (15) | |
| **Platelet count (Upper Limit of Normal = 400 x$10^9$/L)** | ≤ ULN | 64 | 29.3 (12.3) | 0.59 |
| | > ULN | 1 | 22.0 | |

| Variable | Category | Number of patients | Mean fatigue score (SD) | p - value |
|---|---|---|---|---|
| **Did the participant have comorbidities?** | No | 9 | 29.4 (8.7) | 0.966 |
| | Yes | 56 | 29.2 (12.8) | |
| **Did the participant receive any concurrent medication(s)?** | No | 3 | 32.5 (9.3) | 0.641 |
| | Yes | 62 | 29.1 (12.4) | |
| **Interval between cancer diagnosis and initiation of systemic treatment (or recurrence after nephrectomy)** | ≥ 12 months | 18 | 29.6 (11.8) | 0.874 |
| | < 12 months | 47 | 29.1 (12.6) | |
| **Neutrophil count. Upper limit of Normal (ULN) for male and female: 7.5 x $10^9$/L** | ≤ ULN | 63 | 29.5 (12.1) | 0.277 |
| | > ULN | 2 | 19.9 (18.2) | |
| **Duration of treatment with a TKI at inclusion into study** | ≤ 4 treatment cycles | 19 | 27.2 (12.7) | 0.387 |
| | > 4 treatment cycles | 46 | 30.1 (12.1) | |
| **Lactate Dehydrogenase (LDH) levels at baseline.** | ≤ 360 U/L | 61 | 30 (11.7) | **0.034*** |
| | > 360 U/L | 4 | 16.7 (15.8) | |
| **Corrected serum calcium levels at baseline** | ≤ 2.5 mmol/L | 62 | 29.5 (12.3) | 0.445 |
| | > 2.5 mmol/L | 3 | 23.9 (12) | |
| **Thyroid Function Test (TFT); Thyroid Stimulating Hormone. Cycle 2** | > 5 | 8 | 38.4 (8.7) | 0.364 |
| | ≤ 5 | 48 | 29 (12) | |
| **Did the participant receive thyroid replacement therapy? Cycle 2** | No | 38 | 30 (11.1) | 0.299 |
| | Yes | 18 | 31.2 (13.9) | |
| **Thyroid Function Test (TFT); Thyroid Stimulating Hormone. Cycle 3** | > 5 | 9 | 37.8 (8.3) | 0.243 |
| | ≤ 5 | 44 | 29.5 (12.1) | |
| **Did the participant receive thyroid replacement therapy? Cycle 3** | No | 36 | 30.4 (11.1) | 0.445 |
| | Yes | 17 | 32.22 (13.7) | |

| Variable | Category | Number of patients | Mean fatigue score (SD) | p - Value |
|---|---|---|---|---|
| **Thyroid Function Test (TFT); Thyroid Stimulating Hormone. Cycle 4** | > 5 | 9 | 37.8 (8.3) | 0.151 |
| | ≤ 5 | 38 | 30.1 (12.7) | |
| **Did the participant receive thyroid replacement therapy? Cycle 4** | No | 31 | 30.1 (11.2) | 0.28 |
| | Yes | 16 | 34.5 (14.1) | |
| **Haemoglobin levels: Lower Limit Normal (LLN): male: 130 g/L, female: 115 g/L. Cycle 2** | ≥ LLN | 39 | 31.2 (11.9) | 0.746 |
| | < LLN | 17 | 28.6 (12.4) | |
| **Haemoglobin levels: Lower Limit Normal (LLN): male: 130 g/L, female: 115 g/L. Cycle 3** | ≥ LLN | 36 | 32.2 (11.4) | 0.52 |
| | < LLN | 17 | 28.3 (12.8) | |
| **Haemoglobin levels: Lower Limit Normal (LLN): male: 130 g/L, female: 115 g/L. Cycle 4** | ≥ LLN | 29 | 33.5 (12) | 0.907 |
| | < LLN | 18 | 28.6 (12.4) | |
| **Any changes to the comorbidities since the last treatment cycle? Cycle 2** | No | 52 | 30.9 (12) | 0.447 |
| | Yes | 4 | 23.4 (10) | |
| **Any changes to the comorbidities since the last treatment cycle? Cycle 3** | No | 49 | 32.1 (11) | 0.473 |
| | Yes | 4 | 17.5 (15.2) | |
| **Any changes to the comorbidities since the last treatment cycle? Cycle 4** | No | 45 | 32.4 (12) | 0.253 |
| | Yes | 2 | 14.1 (14.1) | |
| **Any changes to the medication since the last treatment cycle? Cycle 2** | No | 47 | 30.8 (12.4) | 0.132 |
| | Yes | 9 | 28.2 (9.5) | |

| Variable | Category | Number of patients | Mean fatigue score (SD) | P - Value |
|---|---|---|---|---|
| **Any changes to the medication since the last treatment cycle? Cycle 3** | No | 41 | 31.3 (11.2) | 0.245 |
| | Yes | 12 | 29.8 (14.5) | |
| **Any changes to the medication since the last treatment cycle? Cycle 4** | No | 37 | 31.4 (12.2) | 0.764 |
| | Yes | 10 | 32.3 (13.1) | |

* $p < 0.05$

Table 17 shows the statistical test results of the impact of the clinical and laboratory variables on the incidence of fatigue (FACIT-F) during treatment with sunitinib and pazopanib TKI drugs. Our results demonstrated that there was no statistically significant impact of either clinical or laboratory variables on the mean fatigue score, apart from lactate dehydrogenase (LDH). Patients with LDH levels of > 360 U/L at baseline had a significantly lower mean fatigue score (SD) of 16.7 (15.8), compared with patients with lactate dehydrogenase levels of ≤ 360 U/L at baseline, who had a mean fatigue score (SD) of 30 (11.7) ($p = 0.034$). Therefore, renal cancer patients with lactate dehydrogenase levels of > 360 U/L at baseline receiving sunitinib or pazopanib showed statistically significantly higher severity of fatigue compared with patients with lactate dehydrogenase levels ≤ 360 U/L.

### 2.3.4 Measuring the Motzer and Heng prognostic survival model scores

The Motzer model is used as a prognostic survival models at baseline with patients in the renal clinic at West of Scotland Beatson Cancer Centre. The Heng score has also been newly approved as a valid prognostic models with renal cancer patients

receiving molecular target agent, such as sunitinib. Therefore, we decided to calculate the individual means of these two prognostic scores in order to compare the prognostic survival rate between these two scores.

**Table 18: Prognostic survival models scores (Motzer and Heng) that are assessed at baseline for all recruited patients.**

| Drug (number of patients) | Motzer score | | Heng score | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| **Sunitinib (22)** | 1.32 | (0.78) | 1.23 | (0.68) |
| **Pazopanib (43)** | 1.28 | (0.88) | 1.3 | (0.88) |
| **Recruited participants (65)** | 1.29 | (0.84) | 1.28 | (0.82) |

Table 18 shows that the mean Motzer score is 1.29 for all 65 recruited participants, which predicts an intermediate survival rate (10 months). However, the Heng score is 1.28, which also predicts an intermediate survival rate (22 months). Therefore, we could say that our research participants on the same degree of survival rate. In addition, we recommend that the Heng score be used as a prognostic survival model in the renal clinic at West of Scotland Beatson Cancer Centre instead of the Motzer score. Furthermore, our results showed that there was no large difference between sunitinib and pazopanib groups based on prognostic scores.

### 2.3.5 Comorbidities and concurrent medications

In order to determine if the presence of comorbidities or any concurrent medication(s) at the baseline and consequent cycles had an influence on fatigue score, we examined the statistical influence of these variables on fatigue score by using independent t-tests. The data in Table 17 demonstrate that there was no statistically significant effect on fatigue score from comorbidities and concurrent medications at the baseline and or at consecutive cycles ($p > 0.05$). Details of medications that were received by participants are reported and attached in Appendix 9.

### 2.3.6 Measuring cancer symptoms

#### 2.3.6.1 Measuring cancer symptoms in renal cancer patients

MDASI is a multi-symptom patient-reported outcome measure for clinical and research use. The MDASI tool includes items that report the “sensory” dimension of symptoms and the “reactive” dimension of symptoms (interference with daily function). In this section, the sensory dimension of symptoms was assessed for the 13 core MDASI symptom items (pain, fatigue, nausea, disturbed sleep, distress, shortness of breath, lack of appetite, drowsiness, dry mouth, sadness, vomiting, difficulty remembering, and numbness or tingling). Table 19 shows the comparison of mean ± SD scores of 13 cancer symptoms assessed by the MDASI tool as well the average worst score for each variable out of 10, among the cycles from cycle one to cycle four for all recruited participants receiving sunitinib or pazopanib.

**Table 19: Mean ± SD for cancer symptoms that were assessed using the MDASI tool for all recruited patients in cycles 1, 2, 3 and 4.**

| MDASI Symptoms (13 items) | Cycle 1 (65 patients) | Cycle 2 (56 patients) | Cycle 3 (53 patients) | Cycle 4 (47 patients) | Overall Mean Score |
|---|---|---|---|---|---|
| **Pain** | 2.11 (2.6) | 2.43 (2.9) | 2.62 (2.6) | 2.57 (2.8) | 2.43 |
| **Fatigue** | 4.22 (2.8) | 4.21 (2.9) | 4.32 (3.1) | 3.96 (2.9) | 4.17 |
| **Nausea** | 2.12 (2.9) | 1.64 (2.5) | 1.87 (2.4) | 1.43 (1.9) | 1.76 |
| **Disturbance of sleep** | 2.72 (2.8) | 2.95 (2.9) | 2.83 (2.8) | 2.72 (2.7) | 2.80 |
| **Distressed (upset)** | 2.06 (2.4) | 1.54 (2.1) | 1.94 (2.4) | 1.66 (2.3) | 1.80 |
| **Shortness of breath** | 1.97 (2.5) | 2.02 (2.4) | 2.19 (2.3) | 2.36 (2.5) | 2.13 |
| **Difficulty remembering** | 1.66 (2.5) | 1.29 (2.1) | 1.42 (1.9) | 1.57 (1.9) | 1.48 |
| **Lack of appetite** | 3.29 (3.5) | 3.04 (3) | 2.75 (2.8) | 2.85 (3) | 2.98 |
| **Drowsiness (sleepy)** | 3.63 (3) | 3.05 (2.9) | 3.57 (2.9) | 3.60 (3.1) | 3.46 |
| **Dry mouth** | 2.74 (3.5) | 2.21 (2.9) | 2.66 (2.9) | 2.94 (3.2) | 2.63 |
| **Sadness** | 1.95 (2.6) | 1.57 (2.4) | 1.72 (2.1) | 1.87 (2.3) | 1.77 |
| **Vomiting** | 1.20 (2.5) | 0.98 (2.1) | 0.92 (1.9) | 0.79 (1.9) | 0.97 |
| **Numbness or tingling** | 1.32 (2.1) | 0.79 (1.5) | 0.96 (1.8) | 0.98 (1.8) | 1.01 |

The study results reveal that fatigue (4.17), drowsiness (3.46), lack of appetite (2.98), sleep disturbances (2.80) and dry mouth (2.63) were rated as the five most severe symptoms among the participants. Tables 20 (A) and (B), show a comparison of mean ± SD scores of 13 cancer symptoms assessed by the MDASI tool as well the average worst score for each variable out of 10, among the cycles from cycle one to cycle four for participants receiving sunitinib (Table 20 A) or pazopanib (Table 20 B).

**Table 20: Mean score with standard deviation for 13 cancer symptoms for sunitinib (A) and pazopanib (B) groups.**

| A | Sunitinib (18 Patients) | | | | |
|---|---|---|---|---|---|
| | Cycle 1 | Cycle 2 | Cycle 3 | Cycle 4 | Overall |
| **Pain** | 2.44 (3.2) | 2.44 (3.4) | 2.5 (2.8) | 2.11 (2.7) | 2.37 |
| **Fatigue** | 3.89 (3.1) | 3.67 (3.2) | 4.72 (3.5) | 3.94 (2.9) | 4.05 |
| **Nausea** | 2.06 (2.7) | 1.94 (2.7) | 2.11 (2.4) | 1.56 (1.8) | 1.91 |
| **Disturbance of sleep** | 2.94 (3.4) | 2.94 (2.8) | 2.94 (2.9) | 2.5 (2.9) | 2.38 |
| **Distressed (upset)** | 1.11 (1.9) | 0.89 (1.5) | 1.83 (2.3) | 1.44 (2) | 1.31 |
| **Shortness of breath** | 1.72 (2.5) | 1.78 (2.1) | 2.67 (2.4) | 2.94 (3) | 2.27 |
| **Difficulty remembering** | 1.5 (2.4) | 1 (1.5) | 1.33 (2) | 1.5 (1.9) | 1.33 |
| **Lack of appetite** | 2.44 (3.3) | 2.5 (3) | 2.56 (2.8) | 2.5 (3.2) | 2.5 |
| **Drowsiness (sleepy)** | 2.89 (3.6) | 3.28 (3.6) | 4.33 (3.5) | 3.33 (3.2) | 3.45 |
| **Dry mouth** | 3.67 (4) | 3.33 (3.3) | 3.17 (2.9) | 3.78 (3.3) | 3.48 |
| **Sadness** | 1.17 (2.2) | 0.83 (1.8) | 1.44 (1.5) | 1.28 (1.4) | 1.18 |
| **Vomiting** | 0.22 (0.54) | 0.67 (1.6) | 0.33 (0.84) | 0.28 (0.75) | 0.37 |
| **Numbness or tingling** | 1.39 (2) | 1.17 (1.7) | 1.56 (1.9) | 1.06 (1.5) | 1.29 |

| B | Pazopanib (29 Patients) | | | | |
|---|---|---|---|---|---|
| | Cycle1 | Cycle 2 | Cycle 3 | Cycle 4 | All |
| **Pain** | 1.86 (2.1) | 2.34 (2.4) | 2.66 (2.5) | 2.86 (2.9) | 2.43 |
| **Fatigue** | 3.69 (2.5) | 4.14 (2.9) | 3.83 (2.9) | 3.97 (2.9) | 3.9 |
| **Nausea** | 1.72 (2.9) | 1.24 (2.2) | 1.38 (2.2) | 1.34 (2) | 1.42 |
| **Disturbance of sleep** | 2.41 (2.5) | 2.79 (2.8) | 2.62 (2.8) | 2.86 (2.6) | 2.67 |
| **Distressed (upset)** | 2.17 (2.3) | 1.79 (2.4) | 1.97 (2.7) | 1.79 (2.5) | 1.93 |
| **Shortness of breath** | 1.66 (2.1) | 2 (2.2) | 2.17 (2.4) | 2 (2.1) | 1.95 |
| **Difficulty remembering** | 1.59 (2.4) | 1.52 (2.3) | 1.66 (2.1) | 1.62 (2) | 1.59 |
| **Lack of appetite** | 3.28 (3.6) | 2.86 (3) | 2.55 (2.9) | 3.07 (2.8) | 2.94 |
| **Drowsiness (sleepy)** | 3.28 (2.6) | 2.97 (2.6) | 3.17 (2.9) | 3.76 (3.1) | 3.29 |
| **Dry mouth** | 1.97 (3) | 1.38 (2.3) | 2.17 (3) | 2.41 (3.1) | 1.98 |
| **Sadness** | 2.31 (2.8) | 1.97 (2.6) | 1.97 (2.5) | 2.24 (2.7) | 2.12 |
| **Vomiting** | 1.07 (2.5) | 0.76 (1.8) | 1 (2.05) | 1.1 (2.3) | 0.98 |
| **Numbness** | 1.17 (2) | 0.48 (1.1) | 0.72 (1.7) | 0.93 (2) | 0.82 |

In sunitinib-treated patients (n = 18), the study results reveal that fatigue (4.05), dry mouth (3.48), drowsiness (3.45), lack of appetite (2.5) and sleep disturbance (2.38) were rated as the five most severe symptoms. On the other hand, in pazopanib-

treated patients (n = 29), fatigue (3.9), drowsiness (3.29), lack of appetite (2.94), disturbance of sleep (2.67) and pain (2.43) were rated as the five most severe symptoms.

#### 2.3.6.2 Evaluating the correlation between mean fatigue score (FACIT-F) and cancer symptoms (MDASI)

To achieve another objective of our research, we evaluated the correlation between the mean fatigue scores that had been assessed by the FACIT-F tool and thirteen cancer symptoms that were assessed by the MDASI tool for all recruited patients using Pearson correlation tests, after approval from the statistician consultant.

**Table 21: Pearson correlation test between mean fatigue score (FACIT-F tool) and cancer symptoms (MDASI tool).**

| Symptoms | Mean | SD | Pearson Correlation (r) | p-value |
|---|---|---|---|---|
| **Pain** | 2.36 | 2.3 | - 0.35 | $p < 0.01$ |
| **Fatigue** | 4.39 | 2.5 | - 0.821 | $p < 0.001$ |
| **Nausea** | 2.00 | 2.3 | - 0.6 | $p < 0.001$ |
| **Disturbance of sleep** | 2.58 | 2.5 | - 0.5 | $p < 0.001$ |
| **Distressed (Upset)** | 1.97 | 2.1 | - 0.6 | $p < 0.001$ |
| **Shortness of breath** | 2.19 | 2.2 | - 0.57 | $p < 0.001$ |
| **Difficulty remembering** | 1.58 | 2.1 | - 0.5 | $p < 0.001$ |
| **Lack of appetite** | 3.18 | 2.7 | - 0.69 | $p < 0.001$ |
| **Drowsiness (Sleepy)** | 3.69 | 2.7 | - 0.74 | $p < 0.001$ |
| **Dry mouth** | 2.76 | 3 | - 0.55 | $p < 0.001$ |

| Symptoms | Mean | SD | Pearson Correlation (r) | p-value |
|---|---|---|---|---|
| **Sadness** | 1.9 | 2.4 | - 0.53 | $p < 0.001$ |
| **Vomiting** | 1.25 | 2.2 | - 0.53 | $p < 0.001$ |
| **Numbness** | 1.16 | 1.7 | - 0.43 | $p < 0.001$ |

A Pearson product-moment correlation coefficient was computed to assess the relationship between 13 cancer symptoms (MDASI tool) and mean fatigue score (FACIT-F tool). As shown in Table 21, the results highlight that all of the 13 cancer symptoms had a significant positive correlation ($p < 0.001$) with the mean fatigue score. Therefore, it could be said that the severity of fatigue rose when patients' cancer symptoms worsen.

### 2.3.7 Evaluating the influence of pazopanib or sunitinib on renal cancer patients' lifestyle based on the MDASI tool

The second available section in the MDSAI tool is the "reactive" dimension of symptoms (interference with daily function). This section answered the last part of the secondary research questions: what is the impact of pazopanib or sunitinib on the quality of life of a renal cancer patient? This part of the tool assessed the interference of TKI agents on six items: general activity, work around the house, walking, mood, relationship with other people and enjoyment of life. The first three items could be called activity items and the other three called mood items. Our results demonstrated that the influence of pazopanib or sunitinib on the lifestyles

was a mild on both, activity items (3.8 out of 10) and mood items (2.55 out of 10) in the daily life of patients (98).

#### 2.3.7.1 Evaluating the interference of sunitinib and pazopanib in patients' daily activity

The first three activity items (general activity, work around the house and walking) were assessed by the MDASI tool using a Split Plot ANOVA test, after discussion with the statistician consultant, to differentiate between the two drug treatment groups: patients received sunitinib or pazopanib, and examining these parameters in cycles 1, 2, 3 and 4. The mean scores ± SD of daily activity items for the sunitinib and pazopanib groups are shown in Table 22.

**Table 22: Mean activity scores with standard deviation for sunitinib and pazopanib groups of patients.**

| Cycles | Drug | Mean | SD | Number of patients |
|---|---|---|---|---|
| **Cycle 1** | **Sunitinib** | 3.1 | 2 | 18 |
| | **Pazopanib** | 4.1 | 2.8 | 29 |
| | **Total** | 3.7 | 2.8 | 47 |
| **Cycle 2** | **Sunitinib** | 2.8 | 2.5 | 18 |
| | **Pazopanib** | 4.2 | 2.8 | 29 |
| | **Total** | 3.7 | 2.8 | 47 |
| **Cycle 3** | **Sunitinib** | 4 | 2.8 | 18 |
| | **Pazopanib** | 4.1 | 3 | 29 |
| | **Total** | 4.1 | 2.9 | 47 |
| **Cycle 4** | **Sunitinib** | 3.3 | 3 | 18 |
| | **Pazopanib** | 4.2 | 2.9 | 29 |
| | **Total** | 3.8 | 2.9 | 47 |

Table 22 shows that mean score of patients in the pazopanib group is higher than patients in the sunitinib group in any given cycle. However, as shown in Table 23, the statistical test comparing the means of these two groups achieved a p-value of

greater than 0.05 (p = 0.264). Therefore, there is no statistically significant difference in measured daily activity items between patients taking TKI agents sunitinib or pazopanib, in any given cycle.

**Table 23: Split-Plot ANOVA test comparing activity score between subjects: sunitinib and pazopanib groups.**

| Source (between subjects) | df | Significance |
|---|---|---|
| **Intercept** | 1 | 0.000 |
| **Type of TKI** | 1 | 0.264 |
| **Error** | 45 | |

df: degrees of freedom.

#### 2.3.7.2 Evaluating the interference of sunitinib and pazopanib in patients' daily mood

Three questions were considered to be mood related items: mood, relationship with people and enjoyment of life. These were assessed by the MDASI tool and analysed using a split plot ANOVA, after discussion with the statistician consultant, to differentiate between two groups: patients receiving sunitinib and those receiving pazopanib in cycles 1, 2, 3 and 4. The mean score ± SD of mood activity items for the sunitinib and pazopanib patient groups are shown in Table 24.

**Table 24: Mean mood score with standard deviation for sunitinib and pazopanib groups of patients.**

| Cycles | Drug | Mean | SD | Number of patients |
|---|---|---|---|---|
| **Cycle 1** | **Sunitinib** | 1.6 | 2.1 | 18 |
| | **Pazopanib** | 3.2 | 2.6 | 29 |
| | **Total** | 2.6 | 2.5 | 47 |
| **Cycle 2** | **Sunitinib** | 1.7 | 1.7 | 18 |
| | **Pazopanib** | 2.8 | 2.3 | 29 |
| | **Total** | 2.4 | 2.1 | 47 |
| **Cycle 3** | **Sunitinib** | 2.5 | 2.1 | 18 |
| | **Pazopanib** | 2.7 | 2.5 | 29 |
| | **Total** | 2.6 | 2.4 | 47 |
| **Cycle 4** | **Sunitinib** | 1.9 | 1.9 | 18 |
| | **Pazopanib** | 3 | 2.5 | 29 |
| | **Total** | 2.6 | 2.3 | 47 |

Table 24 shows that the mean score of the pazopanib group was higher than the sunitinib group in any given cycle. However, as shown in Table 25, the p-value achieved when comparing these two groups statistically was more than 0.05 ($p = 0.105$). Therefore, there is no statistically significant difference in the scores of the measured daily activity items between patient groups receiving TKI agents sunitinib and pazopanib, in any given cycle.

**Table 25: Split plot ANOVA test comparing mood score between subjects: sunitinib and pazopanib patient groups.**

| Source (Between subjects) | df | Significance |
|---|---|---|
| **Intercept** | 1 | 0.000 |
| **Type of TKI** | 1 | 0.105 |
| **Error** | 45 | |

df: degrees of freedom.

## 2.4 Discussion

The growing incidence of cancer coupled with an improvement in survival rates has led to a huge challenge for clinical care teams to identify and manage treatment-related events and maximise quality of life and functional status of cancer patients who are receiving long-term therapies or post-treatment follow-up (101).

Previous studies (34, 48) have confirmed that fatigue is one of the most common side effects of use of the TKI sunitinib and pazopanib, which are recommended first-line targeted therapies for the treatment of metastatic renal cell carcinoma. Therapy-related fatigue has reportedly in some cases impacted upon patients' daily life to such an extent that it has led to patients discontinuing therapy or having treatment breaks. This in turn could lead to disease progression and compromised survival.

The present research was designed to better understand the prevalence and incidence of cancer-related fatigue and its impact on renal cancer patients who are receiving sunitinib or pazopanib over longer time frames than that characterised by earlier studies (34, 48). This study also attempted to examine the confounding factors that might be associated with cancer-related fatigue and measure the impact of sunitinib and pazopanib on patients' daily life. Therefore, it was hoped that the outcome of the study would help the clinical healthcare team to more effectively use sunitinib and pazopanib.

Renal clinics were held twice a week: Monday morning and Thursday afternoon. At the first meeting with the patient, the researcher gave the patient a brief, verbal summary of the study and the reasons for doing it, and possible benefits were described. If patients expressed an interest, they were provided with a patient information leaflet (see Appendix 6) and a consent form (see Appendix 7) to complete if they wished to enter the study. Once they had consented, study participants were asked to complete the 10-minute questionnaires (the FACIT-F and MDASI tools), in a separate room whilst visiting the out-patient clinic which typically lasted 60 minutes.

As described in section 2.3, from January 2015 to February 2016, a total of 70 eligible patients were enrolled in the renal clinic at the Beatson West of Scotland Cancer Centre in Glasgow, United Kingdom. Sixty-five patients agreed to join the study by signing the consent form and 47 participants completed a consecutive four cycles of treatment (Figure 6).

### 2.4.1 Compatibility of patients' characteristics with other studies

Our study included sixty-five patients with metastatic renal cell carcinoma who were treated at the Beatson West of Scotland Cancer Centre, United Kingdom from January 2015 to February 2016. The median patient age was 63.0 years (range 38-83 years), and 42 of them (65%) were ≥ 60 years old. Forty-four were male (68%) while 21 (32%) of the patients were female. This supports the conclusion that renal cell carcinoma is predominantly a disease of older males which is comparable with the latest statistical report from Cancer Research UK in 2016, which showed that

almost 50% of newly diagnosed patients with kidney cancer were males over the age of 60 (4).

Forty-eight patients (74%) were diagnosed with clear cell carcinoma while seventeen (26%) were diagnosed with non-clear cell carcinoma. This is similar to the reported worldwide incidence of clear cell carcinoma (range from 75 to 85% of renal cancer patients (1), and within a clinical trial (102), which showed 80.2% of renal cancer patients are diagnosed with clear cell carcinoma). Similarly, the PISCES trial discussed in Chapter 1 showed a clear cell diagnosis in participants in 87% and 93% of both groups examined (48). Forty patients in our study (62%) had nephrectomy surgery before being diagnosed with metastatic renal cancer and starting sunitinib or pazopanib compared with 83% in the COMPARZ trial and in 85% and 92% of the two groups in the PISCES trial (34, 48). More than half of the patients (54%) in our trial had one metastatic organ affected, with lungs being the most common (55% of patients with metastasis), and again that is compatible with COMPARZ trial findings, showing that the lung was the most common organ to have metastatic deposits. Our result showed that metastatic spread in more than one organ was at a lower rate (54%) than that of the COMPARZ trial, which had 80% of patients experiencing metastases in more than one organ (34).

### 2.4.2 Incidence and severity of fatigue in renal cancer patients

Previous studies proposed a cut-off point in the results obtained by the FACIT-F tool at which cancer-related fatigue in cancer patients can be diagnosed. A clinical study by van Belle et al. (103) examined the validation of the use of the proposed International Statistical Classification of Disease Related Health Problems (10th version) (ICD-10) Criteria for Fatigue through comparison with the FACIT-F subscale and three visual analogue scales to assess fatigue in 834 various types of cancer patients receiving different anticancer treatments regimens in Belgium (103). The ICD-10 criteria are proposed diagnostic criteria for fatigue in cancer patients. A marked decrease in FACIT-F score and visual analogue scale (VAS) score in patients diagnosed with ICD-10 (20 ± 9 vs. 39 ± 8), VAS (34 ± 21 vs. 61 ± 21) was found. This Belgian study also identified a score of < 34 on the FACIT-F subscale as a proposed cut-off point, meaning that mean scores below this value indicate a diagnosis of fatigue (103). Another study, Alexander et al. (104), identified the optimal cut-off for the FACIT-F subscale as < 36. The object of this trial was to diagnose fatigue in two hundred female breast cancer survivors by interview using two questionnaires: The Bidimensional Fatigue Scale (BFS) and the FACIT-F subscale (104). We decided to use the cut-off point < 34 rather than < 36, because the study by van Belle et al. (103) was conducted in a higher number of cancer patients receiving different anticancer treatments regimens than the study by Alexander et al. (104).

Our findings showed that the mean fatigue score of the renal cancer patients participating in our study and receiving sunitinib or pazopanib was less than the

proposed cut-off point of < 34, in the range of 29.5 to 31.8, in four consecutive treatment cycles (see Table 9, section 2.3.2). More specifically, 29 out of 47 participants who completed four consecutive treatment cycles had a fatigue score of < 34, which means 61.7% of our participants could be diagnosed with fatigue.

Thus, it could be concluded that our patients diagnosed with renal cancer and receiving sunitinib or pazopanib are most likely to be diagnosed with fatigue at the time of cancer management. This finding supports the aims of our research to better understand fatigue in renal cancer patients in order to find ways of increasing the tolerability of target agents, sunitinib or pazopanib.

### 2.4.3 Comparison of fatigue scores in our research with fatigue scores in different clinical studies

Fatigue as a side effect has been evaluated in patients diagnosed with different diseases and conditions. Table 26 shows several studies measuring fatigue via the FACIT-F tool in order to compare them with the fatigue score recorded in our study.

**Table 26: Mean fatigue scores (± SD) that were measured using FACIT-F in previous studies and the current research.**

| Source (Reference number) | Group | Mean FACIT-F scale score (± SD) |
|---|---|---|
| **Cella et al. (105)** | General population | 43.6 (± 9.4) |
| | Cancer patients with anaemia (chemotherapy) | 23.9 (± 12.6) |
| | Cancer patients without anaemia (at baseline) | 40.0 (± 9.8) |
| **Tinsley et al. (106)** | Patients with inflammatory bowel disease (IBD) | 38.9 (± 11.0) |
| **Chandran et al. (107)** | Patients with psoriatic arthritis | 35.8 (± 12.4) |
| **Data collected as part of this thesis** | RCC patients treated with pazopanib | 30.2 (± 12.6) |
| | RCC patients treated with sunitinib | 31.5 (± 14.7) |

A clinical study by Cella et al. evaluated fatigue in three groups: healthy people, cancer patients with anaemia, and cancer patients without anaemia. The responses of the group of healthy people were collected through telephone interviews using random digit dialling (105). The study found that the mean score of fatigue was 43.6 in the healthy group, higher than the diagnostic cut-off point identified for diagnosing fatigue using the FACIT-F tool, and explicitly no clinical fatigue (5).

Inclusion criteria for the second group (cancer patients with anaemia) were as follows: they had to have a non-myeloid malignancy and a haemoglobin level less than or equal to 11.0 g/dL, and they had to be receiving concomitant chemotherapy. These patients were identified as having a mean fatigue score of 23.9, rendering these patients clinically and explicitly diagnosed as suffering from fatigue (105). The third group contained cancer patients without anaemia, and to be eligible in this group, patients had to have a cancer diagnosis and no prior chemotherapy or radiation therapy within the previous six months. All baseline assessments were conducted on the first day of chemotherapy with haemoglobin levels > 12 g/dL. The mean fatigue score for cancer patients without anaemia was 40.0, which was not considered a diagnosis of cancer-related fatigue. This study enrolled cancer patients with different stages of disease or patients who had not used any anticancer treatment for six months and who also had not yet started a new regimen of chemotherapy (105).

A clinical study by Tinsley et al. aimed to determine the reliability and validity of the FACIT-F scale in inflammatory bowel disease (IBD) and measured the severity of fatigue in that patient population, because no instrument to measure fatigue had yet been validated in IBD patients (106). Two-hundred and nine patients with IBD completed the 13 items of the FACIT-F (6). Inflammatory bowel diseases are chronic inflammatory conditions of the gastrointestinal tract and are comprised of two major forms: Crohn's disease (CD) and ulcerative colitis (UC). This study identified that the mean fatigue score was 38.9 overall (the score for patients with Crohn's disease was 38.6 and for patients with ulcerative colitis was 39.4) (106). Fatigue is

also an important symptom in psoriatic arthritis (PsA). The third study, by Chandran et al., aimed to determine the reliability and validity of the FACIT-F scale in PsA and examined 135 consecutive patients attending the PsA clinic. This study found that the mean fatigue score in these patients was 35.8 (107).

In conclusion, cancer patients, regardless of the type of cancer, showed a high level of fatigue (less than the cut-off point of 34, which diagnoses fatigue) compared with patients with other diseases or conditions. Psoriatic arthritis and inflammatory bowel disease patients had a fatigue score higher than the cut-off point, which could indicate that these patients do not generally suffer from clinical fatigue. The renal cancer patients treated with TKI agents in our study had less severe fatigue compared with cancer patients treated with chemotherapy in the study by Cella et al., who had a mean score of 23.9 (105).

### 2.4.4 Comparison of fatigue scores of patients receiving sunitinib with patients receiving pazopanib

From literature review, the researcher identified a previous study, the PISCES trial (48), which compared the effects of sunitinib and pazopanib, and measured fatigue scores in week four in each cycle for both agents. However, our research measured the fatigue score at the end of each cycle, at week six. Therefore, we also compared the effects of sunitinib and pazopanib medications. The PISCES trial was a randomised double-blind cross-over patient preference study of pazopanib versus sunitinib therapy in 169 treatment-naïve locally advanced or metastatic renal cell carcinoma patients (48). Participants in this study had metastatic RCC and were

randomly assigned to 50 mg sunitinib per day (four weeks on, two weeks off, four weeks on), and then 800 mg pazopanib per day for 10 weeks, or the reverse sequence of administration. The end point for this trial was patient preference for a specific treatment as assessed by questionnaires at the end of the two treatment periods. The mean fatigue score, measured by the FACIT-F tool, for the sunitinib treatment period was 35.6, and 38.1 for pazopanib. This study concluded that patients favoured pazopanib for various reasons—mainly less fatigue and better overall quality of life—and patients who preferred sunitinib most commonly cited less diarrhoea (48).

In consideration of our results we needed to compare our findings with the PISCES trial which had the same target patients, metastatic renal cancer patients receiving sunitinib or pazopanib, but a different study design. In our study, twenty-nine RCC patients received pazopanib and eighteen patients received sunitinib for four consecutive treatment cycles. After completing four consecutive cycles, the mean fatigue score for the sunitinib group was 31.5 (± 14.7) and for the pazopanib group was 30.2 (± 12.6). Unlike the PISCES trial, we found no statistically significant difference in fatigue scores of patients taking these two agents. Our study also showed that there was no statistically significant difference between the four different cycles ($p > 0.05$; see section 2.3.2).

One potential reason of the lack of a differential in our study could be the time of measurement of fatigue. In our study we measured fatigue in the sunitinib group using the FACIT-F tool at the end of cycle six, after two holiday weeks, compared

with the PISCES trial, which measured fatigue at week four, before the two holiday weeks. The end of week four might be the peak of fatigue for patients receiving sunitinib. Therefore, the sunitinib group of patients in our research demonstrated a lower severity of fatigue compared with the pazopanib group. On the other hand, the PISCES trial showed that sunitinib patients experienced more severe side effects than those in the pazopanib group.

Another difference between our study and the PISCES trial is that mean fatigue was measured in the PISCES trial for two consecutive cycles of treatment, while in our study, mean fatigue was measured for four consecutive cycles. A longer duration of receiving a drug might lead to a more accurate assessment of fatigue. Finally, differences between the findings of our research and those of PISCES trial might be related to different study designs. Patients in the PISCES trial were treatment naïve, compared with the participants in the current study who had received systemic or target agents before enrolling into our research.

### 2.4.5 Fatigue score through treatment time

Because we enrolled patients in the study who were at different stages in treatment cycles, we stratified participants into groups based on the durations of time that treatment was received rather than utilising a baseline such as in previous studies like PISCES and COMPARZ. Patients were therefore stratified into four groups based on treatment periods: 0 – 12 months' treatment duration (year 1), > 12 months but < 24 months (year 2), > 24 months but < 36 months (year 3) and greater than 36 months (year 4). Out of 47 patients who completed questionnaires for four consecutive treatment cycles, twenty-six patients enrolled in the study at year 1 of receiving medication, ten patients at year 2, seven patients at year 3 and four patients at year 4, as described in section 2.3.2.1. Our results showed a similarity in the mean and standard deviation of the fatigue score seen in the first two years for both sunitinib and pazopanib treatment groups (Figure 7). However, at the third year of treatment, we did identify that there was a deviation between these two groups. However, the small sample size meant that it could not be determined whether there was significant difference between the groups, so no conclusions could be made based on this difference.

Four patients were receiving sunitinib in year four, compared with zero patients receiving pazopanib which was because of the later market entry and introduction to clinic practice of pazopanib. Sunitinib was UK market authorized in July 2006, and introduced to clinical practice in April 2009. On the other hand, pazopanib was UK market authorized in June 2010, and introduced to clinical practice in March 2011.

Furthermore, the higher number of recruited participants in years 1 and 2 who were taking sunitinib or pazopanib affected the mean fatigue score, and resulted in greater standard deviations in both groups. The difference in fatigue score in year 3 between sunitinib and pazopanib should be interpreted with caution due to the small numbers involved. Further work in a larger number of patients, which would lead to improved statistical power is warranted.

### 2.4.6 The influence of clinical and laboratory variables on fatigue scores

The causes of cancer-related fatigue are not fully understood, but a variety of factors are believed to contribute to or affect the degree of fatigue experienced by individual renal cancer patients. Contributing factors such as age, physiological conditions (e.g. anaemia), and psychological factors (e.g. depression) have been documented to play a role. In our study, we therefore stratified our patients to examine if there was any influence by either clinical or laboratory variables on fatigue score for renal cancer patients receiving sunitinib or pazopanib. The data were collected at baseline and during the next three consecutive cycles by collection form and then statistically analysed using independent t-tests. However, our results showed that none of the clinical and laboratory variables that were examined significantly influenced the fatigue scores of renal cancer patients ($p > 0.05$), except for lactate dehydrogenase levels at baseline. Four patients, with lactate dehydrogenase levels of > 360 U/L, had a significantly more severe fatigue score of 16.7 compared with 61 patients whose lactate dehydrogenase levels at baseline were ≤ 360 U/L ($p = 0.034$). However, only four patients had lactate

dehydrogenase levels of > 360 U/L, and one of those patients died, and another patient had his medication stopped by the consultant because his disease progressed. Therefore, the mean fatigue scores of patients with lactate dehydrogenase levels at baseline ≤ 360 U/L are more reliable, with a sample size of 61. It is therefore not possible to say with any certainty that lactate dehydrogenase has a direct influence on the fatigue scores of renal cancer patients. This could however be further interrogated if future studies are undertaken with a larger sample size.

Our results (Table 17) showed that some patient categories, such as female patients or non-clear cell carcinoma patients, experienced more severe fatigue than other categories but this was not statistically significant ($p > 0.05$). Therefore, in routine care, these issues do not appear to be a major concern. In conclusion, fatigue in those patients was not directly influenced by clinical and laboratory variables that have been examined in renal cancer patients receiving sunitinib or pazopanib. There have been no previous clinical studies examining the influence of these variables on fatigue score with which to compare our study.

Our results did not appear to be compatible with the hypothesis in the literature that was described in section 2.1.2, that age is a contributing factor to cancer-related fatigue. A clinical study examined cancer patients with different tumours during outpatient treatment with chemotherapy or pamidronate at the H. Lee Moffitt Cancer Centre in the United States (66). They examined 76 varieties of cancer in patients that were over 60 years of age at the time of the study. Fatigue

was assessed using a Fatigue Symptom Inventory, which is a self-report measurement tool, designed to assess the severity, frequency, and daily pattern of fatigue as well as its perceived interference with quality of life. The study reported that 72% of these patients reported fatigue at the time of assessment. However, our results did not demonstrate a significant difference in fatigue score between patients < 60 and patients ≥ 60 years old ($p > 0.05$).

In addition, it has been hypothesised that several physiological factors are thought to contribute to fatigue, including anaemia and hypothyroidism (59). A previous study reported that haemoglobin function is altered in cancer patients, often in response to neoplastic disease and cancer treatments, which change the membrane transport characteristics of erythrocytes through changes in potassium and chloride levels and decreased red blood cells, leading to less oxygen transportation and contributing to fatigue as a consequence (59). However, our results demonstrated that patients had an abnormal level of haemoglobin at baseline, leading to more severe fatigue but no statistically significant differences to those patients with normal haemoglobin ($p > 0.05$).

Hypothyroidism, which often manifests itself with symptoms of fatigue, is one of the most common side effects of treatment with TKI agents, specifically sunitinib and pazopanib (34). Our results demonstrated that this variable does not directly correlate with exacerbated fatigue severity. Prognostic survival criteria like Motzer and Heng, history of cytokine therapy such interferon-alpha and interleukine-2, history of nephrectomy, having comorbidities or receiving concurrent medication(s)

at baseline and for next three consecutive cycles were examined as well. However, none of these variables were shown to directly influence or exacerbate fatigue symptoms in renal cancer patients receiving sunitinib or pazopanib. Therefore, more investigation is recommending in future work for a large number of patients and with different measurement tools in order to be able to accept or reject these hypotheses.

### 2.4.7 Evaluating the score of prognostic survival models, Motzer and Heng.

The Motzer and Heng criteria are two survival prediction models based on clinical and laboratory data in metastatic renal cell carcinoma. The rationale for selecting these models was discussed in section 2.2.7.3. Motzer criteria were derived from 463 mRCC patients treated with previous standard therapy, immunotherapy and interferon-alpha (95). Heng and his colleagues examined 645 mRCC patients treated with the latest group of therapy: target therapy, sunitinib, sorafenib or bevacizumab agents (96). However, the renal clinic at the Beatson West of Scotland Cancer Centre still used Motzer criteria as a prognostic survival model at baseline for newly diagnosed patients.

The recruited participants in our study had a mean Motzer score of 1.29 and a mean Heng score of 1.28 at baseline. Both scores placed patients in an intermediate risk group; Motzer predicts 10 months survival and Heng predicts 22.5 months survival based on these scores. Therefore, we conclude that the Heng model is more appropriate to use at the renal clinic because most diagnosed patients were treated

with targeted therapy and were categorised in the same risk group as the Motzer score, but predicts more survival months.

### 2.4.8 Cancer symptoms experienced and correlated with fatigue score

The MDASI is a multi-symptom, patient-reported outcome measure for clinical and research use. The MDASI includes items that report the "sensory" dimension of symptoms and the "reactive" dimension of symptoms (interference with daily function). Firstly, in our study the sensory dimension of symptoms was assessed for the 13 core MDASI symptom items (pain, fatigue, nausea, disturbed sleep, distress, shortness of breath, lack of appetite, drowsiness, dry mouth, sadness, vomiting, difficulty remembering, and numbness or tingling).

Descriptive statistics of the 13 symptoms of the MDASI scale for renal cancer patients are shown in Tables 20. The most common symptoms that patients experienced during the research were fatigue, drowsiness, lack of appetite, and disturbed sleep in both groups, and dry mouth, which appeared more in the sunitinib group, and pain, which appeared more in the pazopanib group. There was a highly positive correlation between these 13 cancer symptoms (measured by MDASI) and fatigue score (measured by FACIT-F) ($p < 0.01$; see Table 19). This positive correlation means that severity of fatigue score increases when cancer symptoms increases. This could indicate that the severity of 13 cancer symptoms are worsen when cancer-related fatigue increases in renal cancer patients receiving sunitinib or pazopanib.

This conclusion is comparable with a number of previous studies that found an association between the level of symptom distress experienced by cancer patients and the increased severity of fatigue reported (108 - 111). These studies were, however, conducted with participants diagnosed with different types of cancer (breast and liver cancer) who were receiving different types of anticancer treatments. There have been no previous studies examining cancer symptoms in renal cancer patients receiving TKI agents.

Our results also demonstrated that the most unfavorable symptoms caused by the TKI agent are drowsiness, lack of appetite, disturbed sleep, dry mouth, and pain, as well as diagnostic fatigue which was proven in the first section of our results. Therefore, additional clinical intervention may be necessary to manage the adverse effects and increase the tolerability of gold-standard metastatic renal carcinoma therapies, sunitinib or pazopanib.

### 2.4.9 Comparing sunitinib and pazopanib and their influence on quality of life

The second section in the MDASI tool focuses on the "reactive" dimension of symptoms (interference with daily functions). It measures interference based on six items: general activity, work around the house, walking, mood, relationships with other people, and enjoyment of life. The first three items are referred to as activity-related items, and the second three are considered mood-related items. In this study, we evaluated the influence of pazopanib or sunitinib on the lifestyles of renal cancer patients and found that these agents had a mild influence on activity (3.8 out of 10) and mood (2.55 out of 10) in the daily life of patients. These are mild categories according to the MDASI user guide (98). Our results also concluded that no statistically significant difference was identified between sunitinib and pazopanib. However, there was a trend which suggested that, compared with sunitinib treatment, pazopanib treatment had a greater influence on the mean activity, mood and quality-of-life scores. Our results were not consistent with the findings of the COMPARZ trial (34), which compared the efficacy and health-related quality of life of sunitinib and pazopanib patients with treatment-naïve renal cancer patients. The COMPARZ trial evaluated the influence of sunitinib or pazopanib on health-related quality of life after conducted the Supplementary Quality of Life Questionnaire (SQLQ) and Cancer Therapy Satisfaction Questionnaire (CTSQ) (34). The COMPARZ study concluded that sunitinib and pazopanib have similar efficacy, but health-related quality of life favored the clinical use of pazopanib. The

favorability of pazopanib over sunitinib was statistically significant in both assessment tools, at $p < 0.05$ (34). The measurement of health-related quality of life in the COMAPRZ trial was measured on day 28 for the first nine cycles of TKI therapy, then on day 42 for the tenth and subsequent cycles. However, the authors reported a limitation regarding this conclusion as the assessment time for health-related quality of life on Day 28 was biased toward the pazopanib agent because it may not capture the recovery of patients in the sunitinib group during the two-week washout period. However, in our study we measured the influence of both treatments on the patients' quality of life after week six, which means this was measured after the washout period in sunitinib group and that might explain the difference between our research and COMPARZ trial. In addition, the differences in the medical histories of the target populations between our patient cohort and the COMPARZ trial cohort may explain this difference in the results between use of sunitinib and pazopanib (34). We suggest that further comparative research is necessary into health-related quality of life, assuming that the efficacy of both first line treatments, sunitinib and pazopanib, are similar.

## 2.5 Summary and introduction to the next chapter

In summary, we examined the mean fatigue score in renal cancer patients over four consecutive treatment cycles with sunitinib or pazopanib, and diagnosed fatigue at scores of < 34. The standard deviations of the mean fatigue scores for enrolled patients was very high. There were no statistically significant differences between the two TKI agents or between patients over the four different cycles ($p > 0.05$). In addition, at the clinic, it was difficult to interpret or distinguish the fatigue scores obtained from those patients.

Our findings also showed that the FACIT-F score in renal cancer patients was not correlated with age, gender, or clinical and laboratory variables that were examined. In addition, there was a significant statistical correlation between cancer symptom distress and severity of fatigue in renal cancer patients. Finally, there was no statistically significant difference between sunitinib and pazopanib treatment in terms of influence on patients' daily quality of life.

These findings lead us to recommend appropriate consultation at the renal clinic by the health care team to provide patients with appropriate advice on how to manage their fatigue symptoms. "Coping with Fatigue" is a pamphlet produced by the patients' information editorial team at Macmillan Cancer Support (MCS), and it is recommended that renal clinics offer copies of this to all patients receiving sunitinib or pazopanib (112). The MCS booklet is approximately 70 pages in length and provides an overview of the causes and effects of fatigue, a series of coping strategies, and a fatigue diary for users to monitor their symptoms. By contrast, the

locally produced information resource is a succinct two-sided sheet that gives patients only the most common tips to help manage their CRF (112).

The results of this part of the study encouraged the researcher to investigate proposed combination therapies using TKI with other treatments such as radiotherapy, as this may enable dose reduction of individual agents such as sunitinib or pazopanib and thereby minimise the incidence of side effects associated with treatment.

# Chapter 3: An evaluation of combination therapies in renal cancer cell lines

## 3.1 Introduction

As described in Chapter 1, the current treatment for renal cancers includes surgery, immunotherapy, and molecular-targeted therapy. Radiotherapy does not play a central role in renal cancer treatment, although it is usually used in renal cancer patients to control pain in those with metastases (M1) (113). Radiotherapy has also been used as an adjuvant following nephrectomy in patients at high risk for local recurrence, but its role in this setting remains unproven and is generally discouraged (114). Therefore, radiotherapy is used mainly as a palliative therapy for renal cancer patients with metastatic tumour growth.

### 3.1.1 The role of radiation therapy in RCC

Renal cell carcinoma (RCC) has historically been considered a radio-resistant tumour, although this is still controversial, with some studies showing good radiation sensitivity and others showing radiation resistance of renal cancer cells (114, 115). Some studies also suggest that radiation is useful for RCC when used preoperatively, and others suggest efficacy when it is used postoperatively and several other studies have suggested the benefit of using stereotactic irradiation in patients where surgery is not applicable, such as patients living with one functioning kidney (116).

#### 3.1.1.1 Preoperative radiation

Researchers have evaluated the use of radiation therapy preoperatively for RCC retrospectively and prospectively. The one prospective randomised study failed to

identify any benefit of radiotherapy before nephrectomy surgery (117). However, in a retrospective study conducted with eight renal cancer patients who received 45 to 50.4 Gy preoperatively, it was shown that with radiotherapy, four of the patients continued to live without disease progression at 15 to 50 months; one patient died, and three developed metastases (118). Overall, the preoperative use of radiotherapy is not a practical protocol in renal cancer management because no clinical benefit has been approved in prospective clinical trials (116).

#### 3.1.1.2 Postoperative radiation

Postoperative radiation following nephrectomy in renal cancer patients has also been evaluated in several studies. The findings have been similar to those related to preoperative radiotherapy but with slightly better outcomes. For example, in a retrospective study of 147 patients, a group of 56 patients were irradiated with a total dose of 46 Gy, and the other group was not irradiated. The five- and ten-year actuarial survival for the irradiated group was 50% and 44% respectively, and for the non-irradiated group, it was 40% and 32%, respectively (119). The study showed a significant toxicity developed in three patients in the irradiated group. Thus, postoperative radiation might increase the survival rate for renal cancer patients but in a narrower manner and with significant toxicity.

A postoperative randomised trial evaluated the role of adjuvant therapy of radiotherapy in stage II and III renal cancer patients (120). A total of 32 patients received radiotherapy as 55 Gy in 2.5 Gy/fractions after surgery, and 33 patients did not receive radiotherapy. The five-year survival rate for the non-irradiated group

was 63% and for the irradiated group 38%, with more significant increased complications in the irradiated group. Therefore, the study concluded that there was no benefit of using radiotherapy after surgery. However, it is important to realise that these studies used a fractional size that was higher than 1.8 to 2.0 Gy, which may have contributed to increased short and long-term toxicities reported.

#### 3.1.1.3 Stereotactic radiation

Stereotactic body radiation therapy (SBRT) is able to deliver high doses of radiation directly to the tumour without affecting normal tissues. This new radiation technique was initially developed to treat brain tumours and has been used increasingly for extracranial sites including the thorax and abdomen.

SBRT in RCC can be applied where surgical procedures are not applicable, such as in patients who have a single functional kidney, bilateral renal cancer, and/or recurrent tumours after conservative surgical resection (116). A systemic review was carried out in 2012 by two radiation oncologist of the role of SBRT in renal cancer patients. A total of ten publications (seven retrospective and three prospective studies) reported a weighted local control of 93.9% with a range from 84% to 100%, in grade 3 or higher adverse events in 3.8% of the patients. A dose of 40 Gy delivered over five fractions is the most common fractional schedule used. In addition, it can be concluded that SBRT for primary RCC can be delivered with promising rates of local control and acceptable toxicity levels (121).

### 3.1.2 Radio-resistant no more

As mentioned previously, renal cell carcinoma has been considered radio-resistant, but in the past decade, evidence has increased that a high dose given in one or a few fractions (stereotactic body radiotherapy) can overcome resistance. However, most of the evidence in favour of stereotactic body radiotherapy for renal cell carcinoma has not been very robust because no randomised trials have been conducted (122).

Despite the consideration hitherto of RCC carcinoma as a radio-resistant tumour, there is significant evidence that using radiotherapy in more beneficial, novel ways may provide a therapeutic advantage in RCC treatment. Low doses of radiotherapy (1.8 to 3.0 Gy) do not necessarily cause tumour cell death when used as a single therapy; higher radiotherapy doses delivered in fractions actually does conversely destroy tumour blood vessels. It has been hypothesised that this mechanism of action of radiotherapy could impact tumour survival *in vivo* as tumours are heavily dependent on blood vessels induced during tumour angiogenesis for access to nutrients and oxygen to support tumour growth and survival and facilitate metastasis (122). It has furthermore been suggested that radiotherapy could be effective in killing tumour cells either indirectly via inhibiting the angiogenic pathway or via the sphingosine kinase pathway (122).

Enhancing the effect of radiation therapy with the use of radiosensitising agents has provided many promising outcomes in various cancers and is still an area of active research. For example, a clinical study evaluated the sensitising effect of the

bisphosphonate zoledronic acid (ZA) in renal cancer cells *in vitro* and found that this agent has a radio-sensitising effect by potentiating the caspase-3 mediated apoptosis pathway in different renal cell lines including 786-O, 498-A and ACHN. Radiation sensitisation was observed independently of its osteoclastic activity by potentiating the caspase-3-mediated apoptosis pathway (123). The signal transducer and activator of transcription 1 (STAT1) was demonstrated to play a key role in this sensitisation. However, the clinical application of radiation sensitisers in RCC is still an area of active research with little clinical application (123).

#### 3.1.2.1 The importance of tumour vasculature in RCC

Renal cell carcinoma is considered to be comprised of highly vascularised tumour cells because of transcriptional or mutation silencing, hypermethylation, and the von Hippel-Lindau tumour suppressor gene which is mutated in about 60% of RCC cases (124). The VHL gene is a tumour-suppressor gene located on the short arm of chromosome 3. The VHL gene encodes a 213–amino acid normal VHL protein (pVHL) that forms a multiprotein complex. This complex binds to subunits of hypoxia-inducible factor (HIF), a transcription factor that permits cell survival and growth under hypoxic conditions (124). In conditions of hypoxia, which is a condition of low oxygen tension, HIF goes through hydroxylation that leads to pVHL binding and subsequent ubiquitination and proteasomal degradation of HIF. However, in terms of hypoxia, hydroxylation is decreased (125).

When HIF-1 α is not subject to proteolysis, it is constitutively activated, and binds to its partner HIF-1 β and forms a HIF complex that then translocates into the nucleus,

leading to increased transcription of hypoxia-inducible genes (124). This causes overexpression of proteins like vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF). Examination of RCC tumour specimens for VEGF (mRNA transcripts or VEGF protein) has demonstrated VEGF overexpression in most tumours (126, 127). PDGF also has important angiogenic effects and might play an important role in blood vessel formation by recruiting and supporting the growth of pericytes (128).

The Raf/MEK/ERK pathway is another essential downstream signal pathway activated by ligand binding with VEGF and PDGF receptor on endothelial cells, and epidermal growth factor receptor (EGFR) on the cancer cells. Constitutive activation of this pathway leads to tumour cell proliferation, differentiation and survival (129). Angiogenesis is the process by which new blood vessels are formed out of pre-existing vessels, and it is considered one of the key hallmarks of cancer. It is essential for tumour growth, and neovascularisation is necessary for tumours for metastasis to occur (Figure 8) (130). As discussed previously, angiogenesis involves multiple stimulatory and inhibitory factors. Among these factors, the most important is the VEGF receptor. VEGF exerts its biological effect through binding with the extracellular domain of transmembrane tyrosine kinase receptors. These receptors are selectively expressed on multiple cells, including vascular endothelial cells (VEGFR-1 and VEGFR-2) and lymphatics (VEGFR-3) (129). VEGF binding contributes to dimerisation and autophosphorylation of these intracellular receptor tyrosine kinases, which further contributes to activation of a cascade of downstream proteins. This is responsible for induction of endothelial cell division,

enhancement of endothelial cell survival and increased microvascular permeability (125). VEGFR-2 appears to be the main receptor responsible for mediating the proangiogenic effects of VEGF.

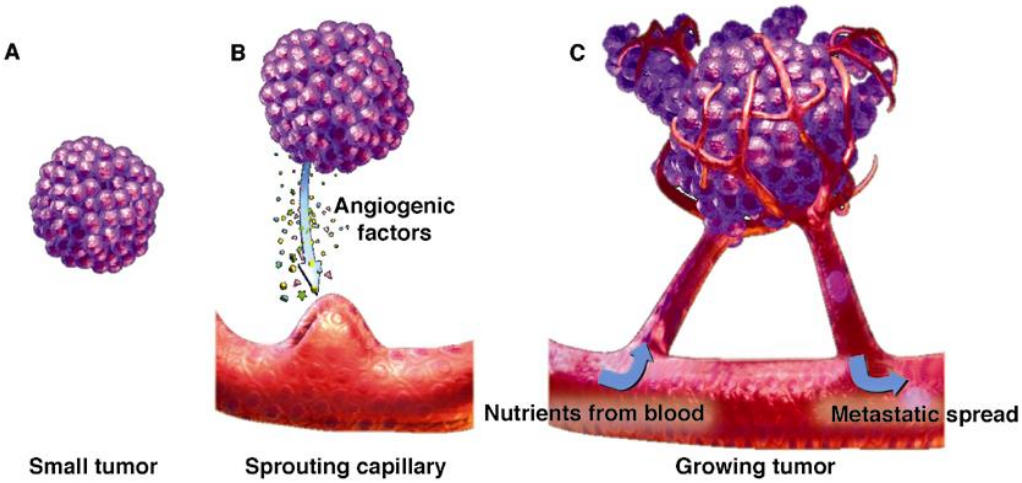


**Figure 8: The development of new vessels in tumour angiogenesis.**

Reproduced from Garrel (130).

### 3.1.3 The role of tyrosine kinase inhibitor (TKI) agents in RCC

The previous section provides compelling evidence that VHL inactivation in most clear cell RCC tumours leads to VEGF and PDGF overexpression and this then drives tumour angiogenesis. Thus, strategies are currently being pursued targeting angiogenesis, including directly targeting VEGF, targeting HIF, and targeting individual ligands that are upregulated in response to HIF and receptors that bind to these ligands, such as the receptor tyrosine kinases VEGFR and PDGF receptor. Another method is to target downstream signalling pathways like the Raf/MEK/ERK

pathway, which is the final common pathway that relays signals from upstream receptor tyrosine kinases (123).

Two of the frequently used angiogenesis inhibitors are sunitinib (Sutent®) and pazopanib (Votrient®), which are described in greater detail in Chapter 1. Sunitinib is a receptor tyrosine kinase inhibitor that targets multiple receptors including vascular endothelial growth factor receptor-1, 2, and 3, platelet-derived growth factor receptor α and β, stem cell growth factor (c-KIT), FMS-like tyrosine kinase receptor 3 (FLT-3), neurotropic factor receptor (RET), and colony-stimulating factor (CSF-1R) (40). The binding of these receptors by the corresponding growth factor normally leads to the activation of multiple signalling pathways that are key in the growth and survival of different cancer cells as well as of endothelial cells, which are the source of new blood vessels. This activation is therefore inhibited by the use of this TKI. Pazopanib is a multi-tyrosine kinase inhibitor; however, as well as the potential indirect anti-angiogenic therapeutic role of both drugs, *in vivo* evidence suggests that these drugs also work directly on tumours, inducing apoptosis directly in tumour cells.

While sunitinib has been explored in the clinical setting mainly for its anti-angiogenic effects, a recent study of tumour biopsies from a gastrointestinal stromal tumour (GIST) patient treated with sunitinib found a marked tumour response in the form of tumour cell necrosis, which was not associated with reduction in tumour vasculature (131). In addition, a further study proved that treatment of renal cancer patients with sunitinib decreased the number of myeloid-

derived suppressor cells (MDSC), increased Th-1 responses, and reduced the number of T regulatory cells in RCC patients (132). However, the underlying mechanism mediating sunitinib-induced tumour cell apoptosis in GIST patients and which reduced immunosuppressive cells in RCC patients remains undetermined (131).

Previous studies have also described the *in vitro* effects of sunitinib on inhibiting the VEGF-induced mitogenic response of human endothelial cells to repel the migration of endothelial cells (133, 134). Sunitinib was also shown to inhibit the ability of human endothelial cells to form capillary-like tubes. Another study also demonstrated that sunitinib induced RCC cell apoptosis and arrested RCC cell growth. The study evaluated sunitinib's effect on RCC cancer cell survival by testing its ability to kill 786-O and RCC4 human RCC cell-lines (135). This study showed that sunitinib could induce tumour cell apoptosis and arrest growth of RCC tumour cells, and that this arrest correlated with signal transducer and activator of transcription-3 (Stat 3) activity inhibition. In conclusion, sunitinib mediated direct effects on RCC tumour cells regardless of VHL tumour suppressor gene status and HIF-2alpha levels. Stat-3 activity reduction led to enhancing the anti-tumour effects of sunitinib, while activated stat-3 rescued tumour cells from death.

In further studies, it was demonstrated that sunitinib could inhibit cell proliferation and induce apoptosis of medulloblastoma tumour cell lines (Daoy) via the activation of caspase-3, which is associated with the inhibition of STAT3 and AKT signalling pathways and their downstream genes involved in tumour cell survival and

proliferation (136). In addition, they concluded that sunitinib increased cleaved caspase-3, the active form of caspase-3, and the active form of PARP levels, in a dose-dependent manner.

Furthermore, an *in vivo* study evaluated the anti-tumour activity of sunitinib using H460 and A431 RCC cell line derived xenograft tumours and proved that a dose of 20 to 80 mg/kg/day of sunitinib caused tumour growth inhibition of 11% to 93% (137). In another study, human glioblastoma xenograft derived cells exposed to sunitinib at a plasma concentration of 50 to 100 ng/ml showed a reduction in density and an increase in apoptosis in micro vessels (138). *In vivo* experiments conducted with sunitinib showed that it could inhibit PDGF receptor phosphorylation and reduce neovascularisation (139). While these results are promising, these drugs as single agents are unlikely to cure cancer alone and will most likely be effective and potentially less toxic in combination with other therapies. Evidence has also suggested that TKI might enhance radiation's anti-tumour effects, and this will be addressed in the following section.

### 3.1.4 Combining radiotherapy with TKI agents

The effects of the TKI agents, sunitinib and pazopanib, on angiogenesis and tumour growth while not extensively studied have been described in previous sections. However, the effects of these agents in combination with radiotherapy have been less well investigated in the literature and have not been fully confirmed. The limited results available indicate that sunitinib enhances the radio response of human prostate cancer cells *in vitro* and *in vivo*; however, the mechanism of this

enhancement may differ in these two model systems (140). There are several reasons why we hypothesise that TKI may enhance radiotherapy.

*In vitro* experiments have shown that using sunitinib in combination with radiotherapy enhances the anti-tumour effect on tumour cell lines, as, generally, many TKI agents including sunitinib inhibit the downstream signalling of growth factor receptors mediated by the PI3K-AKT and ERK pathways (140, 141). The activation of these two pathways occurs frequently in cancer tumour cells and is associated with an increase in the expression of growth factors. In addition, the radioresistance of tumour cells is mediated through the PI3K and ERK pathways. Thus, it was found that TKI agents suppressed the p-ERK pathway and this, when administered in combination with radiotherapy, caused an enhancement effect with respect to cytotoxicity on cancer cells (140). For example, one study concluded that inhibiting the activity of the PI3K-AKT signal pathway is the potential mechanism that may enhance the radiation response by sunitinib (142). Furthermore, another study reported that sunitinib could remodel the tumour cell microenvironment to increase the tumour response to radiation therapy (143).

In the first instance, this refers to the *in vivo* effects on angiogenesis. It has been hypothesised that TKI agents can transiently improve tumour perfusion by normalising the tumour vasculature (144). During this so-called normalisation window, tissue oxygenation is increased, which improves the efficacy of the delivery of drugs into the actual tumour and enhances the efficacy of radiotherapy, which is more effective in oxygenated tissue (144).

#### 3.1.4.1 Preclinical assessment of combining radiotherapy and TKI agents

In a study, sunitinib 100 nM was tested on human prostate cancer cell lines PC3, DU145, and LNCaP *in vitro* in order to evaluate its effectiveness at enhancing the anti-tumour effects of radiation by clonogenic assay (140). This study also evaluated the radio-sensitising effect of sunitinib *in vivo* on tumour xenografts growing in nude mice where the response was assessed by tumour growth delay. The study found that the clonogenic survival of both DU145 and PC3 cells after treatment with 2 Gy and 100 nM sunitinib was reduced from 0.70 and 0.52 in the control to 0.44 and 0.38, with co-treatment with sunitinib respectively. For LNCap prostate cells, radio-sensitisation by sunitinib was not exhibited. It was also demonstrated, however, that combining sunitinib with radiation did not prolong the growth delay of prostate cancer xenografts tumours in the hind limb of nude mice.

Another study examined the *in vitro* effects of sunitinib on the radiation response of endothelial (HUVEC) and breast cancer (MDA-MB-231) cells (143). The authors observed enhanced endothelial cell death as a result of high doses of radiation of 8 and 16 Gy when compared with tumour cells treated in an identical manner, and the administration of sunitinib alone significantly increased HUVEC cell death while having modest additive effects when combined with radiation. In addition, the combination of sunitinib with 8 Gy and 16 Gy radiation also increased tumour cell death compared with tumour cells treated with radiation alone; it was further concluded that sunitinib could enhance tumour radiation response. Finally, the authors found that the clonogenic cell death of tumour cells treated with

combinations of sunitinib and radiotherapy increased in comparison to tumour cells treated with radiation alone, underlining the superior toxicity of the combination therapy.

In another study, the efficacy of combining sunitinib with ionising radiation (IR) on endothelial cells *in vitro* was evaluated (142). Human umbilical-vein endothelial cells (HUVECs) were exposed to IR with or without sunitinib pre-treatment to determine whether sunitinib enhanced the cytotoxic effects of radiation on vascular endothelium. To examine the apoptotic response in apoptosis assays, HUVECs were treated for one hour with or without 1 μM sunitinib before irradiation with 3 Gy. The authors found that a combination therapy of sunitinib and IR significantly increased the number of apoptotic HUVEC cells by 17.6 ± 2.1% ($p < 0.05$) compared with control, sunitinib treatment alone, and IR alone. A clonogenic survival assay showed a significant reduction in colony formation with the combination compared with radiation or sunitinib alone by inhibiting the activity of the PI3K-AKT signal pathway, which may enhance the radiation response by sunitinib.

In addition, another study examined the effects of sunitinib and ionising radiation on human pancreatic adenocarcinoma cell lines. For *in vitro* experiments, human pancreatic adenocarcinoma cell lines were treated with 1 μM sunitinib 1 hour before irradiation (145). Western blot analysis was used to determine the effect of sunitinib on radiation-induced signal transduction. For *in vivo* assays, CAPAN-1 cells were injected into the hind limb of nude mice for tumour volume and proliferation studies. This study showed that sunitinib significantly reduced clonogenic survival

after treatment with radiation ($p < 0.05$) *in vitro* by attenuating radiation-induced phosphorylation of Akt and ERK downstream signalling pathways. Furthermore, their results *in vivo* revealed that sunitinib or radiation when used alone delayed tumour growth; however, when combined, the delay was significantly enhanced. Alternatively, the anti-angiogenic activity of sunitinib may increase tumour hypoxia when administered prior to radiation, thereby decreasing radio-sensitivity and offsetting any radio-sensitising effect of the drug. This possibility is supported by previous reports showing that sunitinib and other angiogenesis-inhibiting agents may enhance the destruction of a tumour's blood vessels during fractionated irradiation.

A study examining the combination of sunitinib and radiotherapy found that this delayed the survival of human umbilical-vein endothelial cells *in vitro* (146). The study found that clonogenic survival decreased and apoptosis increased when sunitinib was used in combination with radiation of up to 6 Gy on endothelial cells. Treatment of cells with sunitinib followed by 6 Gy of radiation demonstrated that the combination had a significantly greater effect than either agent alone ($p < 0.02$) or control cells ($p < 0.001$). The proposed mechanism is that receptor tyrosine kinase antagonists attenuate signalling through viability pathways in tumour vascular endothelium, resulting in enhancement of cytotoxic effects.

After this brief review of previous *in vitro* and *in vivo* studies regarding the combination of sunitinib and radiation, we conclude that sunitinib provides a promising approach with respect to radiosensitisation of RCC cells, which warrants

further investigation. Sunitinib may function as a radio-sensitising agent to increase the effect of radiation on tumour cell lines that are resistant to radiotherapy. This enhancement effect of the combination has been examined in different tumour cell lines but not yet in renal cell lines. Therefore, our study aims to evaluate two TKI agents, sunitinib and pazopanib, alone and in combination with radiotherapy on two different human renal adenocarcinoma cell lines, 786-O and ACHN.

### 3.1.5 Cytotoxicity effect of disulfiram with and without copper on tumour cells

Disulfiram is an inhibitor of aldehyde dehydrogenase and is used for the treatment of alcoholism. In the past few years, several researchers have reported potential anti-cancer properties of disulfiram via the induction of oxidative stress (147, 148), the generation of copper-dependent toxicity (149), and proteasome inhibition (150). Proteasomes are protein complexes inside all eukaryotes and in some bacteria, and their main function is to degrade unneeded proteins by proteolysis. Tumour cells tend to have a higher proliferation rate than normal cells. Therefore, tumour cells are more susceptible to therapies targeting dividing cells, like DNA-damaging agents and anti-angiogenesis agents. As a consequence of the higher metabolic rate, tumour cells have increased oxidative stress compared with normal cells. They are therefore more susceptible to therapy designed to elevate oxidative stress beyond a threshold that will trigger cell death. Oxidative stress is defined as a disturbance between the production of reactive oxygen species (free radicals) and antioxidant defences. In addition, copper content is higher in tumour cells than in

normal cells. As a result, increased oxidative stress, elevated proteasome activity, increased copper levels, and the faster proliferation rate of cancer cells are differences that can be exploited for the targeting of tumour cells over normal cells. Disulfiram is considered an anti-cancer agent due to its ability to interact with multiple components overexpressed in cancer cells and necessary for their survival and metastasis. Disulfiram works in different mechanisms than anticancer agents, via inhibition of proteasome activity and induction of oxidative stress by copper deposition (151).

At present, disulfiram is undergoing a small number of clinical trials for the treatment of various cancer types, including liver, melanoma, lung, and prostate cancers. One of the earlier studies that have explored the cytotoxic effect of disulfiram was conducted in the University of California (148). The authors evaluated the apoptotic effect of disulfiram in human tumour melanoma cell lines c81-46A, c81-61 and c83-2C. The study found that disulfiram at doses of 25 to 50 ng/ml consistently increases apoptosis in these cell lines. The maximum frequency of cell death (the sum of apoptosis and necrosis) ranged from 400% to 600% of the control level, and viability from 28% to 55% of the control.

Further studies evaluated the cytotoxic effect of disulfiram in glioblastoma multiform (GBM) cell lines U251MG, U87MG and U373MG via the MTT cytotoxic assay (152). It was found that disulfiram had a cytotoxic effect on all GBM cell lines in a copper-dependent manner. In addition, they found disulfiram/copper-induced apoptosis in GBM cell lines as detected by annexin staining and flow cytometric

analysis, when compared with cells treated with a single therapy or control cells. In addition, disulfiram had an effect on cancer stem cells, suggesting that disulfiram could target cancer stem cells and reverse resistance to chemotherapy with paclitaxel (PAC), cisplatin (CDDP) and doxorubicin in MDA-MB-$231_{PAC10}$ breast cell lines (153).

The mechanism of action for disulfiram as an anti-cancer agent has not yet been fully elucidated; however, disulfiram-induced cytotoxicity has previously been reported to be mediated by oxidative stress, which may be enhanced by the presence of copper (154). This is further supported by the observation that copper binding drugs have been shown to inhibit its proteasome activity and generate reactive oxygen species (ROS) (154). A potentially significant mechanism of disulfiram-induced cell death involves the inhibition of proteasome activity. Proteasomes degrade unwanted misfolded and superfluous proteins and control many cellular processes involved in differentiation, proliferation, signal transduction, cell cycle progression, and apoptosis, and its activity is increased in cancer cells compared with normal cells. Further underpinning its anti-cancer potential is the fact that disulfiram has been shown to inhibit proteasome activity and induce apoptosis selectively in cancer cells but not in normal cells. The copper-binding activity of disulfiram may also be involved in this mode of action because the formation of organic copper complexes appears to be responsible for pro-apoptotic proteasome inhibition. Other proteasome inhibitors, including the reduced form of disulfiram (diethyldithiocarbamate), have also been shown to have a radio-sensitising effect (154).

### 3.1.6 Combining disulfiram ± copper with radiotherapy

The proteasome is also a key component of the activation pathway of the transcription factor nuclear factor–kappa B (NF-KB) involved in the control of the cell cycle, apoptosis, immune responses, and responses to many other stimuli such as radiation and oxidative stress (151). NF-KB has been demonstrated to be an oxidative stress sensor (155) and is activated in response to radiation to confer radioresistant properties on cells (156, 157). Therefore, the inhibition of the proteasome/NF-KB pathway by disulfiram could explain the radio-sensitising properties of disulfiram.

An *in vitro* study evaluated the cytotoxic effects of disulfiram as a single therapy and combined with copper via clonogenic assay, and examined the radio-sensitising effect of disulfiram in human SK-N-BE neuroblastoma cells and UVW glioma cells (154). The study found that disulfiram as a single agent for treating cells caused a biphasic reduction in the surviving fraction of colonies; less than 4 µM was copper-dependent and more than 10 µM was by oxidative stress. In addition, disulfiram was shown to be a radiosensitiser of human tumour cells in a copper-dependent manner at dose of less than 4 µM of disulfiram.

In summary, as disulfiram has recently been found to have a cytotoxic effect on tumour cell lines, it is the focus of several studies regarding the treatment of liver, lung, and prostate cancers. Our study will also examine the cytotoxic effect of disulfiram and copper in two different renal adenocarcinoma cell lines, 786-O and

ACHN, as well as the radio-sensitising properties of this agent with and without the administration of copper.

### 3.1.7 Aims and objectives

The overall aim of this section of the project is to investigate *in vitro* combination therapies for renal cell carcinoma, which may have improved efficacy and reduced toxicity over current gold standard therapy. The specific aims were to:

- Examine the cytotoxic effect and radio-sensitising properties of TKI agents, sunitinib and pazopanib, as a single therapy and in combination with external beam radiation (XBR) on 786-O and ACHN renal cancer cell lines.
- Examine the mechanism of action of single treatments and combinations via interrogation of the apoptosis activity of 786-O and ACHN cell lines exposed to combination therapy TKI agents, sunitinib and pazopanib, with XBR.
- Examine the cytotoxic effect and radio-sensitising properties of disulfiram/copper as a single therapy and in combination with XBR on 786-O and ACHN renal cancer cell lines.
- Examine the mechanism of action of single treatments and combinations via interrogation of the apoptosis activity of 786-O and ACHN cell line exposed to combination therapy disulfiram ± copper with XBR.

## 3.2 Method and Materials

### 3.2.1 Cell lines

In this study, two different renal cell lines were used: renal cell adenocarcinoma 786-O (ATCC® CRL-1932™) and ACHN (ATCC® CRL1611™). The 786-O cell line (a human renal cell adenocarcinoma) was cultured in Roswell Park Memorial Institute medium (RPMI1640) supplemented with penicillin/streptomycin (100U/ml), Fungizone® (2 µg/ml) and 10% (v/v) foetal calf serum (FCS) (LabTech Int. Ltd, East Sussex, UK). The RPMI-1640 medium was supplemented with 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate, for use in incubators using 5% $CO_2$ in air.

The ACHN cell line (derived from a human renal cell adenocarcinoma) was cultured in Minimum Essential Medium (MEM) (Life Technologies, Paisley) supplemented with penicillin/streptomycin (100 U/ml), Fungizone® (2 µg/ml), 200mM L-glutamine, 10% (v/v), minimum essential medium non-essential amino acids (MEM NEAA), 100 mM sodium pyruvate and 10% (v/v) foetal calf serum (FCS).

Media were obtained from Sigma® (Sigma-Aldrich, Gillingham, UK). Penicillin/streptomycin, Fungizone®, foetal calf serum, sodium pyruvate and MEM NEAA were purchased from Gibco® (Paisley, UK). L-glutamine was purchased from Invitrogen® (Paisley, UK).

### 3.2.2 Culture condition

786-O and ACHN cell lines were cultured in 75 $cm^2$ flasks (Corning B.V, Buckinghamshire, UK) until approximately 80% confluent; medium was then removed and the cells were washed with 4ml of phosphate buffer solution (PBS). Cells were detached by addition of 4ml of 0.05% trypsin (Gibco®, Paisley, UK) and incubation for 5 minutes. Once the cells had detached, 6 ml of fresh media was added to inactivate the trypsin. Various concentrations of cells were then prepared (1:5, 1:10 and 1:20) in three new 75 $cm^2$ flasks containing 20 ml of fresh media to enable continuity of the cell line. The cells were gassed with 5% $CO_2$ and incubated at 37°C in a humidified atmosphere. Stocks of 786-O and ACHN cell lines were P10-18 and passages used in the whole experiments were P12-30.

To freeze cells at -80°C or liquid nitrogen (-196°C), cells were detached from the flasks by the addition of trypsin and following the addition of complete medium (to neutralise the trypsin), were centrifuged at 1500 rpm for 5 minutes and the supernatant removed before the pellets were resuspended in 1 ml of cryoprotectant medium (freezing medium) which was prepared by the addition of 10% DMSO in full medium. The suspension containing the cells were then transferred to labelled cryovials (Thermo Fisher Scientific Inc., Surrey, UK) and stored at -80°C for 24 hrs, before being transferred for storage in liquid nitrogen. To defrost cells for use, vials were warmed in a water bath at 37°C and then immediately transferred to 75 $cm^2$ flasks containing 15 ml medium.

### 3.2.3 Treatments of renal cancer cell lines

#### 3.2.3.1 Treatment of renal cell lines with Tyrosine Kinase Inhibitor (TKI)

Sunitinib (Sutent®, Pfizer) and pazopanib (Votrient®, GlaxoSmithKline) are first line treatments for metastatic renal cell carcinoma. Sunitinib and pazopanib are both orally available tyrosine kinase inhibitor (TKI) primarily of vascular endothelial growth factors receptor VEGFR (1, 2 and 3), platelet derived growth factor receptor PDGFR (α & β) and c-kit growth factors. Both drugs were purchased from Euroasian Chemicals Pvt Ltd. (Mumbai, India).

A 10 mM stock solution was prepared by dissolving drug powder in dimethyl sulfoxide (DMSO) from Sigma (Sigma-Aldrich, Gillingham, UK). The prepared solutions were stored at -20$^{o}$C for both agents. Cell lines 786-O and ACHN were treated with 0-20 µM sunitinib or pazopanib for 24 hours or 48 hours depending on the experimental requirements.

#### 3.2.3.2 Treatment of renal cell lines with external beam radiation (XBR)

For radiosensitisation studies, 786-O and ACHN cells were exposed to external beam radiation (XBR) using a cell irradiation cabinet XRAD 225 (USA) with a 225 keV X-ray beam and dose rate of 2.2 Gray/minute (Gy/min) and current of 13.00mA. For treating cells with radiation as a single therapy, doses of 0 to 4Gy were used. However, a dose range from 0-2 Gy of XBR was used for all combination treatments.

#### 3.2.3.3 Treatment of renal cancer cell lines with disulfiram with/without copper

Clinical trials in the last few years showed that disulfiram has cytotoxic effects on tumor cells. Furthermore, copper when combined with disulfiram showed enhanced cytotoxic effects on cancer cells. A 10 mM stock solution of disulfiram (Fargon Ltd., Newcastle upon Tyne, UK) was prepared in DMSO. A 1 mM copper solution was prepared by dissolving copper dichloride in distilled water. The prepared solutions were stored at 15$^{o}$C for both agents. For treating cells with disulfiram as a single and combination therapy, doses ranging from 0-25 µM were used. Copper was used at a fixed dose of 1 µM in combination therapy. Previous results in the research group had established this as the optimal dose (personal communications).

### 3.2.4 Alamar blue cell viability assay

Alamar blue® dye (Life Technologies, Paisley, UK) is a cell viability and proliferation indicator that is widely used in human and animal cell line cytotoxicity experiments. It contains the dye resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide), a fluorescent blue dye, which when reduced by the mitochondrial NADPH dehydrogenase and NADH dehydrogenase enzymes in live cells only, is converted to the red fluorescent resorufin which can be then quantitatively measured by fluorimetry (158).

#### 3.2.4.1 Alamar blue cell viability assay for the effect of TKI agents, XBR, disulfiram and copper, as single agents

In 25 $cm^2$ flasks, $2x10^5$ 786-O or ACHN cells were cultured in 5 ml of complete medium and incubated at 37°C and 5% $CO_2$. When the cells reached 80% confluence, the medium was removed, the cells washed with PBS and detached using 5 mins incubation with 1 ml of trypsin (0.05%) followed by addition of 2 ml of fresh medium, disaggregated through a 21 G needle and then the cells were enumerated using a haemocytometer. A density of 4000 cells/well cells were then transferred to and cultured in 96 well plates with 100 µl of complete medium. Three replicates were prepared for each experimental condition and incubated at 37°C in 5% $CO_2$ for 48 hours. For each well, medium was replaced with 100 µl of fresh medium containing varying concentrations of sunitinib or pazopanib (0-20 µM), XBR ± 0 to 2Gy, disulfiram (0-25 µM), and copper (1 µM) and incubated at 37°C in 5% $CO_2$. After 24 or 48 hours' incubation with a TKI, the contents of each well was replaced with 100 µl of complete medium containing 10% w/v Alamar blue® and incubated at 37°C and 5% $CO_2$ for 4 hrs. Fluorescence intensity was then measured using a Spectra Max Gemini XS plate reader (CA, USA) with an excitation wavelength of 570 nm and an emission wavelength of 580 nm and processed using SoftMax Pro software (version 4.3).

The results were presented as the mean percentage of cell viability (mean ± SD) compared with untreated control wells of three independent experiments. The results were plotted as % of cell viability against the logarithmic dose, and $IC_{50}$

values were calculated by using non-linear regression curve fitting of cell viability using GraphPadPrism software, version 6.01, 2014 (GraphPad Software Inc, CA, USA).

#### 3.2.4.2 Alamar blue cell viability assay for the effect of TKI agents or disulfiram ± copper, ± XBR

In this experiment, Alamar blue cell viability was carried out to assess the effects of a TKI agent alone and in combination with other agents (disulfiram, copper or XBR) on 786-O and ACHN renal cancer cell lines. A density of 4000 cells were cultured in 96 well plates at 37$^{o}$C in 5% $CO_2$ for 48 hours. At that point, medium was replaced with 100 µl of fresh medium containing a specific concentration of sunitinib or pazopanib (0-20 µM) and incubated for 24 hours at 37$^{o}$C and 5% $CO_2$ followed by exposure to 0 to 2 Gy of XBR. The results were plotted an presented as described in section 3.2.4.1. The same procedure was also performed for disulfiram (0-25 µM) and copper 1 µM doses and cells were incubated for 24 hours followed by exposure to 0 to 2 Gy of XBR.

### 3.2.5 Clonogenic survival assay

The clonogenic survival assay is a cytotoxic assay tool that is used widely to investigate the effect of treatment (chemical or radiation) on mammalian cells. Exposure of cells to toxic agents could affect their normal division and reproduction integrity, which subsequently leads to cell death and therefore inhibits the capacity of cells to further divide and form colonies. However, unaffected cells that retain

the ability to divide and proliferate normally produce large colonies of over 50 cells or more (159).

#### 3.2.5.1 Clonogenic survival assay for the effect of TKI agents as single agents

The clonogenic assay method was conducted as described by Cunningham et al. (160). In brief, 786-O and ACHN cells were cultured in 25 $cm^2$ flasks in 5 ml of complete medium at 37$^oC$ in a 5% $CO_2$ incubator. When incubated cells reached 60-70% confluence, the medium was replaced with 1 ml of fresh medium containing various concentration of TKI agents (0-20 µM) and incubated for 24 hours at 37$^oC$ in 5% $CO_2$. In drug-treated cells, medium was removed and cells were washed with PBS and detached using 1 ml of trypsin (0.05%) followed by addition of 2 ml fresh medium before cells were disaggregated through a 21 G needle and enumerated using a haemocytometer.

Four-hundred cells from each treatment flask were then added to each of three 60 mm Petri dishes containing 5 ml of fresh medium. This triplicate plating was carried out for each experimental condition and control and treatment plates containing the cells were incubated at 37$^oC$ in 5% $CO_2$. Following 10-14 days of incubation, colony formation was assessed to ensure that colonies of over 50 cells had developed in control dishes. The medium was then removed and cells were washed with PBS, fixed with 100% methanol for 10 minutes and stained with 10% Giemsa's stain solution (BDH Laboratory, Dorset, UK) for 10 minutes. The stain was then carefully removed and dishes were rinsed with water and the number of colonies

formed were counted by visual inspection of the dishes. Plating efficiency (PE) and survival fraction (SF) were calculated using the following equations:

(i) PE = number colonies counted / number cells seeded

(ii) Survival fraction = PE treated / PE control.

The experimental results were fitted to a dose-response curve as the mean SF (mean ± SD) versus treatment doses (n = 3 experiments).

### 3.2.6 Caspase-3 activity apoptosis assays

Caspase-3 is known as a marker of early apoptosis activation as a result of DNA damage. It acts by interfering with the normal DNA repair process by breaking down (by proteolysis) the main proteins involved in repair such as poly (ADP-ribose) polymerase (PARP) (161). In this assay, the amino acid sequence site at which PARP is cleaved by caspase-3, DEVD (Asp-Glu-Val-Asp), is occupied by the synthetic tetrapeptide fluorogenic substrate, DEVD-AMC, which contains the amino acid sequence for the PARP cleavage site. Caspase-3 cleaves the tetrapeptide between the DEVD and AMC leading to release of the fluorescent AMC which can be measured by fluorometry (162).

#### 3.2.6.1 Caspase-3 activity apoptosis assays investigating the effect of TKI agents ± radiation

Caspase-3 activity assays were conducted to assess if the TKI agents or disulfiram ± copper induced caspase-3 activity in 786-O and ACHN cells. In 25 $cm^2$ flasks, $2x10^5$

of 786-O or ACHN cells were cultured in 5 ml of complete medium and incubated at 37$^{o}$C in 5% $CO_2$. When cells reached 80% confluence, the medium then was removed and washed with PBS and detached using 1 ml of trypsin (0.05%) followed by 2 mls of fresh medium, disaggregated through a 21 G needle and then counted using a haemocytometer. A density of 4000 cells of 786-O and ACHN cells were cultured in 96 well plates, plating three replicates of each sample, and incubated at 37$^{o}$C in 5% $CO_2$ for 48 hours. For each well, the medium was replaced with 60 µl of fresh medium containing different concentrations of TKI agents (0-5 µM) and ± XBR (2 Gy) and incubated for 24 hours or 4 hours at 37$^{o}$C in 5% $CO_2$. Following incubation, 30 µl of cell lysis buffer containing the Ac-DEVD-AMC caspase-3 fluorogenic substrate mixture were added to each well and plates were incubated for 1 hour. The cell lysis buffer contained 150 mM Hepes, 450 mM NaCl, 150 mM KCl, 30 mM MgCl2, 1.2 mM EGTA, 1.5% Nonidet P40, 0.3% CHAPS and 30% sucrose in distilled $H_2O$, and the pH was adjusted to 7.4. All reagents were purchased from Sigma-Aldrich (Gillingham, UK). Immediately before the assay, the caspase-3 fluorogenic substrate was prepared by adding 30 mM dithiothreitol and 3 mM phenylmethanesulfonyl fluoride to 10 mM DEVD-AMC caspase substrate (BD Bioscience, Oxford, UK).

Fluorescence intensity of free AMC was then determined for each well by using a Spectra Max Gemini XS plate reader (CA, USA) with an excitation and emission wavelength of 360 nm and 460 nm respectively. Data were processed using SoftMax Pro software, version 4.3 (CA, USA). The results were presented as the mean fold increase (mean ± SD) in caspase-3 activity by comparing and normalising

the mean fluorescence intensity for each treated group with the fluorescence intensity of untreated control wells in three independent experiments.

#### 3.2.6.2 Caspase-3 activity apoptosis assays for the effect of disulfiram ± copper ± radiation

These experiments were conducted to test the effect of radiotherapy when combined with TKI agents or disulfiram/copper in order to compare it with single treatment with these agents. A density of 4000 cells of 786-O and ACHN cells were cultured in 96 well plates with three replicates of each sample and incubated at 37$^{o}$C in 5% $CO_2$ for 48 hours. For each well, the medium was replaced with 60 µl of fresh medium containing different concentrations of disulfiram (0-25 µM), ± µM copper and incubated for 24 hours or 4 hours at 37$^{o}$C and 5% $CO_2$ followed by 0-2 Gy of XBR. Following incubation, 30 µl of cell lysis buffer containing the Ac-DEVD-AMC caspase-3 fluorogenic substrate mixture were added to each well and plates were incubated for 1 hour. Plates were read as previously described in section 3.2.6.1.

### 3.2.7 Statistical analysis

All data presented were obtained from at least three independent experiments. All data were statistically analysed using GraphPad Prism software, version 6.0, 2014 (GraphPad Software Inc, CA, USA). The statistical significance of differences between groups was analysed using a one-way analysis of variance (ANOVA)

followed by a Bonferroni post hoc test. A significance level of $p < 5\%$ was selected for all experiments.

## 3.3 Results

### 3.3.1 Clonogenic survival of the 786-O and ACHN cell lines following treatment with sunitinib and pazopanib

Clonogenic survival assays were conducted to evaluate the cytotoxic effect of sunitinib or pazopanib as single agents on clonogenic survival of 786-O and ACHN cells, as described in section 3.2.5. Unfortunately, the effect of sunitinib or pazopanib on the survival fraction of 786-O and ACHN cells following a 24-hours treatment period could not be determined using this assay as the cell lines did not form colonies and were thus unsuitable for this particular assay, as can be seen in Figures 9 A (786-O) and B (ACHN).

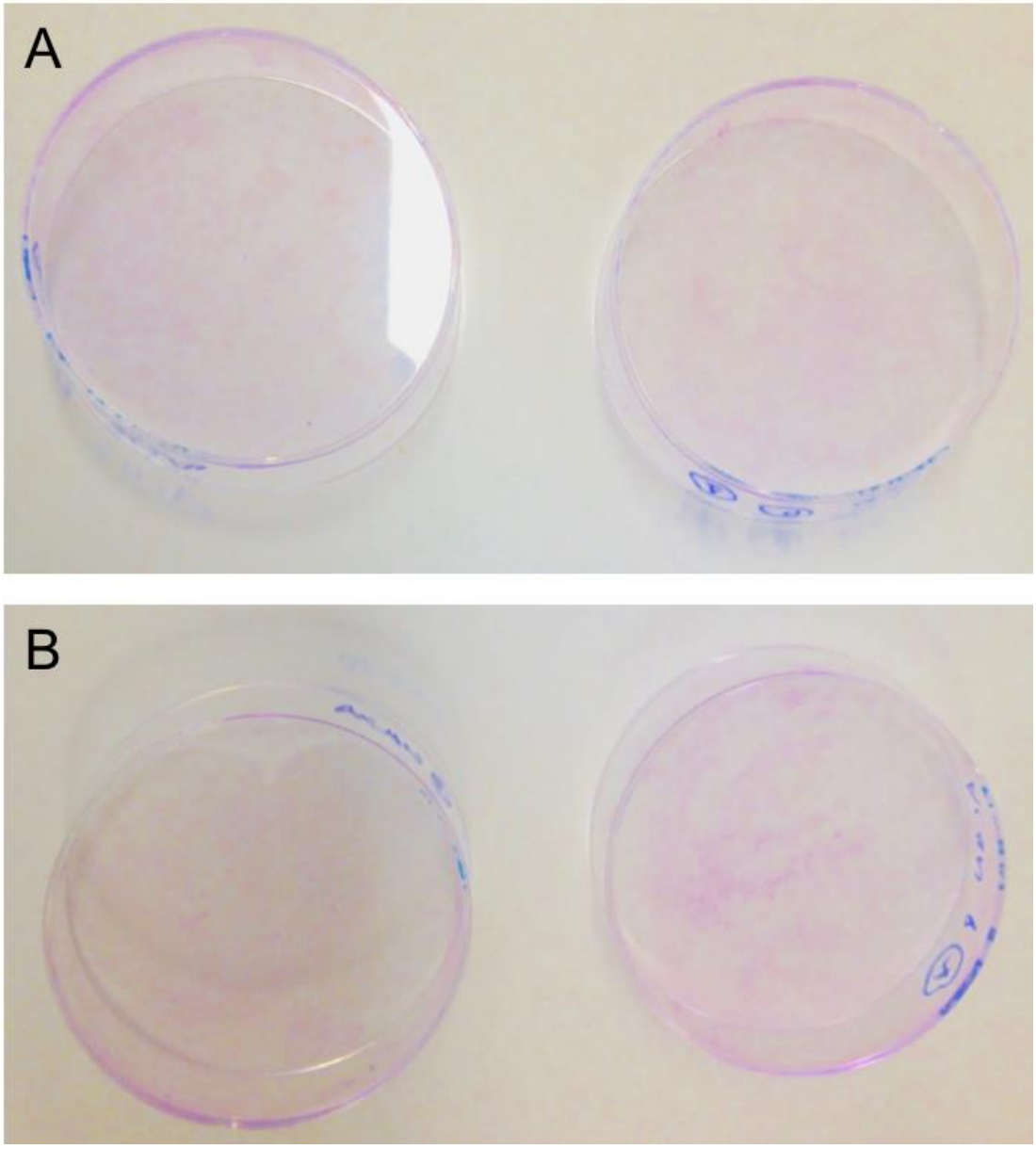


**Figure 9: Representative images of the clonogenic assay results of control 786-O (A) and ACHN (B) cell lines.**

### 3.3.2 Cell viability

#### 3.3.2.1 Cell viability of the 786-O cell line following administration of sunitinib

To evaluate the cytotoxic effect of sunitinib on the cell viability of 786-O, the Alamar blue assay (section 3.2.4) was therefore conducted as an alternative to the clonogenic assay. As is shown in Figure 10 and Table 27, incubation with sunitinib for either 24 or 48 hours reduced cell viability in a dose-dependent manner when compared with control cells ($p < 0.05$). The $IC_{50}$ dose of sunitinib in 786-O cells at 24 hours was 2.45 μM, and 1.02 μM at 48 hours.

The impact of drug incubation times (24 – 48 hours) on cell viability was compared and the results of this comparison are shown in Table 27, which shows that there was a statistically significant difference in cell survival between cells incubated with sunitinib for 24 and 48 hours after treatment with 1.25 μM, 2.5 μM and 5 μM of sunitinib ($p < 0.05$, $p < 0.0001$ and $p < 0.05$, respectively). However, the comparison between 24 and 48 hour incubation times in high doses (10 μM and 20 μM) did not result in a statistically significant difference in cell viability ($p > 0.05$).

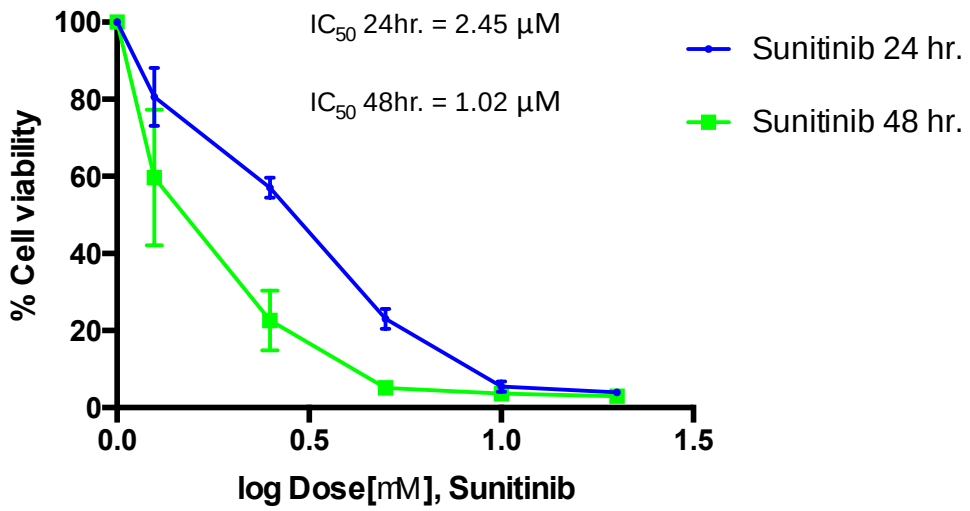


**Figure 10: Effect of sunitinib on cell viability of the 786-O cell line.**

Cells were exposed to 1.25-20 µM of sunitinib for 24 - 48 hours and then cell viability was measured by an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of three separate experiments.

**Table 27: Statistical analysis of the effect of sunitinib on cell viability of the 786-O cell lines after 24 or 48 hours.**

| Dose range (µM) 24 hours | Dose range (µM) 48 hours | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** | **10** | **20** |
| **Control** | | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **1.25** | p < 0.05 | p < 0.05 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **2.5** | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **5** | p < 0.0001 | p < 0.0001 | NS | p < 0.05 | p < 0.05 | p < 0.05 |
| **10** | p < 0.0001 | p < 0.0001 | p < 0.05 | NS | NS | NS |
| **20** | p < 0.0001 | p < 0.0001 | p < 0.05 | NS | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

#### 3.3.2.2 Cell viability of the 786-O cell line following administration of pazopanib

786-O cells were then similarly incubated with varying concentrations (0-20 μM) of pazopanib for 24 or 48 hours. The results are presented in Figure 11 and Table 28. A similar dose-dependent reduction in cell viability was observed when the cells were treated with pazopanib for 24 or 48 hours when compared with control cells ($p < 0.05$). The treatment of 48 hours again showed a greater effect on cell viability than 24 hours' incubation time. The $IC_{50}$ doses for the 24 and 48 hour treatments were 3.11 μM and 1.57 μM, respectively.

The impact of drug incubation times (24 – 48 hours) on cell viability were then compared and the results are shown in Table 28. There was a statistically significant difference in cell survival between cells incubated with pazopanib for 24 and 48 hours after treatment with pazopanib in 2.5 μM and 5 μM ($p < 0.01$). However, the comparison between 24 and 48 hours incubation times in doses of 1.25 μM, 10 μM and 20 μM did not result in statistically significant differences in cell viability ($p > 0.05$).

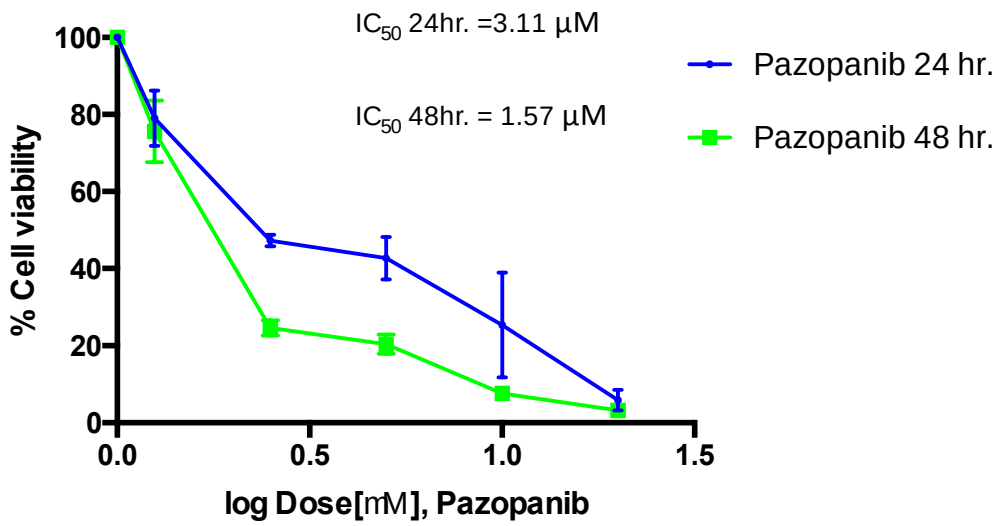


**Figure 11: Effect of pazopanib on cell viability of the 786-O cell line.**

Cells were exposed to 1.25-20 µM of pazopanib for 24 - 48 hours and then cell viability was measured by an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of three separate experiments.

**Table 28: Statistical analysis of the effect of pazopanib on cell viability of the 786-O cell line after 24 or 48 hours**

| Dose range (µM) 24 hours | Dose range (µM) 48 hours | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** | **10** | **20** |
| **Control** | | p < 0.01 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **1.25** | p < 0.01 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **2.5** | p < 0.0001 | p < 0.0001 | p < 0.01 | p < 0.001 | p < 0.0001 | p < 0.0001 |
| **5** | p < 0.0001 | p < 0.0001 | p < 0.05 | p < 0.01 | p < 0.0001 | p < 0.0001 |
| **10** | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS |
| **20** | p < 0.0001 | p < 0.0001 | p < 0.05 | NS | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

#### 3.3.2.3 Cell viability of the ACHN cell line following administration of sunitinib

As is shown in Figure 12 and Table 29, incubation with sunitinib, for either 24 or 48 hours reduced cell viability in a dose dependant manner, and there was a statistically significant difference in cell survival compared with control cells ($p < 0.05$). The $IC_{50}$ doses for the 24 and 48 hour treatments were 2.44 µM and 1.04 µM, respectively.

The impact of drug incubation times (24 – 48 hours) on cell viability was compared and the results of this comparison are shown in Table 29. The table demonstrated that there was a statistically significant difference in cell survival between cells incubated with sunitinib for 24 and 48 hours after treatment with 5 µM sunitinib ($p < 0.01$). However, the comparison between 24 and 48 hours incubation times in doses (1.25 µM, 2.5 µM, 10 µM and 20 µM) did not result in statistically significant difference in cell viability ($p > 0.05$).

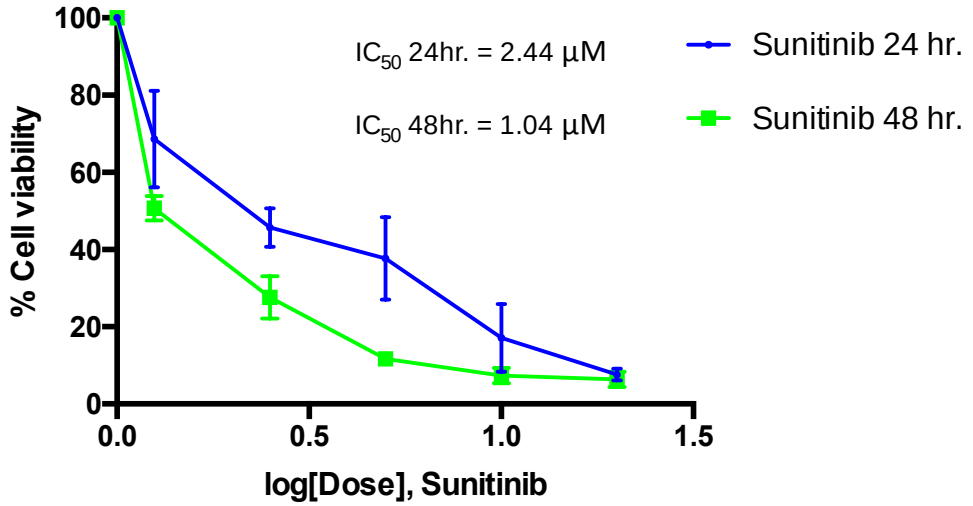


**Figure 12: Effect of sunitinib on cell viability of the ACHN cell line.**

Cells were exposed to 1.25-20 µM of sunitinib for 24 - 48 hours and then cell viability was measured by an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared to the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post-test to compare to control cell. Each value represents the mean ± SD of three separate experiments.

**Table 29: Statistical analysis for the effect of sunitinib on cell viability of the ACHN cell line after 24 or 48 hours.**

| Dose range (µM) 24 hours | Dose range (µM) 48 hours | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** | **10** | **20** |
| **Control** | | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **1.25** | p < 0.001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **2.5** | p < 0.0001 | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **5** | p < 0.0001 | NS | NS | p < 0.01 | p < 0.001 | p < 0.001 |
| **10** | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS |
| **20** | p < 0.0001 | p < 0.0001 | p < 0.05 | NS | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

#### 3.3.2.4 Cell viability of the ACHN cell line following administration of pazopanib

As shown in Figure 13 and Table 30, incubation with pazopanib for either 24 or 48 hours reduced cell viability in a dose-dependent manner, and there was a statistically significant difference in cell survival when compared with control cells ($p < 0.05$).

The impact of drug incubation times (24 – 48 hours) on cell viability was compared and the results of this comparison are shown in Table 30. There was a statistically significant difference in cell survival between cells incubated with pazopanib for 24 and 48 hours after treatment with pazopanib in 2.5 µM and 5 µM ($p < 0.001$). However, the comparison between 24 and 48 hours incubation times in doses of 1.25 µM, 10 µM and 20 µM did not result in statistically significant differences in cell viability ($p > 0.05$).

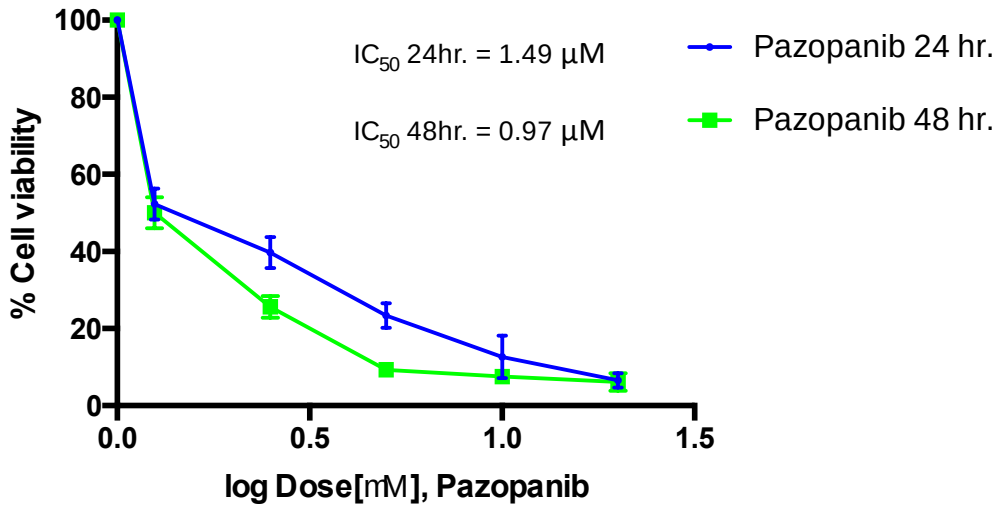


**Figure 13: Effect of pazopanib on cell viability of the ACHN cell line.**

Cells were exposed to 1.25-20 µM of pazopanib for 24 - 48 hours and then cell viability was measured by an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of three separate experiments.

**Table 30: Statistical analysis for the effect of pazopanib on cell viability of the ACHN cell line after 24 or 48 hours**

| Dose range (µM) 24 hours | Dose range (µM) 48 hours | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** | **10** | **20** |
| **Control** | | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **1.25** | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **2.5** | p < 0.0001 | p < 0.05 | p < 0.001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **5** | p < 0.0001 | p < 0.0001 | NS | p < 0.001 | p < 0.001 | p < 0.0001 |
| **10** | p < 0.0001 | p < 0.0001 | p < 0.01 | NS | NS | NS |
| **20** | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.2.5 Cell viability of the 786-O cell line after administration of disulfiram

In 786-O cell lines, the cytotoxic-inhibitory effect of disulfiram on 786-O cell line viability was tested by using the Alamar blue assay and the results are shown in Figure 14 and Table 31. Incubation of cells with 2.5 µM to 25 µM of disulfiram resulted in a statistically significant reduction of cell viability, from 77% cell survival at the lowest dose of 2.5 µM to 8% cell survival at the highest dose of 25 µM, compared with untreated cells. A sharp cell survival reduction occurred with doses of 2.5 µM and 5 µM; a less cytotoxic effect was observed between doses of 7.5- 15 µM and then the cell survival rate decreased again at higher doses of 20 µM and 25 µM. The $IC_{50}$ dose of disulfiram on 786-O cells calculated using GraphPad Prism Software was 3.3 µM.

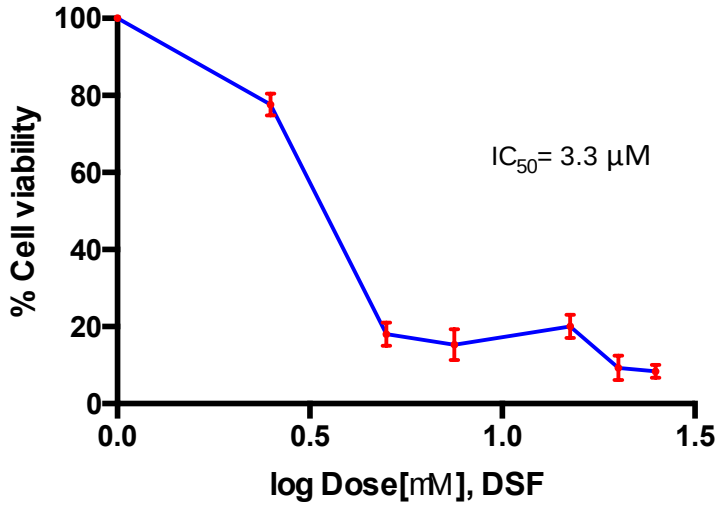


**Figure 14: Effect of disulfiram on cell viability of the 786-O cell line.**

Cells were exposed to 2.5 - 25 µM of 786-O for 24 hours and then cell viability was measured by an Alamar blue assay. Data shown are expressed as percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of three separate experiments.

### 3.3.2.6 Cell viability of the ACHN cell line after administration of disulfiram

In ACHN cell lines, the cytotoxic-inhibitory effect of disulfiram on ACHN cell line viability was tested using the Alamar blue assay and results are shown in Figure 15 and Table 31. Data showed a distinct U-shaped response to disulfiram as seen in Figure 15. Incubation of cells with 2.5 µM of disulfiram resulted in a non-statistically significant reduction in cell viability, with 92% cell survival compared with control cells. Incubation of cells with 5 µM, 7.5 µM, 15 µM, 20 µM and 25 µM of disulfiram resulted in a statistically significant reduction in cell viability, with 42%, 56%, 83%, 77% and 74% cell survival compared with control cells, respectively. The $IC_{50}$ dose of disulfiram in ACHN cells was calculated using GraphPad Prism Software to be 37.7 µM. Our data also concluded that the ACHN renal cell line was more resistant to disulfiram than the 786-O cell line.

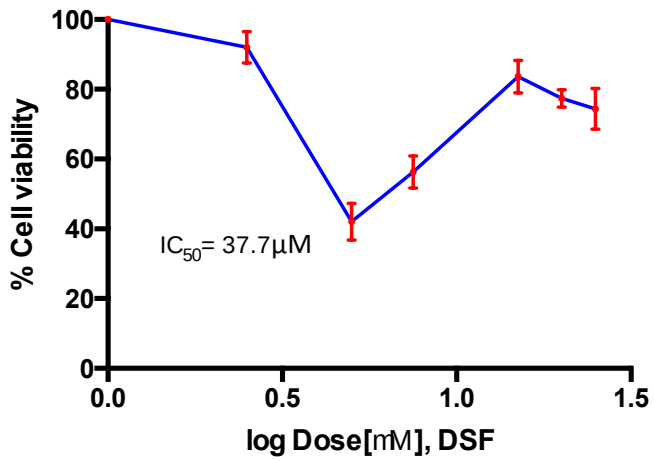


**Figure 15: Effect of disulfiram on cell viability of the ACHN cell line.**

Cells were exposed to 2.5 - 25 µM of ACHN for 24 hours and then cell viability was measured using an Alamar blue assay. Data shown are expressed as percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of three separate experiments.

**Table 31: Statistical analysis of the effect of disulfiram on cell viability of the 786-O and ACHN cell lines after 24 hours**

| Dose range (μM) Disulfiram | Cell lines | |
|---|---|---|
| | 786-O | ACHN |
| **2.5** | $p < 0.0001$ | NS |
| **5** | $p < 0.0001$ | $p < 0.0001$ |
| **7.5** | $p < 0.0001$ | $p < 0.0001$ |
| **15** | $p < 0.0001$ | $p < 0.01$ |
| **20** | $p < 0.0001$ | $p < 0.001$ |
| **25** | $p < 0.0001$ | $p < 0.0001$ |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.2.7 Cell viability of 786-O and ACHN following administration of radiotherapy

Figure 16 and Table 32 show the results of the 786-O and ACHN cell lines being irradiated with 2 or 4 Gy of XBR and then incubated for an additional 24 hours. For the 786-O cell line, there was a significant difference in cell survival when compared with control cells at doses of 2 Gy and 4 Gy ($p < 0.05$ and $p < 0.001$, respectively). In the ACHN cell line, a similar response to XBR was observed with cell death greatest for 4 Gy > 2 Gy > control ($p < 0.05$). The purpose of this experiment was to observe that 2 Gy fractions were cytotoxic as this is what is used in a clinical setting. Because a significant difference in cell survival at the 2 Gy dose for both cell lines when compared with untreated cells was observed, 2 Gy was used in subsequent experiments looking at the radio-sensitisation potential of the TKI agents or disulfiram-copper complexes.

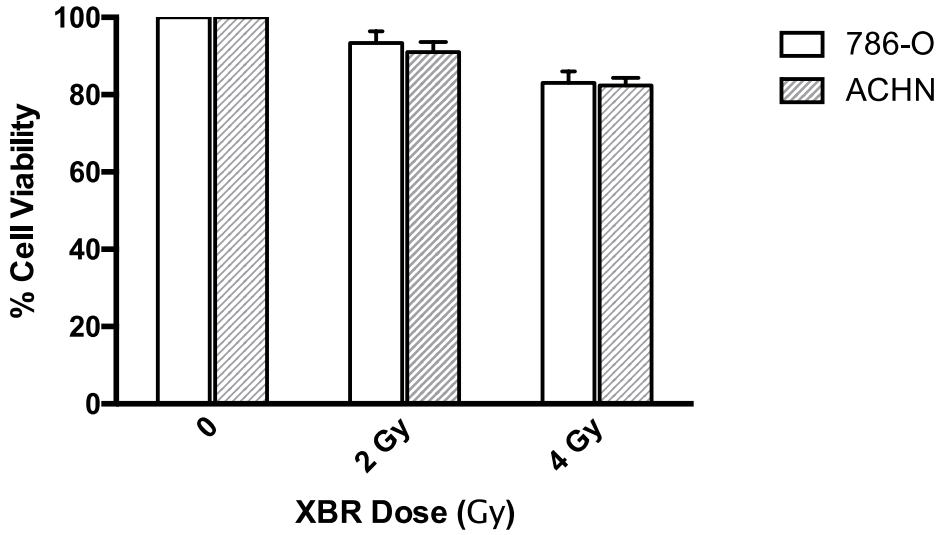


**Figure 16: Effect of external beam radiation (XBR) on cell viability of 786-O and ACHN cell lines.**

786-O and ACHN cells were exposed to 0 Gy, 2 Gy and 4 Gy of XBR for 24 hours and then cell viability was measured using an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of three separate experiments.

**Table 32: Statistical analysis of the effect of XBR on cell viability of the 786-O and ACHN cell lines**

| Dose range (Gy) XBR | Cell lines | |
|---|---|---|
| | **786-O** | **ACHN** |
| **2 Gy** | $p < 0.05$ | $p < 0.01$ |
| **4 Gy** | $p < 0.001$ | $p < 0.0001$ |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.3 Combination therapy

#### 3.3.3.1 Investigation of the effect of combination therapy of sunitinib ± 0 or 2 Gy of XBR exposure on 786-O cell line

As shown in Figure 17 and Table 33, treatment of 786-O cells with sunitinib and 2 Gy XBR had a more pronounced effect on cell viability compared with cells that were incubated with sunitinib alone. The $IC_{50}$ value, determined using GraphPad Prism software was calculated as 2.28 µM for sunitinib alone and 1.18 µM for sunitinib combined with 2 Gy XBR.

We compared the effect of drug treatment (± 0 or 2 Gy of XBR) on cell viability to determine if sunitinib works as a radiosensing agent in the 786-O renal cell line. The results of this comparison are shown in Table 33 which shows that there was a statistically significant difference in cell survival between cells incubated with sunitinib + 0 Gy and cells incubated with sunitinib + 2 Gy of XBR after treatment with 1.25 µM sunitinib ($p < 0.0001$). However, the comparison between single and combination therapy in doses of 2.5 µM, 5 µM, 10 µM and 20 µM of sunitinib did not result in statistically significant differences in cell viability ($p > 0.05$).

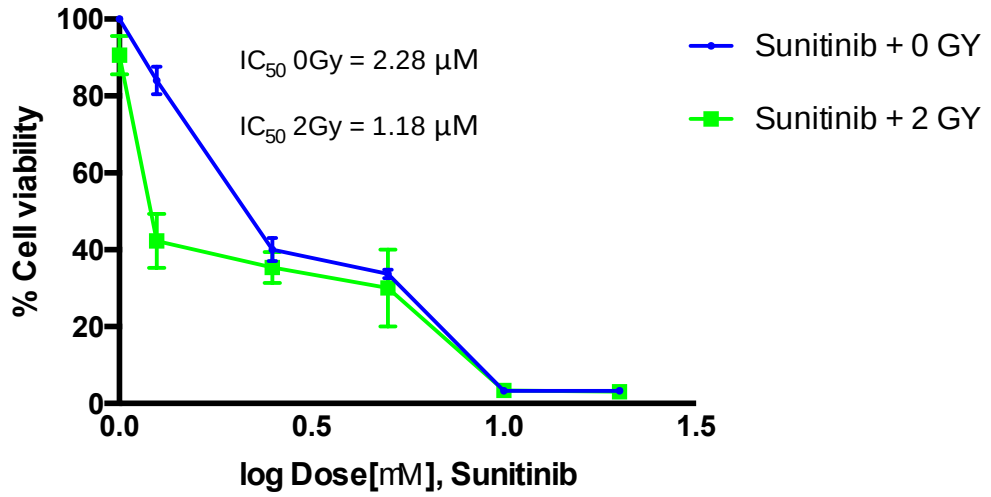


**Figure 17: Effect of sunitinib on cell viability of the 786-O cell line ± 0 or 2 Gy XBR.**

Cells were exposed to 1.25-20 µM of sunitinib ± 0 or 2 Gy of XBR for 24 hours and then cell viability was measured using an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of the three separate experiments.

**Table 33: Statistical analysis of the effect of sunitinib ± 0 or 2 Gy XBR on cell viability of the 786-O cell line.**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** | **10** | **20** |
| **Control** | | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **1.25** | p < 0.01 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **2.5** | p < 0.0001 | NS | NS | NS | p < 0.0001 | p < 0.0001 |
| **5** | p < 0.0001 | NS | NS | NS | p < 0.0001 | p < 0.0001 |
| **10** | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS |
| **20** | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

#### 3.3.3.2 Investigation of the effect of combination therapy of pazopanib ± 0 or 2 Gy of XBR exposure on 786-O cell line

As shown in Figure 18 and Table 34, treatment of 786-O cells with pazopanib and 2 Gy had a more pronounced effect on cell viability compared with cells that were incubated with pazopanib alone. The $IC_{50}$ value, determined using GraphPad Prism software was calculated as 2.12 µM for pazopanib alone and 1.30 µM for pazopanib combined with 2 Gy XRB.

We compared the effect of drug treatment (± 0 or 2 Gy of XBR) on cell viability to determine if pazopanib works as a radiosensitive agent in 786-O renal cell line. The results of this comparison are shown in Table 34 where it is shown that there was a statistically significant difference in cell survival between cells incubated with pazopanib + 0 Gy and cells incubated with pazopanib + 2 Gy of XBR after treatment with in 1.25 µM of pazopanib ($p < 0.0001$). However, the comparison between single and combination therapy in doses of 2.5 µM, 5 µM, 10 µM and 20 µM of pazopanib did not result in statistically significant differences in cell viability ($p > 0.05$).

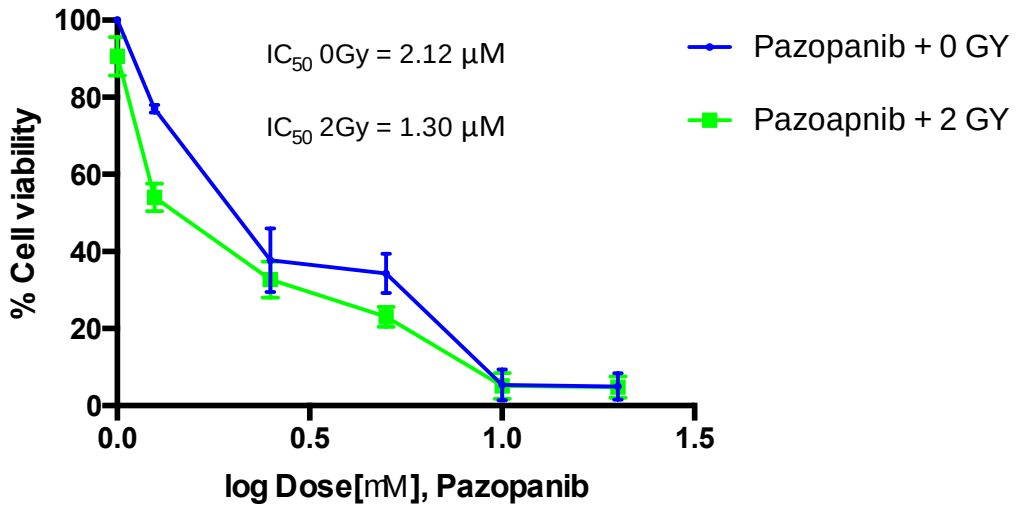


**Figure 18: Effect of pazopanib on cell viability of the 786-O cell line ± 0 or 2 Gy.**

Cells were exposed to 1.25-20 µM of pazopanib ± 0 or 2 Gy of XBR for 24 hours and then cell viability was measured using an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of the three separate experiments.

**Table 34: Statistical analysis of the effect of pazopanib ± 0 or 2 Gy XBR on cell viability of the 786-O cell line.**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** | **10** | **20** |
| **Control** | | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **1.25** | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **2.5** | p < 0.0001 | p < 0.01 | NS | p < 0.05 | p < 0.0001 | p < 0.0001 |
| **5** | p < 0.0001 | p < 0.001 | NS | NS | p < 0.0001 | p < 0.0001 |
| **10** | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.01 | NS | NS |
| **20** | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.01 | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

#### 3.3.3.3 Investigation of the effect of sunitinib ± 0 or 2 Gy of XBR exposure on cell viability of the ACHN cell line

As shown in Figure 19 and Table 35, treatment of ACHN cells with sunitinib and 2 Gy had a more pronounced effect on cell viability compared with cells that were incubated with pazopanib alone. The $IC_{50}$ value, determined using Graphpad Prism software, was calculated as 1.90 µM for sunitinib alone and 1.12 µM for sunitinib combined with 2 Gy XRB.

The effects of drug treatment (± 0 or 2 Gy of XBR) on cell viability were compared to determine if sunitinib works as a radiosensitive agent in ACHN renal cell line. The results of this comparison are shown in Table 35, which demonstrates that there was a statistically significant difference in cell survival between cells incubated with sunitinib + 0 Gy and cells incubated with sunitinib + 2 Gy of XBR after treatment with 1.25 µM and 2.5µM of sunitinib ($p < 0.001$ and $p < 0.0001$, respectively). However, the comparison between single and combination therapy in doses of 5 µM, 10 µM and 20 µM of sunitinib did not result in statistically significant differences in cell viability ($p > 0.05$).

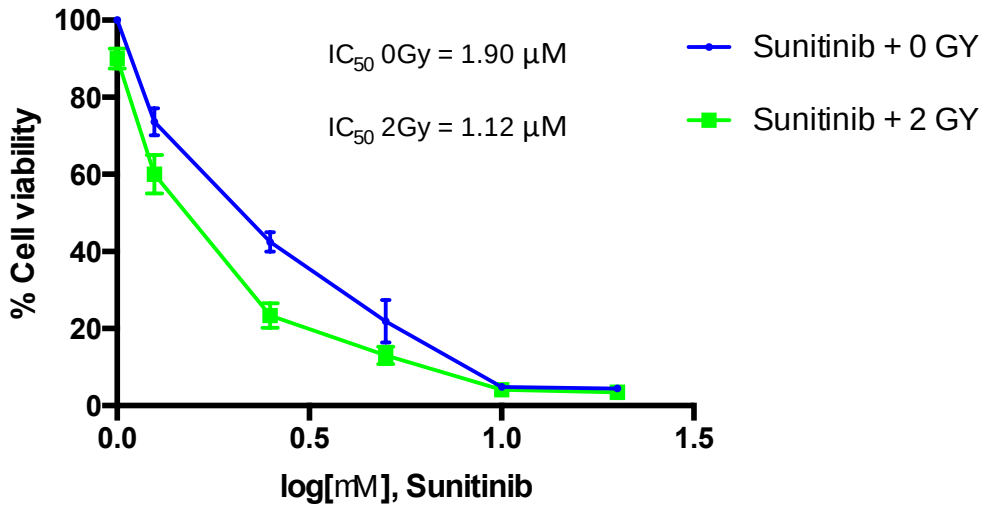


**Figure 19: Effect of sunitinib on cell viability of the ACHN cell line ± 0 or 2 Gy.**

Cells were exposed to 1.25-20 µM of sunitinib ± 0 or 2 Gy of XBR for 24 hours and then cell viability was measured using an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of three separate experiments.

**Table 35: Statistical analysis of the effect of sunitinib ± 0 or 2 Gy XBR on cell viability of the ACHN cell line**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** | **10** | **20** |
| **Control** | | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **1.25** | p < 0.0001 | p < 0.001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **2.5** | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **5** | p < 0.0001 | p < 0.0001 | NS | NS | p < 0.0001 | p < 0.0001 |
| **10** | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS |
| **20** | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

#### 3.3.3.4 Investigation of the effect of pazopanib ± 0 or 2 Gy of XBR exposure on cell viability of the ACHN cell line

As shown in Figure 20 and Table 36, treatment of ACHN cells with pazopanib and 2 Gy XBR had a more pronounced effect on cell viability compared with cells that were incubated with pazopanib alone. The $IC_{50}$ value, determined using GraphPad Prism software was calculated as 1.46 μM for pazopanib alone and 0.89 μM for pazopanib combined with 2 Gy XRB.

The effects of drug treatment (± 0 or 2 Gy of XBR) on cell viability were compared to determine if pazopanib works as a radiosensitive agent in ACHN renal cell line. The results of this comparison are shown in Table 36. The table demonstrated that there was a statistically significant difference in cell survival between cells incubated with pazopanib + 0 Gy and cells incubated with pazopanib + 2 Gy of XBR after treatment with 1.25 μM and 5 μM pazopanib ($p < 0.0001$ and $p < 0.01$, respectively). However, the comparison between single and combination therapy in doses of 2.5 μM, 10 μM and 20 μM of pazopanib did not result in statistically significant difference in cell viability ($p > 0.05$).

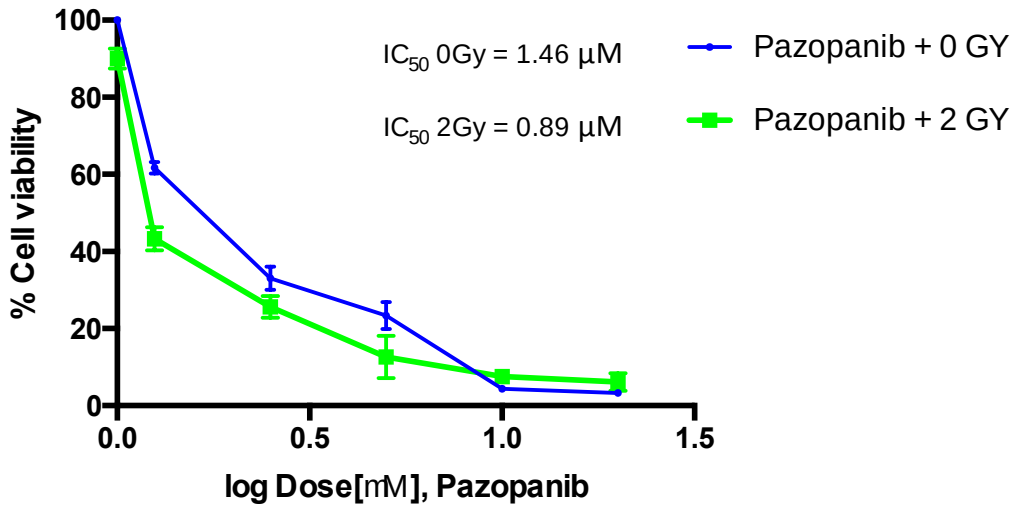


**Figure 20: Effect of pazopanib on cell viability of the ACHN cell line ± 0 or 2 Gy.**

Cells were exposed to 1.25-20 µM of pazopanib ± 0 or 2 Gy of XBR for 24 hours and then cell viability was measured using an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of three separate experiments.

**Table 36: Statistical analysis of the effect of pazopanib ± 0 or 2 Gy XBR on cell viability of the ACHN cell line**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** | **10** | **20** |
| **Control** | | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **1.25** | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **2.5** | p < 0.0001 | p < 0.01 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **5** | p < 0.0001 | p < 0.0001 | NS | p < 0.01 | p < 0.0001 | p < 0.0001 |
| **10** | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS |
| **20** | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.05 | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.3.5 Comparing the $IC_{50}$ values of sunitinib and pazopanib drugs as a single therapy or in combination with XBR across 786-O and ACHN cell lines

From the data above in both cell lines, it is clear that incubation of the cells with the combination therapy (XBR with sunitinib or pazopanib) has a considerablly greater reduction in cell viability compared with incubation of cells with sunitinib or pazopanib as a single therapy. The difference in the curves and the $IC_{50}$ values are noticeable and almost similar in both cell lines, 786-O and ACHN, in sunitinib treatent alone and in combination with XBR (786-O: $IC_{50}$ 2.28 μM vs. 1.18 μM; ACHN: $IC_{50}$ 1.90 μM vs. 1.12 μM). The difference in cells treated with sunitinib as single therapy and in combination therapy with XBR is significant at the lowest dose of 1.25 μM in the 786-O cell line and at concentrations of 1.25 μM and 2.5 μM in the ACHN cell line, showing that the combination of radiation with sunitinib reduced the dose of sunitinib required to achieve the $IC_{50}$. Therefore, it suggests that sunitinib works as a radiosensitive agent in 786-O and ACHN cell lines. Our results also demonstrated that the ACHN cell line was more sensitive to sunitinib than the 786-O cell line, because a lower dose of the agent was needed to cause cell death.

In the pazopanib treatment, the difference between the curves in 786-O cell incubated with pazopanib as a single therapy and in combination therapy with XBR is similar to that of the ACHN cell line (786-O: $IC_{50}$ 2.12 μM vs. 1.30 μM; ACHN: $IC_{50}$ 1.46 μM vs. 0.89 μM). However, treating ACHN with pazopanib as a single and combination therapy resulted in a smaller $IC_{50}$ than in the 786-O cell line, suggesting

that this cell line is more sensitive to pazopanib even as a single therapy. Furthermore, our data showed that the combination of radiation with pazopanib reduced the dose required to achieve the $IC_{50}$. Therefore, this suggests that pazopanib works as a radiosensitive agent in 786-O and ACHN cell lines.

#### 3.3.3.6 Investigation of the effect of disulfiram/copper ± 0 to 2 Gy of XBR exposure on cell viability of the 786-O cell line

Previous studies have demonstrated that the cytotoxicity of disulfiram is copper-dependent or is enhanced in the presence of copper. Therefore, in order to test this hypothesis is renal cell carcinoma cells we performed an Alamar blue assay to evaluate the anti-cancer activity of this complex in renal cancer cell lines. We investigated a dose range of disulfiram (2.5-25 µM) with a fixed dose of 1 µM copper.

Disulfiram alone showed cytotoxicity effect across the selected dose range when incubated with 786-O cells. However, copper alone did not show any statistically significant effect on cell viability compared with untreated control cells ($p > 0.05$). Figure 21 and Table 37 show the statistically significant cytotoxic effect of disulfiram alone at doses of 2.5 µM, 5 µM, 10 µM and 25 µM on 786-O cells compare with the control cells. However, a one-way ANOVA in GraphPad Prism Software showed that when disulfiram and copper were given in combination, copper significantly enhanced the cytotoxic effect of disulfiram only at a low dose of 2.5 µM of disulfiram when compared with the cells treated with disulfiram alone ($p < 0.0001$).

We also investigated the radio-sensitising effect of disulfiram alone, copper alone and disulfiram with copper in combination in 786-O cells. Data analysis (Table 37) showed that there were no statistically significant differences when cells received 2 Gy of XBR in addition to disulfiram alone, copper alone or with the disulfiram-copper combination compared with cells treated with 0 Gy ($p > 0.05$).

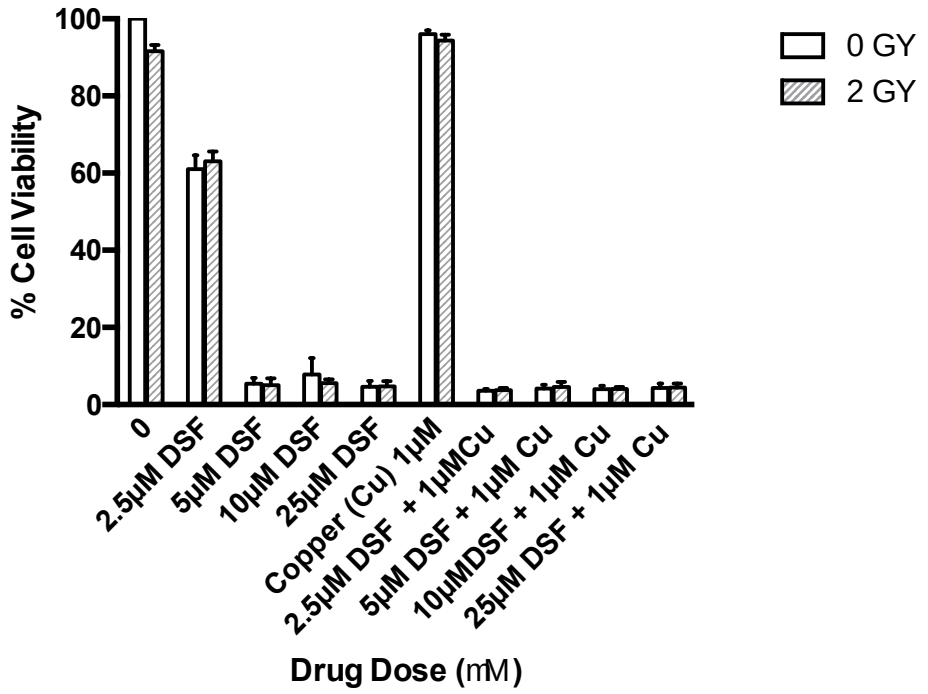


**Figure 21: Effect of disulfiram/copper on cell viability of the 786-O cell line ± 0 or 2 Gy XBR.**

Cells were exposed to 2.5-25 µM of disulfiram for 24 hours followed by ± 0 to 2 Gy XBR exposure using a fixed dose of copper (Cu) of 1 µM, and then cell viability was measured using an Alamar blue assay. Data shown are expressed as a percentage of cell viability compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. Each value represents the mean ± SD of three separate experiments.

**Table 37: Statistical analysis of the effect of disulfiram/copper ± 0 to 2 Gy XBR on cell viability of the 786-O cell line**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 2.5 | 5 | 10 | 25 | Cu | Cu + 2.5 | Cu + 5 | Cu 10 | Cu 25 |
| Control | | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| 2.5 | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| 5 | p < 0.0001 | p < 0.0001 | NS | NS | NS | p < 0.0001 | NS | NS | NS | NS |
| 10 | p < 0.0001 | p < 0.0001 | NS | NS | NS | p < 0.0001 | NS | NS | NS | NS |
| 25 | p < 0.0001 | p < 0.0001 | NS | NS | NS | p < 0.0001 | NS | NS | NS | NS |
| Cu | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| Cu + 2.5 | p < 0.0001 | p < 0.0001 | NS | NS | NS | p < 0.0001 | NS | NS | NS | NS |
| Cu + 5 | p < 0.0001 | p < 0.0001 | NS | NS | NS | p < 0.0001 | NS | NS | NS | NS |
| Cu + 10 | p < 0.0001 | p < 0.0001 | NS | NS | NS | p < 0.0001 | NS | NS | NS | NS |
| Cu + 25 | p < 0.0001 | p < 0.0001 | NS | NS | NS | p < 0.0001 | NS | NS | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.3.7 Investigation of the effect of disulfiram/copper ± 0 to 2 Gy of XBR exposure on cell viability of the ACHN cell line

The cytotoxic effects of disulfiram (2.5-25 µM) with a fixed dose of 1 µM copper in the ACHN cell line was also investigated. Data (Figure 22 and Table 38) indicated that ACHN cells exhibited a U-shape response to disulfiram. In contrast, treatment with copper alone did not show any statistically significant differences in cytotoxicity compared with control cells ($p > 0.05$).

Disulfiram cytotoxicity started from concentrations of > 5 µM to 25 µM. Copper enhanced the cytotoxic effects of disulfiram with concentrations of 2.5 µM and 10 µM of disulfiram being more highly cytotoxic compared with cells treated with disulfiram alone ($p < 0.0001$). Disulfiram alone, copper alone and disulfiram-copper did not act as radiosensitisers when ACHN cells were exposed to 2Gy radiation (Table 38; $p > 0.05$).

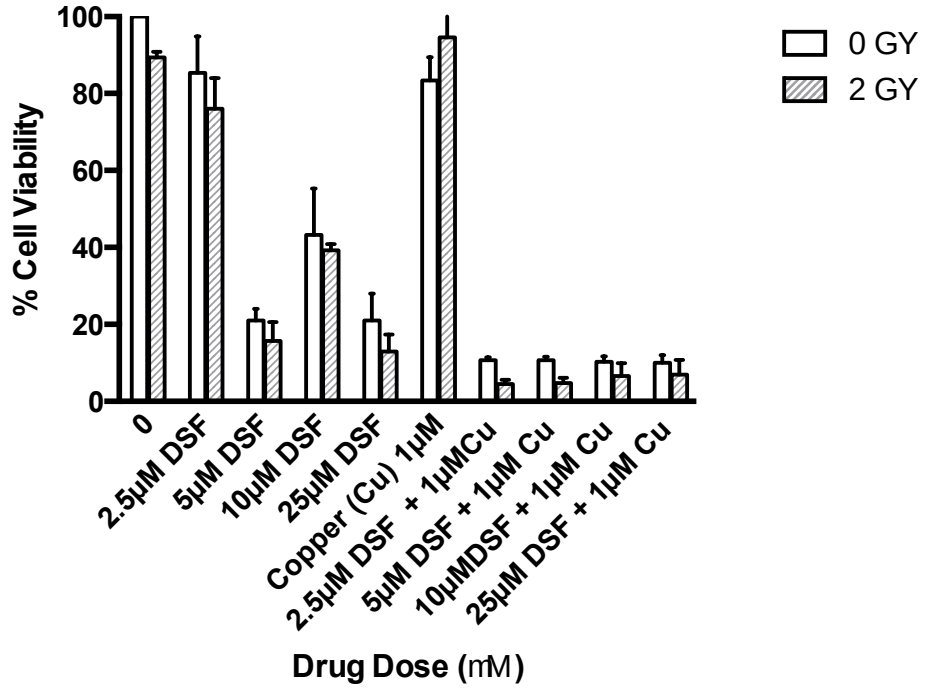


**Figure 22: Effect of disulfiram/copper on cell viability of the ACHN cell line ± 0 to 2 Gy.**

Cells were exposed to 2.5-25 µM of disulfiram for 24 hours followed by +/- 0 to 2 Gy XBR exposure using a fixed dose of copper (Cu) of 1 µM and then cell viability was measured using an Alamar blue assay. Data shown are expressed as percentage of cell viability in treated cells compared with the control group. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cell. Each value represents the mean ± SD of three separate experiments.

**Table 38: Statistical analysis of the effect of disulfiram/copper ± 0 to 2 Gy XBR on cell viability of the ACHN cell line**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **2.5** | **5** | **10** | **25** | **Cu** | **Cu + 2.5** | **Cu + 5** | **Cu 10** | **Cu 25** |
| **Control** | | p < 0.001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **2.5** | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **5** | p < 0.0001 | p < 0.0001 | NS | p < 0.05 | NS | p < 0.0001 | NS | NS | NS | NS |
| **10** | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **25** | p < 0.0001 | p < 0.0001 | NS | p < 0.05 | NS | p < 0.0001 | NS | NS | NS | NS |
| **Cu** | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **Cu + 2.5** | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | NS | p < 0.0001 | NS | NS | NS | NS |
| **Cu + 5** | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | NS | p < 0.0001 | NS | NS | NS | NS |
| **Cu + 10** | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | NS | p < 0.0001 | NS | NS | NS | NS |
| **Cu + 25** | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | NS | p < 0.0001 | NS | NS | NS | NS |

Statistical significance of differences in cell viability in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.4 Investigation of cell death mechanisms resulting from combination treatments.

#### 3.3.4.1 Effect of sunitinib ± 0 or 2 Gy XBR on caspase-3 activation as a marker of apoptosis in the 786-O cell line

Apoptosis (programmed cell death) is one of the major death pathways in mammalian cells. However, suppression of apoptosis in cancer cells is critical in malignancy initiation and progression. Our previous findings (section 3.3.2) demonstrated that sunitinib or pazopanib alone or in combination with 2 Gy XBR showed cytotoxicity against renal cancer cell lines as confirmed by the cell viability Alamar blue assay. It was therefore crucial to assess the mechanism involved in sunitinib-induced cell death. To achieve this, caspase-3 activity as a marker of apoptosis was measured following exposure to mono- and combination therapies. Data in the Alamar blue assay demonstrated that a sunitinib concentration range of 10 to 20 µM resulted in extensive cell kill, with the addition of 2 Gy XBR providing no additional benefit as cell kill had already been maximally achieved. As a consequence, a lower range of sunitinib concentrations (1.25-5 µM) were selected for apoptosis studies and at the drug exposure times of 4 and 24 hours. The effects of sunitinib incubation for 4 hours as a single therapy and when combined with XBR therapy is shown Figure 23 (A) and Table 39. A statistically significant increase in caspase-3 levels was detected when 786-O cells were exposed for 4 hours to 2.5 µM and 5 µM concentrations of sunitinib ($p < 0.05$). The addition of 2 Gy XBR did not

provide any additional increase in caspase expression compared with drugs alone ($p > 0.05$).

A longer incubation period of the 786-O cells in the presence of sunitinib (Figure 23 (B) and Table 39) indicated that sunitinib concentrations as a single therapy did increase expression of caspase-3 significantly compared with the control cells ($p < 0.05$). The addition of 2 Gy XBR to sunitinib treatment did result in caspase-3 expression being significantly greater than in control cells ($p < 0.05$).

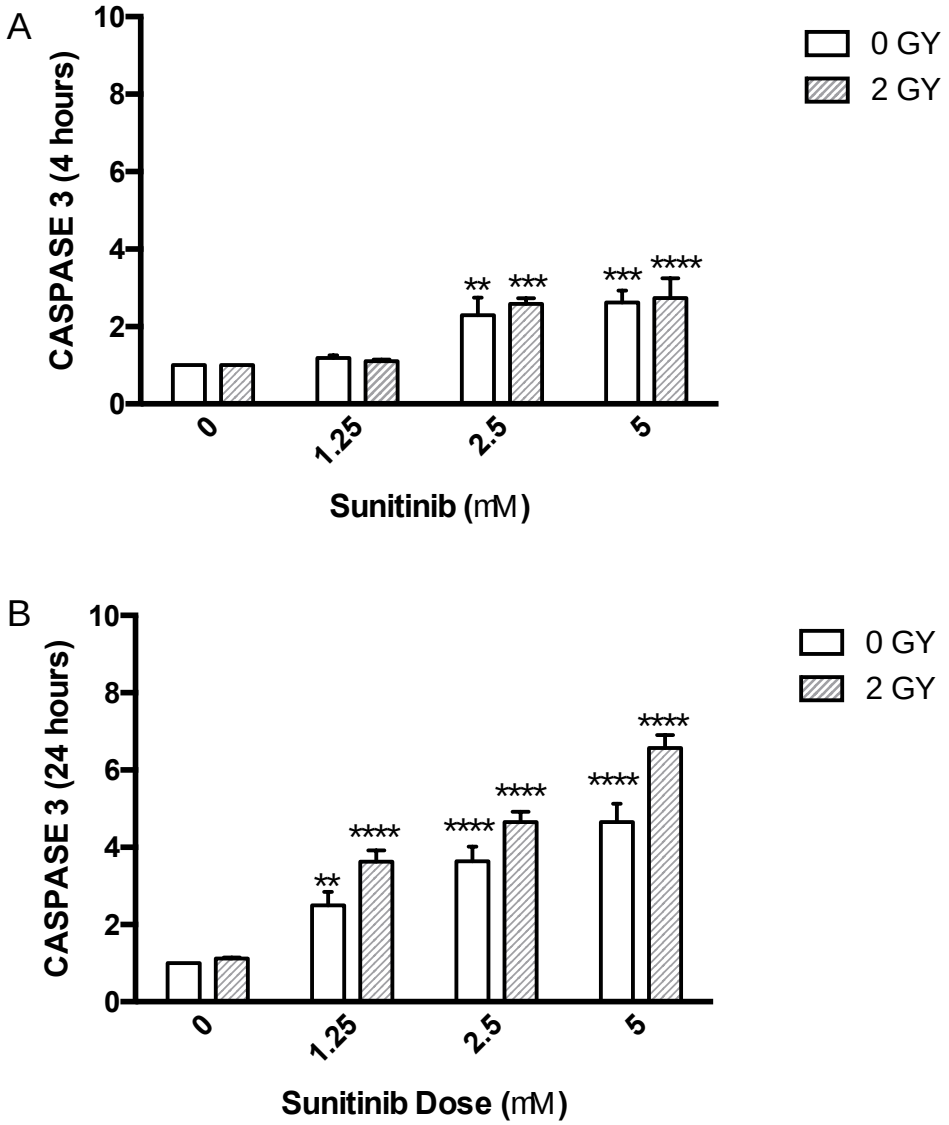


**Figure 23: Effect of sunitinib ± 0 or 2 Gy on caspase-3 activity in the 786-O cell line.**

Cells were treated with different doses of sunitinib (1.25 - 5 µM) for A) 4 hours and B) 24 hours with 0 or 2 Gy and then caspase-3 activity was measured using a caspase-3 fluorometric assay. The chart shows the data as a fold increase following treatment in caspase-3 compared with control cells. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with the control cells. Each value represents the mean ± SD of three separate experiments. **$p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ compared with non-treated control group.

**Table 39: Statistical analysis of the effect of sunitinib ± 0 or 2 Gy XBR on caspase-3 of the 786-O cell line after 4 or 24 hours.**

| Dose range (µM) 0 GY 4 hours | Dose range (µM) 2 GY | | | |
|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** |
| **Control** | | NS | $p < 0.001$ | $p < 0.0001$ |
| **1.25** | NS | NS | $p < 0.001$ | $p < 0.001$ |
| **2.5** | $p < 0.01$ | $p < 0.01$ | NS | NS |
| **5** | $p < 0.001$ | $p < 0.001$ | NS | NS |
| **24 hours** | | | | |
| **Control** | | $p < 0.0001$ | $p < 0.0001$ | $p < 0.0001$ |
| **1.25** | $p < 0.01$ | $p < 0.05$ | $p < 0.0001$ | $p < 0.0001$ |
| **2.5** | $p < 0.0001$ | NS | $p < 0.05$ | $p < 0.0001$ |
| **5** | $p < 0.0001$ | $p < 0.05$ | NS | $p < 0.001$ |

Statistical significance of differences in caspase-3 activation in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.4.2 Effect of pazopanib ± 0 or 2 Gy XBR on caspase-3 activation as a marker of apoptosis in the 786-O cell line

The effects of pazopanib incubation for 4 hours as a single therapy and when combined with XBR therapy is shown Figure 24 (A) and Table 40. There was no statistically significant increase in caspase-3 levels detected when 786-O cells were exposed for 4 hours to 1.25 µM, 2.5 µM and 5 µM concentrations of pazopanib ($p > 0.05$). The addition of 2 Gy XBR did not provide any additional increase in caspase expression compared with the drug alone ($p > 0.05$).

A longer incubation period of the 786-O cells in the presence of pazopanib (Figure 24 (B) and Table 40) indicated that pazopanib concentrations of 2.5 µM and 5 µM as a single therapy did increase expression of caspase-3 significantly compared with the control cells ($p < 0.05$). The addition of 2 Gy XBR to pazopanib treatment at

concentrations of 2.5 µM and 5 µM did result in caspase-3 expression significantly greater than in control cells ($p < 0.05$).

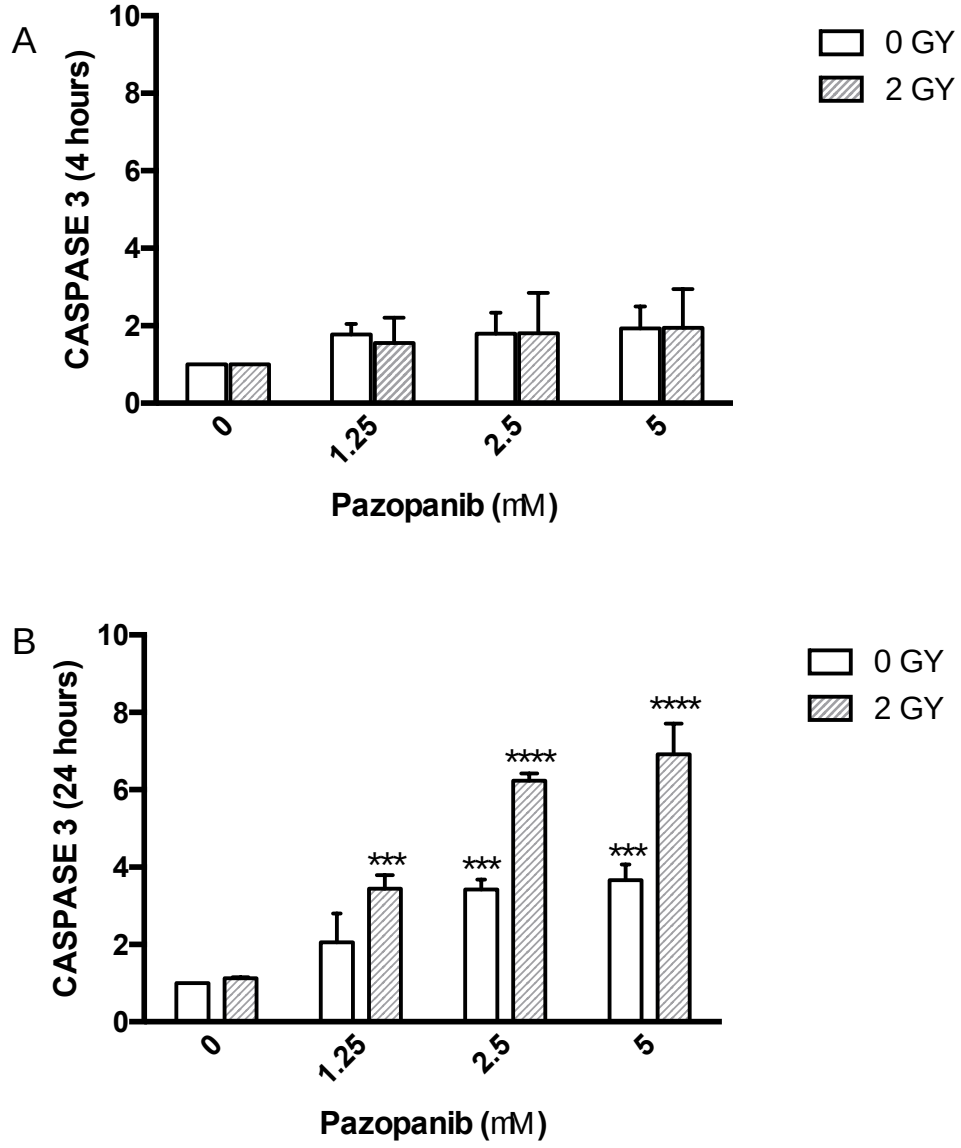


**Figure 24: Effect of pazopanib ± 0 or 2 Gy on caspase-3 activity in the 786-O cell line.**

Cells were treated with different doses of pazopanib (1.25-5 µM) for A) 4 hours and B) 24 hours with 0 or 2 Gy and then caspase-3 activity was measured using a caspase-3 fluorometric assay. The chart shows the data as a fold increase following treatment in caspase-3 level compared with control cells. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with the control cells. Each value represents the mean ± SD of three separate experiments. *** $p < 0.001$ and **** $p < 0.0001$ compared with non-treated control group.

**Table 40: Statistical analysis of the effect of pazopanib ± 0 or 2 Gy XBR on caspase-3 of the 786-O cell line after 4 or 24 hours.**

| Dose range (µM) 0 GY 4 hours | Dose range (µM) 2 GY | | | |
|---|---|---|---|---|
| | Control | 1.25 | 2.5 | 5 |
| **Control** | | NS | NS | NS |
| **1.25** | NS | NS | NS | NS |
| **2.5** | NS | NS | NS | NS |
| **5** | NS | NS | NS | NS |
| **24 hours** | | | | |
| **Control** | | $p < 0.001$ | $p < 0.0001$ | $p < 0.0001$ |
| **1.25** | NS | NS | $p < 0.0001$ | $p < 0.0001$ |
| **2.5** | $p < 0.001$ | NS | $p < 0.0001$ | $p < 0.0001$ |
| **5** | $p < 0.001$ | NS | $p < 0.001$ | $p < 0.0001$ |

Statistical significance of differences in caspase-3 activation in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.4.3 Effect of sunitinib ± 0 or 2 Gy XBR on caspase-3 activation as a marker of apoptosis in the ACHN cell line

The effects of sunitinib incubation for 4 hours as a single therapy and when combined with XBR therapy is shown in Figure 25 (A) and Table 41. There was no a statistically significant increase in caspase-3 levels detected when ACHN cells were exposed for 4 hours to 1.25 µM, 2.5 µM and 5 µM concentrations of sunitinib ($p > 0.05$). The addition of 2 Gy XBR did not provide any additional increase in caspase expression compared with the drug alone ($p > 0.05$).

A longer incubation period of the ACHN cells in the presence of sunitinib (Figure 25 (B) and Table 41) indicated that sunitinib concentrations of 2.5 µM and 5µM as a single therapy did increase expression of caspase-3 significantly compared with the control cells ($p < 0.05$). The addition of 2 Gy XBR to sunitinib treatment at

concentrations of 2.5 µM and 5 µM did result in caspase-3 expression significantly greater than that of control cells ($p < 0.05$).

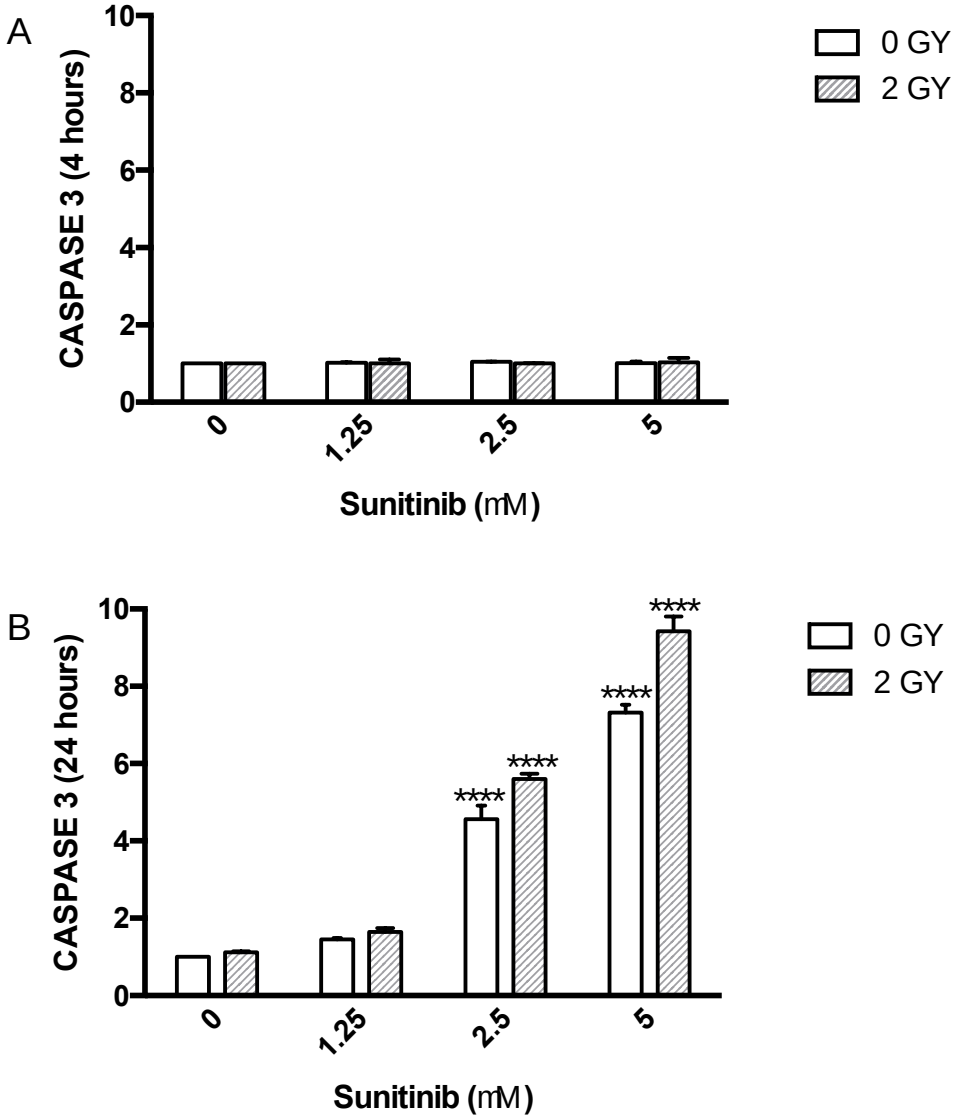


**Figure 25: Effect of sunitinib ± 0 or 2 Gy on caspase-3 activity in the ACHN cell line.**

Cells were treated with different doses of sunitinib (1.25-5 µM) for A) 4 hours, B) 24 hours with 0 or 2 Gy and then caspase-3 activity was measured using a caspase-3 fluorometric assay. The chart shows the data as a fold increase following treatment in caspase-3 level compared with control cells. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with the control cells. Each value represents the mean ± SD of three separate experiments. **** $p < 0.0001$ compared with non-treated control group.

**Table 41: Statistical analysis of the effect of sunitinib ± 0 or 2 Gy XBR on caspase-3 of the ACHN cell line after 4 or 24 hours**

| Dose range (µM) 0 GY 4 hours | Dose range (µM) 2 GY | | | |
|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** |
| **Control** | | NS | NS | NS |
| **1.25** | NS | NS | NS | NS |
| **2.5** | NS | NS | NS | NS |
| **5** | NS | NS | NS | NS |
| **24 hours** | | | | |
| **Control** | | NS | $p < 0.0001$ | $p < 0.0001$ |
| **1.25** | NS | NS | $p < 0.0001$ | $p < 0.0001$ |
| **2.5** | $p < 0.0001$ | $p < 0.0001$ | $p < 0.001$ | $p < 0.0001$ |
| **5** | $p < 0.0001$ | $p < 0.0001$ | $p < 0.0001$ | $p < 0.0001$ |

Statistical significance of differences in caspase-3 activation in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

#### 3.3.4.4 Effect of pazopanib ± 0 or 2 Gy XBR on caspase-3 activation as a marker of apoptosis in the ACHN cell line

The effects of pazopanib incubation for 4 hours as a single therapy and when combined with XBR therapy is shown Figure 26 (A) and Table 42. There was no statistically significant increase in caspase-3 levels detected when ACHN cells were exposed for 4 hours to 1.25 µM, 2.5 µM and 5 µM concentrations of pazopanib ($p > 0.05$). The addition of 2 Gy XBR did not provide any additional increase in caspase expression compared to drug alone ($p > 0.05$).

A longer incubation period of the ACHN cells in the presence of pazopanib (Figure 26 (B) and Table 42) indicated that pazopanib concentrations of 2.5 µM and 5 µM, as a single therapy did increase expression of caspase-3 significantly compared with the control cells ($p < 0.05$). The addition of 2 Gy XBR to pazopanib treatment at

concentrations of 2.5 µM and 5 µM did result in caspase-3 expression significantly greater than that of control cells ($p < 0.05$).

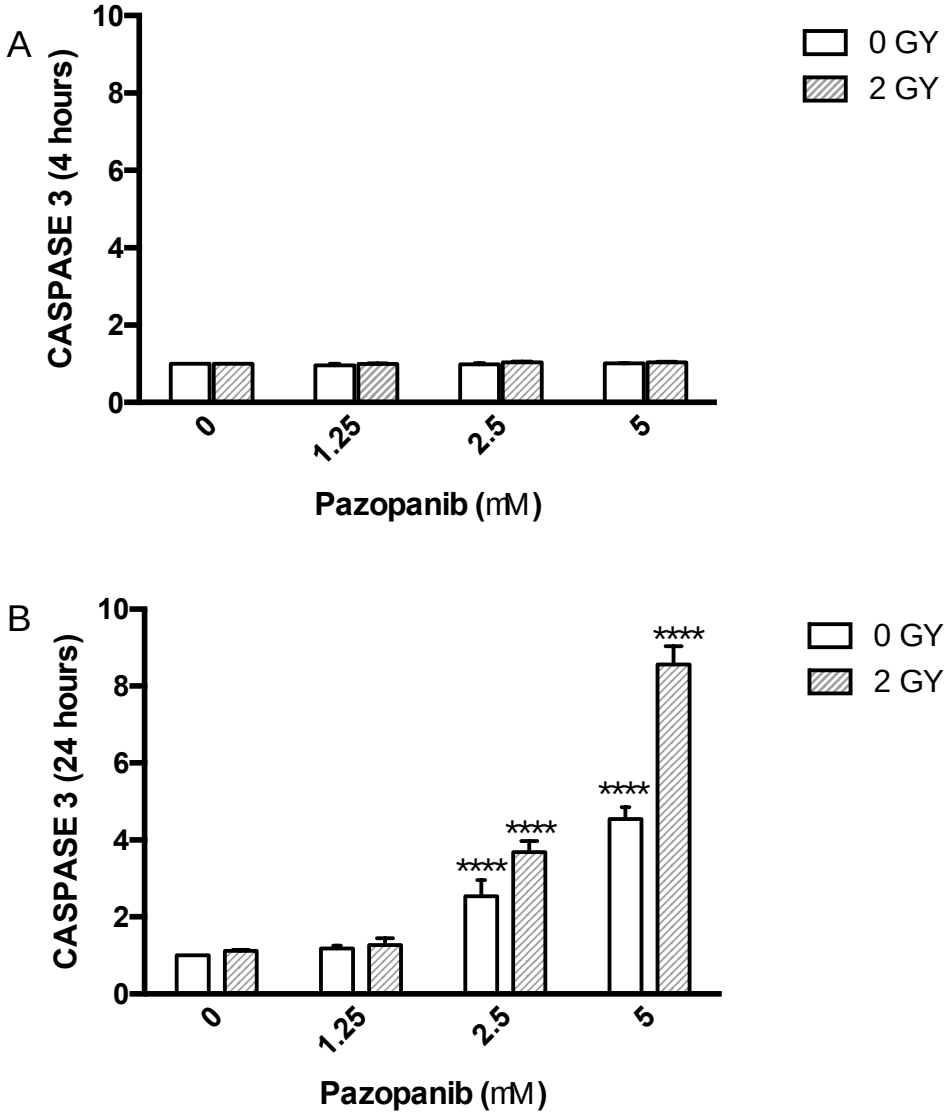


**Figure 26: Effect of pazopanib ± 0 or 2 Gy on caspase-3 activity in the ACHN cell line.**

Cells were treated with different doses of pazopanib (1-10 µM) for A) 4 hours B) 24 hours with 0 or 2 Gy and then caspase-3 activity was measured using a caspase-3 fluorometric assay. The chart shows the data as a fold increase following treatment in caspase-3 level compared with control cells. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with the control cells. Each value represents the mean ± SD of three separate experiments. **** $p < 0.0001$ compared with non-treated control group.

**Table 42: Statistical analysis of the effect of pazopanib ± 0 or 2 Gy XBR on caspase-3 of the ACHN cell line after 4 or 24 hours**

| Dose range (µM) 0 GY 4 hours | Dose range (µM) 2 GY | | | |
|---|---|---|---|---|
| | **Control** | **1.25** | **2.5** | **5** |
| **Control** | | NS | NS | NS |
| **1.25** | NS | NS | NS | NS |
| **2.5** | NS | NS | NS | NS |
| **5** | NS | NS | NS | NS |
| **24 hours** | | | | |
| **Control** | | NS | $p < 0.0001$ | $p < 0.0001$ |
| **1.25** | NS | NS | $p < 0.0001$ | $p < 0.0001$ |
| **2.5** | $p < 0.0001$ | $p < 0.01$ | $p < 0.01$ | $p < 0.0001$ |
| **5** | $p < 0.0001$ | $p < 0.0001$ | NS | $p < 0.0001$ |

Statistical significance of differences in caspase-3 activation in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.4.5 Effect of DSF/copper ± 0 or 2 Gy XBR on caspase-3 activation as a marker of apoptosis in the 786-O cell line

We investigated the potential apoptosis induction of disulfiram alone in 786-O and ACHN cell lines with a range of administered concentrations (2.5-25 µM) and copper alone (1 µM) 4 hours and 24 hours after cell treatment. We also investigated disulfiram/copper as a complex therapy. In addition to all the previous therapies, we also investigated it in combination with XBR therapy 2 Gy for 4 hours and 24 hours. One-way ANOVA was used to test the significance of differences compared with the control cells by using GraphPad Prism Software.

The effects of disulfiram ± copper incubation for 4 hours as a single and complex therapy and when combined with XBR therapy in 786-O cells is shown Figure 27 (A) and Table 43. A statistically significant increase in caspase-3 levels was detected

when 786-O cells were exposed for 4 hours to 2.5 µM, 5 µM, 10 µM and 25 µM concentrations of disulfiram ($p < 0.05$). Copper alone did not result in a statistically significance increase in caspase-3 levels compared with the control cells ($p > 0.05$). Furthermore, copper did not significantly increase caspase-3 levels compared with the cells treated with disulfiram alone ($p > 0.05$). Our results also showed that there were not any statistically significant differences between the doses of disulfiram alone, copper alone and disulfiram/copper complex versus combined with 2 Gy radiotherapy in 786-O cells after incubation for 4 hours.

A longer incubation period of the 786-O cells in the presence of disulfiram ± copper (Figure 27 (B) and Table 44) indicated that disulfiram concentrations of 5 µM, 10 µM and 25 µM as a single therapy and a complex therapy did increase expression of caspase-3 significantly compared with the control cells ($p < 0.05$). Copper alone did not show a statistically significant increase in caspase-3 levels compared with the control cells ($p > 0.05$). Copper also did not significantly increase caspase-3 levels compared with the cells treated with disulfiram alone ($p > 0.05$). Our results also showed that there were not any statistically significant differences between the doses of disulfiram alone, copper alone and the disulfiram/copper complex alone versus when they were combined with 2Gy radiotherapy in 786-O cells after incubation for 24 hours.

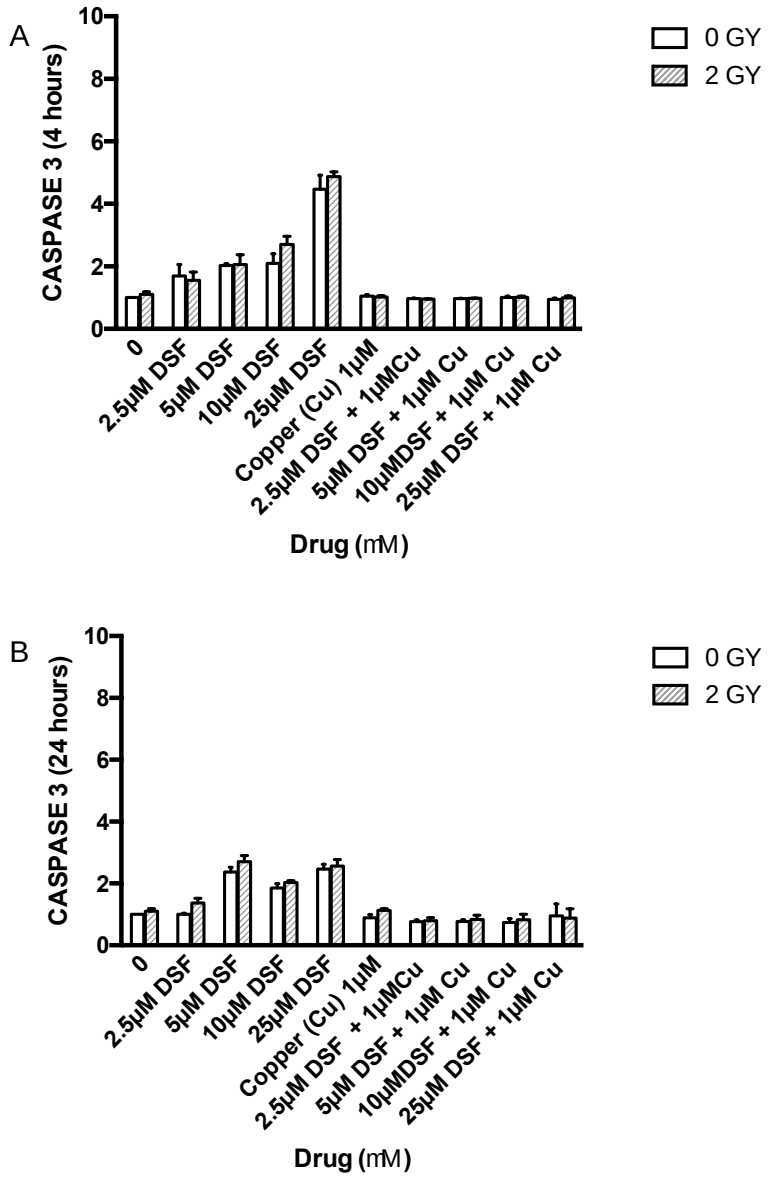


**Figure 27: Effect of disulfiram/copper ± 0 or 2 Gy on caspase-3 activity in the 786-O cell line.**

Cells were treated with different doses of disulfiram 2.5-25 µM for A) 4 hours and B) 24 hours with 0 or 2 Gy using a fixed dose of copper (Cu) of 1 µM and then caspase-3 activity was measured using a caspase-3 fluorometric assay. The chart shows the data as a fold increase following treatment in caspase-3 levels compared with control cells. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with the control cells. Each value represents the mean ± SD of three separate experiments.

**Table 43: Statistical analysis of the effect of disulfiram/copper ± 0 or 2 Gy XBR on caspase-3 of the 786-O cell line after 4 hours**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 2.5 | 5 | 10 | 25 | Cu | Cu + 2.5 | Cu + 5 | Cu 10 | Cu 25 |
| Control | | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| 2.5 | p < 0.05 | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.05 | p < 0.01 | p < 0.01 | p < 0.01 | p < 0.01 |
| 5 | p < 0.0001 | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| 10 | p < 0.0001 | NS | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| 25 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| Cu | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| Cu + 2.5 | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| Cu + 5 | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| Cu + 10 | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| Cu + 25 | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |

Statistical significance of differences in caspase-3 activation in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

**Table 44: Statistical analysis of the effect of disulfiram/copper ± 0 or 2 Gy XBR on caspase-3 of the 786-O cell line after 24 hours**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 2.5 | 5 | 10 | 25 | Cu | Cu + 2.5 | Cu + 5 | Cu 10 | Cu 25 |
| **Control** | | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| **2.5** | NS | NS | p < 0.0001 | p < 0.05 | p < 0.0001 | NS | NS | NS | NS | NS |
| **5** | p < 0.0001 | p < 0.0001 | NS | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **10** | p < 0.0001 | NS | p < 0.0001 | NS | p < 0.001 | p < 0.001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **25** | p < 0.0001 | p < 0.0001 | NS | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 | p < 0.0001 |
| **Cu** | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| **Cu + 2.5** | NS | p < 0.05 | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| **Cu + 5** | NS | p < 0.05 | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| **Cu + 10** | NS | p < 0.01 | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |
| **Cu + 25** | NS | NS | p < 0.0001 | p < 0.0001 | p < 0.0001 | NS | NS | NS | NS | NS |

Statistical significance of differences in caspase-3 activation in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

### 3.3.4.6 Effect of DSF/copper ± 0 or 2 Gy XBR on caspase-3 activation as a marker of apoptosis in the ACHN cell line

The effects of disulfiram ± copper incubation for 4 hours as a single and complex therapy and when combined with XBR therapy in ACHN cells is shown Figure 28 (A) and Table 45. There were no significant elevations in caspase-3 levels in all dose regimens examined with disulfiram, copper, and disulfiram/copper complex and when combined with 2 Gy radiotherapy ($p > 0.05$).

A longer incubation period of the ACHN cells in the presence of disulfiram ± copper (Figure 28 (B) and Table 46) indicated that disulfiram concentrations of 2.5 µM and 10 µM as a single therapy did increase expression of caspase-3 significantly compared with the control cells ($p < 0.05$). Copper alone did not show a statistically significant increase in caspase-3 levels compared with the control cells ($p > 0.05$). Furthermore, copper did not significantly increase caspase-3 levels compared with the cells treated with disulfiram alone ($p > 0.05$). Our results also showed that there were not any statistically significant differences between the doses of disulfiram alone, copper alone and disulfiram/copper complex versus combined with 2 Gy radiotherapy in 786-O cells after incubation for 24 hours.

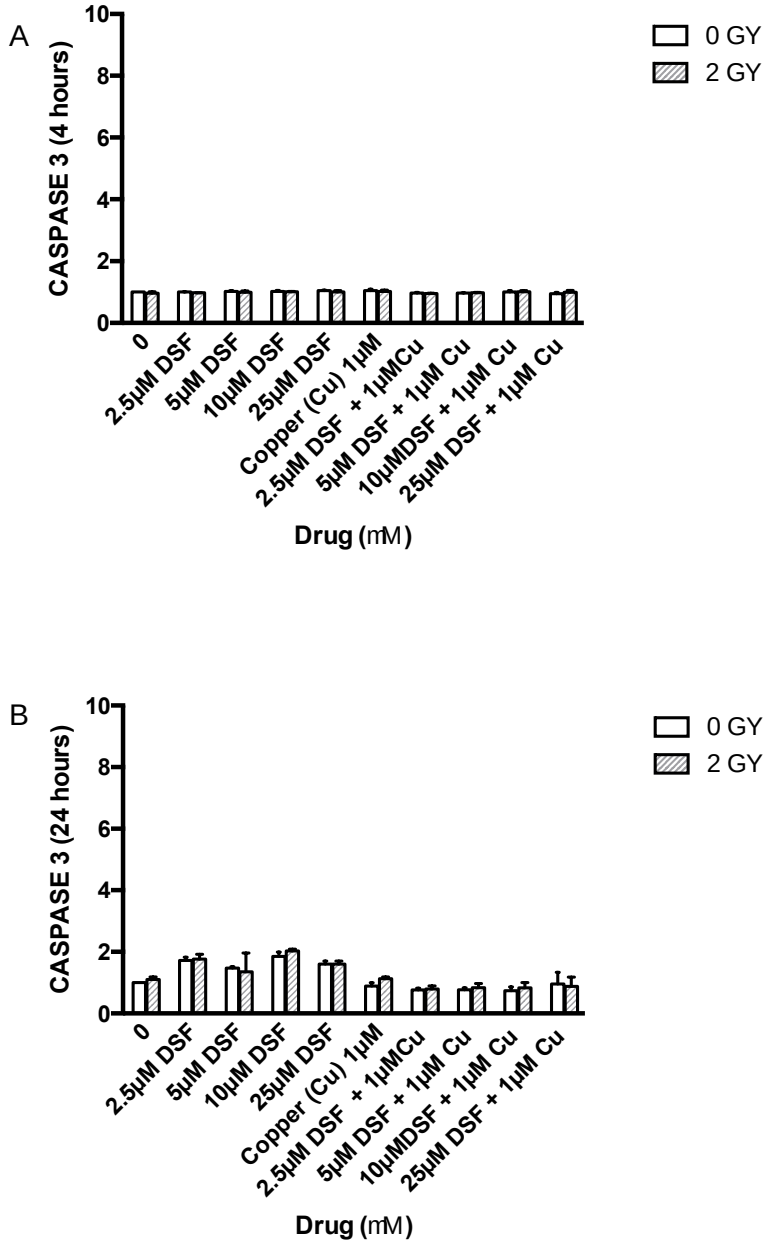


**Figure 28: Effect of disulfiram/copper ± 0 or 2 Gy on caspase-3 activity in the ACHN cell line.**

Cells were treated with different doses of disulfiram 2.5-25 μM for A) 4 hours and B) 24 hours with 0 or 2 Gy using a fixed dose of copper (Cu) of 1 μM and then caspase-3 activity was measured using a caspase-3 fluorometric assay. The chart shows the data as a fold increase following treatment in caspase-3 level compared with control cells. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with the control cells. Each value represents the mean ± SD of three separate experiments.

**Table 45: Statistical analysis of the effect of disulfiram/copper ± 0 or 2 Gy XBR on caspase-3 of the ACHN cell line after 4 hours**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 2.5 | 5 | 10 | 25 | Cu | Cu + 2.5 | Cu + 5 | Cu 10 | Cu 25 |
| **Control** | | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **2.5** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **5** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **10** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **25** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **Cu** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **Cu + 2.5** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **Cu + 5** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **Cu + 10** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **Cu + 25** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

Statistical significance of differences in caspase-3 activation in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

**Table 46: Statistical analysis of the effect of disulfiram/copper ± 0 or 2 Gy XBR on caspase-3 of the ACHN cell line after 24 hours**

| Dose range (µM) 0 GY | Dose range (µM) 2 GY | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **2.5** | **5** | **10** | **25** | **Cu** | **Cu + 2.5** | **Cu + 5** | **Cu 10** | **Cu 25** |
| **Control** | | NS | $p < 0.01$ | NS | $p < 0.0001$ | NS | NS | NS | NS | NS |
| **2.5** | $p < 0.05$ | NS | $p < 0.01$ | NS | NS | NS | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |
| **5** | NS | NS | NS | NS | $p < 0.01$ | NS | NS | NS | NS | NS |
| **10** | $p < 0.01$ | NS | NS | NS | NS | NS | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.01$ |
| **25** | NS | NS | $p < 0.001$ | NS | NS | NS | $p < 0.05$ | NS | NS | NS |
| **Cu** | NS | NS | $p < 0.001$ | NS | $p < 0.0001$ | NS | NS | NS | NS | NS |
| **Cu + 2.5** | NS | NS | NS | NS | NS | NS | NS | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |
| **Cu + 5** | NS | NS | NS | $p < 0.001$ | NS | NS | NS | NS | NS | NS |
| **Cu + 10** | NS | NS | NS | $p < 0.05$ | NS | NS | $p < 0.0001$ | $p < 0.0001$ | NS | $p < 0.001$ |
| **Cu + 25** | NS | NS | NS | NS | NS | NS | $p < 0.05$ | NS | NS | NS |

Statistical significance of differences in caspase-3 activation in the experiment described above. Statistical analysis was carried out using a one-way ANOVA with Bonferroni correction post hoc test to compare with control cells. GraphPadPrism 6.0. NS: not significant.

## 3.4 Discussion

Solid tumours like renal cell carcinomas require angiogenesis for growth. Therefore, inhibition of angiogenesis is one promising strategy for cancer therapy. Sunitinib and pazopanib are TKI whose mechanisms of action include anti-angiogenic activity. These drugs are currently considered the standard first-line therapy for metastatic RCC, a disease that is resistant to traditional chemotherapy and radiotherapy and has long had a very poor patient survival rate. However, several other modes of action beyond the anti-angiogenic activity of TKI may be important, including anti-proliferative and apoptotic induction modes of action have also been reported (135). Overall, the use of sunitinib and pazopanib has more than doubled the progression-free survival of patients with metastatic RCC compared with traditional treatments such as interferon-alpha or interleukin-6 (48).

The present research evaluated the cytotoxic potential of the TKI alone or in combination with external beam irradiation. Furthermore, the research also investigated the cytotoxic effects of disulfiram (with or without radiation) which had not previously been evaluated in renal cell cancer cell lines.

### 3.4.1 The cytotoxic effect of sunitinib and pazopanib as a single therapy

The data demonstrated that the incubation of renal cell line 786-O with sunitinib or pazopanib (1.25 – 20 μM) for either 24 or 48 hours reduced cell viability in a dose-dependent manner. There was a statistically significant difference in cell survival in cells treated with the drug compared with untreated cells ($p < 0.05$). Cell death was

enhanced by the longer incubation time of 48 hours compared with 24 hours for sunitinib concentrations of 1.25 - 5 μM ($p < 0.05$). For the higher concentrations of 10 and 20 μM sunitinib there was no statistically significant difference in cell death between 24 and 48 hours incubation. Likewise, in 786-O cells treated with pazopanib, exhibited a dose-dependant reduction in cell survival for concentrations of 1.25 - 20 μM incubated for 24 or 48 hours compared to untreated control cells ($p<0.05$). Cell death was enhanced by the longer incubation time of 48 hours compared to 24 hours for pazopanib concentrations of 1.25 – 5 μM ($p < 0.05$). However, for the higher concentrations of 10 μM and 20 μM pazopanib there was no statistically significant difference in cell death at 48 and 24 hours incubation.

Likewise, incubation of ACHN cells with sunitinib or pazopanib (1.25 – 20 μM) for either 24 or 48 hours also significantly reduced cell viability in a dose-dependent manner compared with control cells ($p < 0.05$). A significant difference in cell survival at 24 and 48 hours was only observed at the lower concentrations of 2.5 μM and 5 μM ($p < 0.05$).

Our findings are comparable with previous preclinical studies, which examined sunitinib in two human renal cell lines, 786-O and RCC4 (135). This study found that sunitinib could inhibit tumour cell growth for both cell lines in a dose-responsive manner but did not report the $IC_{50}$ value of sunitinib, so that comparison could not be made with our data (135). Another study reported that sunitinib could arrest the growth of human renal cancer cell lines, ACHN and A-498, in a dose-dependent manner (163). The study reported $IC_{50}$ values for sunitinib in the cancer lines of

between 4 – 10 μM which is in the range determined within the thesis (163). The observed differences between the $IC_{50}$ values could be due to the different experimental methods applied. In the previous research (163), cells were serum-starved in media containing only 0.1% FBS overnight before treatment with sunitinib. This is likely to make the cells synchronised, resulting in the need for a high concentration of the drug to kill an equivalent number of cells.

Another study evaluated sunitinib in the prostate cancer cell lines DU145 and PC3 by clonogenic assay and found that sunitinib was able to inhibit cell growth in a dose-dependent manner (140). In contrast, pazopanib examined in multiple myeloma (MM) cells in a bone marrow milieu was reported to decrease growth and survival of MM cells in a dose-dependent manner (164).

### 3.4.2 Evaluating cell viability and apoptosis activity of combination therapy: TKI agents with external beam radiation (XBR)

Sunitinib and pazopanib are currently used in the treatment of RCC with high efficacy but might cause a significant side effect, such as fatigue. Therefore, new approaches of therapy with high efficacy and low toxicity must be considered. Recent evidence has suggested that the labelling of RCC as radio-resistant is perhaps inappropriate and that radiotherapy may be a viable treatment option if used in combination therapy with current gold standard therapies. This project investigated how target therapy could be combined with external beam irradiation to enhance treatment efficacy and reduce potential toxicities such as fatigue (see

section 3.3.3) by potentially reducing the dose of TKI agents required to be administered for a suitable clinical effect.

There is accumulating scientific evidence for the potential therapeutic benefit of combining sunitinib with radiotherapy for different tumour cell lines such as breast and prostate cancer cells (140, 143). For example, one study used breast tumour cells (MDA-MB-231) treated with a single dose of radiation at 2, 4, 8, or 16 Gy alone and in combination with 1 μM of sunitinib (143). The study demonstrated a dose-dependent reduction in cell survival of tumour treated with this combination. In tumour cell line MDA-MB-231, the study demonstrated that sunitinib increased tumour cell death when combined with 8 and 16 Gy compared with cells treated with sunitinib alone ($p < 0.05$ and $p < 0.01$, respectively). However, there was increased tumour cell death when sunitinib was combined with a low dose of radiation (2 Gy and 4 Gy) compared with cells treated with 1 μM of sunitinib alone, but it was not statistically significant ($p > 0.05$) (143). That might be because a low fixed concentration of sunitinib had been combined with radiation.

Another study examined the effect of sunitinib on the survival of the human prostate cancer cell lines DU145, PC3, and LNCaP by clonogenic assay (140). This study reported that combining 100 nM sunitinib with 2 Gy XBR could significantly reduce the survival fraction in DU145 cells treated with the combination compared with cells treated with 2 Gy XBR alone ($p < 0.05$). In PC3, the survival fraction was also reduced but was not statistically significantly lower in cells treated with the combination therapy of 250 nM sunitinib with 2 Gy XBR, compared with cells

treated with 2 Gy XBR alone. However, LNCaP cells were not radio-sensitised by 250 nM sunitinib. The study also examined the mechanism behind this radio-sensitisation and reported that sunitinib could suppress the downstream signalling of growth factor receptors mediated by phosphorylation of both ERK and AKT in DU145 and PC3 cell lines (140).

These previous results revealed that using sunitinib or radiation alone delayed tumour growth. However, when combined, the agents synergised to result in improved cell kill. Therefore, combining TKI agents with radiotherapy in renal cell lines could further improve the efficacy of radiotherapy. Our experiments examined sunitinib and pazopanib (1.25 – 20 μM) in two renal cell lines, 786-O and ACHN, as a single therapy and in combination with 2 Gy of radiation.

In the 786-O cell line, results demonstrated the superior effect of combination therapy over either 1.25-5 μM sunitinib or pazopanib monotherapies ($p < 0.05$). For higher doses of drug, no statistically significant effects were observed. This most likely reflected that maximum cell kill had already been achieved with drugs alone and masked the cytotoxicity of the additional 2 Gy XBR. For future studies, it may be useful to use lower concentrations of TKI to better quantify the interactions between the TKI and XBR. In the ACHN cell line, results demonstrated the superior effects of combination therapy with 1.25 – 2.5 μM sunitinib and 2 Gy XBR ($p < 0.05$). Likewise, a similar effect was observed for pazopanib (1.25 - 5 μM) and 2 Gy XBR ($p < 0.05$).

There have been no previous preclinical studies that examined either sunitinib or pazopanib alone and in combination with radiotherapy in renal cell lines. To our knowledge, this is the first reported study experimenting with this combination using renal cancer cell lines, 786-O and ACHN. Therefore, for future work, conducting long-term response assays, such as a spheroid assays, and using lower dose ranges (< 5μM) of sunitinib and pazopanib will allow greater elucidation of the interactions between TKI and radiation.

The proposed mechanism of action of combination therapy between sunitinib and pazopanib with radiotherapy in radio-resistant cell lines such as renal cell lines is proposed to be via the inhibition of multiple kinase receptors which prevents downstream signalling pathways of these receptors, such as the Ras-Raf-MEK-ERK pathway and the PI3K/Akt pathway (165). Some studies have also reported the importance of these pathways in governing the radiation response in tumour cells (140, 166). Therefore, one of the proposed mechanisms illustrating that sunitinib and pazopanib could enhance the cytotoxicity in renal cell lines is through their ability to inactivate the Akt and ERK pathways.

Numerous preclinical studies have reported this mechanism, such as a study that examined sunitinib administered in combination with a high dose of radiation (8-16 Gy of XRT) in breast tumour cells (MDA-MB-231 cell line) *in vitro* by clonogenic survival and the assessing the apoptotic cell death by *in situ* end labelling (ISEL) assay (143). This study demonstrated that the combination of sunitinib and radiation significantly inhibited colony formation and increased apoptosis of tumour

cells compared with sunitinib alone (143). The study suggested that the activity of the PI3K/Akt signalling pathway was a potential mechanism that could enhance radiation response to sunitinib. In another preclinical study in prostate cancer cell lines DU145 and PC3, researchers reported that sunitinib could suppress the downstream signalling of growth factor receptors mediated by phosphorylation of both ERK and AKT (140). The results of this study suggest this inhibition could play a significant role in enhancing the cytotoxicity of this combination (140). Validation of this mechanism was outside the scope of this project but would be a priority in future work to allow determination of optimal temporal schedules and the composition of any therapies put forward for clinical translation.

Another proposed strategy that might enhance the cytotoxicity of radiotherapy in renal cancer cells is the hypothesis previously mentioned that sunitinib and pazopanib agents can induce normalisation of the functionality and structural integrity of tumour vasculature (167). This mechanism might lead to improving delivery of oxygen inside the tumour cells, which could enhance radiotherapy effects which work better in an oxygenated environment (144).

To explore whether the cytotoxic effect of these combinations resulted in enhanced apoptosis, our project examined the apoptotic response of 786-O and ACHN renal cancer cells to 2 Gy irradiation in combination with either sunitinib and pazopanib (1.25 µM, 2.5 µM, and 5 µM) using the caspase-3 assay.

**Apoptosis activity**

Apoptosis (programmed cell death) is one of the major death pathways in mammalian cells. However, suppression of apoptosis in cancer cells is critical in malignancy initiation and progression and has been identified as one of the key hallmarks of cancer (168). Our previous findings demonstrated that sunitinib and pazopanib, both alone and in combination with XBR, showed cytotoxicity against RCC lines as confirmed by the cell viability Alamar blue assay. Therefore, it was crucial to assess the mechanism involved in sunitinib-induced cell death. To achieve this, caspase-3 activity was used as a marker of apoptosis.

Our results demonstrated that RCC when incubated for 24 hours with sunitinib, as a single therapy or when combined with 2 Gy of XBR radiotherapy, could induce apoptosis in a statistically significant and dose-dependent manner compared with control cells ($p < 0.05$). Radiotherapy (2 Gy) enhanced the level of caspase-3 in a statistically significant way when combined with sunitinib compared with single treatment of sunitinib alone at concentrations of 1.25 µM, 2.5 µM and 5 µM in the 786-O cell line and only 2.5 µM and 5 µM in the ACHN cell line.

In addition, our results demonstrated that when renal cell lines were incubated for 24 hours with pazopanib, as a monotherapy and combination therapy with radiotherapy, could induce apoptosis in a statistically significant and dose-dependent manner compared with control cells ($p < 0.05$). Radiotherapy (2 Gy) enhanced the level of caspase-3 in a statistically significant way when combined with pazopanib compared with the single treatment of pazopanib alone at

concentrations of 2.5 µM and 5 µM in both 786-O and ACHN cell lines. In conclusion, sunitinib and pazopanib as single agents and in combination therapy with radiotherapy could induce apoptosis in both 786-O and ACHN cell lines and act as concentrations of 2.5 µM and 5µM. 2 Gy of XBR as monotherapy did not induce apoptosis in either 786-O or ACHN cells.

Our findings of induced apoptosis are consistent with a study that examined sunitinib in the human medulloblastoma cell line, Daoy (136). This study found that sunitinib induced apoptosis and arrested the growth of medulloblastoma tumour cells by inhibiting the Stat3 and Akt signalling pathways. The Stat3 pathway controls biological processes that include cell cycle progression, apoptosis, and tumour angiogenesis. The study demonstrated that sunitinib could inhibit cell proliferation and induce apoptosis in tumour cells, with associated inhibition of the Stat3 and Akt signalling pathways and their downstream genes involved in tumour cell survival and proliferation. Sunitinib also inhibited the expression of survivin, an anti-apoptotic protein, and downregulated cyclins D2, D3, E, all of which are involved in regulating the cell cycle.

These findings are consistent with a study that examined sunitinib in two human renal cell lines, 786-O and RCC4, to elucidate its mechanism of action of cytotoxicity (135). The study reported that sunitinib-induced tumour cell apoptosis in both renal cell lines and proved that it was correlated with Stat3 activity inhibition. Inhibition of Stat3 activity enhanced the anti-tumour effect of sunitinib. The study used Western blot gene assays to prove that sunitinib could reduce the expression of

several key apoptosis and pro-proliferation genes, including cyclin E, cyclin D, and survivin.

In conclusion, sunitinib and pazopanib could induce apoptosis in RCC lines. Radiotherapy alone did not affect the level of caspase-3, which is the key marker of apoptosis. However, when radiotherapy was combined with sunitinib or pazopanib, significant enhancement of caspase-3 was observed compared with cells treated with sunitinib or pazopanib alone.

### 3.4.3 Evaluating cell viability and apoptosis activity of disulfiram or copper as single therapies and in combination therapy: disulfiram ± copper with external beam radiation (XBR*)*

Disulfiram, an inhibitor of aldehyde dehydrogenase, has been used for several years for the treatment of alcoholism (154). Various preclinical studies have recently demonstrated its promise as an anti-cancer drug and radiosensitiser. The proposed mechanisms of action of the cytotoxic effect of disulfiram in tumour cells are the induction of oxidative stress and inhibition of proteasome activity (154).

The current research was to determine the cytotoxic effect of disulfiram in renal cancer cells. Previous studies have demonstrated that the cytotoxicity of disulfiram is copper-dependent or enhanced in the presence of copper. We therefore evaluated the combination of disulfiram with copper and evaluated the potential of disulfiram to enhance the anti-tumour efficacy of radiotherapy in renal cancer cells.

In order to test this hypothesis, we performed Alamar blue assays to evaluate the anti-cancer activity of this complex.

First, we investigated the cytotoxic effect of disulfiram as a single agent in renal cancer cell lines 786-O and ACHN by incubating the cells for 24 hours with a dose range of disulfiram (2.5 – 25 μM). Our results showed that the cytotoxic effect of disulfiram in the 786-O cell had an initial dose responsive toxicity with a sharp decline in cell survival between administered doses of 2.5 μM (77% cell survival) and 7.5 μM (15% cell survival) observed. At higher administered concentrations of > 7.5 μM to 15 μM the cytotoxicity decreased to an approximate cell survival level of 20%. Cell viability then decreased again at higher doses of 20 μM and 25 μM, with cell survival of less than 10%.

There have been no previous reports of assessment of the cytotoxicity of disulfiram in this cell line. However, in the ACHN cell line, the cytotoxic effect of disulfiram demonstrated a very different cytotoxicity profile. Our data showed a distinct U-shaped response to disulfiram as seen in Figure 15. There was a sharp decline in cell survival after administration of 2.5 μM disulfiram while cell survival was maximal at the administration of 5 μM. Administration of higher concentrations of disulfiram resulted in a rise in cell survival with concentration which plateaued at 15 μM. Our data also concluded that the 786-O renal cell line was more sensitive to disulfiram than the ACHN cell line. The $IC_{50}$ for 786-O was 3.3 μM while the $IC_{50}$ of the ACHN cell line was 37.7 μM. This conclusion is consistent with a previous study which

reported that the ACHN cell line was among the more resistant cell lines of various tumour cell line that had been examined (169).

In addition, we investigated a dose range of disulfiram (2.5 μM, 5 μM, 10 μM and 25 μM) with 1 μM copper in both the 786-O and ACHN renal cell lines. Then, we investigated the radio-sensitising effect of disulfiram alone, copper alone, and a combination of disulfiram with copper in both the 786-O and ACHN cell lines. Our results in both renal cell cancer lines, 786-O and ACHN, demonstrated that copper enhanced the cytotoxic effect of disulfiram only at 2.5 μM in both cell lines, and at 10 μM in ACHN. Therefore, there was no significant enhancement of toxicity of disulfiram afforded by the addition of copper at higher administered doses of disulfiram. Furthermore, our data indicated that copper alone, when incubated with both cell lines, did not demonstrate any cytotoxic effect in renal tumour cells. Our data also showed that there were no statistically significant differences in cytotoxicity in either cell line when 2 Gy of XBR was added with disulfiram relative to treatment with disulfiram alone ($p > 0.05$), with copper alone ($p > 0.05$), or with the disulfiram and copper complex ($p > 0.05$).

From our data, it is apparent that the cytotoxicity of disulfiram is not a simple dose-response in 786-O cells, and the U-shaped survival curve seen in ACHN cells suggests that a more complex biological mechanism underlies the response of cells to this drug. While there have been no previous reports of the activity of disulfiram in 786-O cells, the response observed in our study of 786-O cells treated with disulfiram was demonstrated more obviously in previous preclinical studies using

other cancer cell lines (169). The first such demonstration was in a study by Rae et al. that examined the cytotoxic effect of disulfiram in human neuroblastoma-derived SK-N-Be (2c) cells by clonogenic assay (154). This study reported that the cytotoxic effects of disulfiram had a biphasic response. The study found that the maximum cytotoxic effect of disulfiram was after administration of 1.7 μM, with partial reversal of cytotoxicity up to 10 μM disulfiram. After that, cytotoxicity increased after administration of 17 μM disulfiram (154). In another unpublished study from our group, the cytotoxic effect of disulfiram was examined by clonogenic assay in two human glioma cell lines, UVW and T98g. These data indicated that disulfiram's cytotoxic effect was again a biphasic response in both cell lines (D. Scott, 2016, personal communication).

These responses, which mirror the data presented here, were suggestive of more than one mechanism of action in the disulfiram effect. Rae et al. (154) examined the mechanism of this biphasic cytotoxic effect of disulfiram by treating the cells with 1 mM of the antioxidant N-acetyl-L-cysteine (NAC). They found that the antioxidant could prevent the reduction in clonogenic survival of neuroblastoma cells induced by a dose of disulfiram of more than 10 μM (154). However, there was no significant effect on disulfiram-induced toxicity at administered doses of 10 μM or less. Therefore, the study suggested two mechanisms of disulfiram cytotoxicity: one mechanism of action is reactive oxygen species (ROS)-dependent (as this was perturbed by addition of NAC), and a ROS-independent mechanism of cell kill which predominates at low concentrations of disulfiram. This observation could justify our results, which showed that copper enhanced the cytotoxicity of disulfiram only

at low doses. Previous reports have demonstrated that copper, when combined with disulfiram, can enhance the cytotoxic effect of disulfiram by generating reactive oxygen species via inhibition of proteasome activity (147, 170, 171). Consequently, these data taken together suggest that disulfiram could be cytotoxic even at low concentration and have enhanced cytotoxicity when combined with copper at low concentrations of disulfiram. Further experiments examining full dose curves of *in vitro* and *in vivo* models are recommended.

Additionally, another preclinical study examined the cytotoxic effect of disulfiram alone and with copper on a variety of human glioblastoma (GBM) cell lines (152). The study observed that the cytotoxic effects of disulfiram on GBM cell lines were copper-dependent, but once again this was dependent on the concentration of disulfiram (152). This study demonstrated a similar pattern of both disulfiram alone and disulfiram-copper cytotoxicity as observed in our study with 786-O and ACHN renal cancer cells. The study by Liu et al. (152) suggested that copper plays a crucial role in redox reactions and triggers the generation of reactive oxygen species in human cells. Disulfiram forms a complex with copper called the disulfiram-copper complex and improves the transport of copper into cancer cells when the two are combined. In comparison with Cu, the DS/Cu complex is a stronger ROS inducer. ROS are a group of reactive oxygen-containing chemical species, normally generated from a mitochondrial respiratory chain reaction. High ROS activity can damage DNA, protein, and lipids, leading to cancer cell death (152). The suggested mechanism by Liu et al. (152) may well be identical for the present work in RCC.

Another proposed mechanism for the cytotoxic response of disulfiram has been suggested to occur at concentrations greater than 10 μM disulfiram, which involves the induction of oxidative stress (147, 148). As mentioned in section 3.1.5, tumour cells have increased oxidative stress compared with normal cells (148). Thus, tumour cells will be more susceptible to therapy that is designed to further elevate oxidative stress beyond a threshold that will trigger cell death (151). Therefore, this is another mechanism which could be contributory in our studies at the higher concentrations used. Further experiments are recommended to better understand the cytotoxicity mechanism of disulfiram with and without copper in renal cancer cells.

In the ACHN cell line, disulfiram demonstrated a very different cytotoxicity profile comparing with the 786-O cell line. Our data demonstrated that the response of this cell line to disulfiram was not the classic dose concentration-response correlation. The ACHN cell line exhibited a U-shaped response to disulfiram. Several studies have reported that some molecules are only active at low doses and not at higher doses, exhibiting a U-shape dose responsive curve. Molecules said to exhibit such activities include endostatin, interferon-α, and an integrin ATN-161 molecule in different cancer cell lines (172). A preclinical study demonstrated that the growth of human pancreatic cancer cell lines, BxPC-3 and AsPC-1, were inhibited by a low dose of endostatin, but the therapeutic efficacy was lost once the dose increased (173).

**Combined with radiotherapy (2 Gy of XBR)**

Our results surprisingly showed that there was no statistically significant difference in cell survival with the addition of 2 Gy of XBR to disulfiram alone, copper alone or with the complex (disulfiram and copper) in 786-O and ACHN cell lines. One possible explanation for the lack of radio-sensitisation is the fact that in the absence of radiation the disulfiram plus copper alone resulted in an almost 90% reduction in cell survival in most of the dose range used, rendering it difficult statistically measure additional benefit from the addition of a further cytotoxic insult in the form of radiation. This potential synergism could be better investigated by the employment of more rigorous statistical tools such as combination index analysis as described by the Chou-Talalay method in both two renal cell lines, 786-O and ACHN. This is, therefore, the obvious next step to determine whether there is a true absence of synergism between the three components. Assessment of scheduling dependence is also necessary, as the temporal order in which reagents are given could be crucial to determining synergy as only concomitant administration was examined in our study due to time constraint.

A further possibility, as explained in the introduction to this chapter, is that inhibition of the proteasome/NF-kB pathway by disulfiram plays a key role in causing the radio-sensitising properties of disulfiram. The NF-kB pathway has been shown to be an oxidative stress sensor (155). Furthermore, the NF-KB pathway has been shown to be activated in response to radiation and to confer radio-resistant properties on tumour cells (156, 157). Previous results from our lab and

publications have also demonstrated the radio-sensitising properties of disulfiram in other tumour cell lines. Therefore, a possible explanation for the failure of disulfiram to radio-sensitise is perhaps due to an inability to inhibit this pathway in renal cancer cells 786-O and ACHN, meaning disulfiram's effects may be cell or tissue specific. However, this hypothesis will need to be examined in future studies. The present experiments were performed using an Alamar blue assay. This is a short-term assay, which is less ideal for radiation studies as it is done over a 24-hour period, and it has been reported that the effects of radiation can take longer to accumulate (154). Therefore, for future studies, researchers need to look at other long-term assays such as spheroids and *in vivo* models.

In contrast, the study that examined disulfiram alone and with copper in neuroblastoma cell SK-N-BE (2c) found that disulfiram could work as radio-sensitisation in neuroblastoma cells and that the effects of disulfiram were enhanced by addition of copper, resulting in proteasome inhibition (154). The study showed that radiotherapy alone could decrease the tumour growth in a clonogenic survival assay, whereas in the present study both renal cancer cell lines did not appear to be affected by radiotherapy alone. The study also showed that the cytotoxicity of disulfiram was enhanced when combined with radiotherapy in neuroblastoma cells. This suggested that proteasome/NF-kB pathway inhibitors might be associated with the radiosensitiser effects of disulfiram. Therefore, we could speculate that positive outcomes may occur when radio-sensitivity of disulfiram alone or when combined with copper is associated with tumour types that are sensitive to radiotherapy, such as neuroblastoma.

**Apoptosis activity**

We investigated the apoptosis cell death mechanisms of disulfiram and copper, alone and when combined with radiotherapy (2 Gy of XBR) in 786-O and ACHN cell lines. The time courses used were 4 hours and 24 hours of incubation. Results in both cell lines were comparable with results of those agents in terms of cell viability. In 786-O cells, our data showed that disulfiram alone induced apoptosis by increasing caspase-3 significantly compared with controls after both 4 and 24 hours' incubation. However, in the ACHN cell line, disulfiram-induced apoptosis only significantly elevated caspase-3 at 24 hours compared with the control cells, but in the 786-O cell line, disulfiram induced caspase-3 expression at 4 and 24 hrs. Neither cell line showed any apoptosis effect when administered copper or radiotherapy alone, and there were also no enhancements when copper was added to disulfiram.

The proposed mechanism through which disulfiram could induce apoptosis in renal cancer cell lines is through proteasome inhibition and NF-kB, which are important anti-apoptotic factors (147, 149, 152). The disulfiram has been reported to induce apoptosis selectively in cancer cells, but not in normal cells (149).

The present results that showed that disulfiram could induce apoptosis in renal cancer cells are aligned with previous studies, one of neuroblastoma and one of human glioblastoma cell, in response to disulfiram (152, 154). Additional research has demonstrated that disulfiram induced apoptosis in glioblastoma cells via modulation of the BcI2 family. Another preclinical study demonstrated that disulfiram induced apoptosis in human melanoma cells (148). Although the present

research was inconclusive, additional research is warranted into the use of disulfiram with or without copper as these preliminary results did show some promise.

# Chapter 4: General discussion and conclusions

## 4.1 General discussion

In the United Kingdom, renal cancer accounts for around 3% of adult cancer cases, with more than 11,000 new cases per year and around 4,000 deaths annually (4). The incidence of renal cancer in the UK has been increasing over time. In 1975, there were 5.15 cases per 100,000 compared with 21 cases per 100,000 in 2013 (4). This sharp increase in the incidence of renal cancer might be due in part to the more widespread use of diagnostic imaging techniques with greater resolution which have identified more cases, whilst there has been an increasing prevalence of common risk factors for renal cancer such as obesity and a continuing high incidence of smoking within the population. The growing incidence of renal cancer provides researchers with the impetus to, first, improve the tolerability of first-line therapeutic agents for renal cancer, which have shown enhancements in treatment outcomes compared to previous gold standard treatments, and second, to examine the use of different combinations of these agents to attempt to realise higher efficacy and lower toxicity.

Before 2009, therapeutic options for the treatment of patients with metastatic renal cell carcinoma (mRCC) were largely limited to cytokine therapies, IL-2 and INF-α, which provided modest response rates and significant toxicity (27). Therefore, there was an important need for efficacious and better-tolerated therapeutic options. These arrived in 2009, with the advent of the tyrosine kinase inhibitor sunitinib (Sutent®; Pfizer) for metastatic renal cell carcinoma, followed in 2011 by

pazopanib (Votrient®; GlaxoSmith-Kline), both of which are licensed as first-line treatments in NICE guidelines (42, 46).

Two TKI agents have been approved for the treatment of RCC, including oral sunitinib, at 50 mg/day for four weeks, followed by two weeks' therapy free (wash out period), and continuing oral pazopanib treatment at 800 mg/day. The efficacy of sunitinib in metastatic RCC underwent clinical evaluation in a large-scale, randomised, phase III study that compared sunitinib (n = 375) with IFN-α (n = 375) in renal cancer patients (31). The progression-free survival (PFS) time was longer in the sunitinib group than in those treated with IFN-α (PFS, 11 months vs. 5 months; $p > 0.001$). Pazopanib was assessed in a phase III study of RCC patients undertaken by Sternberg et al., who reported that pazopanib was clinically efficacious, as demonstrated by a longer PFS interval in the overall study population (9.2 months for the pazopanib group vs. 4.2 months for the placebo group; $p > 0.0001$) (47). Although efficacious, sunitinib and pazopanib are associated with significant side effects. Fatigue is considered to be the most common side effect, experienced by around 55% of renal cancer patients receiving these drugs (34). Diarrhoea, nausea, hypertension, and hand-foot syndrome also are highly reported with use of these two agents. Two other TKI agents, sorafenib and axitinib, have also been approved for their efficacy in renal cancer, but are not yet licensed as first line treatment options. The TARGET trial examined the efficacy of sorafenib and proved to be an effective agent for metastatic renal cancer patients compared with a placebo (174). Both axitinib and sorafenib are used as a second-line treatment for mRCC in a clinical setting. However, the AXIS trial compared these two agents as a second line

treatment and proved that axitinib has significantly longer PFS compared with sorafenib in advanced renal carcinoma patients (175). In addition, NICE guidelines recommend axitinib only as a second-line therapy after failure of first-line treatment with a TKI agent (176). Therefore, in the Beatson West of Scotland Cancer Centre, the clinical team used axitinib as a second line option with renal cancer patients receiving sunitinib or pazopanib.

Fatigue continues to be a significant problem associated with the majority of available RCC treatments, especially the newly approved agents, sunitinib and pazopanib, with approximately half of patients reporting all-grade fatigue, and up to one-third of patients reporting fatigue of grade 3 or 4 severity (75). Fatigue is also a significant side effect in second line treatments with axitinib and sorafenib (174, 177). Therefore, the aim of this research was to accurately assess the nature of fatigue in mRCC receiving sunitinib or pazopanib using FACIT-F tool. Furthermore, fatigue might be influenced by many factors that negatively impact the incidence or severity of fatigue. Therefore, this study also examined these confounding factors and determined whether there was a correlation between individual patient's fatigue scores and these factors. Cancer symptoms like depression, sleep disturbance, and lack of appetite might also aggravate the fatigue score. Thus, this study also examined the correlation between fatigue score and cancer symptoms scores. Finally, this study evaluated the impact of pazopanib or sunitinib on the quality of life of renal cancer patients. None of the previous trials discussed the nature of fatigue or even the possibly related confounding factors. This research

was conducted to better understand fatigue side effects to ultimately decrease its negative impact on renal cancer patients receiving sunitinib or pazopanib.

Overall, the data from this study demonstrated that the fatigue score in RCC patients in four consecutive treatment cycles was within a range of 29.5 to 31.8. These fatigue scores are lower than the proposed cut-off point for indication of fatigue as designated by the FACIT-F tool, which is < 34 (103). Therefore, based on the data from this trial, it is possible to conclude that these renal cancer patients receiving sunitinib or pazopanib were most likely to be diagnosed with fatigue when receiving the agent. These fatigue score results are unlike the PISCES trial, which examined the same target patients and reported that patients have less severe fatigue compared to the results of the present study (48). This differentiation might result from the fact that the patients in the PISCES trial were treatment naïve compared with the participants in the current study, who received systemic or target agents before enrolling into the research. Also, the fatigue score in the current study was measured for a longer duration of receiving agent (four consecutive cycles) than in the PISCES trial (two consecutive cycles). It is possible that a longer duration of receiving a drug might lead to a more accurate assessment of fatigue (48).

Based on the literature, fatigue in cancer patients seems to be a multifaceted, personal, and physiological condition influenced by factors of cancer treatment. For example, some previous studies reported that low haemoglobin levels and hypothyroidism might contribute and exacerbate the severity of fatigue (34, 59, 70).

However, as shown in Table 17, the present results demonstrated that none of the examined confounding factors has a significant, direct influence in fatigue score of renal cancer patients.

In addition, this research demonstrated that cancer symptoms measured by the MDASI tool were highly correlated with the severity of fatigue. Previous studies have consistently reported that severity of fatigue increased as cancer symptoms increased (108 - 111). Finally, these results showed that sunitinib and pazopanib have a mild influence on the activity and mood daily life of renal cancer patients, with no statistically significant differences between these two agents. This, however, is inconsistent with the COMPARZ trial, which reported that patients favoured pazopanib over sunitinib in term of quality of life (34). This differentiation could be related to a different assessment time (Day 28), which was biased toward the pazopanib agent, because it may not capture the recovery of patients in sunitinib during the two-week washout period.

As a result, sunitinib and pazopanib as single agents are still associated with significant side effects, including fatigue. Moreover, these two agents are licensed to be administered to renal cancer patients for life. Therefore, there is a clear unmet need to improve the management of cancer-related fatigue in RCC. This improvement can be partially achieved through the use of more efficacious clinical supportive care of the treatment of fatigue in all renal cancer patients before, during, and after treatment to minimise fatigue and enhance patient tolerability of therapy.

Effective counselling regarding how to deal with fatigue is recommended for renal cancer patients who are receiving sunitinib or pazopanib. A 'Coping with Fatigue' booklet produced by the patient information editorial team at Macmillan Cancer Support and a locally produced information sheet to help patients manage their fatigue is recommended for all patients (112).

Results from the present study measured side effects, fatigue, and other cancer symptoms at the end of each cycle, at week six, and after the washout weeks for sunitinib. In contrast, the COMPARZ trial conducted measurements at week four. The present study did not find any significant toxicity difference between sunitinib and pazopanib, but the COMPARZ trial did. Therefore, it appears that week four in patients receiving sunitinib is the peak for fatigue and other side effects. It is recommended that health care professionals educate patients receiving sunitinib about the peak of fatigue, and inform them that the severity of side effects will decrease in the last two weeks (washout weeks). It is preferable to take a break from work or other activities and have more rest time during this period.

An interesting approach to consider is to use systemic therapy intermittently instead of continuously to decrease the incidence and severity of fatigue and lower the impact of sunitinib or pazopanib on patients' quality of life. Patients in a palliative setting receiving intermittent systemic therapy will benefit if survival is unimpaired, toxicities are reduced, and quality of life is improved (178). This theory is being examined in an ongoing study by Frouws et al. (178) in unresectable metastatic colorectal cancer (178). Therefore, it is recommended to examine this

theory in mRCC patients receiving sunitinib or pazopanib in the future to achieve low incidence of diagnostic fatigue and other significant cancer symptoms that might influence quality of life.

Another recommendation that could be applied at the renal clinic in the Beatson West of Scotland Cancer Centre is to use a Heng score, instead of a Motzer score, as an assessment sheet at baseline for the patient to predict survival rate. Data collected at baseline demonstrated that both Heng and Motzer scores, 1.28 and 1.29, respectively, were in an intermediate risk group (intermediate group when scored from 1 to 2). However, the median survival rate for the Heng score is 22.5 months, but 10 months for the Motzer score. Furthermore, the Heng score was determined from a study that examined renal cancer patients receiving target agents, sunitinib, sorafenib, or bevacizumab, while the Motzer score was identified from a study of renal cancer patients receiving cytokine therapy. Most of the new diagnostic renal cancer patients at the clinic started with sunitinib or pazopanib agents. Therefore, using the Heng score sheet to predict survival rate instead of Motzer score sheet is recommended in Beatson West of Scotland Cancer Centre.

On the other hand, the existing TKI agents are not curative, so there is still an urgent need for novel agents with greater efficacy and better safety profiles than the currently approved agents for RCC. Therefore, some new therapies currently under investigation with mRCC patients such as vandetanib tablet (Caprelsa®) kinase inhibitor therapy have been approved for the treatment of symptomatic or progressive medullary thyroid cancer (179). However, an alternative approach to

the costly and time-consuming development of new drugs is to use existing drugs in novel combination schemes where multiple therapeutic approaches are used such as combined radiotherapy with TKI agents. This strategy has advantages by reducing the cost and time involved in development of novel drugs.

Radiotherapy has been used in the management of different types of cancer, but has not gained routine clinical application in renal cancer due to their perceived radio-resistance and has been mainly used as a palliative care therapy or adjuvant therapy following nephrectomy surgery in renal cancer patients (27). Therefore, exploring the possible advantage of a TKI as a radiosensitiser whilst trying to minimise the adverse effects of TKI agents with similar outcomes was worthy of exploration.

In the last few years, several preclinical trials have reported promising results when combining radiotherapy with anti-angiogenesis agents, which inhibit the growth of new tumour vessels, such as target therapy (180). These trials found that sunitinib could enhance the radio-response of prostate and breast cancer cells, *in vitro* and *in vivo* (140, 143). The proven mechanism of this combination was that sunitinib could inhibit the downstream signalling of growth factor receptors mediated by the PI3K-AKT and ERK pathways. These pathways are also associated with radio-resistance of tumour cells (140), so inhibiting the activity of the PI3K/AKT signal pathway is a potential mechanism to enhance the radiation response by sunitinib (140, 141). Therefore, there is a growing interest in examining this combination in RCC patients. The present research examined the combination of sunitinib or pazopanib

with radiotherapy in two renal cell lines, 786-O and ACHN, using Alamar blue assays. This research found that sunitinib and pazopanib could enhance the cytotoxicity of radiotherapy in renal tumour cells and works as a radiosensitive agent. This study also examined the apoptosis activity, which is one of the major death pathways in mammalian cells, of this combination using caspase-3 apoptosis assays. This research also demonstrated that combination therapy significantly enhanced the level of caspase-3 more than single treatment with sunitinib or pazopanib alone in renal tumour cells, 786-O and ACHN.

In addition, the present results proved that combining radiation with the $IC_{50}$, which is the drug concentration required to kill half of the tumour cells, of sunitinib or pazopanib was lower than the $IC_{50}$ of sunitinib or pazopanib used alone. The dose reduction of TKI agents might help avoid the serious side effects that can occur by combined TKI agents with radiotherapy when this combination is adopted. Combining TKI agents with radiation could achieve the high efficacy of cytotoxicity with lower toxicity in tumour cells, and is supported by numerous preclinical trials, such as that carried out by Kleibeuker et al. (181), which examined the effect of combining sunitinib and ionising radiation in a colon cancer cell line, HT29. The study reported an improvement in the therapeutic efficacy of combined sunitinib with radiation, when proper dose scheduling was applied, and showed that combination allows halving the dosage of sunitinib without loss of therapeutic efficacy (181). Another trial, a phase I study of combined sunitinib and image-guided radiotherapy in 21 patients with oligometastases, concluded that decreasing sunitinib doses from 50 mg to 37.5 mg or 25 mg resulted in lower toxicity rates

without losing cytotoxic efficacy (182). Therefore, it is recommended that further research be conducted for this combination in renal cancer cell lines to achieve the optimal treatment regimens and permit dose reduction of TKI agents, which likely reduce the side effects of this combination therapy in a clinical setting.

Disulfiram is an inhibitor of aldehyde dehydrogenase and used for the treatment of alcoholism. In the past few years, several researchers have reported its potential anti-cancer properties (147 – 149) and that it works as a radiosensitiser (151, 153). Therefore, the present study also investigated the cytotoxic effects of disulfiram not previously interrogated in renal cell cancer, alone and in combination with radiation. Results showed that disulfiram as a single therapy has a cytotoxic effect on renal cell lines, 786-O and ACHN. The 786-O cell line was more sensitive to disulfiram than ACHN, which might need to be administered with a higher concentration of disulfiram. Copper was previously shown to enhance the effect of disulfiram (149, 151). In the present study, copper also enhanced the cytotoxic effect of disulfiram only at its lowest dose. This could demonstrate that the disulfiram-copper complex is a stronger ROS inducer leading to cancer cell death (152). At high doses, however, disulfiram has the ability to induce oxidative stress and cause tumour cell death by itself (147 – 148). On the other hand, radiotherapy did not show any benefit when combined with disulfiram or disulfiram-copper complex. In addition, results demonstrated that disulfiram induced apoptosis in both renal cell lines, 786-O and ACHN, in almost the same analogue response in cell viability. Neither copper nor radiotherapy showed any apoptotic effect on these cell

lines. Therefore, more rigorous investigation is recommended in the future, using, for example, Spheroid assays.

To elucidate whether the radio-sensitising potential of TKI agents and dose reduction in the combination of TKI agents with radiotherapy have similar benefits in renal cancer patients, the next phase of examination of this combination in renal cancer cells is warranted. Future studies should examine the radio-sensitising potential of TKI agents, sunitinib and pazopanib, in long term cell viability assays like Spheroid assays and mechanism assays like Western Blot assays, as well as *in vivo* models. Results showed that high concentrations of sunitinib or pazopanib, 10 μM and 20 μM, caused a high level of tumour cell death. Therefore, using lower dose ranges (< 5 μM) of sunitinib and pazopanib will allow greater elucidation of the interactions between TKI and radiation. Examination of the scheduling of combination therapy, TKI with radiation, will achieve better models of these promising combinations in clinical practice.

In addition, our disulfiram experiments demonstrated interesting results in two renal cancer cell lines, both as a single agent and when combined with a low dose of copper. Therefore, more investigations should be conducted (with more dose ranges of disulfiram less than 2.5 μM) using *in vitro* and *in vivo* models to better understand the cytotoxicity mechanism of disulfiram with or without copper in the renal cell line. The present results failed to show radio-sensitising effects of disulfiram in renal cell lines, but this will need to be examined in future studies.

Therefore, for future work, researchers need to look at long-term assays like Spheroid assays.

## 4.2 Conclusions

In conclusion, results demonstrate that fatigue is a significant problem associated with the majority of renal cancer patients receiving sunitinib or pazopanib. The findings also showed that the FACIT-F score in renal cancer patients was not correlated with age, gender, or the clinical and laboratory variables that were examined. In addition, there was a significant statistical correlation between cancer symptom distress and severity of fatigue in renal cancer patients. Finally, there was a mild influence of sunitinib and pazopanib on patients' daily quality of life. As a result, sunitinib and pazopanib as single agents are still associated with significant side effects, including fatigue. Therefore, there is still an urgent need for novel combination with greater efficacy and better safety profiles than the currently approved agents for RCC. In preclinical investigations, these finding showed that TKI agents could improve the radiosensitivity of the renal cell line and showed an interesting option for the management of renal cancer in hope to discover novel combination regimen with the same efficacy and less toxicity. Finally, disulfiram showed potential anti-cancer properties in renal cancer cell lines.

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# 6. Appendix

## 6.1Collection form

**Collection form:** Patient Reference number

| | | | | | |
|---|---|---|---|---|---|
| Sex | Male | Female | | | |
| Age | <60 years | ≥60 years | | | |
| Motzer Criteria | 0 points | 1 point for each of shaded items below | | | |
| **Haemoglobin levels: Lower Limit normal (LLN): male: 130 g/L, female: 115 g/L** | ≥ LLN | < LLN g/L | | | |
| Lactate Dehydrogenase (LDH) levels at baseline | ≤ 360 U/L | > 360 U/L | | | |
| **Corrected serum calcium levels at baseline** | ≤ 2.5 mmol/L | >2.5 mmol/L | | | |
| **World Health Organisation (WHO) Performance Status** | 0 or 1 | ≥ 2 | | | |
| **Interval between cancer diagnosis and initiation of systemic treatment (or recurrence after nephrectomy)** | ≥12 months | <12 months | | | |
| Total Motzer Score | | | | | |
| **Neutrophil count** | ≤ ULN | > ULN | | | |
| **Platelet count** | ≤ ULN | > ULN | | | |
| **Total Heng Score** | **Previous Bold 6 items:** | | | | |
| Tumour stage | I, II | III, IV | | | |
| Histology | Clear cell RCC | Non–clear cell RCC | | | |
| Does the participant have metastatic cancer? | No | Yes, circle site(s) | Brain | Liver | Lung |
| | | | Lymph node | Bone | Other |
| History of nephrectomy? | No | Yes | | | |
| History of cytokine therapy: interferon-alpha or interleukin-2 (IL-2)? | No | Yes | | | |
| Type of TKI | Sunitinib | Pazopanib | | | |
| Duration of treatment on a TKI at inclusion into study | ≤ 4 treatment cycles | > 4 treatment cycles | | | |
| Thyroid Function Test (TFT); Thyroid Stimulating Hormone | TSH > 5 mU/L | ≤ 5 mU/L | | | |
| Did the participant receive thyroid replacement therapy? | No | Yes | | | |
| Did the participant have co-morbidities disease? | No | Yes, record on separate sheet | | | |
| Did the participant receive any concurrent medication(s)? | No | Yes, record on separate sheet | | | |
| Fatigue score | | | | | |

Edited by..... Date / /

Cycle .....

| | | |
|---|---|---|
| Thyroid Function Test (TFT); Thyroid Stimulating Hormone | TSH > 5 mU/L | ≤ 5 mU/L |
| Did the participant receive thyroid replacement therapy? | No | Yes |
| Have there been any changes to the co-morbidities since the last treatment cycle? | No | Yes, record on separate sheet |
| Have there been any changes to the medication since the last treatment cycle? | No | Yes, record on separate sheet |
| Haemoglobin levels: Lower Limit normal (LLN): male: 130 g/L, female: 115 g/L | ≥ LLN | < LLN g/L |
| Fatigue score | | |

Edited by..... Date / /

Cycle .....

| | | |
|---|---|---|
| Thyroid Function Test (TFT); Thyroid Stimulating Hormone | TSH > 5 mU/L | ≤ 5 mU/L |
| Did the participant receive thyroid replacement therapy? | No | Yes |
| Have there been any changes to the co-morbidities since the last treatment cycle? | No | Yes, record on separate sheet |
| Have there been any changes to the medication since the last treatment cycle? | No | Yes, record on separate sheet |
| Haemoglobin levels: Lower Limit normal (LLN): male: 130 g/L, female: 115 g/L | ≥ LLN | < LLN g/L |
| Fatigue score | | |

Edited by..... Date / /

Cycle .....

| | | |
|---|---|---|
| Thyroid Function Test (TFT); Thyroid Stimulating Hormone | TSH > 5 mU/L | ≤ 5 mU/L |
| Did the participant receive thyroid replacement therapy? | No | Yes |
| Have there been any changes to the co-morbidities since the last treatment cycle? | No | Yes, record on separate sheet |
| Have there been any changes to the medication since the last treatment cycle | No | Yes, record on separate sheet |
| Haemoglobin levels: Lower Limit normal (LLN): male: 130 g/L, female: 115 g/L | ≥ LLN | < LLN g/L |
| Fatigue score | | |

Edited by..... Date / /

**Collection form**

Patient Reference number

| | | |
|---|---|---|
| The participant has co-morbidities disease: | | The participant has concurrent medications: |
| Cycle 1 | | |
| Changes of the co-morbidities since last cycle: | | Changes of concurrent the concurrent medications since last cycle: |
| Cycle 2 | | |
| Cycle3 | | |
| Cycle 4 | | |

# 6.2 Functional Assessment Chronic Illness Therapy-Fatigue

**FACIT Fatigue Scale (Version 4)**

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

| | | Not at all | A little bit | Some-what | Quite a bit | Very much |
|---|---|---|---|---|---|---|
| HI7 | I feel fatigued | 0 | 1 | 2 | 3 | 4 |
| HI12 | I feel weak all over | 0 | 1 | 2 | 3 | 4 |
| An1 | I feel listless ("washed out") | 0 | 1 | 2 | 3 | 4 |
| An2 | I feel tired | 0 | 1 | 2 | 3 | 4 |
| An3 | I have trouble starting things because I am tired | 0 | 1 | 2 | 3 | 4 |
| An4 | I have trouble finishing things because I am tired | 0 | 1 | 2 | 3 | 4 |
| An5 | I have energy | 0 | 1 | 2 | 3 | 4 |
| An7 | I am able to do my usual activities | 0 | 1 | 2 | 3 | 4 |
| An8 | I need to sleep during the day | 0 | 1 | 2 | 3 | 4 |
| An12 | I am too tired to eat | 0 | 1 | 2 | 3 | 4 |
| An14 | I need help doing my usual activities | 0 | 1 | 2 | 3 | 4 |
| An15 | I am frustrated by being too tired to do the things I want to do | 0 | 1 | 2 | 3 | 4 |
| An16 | I have to limit my social activity because I am tired | 0 | 1 | 2 | 3 | 4 |

# 6.3 M.D. Anderson Scale Inventory

**Date:** _____ **Institution:**_____

**Participant Initials:** _____ **Hospital Chart #:**_____

**Participant Number:** _____

## M. D. Anderson Symptom Inventory (MDASI) Core Items

**Part I.** How **severe** are your symptoms?

People with cancer frequently have symptoms that are caused by their disease or by their treatment. We ask you to rate how severe the following symptoms have been ***in the past week.*** Please fill in the circle below from 0 (symptom has not been present) to 10 (the symptom was as bad as you can imagine it could be) for each item.

| | **Not Present** 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | **As Bad As You Can Imagine** 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Your **pain** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 2. Your **fatigue (tiredness)** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 3. Your **nausea** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 4. Your **disturbed sleep** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 5. Your feelings of being **distressed (upset)** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 6. Your **shortness of breath** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 7. Your problem with **remembering things** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 8. Your problem with **lack of appetite** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 9. Your feeling **drowsy (sleepy)** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 10. Your having a **dry mouth** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

Date: _____ Institution:_____

Participant Initials: _____ Hospital Chart #:_____

Participant Number: _____

| | Not Present 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | As Bad As You Can Imagine 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11. Your feeling **sad** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 12. Your **vomiting** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 13. Your **numbness or tingling** at its WORST? | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

**Part II. How have your symptoms interfered with your life?**

**Symptoms frequently interfere with how we feel and function. How much have your symptoms interfered with the following items in the last 24 hours:**

| | Did Not Interfere 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Interfered Completely 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 14. **General activity?** | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 15. **Mood?** | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 16. **Work (including work around the house)?** | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 17. **Relations with other people?** | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 18. **Walking?** | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 19. **Enjoyment of life?** | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

## 6.4. Informed consent

**A study to measure the incidence and severity of fatigue in renal cancer patients receiving pazopanib (Votrient®) or sunitinib (Sutent®)**

**Please initial each box**

- I confirm that I have read and understood the information sheet for the above project and the researcher has answered any queries to my satisfaction. ☐
- I understand that my participation is voluntary and that I am free to withdraw from the project at any time, without having to give a reason and without any consequences. ☐
- I understand that I can withdraw my data from the study at any time without giving a reason and without any consequences. ☐
- I understand that any information recorded in the investigation will remain confidential and no information that identifies me will be made publicly available. ☐
- I understand that the results of this study may be published and that any data generated will be used anonymously. ☐
- I consent to being a participant in the project. ☐

| Name of Participant | Date | Signature |
|---|---|---|
| _____ | _____ | _____ |

Version 001 Date 03/09/2014

## 6.5. favourable opinion

Health Research Authority

**NRES Committee London - South East**
Bristol Research Ethics Committee Centre
Level 3, Block B
Whitefriars,
Lewins Mead,
Bristol
BS1 2NT

Telephone: (0117) 3421382

25 November 2014 – Re-issued 05.12.14

Mr. Waleed Altowayan
161 Cathedral Street
Scotland
Glasgow G4 0RE

Dear Mr. Altowayan

| | |
|---|---|
| **Study title:** | **A study to measure the incidence and severity of fatigue in renal cancer patients receiving pazopanib (VotrientÂ®)or sunitinib (SutentÂ®).** |
| **REC reference:** | **14/LO/2135** |
| **IRAS project ID:** | **153491** |

The Proportionate Review Sub-committee of the NRES Committee London - South East reviewed the above application on 21 November 2014.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Mr Wai Yeung, nrescommittee.london-southeast@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

The Sub-Committee has considered and reviewed the project as research and given the ethical opinion detailed below. However, we are of the view that the project would be more appropriately classified as *service evaluation* rather than research. We suggest that you discuss the matter with the sponsor and lead R&D office, and following that discussion advise the REC how it is intended to manage the project. If the sponsor and R&D office advise that the project can now be considered not to be research the application should be withdrawn. If it will continue to be managed as research, the opinion given below will remain in place.

**Ethical opinion**

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

**Conditions of the favourable opinion**

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

*Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.*

*Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.*

*Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.*

*For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.*

*Sponsors are not required to notify the Committee of approvals from host organisations.*

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

**It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).**

**Ethical review of research sites**

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

**Approved documents**

The documents reviewed and approved were:

| *Document* | *Version* | *Date* |
|---|---|---|
| Covering letter on headed paper [Covering page] | | 03 September 2014 |
| Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Indemnity Letter] | | 18 July 2014 |
| IRAS Checklist XML [Checklist_14112014] | | 14 November 2014 |
| Letter from sponsor [PROFESSIONAL INDEMNITY] | | 18 July 2014 |
| Other [MDASI (symptom questionnaire)] | | |
| Other [Collection form] | | 13 November 2014 |
| Participant consent form [Consent Form] | | 03 September 2014 |
| Participant information sheet (PIS) [Patient Information Sheet] | | 03 September 2014 |
| REC Application Form [REC_Form_14112014] | | 14 November 2014 |
| Research protocol or project proposal [Research Protocol ] | | 03 September 2014 |
| Summary CV for Chief Investigator (CI) [C.V. of Chief Investigator ] | | |
| Summary CV for student [C.V. for Student] | | |
| Summary CV for supervisor (student research) [C.V. of Supervisor ] | | |
| Validated questionnaire [FACIT-Fatigue questionnaire ] | 4.0 | 16 November 2007 |

**Membership of the Proportionate Review Sub-Committee**

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**After ethical review**

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

**User Feedback**

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

**HRA Training**

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

| **14/LO/2135** | **Please quote this number on all correspondence** |
|---|---|

Yours sincerely

**Mr Wai Yeung**
**Research Ethics Committee (REC) Assistant**

**pp Professor David Caplin**

Email: nrescommittee.london-southeast@nhs.net

*Enclosures:* *List of names and professions of members who took part in the review*

*"After ethical review – guidance for researchers"*

*Copy to:* *Helen Biagrie, Contracts Manager Research & Knowledge Exchange Services University of Strathclyde*
*Dr. Nathaniel Brittain, Academic Research Co-ordinator-NHS Greater Glasgow and Clyde - Research and Development Central Office-Tennent Institute [Student]*

**NRES Committee London - South East**

**Attendance at PRS Sub-Committee of the REC meeting in correspondence**

**Committee Members:**

| *Name* | *Profession* | *Present* | *Notes* | |
|---|---|---|---|---|
| Professor David Caplin | Physicist | Yes | | |
| Professor Ann Gallagher | Professor of Ethics & Care | Yes | | |
| Professor Robin MacKenzie | Director Medical Law & Ethics | Yes | | |

**Also in attendance:**

| *Name* | *Position (or reason for attending)* | |
|---|---|---|
| Mr Wai Yeung | REC Assistant | |

## 6.6 Protocol timeline

| **Action** | **Timeline** | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 0 | Day 1 (minimum) to enrolment in the research | Day 2 to initiation of cycle 1 | Cycle 1 | Cycle 2 | Cycle 3 | Cycle 4 |
| Patient attends clinic | X | | | | | | |
| Patient provided PIS | X | | | | | | |
| Patient consents to recruitment | | X | | | | | |
| Patient demographics captured | | | x | | | | |
| Patient's biochemistry recorded | | | x | X | X | X | X |
| Patient's medication recorded | | | x | X | X | X | X |
| Patient starts a treatment cycle | | | | X | X | X | X |
| Patient completes questionnaires | | | | X | X | X | X |

## 6.7 Patient Information Sheet

**Strathclyde Institute of Pharmacy and Biomedical Sciences**
**161 Cathedral Street, Glasgow G4 0RE**

# A study to measure the incidence and severity of fatigue in renal cancer patients receiving pazopanib (Votrient®) or sunitinib (Sutent®)

### Information Sheet

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information.

**Who is conducting the research?**

The research is being carried out by Professor Mullen and Mr. Waleed Altowayan from the University of Strathclyde, Institute of Pharmacy and Biomedical Sciences.

**What is the purpose of the study?**

This study will measure the frequency and extent of tiredness experienced when you take sunitinib (Sutent®) or pazopanib (Votrent®) for the treatment of your cancer. This study will help healthcare staff better understand how these medicines may affect you, allowing them to further improve the quality of your care.

Version 001 03/09/2014

**Why have I been invited?**

The drugs that you are receiving are fairly new treatments. We would like to know more about how you feel whilst taking them. This will allow healthcare staff to better understand how these medicines may affect you, allowing them to further improve your quality of your care.

**Do I have to take part?**

No, it is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. You will be asked to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving reason. This would not affect the standard of care you receive or your future treatment.

**What does taking part involve?**

We would like you to complete two questionnaires for four treatment cycles when you visit the renal clinic at the Beatson West of Scotland Cancer Centre. This should take around 10 minutes to complete on each cycle.

**What happens to the information?**

Your identity and personal information will be completely confidential and known only to the researcher. The information obtained will remain confidential and stored within a locked filing cabinet. The data are held in accordance with the Data Protection Act, which means that we keep it safely and cannot reveal it to other people, without your permission. We may use this information in an anonymous way to publish reports. This will allow us to share anything useful that we learn with other healthcare staff so that everyone can benefit from better care.

**What are the possible benefits of taking part?**

It is hoped that by taking part in this research, you will be providing valuable information about how you may feel whilst taking the medicines. This will help us improve how best to use the medicines in people with your type of

cancer. This will allow us to share anything useful that we learn with other healthcare staff so that everyone can benefit from better care in the future.

**What are the possible risks of taking part?**
No risks have been identified in completing the questionnaires.

**Who has reviewed the study?**
This study has been reviewed by the West of Scotland Research Ethics Committee and the Greater Glasgow & Clyde Research and Development office.

**If you have a complaint about any aspect of the study?**
If you are unhappy about any aspect of the study and wish to make a complaint, please contact the researcher in the first instance but the normal NHS complaint mechanisms is also available to you.

**If you have any further questions?**
We will give you a copy of the information sheet and signed consent form to keep. If you would like more information about the study and wish to speak to someone **not** closely linked to the study, please contact Dr Gazala Akram.

**Contacts:**
Dr Gazala Akram
Strathclyde Institute of Pharmacy and Biomedical Sciences
161 Cathedral Street
Glasgow G4 0RE
Telephone: 0141 548 4980
Email: gazala.akram@strath.ac.uk

*Thank-you for your time and interest*

## 6.8 Protocol of the research, no harm individual, anonymity, confidentiality, dissemination, statistical consideration and funding

***Protocol of the research***

Writing a research proposal is the first step in conducting any research, whether you intend to submit the proposal to an ethical committee, a research body or a funding organisation. The protocol reflects your knowledge of how research should be conducted. A protocol should be concise and should have a description of the research questions to be studied, as well as a thorough explanation of why and how the research will answer these questions. This protocol should not be changed in principle while a study is in progress, except in the case of severe or unexpected compliance issues. In this situation, the protocol should then be rewritten from the start if the research question is still relevant. All supervisors and co-workers should agree on the content of the protocol (94).

The written protocol is the detailed plan of the study and every research should have it. The protocol is a written guideline for the team working on the research, and it often helps the investigators clarify their thoughts about all aspects of the study. It is an essential component to getting ethical approval, in cases where the research involves human or animal experiment, and it's also an important component of funding proposals, when necessary (94).

Once the protocol has been developed and approved, then researchers should strictly adhere to it throughout the study. Violations of the protocol can break the whole study, unless the violation is relatively minor.

A well-written protocol should enable the research questions to be answered in a satisfactory way, achieve the study objectives, include a feasible set up for the study, and provide adequate detail that would allow another researcher to do the study and arrive at same conclusion. Rational for the research, objectives, methodology and analysis should also be outlined in the protocol (94).

***No harm individual***

There are no significant risks that participants may face as a result of participation in this study, and there are no tasks other than the questionnaires for participants to complete during the study. It is anticipated that completion of the two questionnaires will take around 6-10 minutes during each of their four consecutive routine clinic visits. The inconvenience to each participant will therefore be minimal, with no impact on the wider aspects of the participant's life anticipated. It is likely that most patients will complete the questionnaires whilst waiting for routine aspects of care during their visit (e.g. waiting to see the prescriber, or waiting for their medication to be dispensed).

***Confidentiality and anonymity***

All study information and data will be kept in strict confidence in accordance with NHS and Caldicott procedures. Information from questionnaires, patient notes and

clinical biochemistry portals will be transferred to an electronic format upon receipt, with the original hard copy (if applicable) stored in a locked cabinet held within a secure room at the Beatson West of Scotland Cancer Centre. Individual encrypted electronic files will be used for each individual patient and stored on an encrypted NHS laptop device. Any data used to generate scientific reports will be anonymised to ensure that participant confidentiality is maintained at all times.

***Dissemination***

It was intended that the results of this research study will be published as a paper in an academic research journal. Access to this paper may require subscription to the journal, or it may be free to access by all members of the public. The results may also be presented in the form of a poster presentation, or as a presentation at a research seminar or conference. The results and findings of our study will also be disseminated to relevant health care organizations and agencies.

***Funding***

This research is being undertaken in part fulfillment of a PhD by Waleed Altowayan, a postgraduate research student at the University of Strathclyde, Glasgow. The studentship is funded by the Al-Qassim University, Buraydah, Kingdom of Saudi Arabia.

## 6.9 Concurrent medications

| Patient ID<br>Drug received | Comorbidities(s) | Concurrent medication(s) |
|---|---|---|
| 011<br>Pazopanib | *Cycle 1:*<br>Asthma, Osteoporosis, Anxiety, Hypothyroidism and Epilepsy | *Cycle 1:*<br>Adcal-D3®, Co-codamol, diazepam, Symbicort® Inhaler, Salbutamol Inhaler, Furosemide, Gabapentin, Lansoprazole, Levothyroxine, Amitriptyline, Oxycodone Hydrochloride and Ranitidine |
| 012<br>Sunitinib | *Cycle 1:*<br>Asthma and Hypertension (HTN) | *Cycle 1:*<br>Omeprazole, Tramadol hydrochloride, Serotide®, Tiotropium bromide, Salbutamol Inhaler, Metoclopramide, Zopiclone, Amlodipine and Trazodone |
| 013<br>Sunitinib | *Cycle 1:*<br>HTN and Hypothyroidism | *Cycle 1:*<br>-Amlodipine and Levothyroxine |
| 018<br>Sunitinib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Candesartan, Lacidipine, Dexamethasone, Omeprazole and Monoxide |
| 019<br>Sunitinib | *Cycle 1:*<br>Hypothyroidism and HTN | *Cycle 1:*<br>Levothyroxine and Amlodipine. |
| 020<br>Sunitinib | *Cycle 1:*<br>Hypothyroidism and Depression | *Cycle 1:*<br>Omeprazole, levothyroxine, Amitriptyline, Amlodipine and Ofloxacin |
| 021<br>Pazopanib | *Cycle 1:*<br>Asthma | *Cycle 1:*<br>Co-codamol, Omeprazole, Morphine and Salbutamol inhaler |
| 022<br>Pazopanib | No comorbidities | *Cycle 1:*<br>Morphine and Omeprazole |
| 026<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Lisinopril |
| 028<br>Pazopanib | *Cycle 1:*<br>Deep Vein Thrombosis (DVT) prophylaxis and Epilepsy | *Cycle 1:*<br>Carbamazepine, Omeprazole, Amlodipine and enoxaparin |
| 032<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Amlodipine |
| 035<br>Pazopanib | No comorbidities | No concurrent medication |
| 044<br>Sunitinib | *Cycle 1:*<br>Depression and Anaemia | *Cycle 1:*<br>Citalopram, Levothyroxine, omeprazole, ferrous sulphate and calcium supplement. |
| 023<br>Pazopanib | No comorbidities | *Cycle 1:*<br>No medication |

| **Patient ID**<br>**Drug received** | **Comorbidities(s)** | **Concurrent medication(s)** |
|---|---|---|
| 027<br>Pazopanib | *Cycle 1:*<br>Diabetes Mellitus (DM), HTN<br>Hypothyroidism and Hyperlipidaemia | *Cycle 1:*<br>Simvastatin, Ramipril, Bendroflurozone and Levothyroxine. |
| 037<br>Pazopanib | *Cycle 1:*<br>HTN and Anxiety | *Cycle 1:*<br>Levomepromazine 6mg, Ramipril.<br>*Cycle 2:* Amlodipine instead of Ramipril.<br>*Cycle 4:* Antibiotic for chest infection. |
| 038<br>Pazopanib | | *Cycle 1:*<br>Omeprazole, Levomepromazine, Bendroflumethiazide<br>*Cycle 3:* Stop Bendroflumethiazide<br>*Cycle 4:* Levomepromazine |
| 041<br>Sunitinib | *Cycle 1:*<br>HTN and Hypothyroidism | *Cycle 1:*<br>Amlodipine and Levothyroxine |
| 040<br>Sunitinib | *Cycle 1:*<br>HTN and Hypothyroidism | *Cycle 1:*<br>Levothyroxine and Amlodipine |
| 036<br>Sunitinib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Losartan and Amlodipine |
| 010<br>Pazopanib | *Cycle 1:*<br>Rheumatic arthritis (RA), Atrial Fibrillation (AF), Ureteric stones and Depression | *Cycle 1:*<br>Aspirin, Bisoprolol, Citalopram, Flecainide, Folic acid, Hydroxychloroquine, Lactulose, Lansoprazole, Morphine, Methotrexate and Prednisolone<br>*Cycle 3:*<br>Morphine |
| 009<br>Pazopanib | *Cycle 1:*<br>HTN, DM and Renal failure | *Cycle 1:*<br>Salicylic Acid, Atorvastatin, Insulin, Cellcept®, Prednisolone, Ezetimibe and Mycophenalate<br>*Cycle 3:*<br>Mycophenolate |
| 014<br>Pazopanib | | *Cycle 1:*<br>Omeprazole, Pregabalin, oxycodone hydrochloride and Dexamethasone. |
| 015<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Amlodipine |
| 033<br>Sunitinib | *Cycle 1:*<br>HTN, Hyperglycaemia and Hypothyroidism | *Cycle 1:*<br>Bendroflumethiazide, Irbesartan, Levothyroxine, Omeprazole, Paracetamol and Simvastatin. |
| 046<br>Pazopanib | | *Cycle 1:*<br>Omeprazole |

| Patient ID<br>Drug received | Comorbidities(s) | Concurrent medication(s) |
|---|---|---|
| 060<br>Sunitinib | *Cycle 1:*<br>HTN and Anaemia | *Cycle 1:*<br>Ramipril, Blood transfusion and Levothyroxine<br>*Cycle 2:*<br>Blood transfusion<br>*Cycle 3:*<br>Blood transfusion<br>*Cycle 4:*<br>Blood transfusion |
| 063<br>Pazopanib | *Cycle 1:*<br>Anaemia and Hyperglycaemia | *Cycle 1:*<br>Zoledronic acid |
| 034<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Atenolol |
| 052<br>Pazopanib | *Cycle 1:*<br>HTN and Gout | *Cycle 1:*<br>-Bendroflumethiazide, Lisinopril, allopurinol and simvastatin |
| 062<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Ramipril and Zelodronic acid |
| 048<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Levothyroxine, Lisinopril and Bendroflumethiazide<br>*Cycle 4:*<br>Stop Bendroflumethiazide |
| 006<br>Sunitinib | | *Cycle 1:*<br>Omeprazole |
| 053<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Amlodipine and Omeprazole |
| 047<br>Pazopanib | *Cycle 1:*<br>DVT and Hypothyroidism | *Cycle 1:*<br>Levothyroxine, Omeprazole and Heparin |
| 008<br>Sunitinib | *Cycle 1:*<br>HTN<br>*Cycle 2:*<br>Chest infection | *Cycle 1:*<br>Bisoprolol, Aspirin and Candesartan.<br>*Cycle 2:*<br>Antibiotic and Corticosteroid tablet |
| 017<br>Pazopanib | *Cycle 1:*<br>Depression | *Cycle 1:*<br>Citalopram |
| 025<br>Sunitinib | *Cycle 1:*<br>Hypothyroidism<br><br>*Cycle 4:*<br>Chest infection | *Cycle 1:*<br>Levothyroxine, Omeprazole, Phosphate and Calcium supplement.<br>*Cycle 4:*<br>Amoxicillin |
| 029<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Ramipril and Felodipine |
| 031<br>Sunitinib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Ramipril |

| **Patient ID**<br>**Drug received** | **Comorbidities(s)** | **Concurrent medication(s)** |
|---|---|---|
| 043<br>Sunitinib | *Cycle 1:*<br>Epilepsy | *Cycle 1:*<br>Omeprazole and Levetiracetam |
| 042<br>Sunitinib | *Cycle 1:*<br>HTN and Hypothyroidism | *Cycle 1:*<br>Ramipril and Levothyroxine |
| 039<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Omeprazole and Ramipril |
| 050<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Amlodipine and Cyclizine |
| 051<br>Pazopanib | Cycle 1:<br>HTN | *Cycle 1:*<br>Losartan |
| 054<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>Omeprazole, Amlodipine, Bendroflumethiazide and Dexamethasone |
| 055<br>Pazopanib | Cycle 1:<br>- HTN | *Cycle 1:*<br>Omeprazole, Cardilopin and Aspirin. |
| 056<br>Pazopanib | *Cycle 1:*<br>- HTN | *Cycle 1:*<br>Lisinopril and Metoclopramide. |
| 057<br>Pazopanib | | *Cycle 1:*<br>Omeprazole |
| 058<br>Pazopanib | *Cycle 1:*<br>HTN and Hypothyroidism | *Cycle 1:*<br>- Bendroflumethiazide, Atorvastatin, Ondansetron, Loperamide, Metoclopramide, oxycodone hydrochloride and Co-codamol. |
| 064<br>Pazopanib | *Cycle 1*<br>-HTN, Hypothyroidism and DM | *Cycle 1:*<br>Amlodipine, Atorvastatin, Bisoprolol, Gliclazide, Metformin, Omeprazole, Perindopril, Pioglitazone and Levothyroxine. |
| 024<br>Pazopanib | *Cycle 1:*<br>Hypothyroidism | *Cycle 1:*<br>Calcium supplement and Levothyroxine |
| 049<br>Sunitinib | *Cycle 1:*<br>HTN and Hypothyroidism | *Cycle 1:*<br>Amlodipine, Codeine, Levothyroxine, Lisinopril, Omeprazole, sandocal® effervescent tablet and magnesium chewable tablets |
| 059<br>Sunitinib | *Cycle 1:*<br>DM, HTN, Hypothyroidism and Anaemia | *Cycle 1:*<br>Metformin, Candesartan, Amlodipine, Levothyroxine, Omeprazole, Atorvastatin and Ferrous sulphate.<br>*Cycle 2:* Blood transfusion<br>*Cycle 3:* Blood transfusion<br>*Cycle 4:* Blood transfusion |

| **Patient ID**<br>**Drug received** | **Comorbidities(s)** | **Concurrent medication(s)** |
|---|---|---|
| 007<br>Pazopanib | *Cycle 1:*<br>HTN and DM | *Cycle 1:*<br>Atenolol, doxazosin, Ramipril,<br>Gliclizide, Co-codamol, Lanzoprazole, Tamsulosin. |
| 061<br>Pazopanib | *Cycle 1:*<br>DM, HTN and<br>Hypothyroidism | *Cycle 1:*<br>- Metoformin, Bendrofluthizide, simvastatin, levothyroxine, amlodipine, dexamethasone.<br>*Cycle 2:*<br>- Amlodipine 10mg |
| 030<br>Sunitinib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>- Amlodipine 10mg |
| 065<br>Pazopanib | *Cycle 1:*<br>- HTN | *Cycle 1:*<br>- Amlodipine, dexamethasone, sevredol, Morphine, omeprazole<br>Zelodronic infusion each cycle |
| 016<br>Sunitinib | *Cycle 1:*<br>Hypothyroidism, HTN and Dyslipidaemia | *Cycle 1:*<br>-Levothyroxine, amlodipine, omeprazole, atorvastatin, cyclize, Acetaminophen, Ibuprofen |
| 045<br>Sunitinib | *Cycle 1:*<br>HTN<br>*Cycle 3:*<br>Urinary tract Infection | *Cycle 1:*<br>Atenolol, Omeprazole and Cyclizine<br>Cycle 3:<br>Antibiotics |
| 001<br>Pazopanib | No comorbidities | Omeprazole |
| 004<br>Sunitinib | *Cycle 1:*<br>DM and Hypothyroidism<br>*Cycle 4:*<br>Respiratory tract infection | *Cycle 1:* Amlodipine, zelodronic acid, Lansoprazole, thyroxin, insulin, fluorocort, hydrocort.<br>*Cycle 4:* Antibiotics |
| 002<br>Pazopanib | *Cycle 1:*<br>DM, HTN *and* Depression<br>*Cycle 3:*<br>Upper respiratory infection | *Cycle 1:*<br>Insulin, Pioglitazone, Duloxetine, simvastatin, metformin,<br>Amitriptyline, omeprazole<br>*Cycle 3:*<br>- Amoxicillin + clavulanic acid<br>- Increase dose of pazopanib from 600 to 800 |
| 003<br>Pazopanib | *Cycle 1:*<br>HTN | *Cycle 1:*<br>- Co-codamol<br>- Amlodipine |
| 005<br>Pazopanib | *Cycle 1:*<br>DM and HTN | *Cycle 1:*<br>Insulin, Oxycodone hydrochloride, Metoprolol, Lisinopril and Indapamide |