The investigation of pressurised intraperitoneal aerosolised therapeutics for peritoneal metastases.

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S Tate L Davies, I Bowler, C Taylor, K Lim, J Torkington. Electrostatic Pressurised Intraperitoneal Aerosol Chemotherapy: An IDEAL introduction in the UK. Colorectal Disease 2019; 21 (Supplement 2) p57

SJ Tate, J Torkington. Pressurized intraperitoneal aerosol chemotherapy: a review of the introduction of a new surgical technology using the IDEAL framework. BJS Open, 2020; 4 (2) p206-215

Abbreviations

αvβ6	Alpha v beta 6 integrin
A20	A20 NAVPNLRGDLQVLAQKVART peptide from FMDV
Ad	Adenovirus
Ad5	Human adenovirus species C type 5
Ad5.GFP	Human adenovirus species C type 5 engineered to express Green Fluorescent Protein as a reporter gene
Ad5.Luc	Human adenovirus species C type 5 engineered to express Luciferase as a reporter gene
AdZ	Homologous recombineering with zero cloning steps
AE	Adverse event
ANOVA	Analysis of variance
ASA	American Society of Anaesthesiologists
ATCC	American Type Culture Collection
BCA	Bicinchoninic acid protein assay
BCLAS	Belgian Council for Laboratory Animal Science
BMI	Body Mass Index
BSA	Bovine serum albumin
BSA	Body surface area
CaNISC	Cancer Network Information System Cymru
CAR	Coxsackie and adenovirus receptor
CAV UHB	Cardiff and Vale University Health Board
CHO	Chinese hamster ovary
CMV	Cytomegalovirus
COSHH	Control of substances hazardous to health
CRS	Cytoreductive surgery
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
Dbait	DNA strand break bait molecule
DC	Direct Current
dH_2O	Distilled water
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
ECACC	European Collection of Authenticated Cell Cultures

ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
EORTC	European Organisation for Research and Treatment of Cancer
EPIC	Early postoperative intraperitoneal chemotherapy
ePIPAC	Pressurised Intraperitoneal Aerosolised Chemotherapy with electrostatic precipitation
EudraCT	European Union Drug Regulating Authorities Clinical Trials Database
5-FU	Fluorouracil chemotherapy
FA	Folinic acid
FBS	Foetal bovine serum
FDA	Food and Drug Administration (United States of America)
FIGO	International Federation of Gynaecology and Obstetrics
FOLFIRI	Folinic acid, fluorouracil, and irinotecan chemotherapy regime
FOLFOX	Folinic acid, fluorouracil, and oxaliplatin chemotherapy regime
GFP	Green fluorescent protein
GOG	Gynaecologic Oncology Group
GRADE	Grading of Recommendations Assessment, Development and Evaluation
hCAR	Human Coxsackie and adenovirus receptor
HIPEC	Heated Intraperitoneal Chemotherapy
HPI	High pressure injector
HR	Hazard ratio
HSPGs	Heparan sulphate proteoglycans
HTA	Human Tissue Authority
ICD 10	International Classification of Diseases Version 10
IDEAL	Idea, Development, Exploration, Assessment, Long-term study
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IMS	Industrial methylated spirit
IP	Intraperitoneal
IPFR	Individual patient funding request
IQR	Inter-quartile range
ISSPP	International Society for the Study of Pleura and Peritoneum
ITT	Intention to treat
IVIS	In Vivo Imaging System
K1	CAR binding ablation mutation
L-Glut	L-glutamine
Luc	Luciferase

MDT	Cancer multidisciplinary team
MEM	Minimum Essential Medium
mRNA	Messenger ribonucleic acid
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4- sulfophenyl)-2H-tetrazolium
NaCl	Sodium chloride/saline
NBOCA	National Bowel Cancer Audit (England and Wales)
NEAA	Non-essential amino acids
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
O/N	Overnight
OD	Optical density
OS	Overall survival
P/S	Penicillin/streptomycin
PBS	Phosphate buffered saline
PCI	Peritoneal Cancer Index
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PFS	Progression free survival
PIPAC	Pressurised Intraperitoneal Aerosolised Chemotherapy
PPE	Personal Protective Equipment
PRGS	Peritoneal Regression Grading System
QoL	Quality of Life
qPCR	Quantitative polymerase chain reaction
RCT	Randomised controlled trial
RLU/mg	Relative light units per mg protein
Rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute
RT	Room temperature
SARS-Cov-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard deviation
SOP	Standard Operating Procedure
TNM	TNM Classification of Malignant Tumours
UKIACR	United Kingdom and Ireland Association of Cancer Registries
vp	Viral particles
WCISU	Welsh Cancer Intelligence and Surveillance Unit
WHSSC	Welsh Health Specialised Services Committee

Summary

Peritoneal metastases are a feature of many cancers. Cancer surveillance statistics in the UK do not record the incidence of specific metastatic locations so it is not possible to define the overall burden of disease. Patient outcomes from peritoneal metastases are generally poor, and current treatments have limitations. Peritoneal metastases therefore represent an area of unmet clinical need, and new therapeutic options are needed. Pressurised Intraperitoneal Aerosolised Chemotherapy (PIPAC) is a recent innovation. It involves a laparoscopic operation to deliver aerosolised chemotherapy into the peritoneal cavity. The systematic investigation of new surgical procedures like PIPAC in clinical trials is challenging. The IDEAL Framework is a paradigm for surgical innovation proposed by the Balliol collaboration to try and address the shortcomings of research in surgery.

A service evaluation of the management of peritoneal metastases from colorectal cancer at Cardiff and Vale University Health Board was performed, allowing the incidence of peritoneal metastases in colorectal cancer patients over the period evaluated, and the treatments provided to be assessed. A systematic review of the literature on PIPAC was carried out, and the evolution of PIPAC, and its introduction to surgical practice was examined with reference to the IDEAL Framework. This work underpinned the introduction of PIPAC to the UK in Cardiff in 2018. An audit of the first cases was carried out using standards identified from the literature. This demonstrates that PIPAC is feasible and can be performed safely in an NHS setting.

Pressurised Intraperitoneal Aerosolisation represents a potential delivery route for other therapeutics. Oncolytic adenoviruses are a promising strategy for cancer therapy. The feasibility of using the technique to administer viral vectors was assessed *in vitro* and *in vivo.* Adenovirus vectors were unaffected by aerosolisation, and retained their ability to transduce cells *in vitro*. Further investigation of this delivery method is warranted.

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1 Introduction

Peritoneal metastases are a feature of the advanced stages of many cancers originating from abdominal organs. They are therefore common. The cancer surveillance statistics in the UK do not record the incidence of specific metastatic locations so it is not possible to define the overall burden of disease accurately. This project focusses on peritoneal metastases from ovarian and colorectal cancer. The International Federation of Gynaecology and Obstetrics (FIGO) staging classification of ovarian cancer incorporates peritoneal involvement, and thus routinely collected cancer registration data can be used to assess the likely incidence of isolated peritoneal disease in ovarian cancer patients. This is not the case in colorectal cancer, where peritoneal metastases are grouped with other distant metastases in stage IV. The results of a service evaluation of the management of peritoneal metastases from colorectal cancer at Cardiff and Vale University Health Board (CAV UHB) will be presented. The evaluation allowed the incidence of peritoneal metastases in colorectal cancer patients to be estimated over the period evaluated, and the treatments provided and results to be assessed.

It is generally acknowledged that the current treatment options for peritoneal metastases are limited and that outcomes are poor. Thus, peritoneal metastases represent a significant area of unmet clinical need, and new therapeutic options are needed. Pressurised Intraperitoneal Aerosolised Chemotherapy (PIPAC) is a novel therapy developed to treat peritoneal metastases. It involves a short laparoscopic operation to deliver aerosolised chemotherapy into the abdominal cavity directly to the peritoneal disease [1]. The technique is possible because of a specially designed laparoscopic nebuliser device (*Micropump*™ Reger Medizintechnik, Rottweil, Germany until 2015 and then *Capnopen*®, Capnomed, Villingendorf, Germany), which allows the injection of the aerosol into the pnuemoperitoneum without increasing the pressure in the insufflated abdomen. The concept was first described in 2000 [2], and the first cases reported in 2013 [3]. There are two key hypotheses behind the proposed benefits of the system. Firstly, that intraperitoneal chemotherapy is superior to intravenous chemotherapy for the treatment of peritoneal metastases. This is because there is the potential to administer a higher concentration of the drug at the site of disease. Secondly, that delivering the chemotherapy solution as an

aerosol into the pressurised pneumoperitoneum confers pharmacodynamic advantages over lavage with liquid chemotherapy solutions. Specifically, that the aerosolisation results in better distribution around the abdominal cavity, and the pressure of the pneumoperitoneum results in improved penetration into the tumour nodules [2].

The systematic investigation of innovative surgical procedures such as PIPAC is challenging. There are several features of surgery that make assessment using conventional trial designs complex. These include the existence of a learning curve for a new intervention, quality variation, and the perception of equipoise. The Balliol collaboration met to try and address the shortcomings of research in surgery in 2009 [4-6]. They proposed a paradigm for innovation to bring a structure to the development of new surgical techniques; the IDEAL Framework [4]. The evolution of PIPAC, and its introduction to surgical practice, will be assessed with reference to the IDEAL Framework. PIPAC was recently introduced to the UK in 2018, and the rationale for this will be described, and an audit of the first cases will be presented.

So far, only chemotherapeutic agents have been delivered using the pressurised aerosolisation technique, however pre-clinical research is ongoing to see if it is a viable delivery method for advanced therapeutics, such as messenger ribonucleic acid (mRNA) complexes [7]. Oncolytic viruses are a promising strategy for cancer therapy. Adenoviruses have several properties that make them good candidate vectors. An adenovirus successfully targeted to infect tumour cells would cause cell lysis, releasing antigens and virus progeny, and therefore amplifying the therapeutic effect at the site of disease. A directed method for the intra-abdominal administration of oncolytic viruses may be therapeutically useful and of interest. Thus far, the PIPAC technique has not been assessed as a delivery method for viruses. The results of a series of experiments to assess the feasibility of using pressurised intraperitoneal aerosolisation to administer oncolytic adenovirus therapy will be presented.

1.1 Peritoneal Metastases

The peritoneum is the mesothelial lining of the abdominal cavity. It is made up of two continuous layers of simple squamous epithelium, which are shown in Figure 1-1. The parietal peritoneum lines the internal surface of the abdominopelvic wall, and the visceral peritoneum invests organs such as the stomach and intestines. The peritoneal cavity is the potential space between the two layers, and is usually empty except for a thin film of fluid that maintains the epithelial surface and enables friction-free movement of the viscera, for example as a result of peristalsis of the gut [8, 9]. The blood supply of the parietal peritoneum is from the abdominal wall vasculature, and originates from the circumflex, iliac, lumbar, intercostal, and epigastric arteries depending on the region, and drains to the corresponding veins [8, 9]. The blood supply of the visceral peritoneum is from the organs it covers. The arterial supply is therefore from the vessels arising from the coeliac axis, superior mesenteric artery, and inferior mesenteric artery, and these are subject to control by the splanchnic nerves of the autonomic nervous system. The venous drainage is via the portal system. This means that any solute absorbed from the peritoneal cavity is subject to first pass hepatic metabolism [8, 9].

The peritoneum may be the site of a primary tumour or, more commonly, becomes involved in malignancy as a secondary site. This is usually spread from an intra-abdominal primary. The most common route of metastasis is via intraperitoneal seeding or direct invasion from a tumour that has extended through the wall of an intra-abdominal viscus, perforated, or been opened or incompletely resected at operation (reviewed in [10, 11]). Shedding of cells from a tumour is thought to occur because of the high interstitial fluid pressure within the tumour, combined with changes in the phenotype of the cells. The tumour cells exhibit 'epithelial to mesenchymal transition', which involves down-regulation of key components in cell-cell adhesion, loss of cell polarity, remodelling of the cell cytoskeleton, and down-regulation of membrane glycoproteins (reviewed in [10, 11]). These changes confer resistance to anoikis, the programmed cell death that normally occurs in detached epithelial cells, and the tumour cells are therefore able to survive and circulate within the peritoneal cavity (reviewed in [12]). To form metastatic nodules, the cells must

be capable of adhesion to the mesothelial surface or extracellular matrix. This is facilitated by abnormal or upregulated expression of surface proteins such as integrins and other glycoproteins [11]. Metastasis is more common in certain locations, because of gravity and the direction of flow of peritoneal fluid, for example, towards the pelvis and right paracolic gutter, or because of the properties of the surface itself. The milky spot immune complexes that are ubiquitous on the omentum are a common site for tumour adhesion and growth, possibly because of the favourable vascular microenvironment [13]. Once attached, the tumour cells invade the submesothelial tissue, either through areas of discontinuity in the mesothelium or by causing apoptosis of mesothelial cells [14]. Haematogenous and lymphatic spread can also be caused by or result from peritoneal metastases. Free tumour cells in the peritoneal cavity can enter the lymphatic system and then the systemic circulation through the subperitoneal lymphatic lacunae that drain peritoneal fluid, and any particulates in it, through stomata between the mesothelial cells [10]. Peritoneal metastases may be diagnosed on cross-sectional imaging or found at operation.

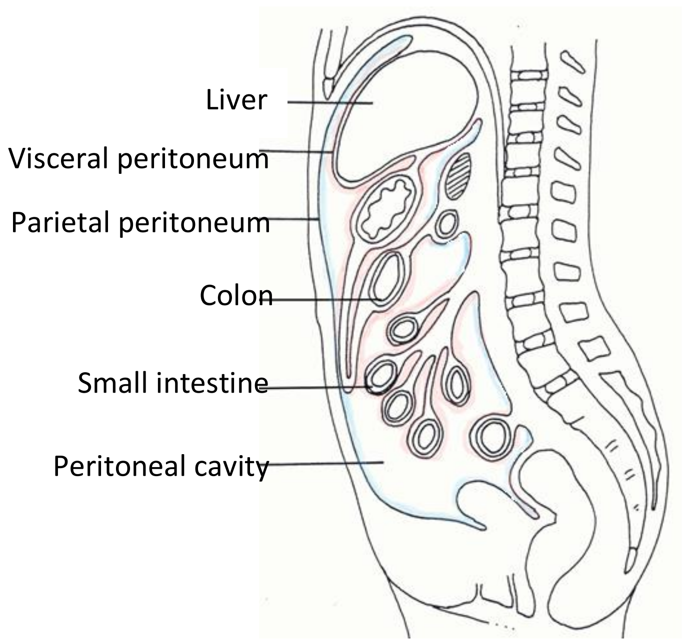


Figure 1-1: Diagram depicting a sagittal section of the abdominal cavity.

Parietal peritoneum is highlighted in blue, visceral peritoneum is highlighted in red. Adapted from an image by Alice Roberts, licensed with CC BY, https://creativecommons.org/licenses/

1.1.1 Incidence

The precise incidence of peritoneal metastases from any given cancer type can be difficult to establish. Whilst peritoneal metastases are included as a specific entity in the staging classification of some cancers, for example ovarian cancer, this is not the case for all cancers where they occur [15]. Additionally, the diagnosis of peritoneal metastases on radiological investigations is notoriously difficult because of the complex anatomy and the small size of the nodules, so some patients may not be staged correctly [16]. The sub classifications of each of the categories of the TNM Classification of Malignant Tumours are not always used when cancers are described on patient records, and this level of detail is not always added when cancers are subsequently registered. In the UK, cancers are registered using the primary site of disease if known and the site of synchronous metastases are not recorded. Recurrent cancer sites are not re-registered, so UK-wide statistics do not capture the incidence of any particular type of metastasis. Thus it is not easy to determine the number of patients with peritoneal metastases specifically. At a population level, the burden of disease must be estimated based on the overall incidence of the primary tumour types, and the expected rate of peritoneal metastases from the literature and other disease specific registries. The estimated incidence of peritoneal metastases in colorectal and ovarian cancer will be discussed further in sections 1.1.5 and 1.1.6.

1.1.2 Treatments

The main treatment options available for peritoneal metastases are anti-cancer therapies, such as chemotherapy drugs and targeted biological agents, which can be administered systemically or into the peritoneal cavity, with or without surgery to remove the peritoneal nodules (reviewed in [17]). The choice of systemic therapy depends on the primary disease and the presence of specific genetic mutations. Surgery for peritoneal metastases is a major undertaking. The aim is to remove all visible (macroscopic) disease. This is termed ‘cytoreductive surgery’ (CRS), and may involve resection of multiple organs as well as stripping away the peritoneum from

the pelvis and diaphragm. It is an 'open' procedure, involving a large incision to access all parts of the abdominal cavity. In an attempt to control any residual disease or free tumour cells that remain at the end of the procedure, the surgery is frequently combined with hyperthermic intraperitoneal chemotherapy (HIPEC) or early postoperative intraperitoneal chemotherapy (EPIC). HIPEC involves perfusing the abdominal cavity with a heated chemotherapy solution for 1-2 hours prior to closure. EPIC involves the administration of intraperitoneal chemotherapy using a catheter inserted into the abdominal cavity. The systematic CRS procedure used for peritoneal metastases in combination with HIPEC was formally described by Paul Sugarbaker in 1995 [18], and is therefore often known as the Sugarbaker technique. The treatment pathways for patients with peritoneal involvement of colorectal and ovarian cancer and the outcomes achieved will be described further in sections 1.1.5.1 and 1.1.6.1.

1.1.3 The peritoneal barrier

It has been observed that patients with peritoneal metastases have a worse prognosis than patients with distant metastases at other sites [19]. One theory that has been put forward to explain why systemic anti-cancer treatments are less effective for peritoneal disease than for metastases at other sites is that the interstitium beneath the mesothelial surface of the peritoneum combined with the endothelium of the capillaries forms a 'barrier' to drug transport [20-22].

The peritoneal mesothelium is supported by a relatively thin layer of submesothelial connective tissue, and together these comprise the peritoneum. This sits on a much thicker layer of connective tissue consisting of parenchymal cells and fibroblasts surrounded by an interstitial matrix of collagen, hyaluronan, and proteoglycans [20]. There are relatively few capillaries, and these are found within the connective tissue some distance from the peritoneal surface [20]. The endothelium of the capillaries, together with the extracellular glycocalyx which coats the intravascular surface represent the final layers of the barrier between the blood plasma and the peritoneal cavity [23]. Additionally, small (i.e. less than 1mm) peritoneal tumour nodules have a poorly developed or absent capillary network and have been shown to be hypoxic as a result [24]. Thus, drug delivery to nodules on the peritoneal surface via the circulation is relatively poor compared to other tissues.

1.1.4 Overcoming chemotherapy resistance in peritoneal disease

Peritoneal metastases are frequently seen in patients who have received or are receiving systemic chemotherapy, and may therefore exhibit resistance to treatment. There are a number of mechanisms by which cancer cells may develop resistance to a chemotherapeutic agent, and they are summarised in Figure 1-2.

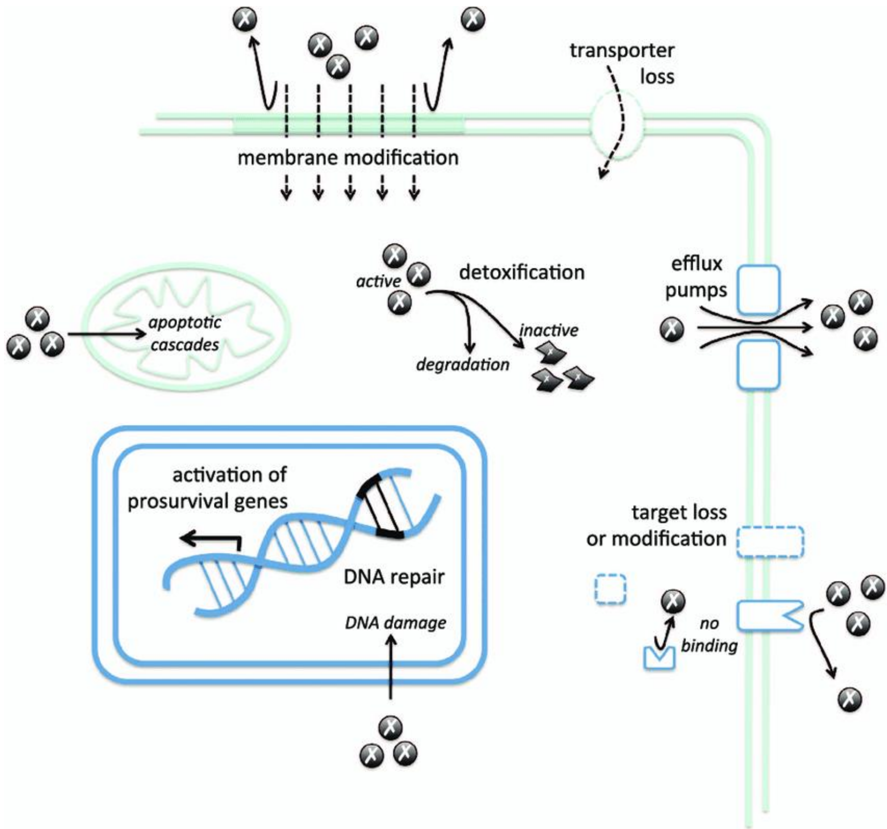


Figure 1-2: General mechanisms of chemotherapy resistance.

Tumour cells can limit accumulation of cytotoxic drugs by a number of mechanisms including; modifying their membrane composition to prevent drug entry by diffusion, downregulating drug transporters and receptors, upregulating efflux pumps, increasing the intracellular capacity for detoxification (e.g. by increasing antioxidants), increasing capacity for DNA repair, and activating anti-apoptotic and pro-survival pathways to prevent drug-initiated apoptosis.

Diagram from Avril *et al* [25] licensed with CC BY 4.0, https://creativecommons.org/licenses/

Targeting these mechanisms may represent a way to improve outcomes. Increasing the concentration of the drug in the extracellular environment may overcome the

resistance conferred by the modification of the cellular membrane, downregulation of drug transporters, and upregulation of efflux pumps. A higher dose entering the cell may counteract the upregulation of detoxification pathways and DNA repair mechanisms. Dose-response studies of platinum compounds in ovarian cancer cells *in vitro*, both cultured from patients with resistant disease and those with induced resistance, have demonstrated that even in cell lines with resistance to platinum-based cytotoxic drugs, high doses will result in cell death [26, 27].

Investigation of the intraperitoneal administration of chemotherapy has shown that it allows a higher concentration of drug to be applied to peritoneal nodules, and it is thought that this may increase drug delivery sufficiently to overcome resistance in some cases. Dedrick et al first described the pharmacokinetic advantages that might be conferred by administering chemotherapy directly into the peritoneal cavity [21]. Transport of small compounds, such as chemotherapeutic agents, into tumour tissue occurs mainly by diffusion. In addition to the size of the molecule, the other key determinants of the rate of diffusion are the concentration gradient, and the surface area of the peritoneum in contact with the solution [28]. In comparison with intravenous delivery, intraperitoneal administration allows in an increased drug concentration at the tumour on the peritoneal surface [29]. Drug clearance from the interstitium is slower than from the plasma. The ‘peritoneal barrier’ minimises systemic absorption and this may reduce systemic chemotherapy side effects compared to intravenous administration [30].

Markman reviewed the results of early trials examining the effect of intraperitoneal instillation of a number of different agents for various pathologies [31]. Whilst a pharmacokinetic advantage in terms of the peak peritoneal cavity/plasma concentration ratio could be demonstrated, this did not always translate to a significant improvement in disease control when compared to standard intravenous regimens [31]. Even with the increased concentration gradient, there is still relatively poor uptake from the peritoneal cavity into the tumour nodules. It is thought that this is because of the higher interstitial pressure in the tissue [32, 33].

Ceelen and Flessner reviewed the evidence on the pharmacodynamics of intraperitoneal chemotherapy and the properties of commonly used cytotoxic drugs [29]. The penetration of individual agents varied from 4-6 cell layers for Doxorubicin, 0.2mm for 5-Fluorouracil, 2mm for Mitomycin C, and up to 3mm for Cisplatin [29]. Surgery has therefore been combined with intraperitoneal chemotherapy, either in a single procedure or sequentially, so that macroscopic tumour nodules that would be incompletely penetrated are removed prior to chemotherapy administration. This type of surgery to remove visible tumour deposits was developed for ovarian cancer but was increasingly used in other cancer types. The stepwise description of the CRS technique by Sugarbaker in 1995 has been widely adopted and used to help standardise and define the procedure [18].

Other adjuncts to improve the penetration and effect of chemotherapeutic agents have been assessed. Administering a drug under hyperthermic conditions has been shown to enhance tumour penetration and cell killing. A number of groups have assessed the effect of hyperthermia on the cytotoxicity of platinum compounds, and found that temperatures up to 41.5°C increase the number of DNA adducts formed, and therefore the sensitivity of cancer cells to platinum compounds *in vitro* [34-36] and *in vivo* [37]. In these studies, heat alone caused an increase in cell death *in vitro*, the effect was enhanced with the addition of cisplatin, and the combination of hyperthermia and cytotoxic agent was more effective than the drug or heat alone [34-36]. The mechanisms of action proposed were that hyperthermia increased permeability of the cell membrane to the drug, as well as increasing membrane drug transport, and that it increased the conversion of the drug into reactive metabolites in the cells. This thermal enhancement of cytotoxicity has been observed in other drug classes which exert their effects on DNA, for example alkylating agents [29]. It has also been suggested that hyperthermia increases the penetration of cytotoxic drugs into tumour nodules. This was initially determined in animal models [37] but has also been demonstrated in humans. Van de Vaart et al studied the depth of cisplatin-DNA adducts from the periphery of peritoneal nodules after exposure to heated cisplatin [34]. The patients in the study were undergoing CRS and HIPEC at 41.5°C, and a single nodule was left *in situ* during the HIPEC part of the procedure

and then collected for analysis before the abdomen was closed. The number of DNA adducts present in the peritoneal biopsies was compared to buccal samples taken at intervals up to 24 hours after the procedures. It was noted that the number of DNA-adducts found at distances between 3-5mm from the surface of the peritoneal nodule was greater than the number found in any of the buccal samples. The authors determined that this must be because of direct drug penetration rather than delivery via the circulation. This penetration distance was further than previously reported figures for cisplatin when used at body temperature, such as those reported by Ceelen and Flessner [29].

The use of pressure to enhance drug penetration and tumour cell killing has also been investigated. Esquis et al assessed the role of increased intra-abdominal pressure on the efficacy of intraperitoneal cisplatin in a rat model of peritoneal carcinomatosis [38]. Intravenous administration of chemotherapy was compared with intraperitoneal lavage (single injection) and raised intra-abdominal pressure (22mmHg) generated using a continuous intraperitoneal infusion. The rats were sacrificed and the cisplatin concentration in various tissues measured after 1 hour. A significantly higher concentration of cisplatin was detected in the tumour nodules and the peritoneal surface of the diaphragm of the rats treated with raised intra-abdominal pressure compared to the isobaric intraperitoneal lavage and intravenous administration groups. The highest drug concentration was found at the periphery of the nodules in all administration protocols and drug concentration decreased towards the centre of the nodules, but the values were significantly higher in the rats in the raised intra-abdominal pressure group indicating better penetration of the drug. There was no significant difference in the concentration of cisplatin in the blood plasma, kidneys, liver, or heart between the different administration methods, suggesting that the raised pressure did not increase systemic absorption. The group also assessed the feasibility of using raised intra-abdominal pressure in pigs, as a more analogous model to human anatomy and physiology [38]. The tolerability of raised intra-abdominal pressure with and without cisplatin was compared. Continuous infusion of solution to achieve maximum pressure of 40mmHg was carried out and then sustained for 2 hours. Various concentrations of

cisplatin were tested, and the pigs were monitored for 4 weeks post procedure. The maximum tolerated dose of cisplatin with raised intra-abdominal pressure was 100mg continuously infused in 10 litres. Doses higher than this resulted in renal failure. The pigs who had an infusion of saline with raised intra-abdominal pressure had no apparent adverse sequelae. This experiment suggested that high concentrations of drug administered with a raised pressure could increase systemic absorption.

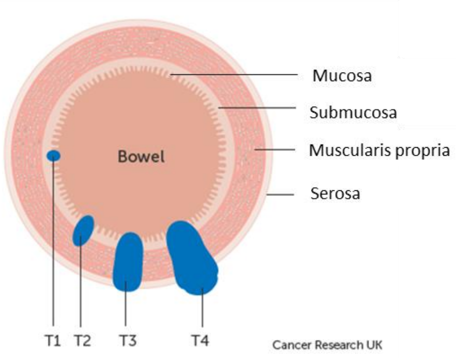
Facy et al [39] assessed the effects of hyperthermia (42 °C) and raised intra-abdominal pressure (25cm H_2O) on the penetration of intraperitoneal oxaliplatin (150mg/L) in an *in vivo* pig model. An open technique was used, with raised pressure generated by using a column of cytotoxic solution. Hyperthermia and increased pressure were assessed individually and in combination, and compared to a control group that had isobaric and normothermic intraperitoneal oxaliplatin. Blood samples were collected throughout the procedure and tissue samples were collected at the end. The procedures were tolerated by the pigs in all groups. There was no significant difference in the plasma concentration of oxaliplatin between groups, suggesting that in this experiment neither hyperthermia nor raised pressure had a major impact on systemic absorption. There were significant differences in the tissue concentrations of oxaliplatin between the control group and the other groups, and the highest concentrations were found in the hyperthermia and high pressure group. The distance that the drug had penetrated was not assessed. In a follow up investigation using the same pig model, administration of intraperitoneal oxaliplatin under pressure using a ‘closed’ technique was compared to the open technique [40]. The closed technique involved the use of tubing to pump in a solution to fill the peritoneal cavity. This experiment found that levels of oxaliplatin in the systemic circulation were increased when the open technique was used, but that the concentration of the drug absorbed by the peritoneum was also higher. The authors suggested that this was because the open technique allowed more movement of the viscera in the chemotherapy solution compared to the closed technique, and therefore a more homogenous distribution and greater surface area in contact with the drug. There was no significant difference detected in the depth of penetration,

which was measured by taking retroperitoneal biopsies and determining the concentration of oxaliplatin present.

1.1.5 Peritoneal metastases in colorectal cancer

The peritoneum is a common site of metastasis in colorectal cancer. The Metastasis (M) 1c category of the TNM staging of colorectal cancer specifically identifies peritoneal disease. The full classification can be seen in Figure 1-3. The TNM categories can be combined to give an overall anatomic stage or prognostic group of disease from I to IV. The presence of distant metastases (M1) means the patient has stage IV disease. Stage IV can be further described as IVa, b, or c depending on the site(s) affected by metastases, in the same way as the M category of the TNM stage. However, the a, b, and c categories are not routinely collected and summarised by cancer registries in the UK. The true incidence of peritoneal disease is therefore not known and can only be estimated by extrapolation. It is thought that 4% of patients presenting with colorectal cancer will have synchronous peritoneal metastases [17]. In addition, 20-40% of patients treated with curative intent will relapse and around 20% of these will have metastatic peritoneal disease [17, 41]. The average incidence of colorectal cancer overall in the UK in 2015-2017 was 42,317 cases per year [42]. If between 4% and 5% of patients presenting with colorectal cancer have synchronous peritoneal disease, then approximately 1900 patients per year will be diagnosed with a new bowel cancer and peritoneal metastases. Approximately 65% of patients in 2015 had stage I-III cancer, representing disease that might be treated with curable intent. If, in addition, 20-40% of patients treated with curative intent relapse, and around 20% of these have peritoneal disease then between 1600 and 2000 of the patients diagnosed each year would go on to develop peritoneal metastases.

Tumour (T)



T0: no evidence of primary tumour
Tis: carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
T1: tumour invades submucosa (through the muscularis mucosa but not into the muscularis propria)
T2: tumour invades muscularis propria
T3: tumor invades through the muscularis propria into the pericolorectal tissues
T4: tumour invades local structures

T4a: tumour invades through the visceral peritoneum
T4b: tumour directly invades or adheres to other adjacent organs or structures

Regional Lymph Nodes (N)

N0 – no spread to lymph nodes

N1 – spread to 1-3 regional lymph nodes
- **N1a:** metastasis in 1 regional lymph node
- **N1b:** metastasis in 2 - 3 regional lymph nodes
- **N1c:** no regional lymph nodes are positive but there are tumour deposits in the subserosa, mesentery or extraperitoneal pericolic or perirectal/mesorectal tissues

N2 – spread to >3 regional lymph nodes
- **N2a:** metastasis in 4 - 6 regional lymph nodes
- **N2b:** metastasis in 7 or more regional lymph nodes

Distant Metastases (M)

M0 – no distant spread

M1a – spread to one other site

M1b – spread to 2 other sites

M1c – spread to the peritoneum

Figure 1-3: The TNM staging classification of colorectal cancer [43]

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The incidence of peritoneal metastases in colorectal cancer has been assessed using large national and regional cancer registries elsewhere in Europe. Thomassen *et al* analysed data from the Eindhoven Cancer Registry (Netherlands) which covers a population of 2.4 million people [44]. 27,632 patients received a new diagnosis of colorectal cancer between 1995 and 2010. Of these, 1304 (4.72%) had peritoneal metastases, with just over half of those (722, 55%) having additional extraperitoneal metastases, and the rest (582, 45%) having isolated peritoneal metastases. Lemmens *et al* used data from 1995 to 2008 from the same registry to determine risk factors for synchronous peritoneal disease and survival after diagnosis [45]. They carried out multiple logistic regression and identified advanced T stage [T4 vs. T1,2: odds ratio (OR) 4.7, confidence limits 4.0-5.6), advanced N stage [N0 vs. N1,2: OR 0.2 (0.1-0.2)], poor differentiation grade [OR 2.1 (1.8-2.5)], younger age [<60 years vs. 70-79 years:

OR 1.4 (1.1-1.7)], mucinous adenocarcinoma [OR 2.0 (1.6-2.4)] and right-sided localisation of primary tumour [left vs. right: OR 0.6 (0.5-0.7)] as risk factors for peritoneal disease.

The Eindhoven Cancer Registry did not record recurrent disease, but a third study followed up all the patients included on the registry between 2003 and 2008 who had surgery with curative intent with their local hospitals to determine the rates of metachronous disease [46]. 1042/5671 (18%) patients had experienced recurrence after a median follow up of 5 years. The liver was the most commonly identified site of metachronous metastases. 19% of patients who had recurrent disease had peritoneal involvement, with over half of these having multiple sites of metastasis. Only 8% patients had recurrent isolated peritoneal metastases. In this study, peritoneal metastases were more common in patients with rectal cancer rather than colon cancer, patients with more advanced primary tumours (T4), node positive primary disease (N1 or N3), and a poor differentiation grade or a positive resection margin in the primary tumour.

A more recent study estimated the incidence of peritoneal metastases in colorectal cancer using the Netherlands Cancer Registry [47]. All patients who were diagnosed with colorectal cancer between January and June 2015 were included, and their outcomes were assessed in 2019. Of the 7233 patients, 409 (5.7%) had synchronous peritoneal metastases, and 166 (2.3%) had isolated synchronous peritoneal metastases. When assessed in 2019, 326/5375 (6.1%) of the patients who had undergone resection of their primary disease had experienced a peritoneal recurrence. With longer follow up, there may have been a higher rate of recurrence. The characteristics of patients presenting with synchronous and metachronous metastases were compared, and multivariable logistic regression was used to determine risk factors for peritoneal disease. Separate analyses found that both synchronous and metachronous peritoneal disease were associated with a histological diagnosis of mucinous adenocarcinoma or signet ring cell carcinoma, a T4 tumour stage, an N1 or N2 node stage, and the presence of extraperitoneal synchronous metastases. Having a rectal tumour and being younger than 75 years old were negatively associated with synchronous disease.

1.1.5.1 Current treatment options and prognosis of colorectal peritoneal metastases

The treatment of peritoneal metastases from colorectal cancer is challenging, and practice varies. In the UK, the NICE guideline recommends offering systemic anti-cancer therapy for metastatic colorectal cancer, including peritoneal metastases [48]. In addition, referral of the patient to a commissioned specialist centre for consideration of CRS and HIPEC should be discussed by an MDT. The choice of agent for first line anti-cancer therapy depends on mutation testing to see if anti-epidermal growth factor receptor (EGFR) targeted therapy is indicated. Patients with previously untreated EGFR-expressing, RAS wild-type metastatic colorectal cancer can be offered cetuximab in combination with folinic acid, fluorouracil, and oxaliplatin (FOLFOX) or folinic acid, fluorouracil, and irinotecan (FOLFIRI). Otherwise, intravenous fluorouracil and folinic acid (5-FU/FA) or oral capecitabine are the recommended first line chemotherapy options. Capecitabine is a fluoropyrimidine carbamate precursor of 5-FU. The final step in the conversion to the active drug is catalysed by thymidine phosphorylase, an enzyme which occurs at higher levels in colorectal cancer, and thus results in higher intra-tumoral drug levels. This reduction in systemic exposure to 5-FU confers an advantage in the rates of side effects observed as a result of treatment [49].

Outcomes have been assessed in both observational and interventional trials. Observational registry studies provide overall data in unselected patients undergoing varied treatment regimes. In the Lemmens *et al* study from the Eindhoven Cancer Registry, the group of patients with isolated peritoneal disease had a poor outcome, with those treated between 2002 and 2008 having a median overall survival of 8 months [45]. The authors also noted that median survival had not really improved over the time period observed. The figure for patients who had presented between 1995 and 2001 was 7 months. This was in contrast to metastases in other solid organ sites, for example the liver. Improvements in surgical and radiologically-guided interventions to treat colorectal liver metastases had increased median survival in the group of patients who presented with isolated liver metastases from 8 months in 1995-2001 to 12 months in 2002-2008.

Franko *et al* analysed individual patient data in the ARCAD database, which collated results from 14 phase 3 randomised trials carried out between 1997 and 2008 [19]. Of the 10,553 patients included, 9178 (87%) had non-peritoneal metastatic disease and 1181 (11%) had peritoneal disease. Of these, 194 (2%) had isolated peritoneal disease. The patients were previously untreated and were enrolled in trials comparing systemic anti-cancer therapies (chemotherapy and/or targeted biological agents). The proportion of patients in each individual trial with peritoneal disease varied from 4% to 25%. When outcomes in patients with only one site of metastasis were compared, those with isolated peritoneal metastases had significantly worse survival outcomes, with a median overall survival (OS) of 16.3 (Inter-quartile Range (IQR) 13.5-18.8) months, compared to those with isolated liver (median OS 19.1 IQR 18.3-19.8 months, Hazard Ratio (HR) 0.75, p=0.0004), lung (median OS 24.6 IQR 22.7-26.4 months, HR 0.53, p=<0.0001), and lymph node (median OS 19.4 IQR 17.0-21.9 months, HR 0.69, p=0.0003) metastases. This effect was seen in trials where all groups were given cytotoxics and was more pronounced when patients received targeted therapies in addition to cytotoxic therapy. It also persisted when results were adjusted for sex, performance status, cancer site, previous chemotherapy, age, and Body Mass Index (BMI). In trials where all treatment arms received only cytotoxic agents, median survival for isolated peritoneal disease was 16.3 (IQR 12.9-19.2) months. In studies where all patients received at least one targeted agent, the median survival for the patients with only peritoneal metastases was 17.1 (13.0-22.1) months. Patients with multiple sites of metastases, but no peritoneal involvement had similar survival to patients with isolated peritoneal metastases. Patients with both peritoneal and extra-peritoneal metastases had the worst outcomes.

CRS and HIPEC has been used extensively for colorectal peritoneal metastases, but it is not universally recommended. It is a major procedure with a high morbidity and an associated mortality, and therefore requires careful patient selection. A study of 117 patients undergoing CRS and HIPEC for colorectal or appendiceal cancer in Australia found that quality of life after surgery was significantly impacted, and did not return to baseline until 3-6 months after the procedure [50]. The median length of stay was 15 days, and 61% of patients experienced a post-operative complication,

of which 30% were classed as severe (requiring hospitalisation and/or life threatening).

A small number of randomised controlled trials (RCTs) have been performed to try and determine whether the risks associated with the procedure are adequately balanced by progression-free survival. Verwaal et al carried out a prospective RCT in the Netherlands, recruiting patients between 1998 and 2001 and completing follow up in 2007 [51]. There were 105 patients who were randomised on a 1:1 basis to receive either CRS and HIPEC with Mitomycin C, followed by adjuvant chemotherapy, or systemic chemotherapy alone. There was a significant benefit of CRS and HIPEC, with the median progression free survival reported to be 12.6 months, versus 7.7 months in the control group (p = 0.02) and the disease-specific overall survival 22.2 versus 12.6 months (p = 0.028). however, there were significant rates of morbidity, and a mortality rate of 7% (4/54 participants), associated with surgery. Figures from the control group were not available for direct comparison which was a limitation of this part of the study.

A more recent trial, PRODIGE 7, compared CRS and HIPEC with oxaliplatin, in combination with pre- and/or post-operative systemic chemotherapy, with CRS alone with pre- and/or post-operative chemotherapy [52]. Overall survival rates in both treatment arms were high after a median follow-up of 63.8 months, with a median OS of 41·7 months (95% CI 36·2–53·8) in the CRS plus HIPEC group and 41·2 months (35·1–49·7) in the CRS-only group. There was no significant difference between the groups (HR 1·00, 0·73 to 1·37). There were 2 deaths within 30 days of surgery in both groups, with an overall 30 day mortality of 1.5%. There were 2 further deaths in the HIPEC group and 1 in the CRS-only group attributable to the intervention at 60 days, with an overall mortality rate of 2.6% at 50 days. The rate of severe complications was similar between the treatment arms at 30 days post-procedure, but by 60 days there was a significantly higher rate of Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or worse events in the CRS and HIPEC group (34/131 [26%] *vs* 20/130 [15%]; p=0·035).

The authors concluded that the study demonstrates that high quality CRS is effective, but questioned the value of HIPEC in addition. Surgeons from other centres have criticised the high-dose oxaliplatin regime used in the trial, suggesting that it may have increased morbidity in the HIPEC arm due to its toxicity compared with other agents that are favoured elsewhere [53]. Additionally, survival in both treatment arms was higher than anticipated. It has been suggested that the effect of CRS has been underestimated in the past and that therefore the PRODIGE 7 trial was underpowered to detect the effect of the addition of HIPEC. There remains ongoing debate as to the value of HIPEC after CRS, but the trial supports the importance of complete tumour removal by an appropriately trained surgeon.

1.1.6 Peritoneal metastases in ovarian cancer

Ovarian cancer classically causes non-specific symptoms such as bloating, nausea, pelvic pain, and urinary symptoms. Consequently, it is often diagnosed at a late stage, particularly in the elderly, and peritoneal metastases at presentation are common. The FIGO staging system for ovarian cancer is depicted in Figure 1-4. Isolated peritoneal metastases are described by stage III, whilst those with stage IV disease may have peritoneal metastases in addition to other distant metastases. The National Cancer Registration and Analysis Service found that in England in 2012-13, 77% of women aged ≥80 were diagnosed at FIGO stage III or IV, compared to 66% of those aged 60-79, and 39% of those aged 15-59. [54]. Overall, between 2013 and 2017, an estimated 31.2% of ovarian cancer cases in England were diagnosed at stage III and 18.3% at stage IV [55]. This is a lower rate than quoted in the literature. Previous studies looking at the incidence of peritoneal metastases specifically have estimated that they are present at diagnosis in 50-80% of patients, and are more frequent in recurrent disease [56]. In 2015-17 there were 7,443 new cases of ovarian cancer per year in the UK and an age-standardised incidence rate (ASR) of 22.2 per 100,000 women [57]. Extrapolating from the UK incidence figures, this would suggest that between 3500 and 6000 women per year are diagnosed with ovarian cancer with peritoneal metastases in the UK.

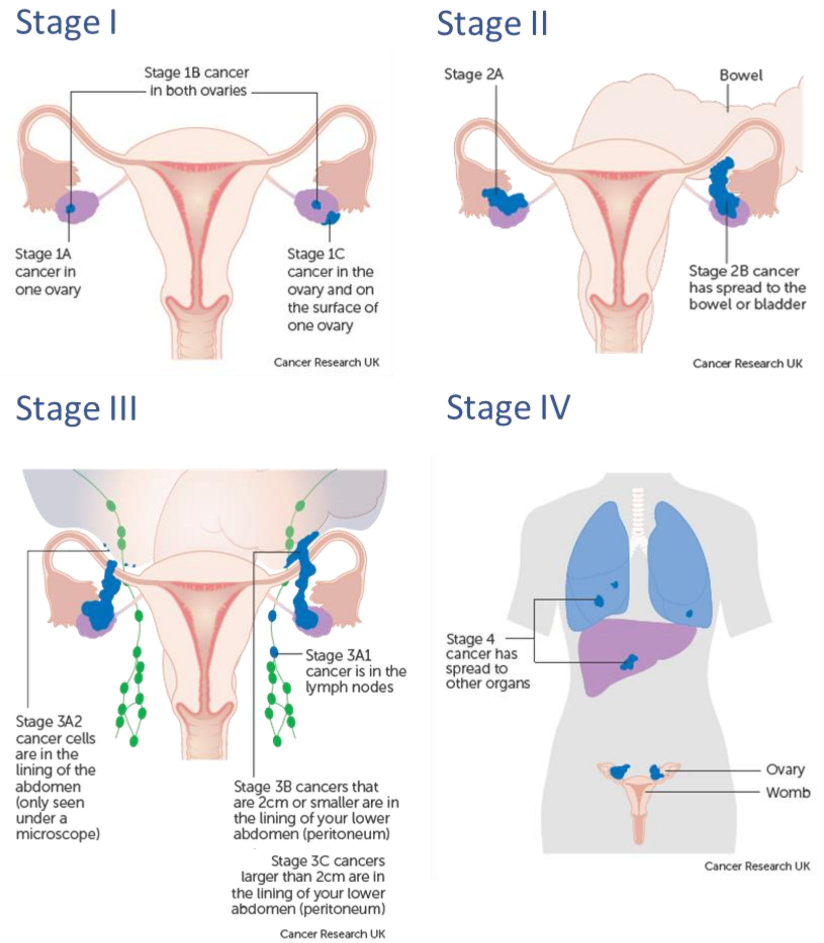

Figure 1-4: The stages of Ovarian Cancer according to the International Federation of Gynecology and Obstetrics (FIGO) system.

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1.1.6.1 Current treatment options and prognosis for peritoneal metastases from ovarian cancer

In the UK, the National Institute for Health and Care Excellence (NICE) guideline recommends that ovarian cancer is treated with optimal cytoreductive surgery [58]. The standard procedure involves surgical staging, and the visual inspection of the abdominal cavity as well as a total abdominal hysterectomy, bilateral salpingo-oophorectomy and infracolic omentectomy, biopsies of any peritoneal deposits, random biopsies of the pelvic and abdominal peritoneum, and retroperitoneal lymph node assessment. This is all that is required if it confirms that the disease is confined

to the ovary. In patients with disease that is FIGO stage II or more, the surgical management may be even more radical, with the aim of removing all tumour deposits that are 1cm or greater. In addition, systemic treatment is indicated, and first-line chemotherapy involves platinum-based agents with or without paclitaxel [58]. This achieves remission in most cases, however more than half of patients with advanced disease will relapse requiring second line chemotherapy [59]. The progression free survival and overall survival benefit achieved by second line chemotherapy is less than by first line treatment, and decreases after each subsequent line of chemotherapy [60].

If ovarian cancer is detected and treated whilst confined to the ovaries, survival is high, with 93.3% of people diagnosed with stage I disease in England between 2013-17 surviving for 5 years or more [55]. However, the prognosis rapidly declines with increasing stage of disease. Only 67.7% of people diagnosed with stage II disease survive 5 years, and this falls to 26.9% for people with stage III disease. The corresponding survival rate for people presenting with stage IV disease is 13.4% [55]. Unfortunately, as already discussed, the majority of women in the UK are diagnosed at stage III or IV [54].

Given the tendency for ovarian cancer to spread within the abdomen, there has been significant interest in intraperitoneal therapies. A systematic review of the benefits of CRS combined with either HIPEC or EPIC for FIGO stage III or IV primary ovarian cancer, or recurrent ovarian cancer, identified 1 non-randomised controlled study and 13 case series [61]. The results from 291 patients were reported overall. The authors deemed meta-analysis inappropriate due to the heterogeneity of the patient populations included and the treatment regimens across the studies. However, after a median follow up of between 13.7 and 30 months, the median OS ranged from 21.9 to 54 months (5 studies), and the mean OS from 31.5 to 48 months (3 studies).

Intraperitoneal chemotherapy following surgery has also been investigated. A Cochrane Collaboration systematic review of randomised controlled trials found that using intraperitoneal chemotherapy in addition to intravenous chemotherapy provided additional survival benefit compared to intravenous chemotherapy alone for people with ovarian cancer of any FIGO stage [62]. Both OS and progression free

survival (PFS) improved in the intraperitoneal chemotherapy group, HR 0.81 (95% CI 0.72-0.90) and 0.78 (95% CI 0.70-0.86) respectively. The quality of the evidence assessed was high according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group classification of evidence. However, intraperitoneal chemotherapy resulted in more adverse effects including pain, infection, fever, and gastrointestinal toxicity. Intraperitoneal chemotherapy was delivered in solution via an indwelling catheter which remained in situ for the duration of treatment, enabling multiple cycles of intraperitoneal therapy. Many of the complications were related to the catheters. Only one of the studies included in the systematic review, the Gynaecologic Oncology Group (GOG) 172 trial, assessed quality of life [63]. This was a randomised phase III trial assessing the impact of intraperitoneal cisplatin and paclitaxel in addition to intravenous paclitaxel for FIGO stage III epithelial ovarian cancer. It found that the patients in the intraperitoneal therapy group had significantly worse patient-reported outcomes for physical and functional wellbeing, neurotoxicity, and abdominal discomfort whilst receiving the treatment and for 3 to 6 weeks afterwards. The difference in the scores for neurotoxicity and abdominal discomfort persisted until the 12 month assessment. However, as per the Cochrane review overall, the patients in the GOG 172 trial who received intraperitoneal chemotherapy had significantly lengthened PFS and OS despite the increase in side effects. Intraperitoneal chemotherapy remains controversial in ovarian cancer treatment. NICE recommends that intraperitoneal chemotherapy should only be used in the UK in the context of a trial [58].

1.2 Pressurised Intraperitoneal Chemotherapy (PIPAC)

PIPAC is a recent innovation in the treatment of peritoneal disease in advanced malignancy [64]. It uses a laparoscopic surgical procedure to deliver intraperitoneal chemotherapy. During laparoscopic surgery the abdominal cavity is insufflated with carbon dioxide, creating a pneumoperitoneum, to allow the surgeon space to work. PIPAC involves aerosolising chemotherapy solutions into the abdominal cavity once the pneumoperitoneum is established using a specially designed laparoscopic nebuliser device (Micropump™ *Reger* Medizintechnik, Rottweil, Germany until 2015 and then *Capnopen®*, Capnomed, Villingendorf, Germany). The pneumoperitoneum

is maintained in a steady state at a pressure of 12mmHg with the aerosol dispersed in the abdominal cavity for 30 minutes. At the end of the procedure, all the gas is removed from the abdominal cavity but any drug solution that has condensed on the peritoneal surface is left in the abdomen. PIPAC can be repeated, with the first descriptions of the technique suggesting that this should be carried out every 4 to 6 weeks [3].

The rationale for PIPAC is that it uses many of the adjuncts discussed in section 1.1.4 to overcome the pharmacokinetic and pharmacodynamic challenges of treating peritoneal disease. It involves delivery of intraperitoneal chemotherapy, allowing higher concentrations of the drug solutions to be used compared to intravenous therapy [3]. The developers hypothesised that delivery into the pneumoperitoneum would be even more effective because the raised intra-abdominal pressure would improve absorption of drugs from the peritoneal cavity and offset the effects of the interstitial pressure in the tumour [2]. Additionally, it was thought that delivering the drug as an aerosol would enable more homogenous distribution through the peritoneal cavity, thus increasing the surface area of disease in contact with the drug [2].

The technique was initially tested *in vitro* and *in vivo.* The first published demonstration of the potential to deliver drugs by aerosolisation into the pneumoperitoneum at laparoscopy was a study in an *in vivo* pig model in 2000 [2]. This, and a subsequent study investigating the technique, used methylene blue dye, allowing a visual assessment of the distribution and penetration of the aerosol versus lavage in the pig model. Solass et al compared the distribution and penetration of methylene blue in the peritoneal cavity of 5 pigs compared to a control animal where the dye was administered by lavage [1]. The distribution of the dye was superior in the aerosolisation cases, although this was assessed by visual inspection rather than any objective measure [1, 2]. The second stage of pre-clinical testing involved an *ex vivo* tissue model to assess the penetration of a therapeutic substance, DNA strand break bait molecules (Dbait), into peritoneal tissue from a patient with metastatic endometrial cancer [65]. Dbait penetration was assessed using immunohistochemistry. Nodules treated with the pressurised aerosol had a more

homogenous drug uptake, and deeper penetration than nodules treated by lavage. Following these pre-clinical experiments, the same team progressed on to human applications [64].

Subsequent pre-clinical studies by other groups have suggested that the first generation of PIPAC technology has limitations. Experiments using chemotherapy agents, where drug uptake can be objectively measured, have found that the drug distribution and penetration in *ex vivo* [66, 67] and post-mortem animal [68] models is heterogenous. Although drug was detected in tissue that was not directly exposed to the aerosol jet, the greatest deposition of the aerosol was opposite the nebuliser. Analysis of the aerosol has shown that the droplet size is heterogenous [69]. Whilst the original CapnoPen design was based on a diesel fuel injector [69], other designs and strategies are now being tested that might overcome some of the limitations of the first generation device. These involve formation of the aerosol outside of the body cavity to enable heating prior to injection and also to ensure that the aerosol delivered is homogenous [70, 71]. Initial animal studies have shown promising results but these devices are not in clinical use.

PIPAC was introduced to clinical practice in Germany as an 'off-label' drug treatment [3]. Patients were accepted for therapy on a case-by-case basis. Case series published in the literature show that formal studies were then undertaken, and the technique was used in Russia, Denmark, Belgium, France, Switzerland, and Italy [64, 72-79]. The clinical research conducted using PIPAC will be reviewed and discussed in Chapter 4.

1.2.1 Electrostatic precipitation as an adjunct to PIPAC

Electrostatic precipitation can be used as an adjunct to the PIPAC procedure. The technology involves the use of a device; the 'Ultravison™ Ion Wand'. This is a stainless steel brush electrode that is inserted into the abdominal cavity during laparoscopic surgery and connected to the electrosurgical system. A Direct Current (DC) voltage (9kV) is applied to the device and the brush electrode releases anions [80]. These collide with any particulate matter in the abdominal cavity, transferring the negative charge. The negatively charged particles are then attracted to the nearest positively charged surface, which because of the patient return electrode, is the peritoneal

surface in the case of laparoscopic surgery. The system was designed to improve vision during laparoscopic surgery by clearing surgical smoke and water vapour generated from the use of electrocautery instruments. The developers of the 'Ion Wand' found that it was a very efficient method of clearing particulates and water droplets from the abdomen. In pre-clinical trials it was capable of precipitating 99.9% of particles ranging from 7nm to 10µm from the pneumoperitoneum [81].

It was hypothesised that the same principle could be used on the aerosol generated by the PIPAC procedures to improve drug deposition. This led to the assessment of electrostatic precipitation during PIPAC in a large animal model. Kakchekeeva et al [82] performed PIPAC with electrostatic precipitation (ePIPAC) and compared it to standard PIPAC, administering a solution containing toluidine blue dye and the drug DT01. There were 3 pigs in each group and a control animal that underwent the ePIPAC procedure with saline. The distribution of the dye was assessed qualitatively. Peritoneal fluid and tissue samples were taken before and after the procedures and the concentration of DT01 between the pre-procedure, post-PIPAC, and post ePIPAC samples was compared. There was a statistically significant difference in DT01 concentration between the samples, with the greatest tissue concentration of DT01 and the lowest peritoneal fluid concentration observed in the ePIPAC group. The authors suggested that ePIPAC may improve both the drug delivery during the procedure, and the safety of the procedure for the operating team. The return electrode attached to the patient's skin means that the entire peritoneal surface is rendered positively charged. In simple PIPAC, the effects of gravity and forward propulsion on the aerosol can cause deposition around the abdominal cavity to be uneven, with less drug deposited on surfaces above the end of the nebuliser, and most drug deposited directly opposite and underneath the CapnoPen [83]. Electrostatic precipitation may help to counteract the effect of these forces, potentially resulting in a more even distribution of the drug. The addition of electrostatic precipitation may also reduce the likelihood of contamination of the theatre environment with cytotoxic drugs, and reduce the chemotherapy content of the waste gas removed at the end of the procedure, by ensuring that a greater proportion of the drug administered is precipitated in the abdomen and absorbed.

The technique was also assessed by Reymond et al [84] and Willaert et al [75] in small patient case series focusing on the clinical safety and the occupational health and safety respectively. Figure 1-5 shows the equipment set up during the administration of chemotherapy by ePIPAC.

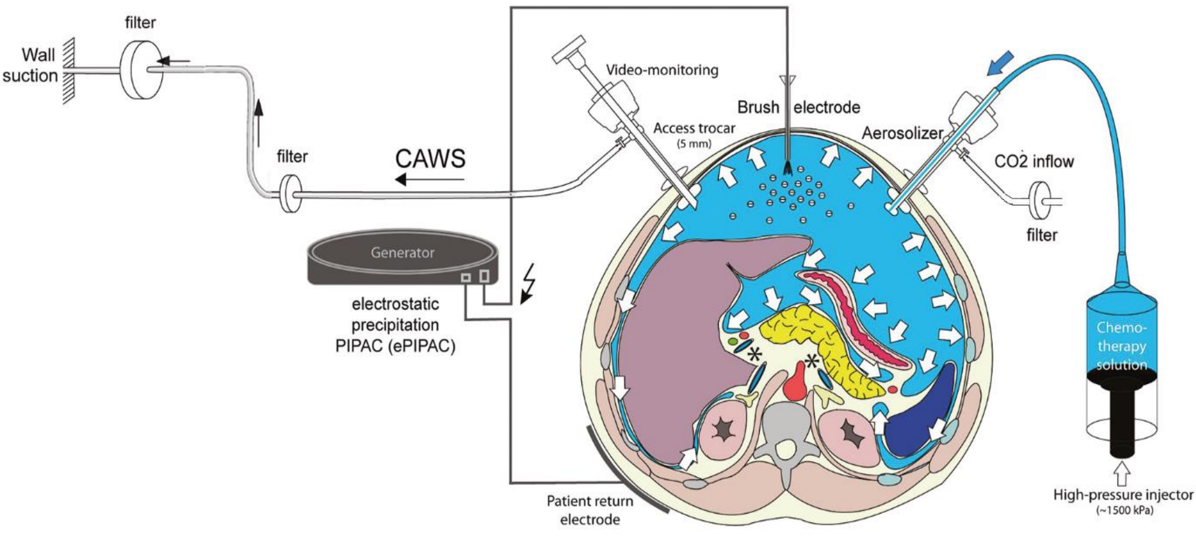

Figure 1-5: A diagram to show the equipment set up for ePIPAC.

Two balloon ports are depicted containing the aerosoliser and the camera for video monitoring of the procedure. The aerosoliser requires a high pressure injector to drive the chemotherapy solution through it. Carbon dioxide is insufflated via one port, and evacuated via the other to wall suction via 2 in-line filters. The brush electrode is inserted percutaneously separate to the other ports, and is connected to the generator. A patient return electrode is also shown. In PIPAC the brush electrode and generator are not used. CAWS = Closed aerosol waste system.

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1.3 Innovation in surgery

There are many ways that innovation in surgery may be described or classified. Krummel (2006) proposed that new developments could be grouped according to impact, starting with ‘simple tool modification’, for example making a small change to an existing instrument to facilitate a new technique, and proceeding to ‘revolutionising tools’ such as the advent of balloon catheters, and ‘revolutionising

technology or science', for example the invention of cardiopulmonary bypass [85]. In 2008 the Society of University Surgeons defined an innovative procedure as one which 'differs from currently accepted local practice, the outcomes of which have not been described, and which may entail risk to the patient' [86]. It was recognised that, as in other areas of medicine, it is necessary for existing procedures and treatments to be modified, and for new techniques to be developed in order to advance the field and improve outcomes. However, they stated that innovation must be conducted ethically and with patient safety in mind. The Society suggested that innovative practice, particularly if it is planned in advance, should still be overseen by a local committee. This group would provide an assessment of the value of the procedure and the outcomes achieved, uphold ethical and patient safety standards, and ensure that progression to formal human subjects research occurs at an appropriate stage [86]. The Society did not provide any direction relating to how this research should proceed. There are a number of hurdles that may need to be overcome in order to successfully complete a program of research in surgery.

1.3.1 The challenges associated with research in surgery

Trials, and in particular, RCTs have historically been scarce in surgery [87]. Traditionally, surgical innovations and new procedures were presented at surgical meetings, or published as case reports. The evolution of a procedure then usually involved large, often retrospective, observational studies or case series. New procedures often had an obvious and pronounced effect, and were adopted without evaluation against a comparator. Surgical practice therefore developed without the tradition of controlled trials [87]. This trend is perpetuated by the fact that there has been a less stringent regulatory requirement for trials prior to the introduction of a novel technique compared to a new drug, particularly if no new devices are required. There has also been less infrastructure and investment within surgery to conduct high quality research [88]. It is still the case that many clinicians feel that surgical trials are too difficult or impractical to undertake [88].

The introduction of a technique in a systematic and evidence-based fashion presents several challenges. There are a number of features of surgical interventions that make the conduct of trials, and RCTs in particular, difficult. The first challenge relates to the standardisation of the intervention. Surgeons frequently make minor adaptations to a procedure to account for the pathology, anatomy, and preference of an individual patient. The way that surgeons are taught, in an apprentice-style fashion, can introduce minor variations as techniques and procedures are demonstrated to and learned by the trainee. This variation can be problematic in the context of a trial. To conduct a RCT, there must be a comparator for which the clinicians and patients involved have an equal, or almost equal, preference. There are many instances where a lack of equipoise has been cited as a reason not to perform randomised trials. An example is breast reconstruction after mastectomy [88]. Theoretically, RCTs would be a good way to determine the optimal type of reconstruction, and the optimal timing, however they have not been conducted because the prevailing opinion in the field is that randomisation to a particular surgical option would be unacceptable to patients and clinicians [88]. Another challenge is that in order to avoid bias, the study subject and the assessor should be blinded to the intervention. There have been blinded surgical trials which have utilised techniques such as standardised dressings, concealment of incisions/scars, independent blinded assessors, and digital alteration of follow-up imaging to mask the type of implant used [89]. However, blinding may not always be possible, particularly in cases where the comparator is a non-surgical intervention, or a markedly different procedure.

New procedures may be unfamiliar or require the use of novel devices. There is usually a learning curve associated with the uptake of a new technique. It has been suggested that this may present a barrier to effective research, since a surgeon’s early cases may not represent the true effect of the procedure [5, 6]. However, it is also problematic to carry out cases without measuring the effect of the intervention until technical proficiency is achieved. If a procedure is shown to be beneficial, there is then the ongoing problem of ensuring that the outcomes remain the same, even as other clinicians take up the technique. It is recognised that the results of randomised

trials may not be generalisable to everyday practice, however this can be more pronounced in surgery since each surgeon that adopts a new practice will have a learning curve to overcome which may impact their early results.

Ethical concerns have been raised about surgical innovation in the past [90]. At the individual patient level, there may be an impact on patient autonomy, because of the difficulty of achieving informed consent for a novel procedure. There may be rare complications that are not identified during the early development of the technique. Surgical interventions are often expensive and operating theatre time is a limited resource. Thus, there is also the ethical problem relating to the allocation of resources in a healthcare system. The need to drive the development of new therapies must be balanced with the provision of standard care.

1.3.2 The IDEAL collaboration paradigm

The IDEAL (Idea, Development, Evaluation, Assessment, Long-term study) Framework is a scheme of investigation for innovative surgical therapeutic interventions that was described by the Balliol Collaboration in 2009 [4-6] and updated in 2019 [91]. The Collaboration was established to formulate a strategy to address concerns regarding shortcomings of research in surgery, with particular reference to novel procedures and practices. It was made up of a group of clinicians and methodologists who met in 2009 and agreed on the recommendations that they termed the IDEAL paradigm and published later that year. The IDEAL paradigm requires that a novel technique is investigated and introduced in a structured way. It is summarised in Table 1-1. The framework ultimately recommends that surgical innovation is carried out in a co-ordinated manner, that investigation should progress to a series of randomised trials, and that the culmination of the process should be an audited clinical registry.

Table 1-1: A summary of the stages of surgical innovation according to the IDEAL paradigm

(adapted from Barkun et al [5], Ergina et al [6], and McCulloch et al in 2009 [4]. A version of this table was included in Tate and Torkington (2020) [92])

Stage of innovation	Description	Number of patients	Proposed method of investigation
Stage 0: Pre-IDEAL	Pre-clinical work in vitro and in animals	None	Varied

Stage 1: Idea	First human applications: proof of concept and small safety studies	Very few	Structured case reports
Stage 2a: Development	Major technical details defined but technique remains experimental	Few, selected	Prospective development studies
Stage 2b: Exploration	Individual learning curves progressing quickly, with a resulting increase in patient accrual and broadening of indication. Effectiveness still not formally demonstrated.	Many, mixed	Research database, explanatory or feasibility RCT
Stage 3: Assessment	Procedure is part of many surgeons' practice and is becoming the standard of care.	Many, variable	RCT
Stage 4: Long Term Study	Procedure is routine practice and long-term outcomes and late/rare complications can be monitored.	Almost all	Registry, rare case reports.

The 'Idea' stage describes the work required for 'proof of concept'. The collaboration suggests that this is likely to be a few first-in-man studies or structured case reports describing the intervention for a small and highly selected cohort of patients. The new procedure would only be carried out by very few 'innovators'. The 'Development' stage describes the work undertaken to refine the new technique and determine the indications, so that it can be replicated in further work. The procedure should be well-described by the end of the Development stage, and the timing, outcomes of interest, and the short-term safety profile should be apparent. The collaboration suggest that the studies carried out should include a relatively small number of patients (approximately 30), should be prospective, and have ethical approval. At this point, a few early adopters would also be performing the technique in addition to the original innovators. The 'Exploration' stage sees a rapid increase in the numbers of patients included. The primary outcomes of the studies conducted may still relate to safety and feasibility, but the overall aim of research is to enable the conduct of a RCT in order to formally determine the efficacy of the technique in comparison to standard care. The short- term efficacy of the intervention is therefore of interest, and patient reported outcomes become more important. The indications for the procedure may expand, and many more surgeons are likely to adopt the technique. The 'Assessment' stage involves the conduct of RCTs to formally determine the efficacy of the new intervention. The procedure is likely to be part of

the early majority of surgeons' practice at this stage, and the indications for the procedure would be well defined. The medium and long term outcomes would become apparent. The cost-effectiveness of the intervention would also be established. The final stage of the paradigm is 'Long term study'. This describes the ongoing surveillance that the collaboration recommends for procedures that are part of routine practice. Registry data, audits, and case reports may be used to determine rare events, long-term outcomes, and to carry out quality assurance.

As well as describing the stages of research, the collaboration also provided recommendations for researchers to ensure that the studies carried out were of a high quality and that progress through the stages was efficient [91]. This included practices such as registering trials and publishing protocols, using standardised reporting for patient characteristics and outcomes, and ensuring that trial results are shared regardless of outcome. They proposed changes to improve the research environment in surgery. They made an appeal to journals to support and encourage the use of the paradigm by publishing the early stage trials and protocols, and requiring that authors adhere to minimum reporting guidelines. They requested that funders support early stage studies as 'pilot' or 'feasibility' for the RCTs that should follow.

The development of surgical research, and the impact of the Framework, was examined by McCulloch et al in 2018 [93]. They assessed adherence to the IDEAL Recommendations as a measure of progress in the field. They identified that there had been an increase in the number of surgical trials carried out between 2000 and 2014. Non-randomised trials were still more common than randomised trials, and there was no clear change in this trend. They also compared two samples of studies, one from 2000-2004 and a second from 2010-2014 to see if there was any change in the compliance with other aspects of the IDEAL paradigm. They found no change in the proportion of papers reporting prospective (as opposed to retrospective) studies. They did however identify an increase in the number of collaborative prospective cohort studies which led on to multicentre RCTs. There was also an increase in the proportion of RCTs that involved blinding or masking, and in the proportion of studies where surgeon learning curves were analysed and taken into account in pre-RCT

studies to address the bias introduced by this phenomenon. However, the IDEAL recommendations were not specifically referenced and the authors acknowledged that the Framework was a work in progress that had not yet been universally adopted. Despite this, there were signs that the research landscape in surgery was improving, and the authors suggested that the Framework may evolve and become more widely incorporated into practice over time.

1.4 Oncolytic viruses

Oncolytic viruses are a promising development in cancer treatment. They are viruses engineered to selectively infect, replicate in, and cause lysis of tumour cells. They therefore have the potential to be more specific and effective than existing cancer treatments. The lysis of the tumour cell generates tumour-derived antigens, thus promoting anti-tumour immunity. In addition to this primary immunotherapeutic action, there are a host of other effects that may be generated by encoding the virus with additional transgenes to enhance the anti-tumour effect. A number of these are summarised in Figure 1-6.

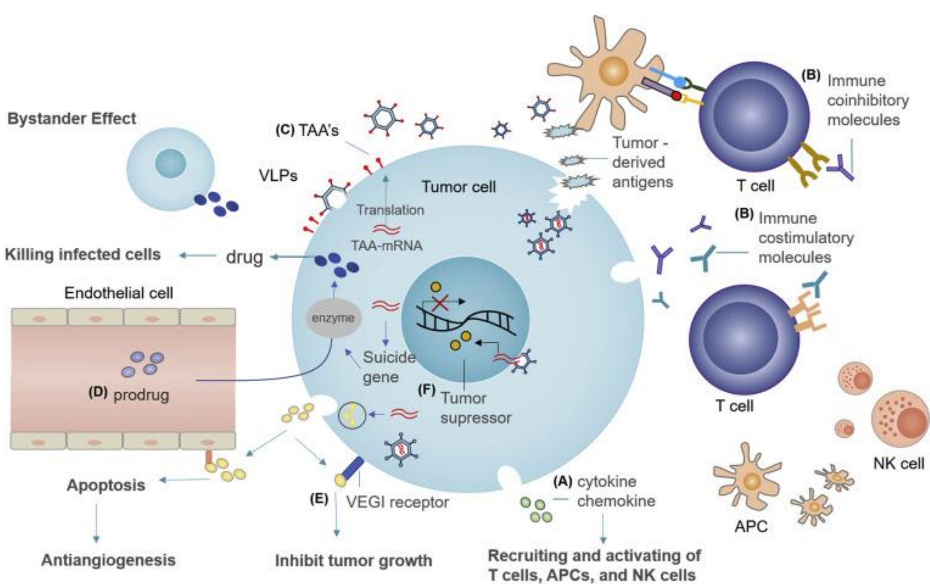

Figure 1-6 Diagram summarising the potential anti-tumour functions of oncolytic viruses

The addition of recombinant transgenes to the oncolytic virus can generate anti-tumour functions in addition to cell lysis.

A. *Stimulation of cytokines and chemokines to recruitment of immune cells in response to viral infection*
B. *Oncolytic virus encoding immune co-stimulatory molecules to activate T cells in the tumour microenvironment*
C. *Tumour-associated antigen encoding oncolytic viruses which cause virus-like particle presentation*
D. *Delivery of a suicide gene encoding an enzyme to convert nontoxic prodrugs to active metabolites inside tumour cells. This method also has a bystander effect since lysis of the tumour cell releases the active drug into the tumour microenvironment.*
E. *Oncolytic viruses encoded with anti-angiogenic transgenes to inhibit endothelial cell proliferation, e.g. vascular endothelial growth inhibitor (VEGI)*
F. *Tumour-suppressor genes to promote tumour regression and apoptosis.*

From Zheng et al [94] under the terms and conditions of the CC BY-NC-ND 4.0 license https://creativecommons.org/licenses/by-nc-nd/4.0/).

Many different viral species have been assessed as potential candidates for oncolytic virotherapy, and there have been clinical trials involving vectors based on adenoviruses, herpes simplex virus, vaccina virus, polioviruses, measles virus, reoviruses, vesicular stomatitis virus, and coxsackie virus (reviewed by Zheng et al [94]). The first virotherapy to be approved by the United States Food and Drug Administration (FDA) is the herpes simplex type1–based talimogene laherparepvec (T-VEC), a licensed oncolytic immunotherapy approved for advanced melanoma [95].

The work in this thesis will focus on adenovirus vectors. Adenoviruses were originally described in 1953 after being isolated from a human adenoid tissue sample [96]. Their potential use in gene therapy has been long recognised. This is both because they are generally considered to be safe, and because knowledge of their structure and biology has allowed modifications to be made to target adenovirus-based vectors to enable tumour-specific infection [97]. They have a number of intrinsic features which make them attractive as a therapeutic option, and these will be discussed further here. They are also an obvious choice for administration by aerosol, since the majority of species identified cause respiratory infections and therefore are evolved to spread via droplets through coughing and sneezing [97].

1.4.1 Structure and biology of adenoviruses

Human adenovirus serotypes were originally classified on the basis of the ability of specific animal antisera to neutralise them. The different adenovirus serotypes have

subsequently been classified into 6 species (A to G) based on their capacity to agglutinate human, rat, and monkey erythrocytes, their oncogenicity in rats, and their neutralisation properties (reviewed by Russell, 2009 [98]). There is some correlation between the different species of human adenovirus, their usage of receptors, their tissue tropism, and the clinical effects of infection. Adenoviruses are non-enveloped, with a double-stranded deoxyribonucleic acid (dsDNA) genome contained within an icosahedral capsid. They are about 90nm in size. Figure 1-7 shows a representation of the structure of the adenovirus, highlighting the hexon, penton base, fiber, and knob domains [99]. There are 240 hexons which make up the 20 faces of the icosahedral capsid. At each of the 12 vertices is a penton, made up of a homopentameric penton base, and a homotrimeric fiber protein which protrudes from each vertex [98]. The fiber has three regions; the tail, shaft, and the knob which is found distally. These proteins vary between species and serotypes, and determine the tropism of the virus. They are also potential sites for modifications. Inside the capsid is the dsDNA, which is associated with five core polypeptides.

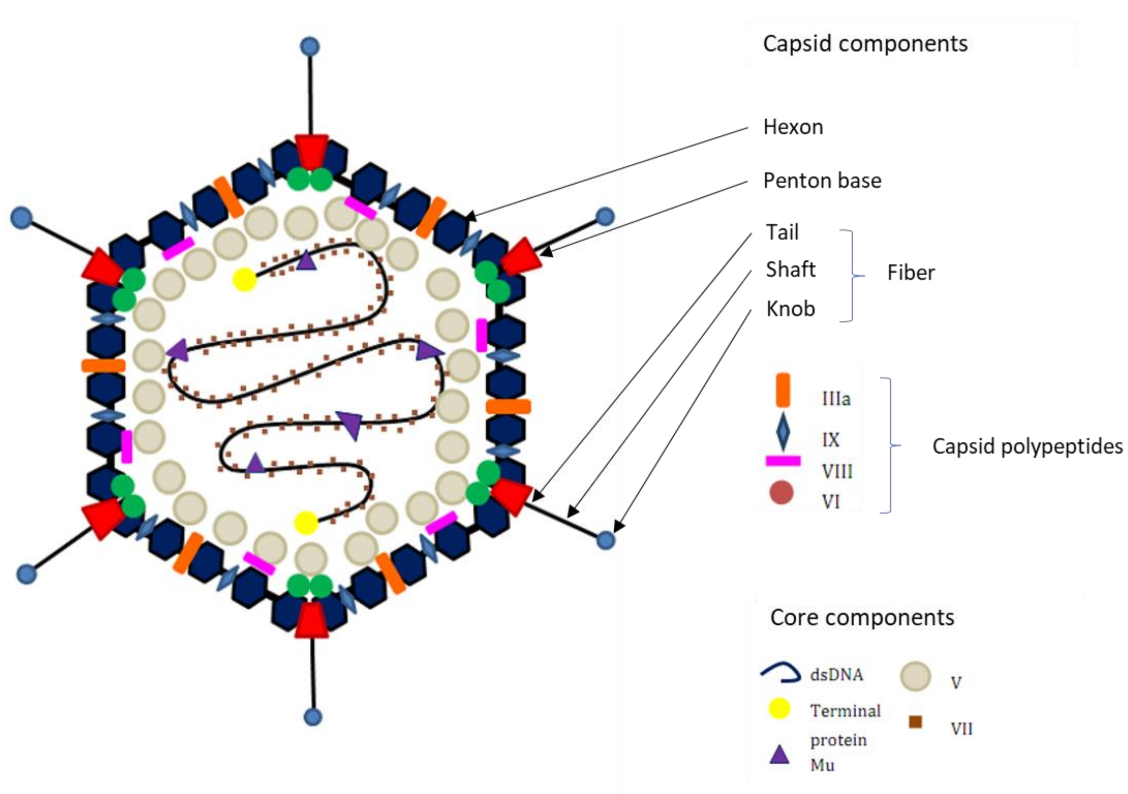

Figure 1-7: Diagram to show the overall structure of the adenovirus.

The major structural proteins of the icosahedral capsid; the hexon, the penton base, and fiber are highlighted. Variations in these structural proteins determine the tropism of the virus.

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Adenoviruses are obligate intracellular parasites, and can infect both dividing and non-dividing cells. They are lytic viruses, causing destruction of the host cell on infection and release of progeny viruses into the tissue. The life cycle of the adenovirus is summarised in Figure 1-8. This replication cycle is fast; the early phase is completed in 6-8 hours and the late phase in 4-6 hours, meaning that from infection to release of the mature new virions takes only 10-14 hours. The virus binds to cell surface proteins through interaction of receptor-binding domains on the knob domain of the fiber. This has shown to be the case for serotype 5 adenoviruses and human Coxsackie and Adenovirus Receptor (CAR) [100], and serotype 26 adenoviruses and sialic acid [101]. Interaction of the penton base with cell surface α*v* integrins is also required to enable internalisation through endocytosis [102]. Once inside the cell, the lowering of the pH in the endosome causes disassembly of the virion, which is beneficial to the virus as it allows escape from the endosome and has also been shown to enhance viral spread by promoting epithelial permeability through interactions with CAR at tight junctions *in vivo* (reviewed in [98]). The virus is then trafficked to the nucleus by the microtubule network, and binds at the nuclear pore complex. This enables release of its genome into the nucleus, and the initiation of the early phase of viral genome transcription and translation of early phase proteins. These promote the transcription of late phase genes and translation of late structural proteins. The new virions are assembled in the nucleus before being released through cell lysis, leading to the death of the host cell.

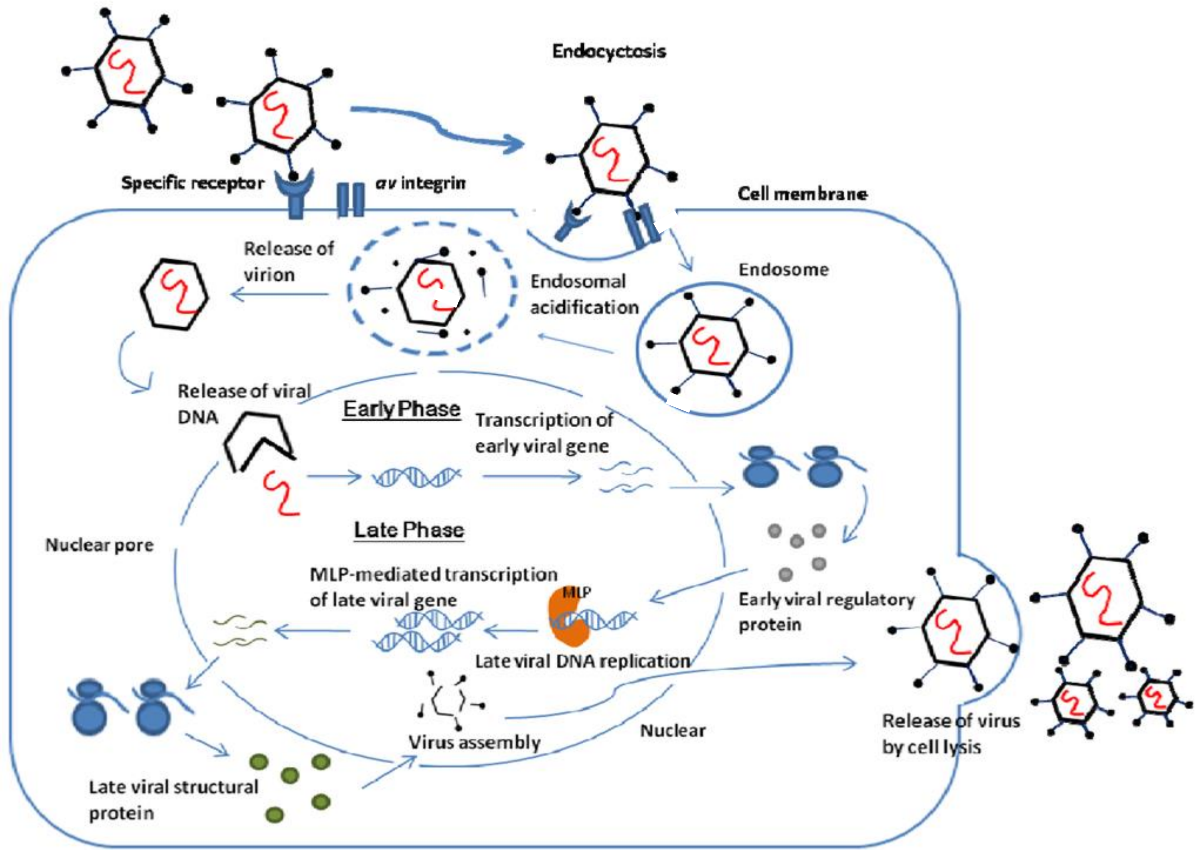

Figure 1-8: Summary of the life cycle of the adenovirus.

The virus binds to specific receptors or cell surface integrins and is internalised by endocytosis. Acidification of the endosome causes partial disassembly of the virion. On its release, it is trafficked to the nuclear pore complex via microtubules, and viral DNA is released. Transcription of early genes takes place first, generating early viral regulatory proteins. In the late phase, there is transcription of the late genes mediated by the Major Late Promoter (MLP). Late viral proteins are translated, and the new virions are assembled. The mature virus progeny are released by cell lysis.

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1.4.2 Adenovirus serotype 5 Vectors

Adenovirus serotype 5 (Ad5) has been a popular vector choice for groups developing biological therapies. Ad5 belongs to species C and uses the human CAR as its primary receptor [103]. It can therefore efficiently transduce a wide range of cell types, since CAR is ubiquitously expressed. The structure and biology of Ad5 is well-understood, including the genome [104]. It is possible to manipulate the Ad5 genome by using simple recombinant DNA techniques, and high levels of expression of foreign DNA inserts have been demonstrated [104]. Like other adenoviruses, Ad5 do not integrate

their DNA into the host genome and so they are not intrinsically oncogenic. High yields of virus can be generated and collected [104]. As discussed in section 1.4.1, infection results in lysis of the host cell, as well as release of progeny. Thus in addition to the initial cell killing occurring as the virus infects cells, the effect is amplified by generation of more virus copies at the target site, and further cell killing by progeny. The lysis of the cancer cells is immunogenic, releasing antigens and recruiting the immune system. These properties have been identified as advantageous for potential use as an adenovirus-based cancer treatment [97].

However, there are still limitations to be overcome. One problem with Ad5 vectors is that existing immunity is a common. There are high seroprevalence rates across the world, with pre-existing humoral immunity in the general population estimated to be around 30% in the UK [105], but as high as 90% or more in some parts of Sub-Saharan Africa [106, 107]. This means that vectors may be neutralised by antibodies before they reach their target tissue. Studies have shown that the humoral response to Ad infection generates antibodies targeted at epitopes on the fiber, penton base, and hexon. These antibodies act synergistically to neutralise the virus on re-exposure [108]. This problem has been circumvented in the past in other vectors by adjusting the method of administration. For example, T-VEC is administered directly into the tumour in order to facilitate entry into cancer cells [95]. Other methods being trialled currently involve substituting parts of the virus capsid, for example the fiber or the fiber knob domain, from another adenovirus serotype with a lower seroprevalence (reviewed in [97]). As well as altering the binding capacity of the engineered vector, this may allow better evasion of existing immunity.

In addition to problems relating to neutralisation, there are also hurdles to be overcome relating the sequestration of Ad5 vectors. Ad5 has been shown to form complexes with coagulation factor X *in vivo* in order to engage heparan sulphate proteoglycans (HSPGs) to enter cells [109]. However, these proteins are expressed abundantly on hepatocytes and this results in the efficient and rapid sequestration of systemically administered Ad5 vectors in the liver. Additionally, the native receptor, human CAR, is ubiquitously expressed within tight junctions throughout the body [110]. Human erythrocytes have also been shown to express CAR and to bind

and inactivate Ad5 in the bloodstream [111]. It is thought this is a mechanism by which Ad5 infection is prevented in humans, independently of antibody status. This means that an Ad5 vector with unmodified tropism may be neutralised rapidly, but could also cause significant off-target effects through infection of other cells expressing CAR or HSPGs. Alterations to the virus may be able to overcome this sequestration by rendering it incapable of interaction with blood factors or native receptors. This is also beneficial for the re-targeting of the virus to specific receptors. CAR expression is frequently altered on tumour cells, and may be up-regulated, down-regulated, or not expressed at all, whilst other proteins may be more common and specific to the tumour [110]. One such example was recently described by Uusi-Kerttula et al [112]. Ad5 $_{NULL}$A20 is an Ad5 vector that has had all native tropisms ablated before being retargeted to the αvβ6 integrin through incorporation of an αvβ6-binding peptide (A20, NAVPNLRGDLQVLAQKVART) within the fiber knob domain HI loop. The αvβ6 integrin was selected as the target of this vector because its expression is restricted to aggressively transformed epithelial cells. In normal tissues, it is usually only expressed during development or after injury during wound healing, and is thought to have a role in the regulation of epithelial cell proliferation, migration, and phenotype [113]. Van Aarsen et al [114] assessed αvβ6 expression in biopsies from a number of epithelial cell tumour types, and found it was strongly upregulated in squamous cell carcinomas. They looked at metastatic deposits from epithelial cell cancers and found that these also expressed αvβ6 integrin, reflecting the tissue type of the primary cancer rather than the site of the metastasis. The Ad5 $_{NULL}$A20 vector has been shown to specifically infect epithelial ovarian cancer cells *in vitro* and *in vivo* [112].

Even with a highly modified and specific vector, there will still be an immune response to the oncolytic virus which may affect its usefulness. In the short term, it is possible that the inflammatory response in the host may reduce the tolerability of the treatment. This was sadly demonstrated in 1999 when an early trial of an adenovirus gene therapy vector resulted in the death of a subject due to a cytokine storm [115]. A huge dose (3.8×10^{13} viral particles [vp]) was administered into the hepatic artery of the 18 year old trial subject, and he developed a fever, coagulopathy, and adult

respiratory distress syndrome, and died 4 days later. The development of vectors has been refined since these events, but it demonstrates the potential for dose-limiting toxicity, and the need to consider the route of administration and potential for off-target effects. Even in cases where the immune response to the vector is tolerated, the generation of antibodies against the virus used may preclude effective repetitive administrations.

1.5 Aims and hypothesis

As stated in the beginning of this introduction, the aims of this project are to investigate the use of pressurised aerosolised therapeutics in the treatment of peritoneal metastases, with a focus on ovarian and colorectal cancer.

The uncertainty regarding the incidence of colorectal peritoneal metastases and the difficulty in separating this patient group using routinely collected outcome data will be managed by a service evaluation of the management of colorectal peritoneal metastases at CAV UHB. This will assess the number of patients treated locally, as well as the therapeutic modalities used, and the overall outcomes achieved.

PIPAC represents an innovative treatment for this patient group. The development and introduction of this technique worldwide will be appraised by conducting a literature search and assessing the clinical studies identified using the IDEAL criteria set out by the Balliol Collaboration in 2009 [4].

The rationale for and the practicalities of the introduction of PIPAC to UK will be discussed, and an audit of the first cases presented. This work on the feasibility of incorporating the technique into UK practice will be essential for the participation in future trials to determine the efficacy of the procedure, and the best indications for its use.

Peritoneal metastases from all cancer types represent an area of unmet clinical need. There are a number of advanced therapeutics in development which may be useful in this clinical scenario. This project will investigate the hypothesis that delivery of oncolytic adenovirus by laparoscopic aerosolisation is feasible. Oncolytic

adenoviruses represent an exciting prospect for the targeted treatment of disseminated cancer, and delivery into the peritoneal cavity may be advantageous in peritoneal disease. Intraperitoneal delivery may circumvent some of the obstacles associated with systemic delivery, such as interactions with blood proteins and circulating antibodies. Ascites is drained as part of the operation, and this may reduce the effect of neutralising antibodies that are present in the ascites of patients who have had previous exposure and therefore acquired immunity to Ad [116]. It may be possible for the dose administered to be reduced if pressurised intraperitoneal administration is more efficient, and this would have benefits for both the cost and safety of treatment.

2 Materials and Methods

2.1 Peritoneal metastases: The management of peritoneal metastases from colorectal cancer – a service evaluation of Cardiff and Vale University Health Board

The service evaluation was registered and approved by the Clinical Director for General Surgery and the Clinical Board Director for surgery (see appendix 8.1). Permission was obtained from the Health Board Caldicott Guardian to access the records relating to the treatment of patients with peritoneal metastases from colorectal cancer using the Cancer Network Information System Cymru (CaNISC) (see appendix 8.2).

Cases were identified by searching the minutes of the colorectal cancer multidisciplinary team (MDT) meeting from January 2014 to December 2019 inclusive. The Cardiff and Vale University Health Board (CAV UHB) Colorectal Multidisciplinary Team Meeting (MDT) Minutes were searched electronically for the term 'perit$' and then the records identified were reviewed. Patients who had a diagnosis of peritoneal metastases from colorectal cancer recorded by the MDT were included. Patients who had metastases from appendiceal cancers were excluded. Patients diagnosed with pseudomyxoma peritonei were excluded. The CaNISC record was used to determine the treatment plan proposed by the MDT for each patient and the treatment received. The potential suitability of patients for CRS and HIPEC was determined from the MDT discussion and outcome recorded. This was corroborated with letters of referral to the Peritoneal Malignancy Institute, Basingstoke, and records of Individual Patient Funding Requests (IPFRs) made by the department. Pathology records, where available, were used to confirm the diagnosis of peritoneal metastases and also of any resectional surgery performed. The patient's age at diagnosis of the peritoneal metastases was determined. Data about the site of the primary tumour and the histological type were recorded, as well as the timing of the peritoneal metastases in relation to the primary tumour (synchronous or metachronous) and the presence of any other metastases. Peritoneal metastases identified at the time of staging for a primary tumour were considered synchronous. Patients who presented as an emergency and had peritoneal metastases diagnosed at operation were also recorded as having synchronous disease. The date of the

investigation or procedure from which the diagnosis of peritoneal metastases was made was recorded and subsequently used to determine survival. Patient status was determined from the hospital record. The final data were collected on 1st October 2020. Patients who had no date of death recorded at this time were censored from the survival analysis using the date of the last entry into the electronic health record.

Data were analysed using Excel and Graph Pad Prism v9.0. Descriptive statistics were determined and survival analyses performed to generate Kaplan-Meier curves for disease, treatment, and age stratified groups.

2.2 The introduction of a new technology: Pressurised Intraperitoneal Aerosolised Chemotherapy assessed by the IDEAL paradigm criteria

2.2.1 Search Strategy

To review the progression of research relating to the use of PIPAC, and appraise this new technology, a search of Medline and Embase was carried out using the terms 'PIPAC', 'ePIPAC', 'aerosol$ adj3 chemotherapy' and 'pressuri$ adj3 chemotherapy' on 08.09.2017. The combination of these search terms is described in Table 2-1. Reymond et al (2000) first described the concept of a 'therapeutic capnoperitoneum' so the search was limited from 01.01.2000-present (8). Additionally, the reference lists of identified papers were screened, and ResearchGate.net was searched for the term 'PIPAC' to identify any other publications. A single reviewer screened the abstracts to identify original research papers relating to PIPAC. Conference abstracts, review articles, editorials, instructional articles associated with videos, and book chapters were excluded, as were errata to articles. Protocol papers were included but were identified as such in the results. Only articles in English were reviewed. The full text was then obtained. To get an up-to-date picture of research activity, ClinicalTrials.gov and the EU Clinical Trials Register were searched to identify trials. The results were cross-referenced with the identified publications. Trials which were not yet reported in the literature were included and assigned a stage in the same way as published protocols.

Table 2-1: Search strategy for database searches

Searches performed	Term
1	PIPAC
2	EPIPAC
3	Aerosol$ adj3 chemotherapy
4	Pressure$ adj3 chemotherapy
5	1 OR 2 OR 3 OR 4 OR 5
6	Remove duplicates from 5 and limit to 01.01.2000 to present

2.2.2 Application of the IDEAL Paradigm to the search results

All the studies identified were graded according to the stages of innovation set out in the IDEAL paradigm. Any studies described in protocol papers or by registration on a trials database were assessed based on the planned inclusion criteria, methods, and outcome measures.

The IDEAL criteria were described in three papers by Barkun et al [5], Ergina et al [6], and McCulloch et al in 2009 [4] and further developed by Hirst et al in 2019 [91]. In order to assess the literature relating to PIPAC, the types of studies corresponding to each IDEAL stage were defined. The criteria used to interpret and assign the stages are summarised in Table 2-2

Stage 0 (Pre-IDEAL) is used to define pre-clinical work *in vitro* and in animals. This stage was assigned to pre-clinical and *in vitro* studies which did not include any data or patient samples from PIPAC cases. Translational *in vitro* work carried out later, for example using samples collected from surgery, were assigned the stage appropriate for the associated clinical part of the research.

Stage 1 (Idea) is used to describe first-in-human applications, with the IDEAL collaboration suggesting that at this point in the development of a technique there would be very few surgeons performing it and they would report their findings in structured case reports, with outcomes relating to proof of concept and feasibility. This stage was assigned to case reports and small case series (in general, less than 10 patients). Studies that evaluated Occupational Health and Safety in the early cases were also included in this stage. For the purpose of this review, data collection could

be prospective or retrospective to be included in this stage. Ethical approval was not a requirement.

Stage 2a (Development) is where the technique is developed further using prospective studies that focus on safety and technical and procedural success. In this review, this stage was assigned to larger case series (in general, more than 10 patients) and single-arm, non-randomised studies of the technique. Formal ethical approval was a defining feature in the IDEAL collaboration description of studies in this stage. For the purpose of this review, data collection could be prospective or retrospective.

Stage 2b (Exploration) is described as a stage of 'learning'. During this stage of research, the number of patients treated and the number of clinicians performing the procedure expand rapidly. The indications for the procedure broaden. The studies performed are designed to evaluate a number of outcomes, ranging from ongoing feasibility, safety, short-term clinical outcomes, and patient-centred or reported/ outcomes. The studies should have ethical approval and data collection should be prospective as part of a research database or an explanatory or feasibility randomised controlled trial (RCT). For the purpose of this review, publications which reported outcomes from patients included in a prospectively maintained database, reports of RCTs, and prospective studies which investigated a new indication for the technique were included.

Stage 3 (Assessment) is reached when there are many surgeons performing the technique and there are well defined indications. Investigation in this stage is by RCT, and the outcomes of interest relate to middle and long-term efficacy, patient-centred or reported outcomes, and cost-effectiveness.

Stage 4 (Long-term study) describes the ongoing surveillance that takes place to monitor a technique that is in mainstream use. This may involve a registry or database. The primary aim is audit and quality assurance. Rare events and long-term outcomes may also be detected. This stage is not yet applicable to PIPAC. The International Registry has the potential to fulfil this role in the future if the technique continues to expand.

Table 2-2: A summary of the stages of surgical innovation according to the IDEAL paradigm.

(adapted from Barkun et al [5], Ergina et al [6], and McCulloch et al in 2009 [4]), with description of the interpretation of these stages in this review relating to the published work on PIPAC. A version of this table was included in Tate and Torkington (2020) [92]

Stage of innovation	**Description**	**Number of patients**	**Proposed method of investigation**	**Studies to be included in this stage in this review**
Stage 0: Idea	Pre-clinical work in vitro and in animals	None	varied	Pre-clinical studies in animals (in vivo and post-mortem) and in vitro.
Stage 1: Idea	First human applications: proof of concept and small safety studies	Very few	Structured case reports	Case reports and small case series. Occupational health & safety studies. Scientific studies of clinical samples. Data presented relate to safety and/or initial feasibility/proof of concept. Prospective or retrospective data collection.
Stage 2a: Development	Major technical details defined but technique remains experimental	Few, selected	Prospective development studies	Larger case series, and single arm non-randomised studies. Scientific studies of clinical samples. Prospective or retrospective data collection.
Stage 2b: Exploration	Individual learning curves progressing quickly, with a resulting increase in patient accrual and broadening of indication. Effectiveness still not formally demonstrated.	Many, mixed	Research database, explanatory or feasibility RCT	Large case series from a prospectively maintained database, and RCTs. Scientific studies of clinical samples. Prospective study relating to a new indication for the technique. Primary outcomes are efficacy related. Prospective data collection.
Stage 3: Assessment	Procedure is part of many surgeons' practice, and is becoming the standard of care.	Many, variable	RCT	RCT with primary outcome relating to efficacy.
Stage 4: Long Term Study	Procedure is routine practice and long-term outcomes and late/rare complications can be monitored.	Almost all	Registry, rare case reports.	Registry or database

2.2.3 Health Technology Appraisal of PIPAC

The primary objective of this aspect of the review was to assess the safety of PIPAC as a treatment for peritoneal metastases from any primary cancer. The secondary objectives were to assess the efficacy of PIPAC, particularly the effect on survival, and the impact on patients' quality of life. These objectives were selected because they are important outcome measures for a treatment used in the palliative setting.

Eligibility criteria for inclusion in the appraisal were defined for each objective. For all objectives, the studies must have reported outcomes from PIPAC treatment in human subjects and described the procedure and the doses of the cytotoxics administered. No specific criteria about the design of the study were set. To be included in the assessment of safety, prospective reporting of adverse events, classified according to a standardised system, must have taken place. For assessment of survival, survival data of a treated group, and the characteristics of the group, had to be described. For assessment of quality of life, a validated assessment tool must have been used before and after treatment. For efficacy, tumour endpoints must have been assessed according to a standardised system before and after treatment.

2.3 ePIPAC at Cardiff and Vale University Health Board: Audit of a pilot program

2.3.1 Pilot development

The protocol for the pilot was developed in accordance with the guidance provided by the faculty at the International Society for the Study of Pleura and Peritoneum (ISSPP) PIPAC training course and considering further evidence available in the literature. The protocol was written with the input of a number of specialists.

2.3.2 Indications and contraindications for treatment

The indications for PIPAC were agreed by a group from the CAV UHB colorectal MDT, including surgeons and oncologists. They were based on the inclusion and exclusion criteria of the available published trials.

Given the experimental nature of the procedure, only adult patients who could provide informed consent were eligible. Prior to the start of the pilot, most of the data available was on the treatment of gynaecological cancers. Peritoneal metastases of ovarian origin were therefore included. The intention was to offer PIPAC as an additional line of treatment. PIPAC was only considered for patients who had platinum resistant disease (relapse within 6 months of treatment with platinum therapy), platinum refractory disease (progression on platinum therapy), or platinum sensitive disease but who had received ≥3 lines of systemic chemotherapy in the metastatic setting. Due to the invasive nature of the procedure, and the requirement for 3 treatments each 6 weeks apart, a life expectancy of 6 months or more was also included in the criteria for treatment. Data were also available for colorectal cancer patients. Again, the aim was to provide an additional treatment modality, and therefore in order to be eligible for PIPAC, patients needed to have a contraindication or be ineligible for CRS and HIPEC, and have progressive disease despite first line systemic chemotherapy in the metastatic setting. It was anticipated that this group of patients would be larger than in other units in England and Scotland because of the lack of funding for CRS and HIPEC for colorectal peritoneal metastases in Wales.

When the pilot was commenced, PIPAC was not generally being performed alongside systemic chemotherapy. PIPAC only treats serosal disease in the peritoneal cavity, and therefore patients with other metastases were ineligible, unless the MDT agreed that those other metastases were stable and not requiring active treatment. Additionally, the patient should not have received chemotherapy in the preceding 3 weeks, radiotherapy in the preceding week, or any other clinical trial drug for a month prior to treatment.

Other criteria related to the potential fitness of the patient to undergo general anaesthesia, an invasive procedure, and receive chemotherapy. Patients were screened using the standard CAV UHB anaesthetic pre-assessment pathway, and parameters were stipulated for haematological and biochemical investigations.

2.3.3 Treatment schedule, drugs, and doses

It was anticipated that each of the three patients treated during the pilot would have three ePIPAC procedures, each 6 weeks apart. If there was evidence of disease progression or development of contraindications to therapy, then treatment would be suspended.

Drug dosing was based on body surface area (BSA), which was calculated using the Dubois and Dubois equation (BSA = (W 0.425 x H 0.725) x 0.007184). Doses were capped at a maximum BSA of $2.2m^2$. Since significant renal or hepatic impairment was considered a contraindication to treatment, there were no planned modifications to the doses.

Cisplatin and doxorubicin were used for ovarian and gastric cancer as per the body of published work available at the start of the pilot. The dose of Cisplatin was $7.5mg/m^2$ in a 150 ml NaCl 0.9% solution delivered as an aerosol into the abdominal cavity during laparoscopic surgery, immediately followed by doxorubicin at a dose of $1.5mg/m^2$ in 50ml NaCl 0.9% solution delivered as an aerosol into the abdominal cavity. Both chemotherapies were delivered at a rate of 30ml/minute and were left in the abdomen for 30 minutes after delivery. Electrostatic precipitation was applied to the gas in the abdomen for one minute at the end of the procedure prior to evacuation of the gas from the abdomen. The timing of the activation was chosen to ensure that chemotherapy delivery was conducted in the same manner as previous reports in the literature. The aim of the electrostatic precipitation was to ensure that the gas being evacuated at the end of the procedure contained as little chemotherapeutic agent as possible, in order to minimise contamination of the theatre environment by gas leak at this stage of the procedure.

For colorectal cancer, Oxaliplatin at a dose of $92mg/m^2$ in a 150 ml 5% glucose solution was delivered as an aerosol into the abdominal cavity. The rate of delivery was 30ml/minute, and again, the aerosol was left in the abdomen for 30 minutes and the electrostatic precipitation activated at the end of the procedure.

The protocol for the pilot cases was approved by the CAV UHB Medicines Management Group, the CAV Cytotoxic Board and the CAV UHB Quality, Safety and Experience Committee, as part of the 'New Procedures' process.

2.3.4 Surgical procedure

ePIPAC was performed according to the protocol described by Reymond et al [84]. A safety checklist was prepared and used to ensure the procedure was standardised (Appendix 8.4). Briefly, under general anaesthesia, laparoscopic access was gained to the abdomen. Balloon ports were used to ensure there was no leak of gas from the pneumoperitoneum. The peritoneal cavity was examined and the extent of disease recording using the Peritoneal Carcinomatosis Index (PCI) score as described by Jacquet and Sugarbaker [117]. Biopsies were taken from each quadrant of the abdomen, with the most suspicious areas sampled. Ascites was drained and a sample sent for histology. The drugs were loaded onto the high-pressure injector and the tubing was connected to the CapnoPen (CapnoMed, Villigendorf, Germany) which was fixed in a 10mm port in the laparoscopic field of view. The camera was also fixed in position. The pressure in the pneumoperitoneum was confirmed, with zero gas flow used to indicate that there was no leak. All staff then left theatre and the injection was initiated remotely. A single staff member re-entered the theatre after 30 minutes had elapsed and activated the electrostatic precipitation (Alesi, Cardiff UK). After a further 60 seconds, the pneumoperitoneum was evacuated using a closed tubing system with in-line filters via the hospital waste gas scavenging system. The abdomen was then closed and the patient recovered from anaesthesia.

2.3.5 Risk assessment, Staff training and safety

A risk assessment was undertaken considering the theatre environment, the sterile services unit, and the ward. The risk to staff on the ward was deemed minimal since the doses being used were small. No specific additional training needs were identified. A training pack was developed for the theatre staff. An initial presentation was given to staff at the perioperative care division monthly quality and safety

training meeting on 13th December 2017. A training manual was developed and was available to all staff who volunteered to take part in the cases. Prior to the first case, a simulation training session was conducted in theatre with the team of staff who would be working on the day. All the procedure-specific equipment was demonstrated. Control of Substances Hazardous to Health (COSHH) training specific to the handling of cytotoxic substances was provided.

As in other centres, staff left the theatre whilst chemotherapy was being administered. There was equipment in place to enable remote monitoring of the patient, and Personal Protective Equipment (PPE) was provided in case re-entry to theatre during delivery of chemotherapy was required. The potential for, and actions to take in the event of a leak or spillage of chemotherapy was discussed with staff and the training session covered the possible scenarios.

Following the first case, a further presentation was made in the anaesthetic monthly quality and safety meeting September 11th 2018 and a specific guide written for anaesthetists who might participate in cases. Two consultant anaesthetists were responsible for the lists used for the PIPAC cases.

2.3.6 Patient safety and clinical governance

A patient information leaflet was developed specifically for the pilot. This acknowledged the innovative nature of the technique and the limitations of the evidence at the time. A standard hospital consent form was used for the procedure itself. The small number of patients involved meant that whilst no patient identifiable data was ever intended to be published or shared, it would potentially be possible for patients to recognise themselves in any future work that used data or images from the pilot. Given the aims of the pilot, it was anticipated that the data from the pilot cases may be used on documents shared outside of the clinical team, e.g. future grant applications or ethics approval applications, or even on publicly available documents. Therefore, a separate and specific consent form was developed and used to ensure that patients were aware how data and images from the cases might be used and had given their permission (Appendix 8.5). A database was populated prospectively (data

collection form shown in Appendix 8.6). This recorded procedural details such as the duration of the cases, as well as data on complications and adverse events relating to the treatment. Any complications that were identified as surgical were graded according to the Clavien-Dindo classification. Complications that were thought to be related to the chemotherapy drug were graded according to the CTCAE v4.0. The grading system is summarised in Table 2-3.

Table 2-3: The grading of adverse events according to the Common Terminology Criteria for Adverse Events v4.0

Grade	Classification	Description
Grade 1	Mild;	asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate	minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
Grade 3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
Grade 4	Life-threatening	Life threatening consequences; urgent intervention indicated
Grade 5	Death	Death

2.3.7 Audit of the pilot:

As described, a prospective database to audit the pilot was maintained. Data on the procedures was captured using a standardised form (see appendix 8.6). The adherence to the planned protocol was recorded as well as feasibility data relating to the procedure. These were compared to the values published in the largest case series.

2.3.7.1 Standards used for the audit of the pilot

The literature review performed before the pilot commenced informed the development of the protocol, and also criteria that were used to benchmark the results. These were updated as further data was published.

Patient characteristics:

The criteria for PIPAC treatment stated in the standard operating procedure are shown in Table 2-4.

Table 2-4: Anticipated patient characteristics for Cardiff ePIPAC pilot:

Patient Characteristic	**Criteria for treatment in pilot**
Primary cancer	Ovarian, colorectal, or appendiceal cancer.
Pre-op Eastern Cooperative Oncology Group (ECOG) performance score	0-2
ASA class at time of procedure	1-3

Procedures:

Giger-Pabst and Tempfer (2018) [118] described the technique used and the results achieved after 1200 cases in 512 patients at a single centre. Alyami et al (2019) [119] conducted a systematic review and meta-analysis and presented the results from 1810 PIPAC procedures in 838 patients. They analysed data from all available studies but separated the results into those from prospective trials and those from retrospective trials. They also excluded any results which appeared to be duplicated reports. Hubner et al [120] presented procedural data on the 127 procedures performed in 58 patients in their consecutive case series from a single centre. These reports were used to provide a benchmark for the data collected on the ePIPAC procedures. Table 2-5 shows the standards that were used to assess procedural data, and Table 2-6 shows the standards that were used to assess safety data.

Table 2-5: Standards used to assess procedural aspects of the Cardiff ePIPAC pilot and source.

Procedural characteristics	**Standard Identified in the literature**
Access to abdomen possible	89.5%-91.5%[2]
Duration to nearest minute (median and range)	Median operating time = 1h38min[3]
Number of laparoscopic ports	2 = 88%, >2 = 12%[3]

Procedural characteristics	Standard Identified in the literature
Number of biopsies taken (median and range)	'Biopsies are taken from all 4 quadrants of the abdomen'[1] Median number of biopsies taken = 3[3]
PCI recorded	Median PCI = 11[3] PCI incomplete = 28%[3]
Volume of ascites drained in millilitres (median and range)	'All ascites is removed'[1] Median = 50ml[3]
Number of ePIPAC procedures	'Goal of 3 cycles per patient'[1] Proportion of patients having 2 or more procedures = 65%[2]
Timing of ePIPAC procedures	42 days (+/- 6 days) between procedures[1]

Notes: 1 = standard identified in Giger-Pabst and Tempfer [118]
2 = standard identified in Alyami et al [119]
3 = standard identified in Hubner et al [120]

Table 2-6: Standards used to assess safety data from the Cardiff ePIPAC pilot and source.

Factor	Standard Identified in the literature	Audit results
Incidents reported	Major = 0% cases[3] Minor = 11% cases[3]	Major = 0 (0%) Minor = 1 (14.3%)
Chemotherapy leaks or spillages	Contained = 5/137 (3.9%)[3] Non-contained = 0[3]	Contained = 0 Non-contained = 0
Complications recorded	Mild to moderate: 33% cases[1], 0-11% patients intra-operatively and 0-6% patients post-operatively[2] Severe or life threatening: 0.3% cases[1], 12-15% patients[2] Death = 0.4% per procedure[1], 1.5%[1] - 2.7%[2] per patient	Mild = 7 (100%) cases Moderate = 3 (43%) cases Severe = 0 Death = 0

Notes: 1 = standard identified in Giger-Pabst and Tempfer [118]
2 = standard identified in Alyami et al [119]
3 = standard identified in Hubner et al [120]

2.4 The Investigation of Pressurised Intraperitoneal Aerosol Therapeutics for Peritoneal Metastases:

2.4.1 Cell culture techniques

Cell lines were obtained from the European Collection of Authenticated Cell Cultures (ECACC) or collaborators. Culture media, with supplements, specific to each cell type

were used. Table 2-7 shows the reagents used and their suppliers. Table 2-8 describes the cell lines used, their source, and the medium used to culture each line.

Cells were cultured in T75 or T150 flasks (Corning UK) in a Human Tissue Authority (HTA) certified cell culture incubator (HERA cell, Thermo Scientific). A humidified atmosphere with 5% carbon dioxide, and a temperature of 37°C was maintained. All materials used were sterile and disposable. Procedures were carried out in a class II cabinet.

Cells were sub-cultured when they reached 70-80% confluency. For fast-growing cells, a ratio of 1:10 was used, and for slow-growing cells a ratio of 1:3. All of the cell lines used are adherent in culture. To split these cells, the cell culture medium was aspirated off, and the cells were then washed with 10ml (T75) or 20ml (T150) of Phosphate Buffered Saline (PBS). Trypsin 0.05% (2.5ml for T75, 5ml for T150 flasks) was then added and the flask rolled to ensure all cells were covered, before incubating at 37°C for 3-5 minutes and rolling the flask to ensure cells were detached.

Table 2-7: Reagents used during cell culture

Reagent	**Abbreviation**	**Supplier**	**Catalogue number**
Dulbecco's Modified Eagle's Medium: Nutrient Mixture F12	DMEM/F12	Sigma, Gillingham, UK	D6421
Roswell Park Memorial Institute 1640 medium	RPMI	Gibco, Paisley, UK	21875-034
Minimum Essential Medium with Hepes	MEM with Hepes	Gibco, Paisley, UK	32360-026
Ham's F12 Nutrient Mix	Ham's F12	Gibco, Paisley, UK	11765-054
Phosphate buffered Saline	PBS	Gibco, Paisley, UK	20012-019
Heat inactivated Fetal Bovine Serum	FBS	Gibco, Paisley, UK	10500-064
L-glutamine 200mM solution	L-glut	Gibco, Paisley, UK	25030-024
Penicillin 100 U/ml, streptomycin 100µg/ml	P/S	Gibco, Paisley, UK	15070-063
Cisplatin 100mg/100ml		Accord-UK Limited	
MEM Non-essential amino acids	NEAA	Gibco, Paisley, UK	11140-035
Sodium Pyruvate		Gibco, Paisley, UK	11360-070

Table 2-8: Cell lines and culture medium requirements

Cell line	Source	Medium	Supplements
Chinese Hamster Ovarian Epithelial (CHO-K1)	Collaborator - Lynda Coughlan, (Oxford)	DMEM/F12	10% FBS P/S L-Glut
Chinese Hamster Ovarian transfected to express hCAR (CHO-CAR)	Collaborator - Lynda Coughlan, (Oxford)	DMEM/F12	10% FBS P/S L-glut
CC1 Wistar Rat Cell Line	ECACC	MEM with Hepes	10% FBS 1% NEAA P/S L-Glut
A2780 cells	Collaborator - Lynda Coughlan, (Oxford)	RPMI	10% FBS P/S L-Glut
A2780/CP70 cells	Collaborator - Lynda Coughlan, (Oxford)	RPMI	10% FBS P/S L-Glut
PEO1 cells	Collaborator – James Cronin (Swansea)	RPMI	10% FBS Sodium Pyruvate P/S L-Glut
PEO4 cells	Collaborator – James Cronin (Swansea)	RPMI	10% FBS Sodium Pyruvate P/S L-Glut
AGS cells	Collaborator - Toby Phesse (ECSCRI, Cardiff)	Ham's F12	10% FBS P/S L-Glut
MKN 28 cells	Collaborator - Toby Phesse (ECSCRI, Cardiff)	RPMI	10% FBS P/S L-Glut
MKN 45 cells	Collaborator - Toby Phesse (ECSCRI, Cardiff)	RPMI	10% FBS P/S L-Glut

2.4.1.1 Long term storage and recovery of cell lines

Long term storage of cell lines was in liquid nitrogen at -130°C. Prior to storage, cells were cultured and prepared as for splitting as described in section 2.4.1. After detachment with trypsin, complete medium was added to neutralise the trypsin. They were then centrifuged at 1200rpm for 3 minutes, the supernatant discarded, and the resulting pellet re-suspended in 3mL of 10% DMSO in FBS. The suspension was aliquoted into 1.5ml cryovials (Corning, UK) and cooled at a rate of -1°C per

minute using a Mr Frosty™ freezing container (ThermoScientific) in a -80°C freezer before transfer to liquid nitrogen storage. Where possible, passage number was recorded on the aliquot.

On recovery of a cell line, a cryovial of cells was removed from the tank, transferred to the Tissue Culture room on ice, and then rapidly thawed in a pre-warmed water bath at 37°C. When the contents of the vial had thawed, a 1ml pipette was used to transfer the cells into a prepared T75 flask containing 15ml of appropriate pre-warmed medium. The flask was then incubated as described above. After 24 hours the medium was changed, and culture continued until cells reached confluency. Sub-culture was then carried out as described in section 2.4.1.

2.4.2 Assessing the response of ovarian cancer cell lines to a cytotoxic drug in vitro

A2780 is an ovarian cancer cell line that was established from tissue from an ovarian endometroid adenocarcinoma tumour in an untreated patient. The A2780/cp70 subline was generated from the A2780 cell line by intermittent exposure to increasing doses of cisplatin up to 70µM *in vitro* [26]. The A2780/CP70 cell line is the subline of cells that survived this process. Behrens et al determined an IC_{50} of 1.1µM for the A2780 cells *in vitro* and noted that there was a right-shift in the dose-response curve in the A2780/CP70 cell line indicating cisplatin resistance.

The PEO cell lines were originally derived from the peritoneal ascites of a patient with a poorly differentiated serous adenocarcinoma. This subtype of disease has a propensity for peritoneal spread. The PEO1 cell line was harvested after treatment with cisplatin, 5-fluorouracil and chlorambucil. The PEO4 cell line was harvested after the patient developed clinical resistance to chemotherapy [121].

The ovarian cancer cell lines were cultured until sub-confluent and then detached from the flasks using trypsin. Complete medium was added to neutralise the trypsin, and then the cells were counted using a haemocytometer. Cells were seeded in flat-bottomed 96-well plates at a density of 20,000 cells per well in complete medium (depending on cell type). Plates were incubated at 37°C and 5% CO_2 overnight to

allow the cells to adhere. The medium was then removed and replaced with 150µl complete medium containing cisplatin at concentrations ranging from 0µM to 500µM. The cisplatin stock solution had a concentration of 1mg/ml, or 3.32×10^{-3}M. To ensure that the conditions in each well were consistent, the stock solution of cisplatin was diluted with sterile isotonic saline so that the dilution of the medium on addition of the drug was the same across all wells. Each well condition was tested in triplicate, and the wells at the perimeter of the plate were left blank and filled with medium.

Plates were then incubated at 37°C and 5% CO_2. Cell viability was assessed using an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay). The method is described in detail in section 2.4.2.1 below. Multiple plates were set up to enable cell viability assessments at 24 hours, 48 hours, and 72 hours.

2.4.2.1 Assessment of cell viability using an MTS assay

Cell viability was assessed using an MTS assay (CellTiter 96® A_{Queous} One Solution Cell Proliferation Assay) using the manufacturer's protocol. The medium was removed from the wells of the 96-well plate and replaced with 100µl of complete medium and 20µl of CellTiter 96® A_{Queous} One Solution Reagent. Three of the blank wells were also filled with medium/reagent to assess the background optical density reading. The remainder of the wells at the perimeter of the plate were filled with 120µl of medium. The plate was then incubated in the dark at 37°C and 5% CO_2 for 2 hours, and the absorbance was read at λ 490nm using a 96-well plate reader (iMark™ Microplate Absorbance Reader Bio-Rad Laboratories Ltd, Hertfordshire, UK). The CellTiter 96® A_{Queous} One Solution Reagent contains a tetrazolium compound (3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent (phenazine ethosulfate; PES). In metabolically active cells, the MTS tetrazolium compound is reduced into a soluble formazan product that is coloured. This is depicted in Figure 2-1. The pyridine nucleotide cofactors NADH and NADPH, produced by

dehydrogenase enzymes, are thought to be the electron donors responsible for the conversion [122]. The quantity of the coloured formazan product measured by absorbance is proportional to the number of viable cells in culture [123]. However, the manufacturer advises that 'a small amount of spontaneous 490nm absorbance occurs in culture medium incubated with CellTiter 96® AQueousOne Solution Reagent'. The optical density readings from the experimental wells were therefore corrected using the average reading from the blank background wells. Readings were expressed as a percentage of the average reading from the untreated wells, which were used as a reference of 100% cell viability.

Figure 2-1: The colorimetric reaction in the The CellTiter 96® AQueous One Solution Cell Proliferation Assay:

3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) is reduced to formazan by NADH and NADPH.

2.4.2.2 *In vitro evaluation of the effect of hyperbaria on the sensitivity of ovarian cancer cells to cisplatin*

Cells were plated in 96 well plates at a density of 20,000 cells per well in complete medium (depending on cell type) and treated with cisplatin as described previously. Immediately after the cisplatin-containing medium was added to the wells, the plate was placed in a hyperbaric apparatus. The apparatus is shown in Figure 2-2. Cisplatin is sensitive to light so the plate was covered with foil. The plates were placed in the airtight bell jar, which was pressurised using a manual sphygmomanometer. Pressures ranging from atmospheric to 40mmHg were tested. The bell jar was

warmed prior to the experiment and kept in a water bath which was maintained at 37°C during the experiment. It was cleaned between experiments and sterilised between uses. After exposure, the plate was removed from the bell jar and incubated at 37°C and 5% CO_2. Cell viability was assessed at 48 hours using an MTS assay (CellTiter 96® A_{Queous} One Solution Cell Proliferation Assay) as described in section 2.4.2.1 above.

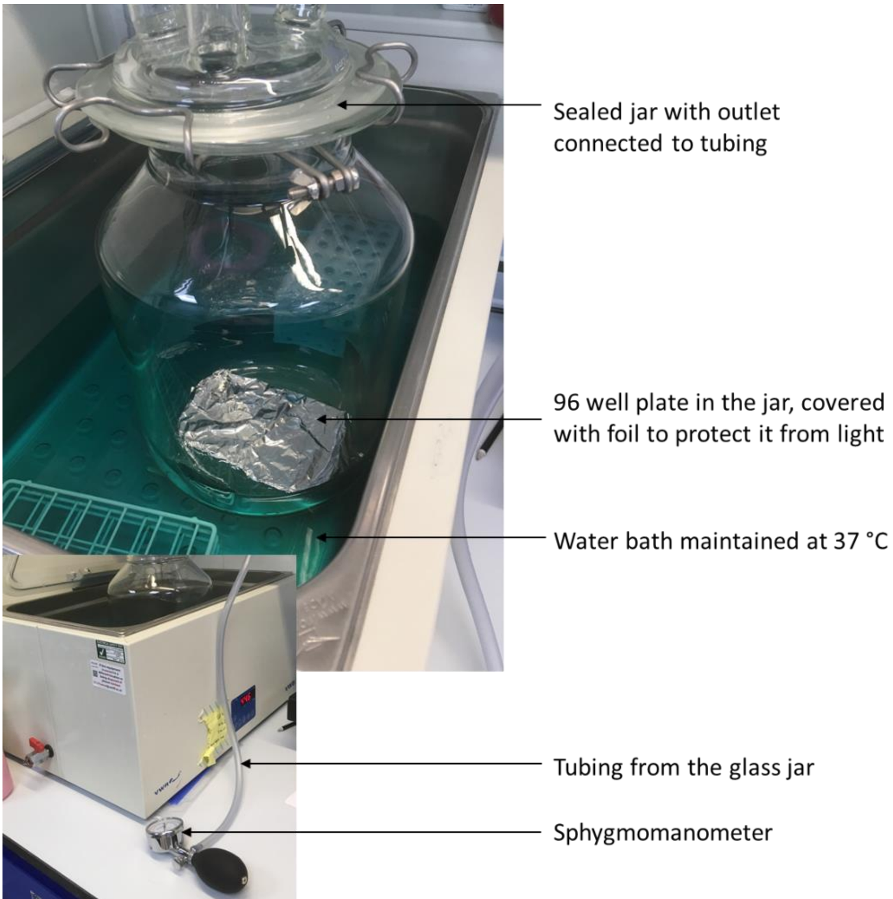


Figure 2-2 The Hyperbaric Apparatus

A glass vessel was chosen as it could be sterilised between uses. It was sealed using the clamp top, and the only outlet was to the tubing of the aneroid sphygmomanometer (Sapphire). The bulb was used to increase the pressure to the desired setting. The apparatus was observed for the duration of the pressure exposure to ensure that a constant reading was maintained.

2.4.2.3 Analysing the dose-response of ovarian cancer cell lines to Cisplatin in vitro

The concentration of cisplatin that inhibited cell survival to 50% (IC_{50}) and the 95% confidence interval were calculated for each set of conditions using non-linear

regression in Graphpad Prism version 9.0 (GraphPad Software Inc., La Jolla, CA, USA). Dose response curves are displayed for each cell type and each pressure tested.

2.4.3 The assessment of aerosolisation as a method for intraperitoneal delivery of oncolytic adenoviruses *in vitro*

Intraperitoneal aerosolisation potentially represents a useful method to deliver therapeutic viruses as it might allow widespread distribution of a small volume of fluid within the abdominal cavity to treat peritoneal disease. We therefore established new methodologies to investigate the feasibility of using this route of administration.

2.4.3.1 The assessment of the ability of adenovirus vectors to survive aerosolisation and transduce cells expressing their native receptor in vitro.

Two Chinese Hamster Ovary (CHO) cell lines were cultured as described in section 2.4.1. CHO CAR cells express human CAR. CHO K1 cells do not. Cells were detached from the flasks when sub-confluent using 0.05% trypsin. Complete medium was added to neutralise the trypsin, and then the cells were counted using a haemocytometer. Cells were seeded in a flat bottomed 96 well plate at 20,000 cells per well and incubated for 12 hours at 37°C and 5% CO_2.

A replication deficient human Ad5 vector engineered to express Green Fluorescent Protein (GFP) as a reporter gene under the control of a short cytomegalovirus (CMV) IE promoter had been previously manufactured by homologous AdZ recombineering [124]. The Ad5.GFP virus was diluted in serum-free media (DMEM) to make a solution containing $2x10^{10}$ viral particles (vp) per millilitre. Ad5 has been shown to engage CAR to enter cells [103], and therefore should be capable of transduction in CHO CAR cells, but not in CHO K1 cells.

Half of the Ad5.GFP solution was reserved to carry out the control experiments. The remaining solution was aerosolised using a High-Pressure Injector (HPI) (Mark 7 Arterion Pedestal, Beyer) connected to a CapnoPen™ aerosolizer (Capnomed GmbH).

A sterile HPI syringe was placed onto the HPI to withdraw the plunger to 18ml. The syringe was then removed from the HPI and, in the Class II cabinet, filled with the prepared Ad5.GFP solution. The injector tubing was connected to the syringe. The sealed syringe was then mounted back onto the injector and the plunger advanced to prime the line. The aerosolizer was sealed inside a clean 50ml falcon tube. The injector was programmed to deliver 8ml and the injection was performed. The aerosolised sample was collected in the 50ml tube. The equipment set up is shown in Figure 2-3.

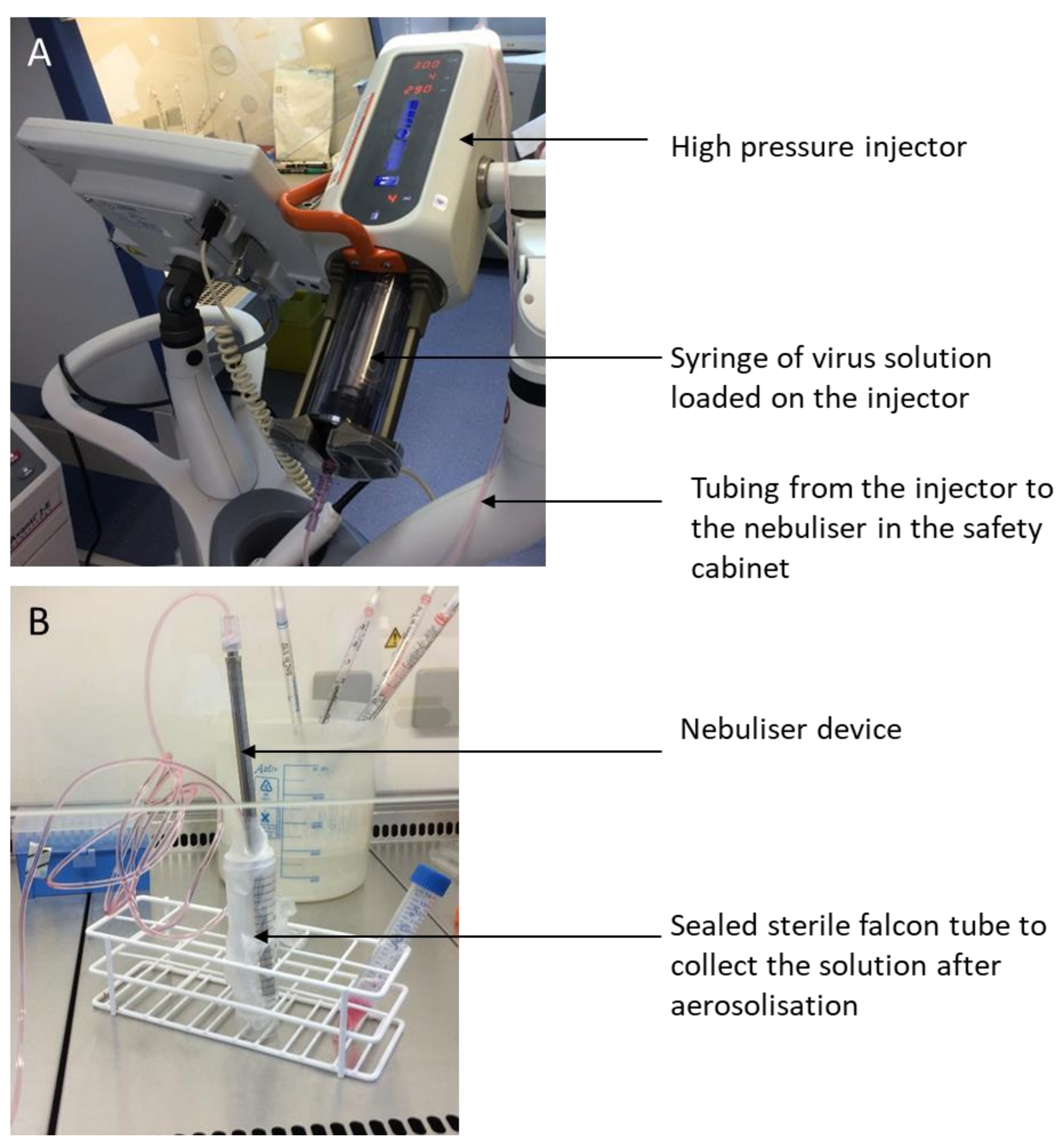


Figure 2-3: Equipment for the aerosolisation of the virus solution in vitro.

A solution of Ad5.GFP in serum free medium was prepared. A sterile HPI syringe was filled with the Ad5.GFP solution. The injector tubing was connected to the syringe. The HPI syringe was then mounted back onto the injector (Mark 7 Arterion Pedestal, Beyer) and the plunger advanced to prime the line. The line was attached to the CapnoPen (Capnomed GmbH) aerosoliser, which was sealed inside a sterile 50ml falcon tube. The injector was programmed the injection was performed. The aerosolised sample was collected in the 50ml tube and subsequently diluted and used in the experiment as described.

A) The HPI with the syringe of Ad5.GFP solution loaded. The injection has completed, and the residual volume of 4 ml displayed.

B) The high-pressure tubing connected to the CapnoPen™ aerosoliser which is sealed in a sterile falcon tube so that the aerosolised solution is collected.

HPI = High pressure injector

Serial dilutions of the aerosolied and non-aerosolised Ad5.GFP solutions were carried out using serum-free medium to make 1x10^{9}vp/ml, 5x10^{8}vp/ml, 2.5x10^{8}vp/ml, and 1x10^{7}vp/ml solutions. These concentrations correspond to a vp/cell concentration of 1000vp/cell, 5000vp/cell and 10,000vp/cell when of 200µl of solution is applied to 20,000 cells. The medium was removed from the cells in the 96 well plate and the Ad5.GFP solutions were added, with aerosolised and non-aerosolised solutions of each concentration applied to both cell types in triplicate. Serum-free media was added to three wells of each cell type as a negative control.

The cells were then incubated at 37 °C with 5% CO_2 for 2 hours. The virus-containing medium was then removed from each well and replaced with 200µl of complete medium (DMEM F12, FBS, L-glut, P/S). The cells were incubated for a further 45 hours.

GFP expression was assessed using flow cytometry. To prepare the cells, the medium was removed from each well and the cells were washed twice with PBS. Trypsin was then added to each well and the cells were incubated for 10 minutes. The trypsin was neutralised by the addition of complete medium to each well, and then after mixing the cells were transferred to a round-bottomed 96 well plate. The plate was spun at 1500rpm for 5 minutes. The supernatant was removed, and the cells were re-suspended in 100µl of 2% paraformaldehyde (PFA). The plate was left on ice for 15 minutes.

Once fixed, the samples were assessed on a BD Accuri C6 (BD Biosciences) flow cytometer. 10,000 events were recorded in channel FL-1. Flow cytometry data were analysed using FlowJo v10. The negative control samples, which had only complete medium and no virus applied, were used to gate the virus-exposed samples. The percentage of cells expressing GFP was calculated and multiple t-tests (Graphpad Prism) were used to compare the aerosolised samples with the non-aerosolised samples.

2.4.3.2 The assessment of the viability of a Wistar Rat Intraperitoneal Aerosolisation Model for testing adenovirus vectors in vitro.

Having established that the virus vectors could survive aerosolisation *in vitro*, we then carried out investigations to establish whether we could test the viruses in a rat model which was being developed at the University of Ghent.

2.4.4 Assessment of the surface receptor expression of cell lines

Immunocytochemistry and flow cytometry were carried out to assess the cell surface expression of CAR and αvβ6 in a number of cell lines. CAR is the native receptor for Ad5, whilst αvβ6 is the target of the $Ad5_{null}A20$ vectors developed by the group for use in cancers of epithelial origin. The Wistar rat hepatocyte cell line CC1 was assessed to determine whether the use of adenovirus vectors would be viable in a rat model. Ovarian and gastric cell lines were also characterised to determine their suitability for use in a xenograft tumour model in athymic rats.

The cell lines were cultured until 70% confluent in a T150. They were detached from the flask using 0.05% trypsin. Appropriate culture media was then added to neutralise the trypsin, and the samples were centrifuged at 1200rpm for 3 minutes. The supernatant was removed, and cell pellets were re-suspended in 10ml of ice cold wash buffer (PBS with 5% FBS). Cell counts were performed using a haemocytometer and 250,000 cell aliquots were prepared for immunocytochemistry.

Antibodies were diluted with wash buffer and the ratios had been previously determined by other team members. Mouse anti-CAR (clone RcmB Millipore, Watford UK) diluted 1:500 and mouse anti- αvβ6 integrin (clone-E7P6 Millipore, Watford UK) diluted 1:100 were used to stain for CAR and αvβ6. A mouse normal IgG (Santa Cruz Biotechnologies, Heidelberg, Germany) diluted 1:200 was used as a positive control. Cells were incubated with 100µl of the primary antibody in solution at 4°C for 1 hour.

Samples were then washed twice with PBS with 5% FBS before incubation with a fluorescent secondary antibody. A goat anti-mouse AlexaFluor 647 antibody (#A21237 Life technologies, Paisley UK) diluted 1:500 was used for all samples. The cells were incubated with 100µl of the secondary antibody solution at 4°C for 1 hour. Samples were then washed three times with PBS with 5% FBS before being fixed with 4% PFA at 4°C overnight.

Once fixed, the samples were assessed on a BD Accuri C6 (BD Biosciences) flow cytometer. 20,000 events were recorded in channel FL-4. Flow cytometry data were analysed using FlowJo v10. Unstained control samples were used to gate the stained samples. The percentage of cells expressing each receptor was calculated.

2.4.4.1.1 Assessment of the ability of Ad5.GFP to transduce CC1 (Wistar rat hepatocyte) cells.

CC1 rat hepatocytes and two Chinese Hamster Ovary (CHO) cell lines were cultured as described in section 2.4.1. CHO CAR cells were used as a positive control since they express human CAR. CHO K1 cells were used as a negative control since they do not. Cells were detached from the flasks when sub-confluent using 0.05% trypsin. Complete medium was added to neutralise the trypsin, and then the cells were counted using a haemocytometer. Cells were seeded in a flat bottomed 96 well plate at 20,000 cells per well and incubated for 12 hours at 37°C and 5% CO_2.

Ad5.GFP was diluted in serum free medium (cell line specific) to achieve concentrations of 5000vp/cell, and 10,000vp/cell in 200µl of medium, and applied to

the wells in triplicate. Serum free medium only was added to the control wells. The plate was incubated at 37°C and 5% CO_2 for 3 hours, and then the virus-containing medium was removed and replaced with complete medium. The plate was incubated for a further 45 hours.

GFP expression was assessed using flow cytometry as outlined in section 2.4.3.1. The percentage of cells expressing GFP was calculated and a two-way analysis of variance (ANOVA) with Tukey's multiple comparison test was used to determine whether there was a difference in expression of GFP in CC1 cells compared to the CHO CAR and CHO K1 cells at the different concentrations of virus.

2.4.4.1.2 *In vitro* assessment of Ad5.Luc as a vector for use in a Wistar Rat Intraperitoneal Aerosolisation model.

Ad5 engineered to express Luciferase as a reporter gene under the control of the short CMV IE promoter (Ad5.Luc) was selected as the best suited vector for use in a rat model *in vivo,* where transgene expression in vivo can be assessed directed using bioimaging technologies (In vivo imaging system; IVIS). A replication deficient human Ad5.Luc vector had been previously manufactured by homologous AdZ recombineering [124]. It was therefore assessed *in vitro*, starting with the effect of aerosolisation on the ability of the virus to transduce cells. CC1 cells were used to assess the viability of this vector in the rat model. CHO CAR and CHO K1 cells were again used as positive and negative controls. PEO1 and PEO4 cells were chosen as a comparator as they had intermediate levels of expression of CAR.

Cells were grown until 60-70% confluent and then harvested, counted, and seeded in a 96 well plate at a density of 20,000 cells per well in complete medium (specific to cell type as per Table 2-8). They were incubated overnight at 37°C and 5% CO_2. Ad5.Luc was diluted in serum free medium (specific to cell type as per Table 2-8) to a concentration of $2x10^9$ vp/ml. Half of each solution was injected through the Capnopen and collected in a 50ml Falcon tube as previously described in section 2.4.3.1, and half was reserved as a non-aerosolised control. The medium was removed from the wells of the 96 well plate and the cells were washed with 200µl

PBS. The virus solutions were then added to the wells, with four replicates per condition (aerosolised and non-aerosolised). 100µl of the solution corresponds to a concentration of 10,000 vp/cell. 100µl of serum free medium was used as a negative control, with four replicates per cell line. The plate was returned to the incubator for 3 hours. The medium was then removed from each well, the cells were washed with 200µl of PBS, and complete medium was added. The cells were incubated for a further 45 hours. At 48hours after the addition of the virus, the medium was removed from the wells and the cells were washed with PBS. Lysis reagent (#E1531, Promega UK Ltd, Southampton, UK) was diluted in water as per the manufacturer's instructions and 100µl was added to each well. The plate was frozen at -80°C.

The expression of the reporter gene was assessed by Luciferase Assay System (#1501, Promega UK Ltd, Southampton, UK) as per the manufacturers' protocol. The plate was thawed, the contents of the wells mixed, and then 20µl of the lysate was transferred to a white 96 well plate. 100µl luciferase reagent was added to each well just prior to measurement of the luciferase activity in Relative Light Units (RLU) on a multimode plate reader (Clariostar). The RLU was then normalised for total cellular protein (RLU/mg). The protein concentration in each well was determined using a bicinchoninic acid (BCA) protein assay (#23227; Pierce™ BCA Protein Assay Kit, Thermo Scientific, Loughborough, UK) as per the manufacturer's instructions. Bovine serum albumin (BSA) was used as a protein standard at concentrations 2.0, 1.5, 1.0, 0.75, 0.5, 0.25, 0.125, 0.025 and 0 mg/mL in PBS. 10µl of each standard, and 10µl of each sample, was transferred to a 96 well plate in duplicate. 200µl of working reagent (A:B = 50:1) was then added to each well, and the plate was incubated for 30 min. The absorbance was measured at λ570 nm on an iMark™ Microplate Absorbance Reader (BioRad, Hertfordshire, UK). The optical density (OD) was normalised in all wells by subtracting the 0mg/ml (PBS only) value from the reading. A standard curve was prepared using the OD obtained from the BSA standards, and the protein concentration in each well was deduced from the equation of the standard curve. A two-way ANOVA with Tukey's multiple comparison test was used to compare the luciferase expression in each cell type and between those infected with aerosolised and non-aerosolised virus.

The effect of different pressure conditions on the ability of Ad5.Luc was also assessed. Plates were prepared in the same way, with CHO K1, CHO CAR, and CC1 cells plated at 20,000 cells per well and incubated for 24 hours in complete medium. A solution of Ad5.Luc in serum-free medium was made and serial dilutions carried out prior to application to the cells in triplicate. The plates were placed in the hyperbaric apparatus described in section 2.4.2.2 and the pressure was maintained for 30 minutes. The control plate was put in the incubator immediately. After 30 minutes, all plates were put in the incubator for a further 1 ½ hours. The virus solution was removed and replaced with complete medium. After a further 48 hours incubation the medium was removed and the cells were washed and frozen in lysis buffer. The protein concentration in each well was determined using a BCA assay, and the expression of luciferase by luminometry after the addition of luciferin as described above. The mean RLU detected was normalised to protein concentration. A two-way ANOVA with Tukey's multiple comparison test was performed in GraphPad Prism 9.0 to compare the results between the different pressure conditions.

2.4.5 In vivo evaluation of the use of an aerosolisation technique to deliver virotherapy for peritoneal carcinomatosis: A comparison of Intraperitoneal injection of adenovirus with intraperitoneal aerosolization.

In vivo experiments were performed in collaboration with Dr Leen Van De Sande and Professor Wim Ceelen at the Department of Experimental Surgery laboratory (University of Ghent, Belgium).

An initial pilot experiment to compare intraperitoneal injection with intraperitoneal aerosolisation was designed. The pilot was to assess the tolerability of the experimental procedures. The dose of Ad5.Luc was selected based on previous experiments in rodent models. All animal experiments were performed under approved protocols by Animal Ethics Committee of Faculty of Medicine and Health Sciences, Ghent University, Belgium (ECD 17-109 and ECD 18-23), and in compliance with Belgian Council for Laboratory Animal Science (BCLAS) guidelines for the Care and Use of Laboratory Animals. The ethics application forms can be found in the

appendix (8.7 and 8.8). The animal experiments were co-funded by the Department of Experimental Surgery. A collaborative visit to Professor Ceelen's laboratory was undertaken for this work to assist with the handling of the adenovirus vectors during the animal experiments, which were performed by Dr Van de Sande in accordance with BCLAS guidelines.

2.4.5.1 A comparison of intraperitoneal injection and intraperitoneal aerosolization for Ad5.Luc in a Wistar rat model.

To minimize variation, and to ensure the size of the rats was appropriate for the aerosolization arm, 8 male Wistar Han rats (Envigo, The Netherlands) were used. Rats were housed in accordance with Belgian Legislation at the animalarium on the Ghent University Hospital campus of Ghent University. The rats were housed in 3 cages, with the rats grouped by treatment allocation. Rat cages were enriched with wood chips and shredded paper. Rats were allowed to acclimatise for 6 days before the experiment began. The experimental schedule is described in this section and the procedures and methods are described in more detail in the sections that follow.

Group A – virus administration by intraperitoneal injection

N = 3 rats

$3x10^{10}$ Ad5.luc viral particles in 5 ml warmed 0.9% NaCl was injected into the peritoneal cavity using standard procedures.

This procedure was repeated for the other two rats in this treatment group.

Group B – virus administration by intraperitoneal aerosolisation

N = 3 rats

$3 x10^{10}$ Ad5.Luc viral particles in 5 ml of 0.9% sodium chloride solution were aerosolized into the peritoneal cavity using the procedure described above.

This procedure was repeated for the other two rats in this treatment group.

Group C – negative controls

Intraperitoneal injection of saline (N = 1 rat)

5 ml warmed 0.9% sodium chloride solution was injected into the peritoneal cavity using standard procedures.

Intraperitoneal aerosolisation of saline (N = 1 rat)

The procedure was the same as for the intraperitoneal aerosolisation interventional group B, but 5ml 0.9% sodium chloride solution was administered rather than a virus solution.

After intervention, the rats were observed for 72 hours. The rats were then imaged to determine the distribution of the Ad5.Luc by *in vivo* luminometry. The rats were sacrificed and *ex vivo* luminometry of individual organs and tissues was performed. Samples of tissue were snap frozen and fixed in PFA for transport back to the UK for further analysis.

2.4.5.2 A comparison of intraperitoneal injection and intravenous injection of Ad5.Luc in a Wistar rat model.

A second experiment was designed in two parts. The first part was a comparison of intraperitoneal and intravenous injection of Ad5.Luc with a dose escalation. If this part was successful, a further comparison of intraperitoneal injection and intraperitoneal aerosolization would take place with the efficacious dose of Ad5.Luc.

To minimize variation, and to ensure the size of the rats would be appropriate for the aerosolization procedure if the experiment progressed, 3 male Wistar Han rats (Envigo, The Netherlands) were used. Rats were housed in accordance with Belgian Legislation at the animalarium at the University of Ghent (University Hospital of Ghent Campus). The rats were housed in 1 cage. Rat cages were enriched with wood chips and shredded paper. Rats were allowed to acclimatise for 72 hours before the experiment began.

Group A – virus injection by intraperitoneal injection (N=1 rat)

1.10^{11}vp Ad5.Luc in 5 ml of warmed 0.9% NaCl solution was injected in to the peritoneal cavity using standard procedures.

Group B – virus injection by intravenous injection (N=1 rat)

1.10^{11} vp Ad5.Luc in 200 µL of 0.9% saline was injected intravenously using standard procedures.

Group C – negative control – 0.9% NaCl solution by intraperitoneal injection (N=1 rat)

5ml warmed 0.9% NaCl solution was injected into the peritoneal cavity using standard procedures.

After intervention, the rats were observed for 72 hours. The rats were then imaged to determine the distribution of the Ad5.Luc by *in vivo* luminometry. The rats were sacrificed and *ex vivo* luminometry of individual organs and tissues was performed. Samples of tissue were snap frozen and fixed in PFA for transport back to the UK for further analysis.

2.4.5.3 Interventions:

2.4.5.3.1 Administration by intraperitoneal injection

The rats were weighed before the start of the procedure (weighing scales, Acculab). General anaesthesia was induced with 8% volume sevofluorane (Sevorane®, Abbvie) and 1200 ml/min O_2. The rat was moved to a class II cabinet. Ad5.Luc viral particles were administered in 5ml of warmed 0.9% NaCl solution. For control experiments, 5 ml of warmed 0.9% NaCl solution was used. This was injected into the peritoneal cavity using standard procedures. The rat was recovered from general anaesthesia and returned to its cage.

2.4.5.3.2 Administration by intraperitoneal aerosolisation

The rats were weighed prior to the start of the procedure (weighing scales, Acculab). General anesthesia was induced with 8% volume sevoflurane (Sevorane®, Abbvie) and 1200 ml/min O_2. The rat was then moved to a class II cabinet. Anesthesia was maintained with 4-5% volume sevofluorane and 800 ml/min O_2. The rat's abdomen was shaved and disinfected with betadine (Meda). The rest of the procedure was carried out under aseptic conditions and under a heat lamp. The first incision was

made on the left side of the abdomen to accommodate a 5mm laparoscopic balloon port. A pneumoperitoneum of 6mmHg was generated by insufflation of carbon dioxide (Olympus UHI-3). A 5mm laparoscope (Olympus) was inserted and then a second incision was made on the right side of the abdomen under direct vision. This was slightly larger, to accommodate a 12mm balloon port. The CapnoPen™ aerosolizer (Capnomed GmBH) was then inserted and the aerosolizer and the camera were fixed and held using a mechanical arm.

After access to the abdomen was secured, the syringe of the HPI (Injektron 82, Medtron) was loaded with the solution to be injected. Ad5.Luc viral particles were administered in warmed 0.9% saline solution.

The solutions were administered using the aerosolizer with a flow rate of 0.5 ml/s, and a maximum injection pressure of 20 bar. The rat was then observed for 30 minutes. The experimental set-up can be seen in Figure 2-4.

After 30 minutes the aerosol was evacuated using a closed aerosol waste system. The ports were removed and the incisions were closed in layers with 4-0 vicryl (Ethicon, USA). Buprenorphine (0.1 mg/kg) was administered subcutaneously and the rat was recovered from general anaesthesia and then returned to its cage.

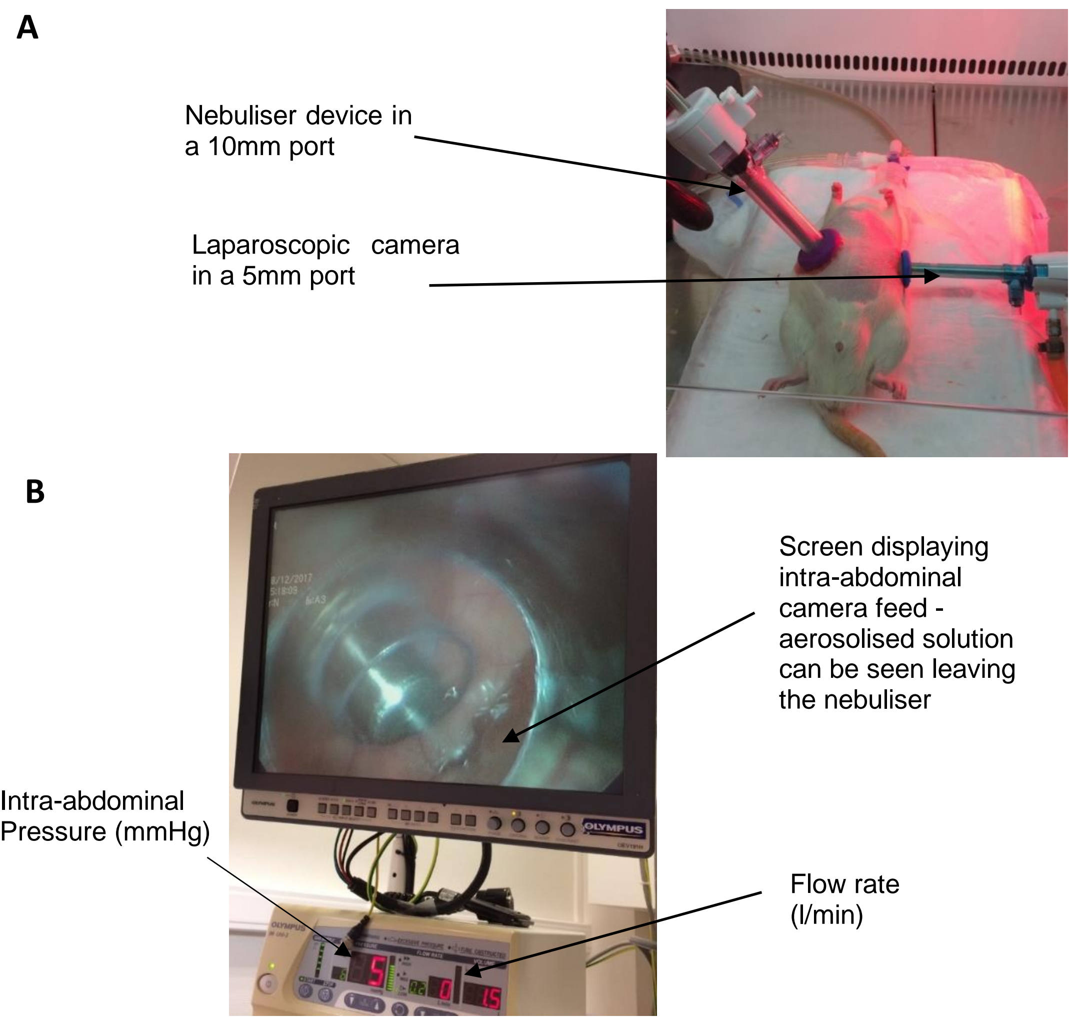


Figure 2-4: Pressurised Intraperitoneal Aerosol delivery of virus in a rat.

A) The procedural set up – the anaesthetised rat has 2 laparoscopic ports in situ. The CapnoPen aerosoliser and the laparoscopic camera are indicated.
B) The camera view of the aerosoliser is seen. The insufflator settings can be seen. The pressure is set at 5mmHg, the flow rate of carbon dioxide into the rat's abdomen is 0 indicating that there is no leak from the pneumoperitoneum.

2.4.5.3.3 Administration by intravenous injection

The rats were weighed before the start of the procedure (weighing scales, Acculab). General anaesthesia was induced with 8% volume sevofluorane (Sevorane®, Abbvie) and 1200 ml/min O_2. The rat was moved to a class II cabinet. Ad5 luciferase particles were administered in 200µl of 0.9% saline solution into a tail vein using standard procedures. The rat was recovered from general anaesthesia and returned to its cage.

2.4.5.4 Monitoring and further procedures following intervention

The animals were returned to the animalarium, where they were housed in cages in groups as before for 72 hours. They were assessed daily using a scoring system and analgesia was administered as per protocol (see Table 2-9).

After 72 hours the rats were imaged to determine the distribution of the Ad5.Luc by luminometry using IVIS. This is described further in section 0. Immediately following live imaging, the rats were sacrificed. General anaesthesia was induced using 8% sevofluorane (Sevorane®, Abbvie) and 1200 ml/min O_2. T-61 (0.3 ml/kg) was then administered intravenously by tail vein injection. Death was confirmed by palpation and auscultation. The rats were dissected and the livers and lungs, and sections of abdominal wall were harvested to assess vector distribution by luminometry using ex vivo imaging (see section 0). The tissues were then divided, with samples fixed in 10% formalin for processing into slides for immunohistochemistry (IHC), and samples snap frozen for DNA extraction and polymerase chain reaction (PCR) analysis.

Table 2-9: Scoring system used to assess animal welfare.

Animals scoring 1 or 2 receive analgesia (0.1 mg/kg buprenorphine). Animals scoring ≥3 requires are referred to the veterinary surgeon of the animal house.

Characteristic	Description	Score
Weight	Normal <10 % weight loss 10 – 15 % weight loss, appetite > 20 % weight loss, no appetite	0 1 2 3
Appearance	Normal Lack of self-care Rough coat (+/- runny nose) Very rough coat, abnormal posture, enlarged pupils	0 1 2 3
Clinical signals	Normal Minor clinical changes Increase in body temperature of 1 – 2 °C Increase in body temperature of > 2 °C	0 1 2 3
Behaviour	Normal Minor behavioural changes Abnormal behaviour, less mobile, less alert, inactive when activity is expected Unsolicited vocalization, self-mutilation	0 1 2 3

Response to extraneous stimuli	Normal Slightly excessive response Moderately excessive response Violent response	0 1 2 3

2.4.5.5 Luminometry:

In Vivo Imaging

To generate bioluminescence D-luciferin, the substrate of luciferase, was administered. Rats were anaesthetized individually using 8% sevofluorane (Sevorane®, Abbvie) and 1200 ml/min O_2, with anaesthesia maintained using 4-5% volume sevofluorane. The abdominal walls of the rats who had received intraperitoneal injections were shaved to ensure consistency with the rats that had undergone an aerosolisation procedure. 1.5ml of luciferin at 15mg/ml was injected into the peritoneal cavity and then after 10 minutes the rat was imaged in the IVIS imager. Images were analysed using Living Image® software (PerkinElmer).

Ex Vivo Imaging

The freshly dissected tissue blocks were placed in 6 well plates with 1.5ml 0.9% saline to prevent the tissue drying out. 1.5ml luciferin at 3mg/ml was added to each well over the tissue blocks, and images were taken in the IVIS imager immediately. Images were analysed using Living Image® software.

2.4.5.6 Immunohistochemistry:

Liver, lung, and abdominal wall tissue samples from each rat were fixed in 10% PFA. Each sample was submerged in 10ml of 10% PFA for 24 hours. The samples were then kept in 70% ethanol until they were processed by the Bioimaging Research Hub Laboratory (School of Biosciences, Cardiff University). Formalin-fixed paraffin-embedded sections were mounted on glass slides. The presence of Ad5 in the tissue was assessed using IHC.

The tissues were re-hydrated by immersion in xylene for three 5 minute washes, followed by 100% industrial methylated spirit (IMS) for two 3 minute washes, and

90% IMS for one 3 minute wash, and distilled water (dH_2O) for one 5 minute wash. The tissue samples for staining were circled with ImmEDGE pen (#H-4000; Vector Laboratories, Peterborough, UK). Antigen retrieval was carried out using proteinase K (#4333793; AB, Foster City, CA, USA) 20µg/ml diluted 1:1000 with PBS. The tissue sections were covered with the proteinase K solution and incubated for 10 minutes at room temperature. This was rinsed off with PBS and then quenched with hydrogen peroxide. The tissue sections were covered with 3% H_2O_2 for 10 minutes before a further rinse with PBS.

A blocking step was then performed. The sections were covered with 2.5% horse serum (#MP-7401; ImmPRESS™ HRP anti-rabbit IgG-HRP polymer detection kit; Vector Laboratories, Peterborough, UK) and incubated at room temperature for 20 minutes at room temperature. This was rinsed off with PBS, and the primary antibody was added. Primary polyclonal rabbit anti-adenovirus type 5 antibody (1 µg/mL; #ab6982, Abcam, Cambridge, UK) was diluted 1:1000 with PBS and 200µl was applied to each slide. Negative controls were also performed to detect any non-specific staining and a rabbit IgG isotype control antibody (1 µg/mL; #GTX35035; GeneTex, Wembley, UK) diluted 1:1000 with PBS was applied to these slides. The slides were incubated in a humidified box at 4°C overnight.

The following day, after a 5 minute wash in PBS, the secondary antibody was applied. The tissue sections were covered with an anti-rabbit secondary (#MP-7401; ImmPRESS™ HRP anti-rabbit IgG-HRP polymer detection kit, made in horse; Vector Laboratories, Peterborough, UK) and incubated in a humidified box for 30 minutes at room temperature. The sections were washed with PBS for 5 minutes and then incubated with DAB substrate (DAB Peroxidase Substrate Cat. No. SK-4100 Vector Laboratories) for 3 minutes. This was rinsed off in tap water, and then the slides were counterstained with haematoxylin for 30 seconds. The slides were rinsed in running tap water until the water was clear. The rehydration steps were performed in reverse to dehydrate the tissues. DPX Mountant for histology (#06522; Sigma Aldrich, Gillingham, UK) was used to mount the slides and coverslips were added before microscopy.

2.5 Data analysis and software used

2.5.1 Statistical analyses

Figures were created and statistical analyses performed in GraphPad Prism version 9.0 (GraphPad Software Inc., La Jolla, California, USA). Unless otherwise stated, data show the mean ± standard deviation (SD) or standard error of the mean (SEM) of n = 3–4 (specific n numbers are indicated in each figure legend). P values were as follows: ns = not statistically significant ($p > 0.05$); * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

2.5.2 Flow cytometry

Analyses of flow cytometry data were performed using FlowJo version 10.0 (FlowJo, BD Life Sciences, Ashland, Oregon, USA).

2.5.3 Luminometry

Images obtained for luminometry using the IVIS were analysed using Living Image® software (PerkinElmer, Waltham, Massachusetts, USA).

3 A Service Evaluation of the Management of Peritoneal Metastases from Colorectal Cancer at Cardiff and Vale University Health Board: Defining The Extent of Unmet Clinical Need.

3.1 Introduction:

Common sites of metastasis for colorectal cancer include the liver, lungs, and peritoneum. The incidence and prevalence of colorectal peritoneal metastases in the UK are not known because data is not routinely collected and summarised as part of national cancer surveillance statistics.

In Wales, the Welsh Cancer Intelligence & Surveillance Unit (WCISU) populates, maintains, and reports on the National Cancer Registry. WCISU is a member of the United Kingdom and Ireland Association of Cancer Registries (UKIACR). This is a network of agencies that work together to ensure that both National and International standards for cancer registration are maintained [125]. Results from multiple bodies within the NHS provide data to WCISU. The reports from the different departments of the Health Boards are collated and validated by WCISU. Whilst this provides a very accurate record that stretches back many years, the level of detail recorded is not sufficient to elicit the incidence of individual metastasis types, for example peritoneal metastases. The annual reports generated contain information on age at diagnosis, sex, Lower Super Output Area (geographical location), International Classification of Diseases version 10 (ICD 10) code, year of diagnosis, and stage at diagnosis [125].

Whilst the development of metastases may be documented locally, the incidence and prevalence of specific sites of metastasis is not collated nationally. In the situation where a patient was diagnosed with synchronous metastases, the registry would just record the primary site if known. Only patients with metastases of unknown origin might be registered using the ICD 10 code for the metastasis type that they had. In cases where metastases are metachronous, any new data about cancer treatment would be added to the register in that patient's original record. The ICD 10 code would remain the original primary diagnosis and the specific site of metastasis might only be inferred from the treatment carried out – for example, a patient having surgery for a liver metastasis.

The number of patients with peritoneal metastases can only be estimated based on studies of similar populations elsewhere. It is thought that approximately 4-5% of

patients with a newly diagnosed colorectal cancer will have peritoneal metastases, and this will be the only site of spread in around 50% of all those with metastases at presentation [44, 126]. In addition, 20-40% of patients treated with curative intent will relapse, and around 20% of these will have peritoneal disease [17, 41]. This potentially represents a significant number of patients.

The management of peritoneal metastases is a controversial area, and the commissioning policy regarding the provision of CRS and HIPEC varies across the UK. The NICE guideline for colorectal cancer recommends that for isolated peritoneal metastases, systemic anti-cancer treatment should be offered, and referral to a commissioned specialist centre for consideration of CRS and HIPEC should be discussed by the MDT [48]. In England, CRS and HIPEC has been commissioned for colorectal metastases in specialist centres without the need for individual funding requests since 2013 [127]. The criteria for treatment require that the patient has a performance status that is sufficient to withstand the surgery, and disease that is amenable to complete or near complete surgical resection. This means that the disease is isolated to the peritoneal cavity, and is not so extensive as to render it unresectable. In Wales, CRS and HIPEC has not been commissioned routinely for colorectal metastases, and requests for funding for CRS and HIPEC must be made to the Welsh Health Specialised Services Committee (WHSSC) on an individual patient basis. The treatment is seen as a low priority in Wales and is not routinely funded [128].

This discrepancy in the management pathway for peritoneal metastases from colorectal cancer, and the ongoing evolution of a potential new treatment option for this cohort of patients, prompted a service evaluation to determine the local incidence of peritoneal metastases from colorectal cancer, and the current management and outcomes of the patient group.

3.2 Incidence of peritoneal metastases from colorectal cancer:

The search of the MDT minutes identified 146 patients who presented with, or developed, peritoneal metastases from colorectal cancer between the start of

January 2014 to the end of December 2019. Of these, 81 had isolated peritoneal metastases, whilst a further 65 patients had additional extraperitoneal metastases. Figure 3-1 shows the number of patients presenting each quarter over the time period evaluated. There was a median of 6 (IQR 4-7) patients diagnosed with peritoneal metastases from colorectal cancer each quarter, with a median of 3 (IQR 2-4) who had isolated peritoneal metastases. These figures include patients diagnosed with peritoneal metastases that were synchronous to their colorectal primary, and those diagnosed with recurrent disease involving the peritoneum which was metachronous to their colorectal primary.

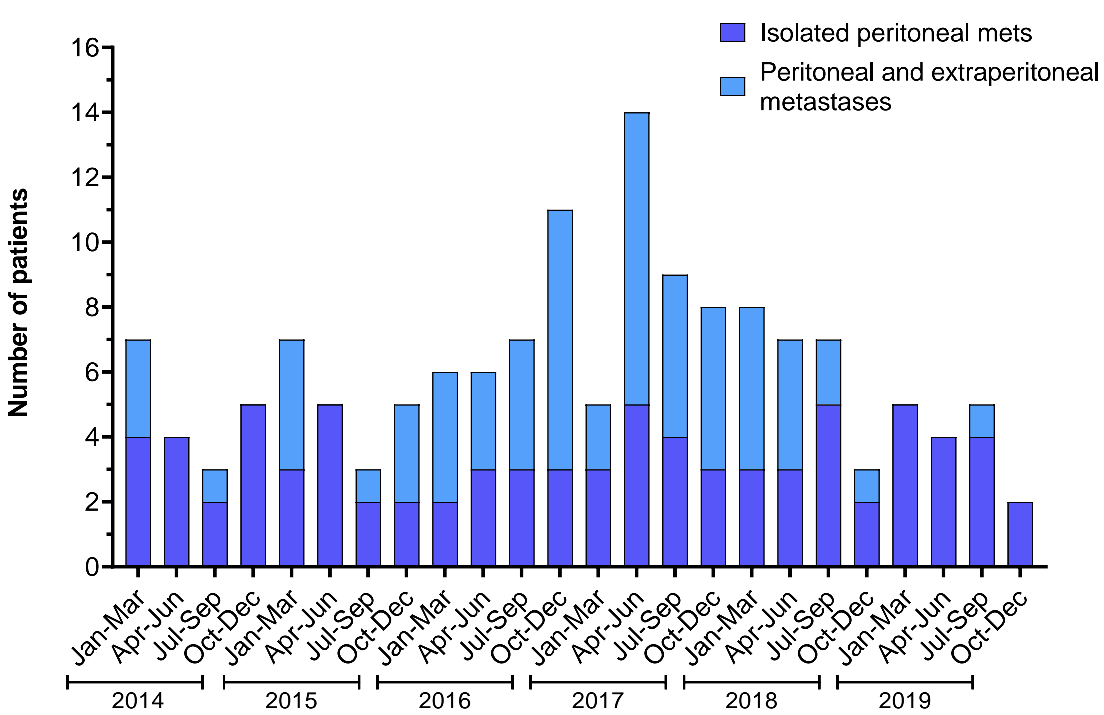


Figure 3-1: Number of patients presenting to the CAV UHB Colorectal MDT with peritoneal metastases from colorectal cancer by quarter from 2014-2019.

All patients who presented to the MDT between January 2014 and December 2019 with peritoneal metastases from colorectal cancer are included. Patients with appendiceal cancers are excluded.

Table 3-1 shows the number of patients presenting to the CAV UHB MDT with colorectal cancer over the 5 year period from March 2014 to April 2019. The date ranges correspond to the annual data collated for the National Bowel Cancer Audit England and Wales (NBOCA). Table 3-1A shows all patients with synchronous peritoneal metastases discussed by the MDT. Some patients were not from the catchment area served by CAV UHB. Table 3-1B shows only the patients from Cardiff and the Vale of Glamorgan. It can be seen that, in total, around 7% of patients referred to this MDT with colorectal cancer have synchronous peritoneal metastases. The peritoneum is the only site of metastasis identified in just under half of these (39/88, 44.3%), representing around 3% of all patients diagnosed with colorectal cancer.

Table 3-1: Diagnosis of new patients presenting to the CAV UHB Colorectal MDT by year.

Patients who presented to the MDT between April 2014 and March 2019 with a new diagnosis of colorectal cancer are included. The date ranges correspond to the annual data collated for the National Bowel Cancer Audit (year Apr-Mar). Patients with appendiceal cancers are excluded.

A All patients with colorectal cancer referred to the MDT

Diagnosis	**2014-2015**		**2015-2016**		**2016-2017**		**2017-2018**		**2018-2019**		**TOTAL**	
	N	*(%)*	N	*(%)*	N	*(%)*	N	*(%)*	N	*(%)*	N	*(%)*
Colorectal cancer	254		218		230		225		293		1220	
Colorectal cancer with synchronous peritoneal metastases	14	*(5.5)*	10	*(4.6)*	19	*(8.3)*	30	*(13.3)*	15	*(5.1)*	88	*(7.2)*
Colorectal cancer with synchronous isolated peritoneal metastases	8	*(3.1)*	6	*(2.8)*	7	*(3.0)*	7	*(3.1)*	11	*(3.8)*	39	*(3.2)*

B Patients living in the CAV UHB catchment area with colorectal cancer referred to the MDT

Diagnosis	**2014-2015**		**2015-2016**		**2016-2017**		**2017-2018**		**2018-2019**		**TOTAL**	
	N	*(%)*	N	*(%)*	N	*(%)*	N	*(%)*	N	*(%)*	N	*(%)*
Colorectal cancer	254		218		230		225		293		1220	

Colorectal cancer with synchronous peritoneal metastases	12	*(4.7)*	9	*(4.1)*	17	*(7.4)*	29	*(12.9)*	14	*(4.8)*	81	*(6.6)*
Colorectal cancer with synchronous isolated peritoneal metastases	6	*(2.4)*	5	*(2.3)*	5	*(2.2)*	6	*(2.7)*	10	*(3.4)*	32	*(2.6)*

Table 3-2 shows the number of patients who were discussed at the MDT having been diagnosed with metachronous peritoneal metastases each year. Table 3-2A shows all patients discussed by the MDT with metachronous peritoneal disease, whilst Table 3-2B shows the patients from the Cardiff and Vale UHB locality. The numbers were lower than those presenting with synchronous disease, but a greater proportion had isolated peritoneal metastases (27/41, 65.9%).

Table 3-2: Patients under the CAV UHB Colorectal MDT diagnosed with recurrent peritoneal disease by year

Patients under the MDT who were diagnosed with recurrent peritoneal disease from colorectal cancer (metachronous peritoneal metastases) between April 2014 and March 2019 are included. The date ranges correspond to the annual data collated for the National Bowel Cancer Audit (year Apr-Mar). Patients with appendiceal cancers are excluded.

A All patients with peritoneal metastases from recurrent colorectal cancer discussed by the MDT

Diagnosis	**2014-2015**	**2015-2016**	**2016-2017**	**2017-2018**	**2018-2019**	**TOTAL**
Colorectal cancer with metachronous peritoneal metastases	6	9	11	9	7	41
Colorectal cancer with metachronous isolated peritoneal metastases	6	5	5	7	4	27

B Patients living in the Cardiff and Vale UHB catchment area with peritoneal metastases from recurrent colorectal cancer discussed by the MDT

Diagnosis	**2014-2015**	**2015-2016**	**2016-2017**	**2017-2018**	**2018-2019**	**TOTAL**
Colorectal cancer with metachronous peritoneal metastases	5	8	9	8	6	36

Colorectal cancer with metachronous isolated peritoneal metastases	5	4	5	6	3	23

3.3 Characteristics of patients with peritoneal metastases

Table 3-3 shows the characteristics of all the patients identified and their disease. There were slightly more female patients in the isolated peritoneal metastases group, and in the cohort overall. The median age at presentation with peritoneal metastases was 69 in all patients, as well as in both groups. There were more patients with right sided primary tumours (61/146, 41.2%) than left sided (49/146, 33.1%) or rectal (18/146, 12.2%) primary tumours. There were also a small number of patients in whom the site of the primary tumour could not be identified, but the peritoneal nodules were histologically consistent with a colorectal primary (4/146, 2.7%). A similar pattern was seen in the patients with isolated peritoneal metastases, but in those with peritoneal and extraperitoneal metastases there were equal numbers of left and right sided tumours. More often peritoneal metastases were synchronous to the primary tumour (98/146, 67.1%) than metachronous (48/146, 32.9%). This was also the case in the patients with isolated peritoneal metastases (48/81, 60.0% had a synchronous presentation) and the group who had multiple sites of metastasis (50/65, 75.8% had a synchronous presentation). As might be expected, most patients presented with locally advanced T4 tumours (97/146, 66.4%). There was an even higher proportion of T4 tumours in the isolated peritoneal metastases group.

Table 3-3: Patients presenting with peritoneal metastases from colorectal cancer to the CAV UHB Colorectal MDT January 2014-December 2019 inclusive.

All patients who presented to the MDT between January 2014 and December 2019 with peritoneal metastases from colorectal cancer are included. Patients with appendiceal cancers are not included.

Characteristic		**Isolated peritoneal metastases**		**Peritoneal and extraperitoneal metastases**		**All patients with peritoneal metastases**	
		n=80 patients, n=82 tumours	%	n=66 patients, n=66 tumours	%	n= 146 patients, n=148 tumours	%
Sex	Female	44	*55.0*	33	*50.0*	77	*52.7*
	Male	36	*45.0*	33	*50.0*	69	*47.3*
Age at diagnosis of peritoneal metastases (years)	<50	10	*12.5*	6	*9.1*	16	*11*
	50-64	23	*28.8*	18	*27.3*	41	*28.1*
	65-74	19	*23.8*	21	*31.8*	40	*27.4*
	75-84	18	*22.5*	12	*18.2*	30	*20.5*
	>84	10	*12.5*	9	*13.6*	19	*13*
Tumour site (all tumours)	Caecum and ascending colon	29	*35.4*	22	*33.3*	51	*34.5*
	Hepatic flexure	7	*8.5*	3	*4.5*	10	*6.8*
	Transverse colon	10	*12.2*	6	*9.1*	16	*10.8*
	Splenic flexure and descending colon	6	*7.3*	6	*9.1*	12	*8.1*
	Sigmoid	17	*20.7*	11	*16.7*	28	*18.9*
	Rectosigmoid	2	*2.4*	7	*10.6*	9	*6.1*
	Rectal	8	*9.8*	10	*15.2*	18	*12.2*
	Unknown site	3	*3.7*	1	*1.5*	4	*2.7*
Histological subtype (all tumours)	Adenocarcinoma	68	*82.9*	59	*89.4*	127	*85.8*
	Mucinous adenocarcinoma	7	*8.5*	1	*1.5*	8	*5.4*
	Signet ring adenocarcinoma	4	*4.9*	1	*1.5*	5	*3.4*
	Not determined (no histology)	3	*3.7*	5	*7.6*	8	*5.4*
Timing of peritoneal metastases to primary disease	Synchronous	48	*60.0*	50	*75.8*	98	*67.1*
	Metachronous	32	*40.0*	16	*24.2*	48	*32.9*
Pre-treatment T stage (largest tumour)	T1	2	*2.5*	0	*0.0*	2	*1.4*
	T2	0	*0.0*	4	*6.1*	4	*2.7*
	T3	12	*15.0*	16	*24.2*	28	*19.2*
	T4	58	*72.5*	39	*59.1*	97	*66.4*
	X	8	*10.0*	7	*10.6*	15	*10.3*
Extraperitoneal metastases present	Liver			52	*78.8*	52	*35.6*
	Lung			24	*36.4*	24	*16.4*
	Liver and Lung			18	*27.3*	18	*12.3*
	Other			11	*16.7*	11	*7.5*

3.4 Treatment of isolated peritoneal metastases

Table 3-4 shows the primary treatment modality for the 80 patients who presented with isolated peritoneal metastases. 15 patients had a comment recorded in the MDT discussion that they were potentially suitable for CRS and HIPEC. 10 referrals were made to the Peritoneal Malignancy Institute. 5 patients were accepted for treatment, secured funding, and went forward to surgery. Of the other 5 patients, two were denied funding because of the perceived benefit of treatment was not good enough, two had funding approved but ultimately declined to have surgery, and one had progression of disease during the workup and funding application and was deemed ineligible.

Of the patients who had CRS and HIPEC, 3 had synchronous peritoneal metastases, and 2 had disease recurrence. There were 20 patients who had a major resection locally for a colorectal primary with synchronous peritoneal metastases and 1 who had a major resection for metachronous peritoneal metastases. 18/21 of these had systemic chemotherapy after the diagnosis of their peritoneal metastases as well as surgery. 38 patients received systemic chemotherapy as their primary anti-cancer treatment. 17 of these had presented with synchronous metastases, and 21 had metachronous peritoneal disease. There were 16 patients who received best supportive care only. 8 of these patients had presented with synchronous peritoneal metastases, and 8 with metachronous peritoneal metastases. There were 6 patients in the chemotherapy and best supportive care groups who had surgery which involved creation of a stoma, or diversion, without a major resection. The median age in the best supportive care group was 82 years, which was older than the median age in the other treatment groups. The median age of patients who received systemic chemotherapy only was lower than the median age of those who had a resection in Cardiff. The small group of patients who underwent CRS and HIPEC at a specialist centre were younger, with a median age of 53 years (IQR 41-62).

Table 3-4: The treatment of patients with isolated peritoneal metastases from colorectal cancer presenting to CAV UHB Colorectal MDT between January 2014 and December 2019.

All patients who presented to the MDT between January 2014 and December 2019 with isolated peritoneal metastases from colorectal cancer are included. Patients with appendiceal cancers are not included. Patients who had CRS and HIPEC had this treatment at The Peritoneal Malignancy Institute, Basingstoke. Patients who had surgery in Cardiff which involved a major resection are included in the local resection group.

Treatment	Number (%)	Age in years (median, interquartile range)
CRS and HIPEC	5 (6.25%)	53 (41-62)
ocal major resection +/- chemotherapy	21 (26.25%)	71 (59-75)
Systemic chemotherapy only	38 (47.5%)	64 (57-72)
Best supportive care only	16 (20.0%)	82 (77-86)
ALL PATIENTS	80	71 (59-75)

The majority of patients with isolated peritoneal metastases (60/80, 75%) received systemic anti-cancer treatment after their diagnosis. 32 (40%) patients received one line of chemotherapy for their peritoneal metastases, 16 (20%) patients received 2 lines of chemotherapy, and 5 (6.3%) patients received 3 lines of chemotherapy. There were 7 patients where the chemotherapy regimen used was not identified. 6 (7.5%) patients received a targeted biological therapy alongside chemotherapy. A number of different chemotherapy regimens were used which included combinations of Fluorouracil and Folinic Acid (modified de Gramont), Oxaliplatin, Capecitabine, and Irinotecan

3.5 Survival of patients diagnosed with isolated peritoneal metastases

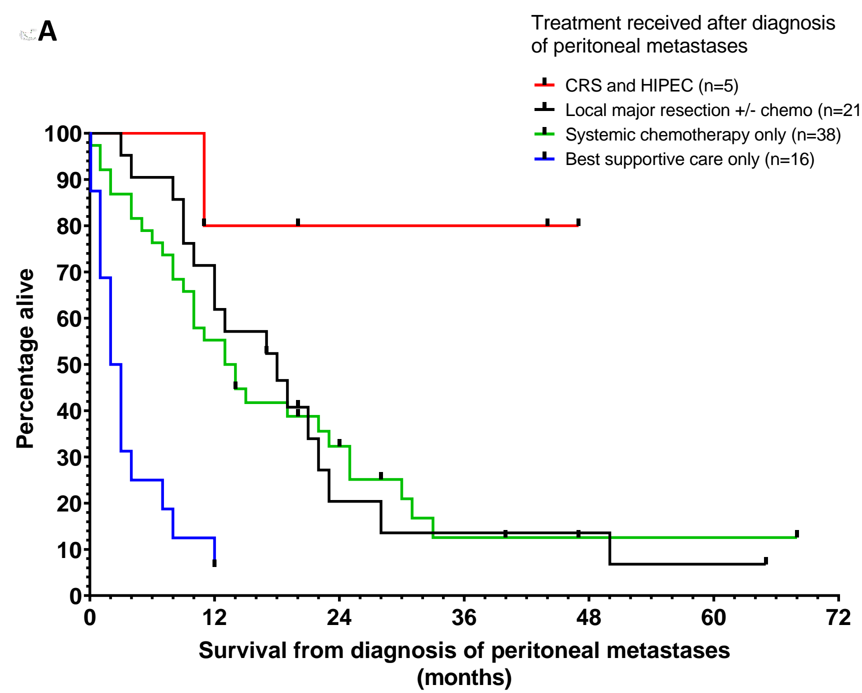
Figure 3-2 shows survival of patients presenting with isolated peritoneal metastases from colorectal cancer grouped by treatment, age, and by the timing of the peritoneal metastases in relation to colorectal primary.

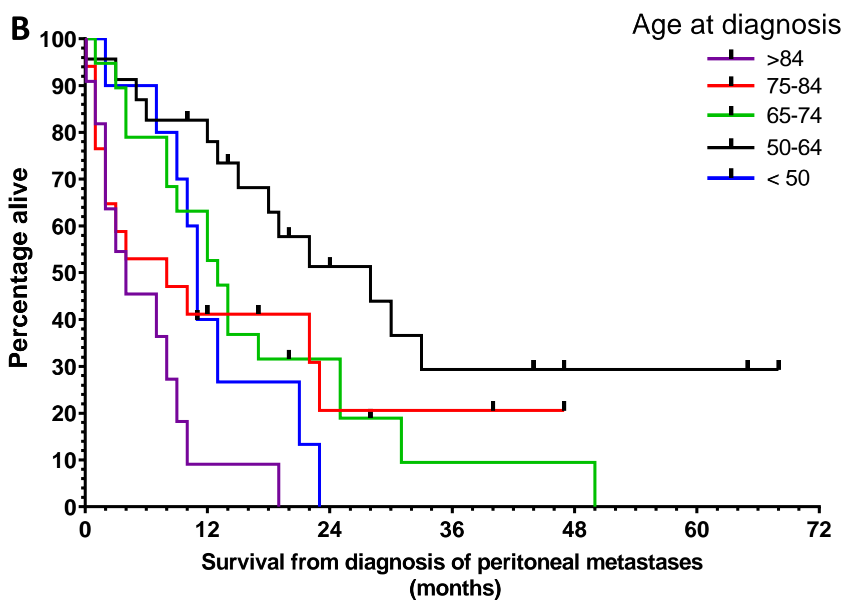
It was not possible to determine the median survival of the patients treated by CRS and HIPEC, since the majority were still alive at the point when outcome was assessed. Whilst it appeared that the survival curve in this group had separated from the other treatment groups, this difference did not achieve statistical significance (log

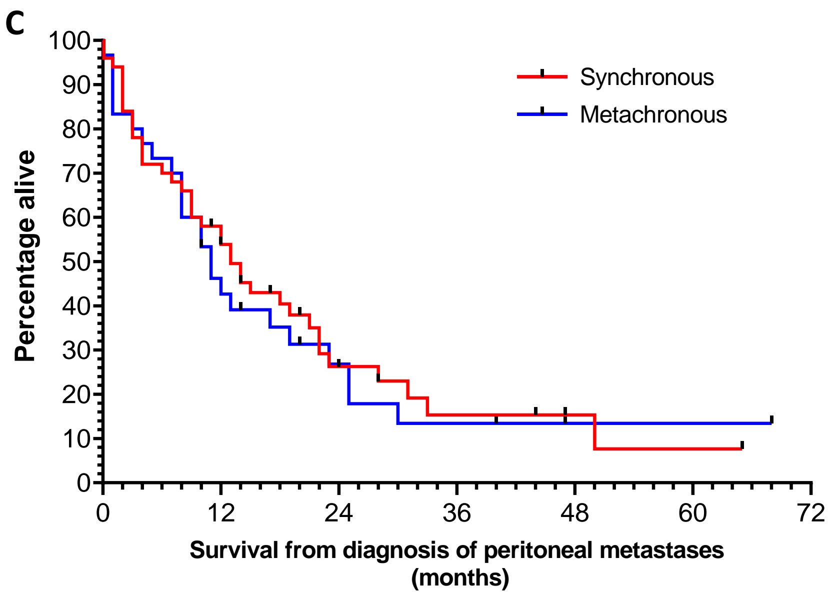
rank Mantel-Cox $\chi^2(2) = 3.88$, p=0.143). Longer follow up from a greater number of patients would be required to determine whether this was a true difference. The median survival in the patients who had a major resection carried out locally was 18 months and the patients who received systemic chemotherapy only was slightly less at 13.5 months. However, there was no significant difference between the survival curves when compared with a log rank (Mantel Cox) test, $\chi^2(1) = 0.023$, p=0.8791. The group who did not receive anti-cancer treatment, and had best supportive care only, had the lowest median survival of 2.5 months. There was a significant difference between the survival curve of this group compared to the curves of the groups who received treatment when compared with a log rank (Mantel Cox) test, $\chi^2(3) = 38.41$, p=<0.0001.

When the patients were grouped by age, the oldest patients had the shortest median survival at 4 months in the >84 year old group and 6 months in the 75-84 year old group. The longest median survival was seen in the 50-64 year old age group at 28 months. The youngest patients (age <50 years at diagnosis) had a median survival of 11 months. A log rank (Mantel Cox) test found a significant difference between the survival curves of the age groups , $\chi^2(4) = 22.15$, p=0.0002.

There was no significant difference between the survival curves plotted when the patients were grouped by the timing of their peritoneal metastases when compared with a log rank (Mantel Cox) test ($\chi^2(1) = 0.1523$, p=0.6963). The median survival in the patients with synchronous disease was 13 months, whilst in the patients with metachronous disease it was 11 months.


A
Treatment received after diagnosis
of peritoneal metastases
CRS and HIPEC (n=5)
Local major resection +/- chemo (n=21)
Systemic chemotherapy only (n=38)
Best supportive care only (n=16)
Percentage alive
100
90
80
70
60
50
40
30
20
10
0
0
12
24
36
48
60
72
Survival from diagnosis of peritoneal metastases
(months)


B
Age at diagnosis
>84
75-84
65-74
50-64
< 50
Percentage alive
100
90
80
70
60
50
40
30
20
10
0
0
12
24
36
48
60
72
Survival from diagnosis of peritoneal metastases
(months)



D

Patient group	Median survival from diagnosis of peritoneal metastases
All (n=80)	12 months
CRS & HIPEC (n=5)	Not determined
Local major resection +/-chemotherapy (n=21)	18 months
Systemic chemotherapy only (n=38)	13.5 months
Best supportive care only (n=16)	2.5 months
Patients aged <50 (n=10)	11 months
Patients aged 50-64 (n=23)	28 months
Patients aged 65-74 (n=19)	13 months
Patients aged 75-84 (n=17)	8 months
Patients aged >84 (n=11)	4 months
Synchronous peritoneal metastases (n=50)	13 months
Metachronous peritoneal metastases (n=30)	11 months

Figure 3-2: Survival in patients presenting with isolated peritoneal metastases from colorectal cancer to the Cardiff and Vale Colorectal MDT January 2014-December 2019 inclusive.

All patients who presented to the MDT between January 2014 and December 2019 with isolated peritoneal metastases from colorectal cancer are included. Patients with appendiceal cancers are excluded. Patients who were lost to follow up or who were still alive on 1st October 2020 are shown as censored results. Survival is measured from the date of the investigation or procedure from which the diagnosis was made.

A: Kaplan Meier graph to show survival of patients grouped by treatment received

B: Kaplan Meier graph to show survival of patients grouped by age

C: Kaplan Meier graph to show survival of patients grouped by the timing of the peritoneal metastases in relation to the colorectal primary.
D: Median survival of patients determined by Kaplan Meier survival analysis

3.6 Discussion

Between the beginning of April 2014 and the end of March 2019, there were 1220 patients who presented to the CAV UHB MDT with colorectal cancer, of whom 88 (7.2%) had synchronous peritoneal metastases. This figure is slightly higher than the 5% reported previously in the literature [44, 126]. It is closer to the figures obtained by a more recent registry study in the Netherlands, which found that 5.7% of the 7233 patients with colorectal cancer included had synchronous peritoneal metastases [47]. The number who had isolated peritoneal metastases in this evaluation was 39 (3.2%), which is a slightly higher proportion than quoted in the literature. If the patients who were referred from outside of the CAV UHB locality are excluded, then 6.6% of patients had synchronous metastases and 2.6% had isolated peritoneal metastases. The number of patients observed here was smaller than in the registry studies where reference figures were derived, so it is possible that these data represent an anomalous finding. Additionally, this is the proportion of patients who were referred to and discussed by the MDT. Whilst most patients would present through this pathway, there may have been other cases of colorectal cancer not recorded by this method. Registry studies would be likely to have a more accurate estimate of the total number of colorectal cancers occurring in the population since their data is generally obtained from multiple different sources within the healthcare system, although for reasons already discussed, they may have less thorough records of specific features such as the number of patients with peritoneal metastases. Registry data for the incidence of colorectal cancer from CAV UHB collated by WCISU were not used for comparison because they are only available until 2017. The publication of more recent data has been delayed because of the pandemic. The WCISU figures are reported by calendar year so cannot be directly compared with the NBOCA figures, but they are slightly higher with between 256 and 312 colorectal cancers registered per year, and a total of 1157 registered between 2014-17 [129]. Using

these figures, the proportion of patients presenting with synchronous peritoneal metastases was 5.9%, and with synchronous isolated peritoneal metastases 2.4%.

It is possible that patients in Cardiff and Vale present with colorectal cancer at a later stage to elsewhere. Data from WCISU shows that the average proportion of patients in Cardiff and Vale presenting with stage 4 disease was 23.9% between 2011-17 [130]. This is higher than the all-Wales average for the same time period, which was 22.2% [130]. Other indicators that late-stage presentation may be more common in Wales, and in Cardiff and Vale in particular, include the fact that bowel cancer screening uptake is lower than in other areas. The uptake recorded in April 2018 to March 2019 in CAV UHB was 56.4% of invited patients [131]. In Wales overall it was 57.3% for the same period, which represented the highest uptake recorded since the initiation of the program [125]. By comparison, the uptake recorded by the English bowel cancer screening program that year was 60.4% [132]. Screening has been demonstrated to improve early detection of colorectal cancer and this translates into better survival outcomes [131]. The lower uptake in the population of interest means that disease is more likely to present symptomatically at a later stage, and by other routes. This is borne out in figures for emergency presentation with a new diagnosis of colorectal cancer, which occurred in 18.7% of patients included in the NBOCA in 2018-19 (England and Wales) but 21% of patients in Wales [133]. Additionally, of the patients who underwent a major resection, 15% in the audit overall had emergency or urgent surgery, whereas this figure in CAV UHB was 32% [133]. These two areas represent an important opportunity to potentially improve the outcome of patients under the MDT. If more disease was detected earlier, then the number of patients presenting with synchronous peritoneal disease would decrease.

The median number of patients diagnosed with isolated peritoneal metastases was 3 per quarter, or around 1 each month. There were 13 patients with isolated peritoneal metastases who were referred to CAV UHB from other Health Boards in Wales. It is unclear what proportion of patients from other centres are being referred to Cardiff, and whether those identified are the ‘best’ candidates in the opinion of the local MDT, or ‘borderline’ candidates for CRS and HIPEC. This pattern may therefore reflect the desire to utilise the experience of the MDT at CAV UHB in the management

of isolated peritoneal disease, and add weight to any IPFR application to access additional treatment such as CRS and HIPEC for good candidates, or to get a second opinion as to whether referral was appropriate. Overall, there were more patients who presented with synchronous peritoneal metastases than metachronous disease, and the same pattern was observed in the numbers of patients in whom the peritoneum was the only site of metastatic disease. The proportion of patients with isolated peritoneal metastases was higher in the group with metachronous disease, perhaps representing a more favourable tumour biology, or the effect of surveillance on the detection of peritoneal disease earlier.

The overall treatment of patients with isolated peritoneal metastases was assessed. The MDT decision-making was easier to interpret retrospectively in this group. Additionally, surgical treatments for peritoneal metastases such as CRS and HIPEC, and more recently PIPAC, are not generally performed in patients with extraperitoneal metastases. There was a very small cohort of patients who underwent CRS and HIPEC. In order to have this treatment they would have needed to have disease which was suitable for surgery, and then an IPFR approved, or private health insurance. The policy position of the WHSSC position group is that funding for CRS and HIPEC for metastatic colorectal cancer should not be routinely provided [128]. The median age of patients who had this treatment was 53. Data surrounding the full medical history of this group was not available in the CaNISC record, but their progression to surgery suggests they had few comorbidities. Due to the small size of the group (n=5), and the fact that most of the patients were still alive at the end of this evaluation, it is not possible to calculate the median survival for comparison. The trend at this point is that this selected group will have improved survival compared to the other treatment groups. Since this data was not collected with the purpose of generalising to other patients, and there is no matched comparison group, it is difficult to draw any firm conclusions. However, the difference in outcome which appears to be emerging supports the routine prospective collection of this data. If there is a continued benefit to CRS and HIPEC observed, then this would support the ongoing provision of this treatment in selected patients.

There were a small group of patients identified who had peritoneal disease which was potentially amenable to CRS and HIPEC but who were refused funding. There were other patients for whom the surgery may have been appropriate, since a comment about referral to England was recorded, but for whom the application was not made. As commissioning for CRS and HIPEC in Wales is different to England, routine collection of the outcome data in patients who would be eligible for treatment if they lived in England would be useful in determining the effect of CRS and HIPEC. There is limited data from randomised controlled trials of CRS and HIPEC, which is one of the reasons that the procedure is not funded in Wales. Such trials are a major undertaking and given that CRS and HIPEC has been available to patients in England and elsewhere in the world for some time, recruitment to a randomised study with a control arm of systemic chemotherapy only would be difficult.

The median age of patients who had treatment in Cardiff, either with a surgical resection or with systemic chemotherapy was greater than those having CRS and HIPEC. There was not an obvious difference in outcome between patients who had a major resection locally and those who did not and received systemic chemotherapy only. Again, the full medical history of these patients was not obtained so it is not possible to determine whether there are differences in the comorbidities of the patients in these groups that may have had an impact. All but one of these patients had synchronous disease, and the resection was of the primary tumour. It was difficult to ascertain the intention of the resectional surgery in these patients from the CaNISC record. In some, there was clearly an attempt to reduce disease burden, since the term ‘CRS’ was used in the description of the operation. However, it was not possible to determine whether this was with the aim of improving survival, or purely to reduce symptoms. It is possible that some of these patients would have been eligible for CRS and HIPEC under the commissioning policy in England, but this was difficult to assess retrospectively from the data available on the electronic record. The benefit of CRS without intraperitoneal chemotherapy was recently assessed in the PRODIGE 7 trial, and no difference was found in progression free or overall survival between the CRS and HIPEC/systemic chemotherapy arm compared to CRS alone/systemic chemotherapy [52]. However, both the NICE interventional

procedures committee, and other authors [53] have made the observation that CRS is a sub-specialist operation, and thus a CRS carried out by a general colorectal surgeon is not comparable to one carried out by a peritoneal specialist. The sites participating in the PRODIGE 7 trial were recognised peritoneal centres [52].

Other patients in this evaluation who had surgery locally after diagnosis of peritoneal metastases are likely to have had a resection in order to treat or prevent obstruction and enable systemic chemotherapy. There may also have been patients who had surgery for symptomatic relief, but who were unfit for or declined chemotherapy post operatively. It is also possible that surgery was used as an adjunct because these patients were not expected to tolerate chemotherapy so well. This may explain the older median age in this group compared to the group who had chemotherapy alone. Again, the routine prospective collection and analysis of this information may help with MDT decision-making in the future about what surgical intervention is appropriate.

The group of patients who received best supportive care only were older and had the poorest outcomes in terms of survival. Further details of the comorbidities, performance status, and also the preferences expressed by the patients in this group would have been useful to confirm the reasons underlying this observation.

The fact that the group of patients who did not receive anti-cancer treatment had a higher median age may partly explain why the older patient groups had the shortest median survival when patients were grouped by age. Other factors that may have influenced this trend are the access of this group to investigations enabling detection and therefore treatment of disease. Patients aged 75 and over are not invited to have bowel cancer screening [131]. Additionally, the gold-standard investigations of colonoscopy or computed tomography (CT) colonoscopy which may be requested if patients are referred through other channels require bowel preparation, and a number of conditions that are more common in older age groups are listed as cautions for these drugs. Access to investigation may also be partly responsible for the lower median survival in the <50 group. Younger patients are not screened for bowel cancer and may have their symptoms attributed to less serious pathology initially if presenting through other channels. They may not meet the criteria for

rapid access clinics so readily since age cut-offs are used in many of the criteria [134]. Disease onset at a younger age is also associated with more aggressive genotypes [135]. All of these factors could result in presentation at a more advanced stage of disease, which is a trend that has been observed across the UK and elsewhere [47, 54]. It may also explain why the youngest and oldest patients are more likely to present as an emergency [54]. Patients <50 would generally be expected to be better candidates for major surgery, since frailty and comorbidities generally increase with age. However, most of the patients in this group did not have CRS and HIPEC because they were found to have peritoneal disease that was not amenable to complete resection.

There was no significant different difference observed in the overall survival between patients with synchronous disease and those with metachronous disease. Research in the past has found that patients with synchronous colorectal liver metastases have a poorer outcome than those with metachronous liver metastases, with the authors suggesting this represented more aggressive tumour biology or later presentation in this group [136]. However, more recent research focusing on the treatment of peritoneal metastases suggests that patients with metachronous peritoneal metastases who have CRS and HIPEC have earlier recurrence than those with synchronous disease treated by the same method [137]. There was no difference when overall survival was examined, but the authors suggested that the early recurrence in the metachronous group of patients may be an important factor to take into consideration when weighing up whether the adverse impact of a major operation such as CRS and HIPEC on quality of life is worthwhile. There did appear to be a difference in the management strategy of these two groups of patients in this department, since many of the patients with synchronous disease had resection of their primary and in a number of cases, other diseased tissue in addition to systemic anti-cancer treatment. Patients with metachronous disease generally only had systemic anti-cancer treatment. It is not possible to say from this observational evaluation whether this difference resulted in the parity seen between the synchronous and metachronous groups. It was also difficult to determine how long disease remained stable after surgery. Again, routine prospective collection of this

data may help to determine this and therefore help to decide whether the approach to the treatment of synchronous disease should be different to metachronous disease.

A major limitation of this evaluation, particularly given the treatment provided in most cases was considered 'palliative', was the fact that patient-reported outcomes were not assessed. Patient experiences are a useful addition to any service evaluation. No patient-reported outcome data in this cohort was routinely collected by the departments involved. This raises the question as to whether quality of life measurement tools should be routinely employed during cancer treatment, particularly if the treatment does not have curative intent. Both systemic chemotherapy and major surgery have the potential to have a significant negative impact on quality of life and understanding the relative effects might be useful for shared decision-making and for service planning in the future.

4 The introduction of PIPAC

4.1 The introduction of a new technology: Pressurised Intraperitoneal Aerosolised Chemotherapy assessed by the IDEAL paradigm criteria

4.1.1 Introduction

Pressurised intraperitoneal aerosol chemotherapy (PIPAC) is a novel technique to administer intraperitoneal chemotherapy to treat peritoneal carcinomatosis. The treatment involves a surgical procedure that is similar to a diagnostic laparoscopy. The peritoneal cavity and the disease present is inspected and biopsies are taken, and then solutions of chemotherapy are delivered to the pneumoperitoneum as an aerosol. PIPAC has been made possible by the development of a laparoscopic nebuliser [2]. There are two key hypotheses underpinning this technology. Firstly, that intraperitoneal chemotherapy is superior to systemically administered chemotherapy for the treatment of peritoneal metastases. Secondly, that delivering the drugs in an aerosolised solution to the pneumoperitoneum at laparoscopy confers pharmacodynamic advantages over liquid intraperitoneal chemotherapy, administered either at operation or by peritoneal catheter. PIPAC is an innovative use of both existing chemotherapeutic agents and existing surgical techniques (laparoscopy), as well as the introduction of a novel device. Laparoscopic surgery has not generally been used as a method for drug delivery in the past.

The Society of University Surgeons define an innovative procedure as one which ‘differs from currently accepted local practice, the outcomes of which have not been described, and which may entail risk to the patient’ [86]. PIPAC certainly fulfils this description. Thus, the introduction of the technology could be expected to fulfil the criteria set out by the IDEAL collaboration. The IDEAL (Idea, Development, Evaluation, Assessment, Long-term study) framework is the scheme of investigation for this type of innovative surgical therapeutic intervention described by the Balliol Collaboration in 2009 [4-6, 91]. The group suggested that a new innovation should progress through a series of investigative stages, starting with the ‘Idea’ stage where first-in-man proof-of-concept reports are generated. The ‘Development’ stage describes the descriptive studies carried out to develop the technique and determine

its safety. There may be adoption of the innovation by a few other surgeons. In the 'Exploration' stage there is an expansion in both the number of patients treated with the technique and in the number of clinicians performing it. Studies in this phase continue to assess the safety and feasibility, but the focus also starts to move to efficacy, and therefore the conduct of feasibility randomised controlled trials. The 'Assessment' stage uses randomised controlled trials or comparable alternatives to determine the effectiveness of the technique when compared to standard treatments. Longer-term outcomes should also be determined. The final stage is the 'Long-term study' stage, which involves ongoing data collection for quality assurance and audit purposes, to ensure that rare events are detected long-term outcomes determined, and that centres and surgeons are performing comparably.

PIPAC was developed in Germany and was introduced to practice there as an 'off-label' drug treatment [64]. Patients were accepted for compassionate therapy on a case-by-case basis. Subsequent to initial reports of the technique, a prospective phase 2 study was carried out in Germany in patients with recurrent platinum-resistant ovarian cancer [138]. A second prospective phase 2 study in Russia in patients with gastric cancer was published in 2016 [139]. Case series showed that by 2017 the technique had also been used in Italy, Denmark, Switzerland, and Belgium [74, 75, 78, 79]. Overall, these publications had reported hundreds of cases of PIPAC, but it had not been used in the UK. We undertook a review of the literature to conduct a technology appraisal of PIPAC and assess the stage of research worldwide to determine how it could be incorporated into UK practice.

4.1.2 Search Results

A search of Medline and Embase was carried out using the terms 'PIPAC', 'ePIPAC', 'aerosol$ adj3 chemotherapy' and 'pressuri$ adj3 chemotherapy'. Figure 4-1 summarises the search results. The search returned 269 records once duplicates were removed. Titles and abstracts were screened by a single reviewer. 56 records were identified and the full text obtained and reviewed for eligibility. All 7 review articles identified were excluded because they did not provide any additional analysis

of the data from the studies reviewed. 49 studies were taken forward to the assessment of the stage of research according to the IDEAL paradigm. 17 of these were pre-clinical studies, 1 was a piece of translational science using biopsies obtained during PIPAC procedures, and 8 were trial registrations or protocols. This left 23 studies that presented data from in-human use of the technique. These were reviewed for a technology appraisal to assess the evidence for safety, efficacy, quality of life during treatment, occupational health and safety, and procedural and logistical details.

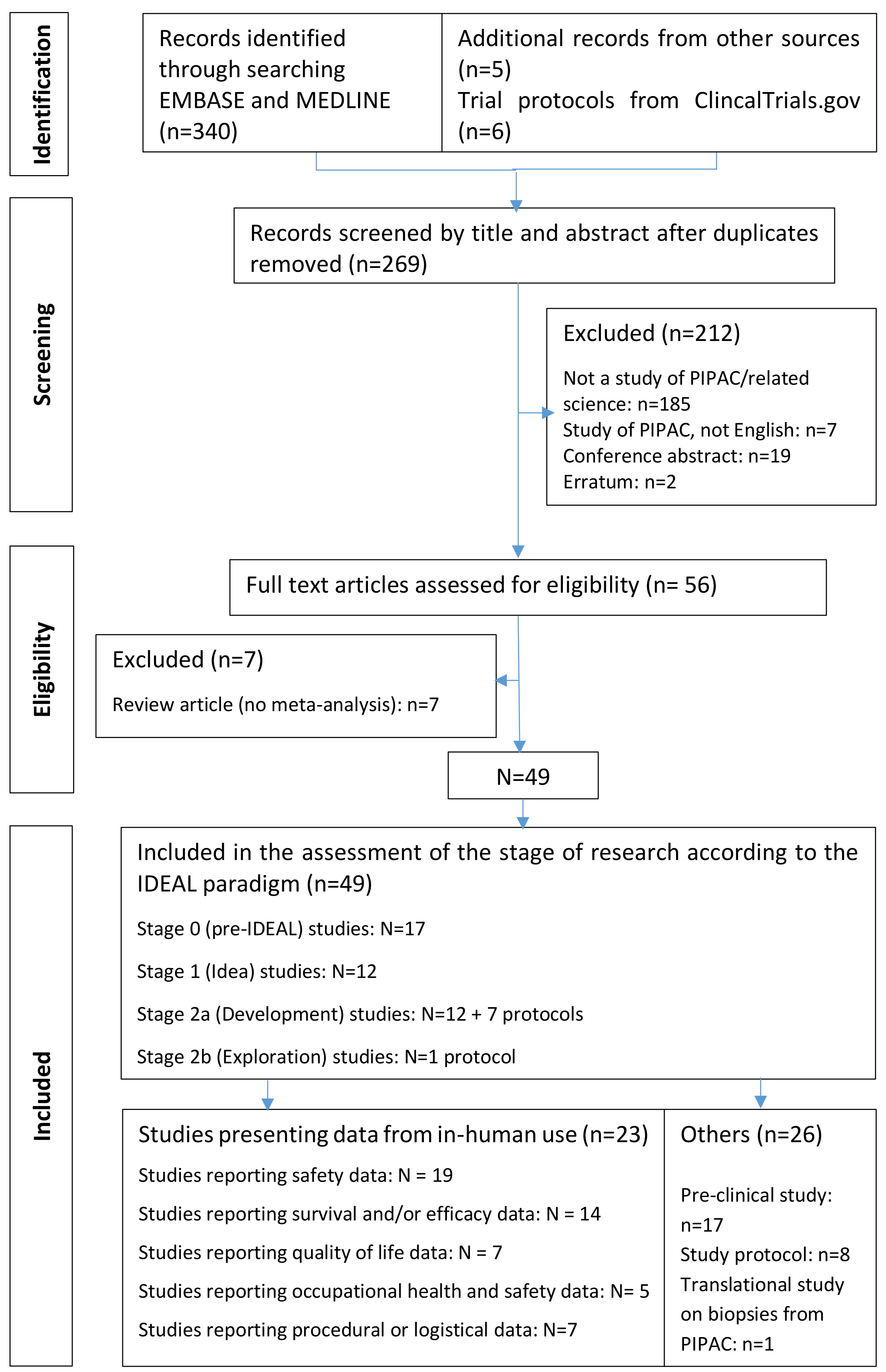


Figure 4-1: Flow diagram of the literature search and selection of articles for review

4.1.3 Summary of results

41 original research papers about PIPAC and/or the related technology, and 9 trial registrations were included in the review of the stage of research worldwide. They were assessed according to the criteria described in Table 2-2 and assigned an IDEAL stage. Overall, the search identified 17 stage 0 'Pre-IDEAL' studies [1, 2, 65-69, 71, 79, 82, 83, 140-147], 12 stage 1 'Idea' studies [64, 74, 75, 84, 138, 148-152], 12 stage 2a 'Development' studies [72, 77, 78, 120, 139, 153-159], seven trial registrations for stage 2a studies (NCT01854255, NCT02735928, NCT03246321/ EudraCT 2017-000927-29, NCT02604784/EudraCT 2015-000866-72 and NCT03124394, EudraCT 2016-003394-18, and EudraCT 2017-001688-20), and 1 trial registration for a stage 2b 'Exploration' study (EudraCT 2017-002637-37). Figure 4-2 shows the evolution of the literature base for PIPAC from the first description of the technique in 2000 to the date of the search in 2017. Study centres are identified by the city where the institution of the lead author or investigator is located. Papers that were available online before being published in print were identified and included by their electronic publication date.

The number of publications increased annually. Prior to 2015, there were few publications each year, but this was followed by an expansion in the literature with 7 publications in 2015, 13 in 2016, and 13 in 2017 up to September. The number of trial registrations also increased. The cumulative number of active centres, in both clinical and pre-clinical research, rose in a similar fashion. In general, there was evidence of progression through the stages of innovation, with increasing numbers of 'Development'-type studies as time passed and then registration of an 'Evaluation'-type study in 2017. Of the centres which had published results from clinical studies, 3 started with an 'Idea'-type study, and 3 with a larger 'Development'-type study. The studies registered on ClinicalTrials.gov and EudraCT were 'Development'-type studies in the main, although some did incorporate a phase 1 element, for example NCT02604784, which included a dose escalation arm. 19/23 studies which presented results from in-human use of the technique reported safety data. 14/23 reported survival and/or data on the efficacy of the treatment measured by some other means, such as histological analysis of repeated biopsies. 5/23 studies

had collected data on the impact of the treatment on patients' quality of life. 5/23 studies reported details about the occupational health and safety aspects of the procedure, and 7/23 presented data on procedural or logistical elements, such as the length of stay or duration of the procedures performed.

Year	2000	2012	2013		2014		2015			2016			2017 (to 8th Sept)				Total by centre
Stage of Innovation	Pre	Pre	I	D	I	D	Pre	I	D	Pre	I	D	Pre	I	D	E	
	0	0	1	2a	1	2a	0	1	2a	0	1	2a	0	1	2a	2b	
Bochum, Germany	1	2	2	P©	2	1	1	1	5	3	1	2	3	1			25 + 1P
Winterthur, Switzerland									P©								1P
Turin, Italy									P©#		2						2 + 1P
Dresden, Germany										1							1
Seoul, South Korea										1							1
Magdeburg, Germany										1							1
Odense, Denmark											1	P#		1		P#	2 + 2P
Moscow, Russia												1					1
Rome, Italy												P©					1P
Ghent, Belgium													1	1	P#		2+ 1P
Paris, France													1				1
Tubingen, Germany													1				1
Essen, Germany													1				1
Lausanne, Switzerland															3		3
Eindhoven, Netherlands															P©#		1P
Total publications by stage	1	2	2	1P	2	1	1	1	5+2P	6	4	3+2P	7	3	3+2P	1P	41+9P
Total publications by year	1	2	2 +1P		3		7 +2P			13 +2P			13 +2P				
Centres conducting pre-clinical research (cumulative)	1	1	1		1		1			4			8				
Centres conducting clinical research (cumulative)	0	0	1		1		3			6			9				

Figure 4-2: Adoption of pressurised intraperitoneal aerosolised chemotherapy according to the IDEAL criteria:

Studies were identified using the search strategy described in Figure 4-1. Included studies were reviewed and assigned a stage of innovation according to the description of the stage and proposed method of investigation suggested by the IDEAL paradigm as described in Table 2-2 from methods. The number of studies published by each centre is shown, broken down by year and stage of innovation. Study centres are described by the city where the institution of the lead author or investigator was located. Publications are described by the year the full text was first available (electronic publication date). An updated version of this figure was used in Tate and Torkington (2020) [92]. Pre= Pre-IDEAL, I= Idea, D= Development, E= Exploration, P= protocol. P©= protocol from registration on ClinicalTrials.gov, P# = protocol from registration on EudraCT.

4.1.4 Stage 0 – Pre-IDEAL Pre-clinical work

The IDEAL collaboration stated that the key outcome of interest in pre-clinical studies of a new technique relate to the efficacy of the procedure in bringing about the desired physical change [91]. Other important outcomes identified related to the safety and reliability, and the identification of any potential problems that might occur in human subjects. Additionally, the value of the new technique and its potential cost-effectiveness should be considered. This work was termed the 'pre-IDEAL' stage of research. Table 4-1 summarises 17 pre-clinical studies that were identified by the search. They assessed PIPAC in a variety of models.

The IDEAL collaboration recommends that pre-IDEAL stage is completed before the first procedure is carried out in humans, in order that any avoidable or predictable risk of failure or harm to the patient is identified [91]. The three studies published before the first human cases were described focused on the safety and feasibility of the administration of a solution using the new nebuliser device. Pigs have been used as an investigation and training model for human surgery for many years because of their comparable size and physiology to humans [160]. The first description of the technique was in 2000 and *in vitro* testing of the device as well as an *in vivo* study conducted in a large animal (pig) model were reported [2]. Two other studies investigating the technique were published in 2012 prior to the publication of the first procedures in patients. Solass et al [1] carried out another 'proof of concept' *in vivo* study comparing the distribution and penetration of methylene blue in the peritoneal cavity of 5 pigs compared to a control animal where the dye was administered by lavage. They concluded that PIPAC achieved a more homogenous and widespread distribution of dye, however this was assessed qualitatively. These two animal studies demonstrated that the procedure was a technically feasible and could be carried out safely, however no data from post-operative observation was presented [1, 2].

The same group also used a fluorescent labelled non-toxic therapeutic agent to assess drug penetration *ex vivo* in peritoneal tissue samples from a patient having CRS for metastatic ovarian cancer [65]. They compared a sample subjected to a simulated PIPAC with one subjected to a simulated lavage. They assessed penetration of the

drug by fluorescent microscopy and found that delivery was improved by the PIPAC technique. The study tested the technique in diseased human peritoneal tissue samples, and was hailed as proof of concept. However the drugs which were later applied in clinical use were not used in this research. Nonetheless, the findings, along with the established body of literature on the intraperitoneal administration of chemotherapy in peritoneal metastases led the group at Bochum to move to clinical use.

There were 14 pre-clinical studies published after the first human cases were described. 6 of these report the investigation of adjuvants to the technique, with Jung et al assessing hyperthermia of the pneumoperitoneum and aerosol [71], Kakchekeeva et al assessing the addition of electrostatic precipitation to PIPAC [82], Khosrawipour et al the effect of irradiation of the peritoneum [141-143], and Minnaert et al assessing the feasibility of using the aerosolisation technique to deliver advanced therapeutics, namely nanoparticles [145]. One study described a method for an *ex vivo* model to investigate PIPAC [146]. A further 7 studies investigated the originally described device and technique. Of these, 4 were from the original centre, Bochum, and a further 2 involved members of the research group from Bochum. This suggests that the pre-IDEAL stage was not complete prior to application of the technique in humans. However, the existing evidence for intraperitoneal chemotherapy (reviewed by Ceelen and Flessner [29]) and the demonstration that the technique was safe in a large animal model was adequate for Institutional Review Board approval for the first cases as an off-label treatment.

The more recent studies have had a greater focus on the efficacy of the technique but have tended to be *in vitro* or *ex vivo* studies on cancer cell lines or tissue samples. There are obvious limitations to the data generated from this work in terms of its generalisability to patients. However, there are few good alternatives for pre-clinical testing. Whilst there are a number of immunocompromised rat and mouse models which can be used to assess treatment of xenograft peritoneal tumours, they are not appropriate for testing the device used in PIPAC because of its size.

Table 4-1: Summary of the Stage 0 (Pre-IDEAL): pre-clinical studies identified

Author, year	Year	Centre	Type	Description	Outcome category
Reymond et al [2]	2000	Bochum, Germany	*In vitro* and *In vivo* large animal model	Development of a device to deliver solutions as an aerosol to the peritoneal cavity and testing in a large animal (pig) model	Safety, feasibility, efficacy
Solass et al [1]	2012	Bochum, Germany	*In vivo* large animal model	Description of a novel device (the CapnoPen) and assessment of the distribution of a dye solution administered in a large animal (pig) model	Safety, feasibility, efficacy
Solass et al [65]	2012	Bochum, Germany	*Ex vivo* tissue model	Comparison of lavage vs PIPAC on the penetration of drug in tissue samples in an *ex vivo* model	Efficacy
Khosrawipour et al [66]	2015	Bochum, Germany	*Ex vivo* tissue model	Assessment of the distribution of the aerosol generated by the CapnoPen device and the penetration of the drug administered into tissue samples in an *ex vivo* model	Reliability, efficacy
Gohler et al [69]	2016	Dresden, Germany	*In vitro*	Characterisation of the aerosol generated by the CapnoPen (droplet size, distribution)	Efficacy
Jung et al [71]	2016	Seoul, South Korea	*In vivo* large animal model	Development and assessment of hyperthermic PIPAC in a large animal (pig) model	Safety, feasibility
Kakchekeeva et al [82]	2016	Magdeburg, Germany	*In vivo* large animal model	Assessment of the feasibility of combining electrostatic precipitation and PIPAC (ePIPAC) in a large animal (pig) model	Safety, feasibility, efficacy
Khosrawipour et al [67]	2016	Bochum, Germany	*Ex vivo* tissue model	Assessment of the position of the CapnoPen device (distance, angle) on the penetration of drug administered by PIPAC in an *ex vivo* model	Reliability, efficacy
Khosrawipour et al [68]	2016	Bochum, Germany	*Ex vivo* large animal model	Assessment of the distribution and penetration of drug into the tissues after PIPAC in a post-mortem pig model	Reliability, efficacy
Khosrawipour et al [142]	2016	Bochum, Germany	*Ex vivo* tissue model	Assessment of the effect of tissue irradiation on the penetration of drug administered by PIPAC in an *ex vivo* model	Efficacy
Bellendorf et al [83]	2017	Essen, Germany	*Ex vivo* large animal model	Comparison of distribution of a radioactive tracer substance administered using PIPAC versus lavage in a post-mortem pig model	Efficacy
Eveno et al [147]	2017	Paris, France	*In vitro*	Assessment of PIPAC versus lavage in colorectal cancer cells *in vitro,*	Efficacy

Author, year	Year	Centre	Type	Description	Outcome category
			Ex vivo tissue model	and tissue samples in an *ex vivo model*.	
Khosrawipour et al [141]	2017	Bochum, Germany	*Ex vivo* tissue model	Assessment of the effect of fractional irradiation of tissue on the penetration of drug administered by PIPAC in an ex *vivo* model	Efficacy
Khosrawipour et al [143]	2017	Bochum, Germany	*Ex vivo* large animal model	Assessment of the effect of fractional irradiation of the abdomen on the tissue penetration of drug administered by PIPAC in a post-mortem pig model	Feasibility, Efficacy
Khosrawipour et al [144]	2017	Bochum, Germany	*In vitro*	Assessment of drug dose and pressure parameters on colorectal cancer cells *in vitro*	Efficacy
Minnaert et al [145]	2017	Ghent, Belgium	*In vitro*	*In vitro* evaluation of aerosolisation as a method to deliver siRNA complexes	Feasibility, Efficacy
Schnelle et al [146]	2017	Tubingen, Germany	*Ex vivo* whole organ model	Description of an *ex vivo* inverted bladder model to test PIPAC	Facilitation of further research

4.1.5 Stage 1 – Idea: first human applications

Table 4-2 summarises the stage 1 'Idea' studies identified. The first in-human applications of PIPAC were carried out between 2011 and 2013 and the first reports were published in 2013 and 2014 [64, 148] . PIPAC was delivered as an off-label therapy to patients for whom 'no satisfactory alternative therapy was available' as a result of progression on systemic treatment, or intolerance of systemic treatment. Patients were treated with doxorubicin 1.5 mg/m^2 in 50ml 0.9% saline, and cisplatin 7.5 mg/m^2 in 150ml 0.9% saline, the doses being arbitrarily set as 10% of the HIPEC doses used at that institution [64, 148]. Ethical approval for off-label use of the drugs involved was sought by the centre in Bochum for their early work. No formal dose escalation study had been performed, although a protocol registered on ClinicalTrials.gov suggested that one was underway in Turin by October 2015 (NCT02604784). The intraperitoneal route of administration meant that patients who also had extraperitoneal metastases were not offered treatment.

The drugs were administered using the nebuliser device (Micropump™ *Reger* Medizintechnik, Rottweil, Germany until 2015 and then *Capnopen*®, Capnomed, Villingendorf, Germany) and then the pneumoperitoneum was left in a steady state for 30 minutes. Regressive histological changes were observed in repeat biopsies from consecutive procedures, suggesting efficacy. Mild, and moderate adverse events were reported, with patients experiencing fatigue, fever, pain and vomiting after surgery. Pharmacokinetic data was collected and it was reported that the systemic absorption of the chemotherapy was low, although only doxorubicin was monitored [64]. Data on the occupational health and safety aspects of the technique were collected, with no evidence of platinum contamination in the operating theatres [149]. As more centres in Europe started performing cases, verification of the occupational health and safety testing of the procedure was performed [75, 151].

The IDEAL collaboration suggests that Stage 1 research should answer the question 'What is the new concept and why is it needed?' [91]. The studies identified do appear to have satisfied this brief. The PIPAC technique was described in detail in the early publications, and in addition the developers carried out demonstrations and training for interested clinicians. This led to a few other centres performing cases. Formal ethical approval was not always sought for these series, and they were performed as off-label drug administrations with the approval of clinicians locally. Minor modifications to the technique were made, for example the use of a single port by Vaira et al [140] and the addition of electrostatic precipitation [84], but overall the concept first described remained unchanged.

The unmet clinical need in malignancy involving the peritoneum was also described. Peritoneal metastases are a common endpoint in many cancers, and though many patients have disease in multiple organ sites, isolated peritoneal metastases are still identified in some. The likely number of eligible patients was not defined, perhaps because this data is not routinely collected in cancer registries. Estimates provided from the literature suggested that pursuing the technique was worthwhile. Additionally, the cases performed and reported suggested that it was acceptable to patients, and that there was a demand for treatment even though the procedure was invasive.

Table 4-2: Summary of the IDEAL stage 1 'Idea' studies identified

Author	Year	Centre	Description	Ethical approval (human studies)	Number of particip-ants	Number of PIPAC cases
Blanco et al [148]	2013	Bochum, Germany	Case series. Safety and toxicity data.	Yes (off-label use)	3	8
Solass et al [149]	2013	Bochum, Germany	Occupational health and safety data	Yes (off-label use)	2	2
Solass et al [161]	2014	Bochum, Germany	Case series	Yes (off-label use)	3	12
Tempfer et al [138]	2014	Bochum, Germany	Case report of a patient with pseudomyxoma peritonei	Yes (off-label use)	1	3
Giger-Pabst et al [156]	2015	Bochum, Germany	Case report of a patient with ovarian cancer	Yes (off-label use)	1	8
Graversen et al [151]	2016	Odense, Denmark	Occupational health and safety data	Yes	2	2
Reymond et al [84]	2016	Bochum, Germany	Case series, first reported use of ePIPAC	Yes (for off-label use, and for a prospective data registry)	3	14
Robella et al [79]	2016	Turin, Italy	Retrospective case series assessing feasibility and safety outcomes	No	14	40
Vaira et al [140]	2016	Turin, Italy	Retrospective case series. Safety and feasibility data.	No	17	29
Graversen et al [74]	2017	Odense, Denmark	Case series of patients with pancreatic cancer	Yes	5	16
Tempfer et al [152]	2017	Bochum, Germany	Case report of a patient with ovarian cancer	No	1	13
Willaert, Sessink, and Ceelen [75]	2017	Ghent, Belgium	Occupational health and safety data	No	2	2

4.1.6 Stage 2a – Development: larger case series

Table 4-3 summarises the stage 2 studies that were identified. The majority of these were 2a 'Development'-type studies. These included further case series of patients with colorectal cancer [154, 156], primary peritoneal cancer [156], gastric cancer [155, 156], and malignant mesothelioma [156] from the original centre, Bochum, in Germany. In gastrointestinal cancers oxaliplatin was used at a dose of 92mg/m^2

[154]. Again, this was arbitrarily derived, representing 20% of the dose used in HIPEC at the centre, and no formal dose-finding study was published. A larger series of patients with ovarian cancer included one patient who sustained a life-threatening bowel perforation, however this occurred when PIPAC was combined with cytoreductive surgery [153]. A PIPAC training programme was developed, and sales of the device required to deliver PIPAC were limited to clinicians who had been certified. Additionally, clinicians were asked to agree to submit data from all cases to an international registry, managed independently by the University of Magdeburg (NCT03210298).

The IDEAL collaboration suggest that stage 2a research should determine whether the new intervention has reached a state of stability sufficient to allow replication by others [91]. The output of work from the group at Bochum was enough to encourage other early adopters to perform cases on an off-label basis and publish their results. This could be interpreted as the completion of stage 2a since it showed that the technique was reproducible. Several more centres registered trial protocols for larger prospective studies and started recruitment. All of these protocols were based on the original technique, particularly with regards the equipment and occupational health and safety aspects. However, the trials that have been developed also propose modifications to the treatment, for example investigation of the administration of other drugs and doses, as well as the combination of systemic chemotherapy and PIPAC concurrently. Thus, it is likely that further stage 2a studies will be developed, since there are many other therapeutics that might be administered using the technique.

4.1.7 Stage 2b – Exploration: expanding the indications

One stage 2b study protocol registration was identified and it is also summarised in Table 4-3. The group at Odense designed a controlled trial to investigate the use of PIPAC as an adjuvant therapy after resection of high-risk colorectal cancers (EudraCT: 2017-002637-37). The trial was opened to treat patients with perforated or Stage pT4 tumours.

The IDEAL paradigm summarises the aims of the 'Exploration' stage of research as addressing any factors that might compromise the conduct of a successful randomised controlled trial for the technique [91]. The main indication described for PIPAC in early studies was a potential barrier to the conduct of randomised clinical trials. A number of different tumour types were included in the studies identified. The treatment was used as a palliative therapy for peritoneal metastases where no other satisfactory treatment existed. Patients in this position, and the clinicians treating them, were unlikely to accept random allocation of PIPAC treatment since the default comparator would be no further treatment. The indication also means that the population being treated is likely to vary from country to country and between tumour type, since it relies on the lack of alternative treatment options rather than a disease definition. This makes the interpretation of results in larger efficacy trials more difficult, since it is unlikely that peritoneal metastases from all cancer types will respond to the drugs chosen initially in the same way. The development of controlled trials to improve the quality of data on the efficacy of PIPAC was therefore an important step in the progression of research. Favourable outcomes in these studies, along with further evidence that treating patients with PIPAC and systemic chemotherapy in mixed regimes will remove many of the barriers to randomised controlled trials, since PIPAC can then be offered in situations where random allocation of treatment is more acceptable.

Table 4-3: Summary of the IDEAL Stage 2 (Development and Exploration) studies identified.

Author	**Year**	**Centre**	**IDEAL stage**	**Description**	**Ethical approval (human studies)**	**Partici-pants (n)**	**PIPAC cases (n)**
NCT01854255	2013	Bochum, Germany	2a	Prospective single arm open label phase 2 clinical trial with feasibility, safety, and efficacy endpoints.	Yes	*50**	*150**
Tempfer et al [153]	2014	Bochum, Germany	2a	Observational cohort study/case series of patients with ovarian cancer.	Yes (off-label use)	18	34

Author	**Year**	**Centre**	**IDEAL stage**	**Description**	**Ethical approval (human studies)**	**Partici-pants (n)**	**PIPAC cases (n)**
Demtroder et al [154]	2015	Bochum, Germany	2a	Retrospective case series of patients with colorectal cancer	Yes (off-label use)	17	45
Nadiradze et al [155]	2015	Bochum, Germany	2a	Retrospective observational cohort study/case series of patients with gastric cancer assessing safety, feasibility, and overall survival	Yes (off-label use)	24	60
Odendahl et al [156]	2015	Bochum, Germany	2a	Retrospective analysis of quality of life data from a case series	Yes (off-label use)	91	158
Tempfer et al [157]	2015	Bochum, Germany	2a	Retrospective cohort study of women with ovarian cancer assessing safety, feasibility and overall survival	Yes (off-label use)	99	252
Tempfer et al [72]	2015	Bochum, Germany	2a	Prospective open label single arm study (Phase 2) assessing safety, efficacy, survival, patient reported QoL.	Yes	64	130
NCT02604784/ EudraCT 2015-000866-72	2015	Turin, Italy	P2a	An Open-label, prospective double-arm, Phase I-II Clinical Trial assessing safety and efficacy of PIPAC, and a parallel dose escalation study	Yes	*100**	*300**
NCT03124394	2015	Winterthur, Switzerland	P2a	A prospective research database of all PIPAC procedures performed	Yes (for prospective database)	Not specified	Not specified
Girshally et al [158]	2016	Bochum, Germany	2a	Retrospective analysis of data from patients who had PIPAC and were down-staged and then went on to have CRS and HIPEC	Yes (for data collection and database)	9	25
Khomyakov et al [139]	2016	Moscow, Russia	2a	Open label phase 2 study of PIPAC in combination with systemic chemotherapy for gastric cancer assessing feasibility, safety, and efficacy.	Yes	31	56

Author	**Year**	**Centre**	**IDEAL stage**	**Description**	**Ethical approval (human studies)**	**Partici-pants (n)**	**PIPAC cases (n)**
Rezniczek et al [159]	2016	Bochum, Germany	2a	Translational study on gene expression changes during treatment using tissue samples from a case series of 63 patients	Yes	63	152
NCT02735928	2016	Rome, Italy	P2a	Single arm prospective phase 1/2 clinical trial of PIPAC in patients with recurrent ovarian cancer with safety and efficacy endpoints.	Yes	*50**	*150**
EudraCT 2016-003394-18	2016	Odense, Denmark	P2a	Single arm prospective phase 2 clinical trial of PIPAC with safety and efficacy endpoints	yes	*137**	*411**
Hubner et al [78]	2017	Lausanne, Switzerland	2a	Retrospective cohort study assessing feasibility and safety	Yes (retrospective approval for the database)	44	91
Hubner et al [120]	2017	Lausanne, Switzerland	2a	Retrospective cohort study assessing logistical and feasibility outcomes	Yes (retrospective approval for the database)	58	127
Teixeira Farinha et al [77]	2017	Lausanne, Switzerland	2a	Retrospective cohort study assessing quality of life data from a case series	Yes (retrospective approval for the database)	42	91
NCT03246321/ EudraCT 2017-000927-29	2017	Eindhoven, Netherlands	P2a	Phase 2 prospective single arm open label study of ePIPAC in colorectal cancer metastases with feasibility, safety, and efficacy endpoints	Yes	*20**	*60**
EudraCT 2017-001688-20	2017	Ghent, Belgium	P2a	Phase 1/2 prospective single arm open label study of PIPAC with Abraxane (albumin-stabilised Paclitaxel nanoparticles) with safety and feasibility outcomes	Yes	*70**	*210**
EudraCT 2017-002637-37	2017	Odense, Denmark	P2b	Prospective open label phase 2 study of PIPAC as an adjuvant treatment for high risk stage T4 colorectal cancer to prevent peritoneal metastases with efficacy primary outcomes, new indication for PIPAC.	Yes	*60**	*180**

P = protocol, *= anticipated numbers

4.1.8 A Technology Appraisal of PIPAC

The data from the in-human studies was then used to assess the evidence available for the safety, efficacy, and effect on patient quality of life of PIPAC treatment. Additionally, any data on the occupational health and safety aspects and the procedural logistics of this novel treatment were summarised. For all objectives, the studies must have tested or reported outcomes from PIPAC in human subjects and described the procedure and the doses of the cytotoxic drugs administered. No specific criteria about the design of the study were set. Twenty-three studies were identified from the search that contained relevant data.

It was not possible to collate the data from the studies identified since there was significant heterogeneity in the primary diagnosis of the patients treated, and of the treatment received. Additionally, since some centres had published more than one case series or study with overlapping time frames, it was not possible to be certain whether data had been re-presented in multiple reports. The total number of PIPAC procedures performed, and the total number of patients treated, was difficult to ascertain for the same reason. If the largest case series from each of the six centres that had reported the use of technique was used to estimate these figures, then there had been at least 563 procedures performed in 235 patients by the time of the search in September 2017 [75, 79, 120, 139, 151, 157].

4.1.8.1 Safety Data

Table 4-4 summarises the safety data that was available from published reports of PIPAC and ePIPAC. There were 19 reports from 5 different centres. The majority were observational case series of PIPAC performed as an off-label compassionate-use therapy, but there were two prospective trials that had been completed [72, 139].

Overall, the incidence of major adverse events (CTCAE grade 3-5) reported varied from 0% (Robella et al [79]) to 37% (Nadiradze et al [155]). Procedure-related mortality was low with 6 in-hospital deaths reported, only 2 of which were deemed 'related' to PIPAC. However, one death attributed to the haemodynamic effects of the removal of massive ascites was deemed 'unrelated' to PIPAC even though the procedure requires drainage of all ascites from the abdomen [155]. It is unclear from

the text whether the patient underwent a further paracentesis in the days following the procedure. Another in-hospital death in the same study was attributed to disease progression since the patient developed bowel obstruction post-operatively. In both cases, it would be difficult to prove that the procedure had no impact. It is pertinent that these two patients had a performance status of ECOG 4 and 3 respectively. This combined with the disease sequelae that would have been present represent potential contra-indications to treatment. In more recent studies, these patients would not have been offered PIPAC.

Most of the studies had followed up patients for some months to capture mortality data to assess survival, but few reported deaths in the context of outcomes that are common in surgical studies such as 30-day mortality. Whilst the palliative indication and repetitive element of the treatment may make this measure more complicated to interpret as a surrogate marker of safety, it is important to ensure that the treatment is performed in an appropriate group of patients. A high mortality rate within 30 days of surgery would suggest that either the procedure was not safe, or that the criteria for treatment needed to be revised.

Some of the series reporting higher rates of serious complications rated CTCAE grade 3 and 4 such as bowel injury and fistula were early in the evolution of PIPAC and CRS was carried out at the same procedure. The authors advised that CRS and PIPAC should not be combined in future studies [153, 157]. Other severe events that were reported are detailed in Table 4-4 and included infection, deranged liver function, abdominal pain, and anaphylaxis to drugs used during the procedure. Minor adverse events, such as mild-moderate abdominal pain and nausea were common in most series. One limitation of the safety data available was that there were discrepancies in the way that data had been collected and reported. Some authors reported the most severe adverse event experienced by each patient, others the total number of each type of adverse event. Some authors attempted to distinguish between complications that had arisen because of the surgical procedure, and complications or side effects that were attributable to the drug administered [74]. Thus, post-operative urinary retention was classified as a 'surgical complication', whilst post-operative pain, nausea and vomiting were considered 'adverse events'. Additionally,

the method that was used to collect adverse event data from patients and the length of adverse event reporting varied, and in many series was not explicitly described. Finally, a significant proportion of the data was reported retrospectively, and this may have affected the accuracy.

4.1.8.2 Efficacy and survival:

Table 4-5 summarises the data available on efficacy and survival. Three centres (Bochum, Moscow, and Odense) have published data on the efficacy of the treatment in the form of histological assessment of repeat biopsies at subsequent PIPAC procedures. In all series there have been cases where histological regression has been demonstrated. The rate of histological response rate (partial or complete) in series with more than 3 patients ranged from 33% to 80% [72, 74, 139, 153-155, 157]. The methods by which histological response was graded were not consistent between all centres and studies. The most commonly used system, favoured by Bochum, is the 'Peritoneal Regression Grading System' (PRGS) described by Solass et al in 2017 [162]. This method of assessing peritoneal biopsies has been shown to be reproducible but the relevance to clinical outcome has not been determined [163]. The prognostic value of such biopsies is likely to be variable. The method of tissue sampling is determined by the surgeon's preference. Thus some may choose to biopsy the most suspicious-looking areas, whilst others may use a systematic approach. Graversen et al described a method of attempting to repeat biopsies from the same location in the abdomen at each procedure by leaving clips in situ but admitted that it was not always successful [74]. Therefore, the experience of the surgeon, and the site and overall volume of disease will all have an impact on the assessment that can be made. Additionally, the repeat biopsies are only available in patients who have had multiple treatments, and this introduces a potential bias to this outcome measure. The reason for cessation of PIPAC treatment was not described in every study, but Tempfer et al stated that treatment was stopped when disease progressed, rather than because patients could not tolerate PIPAC [72]. Consequently, patients who were not responding to treatment were not assessed.

13 studies have published data on the survival of patients after treatment with PIPAC. One further study looked specifically at survival in patients who were down-staged sufficiently from PIPAC to receive CRS [158]. The median OS reported after PIPAC treatment ranged from 6 to 15 months. No studies included data from a comparison group. There was also heterogeneity in the patients treated in terms of their disease, and their prior treatment. It is therefore not possible to determine the overall effect PIPAC on survival.

4.1.8.3 Quality of Life Data

Several studies had assessed the impact of PIPAC treatment on the quality of life (QoL) of patients. The EORTC QLQ C-30 questionnaire was generally used for this purpose. This is a validated tool for assessing the quality of life of patients with cancer [164]. It assesses various domains, including cognitive, physical, emotional, role, and social functioning. The findings are summarised in Table 4-6. No study reported any statistically significant change in QoL scores after PIPAC treatment. Scores were apparently sustained over the course of repeated procedures. Several studies noted a trend towards an increased score after PIPAC treatment was initiated. This represents a promising finding since maintaining a good quality of life is an important aim of treatment in the palliative setting. It could also represent an advantage of PIPAC over systemic chemotherapy in some cases. An American study in patients undergoing systemic chemotherapy reported that patients with good performance status scores prior to starting treatment (ECOG 0 and 1) experienced a decrease in reported QoL scores after treatment commenced [165]. In the same study, the authors noted that the QoL scores reported by patients with poorer performance status scores (ECOG 2 and 3) were not altered by chemotherapy. This suggests that in very advanced disease, the effects of the cancer itself may be the overriding factor that determines overall QoL.

It should also be noted that only patients who continued to undergo repeated PIPAC procedures had data from sequential questionnaires available. This represents a self-selected group who were having the best clinical outcomes on treatment. No study

continued to collect QoL data in patients who stopped having PIPAC treatment, and no study had an ongoing control group for comparison. Farinha et al [77] noted a significant difference between the scores returned by gynaecological cancer patients as opposed to gastrointestinal cancer patients, with the latter having significantly lower scores throughout treatment.

4.1.8.4 Occupational health and safety and logistical aspects:

The studies identified that presented occupational health and safety data are summarised in Table 4-7. The first cases were performed in Germany and a thorough occupational health and safety analysis was carried out. The results of air testing in theatre during 2 cases revealed no detectable platinum compounds [149]. Air testing was repeated by Graversen et al during 2 consecutive PIPAC cases [151], and by Willaert et al during ePIPAC cases [75], and again no platinum was detected on the filters. Willaert et al also carried out extensive testing of surfaces in the theatre, including the gloves of the surgeons, and did not find any platinum contamination. Demtroder et al [166] analysed whether platinum could be detected on the laparoscopic cameras before and after sterilisation following use during PIPAC. They found that small quantities of platinum could be detected on the cameras at the end of the procedure prior to sterilisation (111ng). Following sterilisation, no platinum was detected.

No leaks of the chemotherapy aerosol were reported in any of the studies. There were some instances where liquid chemotherapy was spilled in theatre. Hubner et al [120] reported that 5 leaks/spillages of cytotoxic had occurred at the site of a Y-connecter used to connect the injector syringes to the high-pressure tubing to the Capnopen when carrying out the dual-therapy drug administrations. Liquid chemotherapy was spilt into the plastic sheath enclosing the high-pressure tubing. The risk to staff was therefore deemed to be low provided the appropriate steps had been taken to ensure that the Y connector was enclosed.

Several studies had reported on logistical aspects such as the length of operation and the length of stay, and these data are summarised in Table 4-8. In general, patients

remained in hospital for 3 days post-operatively, however more recent studies report shorter stays, and even day-of-surgery discharge [74]. The average length of the procedure was between 90 and 100 minutes in all but one study.

Table 4-4: Summary of data on the safety of PIPAC from human studies

Author	**Study type**	**Number of Patients (procedures)**	**Comment**	**Rate of adverse events per patient (most severe event counted unless stated)**
Blanco et al 2013 [148]	Case series (prospective data collection)	3 (8)	Only serial blood results reported. 3 PIPAC in 2 patients, 2 PIPAC in one patient. Peripheral venous blood samples pre-op and then daily until 5th day post op. Significant change in liver function noted by day 4 post-op but this was not felt to be clinically relevant and resolved spontaneously. No effect on renal function.	
Solass et al 2014 [64]	Case series (prospective data collection)	3 (12)	Collection of adverse events (AEs) according to CTCAE performed on day 1 and 5 post operatively. One patient had CRS and PIPAC in combination. All patients experienced an adverse event (vomiting, pain, fatigue), 1/3 patient experienced a grade 4 adverse event (bowel perforation). Non-access rate = 0	Grade 1 or 2 = 100% Grade 3 or 4 = 33%
Tempfer et al 2014 [138]	Case report (prospective data collection)	1 (3)	Collection of AEs according to CTCAE but method/duration of reporting not described. Patient experienced grade 1 nausea and grade 2 abdominal pain.	Grade 1 or 2 = 100% Grade 3 or 4 = 0%
Tempfer et al 2014 [153]	Case series (prospective data collection)	18 (34)	8 of the patients had CRS and PIPAC in the same procedure. Collection of AEs according to CTCAE but method/duration of reporting not described. Grade 1 toxicity in 12 patients (abdominal pain, inflammatory response), grade 3 toxicity in 3 patients (fistula, infection), and grade 4 toxicity in 2 patients (bowel injury). No grade 5 AEs but one woman died of disease progression 13 days postop. Non-access rate = 3/21	Grade 1 or 2 = 67% Grade 3 or 4 = 28%
Tempfer et al 2015 [72]	Prospective single arm open label study	64 (130)	Complications of laparoscopy according to CTCAE recorded until hospital discharge. Readmission and death recorded until study end. All patients experienced grade 1 abdominal pain. There were 8 grade 3 adverse events, which included a trocar hernia, a haematoma, and a bowel obstruction. Non- access rate = 11/64	Grade 1 or 2 = 100% Grade 3 or 4 = 13%

Author	**Study type**	**Number of Patients (procedures)**	**Comment**	**Rate of adverse events per patient (most severe event counted unless stated)**
Demtroder et al 2015 [154]	Retrospective case series	17 (48)	Collection of AEs according to CTCAE but method/duration of reporting not described. 16/17 patients had an AE; 12 patients grade 1 events (pain, fever, nausea and vomiting, deranged liver function, deranged renal function), 4 patients grade 3 events (pain, nausea and vomiting, diarrhoea). Secondary non-access rate =6/17	Grade 1 or 2 = 71% Grade 3 or 4 = 24%
Nadiradze et al 2015 ‘[155]	Retrospective case series	24 (60)	Collection of AEs according to CTCAE but method/duration of reporting not described. All patients experienced at least one adverse event. 15 grade 1 events (abdominal pain, inflammatory response, nausea and vomiting), 6 grade 3 events (deranged liver function, pain, bowel injury), 1 grade 4 event (anaphylaxis), and 2 grade 5 events (cardiorespiratory decompensation, bowel obstruction). On further analysis the authors considered the deaths unrelated to surgery. Secondary non-access in 3 patients.	Grade 1 or 2 = 63% Grade 3 or 4 = 29% Grade 5 = 8%
Tempfer et al 2015 [157]	Retrospective case series	99 (252)	Some patients in this study had CRS and PIPAC in the same procedure. Collection of AEs according to CTCAE but method/duration of reporting not described. 57 x experienced grade 1 events (pain, fever), 60 x grade 2 events (pain, anaemia, infection, trocar hernia), 17 x grade 3 events (bowel obstruction, bowel injury, respiratory compromise), and 3 x grade 4 events (bowel injury, fistula, anastomotic leak). Non-access rate 17/99.	Rate per patient (all events recorded): Grade 1 = 58% Grade 2 = 61% Grade 3 = 18% Grade 4 = 4%
Giger-Pabst et al 2015 [150]	Case report	1 (8)	Collection of AEs according to CTCAE but method/duration of reporting not described. 8 x PIPAC performed. Grade 1 nausea and grade 2 abdominal pain after each.	Grade 1 or 2 = 100%

Author	Study type	Number of Patients (procedures)	Comment	Rate of adverse events per patient (most severe event counted unless stated)
Odendahl et al 2015 [156]	Retrospective case series	91 (158)	Collection of AEs according to CTCAE but method/duration of reporting not described. Unclear if all adverse events reported 3 x in-hospital deaths (2 related- bowel injury, 1 due to disease progression) 1 x grade 4 event (anaphylaxis), 8 x grade 3 events (abdominal pain, infection, deranged liver function). Secondary non-access rate 5.5%.	Grade 1 or 2 = not reported Grade 3 or 4 = 10% Grade 5 = 3%
Reymond et al 2016 [84]	Case series (prospective data collection)	3 (14)	Collection of AEs according to CTCAE but method/duration of reporting not described. 2 patients experienced grade 1 abdominal pain, 1 experienced grade 2 abdominal pain.	Grade 1 or 2 = 100%
Tempfer et al 2017 [152]	Case report	1 (13)	Collection of AEs according to CTCAE but method/duration of reporting not described. The patient had 13 treatments and experienced grade 1 abdominal pain and fever 7 times, and a grade 3 pleural effusion twice.	Per procedure: Grade 1 or 2 = 54% Grade 3 or 4 = 15%
Robella et al 2016 [79]	Case series (prospective data collection)	14 (40)	Collection of AEs according to CTCAE but method/duration of reporting not described. Grade 1 (pain) and 2 (nausea) complications observed in 6 and 8 patients respectively. Also measured liver and renal function for 3 days post operatively and found no significant change occurred. Non access rate = 0	Per patient (all events recorded): Grade 1 = 43% Grade 2 = 57%
Vaira et al 2016 [140]	Retrospective case series	17 (29)	Collection of AEs according to CTCAE but method/duration of reporting not described. 29 PIPAC in 17 patients. 6 x grade 1 events (abdominal pain, wound haematoma), 9 x grade 2 events (nausea and vomiting). 0% non-access rate.	Per patient (all events recorded): Grade 1 = 35% Grade 2 = 53%
Farinha et al 2017 [77]	Retrospective case series	42 (91)	Describes ‘complication rate’ of 8.8% but does not classify complications or describe method/length of collection	

Author	Study type	Number of Patients (procedures)	Comment	Rate of adverse events per patient (most severe event counted unless stated)
Hubner et al 2017 [120]	Retrospective case series	58 (127)	Complications recorded but not classified, the method and duration of reporting was not described. 9 post-operative complications, including 1 x death due to arrhythmia 4 days after surgery. Other complications minor. 131 procedures attempted in 60 patients – primary non-access rate 2/60 and secondary non-access rate 1/69 procedures.	
Hubner et al 2017 [78]	Retrospective case series	44 (91)	Complications recorded but not classified, the method and duration of reporting was not described. The overall complication rate was 8 complications after 91 procedures (8.8%), with 7 classified as 'minor complications', and one patient who developed cardiogenic shock 4 days postoperatively and died. No intra-abdominal cause was identified. Non access rate = 3/44	
Khomyakov et al 2016 [139]	Prospective single arm open label study	31 (56)	Collection of AEs according to CTCAE until postoperative day 30 4 adverse events recorded – 3x grade 2 nausea, 1x grade 3 diaphragmatic perforation by biopsy intraoperatively. Non access rate 0.	Grade 1-2 = 10% Grade 3-4 = 3%
Graversen et al 2017 [74]	Case series (prospective data collection)	5 (16)	Collection of AEs according to CTCAE. Assessment prior to discharge and then by telephone call 2 weeks post operatively 0% non-access rate (primary and secondary), 16 procedures performed. All 5 patients experienced mild nausea and vomiting (grade 1). 1 patient died with refractory ascites 1 month after 2nd PIPAC.	Grade 1 = 100%

Table 4-5: Summary of data on efficacy and survival after PIPAC from human studies

Efficacy has been determined using histological assessment and by observing survival. Histological assessment of response is only possible in patients who have had more than one PIPAC procedure.

Author	Study Type	No. of patients (procedures)	Efficacy data	Survival data
Tempfer et al 2014 [153]	Case series (prospective data collection)	18 (34)	Histological assessment made in 8 patients. 6/8 had an objective tumour response, which is 33% of the study cohort. 1 woman had complete response (PIPAC + CRS), 2 had partial response (PIPAC + CRS), 3 had stable disease (PIPAC only), and 12 had progressive disease (7 x PIPAC only, 5 x PIPAC + CRS)	Mean OS in patients with PIPAC + CRS was 486 days, with 74% 400 day survival. Mean OS in patients with PIPAC alone was 268 days, with 57 % 400 day survival.
Tempfer et al 2015 [72]	Prospective single arm open label study	64 (130)	Histological assessment carried out by independent pathologist according to tumour regression grade (TRG). 38/53 (72%) of the intention-to-treat (ITT) population had histological regression (59% of total cohort). 30/34 of the ‘per protocol’ (PP) population had evidence of regression.	Cumulative survival of the ITT population was 50% at one year, with a mean OS of 331 days (95% CI 291-371 days). Mean time to progression was 144 days (95% CI 122-168 days). For the PP population, the figures were cumulative survival of 63% at 1 year, and a mean OS of 407 days (95% CI 347-368 days). Mean time to progression was 174 days (95% CI 150-199).
Tempfer et al 2015 [157]	Case series (retrospective data collection)	99 (252)	Histological assessment according to TRG in 50 patients. 38 had evidence of tumour regression (51% of total cohort).	Median OS after first PIPAC was 14.1 months. Cumulative survival after 12 months was 56%.
Demtroder et al 2015 [154]	Case series (retrospective data collection)	17 (48)	Histological assessment of TRG by an independent pathologist in 14 patients. 12 patients had evidence of tumour regression (71% of total cohort); 7 had complete pathological response, 4 had a major response, 1 a partial pathological response, and 2 had no response.	Median OS was 15.7 months after first PIPAC

Author	Study Type	No. of patients (procedures)	Efficacy data	Survival data
Nadiradze et al 2015 '[155]	Case series (retrospective data collection)	24 (60)	Histological assessment of the PRGS by independent pathologist in 17 patients. 12 patients had histological regression (50% of study cohort); 6 had complete histological response, 6 had a partial response.	Median OS was 15.4 months after first PIPAC. Cumulative survival after 1 year was 52%.
Reymond et al 2016 [84] '	Case series (prospective data collection)	3 (14)	Histology graded according to TRG scale by independent pathologist in 3 patients. Patient 1 had variable regressive changes on histology. Patient 2 had moderate regressive changes on histology. She had resection of the primary tumour between PIPAC treatments. Patient 3: All biopsies at PIPAC procedures were tumour free. All patients had concurrent systemic chemotherapy .	OS in months: Patient 1 = 11. Months, patient 2 = 11.7 months, patient 3 = 22 months.
Khomyakov et al 2016 [139]	Prospective single arm open label study	31 (56)	Histological assessment according to PRGS scale in 15 patients. 9 patients (29% of study cohort) had a histological response; 4 patients had complete pathological response and 5 patients had major pathological response.	Median OS of 13 months.
Graversen et al 2017 [74]	Case series (prospective data collection)	5 (16)	Histological assessment according to PGRS scale in 5 patients. 4 patients (80% of study cohort) demonstrated histological regression in specimens at PIPAC number 2.	Status of patients presented but follow up duration variable (2-11 months).
Solass et al 2014 [64]	Case series (retrospective data collection)	3 (12)	Histological assessment was made but it was unclear if it was standardised or independent. No standardised radiological assessment.	Median OS of 187 days (109-567). Two patients had CRS after commencement of PIPAC.
Tempfer et al 2014 [138]	Case report	1 (3)	Histological assessment was made but was not standardised.	Patient had survived 6 months at time of publication.

Author	Study Type	No. of patients (procedures)	Efficacy data	Survival data
Giger-Pabst et al 2015 [150]	Case report	1 (8)	Histology analysed from each procedure, but no standardised grading system used.	
Girshally et al 2016 [158]	Case series (retrospective data collection)	9 (25)	Histology analysed from each procedure, but no standardised grading system used.	OS presented only for patients who went on to have CRS.
Tempfer et al 2017 [152]	Case report	1 (13)	Objective tumour response, defined as tumour regression on histology, was noted but was a partial response.	
Odendahl et al 2015 [156]	Case series (retrospective data collection)	91 (158)		Median OS of 13.4 months after 1st PIPAC.

TRG = Tumour Regression Grade, PRGS = Peritoneal Regression Grading System, ITT = intention to treat, PP= per protocol, OS = overall survival, CRS = cytoreductive surgery

Table 4-6: Summary of data on quality of life during and after PIPAC treatment from human trials

Author	Study type	Number of patients (procedures)	Comment
Tempfer et al 2015 [72]	Prospective single arm open label study	64 (130)	EORTC QLQ C-30 one day before each procedure. Missing data is a limitation – 7, 4, and 6 patients did not complete the questionnaire at the 3 time points respectively. Overall, there was a trend towards improvement of the global physical health scores, with scores of 52.0, 58.1, and 59.5, but the 95% CIs overlapped.
Robella et al 2016 [79]	Case series (prospective data collection)	14 (40)	EORTC QLQ C-30 and SF-36 used before and after PIPAC procedures. Scores remained stable through PIPAC therapy, however, no figures or analyses were presented.
Farinha et al 2017 [77]	Case series (retrospective data collection)	42 (91)	EORTC QLQ C-30 was used before PIPAC, between treatments, and at post-treatment 4 week follow up consultation. There were no significant changes in QoL score.
Tempfer et al 2017 [152]	Case report	1 (13)	EORTC-QLQ-C30 was used to measure quality of life through the treatment period although the time-points of assessment were not specified. Overall, QoL score 'initially improved' but a transient decline was noted after course 10. Formal statistical analysis of the scores was not presented.
Tempfer et al 2015 [157]	Case series (retrospective data collection)	99 (252)	EORTC QLQ-30+3 questionnaire before each treatment. Missing data is a limitation - 31 patients filled in a second questionnaire, 22 a third, and 9 a fourth. The global scores were sustained (baseline =47.1, after 2nd = 62.4, after 3rd = 53, after 4th = 52.8.
Giger-Pabst et al 2015 [150]	Case report	1 (8)	EORTC QLQ-30 questionnaire before each treatment. Score rose initially between treatments 1 and 2 and then slowly fell back to baseline. No statistical analysis of scores performed.
Odendahl et al 2015 [156]	Case series (retrospective data collection)	91 (158)	EORTC QLQ C-30 questionnaire before each treatment. Comparison of sequential scores in the PIPAC cohort with a reference 'peritoneal carcinomatosis' cohort stratified by survival. Consecutive scores in the PIPAC group appeared stable. Functional and symptom scores in the control cohort deteriorated with as survival decreased. No statistics performed.

Table 4-7: Summary of data surrounding occupational health and safety aspects of PIPAC when used in human trials

Author	**Number of patients (procedures)**	**Air monitoring**	**Leaks**
Varia et al 2016 [140]	17 (29)	No	None detected
Solass et al 2013 [149]	2 (2)	Air sampled during 2 consecutive PIPAC procedures, analysis by an independent centre.	None detected (detection limit<9 ng/m3)
Hubner et al 2017 [120]	58 (127)	No	5 leaks – used a Y connector for sequential injections of cisplatin and doxorubicin and this malfunctioned leading to leak of liquid chemotherapy into the plastic sheath around the tubing.
Graversen et al 2016 [151]	31 (86)	Air sampled during 2 consecutive PIPAC cases.	No aerosol leaks detected, limit of platinum on air filters was 0.0001mg/filter 2 leaks of liquid chemotherapy at the syringe/tubing connection- contained by plastic sheath around the tubing.
Willaert et al 2017 [75]	2 (2)	Air sampled during 2 consecutive ePIPAC procedures	No leaks or platinum contamination of air, surfaces in theatre, or surgeon's gloves detected. Detection limit was 0.02ng/cm^2 for surfaces and 4-27 ng/m^3 for air.

Table 4-8: Summary of data on the procedural and logistical aspects of PIPAC treatment in human trials

Author	**Study type**	**No. of patients (procedures)**	**Median length of stay**	**Mean operating time**
Robella et al 2016 [79]	Case series (prospective data collection)	14 (40)	3 days	
Farinha et al 2017 [77]	Case series (retrospective data collection)	42	3 (range 1 to 20)	
Hubner et al 2017 [78]	Case series (retrospective data collection)	44 (127)	3 (IQR 2-3)	94min (IQR 89–108)
Demtroder et al 2015 [154]	Case series (retrospective data collection)	17 (48)		73 ± 20 minutes
Varia et al 2016 [140]	Case series (retrospective data collection)	17 (29)	3.8 (range 2-5)	96 mins (range 50-145)
Hubner et al 2017 [120]	Case series (retrospective data collection)	58 (127)	Cases 1-20 = 3 days (IQR 2-15) Cases 21-50 = 3 days (IQR 2-10), Cases 51-127 = 2 days (IQR 1-7)	Cases 1-20 = 91 min (IQR 87-103) Cases 21-50 = 93 min (IQR 88-107) Cases 51-127 = 103 min (IQR 91-121)
Graversen et al 2017 [74]	Case series (prospective data collection)	5 (16)	Patients discharged on day of surgery or day 1 post operation.	Median 92 mins (77-107)

4.1.9 Summary and conclusions

The review of the literature ascertained that at the end of 2017, PIPAC was in the 'Development' stage of innovation described by the IDEAL collaboration. There had been a large increase in the number of cases of PIPAC performed in the preceding 2-3 years but there was still a lack of robust prospective data on the efficacy of the treatment.

PIPAC was developed to treat peritoneal metastases and as already described, was only used initially on a compassionate basis in patients who had peritoneal disease which was progressing despite all other conventional therapy [3, 153]. In the early stages of the technique, it was sometimes combined with surgery to debulk peritoneal disease [153, 157]. A number of reports included patients who continued to have systemic chemotherapy alongside PIPAC treatment [79, 84, 139, 154, 155, 167]. Most recommended a 'wash-out' period of at least 2 weeks between administration of a cytotoxic drug systemically and PIPAC. The centre where the technique was developed, as well as those which adopted the technique first, already had an established peritoneal malignancy service.

The following indications for treatment were identified:

- Patients who were unsuitable for cytoreductive surgery/hyperthermic intraperitoneal chemotherapy, either because of the extent of disease or because of other medical problems which made them unfit for CRS and HIPEC.
- Patients who had already had at least one, but usually two lines of chemotherapy in the metastatic setting.
- Patients with no evidence of extra-peritoneal metastases.

Some contraindications for treatment were also identified:

- Symptoms or signs of bowel obstruction or impending bowel obstruction
- ECOG Performance status >2

Patients with peritoneal metastases from various primary cancers had been treated including;

- Ovarian cancer [72, 78, 153, 157, 167]
- Colorectal cancer [78, 154, 167]
- Gastric cancer [78, 167]
- Pancreatic cancer [74]
- Pseudomyxoma peritonei [78, 167]
- Peritoneal mesothelioma [78, 167]
- Cholangiocarcinoma [84]

There had been a thorough assessment of the occupational health and safety of both PIPAC and ePIPAC at three separate centres. The PIPAC technique had been well described, and a training course was available for interested clinicians. Centres performing the technique were sharing their experiences and protocols for maintaining the safety of staff and patients. There had been no reported instances of contamination of theatres with aerosolised cytotoxic drugs. The reported leaks that had occurred were spillages of liquid chemotherapy and these had been contained by the published and recommended safety measures.

There was also a good volume of data on the safety profile for patients. The incidence of serious adverse events reported varied, and in some series was very low. Minor adverse events such as abdominal pain and nausea were common. Treatment with alternative cytotoxic regimes could be expected to result in similar or more frequent adverse outcomes. Data on QoL during treatment was more limited but suggested that EORTC-QLQ-C30 scores were maintained. Thus, if the clinical efficacy of PIPAC treatment could be demonstrated, it would be a reasonable option for this group of patients.

The formal assessment of such a technology in clinical trials is challenging. The first hurdle is that the technique brings together a novel CE marked device (the CapnoPen), a radiographic high-pressure injector potentially being used outside of its CE-marked indication, and licensed drugs being administered in an off-label method of administration. Thus, a trial involving the procedure requires application for

regulatory permissions to conduct a study of an Investigational Medical Product, and this is outside of the usual activity of most surgical research departments. It also requires a higher level of oversight and in combination with the need for multiple surgical interventions for each participant, makes a trial costly. This is perhaps why the technique was initially used extensively in 'compassionate use' circumstances, rather than formal registered trials. This resulted in a situation where, despite hundreds of completed cases, the evidence for efficacy was limited by large variability in the cohort of patients treated and the data available, and the potential confounding factors present.

Having reviewed the literature and ascertained the scope of existing research, a consensus was reached amongst the clinicians involved that a small feasibility or safety trial would represent unnecessary repetition of previous work. It was also acknowledged that given the experimental nature of the technique, it could not be incorporated into routine practice. The procedure had never been performed in the UK before, and the acceptability to UK clinicians and patients had not been established. It was noted that whilst the technique should be within the skill set of a laparoscopically trained surgeon, it was usually performed in a cohort of patients who would not ordinarily have surgery. There was therefore likely to be a learning curve associated with the procedure for both the surgeon and the wider peri-operative team. It was felt that successful delivery of the treatment would be a useful step in enabling participation in future phase II/III trials to establish the procedure's efficacy, either as a UK centre in a multinational trial, or in a UK-based trial. A proposal for a small pilot, involving three patients, was therefore developed. This would enable assessment of the feasibility of delivering the treatment in a UK NHS setting in a carefully monitored and standardised way. The literature review informed the criteria for treatment that were set, and the standards that would be used to benchmark the results. These were updated as further data was published.

4.2 Audit of a pilot program

A standard operating procedure for the ePIPAC pilot was developed by a multidisciplinary group. It was scrutinised by the CAV UHB Quality, Safety and Experience Committee, as well as the CAV UHB Medicines Management Group, the CAV UHB Cytotoxic Board. As part of the 'New Procedures' process, NICE was also notified of the procedure.

The pilot was approved in May 2018 and cases were performed between June 2018 and September 2019.

4.2.1 Patients and accrual

Three patients were identified and treated. An additional three patients were considered but on further investigation were not suitable. It took 48 weeks from approval of the pilot to the start of treatment in the third patient.

The initial scope of the project was to identify patients with metastatic ovarian, colorectal, or appendiceal cancer who were under the relevant MDT in Cardiff. Once the pilot had commenced, a few referrals from other specialties were received. These were assessed on a case by case basis, in discussion with the relevant MDTs, considering the available evidence in each disease. One patient with gastric cancer was treated using the Cisplatin and Doxorubicin protocol as previously published by Nadiradze et al [155]. The patients met all other criteria set out in the standard operating procedure. The median pre-operative ECOG score was 0, and the median ASA class assigned was 2. The characteristics of the patients included in the pilot compared to the criteria set out in the standard operating procedure is summarised in Table 4-9.

Table 4-9: Results of the audit of the ePIPAC pilot: patients treated

Patient Characteristic	Criteria for treatment in pilot	Audit Results
Primary cancer (per patient, N=3)	Ovarian, colorectal, or appendiceal cancer.	Colorectal = 1 Ovarian = 1 Gastric = 1
Pre-op ECOG (median and range) (per procedure, N=7)	0-2	0 (0-2)
ASA class at time of procedure (median and range) (per procedure, N=7)	1-3	2 (1 to 3)

4.2.2 Procedures

Data on the procedural aspects of the ePIPAC pilot and the standards from the literature for comparison are summarised in Table 4-10. Access to the abdomen was possible in all cases. The median operating time was 1h39min (range 1h14-1h50min) This is comparable to the figures identified in the literature. The median total time in theatre once anaesthetic time was also accounted for was 2h10min (1h48-2h30min). This means that at least two ePIPAC procedures could be scheduled on a 2 session list. Three or four ports were used in the procedures. This is more than elsewhere, with Hubner et al completing 88% of procedures using only two trocars. However, the addition of the electrostatic element of the procedure necessitates another small port for the ion wand.

The number of biopsies taken and sent for processing from each case is shown in Table 2-5. The SOP initially stated, in line with other published protocols for the procedure, that biopsies should be taken from each of the 4 quadrants of the abdomen. However, where there was not any identifiable disease present in a quadrant, the additional cost and risk associated with a biopsy, for example bleeding or ingress of chemotherapy into the abdominal wall, was felt to outweigh the benefit and they were not taken. In one case, the patient had only recently undergone diagnostic laparoscopy and biopsies so no further biopsies were taken. In other cases, more than 4 biopsies were taken from areas that were suspicious of disease. The procedure requires any ascites to be drained prior to the application of chemotherapy. The patients in this pilot had low volumes of ascites present. Overall, the number of biopsies required by a protocol, and the potential for future patients

to present with higher volumes of ascites if there was expansion of the provision of the procedure may impact on both the time required in theatre and the resources in terms of support services.

A full course of 3 ePIPAC procedures was delivered in 2 of the 3 patients. This rate is similar to that described in the literature [118, 119]. The interval between cases was longer than planned in three instances. This was because of theatre list availability in 2 cases and a delay for the patient to receive additional treatment in the third case. In a recent review of practice, Alyami et al reported that the duration between treatments reported in the literature was 28-56 days [119], so the intervals in this pilot are comparable to other centres. Theatre scheduling would need to be managed carefully in the context of a trial.

Table 4-10: Results of the audit of the ePIPAC pilot: procedures

Procedural characteristics	**Standard Identified in the literature**	**Audit Results (per procedure, N=7)**
Access to abdomen possible	89.5%-91.5%[2]	7 (100%)
Duration to nearest minute (median and range)	Median operating time = 1h38min[3]	Time in anaesthetic room = 33min (20 to 40 mins) Operating time for ePIPAC procedure = 1h39min (1h14min to 1h50min) Total time in theatre = 2h10min (1h48min to 2h30 min)
Number of laparoscopic ports	2 = 88%, >2 = 12%[3]	3 ports = 2 4 ports = 5
Number of biopsies taken (median and range)	‘Biopsies are taken from all 4 quadrants of the abdomen’[1] Median number of biopsies taken = 3[3]	4 (0-6)
PCI recorded	Median PCI = 11[3] PCI incomplete = 28%[3]	10 (5-24) PCI incomplete = 0%
Volume of ascites drained in millilitres (median and range)	‘All ascites is removed’[1] Median = 50ml[3]	0 (0-50)

Procedural characteristics	Standard Identified in the literature	Audit Results (per procedure, N=7)
Number of ePIPAC procedures	'Goal of 3 cycles per patient'[1] Proportion of patients having 2 or more procedures = 65%[2]	3 procedures: 2 patients (66.6%) ≥2 procedures: 2 patients (66.6%)
Timing of ePIPAC procedures	42 days (+/- 6 days) between procedures[1]	52 days – theatre availability 41 days 50 days – theatre availability 97 days – additional treatment

Notes: 1 = standard identified in Giger-Pabst and Tempfer [118]
2 = standard identified in Alyami et al [119]
3 = standard identified in Hubner et al [120]

4.2.3 Safety

Data on the safety of the ePIPAC procedures performed during the pilot are summarised in Table 4-11. There were no major critical incidents reported during the pilot. One case had to be delayed because the syringe of chemotherapy was damaged in theatre after checking and needed to be replaced by pharmacy. The patient had not been anaesthetised and returned to the ward until the drug was ready. No leaks or spillages of chemotherapy occurred. No unanticipated steps were required during any of the cases.

The patients did experience adverse effects from the treatment but these only required outpatient monitoring and symptomatic treatment. All patients experienced mild pain and nausea. On three occasions, patients reported symptoms that were moderate in severity; diarrhoea, pain, and fever. These all settled spontaneously.

Table 4-11: Results of the ePIPAC pilot audit: safety

Factor	Standard Identified in the literature	Audit results
Incidents reported	Major = 0% cases[3] Minor = 11% cases[3]	Major = 0 (0%) Minor = 1 (14.3%)
Chemotherapy leaks or spillages	Contained = 5/137 (3.9%)[3] Non-contained = 0[3]	Contained = 0 Non-contained = 0

Factor	Standard Identified in the literature	Audit results
Complications recorded	Mild to moderate: 33% cases[1], 0-11% patients intra-operatively and 0-6% patients post-operatively[2] Severe or life threatening: 0.3% cases[1], 12-15% patients[2] Death = 0.4% per procedure[1], 1.5%[1] - 2.7%[2] per patient	Mild = 7 (100%) cases Moderate = 3 (43%) cases Severe = 0 Death = 0

Notes: 1 = standard identified in Giger-Pabst and Tempfer [118]
2 = standard identified in Alyami et al [119]
3 = standard identified in Hubner et al [120]

4.2.4 Discussion

The patients treated in this pilot were comparable to those in other reports in the literature. Approval was initially sought to treat patients with ovarian cancer with Cisplatin and Doxorubicin, and patients with colorectal or appendiceal adenocarcinoma with Oxaliplatin on an off-label use of these chemotherapeutic agents. Intraperitoneal chemotherapy, either alone or in combination with CRS, is in widespread use in these cancers. There is controversy in this area however, and in Wales, intraperitoneal chemotherapy for ovarian cancer, and CRS and HIPEC for colorectal cancer are not part of standard NHS care and are not routinely funded. This is in contrast to the position in England. During the course of the pilot, as further studies of PIPAC were published, and the scope of the pilot was increased to enable treatment of one patient with gastric cancer. In future, it is planned that ePIPAC cases will take place in the context of a trial, and in that instance, the trial protocol would need to be followed with regards the criteria for inclusion, and the drug regimen to be used. The primary outcome measure may well determine how much flexibility is allowed. In trials in the palliative situation, QoL is recognised and valid primary outcome. If the aim of a trial was to determine the effect of PIPAC on QoL then more flexibility might be possible in terms of the indications for treatment. However, key questions remain about the oncological effectiveness of intraperitoneal chemotherapy administered in this way, and in trials assessing this type of outcome, the inclusion criteria and procedure may need to be more standardised.

Whilst surgery is possible in very frail individuals, the requirement to undergo repeated laparoscopy under general anaesthetic in order to have ePIPAC precludes treatment of patients with a poor performance status. The median PCI was 10, and the median volume of ascites drained was 0ml. The procedure has been described in patients with large-volume ascites, however complications such as cardiovascular decompensation and acute kidney injury have been reported where there has been rapid drainage of significant volumes [118]. Giger-Pabst and Tempfer suggest that this can be ameliorated by monitoring such patients in an 'intermediate care unit' for a period of 12-24 hours [118]. This clearly adds a significant additional cost to a course of treatment and the cost-benefit analysis of PIPAC overall is something that has yet to be determined. Given the pressure on beds in higher care settings in NHS centres, even prior to the current situation with Covid-19, this would require careful consideration and would probably not be feasible.

The procedures in this pilot required more laparoscopic ports than those in reports from elsewhere. This is partly explained by the use of the ePIPAC technique, rather than PIPAC. This is an important consideration since each trocar represents a possible site of aerosol leak, which could result in ingress of the cytotoxic drug into the abdominal wall, or contamination of the theatre environment. However, no such complications occurred. The use of two ports in addition to the camera port facilitates safe and thorough inspection of the abdomen by allowing two grasping instruments, and also a two-instrument technique for taking biopsies and removing ascites. These benefits were thought to outweigh the risks of the additional port. ePIPAC was used because of the advantages it confers in terms of the potential for aerosol leak as the abdomen is evacuated, as it helps to ensure that all the aerosol particles are deposited prior to evacuation of the gas from the abdomen. There is also a potential clinical benefit from increasing the deposition of the drug, particularly on the anterior/upper surface of the pneumoperitoneum, however these effects have not been proven in clinical trials yet.

Overall, the procedures were performed safely for both staff and patients. This is important step in determining that a trial involving the technique is feasible at this centre. Training was completed for the group of staff involved. The safety checklist

recommended by the PIPAC training course and used in other centres was employed, and local chemotherapy handling procedures were followed. No leakages or spillages of chemotherapy occurred. The Y connector used to facilitate a dual syringe consecutive injection of Cisplatin and Doxorubicin has been identified as a high-risk site for leakage. Hubner et al reported that this occurred in 5 of the cases they had performed [120]. The connector is enclosed in a plastic sheath, so the risk to staff was deemed low. However, if a significant volume of the drug solution is lost then this type of leak could have an impact on the dose of drug that can be administered to the patient, and therefore the efficacy of the procedure. An alternative to the Y connector is to perform two sequential injections, with one member of staff re-entering the theatre to change the syringe. Given that an aerosol leak has never occurred, this may be a better solution.

There were higher rates of complications in this pilot than reported elsewhere in the literature. All the patients had mild pain and/or nausea following treatment. This was generally managed effectively with oral analgesia and resolved in a timely manner. There were 3 cases where patients suffered side effects that were classed as moderate. These were thought to be attributable to the drug component of the treatment rather than the surgical aspect of it. There are additional serious complications relating to the drugs that have been reported in the literature. Siebert et al reported that 3% patients in their cohort of 132 patients experienced severe hypersensitivity, including anaphylaxis, in response to the platinum-based chemotherapy drugs [168]. The reactions were confirmed as cisplatin or oxaliplatin allergy using skin-prick testing and precluded further use of platinum-based drugs in the affected patients. It is worth noting that all had received platinum-based systemic chemotherapy in the past. An emergency drug box was prepared and kept in theatre during cases for this reason but was not required. The incidence of these types of complications as further data becomes available will be important information for the consent process. It is also important to consider this information when the patient pathways in future trials are designed.

Overall, the pilot demonstrates that ePIPAC treatment is acceptable to patients and staff in an NHS setting. The procedures were carried out in line with other European

centres and the results in terms of the technical and safety outcomes assessed were comparable. The small number of cases performed and the heterogeneity of the patients involved makes it difficult to comment on the effectiveness of the treatment. As already discussed, the existing literature suggests that PIPAC has an acceptable safety profile. However, the efficacy has not been formally investigated in controlled studies. We notified the NICE interventional procedures committee during the planning of the PIPAC pilot, and it has recently published its recommendation that further cases in the UK should only take place in the context of an RCT [191]. The pilot has enabled staff in Cardiff to become familiar with the procedure and has demonstrated that participation in a trial involving ePIPAC or PIPAC would be feasible at this centre and could be delivered safely. The next step should be the development of a UK-based trial or the identification of an international collaborative group to join in order to enable further experience of the technique in line with the NICE recommendation.

5 Investigation of Pressurised Intraperitoneal Aerosol Therapeutics for Peritoneal Metastases

5.1 Introduction

Malignant cells from an intra-abdominal primary tumour may invade the serosal lining of the abdominal cavity causing peritoneal metastases. Peritoneal disease is a feature of many cancers and is therefore common. The cancer surveillance statistics in the UK do not record the incidence of specific metastatic locations so it is not possible to define the overall burden of disease, but the incidence in individual cancer types has been estimated. For example, it is thought that 4% to 5% of patients presenting with colorectal cancer will have synchronous peritoneal metastases [17, 44]. In addition, 20-40% of patients treated with curative intent will relapse, and around 20% of these will have peritoneal disease [17, 41]. Peritoneal metastases are more common in cancers that present late. Ovarian cancer is often diagnosed at a late stage, and peritoneal metastases are present at diagnosis in 50-80% of patients, and are more frequent in recurrent disease [56].

It can be seen by these two examples, that peritoneal metastases represent a significant burden of disease. Unfortunately, the prognosis is generally poor. The reported 5-year survival rate for patients with isolated peritoneal metastases from colorectal cancer varies. In patients treated with systemic chemotherapy figures range from 0-19% [169]. A number of studies have shown that patients with isolated peritoneal metastases have poorer survival outcomes than patients who have isolated metastases in solid organs, such as the liver or lungs, if both groups are treated with systemic chemotherapy [19]. In patients with stage 3 ovarian cancer (spread to the abdominal cavity) the average 5 year survival rate has been reported as 39% [169]. Peritoneal metastases represent an area of unmet clinical need, and new treatment options are needed.

PIPAC is a recent innovation for the treatment of peritoneal disease in advanced malignancy [64]. During laparoscopic surgery, the abdominal cavity is insufflated with carbon dioxide, creating a pneumoperitoneum, to allow the surgeon space to work. PIPAC involves aerosolising chemotherapy solutions into the abdominal cavity once the pneumoperitoneum is established using a specially designed laparoscopic nebuliser. The aerosol allowed to circulate in the pneumoperitoneum, which is

maintained for 30 minutes. The hypothesis behind PIPAC is that the pressure required to maintain the pneumoperitoneum increases absorption of chemotherapy drugs from the abdominal cavity because it offsets the effects of interstitial pressure in the tissues. Pre-clinical studies in animal models have demonstrated that the distribution of solutions delivered using PIPAC is extensive and may be superior to simple lavage of the cavity [1]. *In vitro* testing using human peritoneum demonstrated increased depth of penetration of a fluorescent labelled therapeutic agent applied using PIPAC compared to the same agent applied using lavage [65]. The technique has been used extensively to deliver chemotherapy in phase I/II trials, and the administration of advanced therapeutics is now starting to be assessed *in vitro* and *in vivo* [170]. Investigation of the delivery of nanoparticles [171] and messenger RNA [7] have already been reported. The technique represents a potential delivery method for other novel therapeutics, including oncolytic viruses. This work will focus on adenovirus vectors.

Adenoviruses have long been identified as having the potential to be effective anti-cancer agents. The structure and biology of human Ad5 has been well described and understood [104]. Like other adenoviruses, they are non-enveloped, with a dsDNA genome contained within an icosahedral capsid. They are obligate intracellular parasites and can infect both dividing and non-dividing cells. They are lytic viruses, causing destruction of the host cell on infection and release of progeny viruses into the tissue. The therapeutic effect of an oncolytic virus can be amplified by this mechanism, since further cell killing by progeny takes place in addition to the cell killing from the initial infection of tumour cells. Furthermore, it has been shown that the Ad5 genome can be manipulated by using simple recombinant DNA techniques, and high levels of expression of foreign DNA inserts have been demonstrated [104]. It has been demonstrated that 1.8 to 2.0kb of excess DNA can be inserted into the Ad5 genome to create a recombinant vector. Larger inserts can be accommodated if a corresponding section of the viral genome is deleted. High yields of virus can be generated and collected by infecting a permissive cell line *in vitro* and harvesting and purifying the lysate [104]. As a result of these properties, Ad5 has been a popular vector choice for groups developing biological therapies [97].

However, there are still limitations to be overcome. One problem with Ad5 vectors is that existing immunity is a common because of high seroprevalence rates across the world. Approximately 30% of adults in the UK are immune, but this rises to close to 90% in parts of Sub-Saharan Africa [106, 107]. This means that vectors may be neutralised by pre-existing antibodies before they reach their target tissue. Ad5 has also been demonstrated to form complexes with coagulation factor X *in vivo* in order to enter cells using heparin sulphate proteoglycans, which are abundant on hepatocytes, as well as other cell types [109]. This results in rapid sequestration of systemically administered Ad5 vectors in the liver and may impact uptake in other off-target tissues following other routes of delivery.

As discussed already, one way to overcome this sequestration is to alter the virus so that it is no longer capable of interaction with blood proteins and its usual receptors. This can be achieved by introducing variations or modifications into the structural proteins of the virus; the penton base, hexon, fiber, and knob domains. Modifications to these structural proteins can also result in targeted binding to non-native receptors. The vector developed and described by Uusi-Kerttula et al [172, 173] uses these strategies. Ad5 $_{NULL}$A20 is an Ad5 vector that has had all native tropisms ablated and has been retargeted to the tumour-selective integrin αvβ6 through incorporation of an αvβ6-binding peptide (A20, NAVPNLRGDLQVLAQKVART) within the fiber knob domain HI loop. This vector has been shown to specifically infect epithelial ovarian cancer cells *in vitro [172]* and *in vivo* [112].

Another way of overcoming virus neutralisation is to administer the vector to the target tissue directly. For example, herpes simplextype1–based talimogene laherparepvec (T-VEC), an approved oncolytic immunotherapy approved for advanced melanoma, is injected directly into the tumour in order to maximise entry into cancer cells [95]. The PIPAC technique potentially represents an effective delivery method for oncolytic viruses to the abdominal cavity in peritoneal disease. Intraperitoneal delivery might circumvent some of the obstacles associated with systemic delivery, such as interactions with blood proteins and circulating antibodies. Ascites can be drained as part of the operation, and this may reduce the effect of

neutralising antibodies that are present in the ascites of patients who have had previous exposure and therefore acquired immunity to Ad5 [116].

The aim of the experiments described in this chapter was to assess the use of the aerosolization technique as a method for the delivery of oncolytic adenovirus therapies to treat peritoneal metastases.

A key hypothesis underlying the PIPAC technique is that the pressure generated by the insufflation of gas to maintain the pneumoperitoneum increases the efficacy of the drugs administered. One explanation speculated for this is that the pressure increases absorption of the drug and that this increases cancer cell killing. This was assessed *in vitro* using a pressurised chamber.

The feasibility of intraperitoneal aerosolization as a delivery method for oncolytic virotherapies has not been assessed before, so work was carried out to determine whether adenoviruses can survive aerosolization and go on to transduce their target cells. This was assessed *in vitro* using adenovirus serotype 5 (Ad5) vectors. Following *in vitro* tests, a collaboration was established with a group at the University of Ghent in Belgium to test aerosolization of an adenovirus vector *in vivo* in a rat model to assess the safety and feasibility of this approach.

The oncolytic Ad5 vectors that have been generated by the group have had their native receptor tropism ablated and have been re-targeted to a more tumour-specific peptide, αvβ6 integrin. The expression of αvβ6 in cancer cell lines from tumour types that exhibit peritoneal spread has been assessed to further determine the potential utility of oncolytic Ad5 therapy delivered by the intraperitoneal route.

5.2 Assessing the response of ovarian cancer cell lines to a cytotoxic drug *in vitro*

The sensitivity of ovarian cancer cells to the cytotoxic drug Cisplatin was assessed *in vitro*. Two pairs of human ovarian cancer cell lines were used in these experiments. These were chosen as peritoneal metastases are a common occurrence in ovarian cancer and thus are a target for intraperitoneal treatments.

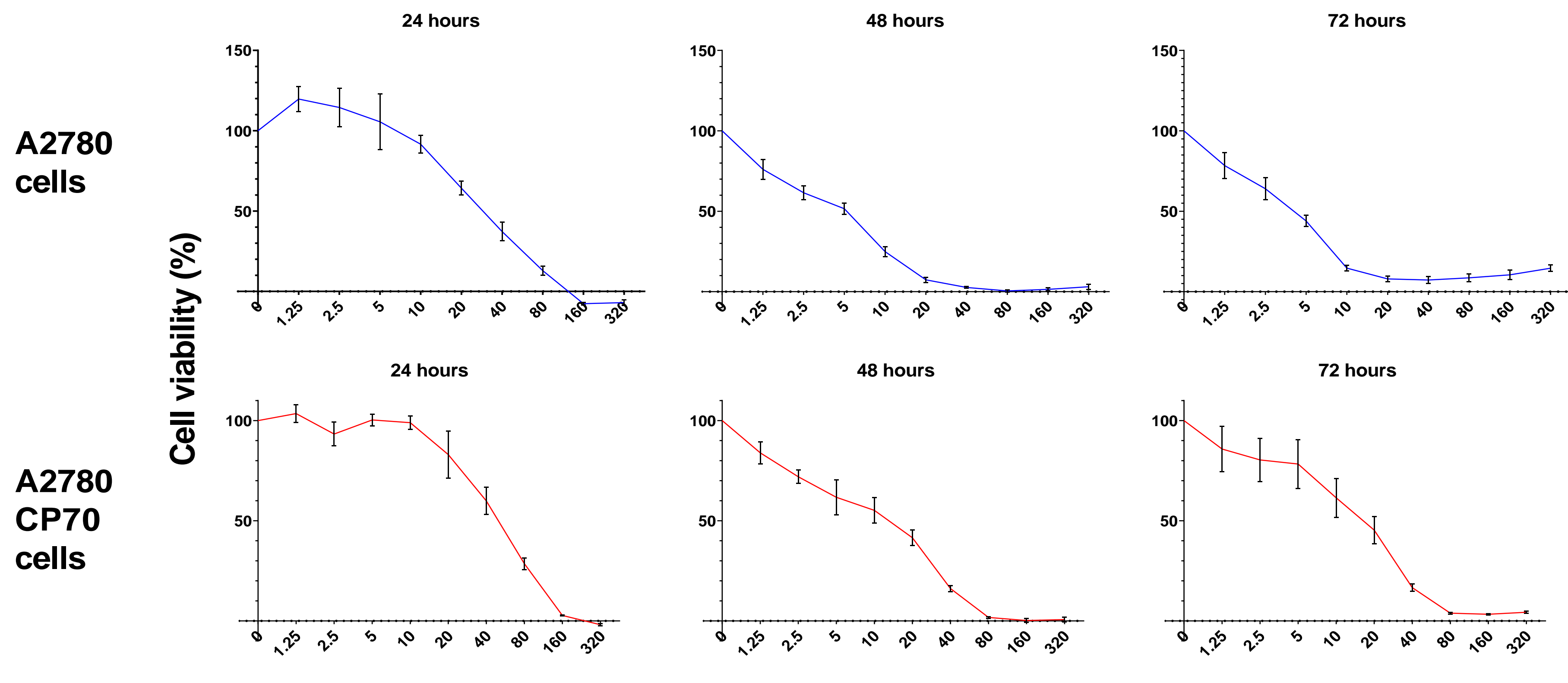
As previously described, A2780 is an ovarian cancer cell line that was established from tissue from an ovarian endometroid adenocarcinoma tumour in an untreated patient. The A2780/cp70 subline is cisplatin-resistant and was generated *in vitro* by intermittent exposure to increasing doses of cisplatin [26]. The PEO cell lines were derived from the peritoneal ascites of a patient with a poorly differentiated ovarian serous adenocarcinoma. The PEO1 cell line was harvested after treatment with cisplatin, 5-fluorouracil and chlorambucil had started and the PEO4 cell line was harvested after the patient developed clinical resistance to chemotherapy [121].

5.2.1 Response to cisplatin with increasing duration of exposure

The sensitivity of the cells to Cisplatin was assessed *in vitro* using a colorimetric assay to determine the viability of cells after exposure to the drug. The first set of experiments assessed the response to cisplatin with increasing duration of exposure. The cisplatin was added to cells in 96 well plates at different concentrations. Each concentration, and the control with complete medium only, was tested in triplicate. The MTS cell viability assay *(CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega)* was carried out at 24, 48, and 72 hours and the optical density was measured after an incubation of 2 hours. The cell viability in each treated well was determined by expressing the optical density as a percentage of the mean optical density of the three untreated control wells on each plate.

Figure 5-1: Assessing the response of ovarian cancer cell lines to Cisplatin with increasing duration of exposure in vitro. Figure 5-1 shows graphs for each cell line displaying the mean of the three repeats for each cisplatin concentration at each time point. At 24 hours, the cell viability determined in the wells treated with the lower cisplatin concentrations was greater than in the control wells in the A2780 and A2780/CP70 cell lines, and the mean percentage cell viability increased above 100%. This effect was seen even with high concentrations of cisplatin in the PEO1 and PEO4 cells. At 48 hours the cell viability recorded in the treated wells has fallen below that in the control wells in all cell lines as all the mean percentage values are below 100%. At 72 hours, the cell viability in the treated wells is still below that in the control wells,

as the mean percentage values remain below 100%, however, there is an increase compared to 48 hours in all but two cisplatin concentrations in the A2780 cell line (5µM and 10µM) and two cisplatin concentrations in the PEO1 cell line (20µM and 40µM). As a result of this pattern, the time point selected for further cisplatin sensitivity experiments was 48 hours.


A2780
cells
A2780
CP70
cells
Cell viability (%)
24 hours
48 hours
72 hours
150
100
50
0
1.25
2.5
5
10
20
40
80
160
320
Cisplatin concentration (micromolar)

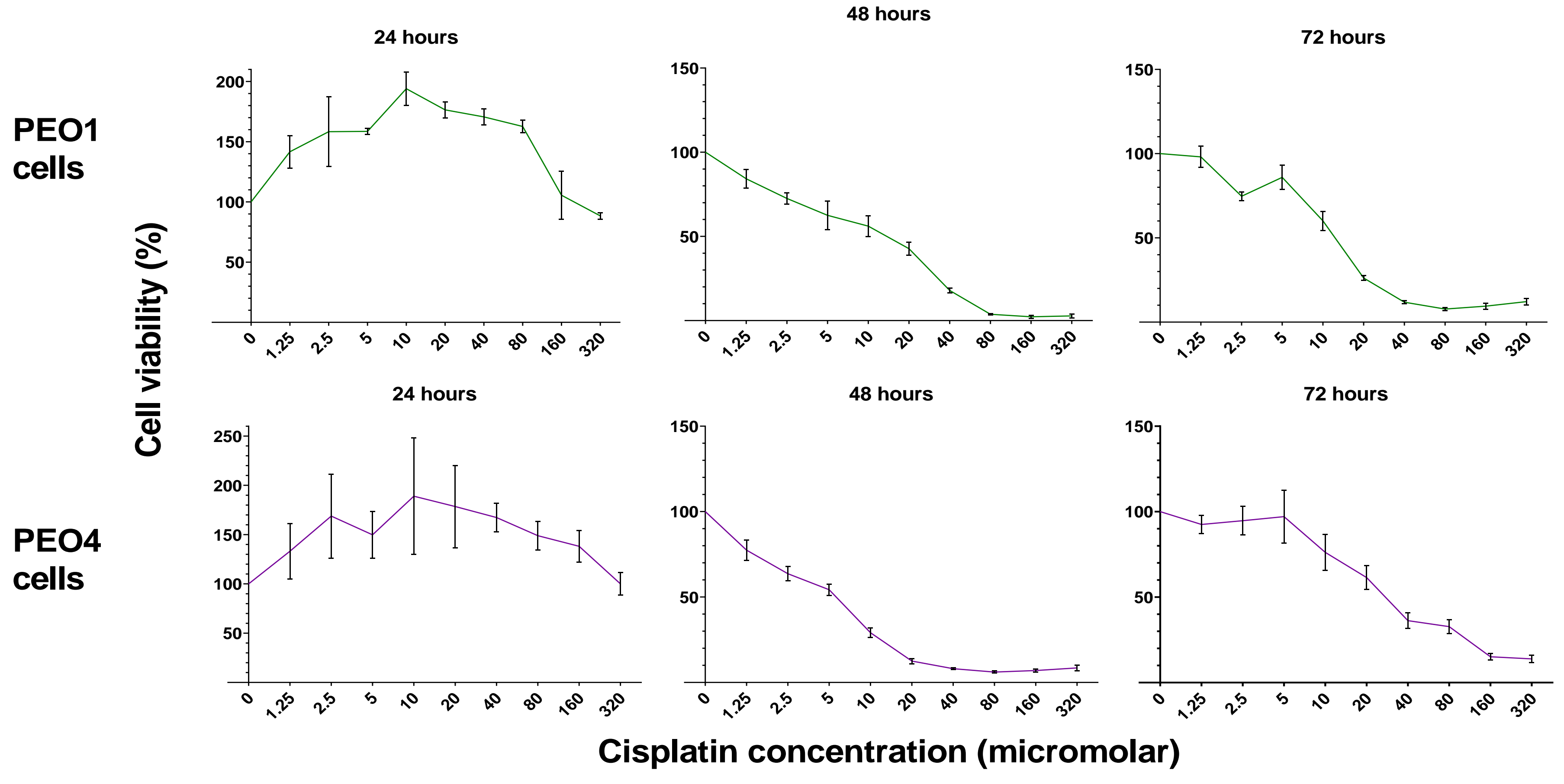


Figure 5-1: Assessing the response of ovarian cancer cell lines to Cisplatin with increasing duration of exposure in vitro.

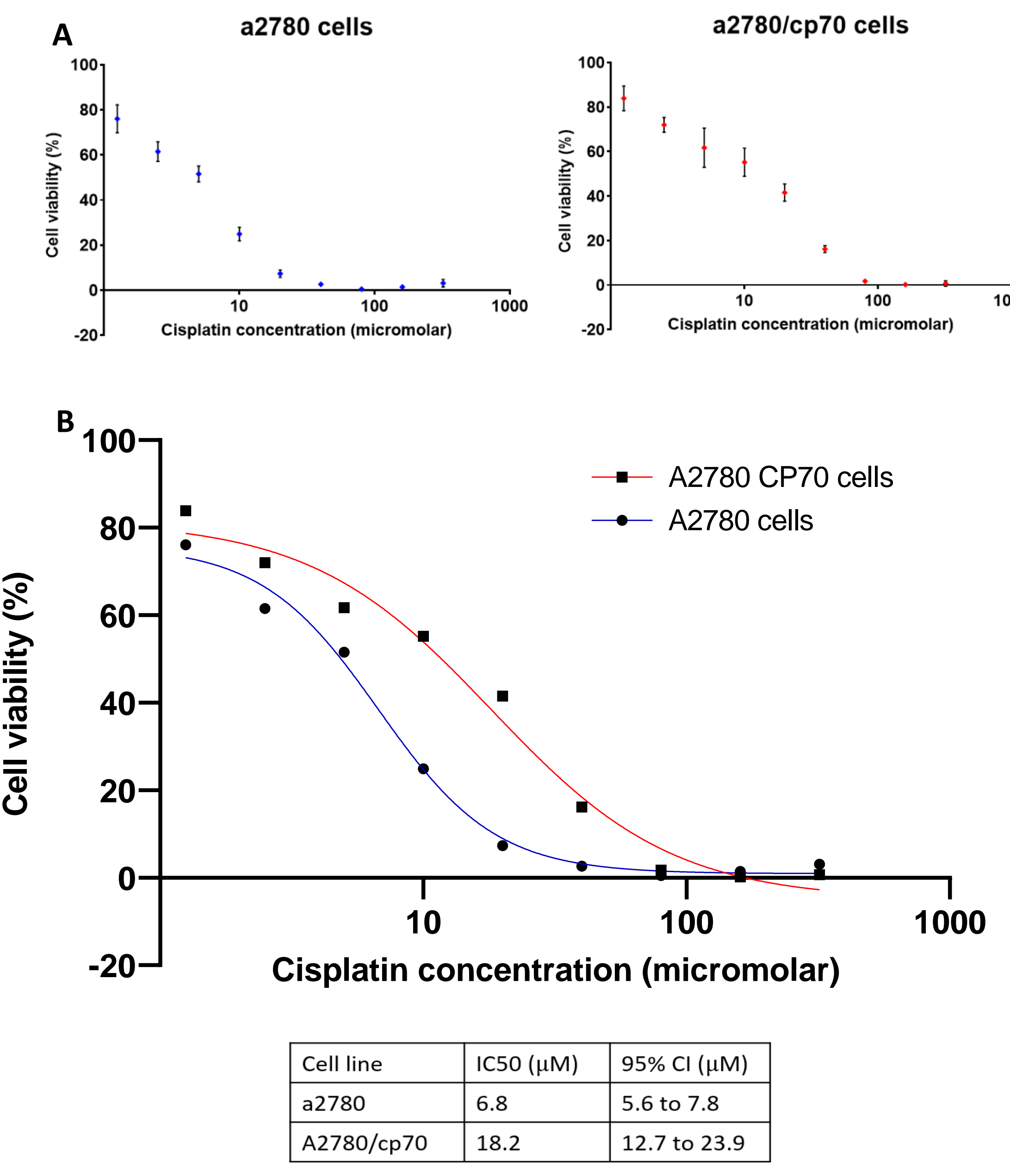
Cells were plated in 96 well plates at a density of 20,000 cells per well in complete medium (depending on cell type) and incubated for 12 hours. The medium was then replaced with complete medium containing cisplatin at concentrations ranging from 0μM to 320 μM. Each concentration was tested in triplicate (n=3). The plates were incubated at 37°C and 5% CO2. Cell viability was assessed at 24 hours, 48 hours, and 72 hours using an MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega) as per the manufacturers protocol. The optical density of each well was measured at 2 hours using a plate reader and readings were corrected for background. The cell viability in each cisplatin-treated well was determined by expressing the optical density as a percentage of the mean optical density of three untreated control wells. The mean cell viability and SD at each cisplatin concentration (μM) for each time point is displayed.

5.2.2 Dose-response of ovarian cancer cell lines to Cisplatin *in vitro*

Dose-response curves from the experiments analysed at 48 hours are displayed in Figure 5-2 and Figure 5-3. Non-linear regression was used to determine the IC_{50} for Cisplatin at 48 hours in each cell line (GraphPad Prism v9.0).

Behrens et al quote an IC_{50} of 1.1µM in their initial description of the A2780-derived cell lines and noted that there was a right-shift in the dose-response curve in the resistant cell lines [26]. The results presented in Figure 5-2 follow this pattern, although the IC_{50} for these cells in this experiment is higher. The A2780/CP70 cells are less sensitive to cisplatin than the A2780 cells, and the 95% confidence intervals (CI) of the IC_{50} for each cell line do not overlap.

In the PEO cell lines (Figure 5-3), the IC_{50} for the PEO1 cells was 18.2µM, and for the PEO4 cells it was 6.8µM. Again, the 95% CIs for these values did not overlap. The results were unexpected since the PEO4 cell line should be more resistant to cisplatin than the PEO1 cell line. This experiment was carried out in triplicate and was repeated but the results were unchanged. One possible explanation for this is that human error has resulted in mislabelling of the cells at some stage. The PEO1 and PEO4 cells were kindly gifted by Dr James Cronin (Swansea University Medical School and they had observed the same pattern of Cisplatin resistance (Personal communication). A second explanation is that there has been drift in the cell lines over time, and their properties with relation to cisplatin resistance. However, both the PEO1 and PEO4 cell lines appeared to exhibit greater resistance to cisplatin than the A2780 cell lines, and therefore were taken forward to the assessment of whether pressure increases the Cisplatin sensitivity of the cells.



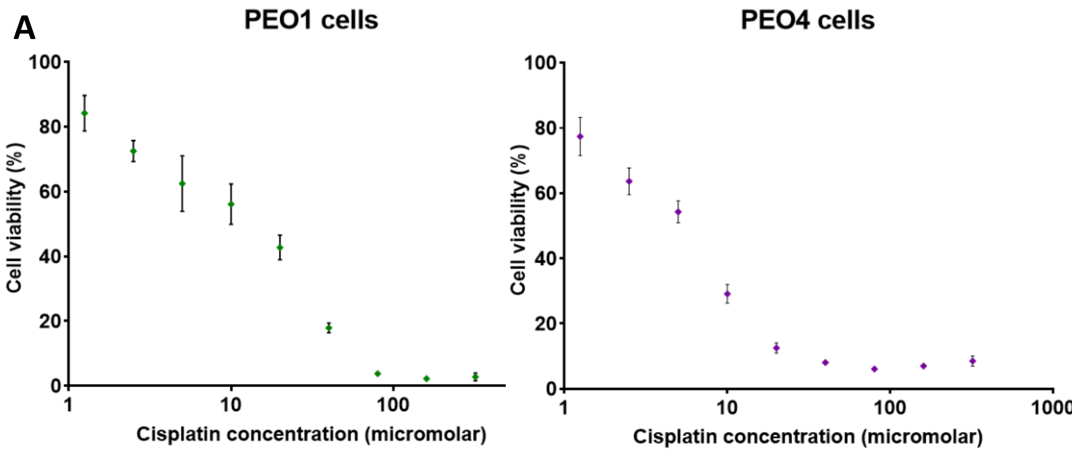
Cell line	IC50 (μM)	95% CI (μM)
a2780	6.8	5.6 to 7.8
A2780/cp70	18.2	12.7 to 23.9

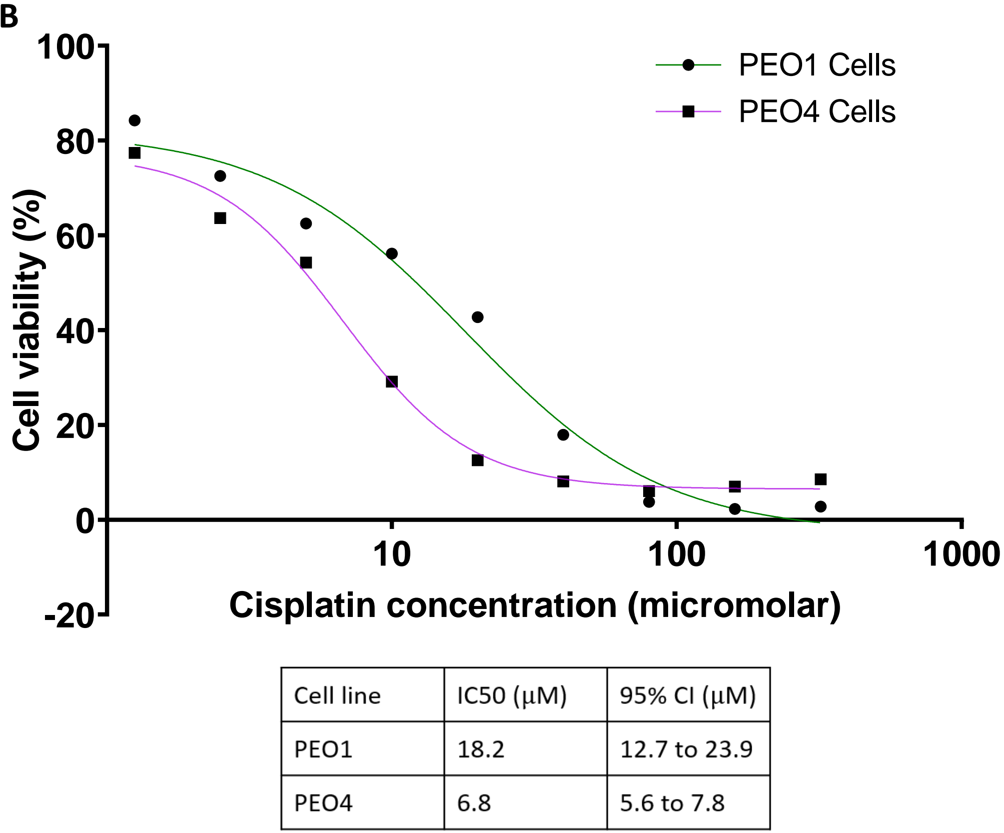
Figure 5-2 The response of A2780 cells and A2780/CP70 cells to Cisplatin in vitro

Cells were plated in 96 well plates at a density of 20,000 cells per well in complete medium (RPMI + L-Glut and 10% FBS) and incubated for 12 hours. The medium was then replaced with complete medium containing cisplatin at concentrations ranging from 0μM to 320 μM. Each concentration was tested in triplicate. The plates were incubated at 37°C and 5% CO2. Cell viability was assessed at 48 hours using an MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega). The optical density of each well was measured using a plate reader and readings were corrected for background. The was determined using non-linear regression

A: Cell viability of A2780 and A2780 CP70 cells after treatment with increasing dose of Cisplatin. The cell viability in each cisplatin-treated well was determined by expressing the optical density as a percentage of the mean optical density of three untreated control wells.

B: Dose response curves and IC_{50} values for A2780 and A2780 CP70 cells determined by non-linear regression (GraphPad Prism).





Cell line	IC50 (μM)	95% CI (μM)
PEO1	18.2	12.7 to 23.9
PEO4	6.8	5.6 to 7.8

Figure 5-3 The response of PEO1 and PEO4 cells to cisplatin in vitro.

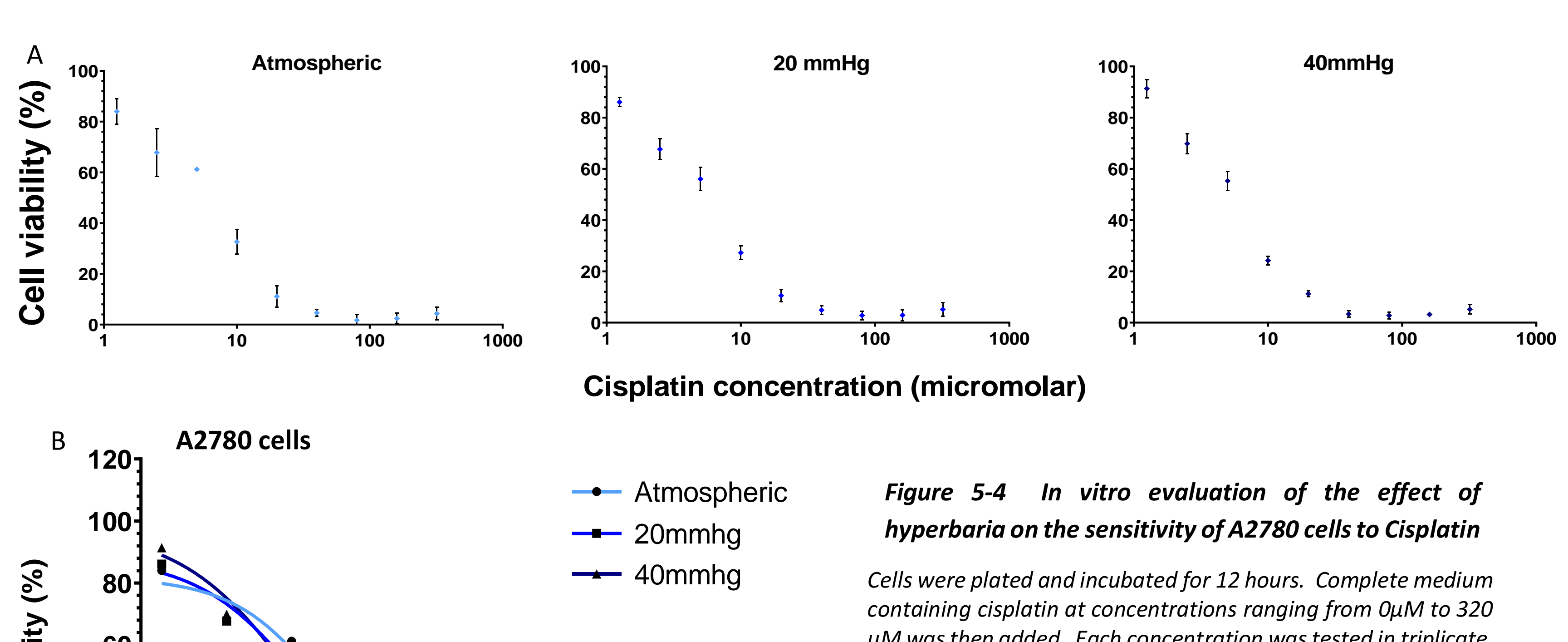
Cells were plated in 96 well plates at a density of 20,000 cells per well in complete medium (RPMI + L-Glut and 10% FBS) and incubated for 12 hours. The medium was then replaced with complete medium containing cisplatin at concentrations ranging from 0μM to 320 μM. Each concentration was tested in triplicate. The plates were incubated at 37°C and 5% CO2. Cell viability was assessed at 48 hours using using an MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega). The optical density of each well was measured using a plate reader and readings were corrected for background.

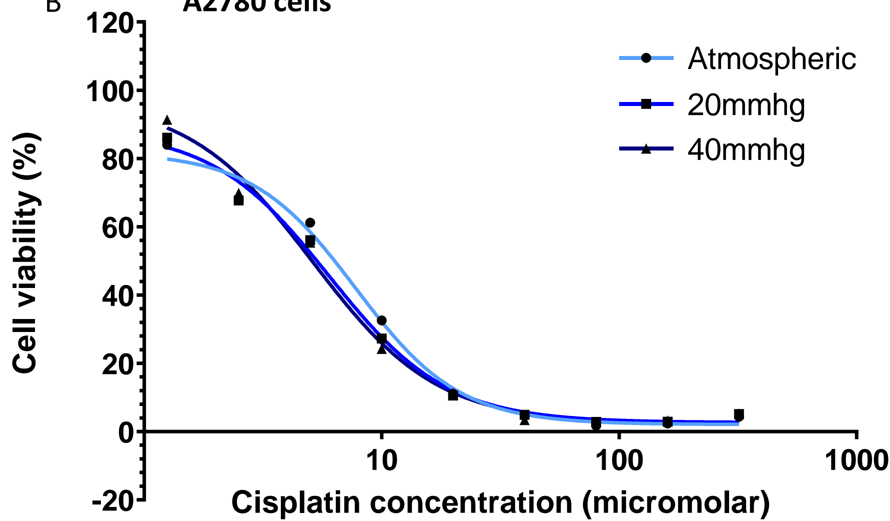
A: Cell viability of PEO1 and PEO4 cells after treatment with increasing dose of Cisplatin. The cell viability in each cisplatin-treated well was determined by expressing the optical density as a percentage of the mean optical density of three untreated control wells.

B: Dose response curves and IC_{50} values for PEO1 and PEO4 cells determined by non-linear regression (GraphPad Prism).

5.2.3 Effect of hyperbaria on the response of ovarian cancer cells to Cisplatin *in vitro*

Apparatus was developed to assess the effect of pressure on the response of the ovarian cancer cells to Cisplatin *in vitro*. In the A2780 and A2780/CP70 cells, the IC_{50} values determined in these experiments were comparable with the initial dose-response analyses performed (Figure 5-4 and Figure 5-5). In the A2780 cell line, there was a trend towards the IC_{50} decreasing with increasing pressure. There was no overlap between the 95% CIs for the IC_{50} at atmospheric pressure and the IC_{50} at 40mmHg. There was overlap between the 95% CI for the IC_{50} at atmospheric pressure and the 95% CI at 20mmHg, the pressure most analogous to the intra-abdominal conditions during laparoscopic surgery. This pattern of response was not observed in the A2780/CP70 cells, and there was overlap between the 95% CIs for the IC_{50} of all three pressures. Similarly, in the PEO1 and PEO4 cells, the 95% CIs for the IC_{50} overlapped in all three pressures tested and there was no obvious correlation between pressure and cisplatin sensitivity (Figure 5-6 and Figure 5-7). Thus, in the experimental model tested here, the pressure generally used during laparoscopic surgery in clinical practice (12-15mmHg) did not have any significant impact on the response of the cells to cisplatin in monolayer culture. Further work to assess the effect of pressure on the delivery of drug to a 3D cell culture or tissue model would be of interest, since pressure may have an effect on the depth of penetration through tissue.





	Atmospheric	20mmHg	40mmHg
IC50	7.779	6.012	5.100
95% CI	6.238 to 9.218	4.850 to 7.049	4.013 to 6.041

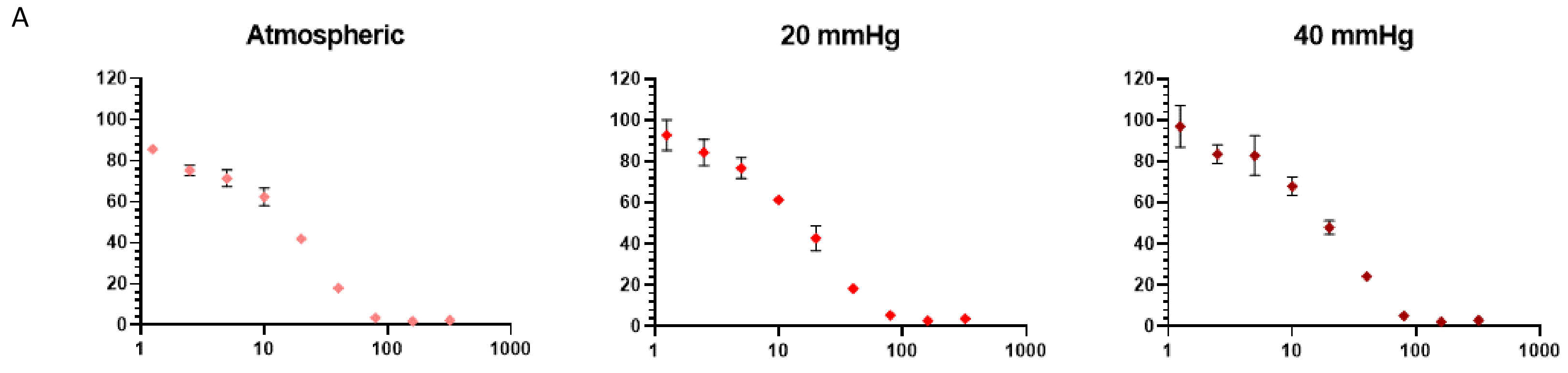
Figure 5-4 In vitro evaluation of the effect of hyperbaria on the sensitivity of A2780 cells to Cisplatin

Cells were plated and incubated for 12 hours. Complete medium containing cisplatin at concentrations ranging from 0µM to 320 µM was then added. Each concentration was tested in triplicate. The plates were placed in the hyperbaric apparatus and the pressure was maintained for 30 minutes. Cell viability was assessed at 48 hours using an MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega). The optical density of each well was corrected for background absorbance. The cell viability in each cisplatin-treated well was determined by expressing the optical density as a percentage of the mean optical density of three untreated control wells.

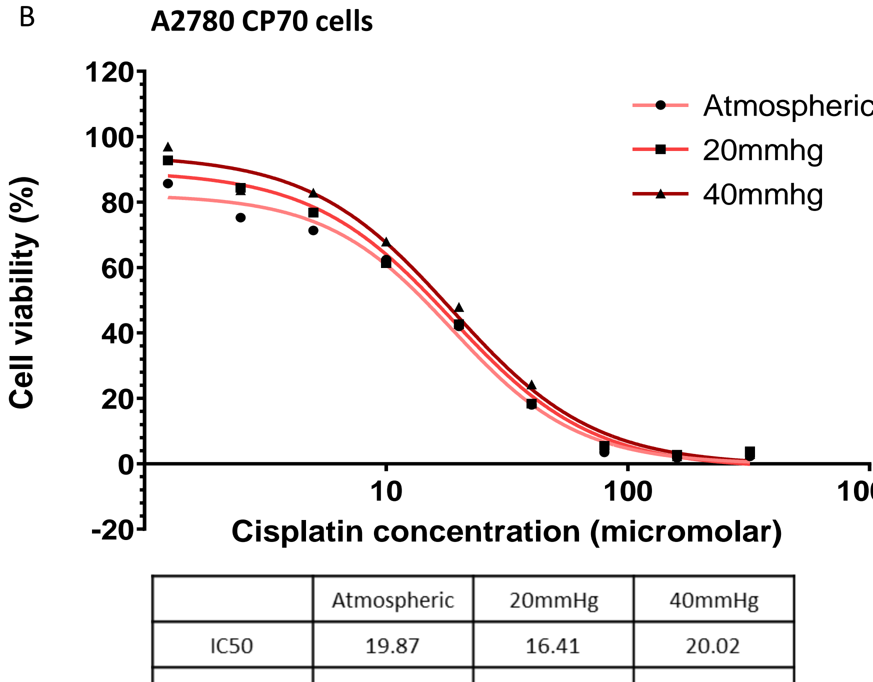
A: Graphs to show the mean cell viability and SD at each cisplatin concentration (µM) for each pressure condition, n=3

B: Dose-response curves for each pressure condition. The IC_{50} *was calculated using linear regression (GraphPad Prism).*

A



B



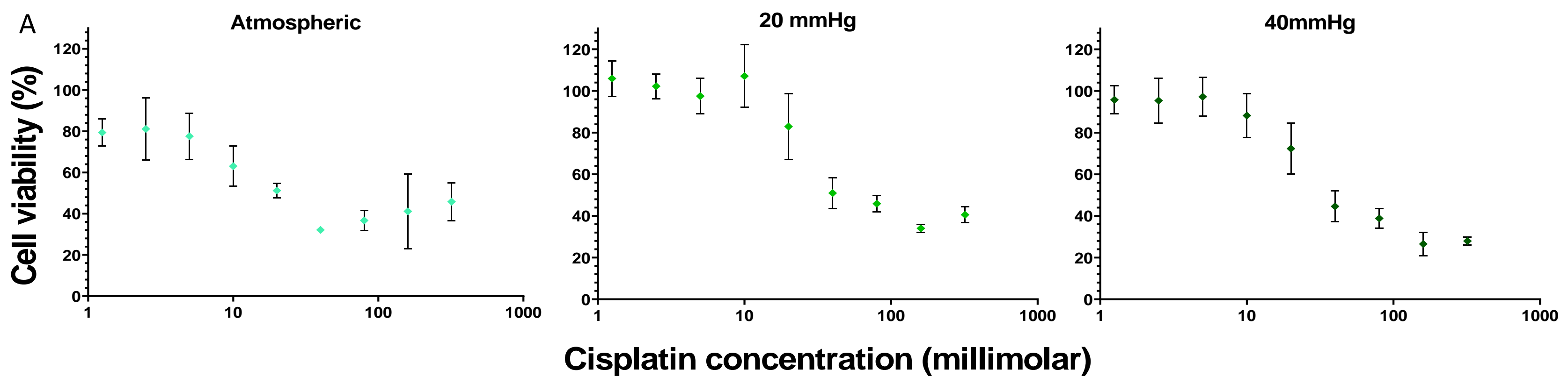
	Atmospheric	20mmHg	40mmHg
IC50	19.87	16.41	20.02
95% CI	17.45 to 22.48	13.71 to 19.34	16.25 to 24.33

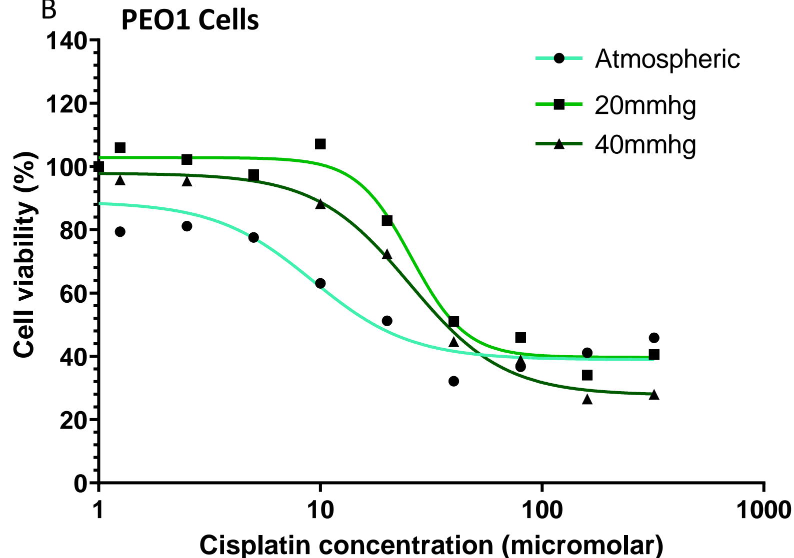
Figure 5-5 In vitro evaluation of the effect of hyperbaria on the sensitivity of A2780 CP70 cells to Cisplatin

Cells were plated and incubated for 12 hours. Complete medium containing cisplatin at concentrations ranging from 0µM to 320 µM was then added. Each concentration was tested in triplicate. The plates were placed in the hyperbaric apparatus and the pressure was maintained for 30 minutes. Cell viability was assessed at 48 hours using an MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega). The optical density of each well was corrected for background absorbance. The cell viability in each cisplatin-treated well was determined by expressing the optical density as a percentage of the mean optical density of three untreated control wells.

A: Graphs to show the mean cell viability and SD at each cisplatin concentration (µM) for each pressure condition, n=3

B: Dose-response curves for each pressure condition. The IC_{50} was calculated using linear regression (GraphPad Prism).





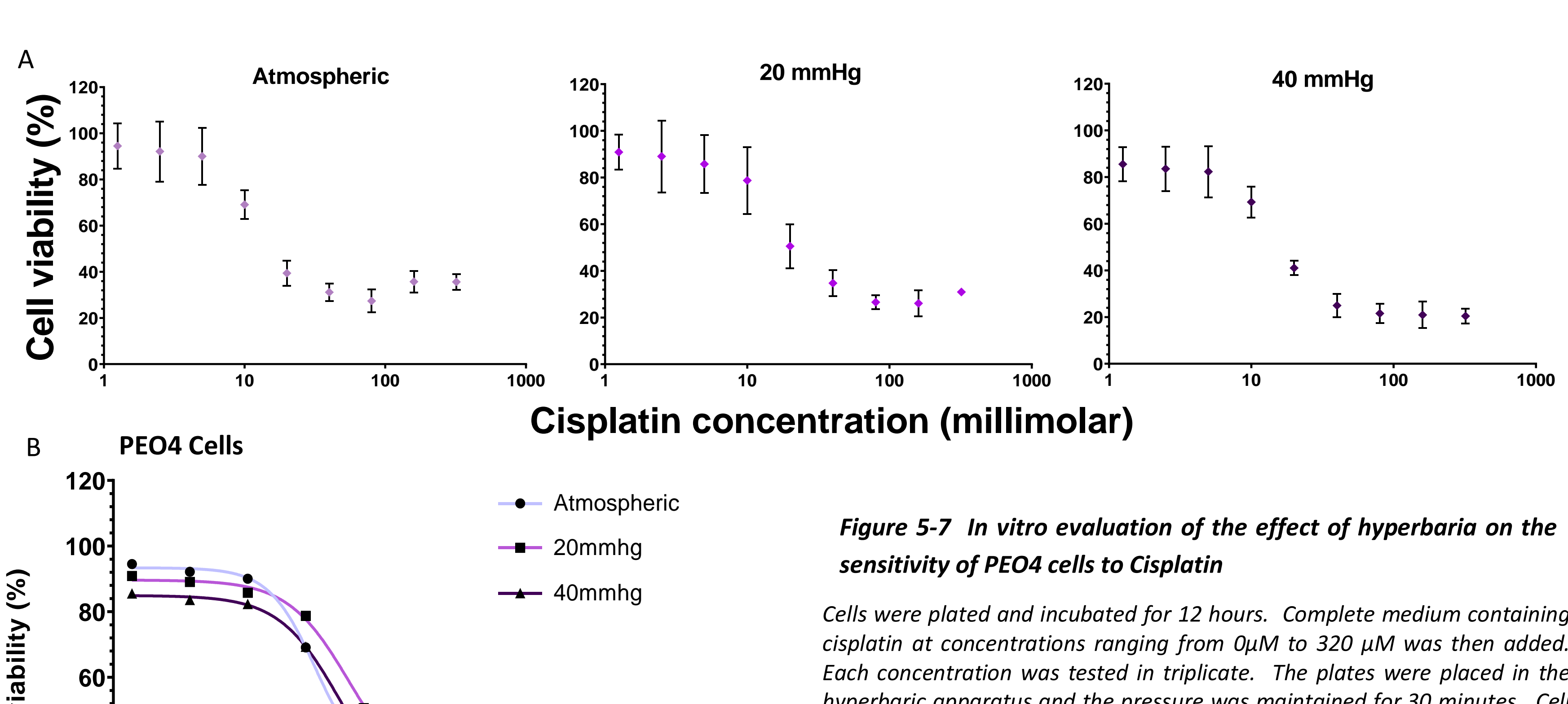
	Atmospheric	20mmHg	40mmHg
IC50	11.87	25.52	25.72
95% CI	6.089 to 20.63	19.49 to 35.21	20.53 to 32.98

Figure 5-6 In vitro evaluation of the effect of hyperbaria on the sensitivity of PEO1 cells to Cisplatin

Cells were plated and incubated for 12 hours. Complete medium containing cisplatin at concentrations ranging from 0µM to 320 µM was then added. Each concentration was tested in triplicate. The plates were placed in the hyperbaric apparatus and the pressure was maintained for 30 minutes. Cell viability was assessed at 48 hours using an MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega). The optical density of each well was corrected for background absorbance. The cell viability in each cisplatin-treated well was determined by expressing the optical density as a percentage of the mean optical density of three untreated control wells.

A: Graphs to show the mean cell viability and SD at each cisplatin concentration (µM) for each pressure condition, n=3

B: Dose-response curves for each pressure condition. The IC_{50} *was calculated using linear regression (GraphPad Prism).*



	Atmospheric	20mmHg	40mmHg
IC50	11.21	16.77	15.12
95% CI	9.288 to 13.59	12.81 to 21.75	12.70 to 17.94

Figure 5-7 In vitro evaluation of the effect of hyperbaria on the sensitivity of PEO4 cells to Cisplatin

Cells were plated and incubated for 12 hours. Complete medium containing cisplatin at concentrations ranging from 0µM to 320 µM was then added. Each concentration was tested in triplicate. The plates were placed in the hyperbaric apparatus and the pressure was maintained for 30 minutes. Cell viability was assessed at 48 hours using an MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega). The optical density of each well was corrected for background absorbance. The cell viability in each cisplatin-treated well was determined by expressing the optical density as a percentage of the mean optical density of three untreated control wells.
A: Graphs to show the mean cell viability and SD at each cisplatin concentration (µM) for each pressure condition, n=3
B: Dose-response curves for each pressure condition. The IC_{50} *was calculated using linear regression (GraphPad Prism).*

5.3 The assessment of aerosolization as a method for intraperitoneal delivery of oncolytic adenovirus vectors

The project then moved on to the assessment of the aerosolization technique to deliver adenovirus vectors.

5.3.1 Adenovirus vectors survive aerosolisation and retain their ability to transduce cells expressing their native receptor *in vitro*

Ad5 transduction can be tested in vitro using a reporter gene transduction assay. The native receptor for Species C adenoviruses, including Ad5, is Coxsackie and Adenovirus Receptor (CAR) [100]. A Chinese Hamster Ovary (CHO) cell line previously generated to overexpress human CAR (CHO CAR) cell line was used for this assay, along with CHO K1, a cell line with no expression of CAR (cell receptor expression data shown later in -A).

Ad5 expressing Green Fluorescent Protein (GFP) reporter gene (Ad5.GFP) was used in the first experiments to assess whether aerosolisation using the CapnoPen affects the ability of the vector to infect and transduce cells *in vitro*. Cells transduced by Ad5.GFP express the GFP reporter gene and fluoresce under light in the blue to ultraviolet range. A solution of Ad5.GFP in serum-free medium was aerosolised using a high-pressure injector (HPI) to push the solution through the CapnoPen device before collection for use in the assay. Flow cytometry was used to determine the level of GFP expression in cells infected with non-aerosolised Ad5.GFP compared with aerosolised Ad5.GFP. Figure 5-8 A and B show the flow cytometry data, and Figure 5-8 C compares the number of cells which expressed GFP from the non-aerosolised virus experiments versus the aerosolised virus experiments. Gating for the flow cytometry was carried out using the control cell populations, which were not exposed to virus, but maintained in complete medium. The results from the non-aerosolised virus experiments demonstrate that, as expected, there was no expression of GFP in the CHO K1 cells. The non-aerosolised Ad5 GFP was able to transduce CHO CAR cells, and expression of GFP was detected in a dose-dependent fashion. The same pattern of GFP expression was seen in the samples infected with aerosolised virus. There was

no expression of GFP in CHO K1 cells, but there was increasing expression in CHO CAR cells with increasing concentration of virus. Multiple t-tests indicated there was no significant difference in expression of GFP in cells inoculated with aerosolised virus when compared to cells inoculated with non-aerosolised virus at any viral titre (1000vp/cell p=0.47, 2500vp/cell p=0.15, 5000vp/cell p=0.46). This confirms that aerosolisation did not have any effect on the ability of the virus vector to infect and transduce the target cells and supports the hypothesis that aerosolisation is a viable delivery method for an adenovirus vector.

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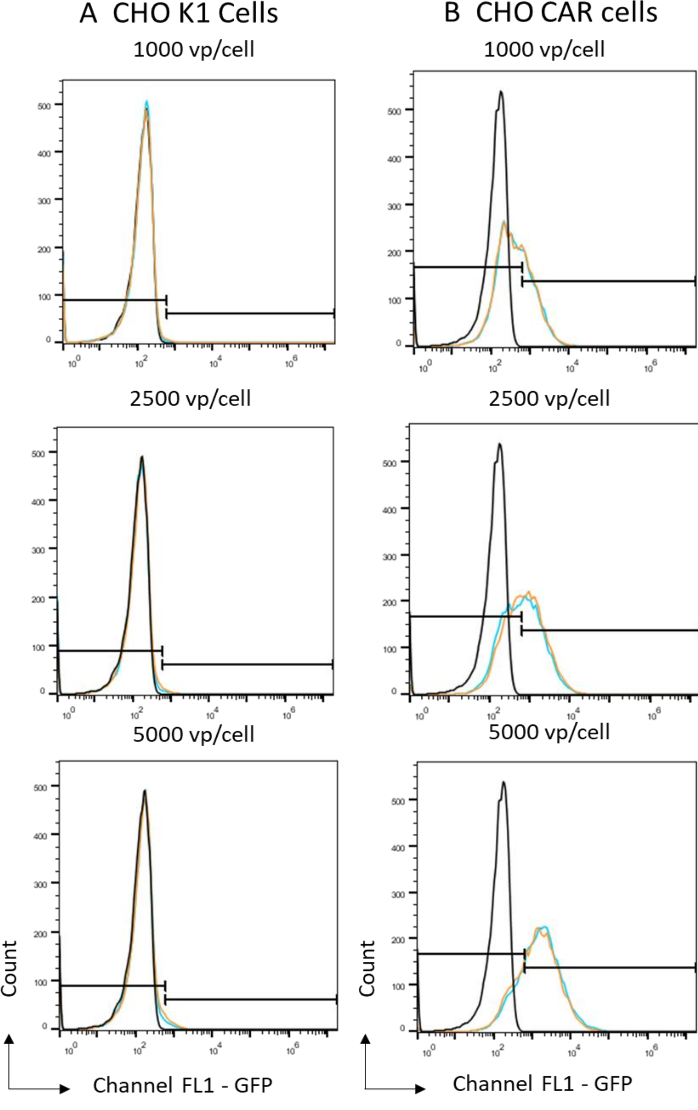


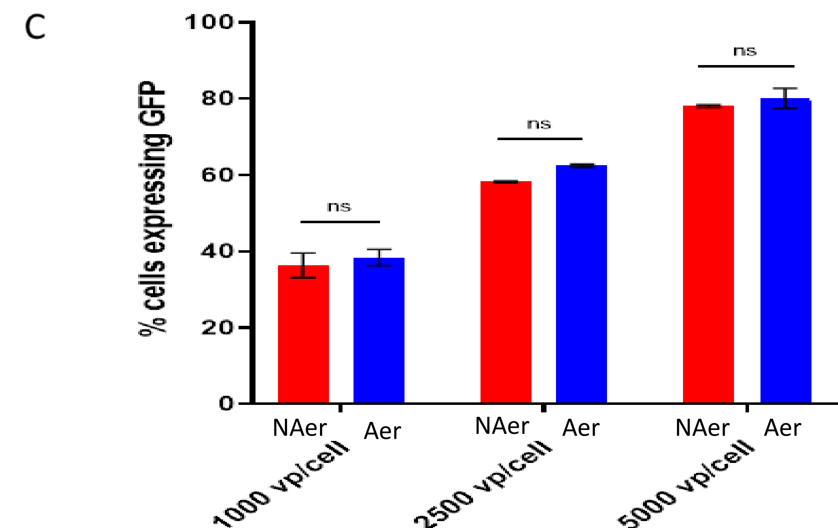


Figure 5-8: Adenovirus vectors survive aerosolisation using the CapnoPen device and retain their ability to transduce cells expressing their native receptor.

CHO K1 and CHO CAR cells were plated at 20,000 cells per well and incubated for 24 hours in complete medium. A solution of Ad5.GFP in serum-free medium was made. Half of the solution was aerosolised using the HPI connected to a CapnoPen™ aerosoliser. The other half was reserved to carry out the comparison experiments. The solutions were diluted to 5000vp/cell, 2500vp/cell, and 1000vp/cell and applied to cells in triplicate. After a 2-hour incubation the virus solution was removed and replaced with complete medium. Flow cytometry was carried out using an Accuri 6 flow cytometer after a further 48h incubation. Control samples (0vp/cell in serum free medium, in triplicate) were used to determine the baseline level of fluorescence intensity. A: virus solutions applied to CHO K1 cells; B: virus solutions applied to CHO CAR cells; C: Graph to show the number of cells expressing GFP after incubation with increasing concentrations of Ad5.GFP. Multiple t tests indicate no significant difference between the values obtained for cells incubated with virus solution which was not aerosolised prior to application (■ NAer) compared with cells incubated with a virus solution that was aerosolised (■ Aer) prior to application at any viral titre (1000vp/cell p=0.47, 2500vp/cell p=0.15, 5000vp/cell p=0.46).

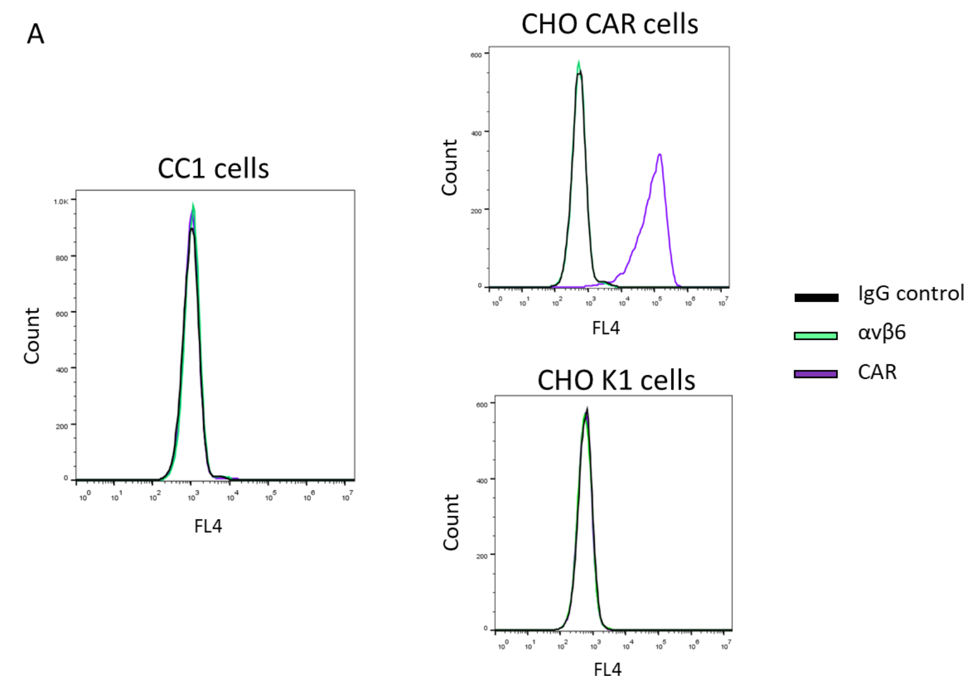
5.3.2 *In vitro* assessment of the viability of a Wistar Rat Intraperitoneal Aerosolisation Model for testing adenovirus vectors

Having assessed the effect of aerosolisation *in vitro*, the next step was to determine whether an *in vivo* model could be used to investigate further. A Wistar rat model of PIPAC had been developed by Van de Sande et al [174] at the University of Ghent. They were approached and were willing to collaborate to test adenovirus vectors in their model. A Wistar rat cell line (CC1, ECACC 93070901) was used to assess the suitability of the model for Ad5 vectors.

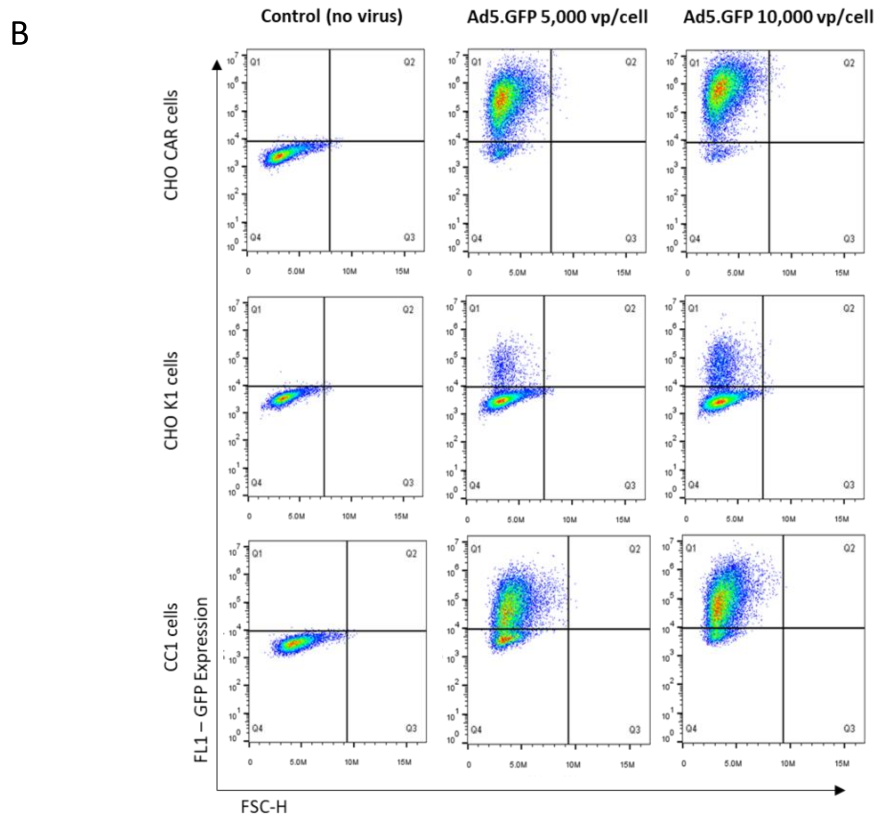
To assess whether the rat would be a suitable model for testing the effects of adenovirus vectors administered by pressurised intraperitoneal areosolisation, we assessed if a rat hepatocyte cell line could be transduced using a human adenoviral vector *in vitro*. The surface expression of receptors in the rat hepatocyte cell line CC1 was assessed using flow cytometry. The primary antibody Anti-CAR Antibody, clone RmcB was used. This is a monoclonal mouse antibody against native human CAR protein, but it is reported to have species reactivity in mice and rats as well as humans. This was to assess the potential for Ad5 vectors to enter the cells using their native receptor. The expression of αvβ6 was also assessed since this protein is the binding site for the $Ad5_{null}A20$ vector. The Anti-αvβ6 E7P6 clone was used, which is raised against human αvβ6. Figure 5-9A shows the results. Channel FL4 is displayed since the secondary antibody was an Alexa Fluor 647 conjugate. No expression of CAR or of αVβ6 was detected in CC1 cells. The results from CHO CAR cells and CHO K1 cells are also displayed, and these cells were used as positive and negative controls for CAR-mediated adenovirus transduction. CHO CAR shows high expression of CAR, whilst CHO K1 has very low/no expression. Previous work has shown that neither of these cell lines express αVβ6, and this was confirmed here.

Whilst CAR is the primary receptor for Ad5, other mechanisms of cell entry have been described. Therefore, the next experiments assessed the ability of Ad5.GFP to transduce CC1 cells. CHO CAR cells were used as a positive control and CHO K1 cells as a negative control. Expression of GFP following incubation with the Ad5.GFP was

assessed using flow cytometry (Figure 5-9B). A two-way ANOVA with Tukey's multiple comparison test was performed to compare the results between the cell lines (Figure 5-9C). The proportion of cells expressing GFP was less in the CC1 cells when compared to the CHO CAR cells at both virus concentrations tested (77% vs 97% cells at 5000vp/cell, p=<0.0001, and 88% vs 97% at 10,000vp/cell, p=<0.0001 respectively). However, the proportion of CC1 cells expressing GFP was higher than the CHO K1 cells. These experiments showed that whilst the native human receptor is not present on the CC1 cells, the human Ad.5 vector is still able to transduce them with good efficiency. The most likely explanation is that the CC1 cells do express rat CAR, but that it was not detected by the RmcB clone antibody that we used, and the virus was able to use the rat CAR to mediate cell entry. Another possibility is that because CC1 is a hepatocyte cell line, the virus was able to bind to an alternative protein or receptor, such as heparan sulphate proteoglycans, which may be expressed.



Cell line	Organ	% expression hCAR	% expression αVβ6
CHO K1	Hamster Ovary	0	0
CHO CAR	Hamster Ovary	99	0
CC1	Rat Liver	0	0



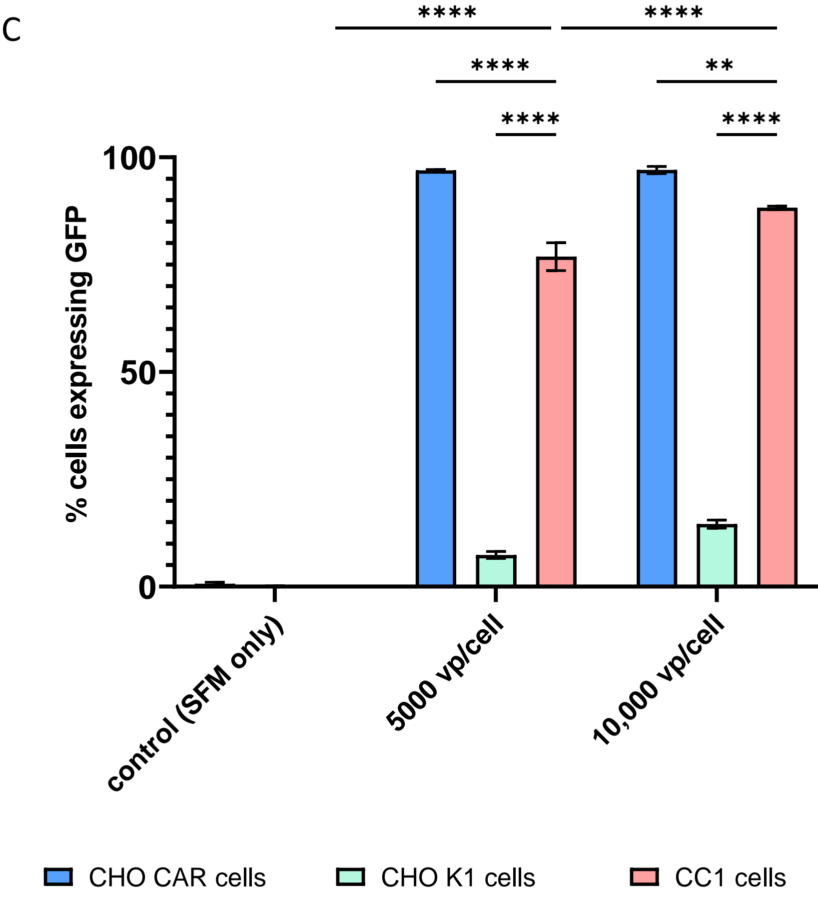


Figure 5-9: Ad5.GFP can transduce Wistar Rat hepatocytes in vitro

A: Flow cytometry to assess the surface receptor expression of CC1, CHO CAR, and CHO K1 cells. 100 000 cells were stained in triplicate with primary antibodies (anti-αvβ6 clone E7P6, anti-CAR clone RmcB) for 1 h on ice, and with secondary AlexaFluor 647 for 30 min on ice. The cells were then washed and fixed using 4% paraformaldehyde. Data were recorded for 10 000–20 000 events after gating with unstained cells using a BD Accuri C6 flow cytometer and analysed using FlowJo software. Normal IgG was used as a control for the primary antibody and the results were compared to determine the percentage of cells expressing each receptor.

B: Flow cytometry to detect GFP expression in cells infected with Ad5.GFP. CHO K1, CHO CAR and CC1 cells were plated at 20,000 cells per well in complete medium in triplicate and incubated for 24 hours. The medium was then removed, and Ad5.GFP diluted in serum-free medium to a concentration of 5000vp/cell in 200µl and 10,000vp/cell in 200µl and applied to cells for 2 hours. The virus solution was removed and replaced with complete medium. After a further 48h incubation cells were washed and fixed in 4% paraformaldehyde. Flow cytometry was performed on a BD Accuri flow cytometer and the data was analysed using FlowJo. The control sample (0vp/cell in serum free medium) was used to determine the baseline level of fluorescence intensity.

C: Graph to show the proportion of cells expressing GFP after incubation with increasing concentration of Ad5.GFP. A two way ANOVA was performed to compare results from the three cell lines

P values: ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$

An Ad5 vector with a firefly luciferase reporter gene (Ad5.Luc) was selected as the best suited to an exploratory *in vivo* study, as it would enable luminometry by *In vivo* Imaging System (IVIS) imaging to be used assess the expression of the reporter gene in the live animals. An experiment was therefore performed to test the ability of Ad5.Luc to transduce CC1 cells *in vitro* after aerosolisation using the CapnoPen device. The results are displayed in Figure 5-10. CHO CAR and CHO K1 cells were again used as positive and negative controls. PEO1 and PEO4 ovarian cancer cells were also included in the assay as they had intermediate levels of CAR expression when assessed using flow cytometry, with 30% and 88% of cells expressing CAR respectively (data shown later in Figure 5-17).

A two-way ANOVA with Tukey's multiple comparison test found there was no significant difference in the RLU detected from cells incubated with aerosolised virus at a concentration of 10,000 vp/cell compared to those incubated with non-aerosolised virus at the same concentration. There was a significant difference in the mean RLU detected in the CC1 samples, compared to the CHO K1 samples, suggesting that the Ad5.Luc was able to infect and transduce these cells, though not as efficiently as the CHO CAR cells. The pattern of luminescence detected in the PEO cell lines appeared consistent with the levels of CAR expression, as the PEO4 cells had a greater RLU/mg protein than the PEO1 cells. However, the p value for these comparisons was greater than 0.05. Similarly, it appeared that the CC1 cells had been transduced more successfully than the PEO cells, but this difference was also not statistically significant. This experiment again supports the hypothesis that aerosolisation is a viable method to administer the Ad5 vectors, since aerosolisation did not have any negative impact on the ability of the virus to infect and transduce the target cells.

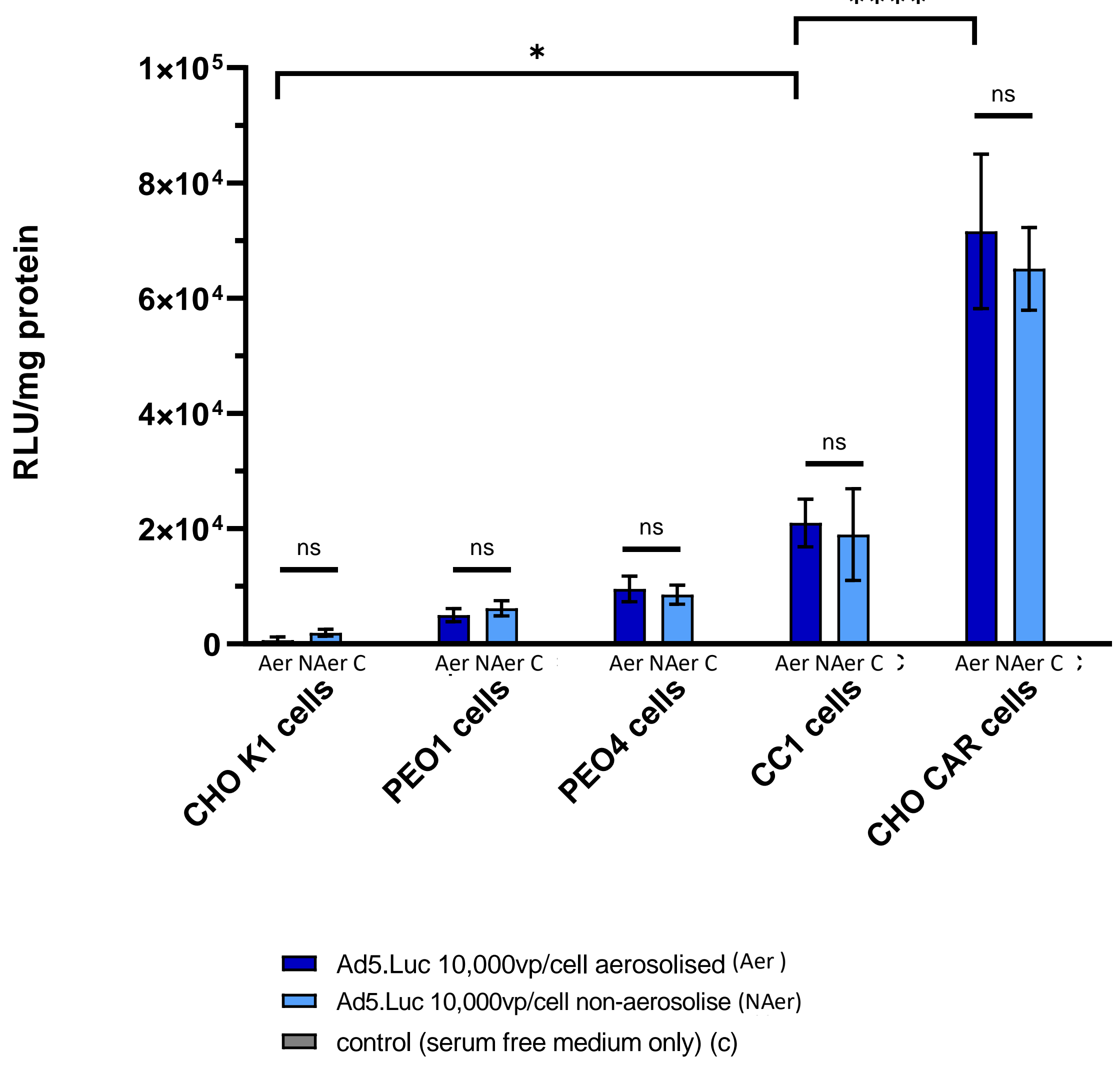


Figure 5-10: Ad5.Luc survives aerosolisation using the CapnoPen device and retains its ability to transduce Wistar Rat hepatocytes in vitro

CHO K1, CHO CAR, PEO1, PEO4 and CC1 cells were plated at 20,000 cells per well and incubated for 24 hours in complete medium. A solution of Ad5.Luc in serum-free medium was made. Half of the solution was aerosolised using the HPI connected to a CapnoPen™ aerosoliser. The other half was reserved to carry out the comparison experiments. The solutions were diluted to 1000vp/cell and applied to cells in triplicate. After a 2 hour incubation the virus solution was removed and replaced with complete medium. After a further 48 hours incubation the medium was removed and the cells were washed and frozen in lysis buffer. The protein concentration in each well was determined using a BSA standards Assay, and the expression of luciferase by luminometry after the addition of luciferin. Graph to show expression of luciferase in various cell lines after incubation with aerosolised Ad5.Luc versus non-aerosolised Ad5.Luc versus control wells. The mean RLU detected normalised by protein concentration, and the standard error of the mean is displayed. A two way ANOVA with Tukey's multiple comparison test was performed in GraphPad Prism 9.0.

The final experiment performed *in vitro* assessed the effect of hyperbaria on the transduction of cells by Ad5.Luc (Figure 5-11). CC1 cells were tested, with CHO CAR and CHO K1 cells as positive and negative controls respectively. The effects of atmospheric pressure, 20mmHg, and 40mmHg in the apparatus were assessed. In the CHO CAR cells, there were differences observed in the expression of luciferase with more luminescence detected in the cells exposed to 20mmHg than those inoculated and incubated at atmospheric and 40mmHg. However this difference was only seen at the highest virus concentration of 5000vp/cell. In the other cell lines and at the other virus concentrations, no significant differences in the luminescence detected were observed, suggesting that the pressure did not have any effect on the infection or transduction of the cells.

A further set of control experiments comparing atmospheric pressure in the apparatus, and standard incubation was also performed to assess the effect of the conditions in the hyperbaric apparatus. There was a trend to increased luminescence in the cells cultured using standard incubation. This was a statistically significant difference in the CHO CAR cells.

These results suggest that the experimental conditions in the hyperbaric apparatus reduce the ability of the virus to infect and transduce the cells. The effect appeared more pronounced in the CHO CAR cells than in the CC1 cells, and there was little transduction in the CHO K1 cells. Given the virus was previously unaffected by injection through the nebuliser device, which involved much higher pressures than those in the hyperbaric chamber, it is likely that this is because the conditions in the apparatus affect the viability of the target cells, rather than the integrity of the viral vector. Further work is needed to clarify the relationship between pressure and transduction.

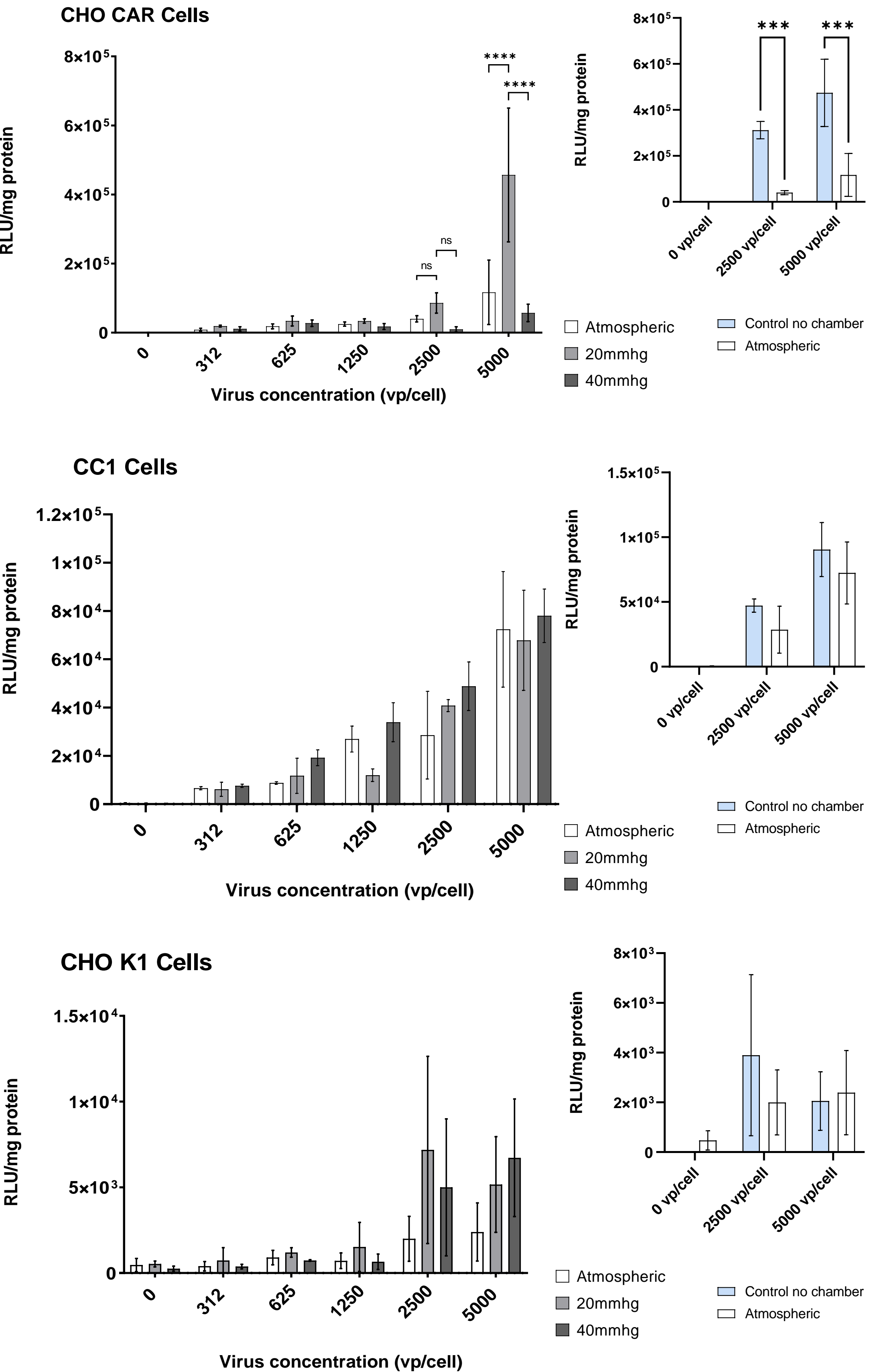

CHO CAR Cells
RLU/mg protein
Virus concentration (vp/cell)
Atmospheric
20mmhg
40mmhg
Control no chamber
CC1 Cells
CHO K1 Cells
0 vp/cell
2500 vp/cell
5000 vp/cell

Figure 5-11: The effect of hyperbaria on the transduction of cells by Ad5.Luc in vitro

CHO K1, CHO CAR, and CC1 cells were plated at 20,000 cells per well and incubated for 24 hours in complete medium. A solution of Ad5.Luc in serum-free medium was made and serial dilutions carried out prior to application to the cells in triplicate. The plates were placed in the hyperbaric apparatus and the pressure was maintained for 30 minutes. The control plate was put in the incubator immediately. After 30 minutes, all plates were put in the incubator for a further 1 ½ hours. The virus solution was removed and replaced with complete medium. After a further 48 hours' incubation the medium was removed and the cells were washed and frozen in lysis buffer. The protein concentration in each well was determined using a BSA standards Assay, and the expression of luciferase by luminometry after the addition of luciferin. Graphs to show expression of luciferase in each cell line at each pressure are displayed. The mean RLU detected normalised by protein concentration, and the standard error of the mean is displayed. A two-way ANOVA with Tukey's multiple comparison test was performed in GraphPad Prism 9.0 to compare the results between the different pressure conditions.

A: CHO CAR Cells, B: CC1 Cells, C: CHO K1 Cells

5.3.3 *In vivo* assessment of the feasibility of pressurised intraperitoneal aerosolised delivery of adenovirus vectors in a Wistar rat model.

Experiments to test the feasibility of pressurised intraperitoneal delivery of Ad5 vectors were carried out in collaboration with Dr Leen Van de Sande and Professor Wim Ceelen at the University of Ghent. These experiments involved a collaborative visit to the Laboratory for Experimental Surgery, Ghent University, to assist with the handling of the adenovirus vectors during the animal experiments, which were performed by Dr Van de Sande in accordance with Belgian Council for Laboratory Animal Science (BCLAS) guidelines. Dr Van de Sande had already assessed the feasibility of using a Wistar rat model to test intraperitoneal therapeutics by performing the PIPAC procedure in an immunocompetent rat [174]. A xenograft peritoneal metastasis model in an immunodeficient rat was in development. The feasibility of pressurised intraperitoneal aerosolised delivery of adenovirus vectors was tested in the immunocompetent rat model using the same experimental protocol as for the rat PIPAC model, but substituting the chemotherapy solution for the virus solution.

5.3.3.1 *A comparison of intraperitoneal injection and intraperitoneal aerosolisation of Ad5.Luc in a Wistar rat model.*

An initial pilot experiment to compare intraperitoneal injection with intraperitoneal aerosolisation was designed. The pilot was to assess the tolerability of the experimental procedures. The dose of Ad5.Luc was selected based on previous experiments in rodent models. All animal experiments were performed under approved protocols by Animal Ethics Committee of Faculty of Medicine and Health Sciences, Ghent University, Belgium (ECD 17-109), and in compliance with BCLAS guidelines for the Care and Use of Laboratory Animals.

The pressurised intraperitoneal aerosolisation procedure was completed successfully in 4 rats. It appeared to cause greater distress to the rats than the intraperitoneal injection, as can be seen by the observations in the 72 hours prior to IVIS Imaging (Table 5-1). By day 2 post procedure, the rats who had undergone IP injection had started to gain weight, whereas the rats who had undergone IP aerosolisation were still losing weight. All rats who underwent an aerosolisation procedure, whether receiving virus solution or saline, required analgesia, whilst the rats who had intraperitoneal injections did not. Overall, however, the procedures carried out were tolerated by the rats in the timeframe observed.

Table 5-1: Rat weights and associated animal welfare scores following intervention.

Scores were assigned based on the animal house's welfare protocol. A score of 0 was assigned if the rat maintained or gained weight. A reduction in weight by <10% was assigned a score of 1 and prompted administration of analgesia if there was an associated behavioural change.

Treatment group	Rat	Day 0	Day 1 post procedure			Day 2 post procedure		
		Weight (g)	Weight (g)	% change	Score	Weight (g)	% change	Score
virus by intraperitoneal injection	A1	343	345	0.6	0	350	1.4	0
	A2	335	337	0.6	0	343	1.8	0
	A3	349	352	0.9	0	353	0.3	0
	B1	342	345	0.9	0	330	-4.3	1

Virus by intraperitoneal aerosolisation	B2	330	330	0.0	0	307	-7.0	1
	B3	340	334	1.2	0	332	-0.6	1
Saline by intraperitoneal injection	C1	337	334	-0.9	1	337	0.9	0
Saline by intraperitoneal aerosolisation	C2	333	324	-2.7	1	317	-2.2	1

In vivo imaging using the IVIS system was carried out 72 hours after administration to assess the level of transduction. The rats were anaesthetised with sevoflurane and injected with luciferin (100mg/kg) into the peritoneal cavity. Images were captured at 10 minutes with a 1-minute exposure. Analysis was carried out by living Image software on a normalised scale (photons/second/cm^2/steradian). Figure 5-12 shows the images captured, with colour indicating the intensity of the luminescence. There was a very slight increase in the luminescence detected from rat A2 (intraperitoneal injection of Ad5.Luc) with 16 photons/second/cm^2/steradian recorded over the abdomen. All the other rats had 13 photons/second/cm^2/steradian or less detected with no sign of localisation, indicating it was likely to represent background signal. Possible explanations for this were considered. Viral transduction may not have occurred, however given the *in vitro* studies, and prior work in the adenovirus field using rats, this seemed unlikely. It is possible therefore that the signal was not detected because either the dose of virus was too low, or the timing of the imaging was not optimal. The experimental protocol with regards the dose of virus and the timing of imaging was based on the group's previous work in mice. However, simply scaling the dose of virus by the relative increase in mass between a mouse and a rat may have been too simplistic.

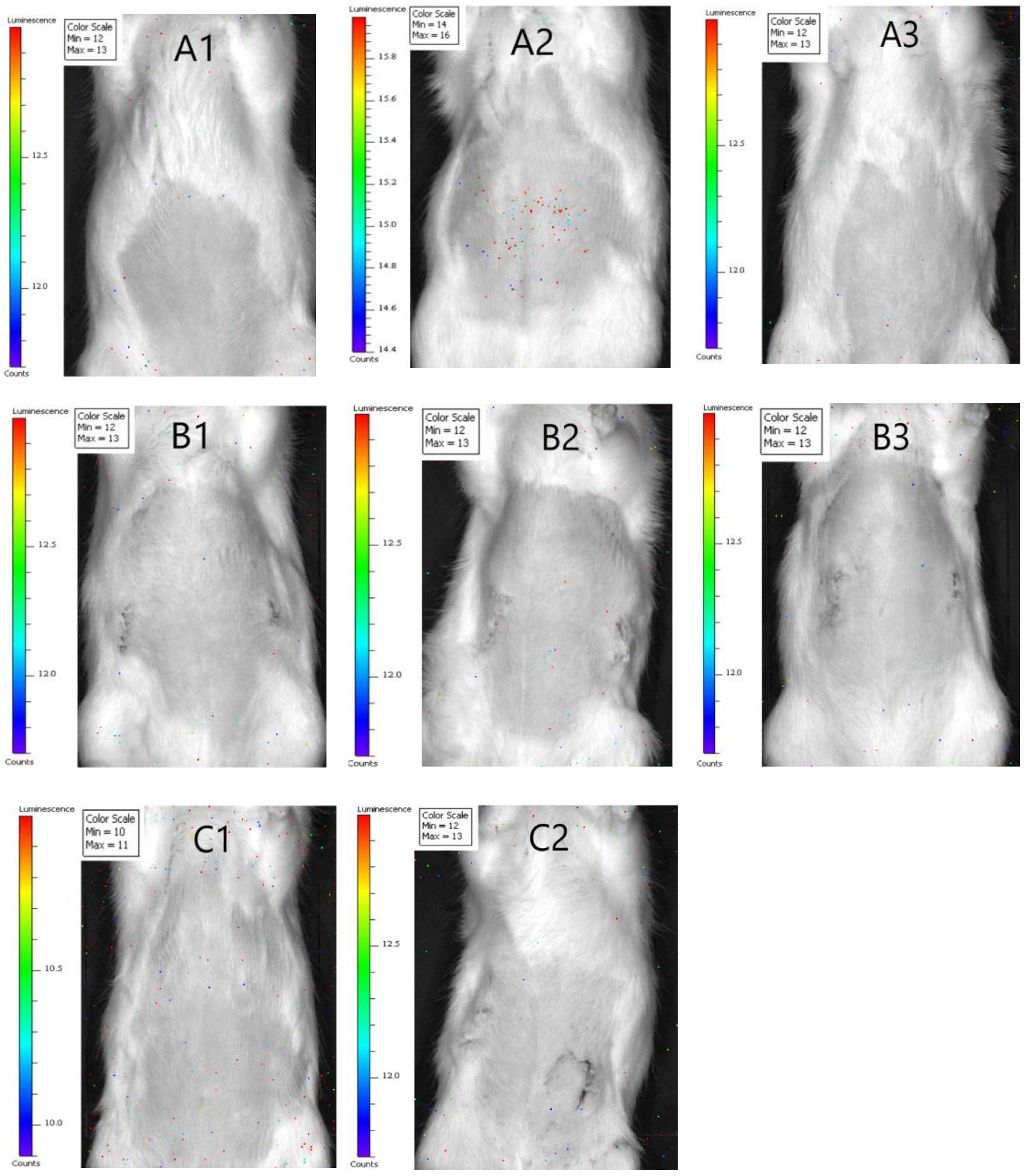


Figure 5-12: IVIS imaging of rats at 72 hours after intervention to determine the adenovirus vector biodistribution.

Rats were anaesthetised by inhalation of sevoflurane and injected with 1.5ml of luciferin at 15mg/ml into the peritoneal cavity. Images were captured at 10 minutes with a 1-minute exposure. Images were analysed by Living Image® software on a normalised scale (photons/second/cm2/steradian). Rats in Group A had in intraperitoneal injection of $3x10^{10}$vp Ad5.Luc in 5 ml 0.9% NaCl. Rats in Group B had intraperitoneal aerosolisation of $3x10^{10}$vp Ad5.Luc in 5 ml 0.9% NaCl. Rat C1 had intraperitoneal injection of 5 ml 0.9% NaCl. Rat C2 had intraperitoneal aerosolisation of 5 ml 0.9% NaCl.

After IVIS imaging the rats were sacrificed and immediately dissected. Tissue blocks from the liver, lung, and abdominal wall were taken and placed in 6 well plates with 1.5ml PBS to prevent desiccation. 1.5ml luciferin at 3mg/ml was then added on top

(final concentration 1.5mg/ml) and imaged immediately. The data was analysed by Living Image® software on a normalised scale (photons/second/cm2/steradian). Figure 5-13 shows the IVIS images of the tissues. Again, there were very low levels of luminescence consistent with background signal, and no difference between the rats in the different interventional groups. As with the biodistribution IVIS imaging, there were a number of possible explanations for this. Further work on tissue samples from the rats was carried out.

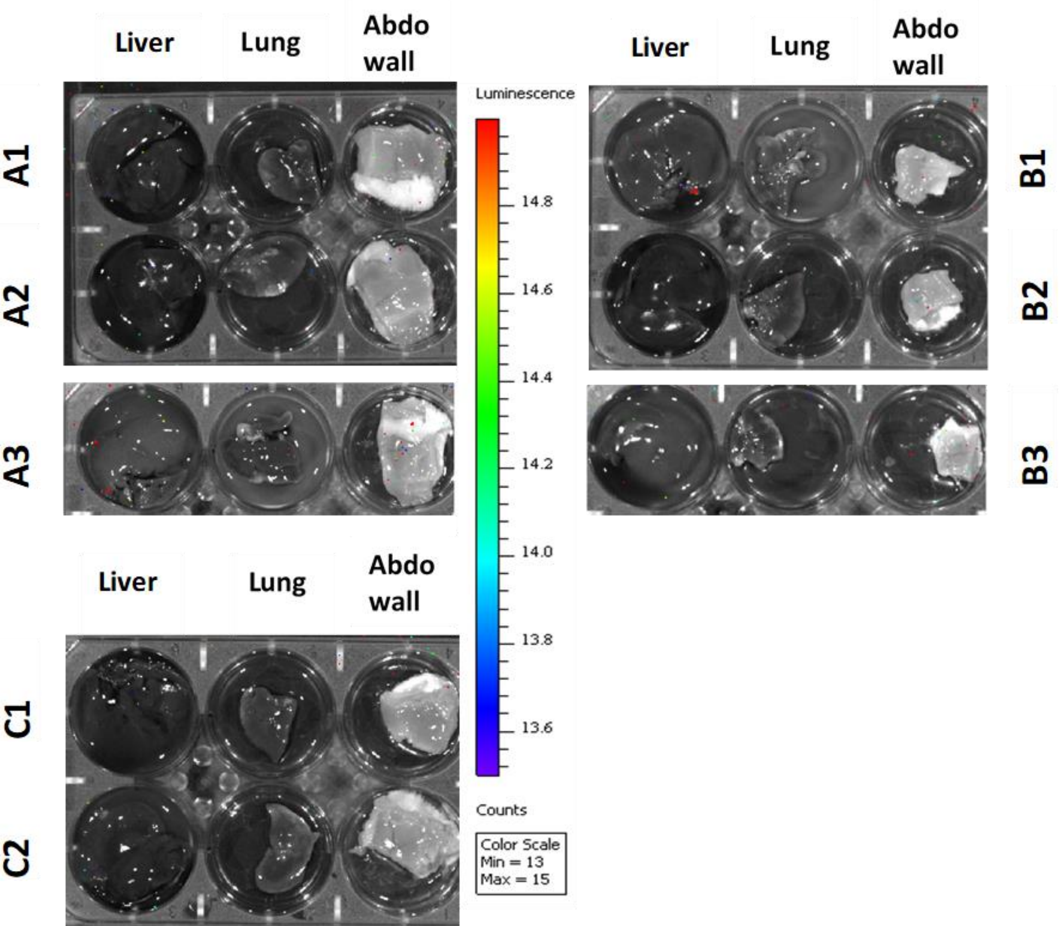


Figure 5-13: IVIS imaging of rat tissue blocks.

Rats were sacrificed and immediately dissected. Tissue blocks from the liver, lungs, and abdominal wall were taken and placed in 6 well plates with 1.5ml PBS. 1.5ml luciferin at 3mg/ml was then added to each well and images were captured immediately and analyzed by Living Image® software on a normalised scale (photons/second/cm2/steradian). Rats in Group A had in intraperitoneal injection of $3x10^{10}$vp Ad5.Luc in 5 ml 0.9% NaCl. Rats in Group B had intraperitoneal aerosolisation of $3x10^{10}$vp Ad5.Luc in 5 ml 0.9% NaCl. Rat C1 had intraperitoneal injection of 5 ml 0.9% NaCl. Rat C2 had intraperitoneal aerosolisation of 5 ml 0.9% NaCl.

Tissue blocks were also taken from the liver, lung, and abdominal wall of each rat and fixed in formalin. The samples were shipped back to Cardiff and were then embedded in paraffin. Sections were mounted and assessed for the presence of Ad5 by immunohistochemistry with an anti-Ad5 primary antibody (primary polyclonal rabbit anti-adenovirus type 5 antibody #ab6982, Abcam, Cambridge, UK). Figure 5-14 shows the images obtained from light microscopy of the stained sections of rat liver. The sections incubated with anti-Ad5 primary antibody are homogenously stained when compared to the sections incubated with the rabbit IgG control. There is little appreciable difference in the staining when the sections from rats from different groups are compared. This suggests that either no Ad5, or very low levels are present in the treated rats, and the anti-Ad5 antibody is exhibiting high levels of background staining.

Tissue blocks from the liver, lung, and abdominal wall were taken from each rat and flash frozen in liquid nitrogen. The intention was to perform qPCR to assess the viral genome copy number in the tissues as a further method to determine whether there was a difference in the Ad5 content of the tissues from the different treatment groups. Unfortunately, the samples were held in customs whilst being shipped back to the UK and had thawed by arrival. They were not suitable for analysis and had to be discarded.

Overall, the experiment showed that whilst the intraperitoneal aerosolisation was technically feasible, there was no evidence of transduction by the Ad5.Luc.

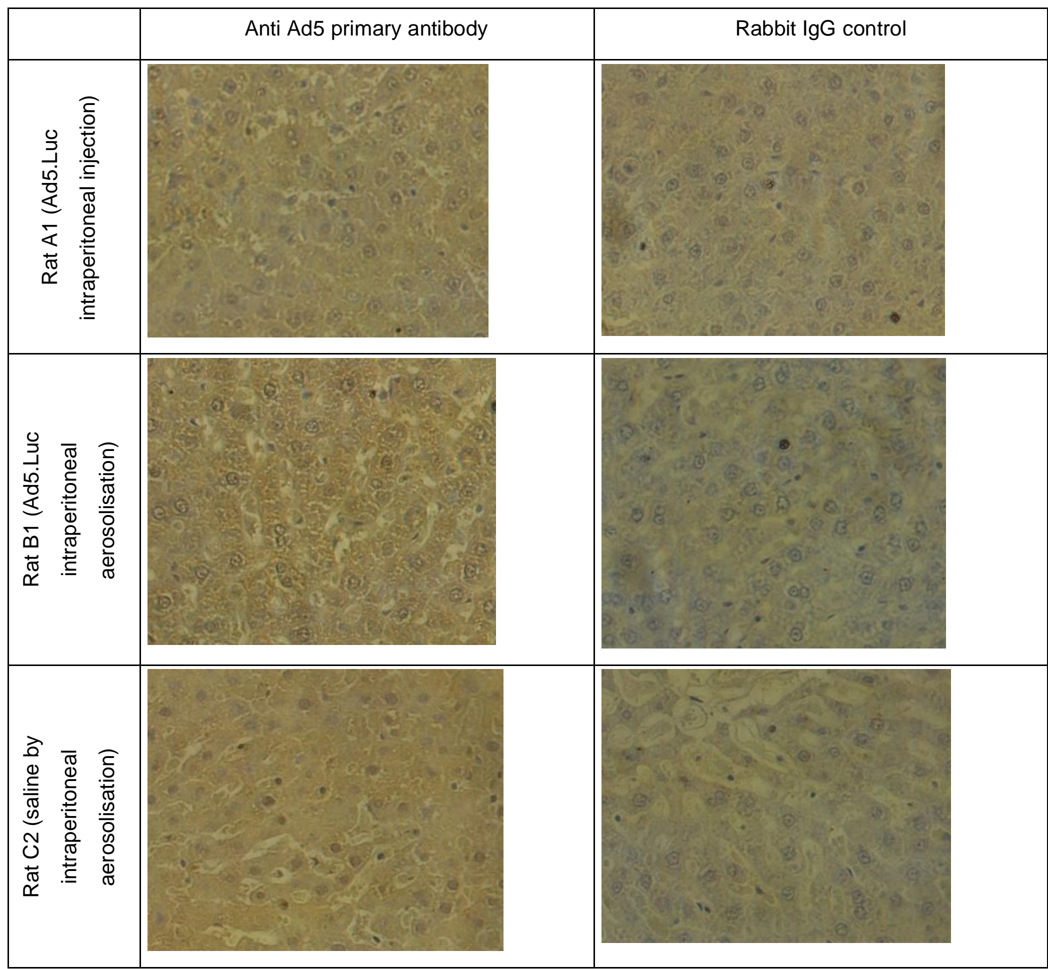


Figure 5-14: Immunohistochemical staining of formalin-fixed paraffin-embedded rat liver sections.

Sections were rehydrated, and an antigen retrieval step using proteinase K followed by H_2O_2 quenching was completed. They were blocked in normal 2.5% horse serum for 1 hour before staining with primary antibody. Anti-Ad5 (1:1000) or rabbit control IgG (1:1000) primary antibodies were added before overnight incubation at 4°C. The secondary antibody was horse anti-rabbit IgG-HRP. Sections were then developed in DAB substrate for 3 min and counterstained with haematoxylin for 30. Images were acquired on a DMi1 light microscope.

5.3.3.2 A comparison of intraperitoneal injection and intravenous injection of Ad5.Luc in a Wistar rat model.

Following the unexpected results of the first *in vivo* study, analysis and consideration of the methodology and results was carried out and discussed with other researchers

in the field. A further study was proposed. This would be in two parts; the first entailing a small dose escalation comparing intravenous and intraperitoneal injection of Ad5.Luc, and the second part again comparing intraperitoneal aerosolisation with intraperitoneal injection, but only if transduction was successfully detected in part 1.

The first part of the study compared intravenous injection of $1x10^{11}$ vp with intraperitoneal injection of $1x10^{11}$ vp. A third rat received an intraperitoneal injection of 0.9% NaCl as a negative control. The intravenous route of administration was included as a positive control since other rodent studies have observed that intravenous injection results in rapid and efficient transduction of the liver with Ad5 based vectors [175, 176]. The intraperitoneal route was chosen for saline administration since it was the result from the intraperitoneal Ad5.Luc injection which was the primary outcome of interest. All animal experiments were performed under approved protocols by Animal Ethics Committee of Faculty of Medicine and Health Sciences, Ghent University, Belgium (ECD 18-23), and in compliance with BCLAS guidelines for the Care and Use of Laboratory Animals.

The higher dose of virus appeared to be tolerated well. Table 5-2 shows the weights of the rats and associated welfare scores. None of the rats required analgesia.

Table 5-2: Rat weights and associated animal welfare scores following intervention (In vivo experiment 2).

Scores were assigned based on the animal house's welfare protocol. A score of 0 was assigned if the rat maintained or gained weight. A reduction in weight by <10% was assigned a score of 1 and prompted administration of analgesia if there was an associated behavioural change.

Treatment group	Day 0	Day 1 post procedure			Day 2 post procedure			Day 3 post procedure		
	Weight (g)	Weight (g)	% change	Score	Weight (g)	% change	Score	Weight (g)	% change	Score
Intraperitoneal Ad5.Luc injection	345	342	0.0	0	350	0.0	0	340	0.0	0
Intravenous Ad5.Luc injection	345	344	0.0	0	347	0.0	0	341	0.0	0
Intraperitoneal 0.9% NaCl injection	346	350	0.0	0	353	0.0	0	341	0.0	0

IVIS imaging to assess expression of the reporter gene was carried out after 72 hours. Figure 5-15 shows the images obtained. Luminescence was observed in both Ad5.Luc injected rats indicating that there had been some successful transduction. There was a higher signal in the IV injected rat (maximum $1x10^6$ photons/second/cm^2/steradian) compared to the intraperitoneal injected rat (maximum $2x10^4$ photons/second/cm^2/steradian). No luminescence was observed in the intraperitoneal 0.9% NaCl injected rat.

The rats were sacrificed and immediately dissected. Tissue blocks from the liver, lung, and abdominal wall were taken and placed in 6 well plates with 1.5ml PBS to prevent desiccation. 1.5ml luciferin at 3mg/ml was then added on top (final concentration 1.5mg/ml) and imaged immediately. The data was analysed by Living Image® software on a normalised scale (photons/second/cm2/steradian). There was luciferase activity detected in the liver of the rat which was treated with an intravenous injection of Ad5.Luc. No activity was detected in the tissues from the rat treated with an intraperitoneal injection of Ad5.Luc, or in the tissues from the rat injected with 0.9% NaCl as a control.

Previous experiments using adenovirus vectors in rodents from this group and others have found that luminescence activity is localised to the liver in similar experiments. Given the pattern seen in the *in vivo* luminescence imaging, some signal in the liver from the intraperitoneal injection of Ad5.Luc was expected. The intensity of the luminescence from the liver of the intravenously treated rat may have been too great relative to the liver of the rat injected via the intraperitoneal route to enable detection, or it may be that the section of liver tissue imaged did not have luciferase activity. Overall, the results demonstrate that intraperitoneal delivery of an Ad5 vector does result in transduction and detectable expression of a reporter gene, albeit at a lower rate than IV delivery. This experiment suggests that the rat is a viable model for the study of intraperitoneal aerosolisation of viral vectors, but at higher doses that first thought. Further work to re-test the pressurised aerosolisation delivery of the higher dose would be the next step.

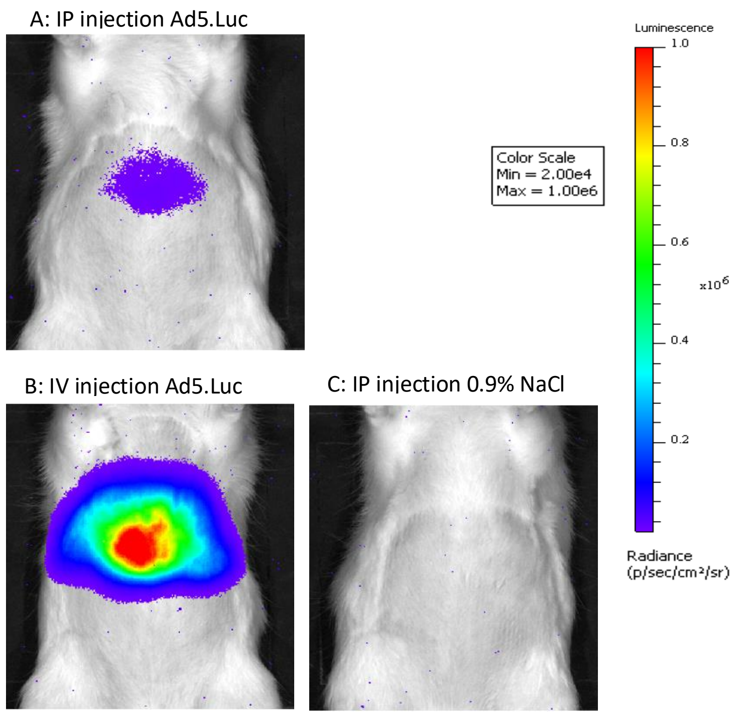


Figure 5-15: IVIS imaging of rats at 72 hours after intervention (In vivo experiment 2).

Rats were anaesthetised and 100mg/kg D-luciferin was injected into the peritoneal cavity using standard procedures. Images were captured 10 minutes after D-luciferin administration. A 10 minute exposure time was used. Images were analysed by Living Image® software on a normalised scale (photons/second/cm2/steradian). Interventions: Rat A = Intraperitoneal injection of Ad5.Luc 1x10^{11} vp in 5 ml 0.9%NaCl. Rat B = Intravenous injection of 1x 10^{11} vp Ad5.Luc in 200 µL of 0.9% NaCl. Rat C = Intraperitoneal injection of 5ml warmed 0.9%NaCl

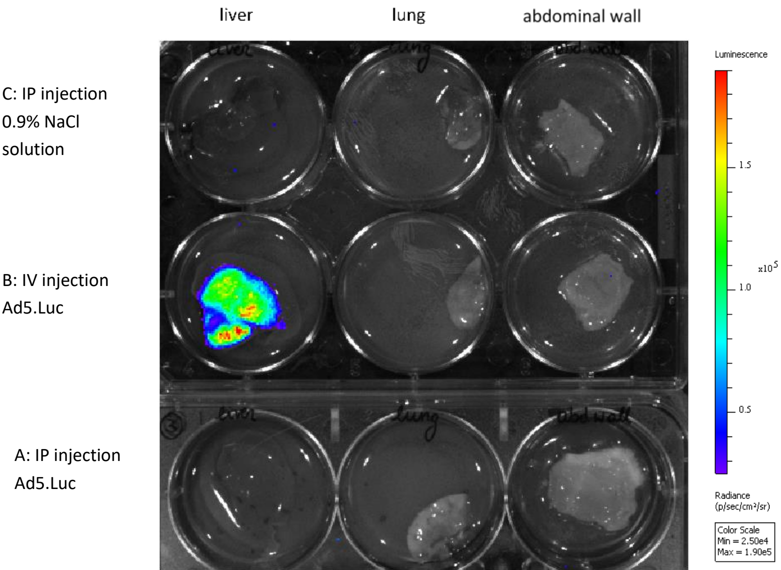


Figure 5-16: IVIS imaging of rat tissue blocks (In vivo experiment 2).

Rats were sacrificed and immediately dissected. Tissue blocks from the liver, lungs, and abdominal wall were taken and placed in 6 well plates with 1.5ml PBS. 1.5ml luciferin at 3mg/ml was then added

to each well and images were captured immediately and analyzed by Living Image® software on a normalised scale (photons/second/cm2/steradian). Interventions: Rat A = Intraperitoneal injection of Ad5.Luc 1x10^{11} vp in 5 ml 0.9%NaCl. Rat B = Intravenous injection of 1x 10^{11} vp Ad5.Luc in 200 µL of 0.9% NaCl. Rat C = Intraperitoneal injection of 5ml warmed 0.9%NaCl

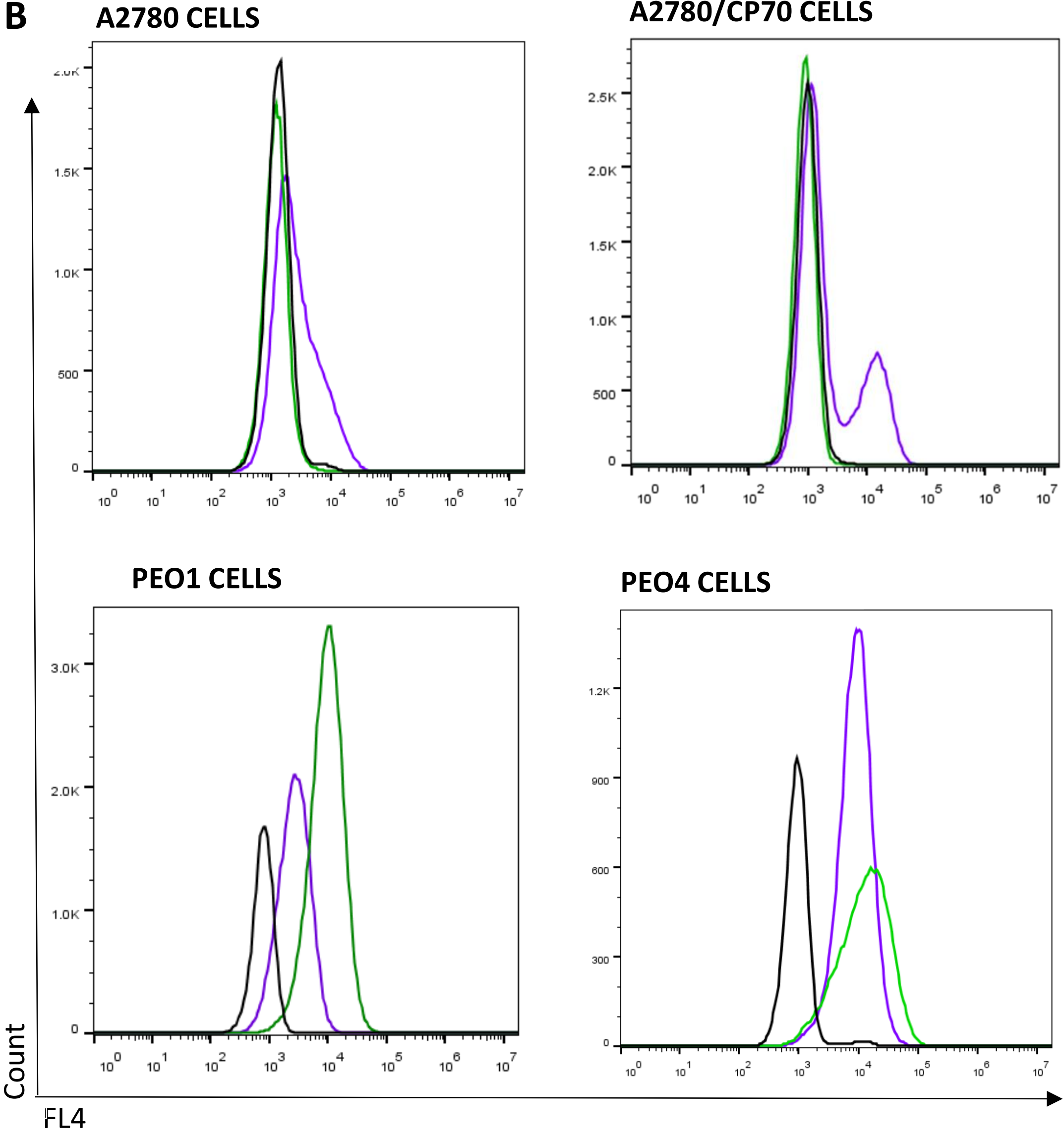
5.4 Characterisation of receptor expression profile of cancer cell lines

The ultimate aim for the rat model, if found to be a viable way to assess intraperitoneal pressurised aerosolisation as an administration method for adenovirus vectors, would be to test cancer-targeted vectors such as $Ad5_{null}A20$. This vector is targeted to αvβ6, an integrin expressed on aggressive epithelial cancers. It was developed to target ovarian cancer metastases using commercially available and patient derived ovarian cancer cell lines [173]. It has been shown to specifically and effectively target ovarian tumours in a patient-derived tumour xenograft mouse model [112]. Since other tumours of epithelial tissues have also been shown to express αvβ6, it's utility in other cancer types is being assessed. The αvβ6 expression profile of a selection of cancer cell lines was determined using flow cytometry. Again, CAR expression was also assessed because it is the native receptor for Ad5 [100].

Flow cytometry was used to assess receptor expression. Four human ovarian cancer cell lines, PEO1, PEO4, A2780, and A2780/CP70, were tested, as well as three human gastric cancer cell lines; AGS, MKN 28, and MKN 45. The results are presented in Figure 5-17. The table in Figure 5-17A shows the mean percentage of cells expressing CAR and αvβ6. CHO CAR and CHO K1 are included again for comparison. CAR expression was variable and differed between the two paired ovarian cancer cell lines. The PEO1 and PEO4, and AGS cells lines expressed high levels of αvβ6. Based on previous studies, it would be expected that the $Ad5_{null}A20$ vector would transduce these cells selectively and with high efficiency. A future direction would be to perform transduction assays *in vitro* to confirm this. A2780 and A2780/CP70 had low αvβ6 expression. These results suggest that investigation of the $Ad5_{null}A20$ vector as a therapy for epithelial cancers generally, rather than just ovarian cancer, is worth pursuing. Additionally, the PEO and AGS cell lines might be useful for generating xenograft models to test the $Ad5_{null}A20$ vector if *in vitro* work confirms that they are efficiently transduced.

A

Cell line	Organ/Condition	% cells expressing Human CAR	% cells expressing Human αVβ6 Integrin
CHO K1	Hamster Ovary	0	0
CHO CAR	Hamster Ovary	99	0
A2780	Human Ovarian Cancer	13	0
A2780 CP70	Human Ovarian Cancer	26	0
PEO1	Human Ovarian Cancer	30	91
PEO4	Human Ovarian Cancer	88	95
AGS	Human Gastric Cancer	79	94
MKN 28	Human Gastric Cancer	2	1
MKN 45	Human Gastric Cancer	21	56



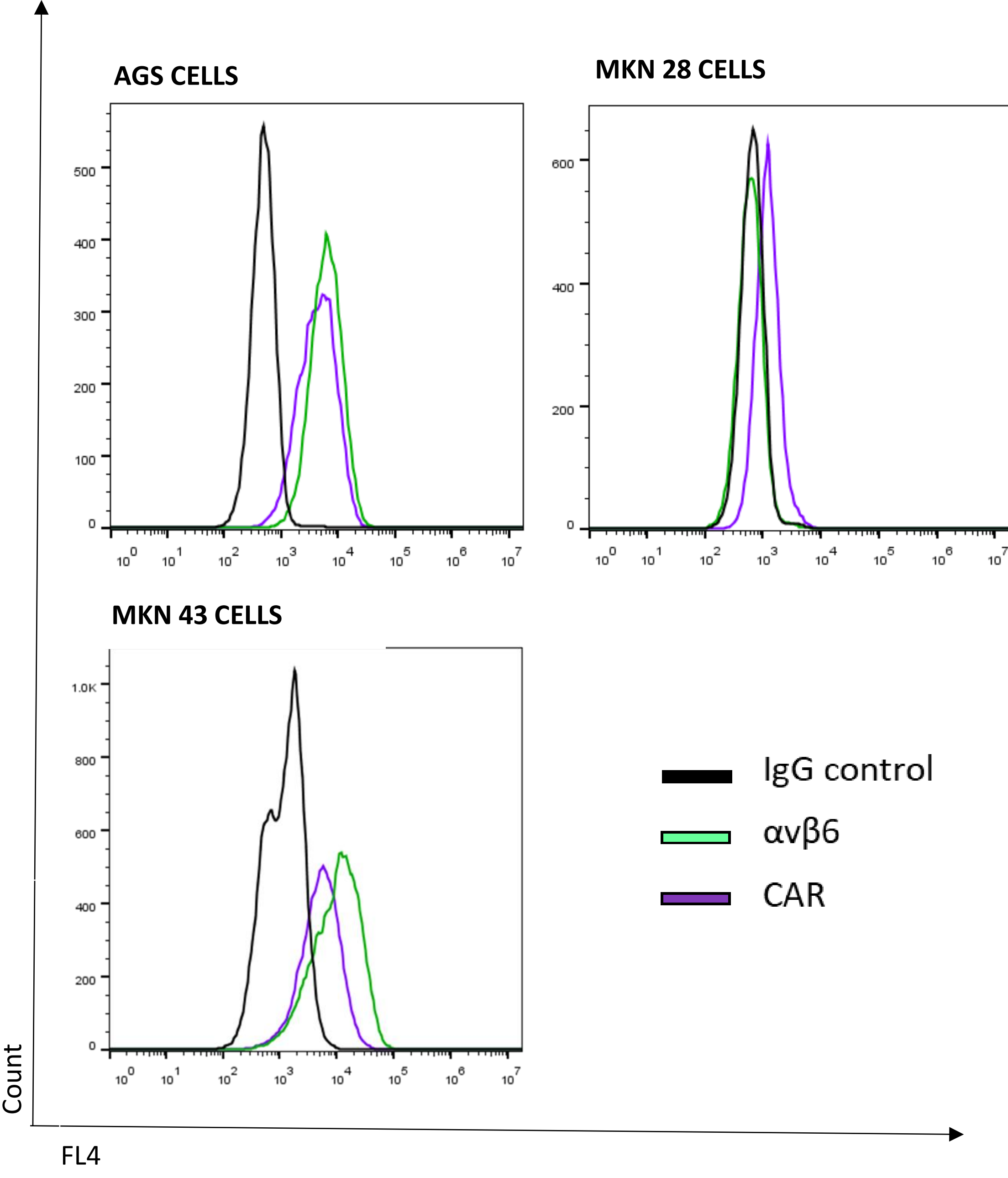


Figure 5-17 Assessment of cell receptor expression profile:

100 000 cells were stained in triplicate with primary antibodies (anti-αvβ6 clone E7P6, anti-CAR clone RmcB; anti-CD46 clone MEM258) for 1 h on ice, and with secondary AlexaFluor 647 for 30 min on ice. Unstained control samples were prepared for gating. Data were recorded for 10 000–20 000 events using a BD Accuri C6 flow cytometer and analysed in FlowJo software. Avβ6, αvβ6 integrin; CAR, coxsackie and adenovirus receptor.
A = Summary table of receptor expression profile
B = flow cytometry data for the human cancer cell lines

5.5 Discussion

5.5.1 The response of ovarian cancer cells to a cytotoxic drug *in vitro*

The initial experiments were carried out to assess the response of ovarian cancer cells to cytotoxic drugs *in vitro*. The ovarian cancer cell lines were characterised by their response to Cisplatin [cis-diamminedichloridoplatinum(II)], so that was the drug chosen for evaluation. Cisplatin is commonly used as first-line treatment in ovarian cancer. It is also one of the drugs commonly used via the intraperitoneal route to treat peritoneal metastases from ovarian cancer in both Heated Intraperitoneal Chemotherapy (HIPEC) and PIPAC procedures. Cisplatin is thought to initiate cell death by several mechanisms. On entry to the cell it is hydrolysed, becoming a potent electrophile. This enables it to cause direct DNA damage by binding to purine residues to create platinum-DNA adducts [177]. This results in intra-strand, and to a lesser extent inter-strand, DNA cross-linking, which is thought to inhibit DNA replication, transcription, and therefore successful cell division [178, 179]. It also induces oxidative stress, with excessive production of reactive oxygen species (ROS). This leads to damage to cellular proteins, and in particular the mitochondria [177]. Ultimately the outcome is initiation of apoptotic cell death.

The cell lines assessed were rapidly growing with doubling times of approximately 37 and 36 hours for the PEO1 and PEO4 cell lines [121], and 25 and 22 hours for the A2780 and A2780/CP70 cell lines [26] respectively. The A2780 cells were originally harvested from the ascites of an untreated patient with ovarian cancer. Cisplatin resistance was then induced by culturing them in the presence of increasing doses of cisplatin [26]. The intracellular level of glutathione has been suggested as a mechanism for resistance to cisplatin in the A2780 and daughter cell lines. Hamilton et al [180] found that glutathione levels correlated with cisplatin resistance, and they also showed that the reduction of glutathione in the cells by the addition of a specific inhibitor of -γ-glutamylcysteine synthetase, an enzyme required for GSH synthesis, made cells more sensitive to cisplatin. Behrens et al also observed this increase in intracellular glutathione in the more resistant cells. Additionally they measured the

capacity of cells to perform unscheduled DNA synthesis in response to drug exposure, and found that only the more resistant cell lines had the capacity for DNA-repair in the presence of Cisplatin [26].

The production of glutathione is an ATP-dependent process, as is DNA repair. Thus, it is possible that the increase in the Formazan end product of the MTS assay in the first 24 hours is an increase in cellular activity as the cells react to the drug, rather than a true increase in cell viability. At 48 hours, the cell viability determined in the cisplatin-treated wells fell below that of the control wells indicating less activity compared to the untreated cells. Cell death induced by DNA damage should be apparent at this time point since all of the cell lines tested had doubling times less than 48 hours. Then at 72 hours, the percentage cell viability started to increase again in many wells, perhaps indicating that the cells remaining are dividing. As a result of this pattern, the time point selected for further cisplatin sensitivity experiments was 48 hours.

The IC_{50} values obtained in the standard culture conditions described here were higher than the values previously described in the literature. The method used to determine cell viability was not the same, so this may partially explain the discrepancy. The MTS assay was chosen for this project because it enabled the viability assay to be repeated using the hyperbaric apparatus to assess the effect of pressure on the dose-response relationship. The main limitation of the MTS assay is that it assumes that the cellular activity detected is a reliable surrogate marker of cell viability. Other methods of determining viability, for example the colony formation in double layer agar system as described by Behrens et al [26] and Hamilton et al [180], do not rely on this assumption and could therefore be considered more accurate. However, these methods would not have been feasible given the number of different doses and conditions tested in the hyperbaric experiments.

Other methods to assess the viability at the end of the assay were assessed. Flow cytometry using LiveDead Aqua staining was attempted. This is a fluorescent dye that reacts with free amines. In living cells, with intact membranes, the dye is restricted to surface amines, whilst in dead cells with compromised membranes it is able to

react with both intracellular amines and surface amines and thus the cells are stained more intensely [181]. However, there was high variability in cell number for each Cisplatin concentration at the end of the experiment, with many more cells in the control and low dose Cisplatin wells compared to the high dose wells. Whilst this is not unexpected, since the Cisplatin causes cell death and therefore arrests cell proliferation, it meant that meant that additional assumptions would be involved in comparing each Cisplatin-treated well with the control well to take into account the lower cell numbers as well as the proportion of live and dead cells (data in Appendix 8.9). This data did support the assumption that the reduction in cellular activity observed with increasing dose of cisplatin at the 48-hour time point was as a result of reduced cell number, rather than just a reflection of cellular activity. Overall, the MTS assay appeared the most appropriate method for the experiments planned.

The experiments assessing the response of cells to Cisplatin under hyperbaric conditions *in vitro* did not find any consistent pattern in relation to increasing pressure. The pressures tested were relatively low. The reasons for this were that firstly, the apparatus could only generate pressures up to 40mmHg consistently. At higher pressures, air started to leak and resulted in variable conditions. Secondly, the experiments were intended to investigate the hypothesis that administering cytotoxic drugs to the pneumoperitoneum during laparoscopic surgery confers an advantage because of the raised pressure in the abdomen. The pressure generally used by surgeons in clinical practice is 12mmHg. Pressures higher than 20mmHg are not tolerated. Therefore, the pressures investigated here were relevant to the clinical application. Similarly, the 30-minute exposure to the pressure conditions was intended to mirror the clinical procedure and test the hypothesis that the pressure of the pneumoperitoneum over the course of a short laparoscopic operation can have an impact on drug delivery. It is possible that a longer exposure to pressure might have different effects and this would be an avenue for further investigation. However, it is unlikely to be feasible to translate such a finding into clinical practice.

It is possible that assessing cell viability at 48 hours is not a sensitive enough measure to detect any variation in response to the pressure. Other methods that could be used to determine the effect on the cells would be assessing the amount of DNA

damage. This could also be carried out immediately after the pressure intervention and would be an area for further investigation in the future.

The use of monolayer cultures of ovarian cancer to test the effect of pressure is a limitation of this work. *In vivo*, the tumour microenvironment is complex, and one reason suggested for the low penetration of drugs into peritoneal nodules in particular is the peritoneal mesothelium and extracellular matrix that surrounds the nodules, and the high tumoral interstitial pressure [22]. The use of pressure has been shown to increase drug delivery *in vivo.* A study in a pig model of open surgery to deliver intraperitoneal chemotherapy demonstrated that raising the pressure in the abdomen using a water column system to deliver the drug resulted in deeper tissue penetration and higher drug concentrations [39]. Similar results were seen in a rat model of peritoneal disease. There were higher drug concentrations in the tumour nodules to a greater depth when the intra-abdominal pressure was raised [38]. The drug solutions in this experiment were administered via an intraperitoneal catheter with the abdomen closed. Treatment with high-pressure intraperitoneal chemotherapy also conferred a survival advantage to a group of rats that were followed up for 120 days post treatment, compared to untreated, intravenously treated, and intraperitoneally (normal pressure) treated rats [38].

The fact that no benefit to increasing the pressure was found in terms of drug delivery to monolayer cells *in vitro* in these experiments suggests that the pressure may have more of an effect on tissue delivery of drug, rather than the pharmacodynamics at a cellular level. The effects of pressure in a tumour have not been fully assessed by these experiments. An important future development to assess the potential of pressurised intraperitoneal aerosolised therapeutics would be to develop and test a 3D tumour model, such as a patient-derived organoid model or a tissue explant *in vitro*. Such a model may also allow assessment of the effect of the drug over a longer time period following treatment.

5.5.2 The assessment of aerosolization as a method for intraperitoneal delivery of oncolytic adenovirus vectors

The experiments *in vitro* demonstrate that aerosolisation using the CapnoPen nebuliser device does not affect the ability of Ad5-based vectors to infect and transduce cells. This was the expected outcome, since Ad5 causes respiratory illness and has therefore evolved to survive transmission by respiratory droplets. However, this is an important finding in determining whether intraperitoneal aerosolisation will be of use as a method of delivery for oncolytic adenoviruses. Two Ad5 vectors were tested here; Ad5.GFP and Ad5.Luc. The $Ad5_{null}A20$ vector, which has been de-targeted from all its native means of uptake, including the CAR receptor, and re-targeted to specifically infect cells expressing αvβ6 integrin was not tested. However, it is anticipated that it would tolerate aerosolisation equally well. Investigation of the ability of the $Ad5_{null}A20$ vector to survive aerosolisation and retain its ability to kill target cells would be an area for further investigation of the feasibility of this technique in future clinical practice.

To ascertain whether the Wistar rat model would be an appropriate way to test intraperitoneal aerosolization of adenovirus vectors, experiments were performed *in vitro* using a Wistar rat hepatocyte cell line (CC1). The Ad5 vector best suited for the rat model, Ad5.Luc was able to transduce the Wistar rat cells *in vitro* with reasonable efficiency, demonstrating that the rat cell line was able to be infected with a human adenovirus and express a transgene. This did not translate into the expected results in the pilot experiment *in vivo* using a dose of $3x10^{10}$ vp of Ad5.Luc. The dose selected was conservative when compared with experiments previously carried out by the group in mice, but we were conscious that the rats were going to undergo the aerosolisation procedure as well as receiving the virus and did not want to induce dose-limiting toxicity. Additionally, we discussed the proposed dose with other groups who used rat models to assess adenovirus-based vectors. Despite this, transduction was not detected. As already discussed, this could have been because the dose of virus was inadequate, or because the imaging protocol was not optimal.

The second *in vivo* experiment therefore tested a higher dose of virus ($1x10^{11}$ vp) to see if transgene expression could be detected. Imaging was still carried out at 72

hours post infection, but a longer exposure time was used. The intraperitoneal route did result in transduction, though at a lower rate than IV delivery. This is perhaps expected given what we know about the intraperitoneal route of administration in humans [29] and other animal models [175, 176]. However, further work should be undertaken to understand whether the reduced signal following intraperitoneal administration is as a result of reduced transduction overall, or whether it represents a more widespread expression of the reporter gene at a lower level compared to the IV delivery where there is a concentration of virus activity and therefore reporter gene expression and detection in the liver. PCR to quantify the number of viral copies in the tissues may be helpful. The immune response of the immunocompetent rat to the vector administration would also be of interest to see whether there is any difference in activation following IV and intraperitoneal administration and the subsequent clearance of the vector.

The same rat model has been used to compare intraperitoneal injection, intraperitoneal aerosolisation, and intravenous injection of mRNA complexes encoding the firefly luciferase protein and found no significant difference in the average overall luminescence detected from rats treated using each of the three administration routes [7]. The authors commented that intraperitoneal aerosolisation and intravenous injection produced less variability in the overall luminescence detected. There was a trend towards greater overall luminescence in the intraperitoneal injection group, but this was not significant because there was more variability between rats treated using that route of administration. When they looked at the distribution of the luminescence, the intraperitoneal aerosolisation technique seemed to produce luminescence from a greater surface area of the abdomen of the imaged rats, however, again, this was not a significant difference because of variability between rats. Intraperitoneal injection and intravenous injection produced much less variable distribution, with the signal concentrated to a smaller area in the upper abdomen.

The detection of transduction in the second *in vivo* study, albeit at a lower level from the intraperitoneal route of administration, supports further investigation in this rat model. The results, combined with the previous finding that the rats were able to

tolerate the intraperitoneal aerosolisation procedure using the lower dose of Ad5.Luc, led to the submission of an amendment to the ethics application for the second stage of the second *in vivo* study. An application to increase in the dose of Ad5.Luc to $3x10^{11}$vp was submitted and approved in January 2020 (ECD 19-53). Ultimately, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic prevented this experiment from going ahead as the first cases in the UK were detected soon after the approval was granted, and the first cases in Belgium followed shortly after. This therefore remains an avenue for future investigation.

5.5.3 Characterisation of receptor expression profile of cancer cell lines

The expression of CAR and αvβ6 varied between cell lines. The expression of CAR was of interest to assess the potential of the cells for transduction by Ad5 vectors with un-modified receptor tropism, for example, the Ad5.Luc used here. The expression of αvβ6 integrin is generally restricted to aggressively transformed epithelial cells. In normal tissues, it is usually only expressed during development or wound healing after injury and is thought to have a role in the regulation of epithelial cell proliferation, migration, and phenotype [113]. Van Aarsen et al [114] assessed αvβ6 expression in biopsies from a number of epithelial cell tumour types, and found it was strongly upregulated in squamous cell carcinomas. They looked at metastatic deposits from epithelial cell cancers and found that these also expressed αvβ6 integrin, reflecting the tissue type of the primary cancer rather than the site of the metastasis.

Of the cells tested in this project, the PEO1 and PEO4, and AGS cells lines expressed high levels of αvβ6. This would fit with their origin, since PEO1 and PEO4 were derived from an epithelial ovarian cancer, and AGS was derived from an epithelial gastric adenocarcinoma. A2780 and A2780/CP70 are derived from an ovarian endometrioid adenocarcinoma and had low αvβ6 expression.

The expression of αvβ6 integrin is of interest to the future direction of this work because of the development of the $Ad5_{null}A20$ vector which can target αvβ6-expressing cells specifically [172]. This has been tested *in vivo* in a mouse xenograft

tumour model, with promising results [112]. The mouse model would not be suitable to assess aerosolisation because of the size of the nebuliser. Van de Sande et al [182] have developed a xenograft rat model of peritoneal metastases using SKOV3 cells, a human ovarian cancer line. The model has been used to assess the efficacy of cisplatin loaded nanoparticles administered via aerosolisation *in vivo* [171]. SKOV3 cells do not express αvβ6 [173], so this model would not currently be appropriate to test the $Ad5_{null}A20$ vector. However, if further investigation using Ad5.Luc found that aerosolisation was a viable method for intraperitoneal administration, the model could potentially be adapted using another cancer cell line. This would enable *in vivo* testing of pressurised intraperitoneal aerosolisation of oncolytic adenovirus therapy. The range of cancer cell lines demonstrating αvβ6 expression in this project suggests that, whilst developed with the aim of targeting ovarian cancer, the $Ad5_{null}A20$ vector is likely to be useful in several other diseases involving epithelial-derived tumours. The unifying feature of all the cancer types tested is that peritoneal metastases are a potential endpoint of disease and are universally challenging to treat. Novel therapeutics would be welcome to address this unmet area of clinical need.

6 Final discussion, future work, and conclusions

6.1 Peritoneal disease: the unmet clinical need

This thesis focused on the management of peritoneal disease from two primary pathologies; colorectal and ovarian cancer. As already discussed, precise figures relating to the burden of peritoneal disease in colorectal cancer are not available. This led to the service evaluation of the management of colorectal peritoneal metastases at CAV UHB described in chapter 3. Analysis of the MDT caseload for the years 2014-2019 confirmed that the incidence rate was comparable to the figures quoted in the literature from other European centres. Whilst we cannot be certain, it seems reasonable to assume that the burden of disease across Wales and the rest of the UK is similar, and that peritoneal disease represents a significant and ongoing problem in colorectal cancer.

Collection of this data retrospectively was challenging. The MDT records reflect the focused clinical discussion that takes place. The lack of options for treatment for peritoneal disease in Wales over the time frame evaluated means that it was difficult to ascertain whether patients might have been eligible for alternatives to palliative chemotherapy if they had presented to other centres in the UK, or at a different time. NICE and WHSCC are both due to update their guidance regarding CRS and HIPEC for peritoneal metastases. If their recommendations regarding treatment change, then it is likely that future MDT records will contain a more accurate picture of peritoneal disease. Overall, it would be better to collect data on the incidence of peritoneal disease and the characteristics of the patients affected, prospectively. The routine collection of this data in the colorectal unit is now being actioned.

The assessment of the true incidence of peritoneal metastases in other subspecialties and diseases would be a further useful development. Whilst the staging system for ovarian cancer allows the incidence of isolated peritoneal metastases to be determined from national statistics, other gastrointestinal cancers such as gastric cancer do not separate peritoneal metastases from other distant disease in the staging classification. Like colorectal cancer, the incidence of peritoneal metastases can only be estimated based on previous observational studies. Prospective disease assessment and collection of data would be required to get an accurate picture of

peritoneal disease. The type and stage of disease, as well as data on the performance status and comorbidities of these patients, would be useful to inform the design and development of trials of novel therapeutics. Increasing numbers of advanced therapeutics, such as oncolytic viruses and new immunotherapies, are predicted to come into mainstream clinical use in the next 5 to 10 years. Whilst a new systemic treatment might be assessed in patients with advanced disease at any site, the outcomes in patients with peritoneal metastases should be of particular interest since this is a group of patients in whom current treatment options are especially limited. Centres with access to accurate data about their patients with peritoneal disease would be in the best position to incorporate the conduct of such trials into their treatment pathways. Collection of data on peritoneal metastases would also provide a better context for the disparities in treatments offered and outcomes in different patient groups across the UK. For example, we can hypothesise about the reasons for the poor outcomes observed in the more elderly patients in the service evaluation, but further clarity would be useful for service planning in future.

Unfortunately, demand for services for advanced disease may continue to rise in the future. An unwelcome consequence of the SARS-CoV-2 pandemic has been the effect on cancer care pathways and screening programs. For example, whilst there was some variability in policy between the four nations, routine screening for bowel cancer was suspended for a significant part of 2020 in the UK [183, 184]. As a result of the pandemic, 600,000 fewer endoscopies were performed in England between March and November 2020, and it is estimated that 3500 fewer people were diagnosed and treated for colorectal cancer than would have been expected [184, 185]. There was a more general trend for reduced numbers of patients presenting to primary care and being referred on for investigation and treatment of cancer, with approximately 40,000 fewer patients starting treatment for cancer in the UK in 2020 compared to 2019 [184]. The pandemic also resulted in modifications to practice. Again, using colorectal cancer as an example, there was a relative reduction of 31% (95% CI 19–42) in the number of patients having surgery overall, and a greater number of rectal cancer patients having neoadjuvant short course radiotherapy [185]. The impact on surveillance programmes for patients who have already had

treatment has not been so well described in terms of numbers, but at the start of the pandemic in the UK the Royal College of Radiologists recommended that routine surveillance investigations should be delayed in the absence of new symptoms [186]. This advice was later changed, however there are likely to be ongoing delays in the conduct and reporting of radiological investigations because the infection control measures required to prevent the spread of SARS-CoV-2 in hospital have significantly reduced capacity in many areas. It is anticipated that over the coming years, this will result in a period of worsened outcomes due to the delays in diagnosis and treatment that will be experienced by patients. The need for treatments for advanced malignancy is likely to be greater than ever.

The service evaluation also confirmed the established view that the outcomes for patients with peritoneal disease are generally poor. Peritoneal disease is less well controlled by standard systemic chemotherapy regimens than metastases in other organs [19, 59]. In colorectal and ovarian cancer, the best outcomes have been reported in patients who have had optimal CRS combined with HIPEC and systemic chemotherapy [17, 59, 187]. The most recent data on the outcomes of CRS and HIPEC in colorectal cancer is from the PRODIGE 7 trial. Outcomes exceeded expectations, with median overall survival in each arm reaching 42 months [52]. This added to the controversy about whether the HIPEC element really confers additional benefit. These results, whilst encouraging, show that CRS and HIPEC cannot be considered curative for colorectal cancer nor other gastrointestinal and gynecological malignancies [52, 59, 187]. It is associated with a significant morbidity and mortality, though these aspects have improved with advances in perioperative medicine. Nonetheless, it has a negative impact on QoL for some months after surgery, and is only suitable for patients with a good pre-operative performance status and few comorbidities [63]. CRS and HIPEC was not generally available to patients in Wales with colorectal cancer during the time period assessed in this project, and therefore the majority of patients from CAV UHB were managed with less extensive primary surgery and systemic chemotherapy. The median survival of the cohort of patients whose care was evaluated was 12 months from diagnosis of peritoneal metastases. This demonstrates that there remains an unmet clinical need in this type of disease.

6.2 PIPAC for peritoneal metastases: the current position and areas for future research

PIPAC was conceived as a method to optimise intraperitoneal delivery of therapeutic agents for peritoneal metastases. It is an interesting concept, and as already discussed, is designed to maximise many of the theoretical advantages of intraperitoneal chemotherapy. Intraperitoneal administration is advocated because of the potential for greater tumour penetration at the peritoneal surface [21, 29, 33]. Studies in animal models and *in vitro* in the past have demonstrated that drug concentration in peritoneal metastases is higher after intraperitoneal administration of drug solutions compared to intravenous administration [33, 176]. With the use of adjuncts such as high pressure, and increased temperature, the depth of penetration is in the region of 1-5mm [29]. This means that intraperitoneal drug therapies can only be used for patients with small volume disease, unless there is some additional mechanism to improve penetration. The inventors of PIPAC suggest that the ability to administer high concentration drug solutions to the peritoneal cavity and maintain a consistent pressure following application, as well as the potential for repeated assessment and administration, results in improved drug delivery [1, 65].

Overall, the clinical evidence suggests that there is a group of patients who benefit from the technique. A recent systematic review of the published reports to date found that there had been promising trends in overall survival in PIPAC studies, as well as evidence of histological and radiological regression of peritoneal disease [119]. The heterogeneity in the patient populations and study methods meant that only some studies were included in the weighted pooled analysis. There was objective evidence of response (partial or complete) in 57.1% in the intention to treat (ITT) group and 73.7% patients the per protocol (PP) group when assessing histological specimens. When Response Evaluation Criteria in Solid Tumours (RECIST) criteria were used, the same figures were 59.0% in the ITT, and 56.4% in the PP group.

The review included studies of advanced peritoneal disease from multiple primary diseases, and these results were also presented separately [119]. There was evidence

of objective clinical response (partial or complete) in 71% of ITT and 86% of PP colorectal cancer patients identified in the review when assessed by histological methods. The median OS in this group was 15.7 months from the start of PIPAC treatment. In ovarian cancer, the corresponding figures were 62-72% in the ITT group assessed histologically, and 62% assessed radiologically using RECIST. In the PP group the histological response rate was 75-76%, and the radiological response rate was 52%. The mean OS was 11 months, the median was 14 months. However, it should be noted that none of trials included were controlled. The fact that not all patients benefit suggests that PIPAC is only partially able overcome the barriers to effective drug delivery in peritoneal disease.

Some key areas that need to be developed and investigated further in the future include the distribution of drug within the peritoneal cavity, the choice of agent and the dose administered.

The development of the protocol for PIPAC procedures, for example the volume of drug used, the pressure applied, the duration that the aerosol is left in situ, has not been fully explained in the literature. The *in vitro* experiments performed in this project did not find any consistent trend towards increased tumour cell sensitivity to a chemotherapeutic agent with increased pressure. As already discussed, this may have been a consequence of the use of a monolayer culture system. However, this represents an important consideration going forwards. The pressure used and the duration that it is applied for may have oncological implications, but might also influence the safety and tolerability of the surgery, as well as the feasibility of performing it in a healthcare system. Investigating the effect of these parameters in pre-clinical models, as well as others such as the timing and duration of electrostatic precipitation, will be important to optimise the procedure going forwards.

Peritoneal metastases and previous surgery are both potential causes of adhesions, which may limit the even distribution of intraperitoneal therapies within the abdominal cavity. Using a laparoscopic technique may mitigate this compared with a closed technique, since some adhesions may be divisible, however it can still be a problem. Early studies of PIPAC suggested that carrying out additional surgery

(resections or CRS) in combination with drug administration conferred a high risk of serious complications [153]. This was worrying and many surgeons do not carry out significant adhesiolysis prior to drug administration in PIPAC as a result [188]. There is the need to balance the risks of visceral injury, made worse by the administration of a cytotoxic, with the need to enable even distribution of the drug within the abdomen. Another source of heterogeneity relates to the aerosol jet generated by the nebuliser device. *In vivo* and *in vitro* studies have shown there is a wide variation in droplet size produced, and a tendency for larger droplets to fall to the peritoneal surface below the end of the nebulizer or precipitate on the surface opposite the nozzle on impact [66, 83]. This has implications for the distribution of drug in the abdominal cavity, and the pharmacodynamics at the peritoneum distant to the nebuliser [69]. The introduction of electrostatic precipitation to the procedure may partly counteract this but further work is needed to understand the ability of the electrostatic forces to overcome gravitational forces acting on the aerosol droplets. Refinement and development of the nebuliser device to generate a finer and more homogenous aerosol may also improve drug delivery.

Cisplatin and doxorubicin are generally used during PIPAC treatment of ovarian cancer. This is the case even in recurrent disease that has previously been treated with platinum-based chemotherapy. Histological regression has been observed in patients with disease that has been classified as platinum resistant [153]. In initial studies, a cisplatin dose of 7.5 mg/m^2, and a doxorubicin dose of 1.5 mg/m^2 were arbitrarily selected, but a recent dose-escalation study suggested that doses of 30 mg/m^2 and 6 mg/m^2 for cisplatin and doxorubicin respectively could be tolerated [189]. Similarly, oxaliplatin is used for PIPAC treatment of colorectal peritoneal metastases, even though it is frequently part of first line chemotherapy regimens [154]. The dose reported initially, and subsequently used most commonly, was 92 mg/m^2, but a maximum tolerated dose of 135 mg/m^2 is reported [189]. The volumes used to administer the drug are small, generally 150-200ml. PIPAC therefore administers very high concentration drug solutions to the peritoneal cavity. As discussed earlier in the introduction, this may be enough to overcome the mechanisms of drug resistance in some tumours. However, the high concentrations

used within the peritoneum are not without side effects. The majority of patients treated with PIPAC report abdominal pain and gastrointestinal upset after treatment [119]. This has been severe in some cases, especially the colorectal patients treated with oxaliplatin. Cases of peritoneal sclerosis have been reported [190], as well as allergic reactions to platinum-based compounds [168]. The optimal dosing schedule will need to balance clinical efficacy with the side effect profile and resulting impact on QoL. Larger prospective controlled trials comparing different dosing regimens and assessing these outcomes will be required. The investigation of other agents would be worthwhile since they may exhibit increased efficacy with a lower dose.

There has been increasing interest and research in treatment protocols combining cycles of PIPAC with systemic chemotherapy [79, 139, 188]. Whilst there is no doubt that some spread to the peritoneum is by local extension of the tumour, e.g. in ovarian cancer, or by direct spread from the surface of a T4 tumour of the colon, there is also spread via lymphatics and by the haematogenous route. The use of intraperitoneal therapeutics risks undertreating disease that has spread by these other routes. The data presented from our own service evaluation demonstrates that isolated peritoneal disease from colorectal cancer is relatively uncommon. Peritoneal metastases were observed in conjunction with other metastases more often. Combining intraperitoneal therapy with systemic therapy is likely to achieve the best overall outcomes. The demonstration that a combined regimen is safe and tolerable will also make randomised trials a more achievable prospect, since it is likely to enable PIPAC to be used earlier in the treatment of peritoneal disease, perhaps as an adjunct to second or even first line chemotherapy in the metastatic setting.

The final area where research is needed relates to the cost of providing PIPAC and the potential cost-effectiveness of treatment. There has been little focus on this aspect of PIPAC to date, but as further evidence on the efficacy of the technique is generated it will be an important step. When using existing chemotherapeutic agents off-label, the major cost incurred during PIPAC relates to the infrastructure and staffing required to deliver the treatment. Intraperitoneal chemotherapy administration generally, but especially using the laparoscopic route as in PIPAC, is more expensive and requires greater technical expertise compared to intravenous

delivery. We modelled the cost of a PIPAC procedure for the pilot we carried out and calculated that in an NHS setting, a course of three treatments cost in the region of £15,000 -£16,000. This included, for each procedure, the cost of a pre-assessment, a 3-day hospital admission, a 90-minute laparoscopic operation, and the nebuliser device and chemotherapy drugs. It does not include the cost of additional equipment that might be required in theatre, or take into account the potential cost of adverse events. The rate of complications requiring hospitalisation after PIPAC (CTCAE grade 3 or more) is probably in the region of 10-15% [119]. This could add significant burden to the cost of treatment. Ultimately, the cost-effectiveness of PIPAC will be determined by the efficacy of the technique in prolonging survival.

Novel agents are usually more expensive. As the cost of the therapeutic itself rises, a method of administration that required a lower dose may become more cost-effective. It is possible that pressurised intraperitoneal administration will allow a lower dose of an advanced therapeutic, for example a virotherapy or a novel immunotherapy, to be administered in the same way that the dose of chemotherapy drugs has been lowered. Pressurised intraperitoneal administration may therefore have a role to play in the future, even if randomised trials and cost-effectiveness analyses find that the current use of the technique is not justified.

6.2.1 The future of PIPAC in the UK

The future of PIPAC in the UK has been determined by the recent interventional procedures guidance from NICE. Following our notification of the procedure to them in 2017, they have published their review of the evidence and their recommendation that further cases in the UK should only take place in the context of an RCT [191]. Two centres in the UK have performed cases to date. The results of an audit of the small pilot performed in Cardiff are presented here. Imperial College London has also performed a small number of cases as part of a feasibility trial (NCT03868228). The trial was paused as a result of the SARS-Cov-2 pandemic, and is unlikely to resume following the advice from NICE. There is an opportunity for these centres to lead an RCT in the UK. Depending on the inclusion criteria and primary outcomes selected,

accrual of sufficient numbers of patients is likely to require multicentre recruitment. The service evaluation we conducted suggests that a centre like Cardiff might be able to recruit one patient with isolated colorectal metastases every 1-2 months, so accrual from other centres would be necessary. There would be advantages to restricting the conduct of PIPAC cases to the two current centres. It would enable both to build on their existing experience and infrastructure and might result in greater consistency of care in the PIPAC arm, which would be beneficial. The disadvantages would be that inclusion in the trial might involve a significant travel burden for patients, and if the trial found a favourable outcome for PIPAC then other centres would not be in a position to offer the treatment to patients immediately.

6.3 Innovation in surgery: PIPAC illustrates the ongoing challenges

The review of the introduction of PIPAC showed that it broadly followed the IDEAL paradigm, as the number of publications increased each year, and the types of study being developed and completed gradually evolved to incorporate more of the Development and Evaluation characteristics. We updated the search and evaluation of the literature after the PIPAC pilot was underway, and found that the pattern observed initially was continuing [92].

However, although the IDEAL paradigm appears to describe the process of research in the evolution of PIPAC, the early investigators did not reference the paradigm. This may be because the IDEAL collaboration was a UK-based initiative and the research output for PIPAC to date has been mostly from Europe. The clinicians involved may not have been aware of the paradigm and its recommendations. Additionally, there are some signs, that on an international level, there are still barriers to following the paradigm in a coordinated manner. The governance and regulations surrounding a procedure such as PIPAC, concerning the novel use of a licensed drug, the use of a new device, and the occupational health and safety legislation covering drug exposure in the workplace, vary from country to country. This may be one reason why there were many stage 1 ‘Idea’-type studies, even though the characteristics

described by the collaboration suggest that this type of study would not usually be repeated.

The IDEAL framework recommends that trial protocols are publicly registered and advocates the reporting of the results from new procedures on online registers available to all surgeons [4]. This is to enable progression through the stages as efficiently as possible. Not all PIPAC centres have followed this recommendation. In our updated search in 2019, we identified 67 clinical studies published, but only 23 registered on ClinicalTrials.gov and Eudract [92]. It may be therefore that because protocols were not available, many of the 'Idea' type studies were conceived in parallel, before the results of previous studies were published. Another possibility is that the work on safety and feasibility was purposely repeated because clinicians had doubts about the existing evidence and its validity or wanted to confirm the results before moving forward. The difficulty of overcoming the learning curve associated with a new procedure has been described as a potential barrier to effective research in surgery in the past [5, 6]. In the case of PIPAC, it may have been that surgeons and their wider multidisciplinary teams wanted to be confident in their ability to deliver the treatment before joining a larger study and saw a feasibility study as a way to achieve this. The technical learning curve in PIPAC can be overcome quickly, as an experienced laparoscopic surgeon will already have the skills required to perform the procedure. The area where a greater learning curve exists in PIPAC relates to patient selection and the perioperative medicine aspects of the procedure since PIPAC involves a cohort of patients that would not usually have surgery, and who may be frail. It is possible that this is a technique where early involvement in a trial will be helpful for new adopters in future, as a well-written protocol would stipulate which patients should be treated and the pre- and post-operative management.

Other reasons for the duplication of small safety and feasibility studies may relate to the availability of funding. Many of the early reports of PIPAC in the literature were small case series of patients treated on a compassionate/off-label basis outside of a formal trial using institutional funds. This may reflect hesitancy from bodies awarding grants to fund a small feasibility and safety study for a new surgical technique. Early trials of new drugs are often supported by industry by the provision of the therapeutic

agent used in the trial. This is often the most expensive element. Surgical trials are different. Whilst the price of the nebuliser necessary for PIPAC is not insignificant, the need for multiple admissions to hospital and the theatre time associated with PIPAC are the major costs involved in treatment. Therefore, even with support from the manufacturer, PIPAC trials are costly and represent a higher risk to an awarding body. The IDEAL collaboration referenced this problem when they appealed to independent funders to factor in the cost of feasibility studies and support new techniques through early research so that later stage trials could take place [91]. It is understandable that government or charity funders are reluctant to invest the money required to deliver a large surgical trial in the early stages of a new technique. However, the result of limited funding is that, as in the case of PIPAC, less powerful studies are duplicated and the effect of the treatment on the outcome of interest may not be determined. This is likely to be an ongoing problem in surgical research.

PIPAC is now moving into the later stages of the IDEAL paradigm. There are still challenges related to achieving the aims stated in the 'Exploration' and 'Assessment' stages. A multicentre approach is likely to be needed in order to recruit adequate patient numbers for an RCT in a timely manner. PIPAC is unusual because it is a technique where multiple variations are possible, for example by changing the drug, dose, and duration of operation, as well as the timing of the intervention in the clinical pathway. Therefore, as well as ongoing approval from regulatory bodies, there will need to be consensus from professional bodies about these elements. PIPAC represents a novel drug delivery system. Future studies may encompass all stages of the IDEAL paradigm depending on whether they are building on evidence for an existing drug, changing the indication for treatment, or investigating a new therapy.

6.4 Pressurised intraperitoneal aerosolisation as a method of administration for oncolytic adenoviruses

This project has examined pressurised intraperitoneal administration of two different therapeutic agents; chemotherapy and oncolytic viruses. Whilst there was a body of

existing pre-clinical and clinical work relating to the administration of chemotherapy using this delivery method, there were no previous reports of the use of the device to administer viral vectors. The finding that the adenovirus vectors tested retained their capacity to infect and transduce cells following aerosolisation using the CapnoPen device is important. We made significant progress towards the assessment of the delivery of adenovirus vectors using pressurised intraperitoneal aerosolisation in a rat model. We were in the process of optimising this model when work was suspended because of the SARS-Cov-2 pandemic. The next experiment that was planned and approved by the ethics committee at the University of Ghent was a further small pilot study comparing the administration of the higher dose ($1x10^{11}$vp) of Ad5.Luc using pressurised intraperitoneal aerosolisation versus a simple intraperitoneal injection. Provided this was tolerated, the aim was to carry out further work using the rat model of peritoneal metastases developed by the University of Ghent team in order to assess the method for the delivery of oncolytic adenoviral vectors.

The comparison of intraperitoneal aerosolisation, intraperitoneal lavage, and intravenous administration is an important step in determining how to take these vectors forward into the clinic. The limitations inherent in an Ad5-based oncolytic therapy relating to pre-existing immunity have already been discussed, as have the problems with systemic administration of therapeutics to target peritoneal disease. Intraperitoneal administration may confer advantages for oncolytic virus therapies in treating peritoneal disease in the same way that it does for chemotherapy.

The relative effects of intraperitoneal administration versus systemic administration in terms of the immune response still need further investigation. Neutralising antibodies are present in the blood and in peritoneal fluid of individuals who have had previous exposure to a virus, and currently this may be overcome by modifying the therapeutic vector [112]. It is not clear whether draining any peritoneal fluid/ascites present would mitigate the effect of such antibodies sufficiently to increase the potential of the virus to enter the target cells. Early clinical trials using other viral vectors, for example the vaccina virus-based GL-ONC1, indicate that application to the peritoneal cavity results in rapid generation (over days) of

neutralising antibodies in patients who were previously naïve to the vector [192]. Further work will be required to determine whether intraperitoneal administration confers any advantage over intravenous delivery for peritoneal disease in terms of the safety profile relating to the systemic inflammatory response, and in the ability to carry out repeated administrations. Much of this work will need to be carried out in clinical trials, since assessment in animal models of intraabdominal cancer is limited by the immunodeficiency required to generate tumour xenografts. Ultimately, the strategies used to engineer the vector, and the properties conferred are likely to be the major determinant of the optimal delivery method.

6.5 Conclusion

Peritoneal metastases are a common problem since they occur in many primary cancer types. They represent a clinical challenge because the anatomy and physiology of the peritoneum renders the mesothelial surface relatively insensitive to systemically administered therapeutics. In the past, the discovery of peritoneal metastases was considered a pre-terminal stage of disease. Modern chemotherapy and the development of other biological therapies, as well as the advancement of surgical techniques and perioperative medicine, mean that disease control is the intention of treatment in more patients with peritoneal disease. However, overall, survival remains poor in most primary tumour types. New therapeutic options are needed.

Intraperitoneal chemotherapy has pharmacodynamic advantages over intravenously delivered chemotherapy for peritoneal disease. PIPAC is a novel technique which may be more effective than simple lavage because of the potential to increase drug penetration, as demonstrated in preclinical trials. There has been a rapid expansion in the literature surrounding the procedure and the administration of specific chemotherapy drugs has been demonstrated to be feasible and safe using the technology. RCTs should now be undertaken to determine the efficacy. A pilot at CAV UHB has demonstrated that PIPAC is acceptable to patients and clinicians in an NHS setting, and the unit is now in a position to be a UK centre for research into the

technique and offer the treatment in the context of a trial, as per the NICE recommendation.

Pressurised intraperitoneal aerosolisation can be regarded as a drug delivery system for the peritoneal cavity. Whilst focus was initially on well-established chemotherapy regimens, there is increasing interest in using the technique to deliver other therapeutics. Oncolytic adenoviruses are a promising strategy for cancer treatment, as they have the potential to be better targeted than standard chemotherapy agents, to amplify the therapeutic effect in the target tissue, both by cell lysis and release of progeny, and by generating tumour antigens and triggering an anti-tumour immune response. Intraperitoneal administration of an oncolytic virus may be advantageous to treat peritoneal disease, so the finding that adenovirus vectors can survive aerosolisation and still transduce their target cells is important. Further work is warranted to investigate pressurised intraperitoneal aerosolisation as an administration method for oncolytic viruses, and other advanced therapeutics.

7 References

1. Solass, W., et al., *Description of a novel approach for intraperitoneal drug delivery and the related device.* Surg Endosc, 2012. **26**(7): p. 1849-55.

2. Reymond, M.A., et al., *Feasibility of therapeutic pneumoperitoneum in a large animal model using a microvaporisator.* Surg Endosc, 2000. **14**(1): p. 51-5.

3. Solass, W., et al., *Intraperitoneal chemotherapy of peritoneal carcinomatosis using pressurized aerosol as an alternative to liquid solution: First evidence for efficacy and tolerability.* Surgical Endoscopy and Other Interventional Techniques, 2013. **1)**: p. S471.

4. McCulloch, P., et al., *No surgical innovation without evaluation: the IDEAL recommendations.* Lancet, 2009. **374**(9695): p. 1105-12.

5. Barkun, J.S., et al., *Evaluation and stages of surgical innovations.* Lancet, 2009. **374**(9695): p. 1089-96.

6. Ergina, P.L., et al., *Challenges in evaluating surgical innovation.* Lancet, 2009. **374**(9695): p. 1097-104.

7. Shariati, M., et al., *High Pressure Nebulization (PIPAC) Versus Injection for the Intraperitoneal Administration of mRNA Complexes.* Pharm Res, 2019. **36**(9): p. 126.

8. Moore KL and Dalley AF, *Clinically Oriented Anatomy*. 4th ed. 1999, Baltimore, USA: Lippincott, Williams, and Wilkins.

9. Solass, W., et al., *Functional vascular anatomy of the peritoneum in health and disease.* Pleura and Peritoneum, 2016. **1**(3): p. 145-158.

10. Ceelen, W.P. and M.E. Bracke, *Peritoneal minimal residual disease in colorectal cancer: mechanisms, prevention, and treatment.* The Lancet Oncology, 2009. **10**(1): p. 72-79.

11. Narasimhan, V., et al., *Colorectal peritoneal metastases: pathogenesis, diagnosis and treatment options - an evidence-based update.* ANZ journal of surgery, 2020. **90**(9): p. 1592-1597.

12. Li, W., et al., *Molecular alterations of cancer cell and tumour microenvironment in metastatic gastric cancer.* Oncogene, 2018. **37**(36): p. 4903-4920.

13. Gerber, S.A., et al., *Preferential attachment of peritoneal tumor metastases to omental immune aggregates and possible role of a unique vascular microenvironment in metastatic survival and growth.* Am J Pathol, 2006. **169**(5): p. 1739-52.

14. Heath, R.M., et al., *Tumour-induced apoptosis in human mesothelial cells: a mechanism of peritoneal invasion by Fas Ligand/Fas interaction.* Br J Cancer, 2004. **90**(7): p. 1437-42.

15. Gospodarowicz, M.K., J.D. Brierley, and C. Wittekind, *TNM classification of malignant tumours*. 2017: John Wiley & Sons.

16. Low, R.N. and R.M. Barone, *Imaging for Peritoneal Metastases.* Surgical Oncology Clinics of North America, 2018. **27**(3): p. 425-442.

17. Spiliotis, J., E. Halkia, and E. de Bree, *Treatment of peritoneal surface malignancies with hyperthermic intraperitoneal chemotherapy-current perspectives.* Curr Oncol, 2016. **23**(3): p. e266-75.

18. Sugarbaker, P.H., *Peritonectomy procedures.* Annals of surgery, 1995. **221**(1): p. 29-42.

19. Franko, J., et al., *Prognosis of patients with peritoneal metastatic colorectal cancer given systemic therapy: an analysis of individual patient data from prospective randomised trials from the Analysis and Research in Cancers of the Digestive System (ARCAD) database.* Lancet Oncol, 2016. **17**(12): p. 1709-1719.

20. Flessner, M.F., *The transport barrier in intraperitoneal therapy.* Am J Physiol Renal Physiol, 2005. **288**(3): p. F433-42.

21. Dedrick, R.L., et al., *Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer.* Cancer Treat Rep, 1978. **62**(1): p. 1-11.

22. Dedrick, R.L. and M.F. Flessner, *Pharmacokinetic problems in peritoneal drug administration: tissue penetration and surface exposure.* J Natl Cancer Inst, 1997. **89**(7): p. 480-7.

23. Alphonsus, C.S. and R.N. Rodseth, *The endothelial glycocalyx: a review of the vascular barrier.* Anaesthesia, 2014. **69**(7): p. 777-784.

24. Li, X.F., et al., *Visualization of hypoxia in microscopic tumors by immunofluorescent microscopy.* Cancer Res, 2007. **67**(16): p. 7646-53.

25. Avril, T., E. Vauléon, and E. Chevet, *Endoplasmic reticulum stress signaling and chemotherapy resistance in solid cancers.* Oncogenesis, 2017. **6**(8): p. e373-e373.

26. Behrens, B.C., et al., *Characterization of a cis-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues.* Cancer Res, 1987. **47**(2): p. 414-8.

27. Johnson, S.W., et al., *Relationship between platinum-DNA adduct formation and removal and cisplatin cytotoxicity in cisplatin-sensitive and -resistant human ovarian cancer cells.* Cancer Res, 1994. **54**(22): p. 5911-6.

28. Flessner, M.F., et al., *A distributed model of peritoneal-plasma transport: tissue concentration gradients.* Am J Physiol, 1985. **248**(3 Pt 2): p. F425-35.

29. Ceelen, W.P. and M.F. Flessner, *Intraperitoneal therapy for peritoneal tumors: biophysics and clinical evidence.* Nat Rev Clin Oncol, 2010. **7**(2): p. 108-15.

30. Elias, D., M. Bonnay, and J.M. Puizillou, *Heated intra-operative intraperitoneal oxaliplatin after complete resection of peritoneal carcinomatosis: Pharmacokinetics and tissue distribution.* Ann Oncol, 2002. **13**.

31. Markman, M., *Intraperitoneal antineoplastic agents for tumors principally confined to the peritoneal cavity.* Cancer Treatment Reviews, 1986. **13**(4): p. 219-242.

32. Heldin, C.H., et al., *High interstitial fluid pressure - an obstacle in cancer therapy.* Nat Rev Cancer, 2004. **4**(10): p. 806-13.

33. Dedrick, R.L., *Theoretical and experimental bases of intraperitoneal chemotherapy.* Seminars in oncology, 1985. **12**(3 Suppl 4): p. 1-6.

34. van de Vaart, P.J., et al., *Intraperitoneal cisplatin with regional hyperthermia in advanced ovarian cancer: pharmacokinetics and cisplatin-DNA adduct formation in patients and ovarian cancer cell lines.* Eur J Cancer, 1998. **34**(1): p. 148-54.

35. Meyn, R.E., et al., *Thermal enhancement of DNA damage in mammalian cells treated with cis-diamminedichloroplatinum(II).* Cancer Res, 1980. **40**(4): p. 1136-9.

36. Miyahara, T., et al., *Hyperthermic enhancement of cytotoxicity and increased uptake of cis-diamminedichloroplatinum(II) in cultured human esophageal cancer cells.* Jpn J Cancer Res, 1993. **84**(3): p. 336-40.

37. Los, G., et al., *A rationale for carboplatin treatment and abdominal hyperthermia in cancers restricted to the peritoneal cavity.* Cancer Res, 1992. **52**(5): p. 1252-8.

38. Esquis, P., et al., *High intra-abdominal pressure enhances the penetration and antitumor effect of intraperitoneal cisplatin on experimental peritoneal carcinomatosis.* Ann Surg, 2006. **244**(1): p. 106-12.

39. Facy, O., et al., *High pressure enhances the effect of hyperthermia in intraperitoneal chemotherapy with oxaliplatin: an experimental study.* Ann Surg, 2012. **256**(6): p. 1084-8.

40. Facy, O., et al., *High pressure does not counterbalance the advantages of open techniques over closed techniques during heated intraperitoneal chemotherapy with oxaliplatin.* Surgery, 2015. **157**(1): p. 72-8.

41. Jeffery, M., B.E. Hickey, and P.N. Hider, *Follow-up strategies for patients treated for non-metastatic colorectal cancer.* Cochrane Database Syst Rev, 2007(1): p. CD002200.

42. Cancer Research UK. *Bowel Cancer*. [cited 2021 2nd February]; Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer.

43. American Joint Committee on Cancer, *AJCC Cancer Staging Manual*. 8th Edition ed. 2016: Springer.

44. Thomassen, I., et al., *Incidence, prognosis, and treatment options for patients with synchronous peritoneal carcinomatosis and liver metastases from colorectal origin.* Dis Colon Rectum, 2013. **56**(12): p. 1373-80.

45. Lemmens, V.E., et al., *Predictors and survival of synchronous peritoneal carcinomatosis of colorectal origin: a population-based study.* Int J Cancer, 2011. **128**(11): p. 2717-25.

46. van Gestel, Y.R., et al., *Metachronous peritoneal carcinomatosis after curative treatment of colorectal cancer.* Eur J Surg Oncol, 2014. **40**(8): p. 963-9.

47. Lurvink, R.J., et al., *Increase in the incidence of synchronous and metachronous peritoneal metastases in patients with colorectal cancer: A nationwide study.* Eur J Surg Oncol, 2020.

48. National Institute for Health and Care Excellence. *Colorectal Cancer NICE guideline NG151*. 2020 [cited 2021 6th February]; Available from: https://www.nice.org.uk/Guidance/NG151.

49. National Institute for Health and Care Excellence, *Guidance on the use of capecitabine and tegafur with uracil for metastatic colorectal cancer. Technology appraisal guidance [TA61]*. 2003.

50. Steffens, D., et al., *Quality of Life After Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy: Early Results from a Prospective Cohort Study of 115 Patients.* Annals of Surgical Oncology, 2020. **27**(10): p. 3986-3994.

51. Verwaal, V.J., et al., *Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer.* J Clin Oncol, 2003. **21**(20): p. 3737-43.

52. Quénet, F., et al., *Cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy versus cytoreductive surgery alone for colorectal peritoneal metastases (PRODIGE 7): a multicentre, randomised, open-label, phase 3 trial.* Lancet Oncol, 2021. **22**(2): p. 256-266.

53. Liberale, G., L. Ameye, and A. Hendlisz, *PRODIGE 7 should be interpreted with caution.* Acta Chir Belg, 2019. **119**(4): p. 263-266.

54. National Cancer Registration and Analysis Service. *Routes to diagnosis 2006-2016*. [cited 2021 6th February]; Available from: http://www.ncin.org.uk/publications/routes_to_diagnosis.

55. Office for National Statistics, *Cancer survival in England 2013-2017*. 2019.

56. Piso, P. and D. Arnold, *Multimodal Treatment Approaches for Peritoneal Carcinosis in Colorectal Cancer.* Deutsches Ärzteblatt International, 2011. **108**(47): p. 802-808.

57. National Cancer Registration and Analysis Service. *Cancer Breakdown by Stage CCG 2015*. 2015 [cited 2017 10th August]; Available from: http://www.ncin.org.uk/publications/survival_by_stage.

58. National Institute for Health and Care Excellence, *Ovarian Cancer: recognition and initial management CG122*. 2011.

59. Al Rawahi, T., et al., *Surgical cytoreduction for recurrent epithelial ovarian cancer.* Cochrane Database of Systematic Reviews, 2013(2).

60. Hanker, L.C., et al., *The impact of second to sixth line therapy on survival of relapsed ovarian cancer after primary taxane/platinum-based therapy.* Annals of Oncology, 2012. **23**(10): p. 2605-2612.

61. Bijelic, L., A. Jonson, and P.H. Sugarbaker, *Systematic review of cytoreductive surgery and heated intraoperative intraperitoneal chemotherapy for treatment of peritoneal*

carcinomatosis in primary and recurrent ovarian cancer. Ann Oncol, 2007. **18**(12): p. 1943-50.

62. Jaaback, K., N. Johnson, and T.A. Lawrie, *Intraperitoneal chemotherapy for the initial management of primary epithelial ovarian cancer.* Cochrane Database of Systematic Reviews, 2016(1).

63. Wenzel, L.B., et al., *Health-related quality of life during and after intraperitoneal versus intravenous chemotherapy for optimally debulked ovarian cancer: a Gynecologic Oncology Group Study.* J Clin Oncol, 2007. **25**(4): p. 437-43.

64. Solass, W., et al., *Intraperitoneal chemotherapy of peritoneal carcinomatosis using pressurized aerosol as an alternative to liquid solution: First evidence for efficacy.* Annals of Surgical Oncology, 2014. **21**(2): p. 553-559.

65. Solass, W., et al., *Therapeutic approach of human peritoneal carcinomatosis with Dbait in combination with capnoperitoneum: proof of concept.* Surgical Endoscopy, 2012. **26**(3): p. 847-852.

66. Khosrawipour, V., et al., *Exploring the Spatial Drug Distribution Pattern of Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC).* Ann Surg Oncol, 2016. **23**(4): p. 1220-4.

67. Khosrawipour, V., et al., *Evaluating the Effect of Micropump (c) Position, Internal Pressure and Doxorubicin Dosage on Efficacy of Pressurized Intra-peritoneal Aerosol Chemotherapy (PIPAC) in an Ex Vivo Model.* Anticancer Research, 2016. **36**(9): p. 4595-4600.

68. Khosrawipour, V., et al., *Distribution pattern and penetration depth of doxorubicin after pressurized intraperitoneal aerosol chemotherapy (PIPAC) in a postmortem swine model.* Journal of Cancer Research and Clinical Oncology, 2016. **142**(11): p. 2275-2280.

69. Gohler, D., et al., *Technical description of the microinjection pump (MIP(R)) and granulometric characterization of the aerosol applied for pressurized intraperitoneal aerosol chemotherapy (PIPAC).* Surg Endosc, 2016.

70. Gohler, D., et al., *Hyperthermic intracavitary nanoaerosol therapy (HINAT) as an improved approach for pressurised intraperitoneal aerosol chemotherapy (PIPAC): Technical description, experimental validation and first proof of concept.* Beilstein Journal of Nanotechnology, 2017. **8**: p. 2729-2740.

71. Jung, D.H., et al., *Feasibility of hyperthermic pressurized intraperitoneal aerosol chemotherapy in a porcine model.* Surg Endosc, 2015.

72. Tempfer, C.B., et al., *Pressurized intraperitoneal aerosol chemotherapy in women with recurrent ovarian cancer: A phase 2 study.* Gynecologic Oncology, 2015. **137**(2): p. 223-228.

73. Khomyakov, V., et al., *Initial experience of pressurized intraperitoneal aerosol chemotherapy (PIPAC) for treatment of peritoneally disseminated gastric cancer.* Annals of Oncology, 2016. **27**: p. 51-51.

74. Graversen, M., et al., *Peritoneal metastasis from pancreatic cancer treated with pressurized intraperitoneal aerosol chemotherapy (PIPAC).* Clinical and Experimental Metastasis, 2017. **34**(5): p. 309-314.

75. Willaert, W., P. Sessink, and W. Ceelen, *Occupational safety of pressurized intraperitoneal aerosol chemotherapy (PIPAC).* Pleura and Peritoneum, 2017. **2**(3): p. 121-128.

76. Sabaila, A., A. Fauconnier, and C. Huchon, *Pressurized intraperitoneal aerosol chemotherapy (PIPAC): A new way of administration in peritoneal carcinomatosis of ovarian cancer.* Gynecologie Obstetrique & Fertilite, 2015. **43**(1): p. 66-67.

77. Teixeira Farinha, H., et al., *Impact of Pressurized Intraperitoneal Aerosol Chemotherapy on Quality of Life and Symptoms in Patients with Peritoneal Carcinomatosis: A Retrospective Cohort Study.* Gastroenterology Research and Practice, 2017. **2017**: p. 1-10.

78. Hubner, M., et al., *Feasibility and Safety of Pressurized Intraperitoneal Aerosol Chemotherapy for Peritoneal Carcinomatosis: A Retrospective Cohort Study.* Gastroenterology Research and Practice, 2017. **2017**(6852749).

79. Robella, M., M. Vaira, and M. De Simone, *Safety and feasibility of pressurized intraperitoneal aerosol chemotherapy (PIPAC) associated with systemic chemotherapy: an innovative approach to treat peritoneal carcinomatosis.* World J Surg Oncol, 2016. **14**: p. 128.

80. Ansell, J., et al., *Electrostatic precipitation is a novel way of maintaining visual field clarity during laparoscopic surgery: a prospective double-blind randomized controlled pilot study.* Surg Endosc, 2014. **28**(7): p. 2057-65.

81. Ansell, J., et al., *The innervision surgical smoke removal system.* International Journal of Surgery, 2012. **10**: p. S43.

82. Kakchekeeva, T., et al., *In Vivo Feasibility of Electrostatic Precipitation as an Adjunct to Pressurized Intraperitoneal Aerosol Chemotherapy (ePIPAC).* Ann Surg Oncol, 2016.

83. Bellendorf, A., et al., *Scintigraphic peritoneography reveals a non-uniform ^{99m}Tc-Pertechnetat aerosol distribution pattern for Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC) in a swine model.* Surgical Endoscopy, 2018. **32**(1): p. 166-174.

84. Reymond, M., et al., *Electrostatic precipitation Pressurized IntraPeritoneal Aerosol Chemotherapy (ePIPAC): first in-human application*, in *Pleura and Peritoneum*. 2016. p. 109.

85. Krummel, T.M., *What is surgery?* Semin Pediatr Surg, 2006. **15**(4): p. 237-41.

86. Biffl, W.L., et al., *Responsible development and application of surgical innovations: a position statement of the Society of University Surgeons.* J Am Coll Surg, 2008. **206**(6): p. 1204-9.

87. Meakins, J.L., *Surgical research: act 3, answers.* Lancet, 2009. **374**(9695): p. 1039-40.

88. Potter, S., et al., *Time to be BRAVE: is educating surgeons the key to unlocking the potential of randomised clinical trials in surgery? A qualitative study.* Trials, 2014. **15**(1): p. 80.

89. Farrokhyar, F., et al., *Randomized controlled trials of surgical interventions.* Annals of surgery, 2010. **251**(3): p. 409-416.

90. Johnson, J., et al., *Ethical challenges of innovative surgery: a response to the IDEAL recommendations.* Lancet, 2010. **376**(9746): p. 1113-5.

91. Hirst, A., et al., *No Surgical Innovation Without Evaluation: Evolution and Further Development of the IDEAL Framework and Recommendations.* Ann Surg, 2019. **269**(2): p. 211-220.

92. Tate, S.J. and J. Torkington, *Pressurized intraperitoneal aerosol chemotherapy: a review of the introduction of a new surgical technology using the IDEAL framework.* BJS Open, 2020. **4**(2): p. 206-215.

93. McCulloch, P., et al., *Progress in clinical research in surgery and IDEAL.* Lancet, 2018. **392**(10141): p. 88-94.

94. Zheng, M., et al., *Oncolytic Viruses for Cancer Therapy: Barriers and Recent Advances.* Molecular Therapy - Oncolytics, 2019. **15**: p. 234-247.

95. US Food and Drug Administration. *IMLYGIC (talimogene laherparepvec).* [cited 2019 5th February]; Available from: https://www.fda.gov/biologicsbloodvaccines/cellulargenetherapyproducts/approvedproducts/ucm469411.htm.

96. Rowe, W.P., et al., *Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture.* Proc Soc Exp Biol Med, 1953. **84**(3): p. 570-3.

97. Uusi-Kerttula, H., et al., *Oncolytic Adenovirus: Strategies and Insights for Vector Design and Immuno-Oncolytic Applications.* Viruses, 2015. **7**(11): p. 6009-42.

98. Russell, W.C., *Adenoviruses: update on structure and function.* J Gen Virol, 2009. **90**(Pt 1): p. 1-20.

99. Waye, M.M.Y. and C.W. Sing, *Anti-Viral Drugs for Human Adenoviruses.* Pharmaceuticals, 2010. **3**(10).

100. Kirby, I., et al., *Identification of contact residues and definition of the CAR-binding site of adenovirus type 5 fiber protein.* J Virol, 2000. **74**(6): p. 2804-13.

101. Baker, A.T., et al., *Human adenovirus type 26 uses sialic acid-bearing glycans as a primary cell entry receptor.* Sci Adv, 2019. **5**(9): p. eaax3567.

102. Wickham, T.J., et al., *Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment.* Cell, 1993. **73**(2): p. 309-19.

103. Bergelson, J.M., et al., *Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5.* Science, 1997. **275**(5304): p. 1320-3.

104. Bett, A.J., L. Prevec, and F.L. Graham, *Packaging capacity and stability of human adenovirus type 5 vectors.* J Virol, 1993. **67**(10): p. 5911-21.

105. Coughlan, L., et al., *Ad5:Ad48 hexon hypervariable region substitutions lead to toxicity and increased inflammatory responses following intravenous delivery.* Mol Ther, 2012. **20**(12): p. 2268-81.

106. Mast, T.C., et al., *International epidemiology of human pre-existing adenovirus (Ad) type-5, type-6, type-26 and type-36 neutralizing antibodies: correlates of high Ad5 titers and implications for potential HIV vaccine trials.* 2010. **28**(4): p. 950-957.

107. Barouch, D.H., et al., *International seroepidemiology of adenovirus serotypes 5, 26, 35, and 48 in pediatric and adult populations.* 2011. **29**(32): p. 5203-5209.

108. Gahéry-Ségard, H., et al., *Immune response to recombinant capsid proteins of adenovirus in humans: antifiber and anti-penton base antibodies have a synergistic effect on neutralizing activity.* J Virol, 1998. **72**(3): p. 2388-97.

109. Parker, A.L., et al., *Multiple vitamin K-dependent coagulation zymogens promote adenovirus-mediated gene delivery to hepatocytes.* Blood, 2006. **108**(8): p. 2554-61.

110. Reeh, M., et al., *Presence of the Coxsackievirus and Adenovirus Receptor (CAR) in human neoplasms: a multitumour array analysis.* British Journal of Cancer, 2013. **109**(7): p. 1848-1858.

111. Carlisle, R.C., et al., *Human erythrocytes bind and inactivate type 5 adenovirus by presenting Coxsackie virus-adenovirus receptor and complement receptor 1.* Blood, 2009. **113**(9): p. 1909-18.

112. Uusi-Kerttula, H., et al., *Ad5NULL-A20: A Tropism-Modified, alphavbeta6 Integrin-Selective Oncolytic Adenovirus for Epithelial Ovarian Cancer Therapies.* Clin Cancer Res, 2018. **24**(17): p. 4215-4224.

113. Häkkinen, L., et al., *Immunolocalization of tenascin-C, α9 integrin subunit, and αvβ6 integrin during wound healing in human oral mucosa.* Journal of Histochemistry & Cytochemistry, 2000. **48**(7): p. 985-998.

114. Van Aarsen, L.A.K., et al., *Antibody-mediated blockade of integrin αvβ6 inhibits tumor progression in vivo by a transforming growth factor-β–regulated mechanism.* Cancer research, 2008. **68**(2): p. 561-570.

115. Lehrman, S., *Virus treatment questioned after gene therapy death.* Nature, 1999. **401**(6753): p. 517-8.

116. Uusi-Kerttula, H., et al., *Incorporation of Peptides Targeting EGFR and FGFR1 into the Adenoviral Fiber Knob Domain and Their Evaluation as Targeted Cancer Therapies.* Hum Gene Ther, 2015. **26**(5): p. 320-9.

117. Jacquet P and Sugarbaker PH, *Clinical research methodologies in diagnosis and staging of patients with peritoneal carcinomatosis.* , in *Peritoneal carcinomatosis: principles of management.*, S. PH, Editor. 1996, Kluwer Academic publishers: Boston. p. 359-374.

118. Giger-Pabst, U. and C.B. Tempfer, *How to Perform Safe and Technically Optimized Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC): Experience After a Consecutive Series of 1200 Procedures.* Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract, 2018. **22**(12): p. 2187-2193.

119. Alyami, M., et al., *Pressurised intraperitoneal aerosol chemotherapy: rationale, evidence, and potential indications.* The Lancet Oncology, 2019. **20**(7): p. e368-e377.

120. Hubner, M., et al., *Pressurized IntraPeritoneal Aerosol Chemotherapy - Practical aspects.* European Journal of Surgical Oncology, 2017. **43**(6): p. 1102-1109.

121. Langdon, S.P., et al., *Characterization and properties of nine human ovarian adenocarcinoma cell lines.* Cancer Res, 1988. **48**(21): p. 6166-72.

122. Berridge, M.V. and A.S. Tan, *Characterization of the Cellular Reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular Localization, Substrate Dependence, and Involvement of Mitochondrial Electron Transport in MTT Reduction.* Archives of Biochemistry and Biophysics, 1993. **303**(2): p. 474-482.

123. Bernabei, P.A., et al., *In vitro chemosensitivity testing of leukemic cells: development of a semiautomated colorimetric assay.* Hematol Oncol, 1989. **7**(3): p. 243-53.

124. Stanton, R.J., et al., *Re-engineering adenovirus vector systems to enable high-throughput analyses of gene function.* Biotechniques, 2008. **45**(6): p. 659-62, 664-8.

125. Public Health Wales. *Welsh Cancer Intelligence and Surveillance Unit (WCISU)*. [cited 2020 21st October]; Available from: https://phw.nhs.wales/services-and-teams/welsh-cancer-intelligence-and-surveillance-unit-wcisu/about-us/.

126. Segelman, J., et al., *Incidence, prevalence and risk factors for peritoneal carcinomatosis from colorectal cancer.* Br J Surg, 2012. **99**(5): p. 699-705.

127. NHS Commissioning Board. *Clinical Commissioning Policy: Cytoreduction surgery for patients with peritoneal carcinomatosis NHSCB/A08/P/a*. 2013 [cited 2021 6th February]; Available from: https://www.england.nhs.uk/commissioning/wp-content/uploads/sites/12/2013/09/a08-p-a.pdf.

128. Welsh Health Specialised Services Committee, *Policy Position: Cytoreductive Surgery with Hyperthermic Intraperitoneal Chemotherapy for Peritoneal Carcinomatosis*. 2015.

129. Welsh Cancer Intelligence and Surveillance Unit (WCISU). *Cancer incidence in Wales, 2001-2017*. 2019 [cited 2021 6th February]; Available from: https://phw.nhs.wales/services-and-teams/welsh-cancer-intelligence-and-surveillance-unit-wcisu/cancer-incidence-in-wales-2001-2017/.

130. Public Health Wales. *Welsh Cancer Intelligence and Surveillance Unit: Cancer incidence by stage at diagnosis*. [cited 2021 6th February]; Available from: https://publichealthwales.shinyapps.io/Cancer_incidence_in_Wales_2001-2017/.

131. Public Health Wales, *Bowel Screening Wales Annual Statistical Report 2018-2019*. 2020.

132. Public Health England. *Cancer Services Data: Demographics, Screening and Diagnostics*. [cited 2021 6th February]; Available from: https://fingertips.phe.org.uk/profile/cancerservices/data#page/0/gid/1938132830/pat/15/par/E92000001/ati/46/are/E39000026/iid/92601/age/280/sex/4/cid/4/page-options/cin-ci-4_ovw-do-0.

133. NBOCA Project Team. *National Bowel Cancer Audit Annual Report 2020*. 2020 [cited 2021 6th February]; Available from: https://www.nboca.org.uk/content/uploads/2020/12/NBOCA-2020-Annual-Report.pdf.

134. Wales Cancer Network. *Colorectal Referral Guidelines*. 2015 [cited 2021 6th February]; Available from: http://www.cancerservicesdirectory.wales.nhs.uk/colorectal-referral-guidelines.

135. Mauri, G., et al., *Early-onset colorectal cancer in young individuals.* Molecular oncology, 2019. **13**(2): p. 109-131.

136. Tsai, M.-S., et al., *Clinicopathological Features and Prognosis in Resectable Synchronous and Metachronous Colorectal Liver Metastasis.* Annals of Surgical Oncology, 2007. **14**(2): p. 786-794.

137. Hentzen, J.E.K.R., et al., *Impact of Synchronous Versus Metachronous Onset of Colorectal Peritoneal Metastases on Survival Outcomes After Cytoreductive Surgery (CRS) with Hyperthermic Intraperitoneal Chemotherapy (HIPEC): A Multicenter, Retrospective, Observational Study.* Annals of surgical oncology, 2019. **26**(7): p. 2210-2221.

138. Tempfer, C.B., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC) with cisplatin and doxorubicin in a woman with pseudomyxoma peritonei: A case report.* Gynecologic Oncology Reports, 2014. **10**: p. 32-35.

139. Khomyakov, V., et al., *Bidirectional chemotherapy in gastric cancer with Peritoneal metastasis combining intravenous XELOX with IntraPeritoneal chemotherapy with low-dose cisplatin and doxorubicin administered as a Pressurized aerosol: An open-label, Phase-2 study (PIPAC-GA2).* Pleura and Peritoneum, 2016. **1**(3): p. 159-166.

140. Vaira, M., et al., *Single-port access for Pressurized IntraPeritoneal Aerosol Chemotherapy (PIPAC): Technique, feasibility and safety.* Pleura and Peritoneum, 2016. **1**(4): p. 217-222.

141. Khosrawipour, V., et al., *Effect of Irradiation on Tissue Penetration Depth of Doxorubicin after Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC) in a Novel Ex-Vivo Model.* Journal of Cancer, 2016. **7**(8): p. 910-914.

142. Khosrawipour, V., et al., *Irradiation does not increase the penetration depth of doxorubicin in normal tissue after pressurized intra-peritoneal aerosol chemotherapy (PIPAC) in an ex vivo model.* In Vivo, 2016. **30**(5): p. 593-597.

143. Khosrawipour, V., et al., *Effect of whole-abdominal irradiation on penetration depth of doxorubicin in normal tissue after pressurized intraperitoneal aerosol chemotherapy (PIPAC) in a post-mortem swine model.* Anticancer Research, 2017. **37**(4): p. 1677-1680.

144. Khosrawipour, V., et al., *Cytotoxic effect of different treatment parameters in pressurized intraperitoneal aerosol chemotherapy (PIPAC) on the in vitro proliferation of human colonic cancer cells.* World Journal of Surgical Oncology, 2017. **15 (1) (no pagination)**(43).

145. Minnaert, A.K., et al., *High-Pressure Nebulization as Application Route for the Peritoneal Administration of siRNA Complexes.* Macromol Biosci, 2017. **17**(10): p. 1616-5187.

146. Schnelle, D., et al., *A new ex vivo model for optimizing distribution of therapeutic aerosols: The (inverted) bovine urinary bladder.* Pleura and Peritoneum, 2017. **2**(1): p. 37-41.

147. Eveno, C., et al., *Experimental pharmacokinetics evaluation of chemotherapy delivery by PIPAC for colon cancer: First evidence for efficacy.* Pleura and Peritoneum, 2017. **2**(2): p. 103-109.

148. Blanco, A., et al., *Renal and hepatic toxicities after pressurized intraperitoneal aerosol chemotherapy (PIPAC).* Annals of Surgical Oncology, 2013. **20**(7): p. 2311-2316.

149. Solass, W., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC): Occupational health and safety aspects.* Annals of Surgical Oncology, 2013. **20**(11): p. 3504-3511.

150. Giger-Pabst, U., et al., *Low-dose Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) as an alternative therapy for ovarian cancer in an octogenarian patient.* Anticancer Research, 2015. **35**(4): p. 2309-2314.

151. Graversen, M., P.B. Pedersen, and M.B. Mortensen, *Environmental safety during the administration of Pressurized IntraPeritoneal Aerosol Chemotherapy (PIPAC).* Pleura and Peritoneum, 2016. **1**(4): p. 203-208.

152. Tempfer, C.B., et al., *Intraperitoneal cisplatin and doxorubicin as maintenance chemotherapy for unresectable ovarian cancer: A case report.* BMC Cancer, 2017. **17**(1): p. 26.

153. Tempfer, C.B., et al., *Activity of Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) with cisplatin and doxorubicin in women with recurrent, platinum-resistant ovarian cancer: Preliminary clinical experience.* Gynecologic Oncology, 2014. **132**(2): p. 307-311.

154. Demtroder, C., et al., *Pressurized intraperitoneal aerosol chemotherapy with oxaliplatin in colorectal peritoneal metastasis.* Colorectal Dis, 2016. **18**(4): p. 364-71.

155. Nadiradze, G., et al., *Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) with Low-Dose Cisplatin and Doxorubicin in Gastric Peritoneal Metastasis.* J Gastrointest Surg, 2016. **20**(2): p. 367-73.

156. Odendahl, K., et al., *Quality of life of patients with end-stage peritoneal metastasis treated with Pressurized IntraPeritoneal Aerosol Chemotherapy (PIPAC).* European Journal of Surgical Oncology, 2015. **41**(10): p. 1379-1385.

157. Tempfer, C.B., et al., *Pressurized Intraperitoneal Aerosol Chemotherapy with Cisplatin and Doxorubicin in Women with Peritoneal Carcinomatosis: A Cohort Study.* Anticancer research, 2015. **35**(12): p. 6723-6729.

158. Girshally, R., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC) as a neoadjuvant therapy before cytoreductive surgery and hyperthermic intraperitoneal chemotherapy.* World J Surg Oncol, 2016. **14**(1): p. 253.

159. Rezniczek, G.A., et al., *Dynamic changes of tumor gene expression during repeated pressurized intraperitoneal aerosol chemotherapy (PIPAC) in women with peritoneal cancer.* Bmc Cancer, 2016. **16**.

160. Swindle, M.M., et al., *Swine as models in biomedical research and toxicology testing.* Vet Pathol, 2012. **49**(2): p. 344-56.

161. Solass, W., et al., *Intraperitoneal Chemotherapy of Peritoneal Carcinomatosis Using Pressurized Aerosol as an Alternative to Liquid Solution: First Evidence for Efficacy.* Annals of Surgical Oncology, 2014. **21**(2): p. 553-559.

162. Solass, W., et al., *Peritoneal sampling and histological assessment of therapeutic response in peritoneal metastasis: proposal of the Peritoneal Regression Grading Score (PRGS).* Pleura Peritoneum, 2016. **1**(2): p. 99-107.

163. Solass, W., et al., *Reproducibility of the peritoneal regression grading score for assessment of response to therapy in peritoneal metastasis.* Histopathology, 2019. **74**(7): p. 1014-1024.

164. Aaronson, N.K., et al., *The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology.* J Natl Cancer Inst, 1993. **85**(5): p. 365-76.

165. Prigerson, H.G., et al., *Chemotherapy Use, Performance Status, and Quality of Life at the End of Life.* JAMA Oncol, 2015. **1**(6): p. 778-84.

166. Demtroder C, et al., *Platinum Contamination of Laparoscopic Instruments During Pressurized Intra Peritoneal Aerosol Chemotherapy (PIPAC).* Journal of Pharmacy and Pharmaceutical Sciences, 2016. **5**(2): p. 8-11.

167. Alyami, M., et al., *Multicentric initial experience with the use of the pressurized intraperitoneal aerosol chemotherapy (PIPAC) in the management of unresectable peritoneal carcinomatosis.* European Journal of Surgical Oncology, 2017. **43**(11): p. 2178-2183.

168. Siebert, M., et al., *Severe hypersensitivity reactions to platinum compounds post-pressurized intraperitoneal aerosol chemotherapy (PIPAC): first literature report.* Cancer Chemotherapy and Pharmacology, 2019. **83**(3): p. 425-430.

169. Lambert, L.A., *Looking up: recent advances in understanding and treating peritoneal carcinomatosis.* CA Cancer J Clin, 2015. **65**.

170. Rahimi-Gorji, M., et al., *Intraperitoneal aerosolized drug delivery: Technology, recent developments, and future outlook.* Adv Drug Deliv Rev, 2020. **160**: p. 105-114.

171. Shariati, M., et al., *Synergy between Intraperitoneal Aerosolization (PIPAC) and Cancer Nanomedicine: Cisplatin-Loaded Polyarginine-Hyaluronic Acid Nanocarriers Efficiently Eradicate Peritoneal Metastasis of Advanced Human Ovarian Cancer.* ACS applied materials & interfaces, 2020. **12**(26): p. 29024-29036.

172. Uusi-Kerttula, H., et al., *Pseudotyped αvβ6 integrin-targeted adenovirus vectors for ovarian cancer therapies.* Oncotarget, 2016. **7**(19): p. 27926-27937.

173. Uusi-Kerttula, H., *Development of ovarian cancer-targeted adenoviral vectors*, in *Division of Cancer and Genetics, School of Medicine*. 2017, Cardiff University.

174. Van De Sande, L., et al., *Establishment of a rat model for pressurized intraperitoneal aerosol chemotherapy.* European Surgical Research, 2017. **58 (Supplement 2)**: p. 32.

175. Meyer, P.T., et al., *Comparison of intravenous and intraperitoneal [123I] IBZM injection for dopamine D2 receptor imaging in mice.* Nuclear medicine and biology, 2008. **35**(5): p. 543-548.

176. Los, G., et al., *Direct diffusion of cis-diamminedichloroplatinum(II) in intraperitoneal rat tumors after intraperitoneal chemotherapy: a comparison with systemic chemotherapy.* Cancer Res, 1989. **49**(12): p. 3380-4.

177. Dasari, S. and P.B. Tchounwou, *Cisplatin in cancer therapy: molecular mechanisms of action.* Eur J Pharmacol, 2014. **740**: p. 364-78.

178. Accord Healthcare Limited. *Cisplatin 1 mg/ml Concentrate for Solution for Infusion SmPC* 2020 [cited 2020 25th November]; Available from: https://www.medicines.org.uk/emc/product/6111/smpc#PHARMACOLOGICAL_PROPS.

179. Gonzalez, V.M., et al., *Is cisplatin-induced cell death always produced by apoptosis?* Mol Pharmacol, 2001. **59**(4): p. 657-63.

180. Hamilton, T.C., et al., *Augmentation of adriamycin, melphalan, and cisplatin cytotoxicity in drug-resistant and -sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion.* Biochem Pharmacol, 1985. **34**(14): p. 2583-6.

181. ThermoFisher Scientific Invitrogen. *LIVE/DEAD™ Fixable Dead Cell Stain Kits*. [cited 2020 10th December]; Available from: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2Flive_dead_fixable_dead_cell_stains_man.pdf&title=VXNlciBHdWlkZTogTElWRS9ERUFEIEZpeGFibGUgRGVhZCBDZWxsIFN0YWluIEtpdHM=.

182. Van de Sande, L., et al., *Establishment of a rat ovarian peritoneal metastasis model to study pressurized intraperitoneal aerosol chemotherapy (PIPAC).* BMC Cancer, 2019. **19**(1): p. 424.

183. Public Health Wales. *News: PHW screening programmes to restart*. [cited 2021 6th March]; Available from: https://phw.nhs.wales/news/public-health-wales-screening-programmes-to-restart/.

184. Cancer Research UK. *Cancer services during Covid-19*. 2021 [cited 2021 6th March]; Available from: https://scienceblog.cancerresearchuk.org/2021/02/02/cancer-services-during-covid-19-40000-fewer-people-starting-treatment/.

185. Morris, E.J.A., et al., *Impact of the COVID-19 pandemic on the detection and management of colorectal cancer in England: a population-based study.* Lancet Gastroenterol Hepatol, 2021. **6**(3): p. 199-208.

186. The Royal College of Radiologists. *RCR advice on non-urgent and cancer imaging during the coronavirus pandemic*. 2020 [cited 2021 March 6th]; Available from: https://www.rcr.ac.uk/college/coronavirus-covid-19-what-rcr-doing/clinical-information/rcr-advice-non-urgent-and-cancer.

187. Passot, G., et al., *What made hyperthermic intraperitoneal chemotherapy an effective curative treatment for peritoneal surface malignancy: A 25-year experience with 1,125 procedures.* J Surg Oncol, 2016. **113**(7): p. 796-803.

188. Nowacki, M., et al., *Multicenter comprehensive methodological and technical analysis of 832 pressurized intraperitoneal aerosol chemotherapy (PIPAC) interventions performed in 349 patients for peritoneal carcinomatosis treatment: An international survey study.* European Journal of Surgical Oncology, 2018. **44**(7): p. 991-996.

189. Robella, M., et al., *A Phase I Dose Escalation Study of Oxaliplatin, Cisplatin and Doxorubicin Applied as PIPAC in Patients with Peritoneal Carcinomatosis.* Cancers, 2021. **13**(5): p. 1060.

190. Graversen, M., et al., *Severe peritoneal sclerosis after repeated pressurized intraperitoneal aerosol chemotherapy with oxaliplatin (PIPAC OX): report of two cases and literature survey.* Clinical and Experimental Metastasis, 2018. **35**(3): p. 103-108.

191. National Institute for Health and Care Excellence. *Pressurised intraperitoneal aerosol chemotherapy for peritoneal carcinomatosis. Interventional procedures guidance [IPG681]*. 2020 [cited 2021 6th March].

192. Lauer, U.M., et al., *Phase I Study of Oncolytic Vaccinia Virus GL-ONC1 in Patients with Peritoneal Carcinomatosis.* Clinical Cancer Research, 2018. **24**(18): p. 4388.

8 Appendices

Appendices:

8.1 Service Evaluation Project Registration Form

1.0 Title of project

The management of peritoneal metastases in South Wales

2.0 Aim of project (the main reason for undertaking the project):

Peritoneal metastases represent the end stage in multiple cancers. Currently the only treatment available in South Wales is cytoreductive surgery (CRS) for patients with metastases of ovarian origin, and systemic chemotherapy for patients with peritoneal metastases from other primary disease sites.

Research into existing alternative treatments, including CRS, and heated intraperitoneal chemotherapy (HIPEC) is ongoing. Additionally there are a number of groups, including some at Cardiff University, working on novel therapeutic strategies for peritoneal metastases. One technique which has been developed and recently introduced in Europe is Pressurised Intraperitoneal Aerosolised Chemotherapy (PIPAC). Cardiff and Vale is currently exploring a pilot program of this technique, with a view to then participating in international multicentre trials.

This service evaluation will enable assessment of the uptake and the outcomes of the current treatment pathways for peritoneal metastases. Additionally it will provide an estimation of the incidence and the prevalence of peritoneal metastases amongst patients with cancer, and the proportion of patients presenting with peritoneal disease, versus those who experience peritoneal metastases during disease recurrence. This data will help to inform the clinical need for alternative therapies, as well as characterising the patient group that any new treatment should be aimed at.

3.0 Objectives: Please detail the objectives in terms which will allow for later evaluation

- Number of patients with peritoneal metastases
- Primary cancer
- Time of diagnosis of peritoneal metastases – synchronous or metachronous
- Treatment of primary disease
- Treatment of peritoneal disease
- Length of survival after diagnosis of peritoneal metastases
- Characteristics of patients with peritoneal metastases – age, ECOG status at diagnosis, comorbidities.

4.0 Proposed Project Start Date **Anticipated End Date**

Appendices:

1/1/18	31/1/18

5.0 Personnel Information

Name of Project Leader: Lt Col Leigh Davies

Job title: Consultant Colorectal Surgeon

Email/phone: Leigh.Davies2@wales.nhs.uk

Directorate/Division: General Surgery

Other Staff involved in the project

Name/Designation	Email/phone number
Miss Sophie Tate, Clinical Fellow, General Surgery	TateSJ@Cardiff.ac.uk, 07919384205
Professor Jared Torkington, Consultant Surgeon	Jared.Torkington@wales.nhs.uk 0292045148

6.0 Methodology: *Describe briefly the project design e.g. population, method of selecting participants, data collection and analysis methodology*.

A retrospective review of cancer Multidisciplinary Team meeting (MDT) data will be performed. A request for retrieval of information from the CANISC database will be submitted. This will retrieve records of patients discussed at the MDT

7.0 Service User involvement: *Describe briefly service user/stakeholder involvement in the project*

Appendices:

Lt Col Davies is a member of the colorectal cancer MDT. Additionally he has an interest in peritoneal surface malignancy, having completed a training fellowship at the Basingstoke Peritoneal Malignancy Institute

8.0

This piece of work will encompass data from Cancer Services in General Surgery and Gynaecology.

pilot program of this technique, with a view to then participating in international multicentre trials.

Prior to the addition of any new service or treatment, it is important to understand the current burden of disease.

Please identify other services which might be affected by this piece of work *(e.g other departments in the UHB, other professional groups)*

9.0 Expected outcome

Training ☐ Restructuring of service ☐ Protocol/Guideline ☐

Patient Information ☐ NICE guideline compliance ☐

Other (please specify) ☑

The outcome of this work will provide baseline information about the current burden of disease and the treatment pathway of patients with peritoneal metastases. This may provide evidence that restructuring of services is required.

10.0

Action Plan

Please list the people who will be involved in the development and implementation of the Action Plan

Appendices:

- Submission of request for retrieval of information from the CANISC database – Miss S Tate
- Analysis of data retrieved from CANISC database – Miss S Tate
- Generation of report – Miss S Tate, Lt Col L Davies, Prof J Torkington
- Dissemination of findings to Colorectal and Gynaecology Cancer MDT - Miss S Tate, Lt Col L Davies, Prof J Torkington

11.0

Dissemination of the project report

Publication/peer review journal ☑

Directorate meeting/neighbourhood meeting ☑ Divisional meeting ☐

LSB meeting ☐ Audit/quality and safety meeting ☐

Electronically via email/internet ☐

Other (please specify) ☐

12.0

Statement by Project leader

I agree to carry out the project as set out in this plan.

I confirm that I have read the UHB Data Protection guidance issued by the UHB and agree to ensure that all data for this project will be collected, collated and stored in accordance with the principles outlined in this guidance.

I agree to ensure that a copy of the findings and recommendations are submitted to the Assistant Director of Innovation and Improvement upon project completion.

Signature page copied below:

Appendices:

Name: LEIGH DAVIES
(Print)

Date: 8th December 2017.

13.0 Statement of Support

I.....GUY BLACKSHAW.....,Clinical/Community Director/Line Manager for ....support this application.

Signature:

Name: G.BLACKSHAW
(Print) 15/12/17.

Date:

I...,Clinical Board Director/ Nurse Director/Head of Operations/ supervisor for ....support this application.

Signature:

Name:
(Print)

Date:

Appendices:

8.2 Request for the retrieval of information from the CaNISC database Form

REQUEST FOR RETRIEVAL OF INFORMATION FROM THE CANISC DATABASE (Version 13)

TO BE COMPLETED BY ORIGINATOR OF REQUEST: (Please print clearly)**

Originator: S. Tate **Phone no./e-mail:** sophie.tate@wales.nhs.uk 07919384205

Originator: L M Davies **Phone no./e-mail:** Leigh.Davies2@wales.nhs.uk 02920 743499

Date: 03/9/2018

Designation (S Tate): Clinical Fellow Organisation: University Hospital of Wales, C&V Health board

Designation (L Davies) : Consultant Surgeon Organisation: University Hospital of Wales, C&V Health board

Dept: General Surgery

Description of Information requested:

The information is requested in order to perform a service evaluation of the treatment of peritoneal metastases.
The aim of the evaluation is to capture how patients with peritoneal metastases are currently managed.
Additionally the evaluation will provide an estimation of the incidence and the prevalence of peritoneal metastases amongst patients with cancer, and the proportion of patients presenting with peritoneal disease, versus those who experience peritoneal metastases during disease recurrence.

Specific date range requested: From: 1/1/17 To: 31/12/17

Specific info requested: (e.g. CRN., Sex, etc.)
Patient – name, hospital number, DOB, sex
Primary diagnosis and date of this diagnosis.
Date of diagnosis of peritoneal metastases/peritoneal cancer
ECOG status at diagnosis of peritoneal metastasis/peritoneal cancer
Medical comorbidities recorded at time of diagnosis.
Treatment – chemotherapy, surgery etc, and dates of treatment.
Date of death if applicable.

Ordered/Grouped by: Date of diagnosis

IF URGENT, deadline date:
(CIU endeavour to process queries as soon as possible, but at least 2 week's notice is appreciated)

Output format: Excel / Word / Other ....

****Data Protection: Are patient identifiers expected in response to request? Yes ■ No ****
If patient identifiers are required, please give reasons (continue on back if necessary)

Patient identifiers are requested because it is expected that a number of patients will have been discussed on multiple occasions by the MDT, when presenting with primary disease and then again with disease recurrence. In order to establish the incidence and the prevalence of disease it is important to understand the timeline of diagnosis. Similarly, in order to understand the current management of this cohort of patients, it is important to be able to ascertain the timeline of treatments.

I agree to abide by the terms of the Data Protection Act, 1998: Signed: ....
(Individuals are reminded of their responsibility to comply with the terms of the Data Protection Act, 1998)

Purpose of request, including where results will be published (if any):

Appendices:

Service evaluation project

- Results will be presented locally.
- Results likely to be used for background information in funding applications for research on peritoneal metastases.

Is this information required for Velindre Cancer Centre Clinical Effectiveness or audit purposes? **

Yes Please submit this form to Clinical Audit Dept, Velindre Hospital
No ■ Please submit this form to National Service Desk, NWIS, Brunel House, Cardiff, CF24 0HA

Is the data to be transferred outside the European Economic Area? **NO**

CALDICOTT GUARDIAN AUTHORISATION
** Originator to obtain signature ***prior*** to submission of request to the National Service Desk **

Signature approving request:	**Date:** 14/9/2015
TO BE COMPLETED BY CANISC SUPPORT TEAM	
Query performed by:	Date:
Query checked by:	Date:
Description of retrieval method:	
Helpdesk Call Number:	
File location:	

Appendices:

8.3 ePIPAC: New interventional procedures form submitted to CAV UHB Quality, Safety, and Experience Committee

New Procedures Procedure Form (ePIPAC)

Section 1 – Submitting Clinician	
Name	Jared Torkington
Status	Consultant
Specialty	General Surgery
Directorate	General Surgery
Address	University Hospital of Wales
Phone/fax	029 20 745148
e-mail	Jared.Torkington@wales.nhs.uk
Section 2 – New Procedure/Technique	
a) Name of procedure (and any alternative names)	Electrostatic Pressurised Intraperitoneal Chemotherapy (ePIPAC)
b) Entirely new procedure, new to UHB, or new to you	Entirely new procedure JT has completed a training workshop in the procedure and a surgeon from Europe experienced in the procedure has been asked to attend in the role of preceptor for the first cases.
c) NICE listed or approved	No
d) Similar to, or different from, established procedure	No
e) Which existing procedure/s might it replace	It would be an additional treatment for peritoneal metastases. ePIPAC may replace a second or third line of systemic chemotherapy, or it may be an additional option after other treatments have been exhausted.
f) Brief description of what is involved in the procedure	ePIPAC is a laparoscopic surgical procedure. The patient is given a general anaesthetic. Two laparoscopic ports are placed to give access to the abdomen. Once the abdomen is insufflated, biopsies are taken of peritoneal nodules (this is to monitor disease – biopsies are compared after serial ePIPAC treatments). An overall disease assessment score is calculated (Peritoneal Carcinomatosis Index). A laparoscopic nebuliser, and an electrostatic precipitation

	ion wand are then inserted into the abdominal cavity, and the laparoscopic camera is fixed so that there is a view of the nebuliser. The nebuliser is connected to the injector and a syringe of liquid chemotherapy is loaded. Colorectal cancer patients are treated with oxaliplatin (92mg/m^2 in a 150 ml 5% glucose solution), whilst ovarian cancer patients are treated with cisplatin (7.5mg/m^2 in a 150 ml NaCl 0.9% solution) and doxorubicin (1.5mg/m^2 in 50ml NaCl 0.9% solution). When all checks are completed the theatre staff leave – the anaesthetist and the surgeon move to the prep room where they have a direct view of the patient and the monitoring equipment. The injector is remotely activated and the chemotherapy is delivered via the nebuliser. The chemotherapy is left in situ for 30 minutes. The surgeon then enters the theatre to activate the ion wand for 60 seconds. The surgical assistant re-enters and the abdomen is exsufflated through a filter to the gas scavenging system. The rest of the staff re-enter and the patient's abdomen is closed and the patient is recovered as per normal procedures. The anticipated length of stay after the operation is 72hours.
Section 3 – Clinicians involved	
a) Which specialties might perform this procedure	General Surgery (colorectal) Gynaecology
b) Individual names/job titles of clinicians proposed	Initially Jared Torkington would be the lead operating surgeon undertaking all cases. Leigh Davies (Consultant Colorectal) and Kenneth Lim (Consultant Gynaecology) will be involved in assisting in cases and care of the patients in their respective specialties.
c) Is training required (how will it be obtained)	The technical aspects of the ePIPAC operation are within the competence of an experienced laparoscopic surgeon. Jared Torkington (Consultant) and Sophie Tate (Clinical Fellow – registrar) have attended PIPAC training courses approved by the manufacturers of the nebuliser device. The procedures defined in the ePIPAC Standard Operating Procedure document are those recommended by the team that developed the technique, and which are used across Europe. All other staff involved in the procedures will be given training in the form of a workshop of simulated cases, and the necessary chemotherapy handling training according to existing Health Board polices/SOPs.

d) Is competence assured (how is it confirmed)	The lead surgeon must have attended a recognised training course (Jared Torkington) A training log will be maintained to ensure that all other staff have attended a workshop of simulated procedures and completed the necessary chemotherapy handling training.
Section 4 – Patients	
a) Which patients are likely to benefit	ePIPAC has been developed as a treatment for peritoneal metastases.
b) The clinical indications for its use	ePIPAC will be offered to patients with colorectal, appendiceal, or ovarian cancer who have isolated peritoneal metastases. A full list of criteria for treatment is provided in the SOP.
c) The reason for introducing this particular intervention	Currently, the only treatment option available in Wales for patients with peritoneal metastases is systemic chemotherapy. In the setting of recurrent disease, systemic chemotherapy is often of limited efficacy. This technique provides a further treatment option for patients where systemic chemotherapy has failed to control their disease.
d) What are the intended health benefits	ePIPAC delivers intraperitoneal chemotherapy to treat peritoneal metastases. The systemic absorption of chemotherapy is low, which means that side effects from the chemotherapy are reduced.
e) Possible adverse effects (and level of risk?)	The risk of having a serious complication of general anaesthesia is 1:10,000 cases, whist the risk of serious complications from a 'diagnostic laparoscopy' (procedure most analogous to ePIPAC) is 1-2:100. In published studies and case series of PIPAC, the incidence of minor adverse events (generally abdominal pain and nausea) has been between 30-100%. The incidence of major adverse events (severe, or life threatening) has been low; in the largest case series rates of 0-4% are reported.
f) Can you estimate numbers/potential impact on NHS	The incidence of colorectal cancer and ovarian cancer has been increasing in the UK. In 2014, figures from Cancer Research UK stated that 2335 of the 41265 cases of colorectal cancer, and 372 of the 7378 cases of ovarian cancer diagnosed in the UK were in Wales [1]. Wales has higher age-standardised incidence and mortality rates for colorectal cancer than the rest of the UK [1]. It is estimated that 4% of patients presenting with colorectal cancer will have synchronous peritoneal metastases [2]. In addition, 20-40% of patients treated with curative intent will relapse and around 20% of these

	will have metastatic peritoneal disease [2, 3]. In ovarian cancer 50-80% patients have peritoneal metastases at diagnosis.[4]. Based on these figures, 400 or more patients each year in Wales may be diagnosed with peritoneal metastasis. A service evaluation is being carried out to ascertain the current situation in the Cardiff and Vale Health Board, in terms of the number of patients with peritoneal metastases currently having treatment, and their outcomes.
Section 5 – Evidence base	
a) Is this procedure in use elsewhere	Yes Cases have been performed, and reported in the literature, in Germany, France, Belgium, The Netherlands, Denmark, Italy, France, Russia, and India.
b) Details of conference proceedings/communications	1. Reymond, M.A., et al., *Efficacy and safety of pressurized intraperitoneal aerosol chemotherapy (PIPAC) in women with recurrent gynaecological cancer and peritoneal carcinomatosis.* Journal of Clinical Oncology. Conference, 2013. **31**(15 SUPPL. 1). 2. Solass, W., et al., *Intraperitoneal chemotherapy of peritoneal carcinomatosis using pressurized aerosol as an alternative to liquid solution: First evidence for efficacy and tolerability.* Surgical Endoscopy and Other Interventional Techniques, 2013. **1)**: p. S471. 3. Reymond, M.A., et al., *Feasibility of pressurized intrathoracic aerosol chemotherapy (PITAC) in the human patient.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S171. 4. Reymond, M.A., et al., *First clinical experience with pressurized intraperitoneal aerosol chemotherapy in patients with gastric cancer and peritoneal carcinomatosis.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S36. 5. Reymond, M.A., U. Giger-Pabst, and J. Zieren, *Pressurized intraperitoneal aerosol chemotherapy (PIPAC): Technical aspects.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S49.

Appendices:

6. Reymond, M.A., et al., *Systemic and local doxorubicin distribution after pressurized intraperitoneal aerosol chemotherapy (PIPAC) in the human patient.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S35.

7. Reymond, M.A., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC): Occupational health and safety aspects.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S72.

8. Reymond, M.A., et al., *Efficacy and safety of pressurized intraperitoneal aerosol chemotherapy in women with recurrent gynaecological cancer and peritoneal carcinomatosis.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S104.

9. Reymond, A., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC) with oxaliplatin as a salvage therapy in patients with peritoneal carcinomatosis of colorectal cancer.* Surgical Endoscopy and Other Interventional Techniques, 2015. **1)**: p. S41.

10. Farinha, H.T., et al., *Short term outcomes of pressurized intraperitoneal aerosol chemotherapy (PIPAC) in patients with peritoneal carcinomatosis.* Colorectal Disease, 2016. **18 (Supplement 1)**: p. 108.

11. Khomyakov, V., et al., *Initial experience of pressurized intraperitoneal aerosol chemotherapy (PIPAC) for treatment of peritoneally disseminated gastric cancer.* Annals of Oncology, 2016. **27 (Supplement 2)**: p. ii51.

12. Reymond, M.A., et al., *Indications and surgical results of pressurized intraperitoneal aerosol chemotherapy (PIPAC) for palliative therapy of peritoneal metastasis after 748 consecutive procedures.* Surgical Endoscopy and Other Interventional Techniques, 2016. **1)**: p. S463.

13. Robella, M., M. Vaira, and M. De Simone, *Pressurized intraperitoneal aerosol chemotherapy (PIPAC) associated with systemic chemotherapy: An innovative approach for peritoneal carcinomatosis.* European Journal of Surgical Oncology, 2016. **42 (9)**: p. S155.

14. Vaira, M., M. Robella, and M. De Simone, *Single-Port access for pressurized intraperitoneal aerosol chemotherapy (PIPAC): Technique, feasibility and safety.* European Journal of Surgical Oncology, 2016. **42 (9)**: p. S155-S156.

15. Etzold, C., et al., *3D-tissue-slices from patients with peritoneal carcinomatosis undergoing a pressurized*

		intraperitoneal aerosol therapy (PIPAC). European Surgical Research, 2017. **58 (5-6)**: p. 295.
	16.	Farinha, H.T., et al., *Inflammatory response and toxicity after pressurized intraperitoneal aerosol chemotherapy.* Colorectal Disease, 2017. **19 (Supplement 2)**: p. 119-120.
	17.	Khomyakov, V., et al., *Bidirectional chemotherapy in gastric cancer (GC) with peritoneal carcinomatosis (PC) combining intravenous chemotherapy with pressurized intraperitoneal aerosol chemotherapy (PIPAC): Results of 103 procedures in 52 patients.* Annals of Oncology, 2017. **28 (Supplement 5)**: p. v225.
	18.	Struller, F., et al., *Pressurized intraperitoneal aerosol chemotherapy with low-dose cisplatin and doxorubicin (PIPAC C/D) in patients with gastric cancer and peritoneal metastasis (PIPAC-GA1).* Journal of Clinical Oncology. Conference, 2017. **35**(4 Supplement 1).
c) Details of peer reviewed papers	1.	Blanco, A., et al., *Renal and hepatic toxicities after pressurized intraperitoneal aerosol chemotherapy (PIPAC).* Annals of Surgical Oncology., 2013. **20**(7): p. 2311-6, 2013 Jul.
	2.	Reymond, M.A., et al., *Efficacy and safety of pressurized intraperitoneal aerosol chemotherapy (PIPAC) in women with recurrent gynaecological cancer and peritoneal carcinomatosis.* Journal of Clinical Oncology. Conference, 2013. **31**(15 SUPPL. 1).
	3.	Solass, W., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC): occupational health and safety aspects.* Annals of Surgical Oncology. , 2013. **20**(11): p. 3504-11, 2013 Oct.
	4.	Solass, W., et al., *Intraperitoneal chemotherapy of peritoneal carcinomatosis using pressurized aerosol as an alternative to liquid solution: First evidence for efficacy and tolerability.* Surgical Endoscopy and Other Interventional Techniques, 2013. **1)**: p. S471.
	5.	Reymond, M.A., et al., *Feasibility of pressurized intrathoracic aerosol chemotherapy (PITAC) in the human patient.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S171.
	6.	Reymond, M.A., et al., *First clinical experience with pressurized intraperitoneal aerosol chemotherapy in*

	patients with gastric cancer and peritoneal carcinomatosis. Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S36. 7. Reymond, M.A., U. Giger-Pabst, and J. Zieren, *Pressurized intraperitoneal aerosol chemotherapy (PIPAC): Technical aspects.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S49. 8. Reymond, M.A., et al., *Systemic and local doxorubicin distribution after pressurized intraperitoneal aerosol chemotherapy (PIPAC) in the human patient.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S35. 9. Reymond, M.A., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC): Occupational health and safety aspects.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S72. 10. Reymond, M.A., et al., *Efficacy and safety of pressurized intraperitoneal aerosol chemotherapy in women with recurrent gynaecological cancer and peritoneal carcinomatosis.* Surgical Endoscopy and Other Interventional Techniques, 2014. **1)**: p. S104. 11. Solass, W., et al., *Intraperitoneal chemotherapy of peritoneal carcinomatosis using pressurized aerosol as an alternative to liquid solution: first evidence for efficacy.* Annals of Surgical Oncology, 2014. **21**(2): p. 553-9, 2014 Feb. 12. Tempfer, C.B., et al., *Activity of Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) with cisplatin and doxorubicin in women with recurrent, platinum-resistant ovarian cancer: preliminary clinical experience.* Gynecologic Oncology., 2014. **132**(2): p. 307-11, 2014 Feb. 13. Tempfer, C.B., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC) with cisplatin and doxorubicin in a woman with pseudomyxoma peritonei: A case report.* Gynecologic Oncology Reports, 2014. **10**: p. 32-5, 2014 Dec. 14. Giger-Pabst, U., et al., *Low-dose pressurized intraperitoneal aerosol chemotherapy (PIPAC) as an alternative therapy for*

ovarian cancer in an octogenarian patient. Anticancer Research. , 2015. **35**(4): p. 2309-14, 2015 Apr.

15. Odendahl, K., et al., *Quality of life of patients with end-stage peritoneal metastasis treated with Pressurized IntraPeritoneal Aerosol Chemotherapy (PIPAC).* European Journal of Surgical Oncology, 2015. **41**(10): p. 1379-85, 2015 Oct.

16. Reymond, A., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC) with oxaliplatin as a salvage therapy in patients with peritoneal carcinomatosis of colorectal cancer.* Surgical Endoscopy and Other Interventional Techniques, 2015. **1)**: p. S41.

17. Sabaila, A., A. Fauconnier, and C. Huchon, *[Pressurized intraperitoneal aerosol chemotherapy (PIPAC): a new way of administration in peritoneal carcinomatosis of ovarian cancer].* Gynecologie, Obstetrique & Fertilite, 2015. **43**(1): p. 66-7, 2015 Jan.

18. Tempfer, C.B., et al., *Pressurized Intraperitoneal Aerosol Chemotherapy with Cisplatin and Doxorubicin in Women with Peritoneal Carcinomatosis: A Cohort Study.* Anticancer Research. , 2015. **35**(12): p. 6723-9, 2015 Dec.

19. Tempfer, C.B., et al., *Pressurized intraperitoneal aerosol chemotherapy in women with recurrent ovarian cancer: A phase 2 study.* Gynecologic Oncology Reports, 2015. **137**(2): p. 223-8, 2015 May.

20. Demtroder, C., et al., *Pressurized intraperitoneal aerosol chemotherapy with oxaliplatin in colorectal peritoneal metastasis.* Colorectal Disease., 2016. **18**(4): p. 364-71, 2016 Apr.

21. Farinha, H.T., et al., *Short term outcomes of pressurized intraperitoneal aerosol chemotherapy (PIPAC) in patients with peritoneal carcinomatosis.* Colorectal Disease, 2016. **18** (Supplement 1): p. 108.

22. Girshally, R., et al., *Pressurized intraperitoneal aerosol chemotherapy (PIPAC) as a neoadjuvant therapy before cytoreductive surgery and hyperthermic intraperitoneal chemotherapy.* World Journal of Surgical Oncology, 2016. **14 (1)** (253).

Appendices:

	23. Graversen, M., P.B. Pedersen, and M.B. Mortensen, *Environmental safety during the administration of Pressurized IntraPeritoneal Aerosol Chemotherapy (PIPAC).* Pleura and Peritoneum, 2016. **1**(4): p. 203-208. 24. Khomyakov, V., et al., *Initial experience of pressurized intraperitoneal aerosol chemotherapy (PIPAC) for treatment of peritoneally disseminated gastric cancer.* Annals of Oncology, 2016. **27** (Supplement 2): p. ii51. 25. Khomyakov, V., et al., *Bidirectional chemotherapy in gastric cancer with Peritoneal metastasis combining intravenous XELOX with IntraPeritoneal chemotherapy with low-dose cisplatin and doxorubicin administered as a Pressurized aerosol: An open-label, Phase-2 study (PIPAC-GA2).* Pleura and Peritoneum, 2016. **1**(3): p. 159-166. 26. Nadiradze, G., et al., *Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) with Low-Dose Cisplatin and Doxorubicin in Gastric Peritoneal Metastasis.* Journal of Gastrointestinal Surgery, 2016. **20**(2): p. 367-73, 2016 Feb. 27. Oyais, A., et al., *[Occupational Health Aspects of Pressurised Intraperitoneal Aerosol Chemotherapy (PIPAC): Confirmation of Harmlessness].* Zentralblatt fur Chirurgie, 2016. **141**(4): p. 421-4, 2016 Aug. 28. Reymond, M., et al., *Electrostatic precipitation Pressurized IntraPeritoneal Aerosol Chemotherapy (ePIPAC): First in-human application.* Pleura and Peritoneum, 2016. **1**(2): p. 79-89. 29. Reymond, M.A., et al., *Indications and surgical results of pressurized intraperitoneal aerosol chemotherapy (PIPAC) for palliative therapy of peritoneal metastasis after 748 consecutive procedures.* Surgical Endoscopy and Other Interventional Techniques, 2016. **1)**: p. S463. 30. Rezniczek, G.A., et al., *Dynamic changes of tumor gene expression during repeated pressurized intraperitoneal aerosol chemotherapy (PIPAC) in women with peritoneal cancer.* BMC Cancer., 2016. **16**: p. 654, 2016 08 19. 31. Robella, M., M. Vaira, and M. De Simone, *Safety and feasibility of pressurized intraperitoneal aerosol chemotherapy (PIPAC) associated with systemic chemotherapy: An innovative approach to treat peritoneal*

	carcinomatosis. World Journal of Surgical Oncology, 2016. **14 (1)** (128). 32. Robella, M., M. Vaira, and M. De Simone, *Pressurized intraperitoneal aerosol chemotherapy (PIPAC) associated with systemic chemotherapy: An innovative approach for peritoneal carcinomatosis.* European Journal of Surgical Oncology, 2016. **42 (9)**: p. S155. 33. Vaira, M., et al., *Single-port access for Pressurized IntraPeritoneal Aerosol Chemotherapy (PIPAC): Technique, feasibility and safety.* Pleura and Peritoneum, 2016. **1**(4): p. 217-222. 34. Vaira, M., M. Robella, and M. De Simone, *Single-Port access for pressurized intraperitoneal aerosol chemotherapy (PIPAC): Technique, feasibility and safety.* European Journal of Surgical Oncology, 2016. **42 (9)**: p. S155-S156. 35. Alyami, M., et al., *Multicentric initial experience with the use of the pressurized intraperitoneal aerosol chemotherapy (PIPAC) in the management of unresectable peritoneal carcinomatosis.* European Journal of Surgical Oncology., 2017. **43**(11): p. 2178-2183, 2017 Nov. 36. Cazauran, J.-B., et al., *Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) Procedure for Non-resectable Peritoneal Carcinomatosis (with Video).* Journal of Gastrointestinal Surgery., 2017. 37. Etzold, C., et al., *3D-tissue-slices from patients with peritoneal carcinomatosis undergoing a pressurized intraperitoneal aerosol therapy (PIPAC).* European Surgical Research, 2017. **58 (5-6)**: p. 295. 38. Eveno, C., et al., *Experimental pharmacokinetics evaluation of chemotherapy delivery by PIPAC for colon cancer: First evidence for efficacy.* Pleura and Peritoneum, 2017. **2**(2): p. 103-109. 39. Farinha, H.T., et al., *Inflammatory response and toxicity after pressurized intraperitoneal aerosol chemotherapy.* Colorectal Disease, 2017. **19** (Supplement 2): p. 119-120. 40. Graversen, M., et al., *Peritoneal metastasis from pancreatic cancer treated with pressurized intraperitoneal aerosol*

	chemotherapy (PIPAC). Clinical & Experimental Metastasis, 2017. **34**(5): p. 309-314, 2017 Jun. 41. Hilal, Z., et al., *Nutritional status, cachexia, and anorexia in women with peritoneal metastasis and intraperitoneal chemotherapy: A longitudinal analysis.* Journal of Gynecologic Oncology, 2017. **28 (6)** (e80). 42. Hubner, M., et al., *Pressurized IntraPeritoneal Aerosol Chemotherapy - Practical aspects.* European Journal of Surgical Oncology, 2017. **43**(6): p. 1102-1109, 2017 Jun. 43. Hubner, M., et al., *Feasibility and Safety of Pressurized Intraperitoneal Aerosol Chemotherapy for Peritoneal Carcinomatosis: A Retrospective Cohort Study.* Gastroenterology research & practice., 2017: p. 6852749, 2017. 44. Khomiakov, V., et al., *Bidirectional chemotherapy in gastric cancer (GC) with peritoneal carcinomatosis (PC) combining intravenous chemotherapy with intraperitoneal chemotherapy with low-dose cisplatin and doxorubicin administered as a pressurized aerosol: An open-label, phase II.* Journal of Clinical Oncology. Conference, 2017. **35**(15 Supplement 1). 45. Khomyakov, V., et al., *Bidirectional chemotherapy in gastric cancer (GC) with peritoneal carcinomatosis (PC) combining intravenous chemotherapy with pressurized intraperitoneal aerosol chemotherapy (PIPAC): Results of 103 procedures in 52 patients.* Annals of Oncology, 2017. **28** (Supplement 5): p. v225. 46. Khosrawipour, T., V. Khosrawipour, and U. Giger-Pabst, *Pressurized Intra Peritoneal Aerosol Chemotherapy in patients suffering from peritoneal carcinomatosis of pancreatic adenocarcinoma.* PLoS ONE [Electronic Resource], 2017. **12**(10): p. e0186709, 2017. 47. Struller, F., et al., *Pressurized intraperitoneal aerosol chemotherapy with low-dose cisplatin and doxorubicin (PIPAC C/D) in patients with gastric cancer and peritoneal metastasis (PIPAC-GA1).* Journal of Clinical Oncology. Conference, 2017. **35**(4 Supplement 1). 48. Teixeira Farinha, H., et al., *Impact of Pressurized Intraperitoneal Aerosol Chemotherapy on Quality of Life and Symptoms in Patients with Peritoneal Carcinomatosis:*

	A Retrospective Cohort Study. Gastroenterology research & practice., 2017: p. 4596176, 2017. 49. Tempfer, C.B., et al., *Intraperitoneal cisplatin and doxorubicin as maintenance chemotherapy for unresectable ovarian cancer: a case report.* BMC Cancer. , 2017. **17**(1): p. 26, 2017 Jan 06. 50. Willaert, W., P. Sessink, and W. Ceelen, *Occupational safety of pressurized intraperitoneal aerosol chemotherapy (PIPAC).* Pleura and Peritoneum, 2017. **2**(3): p. 121-128. 51. Falkenstein, T.A., et al., *First clinical data of pressurized intraperitoneal aerosol chemotherapy (PIPAC) as salvage therapy for peritoneal metastatic biliary tract cancer.* Anticancer Research, 2018. **38**(1): p. 373-378. 52. Farinha, H.T., et al., *Inflammatory response and toxicity after Pressurized Intraperitoneal Aerosol Chemotherapy.* Journal of Cancer, 2018. **9**(1): p. 13-20.
Section 6 – Surveillance	
a) Is the procedure part of a clinical trial	No
b) How will it be audited	A database of cases will be established. This will be maintained locally and will include details of the treatment and any incidents, or complications that occur. Additionally there is an international registry of cases maintained by the University of Magdeburg (NCT03210298). The data submitted to the registry are stored in a SQL-based online database. Patient data are pseudoanonymized. The registry has received approval of the data protection officer of the State of Northrhine-Westphalia. The registry steering committee is blinded towards the identity of the participating institutions. Each participating institution receives an annual report with own data vs. benchmark.
c) What patient information will be provided	A patient information sheet has been generated. It has been reviewed by clinicians
d) Confirm patients will be told status of new procedure	Yes

e) Confirm adverse events will be incident reported	Yes
f) Confirm NICE is aware of procedure/personnel	Notification of Interventional Procedure form has been completed by Sophia Tate (Clinical Fellow).
Section 7 – Resources	
a) Do devices comply with EC standards	Yes
b) Are devices certified for this use	The Capnopen® is CE marked for the laparoscopic delivery of aerosolised liquids. It is designed and certified to be used in conjunction with a high pressure radiographic injector. The Ultravision™ Ion Wand is CE marked for the electrostatic precipitation of vapour and particulate matter from the pneumoperitoneum.
c) Are there cost implications (capital/revenue)	Yes. An estimated costing for the procedure at the University Hospital of Wales is as follows:

Description		**Cost**	**Frequency**
Treatment delivery	Inpatient episode [1]	£3637.07	Per procedure
Drugs	Cisplatin and doxorubicin[2] for 70kg patient, including pharmacy dispensing	£29.56	Per procedure
	Oxaliplatin[3] for a 70kg patient, including pharmacy dispensing	£38.59	Per procedure
Additional Specialist	Capnopen = £1350[4]	£1375.58	Per procedure

equipment (disposables)	High Pressure Injector Tubing = £3.08 High pressure Syringe = £7.50 Ultravision Ion Wand = £15		
Specialist equipment (permanent)	High pressure injector – Accutron HP-D, supplied by Guerbet	£TBC	One off purchase if service continues. For the purpose of the pilot, a machine will be loaned.
	Servicing contract for injector	£TBC	Annual if service continues
	Ultravision generator	Already at UHL	One off
TOTAL	£5042.21 per case for ovarian cancer patients, £15,126.63 for a course of 3 treatments. £5051.24 per case for colorectal cancer patients, £15,123.72 for a course of 3 treatments. Therefore, anticipated cost of 3 patient pilot is £45,379.89 - £45,461.16		

1. This includes a pre-assessment for each treatment, comprising of a band 7 nurse conducting an hour-long assessment for each patient at each visit. The inpatient episode cost is for a 3 day admission for a diagnostic laparoscopy and peritoneal biopsies. This figure is based on a real inpatient episode for this procedure, which is the most analogous to PIPAC or ePIPAC. 30 minutes of extra

	theatre time to enable administration of the chemotherapy has been added to the basic cost. The administration of chemotherapy is the only additional process in the PIPAC/ePIPAC procedure, when compared to a standard diagnostic laparoscopy with peritoneal biopsies. Processing of blood tests and histological samples is included in the basic cost, as is care on a level 1 surgical ward and the associated pharmacy requirements for medications on the ward and at discharge. 2. Cisplatin and doxorubicin administered sequentially at the same procedure (so that both aerosols are in the abdomen at the same time) for ovarian, gastric, and hepatobiliary cancers, and pseudomyxoma peritoneii. 3. Oxaliplatin as a single agent has been used for colorectal cancers. 4. Price correct at time of writing – Device costs 1490€
d) Is a commercial organisation involved	No
e) How will costs be met	Departmental funds
Section 8 – Probity	
a) Could there be any commercial interests	No – No funding is being supplied by commercial organisations and the equipment to be used will be purchased through normal channels.
b) Could there be any intellectual rights	No – the equipment to be used and the procedure that will be carried out are established.
c) Could there be any conflicts of interest	JT is a minor shareholder and clinical advisor for Alesi Surgical.

Appendices:

8.4 Safety checklist for ePIPAC procedure

SAFETY CHECKLIST FOR ePIPAC

Patient Label:	Name Hospital Number Date Of Birth	
Primary cancer	**Procedure Number**	**Date of Procedure**

Initial box to confirm

Prior to start:

1.	**Patient details confirmed?**	
2.	**Surgical procedure confirmed?**	
3.	**Chemotherapy agents in operating theatre and Chemotherapy check complete?** **Lead operating surgeon and anaesthetist to confirm with patient verbally and on their wrist band that identification details (name, address, date of birth) are correct. The surgeon should confirm aloud that the correct drug(s), dose(s) and containers have been supplied in accordance with prescription, and the anaesthetist should document the batch number, expiry date, and time.**	
4.	**Anaesthetic machine checked and facility for remote monitoring available?**	
5.	**Personal Protective Equipment and Spillage Kit available?**	
6.	**All staff in theatre issued with glasses, gloves and protective clothing?**	
7.	**Labelled cytotoxic waste containers available?**	
8.	**Protective sheet on floor under injection system?**	
9.	**Laminar air flow on and working?**	
10.	**Patient consent for video recording?** **- Memory card available to record camera feed?**	

Preparation:

11.	**Access to abdomen gained and 12mm balloon port inserted.**	
12.	**Video recording activated.**	
13.	**Second 12mm balloon port inserted**	
14.	**PCI and ascites documented?**	
15.	**Diagnostic biopsies taken in quadrants 1-4?**	

Appendices:

16.	Ultravision wand inserted and connected to generator unit.	
17.	Intra-abdominal pressure 12mmHg, CO_2 flow 0.0 – 0.2 L/min	
18.	Exsufflation tubing attached to camera port?	
a	Clamp closed?	
b	Port valve closed?	
c	2x filters in-line before connection to gas scavenger outlet?	
19.	CapnoPen inserted into 12mm port; free floating, no bowel contact.	
20.	Laparoscopic camera sheath attached to CapnoPen.	
21.	Chemotherapy syringe(s) loaded into high pressure injector.	
22.	High pressure tubing connected to syringe(s) (+/- Y connector) and plastic sheath secured over connection?	
23.	Confirmation of set up of injector for syringe volume, pressure of 20 bar, and flow rate of 30ml/minute.	
24.	Check remote monitoring devices are all on and functional. Emergency anaesthetic equipment available in theatre/prep room.	
25.	All staff leave operating theatre.	

<u>Application of chemotherapy:</u>

26.	Lead surgeon to confirm that intra-abdominal pressure remains 12mmHg with flow of 0.0-0.2L/min, and that anaesthetist is happy with monitoring parameters. Instigate remote control of application of chemotherapy.	

<u>Completion of procedure:</u>

27.	Wait for 30 minutes.	
28.	Sole member of research team enters operating theatre.	
29.	Ultravision ion wand activated for 60 seconds.	
30.	CO_2 insufflation stopped.	
31.	Activate closed aerosol waste system to exsufflate abdomen.	
32.	Rest of staff re-enter operating theatre.	
33.	All waste (disposable materials and devices) to be packed and labelled as per local guidelines for chemotherapy waste.	
34.	Port sites closed.	
35.	Operation notes completed.	
36.	Audit form for database entry completed – for transfer to database along with video	

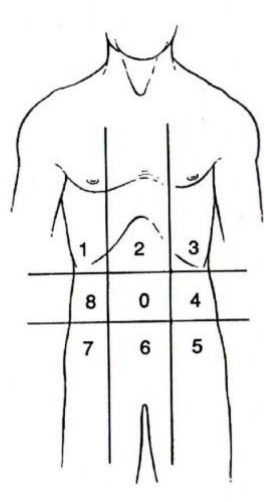
Appendices:

<u>This form should be filed in the patient's hospital notes.</u>

PCI and Acites

Patient Label:	Name Hospital Number Date Of Birth	
Primary cancer	**Procedure Number**	**Date of Procedure**

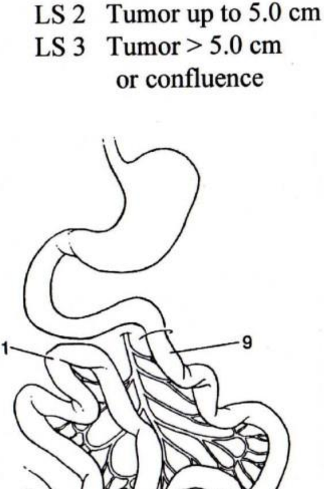
Peritoneal Cancer Index



Regions	Lesion Size
0 Central	____
1 Right Upper	____
2 Epigastrium	____
3 Left Upper	____
4 Left Flank	____
5 Left Lower	____
6 Pelvis	____
7 Right Lower	____
8 Right Flank	____
9 Upper Jejunum	____
10 Lower Jejunum	____
11 Upper Ileum	____
12 Lower Ileum	____
PCI	

Lesion Size Score

LS 0 No tumor seen
LS 1 Tumor up to 0.5 cm
LS 2 Tumor up to 5.0 cm
LS 3 Tumor > 5.0 cm or confluence



Ascites Volume ……………………………………..

This form should be completed and placed in the patient's notes

8.5 Consent form for ePIPAC pilot database and photography

Consent Form

ePIPAC database

Regarding the ePIPAC database: Please sign by one of the following three options:

1. I understand that information about my ePIPAC treatment will be stored in a secure database here at the University Hospital of Wales for the purposes of audit and quality improvement (checking if the service provided is a good standard).

   Signature Print Date

2. In addition to point 1 above, I agree for anonymised information about my ePIPAC treatment to be entered into the international registry for PIPAC to enable comparison of results with other centres worldwide.

   Signature Print Date

3. In addition to points 1 and 2 above, I agree for anonymised information about my ePIPAC treatment to be used in presentations at conferences or medical publications, and am aware that such publications may be available on the internet.

   Signature Print Date

Signature of person taking consent:

Name of person taking consent (PRINT):

Date:

Consent Form

Video recording of the laparoscopic (keyhole) camera feed:

Regarding the video recording of the laparoscopic (keyhole) camera feed (showing the inside of the abdominal cavity, and not including any personal details): Please sign by one of the following three options

A. I understand that the video (or still images from it) will be kept securely here at the University Hospital of Wales as part of the record of my treatment.

Signature | Print | Date

B. In addition to point A above, I agree for the video (or still images from it) to be used for teaching and training of medical, dental, nursing, and healthcare staff and students in the UK and abroad.
I understand that I can withdraw this consent by contacting Professor Jared Torkington (details at the bottom of the form).

Signature | Print | Date

C. In addition to point A and B above, I agree for the video (or still images from it) to be used in presentations at conferences or medical publications, and am aware that such publications may be available on the internet.
I understand that I can withdraw this consent by contacting Professor Jared Torkington (details at the bottom of the form) but that it may not be possible to withdraw images/video clips that have already been published.

Signature | Print | Date

Signature of person taking consent:

Name of person taking consent (PRINT):

Date:

ePIPAC lead Jared Torkington **Contact telephone:** 02920 745148

Consultant Colorectal Surgeon

8.6 ePIPAC audit database form

ePIPAC audit database form:

Addressograph
Name
Address

Please complete this form at the end of the ePIPAC procedure. The data should be entered onto the ePIPAC database on the S drive (secure ePIPAC folder) by the operating surgeon and then this form should be discarded in a confidential waste bin.

Date of procedure	
Number of procedure	
Primary disease	
ASA grade	
BMI	
Pre-operative ECOG performance status	
Pre-operative EORTC-QLQ-C30 score	
If ePIPAC 2 or 3, pre-assessment EORTC-QLQ-C30 score.	
Entry into anaesthetic room	
Start of procedure (knife to skin)	
End of procedure (closure of skin finished)	
Access to abdomen possible?	
Number of ports (please include ultravision ion wand insertion in this number)	
Number of biopsies	
Volume of ascites drained	
PCI index	
Drugs administered and dose	1.
(if only one drug, write N/A)	2.
Any unanticipated steps?	
Blood transfusion	
Conversion to open surgery	
Other (please detail)	

Any incidents reported during this case?	
Spillage of liquid chemotherapy	
Leak of aerosolised chemotherapy	
Deviations recorded on ePIPAC safety checklist?	
Other (please detail)	
Complications reported since last procedure	

8.7 Ethics application: Assessment of intraperitoneal aerosolisation as a delivery method for oncolytic adenovirus therapy in a rat model. Approved ECD 17-109

ANNEX DESCRIPTION OF ANIMAL EXPERIMENTS

REMARKS:

- SINCE A PROJECT CAN CONSIST OF MULTIPLE ANIMAL EXPERIMENTS (that may differ in severity, type of manipulations, animal species, …) THE APPLICANTS SHOULD DUPLICATE THIS ANNEX IN RELATION TO THE AMOUNT OF SPECIFIC EXPERIMENTS (FOR EXAMPLE: IF THE PROJECT ENTAILS THREE SEPARATE EXPERIMENTS THAN THREE FULLY COMPLETED COPIES OF THIS ANNEX SHOULD BE PROVIDED). THE ANNEXES SHOULD BE GROUPED IN ONE SINGLE PDF-DOCUMENT AND ADDED TO THE MAIN APPLICATION FORM.
- THE STRUCTURE OF THIS ANNEX SHOULD NOT BE ALTERED AND ALL QUESTIONS SHOULD BE ANSWERED.

TOTAL AMOUNT OF ANIMAL EXPERIMENTS IN THIS APPLICATION: Complete

Title of animal experiment:

Assessment of intraperitoneal aerosolisation as a delivery method for oncolytic adenovirus therapy in a rat model

a) Detailed description of the animal experiment:

i) Relevance and justification of the animal experiment:

This pilot study will ascertain whether delivery of oncolytic adenovirus by intraperitoneal (IP) aerosolisation is feasible. It will also make a preliminary assessment of whether IP aerosolisation is comparable to IP injection. This will be the first time this technique has been used to deliver a viral vector in vivo. Aerosolisation may offer benefits over IP injection, including increased penetration of the virus into peritoneal tissue, and the ability to use a reduced dose.

ii) Clearly describe the amount of animals needed, the different animal types, amount of experiment repeats, total amount of animals needed, …

Rats will be used. To minimize variation, all rats will be of the same sex. There will be 2 experimental groups: virus administered by IP aerosolisation (n=3 rats), virus administered by IP injection (n= 3 rats). There will also be a control group: IP aerosolisation with saline (n=1 rat) and IP injection of saline (n=1 rat).

iii) Justify how the amount of animals was determined:

This is a pilot study, and as such there is no available data to inform a power calculation. 3 animals in each treatment group should allow the assessment of inter-individual variability in each group, as well as a preliminary inter-group comparison of the effect of the virus therapy. There will be a negative control for both IP injection and IP aerosolisation.

iv) Describe in detail all manipulations that will be done to the animals of every (sub)group. Describe in detail the complete experimental protocol, the chronological order of all manipulations and technical interventions, how you will observe the animals during the experiment to elucidate the pain and discomfort, what action will be undertaken when the predetermined humane endpoints are reached, …

Rats will be given 48 hours to acclimatize before intervention.

Intervention:

Group 1 – negative control injection (n=1 rat) – IP injection of 0.9% NaCl solution

10ml/kg (max 5ml) of warmed 0.9% NaCl will be injected using standard procedures

Group 2 – negative control aerosolisation (n=1 rat) - IP aerosolisation of 0.9% NaCl

As per previously reported experiment, in a class II cabinet, in aseptic conditions under anaesthetic a pneumoperitoneum of 4 mm Hg.will be established. 9 ml of 0.9% NaCl will be administered using the CapnoPen with a flow rate of 0.5 ml/s, and an injection pressure of 20 bar. The deadspace in the injection system is 4ml total, meaning that 5ml will be aerosolised into the peritoneal cavity.

Group 3 – virus by IP aerosolisation (n=3 rats)

In a class II cabinet, in aseptic conditions under anaesthetic a pneumoperitoneum of 4 mm Hg.will be established. 5.4 x10^{10} Ad5 luciferase viral particles in 9ml of 0.9% NaCl will be administered using the CapnoPen with a flow rate of 0.5 ml/s, and an injection pressure of 20 bar. The deadspace in the injection system is 4ml total, meaning that 5ml will be aerosolised into the peritoneal cavity.

Group 4 – virus by IP injection (n=3 rats)

3x10^{10} Ad5 luciferase viral particles in warmed 0.9% NaCl (volume 10ml/kg, maximum 5ml) will be injected using standard procedures.

After intervention, rats will be kept and monitored for 72 hours. Then IVIS imaging will be undertaken to measure the expression of the luciferase reporter gene. The rats will then be sacrificed and intraabdominal organs harvested to assess vector distribution by ex vivo imaging, immunohistochemical staining of paraffin-embedded tissues (IHC-P) and quantification of viral copy number.

b) Severity of the animal experiment

Classification of the severity of the animal experiment: define how the severity grade was assessed. It is advised to consult the document "document on a severity assessment framework" which is available on the website of the European Commission:
http://ec.europa.eu/environment/chemicals/lab_animals/interpretation_en.htm

Class					Amount of animals per class
Terminal	Light	Average	Severe	In vitro	
	8				8

ii) When animals are reused, the applicants should take into account the actual severity of the previous experiment:

Light: ☐

Average ☐

The cumulative effect of the combined experiments is:

Did the person responsible for the health and wellbeing of the animals within your institution/laboratory/animal facility provide a positive recommendation on the reuse of the animals, taking into account the previous manipulations, the current general physical condition of the animal, ? Did the person responsible for the health and wellbeing of the animals within your institution/laboratory/animal facility assess the general well-being and health status of the animal?

iii) Clearly indicate how the well-being of the animal will be followed, assessed and guaranteed during the experiment. Mention how many people are responsible for the daily follow-up of the animals. Mention their name, education/training, function, contact details, and at what specific time points during the experiment they will be involved. A daily inspection of the animals is a legal requirement, also during weekends and holidays.

Is there a protocol from rat model development?

Provide the specifics and criteria that will be used to assess and guarantee the well-being of the animals. If a scoring system is used provide a copy.

Is there a protocol from rat model development?

iv) What methods (analgesia, anesthetics, conditioning/training, …) are used to minimize or eliminate discomfort (pain, suffering, anxiety)?

Is there a protocol from rat model development?

If analgesia, anesthetics, antibiotics or anti-inflammatory medication is used, provide all details (name, type dose, route of administration, dosing period, frequency). Provide references that support the appropriateness of your choice and protocol.

Medication:

Route of administration:

Dose:

Frequency:

Duration:

Who will administer this medication and what is his/her function in the experiment?

Indicate which literature was consulted concerning your choice of analgesia/anesthetics:

☐ Van Zutphen L.F.M., Baumans V., Beynen A.C. 1993. Principles of Laboratory Animal Science: Doses of analgesics for post-operative pain relief ; Rodent, Dog, cat, ferret and larger species, birds, reptiles, amphibians and fish anaesthetic dose rates;

☐ Flecknell P. (1996). Laboratory Animal Anaesthesia - A Practical Introduction for Research Workers and Technicians. 2nd edition. Academic Press, London, p.15-73; p.245-246.

☐ Other:

It is strictly forbidden to execute an animal experiment that leads to severe discomfort, pain and/or anxiety for a prolonged period of time without any intervention that minimizes this severe discomfort, pain and/or anxiety.

If the goals of the study cannot be reached without causing prolonged severe discomfort, pain and/or anxiety, then a very detailed scientifically substantiated motivation should be provided.

n/a

c) Termination of the animal experiment and fate of the animals

i) Endpoints:

Indicate the humane endpoints that will be used to assess discomfort of any kind, and that are adequate to sufficiently early identify this discomfort. The criteria for the evaluation of animal welfare (cf. Animal Experiment: b)/iii)/2nd paragraph) can be used as basis to determine the endpoints.

The rats will be assessed daily after intervention. This will include observations regarding physical state (quality of coat, breathing, stools) and psychological state (grooming behavior, provoked behaviours). Changes will prompt the administration of analgesia. Any severe symptoms will prompt consideration of early euthanasia. All animals will be sacrificed at 72hours post intervention.

- If a pilot study was performed to determine the endpoints of the project then provide the details and the ECD approval number.
ECD number:
Date of approval:

ii) Killing of the animals:

Only the procedures mentioned in Annex 7 of the Royal Decree of May 29, 2013 are allowed to sacrifice the animals.

Describe in detail how the animals will be sacrificed and how their death will be confirmed.

Which animals?	Who?	Method of killing	Confirmation of death
All 8 rats		T-61 Injection	Auscultation, palpation

iii) Animals that will be kept alive:

Only the person responsible for the wellbeing and health status of the animals within your institution/laboratory/animal facility can take the decision to keep the animals alive after the finalization of an experiment.

If already known, what will be the destination of the animals (e.g. reuse, adoption, deliberate release, …)

N/A

If the animals will be kept alive for adoption or deliberate release, provide all details on the evaluation procedure, the type of adoption/release, the used program for social integration, …

Application form for the ethical evaluation of animal experiments

REMARKS:

- THIS FORM COVERS ALL INFOMRATION THAT IS REQUIRED TO ADEQUATELY REVIEW PROPOSED EXPERIMENTS WITH ANIMALS. THESE EXPERIMENTS SHOULD BE PART OF A RESEARCH PROJECT.
- A SEPARATE FORM IS AVAILABLE FOR THE DEVELOPMENT AND MAINTENANCE OF GENETICALLY MODIFIED ANIMALS.
- THE RESPONSIBLE USER (previously designated laboratory director) SHOULD MAKE SURE THAT ALL QUESTIONS ARE ADEQUATELY ANSWERED.
- ALL DOCUMENTS AND COMMUNICATION REGARDING THIS APPLICATION AND ITS EVALUATION SHOULD BE KEPT UNTIL 3 YEARS AFTER THE END OF THE PROJECT.
- THE APPLICATION (This form + the Annexes) SHOULD BE SUBMITTED IN IN A SINGLE PDF-DOCUMENT. THE “NTS” in Dutch, WILL BE SUBMITTED AS AN EXCEL FILE

Title of the project: Assessment of intraperitoneal aerosolisation as a delivery method for oncolytic adenovirus therapy in a rat model

Project code (if applicable) : **not applicable**

User: License number LA **1400072** Responsible USER: **Prof. dr. Wim Ceelen**

Name of the responsible EXPERIMENT LEADER (verantwoordelijke proefleider): **Leen Van de Sande**

Start and end date of the project : **;**

***This application relates to a FWO-Vlaanderen supported project/fellowship*:**

Name promoter or FWO fellow:

Project/fellowship identification code:

Funding period: from to

☒ New project

☐ Adjustment of an approved project with possible negative impact on animal welfare (including an increase (>120%) of the originally foreseen amount of animals). In this case the applicants should provide a **progress report** on the experiments that have already been performed (to be added in a separate PDF-file). Original ECD identification number: ECD

☐ Prolongation of a previously approved project. In this case the applicants should provide a **retrospective analysis** (to be added in a separate PDF-file). Original ECD identification number:
ECD

Project domain (see also section 3):

☐ Fundamental research

☒ Translational or applied research

☐ Regulatory experiments (quality control of products, toxicological or safety experiments, …)

☐ Routine production

☐ Preservation of the environment

☐ Preservation of animal species

☐ Forensic research

☐ Higher education or training

☐ Maintenance of genetically modified animal colonies

Expected severity of the project: if this application contains multiple specific experiments then only indicate the highest level.

terminal ☐ light ☐ in vitro ☐ average ☒ severe ☐

To be completed by the responsible EXPERIMENT LEADER

1. LICENSED LABORATORY (= User)

Laboratory Name:

Experimental Surgery

Address:

UZ Ghent, blok B floor -1, De Pintelaan 185, B-9000 Ghent (Belgium)

License number LA:

LA 1400072

Responsible person of the Licensed Laboratory:

Title and name:

Prof. dr. Wim Ceelen

Phone:

+32 9 332 62 51

E-mail:

Wim.Ceelen@UGent.be

If parts of the animal experiments are being performed at another location (outside the jurisdiction of the Animal Ethics Committee of the UGent Faculty of Medicine and Health Sciences) then provide the details of that partner institution, its license number and the approval of the responsible local animal ethics committee.

Details of the partner institution:

License number LA: Responsible person :

Phone: E-mail:

Research group

Experimental Surgery

Address:

UZ Ghent, blok B floor -1, De Pintelaan 185, B-9000 Ghent (Belgium)

2. STAFF

Responsible Experiment Leader of the project (*The responsible Experiment Leader is responsible for the design and execution of the project and the welfare of the animals)***:**

Name responsible Experiment Leader *(limited to one person)*

Leen Van de Sande

Phone:

9384205

E-mail:

Leen.Vandesande@Ugent.be

Postal address (=**BRIEVENBUSnaam UZGent**):

UZ Ghent, blok B floor -1, De Pintelaan 185, B-9000 Ghent (Belgium)

Staff involved in the project:

Name	Experiment Leader	Active participant	Animal caretaker	Successfully completed specific training (**mention title and date of certificate**); *a copy should be sent to the Animal Ethics Committee*
Leen Van de Sande	☐	☒	☐	Laboratory animal science category C (2015)
Evelien Dierick	☐	☒	☐	Laboratory animal science category B (2017)
Wim Ceelen	☒	☐	☐	Laboratory animal science category C (2006)
Sophia Tate				Observer

Person within the Licensed Laboratory who is responsible for the training and competence of the staff involved in animal experimentation:

Prof. dr. Wim Ceelen

Members of the Animal Welfare Cell (Dierenwelzijnscel) of the Licensed Laboratory:

Prof. dr. Wim Ceelen, Evelien Dierick

Is a member of the Animal Ethics Committee involved in this project? If so, who and how?

(the composition of the Animal Ethics Committee can be consulted at: http://www.ugent.be/ge/nl/raden - login required)

Not applicable

3. PROJECT: description, goals and justification.

Describe in maximal 1.000 words the scientific aspects, rationale and ultimate goals of the project (do not yet mention all experimental details since these should be mentioned in the Annex Description of Animal Experiments).

The project should be described such that it is comprehensible for all members of the Animal Ethics Committee; bearing in mind that the ECD members may not necessarily be experts in

your scientific field. The quality and correctness of the provided information can influence the efficiency of the evaluation process.

The following details are essential:

(i) state-of-the art
(ii) bibliography that supports the justification of the project (provide references to the literature)
(iii) scientific, ethical and social considerations that will facilitate the evaluation of the anticipated results of the project. Mention the scientific unresolved questions and the scientific and/or clinical necessities that justify the execution of this project.

Description, goal and justification:

☐ Fundamental research. *Select the research domain of this project and very briefly describe how your project will contribute to the field. How will the results be communicated? Specify if the project was already evaluated by an external agency. (Select the relevant topic from the drop down list, similar to the report of the statistics)*

...

☒ Translational and applied research. *Precise the added value of your research project and how and when it may contribute to the well being of man and animal (Select the relevant topic from the drop down list, similar to the report of the statistics).*

...

Dr. Alan Parker's research group (University of Cardiff, UK) is studying adenovirus (Ad) vectors for cancer virotherapy applications. The group has generated a number of model replication-deficient (ΔE1/ΔE3) model Ad serotype 5 (Ad5) vectors that are unable to infect normal healthy cells by introducing well characterised ablation mutations in the main proteins on the surface of the virus (hexon, penton base and fiber proteins). The overall aim is to develop a novel therapeutic option for epitheial ovarian cancer (EOC). Thus far, the group has had success in generating a virus that specifically targets αvβ6 integrin, which is found on the EOC cell surface in 1/3 cases, and not on normal peritoneum. The specificity of these vectors has been tested in vitro using primary EOC cell lines. Work has also been carried out in vivo in mouse models of EOC.

The best way to administer this treatment is yet to be determined. Both intraperitoneal (IP) injection and intravenous (IV) injection have been assessed. IP aerosolisation has never been assessed. In animal and human studies where chemotherapy has been administered using

aerosolisation, a reduced dose compared to IV and IP injection has been successful. If the same is true for adenovirus application, it would be beneficial in potentially reducing the immune response, and therefore the side effects of treatment, and it would make the therapy more cost effective if a lower dose is required.

This initial pilot study is using a non-specific adenovirus vector which delivers a luciferase reporter gene to cells. This will enable assessment of the viability of the technique of IP aerosolisation, in comparison with IP injection. Future experiments would then use the rat xenograft model under development to assess the specific oncolytic viruses with the IP aerosolisation technique.

☐ Regulatory tests. *Stipulate the legal basis and regulatory directives if your test is a legal requirement. Select below the type of legal requirement:*

☐ Legislation that meets the requirements of the European Union

☐ Legislation that only meets the national requirements (within the European Union)

☐ Legislation that only meets requirements that do not fall under the jurisdiction of the European Union

...

Subcategories:

- Quality control (including safety and efficacy tests):

n.a.

- Toxicity and other safety tests (including pharmacology)

n.a.

☐ Routine production. *Experiments intending to meet certain needs (blood products, diagnostic kits, …). In this case clarify the need and period in which this need exists. (Select the relevant topic from the drop down list, similar to the report of the statistics)*

...

☐ Preservation of the natural environment with the goal to improve the health and/or well being of man or animal. *Motivate how your project will contribute to the wellbeing or health of man or animal.*

☐ Preservation of animal species. *These experiments usually are part of large multidisciplinary programs. Provide all information that justifies the initiation of such a program.*

☐ Forensic research. *Provide as much details as possible without violating the confidentiality of the investigation and/or privacy of the involved subjects.*

☐ Higher education and/or training. *Describe your goals and explain why these goals cannot be reached using alternative methods. Demonstrate that the use of animals is inevitable for the training and development of competence. If possible the use of alternative methods or cadavers is preferred.*

4. Animal Experiments

Number of different animal experiments that are part of this project application.

1

REMARK: a project can be comprised of different animal experiments (including different manipulations, animal species used, severity of discomfort, …). For each experiment a fully completed version of the form "**ECD_application form_2015_Annex_Animal experiment**"

should be added in as much quantities as the total amount of experiments (Annex Animal Experiment_1, Annex Animal Experiment_2, Annex Animal Experiment_2, …).

Please merge these different Annexes in a single PDF-file and add to this document for your application.

5. ANIMALS

5.1 Species and anticipated amount of animals

Species	Strain	Anticipated amount	Genetic type *			Developmental stage or age of the animals when used	Sex
			Non-genetically modified	Genetically modified without painful/ uncomfortable phenotype	Genetically modified with painful or uncomfortable phenotype		
Rat	Wistar	8	☒	☐	☐	325g	Male

** Genetically modified animals (transgenic, knock-out, …), including natural or induced mutants. The development of a new genetically modified animal line is **always** considered as an animal experiment. Likewise, the breeding of an established genetically modified animal line with painful/uncomfortable phenotype is **always** considered as an experiment.*

All applications for the generation of a new animal line (without any subsequent experimentation) or preservation of an already established animal line should be done using the special application form "xxxxx".

5.2 Origin of the animals

a) Supplier (name, country, certification number):

Animals from external institutes (universities, departments of laboratories) can only be used if that institution has a local certification for the breeding/supply of animals. In case of a Belgian institution the local certification (LA-number) should be available.

Name	Country	Local certification number

Envigo	The Netherlands	GZB/VBB2059853

b) Exemption of use required? (An exemption is required when animals are used that (i) have not specifically been bred for use as laboratory animals but yet are listed in Annex 1 of the Royal Decree of May 29, 2013, (ii) are protected/endangered, (iii) were caught in the wild, (iv) are stray or feral animals or (v) farm animals).

☐ YES. in this case precise for which type of animal an exemption is asked and motivate that your project can only be successfully executed using this type of animals.

☒ No

c) Reuse: (reuse of animals that were used in previous experiments)

☐ Yes (the actual severity of pain/discomfort of the previous animal experiment should be mentioned on "Annex Description of Animal experiments", b)/iii)/2nd paragraph

☒ No

5.3 Housing conditions of the animals

Describe in detail how the animals are housed and whether this is in compliance with Annex 5 of the Royal Decree of May 29, 2013 (use of cage enrichment, group or solitary housing, if solitary housing then mention how long, …). If there is a deviation from the Royal Decree, then explain the potential negative impact for the animals and your actions to minimize this discomfort.

The housing is in accordance with the Belgian legislation. Rat cages are obtained from the animalarium on the UZ Ghent campus. The environment is enriched with wood chips and shredded paper. If possible, the rats are housed in group.

5.4 Animals and the 3R principle

a) The 3R principle should always be applied, with special focus on methods that can replace the use of animals.

i) If alternative techniques exist that do not involve the use of animals, then motivate why this technique is not used in your project. Provide the details of the alternative method and explain why these are not adequate for your specific purpose.

all viruses are initially tested in vitro, but there are not alternatives to in vivo animal testing after this step has been completed.

ii) Which sources have been consulted and when?

Regulatory tests:

- recent list of OESO/OECD-approved alternative methods

(*http://www.oecd.org*) ☐ consulted on:

- recent list of EURL ECVAM approved alternative methods

(*http://ecvam-dbalm.jrc.ec.europa.eu)* ☐ consulted on:

- recent list of European Pharmacopoeia-approved alternative ☐ consulted on:

- Other (specify): ☐ consulted on:

Research:

- Data base and/or published editions of ECVAM or FRAME ☐ consulted on:

- Invittox ☐ consulted on:

- SIS (*http://ihcp.jrc.ec.europa.eu/*)) ☐ consulted on:

- Go3Rs (searches pub med) (*http://www.gopubmed.org/web/go3r/*) ☐
  consulted on:

- Other (specify) : ☐
  consulted on:

Education:

- Norina (*http://www.oslovet.norecopa.no*) ☐
  consulted on:

- NCA (*http://www.nkca.nl/)* ☐
  consulted on:

- Interniche (*http://www.interniche.org/)* ☐
  consulted on:

- Other (specify) : ☐
  consulted on:

iii) Which alternative approach not involving animals could potentially be considered after the initiation of the project.

b) In addition, it is important to design your experiments such that a minimal amount of animals is used (Reduction) while minimizing the distress and pain and maximizing the comfort of the animals (Refinement).

i) Animal species:

Motivate the relevance of the animal species used in your project. The choice of animal species should be in compliance with the requirement to use animals that experience the least amount of pain, suffering or permanent damage, while preserving the reliability of the experiment outcome.

Rats have been selected for the experiment due to the recent demonstration of the feasibility of performing the aerosolisation technique in rats safely. The size of the rats means that IVIS imaging can be used to determine whether the viral vector has successfully delivered the luciferase reporter gene.

ii) Amount:

If possible, demonstrate that the amount of requested animals is in agreement with the amount of animals needed to reach statistical significance.

3 animals have been requested for each treatment group in this pilot study; virus administered by intraperitoneal aerosolisation, virus adminstered by intraperitoneal injection. This is to enable assessment of the variability between individual animals in each group, as well as to estimate the difference between aerosolisation and injection. 2 rats have been requested for control experiments; one rat to have an IP injection of saline, and a second IP aerosolisation of saline.

Which sources have been consulted regarding statistical evaluation (model, previous experience, published data, program, …)?

In case of a statistical analysis, motivate your choice of statistical model and the relevant variables (Power (1-beta), significance (alfa), effect size, …) useful link: http://www.gpower.hhu.de/en.html

In case no statistical analysis is executed then motivate why:

This pilot experiment is to ascertain whether there is evidence of a difference between aerosolised administraton and administration by lavage. It will guide the statistical power calculation used in the design of any future experiments in the event that aerosolisation is feasible, and shows activity that is equivalent or superior to injection.

Is there a possibility to collaborate with an internal or external research group in order to minimize the amount of needed animals? (e.g. by common use of the animals, sharing organs or samples, …).

No ☒

Yes ☐

Which countermeasures have been taken to prevent repetition/duplication of animal experiments?

After a search of pubmed and also after attendance at the recent 4th International PIPAC Congress, Tuebingen October 2017, we are not aware of any other team worldwide that has used this IP aerosolisation system to administer viral vectors.

NTS: Non-confidential, non-technical resume (The person responsible for this project should provide a resume of the project in layman's terms. The target audience of this resume is the general public so avoid the use of technical terms but underscore the importance of your work. The title used in the resume should not necessarily be identical to the one used in the ECD application form. The non-technical resume should be **written in Dutch** using the separate Excel file. Ask assistance to one of your colleagues if you have no knowledge of the Dutch language)

Application form for the ethical evaluation of animal experiments

Repeat the title of your project:

Assessment of intraperitoneal aerosolisation as a delivery method for oncolytic adenovirus therapy in a rat model

Name, **date and signature** of the responsible Experiment Leader, preceded by "read and approved" or "*Gelezen en goedgekeurd*":

Leen Van de Sande

Name, date and signature of the responsible person of the Licensed Laboratory, preceded by "read and approved" or "*Gelezen en goedgekeurd*":

Prof. dr. Wim Ceelen

The completed application form, accompanied by the completed Annex-form should be sent by email to the to attention of Prof. Dr. P. Meuleman, Chairman Animal Ethics Committee (ECD), p/a. ecd.ge@ugent.be

A hard copy (regular internal post) as well as a scan (email) of both signature page should be sent to Prof. Dr. P. Meuleman, Chairman Animal Ethics Committee (ECD), p/a (Dean's office) Decanaat 3K3, De Pintelaan 185, 9000 Gent.

8.8 Ethics application: The assessment of the optimal dose for intraperitoneal administration of adenovirus vectors and the assessment of aerosolisation as an intraperitoneal delivery method in a rat model. Approved ECD 18-23

Application form for the ethical evaluation of animal experiments

remarks:

- *This form covers all information that is required to adequately review proposed experiments with animals. these experiments should be part of a research project.*
- *A separate form is available for the development and maintenance of genetically modified animals.*
- *The responsible Experiment Leader should make sure that all questions are adequately answered.*
- *All documents and communication regarding this application and its evaluation should be kept until 3 years after the end of the project.*
- *The application (this form + signed last page + the annexes (see section 4)) is as a single pdf-document to upload on the ECD-Sharepoint site.*
- *The "NTS" in Dutch, is to upload as an excel file, together with the application form on the ECD-Sharepoint site.*

Title of the project:Assessment of optimal dose for intraperitoneal administration of adenovirus vectors and assessment of aerosolisation as an intraperitoneal delivery method in a rat model.

Project code (if applicable) : **not applicable**

User: License number LA **1400478** Responsible USER: **Prof. Dr. Thierry Bové**

Name of the responsible EXPERIMENT LEADER (verantwoordelijke proefleider): **Leen Van de Sande**

Start and end date of the project : ;

***This application relates to a FWO-Vlaanderen supported project/fellowship*:**

Name promoter or FWO fellow:

Project/fellowship identification code:

Funding period: from to

☒ New project

☐ Adjustment of an approved project with possible negative impact on animal welfare (including an increase (>120%) of the originally foreseen amount of animals). In this case the applicants should provide a **progress report** on the experiments that have already been performed (to be added in a separate PDF-file). Original ECD identification number: ECD

☐ Prolongation of a previously approved project. In this case the applicants should provide a **retrospective analysis** (to be added in a separate PDF-file). Original ECD identification number:
ECD

Project domain (see also section 3):

☐ Fundamental research

☒ Translational or applied research

☐ Regulatory experiments (quality control of products, toxicological or safety experiments, …)

☐ Routine production

☐ Preservation of the environment

☐ Preservation of animal species

☐ Forensic research

☐ Higher education or training

☐ Maintenance of genetically modified animal colonies

Expected severity of the project: if this application contains multiple specific experiments then only indicate the highest level.

terminal ☐ light ☒ average ☐ severe ☐
in vitro ☐

To be completed by the responsible EXPERIMENT LEADER

1. LICENSED LABORATORY (= User)

Laboratory Name:

Experimental Laboratory Surgery

Address:

Corneel Heymanslaan 10, -9000 Gent, -Belgium

License number LA:

1400478

Responsible person of the Licensed Laboratory:

Title and name:

Prof. Dr. Thierry Bové

Phone:

09-3323925

E-mail:

thierry.bové@Ugent.be

If parts of the animal experiments are being performed at another location (outside the jurisdiction of the Animal Ethics Committee of the UGent Faculty of Medicine and Health Sciences) then provide the details of that partner institution, its license number and the approval of the responsible local animal ethics committee.

Details of the partner institution:

License number LA: Responsible person :

Phone: E-mail:

Research group

Not applicable

Address:

Not applicable

2. STAFF

Responsible Experiment Leader of the project (*The responsible Experiment Leader is responsible for the design and execution of the project and the welfare of the animals)***:**

Name responsible Experiment Leader *(limited to one person)*

Leen Van de Sande

Phone:

09 332 15 64

E-mail:

Leen.VandeSande@UGent.be

Postal address **(MAILBOX** (**BRIEVENBUSnaam) UZGent**):

UZ Ghent, blok B floor -1, Corneel Heymanslaan 10, B-9000 Ghent (Belgium)

Staff involved in the project:

Name	Experiment Leader	Active participant	Animal caretaker	Successfully completed specific training (**mention title and date of certificate**)*; a copy should be sent to the Animal Ethics Committee*
Leen Van de Sande	☒	☒	☐	Felasa C (2015)
Sabine De Groote	☐	☒	☒	Felasa B (2016)
	☐	☐	☐	

Person within the Licensed Laboratory who is responsible for the training and competence of the staff involved in animal experimentation:

Prof. dr. Wim Ceelen

Members of the Animal Welfare Cell (Dierenwelzijnscel) of the Licensed Laboratory:

1. Thierry Bové: Doctor in de Genees-heel- en verloskunde, Doctor in de Medische Wetenschappen -09/3323925, thierry.bové@ugent.be

2. Wim Ceelen: Doctor in de Genees-Heel-en verloskunde. Doctor in de Medische Wetenschappen -09/3326251, wim.ceelen@ugent.be

3. Sabine De Groote: 09/3326599, sabine.degroote@ugent.be

4. Lynn De Keyzer: 09/3323607, lynn.dekeyzer@ugent.be

5. Sarah Cosyns: PhD, MSc,09/3321562, sarah.cosyns@Ugent.be

Is a member of the Animal Ethics Committee involved in this project? If so, who and how?

(the composition of the Animal Ethics Committee can be consulted at: http://www.ugent.be/ge/nl/raden - login required)

Not applicable.

3. PROJECT: description, goals and justification.

Describe in maximal 1.000 words the scientific aspects, rationale and ultimate goals of the project (do not yet mention all experimental details since these should be mentioned in the Annex Description of Animal Experiments).

The project should be described such that it is comprehensible for all members of the Animal Ethics Committee; bearing in mind that the ECD members may not necessarily be experts in your scientific field. The quality and correctness of the provided information can influence the efficiency of the evaluation process.

The following details are essential:

(iv) state-of-the art
(v) bibliography that supports the justification of the project (provide references to the literature)

(vi) scientific, ethical and social considerations that will facilitate the evaluation of the anticipated results of the project. Mention the scientific unresolved questions and the scientific and/or clinical necessities that justify the execution of this project.

Description, goal and justification:

☐ Fundamental research. *Select the research domain of this project and very briefly describe how your project will contribute to the field. How will the results be communicated? Specify if the project was already evaluated by an external agency. (Select the relevant topic from the drop down list, similar to the report of the statistics)*

...

☒ Translational and applied research. *Precise the added value of your research project and how and when it may contribute to the well being of man and animal (Select the relevant topic from the drop down list, similar to the report of the statistics).*

PT21 Kanker bij de mens

Dr. Alan Parker's research group (University of Cardiff, UK) is studying adenovirus (Ad) vectors for cancer virotherapy applications. The overall aim is to develop a novel therapeutic option for epitheial ovarian cancer (EOC). The group uses Ad serotype 5 (Ad5) vectors, modifying them by introducing well-characterised ablation mutations in the proteins on the surface of the virus (hexon, penton base and fiber proteins) to abolish the virus' ability to bind with the native Ad receptor on human cells, Coxsackie virus and Adenovirus Receptor. Other mutations are then introduced to promote alternative receptor binding. Thus far, the group has had success in generating a virus that specifically targets αvβ6 integrin, which is found on the EOC cell surface in 1/3 cases, and not on normal peritoneum. The specificity of these vectors has been tested in vitro using primary EOC cell lines. Work has also been carried out in vivo in SKOV 3 mouse models of EOC.

The best way to administer this treatment is yet to be determined. Both intraperitoneal (IP) injection and intravenous (IV) injection have been assessed. IP aerosolisation has never been assessed. In animal and human studies where chemotherapy has been administered using aerosolisation, a reduced dose compared to IV and IP injection has been successful. If the same is true for adenovirus application, it would be beneficial in potentially reducing the immune response, and therefore the side effects of treatment, and it would make the therapy more cost effective if a lower dose is required.

This pilot study is using a standard commercially-available non-replicating (ΔE1/ΔE3) adenovirus vectors. Ad5 luciferase delivers a luciferase reporter gene to cells, and Ad5 β-galactosidase which delivers a β-galactosidase reoprter gene to cells. Initially the feasibility

of using this rat model to test adenovirus vectors. If successful, this will enable assessment of the viability of the technique of IP aerosolisation, in comparison with IP injection. Future experiments would then use the SKOV3 rat xenograft model under development to assess the specific oncolytic viruses with the IP aerosolisation technique.

☐ Regulatory tests. *Stipulate the legal basis and regulatory directives if your test is a legal requirement. Select below the type of legal requirement:*

☐ Legislation that meets the requirements of the European Union

☐ Legislation that only meets the national requirements (within the European Union)

☐ Legislation that only meets requirements that do not fall under the jurisdiction of the European Union

...

Subcategories:

- Quality control (including safety and efficacy tests):

n.a.

- Toxicity and other safety tests (including pharmacology)

n.a.

☐ Routine production. *Experiments intending to meet certain needs (blood products, diagnostic kits, …). In this case clarify the need and period in which this need exists. (Select the relevant topic from the drop down list, similar to the report of the statistics)*

...

☐ Preservation of the natural environment with the goal to improve the health and/or well being of man or animal. *Motivate how your project will contribute to the wellbeing or health of man or animal.*

☐ Preservation of animal species. *These experiments usually are part of large multidisciplinary programs. Provide all information that justifies the initiation of such a program.*

☐ Forensic research. *Provide as much details as possible without violating the confidentiality of the investigation and/or privacy of the involved subjects.*

☐ Higher education and/or training. *Describe your goals and explain why these goals cannot be reached using alternative methods. Demonstrate that the use of animals is inevitable for the training and development of competence. If possible the use of alternative methods or cadavers is preferred.*

4. Animal Experiments

Number of different animal experiments that are part of this project application.

1

REMARK: a project can be comprised of different animal experiments (including different manipulations, animal species used, severity of discomfort, …). For each experiment a fully completed version of the form "**ECD_application form_2015_Annex_Animal experiment**" should be added in as much quantities as the total amount of experiments (Annex Animal Experiment_1, Annex Animal Experiment_2, Annex Animal Experiment_2, …).

Please merge these different Annexes with this form in a single PDF-file to upload on the ECD-Sharepoint site

(Tip: through Athena.ugent.be software as "Foxit PhantomPDF" or "Acrobat Professional"is available to use for merging files into 1 pdf file.)

5. ANIMALS

5.1 Species and anticipated amount of animals

Species	Strain	Anticipated amount	Genetic type *			Developmental stage or age of the animals when used	Sex
			Non-genetically modified	Genetically modified without painful/ uncomfortable phenotype	Genetically modified with painful or uncomfortable phenotype		
Rat	Wistar Han®	12	☒	☐	☐	325 g	M

** Genetically modified animals (transgenic, knock-out, …), including natural or induced mutants. The development of a new genetically modified animal line is **always** considered as an animal experiment. Likewise, the breeding of an established genetically modified animal line with painful/uncomfortable phenotype is **always** considered as an experiment.*

All applications for the generation of a new animal line (without any subsequent experimentation) or preservation of an already established animal line should be done using the special application form "ECD_Aanvraagform_2018_Kweek".

5.2 Origin of the animals

a) Supplier (name, country, certification number):

Animals from external institutes (universities, departments of laboratories) can only be used if that institution has a local certification for the breeding/supply of animals. In case of a Belgian institution the local certification (LA-number) should be available.

Name	Country	Local certification number
Envigo	The Netherlands	GZB/VBB2059853

b) Exemption of use required? (An exemption is required when animals are used that (i) have not specifically been bred for use as laboratory animals but yet are listed in Annex 1 of the

Royal Decree of May 29, 2013, (ii) are protected/endangered, (iii) were caught in the wild, (iv) are stray or feral animals or (v) farm animals).

☐ YES. in this case precise for which type of animal an exemption is asked and motivate that your project can only be successfully executed using this type of animals.

☒ No

c) Reuse: (reuse of animals that were used in previous experiments)

☐ Yes (the actual severity of pain/discomfort of the previous animal experiment should be mentioned on "Annex Description of Animal experiments", b)/iii)/2nd paragraph

☒ No

5.3 Housing conditions of the animals

Describe in detail how the animals are housed and whether this is in compliance with Annex 5 of the Royal Decree of May 29, 2013 (use of cage enrichment, group or solitary housing, if solitary housing then mention how long, …). If there is a deviation from the Royal Decree, then explain the potential negative impact for the animals and your actions to minimize this discomfort.

The housing is in accordance with the Belgian legislation. Rat cages are obtained from the animalarium on the UZ Ghent campus. The environment is enriched with wood chips and shredded paper. If possible, the rats are housed in group. The virus used in this experiment is non-replicating, so normal animal housing and handling methods can be used after administration.

5.4 Animals and the 3R principle

a) The 3R principle should always be applied, with special focus on methods that can replace the use of animals.

i) If alternative techniques exist that do not involve the use of animals, then motivate why this technique is not used in your project. Provide the details of the alternative method and explain why these are not adequate for your specific purpose.

All viruses are initially tested in vitro, but there are not alternatives to in vivo animal testing after this step has been completed.

ii) Which sources have been consulted and when?

Regulatory tests:

- recent list of OESO/OECD-approved alternative methods

(*http://www.oecd.org*) ☐ consulted on:

- recent list of EURL ECVAM approved alternative methods

(*http://ecvam-dbalm.jrc.ec.europa.eu)* ☐
consulted on:

- recent list of European Pharmacopoeia-approved alternative ☐
consulted on:

- Other (specify): ☐
consulted on:

Research:

- Data base and/or published editions of ECVAM or FRAME ☒ consulted on:

13/05/2019

- Invittox ☐
consulted on:

- SIS (*http://ihcp.jrc.ec.europa.eu/*)) ☐
consulted on:

- Go3Rs (searches pub med) (*http://www.gopubmed.org/web/go3r/*) ☒
consulted on: 13/05/2019

- Other (specify) : ☐
  consulted on:

Education:

- Norina (*http://www.oslovet.norecopa.no*) ☐
  consulted on:

- NCA (*http://www.nkca.nl/)* ☐
  consulted on:

- Interniche (*http://www.interniche.org/)* ☐
  consulted on:

- Other (specify) : ☒
  consulted on: 13/05/2019

iii) Which alternative approach not involving animals could potentially be considered after the initiation of the project.

Not applicable

b) In addition, it is important to design your experiments such that a minimal amount of animals is used (Reduction) while minimizing the distress and pain and maximizing the comfort of the animals (Refinement).

i) Animal species:

Motivate the relevance of the animal species used in your project. The choice of animal species should be in compliance with the requirement to use animals that experience the least amount of pain, suffering or permanent damage, while preserving the reliability of the experiment outcome.

Rats have been selected for the experiment due to the recent demonstration of the feasibility of performing the aerosolisation technique in rats safely. The size of the rats also means that IVIS imaging can be used to determine whether the viral vector has successfully delivered the luciferase reporter gene

ii) Amount:

If possible, demonstrate that the amount of requested animals is in agreement with the amount of animals needed to reach statistical significance.

The first part of the experiment is to confirm that our experimental protocol will work in line with work previously published by other groups. Each group has only one rat to minimize the use of animals in this pilot study. The aim is only to confirm adequate detectable reporter gene expression. IP administration of 2 viruses will be tested. One rat will receive the vehicle only as a negative control, and one rat will receive IV virus to act as a positive control.

In the second part, 3 animals have been requested for each treatment group in this pilot study; virus administered by intraperitoneal aerosolisation, virus adminstered by intraperitoneal injection. This is to enable assessment of the variability between individual animals in each group, as well as to estimate the difference between aerosolisation and injection. 2 rats have been requested for control experiments; one rat to have an IP injection of saline, and a second IP aerosolisation of saline.

Which sources have been consulted regarding statistical evaluation (model, previous experience, published data, program, …)?

In case of a statistical analysis, motivate your choice of statistical model and the relevant variables (Power (1-beta), significance (alfa), effect size, …) useful link: http://www.gpower.hhu.de/en.html

Not applicable

In case no statistical analysis is executed then motivate why:

This aim of this pilot experiment is to explore whether there is evidence of a difference between aerosolised IP administraton and administration by lavage IP. However, first it must be confirmed that it is possible to use this rat model to investigate the administration of adenovirus vectors.

If both parts of the study are completed, it will guide the statistical power calculation used in the design of any future experiments in the event that aerosolisation is feasible, and shows activity that is equivalent or superior to injection.

Is there a possibility to collaborate with an internal or external research group in order to minimize the amount of needed animals? (e.g. by common use of the animals, sharing organs or samples, …).

No ☒

Yes ☐

Which countermeasures have been taken to prevent repetition/duplication of animal experiments?

After a search of pubmed and also after attendance at the recent 5th International PIPAC Congress, Paris September 2018, we are not aware of any other team worldwide that is investigating this IP aerosolisation system to administer viral vectors.

NTS: Non-confidential, non-technical resume (The person responsible for this project should provide a resume of the project in layman's terms. The target audience of this resume is the general public so avoid the use of technical terms but underscore the importance of your work. The title used in the resume should not necessarily be identical to the one used in the ECD application form. The non-technical resume should be **written in Dutch** using the separate Excel file. Ask assistance to one of your colleagues if you have no knowledge of the Dutch language)

Application form for the ethical evaluation of animal experiments

Repeat the title of your project:

Assessment of intraperitoneal aerosolisation as a delivery method for oncolytic adenovirus therapy in a rat model

<u>Name</u>, date and signature of the responsible Experiment Leader, preceded by "read and approved" or "*Gelezen en goedgekeurd*":

Leen Van de Sande

<u>Name</u>, date and signature of the responsible person of the Licensed Laboratory, preceded by "read and approved" or "*Gelezen en goedgekeurd*":

Prof. dr. Thierry Bové

These files are to upload on the ECD-Sharepoint site:

- **The completed application form, with the Annex(es) and signed last page <u>as 1 PDF file</u>**
- **<u>NTS (in Dutch)</u> as a separate <u>excel file</u>**

(Tip: through Athena.ugent.be, software as "Foxit PhantomPDF" or "Acrobat Professional" is available to use for merging files into 1 pdf file.)

ANNEX DESCRIPTION OF ANIMAL EXPERIMENTS

REMARKS:

- SINCE A PROJECT CAN CONSIST OF MULTIPLE ANIMAL EXPERIMENTS (that may differ in severity, type of manipulations, animal species, …) THE APPLICANTS SHOULD DUPLICATE THIS ANNEX IN RELATION TO THE AMOUNT OF SPECIFIC EXPERIMENTS

(FOR EXAMPLE: IF THE PROJECT ENTAILS THREE SEPARATE EXPERIMENTS THAN THREE FULLY COMPLETED COPIES OF THIS ANNEX SHOULD BE PROVIDED). THE ANNEXES SHOULD BE GROUPED IN ONE SINGLE PDF-DOCUMENT AND ADDED TO THE MAIN APPLICATION FORM.

- THE STRUCTURE OF THIS ANNEX SHOULD NOT BE ALTERED AND ALL QUESTIONS SHOULD BE ANSWERED.

TOTAL AMOUNT OF ANIMAL EXPERIMENTS IN THIS APPLICATION: Complete (1/1)

Title of animal experiment:

Assessment of optimal dose for intraperitoneal administration of adenovirus vectors and assessment of aerosolisation as an intraperitoneal delivery method in a rat model.

a) Detailed description of the animal experiment:

v) Relevance and justification of the animal experiment:

We conducted a pilot experiment to test delivery of adenovirus by IP aerosolisation compared to IP injection in Wistar Han rats in 2017. The rats tolerated the procedure, but the expression of the reporter gene from the virus was not detected in either the IP aerosolisation or the IP injection group. This was unexpected, since rats have been used as a model for the development and testing of adenovirus vectors by other groups. In vitro experiments have shown that our virus has the capability to infect and deliver a reporter gene in vitro in a Wistar rat cell line (CC1 cell line, ECACC, supplementary data attached). The dose of virus in the 2017 experiment was chosen based on our experience of using adenovirus vectors in mice, and on the doses used in a paper assessing transduction efficiency of adenovirus in rats which tested IP administration (Huard et al 1995). Since then, we have discussed our findings with other groups who work with adenovirus therapies and use a rat model. In the past, they have administered higher doses with success (Denby et al 2004).

This study will first assess whether a higher dose of adenovirus is effective, and tolerated by the rats. If expression of the reporter gene is detected then the second part of the study will reassess whether delivery of adenovirus vectors by intraperitoneal (IP) aerosolisation is feasible. Replication deficient adenoviruses will be used throughout the study.

We are aiming to establish a model with which to assess IP aerosolisation versus IP injection of oncolytic adenoviruses. Peritoneal metastases from ovarian cancer are difficult to treat.

They rapidly become resistant to platinum based chemotherapy regimens, and cannot usually be cured with surgery. Dr Alan Parker's group have been developing adenovirus vectors which specifically target ovarian cancer cells, and could represent an effective novel therapy for metastatic ovarian cancer (Uusi-Kerttula et al 2016). The optimal mode of administration has not been determined. IP aerosolisation has theoretical benefits over IP injection. It has been demonstrated that the distribution and penetration of other drugs administered by this method were better than IP injection (Solass et al, 2012). The aim of this experiment is to establish that aerosolisation is a feasible method to deliver adenovirus vectors, and that this rat model is a viable method to investigate this delivery method further.

Huard, J., et al., *The route of administration is a major determinant of the transduction efficiency of rat tissues by adenoviral recombinants.* Gene Ther, 1995. **2**(2): p. 107-15.

Denby, L., et al., *Adenoviral Serotype 5 Vectors Pseudotyped with Fibers from Subgroup D Show Modified Tropism In Vitro and In Vivo.* Human Gene Therapy, 2004. **15**(11): p. 1054-1064

Uusi-Kerttula, H., et al., *Pseudotyped αvβ6 integrin-targeted adenovirus vectors for ovarian cancer therapies.* Oncotarget, 2016. **7**(19): p. 27926-27937.

Solass W, et al. Therapeutic approach of human peritoneal carcinomatosis with Dbait in combination with capnoperitoneum: proof of concept. Surgical Endoscopy. 2012;26(3):847-52

vi) Clearly describe the amount of animals needed, the different animal types, amount of experiment repeats, total amount of animals needed, …

Rats will be used. To minimize variation, all rats will be of the same sex (males).

Part 1: 4 rats

IP administration of vehicle (saline) – n=1 Wistar Han rat

IV administration of Ad5.luc at a dose of $1x10^{11}$vp - n=1 Wistar Han rat

IP administration of Ad5.luc at a dose of $1x10^{11}$vp - n=1 Wistar Han rat

IP administration of Ad5.βgal at a dose of $1x10^{11}$vp – n=1 Wistar Han rat

Part 2: 8 rats

There will be 2 experimental groups: virus administered by IP aerosolisation (n=3 Wistar Han rats), virus administered by IP injection (n= 3 Wistar Hanrats). There will also be a control group: IP aerosolisation with saline (n=1 Wistar Han rat) and IP injection of saline (n=1 Wistar Han rat).

vii) Justify how the amount of animals was determined:

The first part of the study is to determine whether further investigation using this rat model is feasible. In the previous experiment in 2017 it was anticipated that intraperitoneal administration of Ad5.luc would result in a detectable reporter gene signal. However, this was not the case. The groups in the first part of the experiment will assess the optimal method and virus for administration. A higher dose of virus, in line with Denby et al's work, will be used. The second part of the experiment testing the aerosolisation method for intraperitoneal administration will only go ahead if reliable detection of the expression of the reporter gene after intraperitoneal administration is achieved. One rat will be administered the vehicle only (saline) as a negative control. The remaining 3 rats will be administered virus. The Ad5.luc virus is preferred, since the reporter signal produced is quantified by the In Vivo Imaging System (IVIS) imaging software, giving an immediate determination of transduction. However, a second reporter gene (β-galactosidase) will also be tested. Intraperitoneal administration has been shown to result in a different pattern of transduction to intravenous administration in the past, with higher levels of expression of the reporter gene in a number of tissues (Huard et al 1995). An intravenous administration group is therefore included as a positive control. Each group has only one rat to minimize the use of animals in this pilot study. The aim is only to confirm adequate detectable reporter gene expression.

If expression of the reporter gene is not detected then the study will be discontinued at this point.

The second part of the study will help to confirm tolerability of the dose if it is efficacious, and will have negative controls for both treatment arms. Since this is a pilot study, and the likely difference in expression of the reporter gene between the two groups is not known, a power calculation is not presented. The virus used will be determined by the first part of the experiment.

There will be 3 animals in each treatment group, which will allow assessment of inter-individual variability in each group, as well as a preliminary inter-group comparison of the effect of the virus therapy. There will be one control rat for each administration method, who will receive the vehicle only (saline).

viii) Describe in detail all manipulations that will be done to the animals of every (sub)group. Describe in detail the complete experimental protocol, the chronological order of all manipulations and technical interventions, how you will observe the animals during the experiment to elucidate the pain and discomfort, what action will be undertaken when the predetermined humane endpoints are reached, …

Rats will be given at least 48 hours to acclimatize before intervention.

Part 1

Intervention:

Rat 1. IP injection of max. 5 ml 0.9% NaCl solution of warmed 0.9% NaCl

Rat 2: IV injection of 200microlitres 0.9% NaCl containing $1x10^{11}$ Ad5 luciferase viral particles. 25G needle will be used.

Rat 3: IP injection of max. 5 ml 0.9% NaCl solution of warmed 0.9% NaCl containing $1x10^{11}$ Ad5 luciferase viral particles. Needle of max. 21G will be used (preferably 25G).

Rat 4: IP injection of max. 5 ml 0.9% NaCl solution of warmed 0.9% NaCl containing $1x10^{11}$ Ad5 β galactosidase viral particles. Needle of max. 21G will be used (preferably 25G).

After intervention, rats will be kept for 72 hours and assessed for well-being as described below. Because the virus used is non-replicating, no special animal handling or housing arrangements are required.

Reporter gene analysis: IVIS imaging of rats 1, 2, and 3 will be undertaken at 72 hours to measure the expression of the luciferase reporter gene. The rats will then be sacrificed (first general anesthesia using sevoflurane and then 0.3 ml/kg IV T-61) and intraabdominal organs harvested to assess vector distribution by ex vivo imaging.

Rat 4 will be sacrificed after 72 hours (first general anesthesia using sevoflurane and then 0.3 ml/kg IV T-61), and the intraabdominal organs (liver and spleen) and sections of the abdominal wall will be harvested for transgene expression using β-Gal enzyme-linked immunosorbent assay (ELISA) kit. Liver and spleen from rat 1 will also be processed and tested for expression in the same way.

In all rats, tissue will be harvested for immunohistochemical staining of paraffin-embedded tissues (IHC-P) and quantification of viral copy number using real-time polymerase chain reaction.

If the rats tolerate the procedure, and there is expression of a reporter gene detected, the experiment will proceed to part 2. If the expression of reporter gene is seen in both the Ad5

luciferase rat and the Ad5 β galactosidase rat, then the Ad 5 luciferase virus will be used in part 2.

Part 2:

Intervention:

Group 1 – negative control injection (n=1 rat)

IP injection of max. 5 ml 0.9% NaCl solution of warmed 0.9% NaCl. Needles of max. 21G will be used (preferably 25G).

Group 2 – negative control aerosolisation (n=1 rat)

This intervention is performed in a class II cabinet, in aseptic conditions. General anesthesia is induced with 8%vol sevoflurane and 1200 ml/min O_2. Anesthesia is maintained with 4-5%vol and 800 ml/min O_2. The abdomen is shaved and the skin is disinfected. Two incisions of ± 1 cm are made into the abdominal wall. One balloon trocar per incision is inserted. A pneumoperitoneum of max. 8 mmHg is created. 9 ml of 0.9% NaCl is administered using the CapnoPen with a flow rate of 0.5 ml/s, and a max. injection pressure of 20 bar. The dead space in the injection system is 4 ml total, meaning that 5 ml will be aerosolised into the peritoneal cavity. After 30 min the aerosol is evacuated using a closed aerosol waste system. Peritoneum and muscle wall/skin is sutured. Buprenorphine (0.1 mg/kg) is administered subcutaneously. On day 1-2-3 post-op, 0.05 mg/kg buprenorphine is administered (SC).

Group 3 – virus by IP aerosolisation (n=3 rats)

This intervention is performed in a class II cabinet, in aseptic conditions. General anesthesia is induced with 8%vol sevoflurane and 1200 ml/min O_2. Anesthesia is maintained with 4-5%vol and 800 ml/min O_2. The abdomen is shaved and the skin is disinfected. Two incisions of ± 1 cm are made into the abdominal wall. One balloon trocar per incision is inserted. A pneumoperitoneum of max. 6 mmHg is created. 1.8 $x10^{11}$ viral particles (Ad5 luciferase or Ad 5 β galactosidase) in 9 ml of 0.9% NaCl is loaded into the high pressure injector syringe and the injector set at a flow rate of 0.5 ml/s, and a maximum injection pressure of 20 bar. The dead space in the injection system is 4 ml total, meaning that 5 ml of the solution will be aerosolised into the peritoneal cavity using the CapnoPen, containing a dose of $1x10^{11}$ viral particles (Ad5 luciferase or Ad 5 β galactosidase). After 30 min the aerosol is evacuated using a closed aerosol waste system. Peritoneum and muscle wall/skin is sutured. Buprenorphine (0.1 mg/kg) is administered subcutaneously. On day 1-2-3 post-op, 0.05 mg/kg buprenorphine is administered (SC).

Group 4 – virus by IP injection (n=3 rats)

$1x10^{11}$ viral particles (Ad5 luciferase or Ad 5 β galactosidase) in max. 5 ml warmed 0.9% NaCl is injected IP using standard procedures.

After intervention, rats will be kept for 72 hours and assessed for well-being as described below. Because the virus used is non-replicating, no special animal handling or housing arrangements are required.

Expression of the reporter gene will be assessed as in part 1.

If Ad5 luciferase has been used, then IVIS imaging will be carried out at 72 hours prior to sacrifice (first general anesthesia using sevoflurane and then 0.3 ml/kg IV T-61), and then ex vivo imaging of the abdominal organs, immunohistochemical staining of paraffin-embedded tissues (IHC-P) and qPCR quantification of viral copy number.

If Ad5 β galactosidase is used then rats will be sacrificed (first general anesthesia using sevoflurane and then 0.3 ml/kg IV T-61) at 72 hours and the intraabdominal organs (liver and spleen) and sections of the abdominal wall will be harvested for transgene expression using β-Gal enzyme-linked immunosorbent assay (ELISA) kit. Immunohistochemical staining of paraffin-embedded tissues (IHC-P) and qPCR quantification of viral copy number will also be carried out.

b) Severity of the animal experiment

Classification of the severity of the animal experiment: define how the severity grade was assessed. It is advised to consult the document "document on a severity assessment framework" which is available on the website of the European Commission:
http://ec.europa.eu/environment/chemicals/lab_animals/interpretation_en.htm

Class					**Amount of animals per class**
Terminal	**Light**	**Average**	**Severe**	**In vitro**	
	IV-injections, IP-injections or laparoscopy. We are not anticipating adverse effects from the virus.				12

ii) When animals are reused, the applicants should take into account the actual severity of the previous experiment:

Light: ☐

Average ☐

The cumulative effect of the combined experiments is:

Not applicable

Did the person responsible for the health and wellbeing of the animals within your institution/laboratory/animal facility provide a positive recommendation on the reuse of the animals, taking into account the previous manipulations, the current general physical condition of the animal, ? Did the person responsible for the health and wellbeing of the animals within your institution/laboratory/animal facility assess the general well-being and health status of the animal?

Not applicable

iii) Clearly indicate how the well-being of the animal will be followed, assessed and guaranteed during the experiment. Mention how many people are responsible for the daily follow-up of the animals. Mention their name, education/training, function, contact details, and at what specific time points during the experiment they will be involved. A daily inspection of the animals is a legal requirement, also during weekends and holidays.

Leen Van de Sande – PhD student (follow-up on regular basis)

Leen.VandeSande@UGent.be

+32 9 332 15 64

Provide the specifics and criteria that will be used to assess and guarantee the well-being of the animals. If a scoring system is used provide a copy.

To evaluate animal welfare, a scoreboard is used.

Body mass:

0 normal

1 <10 % weight loss

2 10 – 15 % weight loss, appetite

3 > 20 % weight loss, no appetite

Appearance:

0 normal

1 lack of self-care

2 rough coat (perhaps with runny nose)

3 very rough coat, abnormal posture, pupils enlarged

Clinical signals:

0 normal

1 minor clinical changes

2 rise of body temperature of 1 – 2 °C

3 rise of body temperature of > 2 °C

Behavior:

0 normal

1 minor behavioral changes

2 abnormal behavior, less mobile, less alert, inactive when activity is expected

3 unsolicited vocalization, extreme self-mutation

Response to extraneous stimuli:

0 normal

1 slight excessive response

2 average excessive response

3 violent response

If score 1 or 2 are given on one of the components, pain relief (0.1 mg/kg buprenorphine) is administered. If score 3 is given to one of the components, advice is obtained from the veterinarian of the animalarium. If score ≥ 1 is given on multiple parts, advice is obtained from the veterinarian of the animalarium.

iv) What methods (analgesia, anesthetics, conditioning/training, …) are used to minimize or eliminate discomfort (pain, suffering, anxiety)?

If the scoreboard shows that a rat suffers from pain, buprenorphine is administered subcutaneously. In case of severe pain or in specific ailments, the veterinarian of the animalarium will discuss which medication may be administered for the specific case.

If analgesia, anesthetics, antibiotics or anti-inflammatory medication is used, provide all details (name, type dose, route of administration, dosing period, frequency). Provide references that support the appropriateness of your choice and protocol.

Medication: Sevoflurane

Route of administration: inhalation

Dose: induction 8%vol – maintenance 4-5%vol

Frequency: once (during laparoscopy)

Duration: during laparoscopy

Who will administer this medication and what is his/her function in the experiment? Leen Van de Sande – active participant

Medication: Ketoprofen

Route of administration: subcutaneous

Dose: 5 mg/kg

Frequency: 3 days

Duration: once daily

Who will administer this medication and what is his/her function in the experiment? Leen Van de Sande – active participant

Medication: T-61

Route of administration: IV (tail vene)

Dose: 0.3 ml/kg

Frequency: once

Duration: once

Who will administer this medication and what is his/her function in the experiment? Leen Van de Sande – active participant

Indicate which literature was consulted concerning your choice of analgesia/anesthetics:

☒ Van Zutphen L.F.M., Baumans V., Beynen A.C. 1993. Principles of Laboratory Animal Science: Doses of analgesics for post-operative pain relief ; Rodent, Dog, cat, ferret and larger species, birds, reptiles, amphibians and fish anaesthetic dose rates;

☒ Flecknell P. (1996). Laboratory Animal Anaesthesia - A Practical Introduction for Research Workers and Technicians. 2nd edition. Academic Press, London, p.15-73; p.245-246.

☒ Other: Curtin LI, Grakowsky JA, Suarez M, Thompson AC, DiPirro JM, Martin LBE, e.a. Evaluation of buprenorphine in a postoperative pain model in rats. Comp Med. februari 2009;59(1):60–71.

It is strictly forbidden to execute an animal experiment that leads to severe discomfort, pain and/or anxiety for a prolonged period of time without any intervention that minimizes this severe discomfort, pain and/or anxiety.

If the goals of the study cannot be reached without causing prolonged severe discomfort, pain and/or anxiety, then a very detailed scientifically substantiated motivation should be provided.

Not applicable

c) Termination of the animal experiment and fate of the animals

i) Endpoints:

Indicate the humane endpoints that will be used to assess discomfort of any kind, and that are adequate to sufficiently early identify this discomfort. The criteria for the evaluation of animal

welfare (cf. Animal Experiment: b)/iii)/2nd paragraph) can be used as basis to determine the endpoints.

The rats will be assessed daily after intervention. This will include observations regarding physical state (quality of coat, breathing, stools) and psychological state (grooming behavior, provoked behaviours). Changes will prompt the administration of analgesia. Any severe symptoms will prompt consideration of early euthanasia. All animals will be sacrificed at 72hours post intervention.

- If a pilot study was performed to determine the endpoints of the project then provide the details and the ECD approval number.
ECD number:
Date of approval:

Not applicable

ii) Killing of the animals:

Only the procedures mentioned in Annex 7 of the Royal Decree of May 29, 2013 are allowed to sacrifice the animals.

Describe in detail how the animals will be sacrificed and how their death will be confirmed.

Which animals?	Who?	Method of killing	Confirmation of death
All 12 rats	Leen Van de Sande	T-61 IV	Palpation, ausculation

iii) Animals that will be kept alive:

Only the person responsible for the wellbeing and health status of the animals within your institution/laboratory/animal facility can take the decision to keep the animals alive after the finalization of an experiment.

If already known, what will be the destination of the animals (e.g. reuse, adoption, deliberate release, …)

Not applicable

If the animals will be kept alive for adoption or deliberate release, provide all details on the evaluation procedure, the type of adoption/release, the used program for social integration, …

Not applicable

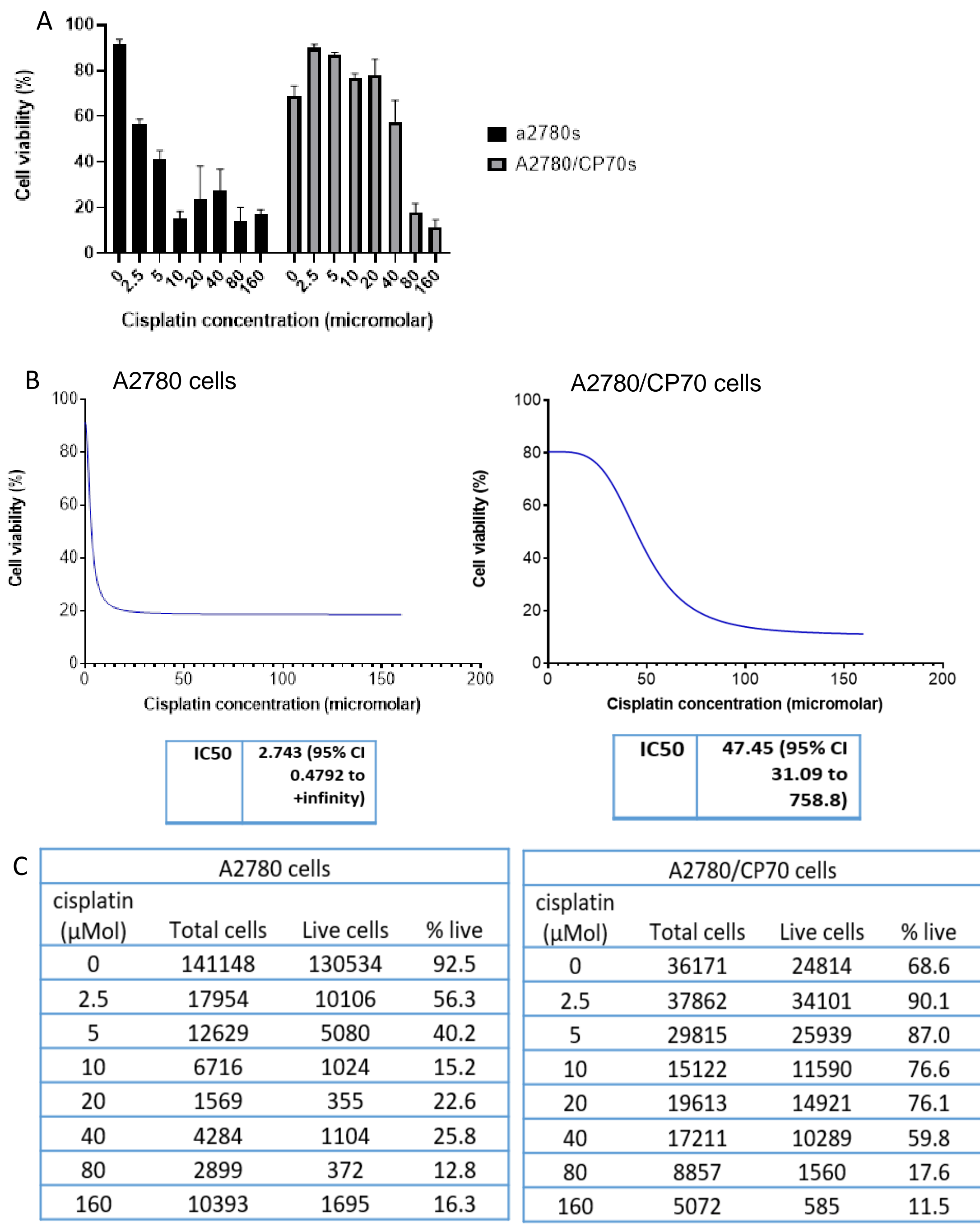
An amendment to this protocol was submitted to change the dose of virus used in the second part of the experiments. The following treatment groups were proposed:

- Negative control IP injection with saline (n=1);
- Negative control nebulization with saline (n=1);
- PIPAV with 3x 10e11 particles (n=3);
- IP injection with 3x 10e11 particles (n=3).

A volume of 5 mL will be administered in each treatment group. For the nebulization groups, you have to take into account a dead volume of 4 mL.

ethical approval was granted: ECD19-53

8.9 Additional figure: Assessing the response of ovarian cancer cell lines to Cisplatin with increasing duration of exposure in vitro using Live Dead aqua staining and flow cytometry.



IC50	2.743 (95% CI 0.4792 to +infinity)

IC50	47.45 (95% CI 31.09 to 758.8)

A2780 cells			
cisplatin (µMol)	Total cells	Live cells	% live
0	141148	130534	92.5
2.5	17954	10106	56.3
5	12629	5080	40.2
10	6716	1024	15.2
20	1569	355	22.6
40	4284	1104	25.8
80	2899	372	12.8
160	10393	1695	16.3

A2780/CP70 cells			
cisplatin (µMol)	Total cells	Live cells	% live
0	36171	24814	68.6
2.5	37862	34101	90.1
5	29815	25939	87.0
10	15122	11590	76.6
20	19613	14921	76.1
40	17211	10289	59.8
80	8857	1560	17.6
160	5072	585	11.5

Appendix Figure 1: Assessing the response of ovarian cancer cell lines to Cisplatin with increasing duration of exposure in vitro using Live Dead aqua staining and flow cytometry.

Cells were plated in 96 well plates at a density of 20,000 cells per well in complete medium (depending on cell type) and incubated for 12 hours. The medium was then replaced with complete medium containing cisplatin at concentrations ranging from 0µM to 320 µM. Each concentration was tested in triplicate (n=3). The plates were incubated at 37°C and 5% CO2. Cell viability was assessed at 48 hours,

using a LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit as per the manufacturers protocol. The number of live cells was then assessed using flow cytometry. The cell viability in each cisplatin-treated well was determined by expressing the number of live cells as a percentage of the total number of cells. A: The mean cell viability and SD at each cisplatin concentration (µM) for each cell line is displayed.

B: Dose response curves and IC_{50} values for A2780 and A2780 CP70 cells determined by non-linear regression (GraphPad Prism)

C: The raw data obtained from flow cytometry. This shows that, in general, the proportion of live cells decreased with increasing cisplatin concentration. However, the total number of cells also decreased.