Image-assisted surgical planning in gynaecological oncology

Pubudu N J Pathiraja

Supervisor: Prof: Ahmed Ahmed



University Of Oxford

Linacre College

MSc (Res) Thesis

Preface:

Thesis Abstract

Major barriers to achieving progress in gynaecological cancer management are limitations in ovarian cancer treatment and morbidity related to lymphadenectomy in early stage cervical, vulval and endometrial cancers.

The main morbidity of treatments for early endometrial, cervical and vulval cancers is surgery. Currently, all patients with high-grade endometrial cancers, cervical cancers and squamous cell cancers of the vulva receive a complete regional lymphadenectomy. Complete lymphadenectomy causes significant morbidity, affecting the long-term quality of life for these patients. There has been an emphasis on sentinel node biopsy to eliminate these morbidities, and some cancer centres already provide sentinel lymph node biopsy using radioactive isotopes, without compromising patient survival. Our study mainly focuses on a novel imaging technique using near infrared (NIR) with compatible dyes. We demonstrated the feasibility and efficacy of using intraoperative NIR for sentinel lymph node biopsy.

Advanced ovarian cancer management involves a combination of surgery and chemotherapy. The aim of surgery is to remove all visible disease before commencing chemotherapy or mid-way during chemotherapy. Limitations to radical surgery are the unpredictability of radicality from available imaging modalities and patient morbidity. Limitations to chemotherapy are the limited response or resistance, mainly due to tumour heterogeneity. Our ovarian cancer pilot study aims to explore methods that could overcome the limitations of surgical planning prior to radical surgery and to identify tumour resistance in specific tumour sites by tracking the tumours during chemotherapy.

Chapter one:

In this chapter, we conducted a pilot study using a Near Infrared imaging technique to isolate sentinel lymph nodes in early stage gynaecological cancers. Therefore, this observational pilot study investigates the feasibility of detecting SLN using near infrared (NIR) fluorescence.

Chapter two:

In this chapter, we conducted a pilot study of the novel approach of using available imaging techniques for an accurate diagnosis and surgical planning in patients who were suspected to have advanced ovarian cancer. This early phase study was registered as OXO-PCR (Oxford Ovarian Cancer – Predict Chemotherapy Response).

Published work form study

Laios A, Volpi D, Tullis I, Woodward M, Kennedy S, Pathiraja P N J

Haldar K, Vojnovic B, Ahmed A A. *A prospective pilot study of detection of sentinel lymph nodes in gynaecological cancers using a novel near infrared fluorescence imaging system.* BMC Res Notes, 2015. **8**: p. 608.

Fluorescence image-guided surgery development and applications

P Pathiraja, D Volpi, IDC Tullis, A Laios, B Vojnovic, A Ahmed. IGCS, Melbourne Australia, November 2014.

Acknowledgements

I offer my sincere thanks to Ahmed Ahmed for providing research funding and the Nuffield Department of Obstetrics and Gynaecology for giving me the opportunity to participate in clinical research. Dr. Ahmed meticulously assessed every possible scenario during my research project to direct me in critical thinking and the use of available resources for my project. He introduced me to the challenging reality of managing patients with advanced ovarian cancer. I learned a few essential laboratory methods; I soon realised the challenges of "lab discipline", and this training was one of the most memorable experiences during the very early stage of my research.

I would like to offer my sincere thanks to Sandra and Mara who guided me in the laboratory and allowed me to orientate myself with various "rules". Without their guidance, I would not have been able to learn the DNA and RNA extraction methods.

I express my gratitude to Alex, Davide and Boris for their contributions and efforts to ensure that the image-guided sentinel lymph node study was successful.

I express my sincere gratitude to Adelyn Wise for her excellent support during the amendments to the clinical trial protocols and for enlightening me about the duties of the clinical trials unit and data management. I offer my sincere gratitude to the early phase trial team, who accommodated my requests and demands with a positive note. I offer my gratitude to Zoe Trail for assisting me with the examination of the CT scans.

I am grateful to my clinical team and the Gynaecology Oncology multi-disciplinary team members for their support.

Table of Contents

Contents:	**Page**
Preface	02
Abstract	02
Acknowledgements	04
List of abbreviations	07
List of figures	08
Introduction	11
Chapter One	
Image-guided surgery for other gynaecological cancers	15
Prospective pilot study of real time near infrared fluorescence imaging for the detection of sentinel lymph nodes in gynaecological cancers. (An Observational Pilot study of Near Infrared (PIONIR) imaging of sentinel lymph nodes in early stage vulval, cervical and endometrial cancers)	
Introduction	17
Materials and Methods	19
Results	26
Discussion and conclusions	30
Chapter Two	35
Tracking ovarian tumours and its clinical implications (A prospective observational study; the Oxford Ovarian Cancer – Predict Chemotherapy Response (OXO-PCR-01) study)	

Introduction

1.1 Ovarian cancer & Present treatment strategies 36

1.2 Aim of the research 39

Patients and Methods 40

Results

Introduction to the study results 45

2.1.1. Patient characteristics 46

2.1.2. Initial Radiological and Laparoscopic staging 48

2.2. Post-chemotherapy assessment 51

2.2.1. Radiological and Laparoscopic response 54

2.2.2. Biopsies obtained with emphasis of the aim of the study 55

2.3. Optimisation of tissue collection for RNA extraction 61

Summary, discussion and future studies

3.1. Summary 66

3.2. Discussion 67

3.3. Further studies 69

Appendix I: Near infrared used in Sentinel nodes detection 70

Appendix II: On going genomic analysis of tumour samples 71

References 72

List of abbreviations:

BMI	Body mass index
Ca	Cancer
CT	Computerised tomography
EORTC	European Organisation for Research and Treatment of Cancer
GI	Gastrointestinal
HGSOC	High grade serous ovarian cancer
ICG	Indocyanine green
IDS	Interval debulking surgery
LGSOC	Low grade serous ovarian cancer
LLQ	Left lower quadrant
LN	Lymph node
LUQ	Left upper quadrant
MB	Methylene blue
MDT	Multi-disciplinary meeting
NACT	Neo-adjuvant chemotherapy
NIR	Near infrared
OCA	Ovarian cancer
RLQ	Right lower quadrant
RUQ	Right upper quadrant
RCT	Randomised controlled trial
UGI	Upper gastrointestinal
USS	Ultrasound scan
SLN	Sentinel lymph node

List of figures:

1. PIONIR. Consort study flow chart

2. PIONIR. Device development

3. PIONIR. External iliac SLN in a patient with endometrial cancer.

4. PIONIR. Subcutaneous SLN visualisation and node sampling in a patient with vulval cancer using the NIR fluorescence device.

5. Schematic representation of the study.

6. Abdominal divisions and common port site.

7. Laparoscopic and CT scan images of diaphragmatic disease.

8. Pre- and post-chemotherapy CT scan showing right peritoneal disease and a partial response.

9. Advanced stage ovarian cancer with omental/large bowel mesenteric disease (Pre-NACT) Pt. A.

10. Advanced stage ovarian cancer after chemotherapy (3 cycles) Pt. A. This image shows an example of stable disease with a minimal response.

11. OCA peritoneal disease and laparoscopic clip application (Pre-NACT) Pt. A. Tumour sampling and mapping of the right pelvic peritoneum.

12. Advanced stage ovarian cancer after chemotherapy (3 cycles) Pt. A.

13. Allele fractions obtained from whole genome sequencing.

List of Tables:

1 PIONIR: Main study outcomes.

2 PIONIR: Histopathological outcomes.

3 OXO-PCR: Radiological and Laparoscopic staging with Histology of screened Patients.

4 OXO-PCR: CT response and CA 125 using the Chi-square test.

5 OXO-PCR: Pre- and Post-NACT CA-125 responses and radiological responses, along with laparoscopic results prior to IDS.

6 OXO-PCR: Laparoscopy, initial biopsy samples, and samples at IDS.

7. PIONIR: Summary of SLN biopsy in gynaecological cancers using NIR fluorescence.

Introduction

Introduction

Ovarian cancer is the sixth most common cancer among females in the UK, and there are 7,300 new diagnoses every year(*1*). More than 53% of cases are diagnosed in women over the age of 65 years. Ovarian cancer is the most lethal gynaecological malignancy(*2*), mainly due to the late diagnosis; most cancers are diagnosed at an advanced stage (stage 3c or 4)(*3*) because of vague symptomatology. There are no methods for screening ovarian cancer. Two major studies investigated screens for ovarian cancers and confirmed the limitations in screening ovarian cancer(*4, 5*).

Current treatments are surgery and chemotherapy for patients who can tolerate radical debulking (surgical removal of all visible tumours)(*6*). Most patients who present advanced stage disease have multiple co-morbidities, and as most of these patients are elderly, they also carry a significant age-related morbidity and mortality(*7*). The administration of neo-adjuvant chemotherapy prior to surgery was an acceptable treatment, with possible lower morbidity, in two recent randomised controlled trials (RCTs)(*8, 9*). During the last few decades concept of surgical management has progressed from the removal of pelvic gynaecological organs to debulking of all visible tumour sites(*10*). Residual disease after surgery is one of the most important independent prognostic factors for survival, which was first reported by Redman et al in 1986 (*9, 11, 12*). One of the major barriers to optimum treatment of ovarian cancer is chemotherapy resistance due to the heterogeneity of the tumour(*13*). The current chemotherapy strategy is two to three decades old and has achieved little success against chemo-resistance. Ongoing translational research is attempting to identify a new-targeted therapy; at present, there are no commercially available agents to combat chemo-resistance. The emphasis on obtaining a good quality tissue sample at diagnosis

and accurate staging at presentation are paramount for efficient management of ovarian cancer(*14*).

Genomic analyses of the tumours and identification of existing mutations and new bio-chemical pathways that allow ovarian cancer cells to survive, metastasise and grow are vital for next generation treatment strategies(*15*).

This early phase study on advanced stage ovarian cancer (OXO-PCR) enabled us to test the concept of intra-operative tumour tracking, tumour sampling and clinical staging. This novel approach of using laparoscopic imaging for accurate diagnosis and surgical planning for suspected advanced ovarian malignancies revealed the common pitfalls of radiological staging, which is the currently accepted method (*16, 17*).

The surgery to treat endometrial, cervical and vulval cancers involves the removal of regional lymph nodes(*18*). The incidences of endometrial and cervical cancers are increasing (*19, 20*). The incidences of vulval cancer appear to have increased in patients below the age of 70 years(*21*). Regional lymphadenectomy for endometrial, cervical and vulval cancers has significant advantages, despite the morbidity. Although a recent RCT of the management of endometrial cancer with or without pelvic lymphadenectomy showed no survival benefit(*22*), this RCT opened an investigation of endometrial cancer surgery. Recent case series and multi-centre studies demonstrate significant benefits of pelvic lymphadenectomy in reducing the morbidity related to pelvic radiotherapy and increasing progression-free survival(*23*). Lymphadenectomy carries significant morbidity that will compromise the patients' quality of life(*24*). The removal of sentinel lymph nodes with radioactive isotopes was introduced in various surgical disciplines to overcome surgery-related morbidity. Groin lymphadenectomy in vulval cancer was one of the first procedures investigated

in a RCT to evaluate the sensitivity and specificity of sentinel node biopsy in gynaecological oncology(*25*). There are no RCTs detecting sentinel nodes in endometrial or cervical cancers. A recent multicentre study of endometrial cancer sentinel node biopsies using technetium and blue dyes challenged the need for pelvic lymphadenectomy(*26*). In cervical cancers, the concept of sentinel node biopsy was evaluated in a multicentre prospective study(*27*).

The aim of our study was to evaluate the use of near infrared imaging (NIR) of lymph nodes to identify sentinel nodes and assess its sensitivity and specificity to diagnose tumour metastasis.

Study Hypothesis

Near Infrared imaging methods for intra-operative sentinel biopsy in gynaecological cancers are feasible and effective.

We propose that a laparoscopic tissue biopsy for initial diagnosis and staging may improve the diagnostic accuracy and enable personalised surgical approaches when treating patients with advanced ovarian cancer.

Aim 1 **PI**lot Study **O**f **N**ear **I**nfra**R**ed **(PIONIR)** – Demonstrate effective sentinel lymph node biopsy of regional lymph nodes using near infrared imaging methods.

Aim 2 Oxford Ovarian Cancer – Predict Chemotherapy Response **(OXO-PCR)** – Demonstrate the feasibility of laparoscopic staging and tumour tracking in patients with advanced stage ovarian cancer.

Chapter One

Image-guided surgery in gynaecological cancers.

Prospective pilot study of near infrared (PIONIR) fluorescence imaging for the detection of sentinel lymph nodes in gynaecological cancers.

Abstract

Lymphadenectomy for cancer staging carries a significant morbidity and consequently reduces the patients' quality of life. Sentinel Lymph Node (SNL) biopsy has been shown to reduce surgical morbidity. Of all gynaecological cancers, SNL biopsy with the radioisotope Technetium 99 (Tc99) is only recommended for vulval cancer. There are significant logistical problems when radioactive tracers are used in clinical practice. Therefore, this observational pilot study investigates the feasibility of detecting SLN with near infrared (NIR) fluorescence.

A custom-built optical system that can detect multiple fluorescent dyes was used in combination with bright-field imaging at laparoscopic and open surgery. This prospective study evaluated 49 women with various gynaecological cancers who were scheduled for complete lymphadenectomy. Commonly used fluorescent contrast agents indocyanine green (ICG) and methylene blue (MB) were used according to the study protocol. Mapping of the lymphatic channels/SLN detection rate and false negative SLN rates were calculated using the NIR fluorescence technique.

Sixty-four SLN were detected in 49 patients who had open or laparoscopy surgery. After a brief optimisation phase, the SLN detection rate reached 100% for all cancer types, without any false negatives. In two patients, co-localisation was identified using both MB and ICG dyes. Percutaneous detection of SLN was also achieved in most women with vulval cancer. No adverse events were noted during the study.

This pilot study confirms the feasibility of detecting SLN in gynaecological cancers with a NIR fluorescence imaging system. This finding would make a case for a non-inferiority study with currently used radioactive agents.

Introduction:

Current surgical treatments for early stage cervical, endometrial and vulval cancers involve the removal of the affected organ or removal of the tumour with an adequate margin of normal tissues and regional lymphadenectomy.

Radical lymphadenectomy for early stage cervical, endometrial and vulval cancers carries significant morbidity, particularly if these patients undergo adjuvant treatment(*28, 29*). There are various complications, ranging from a minor cutaneous loss of sensation to more sinister complications, such as peripheral lymph oedema, lymph cyst formation, venous thrombosis and loss of motor nerve innervation to lower limb muscle compartments(*30, 31*).

SLN is the lymph node that first receives lymph from the region of interest (tumour); hence, the SLN has a greater chance of harbouring tumour cells or micrometastasis(*32*). SLN were first described by Cabanas in cancer surgery; since then, SLN became a routine method for some cancers to avoid major surgical morbidity(*33, 34*). SLN biopsy provides much needed prognostic information and helps to avoid morbidity from overtreatment; moreover, no other method is available to detect micrometastasis(*35*). SLN biopsy is a challenging concept for the gynaecological oncology community, as lymphadenectomy is believed to be a paramount staging surgery, particularly in the management of vulval cancer, despite evolving evidence(*36, 37*). Many dyes are used to detect SLN in cancer treatments, but the most well-established method used in practice is the radioisotope-labelled agent Tc99, which is often combined with fluorescent dyes such as ICG(*38-40*). Many centres and clinical staff have concerns with the use of radioisotopes, not only due to their hazardous nature but also the logistics of their use.

A recent publication by Vahrmeijer et al described the use of near infrared (NIR) fluorescence in surgery, emphasising its advantages as an alternative imaging modality to detect SLN (*38, 41*). Few publications in gynaecological oncology use the indocyanine green (ICG) dye, which has detection rates comparable to radioisotope tracers (*41-44*). As the NIR method of SLN biopsy is evolving, there are a few unanswered questions, mainly regarding its reliability in accurately assessing the tumour site, which is based on the injection site and operator experience(*42*). Despite the study published by Abu-Rustum et al, this topic remains controversial due to the differing opinions on the accuracy of the injection site in uterine endometrial cancer(*45, 46*).

This feasibility study primarily focuses on the use of an in-house developed NIR fluorescence imaging system for mapping SLN in vulval, cervical and endometrial cancers. Our secondary objectives were to determine the rates of SLN detection using NIR fluorescence, the false negative rates and the safety of the procedures.

Material and Methods

Clinical trial

The PIONIR (An observational **PI**lot Study **O**f **N**ear **I**nfra**R**ed) study evaluated the clinical feasibility of the imaging of sentinel nodes in early stage vulval, cervical and endometrial cancers using indocyanine green and methylene blue as fluorophores.

This study was approved by the independent Research Ethics Committee (REC) in Oxfordshire and the Oxford University Hospital NHS Trust (Ethics Ref: 11/SC/0099) on 03 February 2012 and performed in accordance with the ethical standards in the 1975 Declaration of Helsinki.

Study design and participants

The PIONIR study was performed at Churchill Cancer Referral Centre between October 2012 and September 2014. Forty-nine (49) patients with early gynaecological malignancies were recruited (cervical, endometrial and vulval cancer). All these patients were discussed in Gynaecology Oncology multi-disciplinary team meeting prior to recommendation for a lymphadenectomy as part of staging. Of those patients who were scheduled for lymphadenectomy, only patients who met the study inclusion criteria were approached. The exclusion criteria were: age <18 years; (*73*) pregnancy; (3) allergy to fluorescent dyes; (4) previous chemotherapy, radiotherapy or surgery in the LNs of interest; (*1*) patient vulnerability and (6) a lack of capacity to provide informed consent or unwillingness to inform the family doctor about participation in the study. The consort schematic decision tree flow-chart of the study is shown in figure 2 PIONIR.

Fluorescent dyes and injection technique

The clinically approved NIR fluorescent dyes, indocyanine green (ICG) and methylene blue (MB) were used according to the study protocol. ICG (25 mg vials, Pulsion Medical Systems, Munich, Germany) was re-suspended in sterile water to obtain an initial concentration of 5 mg/ml for injection. The dye was freshly prepared on the day of surgery and one vial was used for each procedure. Following the induction of general anaesthesia, 1 ml of ICG was injected immediately before surgery. Dye concentrations and volumes fluctuated during the 'learning phase' until an optimal detection rate (100%) was achieved. MB (Methylthioninium chloride, Provepharm SAS, Marseille, France) was supplied as 'Proveblue' and was already dissolved in water to 5 mg/ml. The injected volume was 4 ml. Patients were continuously monitored for side-effects (evidence of allergy or any delayed hypersensitivity).

For patients with vulval cancer, the intradermal ICG injection was performed with a 27G needle, and the needle was positioned at a ~10° angle relative to the skin. Once the bevel pierced the epidermis, the needle was rotated 180° before injection at each of four peri-scarring/tumoural margins. In women with cervical cancer, the subepithelial ICG injection was initially performed at the 3- and 9-o'clock positions in the submucosal layer and then deep into the stroma on both sides of the cervix using a 27G needle. A cervical injection was performed in women with endometrial cancer, as described above. In two selected cases, a second dye was injected into the uterine fundus (midline subserosa) using a 23G spinal needle. In the first woman, the cervical ICG injection was followed by an MB injection in the uterine subserosa. The second woman received a reversed dye injection (i.e., MB in the cervix and ICG in the uterine subserosa).

Intra-operative NIR fluorescence imaging

A custom-built laparoscopic attachment (Figure 3A PIONIR) and a wide-field imaging head (Figure 3B PIONIR) (described in (*53*)) were used during laparoscopic/open surgeries. These attachments have a common interface (Figure 3C PIONIR) and can be interchanged, if necessary. Two monochromatic (<4 nm bandwidth) sources provided excitation light suitable for imaging ICG and MB fluorescence. A special optical filtering arrangement allowed the real-time, simultaneous detection of bright-field colour images and fluorescence images of multiple dyes using a single camera. Fluorescence imaging was performed after dye(s) injection using both wide-field and laparoscopic imagers, as dictated by the surgical procedure. Live video images were displayed on the operating room monitors and the procedures were recorded. The exposure time during acquisition of the fluorescence images ranged between 40 ms and 320 ms/frame, depending on the emission intensity and imaging depth.

In a subset of patients with vulval cancer, percutaneous NIR fluorescence imaging was performed in inguino-femoral regions prior to the surgical procedure to examine whether subcutaneous SLN were detectable (Figure 4 PIONIR). The surgeon estimated whether the fluorescence signal was sufficient to warrant resection, based on a subjective observation. All intra-operative fluorescence hotspots were verified *ex vivo* using the same system.

Surgical procedures

A routine laparoscopic approach was used as the preferred mode of surgery in patients with cervical and endometrial cancers. Open surgery of these cancers was performed if the patients had any other contraindication for laparoscopy. Pelvic side walls were opened after extra-peritoneal visualisation of the pelvic

nodes to develop the anatomical spaces. Meticulous dissection was undertaken so as not to disturb the lymphatic channels and small blood vessels. Once the pelvic side wall is adequately exposed, the NIR/fluorescence inspection was performed.

All patients with vulval cancer also received a routine inguinal incision, as described for inguino-femoral lymphadenectomy, but depending on the detection of subcutaneous SLN using a surface marking, the first node (SLN) was removed prior to systematic lymphadenectomy along the femoral triangle and 4-6 cm along the femoral vessels.

Specimen handling and Histopathology

The SLN was on the appropriate side was removed as soon as it was identified with NIR/fluorescence hotspots; then, these SLN were accurately labelled and sent for histopathological verification and diagnosis. Next, routine lymphadenectomy specimens were sent for histology, according to standard diagnostic criteria. All lymph nodes were assessed for the diagnosis of metastatic disease. Histopathology was used to determine the presence or absence of lymphatic tissues to confirm SLN and tumour metastasis in the excised specimens. The pathological protocol involved ultra-staging of SLNs.

Statistical analysis

The mean age, number of hotspots identified and anatomical location, but not site, were recorded. The hotspot detection rate was calculated as the ratio between the number of cases in which at least one hotspot was detected and the total number of cases. Consistent with previous studies, the SLN detection rate was calculated as the ratio between (a) the number of cases in which at least one

hotspot was detected and confirmed to be a SLN by histological analysis, and (b) the total number of cases.

The false negative rate was calculated as the number of cases in which at least one LN (non SLN) was positive and the SLN(s) was negative, divided by the total number of cases. The true positive rate was calculated as the number of cases in which at least one fluorescently detected SLN was indeed correctly identified as a cancer-positive LN divided by the total number of cases. Data were presented as percentages or ranges (min-max).

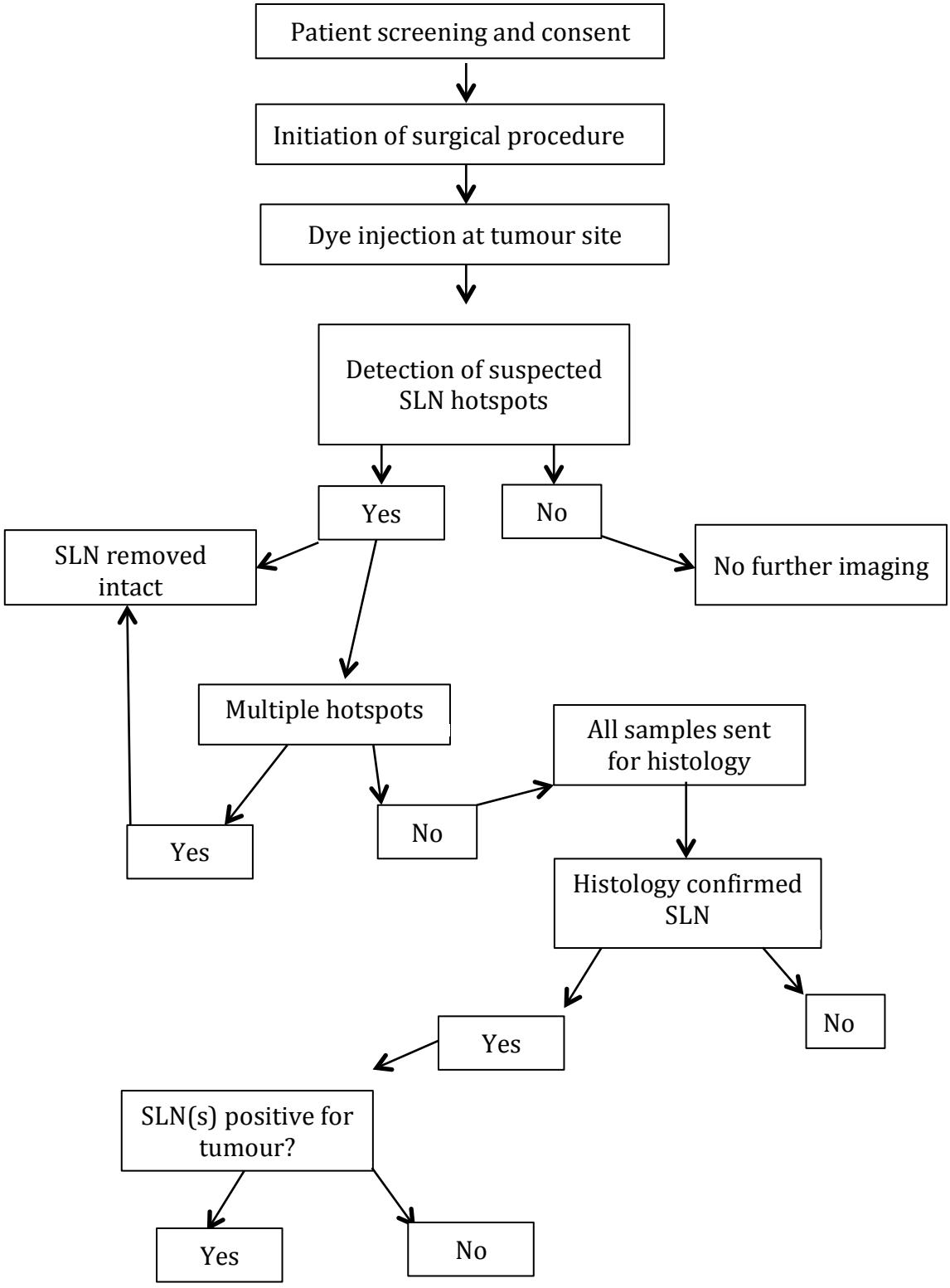


Figure: 1 PIONIR. Consort study flow chart.

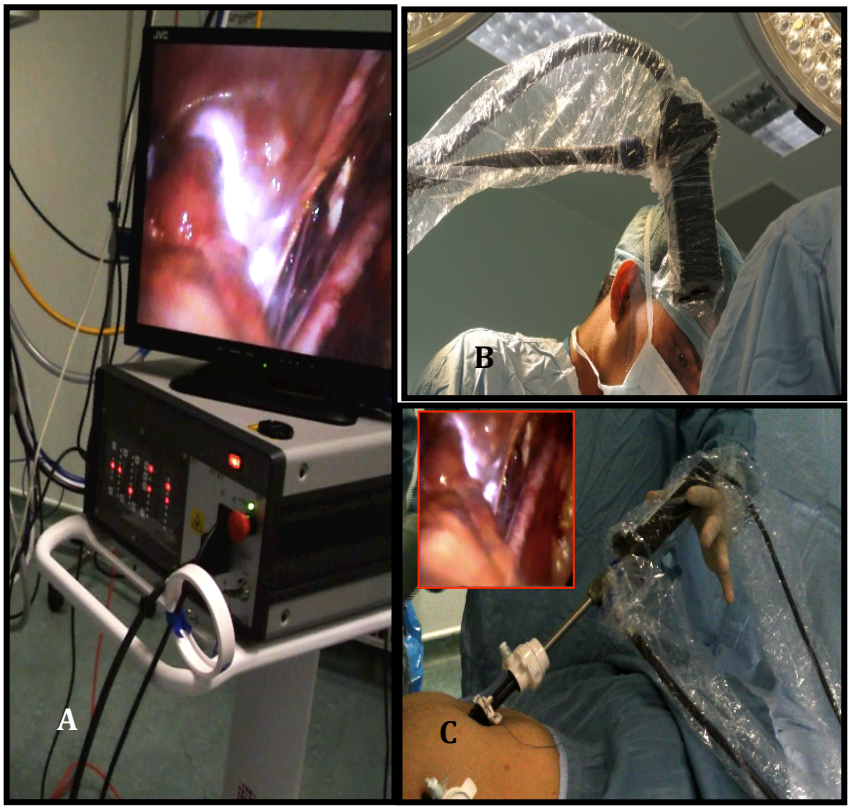


Figure: 2 PIONIR. Device development: *A. Left:* Control unit and display monitor for the imaging system; *B. Top Right:* Hand-held imaging device in use; *C. Bottom right:* Laparoscope, associated imaging device, illumination light guide and inset of surgeon view.

Results

Patients' Characteristics

Forty-nine patients with gynaecological cancers participated in the study; 28 (57.2%) had endometrial cancer, 11 (22.4%) had vulval cancer, and 10 (20.4%) had cervical cancer. The patients' mean age was 61.6 ± 13.2 (range 48-73) years. Only patients with high-grade endometrial cancer participated in the study, according to the local protocol for lymphadenectomy.

SLN detection at surgery

At the beginning of the study, there was a learning phase for the fluorescence detection of SLN, which was overcome by approximately the tenth case of lymphadenectomy. During this phase, the fluorescence hotspots differed slightly from the SLN detection rates, as some hotspots contained adipose tissues. After approximately 30 SLN and lymphadenectomies, the SLN detection rate reached 100%, as shown in Table 1 PIONIR; this detection rate also changed the total number of SLN detected. The dose optimisation procedure was adopted from available studies(*46*) and also by testing various concentrations during the early phase of the study. The optimal injected concentration and volume were 1 mg/ml and 4 ml, respectively (Table 1 PIONIR). In the optimised dose group, the average number of SLNs per case was 2 (1-3) for vulval cancer, 4 (4-4) for cervical cancer and 1.5 (1-3) for endometrial cancer. In endometrial cancers, the most common site of SLN was along the external iliac vessels (Figure: 1 PIONIR). There were no residual hotspots observed at the end of the lymphadenectomy and a fluorescence inspection of the surgical field. No adverse reactions

associated with the fluorescent dyes or intra-operative complications were identified.

	Cancer Type	**All cases (n=49)**	**Optimised dose group (n=16)**
SLN detection rate	Vulval	91%	100%
	Cervical	90%	100%
	Endometrial	68%	100%
	Total	***78%***	***100%***
Average SLNs detected per woman	Vulval	1.1 (0-4)	2 (1-3)
	Cervical	2.3 (0-4)	4 (4-4)
	Endometrial	0.9 (0-3)	1.5 (1-3)
	Total	***1.3 (0-4)***	***1.9 (1-4)***

Table 1 PIONIR: Main study outcomes.

SLN – Sentinel lymph node(s). Note the difference in the rate of SLN detection in all cancer groups during the early phase and optimised stage of the study.

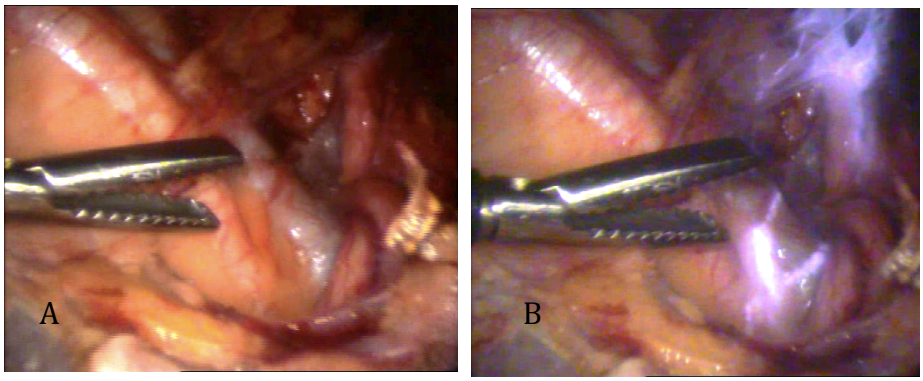


Figure: 3 PIONIR. External iliac SLN in a patient with endometrial cancer. Photograph A demarcates the external iliac artery with small SLN; photograph B highlights the intra-operative fluorescence imaging.

Pre-operative visualisation of possible SLN in vulval cancer

Attempts to detect percutaneous ICG fluorescence using wide-field imaging of the inguino-femoral SLN were successful in 5 out of 7 cases (71%). These SLN were visualised within ~6 min of peritumoural injection of ICG, according to the protocol. A groin incision was made to perform a lymphadenectomy and the SLN biopsy was performed prior to the completion of the lymphadenectomy (Figure: 4 PIONIR). A maximum imaging depth of ~20 mm was noted using a camera exposure time of 80 ms.

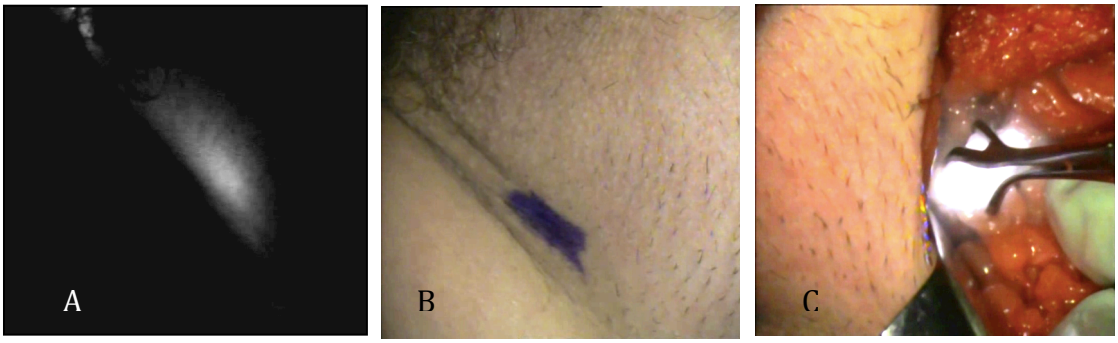


Figure: 4 PIONIR. Subcutaneous SLN visualisation and node sampling in a patient with vulval cancer using the NIR fluorescence device A; demarcation with a marker B; and node sampling using the NIR fluorescence device C.
The image in A shows the subcutaneous visualisation of the possible SLN using the NIR fluorescence imaging device.

Feasibility of Multi-wavelength fluorescence detection in endometrial cancer

Independent fluorescence imaging was demonstrated in two patients with endometrial cancer; ICG and MB were injected into the uterine cervix and uterine fundus. In the first patient, fluorescence co-localisation was identified and the dyes shared common lymphatic channels leading to the external iliac SLN. In the second patient, a para-aortic SLN with only an ICG signal was identified and was correlated with the injection site, as ICG was injected into the uterine fundus and MB was injected to the uterine cervix.

Histopathological analysis

Micro-metastasis of lymph nodes, which include SLN, was detected in 20.4% of patients; the SLN correctly predicted metastasis with no false negative results once the learning phase was excluded from the analysis. The false negative and true positive rates are shown in Table 2PIONIR.

	All patients (n=49)	**Optimised dose group (n=16)**
False negatives	4 (8%)	0 (0%)
True positives	6 (12%)	2 (12.5%)

Table 2 PIONIR: Histopathological analysis.

False positive and negative rates were calculated separately for the procedures performed pre- and post-dose optimisation; note the change in the false negative rates.

Discussion

The gynaecological oncology community is exploring the more effective incorporation of SLN in the routine cancer staging in depth; ESGO has recently published their general consensus (*47*). We decided to conduct a clinical feasibility study to address some of the disadvantages of the existing techniques, minimise the operative morbidity and improve the quality of staging without indiscriminate lymphadenectomy. We successfully demonstrated a custom-built fluorescence imaging system for detecting SLN across all gynaecological cancers where lymphadenectomy is indicated for staging. In some centres in the US, routine NIR fluorescence imaging for the detection of SLN has been shown to improve surgical nodal staging and the diagnosis of micro-metastasis, which is a major prognostic factor(*48*). Following the learning curve required for a new technique, we reported a cumulative SLN detection rate of 100% and no false negatives. The total injected volume and concentration of dye was optimised to 4 ml and 1 mg/ml, respectively. No adverse reactions were observed following dye injection.

One of the most encouraging results was the high SLN detection rate using NIR fluorescence compared to other reported conventional methods based on a blue dye and/or radioactive tracer(*27, 45, 49*). The learning curve indicated a gradual increase in the detection rates following injection, dose optimisation and familiarity with the new technique. Our SLN detection rates agreed with previous studies performed using various NIR fluorescence imaging systems (*42, 50-52*). The detection rates were calculated after the initial 33 cases were excluded. The decrease in detection rate after ~10 cases may be secondary to the subsequent introduction of laparoscopic procedures. This relatively prolonged learning

phase is possibly justified by the disease heterogeneity (vulval, endometrial and cervical cancers), involvement of different surgical teams and the diversity of surgical procedures (open and laparoscopic surgeries) that were considered in this study, but the obvious advantage of detecting SLN using the same imaging modality is worth noting.

The wide-field device in conjunction with a laparoscope proved to be of significant benefit, as fluorescence imaging could be performed independent of the surgery. Moreover, the system allowed simultaneous bright-field and fluorescence visualisation while maintaining a reduced size and weight, making it suitable for hand-held operation. Adequate fluorescence sensitivity was reflected by the high intra-operative detection rates during laparoscopy and the percutaneous SLN detection rates in a large proportion of women (71%) with vulval cancer during open surgery.

Most of the procedures (47/49) described in this study were performed following an injection of ICG alone; the MB fluorescent dye was also used in two patients with endometrial cancer. Independent fluorescence imaging of two different fluorescent dyes (ICG and MB) was achieved using the two separate imaging channels available in our instrument. This unique feature, which is not supported in widely used, commercially available NIR fluorescence systems, justified the development and usage of a novel imaging system. In contrast to previous studies using two different imaging modalities to detect SLN involvement from cervical and hysteroscopic injections (*52*), the use of two fluorescent dyes allowed a more effective comparison and simultaneous examination of differential lymphatic drainage, as the same imaging modality was adopted.

MB has been successfully used as a blue dye for mapping SLN in patients with endometrial cancer (*53*). However, to the best of our knowledge, MB has never been used as a fluorescent dye to detect SLNs. For the first time, we showed that fluorescence SLN visualisation using MB was indeed feasible in a limited number of women (n=2) and provided considerably higher detection sensitivity compared to direct viewing. Nevertheless, further experience with fluorescence imaging of MB is required. In contrast to this argument, many authors recommend using cervical injections for both endometrial and cervical cancers(*47*).

The fluorescently detected SLNs successfully predicted the metastatic status of the other LNs in all but four patients, all of which were included in the learning phase. A possible explanation for the lack of prediction of these cases was the insufficient dose of dye, familiarity with the imaging device or lack of experience. Pathological LN involvement on the ipsilateral side has also been suggested for detecting SLN failure on the tumour side(*45*). The highest SLN detection rate was achieved with an ICG concentration of 1 mg/ml and an injected volume of 4 ml. This finding agreed with a recent meta-analysis of fluorescent SLN biopsy using ICG, which reported a superior detection rate in the sub-group of studies in which the injected volume was ≥ 2 ml and the concentration < 5 mg/ml (*54*).

Our study successfully demonstrated the extracorporeal visualisation of SLN in vulval cancers using ICG and a NIR fluorescence imaging system. Confidence in the subcutaneous detection was accurate up to a depth of ~20 mm below the skin. NIR fluorescence imaging has potential to reduce surgical morbidity in patients with vulval cancer because of the feasibility of using real-time image-guided surgery. However, difficulties were observed in patients with a high BMI

(>30), as the available excitation light intensity could not penetrate the greater tissue thickness. This observation agreed with the findings from a similar study on vulval cancer (*55*).

We were able to demonstrate the independent fluorescence detection of ICG and MB in women with endometrial cancer, which revealed an association between injected anatomical tumour site in the uterus and SLN location; this observation was recorded for the first time in a human subject in this study. To date, there is still widespread controversy regarding the most appropriate injection practice for detecting SLN in endometrial cancer (*46, 51*). Given the limitations in the accessibility of peri-tumoural regions, a common practice is to inject the tracer in the cervical region (*45*). Although this finding was previously reported in studies in which women were either divided into two injection groups (*56*) or assessed using different imaging modalities (*57*), our study is the first in which both sites were concurrently injected in the same woman and the same imaging modality was employed to independently examine the aberrant lymphatic drainage. Using this approach, we eliminated any between patient variability and differences encountered in the sensitivities of detecting the radioactive and blue dyes. We cannot draw a conclusion based on two patients in whom we demonstrated site-specific lymphatic drainage in the uterus, and we plan to re-visit this issue in the future.

I reviewed the available published literature on SLN detection using NIR fluorescence in gynaecological cancers (Table 3 PIONIR). The feasibility of detecting SLN was demonstrated without any disparity in the safety profile. In all but one of the studies, the overall SLN detection rate ranged from 64% to 100% using a variety of imaging devices. However, our study is the only study to report the use of the same imaging system, which can be used to standardise

comparisons of SLN detection across all cancer types. This feature is desirable when the imaging modality strongly relies on the sensitivity of the instrument.

Conclusions

This feasibility study confirms the ability of a custom-built NIR fluorescence imaging device to detect SLN in patients with vulval, cervical and endometrial cancer. We reported a 100% SLN detection rate and maximum specificity and sensitivity following the learning phase and dose optimisation. We observed the use of this system to detect subcutaneous SLN in patients with vulval cancer, as well as the overall safety and versatility of the instruments. Before we use this method in clinical practice, a non-inferiority study with the currently accepted radioisotope tracer agents must be conducted.

Chapter Two

Ovarian tumour tracking and its clinical implications

Abstract.

The aim of this Pilot study was to evaluate the feasibility of establishing a clinical protocol for laparoscopic image-guided surgical planning to link the site-specific chemotherapeutic responses of individual tumours with the underlying genomic alterations. A laparoscopic protocol was assessed in which Intra-operative Video Recording and Tumour tracking (IOVT) was combined with tumour sampling for whole genome sequencing.

Forty-two (n=42) patients were screened for the study, based on a radiological diagnosis of advanced stage ovarian cancer. All patients with suspected stage 3c and stage 4 ovarian cancer were approached. All 42 patients underwent a diagnostic laparoscopy and diagnostic biopsies to evaluate and map specific tumour sites. The patients who were excluded from the study included patients with a non-gynaecological cancer, patients with non-ovarian gynaecological primary disease, patients who were suitable for primary debulking surgery, a patient who moved out of the area, a patient who was not suitable for any treatment option, and another patient who was diagnosed with a rare benign condition. Twenty-two (n=22) patients were selected, based on histology and patient preference.

Nineteen patients (n=19) had interval debulking surgery. Thirteen patients received IDS after 3 cycles of chemotherapy, two patients received IDS after 2 cycles, one patient received IDS after 4 cycles and three patients received IDS after 6 cycles of chemotherapy. Fourteen patients received complete debulking R=0, and five patients received optimal debulking R=0.5 cm.

Samples used to track the tumours were collected at diagnosis and at IDS for genomic studies. Chemotherapeutic responses were evaluated using a post-chemotherapy CT scan and laparoscopy prior to the debulking procedure.

Seventeen patients exhibited a partial response and two patients had stable disease, according to the radiological response. One out of nineteen tissue samples was subjected to whole genome sequencing. The analysis of these data is currently on-going. Based on this study, IOVT combined with whole genome sequencing is a feasible approach to investigate the impact of tumour heterogeneity on chemotherapeutic responses in the management of ovarian cancer. Post-chemotherapy laparoscopic assessments help clinicians to appreciate the changes in tissue morphology that may have been overlooked at surgery.

Introduction:

1.1 Ovarian cancer and present treatment strategy:

Ovarian cancer remains the most lethal gynaecological cancer(*11*) because the majority of ovarian cancers are diagnosed at an advanced stage (70-80%)(*58*). Chemotherapy and surgery are both essential to achieve adequate control of these cancers. The conventional approach for the management of advanced stage disease concentrates on the quality of primary cyto-reductive surgery and chemotherapy(*8, 11, 59*), but long-term control of the disease is mainly determined by the chemotherapeutic response. Ongoing studies are concentrating on individualised, targeted treatments for ovarian cancer (*60*) to overcome the challenge of chemo-resistance due to the heterogenic nature of these tumours (*61*), but there are no commercially available targeted treatments. The identification of prognostic factors and use of various surgical techniques to excise all visible disease has been emphasised in previous publications (*12, 62*) over the last three decades. In recent years, two major randomised controlled trials (RCT) evaluated primary surgery vs interval debulking after neo-adjuvant chemotherapy, but these studies failed to show significant differences between the groups. Nevertheless, the studies showed a benefit of reduced morbidity in patients who received IDS(*8, 11*). Both studies showed the advantage of using NACT to reduce the significant morbidity associated with primary debulking surgery, but tumour resistance remained the main barrier in achieving good survival outcomes. The subjective assessment of “no visible” disease or concept of R=0 (zero residual) disease after surgery and chemotherapy is becoming a challenge due to the unpredictability of the disease response and appearance.

1.2. Aim of the research:

The aim of this Pilot study was to evaluate the feasibility of establishing a clinical protocol for laparoscopic surgical planning to link the site-specific chemotherapeutic responses of individual tumours with underlying genomic alterations.

Laparoscopic staging at the initial diagnosis and Intraoperative Video Recording and surgical planning were the main clinical components of this study.

Intraoperative Video Recording and Tumour tracking (IOVT) combined with tumour sampling for whole genome sequencing was the component of the study that bridged the clinical study with basic science. We hope to evaluate the use of IOVT in surgical planning to optimise surgical outcomes in patients with advanced stage ovarian cancer.

Patients and Methods.

Patients:

All patients with suspected advanced stage ovarian cancers by radiological staging were included after the MDT discussion (whenever possible) prior to histological diagnosis. Patients who met the inclusion criteria and who could undergo the diagnostic laparoscopic procedure were approached during the screening process. The study was discussed with a designated GCP compliant clinician and the patient was provided written information at least 24 hours prior to the laparoscopy.

The diagnostic laparoscopic procedure was standardised for objectivity during initial tumour sampling and mapping with a metal clip. All laparoscopic procedures were recorded in the STROZ-OR system in hospital and a backup copy was made to avoid unintentional removal of the data from the OR system. This recording is also extremely important when assessing the chemotherapeutic response and planning the cyto-reductive surgery at a later stage.

Patients who agreed to participate in the study provided consent on the day of the procedure to the designated person and/or operating surgeon, according to the protocol. During the sample collection process, we emphasised the collection of high quality samples for both diagnosis and research. Tumour mapping was performed using a disposable laparoscopic clip application device, which is inserted into the peritoneal cavity via a 5 mm laparoscopic port.

The biopsy sites were recorded on specimen form and samples were labelled with a unique ID and stored on dry ice until further use.

Patient selection criteria:

Inclusion criteria:

Female ≥ 18 years of age

Newly diagnosed and histologically confirmed primary high-grade serous ovarian cancer, high-grade serous fallopian tube cancer or primary peritoneal carcinoma

Radiological and laparoscopic confirmation of FIGO stage 3C or 4 ovarian cancer

Eastern Cooperative Oncology Group (ECOG) performance score of 0-2

Adequate haematological, hepatic and renal function

Life expectancy ≥ 6 months.

Exclusion criteria:

Pregnant or breast-feeding women

Patients with borderline ovarian tumours

History of previous chemotherapy or radiotherapy to the abdomen or pelvis for ovarian cancer, or ongoing cancer treatment

Patients with medical conditions that make them unfit to receive Paclitaxel and/or standard chemotherapy treatment (e.g., uncontrolled infection, congestive heart failure, and severe liver disease)

Major systemic co-morbidities preventing safe participation

Treatment with any other investigational agent, or participation in another interventional clinical trial within 28 days prior to enrolment

Other active malignancy requiring treatment or preventing read out from this study

Patients who are known to be serologically positive for Hepatitis B, Hepatitis C or HIV

History of previous serious allergic reaction to paclitaxel

Symptomatic peripheral neuropathy if more than grade 1 in severity

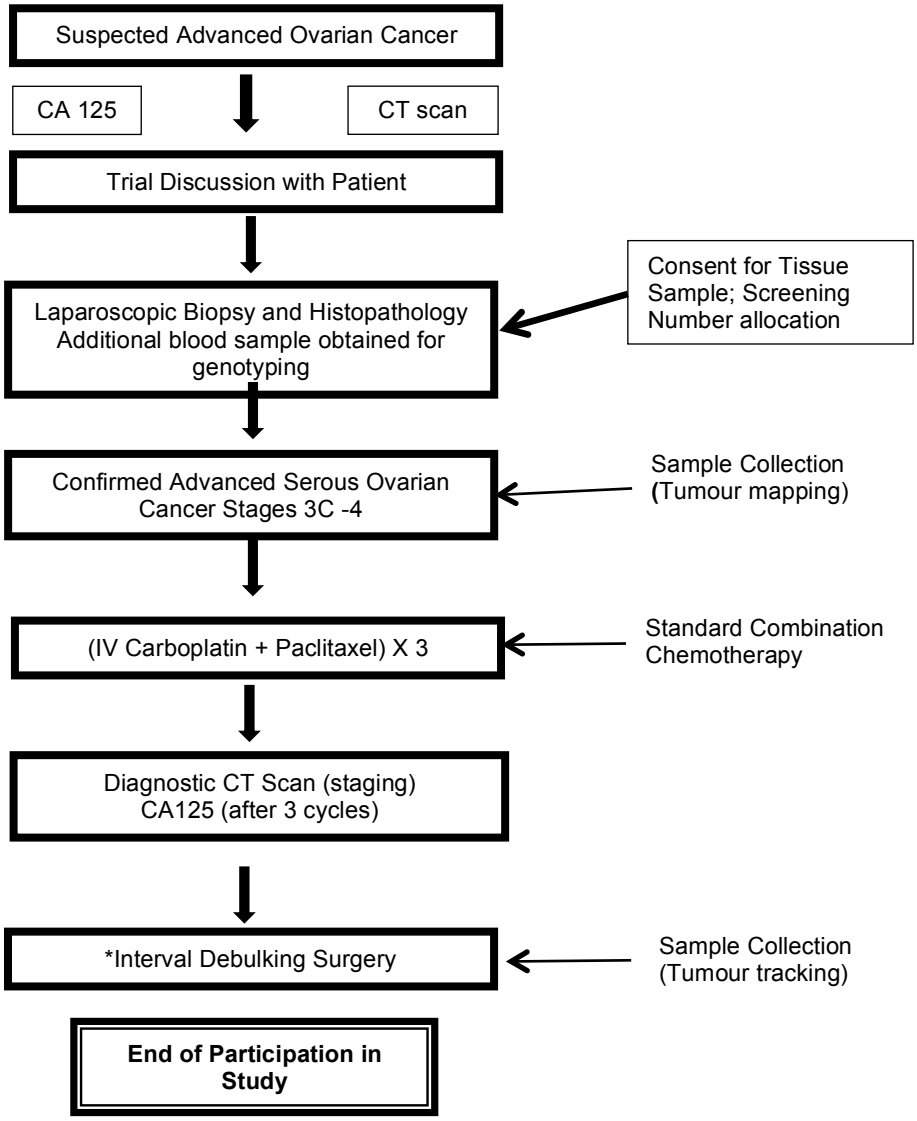


Figure: 5. Schematic representation of the study

Methods:

Laparoscopic procedure and documentation of findings.

Procedure:

Ensure that the laparoscopic instruments are in good working order, with recoding facility and adequate storage on the system.

Primary entry must be from Palmers' point, unless there is evidence of tumour metastasis involving this area. Entry should be the "Open" technique using either re-useable or disposable ports.

Inspection of the laparoscopic entry point and systematic visualisation of the abdominal cavity (pneumoperitoneum) in a 360-degree view. We propose starting from the adjacent site in the left lower quadrant (LLQ), right lower quadrant (RLQ), right upper quadrant (RUQ) and left upper quadrant (LUQ). Ensure that the anatomical *landmarks* of the anterior abdominal wall are adequately visualised and recorded.

Secondary laparoscopic ports (one or two ports) must be inserted under direct vision using re-usable of disposable ports.

Identify the specific tumour sampling area from the anterior abdominal wall peritoneum whenever possible, and prior to tumour sampling, ensure that the measured (width) laparoscopic grasper is placed adjacent to the tumour on the same plane; repeat this procedure for at least one tumour site on each quadrant.

If the tumour is obstructing the view, then the tumour may be mapped on the biopsy sample site of the tumour using a metal clip.

Landmarks:

Lower quadrant landmarks:

1. Median, medial and lateral ligaments,

2. Arcuate line,

3. Umbilicus,

4. Round ligaments.

Right upper quadrant landmarks:

1. Umbilicus,

2. Ligamentum teres and falciform ligament,

3. Lower border of the rib cage.

Left upper quadrant landmarks:

1. Umbilicus,

2. Ligamentum teres and falciform ligament,

3. Lower border of the rib cage.

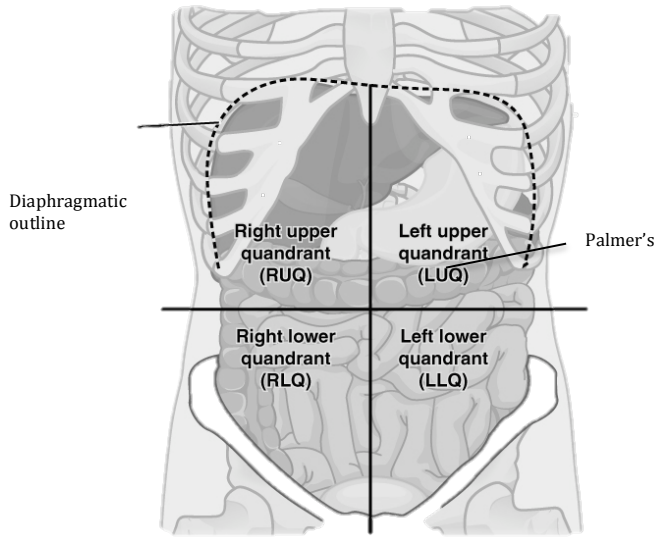


Fig: 6. Abdominal divisions and common port site.

2. Results:

2.1. Introduction to the study results.

This study is a prospective translational study, the Oxford Ovarian Cancer – Predict Chemotherapy Response (OXO-PCR-01), which was approved by the Independent Research Ethics Committee (Ethics number: 12/SC/0404) and the Oxford University Hospital Research & Development Committee. This study was conducted in collaboration with the early phase clinical trials unit at Oxford University. Patients with advanced stage ovarian cancer, based on radiological staging (CT-scan), were screened for the study.

Screening was performed according to study protocol, number OCTO-029, version 4.0. Patients who were suitable for screening provided consent to a clinical research team member to obtain a diagnostic laparoscopy (a minimally invasive procedure where a narrow optical device (laparoscope) is inserted into the abdominal cavity to examine the tissues and obtain biopsies under general anaesthesia). During the laparoscopic procedure, biopsy samples were obtained from multiple sites and the sites were marked with a surgical clip. If the biopsy confirmed high-grade serous ovarian cancer and the laparoscopic assessment revealed advance stage disease (Stage 3C or 4), patients were recruited for the study. Patients who were confirmed to have a lower stage disease by laparoscopy received primary debulking surgery.

Patients who enrolled in the study and who were eligible for interval debulking surgery after three or more cycles of chemotherapy underwent an additional laparoscopic assessment at the time of surgery. If these patients proceed to debulking surgery, repeat biopsies were obtained from sites adjacent to the initial biopsy. Details are provided in the methods section.

2.1.1. Patients' characteristics

Forty-two patients with suspected advanced stage ovarian cancer were screened for eligibility to participate in this study (Table 1 was table 2). Eleven patients had a non-gynaecological histological diagnosis after laparoscopic biopsy and were excluded from the study. Four patients had less advanced disease compared to the disease predicted by radiology and thus proceeded to primary surgery and were not eligible for this study; one patient was diagnosed with a rare benign condition that mimicked omental metastasis. Of the remaining 27 patients, only 19 patients underwent complete chemotherapy and surgical treatment for their disease. We were still awaiting most of the results from the basic science experiments at the time this thesis was written. Thus, the analysis in this report will focus on these 19 patients. Eighteen patients were diagnosed with high-grade serous carcinoma and one patient was confirmed to have low-grade serous cancer. All patients had either Stage IIIC or IV disease. The median age of the patients was 67 (range: 54—78). The final histology of high-grade serous ovarian cancer was confirmed in all but one patient.

Pt. No	Initial CT stage	Laparoscopy	Meet criteria	Completed the study	Histology
1	Stage 3c	Stage 2/3A	No	n/a	Leiomyosarcoma
2	Stage 3c	Stage 3c/4	Yes	n/a	HGAdenoca
3	Stage 3c	Stage 2/3A	No	n/a	HGSOC
4	Stage 3c/4	Stage 3c/4	Yes	n/a	HGSOC
5	Stage 3c	Stage 3c/4	Yes	Yes	HGSOC
6	Stage 3c/4	Stage 3c/4	Yes	Yes	HGSOC
7	Stage 3c	Stage 3c	Yes	No	HGSOC
8	Stage 3c	Stage 3c/4	Yes	Yes	HGSOC
9	Stage 3c	Non-gynae	No	n/a	GI tract tumour
10	Stage 3c	Non-gynae	No	n/q	GI tract tumour
11	Stage 3c	Stage 3c	Yes	Yes	HGSOC
12	Stage 3c	Stage 2/3A	No	n/a	HGSOC
13	Stage 3c	Non-gynae	No	n/a	GI tract tumour
14	Stage 3c	Stage 3c	Yes	Yes	HGSOC
15	Stage 3c	Stage 3c/4	Yes	Yes	LGSOC
16	Stage 3c	Stage 3c/4	Yes	Yes	HGSOC
17	Stage 3c	Stage 3c/4	No	n/a	HGSOC
18	Stage 3c	Stage 3c	No	n/a	HGSOC
19	Stage 3c/4	Stage 3c/4	Yes	Yes	HGSOC
20	Stage 3c	Non-gynae	No	n/a	GI tract tumour
21	Stage 3c	Stage 2/3A	No	n/a	HGSOC
22	Stage 3c	Stage 3c	Yes	n/a	HGSOC
23	Stage 3c	Non-gynae	No	n/a	GI tract tumour
24	Stage 3c	Stage 3c/4	Yes	Yes	HGSOC
25	Stage 3c	Non-gynae	No	n/a	Carcinosarcoma
26	Stage 3c	Stage 3c/4	No	n/a	UGI AdenoCa
27	Stage 3c	Stage 3c	Yes	Yes	HGSOC
28	Stage 3c	Stage 3c	Yes	Yes	HGSOC
29	Stage 3c	Stage 3c/4	Yes	No	HGSOC
30	Stage 3c	Stage 1C3	No	n/a	Clear cell Ca
31	Stage 3c	Stage 3c/4	Yes	No	HGSOC
32	Stage 3c	Non-gynae	No	n/a	LGSOC
33	Stage 3c	Stage 2A	No	n/a	Endometrial
34	Stage 3c	Stage 3c/4	No	n/a	HGSOC
35	Stage 3c	GI Primary	n/a	n/a	Adeno Ca
36	Stage 3c	Stage 3c/4	Yes	No	HGSOC

37	Stage 3C	Stage 3c	No	n/a	Benign
38	Stage 3c	Stage 3c	Yes	No	HGSOC
39	Stage 3c	Stage 3c	Yes	No	HGSOC
40	Stage 3c	Stage 3c	Yes	No	HGSOC
41	Stage 3c	Stage 3c	Yes	No	HGSOC
42	Stage 3c	Stage 3c	Yes	No	HGSOC

Table 3. Radiological and Laparoscopic staging with Histology of screened Patients. HGSOC-High grade serous ovarian cancer, Stage 3c-Tumour of more than 2 cm that metastasised to the omentum and/or abdominal peritoneum, Stage 4-Tumour metastasised within abdominal organs or outside the abdominal cavity. Radiological staging criteria (RECIST)(*17*)

2.1.2. **Initial Radiological and Laparoscopic staging:**

Any patient who was suspected of having advanced stage ovarian cancer after an initial pelvic ultrasound scan (USS), was subjected to a CT scan of the chest, abdomen and pelvis. CT of the chest, abdomen and pelvis is the current recommendation for radiological staging of ovarian cancer(*16, 63*). Currently, radiological staging is accepted as the standard for the initial diagnosis of ovarian/peritoneal or fallopian tube cancers (*9, 17*). If the CT scans and biochemical profiles fit a possible diagnosis of ovarian cancer, then an attempt to perform a tissue diagnosis prior to chemotherapy is recommended. However, if the patients are fit to undergo surgical tumour resection, then most centres would weigh the risks and benefits prior to primary debulking surgery (PDS) procedures. In the UK, the tendency is to offer NACT, as there is robust evidence for PDS in patients with advanced stage ovarian cancer (*16, 64*). The decision to treat seventeen out of forty-two (40%) patients as having advanced stage

ovarian cancer was changed because of diagnostic laparoscopy, and six patients (14%) received primary surgery as a result of the laparoscopic staging. Similar results were reported by recent studies performing laparoscopic evaluations, which emphasise the use of laparoscopy for comprehensive staging (*65*, *66*).

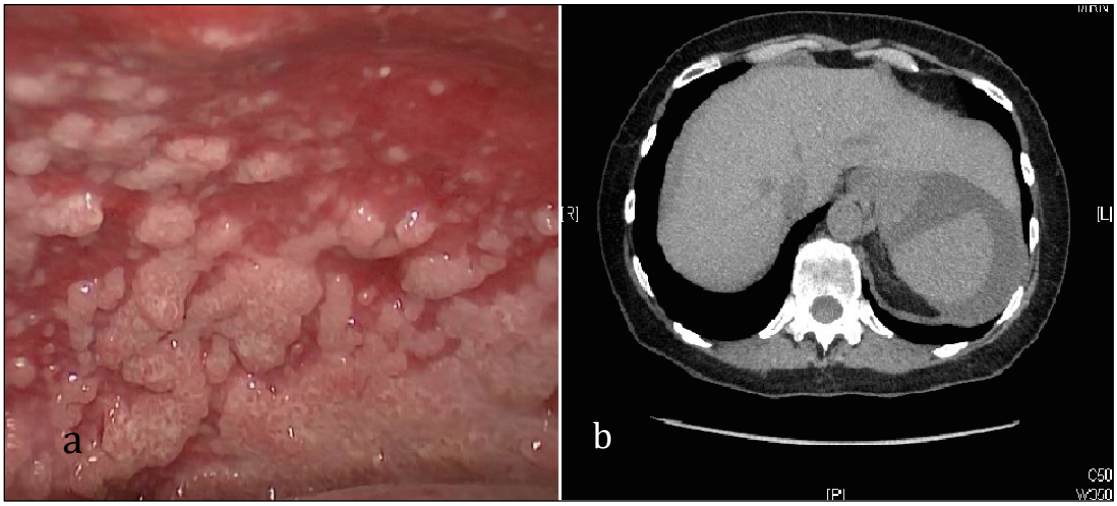


Figure 7: Images of laparoscopy and CT scans of diaphragmatic disease. These pictures illustrate the (a) laparoscopic (visible disease) vs (b) radiological ("no peritoneal carcinomatosis") assessment of the right hemi-diaphragm. The image (a) taken at diagnostic laparoscopy shows carcinomatosis of less than 0.5 cm thickness on the right diaphragmatic peritoneal surface. (b) Cross section CT scan of the same patient at the T11 level. The CT scan failed to recognise the diaphragmatic disease.

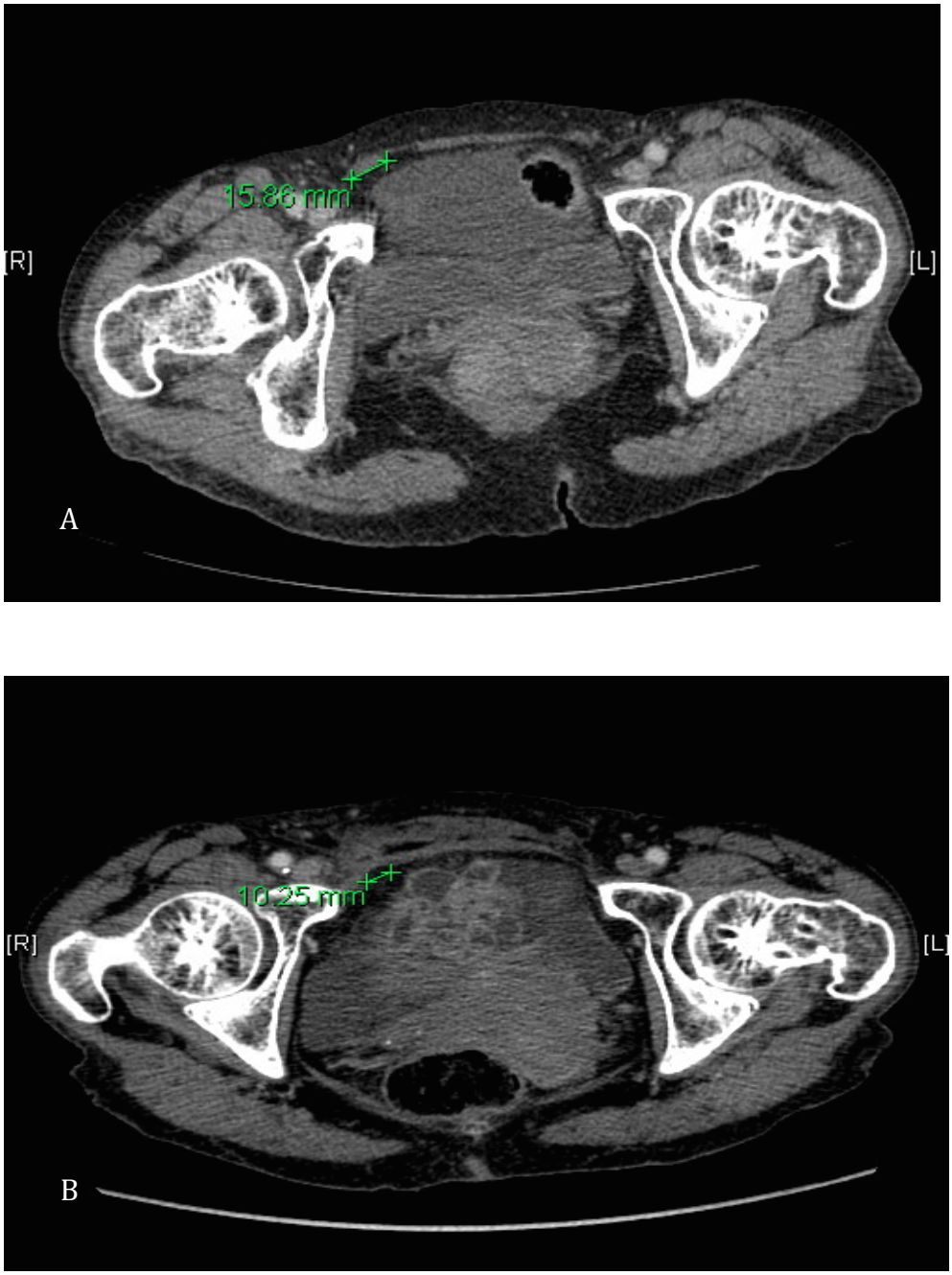


Figure: 8. Pre- and post-chemotherapy CT scan showing right peritoneal disease and a partial response. (A) The pre-chemotherapy CT scan shows the target tumour nodule used to track the response after three cycles of chemotherapy. This nodule measures 15.86 mm at the widest point of reference, and (B) after chemotherapy (3 cycles), this lesion measures 10.25 mm at the widest diameter.

2.2. Post-chemotherapy radiological and laparoscopic assessment:

Prior to interval debulking surgery, the chemotherapeutic response was evaluated biochemically using CA125 measurements and radiologically using CT scans.

The radiological response was assessed based on the established response evaluation criteria in solid tumours (RECIST) (*67, 68*) version 1.1. The response was categorised based on the best-observed response.

A complete response (CR) was reported when there was no visible tumour and when the suspicious lymph nodes measured less than 10 mm on the long axis. None of the patients in this group had CR. A partial response was reported when there was an at least 30% reduction in the mean diameters of the targeted tumours(*28*) (Figure 1). Fifteen patients had PR, and progressive disease was reported when there was a ≥20% increase in the diameter of the targeted tumours. None of the patients had progressive disease. Stable disease (SD) was reported when there was no significant increase and a less than 30% reduction in the diameter of the targeted tumours. One patient had SD.

The biochemical tumour response was assessed according to the Gynaecological Cancer Intergroup (GICG) definition(*68*); briefly, a CA 125 response was defined as an at least 50% reduction in the CA 125 levels compared to the pre-treatment sample. These responses must be confirmed and maintained for at least 28 days. Patients are only evaluated using CA125 if they have a pre-treatment sample that was at least twice the upper limit of the reference range within 2 weeks before starting the treatment. Only fifteen out of the sixteen patients who underwent IDS met this CA 125 criterion, as one patient had a pre-treatment CA125 level that was less than the twice the reference range. Of those fifteen patients, only

twelve patients had an at least 50% reduction in the CA125 levels. Only one sample was obtained prior to IDS and was used in the analysis.

Table 2 presents a summary of the CA 125 and radiological responses; table 3 shows the data for individual patients.

	CT Response PR	CT Response SD	*Marginal Row Totals*
CA125 Optimal	14	2	16
CA125 Sub-optimal	3	0	3
Marginal col Totals	17	2	19 (Total)

Table 4: CT and CA 125 responses; the Chi-square statistic is 0.5769. The P - value is 0.447521. This result is *not* significant at $p < 0.05$. CT, Computerised tomography; PR, partial response; SD, stable disease.

Pre-NACT CA125	Post-NACT CA125	Post-NACT assessment		Rate of Debulking
		Radiology	Laparoscopy	
245	52	PR	Proceed for IDS	0.5
2505	1779	PR	Proceed for IDS	0.0
591	17	PR	Proceed for IDS	0.5
523	92	PR	Proceed for IDS	0.0
169	22	PR	Proceed for IDS	0.0
596	325	PR	Proceed for IDs	0.0
502	54	SD	Proceed for IDS	0.0
1172	73	PR	Proceed for IDS	0.0
98	78	PR	Proceed for IDS	0.0
11392	33	PR	Proceed for IDS	0.0
298	126	SD	Proceed for IDS	0.0
116	44	PR	Proceed for IDS	0.0
1132	249	PR	Proceed for IDS	0.5
325	81	PR	Proceed for IDS	0.0
1375	59	PR	Proceed for IDS	0.0
65	7	PR	Proceed for IDS	0.5
869	11	PR	Proceed for IDS	0.0
3439	23	PR	Proceed for IDS	0.0
650	145	PR	Proceed for IDS	0.5

Table 5. Pre- and Post-NACT CA-125 responses and radiological response with laparoscopic results prior to IDS. NACT-Neo-adjuvant chemotherapy, IDS-Interval debulking surgery. This table illustrates the CT and CA 125 responses after chemotherapy.

2.2.1 **Assessment of heterogeneity in the tumour responses using CT:**

A further detailed analysis of the CT scans was performed to assess whether individual tumour sites from the same cancer varied in their response to chemotherapy. In each patient, two (n = 3 patients) to three (n = 16 patients) individual sites were chosen to measure the tumour responses. A comparison of the CT responses was only feasible for tumours that were more than 1 cm in diameter, as well as solid tumours, which were compared for the purposes of this study. Tumour response was evaluated using RECIST. All patients (100%) had heterogeneous tumour responses on the CT scans.

Based on these results, there is significant heterogeneity in the tumours' responses to chemotherapy. However, the results also highlight the limitations of CT measurements in evaluating the responses of peritoneal and bowel serosal diseases. Obtaining biopsies from multiple tumour sites for research purposes is also difficult.

In addition to the above-mentioned advantages of performing laparoscopy at screening, laparoscopic findings enable us to evaluate the visual clinical response prior to IDS. Currently, we use the laparoscopic procedure for triage patients during IDS, depending on the disease response on the post-chemotherapy CT scans and the extent of disease. Patients with a complete, partial or stable disease response in the post-chemotherapy CT scan receive the laparoscopic evaluation. Our two main criteria for the surgical decisions against IDS are the presence of widespread miliary, including the bowel serosal surface, and multiple hepatic metastases or extra-abdominal disease, which may require multi organ resection. All patients had an adequate clinical response in the CT scan, which was confirmed by laparoscopy, allowing them to proceed with IDS.

The laparoscopic procedure performed during IDS had an advantage of a clinical evaluation of tumour heterogeneity, which was not appreciated by the post-chemotherapy CT scans.

2.2.2 **Biopsies obtained with an emphasis on the aim of the study:**

We obtained samples from multiple tumour sites in all patients prior to chemotherapy and marked those sites using surgical clips or video-laparoscopy. This procedure was followed by chemotherapy and a further laparoscopic evaluation and re-biopsy of the adjacent areas. Twelve patients had matched biopsy samples from an area adjacent to the initial biopsy. These samples will be evaluated using next generation whole genome sequencing methods and areas with different clinical responses will be compared to identify potential drivers of heterogeneity of the chemotherapeutic response. The tumour samples and sites from which they were obtained in these patients are listed in table 4.

Representative images of the biopsy sites from patient "OXO-PCR-1019" before and after chemotherapy are shown in figures 2-4. The corresponding allele fractions obtained from whole genome sequencing of these sample is shown in appendix I (figure 8). Note the laparoscopic appearance of the pre-chemotherapy tumour (figure 2), where the omental tumour appears easily friable. The post-chemotherapy appearance is rather dull and non-friable (figure 3). Figure 4 shows an example of the clip application at the time of laparoscopy prior to chemotherapy and two sites of peritoneal disease (a and b). Figure 5 shows the same sites after three cycles of chemotherapy.

Pt. No.	Laparoscopic biopsy sites	Histology	Biopsy sites at IDS	Outcomes of debulking surgery
1005	1. BP	H. Serous	Not matched	IDS post-NACT x6C
	2. PER			
1006	1. OMR	H. Serous	PEL	Paired
			DP	
			MP	
			OVR1	
			OVR2	
1008	1. FL (Clip 1)	H. Serous	FL1,2,3 (Clip1)	IDS post-NACT x 3C
	2. DR (Clip 2)		DR1,2,3,4 (Clip 2)	R0
			OVL1,2	
			PER1,2	
			OM1,2,3	
			SP1,2	
1011	1. OM	H. Serous	OM	IDS post-NACT x3C
	2. PEL		PE	paired
			DP	R1
			LT (Lig teres)	
1014	1. PE (Clip1)	H. Serous	1. PE1 (Clip1)	IDS post-NACT x3C
	2. OM (Clip2)		2. PE2 (Clip1)	Accurate matching of paired biopsies
			3. OM1 (Clip2)	
			4. OM2 (Clip2)	
			5. OM3 (Clip2)	
1015	1. OM (Clip1)	Low G. serous	OM1 (Clip1)	IDS post-NACT x2
	2. PER (Clip2)		OM2 (Clip1)	Accurate matching of paired biopsies
			OM3 (Clip1)	R0
			PER1 (Clip2)	
			PER2 (Clip2)	
1016	1. OM1 (Clip1)	H. Serous	1. OM1 (Clip 1)	IDS post-NACT x3C
	2. OM2 (Clip2)		2. OM2 (Clip 1)	Accurate matching of paired biopsies
	3. PER (Clip3)		3. OM3 (Clip 2)	R0
			4. OM4 (Clip 2)	
			5. PER (Clip 3)	
1019	1. R-PEL	H. Serous	RPE	IDS matching Bx
	2. L-PEL		Omentum	

	3. Omentum			
1024	1. Diaphragm	H. Serous	Diaphragm	Accurate matching
	2. Omental Bx		Omentum	
	3. R. PE		Rt. PE	
1027	LOM	H. Serous	Omentum	Delay in samples
	DR		Peritoneum	
	Supr. Oment			
1028	ROM X 2	H. Serous	Omentum x 2	Accurately matched
	Cent. Om X 2		Peritoneum	
	Ant Ab. Perito			
1031	Peritoneum Bx	H. serous	Surgery as Private	Delayed samples/ not matched
	Omental Bx			
1036	Pelvic Peritoneum	H. serous	Pelvic Peritoneum	Accurate matching
	R. Diaphragm		Omentum	
	Omentum			
1038	Pelvic Peritoneum	H. Serous	Pelvic Peritoneum	Accurate matching
	Omentum		Omentum	
1039	Pelvic peritoneum	H. Serous	Pelvic peritoneum	Accurate matching
	Omentum		Omentum	
	R. Pelvic peritoneum			

Table 6. Laparoscopy, initial biopsy samples and samples at Interval debulking Surgery - IDS. BP, bladder peritoneum; PER, peritoneum; OMR, omentum right; PEL, pelvis; DP, diaphragmatic peritoneum; DR, right diaphragmatic peritoneum; OVR, ovarian-right; FL falciform ligament.

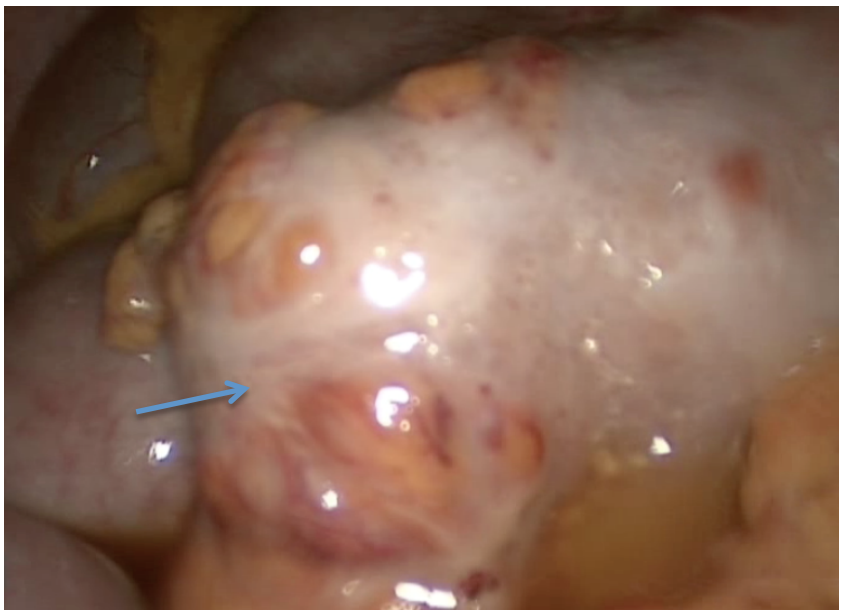


Figure: 9. Advanced stage ovarian cancer with omental/ large bowel mesenteric disease (Pre-NACT).

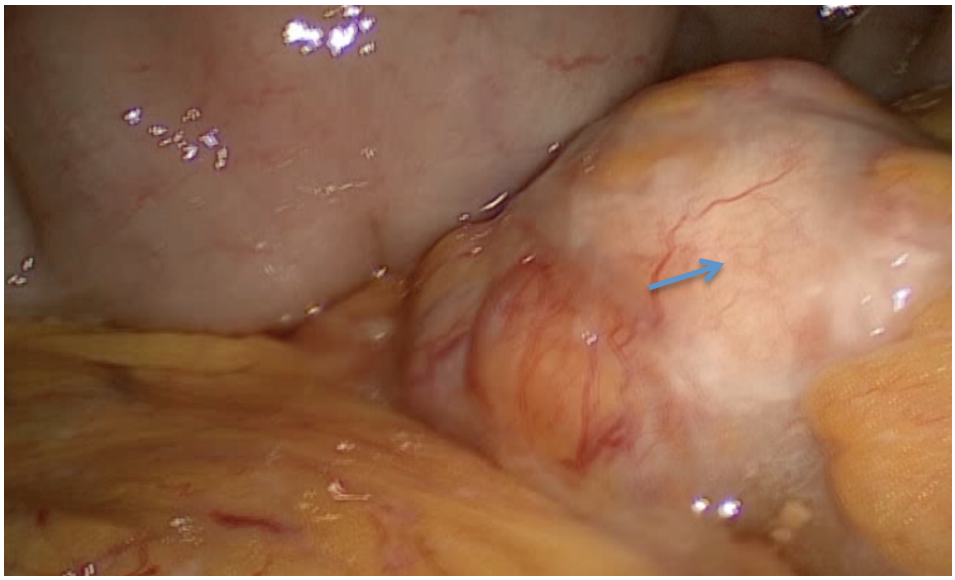
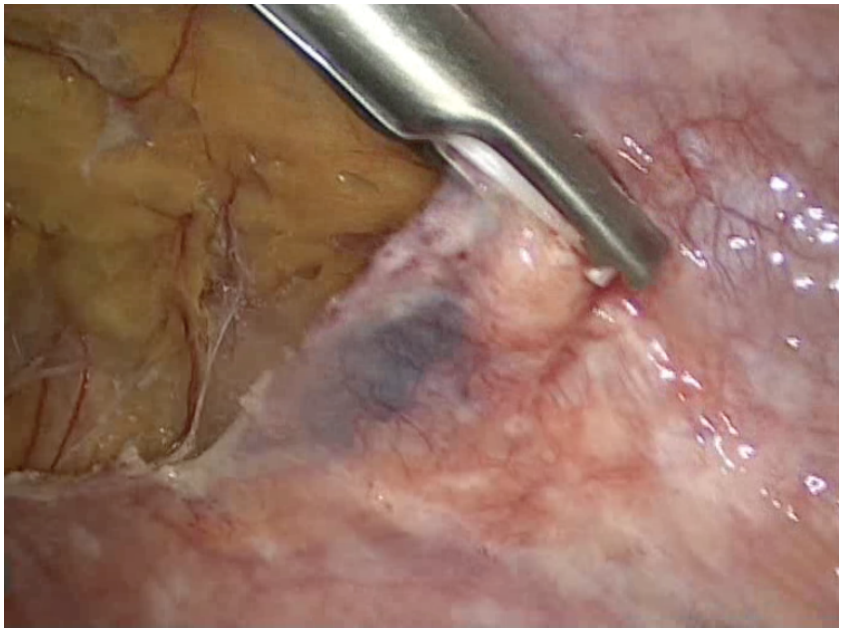


Figure: 10. Advanced stage ovarian cancer after chemotherapy (3 cycles). This image shows an example of stable disease with a minimal response. The arrow indicates the appearance of the tumour after chemotherapy.

(a)



(b)

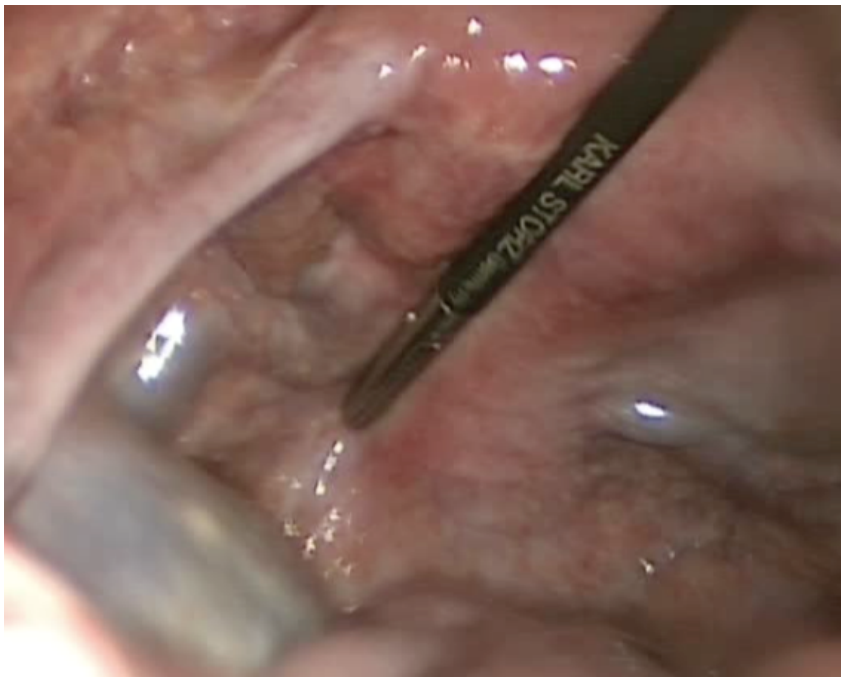
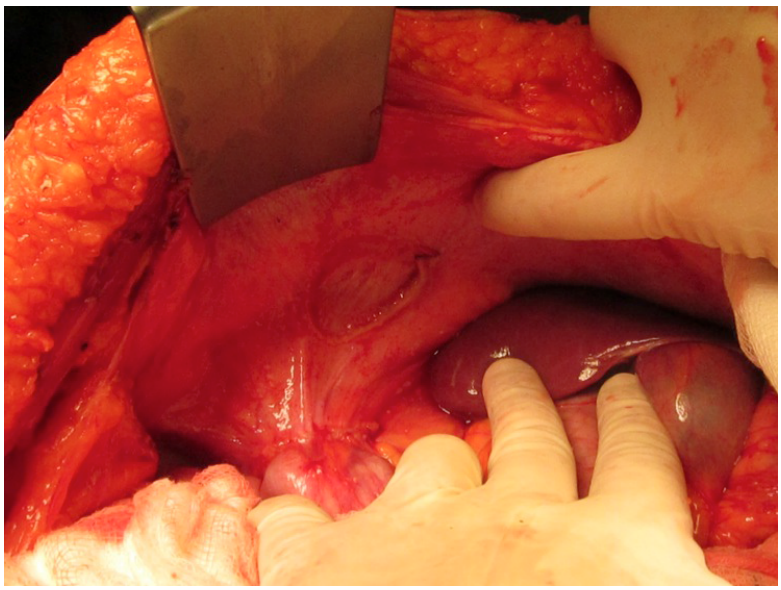


Figure 11: OCA peritoneal disease and laparoscopic clip application (Pre-NACT).

(a) Right pelvic peritoneal tumour sampling and clip application. (b) Left ovarian fossa at initial laparoscopy and biopsy.

(A)



(B)

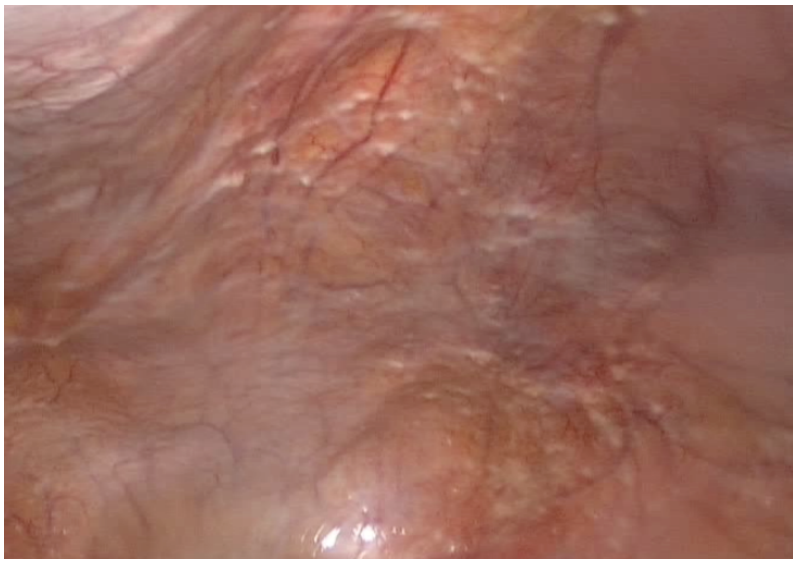


Figure 12; (A) Appearance of the OCA peritoneal disease (post-chemotherapy). This image corresponds to figure 5A: pelvic peritoneal tumour sampling and clip application. The arrow indicates the site of the clip that was placed prior to the chemotherapy. (B) Left pelvic peritoneum post-chemotherapy: small nodules indicate the clinical response to chemotherapy

2.3. **Optimisation of Sample collection for RNA extraction:**

Genomic studies on tumour samples depend on high quality tumour samples from tumour sites; clinicians often pay little attention to the accuracy of the tumour samples collected from various sites for various reasons.

I evaluated the quality of tumour sampling by assessing the quality of the RNA yield, which will directly reflect the availability of high quality genomic material.

I used the filter paper-based lysis and elution method available in QIAGEN kits, which features high throughput capacity.

Tissue samples were collected at the beginning of the procedure.

Sample 1. A small tumour sample (1-2 mm) was immediately placed in RNALater solution during surgery.

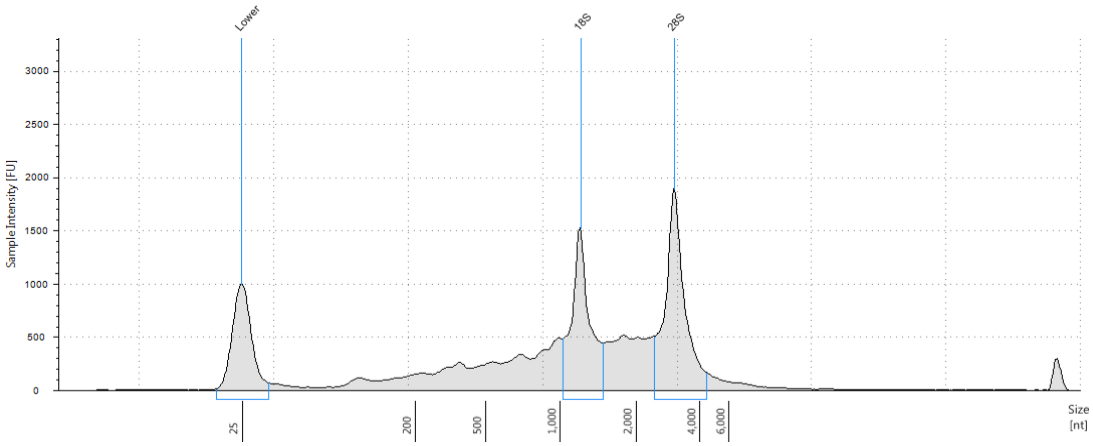
Sample 2. A larger tumour sample (6-7 mm) was immediately placed in RNALater solution during surgery.

Sample 3. A larger tumour sample (6-7 mm) was collected in an empty sample container and RNALater solution was added after 3-4 hours later when the sample was transported to the lab.

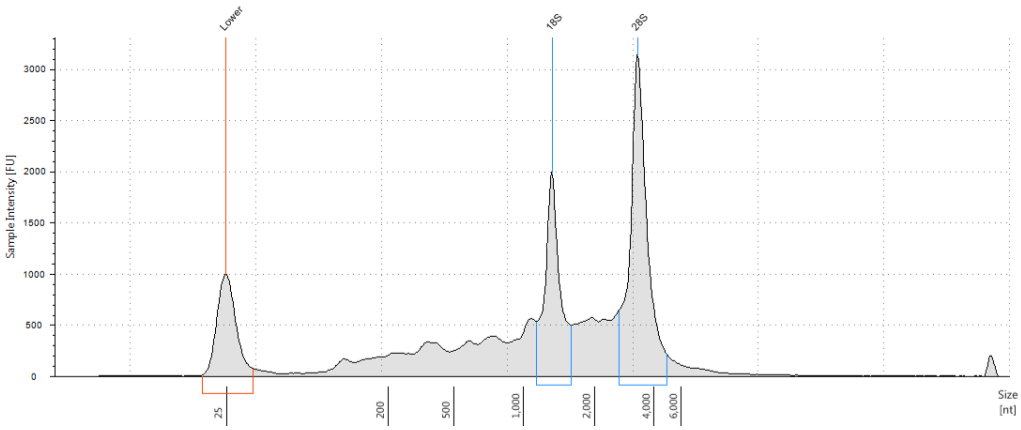
These samples were stored at -80 °C after the RNALater solution was removed. Ten 10-micrometre slices were obtained from samples 2 and 3 for RNA extraction. Sample 1 was used as collected, but without RNALater solution.

All three samples showed good RIN: 6.3, 6.6 and 6.8, respectively, illustrating the potential of using RNALater to optimise the yield of RNA obtained from tumour samples.

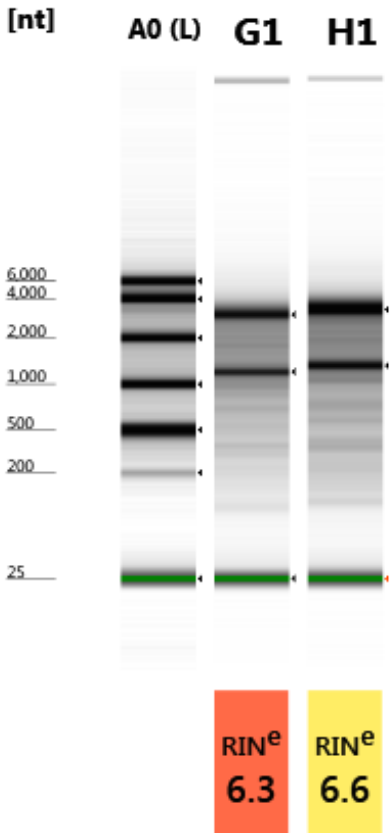
Sample 1:



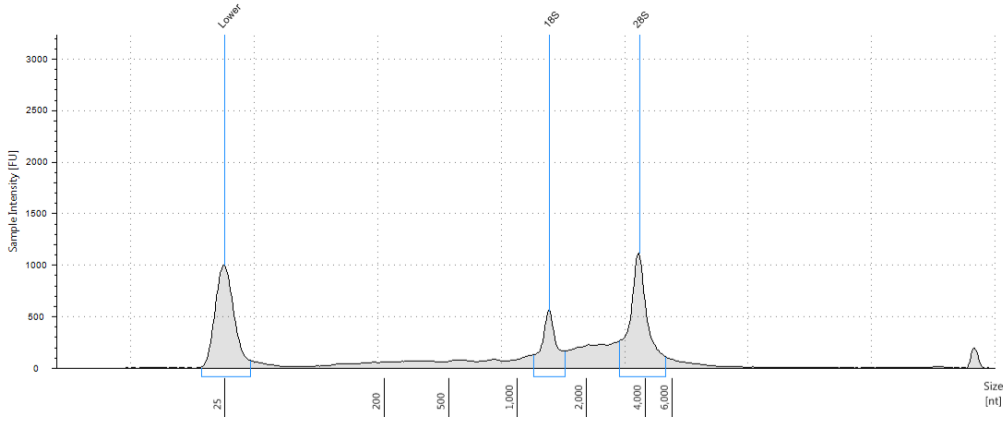
Sample 2:



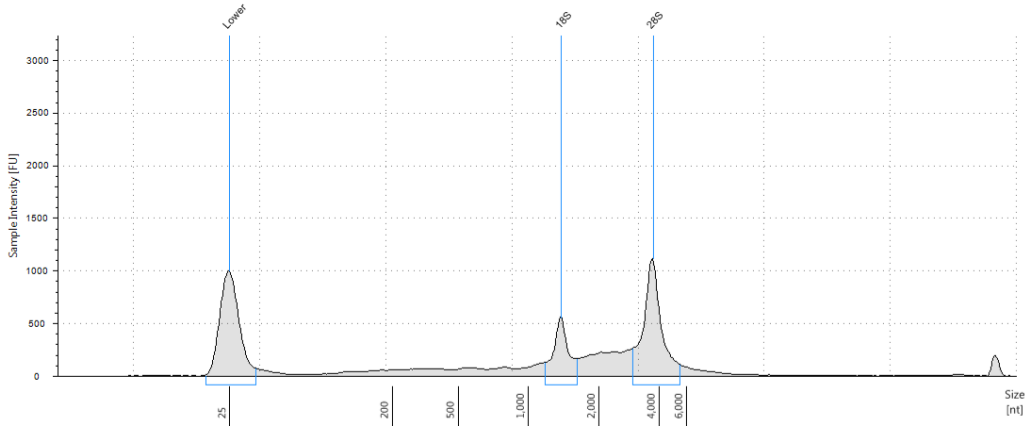
RIN for samples 1 (Small tumour sample with RNALater solution) and 2 (Larger tumour sample (6-7 mm) was collected in an empty sample container and RNALater solution was added after 3-4 hours later):



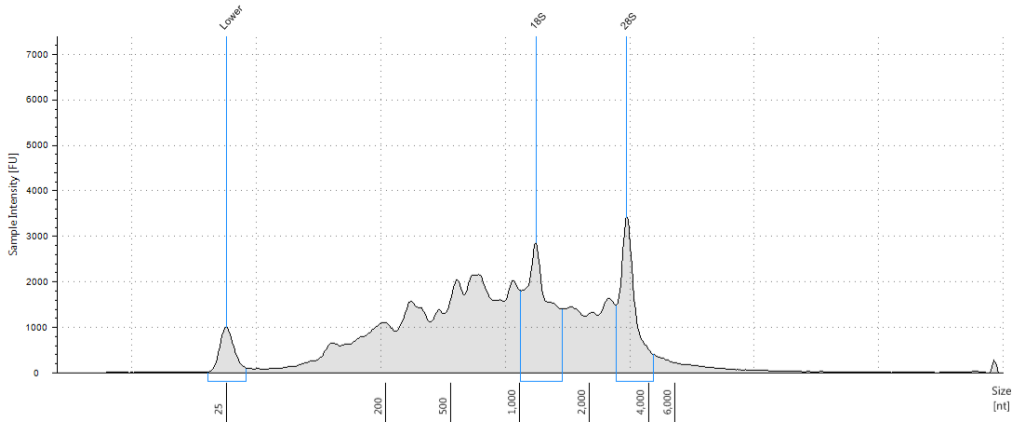
Sample 3: Neat and concentrated samples G2= Neat, G2=1020 pg/µl.



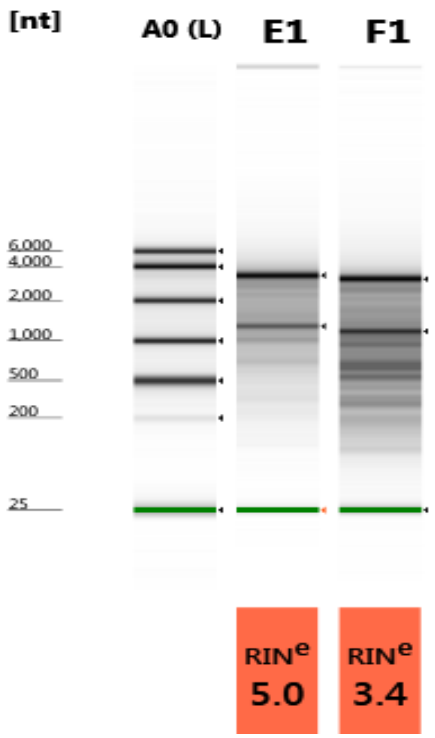
H2= diluted, H2=837 pg/µl.



Compared to the samples shown above, RNA quality was significantly impaired when fresh samples were immediately placed on dry ice, as shown in the sample below.



RIN for this sample was significantly lower: 3.4.



As shown in these examples, the quality of the tissue collected at laparoscopic biopsy has a significant effect on the results.

Summary, Discussion and further studies:

3.1: Summary:

This prospective translational study evaluated the feasibility of monitoring the heterogeneity of ovarian cancer responses to chemotherapy using CT scans and to evaluate the feasibility of assessing clinical responses using intraoperative video laparoscopy. Our data reveals the limitations of the present diagnostic modalities and also highlights the advantages of laparoscopy as diagnostic tool and an efficient research instrument to evaluate, map and obtain several high quality tissue samples.

Post-chemotherapy CT scans only show significant variations in the responses of tumours at multiple sites when the tumour is radiologically detectable. These heterogeneous responses may be due to the genomic heterogeneity and different chemotherapeutic responses of the tumours. However, a significant limiting factor of CT scans is that they fail to detect peritoneal and bowel serosal diseases. One of the limiting factors for optimal tumour debulking is widespread peritoneal disease, and laparoscopic assessments have an obvious advantage of direct clinical evaluation with higher magnification, and, hence, better appreciation of tumour spread, response and heterogeneity.

A continuation of this study to achieve an adequate number of patients to answer some of the questions raised regarding the present clinical practice may contribute to direct positive outcomes, whereas genetic information about tumour heterogeneity will translate into a better understanding of the tumour genome towards the development of targeted treatments for ovarian cancer.

3.2: Discussion:

Initial laparoscopy is useful for obtaining high quality biopsies from the most likely clinical tumour sites, triaging patients for treatment and screening for research. Diagnostic laparoscopy is common practice in many surgical specialties(*69, 70*). Interval laparoscopic assessments did not change the surgical decision, but helped to evaluate the heterogeneous clinical response and to plan surgery.

This heterogeneous response to chemotherapy may be genetically determined. Laparoscopic evaluations and interval biopsies from mapped tumour sites was an important step towards providing answers to these questions. Clinical information collected from the small number of patients who underwent the diagnostic laparoscopic procedure and interval debulking poses more challenging questions for clinicians with regard to the chemotherapeutic response, radiological staging and surgical planning.

Our intra-operative assessment of tumour viability is subjective, and the individual judgement mainly depends on the personnel's experience and willingness to remove 'normal looking' tissues that may not have an apparent tumour. A recent single centre study demonstrated the use of PET/CT to assess chemotherapeutic responses in ovarian cancer and reported limitations; our laparoscopic approach clearly reveals how those limitations could be overcome(*71*). This approach also provides us with opportunities to explore the tumour biology and genetics of ovarian cancer, particularly in chemotherapy-resistant disease. Some have raised concerns regarding the use of diagnostic laparoscopy, due to port-site recurrence(*72*); however, we did not observe any port-site disease recurrence in our cohort of patients. Laparoscopic surgical planning also plays a major role in gathering information for the purpose of

obtaining consent from patients for additional surgical procedures that may not have been possible without visualisation of the tumour spread. In our study, a considerable number of patients would have missed the opportunity for primary surgery at the screening stage if we had not assessed these patients laparoscopically.

Patient recruitment was identified as one of the major challenges of this study. Most of our patients were elderly individuals with multiple co-morbidities, and approximately 70%-80% were suspected to have advanced stage disease at presentation. Moreover, subjecting these patients to a general anaesthetic procedure for an assessment and tissue biopsy was a significant challenge, when this procedure can be performed using less invasive methods. The 42 laparoscopic procedures that were performed for diagnosis and assessment produced extremely valuable information that positively changed the outcomes of these patients because 6 out of 42 patients had less advanced disease and were eligible for primary surgery. This result also confirms the limitations of radiological staging for ovarian cancer. Seven out of 42 patients were diagnosed as having a non-gynaecological pathology and were able to be redirected to relevant specialties after the laparoscopic assessment and biopsy.

The results failed to identify a statistically significant relationship between the rates of tumour debulking with the biochemical response to chemotherapy or clinical response assessed by laparoscopy. The laparoscopic assessment at interval debulking was used to plan the extent of surgery, but did not show a significant change in the rate of debulking. The advantages of the initial diagnostic laparoscopy procedure are less obvious when there are very few patients, as the significance does not translate into meaningfully data for inclusion in clinical practice.

There were other logistical difficulties with regard to video recording, due to failure of computer software/hardware and the loss of patient video/images at the end of a procedure; we are now duplicating the files to avoid a similar situation.

3.3: Further studies:

I am planning to continue this study with a larger number of patients to answer the clinical queries and understand the genetics of these heterogenic tumours.

I will also explore options of combining present radiological tumour response criteria with laparoscopic findings to achieve better tumour staging for ovarian cancer.

Appendix I:

Table 7 PIONIR: Summary of SLN biopsies of gynaecological cancers using NIR fluorescence.

Reference	*Cancer type*	*Surgical procedure*	*Imaging device*	*No. of patients*	*Key results*
Furukawa, 2010 (*7*)	Cervical	Open	Photodynamic Eye	12	At least 1 SLN identified in 83% of the patients
Crane, 2011 (*74*)	Cervical	Open	Custom-made prototype	10	Intraoperative detection rate = 64% relative to total number of *ex vivo* fluorescent SLNs.
Crane, 2011 (*75*)	Vulvar	Open	Custom-made prototype	10	Detection rates relative to gamma probe: fluorescence = 89.7%, patent blue = 72.4%.
Van der Vorst, 2011 (*51*)	Cervical	Open	Mini-FLARE	9	At least 1 SLN identified in each patient. Optimal ICG:HSA dose: 500 μM.
Rossi, 2012 (*44*)	Endometrial/Cervical	Robotic-assisted laparoscopy	SPY scope	20	At least 1 SNL identified in 85% of the cases
Holloway, 2012 (*76*)	Endometrial	Robotic-assisted laparoscopy	Da Vinci NIR fluorescence imaging system	35	At least 1 SLN identified in 97% of the patients with fluorescence and 77% with calorimetric analysis.
Hutteman, 2012 (*42*)	Vulvar	Open	Mini-FLARE	9	Detection rates relative to gamma probe: fluorescence = 100%, patent blue = 71%. Optimal ICG:HSA dose: 750 μM
Schaafsma, 2012 (*43*)	Cervical	Open	Mini-FLARE	18	At least 1 SLN identified in 97% of the patients. No significant difference in signal-to-background ratio between ICG alone and ICG:HSA.
Schaafsma, 2013 (*77*)	Vulval	Open	Mini-FLARE	24	Detection rates relative to gamma probe: fluorescence = 100%, patent blue = 77%. No significant difference in detection rate between ICG alone and ICG:HSA
Rossi, 2013 (*78*)	Endometrial	Robotic-assisted laparoscopy	SPY® scope	29	SLN detection rate: 82% for cervical injection, 33% for hysteroscopic endometrial injection.
Mathéron, 2013 (*79*)	Vulval	Open	Photodynamic Eye	15	SLN detection rate: 98% radioactive, 96% fluorescence, 65% blue dye.
Jewell, 2014 (*49*)	Endometrial/Cervical	Robotic-assisted laparoscopy	PINPOINT®	227	SLN detection rate: 95%. Combined use of ICG and blue dye proved unnecessary.
Sinno, 2014 (*52*)	Endometrial	Robotic-assisted laparoscopy	PINPOINT®	71	Fluorescence ICG superior than blue dye (78.9% vs. 42.4% bilateral detection rate)

Appendix II:

On going whole genomic analysis of paired tumour samples

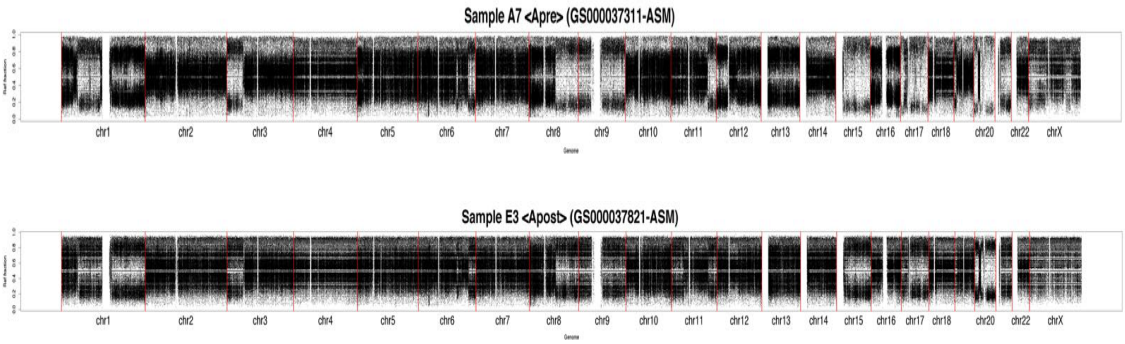


Figure: 13. Allele fractions obtained from whole genome sequencing of Pt. A pre-chemotherapy sample A7 and post-chemotherapy sample E3.

Appendix III:

References:

1. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/ovarian-cancer/incidence 07Oqa527xUYqz8dP.99. (2016).
2. R. C. Bast, Jr., B. Hennessy, G. B. Mills, The biology of ovarian cancer: new opportunities for translation. *Nature reviews. Cancer* **9**, 415 (Jun, 2009).
3. M. Shih Ie, R. J. Kurman, Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *The American journal of pathology* **164**, 1511 (May, 2004).
4. I. J. Jacobs *et al.*, Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. *Lancet* **387**, 945 (Mar 5, 2016).
5. A. N. Rosenthal *et al.*, Results of annual screening in phase I of the United Kingdom familial ovarian cancer screening study highlight the need for strict adherence to screening schedule. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **31**, 49 (Jan 1, 2013).
6. N. F. Hacker, State of the art of surgery in advanced epithelial ovarian cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **24 Suppl 10**, x27 (Dec, 2013).
7. D. Volpi *et al.*, A novel multiwavelength fluorescence image-guided surgery imaging system. *Proc Spie* **8935**, 89350 (2014).
8. I. Vergote *et al.*, Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer. *The New England journal of medicine* **363**, 943 (Sep 2, 2010).
9. S. Kehoe *et al.*, Primary chemotherapy versus primary surgery for newly diagnosed advanced ovarian cancer (CHORUS): an open-label, randomised, controlled, non-inferiority trial. *Lancet* **386**, 249 (Jul 18, 2015).
10. S. M. Eisenkop, R. L. Friedman, H. J. Wang, Complete cytoreductive surgery is feasible and maximizes survival in patients with advanced epithelial ovarian cancer: a prospective study. *Gynecologic oncology* **69**, 103 (May, 1998).
11. J. R. Redman, G. R. Petroni, P. E. Saigo, N. L. Geller, T. B. Hakes, Prognostic factors in advanced ovarian carcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **4**, 515 (Apr, 1986).
12. D. S. Chi *et al.*, Identification of prognostic factors in advanced epithelial ovarian carcinoma. *Gynecologic oncology* **82**, 532 (Sep, 2001).
13. S. Vaughan *et al.*, Rethinking ovarian cancer: recommendations for improving outcomes. *Nature reviews. Cancer* **11**, 719 (Oct, 2011).
14. R. Agarwal, S. B. Kaye, Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nature reviews. Cancer* **3**, 502 (Jul, 2003).
15. G. Coukos, S. C. Rubin, Chemotherapy resistance in ovarian cancer: new molecular perspectives. *Obstetrics and gynecology* **91**, 783 (May, 1998).
16. C. M. Tempany *et al.*, Staging of advanced ovarian cancer: comparison of imaging modalities--report from the Radiological Diagnostic Oncology Group. *Radiology* **215**, 761 (Jun, 2000).

17. E. L. van Persijn van Meerten, H. Gelderblom, J. L. Bloem, RECIST revised: implications for the radiologist. A review article on the modified RECIST guideline. *European radiology* **20**, 1456 (Jun, 2010).
18. A. Ayhan, H. Celik, P. Dursun, M. Gultekin, K. Yuce, Prognostic and therapeutic importance of lymphadenectomy in gynecological cancers. *European journal of gynaecological oncology* **25**, 279 (2004).
19. K. Bhaskaran *et al.*, Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. *Lancet* **384**, 755 (Aug 30, 2014).
20. A. Patel *et al.*, Cervical cancer incidence in young women: a historical and geographic controlled UK regional population study. *British journal of cancer* **106**, 1753 (May 22, 2012).
21. J. Lai *et al.*, Vulval cancer incidence, mortality and survival in England: age-related trends. *BJOG : an international journal of obstetrics and gynaecology* **121**, 728 (May, 2014).
22. A. s. group *et al.*, Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. *Lancet* **373**, 125 (Jan 10, 2009).
23. Y. Todo *et al.*, Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL study): a retrospective cohort analysis. *Lancet* **375**, 1165 (Apr 3, 2010).
24. N. Agar *et al.*, [Morbidity of pelvic lymphadenectomy and para-aortic lymphadenectomy in endometrial cancer]. *Bulletin du cancer* **102**, 428 (May, 2015).
25. M. H. Oonk *et al.*, Size of sentinel-node metastasis and chances of non-sentinel-node involvement and survival in early stage vulvar cancer: results from GROINSS-V, a multicentre observational study. *The Lancet. Oncology* **11**, 646 (Jul, 2010).
26. M. Ballester *et al.*, Detection rate and diagnostic accuracy of sentinel-node biopsy in early stage endometrial cancer: a prospective multicentre study (SENTI-ENDO). *The Lancet. Oncology* **12**, 469 (May, 2011).
27. C. Altgassen *et al.*, Multicenter validation study of the sentinel lymph node concept in cervical cancer: AGO Study Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **26**, 2943 (Jun 20, 2008).
28. G. J. Rustin *et al.*, Definitions for response and progression in ovarian cancer clinical trials incorporating RECIST 1.1 and CA 125 agreed by the Gynecological Cancer Intergroup (GCIG). *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* **21**, 419 (Feb, 2011).
29. P. Bosze, I. Meszaros, L. Palfalvi, L. Ungar, Perioperative complications of 116 radical hysterectomies and pelvic node dissections. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology* **19**, 605 (Dec, 1993).
30. F. Landoni *et al.*, Randomised study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer. *Lancet* **350**, 535 (Aug 23, 1997).
31. J. L. Walker *et al.*, Laparoscopy compared with laparotomy for comprehensive surgical staging of uterine cancer: Gynecologic Oncology

Group Study LAP2. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **27**, 5331 (Nov 10, 2009).
32. M. Asmussen, U. Ulmsten, Effects of radical hysterectomy with lymph nodes dissection on the lower urinary tract. *Zentralblatt fur Gynakologie* **104**, 868 (1982).
33. M. R. Keshtgar, P. J. Ell, Sentinel lymph node detection and imaging. *European journal of nuclear medicine* **26**, 57 (Jan, 1999).
34. R. M. Cabanas, Anatomy and biopsy of sentinel lymph nodes. *The Urologic clinics of North America* **19**, 267 (May, 1992).
35. D. N. Krag, D. L. Weaver, J. C. Alex, J. T. Fairbank, Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. *Surgical oncology* **2**, 335 (Dec, 1993).
36. J. R. Benson, G. Q. della Rovere, G. Axilla Management Consensus, Management of the axilla in women with breast cancer. *The Lancet. Oncology* **8**, 331 (Apr, 2007).
37. M. H. Oonk, H. P. van de Nieuwenhof, J. A. de Hullu, A. G. van der Zee, The role of sentinel node biopsy in gynecological cancer: a review. *Current opinion in oncology* **21**, 425 (Sep, 2009).
38. A. G. Van der Zee *et al.*, Sentinel node dissection is safe in the treatment of early-stage vulvar cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **26**, 884 (Feb 20, 2008).
39. P. J. Bostick, A. E. Giuliano, Vital dyes in sentinel node localization. *Seminars in nuclear medicine* **30**, 18 (Jan, 2000).
40. G. Mariani *et al.*, Radioguided sentinel lymph node biopsy in malignant cutaneous melanoma. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **43**, 811 (Jun, 2002).
41. A. L. Vahrmeijer, M. Hutteman, J. R. van der Vorst, C. J. H. van de Velde, J. V. Frangioni, Image-guided cancer surgery using near-infrared fluorescence. *Nat Rev Clin Oncol* **10**, 507 (Sep, 2013).
42. R. W. Holloway *et al.*, Detection of sentinel lymph nodes in patients with endometrial cancer undergoing robotic-assisted staging: a comparison of colorimetric and fluorescence imaging. *Gynecologic oncology* **126**, 25 (Jul, 2012).
43. M. Hutteman *et al.*, Optimization of near-infrared fluorescent sentinel lymph node mapping for vulvar cancer. *American journal of obstetrics and gynecology* **206**, 89 e1 (Jan, 2012).
44. J. R. van der Vorst *et al.*, Optimization of Near-Infrared Fluorescent Sentinel Lymph Node Mapping in Cervical Cancer Patients. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* **21**, 1472 (Nov, 2011).
45. N. R. Abu-Rustum, Sentinel lymph node mapping for endometrial cancer: a modern approach to surgical staging. *Journal of the National Comprehensive Cancer Network : JNCCN* **12**, 288 (Feb, 2014).
46. M. Frumovitz, R. C. Coleman, P. T. Soliman, P. T. Ramirez, C. F. Levenback, A case for caution in the pursuit of the sentinel node in women with endometrial carcinoma. *Gynecologic oncology* **132**, 275 (Feb, 2014).
47. N. R. Abu-Rustum *et al.*, Sentinel lymph node mapping for grade 1 endometrial cancer: Is it the answer to the surgical staging dilemma? *Gynecologic oncology* **113**, 163 (May, 2009).
48. F. Giammarile *et al.*, The EANM clinical and technical guidelines for lymphoscintigraphy and sentinel node localization in gynaecological

cancers. *European journal of nuclear medicine and molecular imaging* **41**, 1463 (Jul, 2014).
49. H. M. Matheron *et al.*, Multimodal surgical guidance towards the sentinel node in vulvar cancer. *Gynecologic oncology* **131**, 720 (Dec, 2013).
50. S. Kang *et al.*, Sentinel lymph node biopsy in endometrial cancer: meta-analysis of 26 studies. *Gynecologic oncology* **123**, 522 (Dec, 2011).
51. L. M. A. Crane *et al.*, Intraoperative near-infrared fluorescence imaging for sentinel lymph node detection in vulvar cancer: First clinical results. *Gynecologic oncology* **120**, 291 (Feb, 2011).
52. E. L. Jewell *et al.*, Detection of sentinel lymph nodes in minimally invasive surgery using indocyanine green and near-infrared fluorescence imaging for uterine and cervical malignancies. *Gynecologic oncology* **133**, 274 (May, 2014).
53. H. Niikura *et al.*, Tracer injection sites and combinations for sentinel lymph node detection in patients with endometrial cancer. *Gynecologic oncology* **131**, 299 (Nov, 2013).
54. R. Sadeghi, M. Hasanzadeh, Sentinel lymph node biopsy algorithm: Can it be a universal method for midline tumors? *Gynecologic oncology* **132**, 273 (Feb, 2014).
55. L. Xiong *et al.*, Indocyanine green fluorescence-guided sentinel node biopsy: a meta-analysis on detection rate and diagnostic performance. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology* **40**, 843 (Jul, 2014).
56. J. How *et al.*, Accuracy of sentinel lymph node detection following intra-operative cervical injection for endometrial cancer: a prospective study. *Gynecologic oncology* **127**, 332 (Nov, 2012).
57. A. M. Perrone *et al.*, Cervical and hysteroscopic injection for identification of sentinel lymph node in endometrial cancer. *Gynecologic oncology* **111**, 62 (Oct, 2008).
58. W. J. Hoskins, The influence of cytoreductive surgery on progression-free interval and survival in epithelial ovarian cancer. *Bailliere's clinical obstetrics and gynaecology* **3**, 59 (Mar, 1989).
59. K. R. Cho, M. Shih Ie, Ovarian cancer. *Annual review of pathology* **4**, 287 (2009).
60. P. J. Timmers, A. H. Zwinderman, C. Coens, I. Vergote, J. B. Trimbos, Understanding the problem of inadequately staging early ovarian cancer. *European journal of cancer* **46**, 880 (Mar, 2010).
61. C. G. Zorlu *et al.*, Complications of radical hysterectomy: clinical experience of 115 early stage cervical cancers. *Gynecologic and obstetric investigation* **45**, 137 (1998).
62. T. A. Yap, C. P. Carden, S. B. Kaye, Beyond chemotherapy: targeted therapies in ovarian cancer. *Nature reviews. Cancer* **9**, 167 (Mar, 2009).
63. R. Forstner, E. Sala, K. Kinkel, J. A. Spencer, R. European Society of Urogenital, ESUR guidelines: ovarian cancer staging and follow-up. *European radiology* **20**, 2773 (Dec, 2010).
64. I. Vergote *et al.*, Primary surgery or neoadjuvant chemotherapy followed by interval debulking surgery in advanced ovarian cancer. *European journal of cancer* **47 Suppl 3**, S88 (Sep, 2011).
65. D. Quaranta, E. Lambaudie, M. Heinnemann, G. Houvenaeghel, E. Chereau, Evaluation of single-port laparoscopy for peritoneal carcinomatosis

assessment in advanced ovarian cancer. *European journal of obstetrics, gynecology, and reproductive biology* **181**, 60 (Oct, 2014).
66. A. Fagotti *et al.*, Role of laparoscopy to assess the chance of optimal cytoreductive surgery in advanced ovarian cancer: a pilot study. *Gynecologic oncology* **96**, 729 (Mar, 2005).
67. M. J. Rutten, M. M. Leeflang, G. G. Kenter, B. W. Mol, M. Buist, Laparoscopy for diagnosing resectability of disease in patients with advanced ovarian cancer. *The Cochrane database of systematic reviews* **2**, CD009786 (2014).
68. E. A. Eisenhauer *et al.*, New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European journal of cancer* **45**, 228 (Jan, 2009).
69. R. Kumar, R. F. Bamford, D. Kumar, G. Singh-Ranger, Appendagitis following Diagnostic Laparoscopy and Laparoscopic Appendicectomy. *Case reports in surgery* **2016**, 6973061 (2016).
70. U. A. Menakaya, L. Rombauts, N. P. Johnson, Diagnostic laparoscopy in pre-surgical planning for higher stage endometriosis: Is it still relevant? *The Australian & New Zealand journal of obstetrics & gynaecology*, (Jul 28, 2016).
71. H. H. Chung *et al.*, Role of [18F]FDG PET/CT in the assessment of suspected recurrent ovarian cancer: correlation with clinical or histological findings. *European journal of nuclear medicine and molecular imaging* **34**, 480 (Apr, 2007).
72. B. Ataseven *et al.*, Prognostic Impact of Port-Site Metastasis After Diagnostic Laparoscopy for Epithelial Ovarian Cancer. *Annals of surgical oncology*, (Jul 12, 2016).
73. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/ovarian-cancer/incidence 07Oqa527xUYqz8dP.99. (2016).
74. N. Furukawa *et al.*, The usefulness of photodynamic eye for sentinel lymph node identification in patients with cervical cancer. *Tumori* **96**, 936 (Nov-Dec, 2010).
75. L. M. Crane *et al.*, Intraoperative multispectral fluorescence imaging for the detection of the sentinel lymph node in cervical cancer: a novel concept. *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging* **13**, 1043 (Oct, 2011).
76. E. C. Rossi, A. Ivanova, J. F. Boggess, Robotically assisted fluorescence-guided lymph node mapping with ICG for gynecologic malignancies: a feasibility study. *Gynecologic oncology* **124**, 78 (Jan, 2012).
77. B. E. Schaafsma *et al.*, Randomized comparison of near-infrared fluorescence lymphatic tracers for sentinel lymph node mapping of cervical cancer. *Gynecologic oncology* **127**, 126 (Oct, 2012).
78. B. E. Schaafsma *et al.*, Near-infrared fluorescence sentinel lymph node biopsy in vulvar cancer: a randomised comparison of lymphatic tracers. *Bjog-Int J Obstet Gy* **120**, 758 (May, 2013).
79. E. C. Rossi, A. Jackson, A. Ivanova, J. F. Boggess, Detection of sentinel nodes for endometrial cancer with robotic assisted fluorescence imaging: cervical versus hysteroscopic injection. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* **23**, 1704 (Nov, 2013).