CLINICAL STUDY PROTOCOL

A Randomized, Open-label Study to compare Propofol Anaesthesia with Sevoflurane Anaesthesia in terms of Overall Survival in Patients with Surgical Intervention for either Breast-, Colon- or Rectal cancer

Study name: CAN

Prospective, randomised, open label, multinational, multicentre study.

Sponsor Project No:	CAN / CKF-11115
EudraCT number:	2013-002380-25
Investigational Product:	Propofol and sevoflurane
Sponsor:	Ass. Prof. Mats Enlund Centre of Clinical Research (CKF) Västerås Västmanland Hospital Västerås SE-721 89 Västerås, SWEDEN
Sponsor Representative:	Ass. Prof. Mats Enlund Centre of Clinical Research (CKF) Västerås E-mail: *mats.enlund@ltv.se* Phone: +46 (0)21 17 37 75 Fax: +46 (0)21 17 37 33
Project Manager, UCR:	Magnus Ringbom Uppsala Clinical Research Center – UCR UCR/MTC, Dag Hammarskjölds väg 14 B SE-752 37 Uppsala, Sweden E-mail: *magnus.ringbom@ucr.uu.se* Phone: +46 (0)18 611 9565 Fax: +46 (0)18 50 66 38
Coordinating Investigator:	Ass. Prof. Mats Enlund, Västerås

The clinical study will be conducted, and essential documentation archived, in compliance with the requirements of the ICH Guideline for Good Clinical Practice.

SYNOPSIS

Name of Sponsor/Company: **Name of investigational product:** **Name of active ingredient:**	**Individual study table**	(For regulatory authority use only)
Title of study: A Randomized, Open-label Study to compare Propofol Anaesthesia with Sevoflurane Anaesthesia in terms of Overall Survival in Patients with Surgical Intervention for either Breast-, Colon- or Rectalcancer		
Investigator(s): Investigators at up to 20 study centres in Sweden, Poland, Ireland and China. Names and contact details are noted in separate document not included in the protocol.		
Study centre(s): Dept of Surgery/Dept of Anaesthesiology at the study centres.		
Planned study period: Enrollment: Q3/2013 – Q4/Q2019 Follow-up period: Q1/2018 – Q4/2024		**Phase of development:** Not applicable
Objectives: The primary objective is to evaluate whether the one- and five-year survival after radical breast-, or colorectal cancer surgery in general anaesthesia is significantly higher in patients given the intravenously administered hypnotic propofol than in patients given the inhalational hypnotic sevoflurane. The difference is seen as significantly higher and clinically relevant if the absolute difference in five-year-survival is minimum 5%-units.		
Methodology: Prospective, randomised, open label, multinational, multicentre study.		
Number of subjects (planned): 8000		
Diagnosis: Patients with surgical intervention for either breast-, colon- or rectal cancer		
Inclusion criteria: 1. Be informed of the nature of the study and have provided written informed consent 2. At least 18 years of age 3. Patient that is scheduled for elective radical breast- or colorectal cancer surgery in general anesthesia. Radical surgery means that the aim of the surgery is to cure (adjuvant treatment such as chemotherapy and/or radiation therapy seen as part of the curative treatment).		
Exclusion criteria: 1. The surgery that is going to be made is an acute surgical procedure 2. The surgery that is going to be made is palliative surgery 3. Known or suspected hypersensitivity to either propofol or sevoflurane or presence of any contraindication according to the substances' valid SPC 4. Lack of suitability for participation in the trial, for any reason, as judged by the Investigator (e.g. communicative disturbances (language or intellectual))		
Investigational product, dosage and mode of administration: Propofol or sevoflurane prescribed and used according to normal clinical practice and in accordance with the Summary of Products Characteristics for the products available on the market for both these substances.		

Name of Sponsor/Company: **Name of investigational product:** **Name of active ingredient:**	**Individual study table**	(For regulatory authority use only)
Duration of treatment: The hypnotics will be used during the planned surgery.		
Active control, dosage and mode of administration: Not applicable		
Criteria for evaluation: **Efficacy:** Not applicable **Safety:** Survival 1 year and 5 year after surgery Observations made during surgery and up to 365 days post surgery (anaesthesia and surgery related variables)		
Statistical methods: In the present study all endpoints will be evaluated by descriptive methods. All variables will be presented as aggregated data. Categorical variables will be summarised in frequency tables (presenting frequencies and proportions) by type of anaesthesia. The quantitative variables will be summarised by number of observations (n), mean, standard deviation (SD), median, minimum (min) and maximum (max) by anaesthesia. If applicable, separate summaries will be presented for patients receiving one, two or more anaesthesia, as well as pooled summaries. Graphical methods may be used wherever it is regarded as appropriate. Overall survival (OS) and time to progress (TTP) will be presented as Kaplan-Meier curves together with median survival time and time to progression, respectively.		

1 TABLE OF CONTENTS

2 LIST OF ABBREVIATIONS

Abbreviation	Meaning
ALAT	Alanine Aminotransferase
ASA	American Society of Anesthesiologists
ASAT	Aspartate Aminotransferase
BIS	Bispectral Index value
CI	Confidence interval
CKF	Centre of Clinical Research Västerås
CRF / eCRF	Case Report Form / Electronic Case Report Form
CRP	C-reactive protein
DCF	Data Clarification Form
DMP	Data Management Plan
GCP	Good Clinical Practice
ICD	International Classification of Diseases
IEC	Independent Ethics Committee
MAP	Mean arterial pressure
MPA	Medical Products Agency
PI	Principal Investigator
Pts	Patients
RCC	Regional Cancer Centre
SD	Standard deviation
SOP	Standard Operating Procedures
SPC	Summary of Product Characteristics
UCR	Uppsala Clinical Research Centre

3 GENERAL INFORMATION/STUDY ADMINISTRATIVE STRUCTURE

Coordinating Investigator	Assoc. Prof. Mats Enlund Centre of Clinical Research (CKF) Västerås Västmanland Hospital Västerås SE-721 89 Västerås, SWEDEN E-mail: *mats.enlund@ltv.se* Phone: +46 (0)21 17 37 75 Fax: +46 (0)21 17 37 33
Sponsor Representative and primary contact person:	Assoc. Prof. Mats Enlund Centre of Clinical Research (CKF) Västerås Västmanland Hospital Västerås SE-721 89 Västerås, SWEDEN E-mail: *mats.enlund@ltv.se* Phone: +46 (0)21 17 37 75 Fax: +46 (0)21 17 37 33
Project Manager, UCR:	Magnus Ringbom UCR/MTC Dag Hammarskjölds väg 14B MTC-huset, Science Park SE-752 37 Uppsala, Sweden E-mail: *magnus.ringbom@ucr.uu.se* Phone: +46 (0)18 611 95 65 Fax: +46 (0)18-51 55 70 Mob: +46 (0)761 392883
Principal Investigators:	Names and contact details are noted in separate document not included in the protocol.
Statistician:	Anders Berglund Centre of Clinical Research Västerås Västmanland Hospital Västerås SE-721 89 Västerås, SWEDEN
Data Manager, UCR:	Manuela Zamfir UCR/MTC E-mail: *manuela.zamfir@ucr.uu.se* Phone: +46 (0)18-611 95 17 Fax: +46 (0)18-51 55 70

4 SIGNATURE PAGE FOR SPONSOR

Protocol name: CAN
Title: A Randomized, Open-label Study to compare Propofol Anaesthesia with Sevoflurane Anaesthesia in terms of Overall Survival in Patients with Surgical Intervention for either Breast-, Colon- or Rectal cancer

Reviewed and approved by the following:

Mats Enlund, Head of Centre of Clinical Research (CKF), Västmanland Hospital, Västerås,	Date

5 SIGNATURE PAGE FOR INVESTIGATOR

Protocol name: CAN
Title: A Randomized, Open-label Study to compare Propofol Anaesthesia with Sevoflurane Anaesthesia in terms of Overall Survival in Patients with Surgical Intervention for either Breast-, Colon- or Rectal cancer

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practices, local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the conduct of the study.

I will use only the informed consent approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) and will fulfill all responsibilities for submitting pertinent information to the IRB/IEC responsible for this study.

I agree that the Sponsor (Mats Enlund, Centre of Clinical Research (CKF), Västerås) or its representatives shall have access to any source documents from which case report form information may have been generated.

PRINT NAME	ROLE IN THE STUDY and SITE

Signature	Date

6 BACKGROUND INFORMATION AND STUDY RATIONALE

Centre of Clinical Research Västerås, CKF, is an independent unit connected to both the County Council of Västmanland and the disciplinary domain for medicine and pharmacy at Uppsala University. The Centre focuses on clinical research with aim to improve the standard care. Mats Enlund is the Head of CKF until October 2017, thereafter he will be a full time senior researcher.

Inhalational anaesthesia has been gold standard worldwide for long, first with halothane (replacing ether), then isoflurane and enflurane, and later sevoflurane and desflurane. Propofol was introduced in Europe and the USA in late 80's. In few countries, like Belgium, propofol has become the major hypnotic agent, while in most countries, including Sweden; inhalational based anaesthesia has continued to be the main stay.

Based on the data found in literature and a previously completed retrospective study, the Sponsor has a hypothesis that inhalational anaesthetics, which are time- and dose dependently affecting the immune system, and which are also genotoxic, presumably in a dose dependent way, may negatively affect patients' survival after cancer surgery. Theoretically, the opposite may be the case for propofol. This prospective study aims to evaluate whether this hypothesis can be confirmed.

The study will include approximately 8000 patients from up to 20 sites in Sweden, Poland, Ireland and China. The population will be patients that are scheduled for radical breast- or colorectal cancer surgery in general anaesthesia. The patients included in the study will be randomized to either propofol anaesthesia or sevoflurane anaesthesia. Demographic, anaesthesia related, and surgical related variables will be collected. Data about survival will be compiled from the quality registries used within oncology in Sweden. For other countries, there will be local procedures to capture this data.

The study will be completed in accordance with Declaration of Helsinki, Good Clinical Practice (GCP) and applicable regulatory requirements.

6.1 CURRENT STATE OF KNOWLEDGE

6.1.1 IMMUNO-MODULATION

Converging evidence from animal studies and studies of human cell-lines indicate that different anaesthetics have opposite effects on the immune system[1-10]. Commonly used inhalational hypnotics, such as isoflurane and sevoflurane, are in this context pro-inflammatory, whereas the intravenously administered hypnotic agent propofol is anti-inflammatory and also anti-oxidative. A few clinical studies have indicated similar effects in patients[11-15], and a recent review has suggested that "tailoring an anaesthetic plan to patient's needs will become increasingly critical, and immunology should help in this pursuit"[16].

More specifically, previous studies have investigated the immunological effects of different anaesthetics on monocytes, macrophages, natural killer cells, t-cytotoxic cells, and t-helper cells[3,5,6,10,15]. By affecting t-helper cells anaesthetics indirectly

affects the production of anti-inflammatory mediators, such as interleukin-4 and -10. Anaesthetics also affect the production of pro-inflammatory cytokines, such as tumour necrosis factor alpha, and interleukin-1 and -6. Moreover, the effects could be indirect by blocking or non-blocking of the surgical stress response via the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system[17,18]. Thus, stress hormones, such as catecholamines and cortisol, mediates inhibitory effects on immune functions. In a highly complex way, the neuroendocrine system together with both pro-inflammatory- and anti-inflammatory cytokines augments their immune-suppressive effects. Taken together, results from previous research support that inhalational hypnotics are immune-suppressive in mice[3,9,19] as well as in humans[5,6,10].

Earlier findings also indicate other adverse effects of inhalational hypnotics that could be related to immunological processes. For example, inhalational hypnotics seem to increase the occurrence of cancer metastases[1,3,19,20]. These adverse effects have not been found for propofol. In contrast, propofol seem to inhibit tumour growth and reduce the tendency to induce metastases[7,21].

C-reactive protein (CRP) in blood is a marker of systemic inflammation. Elevated CRP is also a marker of poor cancer prognosis at different sites, including breast- and colorectal[22-24]. Hence, CRP may be a phenomenological link between the pro-inflammatory characteristics of inhalational hypnotics to immune-suppression and poorer cancer survival.

The research field of immune-modulation from anaesthetics was recently reviewed by Kurosawa and Kato[25]. They concluded, that "clinical anaesthesiologists should select anaesthetics and choose anaesthetic methods with careful consideration of the clinical situation and the immune status of critically ill patients, in regard to long-term mortality, morbidity, and the optimal prognosis". A key note is, that "many in vitro investigations have elucidated the dose-dependent and time-dependent immunosuppressive effect of volatile (read inhalational) anaesthetics on various immune cells"[25]. It was stressed in another review by Meiler, "that the perioperative process could be responsible for later adverse events", and the necessity to "understand the underlying biology and immunology should be particularly helpful in this pursuit"[26]. Thus, the choice of hypnotic may affect survival after cancer surgery. More specifically, the combined effects of surgical stress and the burden of cancer and perhaps other aggravating circumstances, such as high age and malnutrition, may play a salient role in postoperative morbidity and mortality[16].

There is also other evidence for that anaesthesia may affect survival for patients. Prof Terri Monk, now at Duke University (NC, USA), found in a study at University of Florida, Gainesville (FL, USA), involving an unselected cohort of 1,064 patients, that accumulate time in deep anaesthesia (as defined by a Bispectral Index value (BIS) <40[1]) was an important independent risk factor for death within one-year[27]. In other words, high relative and high absolute doses of anaesthetic drugs were used. Most of their patients (>90%) were anaesthetized with isoflurane, a chemical relative to sevoflurane. Unexpectedly, a majority of the reported mortality was due to malignancies. However, pre-existing malignancies was not controlled for. The authors

[1] BIS (Aspect Medical Systems, Inc., Natic, MA, USA) is based upon a sum of different EEG parameters. Empirically, clinical data has been linked to different index numbers. An index value of 90-100 indicates consciousness, whereas an index value of 40-60 has been recommended as a suitable interval during surgery. An index value <40 is considered as an indicator of unnecessarily deep anaesthetic, i.e. over-dosing.

suggested that, "prolonged deep anaesthesia may alter the inflammatory response in high-risk patients and predispose them to worsened outcomes". Their findings were strengthened by the results in a retrospective Swedish study including 4,087 patients[28]. Patients with pre-existing malignant diagnoses associated with extensive surgery and less favourable prognosis kept a statistical association with low BIS (read relative over-dose). An inhalational hypnotic, probably sevoflurane, was used for 95% of the patients in the referred study.

Data from a group in Dublin, led by Professor Donal Buggy, indicate that immune-competence, measured as the intensity for Naural Killer cells, is better preserved in women with breast cancer given a combination of paravertebral block and propofol, than in women given an opioid-sevoflurane combination[29]. The same group also showed that cancer cell apoptosis is enhanced in patients with breast cancer given the paravertebral-propofol combination compared with patients given the opioid-sevoflurane combination[30].

6.1.2 GENOTOXICITY

Genotoxic agents may negatively affect patients' survival after cancer surgery, as the connection between DNA damage and cancer development is well-known. The potential genotoxicity from inhalational anaesthetic agents in patients and in exposed staff in operating rooms has been studied both in vitro[31,32] and in vivo[33-42]. A dose-response relationship for inhalational agent exposure and DNA damage has been suggested[36,37]. The techniques used, the rate of sister chromatid exchange in lymphocytes and the alkaline comet assay, as indicators of genotoxicity are well validated, and they are frequently used in other contexts. Inhalational agents seem to be consistently genotoxic, whereas the less studied propofol seems not to be so [38,40]. Some Danish studies could, however, not demonstrate a genotoxic effect from inhalational anaesthetics[34]. Those studies are most likely inconclusive, since they mainly included short and/or low concentration exposures. It is notable however, that trace exposure of inhalational anaesthetic agents to operating room staff well below the recommended limits in a more recent study induced sister chromatid exchange in non-smokers to the same extent as in persons smoking 11-20 cigarettes per day[33], which indicates that inhalational anaesthetics are potent genotoxic agents.

6.1.3 HYPOXIA-INDUCIBLE FACTOR AND OTHER PROTEINS

Another possible mechanism, however less studied, is the opposing effect on hypoxia-inducible factor (HIF) from different anaesthetic agents, again with a potentially better outcome from propofol[43-46]. HIF is a defence factor for tumour growth. As oxygen tension becomes critically low in the peri-necrotic core at the centre of a solid tumour, at a far distance from blood vessels, the level of HIF will increase. A number of adaptive changes will then be mediated by HIF, e.g. angiogenesis and a metabolic shift with pH adaption. Cells, which have adapted most successfully after up-regulation of HIF, become more advanced in competing for scarce resources. Such a clone of highly resistant aggressive cancer cells will have heightened ability to invade surrounding tissues and to metastasize. Even when a clear margin of resection is the case during surgery, some marginal cells or micro-metastases may escape removal. As these cells have been exposed to anaesthetics, which by means of interaction with signalling pathways upstream of HIF, the choice of anaesthetic might influence

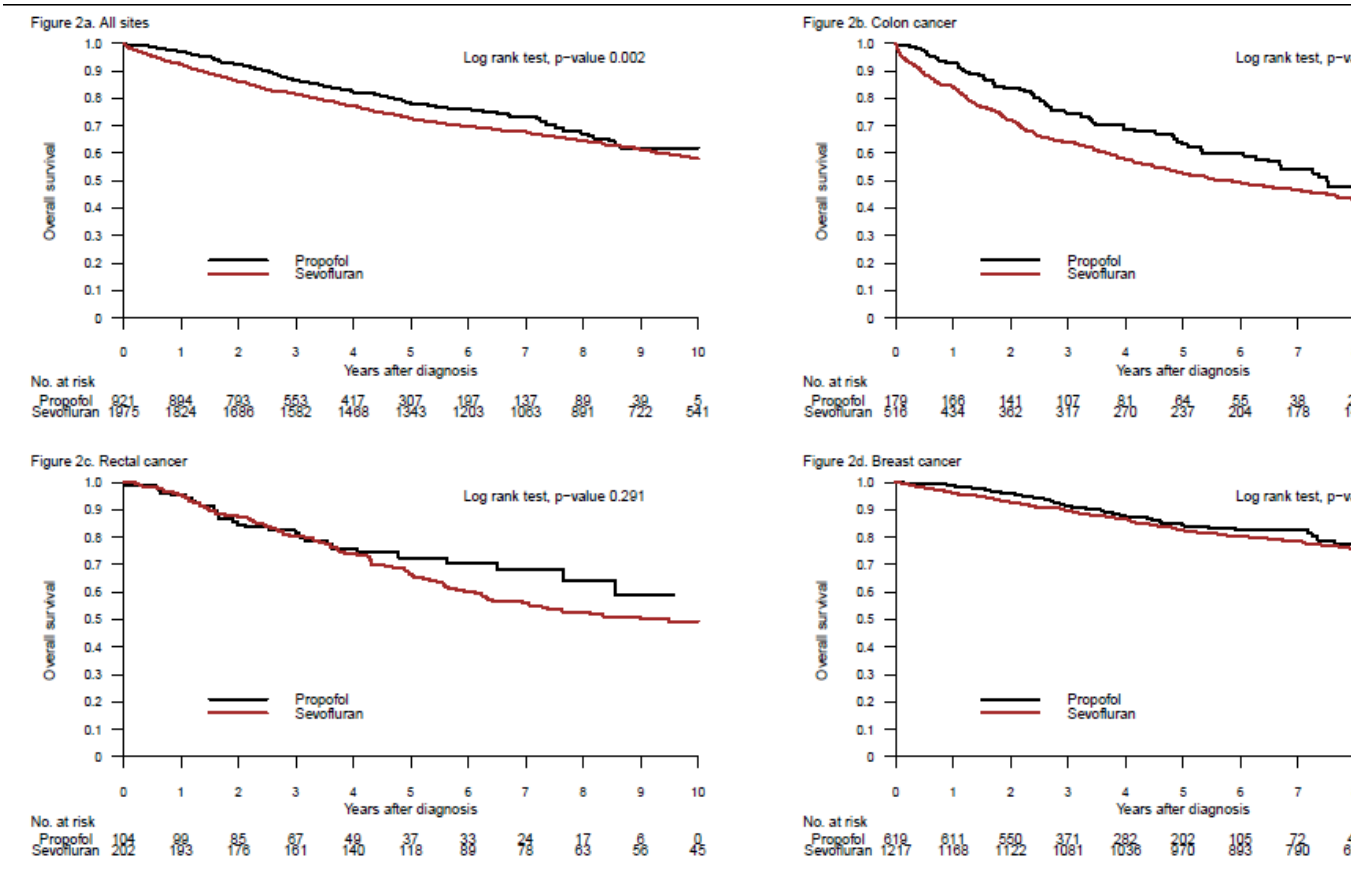
whether or not, the exposed cells may re-establish a secondary tumour or metastases elsewhere. So far it seems as if inhalational anaesthetics up-regulate HIF, whereas propofol down-regulates this factor[43-46].

There are other proteins of interest for cancer dissemination, e.g. Vascular Endothelial Growth Factor C and Transforming Growth Factor β, which behave differently in breast cancer patients dependoing on the choice of anaesthesia[47], again with a potentially better effect from propofol.

6.1.4 DATA FROM RETROSPECTIVE STUDIES

In a retrospective study, "The Choice of Anesthetic and Outcome from Cancer Surgery: A Retrospective Analysis of Surgery for Breast or Colorectal Cancers, Comparing Two Anesthetics", unadjusted data told that survival was higher with propofol anaesthesia, compared with sevoflurane anaesthesia (Fig. 1). However, the retrospective design of this study, with uneven distributions of several confounders and effect modifiers, distorted the picture, so that the statistical significances disappeared after adjustments. The study was found to be under-powered. The p-value for the hazard ratio for sevoflurane was 0.051. Later, a second retrospective study from London, UK, indicated the same result, now with statistical significance[48].

In-text figure 6-1; Overall survival



6.2 STUDY TIME TABLE

The patient inclusion will start once the final approval from the IEC and Regulatory Authorities is available.
Preliminary timetable;

First patient in	3rd - 4th quarter of 2013
Time for enrolment	60 months
Data compilation, 1 year follow-up	4th quarter 2018 – 4th quarter 2020*
Data compilation, 5 year follow-up	2nd quarter 2023– 2nd quarter 2025*

* when updating the protocol, it is obvious that the different parts of the study (breast or colorectal) will have different time frames. The breast cancer cohort has higher recruitment rate than the colo- and rectal cancer cohorts. Accordingly, recruitment of breast cancer patients will be completed earlier.

7 STUDY OBJECTIVES

We hypothesise that one- and five-year survival after radical breast-, or colorectal cancer surgery in general anaesthesia is 5%-units statistically significant higher in patients given the intravenously administered hypnotic propofol than in patients given the inhalational hypnotic sevoflurane.
The hypothesis is based on:
1) The knowledge about the opposite effects on the immune system from the two different forms of anaesthesia and from their different genotoxic potentials.
2) The well-established associations between the state of the immune system and cancer growth, and DNA damage and cancer development, with potential influences on survival.

7.1 PRIMARY OBJECTIVE

The primary objective is to evaluate whether the one- and five-year survival after radical breast-, or colorectal cancer surgery in general anaesthesia is significantly higher in patients given the intravenously administered hypnotic propofol than in patients given the inhalational hypnotic sevoflurane.
The difference is seen as significantly higher and clinically relevant if the absolute difference in five-year-survival is minimum 5%-units.

8 ENDPOINTS

8.2 PRIMARY ENDPOINT

The primary endpoint will be a comparison of overall survival using time to event (i.e. death) analysis. Time for death will be collected from the quality registries used within oncology for the Swedish study population. For other countries, there will be local procedures to capture this data.

9 STUDY DESIGN

9.1 STUDY OUTLINE

This is a prospective, randomized, open label, multinational, multicentre study.

The study will include 8,000 patients. The study will include up to 20 sites in Sweden, Poland, Ireland and China. The population will be patients that are scheduled for radical breast- or colorectal cancer surgery in general anaesthesia. The patients included in the study will be randomized to either propofol anaesthesia or sevoflurane anaesthesia.

In connection with the preoperative anaesthetic procedures (according to local procedures) the patient will be screened and consented for the study. Demographic data will be collected in connection with screening/enrolment. Once enrolled, the patient will be randomized to either propofol anaesthesia or sevoflurane anaesthesia. The anaesthesia is then performed according to standard institutional procedures at each site. Anaesthesia related and surgical related variables during the surgery and during the postoperative course (until 30 days after surgery) will then be collected. Data about survival and other tumour specific data will be transferred from the quality registries used within oncology for the Swedish study population. For other countries, there will be local procedures to capture this data.

The only study specific procedure for this protocol is the randomisation.
Data will then be collected from the Medical Records generated during the patient's normal care (demographics, anaesthesia- and surgical related data).
Tumour-specific and survival data will be transferred via the quality registries used within oncology for patients enrolled in Sweden respectively. For other countries, there will be local procedures to capture this data.

9.2 SCHEDULE OF EVENTS

A summary of study events to be performed each treatment period is presented in the tabular format in Appendix 1.

10 SELECTION AND WITHDRAWAL OF PATIENTS

10.1 SUBJECT INCLUSION CRITERIA

1. Be informed of the nature of the study and have provided written informed consent
2. At least 18 years of age
3. Patient that is scheduled for elective radical breast- or colorectal cancer surgery in general anaesthesia. Radical surgery means that the aim of the surgery is to cure (adjuvant treatment such as chemotherapy and/or radiation therapy seen as part of the curative treatment).

10.2 SUBJECT EXCLUSION CRITERIA

1. The surgery that is going to be made is an acute surgical procedure
2. The surgery that is going to be made is palliative surgery

3. Known or suspected hypersensitivity to either propofol or sevoflurane or presence of any contraindication according to the substances' valid SPC.
4. Lack of suitability for participation in the trial, for any reason, as judged by the Investigator (e.g. communicative disturbances (language or intellectual))

10.3 WITHDRAWAL OF SUBJECTS

As noted in section 17.4, the subjects will be notified of their voluntary participation and of their freedom to withdraw from study. As there are no study specific procedures other than randomization in this study, a withdrawal of a subject will result in that no more data capture in the CRF will be made.

10.4 SUBJECT LOG AND SCREENING OF SUBJECTS

Each study site will recruit patients from their own patient flow.

It is the responsibility of the Investigator at each site that all patients, considered as candidates for the study, are listed in the "Screening and Enrolment log".

Patients will receive a consecutive screening number when signing the informed consent and are thereafter considered to be study patients. Once they have been randomized, they will also get a patient-specific randomization number and they are considered as enrolled in the study.

It is the responsibility of the investigator to keep a patient identification list, identifying each individual study patient.

11 TREATMENT OF SUBJECTS

11.1 TREATMENT ADMINISTRATION

The patient will be randomized to either propofol or sevoflurane anaesthesia. Both these anaesthetics are well established within clinical practice since many years. All sites / Principal Investigators participating in the study have long experience from both inhalational- and propofol based anaesthesia.

The products will be used according to currently available SPC (Summary of Product Characteristics) and according to normal clinical practice at each participating site.

11.2 DESCRIPTION OF INVESTIGATIONAL PRODUCTS

Propofol	registered products with propofol that are available on the markets in the participating countries, e.g. Diprivan®, Propofol-Lipuro®, and Propolipid.
Sevoflurane	registered products with sevoflurane that are available on the markets in the participating countries, e.g. Sevofluran Baxter and Sevorane®.

The protocol allocates which treatment arm that will be used for each patient but not which specific hypnotic product within each treatment arm that will be used. Each participating site can decide this and will use the hypnotic product they have within their normal clinical routines.

More detailed information about the products is available in the SPC of each product.

11.3 PACKAGING AND LABELLING OF INVESTIGATIONAL PRODUCTS

The hypnotic products used in this study will not be provided by the Sponsor (academic Sponsor). The distribution of the products will be made through each site's normal distribution system.

The hypnotic products will not be labelled according to procedures in connection with clinical trials. The products are used according to normal clinical procedures and the study is observational.

11.4 STORAGE AND HANDLING

The products will be stored according to storage conditions noted in the SPC of each product.

11.5 RANDOMISATION AND BLINDING

The study patients will be randomly allocated to either propofol- or sevoflurane-group in a 1:1 ratio. The randomisation list is generated by computer in a permuted block fashion and transferred to a sequence of sealed, opaque, consecutively numbered envelopes. When a patient is considered eligible for the study and has given informed consent, randomisation is performed by opening the next envelope in sequence.
Once assigned a treatment group by randomisation, a subject cannot be "un-randomised" or "de-registered" from the study population. Moreover, "cross-over" between treatment assignments (i.e. propofol to sevoflurane, or vice versa) will not be permitted.

Blinding is not applicable – open label study.

11.6 CONCOMITANT THERAPY

No protocol-specific restrictions in terms of concomitant therapy. Information about re-anaesthesia / re-surgery (if applicable) within 365 days after surgery will be collected.

11.7 COMPLIANCE WITH THE TREATMENT

Not applicable – the products are prescribed and used according to normal clinical practice at each site.

11.8 ACCOUNTABILITY OF INVESTIGATIONAL PRODUCTS

No patient specific drug accountability will be documented. The actual hypnotic product given will be noted in the CRF.

12 ASSESSMENT OF EFFICACY

Not applicable – no efficacy variables are used in this study.

13 ASSESSMENT OF SAFETY

13.1 VARIABLES TO BE COLLECTED

Following variables noted during the care of the patient will be recorded – time for data capture of the different variables is noted in the Flow Chart, Appendix 23.1:

Demographic variables including habits and general health
• Age, Gender
• Place of residence, zip code (proxy for socio-economics)
• Length, Weight
• Smoking (pack years; 1 pack year = 20 cigarettes/day for a year) and Alcohol (standard drinks; 1 standard drink = 12 g pure ethanol)
• ASA-grades
• Co-morbidity
• On-going medication
• Other anaesthetics last year (date, duration, type of)

Study specific variables:
• Randomisation, i.e. allocation to either propofol or sevoflurane-group and confirmation of hypnotic product used during surgery

Anaesthesia related variables
• Duration of current anaesthesia
• Doses of intraoperative opioids during current anaestesia
• Other adjuvant intraoperative treatment such as inotropic drugs
• Accumulated time with mean arterial pressure (MAP) under 65 mmHg or over 130 mmHg
• Accumulated hydration balance at the end of surgery
• Bleeding volume
• Transfusions (red blood cells, plasma)
• Pre-and postoperative laboratory-analyses (see section 13.5 below)
• Complementary regional blockade (which kind of block, type and doses of local anaesthetics)
•Use of Patent blue V
• Doses of postoperative morphine or other opioids
• Antiemetics and anti-inflammatory drugs
• Re-anaesthesia (type of)

Surgical variables
• Cancer location
• Date of surgery
• Duration of surgery
• Surgical (intraoperative) complications
• Postoperative complications / postoperative morbidity related to surgery
• Re-surgery (date, duration, indication)

Following tumour specific variables are not included in the CRF (will be transferred from the cancer quality registries for the Swedish study population. For other countries, there will be local procedures to capture this data):
• Tumour data (i.e. stage, proliferation, hormone status)
• Complementary therapy (e.g. radiation, chemotherapy, anti-hormone, antibody, angiogenesis inhibitors)
• Recurrence/metastasis (localisation, local/regional/ generalized, time point)
• Re-surgery (date, duration, indication)
• Date of death and cause of death, when applicable

13.2 COLLECTION OF COMPLICATIONS

The products used in this study are well established and registered since many years. The manufacturers of the products used are responsible for safety monitoring for pharmacovigilance purposes according to regulations.

In this study, following complications will be collected up to 30 days post-surgery:

- Surgical (intraoperative) complications that receive a ICD-classification number
- Postoperative complications
- Postoperative morbidity related to surgery
- Occurrence of following diagnoses during 30 days after surgery: myocardial infarction, stroke, pulmonary embolism, pneumonia and renal failure

13.3 METHODS FOR ELICITING, RECORDING AND FOLLOW-UP OF COMPLICATIONS

For every complication as noted in section 13.2, only the start and end date will be recorded in the CRF for calculation of the duration of the event.

13.4 FOLLOW-UP OF COMPLICATIONS

The care of the patients is made according to each site's normal clinical practice and this includes the need of follow-up of any complications. There is no study specific procedure. The complications noted in section 13.2 that occur within 30 days after surgery will be noted in CRF.

13.5 LABORATORY ASSESSMENTS

No protocol specific laboratory requirements. Data about CRP, liver enzymes (ASAT and ALAT), Bilirubin, Albumin and Creatinine obtained in normal clinical practice pre- and postoperative will be collected in the CRF.

14 STATISTICS AND DATA MANAGEMENT

14.1 DATA MANAGEMENT

UCR will be responsible for the Data Management of the clinical database and will write a study specific Data Management Plan (DMP) where further details will be specified.

An electronic CRF will be used in this study which also will serve as the clinical database for the study. UCR will be responsible for set-up, support and management of this electronic CRF. All demographic, anaesthesia related and surgical related data will be collected in this database.
This system will also include handling of queries to resolve any inconsistencies detected by the quality control procedures.

Tumor-specific variables including data about survival will not be part of this database but will be transferred from the quality registries used within oncology for the Swedish study population. These quality registries are routinely used within oncology as part of the quality assurance of the care of these patients (the INCA-platform is used). The registries are handled by six Regional Cancer Centers (RCC). The RCC registers includes a quality register for breast cancer, which was started in 1992 but also registers for colon cancer and rectal cancer which was started in 1995 and in 1997 respectively. These registers contain information on mode of detection, histopathology, and stage of cancer at diagnosis, other prognostic markers and complementary treatment given. Hence, complete oncologic- and outcome data will be available for all types of cancer included in the study within the defined period of time. Data on type and stage of the cancer, as well as different prognostic markers recorded in the oncologic registers will be extracted, as well as data on recurrences of disease, vital status and date and cause of death.

In order to be able to link the information in these databases, the patient's full personal identification number will be collected and noted for the Swedish and Polish study population. No personal identification number will be captured for the patients enrolled at the sites in other participating countries. The personal identification number will be deleted after data cleaning and merge of the databases, to ensure anonymity for each person. All analysis made will be on the whole population when the personal identification has been removed.

14.1.1 DATA VALIDATION

CRF data will be subject to both logical computerized checks and manual validation checks against listings in accordance with the study specific DMP. All inconsistencies

detected during these procedures will be resolved through queries, being issued to the monitor or investigational site personnel. The complete procedure will be described in the DMP.

14.1.2 DATABASE CLOSURE

When all patients have been completed (i.e. completion of follow-up 365 days post surgery), all data have been entered into the database, and all queries solved, the Database Closure procedures will start. Decisions will be made how to classify patients into analysis populations, and how to handle protocol violations and deviating or missing data. All decisions will be dated and documented in a Database Closure document. After that the database will be locked. Any changes in the database thereafter will be documented.

14.2 STATISTICAL ANALYSIS

The clinical database will be transferred to the Sponsor by UCR. Tumour-specific data will be transferred to the Sponsor from the RCC quality registers for the Swedish study population. For other countries, there will be local procedures to capture this data and to have the information transferred to the Sponsor. Sponsor will then be responsible for linking these databases and will also do the statistical analysis.

Based on the experience from study start, the breast cancer cohort has higher recruitment-rate than the colo- and rectal-cancer cohorts. Accordingly, recruitment of breast cancer patients will be completed earlier. Due to this, the analysis of the breast cancer cohort will be made earlier than the other two cohorts. Based on the outcome of this analysis, decision will be taken about further recruitment in the study.

In the present study all endpoints will be evaluated by descriptive methods. All variables will be presented as aggregated data. Categorical variables will be summarised in frequency tables (presenting frequencies and proportions) by type of anaesthesia. The quantitative variables will be summarised by number of observations (n), mean, standard deviation (SD), median, minimum (min) and maximum (max) by anaesthesia. If applicable, separate summaries will be presented for patients receiving one, two or more anaesthesia, as well as pooled summaries. Graphical methods may be used wherever it is regarded as appropriate.

Overall survival (OS) and time to progress (TTP) will be presented as Kaplan-Meier curves together with median survival time and time to progression, respectively.

14.2.1 ANALYSIS POPULATION

All patients who have received at least one anaesthesia will be included in the population. Only observed data will be used.

14.3 DETERMINATION OF SAMPLE SIZE

A clinically relevant absolute difference in five-year-survival would be 5%-units. Based on data from our retrospective study, we will have 80% power to detect a difference of 5%-units with a *P*-value of <0.05 if including 7,378 patients (see table below). By adding 8.4% more patients to the inclusion of 8,000 patients, we will have a reasonable safety margin for loss of patients or technical errors.

In-text table 14-1– tumor specific sample size calculation:

80% power and 5% significance demands for	
Breast cancer with an expected 5-year survival of 87% vs 82% a number of:	1 650 pts
Colon cancer with an expected 5-year survival of 60% vs 55% a number of:	3 000 pts
Rectal cancer with an expected 5-year survival of 70% vs 65% a number of:	2 728 pts
Margin for loss of patients or technical errors	622 pts
Total	**8 000 pts**

15 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The Investigator(s)/institution(s) will permit study-related monitoring, audits, IEC review and regulatory inspection(s), providing direct access to source data/hospital records. The Sponsor verifies that each patient has consented in writing to direct access to the original source data/hospital records by the use of written patient information and signed Informed Consent.

In connection with on-site visits, the data recorded in the CRFs by the site will be controlled for consistency with the source data/hospital records by the study monitor (source data verification) for a random sample of patients. Any discrepancies of data will be documented and explained in the monitoring reports. The monitoring procedures including the level of Source Data Verification will be described in a study-specific Monitoring Plan which will be compiled in collaboration with the Sponsor.

16 QUALITY CONTROL AND QUALITY ASSURANCE

16.1 SOURCE DATA

Generally, the Medical Records / laboratory reports / anaesthetic reports will serve as source data. CRF can also be used as source data.
Following general Definition of Source Data location will apply for all sites and thus is no site-specific document made:

- The randomization sheet which is included in the randomization envelope is the source for randomization data. This sheet is filed in Investigators File.
- Date of signed Informed Consent is noted on the originally signed document. This serve as source for data of consent-data.
- Medical Notes is seen as source data for all other variables.

A site-specific Origin of Source Data-log needs to be compiled at the site if there are changes to these general Source Data location procedures.

The following minimum amount of information should be recorded in the hospital records:

- Clinical study number.
- Subject identification.
- Date when patient information was given and when signed Informed Consent was obtained.
- Diagnosis.
- Fulfillment of eligibility criteria.

16.2 MONITORING

In accordance with the principles of Good Clinical Practice (GCP), monitoring of the study will be arranged by the Sponsor. UCR has been appointed by the Sponsor to monitor this study for the Swedish sites and for the central monitoring of the database - see below. For other participating countries, the Sponsor will appoint local monitoring organisation to perform on-site monitoring activities and regular contacts. During the study, the Monitor will have regular contacts with the study site(s), including visits to ensure that the study is conducted and documented properly in compliance with the protocol, GCP and applicable regulatory requirements.

Both centralized monitoring activities and on-site monitoring activities will be used in this project. It has been decided that the major part of the monitoring will be made by centralized methods together with regulary telephone contacts with site but with limited on-site activities. This decision is based on the nature of the study – observational with no other study specific procedures than the randomization. Additionally, the investigational products used are well established within clinical practice since many years and with documented safety profile.

Each site will have a site initiation (can be organised as multi-centre initiation meeting or web-based training) with focus on providing information about study objectives, study procedures and CRF-training. Each site will then have at least one on-site visit during the study. Based on the outcome of centralized monitoring activities, the frequency of on-site monitoring activities may be increased. The Monitor will review source documents for verification of consistency with the data recorded in the eCRFs for a random sample of patients. The Monitor will also provide information and support to the Investigator(s).

The centralized monitoring will be used to detect each site´s level of compliance in terms of completion of CRF generally but also with focus on critical data (e.g. compliance in randomization to propofol or sevoflurane and date of surgery). The outcome of the statistical checks that will be used will indicate whether increased monitoring activities are needed on a specific site.

In terms of review of completed informed consent, only a random sample of the patients will be checked by the monitor in connection with on-site visit. Site will log all completed informed consents on on-going basis. This log needs to be distributed to the monitor on on-going basis and this will serve as monitoring tool of the consent-

process. If there are signs of that the site does not comply with the specified consent-process, extended site-specific training/ increased monitoring activities will be needed.

All monitoring procedures, including both centralized methods and on-site activities, will be described in the Monitoring Plan which will be compiled in collaboration with the Sponsor.

The study centre may also be subject to quality assurance audit by the Sponsor as well as inspection by the Regulatory Authorities. The Investigator and other responsible personnel must be available during the monitoring visits, audits and inspections and should devote sufficient time to these processes.

The Investigator should provide a curriculum vitae (CV) or equivalent documentation of suitability to be responsible for the study. All Investigators and other responsible personnel should be listed together with their function in the study on the signature list.

17 ETHICS

17.1 INDEPENDENT ETHICS COMMITTEE

It is the responsibility of the Coordinating investigator to obtain approval of the study protocol/protocol amendments, the patient information and the Informed Consent from the IEC before enrolment of any subject into the study.

The written approval from the IEC should be dated and have an attached list of those persons (with names and positions) present at the IEC meeting.

If a study stops prematurely at a study centre for any reason, the IEC must be informed. At the end of the study, the Sponsor should notify the IEC. The Sponsor should file all correspondence with the IEC.

The sponsor should file all correspondence with the IEC.

17.2 ETHICAL CONDUCT OF THE STUDY

The study will be conducted in accordance with the protocol, applicable regulatory requirements, GCP and the ethical principles of the Declaration of Helsinki as adopted by the 18th World Medical Assembly in Helsinki, Finland, in 1964 and subsequent versions.

17.3 ETHICAL CONSIDERATIONS INCL RISK EVALUATION

Both propofol and sevoflurane are well established drugs for general anaesthesia with known side effects. The monitoring of the patients during surgery in normal clinical practice is extensive and the study does not add any additional risks for the patient. However, the objective of this study with a potential difference in survival between these hypnotics may worry the patients. It is thus essential that each patient get proper information with time for questions. It is also important to point out that the signs of differences noted in animal studies may potentially not be valid for humans.

There is no specific gain for the individual patient to participate in the study. However, if the hypothesis in this protocol is correct, approximately 370 more patients will reach 5-year-survial in Sweden if propofol is used instead of sevoflurane. On a global basis with 1,600 000 new breast cancer patients annually, the corresponding number will be 80,000. Then, the study results should have a future impact on the hypnotic procedure in connection with cancer surgery.

The study is justified as the risks with the study are limited and there is a potential benefit on population-level for the patients in one of the treatment groups.

17.4 PATIENT INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator to provide each subject with full and adequate verbal and written information about the objectives, procedures and possible risks and benefits of the study. All subjects should be given the opportunity to ask questions about the study and should be given sufficient time to decide whether or not to participate in the study. The written patient information must not be changed without prior discussion with the Sponsor.

The subjects will be notified of their voluntary participation and of their freedom to withdraw from the study at any time and without giving any particular reason. Subjects must also be informed that withdrawing from the study will not affect their future medical care, treatment or benefits to which the subject is otherwise entitled.

The Investigator is responsible for obtaining written Informed Consent from all subjects (or their legally acceptable representatives and/or witnesses, where applicable) prior to enrolment in the study.

The subjects will consent to:
- Participating in the study.
- Personnel concerned at the Sponsor/designee and regulatory authorities to gain full access to hospital records, to control the data collected in the study.
- Recording, collection and processing of data and storing of data in a database.

A copy of the patient information and the Informed Consent form should be given to the subject. The Investigator (or the designated representative) who gave the verbal and written information to the subject shall sign the Informed Consent form. The Investigator should file the signed Informed Consent forms in the Investigator's File for possible future audits and inspections.

18 DATA HANDLING AND RECORD KEEPING

18.1 CASE REPORT FORMS

This study will use eCRF (electronic CRFs). A CRF is required and should be completed for each randomized subject.

The completed CRFs are the sole property of the Sponsor and should not be made available in any form to third parties (except for authorized representatives of appropriate regulatory authorities) without written permission from the Sponsor. A

site-specific pdf-copy of all entered data from that site will be provided to each participating site at study end for filing purposes.

The Investigator is responsible for ensuring the accuracy, completeness and timeliness of the data recorded in the CRFs. The CRFs should be completed in accordance with project specific completion guidelines.

Corrections of the eCRF data will be traceable in audit trail. The person changing the eCRF data must enter a reson for change. The site Investigator will sign off and lock for further changes the completed eCRF to confirm the observations recorded according to ICH GCP.

18.2 RECORD KEEPING

To enable audits and evaluations by the Sponsor and inspections by regulatory authorities, the Investigator shall keep records (essential documents) of the study for 10 years after study completion or longer if required by local law. This includes any original source data related to the study, the subject identification list (with subject numbers, full names and addresses), the original signed Informed Consent forms and pdf-copies of all completed eCRFs.

19 INSURANCE

The Sponsor is responsible to arrange for Insurance in those countries where the patients are not covered by the general Patient and Drug Insurancies available within the health care system in each participating country.

20 PUBLICATION POLICY

After completion of the study, the statistical analyses will be performed by the Sponsor and the results will be presented to the Investigators. Based on these data, the Sponsor, in cooperation with the Investigators, will prepare a clinical study report. The report will be submitted to the Regulatory Authorities and may form the basis for a manuscript intended for publication in a medical/scientific journal. For multicentre studies, the first publication should be a joint publication, reporting the combined results from all centres.

The Sponsor/Coordinating Investigator may initiate the writing of the manuscript. Before publication, the other investigators should be given the opportunity to review and comment upon the manuscript intended for publication in a medical/scientific journal. The time for review should not exceed 3 weeks after receipt of the manuscript.

The procedure for manuscripts also applies for all other publications and presentations (including meeting abstracts).

21 SUPPLEMENTS

21.1 CHANGES OF THE STUDY PROTOCOL

No change in the study procedures shall be effected without the mutual agreement of the Investigator and the Sponsor (except where necessary to eliminate an immediate hazard to subjects). All changes of the final study protocol must be documented by signed protocol amendments. If substantial changes to the study are made, the Regulatory Authorities and the IEC should be notified for review and approval.

21.2 APPLICATION TO REGULATORY AUTHORITIES

If needed according to local regulations, the Sponsor will submit an application for the authorization to conduct the study to the Regulatory Authorities prior to initiating the clinical study.

21.3 STAFF INFORMATION

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study.

21.4 CRITERIA FOR TERMINATION OF THE STUDY

The Sponsor reserves the right to discontinue the study prior to inclusion of the intended number of subjects, but intends to exercise this right only for valid scientific or administrative reasons.

22 REFERENCES

1. Shapiro J, Jersky J, Katzav S, Feldman M, Segal S: Anesthetic drugs accelerate the progression of postoperative metastases of mouse tumors. J Clin Invest 1981; 68: 678-85
2. Inada T, Kubo K, Kambara T, Shingu K: Propofol inhibits cyclo-oxygenase activity in human monocytic THP-1 cells. Can J Anesth 2009; 56: 222-229
3. Melamed R, Bar-Yosef S, Shakhar G, Shakhar K, Ben-Eliyahu S: Suppression of natural killer cell activity and promotion of tumor metastasis by ketamine, thiopental, and halothane, but not by propofol: mediating mechanisms and prophylactic measures. Anesth Analg 2003; 97: 1331-9.
4. Salo M, Pirttikangas CO, Pulkki K: Effects of propofol emulsion and thiopentone on T helper cell type-1/type-2 balance in vitro. Anaesthesia 1997; 52: 341-4
5. Matsuoka H, Kurosawa S, Horinouchi T, Kato M, Hashimoto Y: Inhalation anesthetics induce apoptosis in normal peripheral lymphocytes in vitro. Anesthesiology 2001; 95: 1467-72
6. Loop T, Dovi-Akue D, Frick M, Roesslein M, Egger L, Humar M, Hoetzel A, Schmidt R, Borner C, Pahl HL, Geiger KK, Pannen BH: Volatile anesthetics induce caspase-dependent, mitochondria-mediated apoptosis in human T lymphocytes in vitro. Anesthesiology 2005; 102: 1147-57
7. Kushida A, Inada T, Shingu K: Enhancement of antitumor immunity after propofol treatment in mice. Immunopharmacol Immunotoxicol 2007; 29: 477-86
8. Inada T, Yamanouchi Y, Jomura S, Sakamoto S, Takahashi M, Kambara T, Shingu K: Effect of propofol and isoflurane anaesthesia on the immune response to surgery. Anaesthesia 2004; 59: 954-9
9. Markovic SN, Knight PR, Murasko DM: Inhibition of interferon stimulation of natural killer cell activity in mice anesthetized with halothane or isoflurane. Anesthesiology 1993; 78: 700-6.
10. Woods GM, Griffiths DM: Reversible inhibition of natural killer cell activity by volatile anaesthetic agents in vitro. Br J Anaesth 1986; 58: 535-9.
11. Gilliland H, Armstrong M, Carabine U, McMurray T: The choice of anesthetic maintenance technique influences the antiinflammatory cytokine response to abdominal surgery. Anesth Analg 1997; 85: 1394-8
12. Ke J, Zhan J, Feng X, Wu Y, Rao Y, Wang Y: A comparison of the effect of total intravenous anaesthesia with propofol and remifentanil and inhalational anaesthesia with isoflurane on the release of pro- and anti-inflammatory cytokines in patients undergoing open cholecystectomy. Anaesth Intensive Care 2008; 36: 74-8
13. Schneemilch CE, Bank U: Release of pro- and anti-inflammatory cytokines during different anesthesia procedures. Anaesthesiol Reanim 2001; 26: 4-10
14. Schneemilch CE, Ittenson A, Ansorge S, Hachenberg T, Bank U: Effect of 2 anesthetic techniques on the postoperative proinflammatory and anti-inflammatory cytokine response and cellular immune function to minor surgery. J Clin Anesth 2005; 17: 517-27
15. Kotani N, Hashimoto H, Sessler D, Kikuchi A, Suzuki A, Takahashi S, Muraoka M, Matsuki A: Intraoperative modulation of alveolar macrophage function during isoflurane and propofol anesthesia. Anesthesiology 1998; 89: 1125-32

16. Homburger JA, Meiler SE: Anesthesia drugs, immunity, and long-term outcome. Curr Opin Anaesthesiol 2006; 19: 423-28
17. Ben-Eliyahu S, Shakhar G, Page GG, Stefanski V, Shakhar K: Suppression of NK cell activity and of resistance to metastasis by stress: a role for adrenal catecholamines and beta-adrenoceptors. Neuroimmunomodulation 2000; 8: 154-64
18. Graziola E, Elena G, Gobbo M, Mendez F, Colucci D, Puig N: Stress, hemodynamic and immunological responses to inhaled and intravenous anesthetic techniques for video-assisted laparoscopic cholecystectomy. Rev Esp Anestesiol Reanim 2005; 52: 208-16
19. Lundy J, Lovett Er, Hamilton S, P. C: Halothane, surgery, immunosuppression and artificial pulmonary metastases. Cancer 1978; 41: 827-30
20. Moudgil G, Singal D: Halothane and isoflurane enhance melanoma tumour metastasis in mice. Can J Anesth 1997; 44: 90-4
21. Mammoto T, Mukai M, Mammoto A, Yamanaka Y, Hayashi Y, Mashimo T, Kishi Y, Nakamura H: Intravenous anesthetic, propofol inhibits invasion of cancer cells. Cancer Lett 2002; 184: 165-70
22. Allin K, Bojesen S, Nordestgaard B: Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. J Clin Oncol 2009; 27: 2217-24
23. Catena F, Ansaloni L, Avanzolini A, Di Saverio S, D'Alessandro L, Maldini Casadei M, Pinna A: Systemic cytokine response after emergency and elective surgery for colorectal carcinoma. Int J Colorectal Dis 2009; 24: 803-8
24. Pierce B, Ballard-Barbash R, Bernstein L, Baumgartner R, Neuhouser M, Wener M, Baumgartner K, Gilliland F, Sorensen B, McTiernan A, Ulrich C: Elevated biomarkers of inflammation are associated with reduced survival among breast cancer patients. J Clin Oncol 2009; 27: 3437-44
25. Kurosawa S, Kato M: Anesthetics, immune cells, and immune responses. J Anesth 2008; 22: 2263-77
26. Meiler SE: Long-term outcome after anesthesia and surgery: remarks on the biology of a newly emerging principle in perioperative care. Anesthesiol Clin 2006; 24: 255-78
27. Monk TG, Saini V, Weldon BC, Sigl JC: Anesthetic management and one-year mortality after noncardiac surgery. Anesth Analg 2005; 100: 4-10
28. Lindholm M, Träff S, Granath F, Greenwald S, Ekbom A, Lennmarken C, Sandin R: Mortality within 2 years after surgery in relation to low intraoperative bispectral index values and preexisting malignant disease. Anesth Analg 2009; 108: 508-12
29. Desmond F, McCormack J, Mulligan N, Stokes M, Buggy DJ: Effect of anaesthetic technique on immune cell infiltration in breast cancer: a follow-up pilot analysis of a prospective, randomised, investigator-masked study. Anticancer Res 2015; 35: 1311-9
30. Jaura AI, Flood G, Gallagher HC, Buggy DJ: Differential effects of serum from patients administered distinct anaesthetic techniques on apoptosis in breast cancer cells in vitro: a pilot study. Br J Anaesth 2014; 113 Suppl 1: i63-7
31. Jaloszyński P, Kujawski M, Wasowicz M, Szulc R, Szyfter K: Genotoxicity of inhalation anesthetics halothane and isoflurane in human lymphocytes studied in vitro using the comet assay. Mutat Res 1999; 439: 199-206

32. Karpiński T, Kostrzewska-Poczekaj M, Stachecki I, Mikstacki A, Szyfter K: Genotoxicity of the volatile anaesthetic desflurane in human lymphocytes in vitro, established by comet assay. J Appl Genet. 2005; 46: 319-24
33. Hoerauf K, Wiesner G, Schroegendorfer K, Jobst B, Spacek A, Harth M, Sator-Katzenschlager S, Rüdiger H: Waste anaesthetic gases induce sister chromatid exchanges in lymphocytes of operating room personnel. Br J Anaesth 1999; 82: 764-6
34. Husum B: Mutagenicity of inhalation anaesthetics studied by the sister chromatid exchange test in lymphocytes of patients and operating room personnel. Dan Med Bull 1987; 34: 159-70
35. Wiesner G, Harth M, Hoerauf K, Szulc R, Jurczyk W, Sobczynski P, J. H, Taeger K: Occupational exposure to inhaled anaesthetics: a follow-up study on anaesthetists of an eastern European university hospital. Acta Anaesthesiol Scand 2000; 44: 804-6
36. Wiesner G, Hoerauf K, Schroegendorfer K, Sobczynski P, M. H, Ruediger H: High-level, but not low-level, occupational exposure to inhaled anesthetics is associated with genotoxicity in the micronucleus assay. Anesth Analg 2001; 92: 118-22
37. Wiesner G, Schiewe-Langgartner F, Lindner R, Gruber M: Increased formation of sister chromatid exchanges, but not of micronuclei, in anaesthetists exposed to low levels of sevoflurane. Anaesthesia 2008; 63: 861-4
38. Braz M, Magalhães M, Salvadori D, Ferreira A, Braz L, Sakai E, JR. B: Evaluation of DNA damage and lipoperoxidation of propofol in patients undergoing elective surgery. Eur J Anaesthesiol 2009; 26: 654-60
39. Karabiyik L, Sardaş S, Polat U, Kocabas N, Karakaya A: Comparison of genotoxicity of sevoflurane and isoflurane in human lymphocytes studied in vivo using the comet assay. Mutat Res 2001; 492: 99-107
40. Krause T, Jansen L, Scholz J, Böttcher H, Wappler F, Burmeister M, Schulte am Esch J: Propofol anesthesia in children does not induce sister chromatid exchanges in lymphocytes. Mutat Res 2003; 542: 59-64
41. Krause T, Scholz J, Jansen L, Boettcher H, Koch C, Wappler F, Schulte am Esch J: Sevoflurane anaesthesia does not induce the formation of sister chromatid exchanges in peripheral blood lymphocytes of children. Br J Anaesth 2003; 90: 233-5
42. Sardaş S, Karabiyik L, Aygün N, Karakaya A: DNA damage evaluated by the alkaline comet assay in lymphocytes of humans anaesthetized with isoflurane. Mutat Res 1998; 418: 1-6
43. Benzonana LL, Perry NJ, Watts HR, Yang B, Perry IA, Coombes C, Takata M, Ma D: Isoflurane, a commonly used volatile anesthetic, enhances renal cancer growth and malignant potential via the hypoxia-inducible factor cellular signaling pathway in vitro. Anesthesiology 2013; 119: 593-605
44. Takabuchi S, Hirota K, Nishi K, Oda S, Oda T, Shingu K, Takabayashi A, Adachi T, Semenza GL, Fukuda K: The intravenous anesthetic propofol inhibits hypoxia-inducible factor 1 activity in an oxygen tension-dependent manner. FEBS Lett 2004; 577: 434-8
45. Tanaka T, Takabuchi S, Nishi K, Oda S, Wakamatsu T, Daijo H, Fukuda K, Hirota K: The intravenous anesthetic propofol inhibits lipopolysaccharide-induced hypoxia-inducible factor 1 activation and suppresses the glucose metabolism in macrophages. J Anesth 2010; 24: 54-60

46. Tavare AN, Perry NJ, Benzonana LL, Takata M, Ma D: Cancer recurrence after surgery: direct and indirect effects of anesthetic agents. Int J Cancer 2012; 130: 1237-50
47. Looney M, Doran P, Buggy DJ: Effect of anesthetic technique on serum vascular endothelial growth factor C and transforming growth factor beta in women undergoing anesthesia and surgery for breast cancer. Anesthesiology 2010; 113: 1118-25
48. Wigmore TJ, Mohammed K, Jhanji S: Long-term Survival for Patients Undergoing Volatile versus IV Anesthesia for Cancer Surgery: A Retrospective Analysis. Anesthesiology 2015; 124: 69-79

23 APPENDICES

23.1 STUDY FLOW CHART