University of Zurich[UZH]

UniversityHospital Zurich

Clinical Study Protocol

Does anesthesia technique affect the presence of circulating tumor cells in primary breast carcinoma?

A phase 4, randomized, controlled, double blinded, multicentric trial investigating the effect of anesthetics (sevoflurane versus propofol) on circulating tumor cells positivity in primary breast cancer patients

Sponsor-Investigator and Principal Investigator:	Prof. Dr. med. Beatrice Beck Schimmer
Co-Investigator:	Dr. med. Frédérique Hovaguimian
Local Principal Investigator	Prof. Dr. med. Manfred Seeberger
Study Product:	sevoflurane versus propofol
Protocol Version and Date:	Version 1.4, Date: 20.03.2017
Short title:	CTC_BREAST Study

CONFIDENTIAL

STUDY SYNOPSIS

Sponsor-Investigator	Prof. Dr. med. Beatrice Beck-Schimmer
Study Title:	"Does anesthesia technique affect the presence of circulating tumor cells in primary breast carcinoma?" A phase 4, randomized, controlled, double blind,multicentric trial investigating the effect of anesthetics (sevoflurane versus Propofol) on the presence of circulating tumor cells in primary breast cancer patients
Short Title/Study ID:	CTC_ Breast Study
Protocol Version and Date:	Version 1.4, Date: 20.03.2017
Clinical Phase:	Clinical study phase 4
Methodology:	Prospective, randomized, double blind, controlled trial
Study Duration:	March 2014 to July 2018
Study Center(s):	Multicentric study, University Hospital Zurich Klinik Hirslanden Zurich
Sponsor Investigator:	Prof. Dr. med. Beatrice Beck-Schimmer Institute of Anesthesiology Raemistrasse 100 CH-8091 Zurich Email: beatrice.beck@usz.ch Telefon: +41 44 255 20 32- Fax: +41 44 255 44 09
Local Principal Investigator	Prof. Dr.med Manfred Seeberger Institute of Physicians for Anesthesia and Intensive Care Klinik Hirslanden Wittelikerstr. 40 CH-8032 Zurich Email: manfred.seeberger@hirslanden.ch Phone: +41 44 387 38 80 Fax: +41 44 387 38 85
Objective(s)/ Outcome(s):	**> Primary outcome:** to determine if there is a difference in the mean of blood CTC levels over time among patients exposed to volatile anesthetics (i.e. receiving sevoflurane) compared to patients in the control group (i.e. receiving intravenous anesthesia with propofol) **> Secondary outcomes:** A) to determine if anesthesia interferes with the number and activity of blood natural killer (NK) cells

	B) to examine if CTC levels correlates with the presence of extracapsular spread of tumor cells (ECS) in the histopathological findings in axillary lymph nodes
Number of Subjects:	Total number of 232 patients.
Diagnosis and **Main Inclusion Criteria:**	**Diagnosis**: primary breast cancer **Inclusion Criteria**: > Female > Age 18 to 85 > ASA I-III > Primary breast cancer (TNM stage = T1-3, N0-2, M0) > Primary surgery > Written informed consent
Main Exclusion Criteria:	> Metastatic breast cancer > Other than primary surgery (recurrence, reconstruction) > Pre-operative chemotherapy or radiotherapy > Auto-immune disease, HIV, other active cancer, age>85, ASA IV or V > Concomitant regional anesthesia > Chronic opioids medication > Any systemic immunosuppressive therapy > Known hypersensitivity or suspected allergy to propofol, soya or egg proteins > Known hypersensitivity to volatile anesthetics (malignant hyperthermia) > Pregnancy > Breast feeding > Non German-speaking patients > Enrollment in any other clinical trial during the course of this trial, 30 days prior to its beginning or 30 days after its completion
Study Product, Dose, Route, Regimen:	Sevoflurane will be used in its inhalational form via an endotracheal tube for maintenance of anesthesia. The dose and regimen will be adjusted to keep an adequate depth of anesthesia (MAC 0.8-1.2), according to BIS values between 40 and 60
Duration of administration:	Administration will be limited to the length of the surgical procedure
Reference therapy, Dose, Route, Regimen:	Propofol will be intravenously administered via a target-controlled infusion (TCI) device providing a drug dose ranging from 3.0 to 5.0 mcg/ml. The dose and regimen will be adjusted to keep an adequate depth of anesthesia, attested by BIS values between 40 and 60.
Study Schedule:	March 2014 to July 2018

Statistical Methodology:	Primary analysis : the number of CTC in peripheral blood will be measured before and after exposition to general anesthesia using a linear mixed model with random effects matrix, which accounts for within-subject treatment factor (i.e. anesthesia regimen) Secondary analyses: since most reports refer to CTC levels as a binary outcome (i.e. positive *versus* negative endpoints using a cut-off value of ≥5CTC/7.5 ml blood), data transformation from continuous into a binary variable will be performed using the same cut-off value. This new data set will be analyzed using a logistic regression mixed model with random effects matrix. All primary and secondary analyses will be performed following an *intention-to-treat* (IIT) model. This analysis will subsequently compared to a *per-protocol* analysis to ensure stability of results. Results will be reported as OR, 95% confidence intervals and their associated p-values. A p-value < 0.05 will be considered statistically significant
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, and ICH-GCP as well as all national legal and regulatory requirements.

STUDY SCHEDULE

Study Periods	Screening	Treatment Period		Follow-up	
Visit	1	2	3	4	5
Postoperative day	-1	0a	0b	2	3
Medical history	x				
Physical Examination	x				
Vital signs		x	x	x	x
Concomitant therapy	x	x	x	x	x
In-/Exclusion Criteria	x				
Subject information and informed consent	x				
Pregnancy test	x				
Randomization		x			
Study medication (sevoflurane or propofol)			x		
1° variable: CTC level		x	x	x	x
2° variable A		x	x	x	x
2° variable B*			x		
2° variable C					
Perioperative data	x	x	x	x	x
Safety variables: AE			x	x	x

Visit 1: 24h before surgery
Visit 2 (0a): before induction of anesthesia
Visit 3 (0b): end of surgery, before extubation
Visit 4: 48h after surgery
Visit 5: 72h postoperative, before hospital discharge

Primary variable: assessment of circulating tumor cells in peripheral blood
Secondary variable A: assessment of natural killer (NK) cells (number and activity)
Secondary variable B: extracapsular tumor spread in axillary nodes
* Data will be collected after completion of the study
Perioperative data: demographics and confounding factors

TABLE OF CONTENTS

1. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
ASA	American Society of Anesthesiologists
BIS	Bispectral index
COX	Cyclo-Oxygenase
CRF	Case Report Form
CTC	Circulating Tumor Cells
eCRF	Electronic Case Report Form
ECS	Extra Capsular Spreading
EpCAM	Epithelial Cell Adhesion Molecule
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ISF	Investigator Site File
ITT	Intention to treat
KISIM	Electronic patient chart at the UHZ
MAC	Minimal Alveolar Concentration
NK	Natural Killer
PACU	Post Anesthesia Care Unit
PI	Principal Investigator
PONV	Postoperative nausea and vomiting
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SDV	Source Data Verification
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCI	Target-Controlled Infusion
Th1/Th2	T-helper cell 1 and 2
TMF	Trial Master File
TNF	Tumor Necrosis Factor
TNM stage	Classification of malignant tumors (Tumor; Node; Metastasis)
UHZ	University Hospital of Zürich
VAS	Visual Analog Scale

2. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor-Investigator (Principal Investigator):	Name: Prof. Dr.med. Beatrice Beck Schimmer Address: Institute of Anesthesiology, University Hospital Zurich, Raemistrasse 100, CH-8091 Zürich Email: beatrice.beck@usz.ch Phone: +41 44 255 20 32 Fax: +41 44 255 44 09
Local Principal Investigator Hirslanden	Name: Prof. Dr.med. Manfred Seeberger Institute of Physicians for Anesthesia and Intensive Care Klinik Hirslanden Wittelikerstr. 40 CH-8032 Zurich Email: manfred.seeberger@hirslanden.ch Phone: +41 44 387 38 80 Fax: +41 44 387 38 85
Study Site(s):	Name: University Hospital Zurich Address: Rämistrasse 100, CH-8091 Zurich Name: Klinik Hirslanden Address: Witellikerstr. 40, CH-8032 Zurich
Co-Investigator: UniversitätsSpital Zürich	Name: Dr. med. Frédérique Hovaguimian Address: Institute of Anesthesiology, University Hospital Zurich, Rämistrasse 100, CH-8091 Zurich Email: frederique.hovaguimian@usz.ch Phone: +41 44 255 11 11 Fax: +41 44 255 44 09
Co- Investigator Hirslanden	Name: med. pract. Urs Rölli Institute of Physicians for Anesthesia and Intensive Care Klinik Hirslanden Wittelikerstr. 40 CH-8032 Zurich Email: urs.roelli@hirslanden.ch Phone: +41 44 387 21 11 Fax: +41 44 387 38 85

3. ETHICS

3.1 Independent Ethics Committee (IEC) and Regulatory Authorities

Before this study will be conducted, the protocol, the proposed subject information and consent form as well as other study-specific documents will be submitted to a properly constituted Independent Ethics Committee (IEC) in agreement with local legal requirements, for formal approval. Any amendment to the protocol must be approved by these institutions.

The decision of the IEC concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study.

3.2 Ethical Conduct of the Study

The study will be carried out in accordance with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, and Swiss regulatory authority's requirements.

IEC and regulatory authority will receive annual safety and interim reports and be informed about study stop/ end in agreement with local requirements.

3.3 Subject Information and Informed Consent

The investigator must explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each subject must be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment.

The subject must be informed that his/her medical records may be examined by authorized individuals other than their treating physician.

All subjects for this study will be provided a subject information sheet and a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study.

The subject information sheet and the consent form will be submitted with the protocol for review and approval for the study by the IEC. The formal consent of a subject, using the approved consent form, must be obtained before that subject is submitted to any study procedure.

The subject should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

4. INTRODUCTION

This document is a protocol for a human research study. This study is to be conducted according to international standards of GCP (International Conference on Harmonization guidelines), the current version of the Declaration of Helsinki, and applicable national regulatory authority's requirements.

4.1 Background and Rationale

Breast cancer is the most prevalent tumor affecting adult women in worldwide countries.[1] Each year, 1.38 million of new cases are diagnosed, accounting for an incidence rate of 89.9 per 100'000 women in Western Europe.[2] Despite surgical treatment in more than 90% of newly diagnosed breast cancer patients,[3] 3.5 to 6.3% will face tumor recurrence due to occult metastases.[4] Developing methods for early detection of metastatic disease has thus become a priority in cancer research.

It has been claimed that peripheral blood testing for circulating tumor cells (CTC) may represent a promising tool to identify early relapse and monitor therapy response among cancer patients.[5,6] In a meta-analysis pooling data from more than 6'800 subjects, patients with CTC levels below 5 cells *per* 7.5ml blood were found to have lower risk of disease recurrence and lower mortality rates compared to patients with levels ≥5 CTC/7.5ml blood.[7] These findings were reported for both early-stage and metastatic breast cancer patients, regardless of the method used for CTC detection (CellSearch® assay *versus* reverse-transcriptase polymerase chain reaction (RT-PCR) approach). These results are also consistent with newly published trials, which reported reduced progression-free survival and decreased overall survival with CTC levels as low as 1 cell *per* 7.5ml of blood.[8-10]

Whilst there is sound evidence that the presence of CTC correlates with poor prognosis, uncertainty remains as to the factors triggering CTC release in peripheral blood.[11] Increasing concern about the perioperative period has emerged, since it seems to create conditions promoting metastatic spreading.[12] Tumor manipulation during surgery, for instance, has been claimed to participate in CTC spreading, but in 4 trials, there was no significant change in CTC levels before and after surgical treatment.[13-16] Other authors suggested that pharmacological properties of anesthetic drugs may promote tumor recurrence: (1) either indirectly through immunosuppressive mechanisms, such as decreasing the cytotoxic activity of natural killer (NK) cells (which have a key role in tumor cells destruction and limitation of tumor growth); (2) or by interfering directly with tumor cells biology.[17,18]

Although these effects have been well documented in a large amount of pre-clinical studies, their relevance at the clinical level is still matter of debate. Several factors may account for this lack of consensus: First, the outcome of tumor cells (elimination *versus* further spreading) seems to result from complex immune interactions that have – to date – not been fully elucidated.[18] This delicate interplay between immune and tumor cells might be disrupted by several factors intervening during the perioperative phase, such as surgical stress, pain, hypothermia or blood transfusions.[12] A further complicating aspect in onco-anesthesia research is related to the very experimental nature of *in vitro* studies, which usually target specific

immune or tumor cells, thereby failing to recreate the complex micro-environment seen *in vivo*. Finally, clinical data remain scarce, conflicting and mostly driven by retrospective analyses, which are prone to important methodological limitations. Several authors already pointed out this manifest gap of knowledge and stressed the need for large, well-designed, randomized controlled trials addressing specifically the effects of anesthetics on tumor recurrence.[19,20]

One of the main issues in cancer research resides in the extensive amount of time needed to ascertain tumor recurrence, particularly when considering modestly aggressive tumors such as breast cancer. To overcome this limitation, the use of surrogate markers (i.e. markers predictive of the clinical endpoint) might be of interesting value, especially if they are lying early in the causal pathway. In this respect, CTC levels may represent an ideal surrogate to explore the effect of anesthesia on tumor spreading since they are predictive of tumor relapse, have been described in a variety of oncologic settings,[21] are objectively measured and easily obtained through simple blood test.

Therefore, the aim of this study is to evaluate if findings from pre-clinical onco-anesthetic research are consistent and applicable in a clinical trial setting. To achieve this goal, we will use CTC as a surrogate marker for tumor spreading and evaluate the impact of 2 different anesthetic regimens on CTC levels among patients undergoing surgery for primary breast cancer. More specifically, we will compare the effect of inhalational anesthesia (i.e. sevoflurane) with conventional intravenous anesthesia (i.e. propofol) on perioperative CTC levels measured at 4 different time points.

4.2 Pre-clinical data and evidence from clinical research

We performed 2 systematic searches for relevant reports in the Medline/Ovid database without language or date restriction (last search: November 2014), using both MeSH terms and keywords combined with Boolean operators as follows:
> Pre-clinical data: “exp anesthesia, general”, “exp anesthesia, intravenous”, "anesthesia.tw", "anesthetics.tw", "inhalational.tw", "volatile.tw", "intravenous anesthesia.tw", "propofol.tw", "cell line, tumor", "neoplasms, experimental", "killer cells, natural". We identified 433 reports, of which 356 were excluded after title and abstract screening. Another 47 records were discarded since they were not investigating currently used anesthetics (i.e. isoflurane, sevoflurane or propofol) or were comparing regional with general anesthesia. Thus, we considered 30 studies reporting the effect of propofol and volatile anesthetics on tumor cells (20 studies) [22-41] and on NK cells (10 studies) [42-51].
> Clinical research: “exp anesthesia, general”, “exp anesthesia, intravenous”, "anesthesia.tw", "anesthetics.tw", "inhalational.tw", "volatile.tw", "intravenous anesthesia.tw", "propofol.tw", "neoplastic cells, circulating", "neoplasm recurrence, local", "circulating tumor cells.tw", "CTC.tw", "perioperative.tw". We identified 1586 records, of which most (1564) were excluded after title and abstract screening. From the remaining 22, [52-73] we discarded 19 reports that were not comparing sevoflurane with propofol. Thus, only 3 clinical studies were eventually considered. [57,60,71]

4.2.1 Effects of anesthetics on tumor cells

Table 1 outlines the main results of pre-clinical studies reporting on tumor cells lines exposed to a variety of anesthetics. Although propofol seems to have a protective effect against tumor growth, migration and invasiveness, the heterogeneity in designs, cell lines, interventions and outcomes makes these findings difficult to interpret.

Table 1. Summary of search results: tumor cells studies

Study ID	Design	Comparison (not considered)	Tumor cells	Endpoint(s)	Results
VOLATILE ANESTHETICS *VERSUS* PROPOFOL					
Huang 2014	Prospective, controlled, in vitro	1. Isoflurane 0.5-2% (2hrs) 2. Propofol 0.5–10µg/ml (2hrs) (3. Isoflurane + propofol) 4. Control	Prostate cancer cells line (PC3)	Proliferation, migration, chemoresistance	In cells exposed to isoflurane, markers of proliferation, migration and chemoresistance were significantly increased (HIF-1α pathways). Propofol conveyed protective effects.
VOLATILE ANESTHETICS					
Liang 2013	Prospective, controlled, in vitro	1. Sevoflurane 2.5% (2. Cisplatin) (3. Sevoflurane + Cisplatin) 4. Control	Lung adenocarcinoma cells line A549	Proliferation, apoptosis, invasion	In cells exposed to sevoflurane: - Proliferation and invasion significantly reduced - Apoptosis rates significantly increased
Ecimovic 2013	**Prospective, controlled, in vitro**	**1. Sevoflurane 1, 2, 3, or 4 mM (6hrs) 2. Control**	**Breast cancer cells lines (MCF7 ER(+); MDA-MB-231 ER(-))**	**Proliferation, migration, invasion**	**In cells exposed to sevoflurane: - Significant increase in proliferation and migration (both cell lines) - Significant increase in invasion (MCF7 ER(+))**
Jun 2011	Prospective, controlled, in vitro	1. Isoflurane 2% 3h 2. Isoflurane 2% 6h 3. Control	Head and neck squamous cell carcinoma cells lines (Tca8113; HSC2)	Proliferation, apoptosis, invasion	In cells exposed to isoflurane: - Significant increase in proliferation and invasion - Significant decrease in apoptosis
Kawaraguchi 2011	Prospective, controlled, in vitro	1. Isoflurane 1.2% 2. Control	Colon cancer cells lines (HT29; HCT116)	Apoptosis	No difference between exposed and control groups; In experimental conditions mimicking cellular response to chemotherapy, isoflurane was protective against apoptosis in some cells (HCT116)
Kvolik 2009	Prospective, controlled, in vitro	1. Sevoflurane 3% (1-2hrs) 2. Control	Colon (Caco-2) and laryngeal cancer (HEp-2) cells lines	Apoptosis	Significant increase in apoptosis rates in Caco-2 cells; non significant increase in apoptosis rates in Hep-2 cells.
Kvolik 2005	Prospective, controlled, in vitro	(1. Halothane 1.5%) 2. Sevoflurane 3% 3. Isoflurane 2% (4. Nitrous oxide) 5. Control	Colon (Caco-2), larynx (HEp-2), and pancreatic carcinoma (MIA PaCa-2); metastasis of colon carcinoma (SW-620), normal fibroblasts	Proliferation, DNA/RNA/protein synthesis, apoptosis	Significant growth inhibition in all cell lines exposed to sevoflurane except (MIA PaCa-2). Growth was significantly reduced in some cell lines exposed to isoflurane (HEp-2). Other outcomes not reported for sevoflurane and isoflurane groups.

Studies related to breast cancer research are highlighted in bold. GREEN = may have protective effects against cancer cells; YELLOW = mixed results or no difference; RED: may promote cancer.

Table 1. Summary of search results: tumor cells studies (continued)

Study ID	Design	Comparison (not considered)	Tumor cells	Endpoint(s)	Results
INTRAVENOUS ANESTHETICS: PROPOFOL					
Ecimovic 2014	**Prospective, controlled, in vitro**	**1. Propofol (1-10 µg/ml) (2. Bupivacaine) 2. Control**	**Breast cancer cells lines (MCF7 ER(+); MDA-MB-231 ER(-))**	**Proliferation, migration, invasion**	**In cells exposed to propofol: - no reduction in proliferation - reduction in migration - reduction in invasion (MCF7 only)**
Cui 2014	Prospective, controlled, in vitro	1. Propofol 2, 4, 6, 8 or 10 µg/ml (72hrs) 2. Control	Lung cancer cells line (H460)	Proliferation, apoptosis	Significant inhibition of proliferation in cells exposed to 4-10µg/ml Significant increase in apoptosis in cells exposed to >7.2µg/ml
Cui 2014	In vivo RCT (mice)	Injection of: 1. Cells exposed to propofol 2. Cells exposed to phosphate buffered saline 3. Untreated cells	Lung cancer cells line (H460)	Tumor weight and volume	Significant reduction in tumor weight and volume among mice injected with propofol treated cells
Xu 2013	Prospective, controlled, in vitro	1. Propofol 10, 25, 50 or 100 µmol/L (72 hrs) (2. Propofol 100 µmol/L (8 to 72 hrs)) 3. Control	Esophageal squamous cell carcinoma cells line (Eca-109)	Proliferation, invasion and angiogenesis	Significant inhibition of proliferation in cells exposed to 50 and 100µmol/L Significant increase in apoptosis in cells exposed to 50 and 100µmol/L
Wang 2013	Prospective, controlled, in vitro	1. Propofol 0.1-10 µg/ml (72hrs) (2. Paclitaxel) (3. Propofol + paclitaxel) 4. Control	Ovarian cancer cells lines (HO-8910PM, HO-8910, SKOV-3, OVCAR-3, COC1, ES-2)	Proliferation, apoptosis, invasion	In cell lines resistant to paclitaxel: significant inhibition of proliferation, invasion and apoptosis in cells exposed to 5 and 10µg/ml
Du 2013	Prospective, controlled, in vitro	1. Propofol 10, 25, 50 or 100 µmol/L (72hrs) (2. Gemcitabine) (3. Propofol + gemcitabine) 4. Control	Pancreatic cancer cells line (MIAPaCa-2)	Proliferation, apoptosis	Significant inhibition of proliferation in cells exposed to 50 and 100µmol/L Significant increase in apoptosis in cells exposed to 50 and 100µmol/L
Zhang 2012	Prospective, controlled, in vitro	1. Propofol 10, 20 or 40µmol/L (72hrs) 2. Control	Gallbladder carcinoma cells line (GBC-SD)	Proliferation, apoptosis, invasion	In cells exposed to propofol: - Significant increase in proliferation and invasion - Significant decrease in apoptosis
Wu 2012	Prospective, controlled, in vitro	1. Propofol 2. Control	Lung carcinoma cells line (A549)	Invasion, migration	In cells exposed to propofol, decrease in invasion and migration
Li 2012	**Prospective, controlled, in vitro**	**1. Propofol 2, 5 or 10 µg/ml (24hrs) 2. Control**	**Breast cancer cells line (MDA-MB-231)**	**Invasion, migration**	**Significant inhibition of invasion and migration in cells exposed to 5 and 10µg/ml**
Zhang 2011	Prospective, controlled, in vitro	1. Propofol 2. Control	Esophageal squamous cell carcinoma cells line (Eca-109)	Proliferation, apoptosis, migration, invasion	In cells exposed to propofol, decrease in proliferation, invasion and migration; promotion of apoptosis.
Miao 2010	Prospective, controlled, in vitro	1. Propofol 2, 5 or 8µg/ml (24hrs) 2. Control	Colon carcinoma cells line (LOVO, HT29, SW1116)	Invasion	In cells exposed to propofol, significant decrease in invasion
Kushida 2007	Prospective, controlled, in vivo (mice)	Intraperitoneal injection once/day for 3 weeks: 1. Propofol 50mg/kg (2. Midazolam) (3. Control intralipid) 4. Control saline	Murine thymoma cells (EL4) inoculated subcutaneously at day 3 of experiment	Tumor volume	Significant reduction in tumor volume among mice treated with propofol
Garib 2005	**Prospective, controlled, in vitro**	**1. Propofol 6 µg/mL (20min) (2. Verapamil) (3. Propofol + Verapamil) 4. Control**	**Breast carcinoma cells line (MDA-MB-468)**	**Migration**	**In cells exposed to propofol, significant increase in migration activity**
Melamed 2003	**Prospective, controlled, in vivo (mice)**	**(1. Halothane) (2. Ketamine) (3. Thiopental) (4. Diazepam) 5. Propofol 6. Control**	**Lung metastasis of breast carcinoma cells line (MADB106) inoculated in rats during exposure to anesthetics**	**Lung tumor retention 24h after inoculation**	**No significant difference between volatile, propofol and control groups.**

Studies related to breast cancer research are highlighted in bold. GREEN = may have protective effects against cancer cells; YELLOW = mixed results or no difference; RED: may promote cancer.

4.2.2 Effect of propofol and sevoflurane – clinical evidence

As outlined previously, clinical data remain scarce, conflicting and mostly driven by retrospective analyses. We retrieved only 3 studies comparing the effects of sevoflurane versus propofol on tumor recurrence (Table 2).[57,60,71] The most recent study was a large retrospective register review including data from 2838 patients undergoing surgery for breast or colorectal carcinoma.[57] After adjustment for multiple confounders, there was no difference between groups in terms of survival. In breast cancer patients receiving sevoflurane anesthesia, a beneficial trend towards better survival was identified, although not statistically significant (HR propofol group: 1.33 (0.91–1.94)). These findings were consistent with a small randomized controlled trial that was not able to show any difference in metastasis rates, overall mortality and cancer-related mortality in patients receiving either propofol or sevoflurane anesthesia.[71] Finally, a retrospective chart review of moderate size yielded conflicting results, but several aspects limit the validity of these results.[60]

Table 2. Summary of search results: clinical studies comparing propofol *versus* volatile anesthesia

Study ID	Design	Comparison (nb analysed)	Surgery	Endpoint(s)	Results	Comments
Enlund 2014	Retrospective register review	1. Sevoflurane (1935) 2. Propofol (903)	Breast and colorectal carcinoma	1- and 5-year survival rates	Sevoflurane = reference (HR 1.00): Colon: HR 0.94 (0.71–1.25) Rectal: HR 0.83 (0.52–1.31) Breast: HR 1.33 (0.91–1.94)	Multivariate analysis with adjustment for confounders: no difference in survival rates between groups.
Sofra 2013	RCT	1. Sevoflurane (14) 2. Propofol (14)	Radical cystectomy for primary bladder cancer	Presence of metastasis, mortality (overall and cancer-related)	Metastasis: Sevo 3/14, Propofol 1/14, p=0.28; Overall mortality: Sevo 4/14, propofol 1/14, p= 0.14 Mortality (cancer related): Sevo 5/14, propofol 2/14, p=0.19	No difference between groups; methodology questionable, sample size underpowered.
Forget 2010	Retrospective hospital records review	1. Sevoflurane 2. Propofol (total=319)	Breast cancer	Recurrence-free survival	Thiopental = reference (HR 1.00): Sevoflurane: HR 0.58 (0.0–1.33) Propofol: HR 1.17 (0.87–1.47)	May favour the use of propofol. Univariate analysis, no adjustment for confounders, primary comparison is analgesics (and not anesthetics), unclear why reference = thiopental.

HR: hazard ratio; RCT: randomised controlled trial.

4.2.3 Secondary Outcomes:

4.2.3.1 NK cells and tumor spreading

Under physiologic circumstances, tumor cells are believed to be caught and destroyed through cell-mediated immune responses involving NK cells.[74,75] As mentioned previously, these complex immune interactions are usually impaired during the perioperative period: surgical stress, for instance, has been suggested to induce a generalized inflammatory state that is thought to suppress NK cells activity and therefore enhance tumor spreading.[76,77] Further weakening of the immune response is believed to occur through drug-related effects of anesthetics on NK cells (table 3).The vast majority of these studies were either not controlled or not appropriately controlled, yielding results that are difficult to compare.

Table 3. Summary of search results: NK cells studies					
Study ID	**Design**	**Comparison** (not considered)	**Population** (nb analysed)	**Endpoint(s)**	**Results**
VOLATILE ANESTHETICS *VERSUS* PROPOFOL					
Zhang 2014	RCT	1. Propofol (2. Propofol + Sevoflurane) 3. Sevoflurane	Reconstructive surgery for tongue carcinoma (60)	NK cells, T-lymphocytes, B-lympocytes levels	Significant decrease in all cells levels after exposure to anesthetics in all groups. No difference between groups.
Zancla 1991	Prospective, controlled	1. Propofol 2. Isoflurane	Abdominal surgery	Nk cells cytotoxic activity	No difference between groups
PROPOFOL					
Brand 2003	Prospective, not controlled	All patients received propofol anesthesia.	Elective orthopedic surgery (30)	NK cells, T-lymphocytes, B-lympocytes levels	Significant decrease in NK cells levels after exposure to anesthetics.
VOLATILE ANESTHETICS					
Brand 1997	Prospective, not controlled	All patients received isoflurane anesthesia.	Elective orthopedic surgery (30)	NK cells, T-lymphocytes, B-lympocytes levels	Significant decrease in NK cells levels after exposure to anesthetics.
Kutza 1997	Prospective, not controlled	All patients received isoflurane anesthesia.	Various surgical settings (26)	NK cells (number and cytotoxic activity)	Significant decrease in the cytotoxic activity of NK cells; significant decrease in the relative number of NK cells (% of total white blood cells) but not in absolute numbers.
Markovic 1993	Prospective, controlled, in vivo (mice)	1. Halothane 1.5% (2hrs) 2. Isoflurane 2.1% (2hrs)	Mices (nb not reported)	Nk cells cytotoxic activity	No difference in baseline cytotoxic activity; significant reduction in IFN-induced cytotoxic activity in both exposure groups.
OTHER COMPARISONS					
Miyata 2013	Prospective, controlled, in vivo (dogs)	1. Propofol induction + isoflurane (3hrs) 2. No anesthesia	Dogs (13)	NK cells cytotoxic activity	In dogs exposed to anesthetics, significant decrease in NK cells cytotoxic activity
Richardson 1997	Prospective, not controlled	Most patients received propofol + isoflurane anesthesia	Uveal malignant melanoma (19)	NK cells cytotoxic activity	Increase in perioperative NK cells cytotoxic activity
Pirttikangas 1994	**RCT**	**1. Propofol 2. Thiopental + Nitrous oxide 70%**	**Biopsy for breast cancer (27)**	**NK cells, T-lymphocytes, B-lympocytes levels**	**Significant decrease in NK cells levels in both groups. No difference between groups.**
Bentley 2005	RCT	1. Propofol + Ketamine + isoflurane 2. Propofol + isoflurane	Maxillofacial surgery (59)	NK cells (number and cytotoxic activity)	Significant decrease in cytotoxic activity in both groups, no difference between groups. No difference in the % of NK cells.

Studies related to breast cancer research are highlighted in bold. YELLOW = mixed results or no difference or not adequately controlled

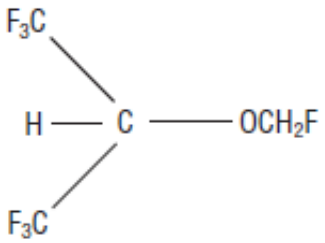
4.2.3.2 CTC and extracapsular spread in lymph nodes

While lymph node status has been regarded as the most important prognostic factor for disease-free and overall survival in breast cancer patients,[78] a substantial amount of node-positive patients will surprisingly remain free of systemic relapse.[9] By contrast, some patients expected to have a favorable outcome will experience the opposite scenario, suggesting that other factors play a role in the metastatic cascade.

Recent findings suggests that extracapsular spread (ECS) of tumor cells observed in axillary lymph node contents might correlate with poorer prognosis in breast cancer patients.[79] According to the yearly statistics of our hospital, axillary node dissection is realized in more than 30% of women undergoing surgery for primary breast cancer. The correlation between CTC positivity and the presence of ECS in the histopathological findings of axillary lymph node contents will be assessed in these patients.

4.3 Investigational Product and Dose Rationale

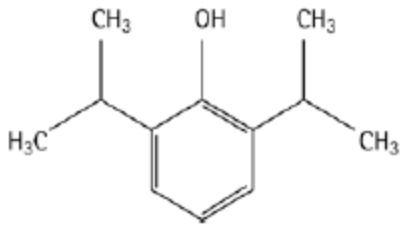
Sevoflurane is a halogenated general inhalation anesthetic drug. It is chemically stable and is neither flammable nor explosive in clinical concentrations. It belongs to the group of halogenated ethers, and its structural formula is:



fluoromethyl 2,2,2,-trifluoro-1-(trifluoromethyl) ethyl ether

Sevoflurane is an inhalational anesthetic agent for induction and maintenance of general anesthesia. The minimum alveolar concentration (MAC) of sevoflurane in oxygen for a 40-year-old adult is 2.1%. Because of its little solubility in blood and tissues its effects are of rapid onset. Recovery of consciousness is usually achieved within 8 to 10 minutes after discontinuation of application. The use of sevoflurane as a general anesthetic is well established, and considered as a safe, routinely prescribed, anesthetic agent.

Propofol is an intravenous hypnotic agent, poorly soluble in water, thus dissolved in a fat emulsion vehicle that contains soya bean oil, triglycerides, egg lecithin, glycerol, sodium oleate and water.



2,6-diisopropylphenol

Its hypnotic effect occurs rapidly (less than 60 seconds), but the effect duration is short, due to rapid redistribution and metabolism. It is a well-established drug, which has been successfully used for general anesthesia or sedation since more than 20 years.

Since the emergence of short acting intravenous anesthetics in the late 80's, an impressive amount of literature comparing volatile with intravenous anesthesia has been produced, focusing on pharmacological effects, patient safety, quality process, patient satisfaction, or organ protection. No consensus regarding the superiority of one technique has been reached so far, mainly because complications related to anesthesia are more the result of multiple factors (surgery, patient specific health problems) rather than due the drug itself. Both sevoflurane and propofol contribute equally to the following systemic effects observed during general anesthesia:

- Cardiovascular: hypotension (fall in systemic resistance), bradycardia.
- Respiratory: reduced minute ventilation, reduced respiratory threshold to hypoxia, increased tolerance to hypercapnia.
- Central nervous system: autoregulation maintained, decrease in cerebral blood flow and oxygen consumption.

If both drugs produce similar physiologic effects, they differ however regarding specific side effects:

- in susceptible patients, Sevoflurane can trigger malignant hyperthermia, a hypermetabolic state due to abnormal calcium release in skeletal muscle (pharmacogenetic disease, autosomal dominant).
- Sevoflurane and other volatile anesthetics are suggested to confer organ protection, a property known as "volatile preconditioning"
- Propofol is known to decrease the incidence of postoperative nausea and vomiting in susceptible individuals
- In soya- or egg-allergic patients, the administration of propofol can induce anaphylactic reactions

The choice between the different regimens of anesthesia maintenance (volatile vs. intravenous) is mostly dictated by habits and local hospital practices.

4.4 Risk/Benefits and Ethical Considerations

By means of this trial, we might be able to identify potential targets playing a crucial role in the metastatic cascade. Being able to find the best (or the least harmful) anesthesia regimen for cancer patients is of primary relevance, and raises important ethical concerns in terms of beneficence and non-maleficence.

5. STUDY OBJECTIVES AND OUTCOMES

We will conduct a prospective, randomized, double blinded, controlled study over a 52-month period to investigate whether commonly used anesthetics (volatile *versus* intravenous) affect the level of circulating tumor cells in patients undergoing surgery for primary breast cancer.

Hence, the **primary objective** is to compare the effects of 2 anesthetic regimens (sevoflurane *versus* propofol) on perioperative CTC levels measured at 4 different time points. Blood levels (i.e. number) of CTC will be determined using the CellSearch® approach, as described below.

Two **secondary objectives** will be determined**:**

A) To determine if anesthesia interferes with NK cells by measuring NK cells number and activity at 4 different time points;

B) To establish if the level of CTC correlates with the presence of ECS in the histopathological findings of axillary lymph nodes (exclusively in patients undergoing breast surgery with axillary dissection).

5.1 Primary Variable: circulating tumor cells (CTC)

Blood CTC levels (i.e. number of CTC per 7.5ml of blood) will be measured before and after the administration of anesthetics using the CellSearch® system, as described elsewhere:[9,80,81] 15ml sample of peripheral blood (necessary amount for 2 CellSearch® tubes) will be collected at 4 pre-defined time points and screened for CTC within 96 hours of blood collection.

5.2 Secondary Variables

5.2.1 Secondary variable A: NK cells (number and activity)

Assessment of NK cells will be performed at 4 pre-definite time points, concomitant to CTC determination, using 2x8ml of peripheral venous blood collected in sterile tubes containing sodium heparin. The number of NK cells will be assessed using flow cytometry, whilst the NK cells activity will be determined *in vitro* by a direct, cell-based cytotoxicity assay against the K562 (human chronic myelogenous leukaemia) cell line with a fluorescent read-out, as previously described.[82,83]

5.2.2 Secondary variable B: extracapsular tumor spread (ECS)

After study completion, pathology reports from patients undergoing surgery with axillary lymph node dissection will be reviewed for extracapsular tumor spread with the aim to establish if blood CTC levels correlates with the presence of ECS.

5.2.3 Other study variables

Demographics and perioperative data will be collected as presented in table 4. Since opioids have been suggested to inhibit the immune function,[84] perioperative analgesia will be administered following a standardized protocol and will be documented thoroughly. Restriction and stratified randomization are expected to minimize the risk of unbalanced confounders (section12). However, since we will perform exploratory analyses whenever there is a need to control for residual confounders, the following variables will be collected: hypothermia, blood transfusion, administration of cyclooxygenase inhibitors, beta-blockers, glucocorticoids and date of last menstruation.[85-87]

5.2.4 Safety variables

Any adverse event (AE) or serious adverse event (SAE) as described below.

Table 4. Demographics and perioperative data to be collected
Patient data
Age
ASA class
Concomitant therapy
Surgery type
Last menstruation date
TNM stage: size T1-T4; pathological node status N0, N1-3, N>3
Tumor type
Histological tumor grade (grade 1-3)
Receptors (E+/P+; E+/P-; E-/P+; E-/P-; HER2+)
Ki67>35%
Extracapsular tumor spreading
Peroperative data
Anesthesia duration (min)
Mean expiratory fraction in group sevoflurane (Exp%)
Total amount of propofol in group propofol (mg)
Mean BIS value
Total amount of fentanyl (mg)
Mean core temperature (°C)
Administration of b-blockers or COX-inhibitors (yes/no)
Administration of PONV prophylaxis (yes/no)
Blood transfusion (number of units)
Postoperative data
Total amount of morphin in PACU (mg)
Total amount of morphin on the ward (mg)
Administration of b-blockers or COX-inhibitors (yes/no)
Administration of PONV prophylaxis (yes/no)
Blood transfusion (number of units)

6. STUDY DESIGN

6.1 General Design

We will conduct a prospective, randomized, double blinded, controlled study over a period of 52 months. We will consider adult female patients undergoing surgery for primary breast cancer. Expecting a dropout rate of 10% over time, we will recruit a total of 232 patients (i.e. 116 in each study group).

Patients will be screened for eligibility according to the inclusion and exclusion criteria on the day prior to surgery. Written informed consent will be subsequently obtained. A pregnancy test will be performed for all included patients.

On the day of operation, the patients will be premedicated with midazolam *per os* (3.75mg to 7.5mg). Randomization will be performed according to a computer-generated anesthesia-assignment (www.randomizer.at) once the patient will be installed for induction of anesthesia. By this means, concealment of random allocation will be ensured.
After preoxygenation, induction of anesthesia will be administrated in both groups similarly, using fentanyl 2-3mcg/kg, Pentothal 4-6 mg/kg and rocuronium 0.6mg/kg intravenously. Maintenance of anesthesia will be conducted following the assignment group:
1) in “Group Sevo”, anesthesia will be maintained with sevoflurane in 80% oxygen, adjusted to keep an adequate depth of anesthesia (minimal alveolar concentration (MAC) between 0.8 and 1.2), attested by bispectral (BIS) index values between 40 and 60.
2) in “Group Propofol” anesthesia will be maintained with a target-controlled infusion (TCI) device providing an intravenous propofol dose adjusted to keep an adequate depth of anesthesia (TCI values: start after induction with 2.0mcg/ml, then ranging from 3.0mcg/ml to 5.0mcg/ml), attested by BIS index values between 40 and 60.
Patients will be ventilated with 80% oxygen in air.
Patients requiring a rapid sequence induction will receive rocuronium 0.9mg/kg instead of 0.6mg/kg. Per- and postoperative analgesia will be provided following a standardized protocol: fentanyl 2mcg/kg (total dose ranging from 5-10mcg/kg) will be used during surgery; surgical wound infiltration with local anesthetics will not be allowed; morphine intravenous 0.02-0.04mg/kg for a VAS <4 will be used in the PACU, whilst subcutaneous morphine (0.1-0.2mg/kg will be prescribed as second line analgesic once patients will be discharged from PACU. Basic postoperative analgesia will be provided using paracetamol and non-steroidal anti-inflammatory drugs. Postoperative nausea and vomiting (PONV) prophylaxis will be prescribed following a strict protocol (no glucocorticoids).

6.2 Primary Study Endpoint: blood CTC levels

The primary endpoint, blood CTC levels, will be assessed at 4 pre-defined time points using the CellSearch® approach: two 7.5ml samples of blood will be collected pre-operatively (visit 2, before induction of anesthesia), at the end of surgery (visit 3, before extubation) and two times postoperatively (visit 4, 48h postoperative, and visit 5, 72h after surgery, before hospital discharge). The number of CTC per 7.5ml of peripheral blood will be determined by the CellSearch® system within 96 hours of blood collection. The analysis will account for the effect of repeated measurements over time (within-subject factor) and the effect of anesthesia regimen (between-subject factor) as described under section 12.

The CellSearch® approach

The CellSearch® assay contains a ferro fluid-based capture reagent and immunofluorescent reagents. The ferrofluid reagent consists of particles with a magnetic core surrounded by a polymeric layer coated with antibodies targeting the epithelial cell adhesion molecule (EpCAM) antigen for capturing CTC. After immunomagnetic capture and enrichment, fluorescent reagents (anti-CK-phycoerythrin, DAPI, and anti-CD45-allophycocyanin) are added for identification and enumeration of CTC. (www.veridex.com)

6.3 Secondary Endpoints

6.3.1 Anesthesia, NK cells and tumor spreading

The assessment of NK cells (mean number and activity) will be performed at 4 pre-definite time points, concomitant to CTC determination. The analysis will account for the effect of repeated measurements over time (within-subject factor) and the effect of anesthesia regimen (between-subject factor) as described under section 12.

6.3.2 CTC positivity and extracapsular tumor spreading

Pathology reports from patients undergoing surgery with axillary lymph node dissection will be reviewed for extracapsular tumor spread with the aim to establish a correlation between CTC positivity and ECS.

6.3.3 Safety Endpoints

Adverse events (AE) or serious adverse events (SAE) will be actively sought and reported. For comprehensive description of AE/SAE, see paragraph 10 below.

6.4 Study procedures (fig.1)

6.4.1 Visit 1 (preoperative day)

- Standard pre-anesthesia visit: medical history, physical examination, demographics (as listed in Table 1)

- Check for eligibility according to the inclusion and exclusion criteria
- Information of patient and written informed consent
- Pregnancy test: if not pregnant, the patient will qualify for the study
- Concomitant therapy* will be administered
- Potential confounders will be documented

6.4.2 Visit 2 (day of surgery – before induction of general anesthesia)

- Vital signs will be recorded, concomitant therapy* will be administered
- Patient randomization to “Group Sevo” (experimental) or to “Group Propofol” (control)
- 2x7.5ml + 2x8ml of blood will be collected and sent for CTC/NK cells assessment (1° variable and 2° variable A)

6.4.3 Visit 3 (day of surgery – end of surgery, before extubation)

- Maintenance of anesthesia following the assignment group (sevoflurane or propofol)
- Vital signs will be recorded, concomitant therapy* will be administered
- 2x7.5ml + 2x8ml of blood will be collected and sent for CTC/NK cells assessment (1° variable and 2° variable A)
- Histopathological findings (2° variable B): data related to this endpoint will be collected after completion of the study.
- Perioperative data collection as listed in Table 3
- AE or SAE will be recorded

6.4.4 Visit 4 and 5 (48h and 72h postoperative)

- Vital signs will be recorded, concomitant therapy* will be administered
- 2x7.5ml + 2x8ml of blood will be collected and sent for CTC/NK cells assessment (1° variable and 2° variable A), concomitant to routine postoperative laboratory tests
- Perioperative data collection as listed in Table 3
- AE or SAE will be recorded

* For medications susceptible to interfere with outcome measure, see paragraph 8.5 below

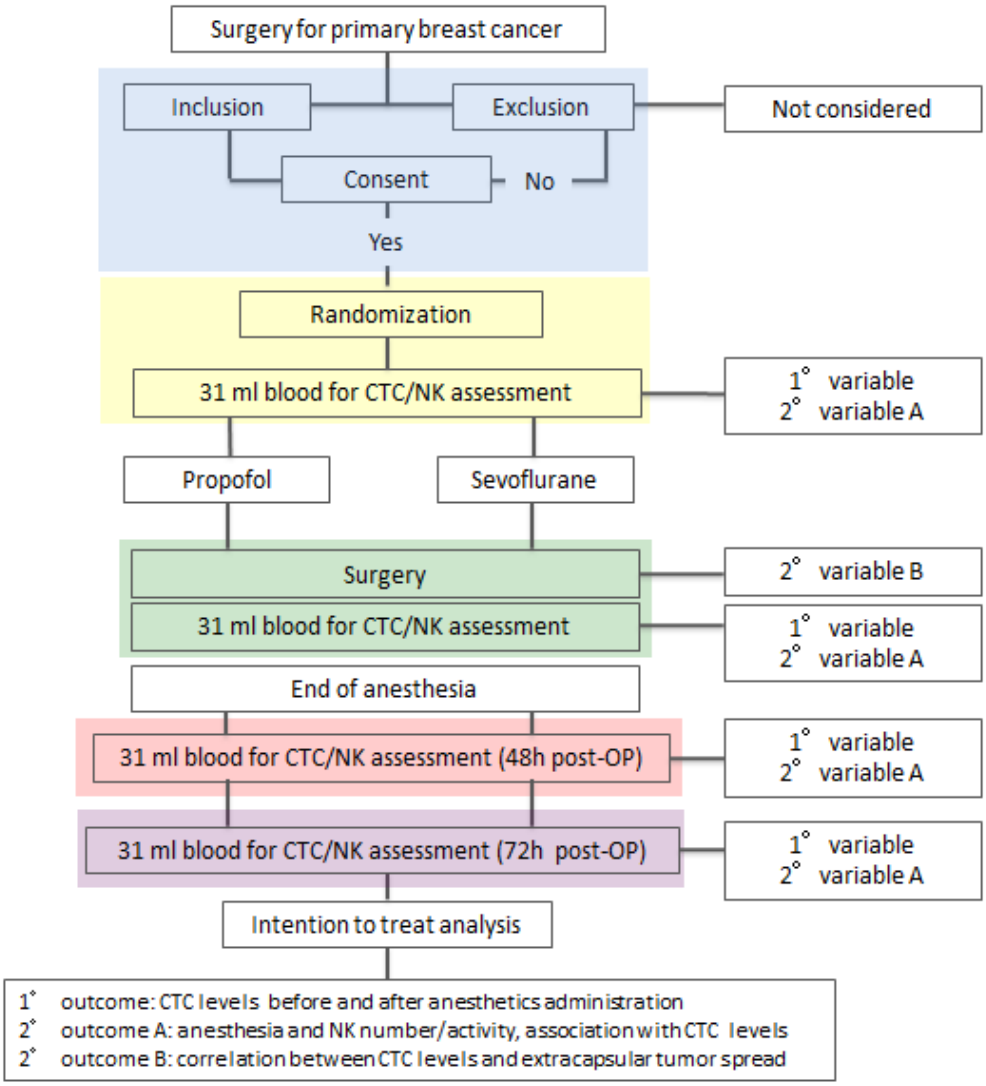


Fig.1 Trial profile. Each visit is highlighted in a different color.

7. SUBJECT SELECTION AND WITHDRAWAL

Patients undergoing surgery for breast cancer will be enrolled at the University Hospital of Zürich (USZ) and at Klinik Hirslanden Zurich. The Department of Gynecology and Breast Cancer Center of the USZ perform about 150-200 operations per year for breast cancer. Based on USZ last year records, we expect to enroll 5-10 patients per month; slightly lower enrollment rates are expected at Klinik Hirslanden, (3-8 patients/month).

Meetings with the anesthesia team USZ and the investigators of Klinik Hirslanden will be organized periodical at least all 6 month, to identify at an early stage potential enrolment or organizational issues. These meetings are also expected to act as a positive feedback and motivational tool.

Patients fulfilling all of the following inclusion criteria will be enrolled in the study:

7.1 Inclusion Criteria

- 18 to 85 years old
- Female patients
- Primary breast cancer without known remote extension
- Scheduled for tumor resection or mastectomy, +/- axillary node dissection
- ASA status I-III, as defined by the American Society of Anesthesiologists
- Capable of giving informed consent

7.2 Exclusion Criteria

The presence of any one of the following exclusion criteria will lead to exclusion of the subject:

- Metastatic breast cancer
- No primary surgery (recurrence, reconstruction)
- Pre-operative chemotherapy or radiotherapy
- Significant immune impairment: auto-immune disease, HIV, other active cancer, age>85, ASA IV-V
- Concomitant regional anesthesia (epidural catheter, paravertebral blockade, wound infiltration with local anesthetics)
- Chronic opioids medication
- Any systemic immunosuppressive therapy (glucocorticoids, cytostatics, antibodies, drugs acting on immunophilins, interferon, TNF bindings proteins, mycophenolate)
- Known hypersensitivity or suspected allergy to propofol, soya or egg proteins

- Known hypersensitivity to volatile anesthetics (malignant hyperthermia)
- Women who are pregnant or breast feeding
- Inability to follow the procedures of the study, e.g. due to language problems, psychological disorders, dementia, etc. of the subject
- Participation in another study with investigational drug within the 30 days preceding and during the present study

Intraoperative radiotherapy is not an excluding criteria.

7.3 Subject Recruitment and Screening

All consecutive patients admitted for breast cancer surgery will be assessed for study eligibility by the anesthesiology staff. The attending anesthetist will inform patients about the study orally and in writing. Patients willing to participate will subsequently provide written informed consent. A pregnancy test will be performed.

Participation is on a strictly voluntary base. No compensation in any form will be granted to the subjects.

7.4 Early Withdrawal of Subjects

Patients will be informed about the possibility to retract their participation to the study at any time. Investigators may withdraw a patient prior to expected completion of the study for safety reasons (for instance as the result of an adverse event (AE) with unacceptable consequence or risk for the patient). In both cases, for patient safety, a final medical examination will be performed. Notification of the discontinuation will be clearly documented on the patient's case report form (CRF).
Since data collection and analysis will be performed following an intention-to-treat model, all consecutive patients will be analyzed according to the initial randomization group, regardless of the study drug administered. These patients will not be withdrawn from analysis.

7.4.1 Criteria for Early Withdrawal of Subjects

Initially included patients meeting unexpectedly exclusions criteria during the course of the study (such as: incidental findings of metastatic cancer, other surgical procedure as initially planned, reoperation within the first 5 postoperative days, patients receiving chemo- or radio- or any immunotherapy during the study course), or patients unable to follow study procedures (for instance, patients transferred to another hospital) will be considered as drop-outs and will not appear in the study analysis.

7.4.2 Data Collection and Follow-up for Withdrawn Subjects

Patients withdrawn because of an adverse event (AE) or patients unexpectedly meeting exclusion criteria will be followed during the study course. Data available through routine care will be recorded in the CRF unless the patient specifically refuses the investigators to access the medical record.

7.5 Early Termination of Study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances:

- ethical concerns,
- insufficient patient recruitment,
- when the safety or benefit of the subjects is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- reaching a positive or negative statistical end point earlier than anticipated.

8. TREATMENTS

8.1 Treatments to be Administered

Sevoflurane group:

Anesthesia will be maintained with sevoflurane (gaseous form via the endotracheal tube) in 80% oxygen, adjusted to keep an adequate depth of anesthesia (minimal alveolar concentration (MAC) between 0.8 and 1.2, corresponding to an age adjusted sevoflurane expiratory concentration ranging from 1.5% to 2.5%), attested by BIS index values between 40 and 60. Drug administration will be limited to the length of the surgical procedure.

Propofol group:

Anesthesia will be maintained with a TCI device providing an intravenous propofol dose adjusted to keep an adequate depth of anesthesia (TCI values: start after induction with 2.0mcg/ml, then ranging from 3.0mcg/ml to 5.0 mcg/ml), attested by BIS index values between 40 and 60. Patients will be ventilated with 80% oxygen in air. Drug administration will be limited to the length of the surgical procedure.

Identity of Investigational Product(s)

Sevoflurane (test group)
Sevorane®, AbbVie, 250ml bottle

Propofol (control group)
Propofol-®Lipuro 1% (10mg/ml), B.Braun, 50ml glass bottle

8.2 Method of Assigning Subjects to Treatment Groups

Fixed randomization based on a 1:1 allocation ratio will be performed. Balanced treatment allocation will be ensured using block sizes of 4. Patients will be stratified according to their ASA class during the randomization process, which will be performed using a web-based interactive allocation platform (www.randomizer.at).

8.3 Treatment Compliance

8.3.1 Study drugs

Because the attending anesthesiologist will provide the administration of study drugs, treatment compliance is not considered as a potential issue.

8.3.2 Medications susceptible to interfere with outcome measure

8.3.2.1 Opioids

Since opioids have been suggested to inhibit the immune function,[84] per- and postoperative analgesia will be provided following a **standardized protocol**:

- Fentanyl 2mcg/kg (total dose ranging from 5-10mcg/kg) will be used during surgery
- Surgical wound infiltration with local anesthetics will not be allowed
- Morphine intravenous 0.02-0.04mg/kg for a VAS <4 will be used in the PACU
- Basic postoperative analgesia will be provided using paracetamol and non-steroidal anti-inflammatory drugs
- Subcutaneous morphine 0.1-0.2mg/kg will be prescribed as second line analgesic on the ward (reserve medication)

With the aim to obtain high protocol compliance, postoperative analgesics in USZ will be prescribed in the patient electronic chart (KISIM) following a predefined schema saved in the KISIM database. The attending anesthesiologist has access to the database and will upload this schema to the electronic patient chart, regardless of the assignment group.
For patients enrolled at Klinik Hirslanden, postoperative analgesics will be prescribed using a standardized paper form specifically designed for this study.

8.3.2.2 Glucocorticoids

Glucocorticoids are hardly prescribed in the perioperative setting but can be used as rescue treatment for postoperative nausea and vomiting, in conjunction with other antiemetics. Thus, PONV medication will be administered following a protocol strictly avoiding the use of glucocorticoids.

- Ondansetron amp 4mg intravenous 2x/day (reserve medication)
- Metoclopramid amp 10mg intravenous 3x/day (reserve medication)
- Meclozin supp 20mg intrarectal 1-2x/day (reserve medication)

Postoperative PONV medication will be prescribed in the patient electronic chart (KISIM) following a predefined schema saved in the KISIM database. The attending anesthesiologist has access to the database and will upload this schema to the electronic patient chart, regardless of the assignment group.
For patients enrolled at Klinik Hirslanden, postoperative PONV medication will be prescribed using a standardized paper form specifically designed for this study.

8.4 Prior and Concomitant Therapy

Patients being on any systemic immunosuppressive therapy (glucocorticoids, cytostatics, antibodies, drugs acting on immunophilins, interferon, TNF bindings proteins, mycophenolate), as well as patients taking chronically opioids, planned for pre-operative chemo- or radiotherapy, or undergoing surgery with concomitant

regional anesthesia (epidural catheter, paravertebral blockade, wound infiltration with local anesthetics) will not be included, as these factors are known to interfere with the immune system.[84,88,89]
Of note is that patients undergoing *intraoperative* radiotherapy with Intrabeam immediately after removal of the tumor are included. This is a new localized intraoperative "standard of care" therapy in breast cancer.

Long-term medications, such as beta-blockers or COX-inhibitors, as well as perioperative blood transfusion or postoperative opioids, have also been suggested to affect the immune system.[84,87] However, because the benefits of these treatments outweigh their presumable detrimental effect on the immunity, and because strict avoidance of opioids in the postoperative phase would lead to increased pain and distress, these treatments will not be interrupted nor avoided. Opioids will be administrated following a strict protocol mentioned previously (8.4.2.1). Adjustment for residual confounders will be performed at the analytical level.

All concomitant and/or rescue treatment(s) have to be recorded in the eCRF, except for perioperative standard medication such as prophylactic antibiotics or heparin.

8.5 Packaging, Labelling and Supply

Both propofol (Propofol-®Lipuro 1% (10mg/ml), B.Braun) and sevoflurane (Sevorane®, AbbVie) are anesthetics used on a daily base in our Anesthesiology department. These products are provided by the Kantonsapotheke and will be labeled as follows:

FOR CLINICAL TRIAL USE ONLY
CTC_BREAST STUDY

Sponsor: Beck Schimmer Beatrice
Study ID:
Propofol (B.Braun) OR sevoflurane (AbbVie)
Charge:
Expiry Date:
Storage: at room temperature
Patient ID:

Study products will be stored in a storage facility accessible exclusively to members of the study group. The study investigators will be in charge of the resupply. Regarding the volatile anesthesia, a vaporizer will be filled with sevoflurane and stored in the locker. This vaporizer will be used exclusively for study patients and will be stored back in the locker at the end of the anesthesia.

8.6 Blinding

Adequate double-blinding will be performed: patients will not be told about their group assignments, the surgical team and nurses on the ward will not be informed during

the perioperative period about the anesthesia regimen provided, anesthesia records will be placed in sealed opaque envelopes at discharge from PACU and kept in a secure locker in the anesthesia office until the patient is discharged from the hospital. The pathologist responsible for histopathological assessment, the lab staff performing the CTC detection and the research fellow in charge for isolation and culture of NK cells (outcome assessors) will not have any access to the operation theatre or to the patient chart. The attending anesthesiologist responsible for randomization and providing the anesthesia and collecting the first two blood samples cannot *per se* be blinded but will not participate in any data collection or analysis. The study research team collecting the data will not have access to patient allocation until the final analysis.

In situations requiring a breach in the blinding process, such as the occurrence of SAEs, an Emergency Code Break will be available to the investigator. This Code Break should be opened only in emergency situations when the identity of the investigational product must be known by the investigator in order to provide appropriate medical treatment.

8.7 Storage Conditions

Both drugs will be stored in the operation theater, at room temperature, in a storage facility with a secured locker.

8.8 Study Drug Accountability

Investigational product supplies will be kept in a secure, limited access storage area under the recommended storage conditions.

The investigator will maintain accurate and adequate records including dates, lot number, doses given, individual usage, etc.

8.9 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug accountability form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

9. STUDY PROCEDURES

9.1 Blood samples for CTC/NK assessment

Blood samples will be collected before induction of general anesthesia (visit 2), at the end of surgery, before extubation (visit 3), 48h (visit 4) and 72h (visit 5) postoperatively.
The CTC detection procedure will be performed as described by the CellSearch® system manufacturer: two 7.5ml samples (i.e. 15ml) of whole blood will be aseptically collected into the CellSave Preservative tubes and will be transported to the University Hospital Zurich; IFA D LAB 26, Sternwarstr. 14, 8091 Zurich for subsequent analysis within 96 hours of blood collection.
Assessment of NK cells will be performed at the same 4 pre-definite time points, concomitant to CTC determination, using 2x8ml of peripheral venous blood collected in sterile tubes containing sodium heparin. After subsequent transport to the ZKF, the number of NK cells will be performed using flow cytometry, while NK cells activity will be determined *in vitro* by a direct, cell-based cytotoxicity assay against the K562 (human chronic myelogenous leukaemia) cell line with a fluorescent read-out as previously described.[82,83]

Tubes for CTC/NK cells detection will be kept in plastic bags specifically labeled with the visit number. Four bags per patient will be available: 2 for the attending anesthetist (visit 2 and 3) and 2 for the attending nursing staff on the ward (visit 4 and 5). The blood samples will be eliminated after subsequent analysis.

9.2 Study drug administration

Sevoflurane or propofol will be administrated according to patient allocation. Because patients scheduled for breast cancer surgery will need a general anesthesia in any case, regardless of their participation to this study, both drugs will be provided by the UHZ.

9.3 Pregnancy test

Patients of childbearing age scheduled for breast surgery are routinely screened for pregnancy one day prior to surgery. After obtaining written informed consent, the attending anesthetist will look for pregnancy test results in the KISIM database. Considered as a routine prescription at the UHZ, the test should not create additional costs.
Patients of childbearing age at Klinik Hirslanden will be screened for pregnancy at enrollment. The UHZ study team will provide the pregnancy tests and will bear the associated costs.

10. SAFETY

10.1 Safety Variables

Serious adverse events occurring within the two study groups during the course of the study will be recorded. The attending anesthetist, the surgeon and the nursing staff on the ward will be instructed to report any (S)AE to the investigators of the study. A dedicated study nurse will retrospectively review the chart of patients after hospital discharge to identify any unreported (S)AE.

10.2 Definition of (Serious) Adverse Events

Adverse events
Adverse events (AEs) are defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal study product, whether or not related to the medicinal study product. An AE may also consist of a new disease, an exacerbation of a pre-existing illness or condition, a recurrence of an intermittent illness or condition, a set of related signs or symptoms, or a single sign or symptom.

AEs observed by the investigator and/or reported by the subject must referring to the HFG, not be reported in the eCRF -

All AEs, qualifying as an SAE , observed by investigator must be reported in the eCRF. For all SAE`s sufficient information will be pursued and/or obtained so as to permit an adequate determination of the outcome of the event and an assessment of the casual relationship between the SAE and the investigational drug or study treatment(s). The causality assessment of the event to the study drug will be made by investigator and Sponsor-investigator, based on criteria listed in the ICH guidelines

Whenever available, the underlying disease or condition for which a therapeutic or diagnostic procedure is required should be reported as the AE term. Surgeries or other invasive procedures that had already been planned prior to the start of the study do not have to be documented as AEs. These planned procedures will be recorded in the eCRF by the investigator at the baseline visit. It is not important if the condition was known before enrolment, only if the procedure was planned before.

Pregnancy per se does not classify as an AE. However, AEs related to a pregnancy have to be reported like any other AEs. Pregnancy should be confirmed by a reliable laboratory test. Pregnant subjects must be immediately withdrawn from the clinical study. All pregnancies occurring during the treatment phase of the study and within 30 days after discontinuation of study medication have to be reported to the

Investigator-Sponsor within one working day of the investigational sites knowledge of the pregnancy on the Initial Pregnancy Report Form. The Sponsor-Investigator will contact the attendant physician by phone during pregnancy and after the estimated date of delivery to enquire about course and outcome of the pregnancy. Course of the pregnancy and health status of the new born child have to be documented on the Follow-Up Pregnancy Report Form.

Serious adverse event

An SAE is any untoward medical occurrence that at any dose results in

- results in death,
- is life-threatening,
- requires subject hospitalization or prolongation of current hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect,
- any important medical event and any event which, though not included in the above, may jeopardise the subject or may require intervention to prevent one of the outcomes listed above.

Any other medically important condition that may be not immediately life-threatening or results in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the outcomes listed above should also usually (i.e. based on medical and scientific judgment) be considered serious. For example: intensive treatment at home for allergic bronchospasm; certain laboratory abnormalities (e.g. blood dyscrasias); convulsions that do not result in hospitalisation; development of drug dependency or drug abuse.

10.3 Recording of (Serious) Adverse Events

Clinical investigators and ultimately the protocol Principal Investigator (PI) have the primary responsibility for AE identification, documentation, grading, and assignment of attribution to the investigational agent/intervention.

Clinical study subjects will be routinely questioned about AEs at study visits. The well-being of the subjects will be ascertained by neutral questioning ("How are you?"). The investigator is responsible for reporting all AEs occurring during the course of the study.

All observed or volunteered adverse drug events (serious or non-serious) and abnormal test findings, regardless of treatment group or suspected causal relationship to the investigational drug or study treatment(s) will be recorded in the patient file and subsequently in the eCRF.

All SAEs, will be fully documented in the appropriate eCRF. For each SAE, the investigator will provide the onset, duration, intensity, treatment required, outcome and action taken with the investigational product.

The intensity of SAEs will be assessed as being

- mild (hardly noticeable, negligible impairment of well-being),
- moderate (marked discomfort, but tolerable without immediate relief), or
- severe (overwhelming discomfort, calling for immediate relief).

The investigator will determine the relationship of the investigational drug to all AEs as defined on the AE page of the eCRF.

10.4 Assessment of Causatility (Serious) Adverse Events

The assessment by the investigator with regard to the study drug relation is done according to the following definitions:

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

10.5 Reporting of Serious Adverse Events

All SAEs must be reported immediately and within a maximum of 24 hours to the Sponsor-Investigator of the study. The Sponsor-Investigator will re-evaluate the SAE and return the form to the site.

The Sponsor-Investigator is responsible for SAE reporting to the IEC, according to the following details:

- Reporting to IEC any SAE which resulted in death:

  - **without delay**, and no later than **7 calendar days**.

- Reporting to IEC of fatal and life-threatening SAEs if evaluated as “drug related” (SADR):

  - **without delay** and no later than **7 calendar days** following awareness that event meets criteria for an SADR.

- Reporting to IEC of fatal and life-threating SAEs if evaluated as “suspected”, “unexpected” and “drug related” (SUSAR)

  - **without delay** and no later than **7 calendar days** following awareness that event meets criteria for an SUSAR.

  - follow-up information regarding the SUSAR within **further 8 calendar days**.

- Reporting to IEC of non-fatal and not life-threatening SAEs if evaluated as “suspected”, “unexpected” and “drug related” (SUSAR):

  - promptly and no later than **15 calendar days** following awareness that event meets criteria for a SUSAR.

- All other SAEs will be summed up in the **annual safety update report**. The annual safety report contains information from all sites including information from all sites. The Sponsor-Investigator prepares it, and then submits it to the participating Investigators. The participating Investigators submit it to the local committees

The Sponsor-Investigator is responsible for SAE reporting to Swissmedic according to the following details:

- Compliance with the regulatory requirements of Swissmedic regarding prompt reporting of unexpected SAEs for which a causal relationship with the study drug or device cannot be ruled out.

- Reporting to Swissmedic of fatal and life-threatening SAEs if evaluated as “suspected”, “unexpected” and “drug related” (SUSAR):

  - **without delay** and no later than **7 calendar days** following awareness that event
    meets criteria for a SUSAR;

  - follow-up information regarding the SUSAR within **further 8 calendar days**.

- Reporting to Swissmedic of non-fatal and not life-threatening SAEs if evaluated as “suspected”, “unexpected” and “drug related” (SUSARs):

  - **promptly** and no later than **15 calendar days** following awareness that event meets criteria for a SUSAR.

- Sending yearly safety reports, starting one year after the date of notification to Swissmedic. These reports should contain:

  - A concise critical summary of the safety profile of the drug studied as well as the safety issues that have arisen;

  - A listing of all SUSARs that have occurred in Switzerland and at international level (if applicable);

- Ideally all adverse drug reactions at international level.
- The accompanying letter provided with the Annual Safety Report should contain a short summary of the status of the clinical trial in Switzerland (number of centers open/closed, number of patients recruited/recruitment closed, and number of SAR/SUSAR.

An unexpected SAE refers to any AE, the nature or severity of which is not consistent with the applicable product information (see Appendix)

10.6 Follow up of (Serious) Adverse Events

Subjects terminating the study (either regularly or prematurely) with

- reported ongoing SAE, or
- any ongoing SAEs of laboratory values or of vital signs being beyond the alert limit

will return for a follow-up investigation. This visit will take place up to 30 days after terminating the treatment period. Follow-up information on the outcome will be recorded on the respective AE page in the eCRF. All other information has to be documented in the source documents. Source data has to be available upon request.

In case of subjects lost to follow-up, efforts should be made and documented to contact the subject to encourage him/her to continue study participation as scheduled. In case of minor AEs a telephone call to the subject may be acceptable.

All new SAE or pregnancies that the investigators will be notified of within 30 days after discontinuation of study medication have to be reported in appropriate report forms and in the eCRF if required.

Follow-up investigations may also be necessary according to the investigator's medical judgment even if the subject has no AE at the end of the study. However, information related to these investigations does not have to be documented in the eCRF but must be noted in the source documents.

11. DATA QUALITY ASSURANCE

The Sponsor-Investigator is implementing and maintaining quality assurance and quality control systems with written SOPs and Working Instructions to ensure that trials are conducted and data are generated, documented (record), and reported in compliance with the protocol, GCP, and applicable regulatory requirement(s).

Monitoring and Audits will be conducted during the course of the study for quality assurance purposes.

11.1 Routine Monitoring

Regular monitoring visits at the investigator's site prior to the start and during the course of the study will help to follow up the progress of the clinical study, to assure utmost accuracy of the data and to detect possible errors at an early time point. The Sponsor-Investigator organizes professional independent monitoring for the study.

All original data including all patient files, progress notes and copies of laboratory and medical test results must be available for monitoring. The monitor will review all or a part of the eCRFs and written informed consents. The accuracy of the data will be verified by reviewing the above referenced documents.

11.2 Audits and Inspections

A quality assurance audit/inspection of this study may be conducted by the regulatory authority or IEC, respectively. The quality assurance auditor/inspector will have access to all medical records, the investigator's study related files and correspondence, and the informed consent documentation that is relevant to this clinical study.

The investigator will allow the persons being responsible for the audit or the inspection to have access to the source data/documents and to answer any questions arising. All involved parties will keep the patient data strictly confidential.

11.3 Specification of Source Documents

The following documents are considered source data, including but not limited to:

• SAE worksheets

• Nurse records, records of clinical coordinators, and

• Medical records from other department(s), or other hospital(s), or discharge letters and correspondence with other departments/hospitals, if subject visited any during the study period and the post study period.

Source data must be available at the site to document the existence of the study subjects and substantiate the integrity of study data collected. Source data must

include the original documents relating to the study, as well as the medical treatment and medical history of the subject.

The following information (at least but not limited to) should be included in the source documents:

• Demographic data (age, sex)

• Inclusion and Exclusion Criteria details

• Participation in study and signed and dated Informed Consent Forms

• Visit dates

• Medical history and physical examination details

• Key efficacy and safety data (as specified in the protocol)

• AEs and concomitant medication

• Results of relevant examinations

• Laboratory printouts

• Dispensing and return of study drug details

• Reason for premature discontinuation

• Randomization number

12. STATISTICS

12.1 Statistical and Analytical Plans

This is a multicentric randomized controlled trial comparing the effect of 2 different anesthetic regimens (sevoflurane *versus* propofol) on perioperative levels of CTC in patients undergoing surgery for primary breast cancer.
Using a strict 4 time-points schedule, the number of CTC in peripheral blood will be measured before and after exposition to general anesthesia (primary outcome).
Using a mixed model analysis, the effect of two independent variables on the mean level of CTC will be explored: a within-subject time factor (i.e. variation of CTC levels over time) and a between-subject treatment factor (i.e. anesthesia regimen).

Fixed randomization based on a 1:1 allocation ratio will be performed using minimization (www.randomizer.at). Patients will be stratified according to their ASA class during the randomization process, which will be performed using a web-based interactive allocation platform (www.randomizer.at).

Accordingly, the risk of allocation and selection bias will be negligible. Restriction and stratified randomization are likely to minimize the risk of unbalanced known and unknown confounders. Blinding of participants and outcome assessors (laboratory staff, pathologist) will prevent measurement/detection bias.

12.2 Null and Alternative Hypotheses

The null hypothesis: there is no difference in the mean CTC levels over time among patients exposed to volatile anesthesia compared to patients in the control group (propofol anesthesia).
The alternative hypothesis: there is a difference in the mean CTC levels over time among patients exposed to volatile anesthesia compared to patients in the control group (propofol anesthesia).

The primary objective of this study is to reject the null hypothesis H_0.

12.3 Planned Analyses

All primary and secondary analyses will be performed following an *intention-to-treat* (ITT) model. This analysis will be subsequently compared to a *per-protocol* analysis to assess the impact of patients who were not treated as randomized. Results will be reported as OR, 95% confidence intervals and their associated p-values. A p-value <0.05 will be considered statistically significant.
Data analyses will be performed after enrolment of the last patient and completion of the study. No interim analyses are planned.

12.3.1 Primary Analyses

The primary endpoint, i.e. blood CTC levels, is a continuous variable. In our study, 2 independent variables may interact with CTC levels: a time factor, which results from repeated measurements over time (i.e. within-subjects factor) and an intervention factor, which is the anesthesia regimen (between subjects factor). Thus, our primary analysis will be performed using a linear mixed model with random effects matrix to account for within-subjects correlated observations as indicated for longitudinal data analysis.

12.3.2 Secondary Analyses

Since most reports refer to CTC levels as a binary outcome (i.e. positive *versus* negative endpoint using a cut-off value of ≥5 CTC/7.5ml blood), data transformation from a continuous into a binary variable will be performed using the same cut-off value as described previously. This new data set will be analyzed using a logistic regression mixed model with random effects matrix.

Provided our sample size is large enough, adequate randomization is likely to reduce imbalance in baseline characteristics between groups. However, further exploratory analyses will be performed whenever there is a need to control for residual confounding.

12.3.3 Interim Analyses

No interim analyses are planned.

12.4 Handling of Missing Data

Longitudinal data analyses are likely to suffer from error and bias due to missing or incomplete data. In the case of dropouts or incomplete dataset, it remains thus essential to collect a maximal amount of information to determine the nature of missing data (data missing completely at random (MCAR), data missing at random (MAR) or data missing not at random (MNAR)). In case of missing randomness we will consider using multiple imputation for analyses where complete data are needed (linear mixed models can deal with missing data without need for imputation), which is considered superior to a *complete case analysis* (assuming that all data are MCAR or MAR) or a *last observation carried forward* analysis.

In our trial, missing data is not expected to exceed 10% for any variable. This trial will be conducted in a university hospital where clinical research is common practice (trained study nurses, research-orientated infrastructure and care, high standards of quality control). Considerable efforts will be made to ensure high protocol compliance (postoperative medication using a predefined schema provided by the electronic patient database; regular meetings with the anesthesia team to identify potential enrolment or organizational issues).

12.5 Determination of Sample Size

Sample size calculation was performed using a method accounting for a longitudinal design with 2 groups, which allows for repeated measurements and subject attrition. Since most studies investigating perioperative CTC only provide positivity rates rather than numerical levels, we were not able to use these data to estimate our sample size. Therefore, we adopted a conservative approach and assumed that the expected effect size between groups would be small (0.3). This should ensure that our estimation is adequately powered to detect even a small difference between groups. Moreover, we estimated a within-subject correlation over time of 0.4. Thus, to detect a 30% difference between groups, with a power of 80% at a significance level of 5% (two-sided) and assuming a dropout rate of 10% over time, we would need 116 patients in each group (i.e. a total of 232 patients).

13. DATA HANDLING AND RECORD KEEPING

The study will strictly follow the protocol. If any changes become necessary, they must be laid down in an amendment to the protocol. All amendments of the protocol must be signed by the Sponsor-Investigator and submitted to IEC and Swissmedic.

The investigators will use electronic case report forms (eCRF), one for each enrolled study participant, to be filled in with all relevant data pertaining to the subject during the study. All subjects who either entered the study or were considered not-eligible or were eligible but not enrolled into the study additionally have to be documented on a screening log. The investigator will document the participation of each study subject on the Enrollment Log.

For data and query management, monitoring, reporting and coding an internet-based secure data base secuTrial® developed in agreement to the Good Clinical Practice (GCP) guidelines provided by the Clinical Trials Center (CTC) Zurich will be used for this study. It is the responsibility of the investigator to assure that all data in the course of the study will be entered completely and correctly in the respective data base. Corrections in the eCRF may only be done by the investigator or by other authorised persons. In case of corrections the original data entries will be archived in the system and can be made visible. For all data entries and corrections date, time of day and person who is performing the entries will be generated automatically.

eCRFs must be kept current to reflect subject status at each phase during the course of study. Subjects must not to be identified in the eCRF by name. Appropriate coded identification (e.g. Subject Number) must be used.

It must be assured that any authorised person, who may perform data entries and changes in the eCRF, can be identified. A list with signatures and initials of all authorised persons will be filed in the study site file and the trial master file, respectively.

Documented medical histories and narrative statements relative to the subject's progress during the study will be maintained. These records will also include the following: originals or copies of laboratory and other medical test results must be kept on file with the individual subject's eCRF.

The investigators assure to perform a complete and accurate documentation of the subject data in the eCRF. All data entered into the eCRF must also be available in the individual subject file either as print-outs or as notes taken by either the investigator or another responsible person assigned by the investigator.

Essential documents must be retained for at least 10 years after the regular end or a premature termination of the respective study (VKlin Art. 25).

Any patient files and source data must be archived for the longest possible period of time according to the feasibility of the investigational site, e.g. hospital, institution or private practice.

14. CONFIDENTIALITY

The investigators are liable to treat the entire information related to the study and the compiled data strictly confidentially. Any passing-on of information to persons that are not directly involved in the study must be approved by the owner of the information.

Data generation, transmission, archiving and analysis of personal data within this study, strictly follows the current Swiss legal requirements for data protection. Prerequisite is the voluntary approval of the subject given by signing the informed consent prior start of participation of the clinical trial.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files.

Data generated as a result of this study are to be available for inspection on request by the monitors, by the IEC and the regulatory health authorities.

15. INSURANCE

Insurance is covered by “Haftpflichtversicherung für den Kanton Zürich betreffend das UniversitätsSpital Zürich“ (Certificate n°1192 ; insurance Policy n°14.970.888).

Any damage developed in relation to study participation is covered by this insurance. So as not to forfeit their insurance cover, the subjects themselves must strictly follow the instructions of the study personell. Subjects must not be involved in any other medical treatment without permission of the principal investigator (emergency excluded). Medical emergency treatment must be reported immediately to the investigator. The investigator must also be informed instantly, in the event of health problems or other damages during or after the course of study treatment.

The investigator will allow delegates of the insurance company to have access to the source data/documents as necessary to clarify a case of damage related to study participation. All involved parties will keep the patient data strictly confidential.

A copy of the insurance certificate will be placed in the Investigator’s Site File.

16. STUDY REGISTRATION

The study will be registered in the local trial registry of the University Hospital Zürich („Studienregister USZ“), the international ClinicalTrials.gov registry (clinicaltrials.gov) and SNTCP N° 0952

17. PUBLICATION POLICY

After the statistical analysis of this trial the sponsor will make every endeavour to publish the data in a medical journal.
The results of this trial will be published irrespective of its findings. Any form of publication bias including selective outcome reporting will be avoided. Third parties with financial or other support will not have any influence in the decision to publish the results of this trial.

18. FINANCIAL DISCLOSURE

This study will be supported by institutional funds (“Betriebsmittelkredit Beck-Schimmer”).

19. SIGNATURES

Sponsor-Investigator (Principal Investigator):

This clinical trial protocol was subject to critical review and has been approved by the Sponsor-Investigator. The information herein is consistent with
- the current risk/benefit evaluation of the investigational product(s)
- the moral, ethical and scientific principles governing clinical research as set out in the current version of the Declaration of Helsinki, Good Clinical Practice and the respective SAMW guidelines.

Prof. Dr. med. Beatrice Beck Schimmer
Institute of Anesthesiology
University Hospital Zurich
Raemistrasse 100
CH-8091 Zurich
Switzerland

Zürich, 23.03.2017

Place/Date	Signature

Co-Investigator:

Dr. med. Frédérique Hovaguimian
Institute of Anesthesiology
University Hospital Zurich
Raemistrasse 100
CH-8091 Zurich
Switzerland

Place/Date	Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site	Klinik Hirslanden
Principal investigator	Prof. Dr. med. Manfred Seeberger

Zürich, 23.03.2017 _____ _____

Place/Date Signature

Investigator :

med. pract. Urs Rölli
Institute of Anaesthesiology and Intensive Care
Klinik Hirslanden
Wittellikerstr. 40
CH-8032 Zurich
Switzerland

_____ _____

Place/Date Signature

20. REFERENCES

1. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *Int J Cancer.* Jul 3 2012.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer.* Dec 15 2010;127(12):2893-2917.
3. Allemani C, Sant M, Weir HK, et al. Breast cancer survival in the US and Europe: A CONCORD high-resolution study. *Int J Cancer.* Jul 20 2012.
4. Karlsson P, Cole BF, Chua BH, et al. Patterns and risk factors for locoregional failures after mastectomy for breast cancer: an International Breast Cancer Study Group report. *Ann Oncol.* Nov 2012;23(11):2852-2858.
5. Coumans FA, Ligthart ST, Terstappen LW. Interpretation of changes in circulating tumor cell counts. *Transl Oncol.* Dec 2013;5(6):486-491.
6. Lianidou ES, Mavroudis D, Georgoulias V. Clinical challenges in the molecular characterization of circulating tumour cells in breast cancer. *British journal of cancer.* Jun 25 2013;108(12):2426-2432.
7. Zhang L, Riethdorf S, Wu G, et al. Meta-analysis of the prognostic value of circulating tumor cells in breast cancer. *Clin Cancer Res.* Oct 15 2012;18(20):5701-5710.
8. Franken B, de Groot MR, Mastboom WJ, et al. Circulating tumor cells, disease recurrence and survival in newly diagnosed breast cancer. *Breast cancer research : BCR.* 2012;14(5):R133.
9. Lucci A, Hall CS, Lodhi AK, et al. Circulating tumour cells in non-metastatic breast cancer: a prospective study. *Lancet Oncol.* Jul 2012;13(7):688-695.
10. Rack B, Schindlbeck C, Juckstock J, et al. Circulating tumor cells predict survival in early average-to-high risk breast cancer patients. *Journal of the National Cancer Institute.* May 2014;106(5).
11. Faltas B. Cornering metastases: therapeutic targeting of circulating tumor cells and stem cells. *Front Oncol.* 2012;2:68.
12. Gottschalk A, Sharma S, Ford J, Durieux ME, Tiouririne M. Review article: the role of the perioperative period in recurrence after cancer surgery. *Anesthesia and analgesia.* Jun 1 2010;110(6):1636-1643.
13. Banys M, Krawczyk N, Becker S, et al. The influence of removal of primary tumor on incidence and phenotype of circulating tumor cells in primary breast cancer. *Breast cancer research and treatment.* Feb 2012;132(1):121-129.
14. Biggers B, Knox S, Grant M, et al. Circulating tumor cells in patients undergoing surgery for primary breast cancer: preliminary results of a pilot study. *Annals of surgical oncology.* Apr 2009;16(4):969-971.
15. Krag DN, Ashikaga T, Moss TJ, et al. Breast Cancer Cells in the Blood: A Pilot Study. *Breast J.* Nov 1999;5(6):354-358.
16. Sandri MT, Zorzino L, Cassatella MC, et al. Changes in circulating tumor cell detection in patients with localized breast cancer before and after surgery. *Annals of surgical oncology.* Jun 2010;17(6):1539-1545.
17. Hiller J, Brodner G, Gottschalk A. Understanding clinical strategies that may impact tumour growth and metastatic spread at the time of cancer surgery. *Best practice & research. Clinical anaesthesiology.* Dec 2013;27(4):427-439.
18. Tavare AN, Perry NJ, Benzonana LL, Takata M, Ma D. Cancer recurrence after surgery: direct and indirect effects of anesthetic agents. *Int J Cancer.* Mar 15 2011;130(6):1237-1250.

19. Cata JP, Kurz A. Challenges in research related to perioperative cancer care and cancer outcomes. *Best practice & research. Clinical anaesthesiology.* Dec 2013;27(4):457-464.
20. Niwa H, Rowbotham DJ, Lambert DG, Buggy DJ. Can anesthetic techniques or drugs affect cancer recurrence in patients undergoing cancer surgery? *Journal of anesthesia.* Oct 2013;27(5):731-741.
21. Bidard FC, Fehm T, Ignatiadis M, et al. Clinical application of circulating tumor cells in breast cancer: overview of the current interventional trials. *Cancer metastasis reviews.* Jun 2013;32(1-2):179-188.
22. Cui WY, Liu Y, Zhu YQ, Song T, Wang QS. Propofol induces endoplasmic reticulum (ER) stress and apoptosis in lung cancer cell H460. *Tumour Biology.* 2014;35(6):5213-5217.
23. Du QH, Xu YB, Zhang MY, Yun P, He CY. Propofol induces apoptosis and increases gemcitabine sensitivity in pancreatic cancer cells in vitro by inhibition of nuclear factor-B activity. *World Journal of Gastroenterology.* 2013;19(33):5485-5492.
24. Ecimovic P, McHugh B, Murray D, Doran P, Buggy DJ. Effects of sevoflurane on breast cancer cell function in vitro. *Anticancer research.* Oct 2013;33(10):4255-4260.
25. Ecimovic P, Murray D, Doran P, Buggy DJ. Propofol and bupivacaine in breast cancer cell function in vitro - role of the NET1 gene. *Anticancer research.* Mar 2014;34(3):1321-1331.
26. Garib V, Lang K, Niggemann B, Zanker KS, Brandt L, Dittmar T. Propofol-induced calcium signalling and actin reorganization within breast carcinoma cells. *European Journal of Anaesthesiology.* 2005;22(8):609-615.
27. Huang H, Benzonana LL, Zhao H, et al. Prostate cancer cell malignancy via modulation of HIF-1alpha pathway with isoflurane and propofol alone and in combination. *British journal of cancer.* Sep 23 2014;111(7):1338-1349.
28. Jun R, Gui-he Z, Xing-xing S, Hui Z, Li-xian X. Isoflurane enhances malignancy of head and neck squamous cell carcinoma cell lines: a preliminary study in vitro. *Oral Oncology.* 2011;47(5):329-333.
29. Kawaraguchi Y, Horikawa YT, Murphy AN, et al. Volatile anesthetics protect cancer cells against tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis via caveolins. *Anesthesiology.* 2011;115(3):499-508.
30. Kushida A, Inada T, Shingu K. Enhancement of antitumor immunity after propofol treatment in mice. *Immunopharmacology & Immunotoxicology.* 2007;29(3-4):477-486.
31. Kvolik S, Dobrosevic B, Marczi S, Prlic L, Glavas-Obrovac L. Different apoptosis ratios and gene expressions in two human cell lines after sevoflurane anaesthesia. *Acta anaesthesiologica Scandinavica.* Oct 2009;53(9):1192-1199.
32. Kvolik S, Glavas-Obrovac L, Bares V, Karner I. Effects of inhalation anesthetics halothane, sevoflurane, and isoflurane on human cell lines. *Life Sciences.* 2005;77(19):2369-2383.
33. Li Q, Zhang L, Han Y, Jiang Z, Wang Q. Propofol reduces MMPs expression by inhibiting NF-B activity in human MDA-MB-231 cells. *Biomedicine & Pharmacotherapy.* 2012;66(1):52-56.
34. Liang H, Wang HB, Liu HZ, Wen XJ, Zhou QL, Yang CX. The effects of combined treatment with sevoflurane and cisplatin on growth and invasion of

human adenocarcinoma cell line A549. *Biomedicine & Pharmacotherapy.* 2013;67(6):503-509.
35. 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. *Anesthesia & Analgesia.* 2003;97(5):1331-1339.
36. Miao Y, Zhang Y, Wan H, Chen L, Wang F. GABA-receptor agonist, propofol inhibits invasion of colon carcinoma cells. *Biomedicine & Pharmacotherapy.* 2010;64(9):583-588.
37. Wang P, Chen J, Mu LH, Du QH, Niu XH, Zhang MY. Propofol inhibits invasion and enhances paclitaxel- induced apoptosis in ovarian cancer cells through the suppression of the transcription factor slug. *European Review for Medical & Pharmacological Sciences.* 2013;17(13):1722-1729.
38. Wu KC, Yang ST, Hsia TC, et al. Suppression of cell invasion and migration by propofol are involved in down-regulating matrix metalloproteinase-2 and p38 MAPK signaling in A549 human lung adenocarcinoma epithelial cells. *Anticancer Research.* 2012;32(11):4833-4842.
39. Xu YB, Du QH, Zhang MY, Yun P, He CY. Propofol suppresses proliferation, invasion and angiogenesis by down-regulating ERK-VEGF/MMP-9 signaling in Eca-109 esophageal squamous cell carcinoma cells. *European Review for Medical & Pharmacological Sciences.* 2013;17(18):2486-2494.
40. Zhang L, Wang N, Zhou S, Ye W, Jing G, Zhang M. Propofol induces proliferation and invasion of gallbladder cancer cells through activation of Nrf2. *Journal of Experimental & Clinical Cancer Research.* 2012;31:66.
41. Zhang LM, Zhang MX, Wang N, et al. [Effect of low dose propofol on biological behavior of esophageal squamous cell carcinoma cell line Eca109]. *Xibao Yu Fenzi Mianyixue Zazhi.* 2011;27(4):402-404.
42. Bentley MW, Stas JM, Johnson JM, Viet BC, Garrett N. Effects of preincisional ketamine treatment on natural killer cell activity and postoperative pain management after oral maxillofacial surgery. *AANA Journal.* 2005;73(6):427-436.
43. Brand JM, Frohn C, Luhm J, Kirchner H, Schmucker P. Early alterations in the number of circulating lymphocyte subpopulations and enhanced proinflammatory immune response during opioid-based general anesthesia. *Shock.* 2003;20(3):213-217.
44. Brand JM, Kirchner H, Poppe C, Schmucker P. The effects of general anesthesia on human peripheral immune cell distribution and cytokine production. *Clinical Immunology & Immunopathology.* 1997;83(2):190-194.
45. Kutza J, Gratz I, Afshar M, Murasko DM. The effects of general anesthesia and surgery on basal and interferon stimulated natural killer cell activity of humans. *Anesthesia & Analgesia.* 1997;85(4):918-923.
46. 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(4):700-706.
47. Miyata T, Kodama T, Honma R, et al. Influence of general anesthesia with isoflurane following propofol-induction on natural killer cell cytotoxic activities of peripheral blood lymphocytes in dogs. *Journal of Veterinary Medical Science.* 2013;75(7):917-921.

48. Pirttikangas CO, Perttila J, Salo M, Vainio O, Liukko-Sipi S. Propofol infusion anaesthesia and immune response in minor surgery. *Anaesthesia.* 1994;49(1):13-16.
49. Richardson PS, Cooke K, Gerrish P, Rees RC, Rennie IG. Natural killer and lymphokine-activated cytotoxicity following anaesthesia in patients with uveal malignant melanoma. *Melanoma Research.* 1997;7(2):129-137.
50. Zancla ME, Rotolo M, Patti R, et al. [Cytotoxic activity of natural killer cells during balanced anesthesia with isoflurane versus total intravenous anesthesia]. *Minerva Anestesiologica.* 1991;57(9):797-799.
51. Zhang T, Fan Y, Liu K, Wang Y. Effects of different general anaesthetic techniques on immune responses in patients undergoing surgery for tongue cancer. *Anaesthesia & Intensive Care.* 2014;42(2):220-227.
52. Biki B, Mascha E, Moriarty DC, Fitzpatrick JM, Sessler DI, Buggy DJ. Anesthetic technique for radical prostatectomy surgery affects cancer recurrence: a retrospective analysis. *Anesthesiology.* Aug 2008;109(2):180-187.
53. Christopherson R, James KE, Tableman M, Marshall P, Johnson FE. Long-term survival after colon cancer surgery: a variation associated with choice of anesthesia. *Anesthesia and analgesia.* Jul 2008;107(1):325-332.
54. Cummings KC, 3rd, Xu F, Cummings LC, Cooper GS. A comparison of epidural analgesia and traditional pain management effects on survival and cancer recurrence after colectomy: a population-based study. *Anesthesiology.* Apr 2012;116(4):797-806.
55. Day A, Smith R, Jourdan I, Fawcett W, Scott M, Rockall T. Retrospective analysis of the effect of postoperative analgesia on survival in patients after laparoscopic resection of colorectal cancer. *British journal of anaesthesia.* Aug 2012;109(2):185-190.
56. de Oliveira GS, Jr., Ahmad S, Schink JC, Singh DK, Fitzgerald PC, McCarthy RJ. Intraoperative neuraxial anesthesia but not postoperative neuraxial analgesia is associated with increased relapse-free survival in ovarian cancer patients after primary cytoreductive surgery. *Regional anesthesia and pain medicine.* May-Jun 2011;36(3):271-277.
57. Enlund M, Berglund A, Andreasson K, Cicek C, Enlund A, Bergkvist L. The choice of anaesthetic--sevoflurane or propofol--and outcome from cancer surgery: a retrospective analysis. *Upsala journal of medical sciences.* Aug 2014;119(3):251-261.
58. Exadaktylos AK, Buggy DJ, Moriarty DC, Mascha E, Sessler DI. Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? *Anesthesiology.* Oct 2006;105(4):660-664.
59. Forget P, Machiels JP, Coulie PG, et al. Neutrophil:lymphocyte ratio and intraoperative use of ketorolac or diclofenac are prognostic factors in different cohorts of patients undergoing breast, lung, and kidney cancer surgery. *Annals of surgical oncology.* Dec 2013;20 Suppl 3:S650-660.
60. Forget P, Vandenhende J, Berliere M, et al. Do intraoperative analgesics influence breast cancer recurrence after mastectomy? A retrospective analysis. *Anesthesia and analgesia.* Jun 1 2010;110(6):1630-1635.
61. Gauthier T, Mollard J, Fermeaux V, Kapella M, Aubard Y. Axillary recurrence after negative sentinel lymph node biopsy under local anesthesia in breast cancer. *European journal of surgical oncology : the journal of the European*

Society of Surgical Oncology and the British Association of Surgical Oncology. May 2009;35(5):464-468.
62. Gottschalk A, Brodner G, Van Aken HK, Ellger B, Althaus S, Schulze HJ. Can regional anaesthesia for lymph-node dissection improve the prognosis in malignant melanoma? *British journal of anaesthesia.* Aug 2012;109(2):253-259.
63. Gottschalk A, Ford JG, Regelin CC, et al. Association between epidural analgesia and cancer recurrence after colorectal cancer surgery. *Anesthesiology.* Jul 2010;113(1):27-34.
64. Gupta A, Bjornsson A, Fredriksson M, Hallbook O, Eintrei C. Reduction in mortality after epidural anaesthesia and analgesia in patients undergoing rectal but not colonic cancer surgery: a retrospective analysis of data from 655 patients in central Sweden. *British journal of anaesthesia.* Aug 2011;107(2):164-170.
65. Ismail H, Ho KM, Narayan K, Kondalsamy-Chennakesavan S. Effect of neuraxial anaesthesia on tumour progression in cervical cancer patients treated with brachytherapy: a retrospective cohort study. *British journal of anaesthesia.* Aug 2010;105(2):145-149.
66. Lacassie HJ, Cartagena J, Branes J, Assel M, Echevarria GC. The relationship between neuraxial anesthesia and advanced ovarian cancer-related outcomes in the Chilean population. *Anesthesia and analgesia.* Sep 2013;117(3):653-660.
67. Lai R, Peng Z, Chen D, et al. The effects of anesthetic technique on cancer recurrence in percutaneous radiofrequency ablation of small hepatocellular carcinoma. *Anesthesia and analgesia.* Feb 2012;114(2):290-296.
68. Lin L, Liu C, Tan H, Ouyang H, Zhang Y, Zeng W. Anaesthetic technique may affect prognosis for ovarian serous adenocarcinoma: a retrospective analysis. *British journal of anaesthesia.* Jun 2011;106(6):814-822.
69. Myles PS, Peyton P, Silbert B, et al. Perioperative epidural analgesia for major abdominal surgery for cancer and recurrence-free survival: randomised trial. *Bmj.* 2011;342:d1491.
70. Retsky M, Rogers R, Demicheli R, et al. NSAID analgesic ketorolac used perioperatively may suppress early breast cancer relapse: particular relevance to triple negative subgroup. *Breast cancer research and treatment.* Jul 2012;134(2):881-888.
71. Sofra M, Fei PC, Fabrizi L, et al. Immunomodulatory effects of total intravenous and balanced inhalation anesthesia in patients with bladder cancer undergoing elective radical cystectomy: preliminary results. *J Exp Clin Cancer Res.* 2013;32:6.
72. Tsui BC, Rashiq S, Schopflocher D, et al. Epidural anesthesia and cancer recurrence rates after radical prostatectomy. *Canadian journal of anaesthesia = Journal canadien d'anesthesie.* Feb 2010;57(2):107-112.
73. Wuethrich PY, Hsu Schmitz SF, Kessler TM, et al. Potential influence of the anesthetic technique used during open radical prostatectomy on prostate cancer-related outcome: a retrospective study. *Anesthesiology.* Sep 2010;113(3):570-576.
74. Yang Q, Goding SR, Hokland ME, Basse PH. Antitumor activity of NK cells. *Immunol Res.* 2006;36(1-3):13-25.
75. Zamai L, Ponti C, Mirandola P, et al. NK cells and cancer. *J Immunol.* Apr 1 2007;178(7):4011-4016.

76. Ben-Eliyahu S, Page GG, Yirmiya R, Shakhar G. Evidence that stress and surgical interventions promote tumor development by suppressing natural killer cell activity. *Int J Cancer.* Mar 15 1999;80(6):880-888.
77. Koda K, Saito N, Takiguchi N, Oda K, Nunomura M, Nakajima N. Preoperative natural killer cell activity: correlation with distant metastases in curatively research colorectal carcinomas. *Int Surg.* Apr-Jun 1997;82(2):190-193.
78. Mirza AN, Mirza NQ, Vlastos G, Singletary SE. Prognostic factors in node-negative breast cancer: a review of studies with sample size more than 200 and follow-up more than 5 years. *Ann Surg.* Jan 2002;235(1):10-26.
79. Dobi E, Bazan F, Dufresne A, et al. Is extracapsular tumour spread a prognostic factor in patients with early breast cancer? *Int J Clin Oncol.* Jul 5 2012.
80. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med.* Aug 19 2004;351(8):781-791.
81. Hayes DF, Cristofanilli M, Budd GT, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res.* Jul 15 2006;12(14 Pt 1):4218-4224.
82. Kane KL, Ashton FA, Schmitz JL, Folds JD. Determination of natural killer cell function by flow cytometry. *Clin Diagn Lab Immunol.* May 1996;3(3):295-300.
83. Valiathan R, Lewis JE, Melillo AB, Leonard S, Ali KH, Asthana D. Evaluation of a flow cytometry-based assay for natural killer cell activity in clinical settings. *Scand J Immunol.* Apr 2012;75(4):455-462.
84. Afsharimani B, Cabot P, Parat MO. Morphine and tumor growth and metastasis. *Cancer metastasis reviews.* Jun 2011;30(2):225-238.
85. Ben-Eliyahu S, Shakhar G, Shakhar K, Melamed R. Timing within the oestrous cycle modulates adrenergic suppression of NK activity and resistance to metastasis: possible clinical implications. *British journal of cancer.* Dec 2000;83(12):1747-1754.
86. Kurz A, Sessler DI, Lenhardt R. Perioperative normothermia to reduce the incidence of surgical-wound infection and shorten hospitalization. Study of Wound Infection and Temperature Group. *N Engl J Med.* May 9 1996;334(19):1209-1215.
87. Melamed R, Rosenne E, Shakhar K, Schwartz Y, Abudarham N, Ben-Eliyahu S. Marginating pulmonary-NK activity and resistance to experimental tumor metastasis: suppression by surgery and the prophylactic use of a beta-adrenergic antagonist and a prostaglandin synthesis inhibitor. *Brain Behav Immun.* Mar 2005;19(2):114-126.
88. Buggy DJ, Smith G. Epidural anaesthesia and analgesia: better outcome after major surgery?. Growing evidence suggests so. *Bmj.* Aug 28 1999;319(7209):530-531.
89. Wada H, Seki S, Takahashi T, et al. Combined spinal and general anesthesia attenuates liver metastasis by preserving TH1/TH2 cytokine balance. *Anesthesiology.* Mar 2007;106(3):499-506.