Effects of Mode of Anaesthesia on Circulating Tumour Cells in Patients Undergoing Inhalational versus Total Intravenous Anaesthesia for Hepatocellular Carcinoma Surgery

A Randomised Controlled Trial.

Introduction

More than 80% of patients with cancer will be exposed to anaesthesia at some point in their treatment. There is increasing evidence that perioperative events, including the type of anaesthesia drugs utilised, have an impact on cancer recurrence and metastases. Although potentially and theoretically curative, surgical resection, manipulation and trauma may disseminate tumour cells and reduce immunity.

There have been a number of suggestions as to why cancer may be, paradoxically, worsened by surgery and what methods may be used to mitigate this. One of these is propofol based total intravenous anaesthesia (TIVA), whereby the traditional inhalational anaesthetic drugs are avoided. Commonly used inhalational drugs, such as sevoflurane and desflurane, are pro-inflammatory. Propofol, however, has anti-inflammatory and anti-oxidative properties, induces apoptosis and has specific inhibitory effects on tumour cell growth in vitro. Laboratory investigations, animal models, retrospective clinical studies and initial clinical research are producing evidence that inhalational anaesthesia facilitates tumour recurrence and metastasis, whilst TIVA can prolong survival.

This randomised, controlled trial will look at the effects on DNA damage and biomarkers of immunity and inflammation of inhalational anaesthesia versus TIVA in patients undergoing surgery for hepatocellular carcinoma, a common tumour in the Southern Chinese population, for whom surgery is potentially-curative. It will focus on subjects undergoing open hepatectomy and investigate changes in biomarkers of inflammation, immunity and gene expression from the patients' blood samples taken before, during and after surgery. Patients will also be followed-up for cancer recurrence, morbidity and five-year mortality. Results could represent a breakthrough in knowledge of how anaesthetic agents impact the results of cancer surgery, and have important implications for a more disease-sensitive approach to improving management and outcomes in these patients.

Aims and Objectives

The aim is to investigate the effect of sevoflurane inhalational versus propofol intravenous anaesthesia on expression of hypoxia-inducible factor 1 (HIF-1), circulating tumour cells, DNA damage and biomarkers of immunity and inflammation in patients with hepatocellular carcinoma undergoing open hepatectomy.

Aim 1: To investigate the changes in gene expression and plasma concentrations of inflammatory biomarkers (HIF-1, IL-6, and TNF-alpha) induced by surgery and the effects of different methods of anaesthesia on this expression.

Aim 2: To investigate the changes in DNA damage induced by surgery and the effects of these two different methods of anaesthesia on this damage.

Aim 3: To investigate changes in circulating tumour cells pre, intra and post-surgery

Aim 4: To follow-up patients post tumour resection for three-year survival and investigate differences in survival between the TIVA and sevoflurane cohorts.

Aim 5: To investigate the difference in post-operative pain and post-operative morphine consumption in patients anaesthetized with propofol versus sevoflurane.

Background of Research

Cancer remains a leading cause of morbidity and mortality, despite continued advances in management and treatment strategies. In 2014, 'malignant neoplasms' was the leading cause of death for both sexes in Hong Kong, accounting for 30.2% of all registered deaths [1]. Of these, liver cancer, defined as malignant neoplasm of the liver and intrahepatic ducts, has been the third leading cause of cancer death in Hong Kong for many years. In 2014, 1,585 patients died from liver cancer, accounting for 11.5% of all cancer deaths [2]. In general, the age-standardized death rate due to liver cancer was higher in males than females, with a decreasing trend in age-standardized death rates in recent years [2]. With the ageing population and the rising incidence of cancer diagnoses, anaesthesiologists are frequently managing patients with cancer in their daily practice.

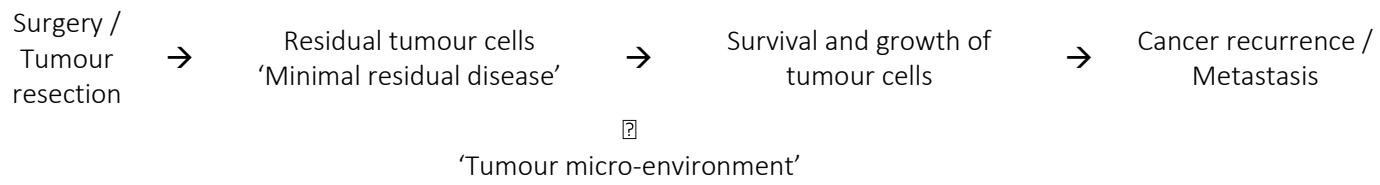
Surgical resection is the main component of treatment for most primary cancers. Primary excision of solid tumours can be curative on its own, or may be accompanied by neoadjuvant or adjuvant chemotherapy or radiotherapy. In hepatocellular carcinoma, the most common type of liver cancer, partial hepatectomy can be potentially curative and is the optimal treatment for patients with adequate liver functional reserve [3]. These include patients who meet the following criteria: a solitary hepatocellular carcinoma confined to the liver that shows no radiographic evidence of invasion of the hepatobiliary vasculature, no evidence of portal hypertension, and well-preserved liver function. Long-term relapse-free survival rates average at 40% or higher in these patients.

Despite significant advances in oncological therapies, post-operative loco-regional recurrence and metastases still remains a leading cause of morbidity and mortality. In recent years, there has been growing interest in the pathophysiological events in the perioperative period that lead to cancer recurrence and metastases, with increasing research showing that anaesthetic and analgesic techniques play a potential role in disease recurrence and progression [4, 5].

Tumour cells are genetically unstable cells that have undergone mutations, making them refractory to regulated cell division, resulting in uncontrolled cellular proliferation. Further steps include evading the host's immune system, ensuring an independent blood supply through angiogenesis, and invasion of surrounding tissues. Metastasis is a

complex process, beginning with the detachment and migration of cancerous cells from the primary tumour, entering of the circulation and evading host immune surveillance, and ending with metastatic proliferation in a separate organ [6]. This deeply intricate process is dependent on a myriad of processes and interactions between the tumour and the host, as well as the metastatic potential of the malignant cells. Successful metastasis requires a suitable 'tumour micro-environment', which consists of surrounding vasculature, inflammatory mediators, growth factors, immune cells and mesothelium, which promote tumour growth and survival.

It is well established that cell-mediated immunity is the initial defence mechanism against invading tumour cells [7]. Cell-mediated immunity is comprised of two types of immune response: the innate, which is a nonspecific system not requiring prior sensitisation, and the adaptive immune responses, which is antigen-specific immune cell mediated. Together, they detect and destroy the presence of tumour cells before cancer becomes clinically detectable [7]. The main cell components include natural killer (NK) cells, cytotoxic T cells, mononuclear cells and dendritic cells. Of these, NK cell activity has been associated with a reduced incidence of cancer, and cytotoxic T cells have also been shown to play an important role in anti-tumour activity.



Many studies have explored the perioperative factors that could potentially influence the process of cancer recurrence and metastasis.

Surgery itself can promote tumour growth and metastasis [8, 9], which has been demonstrated in animal models. For example, laparotomies in mouse models increased the number of liver metastases from ~15 to 34. Proposed mechanisms include the following:

- Angiogenesis

  After surgery, many pro-angiogenetic factors from the tumour are increased, including hypoxia-inducible factors (HIF), vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF- β) [9].

Increased postoperative levels of VEGF were seen in a mouse model after the stress of surgeries including laparotomies and mastectomies, and were also demonstrated in patients undergoing mastectomy for breast cancer, increasing from 806 pg/ml to 1385 pg/ml. Surgery causes the release of TGF- β, which plays a significant role in establishing tumour blood supply and cell proliferation [4].

Angiogenesis is counteracted by anti-angiogenic factors such as angiostatins and endostatins, both of which significantly reduced lung cancer metastasis in a mouse model. Surgery augments angiogenesis by decreasing plasma levels of these factors.

- Liberation of tumour cells

  Surgical manipulation can inadvertently cause dispersal of tumour emboli. In one study analysing peripheral blood and peritoneal fluid in patients undergoing surgery for colorectal cancer, some patients with no detectable tumour cells pre-operation were found to have detectable cells post-surgery, suggesting that surgery had dislodged tumour cells from the primary tumour. Patients with detectable tumour cells in the blood/peritoneal fluid samples post-operatively also had a significantly shorter disease-free survival (43.9 months) compared with patients with none (80.5 months) [5].

- Stress response and suppression of immunity

  Surgical trauma stimulates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, resulting in the release of catecholamines and prostaglandins. This neuroendocrine stress creates a cytokine 'stress' response', which induces a temporary suppression of cell-immunity in the perioperative timing – which is the crucial period that may determine whether metastasis will be established or eradicated [8]. In particular, NK cell activity has been suppressed in experimental and clinical studies.

Even in surgical resections with histologically-negative margins, some tumours cells will remain postoperatively – which, together with micrometastatic deposits, are termed 'minimal residual disease'. Minimal residual disease is defined as clinically undetectable cancer cells that remain despite the surgical removal of macroscopic tumour. It is believed that the interval between the immediate postoperative period and the initiation of supplemental therapeutic treatment coincides with minimal residual disease metastasis, and the persistent presence of tumour cells in the circulation 24 hours after resection is an independent risk factor for recurrence, with the outcome of these cells dependent on the perioperative immune surveillance in the host. As a result, potentially curative surgical resection

may paradoxically create a window of susceptibility, with the perioperative period representing the highest risk for neoplastic metastasis during the course of cancer treatment.

What role does anaesthesia play during this critical period? There has been research exploring the effect of anaesthetic technique on cancer recurrence, raising valid concerns regarding the role of anaesthetic and analgesic techniques in causing regional recurrences and metastases. Data from laboratory and animal experiments suggests that anaesthesia choice may contribute significantly to the pro-tumour environment and affect long-term oncological outcomes after surgery. Certain anaesthetic agents may contribute to post-operative immunosuppression, or even directly interfere with cancer cell biology, influencing cancer recurrence.

Anaesthetic agents are powerful pharmacological tools capable of exerting diverse and potent effects on numerous cellular and organ functions. There are two main classes of drugs used in the maintenance of general anaesthesia – inhaled anaesthetic agents and intravenous anaesthesia. The more commonly used are inhaled anaesthetic agents, also known as “volatile gases”, which are halogen-containing hydrocarbons. Propofol is the most suitable intravenous agent and is administered in combination with an opioid infusion.

Proposed mechanisms by which anaesthetic agents promote or protect against metastasis are compared in the following table:

<table>
<tr><th><u>Inhalational anaesthetic agents</u></th><th><u>Intravenous anaesthetic agents – Propofol</u></th></tr>
<tr><td colspan="2">- Hypoxia-inducible-factors (HIF)</td></tr>
<tr><td colspan="2">HIFs are transcription factors that govern the adaptive response to hypoxia, regulating a variety of genes that control proliferation, angiogenesis and metabolism. Though the HIF system is crucial in the survival of healthy cells, it is also used by cancer cells as a means of promoting their own survival. Many of the genes regulated by HIF-1α and HIF-2α have been shown to have important roles in tumorigenesis and metastasis. Collectively, these genes directly link HIFs to more aggressive phenotypes and poorer clinical prognosis [15].
Clinical evidence for the involvement of HIFs in cancer is extensive. Studies have detected high levels of HIF-1α and HIF-2α in primary tumours and metastases, and a positive relationship between levels of HIF and poor prognosis have been demonstrated in clinical studies of gastric, colorectal, breast, ovarian and hepatocellular carcinomas.</td></tr>
<tr><td>Volatile agents lead to upregulation of hypoxia-inducible-factors (HIF) in tumour cells, which are postulated to stimulate cytoprotective or protumourgenic behaviour in residual cells.
- Isoflurane increases HIF-1alpha and its downstream effectors to promote renal cell carcinoma survival in vitro [16].</td><td>Propofol suppresses HIF-1α protein synthesis in an oxygen tenxion-dependent manner. The most likely mechanism behind propofol’s inhibitory effect on HIF-1 is via impaired translation of mRNA.
- Propofol inhibits HIFs in prostate cancer [17].</td></tr>
<tr><td colspan="2">- Cell-mediated immunity</td></tr>
<tr><td>Volatile agents have been shown to have immunosuppressive effects.
- Isoflurane and halothane inhibit interferon-induced NK cell induction (>90% and 67% respectively) in a mice model [10].</td><td>Propofol facilitates peri-operative immune function
- Propofol aids the preservation of NK cell activity.
- It promotes cytotoxic T cells activity and inhibits lymphoma tumour growth in mice receiving propofol [14].</td></tr>
</table>

- Isoflurane and sevoflurane induce apoptosis in human cytotoxic T cells *in vitro* in a dose-dependent manner [11] - Sevoflurane binds lymphocyte antigens interfering with their activity [12]. - Isoflurane protects cancer cells from tumour necrosis factor (TNF)-induced apoptosis [13].	
- Gene Expression	- Matrix metalloproteinases
Inhaled anaesthetic agents may exert effects on tumour cell gene expression. The propensity for hypoxia in solid malignancies drives a 'genetic switch', which upregulates target genes for a pro-tumour microenvironment, stimulating neutrophil and macrophage infiltration to release cytokines, angiogenic factors and degradation of adhesion molecules to facilitate proliferation and migration of cancer cells. - A study showed that volatile anaesthetic agents could have profound time-dependent effects on gene expression in *ex-vivo* breast and brain tumour cell cultures [18]. - Nitrous oxide has numerous immune-modulating effects; it impairs DNA and purine synthesis via interaction with vitamin B12 and inactivation of methionine synthase. In turn, impaired DNA synthesis limits cell-production and may cause bone-marrow depression.	Propofol decreases the protein expression and activity of matrix metalloproteinases (MMPs). MMPs are proteinase enzymes that enable tumour cells to penetrate the basement membrane layer by degrading the extracellular matrix, which serves as a biological and mechanical barrier to cell movement. MMPs also release growth factors, which work to remodel the extracellular matrix. This mechanism was shown in a study in which Propofol inhibited the invasion of colon carcinoma cells [19].

There have been studies looking at the survival rates for patients undergoing inhalational versus intravenous anaesthesia for cancer surgery. In a retrospective analysis that compared mortality post-cancer surgery in over 7000 patients, mortality was approximately 50% greater with inhalational than with IV anaesthesia, with an adjusted hazard

ratio of 1.46 [20]. There has also been research into the effects of propofol, which has been shown to inhibit the proliferation and migration of oesophageal cancer [21], and also induce apoptosis in non-small cell lung cancer, colon cancer, and ovarian cancer cells.

Many other studies have also looked at other anaesthetic techniques and agents, which will be briefly mentioned below:

- Ketamine and Thiopental significantly reduced NK cell activity and increased the number of retained tumour cells found at autopsy in experiments in rats inoculated with mammary adenocarcinomas. Ketamine in particular had the strongest impact, promoting tumour retention and metastasis more than 2.5 fold.
- Opioids are frequently used in the treatment of oncology patients, both in intraoperative and postoperative settings. Both acute and chronic opioid therapies have been shown to have immunomodulating effects. Conflicting results have been published, with opioids shown to promote and inhibit cancer metastasis in different studies.

  Opioids inhibit cellular and humoral immune function in humans. They suppress NK cell activity, phagocyte function, as well as cytokine and antibody production. Opioids have been shown to increase angiogenesis and promote breast tumour growth in rat models. However, opioids may also reduce the stress response to peri-operative pain, protecting against stress-induced reduction in immunity.
- Regional Anaesthesia. Intense research has been focused in this area, with multiple small studies comparing the effects of general and regional anaesthesia on cancer recurrence with variable outcomes. One postulated mechanism is that RA lowers plasma levels of cortisol and catecholamines during surgical trauma, reducing the immunosuppression caused by surgical stimulation.

In summary, cancer and in particular hepatocellular cell carcinoma is a leading cause of death in Hong Kong. Though surgical resection can be a potentially curative treatment option, patients are still largely susceptible to cancer recurrence and metastasis. There is growing interest in the factors that affect this susceptibility, particularly in the perioperative period – factors including anaesthetic techniques. Can anaesthesiology affect cancer oncological outcomes? The scientific literature cannot yet offer a definite strategy for optimal anaesthetic management in cancer patients. However, the search for the 'anti-cancer anaesthetic' continues, with a need for large, carefully controlled,

prospective trials to attest causation. Though any benefit of one particular anaesthetic technique over another in terms of cancer recurrence is likely to be small, nonetheless, when scaling up to incorporate the growing numbers of patients undergoing cancer surgery, even fairly simple changes in technique could save many lives.

Research Plan and Methodology

All patients aged 18-80 years old with primary hepatocellular carcinoma presenting for elective open hepatectomy surgery in Queen Mary Hospital, Hong Kong, will be invited to join this randomised, controlled trial.

Disease eligibility for partial hepatectomy will be determined according to established surgical criteria. The ideal patient for resection has a solitary hepatocellular carcinoma confined to the liver that shows no radiographic evidence of invasion of the hepatic vasculature, no evidence of portal hypertension, and well-preserved hepatic function. There is no general rule regarding tumour size for selection of patients for resection.

Two main systems are used to stage the disease:

- The American Joint Committee on Cancer (AJCC)/ Union for International Cancer Control (UICC) TNM staging for hepatocellular carcinoma. Surgeons usually consider Stage IIIB or above to be unresectable, which includes:
    - Single / multiple tumours of any size involving a major branch of the portal vein or hepatic vein
    - Tumours with direct invasion of adjacent organs other than the gallbladder, or with perforation of visceral peritoneum
    - Regional lymph node metastasis
    - Distant metastasis
- Child-Pugh classification of severity of cirrhosis – surgical resection is most safely performed in those with class A disease (scoring 5 to 6 points according to the table below), indicating normal bilirubin and well preserved liver function.

Parameters	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Hepatic encephalopathy	None	Grade 1-2	Grade 3-4
Albumin	>35 g/L	28-35 g/L	<28 g/L
Bilirubin	<34.2 mmol/L	34.2-51.3 mmol/L	>51.3 mmol/L
INR	<1.7	1.7-2.3	>2.3

The nature of the study will be explained to all patients by the attending anaesthetist or a research nurse, and they will be given an information sheet to read and decide on whether to participate. All subjects will be asked to sign informed consent. The usual practice in our institution is that the patient will either be seen in an anaesthetic clinic or, if deemed healthy enough, admitted one day before surgery for preoperative assessment, and informed consent for the study will be sought at this time.

Patients will be allocated to one of two groups according to computer-generated random numbers and they will receive either sevoflurane inhalational anaesthesia (SEVO) or intravenous anaesthesia with propofol (TIVA).

Exclusion criteria:

Pre-operative factors	Intra-operative factors
- Contraindication to general anaesthesia - Autoimmune / Chronic inflammatory diseases e.g. Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA) etc. - Chemotherapy in the past year - Steroid therapy - Surgery for tumour removal in the past year - patients post liver transplantation	- Regional Anaesthesia Administration of: - Nitrous oxide - Inhalational agents (with the exception of sevoflurane)

Pre-operative care

Pre-operative assessment will be performed at the preadmission clinic or at the general ward. Patients will be fasted for intake of solid food for 6 hours and clear liquids for 2 hours. Sedative premedication will not be prescribed.

Anaesthesia and intraoperative care

SEVO Group

Patients from the SEVO group will be anaesthetized according to the following protocol:

On arrival at the operation theatre, an intravenous cannula will be inserted. Standard monitoring with pulse oximeter, non-invasive blood pressure, and three-lead electrocardiogram will be applied prior to induction. Non-invasive blood pressure (NIBP) will be checked at least every 5 minutes throughout the operation. An arterial line may be inserted at the discretion of the anaesthetist before or after induction, with invasive blood pressure monitoring in lieu of NIBP if deemed necessary. Additional intravenous cannulas or central lines may also be inserted to facilitate fluid therapy, measure the central venous pressure or administer drugs, also at the discretion of the attending anaesthetist.

Anaesthesia will be induced with titrated, manual propofol injection of 1-2 $mg.kg^{-1}$. Remifentanil infusion will be given for analgesia at a rate of 0.1 – 0.35 $mcg.kg.min^{-1}$ as required, according to haemodynamic parameters. Cisatracurium 0.15 $mg.kg^{-1}$ or atracurium 0.5 $mg.kg^{-1}$ will be used for muscle relaxation. Tracheal Intubation will be performed after induction of general anaesthesia. General anaesthesia monitoring will be used. Sevoflurane at 0.5- 1 MAC, air and oxygen will be used for maintenance of general anaesthesia. Processed EEG (BIS™) will be utilized to monitor depth of anaesthesia, aiming at a BIS value of 40-60. FiO_2 will be kept between 35-50% to maintain and SaO_2 of > 95%. Further muscle relaxants can be given during the operation if required.

In accordance with British consensus guidelines on intravenous fluid therapy for Adult Surgical Patients [22], intraoperative fluid or blood loss will be initially replaced with balanced crystalloid solution, preferably Plasmalyte A (a physiologically balanced electrolyte solution similar to extracellular fluid), for up to 20% of body volume, with additional fluid replacement with colloid or crystalloid at the discretion of the anaesthetist. Intravenous phenylephrine, ephedrine or fluid administration may also be given for management of hypotension. Intravenous anti-hypertensive agents such as beta blockers (e.g. esmolol, labetalol) and glyceryl trinitrate can be given if hypertension occurs.

Patients will receive standardized opioid analgesia of 0.1 $mg.kg^{-1}$ intravenous morphine intra-operatively followed by patient-controlled analgesia (PCA) morphine post-operatively, as well as local anaesthesia (0.5% levobupivacaine) injected in the areas of surgical incision during wound closure for pain control.

Forced air warming blankets will be used with the aim of keeping a core temperature of 35.5-37.5 degrees Celsius. Ondansetron 4mg IV will be given 30 minutes before end of surgery.

Sevoflurane and remifentanil infusion will be switched off at the end of the procedure. Reversal of muscle relaxation can be achieved if required with neostigmine 50 $mcg.kg^{-1}$ IV and atropine 20 $mcg.kg^{-1}$ IV. Patients will subsequently be transferred to the post anaesthetic care unit (PACU) for monitoring for at least 30 minutes.

TIVA Group

Patients in the TIVA group will be anaesthetized according to the following protocol:

Monitoring and other anaesthetic procedures including the management of hypertension and hypotension will be the same as SEVO group. Induction and maintenance of general anaesthesia will be conducted using total intravenous infusion of propofol. Sevoflurane will not be used, and oxygen and air would be given to provide a FiO_2 of 30-50%.

Target controlled infusion (TCI) with a modified Marsh effect site model (Fresenius Kabi) will be used for induction and maintenance of general anaesthesia and titrated to effect. The usual effect site concentration is 1.5-3 $mcg.ml^{-1}$ and BIS monitoring will also be used to produce a value of between 40-60. As with patients in the SEVO group, remifentanil will be infused at a rate of between 0.1-0.3 $mcg.kg.min^{-1}$.

Post-operative care for both groups

In the recovery room after surgery, numerical rating pain score (NRS) will be used to assess level of pain. Patients will select a whole number (0-10 integers) that best reflects the intensity of his/her pain, in which 10 represents the maximal imaginable pain. Boluses of 2 mg intravenous morphine will be given every 5 minutes until the numerical rating scale (NRS) is less than or equal to 4/10. A patient controlled analgesia (PCA) machine will then be connected. The machine will be configured to give 1 mg of morphine whenever the patient demands with the lockout duration set to 5 minutes. No background infusion will be given and the maximum dose limit will be 0.1 $mg.kg^{-1}$ per hour of morphine.

On post-operative day 1, when the patient resumes a fluid diet, oral celecoxib 200 mg will be given twice daily for 3 days.

Whilst on PCA morphine, the patient's respiratory rate, oxygen saturation (SpO_2) and sedation score will be monitored every hour. Heart rate and blood pressure will be checked every 4 hours. NRS pain scores at rest and during cough/movement, cumulative PCA morphine doses, and number of PCA demands/goods delivered, and side effects (nausea, vomiting, dizziness, hypotension, desaturation) will be recorded every 4 hours. Patients will be assessed by a "pain team" every day to determine sufficiency of analgesia, as per usual practice.

Patients will be kept on PCA morphine for at least 2 days. If NRS pain scores during cough/ movement on postoperative day 2 are less than or equal to 3/10, PCA morphine will be stopped. PCA morphine will be continued if NRS is equal or greater than 4, or if the patient remains on a high PCA use. Assessment for analgesia will be conducted daily. Evaluation for complications will be required if NRS pain score is 4 or higher on postoperative day 5. The patient will be further managed at the discretion of the anaesthetist.

After PCA morphine has been withdrawn, NRS pain scores at rest and during cough/ movement, as well as the dose and frequency of rescue analgesia used will be charted once a day until discharge.

Patient data collection

Patient data collected will include: anaesthetic technique, age at the time of surgery, sex, functional classification according to the American Society of Anesthesiologists (ASA) class, severity of surgery, procedure, tumour size, and use of opioids. This data will be obtained from the anaesthetic record and the hospital electronic patient record system.

Patients will be followed up for three-year survival.

Blood-taking *(a total of 15mls on each occasion)* will take place at four time-points:

1. *Pre-operative*

   This is for baseline assessment, and will be taken in the operation theatre from the IV cannula before induction of anaesthesia.

2. *Intra-operative*

   This time point will be up to the discretion of the surgeon. As soon as the surgeon deems the tumour to be resected, the attending anaesthetist will take the blood sample.

3. *Immediately post-operative*

   This will be taken outside the operating theatre 30 mins after arrival in the recovery room.

4. *24 hours post-surgery*

   This will be taken in the general ward or intensive care unit (ICU).

Biochemical assessment of blood samples

Around 15mls of venous or arterial blood will be taken at each time point, and will be analysed for the following biomarkers:

Table 1

Biomarker	Sample	Method
Gene expression (HIF-1, IL-6, TNF-alpha)	RNA/cDNA	PCR
DNA damage (Comet Assay)	White cells	manual
hsCRP	Plasma	Commercial Kit
Glutathione Peroxidase	RBC	Commercial Kit
Superoxide dismutase	"	"
8-oxoGuo, 8-oxodG	Plasma	"
IL-6, TNF-alpha, HIF-1	plasma	ELISA
Circulating tumour cells	Whole blood	Isolation by Size of Tumour Cells

Primary outcome

The primary outcome of this study is the detection of a two-fold difference between the pre-operative blood sample and 24-hour post-operative blood sample in HIF-1 gene expression in the SEVO group, and a less than two-fold difference in the same samples of the TIVA group.

The secondary outcome is a reduction in the incidence of two-year recurrence from 40% in the SEVO group to 20% in the TIVA group.

Other secondary outcomes are listed in Table 1.

Sample size calculation

Using the primary outcome of HIF-1 gene expression of at least 2-fold difference between time points, power of 0.9 and standard deviation (SD) of 0.9, 22 patients are required in each group. To account for drop-outs, 25 patients will be recruited into each group, making a total of 50 patients.

Using the secondary outcome of two-year recurrence after hepatectomy of around 40% and an expected two-year occurrence of 20% in the TIVA group, 108 patients must be recruited into the study (total of 216 in both groups, to account for potential drop-outs 220 patients will be recruited) with an alpha of 0.05 and power of 0.9.

For this current study preliminary analysis and publication of data will be conducted after 50 patients have been recruited. Recruitment will continue until 220 patients have completed the study.

Statistical analysis

Patient demographics, disease stage, and surgery and laboratory biomarker data will be compared between TIVA and SEVO groups using chi-square and *t* tests as appropriate. The Kaplan–Meier method will be used to calculate the overall survival of patients from the date of surgery to the date of death; patients alive will be censored at the follow-up closure date. A Cox proportional hazard regression model will be used to compare the hazard of the two groups by using a univariate model.

Impact of the study and concluding remarks

Thousands of patients with a cancer diagnosis undergo surgery in Hong Kong every year. If it can be shown that anaesthetic technique can impact survival after tumour resection surgery, then this will inform both clinicians and patients regarding the most suitable method of anaesthesia for their particular situation. Through biochemical analysis of blood samples taken pre, intra, and post-surgery, the mechanisms through which anaesthetic techniques may mitigate cancer recurrence after hepatectomy can be elucidated.

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