Journal of Visualized Experiments

An in vivo mouse model of total intravenous anesthesia during cancer resection surgery

--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE62747R1
Full Title:	An in vivo mouse model of total intravenous anesthesia during cancer resection surgery
Corresponding Author:	Erica Sloan Monash University Parkville, VIC AUSTRALIA
Corresponding Author's Institution:	Monash University
Corresponding Author E-Mail:	Erica.Sloan@monash.edu
Order of Authors:	Julia Dubowitz
	Fabian Jost-Brinkmann
	Alexandra Ziegler
	Ryan Gillis
	Bernhard Riedel
	Erica Sloan
Additional Information:	
Question	**Response**
Please specify the section of the submitted manuscript.	Cancer Research
Please indicate whether this article will be Standard Access or Open Access.	Standard Access ($1400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Melbourne, Victoria, Australia
Please confirm that you have read and agree to the terms and conditions of the author license agreement that applies below:	I agree to the Author License Agreement
Please provide any comments to the journal here.	An additional author has been added. Permissions from the other authors for this addition are included in the rebuttal letter.

TITLE:

An *In Vivo* Mouse Model of Total Intravenous Anesthesia during Cancer Resection Surgery

AUTHORS AND AFFILIATIONS:

Julia A Dubowitz[1,2,3], Fabian Jost-Brinkmann[1,4,5], Alexandra I. Ziegler[1], Ryan Gillis[1], Bernhard Riedel[1,2,3,6], Erica K. Sloan[1,2]

[1]Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne VIC 3052, Australia

[2]Department of Anaesthesia, Division of Cancer Surgery, Peter MacCallum Cancer Centre, Melbourne VIC 3000, Australia

[3]Department of Critical Care, Melbourne Medical School, University of Melbourne, Melbourne VIC 3000, Australia

[4]Medical Department, Division of Hepatology and Gastroenterology, Charité – Universitätsmedizin Berlin, Germany

[5]Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

[6]Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne VIC 3000, Australia

Email addresses of co-authors:

Julia A Dubowitz	(Julia.dubowitz@petermac.org)
Fabian Jost-Brinkmann	(fabian.jost-brinkmann@charite.de)
Alexandra I. Ziegler	(alexandra.ziegler@monash.edu)
Ryan Gillis	(ryan.gillis@monash.edu)
Bernhard Riedel	(bernhard.riedel@petermac.org)
Erica K. Sloan	(erica.sloan@monash.edu)

Corresponding Author:

Erica K. Sloan (erica.sloan@monash.edu)

SUMMARY:

This paper describes a method for modeling total intravenous anesthesia (TIVA) during cancer resection surgery in mice. The goal is to replicate key features of anesthesia delivery to patients with cancer. The method allows investigation of how anesthetic technique affects cancer recurrence after resection surgery.

ABSTRACT:

Anesthesia is a routine component of cancer care used for diagnostic and therapeutic procedures. The anesthetic technique has recently been implicated in impacting long-term cancer outcomes, possibly through modulation of adrenergic-inflammatory responses that impact cancer cell behavior and immune cell function. Emerging evidence suggests that propofol-based total intravenous anesthesia (TIVA) may be beneficial for long-term cancer outcomes when compared to inhaled volatile anesthesia. However, the available clinical findings are inconsistent.

Preclinical evidence that provides insight into the underlying mechanisms involved is critically needed to guide the design of clinical studies that will expedite insight. Most preclinical models of anesthesia have been extrapolated from the use of anesthesia in *in vivo* research and are not optimally designed to study the impact of anesthesia itself as the primary endpoint. This paper describes a method for delivering propofol-TIVA anesthesia in a mouse model of breast cancer resection that replicates key aspects of clinical delivery in cancer patients. The model can be used to study mechanisms of action of anesthesia on cancer outcomes in diverse cancer types and can be extrapolated to other non-cancer areas of preclinical anesthesia research.

INTRODUCTION:

More than 60% of patients with cancer receive anesthesia for surgical resection[1]. Currently, there are no specific clinical guidelines that determine the choice of anesthesia used in cancer patients. Surveys of anesthesiologists indicate a preference for volatile-based anesthesia, including during cancer surgery[2,3]. However, there is a growing body of evidence that the use of propofol-based total intravenous anesthesia (TIVA) during cancer surgery may associate with improved postoperative outcomes (disease-free survival, overall survival) when compared to volatile anesthesia[4]. Subsequent clinical studies continue to report contradicting results[5–8]. These findings support the need for preclinical studies to better understand the mechanistic effects of different anesthetic agents on cancer-related outcomes.

However, in *in vivo* studies that model cancer surgery, anesthesia is frequently an incidental part of the procedure. Anesthesia is often not considered in the experimental design, and its impact on cancer-related endpoints may not be evaluated. For example, *in vivo* studies that require maintenance of anesthesia for cancer surgery most commonly use inhaled volatile anesthesia[9]. Where propofol has been used in *in vivo* studies, it has been delivered by single bolus dosing with intraperitoneal delivery, which does not replicate clinical onco-anesthetic protocols[10]. This approach of propofol administration induces light anesthesia that is suitable for rapid procedures. However, it does not allow maintenance of anesthesia that is required for cancer resection surgery which may be protracted. Furthermore, the absorption kinetics of intraperitoneal delivery is distinct to clinical methods of administration.

A model of propofol-based TIVA for cancer resection surgery was developed to address this need. A protocol for sustained maintenance of anesthesia with titration of the anesthetic agent to allow response to the surgical stimulus was developed to replicate key aspects of anesthetic delivery to patients having cancer surgery. The resulting protocol is used with a mouse model of cancer to provide TIVA during cancer resection surgery. The effect on short-term and long-term cancer-related outcomes is evaluated.

PROTOCOL:

All animal studies were undertaken under the approval of the Institutional Animal Care and Use Committee at Monash University. In this study, female Balb/c mice aged 6-8 weeks were used.

1. **Prepare cancer cells**

1.1 Culture tumor cells in medium. Culture 66cl4 murine mammary cancer cells in alpha-MEM containing 10% FBS and 200 mM glutamine. Use cells that are stably transduced to express firefly luciferase for bioluminescence imaging to enable cancer recurrence monitoring after resection surgery[11] (see step 4).

NOTE: The cell line and the medium mentioned above were used in this study.

1.2 Grow cells at 37 °C with 5% CO_2. Passage cells at <80% confluency. Use low-passage cells in the logarithmic growth phase for optimal *in vivo* results.

1.3 Lift the adherent cells with 0.5 mg/mL of trypsin in PBS with 10 mM EDTA; 2 mL for a T75 flask. Count the cells using a hemocytometer. Dilute the cells in PBS for injection. For 66cl4 mammary cancer cells, inject 1 x 10^5 cells in 20 µL of PBS per mouse.

1.4 Place the cells on ice prior to the injection.

2. Generate a mouse model of breast cancer

2.1 Use 4% isoflurane to anesthetize the mouse in an induction chamber. Then, maintain anesthesia with 2%–3% isoflurane using a nose cone. Confirm proper anesthetization by lack of response to toe pinch.

2.2 Prepare the injection site by wiping the fourth left mammary fat pad area using a single-use alcohol swab.

2.3 Draw up tumor cells (see step 1.4) into a 25 µL Hamilton syringe attached to a sterile 27 G hypodermic needle.

2.4 Inject the cells into the fourth left mammary fat pad. Use forceps to secure and lift the skin. Inject approximately 1 mm from the nipple.

2.5 OPTIONAL: If cells are tagged with luciferase, confirm successful injection of tumor cells by bioluminescence imaging. Inject 100 µL of 150 mg/kg D-luciferin into the lateral tail vein of the anesthetized mice using a 0.5 mL insulin syringe with a 30 G hypodermic needle.

2.6 OPTIONAL: Place the mouse in a bioluminescence imaging system with the mammary fat pad facing up. Wait for 2 min from the luciferin injection for optimal tissue uptake of luciferin, then image for 10 s.

2.7 Place the mouse in a clean cage and allow it to recover from anesthesia.

2.8 Continue to monitor animal welfare as per the institutional animal ethics guidelines.

3. Induce stable anesthesia with intravenous delivery of propofol

3.1 Monitor growth of the primary tumor using caliper measurement and calculate the tumor volume using the equation: Volume (mm^3) = (length x $(width)^2 \div 2$).

3.2 Perform tumor resection surgery on mice when the primary tumor reaches the required volume (here, 80–90 mm^3).

3.3 Set up an automated syringe pump with a 30 G 1 mL insulin syringe containing propofol formulation (2% Lipuro propofol) (**Figure 1A**).

3.4 Induce anesthesia of the mouse in an induction chamber with 3% sevoflurane or isoflurane.

NOTE: Here, sevoflurane was used as this is the predominant volatile used clinically.

3.5 Transfer the mouse to a 37 °C heating pad for the duration of the surgery. Briefly maintain anesthesia with 2%–3% sevoflurane using a nose cone.

3.6 To deliver propofol-based TIVA, cannulate the lateral tail vein using a sterile 30 G hypodermic needle attached to a sterile polyurethane catheter. Confirm correct placement by blood flashback into the catheter (**Figure 1B**). Adjust the delivery of sevoflurane as required during intravenous cannulation to maintain a stable depth of anesthesia demonstrated by loss of corneal and pedal reflex, and respiratory rate < 100 breaths per min.

3.7 Commence propofol-TIVA by administering 2% propofol as an initial bolus of 27 mg/kg for over 1 min. Cease sevoflurane administration.

3.8 Continue the infusion of propofol at a maintenance rate of 2.2–4.0 mg/kg/min to maintain a stable depth of anesthesia for the duration of the surgery (**Figure 1C**).

4. Resect the primary tumor

4.1 Apply aqueous lubricant to the eyes to prevent drying. To prepare for surgery, shave the abdomen and prepare the skin for surgery with an iodine-povidone solution.

4.2 Inject 0.05 mg/kg of buprenorphine subcutaneously for analgesia.

4.3 Make a 1 cm incision inferior to the tumor in the region of the left fourth mammary fat pad. Carefully resect the tumor and the left inguinal lymph node using dissection with blunt forceps.

4.4 OPTIONAL: If using luciferase-tagged tumor cells, use bioluminescence imaging to confirm clear surgical margins. Inject 150 mg/kg D-luciferin into the lateral tail vein, wait for 2 min, and

then image for 60 s using a bioluminescence imaging system. If a residual tumor is identified, resect additional tissue from the mammary fat pad and re-image to achieve clear margins.

4.5 Ensure hemostasis at the surgical site and close the skin using 5-0 nylon sutures.

4.6 Wipe the wound with povidone or iodine solution.

4.7 At the conclusion of resection surgery, cease anesthesia. Place the mouse in a clean cage on a 37 °C heating pad and allow it to recover from anesthesia.

4.8 Monitor every 15 min after anesthesia until the mouse has returned to normal alertness. Then, monitor the mouse every 12 h for 48 h after surgery.

4.9 Administer 0.05 mg/kg of buprenorphine subcutaneously every 12 h for 48 h after surgery.

4.10 After 7–10 days, remove sutures using sterile curved stitch cutters under brief sevoflurane or isoflurane anesthesia.

5. Track cancer recurrence with *in vivo* imaging

5.1 Use bioluminescence imaging to track cancer recurrence after resection surgery non-invasively. Use a bioluminescence imaging system to monitor the mice once per week for evidence of primary tumor recurrence or distant recurrence, commencing the week following surgery.

5.2 Induce anesthesia of the mouse in an induction chamber with 4% isoflurane. Then, transfer the mouse to a 37 °C heating pad for the duration of anesthesia and maintain anesthesia with 2%–4% isoflurane using a nose cone.

5.3 Apply aqueous lubricant to the eyes to prevent drying.

5.4 Inject D-luciferin 150 mg/kg into the lateral tail vein. Wait for 2 min, then measure bioluminescence over a 60 s exposure to detect recurrence of the primary tumor or distant metastasis.

5.5 If the primary tumor recurs and becomes palpable, commence monitoring of tumor growth using caliper measurements.

5.6 At the end of the experiment, humanely kill the mice according to the approved protocol. Here, CO_2 was used, followed by cervical dislocation.

REPRESENTATIVE RESULTS:

This method describes a model of total intravenous anesthesia (TIVA) with propofol during cancer resection surgery in mice. Propofol is delivered in this mouse model through an intravenous catheter using a syringe pump (**Figure 1A,B**) to replicate delivery of TIVA in the clinical setting of anesthesia for cancer surgery. Use of the syringe pump minimizes exposure to volatile anesthesia by allowing rapid conversion from initial induction by inhalational anesthesia to intravenous delivery.

After stable anesthesia was achieved by propofol-based TIVA, the primary mammary tumor was resected. *In vivo* bioluminescence imaging was used to confirm complete resection of the primary tumor (**Figure 2**). Regular monitoring of mice by non-invasive *in vivo* bioluminescence imaging identified distant recurrence by luciferase-tagged tumor cells in the lung (**Figure 2**). This method is also suitable to track local recurrence in the mammary fat pad.

In addition to tracking long-term events such as recurrence, the model may be used to assess events that occur during the perioperative period. These early events may provide mechanistic insight into the effects of anesthesia and other surgical factors on cancer-related outcomes. After 24 h of the cancer surgery under propofol, a multiplex enzyme-linked immunosorbent assay was used to quantify circulating plasma cytokines (**Figure 3**). Cytokines were evaluated in 7 mice; the appropriate group size will be influenced by the effect size of the endpoint of interest.

FIGURE AND TABLE LEGENDS:

Figure 1: Experimental set-up for propofol-based TIVA. (**A**) A syringe pump is used to ensure the controlled delivery of propofol from a 1 mL insulin syringe. (**B**) Propofol is delivered into the lateral tail vein by intravenous catheter, connected to a syringe pump, via a 30 G needle. The asterisk shows the needle insertion point. (**C**) Schematic illustrating the plasma concentration of propofol achieved by sequential administration of a bolus followed by a constant infusion using the syringe pump, compared to delivery by bolus or infusion only.

Figure 2: Cancer progression after surgical resection of the primary mammary tumor under propofol-based TIVA. Non-invasive bioluminescence imaging of luciferase-tagged tumor cells was used to track initial growth of the primary tumor growth, successful surgical resection, and subsequent distant recurrence to the lung.

Figure 3: Circulating cytokine levels measured after cancer resection under propofol-based TIVA. Multiplex enzyme-linked immunosorbent assay was used to quantify plasma cytokines 24 h after surgery. Each data point represents data from one mouse. Lines show mean and standard error (N = 4–7).

DISCUSSION:

This study reports on a protocol for administering total intravenous anesthesia (TIVA) with propofol in a mouse model of breast cancer that replicates key aspects of clinical practice for TIVA in patients requiring cancer surgery. The protocol allows investigation of both short-term and long-term clinically relevant outcomes after cancer surgery in a mouse model of cancer progression, including measurement of cytokine levels and cancer recurrence (**Figure 2**, and

Figure 3). The methodology will be useful for the evaluation of the effects of TIVA on cancer-related outcomes and its comparison with other anesthetic techniques such as volatile anesthesia.

In contrast to existing protocols that deliver a single intraperitoneal bolus of propofol for sedation during minor interventions such as blood sampling delivery[10], this protocol allows prolonged intravenous delivery of propofol for maintenance of anesthesia during major interventions such as surgery. Both the induction and maintenance dose of propofol was carefully titrated to optimize anesthetic depth suitable for major surgical interventions while minimizing mortality from hypotension or cardiac arrest. It was found that hypotension could be avoided by using a strict induction dose of 27 mg/kg administered over 60 s, with concurrent down-titration and cessation of inhaled sevoflurane. Maintenance was achieved using an infusion of propofol at 2.2–4.0 mg/kg/min. During major surgical interventions, such as cancer resection, titration within this range was important to respond to and obtund the magnitude of surgical stimulation. This replicates clinical practice and prevents over-dosing anesthesia, which can result in hypotension or death, and under-dosing, which can result in emergence from anesthesia, movement, or surgical stress.

A limitation of the model is the brief use of volatile anesthesia to induce anesthesia prior to canulation initially. This approach was chosen because of the ease of cannulation after induction of anesthesia, allowing the rapid delivery of a therapeutic dose of propofol to continue anesthesia. In addition, mice were briefly anesthetized with volatile anesthesia for tumor cell inoculation, to remove suture, and for imaging. Sevoflurane was used for volatile anesthesia during resection surgery as it is often used in clinical practice. However, isoflurane is also used in clinical practice. Future studies may use a single agent for all episodes of inhalation anesthesia. With experience, less than 2 min elapsed from loss of righting reflex in response to volatile anesthesia exposure to commencement of propofol dosing. Nonetheless, for analyses that intend to compare propofol-based TIVA with inhalational volatile anesthesia techniques, interpretation may be confounded by even the brief use of volatile anesthesia.

An alternative approach to volatile anesthesia induction is to cannulate the lateral tail vein of an awake mouse. While not suitable for all situations, this may provide an alternative to inhalation anesthesia for tumor resection surgery. However, movement of the awake mouse may result in the cannula being dislodged, leading to failure of anesthesia induction. In addition, movement of the needle can result in extravasation of propofol from the vein, which puts the tail at risk of tissue necrosis. This has welfare implications for the mouse, and any associated adrenergic activation as a result of physiological stress may impact the validity of the observed results[11].

An additional potential limitation is the use of buprenorphrine as a postoperative analgesic. Opioids may modulate postoperative inflammatory and immune responses[12,13]. Buprenorphine was chosen for analgesia as its effects on the immune response are less than for other opiates[13]. Nonetheless, future studies might consider the use of non-opioid analgesic agents.

Despite advances in oncological therapy, local and distant cancer recurrence can occur after surgery and remains a dominant cause of mortality in cancer patients. Many patients will be exposed to anesthesia, often multiple times, during diagnostic and therapeutic operative procedures. A growing body of evidence from *in vivo* and *in vitro* models implicates anesthetic agents in modulating the perioperative response to surgery and impacting diverse aspects of tumor cell biology[14]. To better understand the impact of anesthetic agents on cancer progression, the model of intravenous propofol anesthesia developed here will be important in future mechanistic preclinical research. This model may be used to interrogate the mechanisms underlying the effects of anesthetic agents on immunomodulation, perioperative inflammatory response, and tumor cell growth and invasion. Furthermore, this model could be extrapolated for use in non-cancer surgery research where anesthetic agents may have effects on other systems, such as cardiac surgery, trauma research, or critical illness (e.g., sepsis) as propofol is a common sedation used in intensive care units.

ACKNOWLEDGMENTS:

The authors wish to thank members of the Cancer Neural-Immune Laboratory and Dr. Cameron Nowell at Monash Institute of Pharmaceutical Sciences, Monash University, Parkville. This work was supported by grants from National Health and Medical Research Council 1147498, the National Breast Cancer Foundation IIRS-20-025, the Australian and New Zealand College of Anaesthetists (ANZCA), Perpetual and CTC for Cancer Therapeutics.

DISCLOSURES:

The authors declare no competing financial interests.

REFERENCES:

1. Sullivan, R. et al. Global cancer surgery: delivering safe, affordable, and timely cancer surgery. *Lancet Oncology*. **16** (11), 1193–1224 (2015).
2. Lim, J. A. et al. The effect of propofol and sevoflurane on cancer cell, natural killer cell, and cytotoxic T lymphocyte function in patients undergoing breast cancer surgery: an in vitro analysis. *BMC Cancer*. **18** (1), 159 (2018).
3. Pandit, J. J. et al. 5th National Audit Project (NAP5) on accidental awareness during general anesthesia: protocol, methods, and analysis of data. *British Journal of Anaesthesia*. **113** (4), 540–548 (2014).
4. Yap, A., Lopez-Olivo, M. A., Dubowitz, J., Hiller, J., Riedel, B. Anesthetic technique and cancer outcomes: a meta-analysis of total intravenous versus volatile anesthesia. *Canadian Journal of Anesthesia*. **66** (5), 546–561 (2019).
5. Makito, K., Matsui, H., Fushimi, K., Yasunaga, H. Volatile versus total intravenous anesthesia for cancer prognosis in patients having digestive cancer surgery. *Anesthesiology*. **133** (4), 764–773 (2020).
6. Oh, T. K., Kim, H. H., Jeon, Y. T. Retrospective analysis of 1-year mortality after gastric cancer surgery: Total intravenous anesthesia versus volatile anesthesia. *Acta Anaesthesiologica Scandinavica*. **63** (9), 1169–1177 (2019).
7. Lai, H. C. et al. Propofol-based total intravenous anesthesia is associated with better survival than desflurane anesthesia in hepatectomy for hepatocellular carcinoma: a retrospective

cohort study. *British Journal of Anaesthesia*. **123** (2), 151–160 (2019).
8. Hong, B. et al. Anesthetics and long-term survival after cancer surgery-total intravenous versus volatile anesthesia: a retrospective study. *BMC Anesthesiology*. **19** (1), 233 (2019).
9. Flecknell, P. *Laboratory Animal Anaesthesia*. Fourth edition, **Chapter 3**, Special Techniques Elsevier (2015).
10. Cicero, L., Fazzotta, S., Palumbo, V. D., Cassata, G., Lo Monte, A. I. Anesthesia protocols in laboratory animals used for scientific purposes. *Acta Biomedica*. **89** (3), 337–342 (2018).
11. Sloan, E. K. et al. The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Research*. **70** (18), 7042–7052 (2010).
12. Al-Hashimi, M., Scott, S. W. M., Thompson, J. P., Lambert, D. G. Opioids and immune modulation: more questions than answers. *British Journal of Anaesthesia*. **111** (1), 80–88 (2013).
13. DeMarco, G. J., Nunamaker, E. A. A Review of the effects of pain and analgesia on immune system function and inflammation: relevance for preclinical studies. *Comparative Medicine*. **69** (6), 520–534 (2019).
14. Hiller, J. G., Perry, N. J., Poulogiannis, G., Riedel, B., Sloan, E. K. Perioperative events influence cancer recurrence risk after surgery. *Nature Reviews Clinical Oncology*. **15** (4), 205–218 (2018).

Figure 1

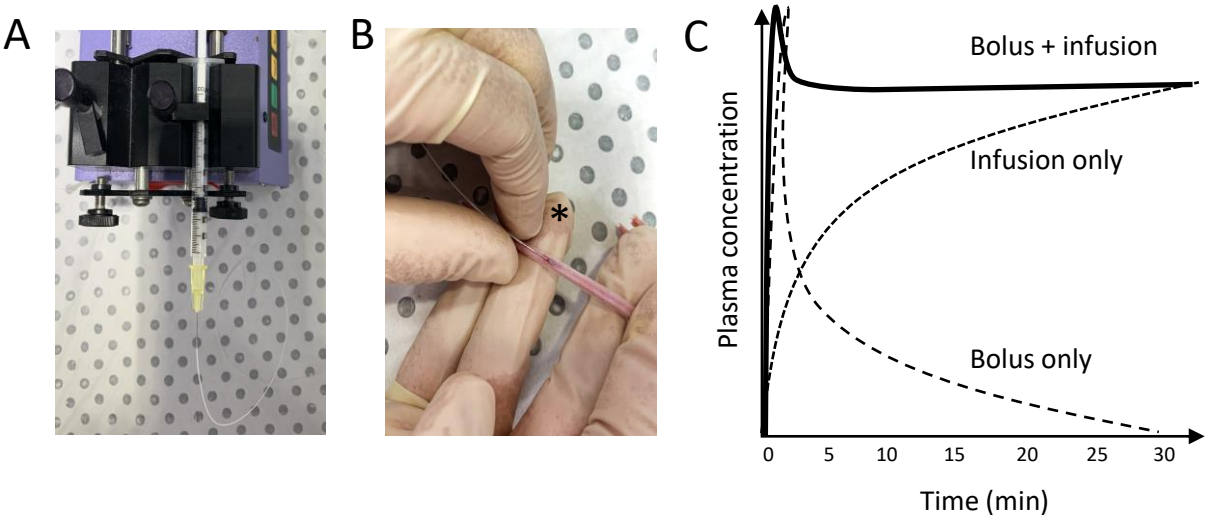
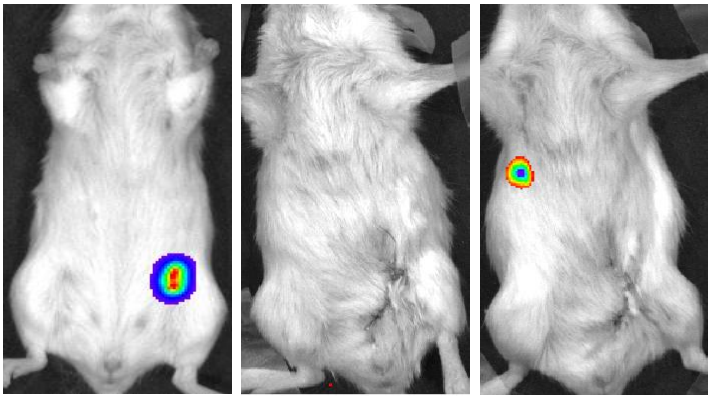
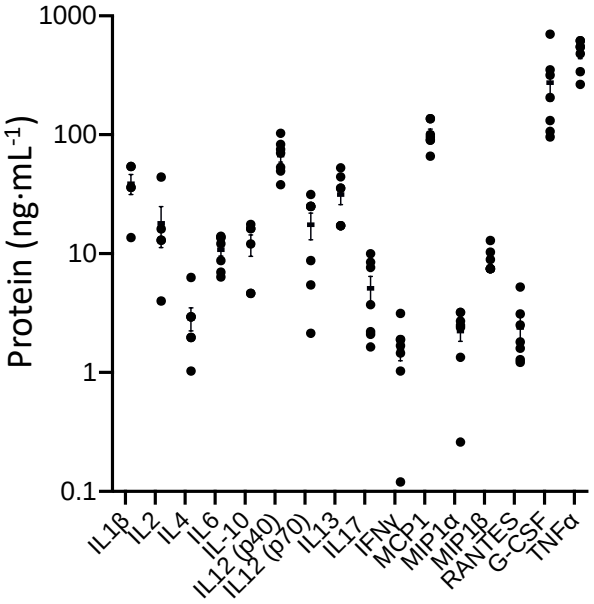


Figure 2



Primary tumor | After resection surgery | Distant recurrence

Figure 3



Name of Material/ Equipment	Company	Catalog number
0.9% saline	Fresnius Kabi	AUST R 197198
Artery forceps	Proscitech	TS1322-140
Buprenorphine	Temgesic	TEMG I
Heated surgical mat	Custom	-
Hypodermic needle (30 G, 1 mL insulin syringe)	Terumo	NN3013R
IVIS Lumina	PerkinElmer	126274
Luciferin	Promega	P1041/2/3
Polyurethane catheter	Intramedic	427401
Povidone Iodine	Betadine	AUST R 29562
Propofol Lipuro, 2%	Braun	3521490
Sevoflurane	Baxter	ANZ2L9117
Sevoflurane vaporiser	Vetquip	VQ1334
Sterile gauze	Multigate Medical	11-600A
Surgical scissors	Proscitech	TS1044
Sutures, 5-0 nylon	Dynek	V504
Syringe pump	Harvard Apparatus	70-4500
Syringes (1 mL)	Terumo	SS+01T

May 21, 2021

Dear Editors,

Thank you for considering our revised article, "An *in vivo* mouse model of total intravenous anesthesia during cancer resection surgery" for publication in JoVE.

We have addressed each of the reviewers' comments as described in the point-by-point response. We include a revised manuscript with the changes highlighted in red.

We now include an additional author, Mr Ryan Gillis. Each of the original authors has given permission for this inclusion (see below). Mr Gillis is a research assistant who is familiar with the model and prepared the revision. He will be involved in filming for the article, as the other authors are not available due to clinical commitments.

We confirm that all authors have approved the manuscript for submission and that the content of the manuscript has not been published or submitted for publication elsewhere.

Thank you for your re-consideration of our manuscript.

Yours sincerely,

Erica Sloan

Permissions for addition of a new author

---------- Forwarded message ---------
From: **Dubowitz Julia** <Julia.Dubowitz@petermac.org>
Date: Mon, 10 May 2021 at 13:24
Subject: Submission
To: Erica Sloan <erica.sloan@monash.edu>
Cc: Riedel Bernhard <Bernhard.Riedel@petermac.org>

Dear Erica
This is to confirm that I agree with addition of Ryan Gillis as a co-author on the manuscript: An *in vivo* mouse model of total intravenous anesthesia during cancer resection surgery.
Yours sincerely,

Dr Julia Dubowitz (Kelly), MBBS, FANZCA
Specialist anaesthetist, Peter MacCallum Cancer Centre
PhD candidate, Monash University
Clinical Fellow, Department of Critical Care, University of Melbourne

E: julia.dubowitz@petermac.org
Ph: 0411 016 828

---------- Forwarded message ---------
From: **Jost-Brinkmann, Fabian** <fabian.jost-brinkmann@charite.de>
Date: Tue, 11 May 2021 at 02:04
Subject: AW: [ext] JoVE publication
To: Erica Sloan erica.sloan@monash.edu

Dear Erica
This is to confirm that I agree with addition of Ryan Gillis as a co-author on the manuscript: An in vivo mouse model of total intravenous anesthesia during cancer resection surgery
Sincerely
Fabian Jost Brinkmann

---------- Forwarded message ---------
From: **Alexandra Ziegler** <alexandra.ziegler@monash.edu>
Date: Mon, 10 May 2021 at 12:01
Subject: JOVE paper Co-author Ryan Gillis
To: Erica Sloan <Erica.Sloan@monash.edu>

Dear Erica,

This is to confirm that I agree with addition of Ryan Gillis as a co-author on the manuscript: An in vivo mouse model of total intravenous anesthesia during cancer resection surgery.
Sincerely
Alex

--
Alexandra Ziegler, PhD
Senior Research Coordinator
Monash Institute of Pharmaceutical Sciences
Faculty of Pharmacy and Pharmaceutical Sciences
Monash University
Room G39, Building 404
399 Royal Parade
Parkville VIC 3052
Australia

T: +61 (3) 9903 9274
Web: https://www.monash.edu/pharm

CRICOS Provider 00008C

---------- Forwarded message ---------
From: **Riedel Bernhard** <Bernhard.Riedel@petermac.org>
Date: Wed, 12 May 2021 at 22:14
Subject: Co-author Approval
To: Erica Sloan <erica.sloan@monash.edu>

Dear Erica

This is to confirm that I agree to add Ryan Gillis as a co-author on the manuscript: An in vivo mouse model of total intravenous anesthesia during cancer resection surgery.

Yours sincerely,
Bernhard

_ _
Professor Bernhard Riedel | MB.ChB. FCA. FANZCA. FASE. MMed. MBA. PhD.
Director, Department of Anaesthesia, Perioperative and Pain Medicine
PETER MAC CALLUM CANCER CENTRE
Phone +61 3 8559 7663 (Julie Reynolds)
Mobile 0438 508 258
Email Bernhard.Riedel@petermac.org

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

- We have proofread the manuscript and believe there are no spelling or grammar issues

2. Please provide an institutional email address for each author.

- Email addresses are provided on the title page.

3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

- Personal pronouns have been replaced in throughout the manuscript.

4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.For example: IVIS Lumina II system, etc.

- IVIS Lumina II system has been replaced throughout with 'bioluminescence imaging system'

5. Line 87: Please specify the volume of trypsin used.

- Section 1.3 has been amended to indicate: Lift adherent cells with 0.5mg/mL trypsin in PBS with 10mM EDTA; 2 mL for a T75 flask.

6. Line 94-95: Please mention how proper anesthetization is confirmed.

- Section 2.1 has been amended to indicate how proper anesthetization is confirmed. The following sentence has been added: Proper anesthetization is confirmed by lack of response to toe pinch.

7. For SI units, please use standard abbreviations when the unit is preceded by a numeral. Abbreviate liters to L to avoid confusion. Examples: 10 mL, 8 µL, 7 cm2 (Lines 88, 100, 107, etc.)

- We have changed SI units to standard abbreviations where indicated.

8. For time units, please use abbreviated forms for durations of less than one day when the unit is preceded by a numeral. Do not abbreviate day, week, month, and year. Examples: 5 h, 10 min, 100 s, 8 days, 10 weeks (Lines 112, 142, 159, 170 etc.).

- We have amended indications of time to use the abbreviations indicted.

9. Please highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

- Steps 1-5 have been highlighted as they show the essential protocol for in vivo work.

10. Please do not use the &-sign or the word "and" when listing authors in the references. Authors should be listed as last name author 1, initials author 1, last name author 2, initials author 2, etc. Title case and italicize journal titles and book titles. Do not use any abbreviations. Article titles should start with a capital letter and end with a period and should appear exactly as they were published in the original work, without any abbreviations or truncations.

- We have updated to JOVE endnote style

11. Figure 1: Please specify in the figure legend what the symbol "*" represents (Figure 1B). Please revise the X-axis legend to "Time (min)" instead of "Time (minutes)".

- We have modified the legend to indicate that the asterisk indicates the needle insertion point. We have revised the X-axis legend to indicate Time (min).

12. Figure 3: Please revise the Y-axis text to "Protein (ng·mL-1)" to place the units within parenthesis. Please note that the symbol used for compound units is a middle dot "(ng·mL-1)" and not a full stop "(ng. mL-1).

- We have revised Figure 3 y-axis as indicated.

13. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please sort the Materials Table alphabetically by the name of the material.

- We have revised the table to include catalog numbers and to list in alphabetical order.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:
The anuors introduce a method to investigate the effect of propofol-based total intravenous anesthesia (TIVA) and inhaled volatile anesthesia on breast cancer outcome.

Major Concerns:
No

Minor Concerns:
1. Please consider use "female athymic nu/nu mice".

- Thank you for pointing out this oversight. An orthotopic model in Balb/c mice were used. We have now noted this under section 2.1: "Here, female Balb/c mice aged 6-8 weeks were used."

2. Did the first week need to check the recurrence or metastasis? Please consider the time to recurrence or metastasis of primary breast cancer.

- This is a good point. We now note in section 5.1 that we commenced imaging the week following surgery. This is important as recurrence may occur at any point.

Reviewer #2:

Manuscript Summary:
This novel murine TIVA model is an extremely useful tool in perioperative research, particularly for longer-term oncological outcomes. As noted in the submitted manuscript, model also useful for preclinical investigation in other areas.

Major Concerns:
None

Minor Concerns:
Some limitations to a valuable model of TIVA which could be noted?

- Thank you for pointing out the limitations below. We agree that describing these limitations in more detail will strengthen the protocol. We have added further discussion to the protocol and discussion sections as described below:

(a) Presumably a relatively homogenous group of animals was used, i.e female mice of a certain strain and age.

- Thank you for identifying this oversight. We now note in section 2.1: Here, female Balb/c mice aged 6-8 weeks were used.

(b) Authors note brief use of inhalational anaesthesia for cannulation to facilitate propofol administration (lines 262 & 263).
However, inhalational anaesthesia is used on three occasions
(1) for inoculation
(2) to facilitate intravenous access for TIVA
(3) to remove sutures.
Accept the rationale for inducing anaesthesia for cannulation regarding welfare and stress but does administration of volatile agents on multiple occasions have implications for analyses examining the impact of TIVA on longer-term oncological outcomes?

- Thank you for pointing this out. We agree it is an important implication that must be acknowledged. We have added a statement to acknowledge the additional inhalation anesthetizations. Furthermore, we note that this may confound interpretation of some analyses with the following statement: *Nonetheless, for analyses that intend to compare propofol-based TIVA with inhalational volatile anesthesia techniques, interpretation may be confounded by even the brief use of volatile anesthesia.* Finally, we discuss alternative approaches including cannulation of the awake mouse. We now note that this may not be suitable for all situations with the following statement: *While not suitable for all situations, this may provide an alternative to inhalation anesthesia for tumor resection surgery.*

(c) Evidence that buprenorphine, though commonly used for analgesia in animal studies, may influence tumour growth. Should non-opioid alternative analgesic agents be considered prior to opioid administration?

- Thank you for noting this important point. We have added a paragraph to the discussion to discuss this limitation:

An additional potential limitation is the use of buprenorphrine as a postoperative analgesic. Opioids may modulate postoperative inflammatory and immune responses. Buprenorphine was chosen for analgesia as its effects on the immune response are less than for other opiates. Nonetheless, future studies might consider the use of non-opioid analgesic agents.

(d) Line 265. "For consistency with clinical practice, we use sevoflurane for volatile anesthesia". Isoflurane is used as the inhalation agent for inoculation and to track cancer. Should a single agent be consistently be used (or suggested) for all episodes of inhalational anaesthesia?

- This is a good point. We now note that: Future studies may use a single agent for all episodes of inhalation anesthesia.

Reviewer #3:
Manuscript Summary:
This short paper describes a protocol for delivering propofol-TIVA anaesthesia in a 66cl4 mouse model of

breast cancer resection. This model is designed to better mimic key aspects of clinical delivery in cancer patients. The authors also demonstrate results on circulating cytokines. The method is well described, the protocol and figures are clear and easy to follow. The discussion is unbiased and limitations are adequately addressed. I do not see any major adverse welfare indications for IV anaesthesia in mice. Normally injectable anaesthetic top ups do not allow for control of depth of anaesthesia but with continuous perfusion via a syringe driver can overcome this allowing steady plasma concentration of anaesthetic to be maintained. I anticipate that this model would be widely used for preclinical cancer research.

Major Concerns:
none

Minor Concerns:
very minor points:
The authors should ideally state if the cells injected are in the logarithmic growth phase.

- We now specify the use of cells in the logarithmic growth phase in section 1.2: *Use low-passage cells in the logarithmic growth phase for optimal in vivo results.*

How long should the mice stay on the heating pad?

- In section 3.4, we now note that use of a heating pad is for the duration of surgery. In section 5.2, we now note that use of a heating pad is for the duration of anesthesia.

How many mice are required to measure circulating cytokines?

- We now describe in the results section: Cytokines were evaluated in 7 mice; the appropriate group size will be influenced by the effect size of the endpoint of interest.